



ESCMID STUDY GROUP  
FOR EPIDEMIOLOGICAL  
MARKERS

European Society of Clinical Microbiology and Infectious Diseases

FINAL PROGRAMME

ESCMID-ESGEM Conference

# 11th International Meeting on Microbial Epidemiological Markers (IMMEM XI)

Navigating Microbial Genomes: Insights from the Next Generation

**Estoril, Portugal**  
**9 – 12 March 2016**



[www.escmid.org/immem11](http://www.escmid.org/immem11)



## Welcome Address

Dear Colleagues and Friends,

It is a great pleasure and honour to welcome you at the eleventh edition of the International Meeting on Microbial Epidemiological Markers (IMMEM XI), now an ESCMID-ESGEM conference.

We are proud of hosting this unique conference in the beautiful city of Estoril. We feel privileged and honoured that some of the world's leading researchers have accepted our invitation to actively participate in this meeting. As is common in IMMEM's tradition we promise you an intense four days with a total of five keynote presentations, 60 oral presentations, divided into 10 plenary sessions, and two posters sessions including 144 abstracts.

Together we will explore the current changes in the understanding of disease transmission brought about by high-resolution molecular typing systems. Given its increasing importance, there will be a particular emphasis on high throughput sequencing and methodologies for complex data analysis. The meeting will also discuss missing links in the technology and bioinformatics pipelines, and how newly opened gaps in knowledge could be bridged. The conference will allow experienced researchers and newcomers to interact and exchange views on this rapidly expanding field, providing the perfect forum to discuss ongoing work and acquire skills which will become crucial for protecting populations from infection.

Inspired by the Portuguese Age of Discovery that brought "new worlds to the world" we invite you to embark with us in **Navigating Microbial Genomes: Insights from the Next Generation**.

We wish you a fruitful meeting and a wonderful stay in Estoril.

João André Carriço  
Hajo Grundmann  
Mario Poljak



## Scientific Committee & Faculty

David Aanensen (UK)  
Frank Møller Aarestrup (DK)  
Bruce Bodowle (US)  
Sylvain Brisson (FR)  
João André Carriço (PT)  
Carolin Colijn (UK)  
Jukka Corander (FI)  
Fernando de la Cruz (ES)  
Gary van Domselaar (CA)  
Edward J Feil (UK)  
Alex Friederich (NL)  
Jeniffer Gardy (CA)  
Peter Gerner-Schmidt (US)  
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Jonathan Green (UK)  
Hajo Grundmann (NL)  
Dag Harmsen (DE)  
Matt Holden (UK)  
Oliver Kurzai (DE)  
Frederic Laurent (FR)  
Nick Loman (UK)  
Martin Maiden (UK)  
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Jacob Moran-Gilad (IL)  
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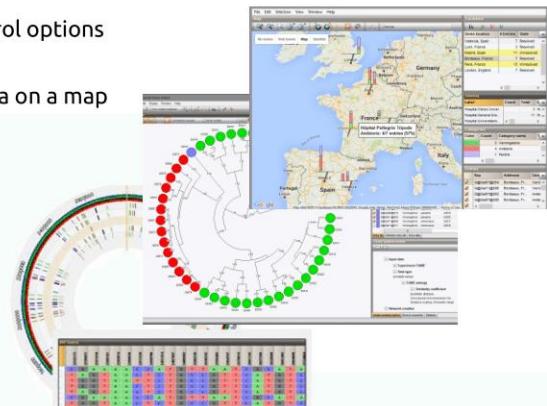


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# Scientific Programme

Wednesday, 9 March 2016

12:00      **Registration**  
14:00

14:00      **Plenary session S1:**  
15:30      Bioinformatics Tools for Genomic Epidemiology

15:30      **Welcome Address**  
16:15            Speakers: Mario Poljak and Hajo Grundmann

16:15      **Keynote:** Origins of diversity in microbial genomes  
17:00            Speaker: Edward J Feil

17:00      Coffee break  
17:30

17:30      **Keynote:** Real world epidemiology meets the genome  
information  
18:15            Speaker: Jennifer Gardy

18:15      **Keynote:** Signals of time and descent in microbial genomes  
19:00            Speaker: Caroline Colijn

19:00      Opening Reception  
20:00

# Scientific Programme

Thursday, 10 March 2016

8:30	<b>Plenary session S2:</b>
10:00	Genomics and adaptation to the host and man-made environments
10:00	
10:30	Coffee break
10:30	<b>Plenary session S3:</b>
12:00	Epidemiology and Public Health – Outbreaks
12:00	
14:30	Lunch and Poster Session I
14:30	<b>Industry Session I1:</b>
15:30	Sponsored Talks by Applied Maths and Biomérieux
15:30	<b>Plenary session S4:</b>
17:00	Food, Zoonotic and Environmental Microbial Risks
17:00	
17:30	Coffee break
17:30	<b>Plenary session S5:</b>
19:00	Novel Diagnostics and Typing methodologies

Friday, 11 March 2016

8:30      **Plenary session S6:**  
10:00     Microbial Population Genomics

10:00  
10:30    Coffee break

10:30      **Plenary session S7:**  
12:00     Antimicrobial resistance and Mobile Genetic Elements

12:00  
13:30    Lunch and Poster Session II

13:30      **Discussion session:**  
14:30     Genomic Epidemiology Ontologies

14:30      **Discussion session:**  
15:30     Need for universal nomenclatures for strain/lineage identification

15:30      **Plenary session S8:**  
17:00     Epidemiology and Public Health: Surveillance

17:00  
17:30    Coffee break

17:30      **Plenary session S9:**  
19:00     Late Breaker Abstracts session

# Scientific Programme

Saturday, 12 March 2016

8:30	<b>Plenary session S10:</b>
10:00	Molecular epidemiology and Public Health
10:00	
10:30	Coffee break
10:30	<b>Keynote:</b> Rapid evolutionary forces: the importance of mobile genetic elements <u>Speaker:</u> Fernando de la Cruz
11:15	
11:15	<b>Keynote:</b> Disruptive technologies in infectious disease epidemiology: the future <u>Speaker:</u> Nick Loman
12:00	
12:00	<b>Closing Session</b>
12:30	
12:30	Beer Break sponsored by Applied Maths
13:30	

## BIOINFORMATICS TOOLS FOR GENOMIC EPIDEMIOLOGY

**Wednesday, 09<sup>th</sup> March 2016, 14:00-15:30**

### **OP01 - Nullarbor: Reads To Reports, Rapidly**

Torsten Seemann<sup>1</sup>; Dieter Bulach<sup>1</sup>; Jason Kwong<sup>1</sup>; Anders Goncalves Da Silva<sup>1</sup>; Benjamin Howden<sup>1</sup>

<sup>1</sup>*University of Melbourne*

### **OP02 - Scalable And User-Friendly Workflows For Molecular Epidemiology Using Enterobase**

Martin Sergeant<sup>1</sup>; Nabil-Fareed Alikhan<sup>1</sup>; Zhemin Zhou<sup>1</sup>; Mark Achtman<sup>1</sup>

<sup>1</sup>*University of Warwick*

### **OP03 - Successes And Challenges In High Throughput Whole Genome Sequencing Of Viruses**

Dan Frampton<sup>1</sup>; Tiziano Gallo Cassarino<sup>1</sup>; Zisis Kozlakidis<sup>1</sup>; Anne Hoppe<sup>1</sup>; Deenan Pillay<sup>2</sup>; Paul Kellam<sup>3</sup>

<sup>1</sup>*Division of Infection and Immunity, UCL;* <sup>2</sup>*Wellcome Trust Africa Centre for Health and Population Studies;* <sup>3</sup>*Wellcome Trust Sanger Institute*

### **SC01 - Whole Genome Sequence Analysis (wgsa.net). An Exemplar Using *Staphylococcus aureus***

David Aanensen<sup>1,2</sup>

<sup>1</sup>*Department of Infectious Disease Epidemiology, Imperial College London.*<sup>2</sup>*Wellcome Trust Sanger Institute, Kingdom*

### **SO05 - The Epiquant Framework For Assessing Epidemiologic And Genetic Concordance: Towards Improved Use Of Genomic Data In Epidemiological Applications.**

Benjamin Hetman<sup>1</sup>; Steven K. Mutschall<sup>2</sup>; Victor P. J. Gannon<sup>2</sup>; James E. Thomas<sup>1</sup>; Eduardo N. Taboada<sup>2</sup>

<sup>1</sup>*Department of Biological Sciences, University of Lethbridge;* <sup>2</sup>*National Microbiology Laboratory at Lethbridge, Public Health Agency of Canada*

### **OP06 - CLIMB: Developing A National Cloud Infrastructure For Microbial Bioinformatics**

Emily Richardson<sup>1</sup>; Nick Loman<sup>1</sup>; Simon Thompson<sup>1</sup>; Matthew Ismail<sup>2</sup>; Sam Sheppard<sup>3</sup>; Thomas Connor<sup>4</sup>; Mark Pallen<sup>2</sup>

<sup>1</sup>*University of Birmingham;* <sup>2</sup>*University of Warwick;* <sup>3</sup>*University of Swansea;* <sup>4</sup>*University of Cardiff*

# S1 - Plenary session

## OP01 - Nullarbor: Reads To Reports, Rapidly

Torsten Seemann<sup>1</sup>; Dieter Bulach<sup>1</sup>; Jason Kwong<sup>1</sup>; Anders Goncalves Da Silva<sup>1</sup>; Benjamin Howden<sup>1</sup>

<sup>1</sup>University of Melbourne

**Background:** The modern public health microbiology laboratory has embraced genome sequencing as the primary assay for pathogen surveillance and outbreak analysis. Here we present Nullarbor, a software pipeline for turning a set of isolate sequence data into a single report summarizing the key information about each isolate and the relationship between isolates.

**Methods:** Nullarbor first adaptor clips and quality trims the reads for each isolate and assesses sequencing depth. Reads are scanned with Kraken to identify the species and any potential contamination or mix-ups.. The reads are de novo assembled into contigs using SPAdes or MegaHit (fast mode) and annotated using Prokka (\*). The contigs are used to infer MLST with the mlst tool (\*) without requiring specification of the scheme. ABRicate (\*) is used to determine the full resistome profile using the ResFinder database (Fig 1). Extra organism-specific *in silico* modules (\*) are available for *N.gonorrhoeae* (NG-MAST), *N.meningitidis* (porA+fetA), *L.monocytogenes* (serotyping, binary typing) and *S.enterica* (MLVA).

Snippy (\*) is used to align each isolate reads to a common reference (can be an assembled isolate) and identify SNPs, and to also compute the core genome alignment, from which a phylogenetic ML tree is generated using FastTree. A key feature of Snippy (\*) is that adding or removing taxa from the alignment can be done very efficiently because no unnecessary recompilation is performed. The pan-genome (Fig 2) is generated by Roary from the annotated assemblies and visualized using roary2svg (\*). The use of the pan-genome augments the core genome SNP analysis with data on mobile and accessory genetic elements, and is a feature unique to Nullarbor amongst the publicly available pipelines.

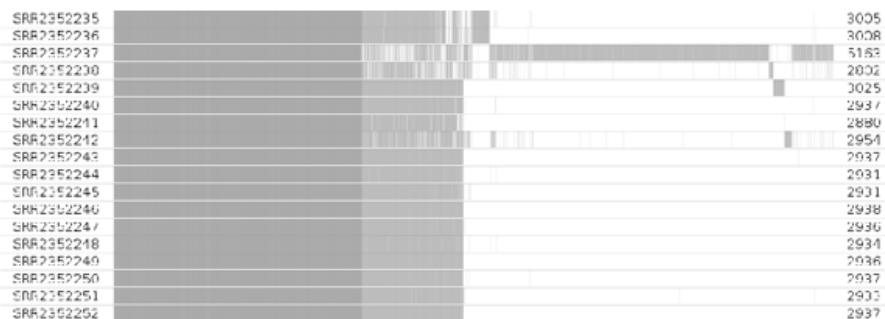
**Results:** The Nullarbor pipeline follows the Unix philosophy of using and combining existing standalone tools in an efficient manner and is parallelizable via the standard Make system. This makes is amenable to provincial public health laboratories with low compute capabilities and slow upload bandwidth to perform their own analyses. The input is a spreadsheet-like text file, and the default output is a clean HTML report. It is currently used by the Microbiological Diagnostics Unit Public Health Laboratory in Australia routinely for regular reporting and all outbreak investigations. An example report is available for viewing at the Nullarbor web site: <https://github.com/tseemann/nullarbor>

**Conclusion:** Nullarbor is open-source software released under a GPLv2 licence and runs on Unix systems. Simple installation of Nullarbor and its dependencies may be achieved via Homebrew Science <https://github.com/Homebrew/homebrew-science>. Future plans include: a virtual machine image for use with Amazon EC, OpenStack and VirtualBox (Jan 2015); a richer, more interactive and colourful report generator called QandongR (Feb 2016); the ability to accept already assembled genomes (Dec 2015); and support for legacy Ion Torrent and Roche 454 data sets (Apr 2016).

(\*) denotes software written by one or more of the authors.

# S1 - Plenary session

isolate	Group	taxa(M)											
		tax(G)	om(B)	om(G)	cap(C27)	cap(C194)	amE	amE	amE	amE	amE	amE	amE
2014-12159	20	✓	✓	✓	✓	✓+35%	✓	✓	✓	✓	✓	✓	✓
2014-12157	12	✓	-	✓	-	✓	-	✓	-	✓	-	✓	✓
2014-12153	12	✓	-	✓	-	✓	-	✓	-	✓	-	✓	✓
2014-12159	12	✓	-	✓+35%	-	✓	-	✓	-	✓	-	✓	✓
2014-12315	12	✓	-	✓	-	✓	-	✓	-	✓	-	✓	✓
2014-12313	11	✓	-	✓	-	✓	-	✓	-	✓	-	✓	✓
2014-12317	11	✓	-	✓	-	✓	-	✓	-	✓	-	✓	✓
2014-12318	12	✓	-	✓	-	✓	-	✓	-	✓	-	✓	✓
2014-12319	12	✓+35%	-	✓	-	✓	+31%	✓	-	✓	-	✓	✓
2014-12320	12	✓	-	✓	-	✓	-	✓	-	✓	-	✓	✓
2014-12321	12	✓	-	✓	-	✓	-	✓	-	✓	-	✓	✓
2014-12322	12	✓	-	✓	-	✓	-	✓	-	✓	-	✓	✓
2014-12323	12	✓	-	✓	-	✓	-	✓	-	✓	-	✓	✓
2014-12324	10	✓	-	✓	-	✓	-	✓	-	✓	-	✓	✓
2014-12325	11	✓	-	✓	-	✓	-	✓	-	✓	-	✓	✓



## S1 - Plenary session

### OP02 - Scalable And User-Friendly Workflows For Molecular Epidemiology Using Enterobase

Martin Sergeant<sup>1</sup>; Nabil-Fareed Alikhan<sup>1</sup>; Zhemin Zhou<sup>1</sup>; Mark Achtman<sup>1</sup>

<sup>1</sup>University of Warwick

**Background:** The decreasing cost of next-generation sequencing can now facilitate the routine sequencing of pathogens, moving closer to real-time molecular epidemiology. There are over 13 billion bacterial nucleotide base-pairs catalogued within GenBank, while pathogens such as *Salmonella enterica* and *Escherichia coli* have over 40,000 and 16,000 sets, respectively, of sequenced reads available. These data could provide a basis for a global perspective of microbial pathogens. However, such analyses are confounded by the unprecedented logistical challenge of corraling, curating and comparing these data. Furthermore, there is a paucity of standardized typing approaches that can reflect subtle genetic variation within outbreaks to deep genealogical structures beyond the species level – revealing both short-term and long-term transmission trends.

**Methods:** Here we present Enterobase, which addresses both challenges by providing novel genotyping methods (cgMLST, CRISPR and SNPs) through a graphical web interface, accessible to a wide audience of clinicians, epidemiologists and bioinformaticians.

Backed by computing resources of the Cloud Infrastructure for Microbial Bioinformatics (<http://www.climb.ac.uk/>), Enterobase offers automatic pipelines to type bacterial strains based on short reads fetched from Sequence Read Archive (SRA) or uploaded by registered users. Data from different sources are integrated and presented in a centralised database, which gives the greatest genetic panoramas so far for several bacterial genera.

**Results:** Enterobase serves over 50,000 genome assemblies from the SRA with metadata and genotyping data (MLST, rMLST and cgMLST), alongside 16,000 legacy records from MLST databases. These assemblies have been screened for contamination, poor sequencing quality and misassemblies and are paired with standardised and curated strain metadata, including geographic, host and temporal information. All sequencing data are processed in a matter of hours and their results are available through the user-friendly web interface.

Enterobase is also designed to be integrated into a data exchange network, in which Enterobase not only offers well established APIs for automatic machine access, but also communicates data with public data archives (EBI), traditional MLST sites (MLST Warwick and pubMLST), and commercial local database interfaces (Bionumerics).

**Conclusion:** Currently Enterobase supports the genera *Salmonella*, *Escherichia/Shigella*, *Moraxella* and the *Yersinia* but further development will see Enterobase as a generic platform for the curation, calculation and communication of sequencing data for all organisms of clinical relevance.

Enterobase is accessible through all modern web browsers, and is available at <http://enterobase.warwick.ac.uk>

### OP03 - Successes And Challenges In High Throughput Whole Genome Sequencing Of Viruses

Dan Frampton<sup>1</sup>; Tiziano Gallo Cassarino<sup>1</sup>; Zisis Kozlakidis<sup>1</sup>; Anne Hoppe<sup>1</sup>; Deenan Pillay<sup>2</sup>; Paul Kellam<sup>3</sup>

<sup>1</sup>Division of Infection and Immunity, UCL; <sup>2</sup>Wellcome Trust Africa Centre for Health and Population Studies; <sup>3</sup>Wellcome Trust Sanger Institute

**Background:** Despite recent advancements human immunodeficiency virus (HIV) remains a significant challenge to global public health. It is a highly diverse virus, exhibiting large genetic variation both at the population level and within individual patients. Importantly, even for treatment-naïve patients the presence of specific drug-resistant minority variants within an individual's viral population can lead to failure of drug therapy. The ability to both identify and quantify minority variants can thus be of direct clinical benefit.

As opposed to traditional Sanger sequencing, next generation sequencing (NGS) enables accurate quantification of minority variants and sequencing of viral sub- and quasi-species. The advent of affordable NGS technology offers a unique opportunity to rapidly process large numbers of clinical samples. Linking genomic and clinical data on this scale should facilitate development of novel clinical diagnostics, prognostics and optimal treatment strategies.

**Materials | Methods:** Within the ICONIC project, we have developed an automated and customizable virus-agnostic high-throughput analysis pipeline to generate viral clinical whole genomes, quantify minority variants and identify drug-resistance mutations (DRMs). Via *de novo* assembly of contigs, concatenation and correction by alignment to reference and iterative remapping of reads, our pipeline derives a consensus sequence and identifies minority variants across each genome within hours.

**Results:** Here we present results from analysis of a set of several hundred HIV samples from both the UK and various cohorts in Africa. Samples were collected and amplified using a pan-HIV1 primer set and sequenced by Illumina MiSeq at the Wellcome Trust Sanger Institute (Cambridge). Despite no prior filtering of samples by viral load or amplification success, our pipeline successfully generated genomes for 384 (91%) of UK samples with a median clinical whole genome coverage across samples of 82%. Pipeline validation was performed by directly comparing *pol* sequences derived by traditional Sanger sequencing to those generated by NGS and our pipeline from the same set of 384 samples.

**Conclusion:** Having applied the pipeline to additional viruses (influenza, norovirus, hepatitis C, respiratory syncytial virus, ebola and coronavirus) on UCL's high performance computing (HPC) cluster, preliminary results suggest this method is of potential clinical utility to a broad range of pathogens.

## S1 - Plenary session

### SC01 - Whole Genome Sequence Analysis (wgsa.net). An Exemplar Using *Staphylococcus aureus*

David Aanensen<sup>1,2</sup>

<sup>1</sup>Department of Infectious Disease Epidemiology, Imperial College London.<sup>2</sup>Wellcome Trust Sanger Institute, Kingdom

### SO05 - The EpiQuant Framework For Assessing Epidemiologic And Genetic Concordance: Towards Improved Use Of Genomic Data In Epidemiological Applications.

Benjamin Hetman<sup>1</sup>; Steven K. Mutschall<sup>2</sup>; Victor P. J. Gannon<sup>2</sup>; James E. Thomas<sup>1</sup>; Eduardo N. Taboada<sup>2</sup>

<sup>1</sup>Department of Biological Sciences, University of Lethbridge; <sup>2</sup>National Microbiology Laboratory at Lethbridge, Public Health Agency of Canada

**Background:** *Campylobacter jejuni* is an important bacterial foodborne pathogen, with an estimated 400 million cases worldwide each year. In Canada and many industrialized countries, *C. jejuni* is the leading cause of bacterially-incited enteritis, with a yearly per capita incidence as high as 1%. Because most cases of campylobacteriosis appear to be non-outbreak related, improved methods for attributing sources of exposure are increasingly critical for public health interventions to reduce this burden of illness. With recent advances in Whole Genome Sequencing (WGS), analysis of *C. jejuni* isolates collected through human and non-human surveillance programs in Canada using WGS is being increasingly used in epidemiologic investigations aimed at the prevention and control of campylobacteriosis.

**Methods:** We have analyzed the genomes of over 3,000 *C. jejuni* isolates from environmental, animal, and clinically-derived sources, with an aim to develop analytical methods that extract critical information from WGS data to facilitate genomic epidemiology for attributing the likely source of human infections. We have developed a model to derive a quantitative summary statistic for the epidemiological similarity (EpiSym) of any two isolates based on parameters derived from basic metadata (date of collection, location, source) collected in routine surveillance. We have also developed a statistical framework (EpiQuant) for assessing the concordance between epidemiological and genomic similarity of isolates in the R environment for statistical computing.

**Results:** The EpiSym statistic allows for an estimate of pairwise similarities between isolates based on basic epidemiological information. This estimate is used in our EpiQuant framework for directly assessing the concordance between pairwise similarity estimates based on epidemiologic data and those derived from genomic data. We have used the EpiQuant framework to optimize similarity thresholds for the interpretation of *C. jejuni* WGS data in the context of epidemiological investigations so as to maximize recovery of epidemiologically and genetically concordant clusters for source attribution. We have also used the EpiQuant framework to identify and investigate cases where genetic similarity estimates deviate from those expected based on underlying epidemiology and show how the genetic and epidemiological linkage between *C. jejuni* isolates can break down under specific circumstances.

**Conclusion:** The EpiSym statistic and the EpiQuant framework allow for direct comparison of epidemiologic and genomic similarity estimates between isolates, paving the way for improved source tracking and source attribution. We are developing an online toolkit to be used in the development of interpretation criteria for WGS in epidemiologic investigations of *C. jejuni* and other high-risk bacterial pathogens.

## S1 - Plenary session

### OP06 - CLIMB: Developing A National Cloud Infrastructure For Microbial Bioinformatics

Emily Richardson<sup>1</sup>; Nick Loman<sup>1</sup>; Simon Thompson<sup>1</sup>; Matthew Ismail<sup>2</sup>; Sam Sheppard<sup>3</sup>; Thomas Connor<sup>4</sup>; Mark Pallen<sup>2</sup>

<sup>1</sup>*University of Birmingham*; <sup>2</sup>*University of Warwick*; <sup>3</sup>*University of Swansea*;

<sup>4</sup>*University of Cardiff*

Whole genome sequencing is transforming public health surveillance of bacterial pathogens. However, the scale of genomic datasets poses new informatics challenges for storing and analysing large aggregate datasets. This need is compounded by a lack of trained bioinformaticians to analyse the data. In 2014 the Medical Research Council made a ~£50m investment in "big data" to support the development of new research infrastructures. The £8.5m CLoud Infrastructure for Microbial Bioinformatics (CLIMB) was the only award to a microbial consortium and is one of the largest investments in microbial genomics bioinformatics ever made. CLIMB provides a national e-infrastructure to deliver bioinformatics infrastructure as a service to the UK academic medical microbial community. CLIMB is hosted across four sites (Birmingham, Cardiff, Swansea and Warwick) and we provide a single sign-on, distributed computing and storage infrastructure. The total investment in hardware is £3.6m, providing 7680 virtual CPUs, 500 terabytes of local high performance storage per site and 7000 terabytes (7PB) of replicated object storage. This facility is sufficient to provide over a thousand simultaneously running virtual machines to users. The service is implemented using the open-source OpenStack framework. Data storage is managed through the CEPH object storage system which also provides redundancy. CLIMB has been made available to early access users mode for the last 6 months and have begun to deploy microbial genomics software to cater for user requirements. In this presentation we demonstrate how CLIMB, and cloud services like it, may impact on the practice of public health genomic surveillance of pathogens. To assist training and ease of use, we have deployed the Genomics Virtual Laboratory system (developed at the University of Melbourne) onto CLIMB. The GVL can help deliver reproducible workflows and assist training of new bioinformaticians through the use of an inbuilt Galaxy server and IPython notebook. This system also reduces much of the burden associated with installing bioinformatics analysis software. Those using genomics for microbial surveillance should be aware of the potential opportunities offered by cloud services for large-scale, integrated data analysis.

## GENOMICS AND ADAPTATION TO THE HOST AND MAN-MADE ENVIRONMENTS

**Thursday, 10<sup>th</sup> March 2016, 08:30-10:00**

### OP07 - Resolving Genotype-Phenotype Maps Of Opportunistic Pathogenicity In *Staphylococcus epidermidis* To Identify Functional Markers Of Infection

Guillaume Méric<sup>1</sup>; Leonardos Mageiros<sup>1</sup>; Samuel K. Sheppard<sup>1</sup>

<sup>1</sup>Swansea University Medical School

### OP08 - Multiomic Approach To Delineate Intralclonal Diversity Of CG14 And CG15 *Klebsiella pneumoniae*

Carla Rodrigues<sup>1</sup>; Val F Lanza<sup>2</sup>; Clara Sousa<sup>3</sup>; Elisabete Machado<sup>4</sup>; João A Lopes<sup>5</sup>; Fernando Baquero<sup>2</sup>; Luísa Peixe<sup>1</sup>; Ângela Novais<sup>1</sup>; Teresa M Coque<sup>2</sup>

<sup>1</sup>REQUIMTE@UCIBIO, Faculdade de Farmácia da Universidade do Porto;

<sup>2</sup>Hospital Universitario Ramón y Cajal and Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS)/CIBERESP; <sup>3</sup>Center of Biological Engineering, University of Minho; <sup>4</sup>REQUIMTE@UCIBIO, Faculdade de Farmácia da Universidade do Porto/FP-ENAS/CEBIMED. Faculdade de Ciências da Saúde, Universidade Fernando Pessoa; <sup>5</sup>iMed, Departamento de Farmácia Galénica e Tecnologia Farmacêutica, Faculdade de Farmácia, Universidade de Lisboa

### OP09 - Evolution Of The Skin Commensal *Staphylococcus epidermidis* In The Hospital Environment: Insights From An Early Collection

Joana Rolo<sup>1</sup>; Rita Sobral<sup>2</sup>; Jesper Boye Nielsen<sup>3</sup>; Peder Worning<sup>3</sup>; Rory Bowden<sup>4</sup>; Samuel Sheppard<sup>5</sup>; Guillaume Méric<sup>5</sup>; Robert Skov<sup>6</sup>; Henrik Westh<sup>3</sup>; Hermínia de Lencastre<sup>7</sup>; Maria Miragaia<sup>1</sup>

<sup>1</sup>Laboratory of Molecular Genetics, Instituto de Tecnologia Química e Biológica António Xavier; Laboratory of Bacterial Evolution and Molecular Epidemiology, Instituto de Tecnologia Química e Biológica António Xavier; <sup>2</sup>Laboratory of Molecular Genetics, Instituto de Tecnologia Química e Biológica António Xavier

; Laboratory of Molecular Microbiology of Bacterial Pathogens, UCIBIO-REQUIMTE, Faculdade de Ciências e Tecnologia - Universidade Nova de Lisboa; <sup>3</sup>Department of Clinical Microbiology, Hvidovre University Hospital;

<sup>4</sup>Wellcome Trust Centre for Human Genetics, University of Oxford; <sup>5</sup>College of Medicine, Swansea University, Institute of Life Science; MRC CLIMB Consortium, Swansea University, Institute of Life Science; <sup>6</sup>Statens Serum Institut; <sup>7</sup>Laboratory of Molecular Genetics, Instituto de Tecnologia Química e Biológica António Xavier; Laboratory of Microbiology and Infectious Diseases, The Rockefeller University

## S2 - Plenary session

### OP10 - Evolution And Adaptation Of *Pseudomonas aeruginosa* Within Patients With Cystic Fibrosis

Rasmus Lykke Marvig<sup>1</sup>

<sup>1</sup>Rigshospitalet, Copenhagen University Hospital

### OP11 - Genomic Diversity Of Carbapenemase-Producing *Klebsiella pneumoniae* In Germany

Laura Becker<sup>1</sup>; Matthias Steglich<sup>1</sup>; Yvonne Pfeifer<sup>1</sup>; Martin Kaase<sup>2</sup>; Stephan Fuchs<sup>1</sup>; Ulrich Nübel<sup>3</sup>; Guido Werner<sup>1</sup>

<sup>1</sup>Robert Koch Institute, FG13 Nosocomial Pathogens and Antibiotic Resistance;

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### SC02 - Genomics Of *Campylobacter* Adaptation To The Host And Man-Made Environments

Samuel K Sheppard<sup>1,2,3</sup>

<sup>1</sup>Department of Zoology, University of Oxford, Oxford, UK; <sup>2</sup>College of Medicine, Institute of Life Science, Swansea University, Swansea, UK; <sup>3</sup>MRC CLIMB

Consortium, Institute of Life Science, Swansea University, Swansea, UK.

### OP07 - Resolving Genotype-Phenotype Maps Of Opportunistic Pathogenicity In *Staphylococcus epidermidis* To Identify Functional Markers Of Infection

Guillaume Méric<sup>1</sup>; Leonardos Mageiros<sup>1</sup>; Samuel K. Sheppard<sup>1</sup>

<sup>1</sup>Swansea University Medical School

**Background:** Effective treatment of nosocomial infections is difficult, as most severe infections can be caused by antibiotic-resistant isolates from bacterial species naturally colonising humans asymptotically. Under certain circumstances, these commensal organisms can become opportunistic pathogens. An example is *Staphylococcus epidermidis*, which lives commensally on human skin and mucous membranes. Although *S. epidermidis* is asymptotically carried, hospitalised patients are often at risk of developing an infection, typically through the contamination of catheters or other surgical implants, and the mechanisms involved in the emergence of pathogenicity in this organism are currently not fully understood.

**Methods:** We present and interpret results of a comparative analysis on 415 whole-genome sequences from genetically diverse *S. epidermidis* from clinical infections as well as asymptomatic isolates. Whole-genome alignments were obtained using a gene-by-gene approach and robust phylogenetic trees were constructed. Groups of genetically related isolates were identified to create a suitable dataset to be analysed using a recently-developed pangenome-wide association study (pan-GWAS) pipeline combined with *in vitro* quantitative phenotyping (immune response in blood and skin cells, biofilm formation, methicillin resistance and cell toxicity).

**Results:** A list of candidate genetic elements statistically associated with opportunistic pathogenicity was obtained. The association signal was then filtered by correlating each element with quantitative scores for phenotypes tested *in vitro* to identify phenotypically-filtered genetic markers of infection.

**Conclusions:** In this study, we present an original and novel approach to identify and filter functionally genetic elements associated with complex ecological lifestyles (opportunistic pathogenicity). The association signal obtained *in silico* is then functionally correlated with *in vitro* quantitative pathogenicity assays. This work provides a clearer understanding of the evolutionary mechanisms underlying the emergence of opportunistic pathogenicity in *S. epidermidis*, as well as a novel and powerful method to resolve genotype-phenotype maps in bacteria.

## S2 - Plenary session

### OP08 - Multiomic Approach To Delineate Intraclonal Diversity Of CG14 And CG15 *Klebsiella pneumoniae*

Carla Rodrigues<sup>1</sup>; Val F Lanza<sup>2</sup>; Clara Sousa<sup>3</sup>; Elisabete Machado<sup>4</sup>; João A Lopes<sup>5</sup>; Fernando Baquero<sup>2</sup>; Luísa Peixe<sup>1</sup>; Ângela Novais<sup>1</sup>; Teresa M Coque<sup>2</sup>

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Project supported by a FEMS Research Grant.

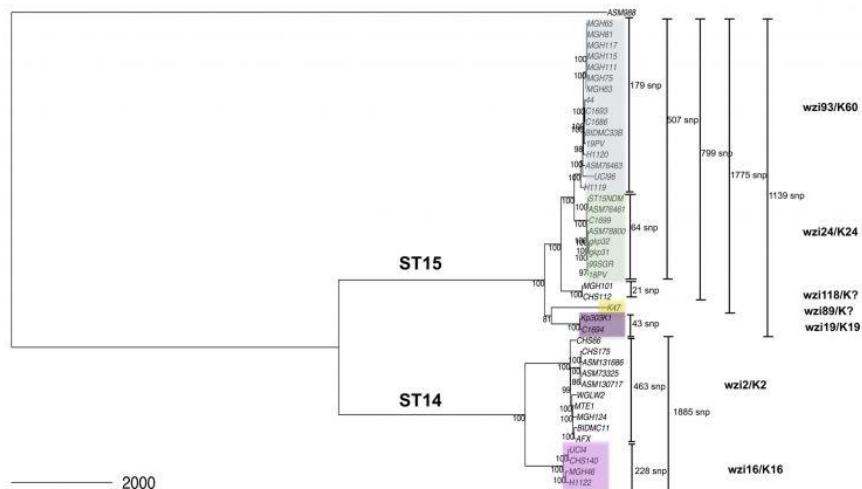
**Background:** Population structure of *Klebsiella pneumoniae* (Kp) cannot be accurately delineated by traditional analysis of MLST data. Recent genome wide genotyping identified 14 clonal groups (CG), with MDR clones largely represented by CG258, CG14 and CG15. Whereas CG258 has been extensively studied, only a few isolates of CG14 and CG15 have been comprehensively analysed at genome level. Hospital and community acquired infections caused by Kp ST14 and ST15 isolates are frequent in Portugal since 2003, and their characterization by molecular genotyping (PFGE, MLST, wzI-based capsule typing) and spectroscopic methods (Fourier transform infrared spectroscopy-FTIR) unveiled congruently the circulation of 1 ST14 and 4 ST15 clones exhibiting specific capsular types. We aimed to delineate the intraclonal diversity of CG14 and CG15 by comparing the genomes from representative circulating clones in Portugal and those available in public databases. Moreover, a comparative analysis of genomics (NGS), proteomics (MALDI-TOF MS) and metabolomics (FTIR) was performed to elucidate differential resolution potential for typing of Kp CG14 and CG15.

**Materials|Methods:** Whole-genome sequencing (WGS) of 9 Kp isolates with different capsular types ST15 (5 K24, 1 K19, 1 K60, 1 wzI89) and ST14 (1 K16) from different origins and variable PFGE-types were obtained using Illumina MiSeq (2x300bp pair-ended runs, ~6Gb/genome, coverage:100x). WGS of ST15 (n=21; USA/Italy/Nepal/Taiwan; 2008-14) and ST14 (n=13; USA/Argentina/Korea/South Africa; 1986-2014) isolates available on NCBI (as from November 2015, all from human infections) were included in the analysis. Assembly was performed using SPAdes and the core genome (>90% similarity and coverage) of Kp isolates was defined using home Perl scripts. Core genes were concatenated and aligned using MUSCLE, and SNPs extraction was performed with Harvest tools. The results were output in a SNP matrix, which was used to generate a phylogenetic tree using the maximum likelihood method (100 bootstraps) in R 3.2.2 (Phangorn package). Spectra (obtained from colony and/or bacterial extracts) were acquired in MALDI-TOF MS and FTIR-ATR, and analysed by PCA/PLSDA using MATLAB 8.3 (PLS toolbox 7.5).

**Results:** Phylogenetic analysis of a 30832 core genome SNPs identified 5 clusters among ST15 and 2 clusters among ST14 isolates, each one of them comprising isolates from different origins that harbour the same capsular type (K19, K24, K60, wzI89 or wzI118 for ST15, and K2 or K16 for ST14) (Fig.1). Phylogenetic relationships among isolates inferred from this tree corroborate previous clonal assignments based on molecular genotyping and FTIR (Fig.2). Within each cluster, a high diversity in ESBL and/or carbapenemase content was observed, suggesting the ability of these lineages to acquire a variable pool of MDR plasmids. In contrast with genomics- or metabolomics-based methods, ST15 or ST14 subpopulations were not discriminated by MALDI-TOF, probably reflecting the high degree of conservation of their corresponding ribosomal proteins.

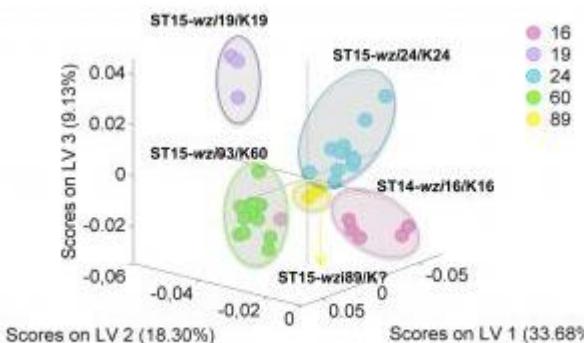
**Conclusions:** WGS and metabolomics are reliable tools to delineate the intraclonal diversity of Kp CG14 and CG15, whereas current MLST scheme or MALDI-TOF are not suitable for an accurate

differentiation. Within these groups, particular widespread subpopulations are linked to specific capsular types, probably reflecting variable host adaptation or pathogenicity potential.



**Fig. 1. Phylogenetic tree of ST15 and ST14 *K. pneumoniae* isolates.**

The tree is based on 30832 core genome SNPs (90% identity and coverage) and 100 bootstrapping replicates. ST14 and ST15 clades are colored according to capsular types founded in Portuguese isolates and assigned on FTIR-ATR: K24 (blue), K19 (violet), K60 (green), wzi89 (yellow) and K16 (pink). Given SNP numbers are approximate averages of individual comparisons.



**Fig. 2. Partial Least Square Discriminant Analysis (PLSDA) of the different ST15 and ST14 *K. pneumoniae* isolates established by analysis of FTIR-ATR spectra.**

## S2 - Plenary session

### OP09 - Evolution Of The Skin Commensal *Staphylococcus epidermidis* In The Hospital Environment: Insights From An Early Collection

Joana Rolo<sup>1</sup>; Rita Sobral<sup>2</sup>; Jesper Boye Nielsen<sup>3</sup>; Peder Worning<sup>3</sup>; Rory Bowden<sup>4</sup>; Samuel Sheppard<sup>5</sup>; Guillaume Méric<sup>5</sup>; Robert Skov<sup>6</sup>; Henrik Westh<sup>3</sup>; Hermínia de Lencastre<sup>7</sup>; Maria Miragaia<sup>1</sup>

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**Background:** One of the strategies of adaptation of *Staphylococcus epidermidis* to the hospital environment has been related with the acquisition and diversification of the staphylococcal cassette chromosome *mec* (SCCmec) that carries *mecA*, the genetic determinant of resistance to methicillin. However, it is not known when SCCmec was first introduced in the genome of *S. epidermidis* and what additional evolutionary mechanisms could have been associated to the adaptation of these bacteria to the nosocomial setting and to a more pathogenic lifestyle. In this study, we aimed to identify the molecular events that allowed for the recent emergence of *S. epidermidis* as a pathogen, through the study of its genomic evolutionary history.

**Materials&Methods:** We compared the whole genomes of 21 early *S. epidermidis* strains isolated in a hospital in Denmark in 1965 with the genomes of 14 contemporary nosocomial *S. epidermidis* collected in the same country in 1997/1998. Genomes were sequenced using HiSeq technology and reads were assembled *de novo* with VELVET. Multilocus sequence types were extracted from the contigs. In addition, the core genome of all strains was predicted with Mugsy and aligned with Mauve. Finally, screening of virulence genes was performed by BLAST analysis and the presence of intact prophages, insertion sequences and antibiotic resistance genes in the contigs was assessed using online databases of specific bacterial sequences.

**Results:** We found that the great majority of early (67%) and contemporary (79%) strains belonged to the main *S. epidermidis* clonal lineage, clonal complex 2 (CC2). In addition, our analysis revealed that the core genome of early and contemporary isolates was conserved but their mobilome showed distinctive features. Early isolates carried genes associated to resistance to several different antibiotics commonly used in clinical practice in the 1960s; some were extremely prevalent (*blaZ*, 67%, *fosA*, 90%; *norA*, 100%) and others were found in a low frequency (*aaD*, 9.5%; *ermA*, 9.5%, *fusB*, 9.5%; *cat*, 14.3%; *tet(K)*, 14.3%). Of note, a single isolate carried the *mecA* gene, disrupted by IS431, within a SCCmec IV-like element. In addition, we found that early strains carried biofilm-associated factors, such as *ica* (5%), *bhlP* (28.5%) and *aap* (38%) and the arginine catabolic mobile element, ACME (types I and II equally represented, 38%). Moreover, a high frequency of intact prophages (95%) and as many as 10 different insertion sequences (IS) were found in early genomes. In contrast, resistance determinants (including SCCmec IV, 57%), biofilm-associated genes (*ica*, 21%; *aap*, 64%) and ACME-I (57%) were found in higher frequency among contemporary isolates. Moreover, additional genes associated to biofilm production (*bap*, 7%; *sdrF*,

14%) and IS, presumably originated in other bacterial species, were found in contemporary isolates. However, the frequency of prophages decreased dramatically (to 50%) in this population.

**Conclusion:** Our results suggest that the accumulation of genetic determinants associated to antibiotic resistance, colonization, biofilm formation and genome plasticity as well as phage loss have contributed to the adaptation of *S. epidermidis* to the hospital environment and to its emergence as a major nosocomial pathogen. Also, our data suggest that SCCmec IV acquisition and spread in *S. epidermidis* population was a relatively recent event (<50 years).

## S2 - Plenary session

### OP10 - Evolution And Adaptation Of *Pseudomonas aeruginosa* Within Patients With Cystic Fibrosis

Rasmus Lykke Marvig<sup>1</sup>

<sup>1</sup>Rigshospitalet, Copenhagen University Hospital

Bacterial whole genome sequencing of longitudinally collected isolates enables the investigation of evolutionary trajectories, which may inform both the prevention and treatment of human-associated pathogen infections. Chronic infection with *Pseudomonas aeruginosa* is a major cause of morbidity and mortality in cystic fibrosis (CF) patients, and a more complete understanding of *P. aeruginosa* within-host genomic evolution, transmission, and population genomics may provide a basis for improving intervention strategies.

By genome sequencing of >600 *P. aeruginosa* isolates sampled over 40 years from 50 patients, we elucidated the within-host evolution of clonal lineages in each individual patient. Many of the identified mutations were located in pathoadaptive genes associated with host adaptation, and we correlated mutations with changes in CF-relevant phenotypes such as antibiotic resistance. Considerable genome reduction was detected, suggesting that host adaptation is characterized by the reduction of genomic repertoire rather than acquisition of novel functions. Furthermore, the genomic analysis revealed transmission of *P. aeruginosa* between patients, and we show that patient-to-patient transmission may be facilitated by temporal overlap in the respective patients' visits to the hospital.

Comparison of the genomes of multiple isolates from the same patients showed extensive within-patient genomic diversification; the populations were composed of different sub-lineages that had coexisted for many years since the initial colonization of the patient and occupied distinct niches. Thus, our results suggest that the spatial heterogeneity in CF airways plays a major role in relation to the generation and maintenance of population diversity and emphasize that a single isolate in sputum may not represent the entire pathogen population in the infected individual.

Although there are other studies encompassing several hundreds of whole genome sequenced isolates of bacterial pathogens, our study is unique in its focus on an opportunistic pathogen causing long-term infections. In addition, whereas many other studies are focusing on single clonal lineages or complexes of a pathogenic species, our collection compares the within-host evolution of genetically different strains of the same species. This is an important issue, as mutations or genes associated with pathogenicity in one bacterial strain might not be predictive of pathogenicity in other strains.

In conclusion, we present the largest collection of whole genome sequenced *P. aeruginosa* isolated from CF patients, and together with both phenotypic and clinical information this dataset facilitates a more detailed understanding of *P. aeruginosa* within-host genomic evolution, transmission, and population genomics.

### OP11 - Genomic Diversity Of Carbapenemase-Producing *Klebsiella pneumoniae* In Germany

Laura Becker<sup>1</sup>; Matthias Steglich<sup>1</sup>; Yvonne Pfeifer<sup>1</sup>; Martin Kaase<sup>2</sup>; Stephan Fuchs<sup>1</sup>; Ulrich Nübel<sup>3</sup>; Guido Werner<sup>1</sup>

<sup>1</sup>*Robert Koch Institute, FG13 Nosocomial Pathogens and Antibiotic Resistance;*

<sup>2</sup>*National Reference Laboratory for multidrug-resistant gram-negative bacteria, Department for Medical Microbiology, Ruhr-University Bochum;* <sup>3</sup>*Leibniz Institute DSMZ*

**Background:** Carbapenem-resistant *Klebsiella pneumoniae* are dreaded nosocomial pathogens. In Germany, the carbapenemases KPC-2, KPC-3, OXA-48 and NDM-1 display the highest prevalence in carbapenem-resistant *K. pneumoniae*. As a basis for future next generation sequencing (NGS)-based outbreak analyses and the elucidation of dynamics of epidemic strains, we analysed the genomic diversity of carbapenemase-producing *K. pneumoniae* occurring in German hospitals. In cooperation with the National Reference Laboratory for multidrug-resistant gram-negative bacteria, a representative collection of more than 100 carbapenem-resistant isolates from both outbreaks and single cases which had been collected between 2009 and 2014 allover Germany was included in our analysis.

**Material/Methods:** All isolates were characterised previously by antimicrobial susceptibility testing and by determination of the carbapenemase gene. For whole genome sequencing, bacterial DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen). Sequencing libraries were prepared using the Nextera XT DNA Sample Preparation Kit. Sequencing was performed on a MiSeq machine (Illumina). Sequencing reads were trimmed (Trimmomatic) and mapped (BWA-SW) onto a reference genome. Based on single nucleotide polymorphisms (SNPs) a maximum likelihood tree was constructed. Further, whole genome sequence data were used to determine the content of virulence and resistance genes.

**Results:** Whole genome sequence analysis revealed the presence of four major clusters, representing several carbapenem-resistant *K. pneumoniae* multilocus sequence types (ST) known to be distributed internationally: KPC-3-producing ST512, KPC-2-/KPC-3-producing ST258, OXA-48-producing ST101 and OXA-48-producing ST147. Genomic diversity within these groups was extremely low, reflecting the clonal spread of distinct carbapenem-resistant *K. pneumoniae* strains in Germany.

**Conclusion:** Our baseline genomic sequence dataset provides a comprehensive insight in the diversity of carbapenemase-producing *K. pneumoniae* in Germany and will enable rapid identification and phylogenetic clustering of emerging strains.

## S2 - Plenary session

### SC02 - Genomics Of Campylobacter Adaptation To The Host And Man-Made Environments

Samuel K Sheppard<sup>1,2,3</sup>

<sup>1</sup>*Department of Zoology, University of Oxford, Oxford, UK;* <sup>2</sup>*College of Medicine, Institute of Life Science, Swansea University, Swansea, UK;* <sup>3</sup>*MRC CLIMB Consortium, Institute of Life Science, Swansea University, Swansea, UK.*

## EPIDEMIOLOGY AND PUBLIC HEALTH - OUTBREAKS

**Thursday, 10<sup>th</sup> March 2016, 10:30-12:00**

### **SC03 - Modern Interventional Microbiology For Infection And Resistance Control**

Alexander W. Friederich<sup>1</sup>

<sup>1</sup>Department of Medical Microbiology, University of Groningen, University Medical Center Groningen, Groningen, Netherlands.

### **OP12 - Utility Of The *Salmonella* In Silico Typing Resource (SISTR) To Outbreak Investigations**

James Robertson<sup>1</sup>; Catherine Yoshida<sup>1</sup>; Peter Kruczakiewicz<sup>1</sup>; Eduardo N. Taboada<sup>1</sup>; John H.E. Nash<sup>1</sup>

<sup>1</sup>Public Health Agency of Canada

### **OP13 - Analyzing Vancomycin-Resistant *Enterococcus faecium* Outbreaks By Core Genome Multi Locus Sequence Typing**

Xuewei Zhou<sup>1</sup>; Jan P. Arends<sup>1</sup>; Sigrid Rosema<sup>1</sup>; Erik Bathoorn<sup>1</sup>; Alexander W. Friederich<sup>1</sup>; John W.A. Rossen<sup>1</sup>

<sup>1</sup>University Medical Center Groningen

### **OP14 - Phylogenomic Characterization Of The Causative Strain Of One Of The Largest Worldwide Outbreaks Of Legionnaires' Disease Occurred In Portugal In 2014**

Vítor Borges<sup>1</sup>; Alexandra Nunes<sup>1</sup>; Daniel A Sampaio<sup>1</sup>; Luís Vieira<sup>1</sup>; João P. Gomes<sup>1</sup>

<sup>1</sup>National Institute of Health Dr. Ricardo Jorge

### **SC04 - WGS-Based Approaches For Cluster Investigation Of Legionnaires' Disease: Current Experience And Future Direction**

Jacob Moran-Gilad<sup>1-4</sup>

<sup>1</sup>Chair, National Programme for Legionellosis Prevention, Public Health Services, Ministry of Health, Jerusalem, Israel; <sup>2</sup>Professor of Clinical Microbiology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel; <sup>3</sup>ESCMID Study Group for Legionella Infections (ESGLI); <sup>4</sup>ESCMID Study Group for Molecular Diagnostics (ESGMD)

### **SC05: - Whole Genome-Based Population Biology And Global Epidemiological Surveillance Of *Listeria monocytogenes***

Sylvain Brisse<sup>1</sup>

<sup>1</sup>Institut Pasteur, Genotyping of Pathogens and Public Health, Paris, France

## S3 - Plenary session

### SC03 - Modern Interventional Microbiology For Infection And Resistance Control

Alexander W. Friederich<sup>1</sup>

<sup>1</sup>*Department of Medical Microbiology, University of Groningen, University Medical Center Groningen, Groningen, Netherlands.*

### OP12 - Utility Of The *Salmonella* In Silico Typing Resource (SISTR) To Outbreak Investigations

James Robertson<sup>1</sup>; Catherine Yoshida<sup>1</sup>; Peter Kruczakiewicz<sup>1</sup>; Eduardo N. Taboada<sup>1</sup>; John H.E. Nash<sup>1</sup>

<sup>1</sup>Public Health Agency of Canada

**Background:** Whole genome sequencing (WGS) costs and processing time have decreased dramatically over the last few years to the point that they can be implemented in routine surveillance, outbreak detection and source attribution. There has been an explosive growth in the number of Next Generation Sequencing runs submitted to the Short Read Archive (SRA) with more than 22,000 *Salmonella* runs submitted in 2015. Using WGS sequencing researchers can classify isolates to a serotype and distinguish between highly clonal samples. The *Salmonella* in silico typing resource (SISTR) <https://ifz.corefacility.ca/sistr-app/> addresses the problems of scalability and portability of WGS results by implementing a core genome MLST (cgMLST) scheme of 330 genes that can reliably assign draft assemblies to a serotype with ~94.6% accuracy on a dataset of 3,727 genomes.

**Materials and Methods:** A total of 10,984 *Salmonella* WGS runs were downloaded from the SRA and the serotype information was manually curated to extract the information where it was absent from the species name and to clean up synonyms, typos and other data errors in the input. Illumina reads were assembled using SPAdes and 454 reads were assembled using Mira. The genomes were then uploaded to SISTR using the API, and genomes with fewer than 300 complete core genes were checked separately for contamination and assembly quality. Assembly metrics (e.g. N50, largest contig, number of contigs > 1000 bp, etc.) were computed using QUAST (v3.1).

**Results:** A candidate list of 10,948 genomes was selected for assembly, with preference given to serotypes of lower representation in the database to increase confidence in cgMLST for these serovars. It was found that the 330 core genes had a very high recovery rate from draft assemblies with ~92% of the drafts having the complete sequence coverage for the genes and ~99% with at least 300 complete genes. The remaining ~1% of assemblies either had poor assembly metrics or originated from non-*Salmonella* genomes. After the addition of the new genomes, SISTR has 505 serotypes represented in the database with 90 of the Canadian top 100 reported *Salmonella* serotypes represented by at least one genome in SISTR. After curation of synonyms and monophasic types of *Salmonella* serotypes there was 98.5% agreement between the NCBI reported serotype and the SISTR reported serotype.

SNP MST trees from (Quick et al., 2015) were visually compared to the trees produced by SISTR using cgMLST. The cgMLST tree was highly concordant with the SNP tree from the (Quick et al., 2015), which demonstrates that for the serotype Enteriditis the cgMLST scheme captured similar amounts of diversity compared to the SNP tree.

**Conclusion:** Using draft genome assemblies, SISTR is able to reliably determine the serotype of isolates with 98.5% accuracy. The core genes selected in this scheme were easily extracted from the majority of assemblies with ~92% of the 10,948 genomes having the complete complement of the 330 genes. Furthermore, the cgMLST scheme contains enough variability to distinguish between geographically distant isolates. It also accurately portrays serotypes of a polyphyletic nature such the Newport serotype. The broad applicability of SISTR to different *Salmonella* serotypes means that it could be deployed in public health laboratories for serotyping and rough source attribution of isolates without any specialized bioinformatics training.

## S3 - Plenary session

### OP13 - Analyzing Vancomycin-Resistant *Enterococcus faecium* Outbreaks By Core Genome Multi Locus Sequence Typing

Xuewei Zhou<sup>1</sup>; Jan P. Arends<sup>1</sup>; Sigrid Rosema<sup>1</sup>; Erik Bathoorn<sup>1</sup>; Alexander W. Friedrich<sup>1</sup>; John W.A. Rossen<sup>1</sup>

<sup>1</sup>University Medical Center Groningen

Keywords: *Enterococcus faecium*, VRE, cgMLST, whole-genome sequencing, outbreak

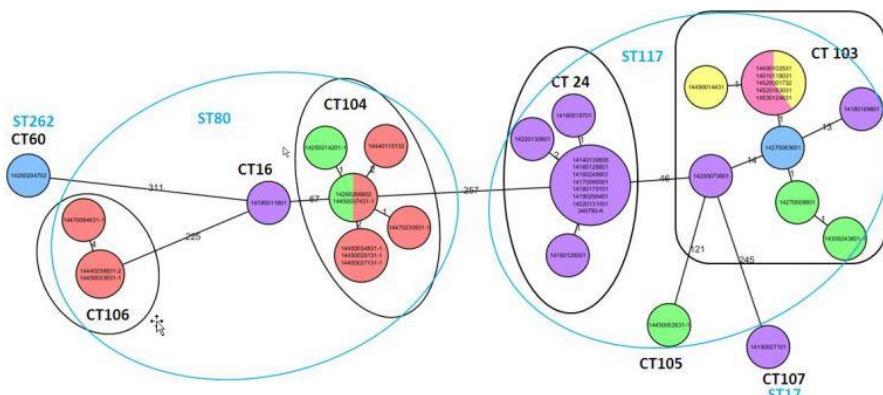
**Introduction:** *Enterococcus faecium* has emerged as a nosocomial pathogen worldwide. Outbreaks are mainly caused by vancomycin resistant *E. faecium* (VREfm) that acquired the *vanA* or *vanB* gene. Multilocus sequence typing (MLST) has been the main tool to study the genetic relatedness and epidemiology of *E. faecium* isolates. The discriminatory power of MLST may not be sufficient to analyse nosocomial outbreaks. Recently, a core genome (cg)MLST scheme for *E. faecium* was developed (1). Here we evaluated previously sequenced VREfm isolates in our laboratory originating from outbreaks between 2010 and 2015 according to this cgMLST scheme.

**Methods:** In total 63 VRE isolates from 2010-2015 were evaluated. From 2014, 37 samples were included and six episodes of small and larger outbreaks were defined, based on epidemiological data. GenomicDNA was extracted using the MO BIO Ultraclean Microbial DNA Isolation Kit. A DNA library was prepared using the Nextera XT kit and then run on a MiSeq instrument (Illumina) for generating paired-end 250-bp reads, aiming at a coverage of at least 60-fold. *De novo* assembly was performed by CLC Genomics Workbench v7.0.4 (QIAGEN, Hilden, Germany) after quality trimming ( $Qs \geq 28$ ) with optimal word sizes. MLST and cgMLST were performed by Ridom SeqSphere.

**Results:** The 37 isolates from 2014 belonged to sequence types ST117 (n=23), ST80 (n=12), ST262 (n=1) and ST17 (n=1) (Fig 1). *E. faecium* cluster types (CT) among these isolates were CT103 (n=11), CT24 (n=11), CT104 (n=8), CT106 (n=3), CT107 (n=1), CT105 (n=1), CT60 (n=1) and CT16 (n=1) (Fig 1). Patients belonging to the November 2014 D3 (orange) outbreak within CT106 harboured both *vanA* and *vanB* VRE and transmitted the *vanA* and/or *vanB* gene(s) to other patients within CT104. Epidemiologically, 14 isolates belonged to the April 2014 VRE vanB C3 outbreak (purple). However, patient \*9801, was already VRE positive before and cgMLST analysis showed he belonged to the outbreak of 2013 (CT103). The index patient (\*790K) of the 2014 outbreak appeared to come from another regional hospital. Eventually, 11 patients within CT24 were epidemiologically and genotypically related. Patients from the July 2014 C3, November 2014 C4 and December 2014 E1IC outbreaks were genotypically related and this relation could also be confirmed by data of admittance and transfers to 4 common wards.

**Conclusion/discussion:** The cgMLST scheme provided a higher discriminatory power in the epidemiologically related and unrelated *E. faecium* isolates compared to MLST. It improved the epidemiological insight in the outbreaks and allows inter-laboratory exchange of typing data. However, it also created new questions regarding transmission pathways for which deeper epidemiological insight in patient transfer is required. Therefore, to create a complete picture of the outbreaks, all patients should be included in the cgMLST analysis. In addition, one should keep in mind that a patient can have several types of VRE over time.

**Reference:** 1. de Been, M, Pinholt, M, Top, J, Bletz, S, Mellmann, A, van Schaik, W, Brouwer, E, Rogers, M, Kraat, Y, Bonten, M, Corander, J, Westh, H, Harmsen, D, Willems, RJ. 2015. A core genome MLST scheme for high-resolution typing of *Enterococcus faecium*. *J. Clin. Microbiol.*



- Epi-event dec 2014 VRE vanB E1IC
- Outbreak apr 2014 VRE vanB C3
- Outbreak jul 2014 VRE vanB D4/E2/I/C3
- Outbreak jul 2014 VRE vanB D4/E2/I/CB
- Outbreak nov 2014 VRE vanB C4
- Outbreak nov 2014 VRE vanB D3

CT= cluster type (black), ST=sequence type (blue)  
 Numbers between lines reflects allele differences between the connecting isolates.  
 Epi-event = "small" outbreak  
 Outbreak = "large outbreak"  
 The last characters of the outbreak descriptions reflects the different wards.

## S3 - Plenary session

### OP14 - Phylogenomic Characterization Of The Causative Strain Of One Of The Largest Worldwide Outbreaks Of Legionnaires' Disease Occurred In Portugal In 2014

Vítor Borges<sup>1</sup>; Alexandra Nunes<sup>1</sup>; Daniel A Sampaio<sup>1</sup>; Luís Vieira<sup>1</sup>; João P. Gomes<sup>1</sup>

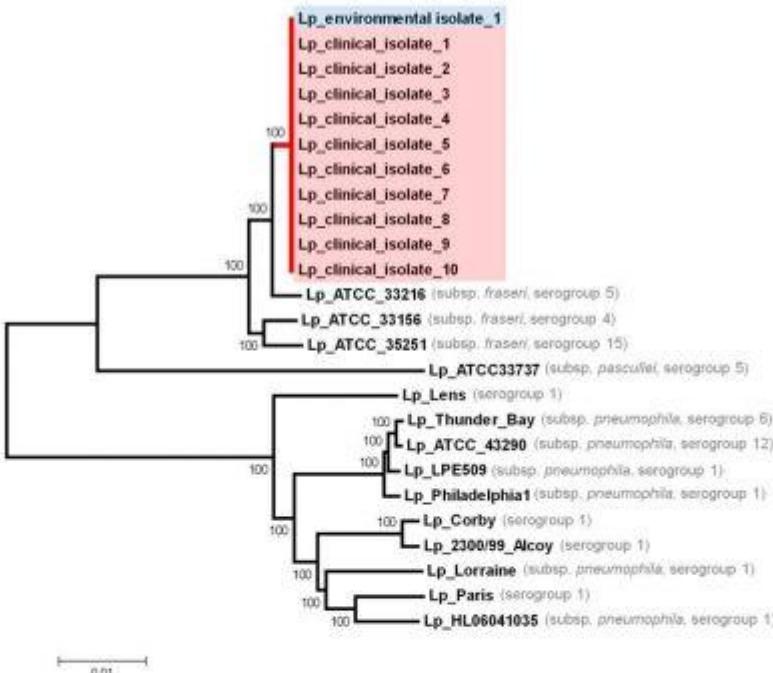
<sup>1</sup>National Institute of Health Dr. Ricardo Jorge

**Background:** Legionnaires' disease (LD) is a severe pneumonia with a case fatality rate of 8–12%. Transmission usually occurs by inhalation of aerosols or aspiration of water containing *Legionella* spp, where *L. pneumophila* serogroup 1 accounts for most of the occasional outbreaks. The world's second largest outbreak of LD occurred in 2014 in Vila Franca de Xira, Portugal, yielding more than 400 cases and 14 deaths. The *L. pneumophila* causative strain is from serogroup 1 and displays the novel sequence type (ST) ST1905. Here, we described how whole-genome sequencing (WGS) / Bioinformatics was used for real-time investigation of this LD outbreak as well as for integrating the genetic backbone of the causative strain in the frame of the species phylogeny and diversity.

**Materials | Methods:** To investigate the genetic relatedness of ST1905 isolates and to attribute the source of LD Portugal outbreak, 10 clinical isolates and one environmental isolate obtained from an industrial cooling tower were selected for WGS on a MiSeq Illumina instrument (depth of coverage >100-fold). Genome assembly was carried out using Velvet. Phylogenetic inferences using alignments of core-genome sequences from the 11 outbreak-associated *L. pneumophila* serogroup 1 isolates and/or multiple publicly available *L. pneumophila* genomes were performed through MEGA5 by using the neighbor-joining method with bootstrapping (1000 replicates).

**Results:** By using more than 99% of each draft genome sequence, we found no nucleotide differences between each clinical isolate and the environmental isolate, thus suggesting the suspected industrial cooling tower as the source of the LD outbreak. Unexpectedly, the outbreak causative strain phylogenetically clustered apart from the most worldwide studied outbreak-associated *L. pneumophila* serogroup 1 strains (Philadelphia-1, Paris, Lens, Corby, and 2300/99 Alcoy), being more closely related to *L. pneumophila* subsp. *fraseri* strains from serogroups other than serogroup 1 (Figure 1). We found that the novel outbreak strain harbors multiple genomic islands potentially related to virulence and/or adaptation, and carries an exclusive ~38kb genomic region that was only detected so far in one *L. oakridgensis* strain, a species that rarely causes LD. Of note, confirmation of the ST1905 allelic profile highlighted a bias associated with the *in silico* extraction of the allele sequence from WGS data, since the studied strain displayed non-matching *mompS* copies. This issue would hamper a proper ST attribution if the allelic profile was exclusively determined *in silico*.

**Conclusion:** WGS enabled an in-depth investigation of the recent LD outbreak that occurred in Portugal, as it allowed attributing, with a greater level of certainty, the source of the epidemic to an industrial cooling tower. Given the unusual genetic features apparently displayed by this outbreak causative stain, it is of the utmost importance to dissect the transmission skills and virulence for this particular strain in a near future, as those traits may justify the magnitude of the outbreak.



**Figure 1. Phylogenetic tree of *Legionella pneumophila* strains.** The phylogenetic reconstruction enrolls the draft genomes of 11 outbreak-associated *L. pneumophila* serogroup 1 isolates (ten clinical isolates plus one environmental isolate), ten finished chromosome sequences (including the ones from the most studied outbreak-associated *L. pneumophila* serogroup 1 strains: Lp Philadelphia-1, Lp Paris, Lp Lens, Lp Corby, and Lp 2300/99 Alcoy) and four draft genomes from subspecies other than the subsp. *pneumophila* available at GenBank. Sequences were aligned using the progressive algorithm of Mauve software (version 2.3.1), and a core-alignment (enrolling ~75% of each sequence) was extracted by keeping regions where all chromosome sequences aligned over at least 500 bp. Phylogenetic estimations were carried out through MEGA5 by using the neighbor-joining method with bootstrapping (1000 replicates) (values are shown next to the branch nodes), where the Kimura two-parameter method was employed to estimate the evolutionary distances.

## S3 - Plenary session

### SC04 - WGS-Based Approaches For Cluster Investigation Of Legionnaires' Disease: Current Experience And Future Direction

Jacob Moran-Gilad<sup>1-4</sup>

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<sup>4</sup>ESCMID Study Group for Molecular Diagnostics (ESGMD)

In recent years, development and implementation of molecular methods for *Legionella pneumophila* (Lp) have improved the detection of Lp in clinical and environmental samples as well as Legionnaires' disease (LD) investigation, with the 7-loci sequence-based typing (SBT) scheme being the current gold standard. Next-generation sequencing (NGS) is rapidly transforming public health microbiology and has proved to be a powerful tool for bacterial characterisation and typing. Whilst whole genome sequencing (WGS) is thus a promising modality for LD investigation, bioinformatics analysis approaches are neither standardised, nor agreed and there is a growing need for the development and adoption of a robust and widely accepted WGS-based Lp typing scheme. The recent application of WGS for LD investigation with emphasis on the recently proposed cgMLST scheme for Lp (Moran-Gilad et al., Eurosurveillance 2015) will be presented and discussed. WGS appears to resolve several known limitations of SBT, but at the same time, difficulties in ensuring backward compatibility need to be addressed. An international working group for Lp WGS has recently been set up on behalf of ESGLI and its activities in the near future will underpin the standardised implementation of WGS in the field of LD.

**SC05: - Whole Genome-Based Population Biology And Global Epidemiological Surveillance Of *Listeria monocytogenes***

Sylvain Brisse<sup>1</sup>

*<sup>1</sup>Institut Pasteur, Genotyping of Pathogens and Public Health, Paris, France*



**Thursday, 10<sup>th</sup> March 2016, 14:30-15:30**

**IP01 - Development And Validation Of A Whole Genome MLST Schema For *Salmonella* Surveillance**

Johan Goris, Hannes Pouseele, Katrien De Bruyne, Bruno Pot and Koen Janssens

*Applied Maths NV, Belgium*

**IP02 - Combined Genomic Antimicrobial Susceptibility Testing And Epidemiological Typing**

Alex van Belkum

*bioMerieux, La Balme Les Grottes. France*

# I1 - Industry session

## IP01 - Development And Validation Of A Whole Genome MLST Schema For *Salmonella* Surveillance

Johan Goris, Hannes Pouseele, Katrien De Bruyne, Bruno Pot and Koen Janssens

Applied Maths NV, Belgium

**Introduction:** The standard for molecular surveillance of bacterial foodborne diseases has been PFGE for many years. With the advent of high-throughput Whole Genome Sequencing (WGS), the key challenge remains the rapid and automated processing of WGS data, obtaining higher resolution compared to traditional methods. Whole genome Multi Locus Sequence Typing (wgMLST) and the availability of reliable and easy to use workflows allow for outbreak cluster detection in WGS-based surveillance. Similar to MLST, the wgMLST method requires a schema describing the loci and alleles. In this study, we report on the development and validation of a species-wide wgMLST schema for *Salmonella enterica*.

**Material and methods:** The *Salmonella enterica* wgMLST schema was created based on 163 genome and plasmid sequences, representing the major serotypes. An in-house developed schema creation procedure used a sampling-based multi-reciprocal BLAST search to determine those sets of alleles that make up the stable loci in the accessory, i.e. pan genome. Next, a per-locus allele assessment procedure determined the central prototype allele and thus the locus definition. The final schema contained 11636 loci, covering 94% on average of the CDS defined on the reference genomes.

Allele calls for each of the loci in the schema were a consensus of two approaches: (1) an assembly-based allele calling, done through a BLAST based procedure on de novo assembled genomes and (2) an assembly-free allele calling, using a k-mer approach directly on the reads.

Validation of the wgMLST schema was performed on publicly available whole genome sequences, described in outbreak reports or WGS studies. Since in the latter studies mainly whole genome single nucleotide polymorphism (wgSNP) based approaches were used, we re-analyzed the data with an in-house developed wgSNP tool as additional reference.

**Results:** The *Salmonella enterica* schema validation includes analysis of CDS and base recovery rates, and within and between locus diversity statistics. After validation, wgMLST analysis is performed on data from public studies and outbreak reports. Without exception, this analysis reliably identified isolates belonging to documented *Salmonella* outbreaks. wgMLST overall exhibited a very high resolution, obviously much higher than PFGE but also surpassing core SNP approaches. Moreover, the resolution of wgMLST can be tuned by switching to (local) typing schemes e.g. a core MLST schema or even the traditional 7-gene MLST schema. Using a set of quality criteria, problematic WGS runs could be clearly identified. The allele calling procedure proved to be robust, since the number of called alleles remains relatively unaffected by noticeable drops in sequence coverage or N50. For a few samples, the analysis provided clear evidence for contamination (mixed cultures).

A good correlation was observed between wgMLST and wgSNP results, with the resolution of wgSNP only surpassing that of wgMLST when a cluster-specific reference sequence was chosen.

**Conclusion:** The developed wgMLST schema for *Salmonella* offers ample resolution for outbreak cluster detection and, in combination with BioNumerics' analysis tools, enables a species-wide sample comparison in routine surveillance. While wgMLST and wgSNP essentially capture the same signal, wgMLST has the advantage of being reference-independent and hence can form the basis for fast screening and more important, for a stable nomenclature to be used internationally.

## IP02 - Combined Genomic Antimicrobial Susceptibility Testing And Epidemiological Typing

Alex van Belkum

*bioMerieux, La Balme Les Grottes. France*

Classic antimicrobial susceptibility testing (AST) mostly employs culture based technologies but over the past decades a variety of alternative methodologies have been presented in international literature. Essentially, any method that can distinguish dead or dying cells from growth-arrested or fully viable organisms is potentially suited as an AST system. Examples of such methods are spectroscopy, spectrometry and otherwise physics and/or (bio-)chemistry based procedures. Several of these innovative technologies have now been adopted by spin-off companies that are seeking accreditation of such tests. Obviously, combined susceptibility values of individual bacterial strains towards a panel of antimicrobials has functioned and will continue to function as the basis of epidemiological type designation.

The most extensive AST innovation has been in the domain of high-throughput sequencing technology. Whereas the role of next generation sequencing (NGS) in straightforward sequence-based epidemiological monitoring and source tracking has been presented convincingly, the use of NGS for AST has been a bit more controversial. The presentation will cover an assessment of the NGS AST state of affairs and the impact of AST on the epidemiological use of genomic AST data.

Genomic AST can be used in three different fields of research. The technology allows for the definition of new resistance mechanisms and this will be exemplified by research we performed with strains of *E. coli* being heterogeneous for TZT resistance. Beta-lactamase resistance was amplified during selection of resistant isolates and the mechanisms leading to this amplification has now been partially elucidated. NGS can also be used to build predictive models where a genome sequence can be used to propose a molecular antibiogram. Examples of such work for *Staphylococcus aureus* and some enterobacterial species have been published and our own work will cover phenotype-genotype correlations in *Pseudomonas aeruginosa*. Finally, resistome wide association studies can lead to detection of (new) molecular markers associated with specific levels of resistance. Obviously, all of this work requires the construction of adequate reference databases and the use of solid sequencing data.

The presentation will focus on the genomics of AST but will also correlate genomic AST data with epidemiological fine-typing. Test validation and the use of open databases will be addressed as will be the need for coordination of data interpretation in light of regulatory accreditation of the tests and their ultimate application in the setting of the routine clinical microbiology laboratory. Perspectives are good but there are many issues in the clinical perimeter that still need to be resolved.



## FOOD, ZOONOTIC AND ENVIRONMENTAL MICROBIAL RISKS

**Thursday, 10<sup>th</sup> March 2016, 15:30-17:00**

### OP15 - Comparative Phenotypic and Genomic Analysis of Food Borne Isolates Involved In Multistate Outbreaks Of Atypical *Salmonella enterica* Serovars

Daniel Hurley<sup>1</sup>; Maria Hoffmann<sup>2</sup>; Marta Martins<sup>1</sup>; Eric W. Brown<sup>2</sup>; Séamus Fanning<sup>1</sup>

<sup>1</sup>UCD Centre for Food Safety, University College Dublin; <sup>2</sup>Center for Food Safety and Nutrition, Division of Microbiology, Office of Regulatory Science, U.S. Food and Drug Administration

### OP16 - A Single Clonal Lineage Of Group B *Streptococcus* Is Causing Bovine Mastitis In The North Of Portugal

Alexandre Almeida<sup>1</sup>; Cinthia Alves-Barroco<sup>2</sup>; Elisabeth Sauvage<sup>3</sup>; Ricardo Bexiga<sup>4</sup>; Pedro Albuquerque<sup>5</sup>; Fernando Tavares<sup>5</sup>; Ilida Santos-Sanches<sup>2</sup>; Philippe Glaser<sup>3</sup>

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### OP17 - Genomic Epidemiology And Cross-Border Transmission Of *Salmonella choleraesuis* var. *kunzendorf* In European Pigs And Wild Boar

Pimlapas Leekitcharoenphon<sup>1</sup>; Frank M. Aarestrup<sup>1</sup>; Rene S. Hendriksen<sup>1</sup>

<sup>1</sup>Technical University of Denmark

### OP18 - Whole Genome Sequencing Of The Leprosy Agent *Mycobacterium lepromatosis* In Infected Red Squirrels From Scotland

Charlotte Avanzi<sup>1</sup>; Andrej Benjak<sup>1</sup>; Philippe Busso<sup>1</sup>; Chloé Loiseau<sup>1</sup>; Karen Stevenson<sup>2</sup>; Joyce McLucky<sup>2</sup>; Jorge Del Pozo<sup>3</sup>; Lucio Vera Cabrera<sup>4</sup>; Anna Meredith<sup>3</sup>; Stewart T. Cole<sup>1</sup>

<sup>1</sup>Ecole Polytechnique Fédérale de Lausanne; <sup>2</sup>Moredun Research Institute;

<sup>3</sup>The University of Edinburgh; <sup>4</sup>Servicio de Dermatología, Hospital Universitario

## S4 - Plenary session

### OP19 - Tracing Human *Campylobacter jejuni* Isolates To Chicken Slaughter Batches And Swimming Water Using Whole-Genome Multilocus Sequence Typing

Sara Kovanen<sup>1</sup>; Rauni Kivistö<sup>1</sup>; Ann-Katrin Llarena<sup>1</sup>; Ji Zhang<sup>1</sup>; Ulla-Maija Kärkkäinen<sup>2</sup>; Tamara Tuuminen<sup>3</sup>; Jaakko Uksila<sup>4</sup>; Marjaana Hakkinen<sup>5</sup>; Mirko Rossi<sup>1</sup>; Marja-Liisa Hänninen<sup>1</sup>

<sup>1</sup>University of Helsinki; <sup>2</sup>Eastern Finland Laboratory Centre Joint Authority Enterprise (ISLAB), Kuopio District Laboratory; <sup>3</sup>Eastern Finland Laboratory Centre Joint Authority Enterprise (ISLAB), Mikkeli District Laboratory; <sup>4</sup>Keslab Laboratory, Department of Clinical Microbiology, Central Finland Health Care District,; <sup>5</sup>Finnish Food Safety Authority

### OP20 - Spread Of Fluoroquinolone Resistant CTX-M-15-Producing *Escherichia coli* ST410 In Humans And Animals

Linda Falgenhauer<sup>1</sup>; Can Imirzalioglu<sup>1</sup>; Hiren Ghosh<sup>1</sup>; Konrad Gwozdzinski<sup>1</sup>; Judith Schmiedel<sup>1</sup>; Katrin Gentil<sup>1</sup>; Trinad Chakraborty<sup>1</sup>

<sup>1</sup>Institute of Medical Microbiology, University Hospital Giessen and German Center for Infection Research (DZIF), partner site Giessen-Marburg-Langen

### OP15 - Comparative Phenotypic and Genomic Analysis of Food Borne Isolates Involved In Multistate Outbreaks Of Atypical *Salmonella enterica* Serovars

Daniel Hurley<sup>1</sup>; Maria Hoffmann<sup>2</sup>; Marta Martins<sup>1</sup>; Eric W. Brown<sup>2</sup>; Séamus Fanning<sup>1</sup>

<sup>1</sup>UCD Centre for Food Safety, University College Dublin; <sup>2</sup>Center for Food Safety and Nutrition, Division of Microbiology, Office of Regulatory Science, U.S. Food and Drug Administration

**Background:** *Salmonella* is a zoonotic pathogen responsible for illness on a global scale due to its ability to invade a broad range of hosts causing both acute and chronic infection. The pathogenesis of *S. Typhimurium* is well researched and has traditionally been studied using *in vivo* models and murine cell lines. However, there are over 2,600 *Salmonella* serovars and fewer studies in a human relevant model are available for non-typhoidal *S. enterica* serovars which contribute significantly to clinical cases of salmonellosis. In this study, 10 food borne isolates of atypical *S. enterica* serovars cultured from multistate, US outbreaks were characterised both phenotypically using *in vitro* and infection biology techniques as well as genotypically by whole genome sequencing and comparative bioinformatic analyses to determine the underlying virulence factors behind these outbreaks.

**Materials | Methods:** To emulate the challenges ingested salmonellae encounter along the alimentary canal during infection, all isolates were characterised phenotypically by *in vitro* acid survival, swim/swarm motility and bile salt tolerance assays. Pathogenicity was evaluated *ex vivo* by gentamicin protection assays in both murine RAW 264.7 and human THP-1 macrophages using *S. Typhimurium* ST4/74 as a reference strain. The host response to infection between isolates was characterised by quantifying proinflammatory cytokine and infection relevant chemokine release from infected macrophages. Whole genome sequencing of the isolates was performed using both the Illumina MiSeq and Pacific Biosciences RS II sequencing platforms.

**Results:** In human THP-1 macrophage, a number of isolates survived for up to 168 Hours Post Infection (HPI) (7 days) whereas *S. Typhimurium* ST4/74 was unrecoverable beyond 96 HPI. *S. Tennessee* CFSAN1387 displayed the lowest reduction in intracellular bacteria between 2 and 168 HPI highlighting the ability of this isolate to persist comfortably within macrophages. *S. Weltevreden* CFSAN001415 displayed a 2-log reduction in viable intracellular bacteria between 2 and 168 HPI, surviving for 7 days and stimulated increased release of multiple chemokine targets (including IP-10, MCP-1 and MIP-1 $\alpha/\beta$ ).

*Salmonella* Pathogenicity Island (SPI) gene content in addition to known *Salmonella* virulence factors of the isolates were compared identifying distinct variable regions and/or absence of key effector proteins in specific isolates. *S. Anatum* CFSAN003959 possesses an altered SPI-2 SsaB protein and associated defective virulence phenotype rendering the isolate unrecoverable early during infection. *S. Heidelberg* CFSAN002063 harbours an altered SptP protein stimulating high levels of TNF- $\alpha$  release compared to all other isolates. The SseBCD translocon for the secretion of effector proteins by *Salmonella* is integral intracellular survival. *S. Tennessee* CFSAN001387 displays highly altered SseBCD proteins in comparison to *S. Typhimurium* ST4/74 which has given clues as to the ability of this isolate to survive within THP-1 macrophages for extended periods of time.

**Conclusion:** Taken together, these phenotypic observations extend our understanding of the bacterial host-cell response and coupled with the depth of scope provided by whole genome sequencing has enabled the identification of features that may contribute to the ability of these isolates to cause food borne outbreaks.

## S4 - Plenary session

### OP16 - A Single Clonal Lineage Of Group B *Streptococcus* Is Causing Bovine Mastitis In The North Of Portugal

Alexandre Almeida<sup>1</sup>; Cinthia Alves-Barroco<sup>2</sup>; Elisabeth Sauvage<sup>3</sup>; Ricardo Bexiga<sup>4</sup>; Pedro Albuquerque<sup>5</sup>; Fernando Tavares<sup>5</sup>; Ilda Santos-Sanches<sup>2</sup>; Philippe Glaser<sup>3</sup>

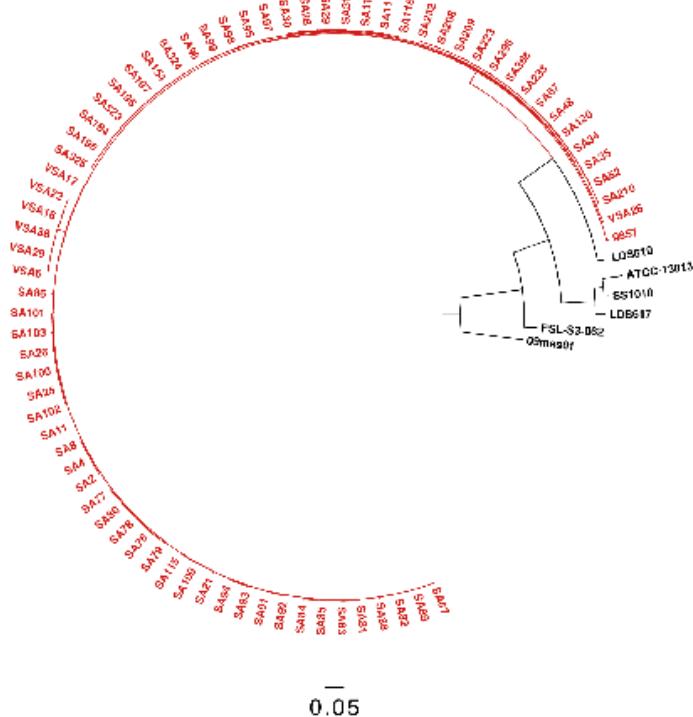
<sup>1</sup>Institut Pasteur, Unité Evolution et Ecologie de la Résistance aux Antibiotiques; CNRS UMR 3525; Université Pierre et Marie Curie; <sup>2</sup>UCIBIO, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa; <sup>3</sup>Institut Pasteur, Unité Evolution et Ecologie de la Résistance aux Antibiotiques; CNRS UMR 3525; <sup>4</sup>Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa; <sup>5</sup>Faculdade de Ciências, Departamento de Biologia, Universidade do Porto; Centro de Investigação em Biodiversidade e Recursos Genéticos

**Background:** Group B *Streptococcus* (GBS) is a leading cause of neonatal infections. However, it was first described by Nocard-Mollereau in 1887 as a cause of bovine mastitis. Particularly in Portuguese dairy farms, GBS remains a serious concern as one of the species most frequently isolated from animals with mastitis. The purpose of this work was to understand the persistence of GBS mastitis in Portugal and to gain new insights into the evolutionary processes behind its specific adaptation to the bovine environment.

**Materials and Methods:** We have analysed a set of 203 GBS isolated from the north and southwest of Portugal between 2002 and 2014, originating from 16 farms, together with eight strains from the southwest of France collected between 1996 and 1997. A CRISPR typing was performed for epidemiological assessment and 73 representative strains were subsequently selected for whole-genome sequencing. Core-genome phylogenies were inferred using both a maximum-likelihood (RAxML) and a Bayesian framework (BEAST). Furthermore, pan-genome analyses were carried out to compare the genetic content across the samples selected, as well as dN/dS calculations to look for positive selection of particular mutations.

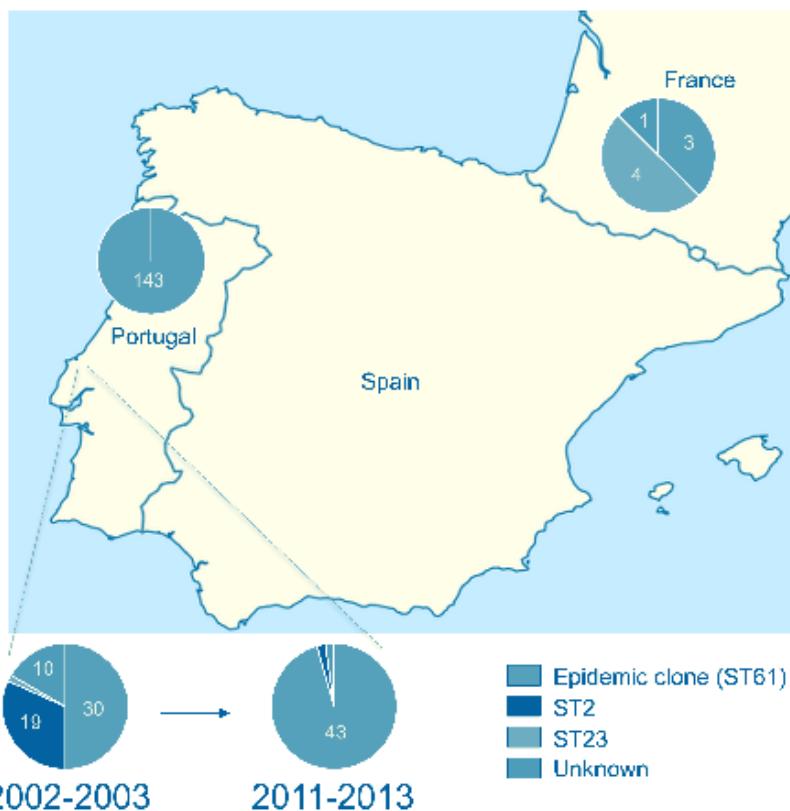
**Results:** CRISPR typing revealed that all GBS strains sampled from the north belong to clonal complex 61. Surprisingly, phylogenetic inference with other publicly available ST61 genomes revealed that the entire GBS population infecting the north of Portugal was the result of the introduction and subsequent dissemination of a single clone (Figure 1). BEAST analyses estimated that this expansion started between 1971 and 1995, a period in which Portugal underwent significant political and economical changes followed by the expansion of national dairy herds and an increase in individual herd size. 50% of the strains from the southwest of Portugal collected between 2002 and 2003 also belong to this lineage. Strikingly, preliminary assessment of a more recent GBS collection from this region revealed an ongoing replacement of the local GBS population by this epidemic clone (Figure 2). The accessory genome of strains from this clonal lineage showed a dynamic flux of mobile genetic elements shared among other animal-adapted streptococcal species. These included 40 genes involved in drug production or resistance, and 21 responsible for sugar metabolism. Adaptation of these strains to the bovine udder and milk resulted in a directed, albeit limited, loss of functional traits, some of which were shown to be important for GBS colonization and infection of the human host. The presence of recurrent nonsynonymous mutations in five transcriptional regulator genes suggests parallel adaptive evolution, possibly reflecting important selective forces for the bovine/milk-specific adaptation.

**Conclusion:** Here, we show that GBS infected farms in the north of Portugal are all colonized by one particularly well-adapted clone that is also taking over other dairy farms in the country. The loss of dispensable traits more important for virulence and adaptation to humans suggests that once GBS undergoes extensive adaptive changes in bovines, it might not be able to as efficiently recolonize and infect the human host. New strategies will need to be applied to control GBS mastitis in this region and circumvent the particular affinity of this epidemic clone to the bovine host.



**Figure 1.** Core-genome phylogeny of the 73 strains selected for whole-genome sequencing (in red) together with other ST61 genomes publicly available (black). Strain 09mas01 belonging to ST1 was used as an outgroup.

## S4 - Plenary session



**Figure 2.** Number of strains found to belong to the ST61 epidemic clone or other sequence types (STs), according to the figure key. Genotyping was performed by CRISPR sequencing (Lopez-Sanchez et al. 2012).

### OP17 - Genomic Epidemiology And Cross-Border Transmission Of *Salmonella choleraesuis* var. *kunzendorf* In European Pigs And Wild Boar

Pimlapas Leekitcharoenphon<sup>1</sup>; Frank M. Aarestrup<sup>1</sup>; Rene S. Hendriksen<sup>1</sup>

<sup>1</sup>Technical University of Denmark

**Background:** *Salmonella Choleraesuis* is a relative infrequent serovar adapted to pigs but also have a propensity to cause extraintestinal infections in humans. *S. Choleraesuis* var. *Kunzendorf* are responsible for the majority of outbreaks among pigs. In Europe, *S. Choleraesuis* is a relatively rare serovar, both in slaughter pigs and in breeding herds. In Denmark, only a few outbreaks have been reported among pig herds within the last decade; 1999 – 2000 and 2012 – 2013 and in both cases it has been impossible to identify the route of transmission and source of infection. With the advance in whole genome sequencing, we have sequenced 108 retrospective *S. Choleraesuis* isolates from 1991 – 2013 isolated from pig and wild boar from 11 European countries and USA in order to understand transmission and epidemiology of *S. Choleraesuis*.

**Materials | Methods:** We applied SNP-based phylogenetic methods based on whole genome sequences (sequenced by Illumina MiSeq) to identify the population structure. We used Bayesian phylogeny to estimate dates of divergence and phylogeographic analyses of lineages by using BEAST with Bayesian Skyline model of population size change and relaxed uncorrelated lognormal clock as the molecular clock.

**Results:** All 108 swine isolates from 12 countries were aligned to the reference genome and 5,418 SNPs were determined. The maximum parsimony tree of all isolates consisted of distantly separated clusters according to ST types; ST-1858, ST-139, ST-1857 and ST-1804. We selected 94 isolates from a complex cluster of ST-1857 and ST-1804 for further analysis. The 94 isolates yielded 2,428 SNPs. These SNPs were subjected to temporal phylogenetic reconstruction using BEAST. Figure 1 showed the Bayesian maximum clade credibility tree for the 94 isolates. The mutation rate was estimated to be  $1.58 \times 10^{-6}$  SNPs/site/year. The most recent common ancestor was estimated to have emerged in 1946 (95% credible interval, 1872 - 1981). The isolates were divided into two complex clusters and they resided in sub-clusters according to countries and neighbour countries of isolation. The exceptions were isolates from Germany, Poland and Ireland that sporadically resided through out the tree. According to the source of isolation, the wild boar isolates from Austria were clustered together but the wild boar isolates from Germany, Hungary and Estonia were clustered with strains from pig. ST type supported the clustering of isolates from Estonia. They all had ST-1857. The majority of isolates had ST-1804 except for an isolate from the US having ST-1860 that was clustered closely with ST-1804 isolates as these two ST types have only one allele difference. The Danish outbreak isolates were distantly divided into two groups according to outbreak period. The recent outbreak isolates (2012-2013) contained an extra Q1 replicon, whereas some earlier outbreak isolates (1991-2000) had an extra I1 replicon. Some of the earlier outbreak strains were isolated from different organs from the same animal. Those isolates clustered by the pig where they were isolated from.

**Conclusion:** These results provide the advantage of using WGS for elucidating evolution and transmission of *S. Choleraesuis* and emphasize the usefulness of the phylodynamic approaches to monitor the emergence and spread over time of these particular strains. These findings may help to promote and establishing future prevention and control measurement of similar successful clones.

## S4 - Plenary session

### OP18 - Whole Genome Sequencing Of The Leprosy Agent *Mycobacterium lepromatosis* In Infected Red Squirrels From Scotland

Charlotte Avanzi<sup>1</sup>; Andrej Benjak<sup>1</sup>; Philippe Busso<sup>1</sup>; Chloé Loiseau<sup>1</sup>; Karen Stevenson<sup>2</sup>; Joyce McLucky<sup>2</sup>; Jorge Del Pozo<sup>3</sup>; Lucio Vera Cabrera<sup>4</sup>; Anna Meredith<sup>3</sup>; Stewart T. Cole<sup>1</sup>

<sup>1</sup>Ecole Polytechnique Fédérale de Lausanne; <sup>2</sup>Moredun Research Institute;

<sup>3</sup>The University of Edinburgh; <sup>4</sup>Servicio de Dermatología, Hospital Universitario

Keywords: *M. lepromatosis*, *Sciurus vulgaris*, genomics

**Background:** Leprosy is a chronic mycobacterial infection caused by *Mycobacterium leprae* and the newly described species *M. lepromatosis*. While *M. leprae* is commonly found in humans in endemic countries worldwide, *M. lepromatosis* in humans is mainly reported in Mexico and the Caribbean region. In contrast to *M. leprae*, the existence of environmental reservoirs of *M. lepromatosis* has not yet been described. However, this species was recently reported in diseased red squirrels (*Sciurus vulgaris*) in Scotland. The goal of this study was to compare the strains of *M. lepromatosis* from Scotland to those from Mexico using whole genome sequencing.

**Materials/Methods:** We received tissue samples from four infected red squirrels from Scotland and one skin biopsy sample from a Mexican patient. DNA was extracted from tissue samples by an in-house optimized method. Illumina libraries were prepared (Kapa hyper prep kit, Kapabiosystem) and sequenced in multiplex on HiSeq according to established procedures. Reads were mapped to the *M. lepromatosis* reference genome (AN: JR PY01000001) and single nucleotide polymorphisms (SNPs) were identified using Freebayes. The phylogenetic tree was calculated using MEGA6.

**Results:** We obtained high average genome coverage for all samples, ranging from 80 to 376X. Comparative analysis revealed that 472 polymorphisms were common to the 4 strains from Scotland, compared to the Mexican strains. There is low variation between strains from Scotland (7 to 9 polymorphisms) as well as between the two Mexican strains (15 polymorphisms).

**Conclusion:** We have confirmed the presence of *M. lepromatosis* in red squirrels in Scotland using shotgun sequencing. Genetic variation within *M. lepromatosis* populations is very low, similar to *M. leprae*. In addition, our findings show that strains from Scotland belong to a distinct lineage compared to Mexican strains but this is more diverse compared to *M. leprae* strains. Further research is needed to estimate the time of introduction of *M. lepromatosis* into Scotland, as well as to assess possible risk factors for transmission to humans.

### OP19 - Tracing Human *Campylobacter jejuni* Isolates To Chicken Slaughter Batches And Swimming Water Using Whole-Genome Multilocus Sequence Typing

Sara Kovanen<sup>1</sup>; Rauni Kivistö<sup>1</sup>; Ann-Katrin Llarena<sup>1</sup>; Ji Zhang<sup>1</sup>; Ulla-Maija Kärkkäinen<sup>2</sup>; Tamara Tuuminen<sup>3</sup>; Jaakko Uksila<sup>4</sup>; Marjaana Hakkinen<sup>5</sup>; Mirko Rossi<sup>1</sup>; Marja-Liisa Hänninen<sup>1</sup>

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**Background:** Campylobacteriosis is the most commonly reported cause of bacterial gastroenteritis in the European Union and handling of raw or eating improperly cooked poultry meat have been identified as major risk factors for acquiring the infection. Although several studies have highlighted chickens as an important reservoir for human infections, few have attempted to trace human cases directly to slaughter batches. Thus, the aim of this study was to assess the ability of whole genome MLST (wgMLST) to directly trace human *C. jejuni* domestically acquired infections to Finnish chicken.

**Methods:** The human patient *C. jejuni* isolates (n=95) included represent all recovered isolates from domestically acquired campylobacteriosis cases collected during the seasonal peak (June to September) from three hospital districts in Central Finland in 2012. The included chicken-derived *C. jejuni* isolates (n=83) were collected during the same time period through the Finnish *Campylobacter* monitoring program for poultry. In tracing the patient *C. jejuni* isolates to chicken slaughter batches we used the following temporary relationship estimations: the chicken isolates originating from the positive slaughter batches were considered a potential source of human infection if the slaughter preceded the illness by two to 23 days (the time between the date of slaughter and time of patient sampling). Draft genome sequences were determined using Illumina HiSeq sequencing technology and assembled using SPAdes 3.2.1. MLST types were assigned using the *Campylobacter* pubMLST database. Further, wgMLST was performed using Genome Profiler. To identify single nucleotide polymorphisms (SNPs), alignments of loci having multiple allele types were manually inspected and ≤ 5 SNPs was used as a cut-off value to indicate genetically highly related isolates.

**Results:** At MLST level, 79% of the sequence types (STs) of the human isolates overlapped with chicken STs thereby confirming chicken as an important reservoir. Four STs, the ST-45, ST-230, ST-267 and ST-677, covered 75% of the human and 64% of the chicken isolates. Further wgMLST analysis of the isolates within these STs and their temporal relationship revealed that 22 of the human isolates (24%) were traceable back to 12 of a total of 69 chicken batches.

**Conclusion:** These results illustrate the power of whole genome analysis to reconstruct the transmission pathway from chicken slaughter batches to humans. Further, our study point out the necessity of identifying other potential transmission routes for campylobacteriosis as to successfully implement effective public health intervention strategies. Additionally, the results showed that the high discriminatory power of wgMLST supports its use as an accurate method for comparison of temporarily associated isolates. However, studies on the genomic stability and interpretation of genetic changes between *C. jejuni* isolates are needed.

## S4 - Plenary session

### OP20 - Spread Of Fluoroquinolone Resistant CTX-M-15-Producing *Escherichia coli* ST410 In Humans And Animals

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**Background:** Multidrug-resistant *Escherichia coli* from humans, companion animals, and livestock frequently harbour extended-spectrum beta-lactamase genes, thereby impairing the treatment options in case of an infection with bacteria carrying these enzymes. In particular, CTX-M-type ESBL enzymes isolates are often isolated from humans, companion animals, livestock, wild animals and the environment, raising concern regarding exchange and spread of isolates among these populations. To address this question, we performed detailed molecular epidemiological analysis of ESBL-producing *E. coli* in animal and human populations in Germany.

**Material and Methods:** Whole genome sequencing was performed for 94 CTX-M-15-producing *E. coli* isolates from humans (n=47), companion animals (n=26), livestock (n=17), and farm environments (n=4). The sequence type (ST) of these isolates was identified. Phylogeny and single nucleotide polymorphisms (SNPs) of sequenced isolates were assessed. Virulence genes and plasmid properties were identified.

**Results:** 26 STs were detected among the CTX-M-15-producing *E. coli* isolates. ST410 was the most frequent ST found and was isolated from humans (n=9), companion animals (n=4), livestock (n=8), and the farm environment (n=3). Within the ST410 isolates, we identified five clades (A-E). Isolates of clade B were present in all four populations and their core genomes differed by less than 75 SNPs from each other. In addition, isolates of clade B and C were clonally marked by chromosomal insertion of *bla*<sub>CTX-M-15</sub> genes either in the *rhsE* locus (clade B) or in a defective lambdoid bacteriophage (clade C).

**Conclusion:** Our data provides strong evidence for clonal dissemination of CTX-M-15-producing *E. coli* ST410 between human and animal populations.

## NOVEL DIAGNOSTIC AND TYPING METHODOLOGIES

**Thursday, 10<sup>th</sup> March 2016, 17:30-19:00**

### **OP21 - Molecular Divergence Of Vancomycin-Resistant *Enterococcus faecium* Isolated In Belgium And Denmark Dissected By Whole Genome Mapping**

Jasmine Coppens<sup>1</sup>; Basil Britto Xavier<sup>1</sup>; Katherine Loens<sup>1</sup>; Christine Lammens<sup>1</sup>; Lotte Jakobsen<sup>2</sup>; Anette M. Hammerum<sup>2</sup>; Herman Goossens<sup>1</sup>; Surbhi Malhotra-Kumar<sup>1</sup>

<sup>1</sup>Department of Medical Microbiology, Vaccine & Infectious Disease Institute, Universiteit Antwerpen; <sup>2</sup>Department of Microbiological Surveillance and Research, Statens Serum Institut

### **OP22 - Mitigating The Effects Of Sequence Data Quality On Strain Typeability: Towards The Development Of Robust Core Genome MLST (cgMLST) Schemes**

Dillon O.R. Barker<sup>1</sup>; Peter Kruczakiewicz<sup>2</sup>; James E. Thomas<sup>1</sup>; Chad Laing<sup>2</sup>; Victor P. J. Gannon<sup>2</sup>; Eduardo N. Taboada<sup>2</sup>

<sup>1</sup>Department of Biological Sciences, University of Lethbridge; <sup>2</sup>National Microbiology Laboratory at Lethbridge, Public Health Agency of Canada

### **OP23 - Development Of A Rapid Diagnostic Test For The Identification Of Pathogenic *Campylobacter jejuni* Isolates**

Cody Buchanan<sup>1</sup>; Peter Kruczakiewicz<sup>2</sup>; Steven K. Mutschall<sup>2</sup>; Benjamin Hetman<sup>3</sup>; James E. Thomas<sup>3</sup>; Victor P. J. Gannon<sup>2</sup>; G. Douglas Inglis<sup>4</sup>; Eduardo N. Taboada<sup>2</sup>

<sup>1</sup>Animal Diseases Research Institute, Canadian Food Inspection Agency;

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<sup>4</sup>Lethbridge Research Centre, Agriculture and Agri-Food Canada

### **OP24 - Proteotyping Of *Streptococcus pneumoniae*, Using Tandem Mass Spectrometry For Identification Of Biomarkers For Species And Strain Differentiation**

Hedvig Engström Jakobsson<sup>1</sup>; Lucia Gonzales Siles<sup>1</sup>; Roger Karlsson<sup>2</sup>; Fredrik Boulund<sup>3</sup>; Francisco Salvà-Serra<sup>1</sup>; Erik Kristiansson<sup>3</sup>; Edward R.B. Moore<sup>1</sup>

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## S5 - Plenary session

### OP25 - The Evaluation Of Whole Genome Sequencing For The Epidemiological Typing Of *Legionella pneumophila*

Sophia David<sup>1</sup>; Martin Aslett<sup>1</sup>; Rediat Tewolde<sup>2</sup>; Simon Harris<sup>1</sup>; Massimo Mentasti<sup>2</sup>; Baharak Afshar<sup>2</sup>; Anthony Underwood<sup>2</sup>; Julian Parkhill<sup>1</sup>; Timothy Harrison<sup>2</sup>

<sup>1</sup>Wellcome Trust Sanger Institute; <sup>2</sup>Public Health England

### OP26 - Tracking A *Mycobacterium tuberculosis* MDR Strain In Equatorial Guinea Based On ASO-PCR Targeting Specific SNPs Obtained From WGS Data

Laura Pérez-Lago<sup>1</sup>; Griselda Tudó<sup>2</sup>; Iñaki Comas<sup>3</sup>; María Carcelén<sup>1</sup>; Marta Herranz<sup>1</sup>; Santiago Izco<sup>1</sup>; María Jesus Ruiz Serrano<sup>1</sup>; Juliá Gonzalez<sup>2</sup>; Juan Eyene<sup>4</sup>; Emilio Bouza<sup>1</sup>; Darío García de Viedma<sup>1</sup>

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### OP21 - Molecular Divergence Of Vancomycin-Resistant *Enterococcus faecium* Isolated In Belgium And Denmark Dissected By Whole Genome Mapping

Jasmine Coppens<sup>1</sup>; Basil Britto Xavier<sup>1</sup>; Katherine Loens<sup>1</sup>; Christine Lammens<sup>1</sup>; Lotte Jakobsen<sup>2</sup>; Anette M. Hammerum<sup>2</sup>; Herman Goossens<sup>1</sup>; Surbhi Malhotra-Kumar<sup>1</sup>

<sup>1</sup>Department of Medical Microbiology, Vaccine & Infectious Disease Institute, Universiteit Antwerpen; <sup>2</sup>Department of Microbiological Surveillance and Research, Statens Serum Institut

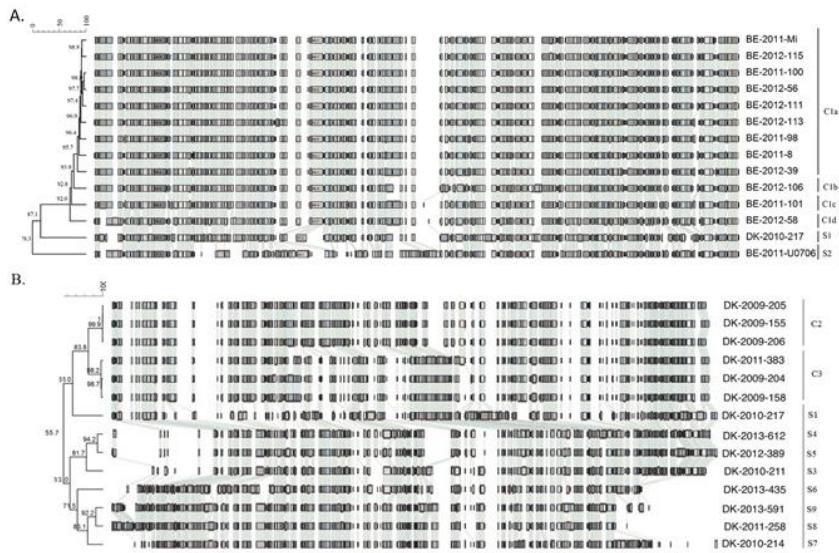
**Introduction:** Vancomycin-resistant *E. faecium* (VRE) is a prominent cause of nosocomial outbreaks worldwide. Molecular strain typing plays a crucial role in outbreak investigations and hospital epidemiological surveillance. Here we report, for the first time, a whole genome mapping (WGM) based-typing approach to study VRE isolated from two different geographic areas in Europe, Belgium (BE, Belgian National Reference Centre for invasive Group A Streptococci, n=13) and Denmark (DK, Skejby Aarhus University Hospital, n=14).

**Methods:** Twenty-seven VRE strains isolated during 2009-2013 from blood (n=8), catheter (n=2), urine (n= 8), wound (n=2), throat (n=2), faeces (n=1), abdominal secretions (n=1), bronchial/tracheal secretions (n=1), and from other undetermined sites (n=2) were typed by MLST, PFGE and WGM. Restriction analysis for PFGE was performed using *Sma*I using a 7-band difference cut-off. WGMs were generated using *Nco*I restriction and the Argus® system (Opgen, Gaithersburg, USA), and data were analysed using Bionumerics (Applied Maths NV).

**Results:** According to MLST, all typed *E. faecium* belonged to CC17 and were divided into 5 ST types. All BE and one DK (217) strains were ST203. The other 13 DK strains were ST18 (n=6), ST192 (n=3), ST117 (n=3) and ST260 (n=1) (Table 1). All BE strains (ST203) were grouped into 1 PFGE type further subdivided into 2 subtypes, while DK strains were divided into 8 PFGE types and 10 subtypes (Table 1). Twelve of the 14 ST203 strains formed one WGM cluster (95% similarity co-efficient), while the 2 singleton strains (BE-U0706 and VRE217) showed only 78.3% similarity to the major cluster (Fig 1a). Using similar criteria, WGMs of the 13 DK strains were divided into 2 clusters and 7 singletons (Fig 1b). Application of the adjusted Wallace index showed that both WGM and PFGE types were strongly predictive of MLST types (Wallace co-efficient of 1.00 for both methods). The Simpson's diversity index showed that discriminatory power of WGM (0.871, 95% CI: 0.758-0.984) was slightly higher than that of PFGE (0.843, 95% CI: 0.716-0.971).

**Conclusion:** This study revealed high genomic heterogeneity among the typed CC17 *E. faecium* clinical isolates. Notwithstanding the obvious advantage of WGM in allowing comparisons to whole genome sequenced isolates to detect deletions/insertions in the tested strains, our comparison of typing methods showed WGM to be a superior approach to PFGE for analyzing rapidly evolving clones like the *E. faecium* CC17.

## S5 - Plenary session



**Figure 1.** Comparison of WGMs of ST203 (BE and DK) (A), and of STs 18, 192, 117 and 260 (B). Cluster algorithm UPGMA was used to generate dendrogram with similarity co-efficient of 95% in Bionumerics. Reference WGMs were BE-2011-Mi (A) and DK-2009-205 (B).

Table 1

Strain name	ST type	PFGE type	WGM cluster
BE-2011-8	203	130a	C1a
BE-2011-Mi	204	130a	C1a
BE-2011-U0706	203	130b	S2
BE-2012-39	203	130a	C1a
BE-2012-56	203	130a	C1a
BE-2012-58	203	130a	C1d
BE-2011-98	203	130a	C1a
BE-2011-100	203	130a	C1a
BE-2011-101	201	130a	C1c
BE-2012-106	204	130a	C1b
BE-2012-111	203	130a	C1a
BE-2012-113	203	130a	C1a
BE-2012-115	201	130a	C1a
DK-2010-217	203	90a	S1
DK-2009-155	18	86a	C2
DK-2009-205	18	86a	C2
DK-2009-206	18	86a	C2
DK-2009-158	18	86b	C3
DK-2009-204	18	86b	C3
DK-2011-381	18	86c	C3
DK-2010-214	117	91a	S7
DK-2011-258	117	91a	S8
DK-2013-591	117	95a	S9
DK-2010-211	192	101a	S3
DK-2012-389	192	101a	S5
DK-2013-612	192	102a	S4
DK-2013-435	260	98a	S6

Note: C: cluster, S: singleton, BE: Belgium, DK: Denmark

## S5 - Plenary session

### OP22 - Mitigating The Effects Of Sequence Data Quality On Strain Typeability: Towards The Development Of Robust Core Genome MLST (cgMLST) Schemes

Dillon O.R. Barker<sup>1</sup>; Peter Kruczakiewicz<sup>2</sup>; James E. Thomas<sup>1</sup>; Chad Laing<sup>2</sup>; Victor P. J. Gannon<sup>2</sup>; Eduardo N. Taboada<sup>2</sup>

<sup>1</sup>Department of Biological Sciences, University of Lethbridge; <sup>2</sup>National Microbiology Laboratory at Lethbridge, Public Health Agency of Canada

**Background:** Core Genome Multi-Locus Sequence Typing (cgMLST) is a sequence typing method in which the core genome, which is conserved and shared by all members of a taxonomic group, is used in a conceptual extension of Multi-Locus Sequence Typing (MLST). By expanding from 7-9 loci used in MLST to hundreds or thousands of loci, cgMLST promises a dramatic increase in discriminatory power. Moreover, because it incorporates essential features for public health surveillance such as stable type definitions that can be centralized, curated, and universally shared, cgMLST represents a viable approach for global genomic epidemiology efforts. A current problem hindering the development of robust cgMLST schemes relates to incomplete loci in draft genome assemblies, which is exacerbated as the number of genomes analyzed increases. Our aim was to examine approaches to mitigate the effects of data loss on strain typeability in the design cgMLST schemes.

**Materials and Methods:** A prototype scheme based on 732 core genes was designed for the foodborne pathogen *Campylobacter jejuni*, one of the leading cause of enteritis worldwide. This scheme was used to examine a set of 2,585 draft genome assemblies. Assignable loci were determined; incomplete loci due to contig truncations were identified and the location of truncations catalogued. The global and local information content at all loci was analysed using Shannon Entropy. A subset of genomes with complete data at all loci (n=1,464) was used to examine the effect of incomplete loci on strain typeability by introducing varying levels of synthetic contig truncations. Three different strategies for mitigating the effects of incomplete loci were evaluated: loci based on optimised partial gene sequences; imputation of incomplete loci based on linkage disequilibrium; and use of reduced numbers of target loci.

**Results:** The overall levels of incomplete loci observed in the dataset were approximately 3.5%. Although most genomes did not present contig truncations, 713 genomes (27.6%) had one or more incomplete loci; a significant number of genomes (n=107; 4.1%) had greater than 1% incomplete loci. Similarly, although most loci possessed low probability of contig truncation, some loci (n=19; 2.6%) had incomplete data in 1% or more of the genomes analyzed. Such loci can be utilized in a cgMLST scheme provided that partial sequences avoiding regions of high truncation probability and optimised for high information content are used. For most loci with low levels of incomplete data, data imputation based on linkage disequilibrium and knowledge of alleles at flanking loci could correctly estimate the allele with above 95% accuracy despite the high recombinogenic potential of *C. jejuni*. Moreover, 95% of cgMLST schemes based on 200-250 randomly selected loci were able to retain most of the information content captured by the assay based on 732 loci while concomitantly decreasing exposure to incomplete loci.

**Conclusions:** Our results suggest that empirically observed levels of incomplete loci in draft WGS data can have a significant impact on the typeability of strains. However, the application of one or more strategies tested in this study can be used to mitigate these adverse effect and can help inform the development of reliable and robust approaches to cgMLST.

### OP23 - Development Of A Rapid Diagnostic Test For The Identification Of Pathogenic *Campylobacter jejuni* Isolates

Cody Buchanan<sup>1</sup>; Peter Kruczakiewicz<sup>2</sup>; Steven K. Mutschall<sup>2</sup>; Benjamin Hetman<sup>3</sup>; James E. Thomas<sup>3</sup>; Victor P. J. Gannon<sup>2</sup>; G. Douglas Inglis<sup>4</sup>; Eduardo N. Taboada<sup>2</sup>

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**Background:** *Campylobacter jejuni* is an important bacterial foodborne pathogen, with an estimated 400 million cases worldwide each year. In Canada and many industrialized countries, *C. jejuni* is the leading cause of bacterially-incited enteritis, with a yearly per capita incidence as high as 1%. Deducing the epidemiology of campylobacteriosis towards the development of efficacious prevention and control measures is complicated by the fact that most cases of campylobacteriosis appear to be sporadic in nature. *C. jejuni* has a broad ecological distribution and high prevalence in wild and domesticated animals, including in most companion and food production animals, and environmental sources such as water, and soil, complicating efforts in source tracking and source attribution. Evidence from previous molecular epidemiology efforts by our group suggests that many genetic lineages are rarely associated with disease in humans (i.e. "non-clinically associated" or NCA). Moreover, these data also suggest that a small number of genetic lineages is responsible for a disproportionate number of human campylobacteriosis cases (i.e. "clinically associated" or CA). Our aim was to perform a genome-wide association study to identify genetic markers associated with CA lineages in order to develop a molecular assay for rapidly identifying strains likely to pose a risk to public health.

**Methods and Materials:** The Canadian National Comparative Genomic Fingerprinting database, which contains subtyping data for >20,000 *C. jejuni* isolates was used to select a set of 166 isolates representative of prominent CA and NCA lineages in Canada for whole genome sequencing. Comparative genomic analysis of gene content was used to identify genes with statistically significant differences in carriage between CA and NCA lineages. Candidate genes were subjected to primary validation *in silico* against a panel of over 2,000 publicly-available *C. jejuni* genomes. Leading candidate markers were subjected to secondary validation against a set of 376 isolates, including cohorts of 192 CA and 184 NCA isolates, and tertiary validation against a cohorts of 557 CA and 356 NCA isolates.

**Results:** From a total of 730 statistically significant genes identified in the first stage of the analysis, a total of 33 high quality markers were identified based on primary *in silico* validation against the expanded set of *C. jejuni* genomes. Among these, 11 markers maintained extreme differences in carriage ( $p < 10^{-20}$ ) between CA and NCA isolates after tertiary validation. These included operons for iron acquisition and vitamin B<sub>5</sub> biosynthesis, and several putative and hypothetical open reading frames. These markers, when used in combination were present in 97% versus 46% of strains from CA and NCA lineages, respectively and a subset of 5 markers has been used to develop of a PCR assay for rapid and high-throughput identification of potentially pathogenic strains of *C. jejuni*.

**Conclusions :**Effective mitigation strategies will require identification of reservoirs with the highest prevalence of strains posing an increased risk to human health. Employing a comparative genomic approach on CA (i.e. "pathogenic") and NCA (i.e. "non-pathogenic") lineages, we have developed a PCR-based risk assessment tool for public health authorities to rapidly identify *C. jejuni* strains that are potentially pathogenic to the human population.

## S5 - Plenary session

### OP24 - Proteotyping Of *Streptococcus pneumoniae*, Using Tandem Mass Spectrometry For Identification Of Biomarkers For Species And Strain Differentiation

Hedvig Engström Jakobsson<sup>1</sup>; Lucia Gonzales Siles<sup>1</sup>; Roger Karlsson<sup>2</sup>; Fredrik Boulund<sup>3</sup>; Francisco Salvà-Serra<sup>1</sup>; Erik Kristiansson<sup>3</sup>; Edward R.B. Moore<sup>1</sup>

<sup>1</sup>Department of Infectious diseases, Gothenburg University; <sup>2</sup>Department of Infectious diseases, Gothenburg University and Nanoxis Nanoxis Consulting AB; <sup>3</sup>Department of Mathematical Statistics, Chalmers University of Technology

**Background:** *Streptococcus pneumoniae* (pneumococcus) is the leading cause of community-acquired pneumonia and a major cause of morbidity and mortality worldwide. *S. pneumoniae* belongs to the *S. mitis*-group (viridans streptococci) and is phenotypically and genotypically similar to commensal species of the upper respiratory tract of the *S. mitis*-group such as *S. mitis*, *S. oralis*, and *S. pseudopneumoniae*. This fact causes problems of identification in clinical microbiology laboratories. In this project, we are applying state-of-the-art proteomics techniques for *Streptococcus* spp. proteotyping; to detect and characterize expressed protein biomarkers for species-level identification, determine antibiotic resistance and virulence factors and perform strain typing for epidemiological analyses.

**Material and methods:** The proteins of intact bacteria or cell fractions are bound to a membrane surface, using patented (WO2006068619) Lipid-based Protein Immobilization (LPITM) technology. Peptides are generated from the bound proteins using enzymatic digestion and then separated and analysed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The mass spectra profiles are compared to a database of reference peptide sequences, consisting of all complete genomes of the NCBI Reference Sequence (RefSeq) Database. In this study, the type strains of the closely-related *mitis* complex species *S. pneumoniae* (CCUG 28588<sup>T</sup>), *S. mitis* (CCUG 31611<sup>T</sup>), *S. oralis* (CCUG 13229<sup>T</sup>), *S. psedopneumoniae* (CCUG 49455<sup>T</sup>) and the more distantly-related *S. pyogenes* (CCUG 4207<sup>T</sup>) were analysed individually and in mixtures, to demonstrate the capabilities of proteotyping to differentiate closely related species. Additionally, mixes containing different *S. pneumoniae* strains were analysed.

**Results:** Using proteotyping protocols, it was possible to detect and correctly identify *S. pneumoniae* from the closely related bacterial species, *S. mitis*, *S. oralis*, *S. psedopneumoniae* and *S. pyogenes*, as well as different strains of *S. pneumoniae* by identification of unique discriminatory peptides. For successful proteotyping, a comprehensive and accurate genomic database is the key to obtaining reliable proteotyping data. Importantly, since there is a high rate of misclassified genomes in the public databases, the taxonomic classification of the genome sequences should be verified for accurate identification of biomarkers. In this study, hundreds of genomes of the *S. mitis*-group in GenBank were analysed against type strain genomes by calculating the Average Nucleotide Identity using BLAST (ANIB). *S. pneumoniae* were well classified, while almost half the *S. mitis* were misclassified. Also, *S. mitis*-group strains that could not be identified to the species level, using standard genotypic and phenotypic approaches, were characterized by proteotyping and whole genome sequencing to describe their taxonomy and to improve the database matching.

**Conclusion:** Proteotyping enabled the differentiation and identification of pneumococcus from its closely related species as well as sub-species-level strain discrimination, all from single MS analyses. The whole method will enhance the identification and characterization of microorganisms, allowing high-resolution discrimination of closely related species through the confident identification of new biomarkers, ultimately for cultivation-independent analyses of clinical samples.

### OP25 - The Evaluation Of Whole Genome Sequencing For The Epidemiological Typing Of *Legionella pneumophila*

Sophia David<sup>1</sup>; Martin Aslett<sup>1</sup>; Rediat Tewolde<sup>2</sup>; Simon Harris<sup>1</sup>; Massimo Mentasti<sup>2</sup>; Baharak Afshar<sup>2</sup>; Anthony Underwood<sup>2</sup>; Julian Parkhill<sup>1</sup>; Timothy Harrison<sup>2</sup>

<sup>1</sup>Wellcome Trust Sanger Institute; <sup>2</sup>Public Health England

**Background:** Human infections with *Legionella pneumophila* are acquired directly from a contaminated environmental source. The characterisation of clinical and epidemiologically linked environmental isolates is crucial for locating the source and determining the extent of infection. The cost and turn-around time of whole genome sequencing (WGS) has decreased significantly in recent years making it a viable tool for outbreak investigations by public health reference laboratories. We compared a range of WGS-based methods for the epidemiological typing of *L. pneumophila* and compared their performance to that of current typing techniques.

**Materials & Methods:** A number of methods for analysis of WGS data were tested including SNP/mapping-based, kmer-based, gene presence/absence-based, and scaled-up multi-locus sequence typing (MLST) approaches. Five different MLST schemes were created using 53 ribosomal genes and 50, 100, 500 and 1455 core genes. These different approaches were tested using WGS data from the European Society for Clinical Microbiology Study Group on Legionella Infections (ESGLI) standard typing panel comprising 106 isolates and 17 sets of epidemiologically related isolates. A further 206 isolates, comprising five major disease-associated sequence types (STs) as defined by the current gold standard 7-gene MLST, were used to further test the discriminatory power of the different methods. A number of isolates were also sequenced multiple times at different sequencing centres, using different platforms, and in the same and different sequencing runs, to establish the reproducibility of the methods.

**Results:** All WGS-based methods provide high levels of discrimination when applied to epidemiologically unrelated isolates from the ESGLI typing panel. When only a single difference is required for isolates to be considered different "types", the WGS-based methods achieved indices of discrimination of between 0.980 and 1. However, the most discriminatory methods also detect differences between epidemiologically related isolates. For each method, we therefore defined a higher number of differences permitted for isolates to be considered the same "type" in order to maintain epidemiological concordance.

The WGS-based methods mostly offered high discrimination within some of the common disease-associated STs (e.g. ST1). However, the clonal nature of some *L. pneumophila* strains means that isolates belonging to some STs (e.g. ST47) remain difficult to differentiate, even using the most discriminatory methods. Indeed many epidemiologically unrelated isolates are genetically identical and thus total differentiation between epidemiologically related and unrelated isolates is not achieved with any method.

Finally, we show that reproducibility is intrinsically linked to sequence data quality and that high quality sequence data provides reproducibility scores approaching 100% for all methods.

**Conclusion:** WGS-based methods achieve higher discrimination than current typing techniques and will be particularly useful when investigating outbreaks involving common strains such as ST1. While the different WGS-based methods have their own advantages and disadvantages, we conclude that a typing scheme based on a scaled-up MLST approach offers the best means of achieving an appropriate balance between discrimination and epidemiological concordance. Importantly, it is also amenable to standardisation and the easy exchange of data between laboratories.

## S5 - Plenary session

### OP26 - Tracking A *Mycobacterium tuberculosis* MDR Strain In Equatorial Guinea Based On ASO-PCR Targeting Specific SNPs Obtained From WGS Data

Laura Pérez-Lago<sup>1</sup>; Griselda Tudó<sup>2</sup>; Iñaki Comas<sup>3</sup>; María Carcelén<sup>1</sup>; Marta Herranz<sup>1</sup>; Santiago Izco<sup>1</sup>; María Jesus Ruiz Serrano<sup>1</sup>; Juliá Gonzalez<sup>2</sup>; Juan Eyene<sup>4</sup>; Emilio Bouza<sup>1</sup>; Darío García de Viedma<sup>1</sup>

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**Background:** Molecular epidemiology studies in TB are generally based on the application of the same fingerprinting tool, namely MIRU-VNTR, to all the *Mycobacterium tuberculosis* (MTB) isolates in a population. In some cases, specific PCRs have been developed to track selected strains, but this was only possible when a specific genetic feature made it possible to target them. Whole genome sequencing (WGS) enables us to identify singular features (strain-specific SNPs) for any MTB strain of interest and to develop allele-specific-oligonucleotide (ASO)-PCRs tailored to detect relevant strains. We evaluated the efficiency and flexibility of this new strategy to track prevalent multidrug-resistant (MDR) MTB strains.

**Materials and Methods:** We focused on a MDR MTB strain (EG-MDR) suspected to be prevalent in Equatorial Guinea (EG). One isolate from an immigrant case infected with this strain, identified by MIRU-VNTR, was analyzed by WGS to identify strain-specific SNPs. An allele-specific PCR (ASO-PCR) was designed to target 4 of these strain-specific SNPs. The ASO-PCR was prospectively applied on the left-overs of the diagnostic molecular tests (GenXpert or Genotype MDRplus) performed on the specimens obtained in EG in the period February-April 2015, which were delivered to our laboratory in Spain.

**Results:** The ASO-PCR was applied to 161 positive specimens for MTB and a result was obtained in 139. The EG-MDR strain was identified in 18 isolates. All the isolates identified as EG-MDR strain by ASO-PCR were confirmed by MIRU-VNTR analysis and all corresponded to resistant strains according to the molecular diagnostic tests. The EG-MDR isolates constituted 51.4% of all the resistant strains detected in EG. To track retrospectively the presence of the EG-MDR strain in EG, the ASO-PCR was applied on a sample of stored isolates obtained in EG in 1999-2001, as part a molecular epidemiology study. The analysis was performed directly on the crude boiled extracts of 93 strains and the EG-MDR strain was identified in two cases. Both corresponded to MDR cases, one of them a new case and the other a previously treated case.

**Conclusion:** PCRs targeting strain-specific SNPs is a novel strategy for monitoring relevant MTB strains. It is based on low-cost, rapid, and transferable tests tailored to the challenges of different populations. Its prospective application allowed to track a MDR strain, which is responsible for half of the current MDR cases in EG, and to document retrospectively its presence in the country 15 years ago.

## MICROBIAL POPULATION GENOMICS

**Friday, 11<sup>th</sup> March 2016, 08:30-10:00**

### OP33 - Pan-Genome Approaches To Identify Candidate Genetic Markers In Clinical *Staphylococcus* Species

Guillaume Méric<sup>1</sup>; Leonardos Mageiros<sup>1</sup>; Samuel K. Sheppard<sup>1</sup>

<sup>1</sup>Swansea University Medical School

### OP34 - Biomarker Discovery For Phenotypic Groups In *Escherichia coli* Using Superphy

Chad Laing<sup>1</sup>; Eduardo Taboada<sup>1</sup>, Akiff Manji<sup>2</sup>; Vic Gannon<sup>1</sup>

<sup>1</sup>Public Health Agency of Canada; <sup>2</sup>The University of British Columbia

### OP35 - Clostridium Difficile - Heterogeneity Of Pathogenicity Locus and Characterisation Of Atypical Paloc-Replacing Sequences In Non-Toxigenic Strains

Sandra Janezic<sup>1</sup>; Kate Dingle<sup>2</sup>; Derrick W. Crook<sup>2</sup>; Maja Rupnik<sup>1</sup>

<sup>1</sup>National Laboratory for Health, Environment and Food and University of Maribor, Faculty of Medicine; <sup>2</sup>Oxford University, John Radcliffe Hospital

### OP36 - Exploring The Key Factors For The Success Of Clonal Complex 2 (CC2), The Main *Staphylococcus epidermidis* Lineage

Diana Espadinha<sup>1</sup>; Rita Sobral<sup>2</sup>; Guillaume Méric<sup>3</sup>; Samuel Sheppard<sup>3</sup>; Hermínia de Lencastre<sup>4</sup>; Maria Miragaia<sup>1</sup>

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<sup>2</sup>Laboratory of Molecular Microbiology of Bacterial Pathogens, UCIBIO@REQUIMTE, FCT-UNL; <sup>3</sup>College of Medicine, Institute of Life Science, Swansea University; <sup>4</sup>Laboratory of Microbiology and Infectious Diseases, The Rockefeller University

### OP37 - Insertion Sequences Play A Key Role In The Phenotypic And Genotypic Plasticity Of The Most Frequent *Staphylococcus haemolyticus* Clonal Lineage

Ons Bouchami<sup>1</sup>; Herminia de Lencastre<sup>2</sup>; Maria Miragaia<sup>1</sup>

<sup>1</sup>Laboratory of Molecular Genetics, Instituto de Tecnologia Química e Biológica (ITQB) António Xavier, Oeiras, Portugal and Laboratory of Bacterial Evolution and Molecular Epidemiology; <sup>2</sup>Laboratory of Molecular Genetics, Instituto de

Tecnologia Química e Biológica (ITQB) António Xavier, Oeiras, Portugal and Laboratory of Microbiology and Infectious Diseases, The Rockefeller University, New York, New York, United States of America

## S6 - Plenary session

### OP38 - Novel Lineage Of Non-European CC80 Community-Acquired MRSA

Sofie Edslev<sup>1</sup>; Paal Skytt Andersen<sup>1</sup>; Henrik Westh<sup>2</sup>; Nobumichi Kobayashi<sup>3</sup>; Mette Bartels<sup>2</sup>; Peder Worming<sup>2</sup>; Robert Skov<sup>1</sup>; Anders Rhod Larsen<sup>1</sup>; Marc Stegger<sup>1</sup>

<sup>1</sup>Statens Serum Institut; <sup>2</sup>Hvidovre Hospital; <sup>3</sup>Sapporo Medical University School of Medicine

### OP33 - Pan-Genome Approaches To Identify Candidate Genetic Markers In Clinical *Staphylococcus* Species

Guillaume Méric<sup>1</sup>; Leonardos Mageiros<sup>1</sup>; Samuel K. Sheppard<sup>1</sup>

<sup>1</sup>Swansea University Medical School

**Background:** Opportunistic pathogens from the genus *Staphylococcus* are associated with increased mortality and morbidity, especially in the hospital environment. In the clinical laboratory, *S. aureus* and other *Staphylococcus* sp. including *S. epidermidis* are routinely discriminated using growth on blood agar, latex fixation tests, MALDI-TOF, or coagulase assays. However, it remains challenging to identify other non-*aureus* and non-*epidermidis* staphylococcal species carried by humans, some of which can also cause disease. The decreasing cost of high-throughput sequencing and the increasing availability of user-friendly bioinformatic analysis methods can help solve this problem. Additionally, there is a need to identify markers that are relevant for human infection in order to improve diagnostics.

**Methods:** We present here a comparative genomics pipeline based on the gene-by-gene approach to compare more than 500 whole genome sequences from more than 25 staphylococcal species to characterize core and accessory genome variation, and find species-specific epidemiological markers. This approach is based on the creation of a reference pan-genome of more than 21,000 genes from many strains of all species in our dataset. It provides an alternative to classical methods which fail to capture species-specific markers that are absent from a single-strain reference genome.

**Results:** A robust phylogeny of clinically-important *Staphylococcus* species was obtained and analysed. Additionally, we identified in 14 *Staphylococcus* sp. a total of 2942 genes that were species-specific (i.e., present in one species but absent in all other species), amongst which a total of 1543 were core to individual species (i.e., present in all isolates from a given species but absent from all other species).

**Conclusions:** Possible applications are discussed, including the creation of a gene “scheme” which can allow the rapid discrimination of most human-carried species of *Staphylococcus* after high-throughput sequencing in the clinical environment.

## S6 - Plenary session

### OP34 - Biomarker Discovery For Phenotypic Groups In *Escherichia coli* Using Superphy

Chad Laing<sup>1</sup>; Eduardo Taboada<sup>1</sup>, Akiff Manji<sup>2</sup>; Vic Gannon<sup>1</sup>

<sup>1</sup>Public Health Agency of Canada; <sup>2</sup>The University of British Columbia

**Background:** Shiga-toxin producing *Escherichia coli* (STEC) are associated with food- and water-borne human disease outbreaks and sporadic infections. Symptoms can range from diarrhea to the life-threatening hemolytic uremic syndrome. Among STEC, certain serotypes and lineages within serotypes are more frequently associated with human disease than others. Further, they may differ in their ability to colonize animals and survive in the environment. Despite the importance of STEC, the link between phenotype and genotype is still largely unknown. The omnilog system by Biolog is a phenotypic microarray (PM) that tests nutrient utilization and chemical sensitivity of microorganisms, in a high-throughput manner. We have previously developed SuperPhy, an online predictive genomics platform (<<http://lfz.corefacility.ca/superphy/>>) for \**Escherichia coli*\*, which can be used to determine statistically predictive markers for groups of genomes. In this study we examined the whole-genome sequence and phenotypic profile of 143 STEC of a variety of serotypes isolated over a period of 20 years, from bovine, human, and environmental sources.

**Materials and Methods:** Genomes were sequenced using the Illumina MiSeq at Genome Quebec or the National Microbiology Laboratory in Winnipeg, Canada. Sequences were assembled using Spades. Comparative genomics were performed using Panseq. Phylogenetic trees were created using FastTree2. PM analyses were performed using the Biolog Omnilog system, and the opm package for R. Statistical analyses were performed using methods from the SuperPhy platform, in R. The PM data were hierarchically clustered with the Euclidean distance measure using complete linkage.

**Results:** Hierarchical clustering of the 143 strains based on phenotypic differences generated a tree that was broadly split into two groups: one comprised of O157:H7 strains and the other of non-O157:H7 strains. Within the non-O157 group, four large clusters were observed, each with strains of multiple serotypes. Within each of these four large clusters, smaller serotype-specific clades were identified. The large O157:H7 specific group was divided into three main clusters that were largely specific to the three major lineages of the serotype. Phenotypic utilization profiles capable of statistically predicting the observed clades, as well as serotype-specific clusters were also identified. The phylogeny based on single nucleotide polymorphisms (SNPs) among the 143 genomes produced clusters of strains that were largely concordant with those based on the PM data, showing that most clades were serotype specific with occasional outliers clustering apart from other strains of the same serotype.

**Conclusion:** The tree based on phenotypic markers recapitulated the groupings of bacterial strains obtained through SNP whole-genome phylogeny. Known genetic lineages such as those within serotype O157:H7 were distinguished based on phenotypic profile and sets of markers predictive of these subgroups were identified. The results likely reflect a similar physiology among related strains, which could indicate a similar pathogenicity to humans, or environmental niche. Potential implications of this work include the development of selective media for particular serotypes, clades and lineages, or biochemical tests to identify specific bacterial sub-groups most frequently associated with human disease.

### OP35 - Clostridium Difficile - Heterogeneity Of Pathogenicity Locus and Characterisation Of Atypical Paloc-Replacing Sequences In Non-Toxigenic Strains

Sandra Janezic<sup>1</sup>; Kate Dingle<sup>2</sup>; Derrick W. Crook<sup>2</sup>; Maja Rupnik<sup>1</sup>

<sup>1</sup>National Laboratory for Health, Environment and Food and University of Maribor, Faculty of Medicine; <sup>2</sup>Oxford University, John Radcliffe Hospital

**Background:** A Gram-positive, anaerobic, sporogenic, bacterium *Clostridium difficile* is the most common pathogen associated with nosocomial intestinal infections in humans. The clinical manifestations of *C. difficile* infection (CDI) range from mild diarrhoea to pseudomembranous colitis and even death. Although, majority of CDI are still healthcare-associated, increasing number of studies describe isolation of *C. difficile* from community CDI cases, from animal hosts and from different non-hospital environments, which may represent important reservoirs. Toxin A and B are responsible for onset of the disease and are encoded within the 19.6 kb pathogenicity locus (PaLoc) together with three accessory genes (*tcdC*, *tcdE* and *tcdR*). In the majority of toxigenic strains, the PaLoc is found to be inserted in the same chromosomal site and in non-toxigenic strains replaced by conserved 115/75 bp non-coding region. Recently, a 7.2 kb sequence of unknown origin have been found to be inserted at the PaLoc insertion site and strains with the PaLoc integrated in unusual chromosomal sites have been described by different authors. Genetic variation of the PaLoc has been assessed by toxinotyping, a PCR-RFLP based typing method, in which strains with different PaLoc variants are distributed into different toxinotypes. In this study, we used whole genome sequencing to explore variations in *tcdA* and *tcdB*, and to investigate phylogenetic relationship of different toxinotypes. Furthermore, we used genomic sequencing to explore genomic organization in a group of non-toxigenic strains where the 115 bp sequence could not be PCR-amplified.

**Materials/methods:** Genomes of 25 representative *C. difficile* toxinotypes and 12 non-toxigenic isolates (2 human and 10 soil isolates) were sequenced using Illumina technology and *de-novo* genome assemblies were created using Velvet.

**Results:** Sequence analysis confirmed earlier observations that single nucleotide polymorphisms (SNPs) are more common in *tcdB*. Four different patterns of point mutations were differentiated in TcdB, which correlated well with phylogenetic lineages and type of cytopathic effect of TcdB on cultured cells, suggesting evolution of TcdB from different ancestors. The *tcdA* is more conserved with mostly deletions and insertions of larger sequences present in catalytic and binding domain, respectively. In some non-toxigenic strains, insertions up to 9 kb in size were found. *In silico* MLST analysis showed that toxinotypes were distributed across all five toxigenic clades, so far described for *C. difficile*. One strain of toxinotype XXXII (with variant form of PaLoc and atypical PaLoc insertion site) occupied a distinct branch and was therefore designated as a putative novel lineage. Strains with atypical insertions replacing the PaLoc were found in three distinct and highly divergent clades, of which only one was described previously (C-I) (figure 1).

**Conclusion:** Characterization of new *C. difficile* variants and screening for changes in toxin coding regions are important in development of molecular diagnostic tests, vaccines and have also implications in understanding the evolution of PaLoc variants.

## S6 - Plenary session

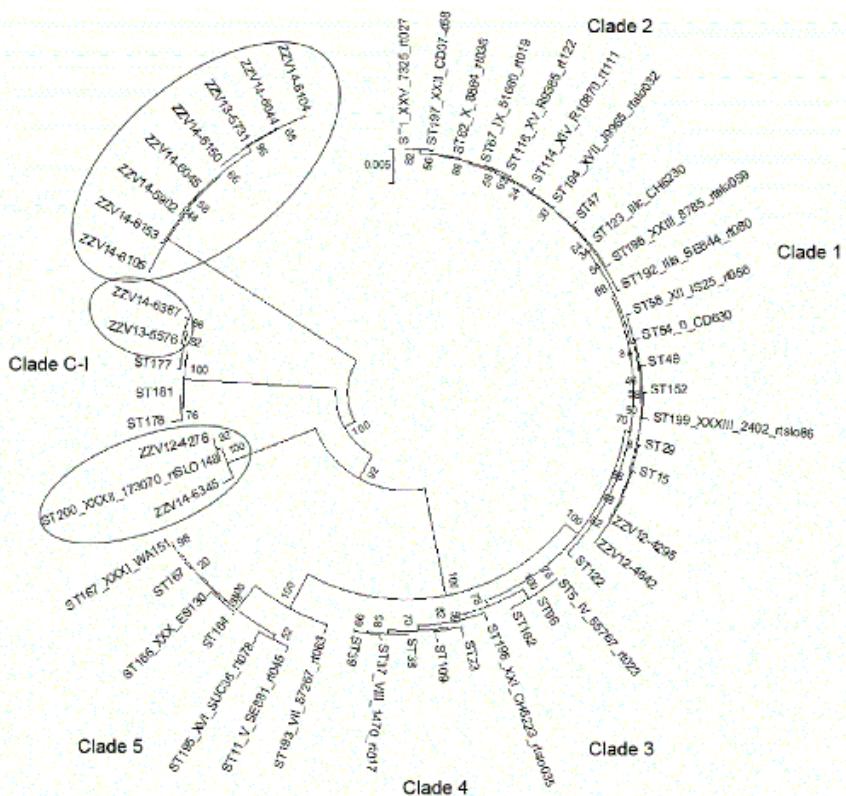


Figure 1. Phylogenetic organisation of *C. difficile* strains. Neighbour-Joining trees illustrating relationships based on MLST data. Non-toxigenic strains with atypical insertions in the PaLoc integration site are marked with circles.

### OP36 - Exploring The Key Factors For The Success Of Clonal Complex 2 (CC2), The Main *Staphylococcus epidermidis* Lineage

Diana Espadinha<sup>1</sup>; Rita Sobral<sup>2</sup>; Guillaume Méric<sup>3</sup>; Samuel Sheppard<sup>3</sup>; Hermínia de Lencastre<sup>4</sup>; Maria Miragaia<sup>1</sup>

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**Background:** *Staphylococcus epidermidis* is the main colonizer of the human skin and the most frequent opportunistic pathogen associated to device-related infections. The most prevalent lineage found in the community and hospital settings is clonal complex 2 (CC2), a highly genetically diverse lineage. The factors underlying CC2 success are not well understood yet.

**Material/Methods:** In order to explore the genetic and phenotypic factors responsible for the success of CC2 lineage, 83 *S. epidermidis* clinical strains representative of the population from the hospital and community settings were sequenced using HiSeq. Whole genome sequencing (WGS) data were searched for virulence and antimicrobial resistance genes by BLAST analysis. Strains were also characterized for phenotypic traits such as the ability to form biofilm, growth in acidic and osmotic stress conditions and resistance to β-lactams (oxacillin).

**Results:** We observed that in a range of pH conditions (4.5-7.4) and high osmotic stress (2 M NaCl) the growth rates were similar for CC2 and non-CC2 strains. However, non-CC2 strains showed a longer lag phase and did not reach optical density values as high as CC2-strains at the stationary phase. Moreover, whereas the CC2 group presented a variable ability to form biofilm, none of the non-CC2 strains produced biofilm. No differences were observed regarding resistance to oxacillin. Additionally, WGS analysis showed that some hypothetical proteins were exclusively found within CC2 and non-CC2 groups. Genes involved in the formation of biofilm, adhesion and colonization were significantly more represented in CC2 strains, whereas genes involved in anaerobiosis and resistance to oxidative stress were exclusively found among non-CC2 strains.

**Conclusions:** Our results suggest that CC2 strains have a higher fitness than non-CC2 strains under conditions of biological stress and that their success may rely on specific genetic determinants related to biofilm formation, adhesion and colonization.

## S6 - Plenary session

### OP37 - Insertion Sequences Play A Key Role In The Phenotypic And Genotypic Plasticity Of The Most Frequent *Staphylococcus haemolyticus* Clonal Lineage

Ons Bouchami<sup>1</sup>; Herminia de Lencastre<sup>2</sup>; Maria Miragaia<sup>1</sup>

<sup>1</sup>Laboratory of Molecular Genetics, Instituto de Tecnologia Química e Biológica (ITQB) António Xavier, Oeiras, Portugal and Laboratory of Bacterial Evolution and Molecular Epidemiology; <sup>2</sup>Laboratory of Molecular Genetics, Instituto de Tecnologia Química e Biológica (ITQB) António Xavier, Oeiras, Portugal and Laboratory of Microbiology and Infectious Diseases, The Rockefeller University, New York, New York, United States of America

Keywords: Methicillin-resistance, Insertion sequences, Evolution.

**Background:** *Staphylococcus haemolyticus* is one of the most important nosocomial human pathogens frequently isolated in bloodstream and medical devices-related infections. This species is notorious for its multidrug resistance and genome plasticity, which is believed to result from the multiple insertion sequences (IS) elements contained in its chromosome. However, the impact of IS in the evolution and adaptation of *S. haemolyticus* is not clear. This study was undertaken to understand how IS modulate chromosomal and phenotypic diversity in *S. haemolyticus*.

**Material and Methods:** With this purpose, we analyzed a strain belonging to the most epidemic *S. haemolyticus* clonal type (ST3) for genetic and phenotypic stability after serial passage *in vitro* (n=34 days). Cultures were characterized by PFGE and Southern hybridization of Smal patterns with a probe for IS1272 and also for growth, oxacillin and cefoxitin susceptibility, hemolysis, mannitol fermentation and biofilm production. To assess variability within the cell population, five colonies at 7 time points during stability assays (n=35 colonies) were analyzed for phenotypic characteristics and whole genome sequencing (WGS) using MiSeq.

**Results:** A high instability was observed both in Smal PFGE and IS 1272 hybridization patterns during serial passage *in vitro*. Most of the changes in the Smal-IS 1272 patterns paralleled those observed in Smal-PFGE profiles and alterations in mannitol fermentation, hemolysis, biofilm formation were also identified. Phenotypic and genotypic variants were detected within individual colonies taken from the same population at the same time point. Analysis of single colony WGS data showed the occurrence of 11 alterations in IS insertion sites. Alterations were associated with IS 1272 loss and/or deletion of the regions flanking IS 1272 elements. Variant IS sites were in the vicinity of genes related to respiratory chain, DNA replication, mRNA degradation, iron sequestration, resistance to nitrosative stress and metal and aminoacid transport. Also, alteration in the number of viable cells and population growth rates were observed from day to day.

**Conclusion:** Our findings suggest that the high genetic and phenotypic variability observed in the most epidemic *S. haemolyticus* clonal type appears to be promoted by IS transposition. Overall, our results suggest that *S. haemolyticus* populations are composed of subpopulations of transposition variants that might be affected in their growth, gene expression level, stress resistance, nutrient availability and virulence. The maintenance of subpopulations of cells in different physiological states might be a strategy to adapt rapidly to environmental stresses imposed by host or hospital environment.

### OP38 - Novel Lineage Of Non-European CC80 Community-Acquired MRSA

Sofie Edslev<sup>1</sup>; Paal Skytt Andersen<sup>1</sup>; Henrik Westh<sup>2</sup>; Nobumichi Kobayashi<sup>3</sup>; Mette Bartels<sup>2</sup>; Peder Worming<sup>2</sup>; Robert Skov<sup>1</sup>; Anders Rhod Larsen<sup>1</sup>; Marc Stegger<sup>1</sup>

<sup>1</sup>Statens Serum Institut; <sup>2</sup>Hvidovre Hospital; <sup>3</sup>Sapporo Medical University School of Medicine

Keywords: CA-MRSA, Evolution, SCCmec

**Background:** We recently reconstructed the evolutionary history of the PVL-positive European community-acquired *Staphylococcus aureus* clonal complex (CC) 80 lineage (Stegger *et al.* mBio 2014). This revealed a Sub-Saharan origin with distinct genetic features associated with the endemic CA-MRSA clone, and with indications that selective use of clinical antimicrobials had driven the expansion across Europe, the Middle East and Northern Africa. However, a recent discovery of PVL-negative, fusidic acid-susceptible MRSA isolates in Bangladesh led us to investigate the genetic features of these isolates and their presence outside this geographic region.

**Materials | Methods:** This study comprises 231 CC80 isolates; 97 isolates (23 MSSA and 74 MRSA) from an international collection (Stegger *et al.*); three MRSA isolates from Bangladesh (Paul *et al.* Microb Drug Resist, 2014); and 131 Danish isolates that include all PVL-negative CC80 MRSA isolates identified in Denmark, and all identified CC80 MRSA isolates from the greater capital region of Denmark from 2013 onwards (Bartels *et al.* Euro Surveill, 2015). All isolates were whole-genome sequenced using Illumina, and their phylogenetic relationships were inferred using SNPs identified in the core genome. Genetic typing of the SCCmec element and encoding of resistance and virulence genes (*lukS/F*, *mecA*, *fusB* and *tet(K)*), was performed using CLCbio's Genomics Workbench.

**Results:** Phylogenetic analyses revealed the existence of three distinct clades in the CC80 complex, including the previously identified basal clade encompassing sub-Saharan African isolates and the derived clade that as a whole define the European CA-MRSA clone. The new 3<sup>rd</sup> clade, containing 20 MRSA isolates, include the three Bangladesh isolates and 17 Danish isolates. All isolates in this new unrelated clade carried an SCCmec type IVa element, contrary to the type IVC found exclusively in the European CA-MRSA clone. No isolates in the new cluster harboured the PVL-encoding genes *lukS* and *lukF*, but all carried *ΦSa2* remnants. All isolates in the novel clade lacked the fusidic acid resistance determinant *fusB*, whereas four isolates carried pT181-like plasmids encoding *tet(K)*.

**Conclusion:** Our investigation provides new insight into the emergence and spread of CA-MRSA. We find that CC80 MRSA isolates are more diverse than previously thought, and include a novel PVL-negative, fusidic acid-susceptible sublineage with a distinct SCCmec type IVa element. All isolates in this cluster carry remnants of the PVL-encoding prophage, indicating that the ancestor of the CC80 lineage originally carried the prophage. Investigation of the Danish isolates, showed this clone to be present from 2009 onwards, with >10% of the isolates as part of this novel sublineage. Preliminary analyses of the epidemiological information of the patients indicate younger age, skin and soft-tissue infections and primarily largely related to import from Asia. Combined, these new data show that two distinct sublineages of CC80 MRSA evolved simultaneously; i) the European CA-MRSA from a subpopulation originating in sub-Saharan Africa, and ii) a secondary unrelated acquisition of the SCCmec element into a PVL-negative CC80 variant unrelated to the sub-Saharan clone.



## ANTIMICROBIAL RESISTANCE AND MOBILE GENETICS ELEMENTS

**Friday, 11<sup>th</sup> March 2016, 10:30-12:00**

### OP39 - Targeted Metagenomics To Track And Characterize The Antimicrobial Resistome (RESCAP1.0)

Val Fernandez Lanza<sup>1</sup>; Irene Rodriguez<sup>2</sup>; Ana P. Tedim<sup>2</sup>; Maria de Toro<sup>3</sup>; Fernando de la Cruz<sup>3</sup>; Rafael Cantón<sup>2</sup>; Fernando Baquero<sup>2</sup>; Teresa M. Coque<sup>2</sup>

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### OP40 - A Pilot Study Using WGS To Detect The Prevalence And Mechanism Of Azithromycin Resistance In A U.K Population Of Non Typhoidal *Salmonella*

Satheesh Nair<sup>1</sup>; Philip Ashton<sup>1</sup>; Gauri Godbole<sup>1</sup>; Michel Doumith<sup>1</sup>; Steve Connell<sup>1</sup>; Anais Painset<sup>1</sup>; Elizabeth de Pinna<sup>1</sup>; Martin Day<sup>1</sup>

<sup>1</sup>Public Health England

### OP41 - Molecular Epidemiology Of Carbapenemase-Producing Gram-Negatives In A Pediatric Hospital In Angola

Laurent Poirel<sup>1</sup>; Nicolas Kieffer<sup>1</sup>; Marta Aires-De-Sousa<sup>2</sup>; Patrice Nordmann<sup>1</sup>

<sup>1</sup>University of Fribourg; <sup>2</sup>Escola Superior de Saúde da Cruz Vermelha Portuguesa

### OP42 - Investigation Of Potential Sharing Of Carbapenem Resistance Plasmids Between Members Of The Enterobacteriaceae Using MinION Technology

Sandra Reuter<sup>1</sup>; Kim Judge<sup>1</sup>; Simon R Harris<sup>2</sup>; Catherine Ludden<sup>1</sup>; Theodore Gououris<sup>1</sup>; Sharon J Peacock<sup>1</sup>

<sup>1</sup>University of Cambridge; <sup>2</sup>Wellcome Trust Sanger Institute

### OP43 - Genomic Epidemiology Of Vancomycin Resistant *Enterococcus faecium* And Dissemination Of The VanA Transposon (Tn1546) In Copenhagen 2012-14

Mette Pinholt<sup>1</sup>; Jesper Boye Nielsen<sup>1</sup>; Heidi Gumpert<sup>1</sup>; Michael Pedersen<sup>2</sup>; Veronika Vorobieva<sup>3</sup>; Peder Worning<sup>1</sup>; Henrik Westh<sup>1</sup>

<sup>1</sup>Department of Clinical Microbiology, Hvidovre University Hospital; <sup>2</sup>Department of Clinical Microbiology, Herlev University Hospital; <sup>3</sup>Department of Clinical Microbiology, Copenhagen University Hospital, Rigshospitalet

## S7 - Plenary session

### SC06 - Genomic Epidemiology Of Vancomycin-Resistant *Enterococcus faecium* (VRE) During An Epidemic Rise Of VRE In Dutch Hospitals

Malbert Rogers<sup>1</sup>; Anita Schürch<sup>1</sup>; Iris Braat<sup>1</sup>; Ellen Brouwer<sup>1</sup>; Janetta Top<sup>1</sup>; Marc Bonten<sup>1</sup>; Jukka Corander<sup>2</sup>; Rob Willems<sup>1</sup>

<sup>1</sup>UMC Utrecht; <sup>2</sup>University of Helsinki

## OP39 - Targeted Metagenomics To Track And Characterize The Antimicrobial Resistome (RESCAP1.0)

Val Fernandez Lanza<sup>1</sup>; Irene Rodriguez<sup>2</sup>; Ana P. Tedim<sup>2</sup>; Maria de Toro<sup>3</sup>; Fernando de la Cruz<sup>3</sup>; Rafael Cantón<sup>2</sup>; Fernando Baquero<sup>2</sup>; Teresa M. Coque<sup>2</sup>

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**Background:** Metagenomics has recently been used to analyze the ensemble of antimicrobial resistance genes in a particular environment (resistome). All metagenomic open and closed formats exhibit low sensitivity and lack of specificity to uncover the size and diversity of minor fractions of total metagenomes. To achieve better sensitivity and specificity in resistome mining, we developed a targeted sequence capture panel (ResCap v.01) based on SeqCapEZ technology (NimblegeneRoche), designed to enrich sequences from genes encoding resistance to antibiotics, biocides and heavy metals.

**Material & Methods:** ResCap is composed by a panel of approximately 79,000 non-redundant genes (80 Mb) including 7,963 well-known antibiotic resistance genes, 30,740 biocide & heavy metal resistance genes, and 2,517 relaxases, signature proteins of plasmid conjugation. Design of the probes was based on a non-redundant database (DB), constructed by mixing curated DBs for antibiotic resistance (CARD, ARG-ANNOT and RED-DB). To improve the detection of "novel" AbR genes, a Hidden Markov Model (HMM) from each AbR family was constructed for detection of homologous sequences by using hammer3 software against Uniref100 DB. Sequences coding for resistance to heavy metals and antiseptics were recruited from BacMet DB, which includes both experimentally confirmed and predicted antibacterial biocide- and metal-resistance genes. Relaxases were retrieved from the ConjDB repository. ResCap workflow consisted on i) whole-metagenome shotgun library construction, ii) hybridization, and iii) capture. All steps were performed according Nimblegene standard protocols for Illumina platforms. To evaluate ResCap efficiency, samples were sequenced before and after capture. ResCap was validated by analysing fecal samples from 9 humans and 8 swine. Robustness of the platform was tested by comparative analysis of two technical replicates of two samples. Bioinformatic analysis was performed by mapping sequenced reads against well-known gene DBs (ARG-ANNOT, BacMet Experimental and ConjDB) using Bowtie2 with the output option of all hits (-a). All statistical operations were performed using R and homemade Perl scripts.

**Results:** An average of  $1.9 \times 10^7$  reads were obtained from the ResCap pool ( $9.2\text{--}32 \cdot 10^6$ ). The average on-target hits was 0.11% (0.07%–0.18%) for pre-captured samples and 30% (20%–41%) for post-captured sequences. The enhanced sensitivity of ResCap represents a gain of 279 fold (170–480 fold). The diversity of genes encoding antimicrobial resistance and relaxases was 627 (436–937) in pre-capture samples and 1367 (1071–1616) in samples captured with ResCap (Figure 1). Reproducibility was addressed by the correlation between replicates, showing R factors of 0.81 and 0.96, respectively.

**Conclusion:** ResCap v0.1 substantially enhances the sensitivity and specificity of metagenomics shotgun sequencing for analyzing the resistome. Moreover, the ability to accurately detect low abundant or rare populations and its robustness, make ResCap a promising tool for a wide number of research and diagnostic applications and suitable for being used in both longitudinal and cross-sectional studies. The platform constitutes one of the first examples of targeted metagenomics for analysing bacterial populations.

## S7 - Plenary session

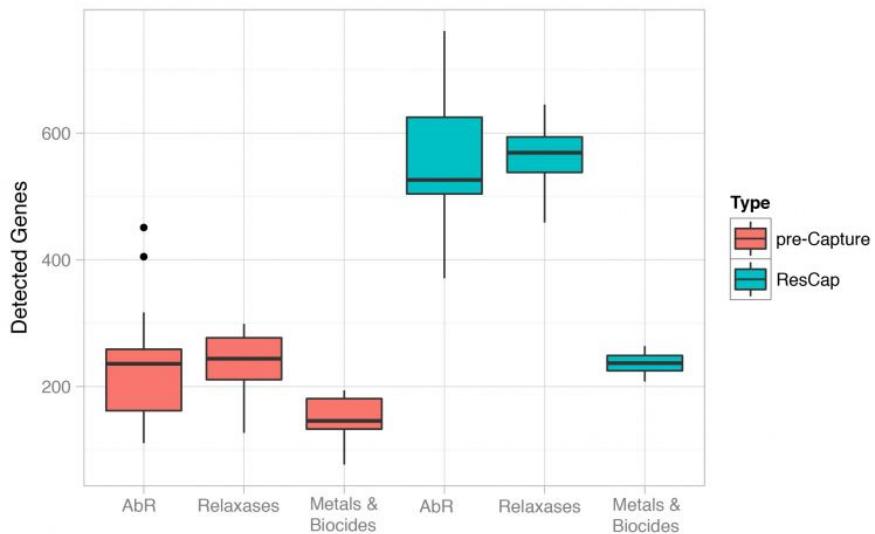


Figure 1. Boxplot of metagenomic diversity of samples pre-captured and targeted captured with ResCap

### OP40 - A Pilot Study Using WGS To Detect The Prevalence And Mechanism Of Azithromycin Resistance In A U.K Population Of Non Typhoidal *Salmonella*

Satheesh Nair<sup>1</sup>; Philip Ashton<sup>1</sup>; Gauri Godbole<sup>1</sup>; Michel Doumith<sup>1</sup>; Steve Connell<sup>1</sup>; Anais Painset<sup>1</sup>; Elizabeth de Pinna<sup>1</sup>; Martin Day<sup>1</sup>

<sup>1</sup>Public Health England

**Background:** Multidrug resistance especially to first line antibiotics ,fluoroquinolones and 3<sup>rd</sup> generation cephalosporins in *Salmonella* are an emerging threat. Resistance to azithromycin has also been described recently in the Enterobacteriaceae population. Whole genome sequencing (WGS) and phenotypic methods were used in this study to determine the prevalence of azithromycin resistance in *Salmonella enterica* isolates from the UK and identify the underlying mechanisms of resistance.

**Methods:** WGS by Illumina HiSeq was carried out on 683 isolates of *Salmonella* spp isolated between 2012-2015 from the Gastrointestinal Bacteria Reference Unit (GBRU), Public Health England (PHE). Detection of known acquired resistance genes, including those previously associated with azithromycin resistance were determined from WGS using a mapping-based approach and presence of those encoding β-lactamases were confirmed by PCR. Characterization and location of macrolide/azalide resistant determinants were assessed by BANDAGE, BLAST and ARTEMIS genome browser analysis. Susceptibility testing was in accordance with the EUCAST methodology (MIC breakpoint ≤16mg/L).

**Results:** Nine isolates of non-typhoidal *Salmonella enterica* (NTS) belonging to serovars S.Blockley, S. Typhimurium, S. Thompson, S. Ridge and S. Kentucky showed resistance or decreased susceptibility to azithromycin (from 6 to >16mg/L) due to the presence of macrolide resistance genes *mphA*, *mphB* or *mefB*. These genes are either plasmid or chromosomally mediated. None of the isolates had the *ermB* gene associated with resistance to Erythromycin. All nine isolates were also resistant to multiple classes of antibiotics, including ampicillin, aminoglycosides, trimethoprim, tetracycline, sulphonamide ciprofloxacin and third generation cephalosporins.

Characterisation of a further 16 S. Blockley isolates showed the presence of *mphA* in six unrelated isolates. All nine S. Blockley isolates harbour the macrolide inactivation gene cluster *mphA-mrx-mphr(A)* in the same chromosomal location. To our knowledge this is the first novel chromosomally mediated *mphA* gene cluster in Salmonellae. Phylogenetic analysis based on single nucleotide polymorphisms (SNP) of all the S. Blockley isolates indicated that S. Blockley isolates harbouring *mphA* gene formed a single lineage, distinct from S. Blockley's without the *mphA* gene.

The azithromycin MICs of the 15 *Salmonella* spp. isolates showed that the presence of the *mphA* gene was associated with MIC≥16mg/L, while presence of *mefB* or *mphB* was not associated with MIC≥16 mg/L.

**Conclusion:** The study showed that resistance to azithromycin, entirely due to acquisition of known macrolide resistance genes was seen in four different *Salmonella* serovars and can be either plasmid or chromosomal encoded. Even though the numbers of azithromycin resistance in *Salmonella* spp. from the UK remained low (15/683 isolates studied) this does not rule out the possibility of spread or increase of azithromycin resistance in the future and in turn requires the re-evaluation of azithromycin treatment for Salmonellosis.

## S7 - Plenary session

### OP41 - Molecular Epidemiology Of Carbapenemase-Producing Gram-Negatives In A Pediatric Hospital In Angola

Laurent Poirel<sup>1</sup>; Nicolas Kieffer<sup>1</sup>; Marta Aires-De-Sousa<sup>2</sup>; Patrice Nordmann<sup>1</sup>

<sup>1</sup>University of Fribourg; <sup>2</sup>Escola Superior de Saúde da Cruz Vermelha Portuguesa

**Background:** Acquired resistance to carbapenems in Gram negatives nowadays represents a major public health threat. Resistance to carbapenems is mainly due to production of acquired carbapenemases. The class B carbapenemase NDM-1 and the class D carbapenemase OXA-48 are increasingly identified worldwide, being mostly found in Enterobacteriaceae. No epidemiological data are available regarding the occurrence of carbapenemases in Angola. We therefore initiated a prospective study in order to evaluate the rate of colonization of patients by carbapenemase producers at a Pediatric hospital in Luanda.

**Methods:** Rectal swabs were collected from children being either inpatients or outpatients during a one-week screening period, May 2015. After a pre-enrichement in broth supplemented withertapenem 0.25 µg/ml, samples were screened for the occurrence of carbapenem-resistant enterobacterial isolates using selective agar plates including the Chrom ID Carba SMART (bioMérieux). Then colonies were tested with the Rapidec Carba NP test (bioMérieux) for detection of carbapenemase production. PCR experiments were further performed using primers specific for all carbapenemase genes (KPC, NDM and OXA-48-like).

Conjugation experiments were realized by broth mating method in order to detect the possible transfer of the carbapenemase genes. Genotyping was performed by PFGE analysis and also by MLST.

**Results:** A total of 157 samples were collected from children being 3 months to 13 years-old. A total of 90 carbapenem-resistant Gram negative isolates were recovered, the majority being *Klebsiella pneumoniae* (n=34) and *Escherichia coli* (n=37) isolates. A total of 74 carbapenemase producers were identified, including 34 *K. pneumoniae*, 33 *E. coli*, and 4 *Acinetobacter baumannii*. The most common carbapenemase identified was OXA-181 (a derivative of OXA-48) found in 39 32 *K. pneumoniae*, 29 *E. coli*, and a single *Enterobacter cloacae*. Nine NDM-1-producing isolates were identified, being 4 *E. coli*, 2 *K. pneumoniae*, a single *Providencia stuartii*, a single *Providencia retgeri*, and a single *A. baumannii*. PFGE analysis indicated that eleven different clones were identified among the 32 *K. pneumoniae* isolates. Seventeen different clones were identified among the 31 *E. coli* isolates. MLST indicated that Among the *E. coli* isolates, the most frequent Sequence Type was ST5692. Among the *K. pneumoniae* isolates, the most frequent Sequence Type was a new ST.

**Conclusion:** This study identified for the first time carbapenemase producers in Angola. Noteworthy, those isolates were recovered from children. There was a significant number of NDM-1 producers, there was a large majority of OXA-181-producing isolates. This is remarkably considering that so far there have been only scattered reports of OXA-181 producers in the world, even though the Indian subcontinent is supposed to be the main reservoir nowadays, as it is for NDM-1 producers. The source of contamination could not be linked to India so far, and therefore remains unknown. According to the very high rate of carbapenemase-producing isolates identified here, it seems that Angola can be now considered as an endemic area.

### OP42 - Investigation Of Potential Sharing Of Carbapenem Resistance Plasmids Between Members Of The Enterobacteriaceae Using MinION Technology

Sandra Reuter<sup>1</sup>; Kim Judge<sup>1</sup>; Simon R Harris<sup>2</sup>; Catherine Ludden<sup>1</sup>; Theodore Gououris<sup>1</sup>; Sharon J Peacock<sup>1</sup>

<sup>1</sup>University of Cambridge; <sup>2</sup>Wellcome Trust Sanger Institute

This abstract presents independent research supported by the Health Innovation Challenge Fund (WT098600, HICF-T5-342), a parallel funding partnership between the Department of Health and Wellcome Trust.

Keywords: Carbapenem resistance, plasmids, MinION

**Background:** Carbapenem resistance in Enterobacteriaceae is increasing worldwide, leading to limited treatment options. This resistance may arise through different mechanisms, the most worrying being the acquired carbapenemases transferrable via mobile genetic elements such as plasmids. During investigations of wastewater in East Anglia, England, two carbapenem resistant isolates (one *Raoultella ornithinolytica*, one *Enterobacter kobei*) were recovered that shared a similar phenotypic resistance pattern and with the same carbapenemase gene. We sought to investigate the potential sharing of plasmids between these isolates.

**Methods:** Untreated wastewater was obtained from a treatment plant in East Anglia. Carbapenem-resistant Enterobacteriaceae were identified using MALDI-TOF mass spectrometry, and minimum inhibitory concentrations to meropenem, ertapenem, and imipenem were determined by the Etest. We undertook sequencing using a combination of Illumina and MinION technology to address the technical challenge of assembling short reads associated with regions of repeats and insertion sequence elements. We aimed to scaffold the bacterial genomes in as few contigs as possible, and obtain single-contig plasmids.

**Results:** Single technology assemblies were performed for Illumina and the MinION data, and compared with hybrid assembly. Shared plasmids were not detectable using the Illumina data alone, but analysis confirmed the presence of plasmids in both bacterial isolates contributing to the antimicrobial resistance observed. Betalactamase genes found in both isolates were *bla<sub>OXA-48</sub>* conferring carbapenem resistance, *bla<sub>SHV-12</sub>* for an extended-spectrum betalactamase (ESBL) phenotype, and *bla<sub>TEM-1</sub>*. Resistance to other antimicrobial resistance classes were also detected, including aminoglycosides (*aac<sup>6'-IIC</sup>*), erythromycin (*ereA2*), sulphonamide (*sul1*), as well as heavy metal resistance (arsenic, copper, tellurite, mercury, cadmium).

**Conclusion:** The dissemination of carbapenemase genes in wastewater is an important public health concern. As carbapenemases can be spread via plasmids between organisms, it is imperative that suitable methods are developed for detection of such plasmids in order to fully investigate the global epidemiology. Plasmids and their dissemination through different bacterial species is difficult to track using short-read data, with long-read options such as MinION opening new investigation avenues, either using a hybrid or long-read only assembly approach.

This abstract presents independent research supported by the Health Innovation Challenge Fund (WT098600, HICF-T5-342), a parallel funding partnership between the Department of Health and Wellcome Trust.

## S7 - Plenary session

### OP43 - Genomic Epidemiology Of Vancomycin Resistant *Enterococcus faecium* And Dissemination Of The VanA Transposon (Tn1546) In Copenhagen 2012-14

Mette Pinholt<sup>1</sup>; Jesper Boye Nielsen<sup>1</sup>; Heidi Gumpert<sup>1</sup>; Michael Pedersen<sup>2</sup>; Veronika Vorobieva<sup>3</sup>; Peder Wørnig<sup>1</sup>; Henrik Westh<sup>1</sup>

<sup>1</sup>Department of Clinical Microbiology, Hvidovre University Hospital; <sup>2</sup>Department of Clinical Microbiology, Herlev University Hospital; <sup>3</sup>Department of Clinical Microbiology, Copenhagen University Hospital, Rigshospitalet

Keywords: VRE, whole genome sequencing, Tn1546 transposon

**Background:** Vancomycin resistant *Enterococcus faecium* (VREfm) is an emergent nosocomial problem. From 2012 - 2014 a ten-fold increase of *vanA* VREfm isolates was observed in Copenhagen. *E. faecium* is inherently resistant to cephalosporins and has the capacity to acquire and disseminate mobile genetic elements containing virulence and resistance genes. The *vanA* transposon (Tn1546) consists of two transfer-related genes (*orf1* and *orf2*), two regulatory genes (*vanR* and *vanS*), four vancomycin resistance genes (*vanH*, *vanA*, *vanX* and *vanY*) and a *vanZ* gene of unknown function. Variations in the Tn1546 transposon structure among clinical VREfm isolates are well described. The objective of this study was to determine the clonal structure of the *vanA* VREfm isolates in Copenhagen. Furthermore, all *vanA* transposons have been characterised to assess their similarity.

**Methods:** All *vanA* VREfm from Copenhagen (The Capital Region of Denmark, 1.7 M inhabitants) submitted to Hvidovre University Hospital from January 2012 through December 2014 for whole genome sequencing were included in the study.

All isolates were sequenced on the Illumina MiSeq platform. 2 x 150 bp paired-end reads were produced. Reads were mapped with Stampy to AUS004 (NC-017022). SNP calling was performed using Samtools. Phylogeny was inferred by the neighbor-joining algorithm with bootstrapping in SplitsTree4. Sequence reads were assembled *de novo* using Velvet. All isolates were annotated by RAST to determine the presence and location of *orf1*-*orf2*, *van* genes and IS elements. Multiple alignments of transposon elements were performed by Muscle to identify point mutations and deletions in genes and intergenic regions.

**Results:** Four hundred and ninety five *vanA* VREfm isolates were whole genome sequenced. The SNP-tree revealed a picture of genetic diversity where the isolates were divided into 19 distinct groups and 25 singletons. Using a similarity threshold of ≤4 SNPs demonstrated that many VREfm isolates were genetically closely related. A total of 412 (83%) VREfm isolates differed by ≤4 SNPs to at least one VREfm isolate and 318 (64%) differed by ≤4 SNPs to at least five VREfm isolates.

Four different transposon structures were identified. The dominant Tn1546-like transposon (81%) had a deletion of *orf1* and an insertion of a transposase IS1251 in the intergenic region between *vanS* and *vanH*. Eleven percent had a similar structure including a deletion of *orf2*. The original Tn1546 structure was present in 4% of the isolates and another 4% had a deletion of *orf1* and *orf2*. Multiple alignments demonstrated that the transposon elements were highly conserved. Only two positions in the transposon were affected by point mutations. Both mutations were non-synonymous and they only affected a single codon.

**Conclusion:** Genomic epidemiology has revealed a polyclonal structure of the VREfm isolates. However, within the groups the isolates are genetically closely related. The majority of the isolates harbour an identical Tn1546-like transposon. This suggests that dissemination of the Tn1546-like transposon by horizontal transfer into ampicillin resistant, vancomycin susceptible *E. faecium*, followed by clonal spread of VREfm in the hospitals have contributed to the increase and diversity of VREfm in Copenhagen.

**SC06 - Genomic Epidemiology Of Vancomycin-Resistant *Enterococcus faecium* (VRE) During An Epidemic Rise Of VRE In Dutch Hospitals**

Malbert Rogers<sup>1</sup>; Anita Schürch<sup>1</sup>; Iris Braat<sup>1</sup>; Ellen Brouwer<sup>1</sup>; Janetta Top<sup>1</sup>; Marc Bonten<sup>1</sup>; Jukka Corander<sup>2</sup>; Rob Willems<sup>1</sup>

<sup>1</sup>UMC Utrecht; <sup>2</sup>University of Helsinki



## **GENOMIC EPIDEMIOLOGY ONTOLOGIES**

***Friday, 11<sup>th</sup> March 2016, 13:30-14:30***

### **DS1 - IRIDA's Genomic Epidemiology Application Ontology: Genomic, Clinical And Epidemiological Data Standardization And Integration**

Emma Griffiths<sup>1</sup>; Melanie Courtot<sup>2</sup>; Damion Dooley<sup>3</sup>; Josh Adam<sup>4</sup>; Franklin Bristow<sup>4</sup>; Joao A Carrico<sup>5</sup>; Bhavjinder K. Dhillon<sup>1</sup>; Alex Keddy<sup>6</sup>; Matthew Laird<sup>2</sup>; Thomas Matthews<sup>4</sup>; Aaron Petkau<sup>4</sup>; Julie Shay<sup>1</sup>; Geoff Winsor<sup>1</sup>; IRIDA Ontology Advisory Consortium<sup>7</sup>; Robert Beiko<sup>6</sup>; Lynn M Schriml<sup>8</sup>; Eduardo Taboada<sup>4</sup>; Gary Van Domselaar<sup>4</sup>; Morag Graham<sup>4</sup>; Fiona Brinkman<sup>1</sup>; William Hsiao<sup>3</sup>

<sup>1</sup>*Simon Fraser University*; <sup>2</sup>*European Bioinformatics Institute*; <sup>3</sup>*BC Public Health Microbiology and Reference Laboratory*; <sup>4</sup>*National Microbiology Laboratory, Public Health Agency of Canada*; <sup>5</sup>*University of Lisbon*; <sup>6</sup>*Dalhousie University*; <sup>7</sup>*BC Centre for Disease Control*; <sup>8</sup>*University of Maryland School of Medicine*

## **NEED FOR UNIVERSAL NOMENCLATURES FOR STRAIN/LINEAGE IDENTIFICATION**

***Friday, 11<sup>th</sup> March 2016, 14:30-15:30***

**Speakers Panel:** Dag Harmsen, Hajo Grundmann and James McInerney

## Discussion sessions

### DS1 - IRIDA's Genomic Epidemiology Application Ontology: Genomic, Clinical And Epidemiological Data Standardization And Integration

Emma Griffiths<sup>1</sup>; Melanie Courtot<sup>2</sup>; Damion Dooley<sup>3</sup>; Josh Adam<sup>4</sup>; Franklin Bristow<sup>4</sup>; Joao A Carrico<sup>5</sup>; Bhavjinder K. Dhillon<sup>1</sup>; Alex Keddy<sup>6</sup>; Matthew Laird<sup>2</sup>; Thomas Matthews<sup>4</sup>; Aaron Petkau<sup>4</sup>; Julie Shay<sup>1</sup>; Geoff Winsor<sup>1</sup>; IRIDA Ontology Advisory Consortium<sup>7</sup>; Robert Beiko<sup>6</sup>; Lynn M Schriml<sup>8</sup>; Eduardo Taboada<sup>4</sup>; Gary Van Domselaar<sup>4</sup>; Morag Graham<sup>4</sup>; Fiona Brinkman<sup>1</sup>; William Hsiao<sup>3</sup>

<sup>1</sup>*Simon Fraser University*; <sup>2</sup>*European Bioinformatics Institute*; <sup>3</sup>*BC Public Health Microbiology and Reference Laboratory*; <sup>4</sup>*National Microbiology Laboratory, Public Health Agency of Canada*; <sup>5</sup>*University of Lisbon*; <sup>6</sup>*Dalhousie University*; <sup>7</sup>*BC Centre for Disease Control*; <sup>8</sup>*University of Maryland School of Medicine*

**Background:** Bacterial whole-genome sequencing (WGS) applications are rapidly moving from proof-of-concept studies to clinical implementation. Successful adaptation of genomics as a tool for microbial typing, surveillance and outbreak investigation requires access to contextual information in order to validate genomic based metrics. While sequence data usually adheres to a few standardized formats (such as FASTQ), contextual data such as surveillance data and exposure information are mostly unstructured and without interoperable standards. Canada's Integrated Rapid Infectious Disease Analysis (IRIDA) platform will equip public health workers with user-friendly tools for incorporating WGS into isolate typing and epidemiological pipelines to support real-time infectious disease investigation. IRIDA is working to develop a Genomic Epidemiology Ontology, a well-defined and standardized vocabulary interconnected by logical relationships, which is crucial for providing the framework for integrating these diverse data types.

**Materials | Methods:** In order to understand the practical requirements for implementing a Genomic Epidemiology Ontology within the Canadian health care network, a needs assessment was conducted by interviewing public health stakeholders and domain experts. User activities, lab management software, information and work flows, exposure tracking and reporting systems were profiled to better characterize users' needs. Community standards were reviewed to determine the utility of different ontologies for fulfilling the identified requirements. Laboratory and epidemiological materials (LIMS, MiSeq input/output, line lists, repository requirements, outbreak questionnaires, policies and legislation) were mined for important fields, terms and descriptors. The IRIDA application ontology was tested using de-identified public health data.

**Results:** No single ontology currently is sufficient to cover all attributes required for a genomic epidemiology program and the very breadth of many ontologies hinders their practical use in real-time by users with little bioinformatics expertise. With this in mind, user profiles and data requirements were harmonized with different ontological standards to create a single resource. An OWL file containing metadata fields and terms describing isolate source attribution (food, environment, host body products), clinical data, sequencing/assembly/annotation processes, quality metrics, patient demographics/histories/comorbidities and exposures was created adhering to the best practices of the Open Biomedical and Biological Ontology (OBO) Consortium. The application was made more robust through testing in different pathogen surveillance initiatives. Key gaps in domain vocabulary requiring expansion were also identified eg NGS parameters and food exposures.

**Conclusion:** IRIDA's ontology-based platform is being developed for integrating important laboratory, clinical and epidemiological data fields. Federated data integration will promote interoperability between different public health systems, improve querying and facilitate automation of many processes. To promote international standards in outbreak investigations and surveillance activities, IRIDA is calling for the formation of an International Genomic Epidemiology Ontology Consortium to build partnerships and solicit domain expertise. We anticipate that members of the consortium will have a wide range of interests and use cases.

## EPIDEMIOLOGY AND PUBLIC HEALTH - SURVEILLANCE

***Friday, 11<sup>th</sup> March 2016, 15:30-17:00***

### **OP27 - Trace And Control The Regional Expansion Of Extended-Spectrum B-Lactamase Producing ST15 *Klebsiella pneumoniae***

John W. A. Rossen<sup>1</sup>; Mariette Lokate<sup>1</sup>; Ruud H. Deurenberg<sup>1</sup>; Marga Tepper<sup>1</sup>; Jan P. Arends<sup>1</sup>; Erwin G. C. Raangs<sup>1</sup>; Jerome Lo-Ten-Foe<sup>1</sup>; Hajo Grundmann<sup>1</sup>; Kai Zhou<sup>1</sup>; Alexander W. Friedrich<sup>1</sup>

<sup>1</sup>*University of Groningen, University Medical Center Groningen*

### **OP28 - Identification Of Multiple Silent Introductions And Household Transmission Of Methicillin-Resistant *Staphylococcus aureus* USA300 In The United Kingdom**

Michelle S. Toleman<sup>1</sup>; Sandra Reuter<sup>1</sup>; Francesc Coll<sup>1</sup>; Ewan Harrison<sup>1</sup>; Estee Torok<sup>1</sup>; Nicholas Brown<sup>1</sup>; Beth Blane<sup>1</sup>; Julian Parkhill<sup>2</sup>; Sharon Peacock<sup>3</sup>

<sup>1</sup>*University of Cambridge*; <sup>2</sup>*Sanger Institute*; <sup>3</sup>*London School of Hygiene and Tropical Medicine*

### **OP29 - Five Years Of Genomic Data From The Meningitis Research Foundation Meningococcal Genome Library (MRF-MGL) – UK And Ireland 2010-2015**

Charlene Rodrigues<sup>1</sup>; Carina Brehony<sup>2</sup>; Richard Moxon<sup>1</sup>; Martin Maiden<sup>1</sup>

<sup>1</sup>*University of Oxford*; <sup>2</sup>*University Hospital Galway*

### **OP30 - Expansion Of Previously Circulating Clones Underlies The Replacement Of Pneumococcal Serotypes In Adult IPD Following Introduction Of PCV7 In Portugal**

Andreia Neves Horácio<sup>1</sup>; Catarina Silva Costa<sup>1</sup>; Jorge Diamantino Miranda<sup>1</sup>; Joana Pimento Lopes<sup>1</sup>; Mário Ramirez<sup>1</sup>; José Melo Cristina<sup>1</sup>

<sup>1</sup>*Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa*

### **OP31 - Insight Into *Salmonella* Epidemiology From Whole Genome Sequencing**

Philip Ashton<sup>1</sup>; Satheesh Nair<sup>1</sup>; Elizabeth de Pinna<sup>1</sup>; Richard Elson<sup>1</sup>; Philip Monk<sup>1</sup>; John Mair Jenkins<sup>1</sup>; Sanch Kanagarajah<sup>1</sup>; Isidro Carrion<sup>1</sup>; Kathie Grant<sup>1</sup>; Tim Dallman<sup>1</sup>

<sup>1</sup>*Public Health England*

## S8 - Plenary session

**OP32 - Next Generation Sequencing (NGS) Reveals Nosocomial Transmission Of Livestock-Associated Methicillin Resistant *Staphylococcus aureus* In Humans**

Thijs Bosch<sup>1</sup>; Sandra Witteveen<sup>1</sup>; Martijn van Luit<sup>1</sup>; Fabian Landman<sup>1</sup>; Anja Haenen<sup>1</sup>; Leo Schouls<sup>1</sup>

<sup>1</sup>National Institute for Public Health and the Environment

### OP27 - Trace And Control The Regional Expansion Of Extended-Spectrum B-Lactamase Producing ST15 *Klebsiella pneumoniae*

John W. A. Rossen<sup>1</sup>; Mariette Lokate<sup>1</sup>; Ruud H. Deurenberg<sup>1</sup>; Marga Tepper<sup>1</sup>; Jan P. Arends<sup>1</sup>; Erwin G. C. Raangs<sup>1</sup>; Jerome Lo-Ten-Foe<sup>1</sup>; Hajo Grundmann<sup>1</sup>; Kai Zhou<sup>1</sup>; Alexander W. Friedrich<sup>1</sup>

<sup>1</sup>University of Groningen, University Medical Center Groningen

**Background:** *Klebsiella pneumoniae* has emerged as an important nosocomial pathogen, known as one of the “ESKAPE” pathogens. Especially, the prevalence of multi-drug resistance *K. pneumoniae* increased dramatically in recent years. This limits efficient clinical treatment tremendously resulting in undesirable treatment outcomes. In this study, we present our experience in using whole-genome sequencing (WGS) and further developing an outbreak clone-specific PCR for tracing and controlling a regional and inter-institutional outbreak caused by a ST15 CTX-M-15-KP clone between May 2012 and September 2013 in the north of Netherlands.

**Materials | Methods:** In total, 19 *K. pneumoniae* isolates obtained from patients and environment were included. Genomes were manually curated after performing automatic annotation on the RAST server. Acquired antimicrobial resistance genes were identified by uploading assembled genomes to the Resfinder server. Other genes relating to resistance and virulence were detected using the mapping unit of CLC Genomics Workbench to map and/or blast assembled genomes to a pseudomolecule generated by concatenating a set of target genes. SNPs were detected using CLC Genomics Workbench by mapping genomes to the finished genome of *K. pneumoniae* PMK1. High-quality SNPs were used for SNP-based phylogenetic reconstruction by RAxML v7.4.2. Assembled genomes and genomes retrieved from GenBank were aligned by ProgressiveMauve. Fragments ( $\geq 500$  bp) shared by all genomes were collected and concatenated resulting in a pseudomolecule defined as the core genome. Alignment of core genomes was used for estimating the maximum likelihood (ML) phylogeny by RAxML v7.4.2. The core genome of outbreak isolates was blasted against our local *K. pneumoniae* genome database (76 genomes with diverse sequence types). The unique fragments were extracted and blasted against GenBank. The non-match fragments not related to mobile genetic elements (e.g. phages, plasmids, and transposons) were considered as the DNA signatures for the outbreak clone. Primers for multiplex PCR specific to DNA signatures were designed by MPprimer.

**Results:** A suspected epidemiological link between clinical *K. pneumoniae* isolates was supported by patient contact tracing and genomic phylogenetic analysis from May to November 2012. By May 2013, a patient treated in three institutions in two cities was involved in an expanding cluster caused by this high-risk clone (HiRiC) (local expansion, CTX-M-15 producing, and containing hypervirulence factors). A clone-specific multiplex PCR was developed for patient screening by which another patient was identified in September 2013. Genomic phylogenetic analysis resolved a close homology with isolates previously found in the USA, and split ST15 strains into two clades. Two serotypes (K24 and K60) were carried by the two clades, respectively. Environmental contamination and lack of consistent patient screening were identified as being responsible for the clone dissemination.

**Conclusion:** The investigation addresses the advantages of WGS in the early detection of HiRiC with a high propensity of nosocomial transmission and prolonged circulation in the regional patient population. It shows the added value of designing a clone-specific multiplex PCR for effective and adequate infection control management. Our study suggests the necessity for inter-institutional/regional collaboration for infection/outbreak management of *K. pneumoniae* HiRiCs.

## S8 - Plenary session

### OP28 - Identification Of Multiple Silent Introductions And Household Transmission Of Methicillin-Resistant *Staphylococcus aureus* USA300 In The United Kingdom

Michelle S. Toleman<sup>1</sup>; Sandra Reuter<sup>1</sup>; Francesc Coll<sup>1</sup>; Ewan Harrison<sup>1</sup>; Estee Torok<sup>1</sup>; Nicholas Brown<sup>1</sup>; Beth Blane<sup>1</sup>; Julian Parkhill<sup>2</sup>; Sharon Peacock<sup>3</sup>

<sup>1</sup>University of Cambridge; <sup>2</sup>Sanger Institute; <sup>3</sup>London School of Hygiene and Tropical Medicine

**Background:** The USA300 MRSA clone is widely disseminated across the United States (USA). This contrasts sharply with Europe, where only sporadic cases or small outbreaks have been described. The prevalence of USA300 in the United Kingdom is unknown. We conducted prospective surveillance for USA300 in the East of England.

**Methods:** A one-year prospective cohort study was performed between 2012 and 2013, where all cases with MRSA isolated in the microbiology laboratory at a tertiary care hospital in the east of England were identified and at least one MRSA isolate from each case whole-genome sequenced. This captured hospital and community healthcare isolates. Epidemiological and clinical information was collected on each case. Phylogenetic reconstruction of the collection was performed and compared to USA300 genomes from the USA.

**Results:** We processed samples from 1489 patients and sequenced 2307 isolates associated with carriage and/or disease. The USA300 MRSA clone was isolated from 1.6% (n=24) of cases. The majority of first positive samples were submitted from first-opinion services (emergency department (8/24, 33%), general practitioners (5/24, 21%)). Half of the cases had USA300 isolated from carriage screens alone, while 10 cases had skin and soft tissue infection and two cases had invasive infection. Cases were distributed throughout the 12-month period. Phylogenetic analyses identified multiple separate introductions and household transmission of USA300.

**Conclusions:** This provides evidence that USA300 was repeatedly introduced into the East of England in 2012-13. Clinical and epidemiological investigation revealed the same characteristics as the US epidemic (household transmissions and a high proportion of skin and soft tissue infection). In the absence of systematic surveillance, we may miss the emergence of novel endemic MRSA lineages in England.

### OP29 - Five Years Of Genomic Data From The Meningitis Research Foundation Meningococcal Genome Library (MRF-MGL) – UK And Ireland 2010-2015

Charlene Rodrigues<sup>1</sup>; Carina Brehony<sup>2</sup>; Richard Moxon<sup>1</sup>; Martin Maiden<sup>1</sup>

<sup>1</sup>University of Oxford; <sup>2</sup>University Hospital Galway

Keywords: *Neisseria meningitidis*, meningococcal disease, molecular epidemiology

**Background:** Invasive meningococcal disease (IMD) can manifest as either septicaemia, meningitis or both with significant morbidity (20%) and mortality (3-10%). Meningococcal conjugate serogroup C vaccines were implemented in the UK in 1999 and Ireland in 2000, resulting in a dramatic fall in incidence. Since then there has been a declining but persistent disease burden, predominantly serogroup B. The recent surge in serogroup W cases resulted in the introduction of conjugate ACWY vaccine for teenagers in the UK, importantly MRF-MGL data helping inform national vaccine policy. Multi-component protein vaccine Bexsero® has also recently been implemented into the infant UK national immunisation schedule (September 2015). With this rapidly changing landscape, it is important to study population structure of *Neisseria meningitidis* using whole genome sequencing methodology.

**Methods:** The MRF-MGL was developed by Public Health England, Scottish Haemophilus, Legionella, Meningococcus and Pneumococcus Reference Laboratory, Wellcome Sanger Institute and University of Oxford as a collaboration. The project is funded by Meningitis Research Foundation. In addition we used the Irish Genome Library (Epidemiology and Molecular Biology Unit and Irish Meningitis and Meningococcal Reference Laboratory and University of Oxford). Genomic data from IMD isolates from five epidemiological years, 2010-11 to 2014-15, from UK and Republic of Ireland were studied. Analyses were performed using embedded analysis tools and R version 3.2.2.

**Results:** Between 2010-11 and 2014-15 there were 2599 cases of culture-confirmed IMD (UK n=2428, Ireland n=171) sequenced in the pubMLST database. Disease is predominantly caused by serogroup B, though this was highest in 2010-11 (n=451) with a progressive decline to 2014-15 (n=276). There was an increase in serogroup W from 2010-11 (n=27) to 2014-15 (n=166). This increase was also seen as a clonal expansion of ST-11/ET-37 clonal complex (cc) contributing to 30.6% of all disease in 2014-15 compared to 3.8% in 2010-11 (Figure 1). Using multilocus sequence typing (MLST), there were 84 different sequence types (ST) in Ireland and 568 in UK. With higher resolution typing, ribosomal MLST (rST), there were 108 rST in Ireland and 805 in the UK, but 2 rST containing 257 and 192 isolates each relating to ST-11/ET-37 complex (serogroup W) and ST-23 complex/Cluster A3 (serogroup Y) respectively. On analysis of vaccine antigens, there were 569 different Bexsero® Antigen Sequence Typing (BAST) profiles. No isolates had matches at 5 (BAST1) or 4 vaccine antigens. There were 231 that matched BAST1 profile at 3 antigens (NHBA, PorA VR1 and PorA VR2), 96.5% in ST-41/44 complex/lineage 3.

#### Discussion:

This unique MRF-MGL, serves as a valuable resource for studying IMD in the UK and Ireland, where vaccinations are likely to impact on *N. meningitidis* population. Prior to both vaccines being introduced in autumn 2015, it is evident that there is great diversity amongst all isolates, but only 9 cc make up the majority of cases (90%). Serum bactericidal assays are the accepted correlate of protection in IMD, and vaccine coverage has been estimated at 73%. However, coverage of Bexsero® vaccine antigens is key, and in this cohort no isolates matched at 4 or 5 loci, data that needs interpreting in conjunction with Meningococcal Antigen Typing System assay outputs over coming months.

## S8 - Plenary session

### OP30 - Expansion Of Previously Circulating Clones Underlies The Replacement Of Pneumococcal Serotypes In Adult IPD Following Introduction Of PCV7 In Portugal

Andreia Neves Horácio<sup>1</sup>; Catarina Silva Costa<sup>1</sup>; Jorge Diamantino Miranda<sup>1</sup>; Joana Pimenta Lopes<sup>1</sup>; Mário Ramirez<sup>1</sup>; José Melo Cristino<sup>1</sup>

<sup>1</sup>*Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa*

**Background:** The 7-valent conjugate vaccine (PCV7) was available for children through the private sector in Portugal from 2001 onwards until it was replaced in the beginning of 2010 by the 13-valent conjugate vaccine (PCV13). In 2012, PCV13 received approval for use also in adults > 50 years of age with an extension being made to all ages in 2013. The aim of this study was to identify the clones causing invasive pneumococcal disease (IPD) in adults between 2008 and 2011, a period when significant changes in serotypes have occurred and before the availability of PCV13 for adults.

**Materials/Methods:** Among the 1660 isolates recovered from adult (>=18 yrs) invasive pneumococcal disease (IPD) in 2008-2011, a random sample of 50% of each serotype was chosen for MLST analysis and evaluation of the presence and type of the pilus islands (n=871). The initial collection had been previously characterized regarding serotype distribution and antimicrobial susceptibility to different classes of antibiotics.

**Results:** The genetic diversity was high with 206 different sequence types (STs) detected (SID=0.971, CI95%: 0.967-0.976). Among these, 39 (4.5%) were new alleles or combinations of alleles, while 587 (67.3%) were related to 29 of the 43 Pneumococcal Molecular Epidemiology Network (PMEN) recognized clones. The different STs organized into 80 clonal complexes (CCs) according to goeBURST (SID=0.948, CI95%: 0.942-0.953). There was a strong correlation between CC and the vaccine serotype groups (AW=0.810, CI95%: 0.763-0.857), with the six most prevalent CCs being mainly composed of isolates presenting vaccine serotypes (95.5%) – CC156 (serotypes 14, 9V and 23F), CC191 (serotype 7F), CC180 (serotype 3), CC306 (serotype 1), CC62 (serotypes 8 and 11A) and CC230 (serotype 19A). Clonal expansion of previously circulating clones was the main trigger of serotype replacement that occurred in adult IPD after the introduction of the seven-valent pneumococcal conjugate vaccine (PCV7), while capsular switching was infrequent and not related with vaccine use. However, five isolates related to the PMEN clone Denmark<sup>14-230</sup> expressed the non-PCV13 serotype 24F. The overall proportion of isolates positive for any of the pilus islands was small (31.9%) and declined gradually during the study period (only 26.6% in 2011) due to the significant decline of serotype 1, which is associated with pilus island 2. The reduction of IPD caused by PCV7 serotypes in the years following PCV7 implementation did not result in a decline of antimicrobial resistance in part due to the selection of resistant genotypes among serotypes 14 and 19A.

**Conclusion:** The dominant clonal complex causing invasive disease in Portugal still expressed PCV7 serotypes. Significant changes in clonal composition are expected with continued vaccine use, particularly following its adult indication in 2012 and its introduction in the National Immunization Program for children born in January 2015 onwards.

### OP31 - Insight Into *Salmonella* Epidemiology From Whole Genome Sequencing

Philip Ashton<sup>1</sup>; Satheesh Nair<sup>1</sup>; Elizabeth de Pinna<sup>1</sup>; Richard Elson<sup>1</sup>; Philip Monk<sup>1</sup>; John Mair Jenkins<sup>1</sup>; Sanch Kanagarajah<sup>1</sup>; Isidro Carrion<sup>1</sup>; Kathie Grant<sup>1</sup>; Tim Dallman<sup>1</sup>

<sup>1</sup>Public Health England

Public Health England have been using whole genome sequencing (WGS) as the primary test for *Salmonella* identification and typing since April 1<sup>st</sup> 2015. Since that time, there have been numerous outbreaks and incidents in which WGS has been an invaluable tool. In this presentation, we will present some of the most interesting examples alongside the methods we have developed to identify clusters and communicate with diverse public health teams.

Firstly, there was a 'slow-burn' *Salmonella* Enteritidis Phage Type 8 cluster that was identified using WGS. There were an average of 8 cases per month between April 2014 and July 2015, which varied between 5% and 90% of the Enteritidis PT8 received in each of those months. Without sequencing it would not be possible to link these cases. On investigation, 81% of cases had contact with a snake, compared with 0.5% of controls. Targeted investigations based on this identified *Salmonella* Enteritidis from the same SNP cluster in mice fed to the snakes.

Secondly, there was an outbreak of *Salmonella* 4, 5, 12:i:- sequence type 34 (aka monophasic Typhimurium) associated with a restaurant that started as a typical point source outbreak. However, there were further cases within the same SNP cluster associated with the restaurant continuing for 30 weeks after the original peak, despite stringent controls put in place by the operator. One of the non-synonymous mutations that defines the outbreak was in the *mutS* gene which encodes a DNA replication fidelity protein, we will present data on the impact of this mutation on mutation rate.

Finally, an example of how valuable the accuracy of WGS can be in determining true links between exposures and cases. A case was exposed at a restaurant which typed as *Salmonella* Enteritidis PT 14b, an isolate was then isolated from chicken at the restaurant which was phage type RDNC (reacts but does not conform). If you want to know whether the chicken and the case were linked, you will have to come to the talk!

## S8 - Plenary session

### OP32 - Next Generation Sequencing (NGS) Reveals Nosocomial Transmission Of Livestock-Associated Methicillin Resistant *Staphylococcus aureus* In Humans

Thijs Bosch<sup>1</sup>; Sandra Witteveen<sup>1</sup>; Martijn van Luit<sup>1</sup>; Fabian Landman<sup>1</sup>; Anja Haenen<sup>1</sup>; Leo Schouls<sup>1</sup>

<sup>1</sup>National Institute for Public Health and the Environment

**Background:** In the Netherlands, LA-MRSA CC398 isolated from humans is the predominant MRSA clade among isolates submitted for typing in the Dutch MRSA surveillance. Despite its high prevalence, the capability of LA-MRSA to transmit between humans has been a subject of debate. In this study, we performed NGS on 126 LA-MRSA isolates to assess presumed transmission of LA-MRSA between humans in Dutch healthcare settings.

**Methods:** To assess cut-off values in the NGS analysis, 62 LA-MRSA isolates from well-documented transmission events were used. Forty isolates originated from livestock veterinarians and their household members (HHM) and 12 isolates originated from an outbreak in a Dutch nursing home. In addition, four confirmed transmission events ( $n=8$ ) in other Dutch healthcare facilities were selected.

Nosocomial LA-MRSA transmission was studied using 12 LA-MRSA isolates from a presumed outbreak in a Dutch hospital and 54 isolates from 23 presumed LA-MRSA nosocomial transmission events. All isolates were sequenced using Illumina technology.

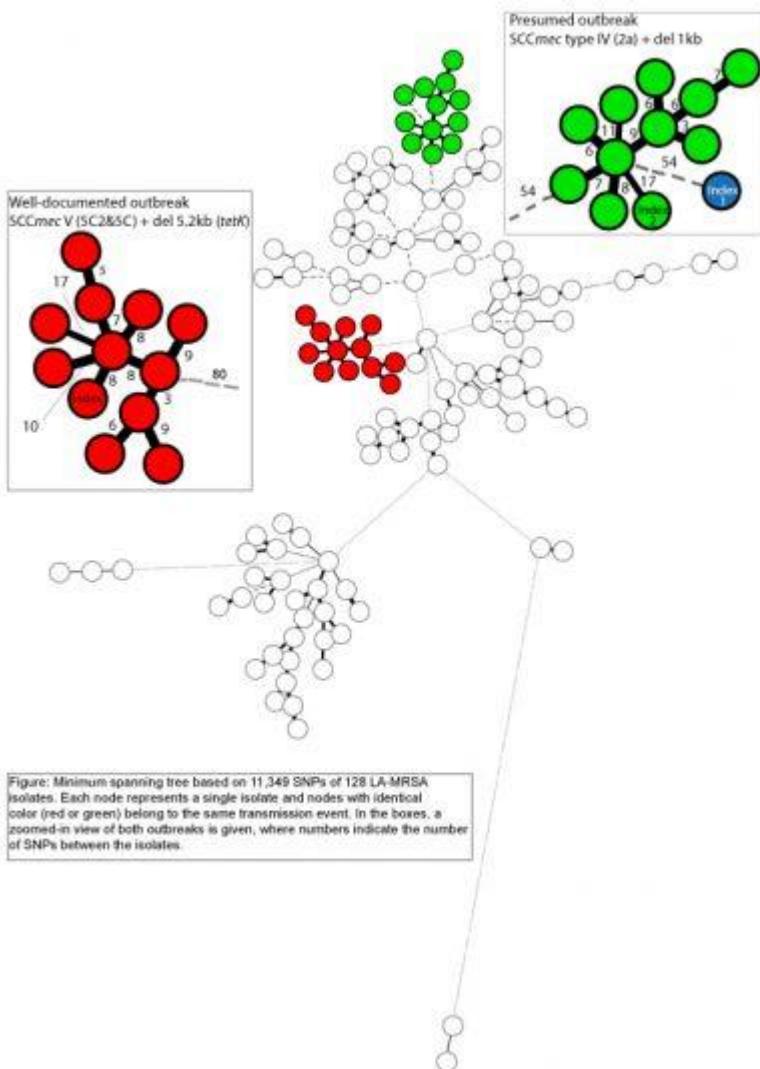
The SCCmec region was identified and classified based on the currently known SCCmec-types. For single nucleotide polymorphism (SNP) analysis, 11,439 SNPs were identified based on the core genome of the Dutch LA-MRSA isolate RIVM-1295, previously sequenced in our laboratory.

**Results:** SNP analysis revealed a maximum of 12 SNPs between isolates obtained from veterinarians and their HHM. NGS of 12 isolates belonging to the outbreak in a nursing home showed a maximum of 17 SNPs between the outbreak isolates (Figure 1). SCCmec of latter isolates revealed a unique type V (5C2&5C) variant, from which a 5.2kb fragment, including *terK*, was deleted. All four confirmed transmission events in Dutch healthcare facilities showed less than 20 SNPs between the isolates and carried identical SCCmec types. These results led to a cut-off value of  $\geq 20$  SNPs for likely transmission, whereas isolates yielding between 20-50 SNPs were regarded as possible transmission events. Transmission was not likely when  $\geq 50$  SNPs were identified between isolates.

Twelve isolates of the presumed LA-MRSA outbreak in a Dutch hospital differed in 54 SNPs. This was caused by a single isolate taken from a different sampling site of the index patient. However, another isolate obtained from the same index patient only differed in eight SNPs from its nearest neighbor.

Of the presumed transmission events, transmission likely occurred in 14 of the 23 events, with  $\geq 20$  SNPs and identical SCCmec types between the isolates. In seven presumed transmissions 20-50 SNPs were found and each pair carried the same SCCmec region. In the remaining two events, transmission was highly unlikely. In one event, isolates differed in 51 SNPs and carried different SCCmec regions. In the remaining event (three isolates) transmission was likely in one case (4 SNPs), but not in the other (124 SNPs, different SCCmec).

**Conclusion:** Our study strongly supports the assumption that transmission of LA-MRSA in Dutch healthcare settings does occur. NGS could confirm transmission in well-documented events and indicated that transmission was unlikely in two presumed transmissions. SCCmec proved to be a valuable addition to SNP analysis, but other mobile genetic elements, such as plasmids, may provide additional information on the transmission routes of this pathogen.





## LATE BREAKER ABSTRACT SESSION

**Friday, 11<sup>th</sup> March 2016, 17:30-19:00**

### **SC07 - Whole-Genome Linkage Analysis Reveals Co-Evolutionary Mechanisms Of Antibiotic Resistance In The Pneumococcus**

Jukka Corander<sup>1</sup>

<sup>1</sup>*Department of Mathematics and Statistics, University of Helsinki*

### **OP04 - How To Compare And Cluster Every Known Genome In About An Hour**

Adam M. Phillippy<sup>1</sup>; Brian D. Ondov<sup>2</sup>; Todd J. Treangen<sup>2</sup>; Sergey Koren<sup>1</sup>

<sup>1</sup>*National Human Genome Research Institute, National Institutes of Health;*

<sup>2</sup>*National Biodefense Analysis and Countermeasures Center*

### **OP45 - Whole Genome Sequence Analysis Suggests That A Remarkable Spread Of Anthrax Occurred In France During The Hundred Years War With England**

Gilles Vergnaud<sup>1</sup>; Guillaume Girault<sup>2</sup>; Simon Thierry<sup>2</sup>; Christine Pourcel<sup>1</sup>; Nora Madani<sup>2</sup>; Yann Blouin<sup>3</sup>

<sup>1</sup>*University Paris-Saclay;* <sup>2</sup>*ANSES, University Paris-Est;* <sup>3</sup>*DGA*

### **OP46 - MLST And CGF40 For Characterization Of *Campylobacter jejuni* Isolates From The Poultry Chain Production And From Clinical Cases In France**

Katell Rivoal<sup>1</sup>; Amandine Thépault<sup>1</sup>; Valérie Rose<sup>1</sup>; Ségolène Quesne<sup>1</sup>; Francis Mégraud<sup>2</sup>; Marianne Chemaly<sup>1</sup>

<sup>1</sup>*Anses;* <sup>2</sup>*CNR Campylobacters and Hélicobacters*

### **OP47 - TAXONOMER, An Integrated Ultra-Fast Tool For Metagenomic Sequence Analysis**

Robert Schlaberg<sup>1</sup>; Guochun Liao<sup>2</sup>; Stephen Flygare<sup>2</sup>; Evan Buss<sup>2</sup>; Keith Simmon<sup>3</sup>;

Chase Miller<sup>1</sup>; Yi Qiao<sup>1</sup>; EJ Osborne<sup>1</sup>; Karen Eilbeck<sup>1</sup>; Gabor Marth<sup>1</sup>; Mark Yandell<sup>1</sup>

<sup>1</sup>*University of Utah;* <sup>2</sup>*DbyDNA Inc.;* <sup>3</sup>*Institute for Clinical and Experimental Pathology, ARUP Laboratories*

### **OP48 - Tracking Resistance Determinants Encoded Within The Chromosomes Of *Pasteurellaceae* Species**

Stephen Douthwaite<sup>1</sup>; Benoit Desmolaize<sup>1</sup>; Anders S. Olsen<sup>1</sup>; Michal Beker<sup>1</sup>; Simon Rose<sup>1</sup>

<sup>1</sup>*University of Southern Denmark*

## S9 - Plenary session

**SC07 - Whole-Genome Linkage Analysis Reveals Co-Evolutionary Mechanisms Of Antibiotic Resistance In The Pneumococcus**

Jukka Corander<sup>1</sup>

*<sup>1</sup>Department of Mathematics and Statistics, University of Helsinki*

**OP04 - How To Compare And Cluster Every Known Genome In About An Hour**

Adam M. Phillippy<sup>1</sup>; Brian D. Ondov<sup>2</sup>; Todd J. Treangen<sup>2</sup>; Sergey Koren<sup>1</sup>

<sup>1</sup>National Human Genome Research Institute, National Institutes of Health;

<sup>2</sup>National Biodefense Analysis and Countermeasures Center

The rapid growth of genomic data has begun to outpace traditional methods for sequence clustering and search. Given a massive collection of sequences, it is infeasible to perform pairwise alignment for basic tasks like sequence clustering and search. To address this problem, we demonstrate that the MinHash technique, first applied to clustering web pages, can be applied to biological sequences with similar effect, and extend this idea to include biologically relevant distance and significance measures. Our new tool, Mash, uses MinHashing to reduce large sequences to a representative sketch and rapidly estimate pairwise distances between genomes or metagenomes. Using Mash, we explored several use cases, including a ~7,000-fold size reduction and clustering of all ~55,000 NCBI RefSeq genomes in 46 CPU hours. The resulting 93 MB sketch database includes all RefSeq genomes, effectively delineates known species boundaries, reconstructs approximate phylogenies, and can be searched in seconds using assembled genomes or raw sequencing runs from Illumina, Pacific Biosciences, and Oxford Nanopore. For metagenomics, Mash scales to thousands of samples and can replicate Human Microbiome Project and Global Ocean Survey results in a fraction of the time. Other potential applications include any problem where an approximate, global sequence distance is acceptable, e.g. to triage and cluster outbreak genomes, assign species labels, identify mis-tracked samples, and rapidly search massive genomic databases. In addition, the Mash distance metric is based on simple set intersections, which are compatible with privacy-preserving tests via homomorphic encryption schemes. To facilitate integration with other software, Mash is implemented as a lightweight C++ toolkit and freely released under a BSD license at <https://github.com/marbl/mash>.

## S9 - Plenary session

### OP45 - Whole Genome Sequence Analysis Suggests That A Remarkable Spread Of Anthrax Occurred In France During The Hundred Years War With England

Gilles Vergnaud<sup>1</sup>; Guillaume Girault<sup>2</sup>; Simon Thierry<sup>2</sup>; Christine Pourcel<sup>1</sup>; Nora Madani<sup>2</sup>; Yann Blouin<sup>3</sup>

<sup>1</sup>University Paris-Saclay; <sup>2</sup>ANSES, University Paris-Est; <sup>3</sup>DGA

**Background:** *Bacillus anthracis*, the highly dangerous zoonotic bacterial pathogen species responsible for anthrax is naturally present in many places worldwide. In France, previous investigations have demonstrated that the majority of sporadic strains belong to the so-called A.Br.011/009 lineage. Whole genome sequencing of more than 120 french strains further demonstrated that the A.Br.011/009 strains defined a very remarkable polytomy, i.e. a star-shape pattern of evolution with six branches. Here we explore the significance of this polytomy by comparing the French *B. anthracis* lineages to worldwide lineages.

**Materials and methods:** We take advantage of whole genome sequence data previously determined for 122 French strains and included publicly available sequence data from 45 strains of various origins. Single nucleotide polymorphisms (SNPs) were identified by mapping each genome on the Ames strain genome sequence.

**Results:** A total of 6690 SNPs was identified among the available dataset and used to draw the phylogeny. The data clearly demonstrates that the currently predominant *B. anthracis* lineage in North America, called WNA for Western North American, and another lineage frequently encountered in former French colonies in West Africa are both derived from one of the six branches of the A.Br.011/009 polytomy predominant in France. The position of the split along the French lineage, together with the consideration of French history can then be used to provide a tentative dating for the most recent common ancestor (MRCA) of the A.Br.011/009 polytomy. The Hundred Years period, 1350-1450, appears to be the most likely candidate. However, the wide range of observed substitution rates along the different *B. anthracis* lineages illustrated by the present data, with ratios up to forty, complicates such dating attempts.

**Conclusion:** The present work extends the range of observed substitution rate heterogeneity within *B. anthracis*, in agreement with its ecology and in contrast with some other pathogens. The population structure of A.Br.011/009 in France, diversity of branch length, and comparison with two derived lineages, suggests that the MRCA of the six branches polytomy identified in France dates back to the Hundred Years' war between France and England started in the mid-fourteenth century. These events were associated in France with deadly epidemics and major economic and social changes. This suggestion will need to be confirmed by sequencing additional A.Br.011/009 of diverse origins.

### OP46 - MLST And CGF40 For Characterization Of *Campylobacter jejuni* Isolates From The Poultry Chain Production And From Clinical Cases In France

Katell Rivoal<sup>1</sup>; Amandine Thépault<sup>1</sup>; Valérie Rose<sup>1</sup>; Ségolène Quesne<sup>1</sup>; Francis Mégraud<sup>2</sup>; Marianne Chemaly<sup>1</sup>

<sup>1</sup>Anses; <sup>2</sup>CNR Campylobacters and Hélicobacters

**Background:** Intestinal campylobacteriosis is the leading bacterial zoonosis in Europe and worldwide. The main causative agent of this foodborne acute gastroenteritis is *Campylobacter jejuni*. *C. jejuni* contaminated poultry meat and meat products are considered the most important sources of disease in humans. In France, 76% of chicken meat at the retail level is contaminated by *Campylobacter*. The aim of this study is to trace by Multilocus Sequence Typing (MLST) and comparative genomic fingerprinting (CGF) using a 40-gene assay (CGF40) *C. jejuni* strains circulating along the French poultry chain production from farms to retail stores and to determine a link with human cases.

**Materials and Methods:** Isolates from farms (176) and slaughterhouses (295) were collected during a survey representative of the French broiler production (Rivoal et al., 2011; Guyard-Nicodème et al., 2015). Retail meat isolates (174) were collected in 10 French departments representing the most significant consumption patterns over one year. Human isolates (143) were collected during the same year from patients living in the same areas in order to study the overlap between broiler and human isolates. All the 788 isolates were typed using both MLST and CGF40 according to Taboada et al. (2012). For the CGF40, isolates were categorized into types based on more than 90% CGF40 fingerprint similarity (CGF-90%). The results were analyzed using BioNumerics software and the genetic diversity of the different strain populations was evaluated using the Simpson Index of Similarity (ID).

**Results:** It appears that the CGF40 presents a good congruence with MLST and a higher discriminating power. A high genetic diversity was observed with 90 different CGF-90% types and 82 Clonal Complex (CC) among the 788 isolates typed, corresponding to indices of 0.957 and 0.925 respectively. Within the clinical isolates, a lower diversity in *C. jejuni* population was observed with indices of 0.909 (CGF40) and 0.843 (MLST). Nevertheless, almost 50% of human isolates were divided into few CGF40 types or CC all found in chicken isolates. These results confirmed the strong link between poultry and campylobacteriosis (Sheppard et al., 2009; Strachan et al. 2012; Abay et al., 2014). Nevertheless some isolates highly prevalent among the poultry isolates were not isolated from clinical isolates supporting thus the hypothesis of different virulent potential between poultry isolates (Schouls et al., 2003; Guyard-Nicodème et al., 2015). On the other hand, 9% and 6% of *C. jejuni* isolates issued from human campylobacteriosis cases found respectively by CGF40 and MLST were not present in chicken population supporting in that case the implication of other sources of campylobacteriosis.

**Conclusion:** This work reveals the great diversity among *C. jejuni* isolates and the implication of the poultry meat production in human contamination. However, other animal reservoirs must be investigated to elucidate the part not attributed to poultry.

## S9 - Plenary session

### OP47 - TAXONOMER, An Integrated Ultra-Fast Tool For Metagenomic Sequence Analysis

Robert Schlaberg<sup>1</sup>; Guochun Liao<sup>2</sup>; Stephen Flygare<sup>2</sup>; Evan Buss<sup>2</sup>; Keith Simmon<sup>3</sup>; Chase Miller<sup>1</sup>; Yi Qiao<sup>1</sup>; EJ Osborne<sup>1</sup>; Karen Eilbeck<sup>1</sup>; Gabor Marth<sup>1</sup>; Mark Yandell<sup>1</sup>

<sup>1</sup>*University of Utah;* <sup>2</sup>*DbyDNA Inc.;* <sup>3</sup>*Institute for Clinical and Experimental Pathology, ARUP Laboratories*

Metagenomics, the genomic analysis of a population of microorganisms, makes possible the profiling of microbial communities in the environment and the human body at unprecedented depth and breadth. Enrichment-independent, high-throughput sequencing allows for unbiased, hypothesis-free detection and molecular typing of a theoretically unlimited number of common and unusual pathogens. Rapid, accurate, and user-friendly data analysis is limiting adoption.

Taxonomer is an integrated, ultra-fast tool for metagenomic sequence analysis. Taxonomer enables novel analysis modalities of unmatched complexity in an easy-to-use format including: (1) comprehensive panmicrobial detection and discovery, (2) host-response profiling, (3) interactive result visualization, and (4) access through an IOBIO-based web user interface, which eliminates the need for specialized hardware or expertise.

Taxonomer computes at speeds comparable to the fastest existing search tools, but supports both nucleotide and protein-based classification using a single integrated algorithmic framework using the same k-mer weighting-based approach and classification algorithm. The result is greater tolerance for sequencing errors, greater sensitivity, more accurate microbial abundance estimates, and execution times exceeding the fastest published protein search tools. This speed and breath of functionality is crucial, as many samples contain complex mixtures of bacterial, fungal and viral taxa.

Here we present analyses of patient samples harboring viral, bacterial, and fungal pathogens, including pathogens of great public health concern, demonstrating that Taxonomer provides effective means for rapid pathogen detection for patient care and discovery in public health emergencies. As costs and turn-around times for high-throughput sequencing continue to fall, Taxonomer will enable a rapidly growing number of laboratories with access to sequencing instruments to analyze data in a meaningful timeframe without having to invest in computational infrastructure or bioinformatics expertise.

**OP48 - Tracking Resistance Determinants Encoded Within The Chromosomes Of Pasteurellaceae Species**

Stephen Douthwaite<sup>1</sup>; Benoit Desmolaize<sup>1</sup>; Anders S. Olsen<sup>1</sup>; Michal Beker<sup>1</sup>; Simon Rose<sup>1</sup>

<sup>1</sup>University of Southern Denmark

**Background:** The bacterial pathogens *Mannheimia haemolytica* and *Pasteurella multocida* cause respiratory disease in a range of hosts including poultry, swine and cattle. Treatment with one of several groups of antibiotics is generally effective, although therapeutic regimens become complicated in cases where the pathogens have acquired resistance (1-4). Tracking the occurrence of resistance in field isolates together with the mechanisms of resistance and their means of dissemination are important not only for the farming industry but also for human health.

**Materials & Methods:** Independent field isolates of *P. multocida* and *M. haemolytica* were screened for resistance to a range of antibiotics. Over 40 resistant strains were analyzed by a combination of next generation sequencing and multiplex PCR approaches. The occurrence and composition of integrative conjugative elements (ICEs) were determined.

**Results:** One or more of the resistance determinants *erm(42)*, *msr(E)* and *mph(E)* were found in the genomes of over 40 strains and conferred resistance to different sets of macrolide antibiotics. The macrolide resistance genes were confined to ICEs within the *M. haemolytica* and *P. multocida* chromosomes, and were often associated with genes conferring resistance to aminoglycosides and less frequently with genes conferring resistance to sulfonamides, chloramphenicol, tetracyclines and beta-lactams. In a subset of strains that were highly-resistant to macrolides, ICE sequences were absent, and in these cases, macrolide resistance was shown to be conferred by mutations in all six copies of the *rrn* operons encoded 23S rRNA.

**Conclusion:** Resistance to macrolides, sulfonamides, chloramphenicol, tetracyclines and beta-lactams was associated with chromosomally encoded ICEs sequences, and the composition of ICEs varies greatly between isolates. Macrolide resistance can additionally arise from 23S rRNA mutations (Figure) in the absence of an ICE. The data have been used to design more comprehensive multiplex PCR tools for screening for resistance determinants in the *Pasteurellaceae* including the human pathogen *Haemophilus influenzae*.

**References:** 1. Desmolaize B et al. (2011) *Mol. Microbiol.* **80**: 184-94; 2. Desmolaize B et al. (2011) *Antimicrob. Agents Chemother.* **55**: 4128-33; 3. Michael GB et al. (2012) *J. Antimicrob. Chemother.* **67**: 84-90; 4. Olsen AS et al. (2015). *J. Antimicrob. Chemother.* **70**: 420-423.

## S9 - Plenary session

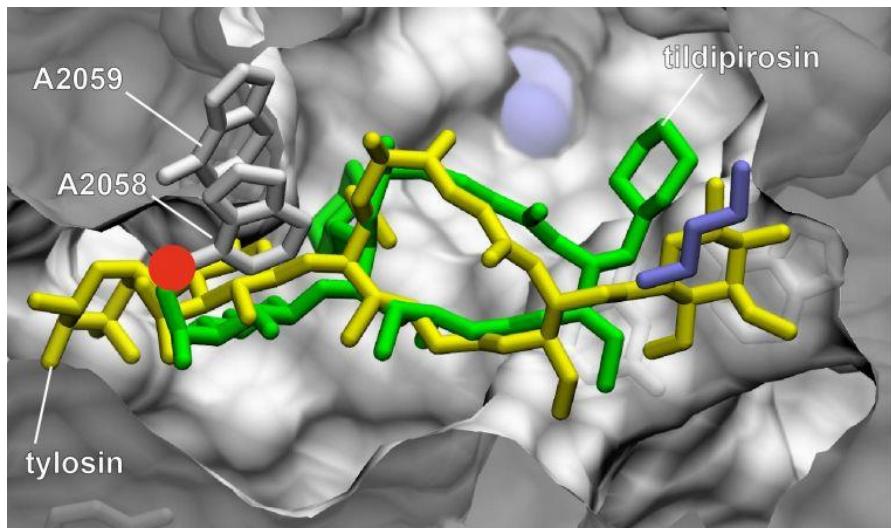


Figure legend: The macrolide binding site in the bacterial ribosome (grey) showing the orientations of the 16-membered ring compounds tylosin (yellow) and tildipirosin (green). Resistance to both drugs is conferred in *P. multocida* and *M. haemolytica* by mutations at the 23S rRNA nucleotides A2058 or A2059 (4), or by methylation at A2058 (red sphere) by Erm(42) (1).

**SC07 - Whole-Genome Linkage Analysis Reveals Co-Evolutionary Mechanisms Of Antibiotic Resistance In The Pneumococcus**

Jukka Corander<sup>1</sup>

*<sup>1</sup>Department of Mathematics and Statistics, University of Helsinki*



**MOLECULAR S10 - PLENARY SESSION: MOLECULAR EPIDEMIOLOGY AND PUBLIC HEALTH**

**Saturday, 12<sup>th</sup> March 2016, 08:30-10:00**

**OP49 – IRIDA: A Federated Bioinformatics Platform Enabling Richer Genomic Epidemiology Analysis In Public Health**

Franklin Bristow<sup>1</sup>; Josh Adam<sup>1</sup>; João André Carriço<sup>2</sup>; Mélanie Courtois<sup>3</sup>; Bhavjinder Dhillon<sup>4</sup>; Damion Dooley<sup>5</sup>; Emma Griffiths<sup>4</sup>; Judy Isaac-Renton<sup>5</sup>; Alex Keddy<sup>6</sup>; Peter Kruczakiewicz<sup>1</sup>; Thomas Matthews<sup>1</sup>; Aaron Petkau<sup>1</sup>; Lynn Schriml<sup>7</sup>; Julie Shay<sup>4</sup>; Eduardo Taboada<sup>1</sup>; Patrick Tang<sup>8</sup>; Joel Thiessen<sup>1</sup>; Geoff Winsor<sup>4</sup>; Robert Beiko<sup>6</sup>; Morag Graham<sup>1</sup>; Gary Van Domselaar<sup>1</sup>; William Hsiao<sup>9</sup>; Fiona Brinkman<sup>4</sup>

<sup>1</sup>National Microbiology Laboratories; <sup>2</sup>University of Lisbon; <sup>3</sup>European Bioinformatics Institute; <sup>4</sup>Simon Fraser University; <sup>5</sup>University of British Columbia; <sup>6</sup>Dalhousie University; <sup>7</sup>University of Maryland School of Medicine; <sup>8</sup>Sidra Medical and Research Center; <sup>9</sup>British Columbia Public Health Laboratory

**OP50 - The NCBI MLST Proposal For Supporting Surveillance Of Bacterial Foodborne Pathogens**

Richa Agarwala<sup>1</sup>; Joshua Cherry<sup>1</sup>; Sergey Shiryev<sup>1</sup>; Alejandro Schaffer<sup>1</sup>; Arjun Prasad<sup>1</sup>; Mike DiCuccio<sup>1</sup>; William Klimke<sup>1</sup>; James Ostell<sup>1</sup>; David Lipman<sup>1</sup>  
<sup>1</sup>NCBI/NIH

**OP51 - PulseNet International Vision For The Implementation Of Whole Genome Sequencing For Global Foodborne Disease Surveillance**

Celine Nadon<sup>1</sup>; PulseNet International Steering Committee<sup>2</sup>  
<sup>1</sup>Public Health Agency of Canada; <sup>2</sup>PulseNet International

**OP52 - Surveillance And Outbreak Investigation Of Shiga Toxin-Producing And Enteroinvasive *Escherichia coli* Using Whole Genome Sequencing-Time For A Change!**

Marie Anne Chattaway<sup>1</sup>; Neil Perry<sup>1</sup>; Amy Gentle<sup>1</sup>; Sophie Long<sup>1</sup>; Philip Ashton<sup>1</sup>; Derren Ready<sup>1</sup>; Tim Dallman<sup>1</sup>; Claire Jenkins<sup>1</sup>  
<sup>1</sup>Public Health England

**SC08 - Implementation Of Genomics In Public Health Surveillance In The United States**

Peter Gerner-Smidt<sup>1</sup>

<sup>1</sup>Enteric Diseases Laboratory Branch, Centers for Disease Control & Prevention (CDC), Atlanta, USA.

## S10 - Plenary session

### SC09 - eCDC Strategy And Roadmap For EU Genomic Surveillance And Epidemic Preparedness

Marc Struelens<sup>1</sup>

<sup>1</sup>*European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden.*

**OP49 – IRIDA: A Federated Bioinformatics Platform Enabling Richer Genomic Epidemiology Analysis In Public Health**

Franklin Bristow<sup>1</sup>; Josh Adam<sup>1</sup>; João André Carrizo<sup>2</sup>; Mélanie Courtot<sup>3</sup>; Bhavjinder Dhillon<sup>4</sup>; Damion Dooley<sup>5</sup>; Emma Griffiths<sup>4</sup>; Judy Isaac-Renton<sup>5</sup>; Alex Keddy<sup>6</sup>; Peter Kruczakiewicz<sup>1</sup>; Thomas Matthews<sup>1</sup>; Aaron Petkau<sup>1</sup>; Lynn Schriml<sup>7</sup>; Julie Shay<sup>4</sup>; Eduardo Taboada<sup>1</sup>; Patrick Tang<sup>8</sup>; Joel Thiessen<sup>1</sup>; Geoff Winsor<sup>4</sup>; Robert Beiko<sup>6</sup>; Morag Graham<sup>1</sup>; Gary Van Domselaar<sup>1</sup>; William Hsiao<sup>9</sup>; Fiona Brinkman<sup>4</sup>

<sup>1</sup>National Microbiology Laboratories; <sup>2</sup>University of Lisbon; <sup>3</sup>European Bioinformatics Institute; <sup>4</sup>Simon Fraser University; <sup>5</sup>University of British Columbia; <sup>6</sup>Dalhousie University; <sup>7</sup>University of Maryland School of Medicine; <sup>8</sup>Sidra Medical and Research Center; <sup>9</sup>British Columbia Public Health Laboratory

**Background:** Whole genome sequencing (WGS) is revolutionizing molecular typing methodologies for microbial infectious disease surveillance and outbreak investigation. Beyond providing the highest possible resolution of the genetic relationship of sampled microbial populations, WGS provides additional information about these disease causing microbes such as virulence factors, antimicrobial resistance profiles, persistence in hospital and food processing facilities, and more. Bioinformatics pipelines for genomic epidemiology that process the “Big Data” sets generated by WGS are proliferating, but they vary considerably in the technical prowess required to use them effectively. In addition, most public data analysis tools/pipelines for genomic epidemiology require uploading of data to public servers for data crunching and do not integrate rich, sometimes sensitive, epidemiologic data. These limitations provide a barrier to the widespread adoption and use of genomics by public health workers. Canada’s Integrated Rapid Infectious Disease Analysis (IRIDA) web-based platform provides a secure environment for integrating genomic data with rich epidemiological data, and bioinformatics pipelines for the analysis of that data.

**Materials | Methods:** The IRIDA development team comprises five interconnected working groups: 1) Ontology and Database; 2) Microbial Typing; 3) Architecture and API; 4) Tools Development; 5) User Experience. Teams are embedded in Canadian national and provincial public health agencies, and in academia, to engage end users and stakeholders during design and implementation phases of the project. IRIDA has implemented secure storage of WGS data, epidemiological and application metadata, data analysis pipelines, visualization of results, and a federated data sharing model intended to facilitate secure communication within and between provincial and federal public health institutions in Canada. Metadata has been represented using an application ontology following community recognized standards and extending existing OBO domain ontologies (<http://www.obofoundry.org/>) to promote interoperability. Data analysis pipelines, execution provenance, and data QA/QC are transparently implemented using Galaxy, and federated data sharing and analysis is realized with a common REST API across platform instances. Data analysis tools developed and in development include phylogeographic analysis, *in silico* microbial typing capability for *Salmonella* and *E. coli*, whole genome and core genome MLST, and visualization tools for antimicrobial resistance, virulence factor, and genomic island analysis.

**Results:** An initial IRIDA version is being tested in the Canada’s public health agencies using current outbreak data, enabling further refinement of ontology and tool development. Linkage with other international genomic epidemiology initiatives, involving public genomic data release with more limited metadata, is also envisaged. A publicly available academic version that does not provide access to patient and clinical metadata will provide IRIDA’s analysis tools for wider research use.

**Conclusion:** IRIDA is free, open-source software that may be a useful platform for other countries with autonomous health regions that wish to empower their public health workers’ genomic analysis capabilities. See <http://www.irida.ca> for more information.

## S10 - Plenary session

### OP50 - The NCBI MLST Proposal For Supporting Surveillance Of Bacterial Foodborne Pathogens

Richa Agarwala<sup>1</sup>; Joshua Cherry<sup>1</sup>; Sergey Shiryev<sup>1</sup>; Alejandro Schaffer<sup>1</sup>; Arjun Prasad<sup>1</sup>; Mike DiCuccio<sup>1</sup>; William Klimke<sup>1</sup>; James Ostell<sup>1</sup>; David Lipman<sup>1</sup>

<sup>1</sup>NCBI/NIH

Keywords: GMI, outbreaks, MLST

**Background:** The vision of Global Microbial Identifier (GMI) initiative is to develop a global system to aggregate, share, mine and use microbiological genomic data to address global public health and clinical challenges. NCBI has been an active participant in the GMI initiative in the area of identification of common sources of contamination from food products that cause disease and sickness in people and for providing assistance in quickly resolving such outbreaks. For bacterial pathogens, the highest resolution is obtained when using the whole genome sequence to cluster isolates that come from a common source. Public Health England in Europe and a number of agencies in the US, including FDA-CFSAN, CDC, USDA-FSIS, and state and public health laboratories have started national genome sequencing projects to improve surveillance methods. This includes sequencing both retrospective and real time isolates for foodborne pathogens. NCBI has collaborated with these agencies to provide database and analytical support for surveillance.

**Methods:** Once sequencing is completed, data deposited to NCBI include the raw sequence reads to Sequence Read Archive and the sample metadata to the Biosample database using a template to describe foodborne pathogens. The data are integrated globally from all public submissions by the NCBI pipeline to produce: 1) assemblies generated from the raw reads, 2) a distance tree based on comparison of kmers extracted from the assemblies in GenBank and those submitted from the public health agencies to obtain rough clusters, 3) tight clusters based on SNPs for closely related isolates. Preliminary work using multilocus sequence typing (MLST) has shown that for pairs of isolates relevant for outbreak detection, there is a high level of concordance between SNP counts and number of allele differences using a MLST scheme when confounding issues, such as, genome quality and contamination are accounted for correctly. To find tight clusters quickly, NCBI plans to develop software that will generate MLST schemes for bacterial species relevant for outbreak detection, utilize quality assessment checks for assemblies, and compute tight clusters using allele differences.

**Results:** As of Sept 5, 2014, 8810 samples were sequenced and the raw reads submitted to NCBI including 1) 1775 *Listeria*, 2) 6694 *Salmonella*, 3) and 341 *E. coli*. The number of isolates for the species analyzed by the NCBI pathogen pipeline as of November 24, 2015 exceeds 48000 and are distributed as: 1) 5717 *Listeria*, 2) 33706 *Salmonella*, 3) 8027 *E. coli*, and 4) 1132 *Campylobacter*. Using MLST can speed the initial clustering for our SNP processing and also provide another consistency check for the groupings of isolates. Furthermore, since MLST is an important and popular classification method in epidemiology, our goal is to make our resources more useful to many of our collaborators.

**Conclusion:** Use of whole genome sequencing to speed up epidemiological investigations and of markers for classifying species are well established. Merging the two for scaling current methods to volumes of data being generated without compromising quality is warranted.

**OP51 - PulseNet International Vision For The Implementation Of Whole Genome Sequencing For Global Foodborne Disease Surveillance**Celine Nadon<sup>1</sup>; PulseNet International Steering Committee<sup>2</sup><sup>1</sup>*Public Health Agency of Canada; <sup>2</sup>PulseNet International*

PulseNet International is a global network dedicated to laboratory-based subtyping surveillance for foodborne diseases world-wide. The network is comprised of the national and regional laboratory networks of USA, Canada, Latin America & Caribbean (16 countries and one subregional laboratory for 20 countries), Europe, Africa (11 countries), Middle East (10 countries) and Asia Pacific (13 countries). The use of internationally standardised molecular subtyping and analysis methods in real-time coupled with national/regional databases has enabled the immediate, direct comparisons of subtyping data from across the world. This has facilitated rapid outbreak detection and response within countries, throughout regions, and worldwide. The vision of PulseNet International is the use of whole genome sequencing (WGS) in all public health laboratories in the world to identify, characterise and subtype foodborne bacterial pathogens, replacing existing phenotypic and molecular methods in support of foodborne disease preparedness and response to save lives and reduce global social and economic loss.

The cost of WGS is now approaching or is less than current bacterial characterisation and subtyping methods. It brings higher resolution and accuracy for laboratory-based surveillance, and many countries and/or networks are in the process of implementing WGS for outbreak response and surveillance. Parallel to advances in genomics technology have been the development of bioinformatics pipelines for the analysis of results; there are many bioinformatics tools now available worldwide providing solutions for diagnostics, surveillance, and population-based genomic epidemiology. Many of the tools are open-source and freely available for use.

To meet the needs of real-time surveillance, the PulseNet International network will standardise subtyping via WGS using the gene-by-gene approach, i.e., whole genome MLST. This approach delivers optimal resolution and epidemiological concordance for the purposes of surveillance whilst providing unambiguous nomenclature; in addition, it is computationally efficient and thereby realistic for all public health laboratories to use on a daily basis. Standardised protocols, validation studies, quality control programs, database and schema development, and training materials all must support the implementation and decentralisation of any new technique. The development of these are currently underway using established PulseNet International processes.

In order to truly standardise foodborne disease subtyping across the world, a public WGS-based nomenclature, curated where necessary, must be available. In addition, contrary to e.g. pulsed field gel electrophoresis molecular subtyping data, WGS data are not only suitable for surveillance and outbreak purposes, but also for answering other scientific questions such as source attribution, antimicrobial resistance, transmission patterns, and population structure. Therefore, in addition to the WGS nomenclature, the WGS data itself should also be publicly available. To fully realise the vision, technical and political challenges must be overcome, particularly around the shift to publicly available data. The latter should not be used as nontariff barriers to trade or to scientific advancements that will provide the best possible surveillance and outbreak response at all levels, and enable the protection and improvement of public health with respect to foodborne disease.

## S10 - Plenary session

### OP52 - Surveillance And Outbreak Investigation Of Shiga Toxin-Producing And Enteroinvasive *Escherichia coli* Using Whole Genome Sequencing-Time For A Change!

Marie Anne Chattaway<sup>1</sup>; Neil Perry<sup>1</sup>; Amy Gentle<sup>1</sup>; Sophie Long<sup>1</sup>; Philip Ashton<sup>1</sup>; Derren Ready<sup>1</sup>; Tim Dallman<sup>1</sup>; Claire Jenkins<sup>1</sup>

<sup>1</sup>Public Health England

**Background:** Implementation of commercial PCRs for the detection of Gastrointestinal pathogens at local hospital laboratories in the United Kingdom is increasingly common. As these kits include primers targeting the genes encoding Shiga toxin (stx) this has led to an increase in the number of Shiga toxin-producing *Escherichia coli* (STEC) other than serogroup O157 (non-O157 STEC) isolated in England. Enteroinvasive *E. coli* (EIEC) is currently only detected by the national reference laboratory, in 2014 the largest UK EIEC outbreak occurred.

**Methods:** At the Gastrointestinal Bacteria Reference Unit (GBRU) at Public Health England, we evaluated the use of whole genome sequencing (WGS) for serotyping, detection of *E.coli* pathogenic markers, stx subtyping and outbreak investigation of non-O157 STEC and a large EIEC outbreak in 2014. Between January 2012 and December 2014, GBRU received 218 isolates of non-O157 STEC from human cases, including 21 (9.6%) with Haemolytic Uraemic Syndrome (HUS). This comprised 42 different serogroups with the most common being O26, O146, O55 (16 cases representing a community outbreak) and O103. Of the 218 strains, 159 underwent WGS, phenotypic serotyping and stx subtyping by PCR were compared to an *in silico* approach. In addition 1393 non-O157 STEC strains from 2005-2014 were WGS and common sequence types (ST) assessed. During the EIEC outbreak, GBRU received 92 faecal samples and 41 environmental *E.coli* isolates for detection, isolation and typing of EIEC.

**Results:** Of the 159 isolates sequenced, a serogroup was identified in 156 (98%) isolates using *in silico* WGS compared to 108 (68%) by phenotypic serotyping. There were eight mismatches, six of which were caused by errors in the original phenotypic serotyping. Stx subtyping by PCR was available for 90 isolates and were 100% concordant when compared to PCR subtyping results. Common non-O157 STEC ST over the past 9 years were ST21 (O26), ST442 (O146), ST335 (O55), ST17 (O103, O71), ST33 (O91), ST675 (O76), ST10 (O2,O113,O38) and ST342 (O177). EIEC WGS analysis indicated a ST99 (O96) strain to be responsible for the outbreak and related to the strain from the outbreak in Italy in 2012. The WGS approach also provided data on the presence and absence of a wide range of virulence genes encoding toxins, adherence and invasion mechanisms. Furthermore, analysis of the single nucleotide polymorphism (SNPs) in the core genome provided highly discriminatory typing data that facilitated the investigation of multiple community outbreaks.

**Conclusions:** WGS is a rapid, robust, reliable and cost effective approach to characterise all *E.coli* for routine surveillance and outbreak investigation. WGS has now been implemented as a routine service at GBRU for *E.coli* since October 2015.

**SC08 - Implementation Of Genomics In Public Health Surveillance In The United States**

Peter Gerner-Smidt<sup>1</sup>

*<sup>1</sup>Enteric Diseases Laboratory Branch, Centers for Disease Control & Prevention (CDC), Atlanta, USA.*

## S10 - Plenary session

### SC09 - eCDC Strategy And Roadmap For EU Genomic Surveillance And Epidemic Preparedness

Marc Struelens<sup>1</sup>

<sup>1</sup>*European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden.*

## BIOINFORMATICS TOOLS FOR GENOMIC EPIDEMIOLOGY

**Thursday, 10 March 2016, 12:00-14:30**

### **PO1 - Oligonucleotide-based Fast Clustering Of Bacterial Genomes For Epidemiology And Phylogenetics**

Rosario San Millan<sup>1</sup>; Iñaki Martínez-Ballesteros<sup>2</sup>; Lorena Laorden<sup>2</sup>; Javier Garaizar<sup>2</sup>; Joseba Bikandi<sup>2</sup>

<sup>1</sup>University of the Basque Country (EHU/UPV). Faculty of Medicine and Odontology, <sup>2</sup>University of the Basque Country (EHU/UPV). Faculty of Pharmacy

### **PO2 - Russian Phage Roulette In Reference Genomes**

Jonas T. Björkman<sup>1</sup>; Kristoffer Kiil<sup>1</sup>; Eva Møller Nielsen<sup>1</sup>

<sup>1</sup>Statens Serum Institut

### **PO3 - SCCmec Typing Of MRSA Genomes**

Peder Worning<sup>1</sup>; Jesper Boye Nielsen<sup>1</sup>; Heidi Gumpert<sup>1</sup>; Mette Bartels<sup>1</sup>; Henrik Westh<sup>1</sup>

<sup>1</sup>Hvidovre University Hospital

### **PO4 - Towards NGS As Frontline Tool For Routine Typing Of *S. aureus* At The German Reference Center For Staphylococci And Enterococci?**

Birgit Strommenger<sup>1</sup>; Sophie Ziegler<sup>1</sup>; Stephan Fuchs<sup>1</sup>; Jennifer Bender<sup>1</sup>; Franziska Layer<sup>1</sup>; Guido Werner<sup>1</sup>

<sup>1</sup>Robert Koch Institute

### **PO5 - Accnet: New Tool For Accessory Genome Analysis Using Bipartite Networks.**

Val Fernandez Lanza<sup>1</sup>; Irene Rodriguez<sup>1</sup>; Ana P. Tedim<sup>1</sup>; María de Toro<sup>2</sup>; Fernando de la Cruz<sup>2</sup>; Rafael Cantón<sup>1</sup>; Fernando Baquero<sup>1</sup>; Teresa M. Coque<sup>1</sup>

<sup>1</sup>CIBER-ESP. Hospital Universitario Ramón y Cajal and Instituto Ramón y Cajal de Investigación Sanitaria IRYCIS, Instituto de Biomedicina y Biotecnología de Cantabria IBBTEC UC-SODERCAN-CSIC

### **PO6 - The Contaminated Genome**

Peder Worning<sup>1</sup>; Heidi Gumpert<sup>1</sup>; Jesper Boye Nielsen<sup>1</sup>; Henrik Westh<sup>1</sup>

<sup>1</sup>Department of Clinical Microbiology, Copenhagen University Hospital, Hvidovre Hospital

## S1 - Poster session I

### PO7 - Genome-based Epidemiological Surveillance Of *Listeria monocytogenes* At A Global Scale

Alexandra Moura<sup>1</sup>; Hannes Pouseele<sup>2</sup>; Alexis Criscuolo<sup>1</sup>; Mylène Maury<sup>1</sup>; Marie Touchon<sup>1</sup>; Alexandre Leclercq<sup>1</sup>; Viviane Chenal-Francisque<sup>1</sup>; Hélène Dieye<sup>1</sup>; Thomas Cantinelli<sup>1</sup>; Louis Jones<sup>1</sup>; Elise Larsonneur<sup>1</sup>; Cheryl Tarr<sup>3</sup>; Heather Carleton<sup>3</sup>; Zuzana Kucerova<sup>3</sup>; Lee Katz<sup>3</sup>; Steven Stroika<sup>3</sup>; Jonas T. Larsson<sup>4</sup>; Aleisha Reimer<sup>5</sup>; Matthew Walker<sup>5</sup>; Celine Nadon<sup>5</sup>; Eva M. Nielsen<sup>5</sup>; Tim Dallman<sup>6</sup>; Kathie Grant<sup>6</sup>; Peter Gerner-Smidt<sup>3</sup>; Bruno Pot<sup>2</sup>; Marc Lecuit<sup>1</sup>; Sylvain Brisson<sup>1</sup>

<sup>1</sup>Institut Pasteur <sup>2</sup>Applied-Maths <sup>3</sup>Centers for Disease Control and Prevention

<sup>4</sup>Statens Serum Institut, <sup>5</sup>Public Health Agency of Canada, <sup>6</sup>Public Health England

### PO8 - Fimtyper; A Web Tool For *E. coli* fimH Genotyping

Evgeni Sokurenko<sup>1</sup>; Veronika Tchesnokova<sup>1</sup>; Mariya Muradova<sup>1</sup>; Sujay Chattopadhyay<sup>1</sup>; Johanne Ahrenfeldt<sup>2</sup>; Rosa Allesøe<sup>2</sup>; Frank Hansen<sup>3</sup>; Anette Hammerum<sup>3</sup>; Henrik Hasman<sup>3</sup>

<sup>1</sup>University of Washington, <sup>2</sup>Technical University of Denmark, <sup>3</sup>Statens Serum Institut

### PO9 - Examining Gene-by-gene Diversity Across Thousands Of Whole Genomes To Determine Suitable Targets

Sofia Hauck<sup>1</sup>

<sup>1</sup>University of Oxford

### PO10 - Core Genome Typing: A Useful Tool For Micro-epidemiological Investigations Involving *Staphylococcus aureus*?

Bruno Pichon<sup>1</sup>; Michel Doumith<sup>1</sup>; Neil Woodford<sup>1</sup>; Angela M. Kearns<sup>1</sup>

<sup>1</sup>Public Health England

### PO11 - Genetic Diversity Of *Salmonella typhimurium* And *Salmonella enteritidis* Isolated From Ducks And Chickens Using Random Amplified Polymorphic DNA (RAPD)

Frederick Adzitey<sup>1</sup>; Courage Kosi Setsoafia Saba<sup>2</sup>; Raja Arief Deli<sup>3</sup>

<sup>1</sup>University for Development Studies, Animal Science Department, <sup>2</sup>University for Development Studies, Department of Biotechnology, <sup>3</sup>Food Technology Research Centre, Malaysian Agriculture Research and Development Institute

### PO12 - Application Of PCR-RFLP Provides As Discrimination As Total FLAA Sequence Analysis Among *C. jejuni* Isolates Of Clinical Origin

Mahdi Ghorbanalizadgan<sup>1</sup>; BitaBakhshi<sup>2</sup>; ShahinNajar-Peerayeh<sup>2</sup>

<sup>1</sup>Department of Bacteriology, Faculty of Medical Sciences, TarbiatModares University, Tehran, IR Iran, <sup>2</sup>Department of Bacteriology, Faculty of Medical Sciences, Baqiyatallah University, Tehran, IR Iran

**PO13 - A Unified Database For *Acinetobacter baumannii* Genomic And MLST Sequences Enables Comparison Of Pasteur And Oxford 7-gene MLST Nomenclatures**

Raffaele Zarrilli<sup>1</sup>; Virginie Passet<sup>2</sup>; Eliana Pia Esposito<sup>1</sup>; Keith A Jolley<sup>3</sup>; Sylvain Brisson<sup>2</sup>

<sup>1</sup>Department of Public Health, University of Naples "Federico II", Naples,

<sup>2</sup>Italy, Institut Pasteur, Microbial Evolutionary Genomics, Paris, France, <sup>3</sup>Department of Zoology, University of Oxford, Oxford, UK

**PO14 - PHYLOViZ Online: Web-based Phylogenetic Data Analysis And Visualization For Allelic Profiles And SNP Data**

Bruno Ribeiro-Gonçalves<sup>1</sup>; Alexandre Francisco<sup>2</sup>; Cátia Vaz<sup>3</sup>; Mário Ramirez<sup>1</sup>; João André Carriço<sup>1</sup>

<sup>1</sup>Instituto de Microbiologia, Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, <sup>2</sup>Instituto Superior Técnico, Universidade de Lisboa, <sup>3</sup>Instituto Superior de Engenharia de Lisboa <sup>4</sup>Instituto Politécnico de Lisboa

<sup>1</sup>Instituto de Microbiologia, Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, <sup>2</sup>Instituto Superior Técnico, Universidade de Lisboa, <sup>3</sup>Instituto Superior de Engenharia de Lisboa <sup>4</sup>Instituto Politécnico de Lisboa

**PO15 - Comparative Analysis Sequencing-based Typing Methods For Epidemiological Study Of *C. trachomatis***

Zhanna Amirbekova<sup>1</sup>; Dmitriy Babenko<sup>1</sup>; Anar Turmuhambetova<sup>1</sup>

<sup>1</sup>Karaganda State Medical University

**PO16 - Appraisal Of Complementary Strategies For Refinement Of A Core Genome Multi-locus Sequence Typing Scheme For Typing Of *Acinetobacter baumannii***

Mickael Silva<sup>1</sup>; João André Carriço<sup>1</sup>; Nir Gilad<sup>2</sup>; Michal Gordon<sup>2</sup>; Mario Ramirez<sup>1</sup>; Vered Chalifa-Caspi<sup>2</sup>; Jacob Moran-Gilad<sup>2</sup>

<sup>1</sup>Faculdade de Medicina, Instituto de Microbiologia, Instituto de Medicina Molecular, Universidade de Lisboa, <sup>2</sup>Surveillance and Pathogenomics Israeli Centre of Excellence, National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev

**PO17 - MLVA For The Genotyping Of *Acinetobacter baumannii*: Towards A Consensus For Interpretation Of Data And The Development Of International Databases**

Christine Pourcel<sup>1</sup>; Yolande Hauck<sup>1</sup>; Charles SOLER<sup>2</sup>; Gilles Vergnaud<sup>1</sup>

<sup>1</sup>University Paris-Saclay, <sup>2</sup>Percy military hospital

**PO18 - Straintracer-db: A Flexible Analysis And Persistent Storage System For Sequence And Epidemiological Data**

Jonas Evensen<sup>1</sup>; Sveinung Gundersen<sup>2</sup>; Anja B. Kristoffersen<sup>3</sup>; Karin Lagesen<sup>4</sup>

<sup>1</sup>Department of Informatics, University of Oslo; <sup>2</sup>ELIXIR.NO / Department of Informatics, University of Oslo; <sup>3</sup>Norwegian Veterinary Institute; <sup>4</sup>Norwegian Veterinary Institute/Department of Informatics, University of Oslo

## S1 - Poster session I

### **PO19 - MicrobesNG: A Microbial Sequencing Facility Designed To Integrate Myriad Of Sequenced Whole Genomes With Their Samples**

Pablo Fuentes Utrilla<sup>1</sup>; Emily Richardson<sup>1</sup>; Joshua Quick<sup>1</sup>; Jennie Law<sup>1</sup>; Andrew Smith<sup>1</sup>; Ian Henderson<sup>1</sup>; Roy Chaudhuri<sup>2</sup>; Nick Loman<sup>1</sup>; Tim Wells<sup>1</sup>

<sup>1</sup>University of Birmingham, <sup>2</sup>University of Sheffield

### **PO20 - A Scripting Language For Standardized Evaluation Of Quality Metrics In Galaxy And Command-line Driven Workflows**

Damion M. Dooley<sup>1</sup>; Aaron J. Petkau<sup>2</sup>; Franklin Bristow<sup>2</sup>; Gary Van Domselaar<sup>2</sup>; William W.L. Hsiao<sup>3</sup>

<sup>1</sup>Department of Pathology, University of British Columbia, <sup>2</sup>National Microbiology Laboratory, Public Health Agency of Canada, <sup>3</sup>Department of Pathology, University of British Columbia & BC Public Health Microbiology and Reference Laboratory

### **PO21 - GTFinder Workflow To Detect Exclusively Conserved Region Across A Large Genome Dataset: *Salmonella* spp. Study**

Arnaud Felten<sup>1</sup>; Laurent Guillier<sup>1</sup>; Fabrice Touzain<sup>2</sup>; Michel-Yves Mistou<sup>1</sup>; Renaud Lailler<sup>1</sup>; Nicolas Radomski<sup>1</sup>; Sabrina Cadel Six<sup>1</sup>

<sup>1</sup>Université PARIS-EST, Anses, Laboratory for food safety, <sup>2</sup>Anses, Genomic platform

### **PO22 - MOST: A Modified MLST Typing Tool Based On Short Read Sequencing**

Rediat Tewolde<sup>1</sup>; Tim Dallman<sup>1</sup>; Ulf Schaefer<sup>1</sup>; Carmen Sheppard<sup>1</sup>; Philip Ashton<sup>1</sup>; Bruno Pichon<sup>1</sup>; Craig Swift<sup>1</sup>; Jonathan Green<sup>1</sup>; Anthony Underwood<sup>1</sup>

<sup>1</sup>Public Health England

### **PO23 - The *Brucella* MLVA Database**

Gilles Vergnaud<sup>1</sup>; Yolande Hauck<sup>1</sup>; Christine Pourcel<sup>1</sup>; Isabelle Jacques<sup>2</sup>; Axel Cloeckaert<sup>2</sup>; Michel Zygmunt<sup>2</sup>

<sup>1</sup>University Paris-Saclay, INRA

## PO1 - Oligonucleotide-based Fast Clustering Of Bacterial Genomes For Epidemiology And Phylogenetics

Rosario San Millan<sup>1</sup>; Iñaki Martínez-Ballesteros<sup>2</sup>; Lorena Laorden<sup>2</sup>; Javier Garaizar<sup>2</sup>; Joseba Bikandi<sup>2</sup>

<sup>1</sup>University of the Basque Country (EHU/UPV). Faculty of Medicine and Odontology, <sup>2</sup>University of the Basque Country (EHU/UPV). Faculty of Pharmacy

**Background:** Comparing the oligonucleotide frequencies of genomes to find phylogenetics relationships has a long history. In most cases, very short oligonucleotides, often tetranucleotides, have been researched. Similar frequencies were observed in phylogenetically-related genomes, such as members of the same species or family. In this work, we selected some types of frequencies and distances from the literature to compare oligonucleotide frequencies. We then studied their behavior in different situations. We wanted to know whether oligonucleotides over four bases long could be used to cluster together phylogenetically-related genomes, and whether the clustering method could be applied to members of the same species for epidemiology.

**Materials | Methods:** Sequences and other related files were obtained from NCBI or *Ensembl Genomes*. Tools to assess the best comparison algorithm were developed with PHP running on an Apache server with a Linux operating system. An online service was also developed that uses C programs for some computationally intensive tasks. The basic code used in the service is provided.

**Results:** Different statistical methods were searched to determine which would yield the best clustering results for oligonucleotides. The best results were obtained by comparing longer oligonucleotides (octanucleotides) and hamming distance. The selected procedure was applied to several complete sequenced genomes from *Ensembl Genomes*. The proposed method was able cluster together phylogenetically-related genomes. In addition, epidemiologically-related genomes (as, for example, members of the same *Salmonella* or *E. coli* serotypes) clustered together.

**Conclusion:** Usage of oligonucleotide frequencies for fast comparison and clustering of complete bacterial genomes has great potential for both phylogenetics and epidemiology. This method may be used for many species. A freely accessible website was developed for evaluation of the proposed comparison methods (<http://gscompare.ehu.eus>). The service allows uploading of sequences and comparisons of them or with sequences already in our database of more than 20,000 complete genomes.

## S1 - Poster session I

### PO2 - Russian Phage Roulette In Reference Genomes

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<sup>1</sup>Statens Serum Institut

For outbreak analysis, you often would like the maximum discriminatory power, resulting in that you resort to SNP analysis with a closely related strain as mapping reference. We have analysed outbreaks, clusters and closely related isolates of *Listeria monocytogenes* from a ten-year period in Denmark and assessed the impact on the cluster analysis when using reference genome with, or without prophages.

Prophages were identified using PHAST and by looking at SNP density over the genome. In some isolate clusters, certain positions in the genome were always occupied by a phage. The phages did not necessarily have a common phylogenetic origin with the host strain, but all the phages were related enough to contribute SNPs, that our standard method of removing horizontally transferred genetic material did not catch.

We found there could be a large variation of related phages in the same genomic site. The individual phage variants showed very few internal SNPs but the different phage variants had enough similarity to cause a large impact on the tree creation and cluster analysis.

The effect of using the reference genome with and without removal of phage varied from no impact at all, to relatively small with mostly elongated branches, and finally to a strong impact, where the phages accounted for more than 90 % of the variation and caused rearrangements of the trees.

Our recommendation is that you (at least for *Listeria*) should not use a reference genome with a possibly mobilisable prophage present. Since the task of properly identifying all prophages in a genome might not be trivial, ideally this should be done thoroughly once on reference genomes, that subsequently could be shared throughout the world.

## PO3 - SCCmec Typing Of MRSA Genomes

Peder Worning<sup>1</sup>; Jesper Boye Nielsen<sup>1</sup>; Heidi Gumpert<sup>1</sup>; Mette Bartels<sup>1</sup>; Henrik Westh<sup>1</sup>

<sup>1</sup>Hvidovre University Hospital

Keywords: SCCmec Type, MRSA, Whole Genome Sequencing.

**Background:** Since 2013 all methicillin resistant *Staphylococcus aureus* (MRSA) isolates from the Capital Region of Denmark have been whole genome sequenced on an Illumina MiSeq. We have made a SCCmec typing tool as a part of our MRSA pipeline, which also includes spa-, dru- and MLST-typing. We have determined SCCmec types for 3,052 different isolates (by October 2015). The 3,052 isolates contains 337 different spa-types and 109 different MLST types.

**Methods:** The main SCCmec types (I – XI) are determined by blasting the DNA sequence in question against a list of known SCCmec genes to determine which versions of the SCCmec genes the sequence contains. We use *mecA*, *mecR*, *mecI* and *mecC* from the *mec* complex, *ccrA1-4*, *ccrB1-4,6*, *ccrC* from the *ccr* complex plus the two IS elements IS431 and IS1272. To find SCCmec type XI we use alternative versions of the *mec*- and the *ccr*-genes, namely *mecC*, *mecRx1*, *mecIx1*, *ccrAx1* and *ccrBx3*. For SCCmec type IV we use six sets of primers and a virtual PCR routine to determine the sub-type. Here we can resolve the subtypes IVa, IVb, IVd, IVg, and IVh, but we cannot separate IVc from IVe, so we report them as IVc/e.

**Results:** By far we see SCCmec type IV the most at 76%, followed by type V at 13.2%, type IX at 1.6 % and type II at 1.4 %. We perform subtyping of type IV into six different subtypes: IVa, IVb, IVc/e, IVd, IVg and IVh, where IVa and IVc/e are the most common. We will bring a computer with the software installed, so guests at IMMEM XI can bring their favorite MRSA genome on a stick in fasta format to get it typed. The SCCmec typer is a work in progress and we are very interested in collaboration to make the determinations of the SCCmec types more complete. It is our plan to host the validated SCCmec typer online.

**Conclusion:** We can type the 11 main SCCmec types and six SCCmec IV sub-types, from DNA sequences. We see by far most SCCmec type IV 76 %, followed by type SCCmec V at 13%. Our dataset contains 3,052 isolates representing 337 spa types and 109 MLST types.

## S1 - Poster session I

### PO4 - Towards NGS As Frontline Tool For Routine Typing Of *S. aureus* At The German Reference Center For Staphylococci And Enterococci?

Birgit Strommenger<sup>1</sup>; Sophie Ziegler<sup>1</sup>; Stephan Fuchs<sup>1</sup>; Jennifer Bender<sup>1</sup>; Franziska Layer<sup>1</sup>; Guido Werner<sup>1</sup>

<sup>1</sup>Robert Koch Institute

**Background:** The rapid progress of Next generation sequencing (NGS) technology has dramatically changed our vision of clinical microbiological diagnostics, strain characterization and typing during recent years. Although the constant decrease in cost nowadays enables the implementation of NGS technology in molecular surveillance activities, detailed bioinformatic analysis of genome data still remains a challenge. Therefore this study was initiated to evaluate feasibility and benefit of NGS for the staphylococcal molecular typing service provided by the National Reference Centre (NRC) for staphylococci and enterococci in comparison to typing tools used to date.

**Materials and methods:** We selected a total of 282 *S. aureus* isolates sent to the NRC throughout February 2014. All isolates passed the routine typing workflow at our laboratory including phenotypic susceptibility testing, spa-typing (Sanger sequencing) and PCR-based *mecA*-detection. Depending on the initial strain report additional resistance and virulence determinants were investigated by phenotypic and genotypic methods, respectively. Sequencing was carried out on a MiSeq instrument aiming at a theoretical coverage of app. 120-fold. Initial data analysis was performed using the Ridom SeqSphere 2.4 pipeline including Velvet as assembler. Based on the finished assemblies SeqSphere extracted spa-type and MLST from the whole genome data for each isolate. For in-depth-typing a predefined core genome MLST scheme (cgMLST) was used which analyzes sequence diversity of 1861 *S. aureus* core genome loci. To extract further information concerning the presence of a variety of relevant resistance and virulence determinants, user-defined sets of corresponding genetic loci were added to the SeqSphere queries.

**Results:** Out of 282 genome datasets 278 yielded satisfactory results ( $\geq 95\%$  of the 1861 MLST<sup>+</sup> target genes with good quality) in the SeqSphere pipeline, thus highlighting the importance of high quality raw data and an average coverage of at least 70-fold. Typing results extracted from NGS data were overall concordant with those generated by currently used genotypic methods (spa-typing, MLST). However, concordance with phenotypic methods (e.g. antibiotic resistance) was lower than expected (app. 75% in initial analyses). Molecular typing based on cgMLST data, as expected, yielded significantly higher discrimination, especially for endemic *S. aureus* lineages and enabled the support of outbreak investigations in several institutions.

**Conclusion:** In conclusion, whole genome sequencing was successfully established for molecular typing purposes at the NRC. Future work will focus on the clarification of contradictory results and on the comparison of results obtained from the combination of different available bioinformatic tools.

## PO5 - Accnet: New Tool For Accessory Genome Analysis Using Bipartite Networks.

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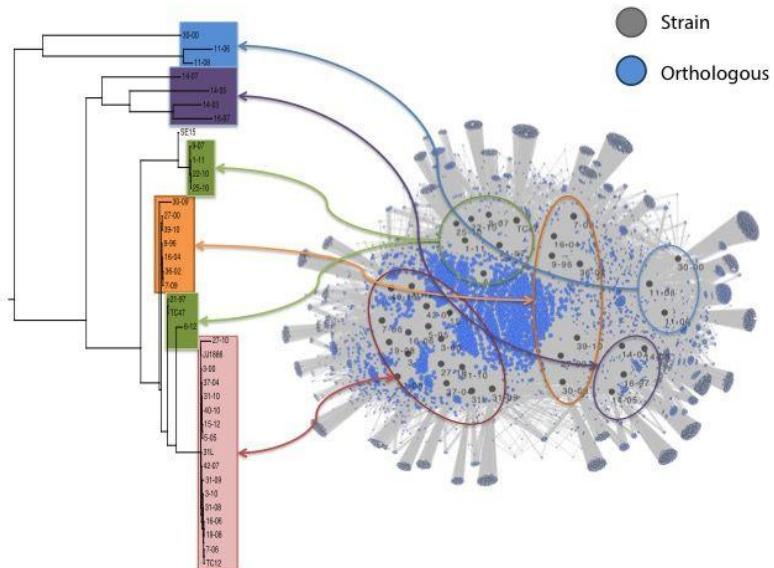
**Background:** Lateral gene transfer plays an essential role in the bacterial evolution. The genomes of most opportunistic pathogens differ in their accessory genome which include determinants associated with virulence, colonization or antibiotic resistance, among other adaptive traits. It exists a wide range of bioinformatics tools for typing, core genome analysis or phylogenetics but only a few tools for analysing the accessory genomes. We present AccNET (Accessory Constellation Network), a new tool, non-computational expensive, based on bipartite networks that is able to analyse simultaneously tens of genomes.

**Material & Methods:** AccNET uses the kClust software to customly detect highly-related homologous genes, by modifying the identity threshold. The core genome, defined as the subset of proteins present in all genomes, is removed from the analysis. Then, AccNET builds a bipartite network of the accessory genome with two kind of nodes: genomes and proteins. The edges that link the nodes represent the presence of a protein in a given genome. The edge-weight is determined as a phylogenetic distance between the proteins included in the cluster. To calculate such distance, we use a pipeline: First, we align all the proteins belonged to the cluster using MUSCLE. Then, we optimize the multiple alignment using Trimal and finally we calculate the distance matrix using ProtDist, (PHYLIP package). For each protein the phylogenetic distance for edge-weight is determined by the mean of the distance with all of the remaining proteins. Final output network is compatible with most of the network viewer software such as Cytoscape or Gephi. We used ForceAtlas2 layout from Gephi to arrange networks. To compare the structure of the accessory distribution with the phylogenetic relationships, we build the core-genome phylogenetic tree using the previous defined core-genome. First, we aligned each one of the core genes using MUSCLE. Then, we concatenated all the multiple alignments and extract the SNPs using HarvestTools. Finally, we built a Maximum Likelihood phylogenetic tree using FastTree2 with the resulting SNPs. To demonstrate AccNET capabilities, we have used two datasets that comprises genomes of the polyclonal group ST131 of *E. coli* (n=37) and the phylogenomic group BAPS 2.1 of *E. faecium* (n=25). Both include specific clonal variants worldwide disseminated.

**Results:** The resulting network from *E. coli* dataset comprises 6973 nodes and 41585 edges while that of *E. faecium* has 3326 nodes and 20041 edges. A high correlation was noted between the clusters in the network of accessory genomes and the branches in the phylogenetic tree of the core genomes. However, the networks disclose specific interactions between genome clusters that are remote in the phylogenetic tree. Besides, both network shown specific protein clusters that belong to genome clusters associated with epidemic multidrug resistant variants such as *E. coli* ST131-fimH30 or *E. faecium* ST117. Fig1 show a snapshot of the network arrangements obtained by using AccNET and the core-phylogenetic tree applied to the *E. coli* dataset.

**Conclusion:** We have developed an specific software tool for the analysis of accessory genome. This software is able to analysis tens of genomes with high precision. User friendly environment as Cytoscape allow us comprehensively exploring the accessory genome, and identifying specific genes/gene clusters of interest in evolutionary biology and epidemiological surveillance.

## S1 - Poster session I



**Figure 1.** AccNET network and core-genome phylogenetic tree of *E. coli* dataset. The correspondence between monophyletic branches and genome clusters is highlighted. The large orthologous cluster coloured in blue represents a pseudo-core genome, a set of proteins shared by most of the genomes (>95%).

## PO6 - The Contaminated Genome

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Keywords: Contaminated Samples, Mixed Assembly, Whole Genome Sequencing

**Background:** Whole genome sequencing is employed for every methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecium* (VREfm) isolate that passes through the Department of Clinical Microbiology at Hvidovre Hospital (KMA-Hvidovre) as part of the daily routine. Now and then we observe assemblies that contain more DNA, i.e. a larger genome, than we would expect for these species. The larger assembly size is interpreted as a sign of a contaminated sample. Yet, questions such as how different do mixed isolates have to be to make a larger assembly, and would a mixture of two different isolates of the same species result in an assembly which is significantly larger than a clean sample, remain to be answered.

**Methods:** We have made combined assemblies of reads from different isolates and analyzed the size of the combined assembly as a function of the SNP distance between the involved isolates. Sequences were mixed in a ratio of 1:1 with an equal coverage of about 50 fold. The isolates have been sequenced on an Illumina MiSeq using 150 bp paired-end reads. Reads have been assembled using Velvet and VelvetOptimiser. Reference genomes for calculating SNP-distances used are USA300-THC1415 (NC\_010079) for the MRSA samples and AUS004 (NC\_017022) for the VREfm samples.

**Results:** We observe that the size of the combined assemblies increases linearly with the SNP distance, and we see a significantly larger assembly already when the distance between the two isolates is as small as 1,000 SNPs. 1,000 SNPs is equal to the diameter of a typical *S. aureus* clonal complex (CC). The size of the combined assemblies saturates abruptly when the size is equal to the sum of the sizes of the two isolates. This happens at a SNP distance on the order of 35-45,000 SNPs. This means that when we mix reads from two genomes with a SNP distance larger than 45,000 SNPs, we get the two complete sequences from our assembly. 45,000 SNPs are further apart than many CC are in *S. aureus*, but still inside the same species.

When we compare the two species included in this study, we observe that the size of the combined assembly increases faster with the SNP distance for VREfm than for MRSA. The size of the combined VREfm assemblies saturates at a SNP distance of approximately 35,000 SNPs, while the size of the MRSA assemblies saturates at approximately 45,000 SNPs.

**Conclusion:** Contaminated samples shows up as to large genomes when the reads are assembled into contigs. The size of the contaminated assembly increases linearly with the SNP distance and significantly larger assemblies are observed when the distance between the two isolates is as small as 1000 SNPs. This means that contamination with isolates from a different clonal complex of the same species will result in enlarged assembled genomes.

## S1 - Poster session I

### PO7 - Genome-based Epidemiological Surveillance Of *Listeria monocytogenes* At A Global Scale

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*Listeria monocytogenes* (*Lm*) outbreaks have been reported worldwide, causing numerous deaths and reinforcing the need for discriminatory typing methods for surveillance and outbreak investigations. Here we report a standardized genome-wide genotyping system based on core genome multi-locus sequencing typing (cgMLST) of 1748 highly conserved genes, representing 62% of the *Lm* genome. The *Lm* cgMLST scheme was developed and validated based on a large set of genomes ( $n=957$ ) representative of the clonal diversity within *Lm* lineages. Robustness of genotyping was tested using assembly-free and assembly-based allele detection methods and different assembly pipelines. Epidemiological and phylogenetic relevance of cgMLST profiles was assessed using genomes collected within the frame of national surveillance programs in USA, Canada, England, Denmark and France ( $n=1696$ ). cgMLST showed highly reproducibility of profiles using both assembly-free and assembly-based calls as well as different assemblers. Allelic profiles reflected the phylogenetic relationships between isolates and were highly conserved within single outbreaks. Moreover, the discriminatory power of cgMLST was significantly higher compared to the current standardised pulsed-field gel electrophoresis (PFGE) typing method, allowing to narrow clusters of closely related isolates with identical PFGE profiles and to improve epidemiological investigations. To facilitate global communication, a bioinformatics platform and a unified nomenclature of sublineages and cgMLST types were implemented. This study shows that cgMLST is a practical and robust approach for international collaboration on research and public health surveillance of *Lm*, enhancing disease surveillance and response efforts.

**PO8 - Fimtyper; A Web Tool For *E. coli* fimH Genotyping**

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<sup>1</sup>University of Washington, <sup>2</sup>Technical University of Denmark, <sup>3</sup>Statens Serum Institut

**Background:** The *fimH* gene is part of a *fim* operon which encodes a surface organelle called Type 1 fimbriae found in most *E. coli* strains. The FimH protein is localized at the tip of the fimbrial structure and serves as a D-mannose specific adhesin, which aids to immobilize the bacterium on both biotic and abiotic surfaces. Studies have shown great allelic variation in the *fimH* gene, which have an influence on binding characteristics of the fimbriae. Until now, almost 500 different alleles of *fimH* have been identified. In addition to being used to study binding abilities of the FimH protein variants, these *fimH* alleles can also be used for subtyping of different *E. coli* clonal variants. The relevance of this *fimH* sub-typing has been especially emphasized within the highly virulent sequence type (ST) 131 clonal clade, where resistant and multi-resistant sub-clades carrying *fimH30* allele have been identified. As *E. coli* ST131 is the most dominant human pathogenic clonal group, the need to be able to perform *fimH* sub-typing is increasing. Traditionally, typing of the *fimH* allele has been performed by specific *fimH* PCR followed by Sanger sequencing and alignment to a *fimH* allele database containing 477 different *fimH* alleles. With the rapid increase in the use of Whole Genome Sequencing (WGS) to type and analyze bacteria, a need to develop a system to handle WGS data in relation to *fimH* typing has emerged.

**Materials and Methods:** The Centre for Genomic Epidemiology has been developing freely available stand-alone web-tools for handling WGS information for outbreak investigation, epidemiological surveillance and diagnostics based on BLAST analysis towards standardized and curated gene databases. A new stand-alone web-tool called FimTyper was developed. Here, a FASTA database containing all 477 *fimH* alleles is used to perform BLAST searches within WGS data. These WGS data can either originate directly from sequencing platforms such as Illumina, Ion Torrent or 454, or they can consist of *de novo* assembled draft (or complete) genomes. The best hit to a given *fimH* allele from the database is given as output including length of the hit and percent identity between the hit in the genome and in the database. To test the database, 245 Extended-Spectrum Beta-Lactamase (ESBL) producing *E. coli* draft genomes originating from blood infections and submitted to Statens Serum Institut in 2014 as part of the routine surveillance were analyzed using the FimTyper tool available at <https://cge.cbs.dtu.dk/services/FimTyper-1.0/>.

**Results:** The FimTyper tool successfully analyzed all 245 draft genomes. FimTyper was able to identify a *fimH* allele (more than 80% identity to any target in the database) in 232 of the 245 genomes giving a typability of 95%. In total, 33 different alleles were identified in the dataset including 2 new alleles with the most dominant being the *fimH30* allele (38%).

**Conclusion:** We have developed a fully functional and freely available web tool called FimTyper to identify *fimH* alleles in WGS data from *E. coli* genomes, thus enabling researchers and primary investigators to rapidly detect the *fimH* allele in their WGS datasets. The service is available at <https://cge.cbs.dtu.dk/services/FimTyper-1.0/>. It is the aim to include this in a newly developed web-based WGS pipeline combining all CGE tools for microbial typing including Serotyping, resistance gene typing and Multilocus sequence typing (MLST) already available at [www.genomicepidemiology.org](http://www.genomicepidemiology.org).

## S1 - Poster session I

### PO9 - Examining Gene-by-gene Diversity Across Thousands Of Whole Genomes To Determine Suitable Targets

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**Background:** With the rise of high throughput whole genome sequencing, thousands of genomes for the most epidemiologically important bacterial species have become available. While many of these genomes will have poor coverage or will not assemble well, in combination they are a useful resource for exploring the diversity of a species at a gene-by-gene level. This allows for the discovery of both highly conserved and highly diverse genes, a key step in choosing markers for epidemiological studies.

**Materials & Methods:** Whole genomes from public archives such as the Sequence Read Archive (SRA) were added to the PubMLST database, following assembly via a Velvet optimiser pipeline. This database runs the BIGSdb (Bacterial Isolate Genome Sequence database) software, which allows for genome annotation based on a set of reference sequences that defines genes, and an iterative scanning and definition process for new alleles of those genes. A set of simple Perl scripts then finds measures of diversity from the length, count of unique alleles (in both nucleotide and amino acid format), and ratio of variable sites for each defined locus, using the mean for the genome as the point of comparison

**Results:** This method was first tested on a dataset of 7681 *Mycobacterium tuberculosis* genomes, using the nearly four thousand defined genes in the H37Rv annotation as the reference sequences. This allowed us to identify genes both in the conserved and diverse ends of the spectrum, on all measures. Of special interest were genes in the ESAT-6 family which were genetically diverse (a high number of alleles found) but functionally conserved (a low ratio of amino acid to nucleotide unique sequences), suggesting perhaps two stages of evolution with different pressures. We are currently running the same methods on *Staphylococcus aureus*, *Neisseria gonorrhoeae* and *Salmonella enterica* data sets of similar sizes, and hope to have results ready for presentation in March.

**Conclusion:** High throughput whole genome sequencing allows for thousands of genomes to be gathered and analysed together. Together with annotated reference genomes, this means genes can be explored as "gene pools" that can be compared to one another in order to find markers according to need. For example, if a highly reliable species identification is necessary, a highly conserved gene can be chosen. If typing down to individual strains is more important, a often present but highly diverse gene might be preferred. This method allows to simple and rapid evaluation of gene targets across an entire genome using a large number of isolates so as to avoid sampling bias.

## PO10 - Core Genome Typing: A Useful Tool For Micro-epidemiological Investigations Involving *Staphylococcus aureus*?

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<sup>1</sup>Public Health England

**Background:** Recent advances in sequencing technologies provide unprecedented insights into the relatedness of bacteria such as *S. aureus*. SNP analyses based on whole genome sequence (WGS) data are commonly used to infer the phylogenetic relatedness, but results are dependent on the reference genome used, the bioinformatics pipeline and user expertise in phylogenetic interpretation. We have explored an alternative approach based on sequence analysis of core genome loci.

**Methods:** Conserved gene sequences (n=1334) in 54 complete genomes available from Genbank were identified by BLAST search. Combinations of allelic profiles were assigned to an identifier dubbed a core genome cluster (CGC) using an in-house hierarchical clustering approach based upon pairwise homology, and were populated in a reference database. Further validation was performed on 114 study genomes, newly sequenced, and representing i) a broad range of lineages (human and animal clades); ii) suspected clusters (2 HA-MRSA ST22 and one CA-MRSA ST59); iii) a representative of *S. argenteus* (ST2250). Genomes were assembled *de novo* and loci sequences were extracted by BLAST. New alleles were confirmed using mapping-based approach before being added to the database. Whole genome variant calling pipeline used BWA-mem and GATK2 using ST22MRSA H050960412 and SA40 Asian Pacific Clone as reference strains for respectively the ST22 and ST59 case cluster analysis.

**Result:** Fifty four allelic profiles were identified from the analysis of published genomes. The number of alleles per locus ranged from 2 to 30 (mean of 15.8). Newly sequenced genomes were grouped into 97 CGCs and corroborated with MLST lineages. Core genome typing grouped those with known epidemiological links and differentiated them from the background population belonging to the same MLST lineage. Genomes belonging to the same CGC differed by 0 to 5 WGS SNPs. CGCs of linked isolates differed from those representing background isolates by 34 or more alleles compared to 50 or more WGS SNPs by phylogenetic analysis.

**Conclusion:** We designed and applied a core genome typing approach and applied it to a broad range of *S. aureus* lineages including *S. argenteus*. The technique was able to assign a CGC to all reference and study genomes. Core genome clustering was able to resolve the molecular epidemiology with a discriminatory power comparable, to a certain extent, to SNP-based phylogenetic analyses without requiring the construction and interpretation of a phylogenetic tree. Core genome clustering is a promising approach for micro-epidemiological investigations involving MSSA/MRSA.

## S1 - Poster session I

### PO11 - Genetic Diversity Of *Salmonella typhimurium* And *Salmonella enteritidis* Isolated From Ducks And Chickens Using Random Amplified Polymorphic DNA (RAPD)

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**Background:** Genetic characterization of *Salmonella* species of food animal origin is important for determining their relationships. Such relationships are used to establish the similarities and differences between bacterial isolates. This study investigated the genetic diversity between *Salmonella* Typhimurium *Salmonella* Enteritidis strains isolated from chicken and ducks using Random Amplified Polymorphic Deoxyribonucleic Acid (RAPD)-PCR.

**Materials | Methods:** DNA of 36 pure *Salmonella* colonies of chicken and duck origins were extracted using Wizard® Genomic DNA Purification Kit following the manufacturers' instructions. The C-05 (10-mer) primer 5'-GATGACCGCC-3' was selected for RAPD-PCR after a panel of 8 random primers (designed and manufactured by 1st BASE, Singapore) had been screened. The PCR and Amplifications were performed according to Adzitey et al. (2013). Clustering was defined at a coefficient of 0.85. *Salmonella* serovars not belonging to any particular cluster were referred to as singletons (single isolates). Discriminatory index was calculated according to Hunter and Gaston (1988) based on the number of clusters and singletons identified.

**Results:** Analysis of the *Salmonella* strains by RAPD-PCR produced DNA bands that ranged from 242 to 3189 bp *Salmonella* Typhimurium and 252 to 2756 bp for *Salmonella* Enteritidis. Cluster analysis at a coefficient of 0.85 grouped the *Salmonella* Typhimurium into 4 clusters and 1 singleton at a discriminatory index of 0.85. *Salmonella* Enteritidis were grouped into 2 clusters and 2 singletons at a discriminatory index of 0.64. One *Salmonella* Typhimurium isolated from chicken carcass was not characterized as the RAPD-PCR employed failed to produce any DNA band from that isolate. It was evident from this study that some *Salmonella* strains circulating between chickens and ducks were similar while others were not. Characterizing *Salmonella* serovars from different sources is important to determine their genetic relatedness, and source of contamination and spread.

**Conclusion:** This study compared the genetic relatedness of chicken and duck *Salmonella* serovars using RAPD-PCR. RAPD-PCR analysis of the *Salmonella* serovars resulted in the characterization of the various *Salmonella* strains isolated from chickens and ducks and provided a means of determining the genetic relatedness among the serovars. Some *Salmonella* strains were genetically closely related but not others.

**PO12 - Application Of PCR-RFLP Provides As Discrimination As Total FLAA Sequence Analysis Among *C. jejuni* Isolates Of Clinical Origin**

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Keywords:*Campylobacter jejuni*,flaAsequence, typing

The aim of this study was to determinethegenetic relatedness amongtwenty clinical *Campylobacterjejuni*isolated from children with diarrhea in Iran, and to introduce the best discriminatory method based on *flaA* sequence divergence.A total of 400 stool specimens were obtained from children under five years of age during July 2012 to June 2013. Primers were designedbased on conserved sequences flanking the *flaA* gene which encompass and amplify entire *flaA*genefollowed bysequencing and analysis with MEGA version 6.0.6 software.Ninetyamino acid versus 560 nucleotidepolymorphic sequenceswere detectedwithin 1681bp of *flaA* sequence among which 43 (2.5% and 12 (0.7%) were singleton.New repeat boxes within *flaA* sequences were found in this study.

UPGMA dendrogram based on nucleotides of full length *flaA*gene,*flaA* SVR gene and in silicoflaA phylogenetic tree of *DdeI* RFLP profilesproduced very similarclustering with diversity indexof 0.86 for each of three methods. It can be concluded that *flaA* typing based on *DdeI* RFLP of the PCR products, can be applied as cheap, rapid and reliable method for epidemiological study of *C. jejuni* isolates of clinical origin in resource limiting regions or in large scale population

## S1 - Poster session I

### PO13 - A Unified Database For *Acinetobacter baumannii* Genomic And MLST Sequences Enables Comparison Of Pasteur And Oxford 7-gene MLST Nomenclatures

Raffaele Zarrilli<sup>1</sup>; Virginie Passet<sup>2</sup>; Eliana Pia Esposito<sup>1</sup>; Keith A Jolley<sup>3</sup>; Sylvain Brisse<sup>2</sup>

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**Background:** *Acinetobacter baumannii* is a Gram-negative bacteria responsible for a large number of hospital-acquired infections, which are usually caused by a limited number of multidrug-resistant epidemic clones assigned to distinct clonal lineages. The population structure of *A. baumannii* has been studied using two MLST nomenclatures, which complicates advances of the epidemiological and population biology knowledge of this important pathogen. The aim of the present study was to create a unified nomenclature of *A. baumannii* clones by taking advantage of publicly available genome sequences.

**Methods:** The *Acinetobacter baumannii* PubMLST website hosted at the University of Oxford and powered by the Bacterial Isolate Genome Sequence Database (BIGSdb) software was used to integrate the MLST databases based on the "Oxford" scheme and the "Pasteur" scheme into a single database. The *Acinetobacter* isolates database was populated with genome data from 765 strains of the *Acinetobacter calcoaceticus*-*baumannii* (Acb) complex and automated tagging and assignment of alleles for both MLST schemes was performed. Whole genome sequence (WGS) assemblies of 196 out of 765 *Acinetobacter* isolates were uploaded to the isolates database. *A. baumannii* ATCC 17978 and ACICU WGS were selected as reference genomes. The universal ribosomal MLST scheme based on 53 loci (Jolley et al., *Microbiology* 158, 1005, 2012) was used to assign rMLST genotypes to all genome sequences.

**Results:** ST assignments for a large number of genomes generated a convenient and expandable table of correspondence between the two MLST schemes. MLST-based species identification was performed using the "Pasteur" MLST scheme by neighbor-joining tree analysis of concatenated allele sequence alignments and comparison with reference STs. The species field was imported in to the PubMLST database for all STs with no ambiguous clustering (> 98% of them). The "Pasteur" scheme was less discriminant, but possibly more appropriate for population biology. On the other hand, the "Oxford" scheme was able to identify additional genotypes and to differentiate isolates belonging to international clone II into two distinct clonal lineages, but suffered from elevated variability due to high recombination at the gpi and gdhB loci. rMLST showed improved resolution over both 7-gene MLST schemes and provided a useful subtyping approach.

**Conclusion:** The availability of a single database hosting both widely-used classical MLST schemes will facilitate epidemiology and population biology research of *Acinetobacter*. The publicly accessible database can be expanded to allow rapid extraction of biologically and epidemiologically relevant information from *Acinetobacter* genome sequences. It may also host in the future, a genome-wide MLST scheme that will provide the improved resolution needed for small scale epidemiology and for precise strain classification into *A. baumannii* clonal groups.

## PO14 - PHYLOViZ Online: Web-based Phylogenetic Data Analysis And Visualization For Allelic Profiles And SNP Data

Bruno Ribeiro-Gonçalves<sup>1</sup>; Alexandre Francisco<sup>2</sup>; Cátia Vaz<sup>3</sup>; Mário Ramirez<sup>1</sup>; João André Carriço<sup>1</sup>

<sup>1</sup>*Instituto de Microbiologia, Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, <sup>2</sup>Instituto Superior Técnico, Universidade de Lisboa, <sup>3</sup>Instituto Superior de Engenharia de Lisboa <sup>4</sup>Instituto Politécnico de Lisboa*

**Background:** Next Generation Sequencing methods gave rise to a paradigm shift in microbial typing. The ability to sequence the genomes of hundreds of strains created the need for effective ways to represent the phylogenetic relationships between isolates that are scalable and robust. Single Nucleotide Polymorphism (SNP) analysis and core/whole genome MLST approaches create profiles with up to thousands of loci that can be used for outbreak investigation, surveillance and bacterial population or evolutionary studies. The analysis of these profiles can be done with traditional phylogenetic algorithms or Minimum Spanning Tree (MST) like approaches, the latter being in particularly suited to cope with the increasing number of isolates that are used in each study. The software PHYLOViZ allows using epidemiological data to annotate the resulting tree, but lacks options to share visualizations between users or provide public access to a given dataset.

With the aim of overcoming these limitations, we have developed PHYLOViZ Online, a user-friendly web application for profile based data analysis, visualization and sharing.

**Methods:**PHYLOViZ Online is an online application written in node.js in a modular perspective, separating visualization from data processing. Input files formats can be FASTA, tab separated text or Newick. The goeBURST algorithm is used to define the MST that is displayed in a force directed-layout on WebGL rendering engine, which allows displaying simultaneously thousands of isolates on a tree in a web browser.

Novel features of this online version are: 1) an interactive heat map visualization of distances of selected nodes on the tree, where information and heat map ordering can be selected according to available auxiliary data; and 2) N Locus Variant (NLV) graph analysis, which generates clusters by modifying the MST and linking all profiles up to a given distance, allowing a different visualization of how large datasets are related.

PHYLOViZ Online also offers user-specific areas where users can store private datasets for future access while providing a way to share datasets through the creation of public links, allowing other researchers to access – but not modify – private data. A RESTful API provides authenticated programmatic access to dataset creation, which can then be accessed using the web interface.

**Results:** PHYLOViZ Online allows the online analysis of allelic profile or Single Locus Polymorphism (SNP) through the use of a web-browser. With the integration of auxiliary data and by analyzing the distances in a heat map, users can determine which factors can be relevant in an epidemiological perspective. Through the user-specific area, researchers have now the ability to share data, facilitating the exchange of relevant epidemiological information. The RESTful API facilitates the integration with novel visual and data analysis applications. This allows the seamless integration of PHYLOViZ Online with existing databases and other online resources. All data can be exported in CSV or PDF format and images can be exported in PNG format.

**Conclusions:** The PHYLOViZ Online web-application provides a resource allowing MST analysis of profiles and SNP data without the need to install specific software. PHYLOViZ Online can be found at <http://node.phyloviz.net> and the source code is freely available at <https://github.com/bfrgoncalves/Online-PhyloViZ>.

# S1 - Poster session I

## PO15 - Comparative Analysis Sequencing-based Typing Methods For Epidemiological Study Of *C. trachomatis*

Zhanna Amirkelkova<sup>1</sup>; Dmitriy Babenko<sup>1</sup>; Anar Turmuhambetova<sup>1</sup>

<sup>1</sup>Karaganda State Medical University

**Background:** Chlamydia trachomatis is a leading cause of infectious blindness and sexually transmitted disease worldwide (Gerbaise et al. 1998) causing in a variety of body sites including the eye and the urogenital tract and leading to life-threatening conditions (Peipert JF, 2003).

Several genetic approach for typing C.trachomatis have been developed including MLST, MLVA, PFGE, PCR-based omp-gene typing and others which have advanced from conventional typing using antibodies (Pedersen et al., 2009).

The aim of present study was the comparison of sequence-based typing methods for C.trachomatis.

**Materials | Methods:** The collection of 127 C.trachomatis genomes have been downloaded from Genbank (<http://www.ncbi.nlm.nih.gov/genome>) and have been analyzed with SeqSphere v.3 (Ridom Software) to extract alleli for MLST (for Chlamydiales and Uppsala scheme), wgMLST and MOMP gene complex. Diversity and concordance between typing methods were calculated using Simpson index and adjusted Wallace coefficients (<http://www.comparingpartitions.info>). Comparison of dendrogram have been performed with dendextend packages (R statistics).

**Results:** Using 127 whole genomes of C. trachomatis two MLST types (7 alleli for MLST Chlamydiales and 5 alleli for Uppsala scheme), wgMLST (933 genes) and alleli for MOMP gene complex (ompH, ompB and omcB genes) have been determined. Characteristics such as resolution power and concordance of these typing are presented in table1.

Table 1. Resolution ability and concordance between sequence-based typing methods for C.trachomatis.

Typing method	# Different types	Simpson's index		Adj.Rand coefficient	Wallace coefficient					
		Discriminatory index [95% CI]	MLST (Chlamydiales)		MLST (Uppsala scheme)	wgMLST	MOMP complex	MLST (Chlamydiales)	MLST (Uppsala scheme)	wgMLST
MOMP complex	23	0.888 [0.861 - 0.915]	0.603	0.344	0.01	1	0.653	0.257	0.006	
MLST (Chlamydiales)	33	0.886 [0.852 - 0.921]	1	0.363	0.01	0.642	1	0.268	0.005	
MLST (Uppsala scheme)	47	0.961 [0.948 - 0.974]	0.344	1	0.03	0.74	0.785	1	0.016	
wgMLST	123	0.999 [0.998 - 1.0]	0.01	0.03	1	1	1	1	1	

Clustering rates for MOMP complex, MLST (chlamydiales), MLST (Uppsala) and wgMLST were 0.795, 0.74, 0.63 and 0.031 respectively.

Clonal complex structure based on burst algorithm showed that there was 1 complex with 119 samples for MLST Uppsala, 1 CC with 127 Samples for MOMP gene set, 3CC with 115 Samples for MLST Chlamydiales and 12 CC with 106 Samples for wgMLST.

Dendrogram comparing and entanglement parameter is shown in figure 1.

**Conclusion:** wgMLST as perspective typing approach based on whole genome sequence showed the ultimate level of discriminatory power – 99,9%. Furthermore, wgMLST is able to predict well the other sequence type (Wallace coef was 100%).

Generally, there was a poor/moderate concordance between typing methods using several genes/alleli – no more 75%.

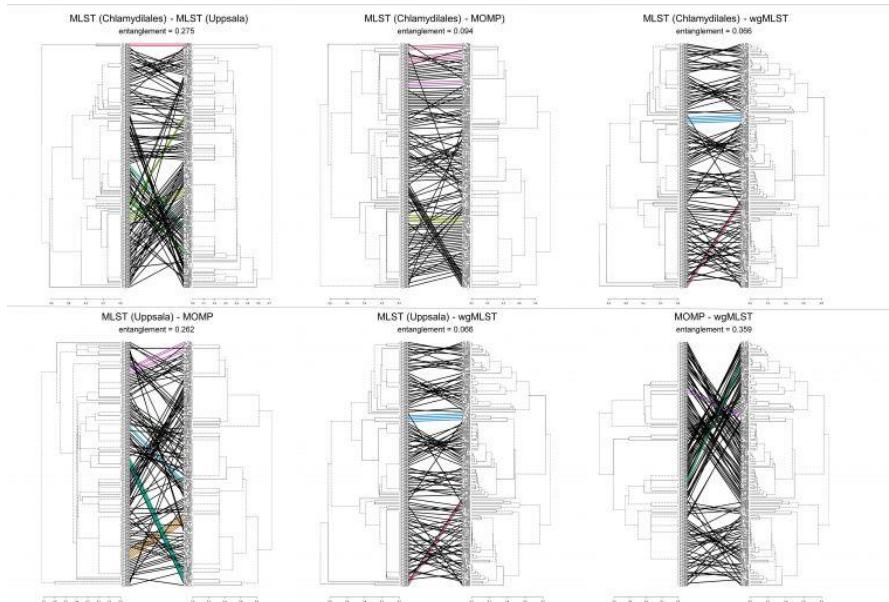


Figure 1. Comparison of dendograms created on different typing data

## S1 - Poster session I

### PO16 - Appraisal Of Complementary Strategies For Refinement Of A Core Genome Multi-locus Sequence Typing Scheme For Typing Of *Acinetobacter baumannii*

Mickael Silva<sup>1</sup>; João André Carriço<sup>1</sup>; Nir Gilad<sup>2</sup>; Michal Gordon<sup>2</sup>; Mario Ramirez<sup>1</sup>; Vered Chalifa-Caspi<sup>2</sup>; Jacob Moran-Gilad<sup>2</sup>

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**Objectives:** *Acinetobacter baumannii* is a therapeutic and infection control challenge worldwide. Microbial whole genome sequencing (WGS) currently allows bacterial typing based on a gene-by-gene approach using a large set of core genome genes as compared to only seven housekeeping genes in traditional MLST, thus achieving a more refined population structure analysis and increased discriminatory power. Here we propose a novel core genome MLST (cgMLST) typing scheme for *A. baumannii* complex.

**Methods:** For the construction of the wgMLST scheme we downloaded from NCBI, 16 closed *A.baumannii* genomes. Processing via an in-house bioinformatics pipeline identified a set of single copy genes, present in at least one strain. To validate the scheme, a total of 1042 genomes were analyzed. This included the majority of strains annotated in ENA as *A.baumannii* and sequenced by Illumina technologies (987 reads sets). Draft assemblies were generated using SPAdes . An allele calling algorithm was developed and applied to define standardized alleles for the 1042 genomes. Considering the issue of missing data and possible assembly problems, a method was developed to identify the core genome by removing strains with defined levels of missing data. The algorithm considers an exclusion threshold (*et*) at which a genome with loci with missing data over the *et* will be excluded by analyzing loci present in over 99% of the whole set of genomes. This algorithm runs iteratively for each *et* in order to maximize the number of cgMLST loci. Higher *ets* will provide a higher typeability, with more included genomes and less cgMLST loci, while lower *ets* will provide a higher discriminatory power, with less genomes included in the analysis but more cgMLST loci.

**Results:** The initial wgMLST gene set derived from reference genomes consisted of 5,266 loci. Running the algorithm to identify the core genome we were able to generate two schemes. A restrictive core (RS) genome scheme at a *et* of 60 and an inclusive (IS) at *et* of 190. RS classified 974 strains with 718 unique profiles and using 942 loci, whereas IS classified 997 strains into 593 unique profiles using 551 loci.

**Conclusion:** Both proposed cgMLST schemes offered a high discrimination between *A.baumannii* strains and allowed the differentiation of strains belonging to other *A.baumannii* complex species, wrongly submitted to public databases as *A.baumannii*. However each scheme demonstrated its own advantages and caveats that have to be considered prior to their usage. The cgMLST approaches can allow standard common allelic nomenclature and easy implementation in automated analysis pipelines, and is thus expected to enable the adoption of draft genome sequencing as a mainstream procedure in clinical microbiology.

## PO17 - MLVA For The Genotyping Of *Acinetobacter baumannii*: Towards A Consensus For Interpretation Of Data And The Development Of International Databases

Christine Pourcel<sup>1</sup>; Yolande Hauck<sup>1</sup>; Charles SOLER<sup>2</sup>; Gilles Vergnaud<sup>1</sup>

<sup>1</sup>University Paris-Saclay, <sup>2</sup>Percy military hospital

**Background:** Infections by *A. calcoaceticus*-*A. baumannii* (ACB) complex isolates represent a serious threat for wounded and burn patients. Three international multidrug-resistant (MDR) clones (EU clone I-III) are responsible for a large proportion of nosocomial infections with *A. baumannii* but other emerging strains with high epidemic potential also occur. Different genotyping techniques that not always allow differentiation of strains inside clones have been described. Multiple locus VNTR analysis (MLVA) based upon variable number of tandem repeats (VNTR) polymorphism is among the most promising methods owing to its low cost, discriminatory power, and the possibility to create shared databases over the internet. Since the initial report of VNTRs in *Acinetobacter baumannii* in 2011, and the proposition of an MLVA assay, hundreds of additional strains have been genotyped worldwide, and the corresponding data has been made public. In parallel, additional strains have been fully sequenced. We here take advantage of this context to update the available internet MLVA database and MLVA assay.

**Materials and methods:** The <http://mlva.u-psud.fr> resource and associated tools was used to create a MLVA cooperative database, from published and unpublished data set. The experimental genotyping data set was complemented by *in silico* deduced MLVA genotypes using available complete genome sequences (downloaded from sequence databases accessed via the NCBI resources). PubMed was searched to identify MLVA publications.

**Results:** Five studies using MLVA to genotype *Acinetobacter baumanii* published since the initial 2011-2012 MLVA reports were identified. Altogether, more than 800 strains were genotyped, allowing the critical assessment of MLVA. The full genome from more than 30 strains was *in silico* analysed to deduce their MLVA genotype. The data was used, together with data from the current MLVA database based upon the 2012 published data to create a new cooperative database comprising seven elementary databases for a total of more than 1000 strains. Different combinations of markers have been proposed by different groups, they are all represented in the new database.

**Conclusion:** MLVA assays, such as MLVA8<sub>Orsay</sub> which employs 4 VNTRs with low level of variability (the L markers) and 4 VNTRs with a high level of polymorphism (the S marker) are fast and cheap and can be applied to different epidemiological investigations made necessary by the increasing occurrence of *A. baumannii* infections in the hospital. The new version of the MLVA database which will be presented should facilitate such investigations.

## S1 - Poster session I

### PO18 - Straintracer-db: A Flexible Analysis And Persistent Storage System For Sequence And Epidemiological Data

Jonas Evensen<sup>1</sup>; Sveinung Gundersen<sup>2</sup>; Anja B. Kristoffersen<sup>3</sup>; Karin Lagesen<sup>4</sup>

<sup>1</sup>*Department of Informatics, University of Oslo;* <sup>2</sup>*ELIXIR.NO / Department of Informatics, University of Oslo;* <sup>3</sup>*Norwegian Veterinary Institute;* <sup>4</sup>*Norwegian Veterinary Institute/Department of Informatics, University of Oslo*

**Background:** Traditionally, the transmission pathways of infectious diseases have been tracked using epidemiological data, such as time, location and contact information. Today, new technologies allow for direct analysis of the genome sequence of an infectious agent. Based on differences detected between the genomes of different isolates, the transmission pathways of the agent can potentially be elucidated. In addition, inferences of how an agent moves in time and space can be greatly strengthened by combining epidemiological data with sequence information. Systems for managing and analysing such data need to satisfy a range of requirements. For instance, it may not be desirable or permissible to share the data outside of the host institution; hence the system should be locally installable and usable. Also, bioinformatics analyses are often very resource intensive, thus such a system should be able to run on compute clusters. In addition, it should allow for storing already processed results to enable easy comparison with new results. The system should also allow its users to create new analyses to enable them to explore the data in novel ways.

**Methods and Materials:** In this project we combine Galaxy a web-based bioinformatics analysis platform, with a postgres database for persistent storage of epidemiological data, sequence information, analysis metadata and results. Galaxy gives users access to many different tools and allows users to install new tools themselves. Tools can be chained into reusable workflows, thus making results from different runs comparable. Additionally, Galaxy can be installed and used on a local PC as well as on large compute clusters.

**Results:** StrainTracer-DB is a flexible analysis and persistent storage system for sequence and epidemiological data. StrainTracer-DB is a collection of tools inside Galaxy that allows the combination of many useful features of Galaxy with a database designed to store data produced throughout all steps necessary for an analysis. StrainTracer-DB provides tools for uploading sequence data for specific biological agents. Within the tool, the metadata that should be associated with the sequence information is specified. A Galaxy workflow is also included in the tool, and once that is run, the results including run information is stored in the database. The database storing the results is designed to be very flexible. Thus the workflows processing the data can provide SNP data, "shared gene" data, allele data and more as their results. StrainTracer-DB includes tools that allow for accessing previous results from the database, so that results based on new data can be compared to those already processed. All tools and data sets are under access control, so that only authorised users have access and modification rights to specific tools and workflows, and so that different groups of users can share the same system.

**Conclusion:** StrainTracer-DB offers an easy-to-use system for managing and analysing sequence and epidemiological data. We envision that the system will make it easier and faster to trace the transmission pathways and origins of infectious diseases.

## PO19 - MicrobesNG: A Microbial Sequencing Facility Designed To Integrate Myriad Of Sequenced Whole Genomes With Their Samples

Pablo Fuentes Utrilla<sup>1</sup>; Emily Richardson<sup>1</sup>; Joshua Quick<sup>1</sup>; Jennie Law<sup>1</sup>; Andrew Smith<sup>1</sup>; Ian Henderson<sup>1</sup>; Roy Chaudhuri<sup>2</sup>; Nick Loman<sup>1</sup>; Tim Wells<sup>1</sup>

<sup>1</sup>University of Birmingham, University of Sheffield

Improvements in sequencing technology have driven down the cost of sequencing bacterial genomes. However, it remains time consuming and expensive to establish efficient high-throughput sequencing in non-specialist laboratories. MicrobesNG is a BBSRC-funded facility to provide sequencing of microbial genomes. The facility aims to i) advance sequencing protocols to improve data quality ii) reduce cost and decrease processing time, iii) set up a repository for the storage and delivery of bacterial strains, iv) develop pipelines for processing sequencing data and providing basic bioinformatics v) develop tools with user friendly interfaces that allow scientists to not only analyse their own data but to see it in the context of all relevant publicly available strains.

MicrobesNG accepts either genomic DNA or individual bacterial strains, deposited on bead tubes. We have established laboratory automation protocols that are capable of generating 480 samples per week using the Hamilton STAR robotics platform running a modified Nextera XT protocol, with libraries sequenced on 250bases paired-end on Illumina HiSeq/MiSeq instruments. Efforts have been expended in ensuring the longest possible insert sizes are produced with this protocol in order to give the most contiguous assemblies. These protocols have also been optimised for speed and are available for others to reuse. In order to encourage sharing and reuse of data, to access the service at its lowest subsidised price we mandate a policy of strain and data sharing, with all data being uploaded to the European Nucleotide Archive within one year of sending strains for sequencing. We have established bioinformatics protocols for quality control of data.

MicrobesNG currently utilises three main pipelines, QC, assembly and variant calling. The QC pipeline uses Kraken to identify the closest reference genome, to which the reads are then aligned to. The Kraken output is also used to assess contamination. The assembly pipeline uses SPAdes to assemble the genomes and PROKKA to annotate. The variant pipeline uses BWA-MEM to align to the reference, samtools to process the alignment, Varscan to call variants and SnpEff to add annotation context to the results. Two types of variant calling are performed, sensitive (variant present in >90% of depth) and specific (variant present in >10% depth). In order to visualise variants, a custom interface has been built using the Dalliance browser (figure 1).

So far we have sequenced 4000 isolates using this system. Currently we are able to sequence a microbial genome for a consumable cost of < £45 (\$65). Through further anticipation we anticipate that this cost can be halved by the end of 2016.

## S1 - Poster session I



## PO20 - A Scripting Language For Standardized Evaluation Of Quality Metrics In Galaxy And Command-line Driven Workflows

Damion M. Dooley<sup>1</sup>; Aaron J. Petkau<sup>2</sup>; Franklin Bristow<sup>2</sup>; Gary Van Domselaar<sup>2</sup>; William W.L. Hsiao<sup>3</sup>

<sup>1</sup>Department of Pathology, University of British Columbia, <sup>2</sup>National Microbiology Laboratory, Public Health Agency of Canada, <sup>3</sup>Department of Pathology, University of British Columbia & BC Public Health Microbiology and Reference Laboratory

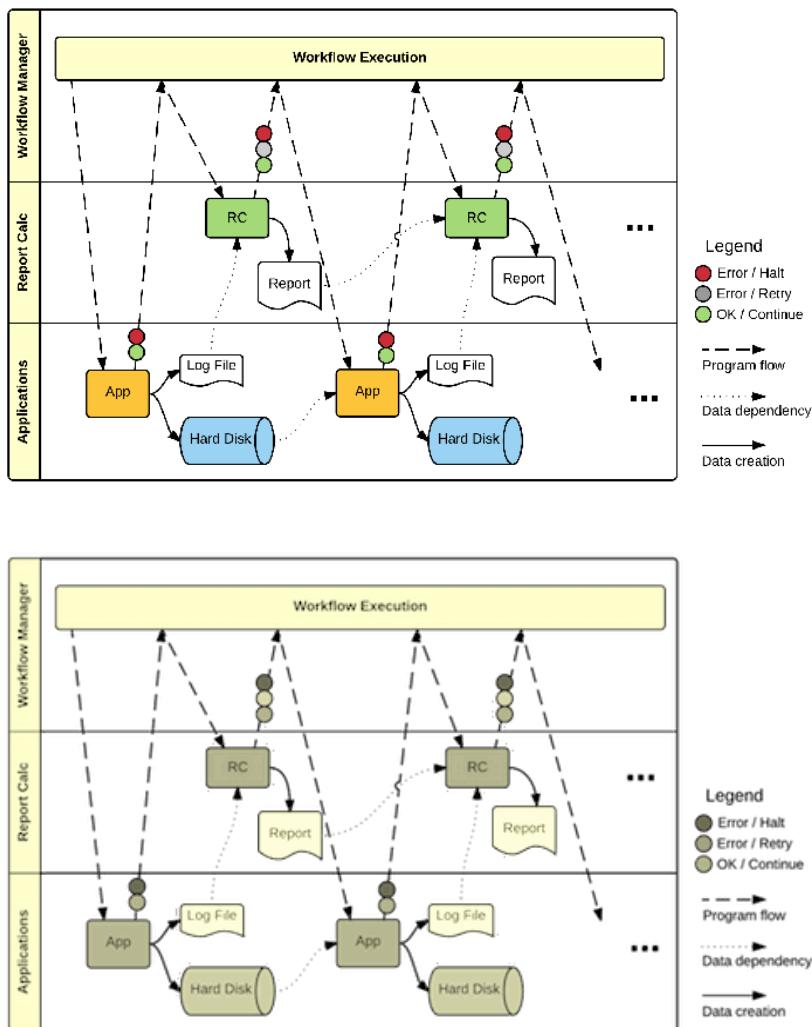
Keywords: Quality control - Metrics - Pipeline - Workflow - Whole genome sequencing

**Background:** Quality control (QC) metrics in a genomics assembly and annotation pipeline are essential for routine lab sample processing, and for comparing the performance of pipeline optimizations or alternatives. Ideally sequence run read metrics and other assembly or annotation stats can be collected and analyzed in real-time to enable low QC scores to stop otherwise unnecessary processing and manual analysis. QC reporting can also enable retrospective tracing of problems that arise. Unfortunately QC metrics are a complicated affair influenced by the subject organism, sample quality, sequencing technology and preparation, and the mix of software components that are brought together in a workflow. The challenge is to transform QC reporting from a manual process of perusing a workflow's disparate and often opaque application log files, into an intermediate and automated system of reporting and decision making that can control workflow execution, and that can be adjusted by researchers and system administrators who are not expert programmers.

**Results:** We have developed a general purpose text-mining and reporting application called Report Calc for Quality Control (RCQC) that works directly within command-line scripts, or as a tool in Galaxy (an interactive bioinformatics platform and workflow engine). Our test case is an assembly and annotation pipeline used in the IRIDA.ca (Interactive Rapid Infectious Disease Analysis) project, one that combines command-line Flash, Spades, and Prokka tools. For a given workflow job, RCQC extracts QC variables from various pipeline log files, and it implements a variety of rules that can appropriately trigger a warning or failure status in the workflow at large.

**Conclusions:** The Report Calc for Quality Control system provides a transparent and adjustable ruleset that reduces the skill needed to make concise reports and process adjustments based on QC metrics. We were able to standardize many aspects of QC reporting and job handling while remaining within the limits of Galaxy's workflow engine. A few standard assembly pipeline QC metrics are introduced which provide a blueprint for the way QC components could be shared amongst NGS sequencing pipelines. Further information, including source code, is available at <https://github.com/Public-Health-Bioinformatics/rcqc>.

# S1 - Poster session I



**PO21 - GTFinder Workflow To Detect Exclusively Conserved Region Across A Large Genome Dataset: *Salmonella* spp. Study**

Arnaud Felten<sup>1</sup>; Laurent Guillier<sup>1</sup>; Fabrice Touzain<sup>2</sup>; Michel-Yves Mistou<sup>1</sup>; Renaud Lailler<sup>1</sup>; Nicolas Radomski<sup>1</sup>; Sabrina Cadel Six<sup>1</sup>

<sup>1</sup>Université PARIS-EST, Anses, Laboratory for food safety, <sup>2</sup>Anses, Genomic platform

Keywords: exclusively conserved regions, *de novo* assembly, k-mer clustering, *Salmonella*, bacterial genomics.

**Background:** The modern molecular microbiologists are today in front of massive databases of DNA sequences produced by high-throughput sequencing, and need to develop their own *in silico* tools according to their specific objectives. In a context of food safety, the *Salmonella* serotyping method based on sero-agglutination is cost prohibitive, and leads to propose less expensive molecular targets to identify *Salmonella* serotypes by PCR-based methods. Consequently, we propose in the present manuscript a workflow called 'Genome Target Finder' (GTFinder) which has been designed to detect exclusively conserved k-mers across a large genome dataset of *Salmonella* serovars.

**Results:** Based on stringent and tolerant clustering algorithms implemented in the CD-HIT programs, the GTFinder workflow includes Python scripts driving k-mers through redundancy, specificity, sensitivity, and combination filtrations, successively. Among 526 genomes from NCBI, as well as 211 newly sequenced and assembled genomes, 90 serovars of *Salmonella* spp. were taken into account, including 48 and 31 serovars represented by only one genome and more than four genomes, respectively. Concerning the 31 serovars represented by more than four genomes, unique k-mers were detected as exclusively conserved into 18 serovars, and combined k-mers were selected to identify the 13 remaining serovars.

**Conclusion:** The unique and combined k-mers identified by GTFinder workflow as specific and sensitive into 31 serovars, will be used to design PCR primers in order to check their *in vitro* specificity and sensitivity by the Access Array system from Fluidigm. The GTFinder workflow could also be applied to other bacterial genus, species, and subspecies in order to discover regions distinguishing between genotypes or phenotypes.

## S1 - Poster session I

### PO22 - MOST: A Modified MLST Typing Tool Based On Short Read Sequencing

Rediat Tewolde<sup>1</sup>; Tim Dallman<sup>1</sup>; Ulf Schaefer<sup>1</sup>; Carmen Sheppard<sup>1</sup>; Philip Ashton<sup>1</sup>; Bruno Pichon<sup>1</sup>; Craig Swift<sup>1</sup>; Jonathan Green<sup>1</sup>; Anthony Underwood<sup>1</sup>

<sup>1</sup>Public Health England

**Background:** Multilocus sequence typing (MLST) is one of the most commonly used typing methods used for surveillance and identification of outbreaks. The conventional MLST technique involves Polymerase Chain Reaction (PCR) amplification of 5-10 housekeeping genes followed by Sanger DNA sequencing. Public Health England (PHE) is in the process of replacing the conventional MLST methodology used by the reference services with Whole Genome Sequencing (WGS). In order for PHE to replace the conventional method, validation tests should demonstrate concordance between the WGS outputs and the conventional assay in conjunction with quantitative quality metrics for ongoing assurance purposes.

**Materials | Methods:** This presentation compares the reliability and quality of MLST results derived from WGS data using de novo assembly/blast-based and mapping based approaches to MLST results derived from the conventional Sanger sequencing method. Sensitivity and specificity of the two WGS based methods were further investigated with a set of mixed and low coverage genomic data. The initial set of isolates used for the validation included 120 *Campylobacter sp.* (*Coli* and *jejuni*) , 121 *Staphylococcus aureus* and 99 *Streptococcus pneumoniae* isolates. These were all characterized by conventional and WGS based MLST methods . A further 26 mixed strain samples and 29 low coverage samples were used to investigate sensitivity and specificity of the WGS based MLST methods.

**Results:** The assembly and mapping approaches obtained a full MLST profile more often than the conventional method. The concordance between the conventional and both WGS methods was 100%. For both mixed strain and low coverage samples the mapping based methodology obtained a full MLST profile more often than the assembly approach.

**Conclusion:** In conclusion, deriving MLST from WGS is sensitive as well as being less labour intensive than the conventional method. In addition, the mapping based approach assigns quality metrics that flag mixed and low sequence coverage genomic data from bacterial populations, something that is difficult to determine using both conventional methodologies and WGS assembly based approaches.

## PO23 - The *Brucella* MLVA Database

Gilles Vergnaud<sup>1</sup>; Yolande Hauck<sup>1</sup>; Christine Pourcel<sup>1</sup>; Isabelle Jacques<sup>2</sup>; Axel Cloeckaert<sup>2</sup>; Michel Zygmun<sup>2</sup>

<sup>1</sup>University Paris-Saclay, INRA

Polymorphic tandem repeats have proved to be sufficiently numerous in the *Brucella* genome to allow the development of Multiple Loci VNTR (Variable Number of Tandem Repeats) Analysis (MLVA) assays. MLVA assays are interesting because they rely on widely available technologies, i.e. PCR amplification and DNA fragment length measurement. They do not require the manipulation of the live agent, and can be run at low cost in any laboratory. DNA fragment lengths are subsequently converted into a number of repeat units and the resulting genotypes can be easily stored, compared, shared, and made accessible in databases via the internet.

The most widely used *Brucella* MLVA assay is composed of 16 loci, eight with large repeat units which can be analysed with many different equipment including regular agarose gels, and eight with shorter repeat units. The 16 loci can be typed in four multiplex PCRs if the appropriate equipment is available.

An MLVA database can be accessed at <http://mlva.u-psud.fr>. It contains genotypes from 2500 strains, organized in nine contributing databases contributed by different laboratories.

We will present a new version of the database, with extensive improvements of the underlying software and inclusion of new data from approximately 2000 strains. Representatives from the newly described *Brucella* species are included. The new version contains genotypes from more than 4000 strains. This central repository also enables the naming of new genotypes in a coordinated manner.



## GENOMICS OF ADAPTATION TO THE HOST AND MAN-MADE ENVIRONMENTS

**Thursday, 10 March 2016, 12:00-14:30**

### PO24 - Comparative Genomic Analysis Of Two NetF-positive *Clostridium perfringens* Isolates Associated With Foal And Canine Necrotizing Enteritis

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### PO25 - Population Profiling Of *Enterococcus faecium*: A Comparative Study Of Diversity And In-vivo Gene Transfer Events Within The Human Bowel

S. A. Ballard<sup>1</sup>; S. Xie<sup>1</sup>; A. Mahony<sup>1</sup>; A. Buultjens<sup>2</sup>; E. A. Grabsch<sup>3</sup>; M. L. Grayson<sup>1</sup>; T. Seemann<sup>4</sup>; B. P. Howden<sup>5</sup>; T. Stinear<sup>6</sup>; P. D. R. Johnson<sup>1</sup>

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### PO26 - Elucidation Of Transmission Events Of Vancomycin Resistant *Enterococcus faecium* Isolates In Intensive Care Units Of A German Tertiary-care Hospital

Jennifer Bender<sup>1</sup>; Carola Fleige<sup>1</sup>; Torsten Semmler<sup>1</sup>; Sandra Schneider<sup>2</sup>; Petra Gastmeier<sup>2</sup>; Guido Werner<sup>1</sup>

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### PO28 - Lack Of Genetic Isolation By Distance And Persistence Of Monomorphic Genotypes In Generalist *Campylobacter jejuni* Strains

Mirko Rossi<sup>1</sup>; Ji Zhang<sup>1</sup>; Ann-Katrin Llarena<sup>1</sup>; Minna Vehkala<sup>1</sup>; Niko Välimäki<sup>1</sup>; Jukka Corander<sup>1</sup>

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### PO29 - Virulence Differences Among Type AI *Francisella tularensis* Strains Linked To Genetic Mutations Found By Whole Genome Sequencing

Caroline Öhrman<sup>1</sup>; Jeannine Petersen<sup>2</sup>; Luke Kingry<sup>2</sup>; Kiersten Kugeler<sup>2</sup>; Claudia Molins<sup>2</sup>; Laurel Respicio/Kingry<sup>2</sup>; Andreas Sjödin<sup>1</sup>; Mats Forsman<sup>1</sup>; Dawn Birdsell<sup>3</sup>; Paul Keim<sup>3</sup>; Dave Wagner<sup>3</sup>; Anders Johansson<sup>4</sup>

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## S2 - Poster session I

### PO31 - Using Whole Genome Sequencing To Understand Host-nontuberculous Mycobacteria Interaction

Sonia Faria<sup>1</sup>; Vitor Borges<sup>2</sup>; Catarina Carneiro<sup>2</sup>; Luis Vieira<sup>2</sup>; Joao Paulo Gomes<sup>2</sup>; Luisa Jordao<sup>2</sup>

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### PO32 - Adaptive Evolution Of *Burkholderia cenocepacia* Within The Lungs Of A Chronically Infected Cystic Fibrosis Patient

Sandra C. dos Santos<sup>1</sup>; Carla P. Coutinho<sup>1</sup>; Marcus Dillon<sup>2</sup>; Ana S. Moreira<sup>1</sup>; Rita F. Maldonado<sup>1</sup>; Vaughn S. Cooper<sup>2</sup>; Isabel Sá-Correia<sup>1</sup>

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### PO33 - Comparative Genomics Of An Endemic Strain Of Multidrug-resistant *Pseudomonas aeruginosa* In A Pediatric Cystic Fibrosis Population

Ruth Ann Luna<sup>1</sup>; Jennifer K. Spinler<sup>1</sup>; Anthony M. Haag<sup>1</sup>; Miriam Balderas<sup>1</sup>; Prapaporn Boonma<sup>1</sup>; Abria R. Magee<sup>1</sup>; Peter W. Hiatt<sup>1</sup>; James Versalovic<sup>1</sup>

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### PO34 - Genomic Epidemiology Of The Gaza Community-acquired Methicillin-resistant *Staphylococcus aureus* Clone: A Unique ST22 Clone

Qizhi Chang<sup>1</sup>; Asaf Biber<sup>2</sup>; Izzeldin Abualais<sup>3</sup>; Hanaa Jaber<sup>2</sup>; Galia Rahav<sup>2</sup>; William P Hanage<sup>1</sup>; Gili Regev-Yochay<sup>2</sup>

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### PO35 - Next Generation Sequencing: The Key To Understood *Klebsiella pneumoniae* Biofilms?

Luisa Jordão<sup>1</sup>; Vitor Borges<sup>1</sup>; Luis Vieira<sup>1</sup>; João Paulo Gomes<sup>1</sup>; Aida Duarte<sup>2</sup>

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### PO36 - Whole Genome Sequencing Of Clonal Complex 8 MRSA Isolates Shows Several Clusters Of USA300-like Isolates

Mette Damkjær Bartels<sup>1</sup>; Peder Worning<sup>1</sup>; Jesper Boye Nielsen<sup>1</sup>; Henrik Westh<sup>1</sup>

<sup>1</sup>Hvidovre University Hospital

### PO37 - *Mycobacterium tilburgii*: The First Sequenced Genome

Jan Zoll<sup>1</sup>; Jakko van Ingen, Saskia Kuipers, and Willem Melchers

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**PO24 - Comparative Genomic Analysis Of Two NetF-positive *Clostridium perfringens* Isolates Associated With Foal And Canine Necrotizing Enteritis**

Iman Mehdizadeh Gohari<sup>1</sup>; Andrew M. Kropinski<sup>1</sup>; Scott J. Weese<sup>1</sup>; Valeria R. Parreira<sup>1</sup>; Ashley E. Whitehead<sup>2</sup>; Patrick Boerlin<sup>1</sup>; John F. Prescott<sup>1</sup>

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**Background:** *Clostridium perfringens* type A-associated diarrheal and enteric disease in foals and dogs is not well characterized. However, recently, our group described three novel putative toxin genes encoding proteins related to the pore-forming Leukocidin/Hemolysin Superfamily; these were designated *netE*, *netF*, and *netG*. *NetF* has been implicated as the primary virulence factor of foal necrotizing enteritis and canine haemorrhagic gastroenteritis. This study presents the complete genome sequence of two *netF*-positive strains, JFP55 and JFP838, which were recovered from cases of foal necrotizing enteritis and canine haemorrhagic gastroenteritis, respectively. Work is continuing on sequencing 30 additional *netF*-positive strains from dogs and horses that will be used for epidemiological analysis.

**Materials and Methods:** The genomic DNA of the samples was extracted using a modified version of the Qiagen bacterial DNA extraction protocol. Single Molecule, Real-Time (SMRT) technology-PacBio and Illumina Hiseq2000 PE100 were used for both samples. *De novo* assembly was done using DNASTAR's SeqMan NGen12 software. Subsequently, the complete chromosome sequences were annotated by the prokaryotic genome annotation pipeline. In addition, complete plasmid sequences were annotated by MyRAST software, the next generation of Rapid Annotation using Subsystem Technology. Sequencing of an additional 30 strains is proceeding with Illumina Hiseq2000 PE100.

**Results:** The JFP55 and JFP838 genomes include a single 3.34 Mb and 3.53 Mb chromosome, respectively, and both genomes include five circular plasmids. The *C. perfringens* JFP55 chromosome carries 2,825 protein-coding genes whereas the chromosome of JFP838 contains 3,014 protein-encoding genes. Comparison of these two chromosomes with three available reference *C. perfringens* chromosome sequences identified 48 (~ 247 kb) and 81 (~ 430 kb) regions unique to JFP55 and JFP838, respectively. Sixteen of these unique chromosomal regions (~69 kb) were shared between the two isolates. Five of these shared regions formed a mosaic of plasmid-integrated segments, suggesting that these elements were acquired early in a clonal lineage of *netF*-positive *C. perfringens* strains. These shared chromosomal regions could possibly represent epidemiology markers for this clonal lineage. In addition, plasmid annotation revealed that both *netF*-positive *C. perfringens* strains, JFP55 and JFP838, harbour three plasmids in common, including a NetF/NetE toxins-encoding plasmid, a CPE/CPB2 toxins-encoding plasmid and a putative bacteriocin-encoding plasmid. The common plasmid profiles may also represent valuable epidemiological markers for *netF*-positive *C. perfringens* strains. Sequencing of an additional 30 canine and equine *netF*-positive using JFP55 and JFP838 as scaffolds will be used for epidemiologic purposes to examine whether there are canine and equine specific strains, the presence of conserved plasmids, and the evolution of this lineage(s) of *C. perfringens*.

**Conclusion:** These results provide potential useful molecular markers for *netF*-positive *C. perfringens* by identifying unique chromosomal regions shared by *netF*-positive strains as well as common plasmids profiles.

## S2 - Poster session I

### PO25 - Population Profiling Of *Enterococcus faecium*: A Comparative Study Of Diversity And In-vivo Gene Transfer Events Within The Human Bowel

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**Background:** Using large scale whole genome sequencing we have shown previously that acquisition of vancomycin resistant *E. faecium* (VREfm) is frequently driven by *de-novo* acquisition of the Tn 1549 element and that these events may obscure the true rate of nosocomial transmission of VREfm. Moreover, the population of *E. faecium*, *a priori*, to these events appears to be of significance and likely drives the evolution of clinically important vancomycin resistant lineages. This longitudinal study uses genomics to explore *E. faecium* diversity within and between patients, to provide insights into the frequency and diversity of vancomycin susceptible populations within the fecal microbiota in attempt to identify factors driving the development of new VREfm strains and their expansion.

**Materials & Methods:** A large prospective cohort of patients at risk of acquisition of VRE was recruited from Austin Health, Heidelberg, Australia. Faecal samples were collected on admission and up to seven occasions looking for patient conversion to VREfm. Faeces were screened for VREfm and without selection for vancomycin sensitive *E. faecium* (VSEfm) both directly and by enrichment broth to improve the isolation of minority populations. Subsequently all isolates were subjected to whole genome sequencing, their resistance phenotype was confirmed, an *in silico* determination of MLST made and the core genome phylogeny of isolates examined.

**Results:** Of the 186 patients recruited, 25 transitioned to VRE carriage; 1/25 demonstrated probable *de novo* generation of VREfm via transfer of *vanB* between enterococci. A total of 1188 *E. faecium* isolates were obtained along with 2 *vanB*-Tn1549 anaerobes. VREfm isolates represented 7 different MLSTs. In contrast there was exceptional diversity in MLST amongst VSEfm with 92 different STs and up to 30 uncharacterised STs, highlighting the potential depth of minority populations of VSEfm. This diversity was also observed within patients, with up to 7 different VSEfm clones isolated from a single patient specimen. Complex antibiotic exposure and chemotherapy lead to loss of diversity and acquisition of the dominant hospital clone.

**Conclusion:** In conclusion, acquisition of VRE from the hospital environment is still an important mechanism of transmission and antibiotic selection pressure appears to play an important role in the acquisition of hospital adapted VREfm. However, whilst periodic selection may place limits on genetic diversity of hospital populations of *E. faecium* it does not prevent divergence nor limit diversity. The cloud of VSEfm clones within the human bowel, the plasticity of their genome and the potential for lateral gene transfer likely contribute significantly to the evolution of new VRE strains.

**PO26 - Elucidation Of Transmission Events Of Vancomycin Resistant *Enterococcus faecium* Isolates In Intensive Care Units Of A German Tertiary-care Hospital**

Jennifer Bender<sup>1</sup>; Carola Fleige<sup>1</sup>; Torsten Semmler<sup>1</sup>; Sandra Schneider<sup>2</sup>; Petra Gastmeier<sup>2</sup>; Guido Werner<sup>1</sup>

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**Background:** According to the recent EARS-Net report, Germany is one of the few European countries where numbers of vancomycin resistant enterococci (VRE) isolated from bloodstream infections have constantly increased since 2002. Also, data from the German National Nosocomial Surveillance System report a significant increase of VRE frequency in intensive care units (ICUs) (Gastmeier *et al.*, doi: 10.1093/jac/dku035). Further molecular typing of isolates received by the National Reference Centre for Staphylococci and Enterococci at the Robert Koch Institute revealed distinct sequence types highly prevalent in German hospitals. However, little is known about the clonal relatedness and success of dominant lineages in ICUs with a persistent burden of VRE. Thus, we set out to characterize 176 VRE derived from a study on Surveillance of Antibiotic Usage and Bacterial Resistance (SARI-IQ) from 6 ICUs with respect to population structure and dynamics.

**Methods:** *Enterococcus faecium* isolates were collected by the Charité hospital between 2014 and 2015 and sent to the NRC for detailed molecular analyses. The entire set of strains was analyzed by the rep-PCR-based method DiversiLab® and whole genome sequencing (WGS) utilizing Illumina technology. WGS data were used to infer phylogenies by means of a mapping and SNP-based approach, a comparison of the maximum common genome and by utilizing the commercially available software SeqSphere (Ridom, Münster, Germany).

**Results:** Initial MLST analyses proved to be insufficient to allocate specific isolates to entry or transmission events across ICUs due to limited discriminatory power. Conflicting results were obtained by analyzing the population structure derived from DiversiLab® results. As an example, several smaller ( $n < 10$ ) or larger ( $n > 25$ ) DL clusters combined obviously different VRE isolates demonstrating *vanA* and *vanB* genotypes, varying presence of virulence determinants and different antibiotic susceptibility profiles. In addition, some DL clusters contained isolates with up to 4 different MLST types. Hence, whole genome sequencing was applied for maximal resolution to discriminate entry, transmission and persistence events (ongoing analyses).

**Conclusion:** WGS provides maximal discriminatory power to monitor entry and/or transmission events between hospital wards which is of utmost importance in order to reduce the possibility of VRE infections in high risk patients.

## S2 - Poster session I

### PO28 - Lack Of Genetic Isolation By Distance And Persistence Of Monomorphic Genotypes In Generalist *Campylobacter jejuni* Strains

Mirko Rossi<sup>1</sup>; Ji Zhang<sup>1</sup>; Ann-Katrin Llarena<sup>1</sup>; Minna Vehkala<sup>1</sup>; Niko Välimäki<sup>1</sup>; Jukka Corander<sup>1</sup>

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**Background:** Identifying spatiotemporal genetic changes in the pathogen populations circulating in a given area is critical for forecasting the epidemiological relationship between isolates. *C. jejuni* ST-45 clonal complex (cc) is a genotype frequently detected in human infections and colonize chicken flocks worldwide. However, little is known on the population stability over time and space. Thus, the aim of this study was to explore spatiotemporal patterns of genetic diversity within ST-45cc by phylogenomic and genome-wide association studies (GWAS).

**Methods:** The genomes of a total of 340 isolates obtained from four European countries (Finland, UK, Lithuania and Estonia) during 14 years were selected. Genealogy was reconstructed using maximum likelihood phylogenetic analysis (RAxML) of 1,043 core genes after deleting recombination regions using BRATNEXGEN. Population structure was inferred with BAPS. RAxML was also used to cluster strains based on distribution of 1,281 accessory genes. GWAS was facilitated by first running a distributed string mining method on both core and accessory genomic elements, and then testing for association among the resulting genetic features. Pan-genome allele profile of a single BAPS lineage was generated using Genome Profiler and goeBURST was used to infer microevolution of isolates. The effect of time on the genetic diversity was estimated using BEAST v1.8.2.

**Results:** We identified two major clades of ST-45cc characterized by a large variation in r/m ratio (from 0.9 up to 22). With few exceptions, STs did not form monophyletic lineages and were composed by numerous BAPS populations characterized by a clonal structure, limited genetic variation and presence or absence of specific features regardless origin or year of isolation. However, when accessory genome was included in the analysis, GWAS identified short genetic signals over-represented in either Finnish or British isolates. Supporting the results of GWAS, RAxML tree based on distribution of accessory genes clustered isolates with identical core sequence according to origin, regardless the year of isolation. Nevertheless, microevolutionary analysis with goeBURST of a single BAPS lineage, having the highest Shannon and Simpson diversity index in term of countries and year of isolation, did not allow a clear separation of the isolates according to origin even at lower LV values. We further attempted to estimate divergence times between Finnish isolates belonging to a single BAPS lineage sampled serially between 2000 and 2012. A linear regression of the root to tip distance versus date of isolation was not possible ( $R^2 = 0.12$ ), suggesting absence of temporal signal. Moreover, calibrating the clock with mutation rate calculated using MLST data ( $3.2 \times 10^{-5}$ ; Wilson et al., 2009), we estimated the time for TMRCA to be 2011, clearly not reflecting the true sampling time of the isolates.

**Conclusion:** Our genomic study supported the theory of homogeneous global distribution of *C. jejuni* genotypes. Contrary to what expected, several lineages within ST45cc appear to be genetically monomorphic pathogens. They evolved slower than previously thought and were persistently detected over the years independently from geographical origin. We observed a lack of genetic isolation by distance suggesting that proximity-by-mobility due to rapid animal and/or human movement eroded the effect of geographical separation on the fine-scale genetic structure of *C. jejuni* ST-45cc.

## PO29 - Virulence Differences Among Type AI *Francisella tularensis* Strains Linked To Genetic Mutations Found By Whole Genome Sequencing

Caroline Öhrman<sup>1</sup>; Jeannine Petersen<sup>2</sup>; Luke Kingry<sup>2</sup>; Kiersten Kugeler<sup>2</sup>; Claudia Molins<sup>2</sup>; Laurel Respicio/Kingry<sup>2</sup>; Andreas Sjödin<sup>1</sup>; Mats Forsman<sup>1</sup>; Dawn Birdsell<sup>3</sup>; Paul Keim<sup>3</sup>; Dave Wagner<sup>3</sup>; Anders Johansson<sup>4</sup>

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**Background:** *Francisella tularensis* subspecies *tularensis* is one of three subspecies of *Francisella tularensis* causing the potentially severe disease tularemia. This subspecies, which is also known as type A and found in North-America, is the most virulent and was historically stockpiled as a biological weapon. In 2004, multiple locus variable-number tandem repeat analysis (MLVA) identified two major genetic subpopulations among type A strains (AI and All) and in 2009, analysis by pulsed-field gel electrophoresis (PFGE) identified a further genetic subdivision of AI strains into A1a and A1b. Strikingly, comparison with epidemiological surveillance data showed there were pronounced differences in clinical outcomes in infections by different type A genetic subpopulations. Despite that all type A strains shares an extensive genetic similarity, mortality among humans was strongly associated with A1b strains. Here, we used whole-genome sequencing to identify mutations that may explain virulence differences among type AI strains.

**Material/Methods:** Whole genome sequencing with paired end Illumina technology was used to sequence AI strains ( $n=13$ ) with a subset of strains ( $n=6$ ) selected for long pair inserts, so called mate pair sequencing. The genomes were assembled and single nucleotide polymorphisms (SNPs), insertion deletion events (INDELs), and genomic rearrangements were identified. Using this genomic data, we recently set up a typing system based on assaying canonical whole genome SNPs (canSNPs) identifying three distinct genetic subpopulations (A.I.3, A.I.8 and A.I.12) among *F. tularensis* AI strains and in this work, typing results were analyzed in relation to clinical outcomes and whole genome differences.

**Results:** Typing of 106 clinical strains from infected humans by canSNP assays revealed there was 27 percent mortality in infections from strains assigned to the genetic subpopulations A.I.3 ( $n=4/15$ ) or A.I.8 ( $n=6/22$ ) but only 6 percent mortality from A.I.12 strains ( $n=4/69$ ). For two strains from each of the three subpopulations, the genomes were completed including determination of gene orders across insertion sequence elements. Comparing the three subpopulations, twenty-seven SNPs and two INDELs were unique to the A.I.3 group and nine SNPs and two indels to the A.I.8 group. Five SNPs were shared between A.I.12 and A.I.8 strains that thereby formed sister clades in a phylogenetic whole-genome analysis. Thirty-eight SNPs, seven INDELs, and one 23,100 bp genomic inversion were unique to the A.I.12 subpopulation. The genomic inversion fully explained the unique banding pattern previously denoted A1a by PFGE typing using the restriction enzyme *Pmel*.

**Conclusions:** The analysis suggests virulence differences among type AI *F. tularensis* strains evolved by a decrease in virulence along the A.I.12 whole-genome phylogenetic tree branch and we have identified 46 mutation candidates in the genome to explain this. Gene order differences caused by one genomic inversion event explains the previously observed distinct PFGE pattern for type AI strains with decreased virulence.

## S2 - Poster session I

### PO31 - Using Whole Genome Sequencing To Understand Host-nontuberculous Mycobacteria Interaction

Sonia Faria<sup>1</sup>; Vitor Borges<sup>2</sup>; Catarina Carneiro<sup>2</sup>; Luis Vieira<sup>2</sup>; Joao Paulo Gomes<sup>2</sup>; Luisa Jordao<sup>2</sup>

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**Background:** Nontuberculous mycobacteria (NTM) are a large group of *Mycobacterium* species that don't belong to the *Mycobacterium tuberculosis* (Mtb) complex. These bacteria are mostly environmental being regarded as etiological agents of opportunistic infection in humans mainly immunocompromised. Distinguishing NTM from Mtb is still a challenge and identification at the species level is essential for an accurate diagnostic and effective treatment. The growth rate of NTM is very important for the onset of treatment being these bacteria divided into rapidly growing mycobacteria (RGM) and slowly growing mycobacteria (SGM). *Mycobacterium fortuitum*, *M. abscessus* and the model organism *M. smegmatis* are RGM whereas *M. avium* is SGM that take more than 7 days to form CFU on growth media. The knowledge of NTM infections is still reduced being needed more studies to understand the host-pathogen interaction during the infectious process in order to establish more effective therapeutic schemes. Recently our group conducted a study using human alveolar macrophages as a model. The obtained data showed that both RGM (747/08) and SGM (60/08) were able to persist and even replicate within this macrophages whereas others do not (*M. smegmatis* and *M. abscessus*)<sup>1</sup>. Here we used NGS as a tool to identify bacterial factor responsible for the observed outcome.

**Materials and Methods:** Three reference strains (*M. fortuitum* ATCC6841, *M. avium* ATCC25291, *M. smegmatis* ATCC700084) and 3 strains from Ricardo Jorge mycobacterial collection isolated from patients (*M. fortuitum* 747/08, *M. avium* 60/08 and Mtb70/09) were used. For DNA extraction bacteria were grown in Middlebrook 7H9 supplemented with 10% OADC and 0.05% Tween80.<sup>2</sup>

Full genome sequence was performed using NGS platform MiSeq (Illumina Inc., San Diego, CA, USA) according to the manufacturer's instructions.

Data analysis: RAST ([www.rast.nmpdr.org](http://www.rast.nmpdr.org)) and MAUVE platforms were used for annotation and multiple alignments, respectively.<sup>3</sup>

**Results:** Data analysis suggest a link between mycobacteria growth rate and genome size with RGM (6.7 million bp) having longer genomes than SGM (4.8 million bp) what might reflect bacteria adaptation to the host(s). Mtb with an exclusive host has a shorter genome (4.3 million bp) than NTM which exhibit a wider host tropisms and the ability to persist within the environment. Therefore, we can find genes associated to the Lactate fermentation like MAV\_2543 and Methanogenesis xfp that cannot be found in Mtb. The two NTM (747/08 and 60/08) strains sequenced and that show intramacrophage persistence displayed also a longer size, which is associated to the persistence related genes.

In specific gene subsystems were observed intra-species differences between clinical and reference NTM. Fatty acid metabolism cluster and cell envelope related genes subsystems illustrate this result.

**Conclusion:** The preliminary results suggest a link between genome size and intracellular persistence and support the fact that clinic NTM share virulence factors with Mtb. However, only a transcriptional analysis could ensure the evolvement of these genes in intracellular persistence.

**References:** Sousa S et al. *Int J Mycobacteriology*. 2015;4(1):36-43. Benjak A et al. *Methods Mol Biol*. 2015;1285:1-16. Darling ACE et al. *Genome Res*. 2004;14(7):1394-1403.

## PO32 - Adaptive Evolution Of *Burkholderia cenocepacia* Within The Lungs Of A Chronically Infected Cystic Fibrosis Patient

Sandra C. dos Santos<sup>1</sup>; Carla P. Coutinho<sup>1</sup>; Marcus Dillon<sup>2</sup>; Ana S. Moreira<sup>1</sup>; Rita F. Maldonado<sup>1</sup>; Vaughn S. Cooper<sup>2</sup>; Isabel Sá-Correia<sup>1</sup>

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**Background:** Chronic pulmonary infections are the prominent cause of high morbidity and mortality in cystic fibrosis (CF). *Burkholderia cepacia* complex (Bcc) bacteria are particularly threatening since they are associated with a worse prognosis, are inherently resistant to most clinical antibiotics and antimicrobials of the immune system, and are able to evolve and adapt to multiple selective pressures, significantly hindering their eradication.

**Methods and Materials:** In order to uncover key features in the pathogenic potential, persistence and virulence of *B. cenocepacia* during progressive lung disease, a comparative genomic analysis was performed for 11 sequential clonal variants, obtained from a single patient followed at the major Portuguese CF Center at Hospital de Santa Maria (HSM) in Lisbon [1]. This work is anchored on a 2 decade-long collaboration with this CF Centre for microbiological surveillance of Bcc respiratory infections, resulting in a collection of over 800 Bcc clinical isolates with accompanying patient clinical data [2]. Sputum samples were collected from the onset of infection until the patient's death 3.5 years later with a fatal necrotizing pneumonia known as cepacia syndrome. The complete genome of all isolates was sequenced using Illumina to generate short paired-end reads, while the first isolate collected and believed to have initiated the infection (IST439) was sequenced with PacBio, allowing the assembly of a complete draft genome to be used as reference. Different computational approaches were used for the identification of indels and major genomic rearrangements in the short-read sequencing data, specifically Breseq (made available by the Barrick Lab), and an in-house computational pipeline developed jointly at the Cooper Lab.

**Results:** IST439 has a total genome size of 7.63 Mb arranged in 3 chromosomes. Overall, 59 single nucleotide polymorphisms were detected (8 synonymous, 46 non-synonymous, 5 intergenic), as well as 11 deletion events. The altered genes were analyzed regarding biological function, and include encoded proteins involved in response to low iron conditions, oxidative and heat stress, antimicrobial treatment, lipopolysaccharide biosynthesis, microaerophilic growth, and membrane transport. Most remarkably, one isolate recovered near the end of the infection exhibited *in vivo* loss of chromosome 3. Additional fitness and virulence studies are consistent with the suggested role of this chromosome as a virulence encoding and stress response non-essential megaplasmid.

**Conclusion:** Comparative genomic studies possess a paramount importance in the identification of genomic modifications underlying the dynamics of adaptive evolution that promote successful colonization and long-term survival of pathogenic bacteria in a given host. Here we present a number of specific mechanisms of convergent evolution and adaptation of Bcc bacteria in the CF lung, representing potential targets for inactivation to enhance antimicrobial activity and to limit persistent infections by these and other Gram-negative bacteria.

**References:** [1] Coutinho et al., Infection and Immunity 79(7): 2950-60, 2011. [2] Coutinho et al., Front Cell Inf. Microbiol, 1:1-11, 2011.

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## S2 - Poster session I

### PO33 - Comparative Genomics Of An Endemic Strain Of Multidrug-resistant *Pseudomonas aeruginosa* In A Pediatric Cystic Fibrosis Population

Ruth Ann Luna<sup>1</sup>; Jennifer K. Spinler<sup>1</sup>; Anthony M. Haag<sup>1</sup>; Miriam Balderas<sup>1</sup>; Prapaporn Boonma<sup>1</sup>; Abria R. Magee<sup>1</sup>; Peter W. Hiatt<sup>1</sup>; James Versalovic<sup>1</sup>

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**Background:** Multi-year molecular epidemiological surveillance of the multidrug-resistant *Pseudomonas aeruginosa* (MRPA) isolates obtained from a pediatric cystic fibrosis (CF) population led to the identification of an endemic strain (Houston-1). Acquisition of the Houston-1 strain correlated strongly with recent hospitalization, and subsequent infection with the Houston-1 strain was associated with higher hospitalization rates and greater declines in lung function and nutritional status compared to infection with other MRPA strains. Improvements in infection control procedures and compliance have successfully mitigated ongoing transmission of the Houston-1 strain, but it persists in the pediatric population as well as in the patients transitioning to local adult CF care centers. Comparative genomics between this endemic MRPA strain with other MRPA strains isolated from our pediatric CF population as well as publicly available *P. aeruginosa* genomes will enable further investigation into underlying factors related to potential increased transmissibility and virulence of the Houston-1 strain.

**Methods:** Eight archived MRPA isolates obtained from patients of the pediatric CF center and previously classified via molecular strain typing were selected based on their clonal group as well as their antibiotic susceptibility profiles. The genomes of these eight MRPA isolates, which included a total of 5 different MRPA strains (4 Houston-1 and 4 unique isolates), were obtained via next-generation sequencing (Roche 454). Circular BLASTn-based representations of the MRPA genomes were constructed using BLAST Ring Image Generator. The newly generated clinically-relevant genomes were compared to eight other publicly available genomes from *P. aeruginosa* isolates associated with CF. Multi-locus sequence typing was carried out using the *Pseudomonas aeruginosa* MLST database. In addition, selected reaction monitoring (SRM) was implemented to determine the rate at which these MRPA strains degrade five classes of antimicrobials.

**Results:** The genome sizes of the newly sequenced MRPA genomes range from 6-7 Mbp and contain an average of 6,500 genes per genome. The four isolates identified as the Houston-1 strain distinctly clustered into a clade separate from other MRPA isolates, including the other four MRPA isolates/strains obtained from the same pediatric CF population. Based on MLST data, the Houston-1 strain represents a novel sequence type. Antibiotic degradation monitoring by SRM also indicated rapid degradation of penicillin class antibiotics by the Houston-1 strains as compared to unique MRPA strains.

**Conclusions:** Comparative genomics analysis has emphasized the distinct evolution of the Houston-1 strain that was found to be endemic in our pediatric CF population. Further characterization of these strains will highlight virulence determinants important for their evolution and persistence. Longitudinal SRM monitoring of MRPA isolates in this patient population may allow for the rapid detection of antibiotic resistance and assist in effective clinical management and minimized risk of newly emergent antibiotic resistant strains. In addition, investigation into genetic variations identified in the Houston-1 strain may provide additional insight into the perceived increased transmissibility of this MRPA strain.

**PO34 - Genomic Epidemiology Of The Gaza Community-acquired Methicillin-resistant *Staphylococcus aureus* Clone: A Unique ST22 Clone**

QiuZhi Chang<sup>1</sup>; Asaf Biber<sup>2</sup>; Izzeldin Abualaish<sup>3</sup>; Hanaa Jaber<sup>2</sup>; Galia Rahav<sup>2</sup>; William P Hanage<sup>1</sup>; Gili Regev-Yochay<sup>2</sup>

<sup>1</sup>Harvard School of Public Health <sup>2</sup>Sheba Medical Center <sup>3</sup>Toronto University

**Introduction:** Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has become a major global problem. We recently reported an abnormally high carriage prevalence of CA-MRSA in healthy children and adults in the Gaza. A particular ST22 clone, associated with HA-MRSA EMRSA-15, accounted for over 60% of all MRSA isolates. Using whole genome sequencing we provide detail on virulence properties, the unique resistance pattern, and the evolutionary history of the Gaza MRSA clone.

**Methods:** During 2009, a cross-sectional surveillance of *S. aureus* nasal carriage was conducted among healthy individuals in the Gaza, in the framework of the Palestinian-Israeli Collaborative Research (PICR). Isolates collected were assessed for antibiotic susceptibility using the VITEK2 system. Any unexpected susceptibility patterns were further assessed using disk diffusion and E-test. High quality draft Genomes of 69 ST22 *S. aureus* isolates were assembled from Illumina short read data using Velvet. Together with 175 additional ST22 genomes from global sources, we reconstructed the phylogeny of the ST22 lineage to define the origin and evolution of the Gaza clone.

**Results:** The Gaza isolates did not group with typical HA ST22s and were in fact two distinct closely related clades, one of which was characterized by the presence of staphylococcal cassette chromosome *mec* element (SCCmec) IV and the other by SCCmecV. Nearly all Gaza isolates in both clades contained the toxic shock syndrome toxin 1 (*tsst-1*) gene but the two clades had distinct antibiotic resistance genes. Only SCCmecIV clade isolates contained the co-trimoxazole resistant gene *dfrA*. These isolates showed a contradictory resistance phenotype, detected as resistant by automated machines, but susceptible by disk-diffusion, E-test and broth microdilution. The diversity of both clades is not consistent with recent emergence and shows evidence of changes in population structure.

**Discussion:** The 'Gaza clone' is in fact two clones that group with the diverse and predominantly methicillin-susceptible *S. aureus* ST22 isolates from which EMRSA15 emerged. This suggests two separate acquisitions of resistance by the same lineage, followed by diversification in other resistance determinants. In the absence of phenotypic testing, the *dfrA* variant would have been erroneously flagged as highly resistant, showing the importance of accurately linking phenotype to genotype.

## S2 - Poster session I

### PO35 - Next Generation Sequencing: The Key To Understood *Klebsiella pneumoniae* Biofilms?

Luisa Jordão<sup>1</sup>; Vitor Borges<sup>1</sup>; Luis Vieira<sup>1</sup>; João Paulo Gomes<sup>1</sup>; Aida Duarte<sup>2</sup>

<sup>1</sup>Instituto Ricardo Jorge Faculdade de Farmácia ULisboa

**Background:** The incidence of healthcare-associated infections (HAI) is determined by underlying disease conditions and exposure to high risk medical interventions. In Portugal since 1980s *K. pneumoniae* is a recognized etiological agent of epidemic and endemic infections in healthcare units. An increasing rate of *K. pneumoniae* strains resistant either to extended cephalosporins or carbapenems has been observed and one of the mechanisms responsible for the emergence of drug resistance could be the biofilm assembly. The capacity of *K. pneumoniae* to form biofilm was first described in the 1980s for abiotic surfaces and ten years later on biotic surfaces. The antibiotic failure to penetrate through the biofilm layers, the emergence of mutations which might be easily transferred horizontally, and quorum sensing have been pointed as responsible for the increased antibiotic resistance of bacteria within biofilms. The main objective was to study the biofilm structure and the kinetic assembly associated to antibiotic resistance profile in *K. pneumoniae* strains capsulate or not, and identify the genes involved in biofilm assembly on full genome sequencing of studied strains.

**Material and Methods:** Three *K. pneumoniae* isolates collected in 1980 (Kp45, Kp703) and one (Kp2948) in 2011 were studied. Kp703 was encapsulated and the remaining had capsular type K:2. The bacterial ability to assemble biofilms on cell culture plates was evaluated. For SEM analysis, biofilms were allowed to form on six wells cell culture plates (Nunc) for 12h at 37°C. DNA was extracted using QiAamp DNA mini kit following the manufacturer's instructions. Full genome sequence was performed using next-generation sequencing platform MiSeq (Illumina Inc., San Diego, CA, USA) according to the manufacturer's instructions. The RAST platform was used for annotation and MAUVE platform for multiple alignments.

**Results:** The three isolates were able to assemble biofilms although following different kinetics. *K. pneumoniae* strains Kp703 and Kp45 followed similar kinetics with identical biomass increase nevertheless these bacteria differ in capsule expression. Full-sequencing and annotation of genomes of isolates was performed in order to explain the differences found in biofilm assembly. Preliminary data already revealed that the *K. pneumoniae* strains displaying enhanced biofilm-forming ability is genetically different from the others, and, in particular, present some specific features enrolling genomic regions believed to be biofilm-related in other bacteria (an intact prophage, genes coding for a filamentous haemoagglutinin, a haemolysin expression modulating protein and an YdeA protein).

**Conclusion:** *K. pneumoniae* lacking capsule, regarded as less virulent, have a better performance as biofilm assembler and exhibited the highest increase in antibiotic resistance when organized within biofilms. The analysis of the full genome sequence will allow reaching on *K. pneumoniae* biofilms and provide novel opportunities to exploit the overall fitness of *K. pneumoniae* under antibiotic stress.

## PO36 - Whole Genome Sequencing Of Clonal Complex 8 MRSA Isolates Shows Several Clusters Of USA300-like Isolates

Mette Damkjær Bartels<sup>1</sup>; Peder Worning<sup>1</sup>; Jesper Boye Nielsen<sup>1</sup>; Henrik Westh<sup>1</sup>  
<sup>1</sup>Hvidovre University Hospital

**Background:** MRSA strains belonging to clonal complex (CC) 8 are common worldwide. Among the CC8 clones is the USA300 clone which usually is *spa* type t008 and contains both Panton Valentine Leukocidin (PVL) genes and the arginine catabolic mobile element (ACME). In 2014, t008 was the sixth most common *spa* type in Denmark. Another CC8 clone, t024, PVL negative and ACME positive, was spreading in nursing homes and local hospitals of the Capital Region of Denmark in the beginning of the millennium. We have previously shown that PFGE could not distinguish between t008 and t024 (1). In a study on sequence polymorphisms within 112 housekeeping loci, we could divide 174 geographically and time spread *S. aureus* isolates into 9 clusters (2) thereby separating t008 from t024. Whole genome sequencing (WGS) of MRSA isolates has been a routine analysis in our laboratory since 2013, and the purpose of this study was to obtain a more detailed insight into the evolution and spread of CC8 in the Capital Region of Denmark.

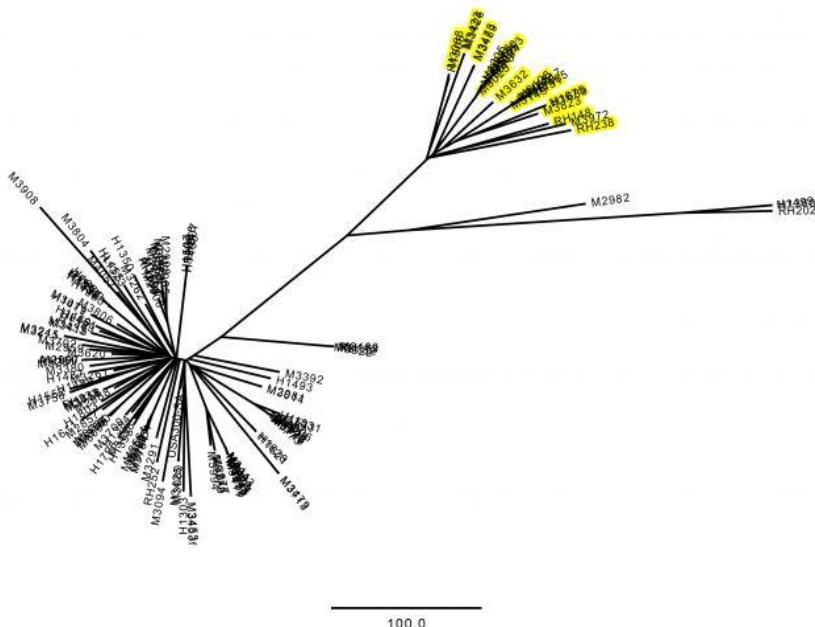
**Material and methods:** In the present study we included 263 CC8 isolates from the Capital Region of Denmark. All isolates were whole genome sequenced on an Illumina MiSeq. Isolates were sequenced with 2 x 150 bp paired-end reads. An in-house script analysed the genomes for *mecA*, *mecC*, *nuc*, *ccr* genes, *spa*-type, MLST, *dru* types, PVL, ACME and SCC*mec*. Reads were assembled using Velvet and Velvet optimizer. The reads were mapped to a reference genome (US300\_TCH1516) using Stampy and single nucleotide polymorphisms (SNPs) were called using Samtools.

**Results:** A distinct cluster of t024/ST8/ACME+/PVL- isolates was seen as well as a large cluster of t008/ST8/ACME+/PVL+ isolates. In addition, a minor cluster of 28 ACME-/PVL+ isolates was seen (yellow isolates in Fig. 1, a subtree of 149 isolates). The ACME-/PVL+, isolates were *spa* type t008 except for one isolate that was a *spa* type t024. Among the 28 isolates some isolates had less than 10 SNPs between them, but an epidemiological link was not always present. However, the majority of the 28 ACME-/PVL+ isolates had more than 100 SNPs between them indicating that it was not a large outbreak. In most cases, there was no information on travel abroad and the majority of patients were ethnic Danes. Finally, among the t008/ ACME+/PVL+ isolates, SNP analysis revealed previously unrecognized relations between several patients, e.g. two mothers that have given birth few days apart and had overlapping hospitalization in the same department.

**Conclusion:** WGS gives us a detailed insight into the evolution and spread of CC8 MRSA isolates in the Capital Region of Denmark. The SNP analysis shows that the typical USA300 clone has diversified into a subgroup of ACME negative isolates. Whether the loss of ACME changes the pathogenicity of this clone is unknown. SNP analysis also revealed unrecognized spread of MRSA in some cases.

**References:** Larsen AR et al, J Clin Microbiol. 2009 Nov;47(11):3765-8. Strommenger B et al, J Antimicrob Chemother. 2014 Mar;69(3):616-22.

## S2 - Poster session I



**PO37 - *Mycobacterium tilburgii*: The First Sequenced Genome**

Jan Zoll<sup>1</sup>; Jakko van Ingen, Saskia Kuipers, and Willem Melchers

<sup>1</sup>Radboud University Medical Center

**Background:** *Mycobacterium tilburgii* is a recently discovered *Mycobacterium* species causing serious infections with high mortality in immunocompromised hosts, specifically in North-West Europe. *M. tilburgii* cannot be grown in vitro. Therefore, resistance cannot be determined phenotypically and proper treatment of the infection is hampered. Until now, *M. tilburgii* could only be diagnosed by microscopy and 1S6 rDNA sequence analysis.

**Materials and methods:** In this study, we determined the full genome of *M. tilburgii* directly from an autopsy sample of the spleen from a patient with disseminated *M. tilburgii* disease. Depletion of human DNA was a prerequisite for successful determination of the *Mycobacterium* genome. Therefore, human cells were selectively lysed and treated with nucleases using the Molzym Molysis system. The *Mycobacterium* DNA isolated from the samples was used for whole genome sequencing using Illumina NextSeq500 technology.

**Results and conclusion:** *De novo* assembly using CLC Genomic Workbench resulted in 173 contigs ranging from 500 to 220.000 bp, covering almost the entire bacterial genome. Phylogenetic analysis showed a close relationship with *Mycobacterium simiae*. Whole genome sequencing was required to obtain extra information on antibiotic susceptibility. Genomic characteristics and genetic relationships with other *Mycobacterium* species will be presented.



## EPIDEMIOLOGY AND PUBLIC HEALTH - OUTBREAKS

**Thursday, 10 March 2016, 12:00-14:30**

### PO38 - Description Of A Fatal Outbreak In A Neonatal Intensive Care Unit (NICU) Caused By The Paediatric Clone (ST5-IVa) Of MRSA

Nuno Alexandre Faria<sup>1</sup>; Maria Miragaia<sup>1</sup>; Edgar Botelho-Moniz<sup>2</sup>; M. José Espinar<sup>2</sup>; Susana Pissarra<sup>2</sup>; Carlos Alves<sup>2</sup>; M. Manuela Ribeiro<sup>2</sup>; Hermínia de Lencastre<sup>3</sup>

<sup>1</sup>Instituto de Tecnologia Química e Biológica António Xavier,<sup>2</sup> Centro Hospitalar São João,<sup>3</sup> Laboratory of Microbiology and Infectious Diseases, The Rockefeller University

### PO39 - Application Of Whole Genome Sequencing To Investigate A Two Year's *Pseudomonas aeruginosa* Outbreak

Bárbara Magalhães<sup>1</sup>; Mohamed M. H. Abdelbary<sup>1</sup>; Frédéric Tissot<sup>1</sup>; Patrick Basset<sup>1</sup>; Mette Berger<sup>2</sup>; Yok-Ai Que<sup>2</sup>; Philippe Eggimann<sup>2</sup>; Guy Prod'Hom<sup>3</sup>; Gilbert Greub<sup>3</sup>; Giorgio Zanetti<sup>1</sup>; Laurence Senn<sup>1</sup>; Dominique S. Blanc<sup>1</sup>

<sup>1</sup>Service of Hospital Preventive Medicine, Lausanne University Hospital,<sup>2</sup> Intensive Care Service, Lausanne University Hospital,<sup>3</sup> Institut of Microbiology, Lausanne University Hospital

### PO40 - KPC-2 Carbapenemase Producing *Enterobacteriaceae* At A Multi-species Nosocomial Outbreak: Genomic Insights Of A Novel IncN Plasmid And Its Dissemination

Yancheng Yao<sup>1</sup>; Can Imirzalioglu<sup>1</sup>; Yalda Rezazadeh<sup>1</sup>; Konrad Gwozdzinski<sup>1</sup>; Linda Falgenhauer<sup>1</sup>; Jörg Overmann<sup>2</sup>; Boyke Bunk<sup>2</sup>; Alexander Goesmann<sup>3</sup>; Soeren Gatermann<sup>4</sup>; Martin Kaase<sup>4</sup>; Torsten Hain<sup>1</sup>; Eugen Domann<sup>1</sup>; Trinad Chakraborty<sup>1</sup>

<sup>1</sup>Institute for Medical Microbiology, Justus-Liebig-University Giessen and German Centre for Infection Research (DZIF), partner site Giessen-Marburg-Langen, Campus Giessen, Germany,<sup>2</sup> Leibniz Institute DSMZ- Bioinformatics, German Collection of Microorganisms and Cell Cultures, Braunschweig, and German Center for Infection Research (DZIF), partner site Hannover-Braunschweig, Germany,<sup>3</sup> Bioinformatics Computational Biology, Justus-Liebig-University Giessen and German Centre for Infection Research (DZIF), partner site Giessen-Marburg-Langen, Campus Giessen, Germany,<sup>4</sup> Institute for Hygiene and Medical Microbiology, Ruhr University, Bochum, Germany

## S3 - Poster session I

### PO41 - Real-time Whole-genome Sequencing For Investigating A Vancomycin-resistant *Enterococcus faecium* Outbreak At A Tertiary Care Hospital

Mohamed M. H. Abdelbary<sup>1</sup>; Claire Bertelli<sup>2</sup>; Gilbert Greub<sup>2</sup>; Delphine Hequet<sup>1</sup>; Giorgio Zanetti<sup>1</sup>; Laurence Senn<sup>1</sup>; Dominique S. Blanc<sup>1</sup>

<sup>1</sup>Service of Hospital Preventive Medicine, Lausanne University Hospital,<sup>2</sup> Institute of Microbiology, Lausanne University Hospital

### PO42 - Fast Update, Supported On ASO-PCR Based On WGS Of The Prevalence Of A *Mycobacterium tuberculosis* Outbreak-strain 20 Years After Its Emergence

Laura Pérez-Lago<sup>1</sup>; Estefanía Abascal<sup>2</sup>; Diana Maricela Herrera<sup>2</sup>; Iñaki Comas<sup>3</sup>; Sofía Samper<sup>4</sup>; María Isolina Campos-Herrero<sup>5</sup>; Rodolfo Copado<sup>6</sup>; Laura Sante<sup>7</sup>; Emilio Bouza<sup>1</sup>; Darío García de Viedma<sup>1</sup>

<sup>1</sup>Instituto de Investigación Sanitaria Gregorio Marañón,<sup>2</sup> CIBER Enfermedades Respiratorias, CIBERES,<sup>3</sup> Instituto de Investigación Sanitaria Gregorio Marañón,<sup>4</sup> Genomics and Health Unit, FISABIO Public Health,<sup>5</sup> CIBER Salud Pública CIBERESP,<sup>6</sup> Instituto de Investigación Sanitaria Aragón,<sup>7</sup> Hospital Universitario Miguel Servet,<sup>8</sup> CIBER Enfermedades Respiratorias, CIBERES,<sup>9</sup> Hospital Universitario de Gran Canaria Dr. Negrín,<sup>10</sup> Hospital Dr. José Molina Orosa,<sup>11</sup> Hospital Universitario de Canarias

### PO43 - Microevolution And Persistence Of *Listeria monocytogenes* In The Environment And Links To A Foodborne Outbreak

Vítor Borges<sup>1</sup>; Carla Maia<sup>1</sup>; Maria João Barreira<sup>1</sup>; Leonor Silveira<sup>1</sup>; Catarina Silva<sup>1</sup>; Silvia Duarte<sup>1</sup>; Luis Vieira<sup>1</sup>; Luis Lito<sup>2</sup>; Mónica Oleastro<sup>1</sup>; João Paulo Gomes<sup>1</sup>

<sup>1</sup>National Institute of Health, Portugal,<sup>2</sup> Laboratory of Microbiology, Department of Clinical Pathology, Centro Hospitalar de Lisboa Norte

### PO44 - Tracing Of Influenza A H3N2 Outbreaks In Two Nursing Homes And A Hospital In Bavaria By Whole Genome Sequencing Within The Bavarian Influenza Sentinel

Dominik Meinel<sup>1</sup>; Susanne Heinzinger<sup>2</sup>; Ute Eberle<sup>2</sup>; Helmut Blum<sup>3</sup>; Katharina Schönberger<sup>2</sup>; Andreas Sing<sup>2</sup>

<sup>1</sup>Unispital Basel,<sup>2</sup> Bavarian Health and Food Safety Authority,<sup>3</sup> Gene Center

### PO45 - Large-scale Survey Of *Bacillus cereus*-induced Food-borne Outbreaks: Epidemiological Data And Genetic Diversity

Benjamin Glasset<sup>1</sup>; Anne Brisabois<sup>1</sup>; Sabine Herbin<sup>1</sup>; Jacques-antoine Hennekinne<sup>1</sup>; Nalini Ramarao<sup>2</sup>

<sup>1</sup>ANSES,<sup>2</sup> INRA

## PO38 - Description Of A Fatal Outbreak In A Neonatal Intensive Care Unit (NICU) Caused By The Paediatric Clone (ST5-IVa) Of MRSA

Nuno Alexandre Faria<sup>1</sup>; Maria Miragaia<sup>1</sup>; Edgar Botelho-Moniz<sup>2</sup>; M. José Espinar<sup>2</sup>; Susana Pissarra<sup>2</sup>; Carlos Alves<sup>2</sup>; M. Manuela Ribeiro<sup>2</sup>; Hermínia de Lencastre<sup>3</sup>

<sup>1</sup>Instituto de Tecnologia Química e Biológica António Xavier, <sup>2</sup>Centro Hospitalar São João, <sup>3</sup>Laboratory of Microbiology and Infectious Diseases, The Rockefeller University

**Background:** *Staphylococcus aureus* is a major nosocomial pathogen worldwide and one of the main causes of outbreaks in neonatal intensive care units (NICU). In Portuguese hospitals, MRSA prevalence reached 47.4% in 2014, the second highest rate in Europe, and a single clone, EMRSA-15 accounts for more than 70% of nosocomial infections caused by MRSA. In this study we characterized MRSA isolates responsible for a large outbreak in a NICU, defined their clonal structure and origin and established their relation with the known Portuguese MRSA epidemiology.

**Methods:** Between November 2013 and April 2014, a MRSA outbreak occurred at the NICU of a major Portuguese hospital that caused two deaths in newborn babies. A total of 35 isolates were recovered from 12 patients and one healthcare worker. Isolates from four patients were recovered from infection (hemoculture and sputum, *n*=10) and colonization (nasal, perineal and umbilical swabs, *n*=9), while isolates from eight patients and one healthcare worker were collected from colonization (*n*=16). All isolates were characterized by PFGE. Representative isolates of each PFGE subtype per patient were characterized by spa typing and MLST. Detection of Panton-Valentine leukocidin (PVL) and staphylococcal cassette chromosome *mec* (SCCmec) typing was performed by PCR. Representative isolates were screened for vancomycin resistance by population analysis profiles (PAP). Whole genome sequencing (WGS) was performed for key isolates to establish outbreak relation with hospital flora.

**Results:** During the NICU outbreak, 33 MRSA and 2 MSSA were recovered. Molecular characterization identified two clonal types. The majority of the isolates belong to PFGE type A (subtype A1), spa type t311, ST5-SCCmec IVa (*n*=31) (Paediatric clone related); to subtype A3, spa type t586, ST5-MSSA (*n*=1); and to subtype A4, spa type t010, ST5-MSSA (*n*=1). The remaining isolates belong to PFGE B1, spa type t032, ST22-IVh (*n*=2) (EMRSA-15), and considered as outbreak independent. All isolates were PVL negative. Preliminary PAP results demonstrate that strains belonging to ST5-IVa contain subpopulations with decreased susceptibility to vancomycin, having the capacity to grow up to 12.5 µg/ml of vancomycin (MIC, *R*>2 mg/L). The Paediatric clone was detected in four infected babies, colonizing other four babies and in a healthcare worker, the majority at the end of the study period, suggesting cross transmission and persistence of the clone in the NICU environment and the HCW as possible vehicle of MRSA dissemination.

The Paediatric clone and EMRSA-15 were previously described in this hospital as part of a national surveillance performed in 2011. Back then EMRSA-15 was the major clone, accounting for more than 50% of the MRSA population, whereas the Paediatric clone was a sporadic isolate. WGS analysis is being performed to assess relations between outbreak and previous minor clones, and to evaluate the role of the HCW in the transmission chain during the outbreak.

**Conclusions:** This is the first description of an outbreak caused by the Paediatric clone in a NICU in Portugal. Decreased susceptibility to vancomycin might have been the reason behind therapeutic failure and newborn deaths. This can be indicative of the clonal replacement of EMRSA15 by the Paediatric clone in the hospital and directs attention to the potential clinical relevance of minor clones in high-risk wards and the need of continuous surveillance of MRSA and vancomycin resistance.

## S3 - Poster session I

### PO39 - Application Of Whole Genome Sequencing To Investigate A Two Year's *Pseudomonas aeruginosa* Outbreak

Bárbara Magalhães<sup>1</sup>; Mohamed M. H. Abdelbary<sup>1</sup>; Frédéric Tissot<sup>1</sup>; Patrick Basset<sup>1</sup>; Mette Berger<sup>2</sup>; Yok-Ai Que<sup>2</sup>; Philippe Eggimann<sup>2</sup>; Guy Prod'Hom<sup>3</sup>; Gilbert Greub<sup>3</sup>; Giorgio Zanetti<sup>1</sup>; Laurence Senn<sup>1</sup>; Dominique S. Blanc<sup>1</sup>

<sup>1</sup>Service of Hospital Preventive Medicine, Lausanne University Hospital,<sup>2</sup>Intensive Care Service, Lausanne University Hospital,<sup>3</sup>Institute of Microbiology, Lausanne University Hospital

**Background:** From 2010 to 2012, an increase in *P. aeruginosa* incidence was observed in the ICUs at the University Hospital of Lausanne. A total of 689 isolates from 254 patients were typed using Double Locus Sequence Typing (DLST), and subsequently grouped into 46 DLST clusters. Cluster DLST 1-18 affected the highest number of patients (24 out of 254), mostly hospitalized due to burn injuries. Isolates of the same DLST type were found in the environment of the burn unit's hydrotherapy room, suggesting this location was the source of the outbreak. To investigate the suspected transmission events, and to infer the outbreak isolates' phylogeny, we decided to complement this investigation with the high discriminatory power of whole genome sequencing (WGS).

**Material and Methods:** DLST cluster 1-18 incorporates 106 isolates, including clinical specimens of 24 patients, as well as environmental isolates. In this preliminary study, we performed WGS on ten clinical isolates randomly selected between March 2010 and October 2012. The isolates' sequence type (ST) was assigned from the short reads data. Core genome alignment of all ten isolates was acquired with Snippy. This alignment was subsequently used as an input for maximum likelihood tree construction with Gubbins, excluding regions of high SNP density suggestive of recombination.

**Results:** All ten sequenced isolates belonged to *P. aeruginosa* ST 1076. Phylogenetic analysis demonstrated the occurrence of a major ICU clade, clearly separated from one isolate retrieved from a patient possibly infected before its admission to the ICU. The ICU clade was further divided into subclade A, including six isolates from patients hospitalized in ICU 3 (burn unit), and subclade B, which comprises three isolates from other ICUs (ICU 2, 4, and 6). WGS results confirmed the occurrence of two suspected epidemiological links in the burn unit (hospitalized at the same time), while determined the close relatedness of two isolates retrieved two years apart, suggesting an environmental source.

**Conclusion:** Preliminary results showed a clear differentiation between *P. aeruginosa* DLST 1-18 isolates present in the burn unit, and isolates retrieved in other ICUs or hospital units. This suggests that different routes of transmission may have occurred during this outbreak. These results prompt us to further investigate this outbreak by sequencing isolates from other patients and from the environment in order to have a broader representation of transmission events and probable outbreak sources.

## PO40 - KPC-2 Carbapenemase Producing *Enterobacteriaceae* At A Multi-species Nosocomial Outbreak: Genomic Insights Of A Novel IncN Plasmid And Its Dissemination

Yancheng Yao<sup>1</sup>; Can Imirzalioglu<sup>1</sup>; Yalda Rezzazadeh<sup>1</sup>; Konrad Gwozdzinski<sup>1</sup>; Linda Falgenhauer<sup>1</sup>; Jörg Overmann<sup>2</sup>; Boyke Bunk<sup>2</sup>; Alexander Goesmann<sup>3</sup>; Soeren Gatermann<sup>4</sup>; Martin Kaase<sup>4</sup>; Torsten Hain<sup>1</sup>; Eugen Domann<sup>1</sup>; Trinad Chakraborty<sup>1</sup>

<sup>1</sup>Institute for Medical Microbiology, Justus-Liebig-University Giessen and German Centre for Infection Research (DZIF), partner site Giessen-Marburg-Langen, Compus Giessen, Germany,<sup>2</sup> Leibniz Institute DSMZ- Bioinformatics, German Collection of Microorganisms and Cell Cultures, Braunschweig, and German Center for Infection Research (DZIF), partner site Hannover-Braunschweig, Germany,<sup>3</sup> Bioinformatics Computational Biology, Justus-Liebig-University Giessen and German Centre for Infection Research (DZIF), partner site Giessen-Marburg-Langen, Compus Giessen, Germany,<sup>4</sup> Institute for Hygiene and Medical Microbiology, Ruhr University, Bochum, Germany

**Background:** Carbapenem-resistant *Enterobacteriaceae* are an increasing cause of nosocomial infections and hospital outbreaks. An outbreak involving 132 patients occurred in a single hospital in Germany from October 2013 to September 2014. The outbreak was unusual as it involved many different bacterial species and sub-species *Enterobacteriaceae* all exhibiting KPC-2 carbapenem-resistance. It could be demonstrated that all isolates harboring a *bla*<sub>KPC-2</sub> carrying plasmid. We describe the determination and comparison of the genomes and plasmids of the carbapenemase encoding isolates to discover horizontal carbapenemase transfer between bacteria of different species, as well as to find evidence for dissemination of a distinct *bla*<sub>KPC-2</sub> carrying plasmid in Germany.

**Material and Methods:** We analysed 36 outbreak isolates, including *Citrobacter freundii* (n=13), *Escherichia coli* (n=7), *Klebsiella oxytoca* (n=6), *K. pneumoniae* (n=3), *Enterobacter aerogenes* (n=3), *C. amalonaticus*, *C. braakii*, *C. koseri* and *E. cloacae* from patients and the outbreak hospital as well as 7 non outbreak isolates from three other hospitals by whole genome sequencing (Illumina MiSeq). A subset of 10 isolates was additionally sequenced with single-molecule-real-time sequencing (PacBio RSII) for complete genome assembly. S1-nuclease digestion followed by pulsed-field gel electrophoresis was carried out to investigate the plasmids. Phylogenetic and comparative analysis was performed.

**Results:** The phylogenetic analysis and *in silico* MLST typing revealed that the isolates of *K. oxytoca* belonged to a distinct clone, while all other outbreak related species such as *C. freundii*, *E. coli*, *K. pneumoniae* and *E. aerogenes* were not clonally related and displayed different ST's. The non-outbreak isolates were phylogenetically not related to the outbreak isolates. The carbapenem resistance gene *bla*<sub>KPC-2</sub> was located adjacent to *bla*<sub>TEM-1</sub> in a 9,571 bp unique genetic environment on an IncN plasmid. One isolate harboured *bla*<sub>KPC-2</sub> on an IncFI plasmid. The IncN plasmids encoded CDS required for replication, conjugation transfer and a multi drug resistance (MDR) region, but varied considerably in size, from 58,447 to 78,849 bp mainly due to variations in the MDR region. The most frequently occurring plasmid comprises 78,021 bp encoding 100 CDS and including 13 different antibiotic resistance genes in a single multi drug resistance region of 40,501 bp. Plasmids were transferrable to *E. coli* J53 by conjugation but also showed size variation following transfer indicating that both mobility and genetic variability are inherent properties of these plasmids.

**Conclusion:** Complete genome sequence analysis supports the finding of promiscuous plasmid transmission between the carbapenem-resistant multi-species isolates involved in the described nosocomial outbreak. The outbreak was caused by the efficient and dynamic transfer of a novel IncN plasmid. This plasmid does not only occur locally as in the hospital outbreak but could be demonstrated in independent isolates from different regions in Germany, hinting towards an

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endemic situation in healthcare settings with the potential for new outbreaks. The presence of the unique *bla*<sub>KPC-2</sub> in direct genetic vicinity with *bla*<sub>TEM-1</sub> in all outbreak strains and non-outbreak strains can be used as a unique molecular probe for detecting this plasmid in outbreak situations and epidemiologic investigations.

**PO41 - Real-time Whole-genome Sequencing For Investigating A Vancomycin-resistant *Enterococcus faecium* Outbreak At A Tertiary Care Hospital**

Mohamed M. H. Abdelbary<sup>1</sup>; Claire Bertelli<sup>2</sup>; Gilbert Greub<sup>2</sup>; Delphine Hequet<sup>1</sup>; Giorgio Zanetti<sup>1</sup>; Laurence Senn<sup>1</sup>; Dominique S. Blanc<sup>1</sup>

<sup>1</sup>Service of Hospital Preventive Medicine, Lausanne University Hospital,<sup>2</sup>Institute of Microbiology, Lausanne University Hospital

**Background:** Between January and March 2015, an outbreak of vancomycin-resistant *Enterococcus faecium* (VREfm) occurred at the University Hospital of Lausanne. We performed whole-genome sequencing (WGS) to determine characteristic genetic signatures in the outbreak isolates for developing a specific multiplex PCR that could be implemented in the outbreak control measures. Furthermore, we investigated the relatedness, molecular features and the transmission events of the outbreak isolates.

**Materials and Methods:** In total, twenty-six VREfm isolates that were retrieved from clinical samples and rectal swabs were sequenced using the Illumina MiSeq platform. Genome sequences were subjected to *in silico* multi-locus sequence typing (MLST) and the identification of genetic determinants of antibiotic resistance. Comparative genome analysis of the assembled ordered draft genomes was performed using the Mauve software. To infer the isolates' phylogeny, all paired-end reads were mapped to the most closely related reference genome and, subsequently, a maximum likelihood tree was constructed using single nucleotide polymorphisms (SNPs) from a core genome alignment.

**Results:** The *in silico* MLST analysis revealed that twenty-five of the sequenced isolates represented the multilocus sequence type (ST) 17, whereas the remaining isolate belonged to ST80. Using the comparative genomics approach, we identified genetic loci responsible for polysaccharide biosynthesis that were specific to all ST17 outbreak isolates. These loci were used to develop a multiplex PCR to differentiate between outbreak and non-outbreak isolates, which preceded and followed the outbreak period. This specific PCR revealed that three additional patients were part of the outbreak; two of them were hospitalized in November 2014, while the remaining patient was admitted in late June 2015. Furthermore, we were able to exclude four patients from the outbreak. The phylogenetic analysis demonstrated that outbreak isolates were very closely related with only 0-4 SNPs differences. It also revealed that none of our isolates could be considered as the initial source of the outbreak, suggesting that previous transmission events occurred in the hospital.

**Conclusion:** WGS analysis provided comprehensive evidence of a clonal outbreak of VREfm, and was able to precisely distinguish outbreak from non-outbreak isolates. Furthermore, it suggests that there were some undiscovered cases prior to the index case; and that VREfm was introduced from a point source with secondary transmissions. WGS demonstrates the prospect to substitute currently used phenotypic and molecular typing approaches to investigate outbreaks.

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### PO42 - Fast Update, Supported On ASO-PCR Based On WGS Of The Prevalence Of A *Mycobacterium tuberculosis* Outbreak-strain 20 Years After Its Emergence

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**Background:** Standard genotypic analysis allowed the identification of a severe outbreak of tuberculosis in Gran Canaria (GCI), Canary Islands, Spain. It involved a Beijing strain (GCI strain), which was imported by a Liberian immigrant in 1993 and it spread until being responsible for 25% of the total number of cases in GCI. Since 2008, no new data about the prevalence of the GCI strain in the island have been obtained. A recent tracking of the GCI strain in Madrid allowed to identify it from two immigrant cases recently diagnosed (2014) after arriving from Guinea Conakry, a country sharing borders with Liberia, which was the origin of the first importation, suggesting that the GCI strain might be prevalent in West Africa. Our study aimed to i) update the current situation of the GCI strain in the Canary Islands using a simple, fast and low cost tool based on targeting strain-specific SNPs detected by whole genome sequencing (WGS) and ii) characterize the potential microevolution of this strain.

**Materials/Methods:** WGS was used to identify the specific SNPs for the GCI strain. We optimized a multiplex allele-specific PCR (ASO-PCR) targeting 4 strain-specific SNPs. It was applied directly on boiled crude extracts from the stored collections (period 2013-2014) from 3 hospitals which collect strains from four of the seven Canary Islands, Gran Canaria, La Palma, Lanzarote and Tenerife (these islands are representative of the two provinces of the archipelago). MIRU-VNTR was applied to track the emergence by microevolution of clonal variants of the GCI strain.

**Results:** The ASO-PCR specific for the GCI strain was applied to 145 isolates from 4 of the 7 Canary Islands. ASO-PCR results were available within the same day in which the isolates were received. The amplification pattern expected for the GCI strain was identified in 32 isolates (22%). Among them, a pattern consistent with a co-infection involving a GCI strain and another strain was found in two cases. 4 clonal variants, with variations in a single MIRU-VNTR locus each, were detected.

**Conclusion:** We could fastly update the situation of the GCI outbreak strain by applying a simple, fast and low-cost strategy based on ASO-PCR targeting strain-specific SNPs. This strategy also allowed to identify mixed infections involving the GCI strain and another non-GCI strain. 22% of the current TB cases in the Canary Islands are still infected by this strain. The high prevalence of the GCI strain is not restricted to the island where the outbreak was described, Gran Canaria, but it was also found in other islands. Several microevolved clonal variants have emerged from the parental outbreak strain and are being also transmitted.

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## PO43 - Microevolution And Persistence Of *Listeria monocytogenes* In The Environment And Links To A Foodborne Outbreak

Vítor Borges<sup>1</sup>; Carla Maia<sup>1</sup>; Maria João Barreira<sup>1</sup>; Leonor Silveira<sup>1</sup>; Catarina Silva<sup>1</sup>; Silvia Duarte<sup>1</sup>; Luis Vieira<sup>1</sup>; Luis Lito<sup>2</sup>; Mónica Oleastro<sup>1</sup>; João Paulo Gomes<sup>1</sup>

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**Background:** *Listeria monocytogenes* is a foodborne human pathogen associated with a high mortality rate. Given the remarkable ability of this bacterium to persist in food processing facilities, ready-to-eat food products are common vehicles of *L. monocytogenes*. In the present study, we report the use of whole-genome sequencing (WGS) to investigate the diversity among *L. monocytogenes* isolates associated with a hospital outbreak of listeriosis occurred in Portugal.

**Materials | Methods:** To investigate the potential source of 3 cases of hospital-acquired *L. monocytogenes* infections, we performed WGS (on a MiSeq Illumina instrument) on strains isolated from: patient (n=3); environment, collected by swabbing surfaces in the establishment that produces the foodstuffs for the hospital (n = 20); and ready-to-eat foods prepared in this establishment and served in the hospital (n = 4). Samples from the environment included surfaces of the processing area and equipment, and were collected spaced by 2 weeks, with cleaning and disinfection procedures performed during this interval. To infer the genetic relatedness among all the strains isolated, we performed reference-based mapping followed by the identification of bona fide core Single Nucleotide Polymorphisms (SNPs) (using samtools/bcftools). SNP-based phylogenetic relationships among the isolates were visually depicted in a minimum spanning tree constructed using the goeBURST algorithm implemented in the PHYLOViZ platform.

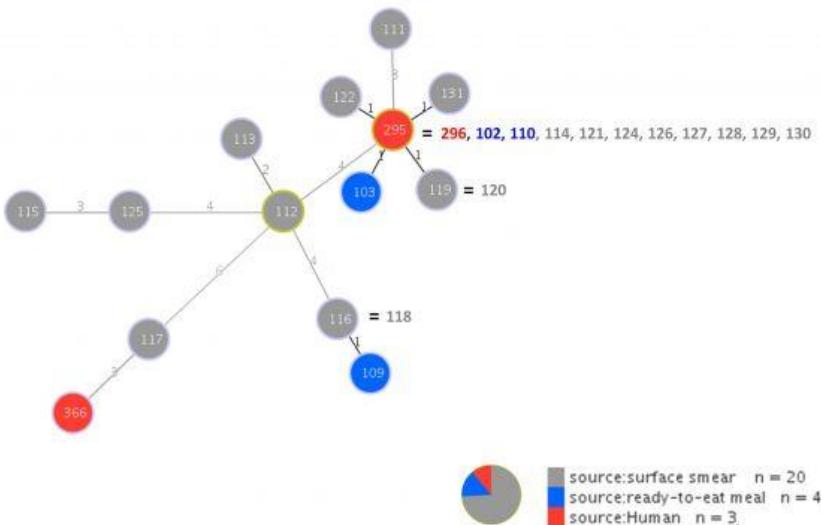
**Results:** *L. monocytogenes* infections were confirmed in 3 patients admitted to a central hospital in Portugal, being the meals prepared in a food establishment and distributed in the hospital the presumable pathogen's vehicle. WGS-based investigation of the relatedness between clinical isolates and multiple isolates collected from that establishment and ready-to-eat foods (Figure 1) revealed:

- i) a high degree of genetic homogeneity between clinical and environmental isolates, with a maximum SNP distance among all isolates of only 12 SNPs;
- ii) 14 unique sequences profiles, indicating that at least 14 distinct clones coexisted in the outbreak' source;
- iii) that 2 clinical cases genetically match to several clones collected from diverse locations in the establishment that produces the foodstuffs for the hospital, whereas the third patient isolate was closely linked with a single location;
- iv) a repertoire of loci likely involved in microevolutionary events (where non-synonymous mutations are overrepresented) potentially driving *L. monocytogenes* environmental persistence;
- v) that clones isolated from ready-to-eat foods perfectly matched to clones circulating in the surfaces of the processing area and equipments, corroborating that cross-contamination may still occur.

Further analyses are underway to investigate the potential existence of mobile genetic elements in order to increase the resolution power, and perhaps, to establish the genetic link between the two indistinguishable clinical isolates and a particular location in the outbreak source.

**Conclusion:** In this study, we show how the high discriminatory power of WGS may identify potential links within outbreak scenarios and unveil how *L. monocytogenes* microevolution may pose some challenge in attributing meaningful similarity thresholds for food source attribution. It can also contribute to identify genes targeted by microevolution that could contribute to the *L. monocytogenes* persistence in food processing facilities.

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**Figure 1. WGS-based phylogenetic relationships of the 27 *Listeria monocytogenes* isolates associated with a hospital outbreak of listeriosis.** The minimum spanning tree was constructed using the goeBURST algorithm implemented in the PHYLOVIZ platform, and is based on a core of 30 bona fide Single Nucleotide Polymorphisms (SNPs). Each circle contains the isolate's name and represents a unique given clone / sequence type. Samples indistinguishable for particular clones are listed nearby the respective circles. The different sample origins (human, ready-to-eat foods and surface swabs) are distinguished by different colors. The numbers on the connecting lines represent the SNP distances between clones.

**PO44 - Tracing Of Influenza A H3N2 Outbreaks In Two Nursing Homes And A Hospital In Bavaria By Whole Genome Sequencing Within The Bavarian Influenza Sentinel**

Dominik Meinel<sup>1</sup>; Susanne Heinzinger<sup>2</sup>; Ute Eberle<sup>2</sup>; Helmut Blum<sup>3</sup>; Katharina Schönberger<sup>2</sup>; Andreas Sing<sup>2</sup>

<sup>1</sup>Unispital Basel,<sup>2</sup> Bavarian Health and Food Safety Authority,<sup>3</sup> Gene Center

**Background:** Annually the influenza epidemics affect several thousand people in Europe. Morbidity, mortality and associated healthcare costs are high. Many countries conduct surveillance programs collecting epidemiological data about the influenza season, however only very little large-scale genomic data is available regarding the molecular epidemiology of an influenza season helping to understand transmission routes. Here we describe a fast and straight forward tool to investigate transmission routes of Influenza A in surveillance programs by NGS.

**Methods:** We established a fast RT-PCR strategy starting directly from patient material (swabs) to generate influenza A whole genome sequences. The patient swabs were obtained within the Bavarian influenza Sentinel and extracted using a routine laboratory Hamilton robotic device. The entire Influenza A virus genome was amplified in one multiplex RT-PCR reaction with an RT and a PCR step. DNA was sequenced using an Illumina MiSeq with 50x multiplexing of samples. Data analysis was carried out using a local Galaxy server, R and Mega 6.0. The analysis included quality trimming, mapping (Bowtie 2.0), variant calling (VarScan) and phylogenetic analysis with various algorithms (e.g. Neighborhood joining).

**Results:** We report the genomic sequences for 50 influenza A viruses from the H3N2 type which was the dominating virus in the season 2014/15 with a very high coverage ( $> 350\times$  fold). The dataset included randomly sampled isolates from all over Bavaria (Germany) and spiked in isolates, which were derived from three suspected influenza A H3N2 outbreaks in two nursing homes and one hospital in 2014/15. The samples were temporally and spatially matched. We were able to identify highly robust the outbreak isolates, which were spiked into the randomly selected dataset, based on the very high sequence identity within the isolates belonging to each outbreak. We show that WGS is superior in resolution compared to analysis of single segments, such as HA or NA. Furthermore, we found one of the suspected outbreak isolates to be genetically unrelated to the other samples of the same outbreak, suggesting an independent infection and illustrating the high discriminatory power of WGS. Additionally, we detected manifestation of substantial amounts of viral quasispecies in several patients, carrying mutations varying from the dominant virus in each patient.

**Conclusions:** Our rapid whole genome sequencing approach for influenza A virus shows that WGS can effectively be used to detect and understand outbreaks in communities. Additionally, the genome data provides details about the circulating strains within a community and will help to detect mutations of the virus within one season thus contributing to our in depth understanding of transmission pathways and evolution of the Influenza virus.

## S3 - Poster session I

### PO45 - Large-scale Survey Of *Bacillus cereus*-induced Food-borne Outbreaks: Epidemiological Data And Genetic Diversity

Benjamin Glasset<sup>1</sup>; Anne Brisabois<sup>1</sup>; Sabine Herbin<sup>1</sup>; Jacques-antoine Hennekinne<sup>1</sup>; Nalini Ramarao<sup>2</sup>

<sup>1</sup>ANSES,<sup>2</sup> INRA

**Background:** *Bacillus cereus* is a ubiquitous spore-forming bacterium responsible for food-borne outbreaks (FBOs). *B. cereus* can induce diarrhoeal and emetic syndromes depending on the type of strains, the first due to production of enterotoxins (diarrhoeal strains) and the second to ingestion of cereulide (emetic strains). Historically, *B. cereus* has often been regarded as a surface contaminant, but the increasing number and gravity of suspected *B. cereus* FBOs highlighted the importance of a thorough characterisation of strains in relation to the corresponding epidemiological data. Indeed, *B. cereus* is now estimated as the fourth agent responsible for FBOs in the European Union and the second in France.

**Materials & Methods:** The aim of this study was therefore to identify and characterise *B. cereus* strains from a unique national collection of 564 strains closely related to 140 FBOs that occurred in France during the 2007-2014 period in order to improve the monitoring and investigation of *B. cereus* FBOs, assess the risk of emerging clusters of strains and identify strain variability. The epidemiological data related to each FBO were collected through interviews or questionnaires by local health authorities. Characterization of the strains was based on phenotypic and genotypic methods. Diversity of isolates in samples within a same FBO was studied using M13-PCR derived from an RAPD technique. An extensive molecular characterisation study was conducted on already known virulence markers: the genes encoding cereulide, diarrhoeic toxins (Nhe, Hbl, CytK1 and CytK2) and Haemolysin HlyI, as well as *panC* phylogenetic classification.

**Results:** Starchy food and vegetables were the most frequent food vehicles identified and 82% of human cases occurred in institutional catering contexts. A significant difference was observed for incubation periods before the onset of symptoms according to the type of strain. The combination of the presence or absence of the virulence markers allowed to cluster the strains into 12 "genetic signatures" (GS), GS1 to GS12. The most frequent GS encountered in the collection was GS1, which accounted for 22% of strains. Several genetic signatures defined in this study were associated to a single *panC* phylogenetic group ie: GS2 (IV), GS3 (III) GS7 (VII), GS8 (VI), GS11 (III) and GS12 (II). 84% of the strains belonged in GS1 to GS6 thus showing that there were main profiles of *B. cereus* strains involved in food poisoning. That allowed us clustering strains into several features and determined the virulence marker combinations of the strains responsible for FBOs.

**Conclusion:** This study highlighted the virulence potential of each strains and the genetic diversity among *B. cereus* in FBOs. Indeed, certain toxin gene profiles were significantly more encountered and seem to play a greater role in food poisoning. Taken together, this study provided an accurate and extensive report of FBOs associated with *B. cereus* and provided insights into molecular characterisation and genetic diversity of the *B. cereus* strains responsible for FBOs. We show that a scheme should be followed by food safety authorities to better investigate the FBOs suspicion associated with *B. cereus*, which is a real food pathogen with a strong impact on human health. Further studies are now necessary to precisely characterize the virulence determinants able to predict the pathogenicity and virulence of strains isolated by food companies to avoid risks of FBOs.

## FOOD, ZOONOTIC AND ENVIRONMENTAL MICROBIAL RISKS

**Thursday, 10 March 2016, 12:00-14:30**

### PO46 - Clonal Dynamics Of Diverse *Salmonella enterica* Serotypes From Traded Food Products In Portugal

Joana Campos<sup>1</sup>; Joana Mourão<sup>1</sup>; Leonor Silveira<sup>2</sup>; Jorge Machado<sup>2</sup>; Carla Novais<sup>1</sup>; Luísa Peixe<sup>1</sup>; Patrícia Antunes<sup>3</sup>

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### PO47 - Antibiotic Resistance And Molecular Characterization Of *Staphylococcus pseudintermedius* Strains Isolated From Companion Animals In Russia

A. Balbutskaya<sup>1</sup>; O. Dmitrenko<sup>2</sup>; V. Skvortsov<sup>1</sup>

<sup>1</sup>Belgorod department of «All-Russian scientific research institute of experimental veterinary medicine named after Ya.R.Kovalenko»,<sup>2</sup> Federal State Institution «N.F. Gamaleya Federal Scientific Center of Epidemiology and Microbiology» Ministry of Health RF

### PO48 - Isolation Of *Arcobacter* spp. Quinolone Resistant From Fresh Vegetables

Isidro Favian Bayas Morejón<sup>1</sup>; Ana González Pellicer<sup>1</sup>; María Antonia Ferrús Pérez<sup>2</sup>

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### PO49 - Study Of Population Diversity Of *Toxoplasma gondii* Human Strains In Portugal

Anabela Vilares<sup>1</sup>; Idalina Ferreira<sup>1</sup>; Susana Martins<sup>1</sup>; Maria João Gargaté<sup>1</sup>

<sup>1</sup>National Institute of Health Dr Ricardo Jorge, Department of Infectious Diseases, Reference Unit for Parasitic and Fungal Infections

### PO50 - Identification Of Potential Amplicon Sequencing Targets For Identification And Strain-level Characterization Of Shiga Toxin-producing *Escherichia coli*

Lori Michelle Gladney<sup>1</sup>; Daniel Fasulo<sup>2</sup>; Alexandra Mercante<sup>1</sup>; Lisley Garcia-Toledo<sup>1</sup>; Rebecca Lindsey<sup>1</sup>; Eija Trees<sup>1</sup>; Nancy Strockbine<sup>1</sup>; Efrain Ribot<sup>1</sup>; Heather Carleton<sup>1</sup>; John Besser<sup>1</sup>

<sup>1</sup>Centers for Disease Control and Prevention,<sup>2</sup> Pattern Genomics

## S4 - Poster session I

### PO46 - Clonal Dynamics Of Diverse *Salmonella enterica* Serotypes From Traded Food Products In Portugal

Joana Campos<sup>1</sup>; Joana Mourão<sup>1</sup>; Leonor Silveira<sup>2</sup>; Jorge Machado<sup>2</sup>; Carla Novais<sup>1</sup>; Luísa Peixe<sup>1</sup>; Patrícia Antunes<sup>3</sup>

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Keywords: *Salmonella* clones, antimicrobial resistance, metals.

**Background:** Non-typhoidal *Salmonella* infections represents a global public health burden, being the identification and tracking of emerging serotypes/clones in the food chain crucial to contain their spread. In this study we assessed the serotype/clonal distribution trends and their association with antimicrobial resistance in *Salmonella* isolates from foodstuffs traded in Portugal. Results were also compared with previous data from human clinical isolates (2009-2015).

**Materials/Methods:** *Salmonella* isolates (n=250; 13 serotypes), resulting from food safety and quality control, from diverse national/imported food sources (pork/Pk-191, poultry/Py-42, clam-10, beef/Bf-6, cooked food/Cf-1) recovered in different regions of Portugal (2014-2015) were studied. In these isolates, sulfametoazole resistance (*sul1/sul2/sul3*) and other genes linked with class 1 integrons (*intI1*/antibiotic resistance gene cassettes) were screened by PCR. Then, in representative isolates detection of other antibiotic resistance, as well as metals (copper-*pcoD*, copper/silver-*siA*) tolerance genes by PCR, susceptibility tests to 12 antibiotics (ampicillin-A; chloramphenicol-C; ciprofloxacin; gentamicin-G; kanamycin; meropenem; nalidixic acid-Na; pefloxacin-P; streptomycin-S; sulfametoazole-Su; tetracycline-T; trimethoprim-Tr) and beta-lactamase production by disk diffusion (amoxicillin-clavulanic acid; cefepime; ceftazidime-Cz; cefotaxime-Cx; Cefoxitin-Fx) (CLSI/EUCAST) and clonal relatedness (PFGE/MLST) were performed.

**Results:** In *S. 4,[5],12:i-* we detected the two major clones circulating in Europe, with the predominance of multidrug-resistant (MDR) "European clone" (86%-85/99; Pk+Py+Bf+Cf; *sul2±intI1/pcoD+siA*; mostly ASSuT) with PFGE-types similar to those emerging in clinical sources. The frequency of the "Spanish clone" (*intI1+sul1+sul2+sul3/siA*; ACSSuTTr) is decreasing (5%-5/99; Pk+Py+Bf) comparing to previous years, as occurring in human cases. In *S. Typhimurium*, "DT104" (32%-20/62; Pk+Py; *intI1+5'CS-aadA2+5'CS-bla<sub>PSE-1</sub>+sul1*) and "OXA-30-producing" (15%-9/62; Pk; *intI1+5'CS-bla<sub>OXA-30</sub>+sul1*) clones were the most frequent and presented identical ACSSuT and PFGE profiles to those described in humans. "*S. Typhimurium European clone*" (8%-5/62; Pk; *sul2/pcoD+siA*), which is emerging in clinical sources, presented similar MDR (mostly ASSuT) and related PFGE-types to *S.4,[5],12:i-* "European clone". In *S. Rissen* stands out the maintenance of the MDR clone frequently associated with class 1 integrons (36%-11/31; Pk; *intI1+sul1±sul3/pcoD+siA*). Noteworthy, a MDR-ACzCxRx(K)PNaSuT *S. Heidelberg* clone/ST15 (80%-4/5; imported Py gizzards) CMY-2-producing was detected.

**Conclusions:** In the analysed *Salmonella* collection we detected in different food sources the persistence of serotypes/clones frequently associated with human infections, contributing for the high rates of MDR. Noteworthy is the expansion of MDR *S. 4,[5],12:i-* "European clone" and *S. Rissen* clone from pork foods enriched with copper/silver tolerance genes and the detection of MDR *S. Heidelberg*-CMY-2 producing clone from imported poultry. The acquisition of antimicrobial resistance genes (antibiotics/metals) seems to have a relevant role for the success of these clones. However, understanding all the adaptive factors promoting their survival/persistence is crucial for implementing effective strategies in food safety at global level.

## PO47 - Antibiotic Resistance And Molecular Characterization Of *Staphylococcus pseudintermedius* Strains Isolated From Companion Animals In Russia

A. Balbutskaya<sup>1</sup>; O. Dmitrenko<sup>2</sup>; V. Skvortsov<sup>1</sup>

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**Background:** Coagulase-positive *Staphylococcus pseudintermedius* has emerged as a cause of wide range infections in many kinds of animal species, predominantly in dogs and cats. There is evidence of transmission of this pathogen between companion animals and their owners. *S. pseudintermedius* has been also identified as a causative agent of severe bacterial infections in humans. Acquisition of staphylococcal cassette chromosome (SCC) *mec* elements and various resistance genes by several clonal lineages of *S. pseudintermedius* and rapid spread of multidrug resistant methicillin-resistant (MDR MRSP) clones in many countries leaded to treatment challenges to veterinary medicine and public health issue. This study was performed to evaluate prevalence of MRSP and MDRSP among companion animals in the Central region of Russia and to characterize colonizing and infecting strains.

**Materials/Methods:** Oral, nasal and skin swabs samples were taken from 53 healthy dogs. 87 dogs and 29 cats with various pyoinflammatory diseases were also sampled. A total of 30 *S. pseudintermedius* isolates were obtained from healthy dogs and 57 isolates from diseased animals were collected. The identity of the isolates was confirmed by multiplex PCR as described Sasaki T. et al. (2010) and amplification and partial sequencing of *kat* gene with primers designed by BlaiottaG. et al. (2010). Susceptibility to 8 antimicrobials was determined by disc testing. The molecular characteristic of the isolates was carried out by PCRs to determine *mecA* gene, SCCmec types and sequencing *spa* according Kondo Y. et al. (2007) and Moodley A. et al. (2009), respectively.

**Results:** Frequency of resistance to antimicrobials was as follow (healthy/diseased animals (%)): clindamycin (0/38,6), erythromycin (0/38,6), doxycycline (10/29,8), chloramphenicol (0/21), gentamycin (0/21), oxacillin (0/8,8), ciprofloxacin, (0/3,5) and fusidic acid (0/0). Overall, 23% of *S. pseudintermedius* isolates were MDR. The molecular characterization has shown that 5 isolates were *mecA* positive. A total of 8 different *spa* types were found in 14 *S. pseudintermedius* isolated from diseased dogs and 1 isolate from healthy dog. 4 MRSP isolates belonged to SCCmec type IV and presented the additional *ccr1* gene complex, 2 of them had *spa* type t03 and t05. The remaining isolate belonged to SCCmec type III and had *spa* type t02. The combination of SCCmec type III and *spa* t02 is the most common for European epidemic MRSP clone ST71. Methicillin-susceptible *S. pseudintermedius* isolates were identified harboring the most frequent *spa* type t71 (n=7) and single *spa* types t02, t05, t09, t15, t17.

**Conclusion:** This study shows the high prevalence (57%) of *S. pseudintermedius* carriage in healthy dogs in Russia. The pathogen caused disease in 10% of examined cats and 63% of dogs. MRSP was detected from five (5,7%) diseased dogs. All of MRSP were MDR. To our knowledge, this is the first report of canine infections caused by MRSP in Russia, suggesting that MRSP ST71 is involved. *Spa* gene was not amplified in 83% of *S. pseudintermedius*, suggesting rearrangements in the *spa* or lacking of *spa* in this isolates. Additional studies are needed to modify *spa*-typing protocol to amplify non-typeable samples of our population. Molecular typing is required to determinate circulation of epidemic clones in different geographic areas.

## S4 - Poster session I

### PO48 - Isolation Of *Arcobacter* spp. Quinolone Resistant From Fresh Vegetables

Isidro Favian Bayas Morejón<sup>1</sup>; Ana González Pellicer<sup>1</sup>; María Antonia Ferrús Pérez<sup>2</sup>

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**Background:** *Arcobacter* is considered an emergent foodborne and waterborne enteropathogen. At the present, the genus *Arcobacter* includes 23 species, some of them isolated from food. The species *A. butzleri*, *A. skirrowii*, *A. cryaerophilus* and *A. cibarius* have been directly associated with animal and human infections. Besides, *A. butzleri* and *A. cryaerophilus* are the most common species detected in food, and the number of resistant strains to quinolones is increasing, which represents an enormous challenge for public health. Nowadays the consumption of vegetables has grown because they are considered healthy food but the presence of *Arcobacter* in them is little known. All these factors motivated us to study the presence of *Arcobacter* in vegetables and the susceptibility to quinolones of the obtained isolates.

**Materials/Methods:** One-hundred vegetable samples were studied: 41 lettuces, 34 chard, 21 spinach and 4 cabbage. Selective enrichment of the samples was carried out using *Arcobacter* Broth supplement with Cephoperazone-AmphotericinB-Teicoplanin. After 48h incubation at 37°C under microaerophilic conditions, 100µl of the enrichment broth were placed onto the surface of *Arcobacter* agar plates supplemented with 5% sheep blood using a membrane filter. The plates were incubated at 30°C in aerobic conditions, finally the filters were removed and the plates were incubated at 37°C under microaerophilic conditions. Presumptive arcobacters were identified by specific genus PCR that amplifies a 331-bp fragment of 23S rRNA gene. Species identification was performed using the 16S rDNA-RFLP method described by Figueras et al. (2012). *Arcobacter* susceptibility to ciprofloxacin and levofloxacin were determined with the disc diffusion and E-test strips methods. The diameter of the inhibition zones in discs and the MICs were determined. Additionally, to clarify the mechanism of quinolone resistance, we analysed the region QRDR of *gyrA* gene because mutations in the *gyrA* gene of Gram-negative bacteria results in resistance to fluoroquinolones. A 344-bp fragment of this region was amplified, purified and then sequenced by IBMCP-UPV.

**Results:** *Arcobacter* spp. were isolated in 17 samples over the 100 vegetables samples. They were most detected in spinaches (8 of 21 samples) followed by chard (4 of 34), lettuces (4 of 41), and cabbages (1 of 4). A total of 25 isolates were obtained, 13 from spinach, 6 chard, 5 lettuces and 1 cabbage. By PCR, 17 of the isolates were identified as *A. butzleri* and 8 as *A. cryaerophilus*. All the *A. cryaerophilus* isolates and 15 *A. butzleri* isolates were susceptible to quinolones. However, two *A. butzleri* isolates showed antimicrobial resistance by disc diffusion method and E-test strips. The sequencing of the PCR product revealed the presence of a mutation in position 254 of *gyrA* gene (C-T transition) in the resistant isolates, while the sensitive isolates and the reference strain *A. butzleri* DSM 8739 showed no mutation.

**Conclusion:** The results of this study indicate high levels of *Arcobacter* contamination in vegetables. In addition, two of the isolates of *A. butzleri* were resistant to quinolones. This could pose a risk to public health because they are generally eaten raw.

**PO49 - Study Of Population Diversity Of *Toxoplasma gondii* Human Strains In Portugal**

Anabela Vilares<sup>1</sup>; Idalina Ferreira<sup>1</sup>; Susana Martins<sup>1</sup>; Maria João Gargaté<sup>1</sup>

<sup>1</sup>National Institute of Health Dr Ricardo Jorge, Department of Infectious Diseases, Reference Unit for Parasitic and Fungal Infections

**Background:** *Toxoplasma gondii* is an obligate intracellular protozoan parasite, which is responsible for toxoplasmosis in different species, including humans. It has been estimated that one third of the world population has been infected. In most human adults it does not cause serious illness, however, blindness and mental retardation can be caused in congenitally infected children and severe diseases or death in those with compromised immunity. *T. gondii* have a distinct clonal population structure composed of type I, II and III lineages in North America and Europe. But more recent studies demonstrated high diversity, recombinant and atypic strains (more virulent) in South America. The genotype *T. gondii* scenario is lacking in Portugal, except for three limited evaluations, not even the classical typing studies have been performed before in humans. In order to achieve this knowledge we studied the genetic diversity of *T. gondii* isolated from human products.

**Methods:** We studied 47 strains from congenital toxoplasmosis and one from a positive HIV with cerebral toxoplasmosis. Most isolates were from the central region of Portugal; however 25% were from north, 6.3% from south and 2% from Azores islands. All samples belong to the strain collection (since 1994 until 2013) of the National Reference Laboratory of Parasitic and Fungal Infections, Portuguese National Institute of Health (NIH). We performed 5 microsatellites (B17, B18, TgM-A, W35 and Tub2) multiplex PCR and a combination of Sanger sequencing and Next Generation Sequencing (NGS) of several loci likely responsible for virulence (Sag2, CB21-4, PK1, L363, Sag1, Gra6 and Sag3).

**Results:** We successfully sequenced the two ends of Sag2 (I, II, III) gene and this allowed the differentiation among the three "classical" *T. gondii* strain types. Thirty-five (73%) strains were type II and thirteen (27%) were type I. PCR multiplex microsatellites analysis and NGS were applied to all samples and were highly efficient. Microsatellites analysis identified ten (21%) recombinant strains and NGS approach allowed the identification of new and unexpected recombinant strains, also increased the extension of the diversity in *T. gondii* strains isolated in Portugal.

**Conclusion:** This work showed the first evidence of recombinant strains circulating in Portugal isolated from humans and also the first report of NGS in *T. gondii* strains isolated in Portugal. The combination of data and the high resolution genetic characterization, enhance our understanding of the molecular epidemiology and genetic variation of *T. gondii*, which will be ultimately beneficial for the control of *T. gondii* transmission.

## S4 - Poster session I

### PO50 - Identification Of Potential Amplicon Sequencing Targets For Identification And Strain-level Characterization Of Shiga Toxin-producing *Escherichia coli*

Lori Michelle Gladney<sup>1</sup>; Daniel Fasulo<sup>2</sup>; Alexandra Mercante<sup>1</sup>; Lisley Garcia-Toledo<sup>1</sup>; Rebecca Lindsey<sup>1</sup>; Eija Trees<sup>1</sup>; Nancy Strockbine<sup>1</sup>; Efrain Ribot<sup>1</sup>; Heather Carleton<sup>1</sup>; John Besser<sup>1</sup>

<sup>1</sup>*Centers for Disease Control and Prevention, <sup>2</sup> Pattern Genomics*

Shiga toxin-producing *Escherichia coli* (STEC) is an important foodborne pathogen. STEC infections have been diagnosed by culture and monitored by public health agencies using a variety of culture-based subtyping methods. Recently, culture-independent diagnostic tests (CIDTs) have been introduced in many clinical labs. CIDTs are rapid and cost-effective, but do not result in an isolate. To maintain public health surveillance activities, we are developing a PCR-based amplicon sequencing assay that may be used directly with complex samples such as stool.

A total of 342 genomes (104 STEC, 201 other *E. coli*, and 37 other *Enterobacteriaceae*) were screened to identify PCR targets that are conserved in, and unique to O157:H7 and present in single copy using Daydreamer™ software (Pattern Genomics).

Next, the O157:H7 Shiga toxin (*stx*) phage genome was explored to identify heterogeneous regions flanked by stable loci that may serve as potential amplifiable subtyping markers. A specific region of the phages was extracted from the antiterminator (N) to the phage lysis gene (R). Sequence alignment of the phages was performed using Mega5. Then, the alignments were trimmed to conserved ends (repressor CI to lysis gene S) and data were concatenated for strains that contain multiple phages. Phylogenetic trees were created using Mega5 and kSNP3.0. Strain differences were calculated using MuMmer 3.23. Lastly, the whole genome phylogeny of the host strains was compared to the phage genome phylogeny to see if their topologies were concordant.

We identified a single copy PCR target that is specific to O157:H7. The function is unknown; however, the target resides in an iron-sulfur cluster. We designed primers for three sets of amplicons that range from 67-91 bp. We performed *in silico* PCR on 854 genomes (including 145 O157) to access the overall successfulness of the design. The coverage of our primers was 97% while the accuracy was 100%. Four strains failed to amplify because they did not contain the target region.

We observed that the phylogeny based on the Shiga toxin phage regions (12-16 kb) in the O157:H7 strains does reflect the topology of the whole genome phylogeny and contains reasonable differences among strains (2-310 SNPs). There are also conserved regions to place primers which flank heterogeneous regions and at reasonable amplicon lengths. Primers can be designed so that amplicons overlap, are linked to *stx*, and may be sequenced using next generation sequencing. In addition, where there are multiple copy phages in a single host strain, they appear different enough that they would be unlikely to collapse with assembly.

Our data suggest that targets are present in the O157 phage genome that can be directly linked to *stx* and contain conserved and heterogeneous regions that can be exploited in the development of a culture-independent subtyping assay. We also report a single copy O157:H7 marker that can be used to infer mixed STEC infections and separate normal flora background through a statistical association of O157 copy numbers with virulence and antimicrobial resistance markers. Next, we plan to evaluate the heterogeneity in adjacent regions to *stx* which may be used to differentiate strains. The region with genetic differences that reflect epidemiological associations and the least number of amplicons will be selected. Lastly, we plan to evaluate the specificity of the conserved phage regions to all STEC and the lambdoid Shiga toxin phages.

## NOVEL TYPING AND DIAGNOSTIC METHODOLOGIES

**Thursday, 10 March 2016, 12:00-14:30**

### PO51 - Evaluation Of A Core Genome MLST Scheme For Typing Of Vancomycin-resistant Enterococci Using Pulsed-field Gel Electrophoresis As The Reference Method

Meyke Gillis<sup>1</sup>; Johanna Tien<sup>1</sup>; Danuta Stefanik<sup>1</sup>; Bozica Licanin<sup>1</sup>; Daniela Tacke<sup>2</sup>; Maria J.G.T. Vehreschild<sup>3</sup>; Paul G. Higgins<sup>1</sup>; Harald Seifert<sup>1</sup>

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### PO52 - Real-time Identification Of Carbapenem-resistant *Klebsiella pneumoniae* Outbreak Isolates By Fourier Transform Infrared (FTIR) Spectroscopy

Liliana Silva<sup>1</sup>; Carla Rodrigues<sup>2</sup>; Clara Sousa<sup>3</sup>; Agostinho Lira<sup>4</sup>; Mariana Leão<sup>4</sup>; Margarida Mota<sup>4</sup>; Paulo Lopes<sup>4</sup>; Angelina Lameirão<sup>4</sup>; Gabriela Abreu<sup>4</sup>; João Lopes<sup>5</sup>; Ângela Novais<sup>2</sup>; Luísa Peixe<sup>6</sup>

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### PO53 - New MLVA Schemes For *Streptococcus pyogenes* And *Streptococcus anginosus* Typing.

Katarzyna Obszańska<sup>1</sup>; Izabella Kern-Zdanowicz<sup>1</sup>; Izabela Sitkiewicz<sup>2</sup>

<sup>1</sup>Institute of Biochemistry and Biophysics, Polish Academy of Sciences,<sup>2</sup> National Medicines Institute

### PO54 - Use Of An Early Access MinION To Determine The Context Of Resistance Genes In *Escherichia coli* And *Acinetobacter baumannii*

Jane Turton<sup>1</sup>; Claire Perry<sup>1</sup>; Katie Hopkins<sup>1</sup>; Neil Woodford<sup>1</sup>

<sup>1</sup>Public Health England

## S5 - Poster session I

### PO55 - Metabolic Profiles Of ST131 *Escherichia coli* Subclones Depicted By Fourier-transform Infrared Spectroscopy Unveil Variability Among The H30 Subclone

Angela Novais<sup>1</sup>; Carla Rodrigues<sup>1</sup>; Clara Sousa<sup>2</sup>; Ana Constança Mendes<sup>3</sup>; João A Lopes<sup>4</sup>; Teresa M Coque<sup>5</sup>; Luísa Peixe<sup>1</sup>

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### PO56 - Recovering Full-length Human Papillomavirus Genomes From Cervical Swab Samples Using Benchtop Next Generation Sequencing.

Joël Mossong<sup>1</sup>; Jessica Tapp<sup>1</sup>; Ardashel Latsuzbaia<sup>1</sup>; Steven Weyers<sup>2</sup>; Marc Arbyn<sup>3</sup>; Trung Nguyen<sup>1</sup>; Marc Fischer<sup>1</sup>; Dag Harmsen<sup>4</sup>; Catherine Ragimbeau<sup>1</sup>

<sup>1</sup>Laboratoire National de Santé,<sup>2</sup> Ghent University Hospital,<sup>3</sup> Scientific Institute of Public Health,<sup>4</sup> University Münster

### PO58 - INNUENDO: A Cross-sectorial Platform For The Integration Of Genomics In Surveillance Of Food-borne Pathogens

Mirko Rossi<sup>1</sup>; João Carriço<sup>2</sup>; Javier Garaizar<sup>3</sup>; Friederike Hilbert<sup>4</sup>; Saara Salmenlinna<sup>5</sup>; Marjaana Hakkinen<sup>6</sup>; Monica Oleastro<sup>7</sup>; Mihkel Mäesaar<sup>8</sup>; Aivars Bērziņš<sup>9</sup>; INNUENDO consortium<sup>10</sup>

<sup>1</sup>University of Helsinki,<sup>2</sup> University of Lisbon,<sup>3</sup> Universidad del País Vasco/Euskal Herriko Unibertsitatea,<sup>4</sup> University of Veterinary Medicine, Vienna,<sup>5</sup> National Institute for Health and Welfare,<sup>6</sup> Finnish Food Safety Authority,<sup>7</sup> Instituto Nacional de Saúde Dr. Ricardo Jorge,<sup>8</sup> Veterinary and Food Laboratory,<sup>9</sup> Pārtikas drošības, dzīvnieku veselības un vides zinātniskais institūts,<sup>10</sup> na

### PO59 - Comparison Of Typing Methods For Hospital-associated *Acinetobacter baumannii* Clones Isolated In Greece And India

Basil Britto Xavier<sup>1</sup>; Jasmine Coppens<sup>1</sup>; Lavanya Vanjari<sup>2</sup>; Konstantina Dafopoulou<sup>3</sup>; Julia Sabirova<sup>1</sup>; Christine Lammens<sup>1</sup>; Olympia Zarkotou<sup>4</sup>; Trevor Wagner<sup>5</sup>; Athanassios Tsakris<sup>3</sup>; Spyros Pournaras<sup>6</sup>; Herman Goossens<sup>1</sup>; Surbhi Malhotra-Kumar<sup>1</sup>

<sup>1</sup>University of Antwerp,<sup>2</sup> National Institute of Tuberculosis,,<sup>3</sup> Department of Microbiology, University of Athens,,<sup>4</sup> Department of Microbiology, Tzaneio General Hospital,,<sup>5</sup> OpGen, Inc.,<sup>6</sup> Department of Microbiology, Medical School, National and Kapodistrian University of Athens

**PO61 - Development And Evaluation Of Double Locus Sequence Typing For Molecular Epidemiological Investigations Of *Clostridium difficile***

Miloš Stojanov<sup>1</sup>; Bàrbara Magalhaes<sup>2</sup>; Valery Terletsky<sup>1</sup>; Patrick Basset<sup>1</sup>; Guy Prod'hom<sup>1</sup>; Gilbert Greub<sup>1</sup>; Laurence Senn<sup>1</sup>; Dominique Blanc<sup>1</sup>

<sup>1</sup>Lausanne University Hospital,<sup>2</sup> Valery Terletsky

**PO62 - Mass Spectrometry Proteotyping For Detection, Identification Characterization And Diagnostics Of Infectious Bacteria In Clinical Respiratory Samples**

Lucia Gonzales Siles<sup>1</sup>; Roger Karlsson<sup>1</sup>; Chantal van Houten<sup>2</sup>; Louis Bont<sup>2</sup>; Fredrik Boulund<sup>3</sup>; Erik Kristiansson<sup>3</sup>; Edward Moore<sup>1</sup>

<sup>1</sup>University of Gothenburg,<sup>2</sup> University Medical Center Utrecht,<sup>3</sup> Chalmers University

**PO63 - Comparison Of MLST, rMLST And wgMLST As Global Typing Schemes For *S. Enterica***

Madison Pearce<sup>1</sup>; Martin Maiden<sup>2</sup>; Kathie Grant<sup>3</sup>; James Bray<sup>2</sup>; Keith Jolley<sup>2</sup>; Claire Jenkins<sup>3</sup>; Tim Dallman<sup>3</sup>; Philip Ashton<sup>3</sup>

<sup>1</sup>University of Oxford/Public Health England/ NIHR HPRU,<sup>2</sup> University of Oxford,<sup>3</sup> Public Health England

**PO64 - On The Road Of Transition – From PFGE To NGS Based Strain Typing**

Elke Brockmann<sup>1</sup>; Jacob Bælum<sup>1</sup>; Mads Bennedsen<sup>1</sup>; Annette Møller<sup>1</sup>; Birgitte Stuer-Lauridsen<sup>1</sup>; Martin Abel-Kilstrup<sup>1</sup>; Anette Wind<sup>1</sup>

<sup>1</sup>Chr. Hansen A/S

**PO65 - Whole Genome Sequencing Of Fecal Samples As A Tool For The Diagnosis And Genetic Characterization Of Norovirus**

Herjan Bavelaar<sup>1</sup>; Janette Rahamat-Langendoen<sup>1</sup>, Hubert G.M. Niesters<sup>2</sup>, Jan Zoll<sup>1</sup>, Willem J.G. Melchers<sup>1</sup>

<sup>1</sup>Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, The Netherlands,<sup>2</sup> Department of Medical Microbiology, Division of Clinical Virology, University Medical Center Groningen, Groningen, The Netherlands

**PO66 - Plasmablasts After Antigen Challenge - A Diagnostic Tool For Assessing Response To Influenza Vaccination In Patients With Chronic Lymphocytic Leukemia**

Ewelina Grywalska<sup>1</sup>

<sup>1</sup>Medical University of Lublin

## S5 - Poster session I

### PO67 - Discrimination Of Vancomycin-resistant *Enterococcus faecium* Clones By MLST (eBURST/BAPS) And Spectroscopic Techniques (MALDI-TOF MS And FTIR-ATR)

Ana R. Freitas<sup>1</sup>; Clara Sousa<sup>2</sup>; Jan Baylovič<sup>3</sup>; Liliana Silva<sup>4</sup>; Carla Novais<sup>1</sup>; Teresa M. Coque<sup>5</sup>; João Lopes<sup>6</sup>; Luísa Peixe<sup>1</sup>

<sup>1</sup>UCIBIO/REQUIMTE. Departamento de Ciências Biológicas. Laboratório de Microbiologia. Faculdade de Farmácia. Universidade do Porto.,<sup>2</sup> CEB-Centro de Engenharia Biológica, Universidade do Minho.,<sup>3</sup> Faculty of Pharmacy in Hradec Králové, Charles University, Prague and Faculty of Military Health Sciences, University of Defence, Brno, Czech Republic,<sup>4</sup> UCIBIO/REQUIMTE. Departamento de Ciências Biológicas. Laboratório de Microbiologia. Faculdade de Farmácia. Universidade do Porto,<sup>5</sup> ESALD, Instituto Politécnico de Castelo Branco,<sup>6</sup> Servicio de Microbiología. Hospital Ramón y Cajal. Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS),<sup>7</sup> iMed, Departamento de Farmácia Galénica e Tecnología Farmacéutica, Faculdade de Farmácia, Universidade de Lisboa

### PO68 - Development Of A Conventional Multiplex PCR For Identification Of *Escherichia coli*, *Escherichia fergusonii*, And *Escherichia albertii*

Garcia Toledo, Lisley<sup>1</sup>; Gladney, L.M<sup>2</sup>; Fasulo, D.<sup>3</sup>; Strockbine, N<sup>4</sup>; Carleton, H<sup>4</sup>; Lindsey, R. L.<sup>4</sup>

<sup>1</sup>Oak Ridge Institute for Science and Education,<sup>2</sup>Centers for Disease Control and Prevention,<sup>3</sup> IHRC, Inc,<sup>4</sup> Centers for Disease Control and Prevention,<sup>5</sup> Pattern Genomics, LLC,<sup>6</sup> Enteric Diseases Laboratory Branch, Centers for Disease Control and Prevention

### PO69 - A Comparison Of Diversilab rep-PCR And cgMLST For Molecular Typing Of Carbapenem-resistant *Acinetobacter baumannii* Isolates From Greece And Spain

Paul G Higgins<sup>1</sup>; Stefanie Gerson<sup>1</sup>; Jennifer Nowak<sup>1</sup>; Esther Zander<sup>1</sup>; Harald Seifert<sup>1</sup>

<sup>1</sup>University of Cologne

### PO70 - Multilocus Sequencing Typing As A Tool To Investigate Childhood *Haemophilus influenzae* Invasive Disease In Portugal

Célia Bettencourt<sup>1</sup>; Vitor Borges<sup>1</sup>; José Gonçalo Marques<sup>2</sup>; Florbela Cunha<sup>3</sup>; Maria Paula Bajanca-Lavado<sup>1</sup>

<sup>1</sup>Instituto Nacional de Saúde Dr. Ricardo Jorge,<sup>2</sup> Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, EPE,<sup>3</sup> Hospital de Vila Franca de Xira

### PO71 - Cost Effective Strategy For Typing *Brucella abortus* In An Endemic Scenario

Giuliano Garofolo<sup>1</sup>; Ilenia Platone<sup>1</sup>; Katiuscia Zilli<sup>1</sup>; Anna Abass<sup>1</sup>; Elisabetta Di Giannatale<sup>1</sup>

<sup>1</sup>Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise

**PO73 - Mixed *Mycobacterium tuberculosis* Infections In Switzerland Identified With Whole Genome Sequencing**

David Stucki<sup>1</sup>; Marie Ballif<sup>2</sup>; Hans-Jakob Furrer<sup>3</sup>; Kathrin Zürcher<sup>2</sup>; Matthias Egger<sup>2</sup>; Sébastien Gagneux<sup>1</sup>; Lukas Fenner<sup>4</sup>

<sup>1</sup>Swiss Tropical and Public Health Institute, University of Basel,<sup>2</sup> Institute of Social and Preventive Medicine, University of Bern,<sup>3</sup> Clinic for Infectious Diseases, Bern University Hospital, and University of Bern,<sup>4</sup> Institute of Social and Preventive Medicine, University of Bern, and Swiss Tropical and Public Health Institute, University of Basel

## S5 - Poster session I

### PO51 - Evaluation Of A Core Genome MLST Scheme For Typing Of Vancomycin-resistant Enterococci Using Pulsed-field Gel Electrophoresis As The Reference Method

Meyke Gillis<sup>1</sup>; Johanna Tien<sup>1</sup>; Danuta Stefanik<sup>1</sup>; Bozica Licanin<sup>1</sup>; Daniela Tacke<sup>2</sup>; Maria J.G.T. Vehreschild<sup>3</sup>; Paul G. Higgins<sup>1</sup>; Harald Seifert<sup>1</sup>

<sup>1</sup>Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne,<sup>2</sup> Department of Anesthesiology and Intensive Care Medicine, University Hospital of Cologne,<sup>3</sup> Department I for Internal Medicine, University Hospital of Cologne

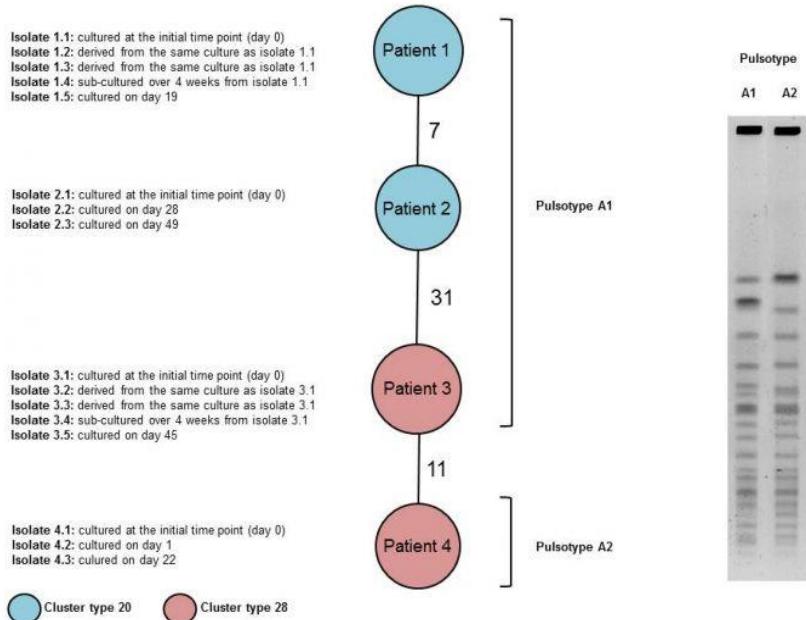
**Background:** Colonization and infection with vancomycin-resistant enterococci (VRE) is an emerging problem in patients suffering from hematological malignancies and among ICU patients. To better control the nosocomial spread of VRE transmission analysis among VRE colonized and infected patients is of fundamental importance. However, VRE has shown a propensity for recombination which makes whole-genome sequencing (WGS) and single nucleotide polymorphism (SNP) analysis difficult to interpret. A core genome MLST (cgMLST) scheme for typing of *Enterococcus faecium* has been recently published (doi:10.1128/JCM.01946-15) and the objective of this study was to evaluate it among VRE isolates presenting with similar PFGE-patterns.

**Materials | Methods:** VRE isolates were collected from colonized patients during active surveillance (rectal swabs/stool specimens) at the University Hospital Cologne. According to PFGE patterns, VRE isolates were selected for WGS and cgMLST analysis. Overall we evaluated 16 VRE isolates from 4 different patients: Patients 1 and 3 had 5 isolates each. Three isolates were derived from the same sample (i.e., 3 VRE colonies were picked from the same agar plate). One isolate was collected at a distinct time point. The remaining isolate was obtained from the initial culture and sub-cultured over a 4-week period at one-week intervals. Patients 2 and 4 had 3 isolates each taken at different time points. Patients 1, 2 and 3 displayed an identical pulsotype (pulsotype A1). Patient 4 presented with a slightly different pulsotype (pulsotype A2; i.e., 4-5 band differences). To identify clonally related VRE isolates cgMLST using SeqSphere<sup>+</sup> version 3.0.0 was interpreted by the following criteria: 0 allelic differences = isolates indistinguishable; 1-20 differences = closely related; 21-40 differences = possibly related; >40 differences = most likely unrelated.

**Results:** Results of cgMLST are summarized in figure 1. In intra-individual isolates, no allele differences were detected at the same time point, at different time points and after being subcultured. With regard to VRE isolates from different patients, isolates from patients 1 and 2 showed 7 allele differences, whereas isolates from patient 3 exhibiting identical PFGE patterns to those from patient 2 displayed 31 allele differences when compared to isolates from patient 2. Of note, isolates from patient 4 were separated by only 11 allelic differences from those of patient 3, although displaying a slightly variant pulsotype compared to the isolates recovered from other patients. Moreover, cgMLST divided isolates into 2 different cluster types based on allelic differences: Isolates from patients 1 and 2 belonged to cluster type 20, while isolates from patients 3 and 4 corresponded to cluster type 28.

**Conclusion:** Intra-individually, cgMLST correlated well with PFGE patterns. However, when comparing both methods inter-individually, allelic differences seen by cgMLST were not necessarily reflected by different PFGE patterns: VRE isolates were interpreted as only possibly related according to the number of allelic differences but showed indistinguishable PFGE patterns. We therefore conclude that the cgMLST scheme evaluated in our study allows us to further subdivide VRE isolates within the same pulsotype and therefore might potentially provide a more accurate analysis of clonal relatedness among clinical VRE when compared to conventional PFGE analysis.

Figure 1: Minimum spanning tree built from cg MLST analysis of 16 clinical VRE isolates compared to their PFGE patterns.



## S5 - Poster session I

### PO52 - Real-time Identification Of Carbapenem-resistant *Klebsiella pneumoniae* Outbreak Isolates By Fourier Transform Infrared (FTIR) Spectroscopy

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**Background:** The rapid emergence and spread of multidrug resistant (MDR) bacteria causing hospital-acquired infections demands a rapid and accurate control of nosocomial outbreaks. DNA-based approaches (mainly PFGE, MLST or whole genome sequencing) are commonly used for bacterial typing, nonetheless these methods are still expensive and/or time-consuming. We recently demonstrated that FTIR-ATR (attenuated total reflectance) spectroscopy coupled with multivariate data analysis (MDA) has a very good potential for strain discrimination in different species, including in *K. pneumoniae* [1]. Thus, we aimed to assess the ability of this methodology to assist infection control in real-time, in the context of a nosocomial outbreak of carbapenemase-producing *K. pneumoniae*.

**Materials/Methods:** We received 24 *K. pneumoniae* from infected or colonized patients of different wards of a Portuguese hospital. Clonal relationships were established by FTIR-ATR spectroscopy and further compared with reference methods (*Xba*I-PFGE, MLST). Isolates were grown on Mueller-Hinton agar (37°C, 18h) and directly applied on the ATR crystal. Spectra comparison (each other and with those included in an in-house MDR *K. pneumoniae* clones database) was performed by principal component analysis (PCA), considering the phospholipids/DNA/RNA/carbohydrates region (1500-900 cm<sup>-1</sup>). Carbapenemase production was assessed by Blue-Carba, and confirmed by PCR and sequencing. Genetic context of *bla*<sub>KPC</sub>, plasmid profile and capsule typing (*wzi*) were investigated by PCR and sequencing. Antimicrobial susceptibility patterns were assessed by disc diffusion/agar microdilution methods, according to the EUCAST/CLSI guidelines.

**Results:** Using FTIR spectroscopy analysis we were able in less than 48h to assess clonal relatedness of isolates involved in the outbreak: 22 *K. pneumoniae* isolates and the international ST147 clone clustered together, whereas the remaining 2 strains were unrelated. These results were corroborated by PFGE (identical PFGE-patterns) and MLST (ST147), with all outbreak isolates carrying *wzi*64 (capsule type K14.K64). Additional characterization revealed that ST147 isolates produced KPC-3 (linked to Tn4401d transposon variant and IncFIA plasmid). The other two clones depicted by FTIR spectroscopy corresponded to a *K. pneumoniae* ST336 carrying *wzi*150 producing KPC-3 (linked to Tn4401b transposon variant) and a *K. pneumoniae* ST348 harboring *wz*94 which is a non-carbapenemase producer (Blue-Carba negative). All isolates exhibited MDR phenotypes and were susceptible, at least, to amikacin and colistin.

**Conclusion:** In this study, a complete concordance was observed between FTIR-ATR spectroscopy coupled with MDA and the reference typing methods (MLST, PFGE) for strain discrimination. Our results suggest that this methodology is suitable for a reliable, quick and low cost identification and

typing of MDR *K. pneumoniae* clones that could revolutionize the speed of bacterial typing in the context of nosocomial outbreaks and hospital surveillance, and consequently have a strong impact on the efficiency of infection control measures and therapeutic choices.

**Reference:** Rodrigues C, Branquinho R, Sousa C, Andrade LN, Machado E, Darini AL, Novais Â, Peixe L. Identification of diverse capsular types in multidrug-resistant (MDR) *Klebsiella pneumoniae* clones using *wzi* sequencing and FTIR. 6th Congress of European Microbiologists (FEMS 2015), Maastricht, 2015.

## S5 - Poster session I

### PO53 - New MLVA Schemes For *Streptococcus pyogenes* And *Streptococcus anginosus* Typing.

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**Background:** Molecular typing methods – from general, to methods of high resolution, are very important epidemiological tools. They can be used to track spreading of global pathogenic clones, as well as clones within outbreaks. One of the high resolution methods is PCR based detection of variable number tandem repeats (VNTR) such as MLVA/MLVF method. Number of tandem repeats detected for each of several loci is characteristic trait of a strain and analysis of multiple loci can be used to determine relation between strains. It has been successfully adopted for both Gram-positive and Gram-negative bacteria. The analysis is easy to perform, fast and interpretation of the results is rather straightforward.

Many streptococcal species, including *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae* and *Streptococcus anginosus* are human pathogens which cause life threatening infections. For *S. pneumoniae* and *S. agalactiae*, MLVA/MLVF methods were already introduced. The aim of this work was development of *S. pyogenes* and *S. anginosus* MLVA/MLVF typing methods.

**Methods:** Genomes of *S. pyogenes* and of *S. anginosus*, available at NCBI and provided by our sequencing laboratory, were explored using Tandem Repeats Finder (<http://tandem.bu.edu/trf/trf.html>). Search generated over 250 putative loci for each species that could be polymorphic. Putative VNTR loci were screened *in silico* to fulfill set criteria (longer than 15 bp and more than 2 repeated units). As a next step we screened by PCR our collections of *S. pyogenes* and of *S. anginosus* to experimentally detect loci exhibiting size variants. After PCR screen we selected seven and nine primer pairs, for *S. pyogenes* and *S. anginosus*, respectively, to compose multiplex PCR reactions. Using created multiplexes we examined over 700 *S. pyogenes* and 90 *S. anginosus* strains to assess the method resolution.

**Results:** Each of selected primer pairs detected from two to fourteen size variants. Theoretically, based on typing using seven and nine loci, we are able to uncover over forty thousand and over half a million different patterns, for *S. pyogenes* and for *S. anginosus*, respectively. Practically, we found almost 150 patterns among *S. pyogenes* and 70 patterns among *S. anginosus* strains. We detected distinct patterns among quite homogenous groups of strain such as M3 *S. pyogenes* isolates, and also different variants of M1 strains that typed as identical using other methods. *S. anginosus* strains, that were similar as shown by RFLP-PFGE patterns, also clustered into similar groups using MLVF analysis.

**Conclusion:** We developed simple, little time-consuming and interpretation-friendly molecular typing methods for two pathogenic streptococcal species, *S. pyogenes* and *S. anginosus*. Designed methods have discriminatory power as high as or even higher than RFLP-PFGE.

The work was supported by grant N N401 535940 and grant N N401 536140 from National Science Center (NCN), Internal funding (DS 5.67 and DS 5.82), NPOA-Moduł1 grant from Ministry of Health and SPUB-MIKROBANK2 grant from Ministry of Science and Higher Education for strain collection and maintenance.

**PO54 - Use Of An Early Access MinION To Determine The Context Of Resistance Genes In *Escherichia coli* And *Acinetobacter baumannii***Jane Turton<sup>1</sup>; Claire Perry<sup>1</sup>; Katie Hopkins<sup>1</sup>; Neil Woodford<sup>1</sup><sup>1</sup>Public Health England

**Background:** Resistance genes are often in close proximity to insertion sequences and transposons that are found in multiple places in the genome, making determination of their context difficult using PCR and short-read sequencing methods. Nanopore sequencing provides long reads (often  $\geq 10$  kb and up to 65 kb or more) giving the potential to definitively link the elements in resistance islands or plasmids and sometimes sequence across the whole element in a single read. Here we used an early access minION to determine the context of *bla*<sub>OXA-48</sub> in representatives of *Escherichia coli* belonging to ST38 and of *bla*<sub>OXA-23</sub> in representatives of *Acinetobacter baumanii* belonging to 'OXA-23 clone 1', a sub-lineage of international clone II, both of which are currently prevalent types carrying these genes in the United Kingdom.

**Materials | Methods:** Libraries were prepared from genomic DNA as recommended by Oxford Nanopore Technologies using a SQK-MAP005 Genomic Sequencing kit and New England Biolabs reagents and loaded onto an early access minION. Reads were basecalled by Metrichor™ analysis and the '2D' pass reads combined with available Illumina reads in a SPAdes 3.1.1 hybrid assembly. The position of resistance genes was determined from the contigs generated and from individual long reads. The context of these genes in further representatives was determined using primers designed from chromosome/resistance element junctions of sequenced isolates.

**Results:** The nanopore sequencing runs provided 2D pass yields of up to 1108 Mb in a single 48 h run with read lengths of up to 65 kb. Contigs generated from hybrid minION/Illumina assemblies containing the resistance genes sought were up to 317 kb in length. It was clear from the long reads that the two representatives of *E. coli* ST38 sequenced carried IS1R-mediated chromosomally integrated fragments of the commonly found pOXA-48a IncL/M plasmid, rather than the plasmid itself. PCR of the chromposome/plasmid fragment junctions in further representatives ( $n=43$ ) confirmed that the insertion site and length of plasmid fragment varied between isolates, but was consistent among many representatives of a homogenous cluster that had originally been identified by pulsed-field gel electrophoresis and confirmed by SNP analysis and had been referred from 25 hospital laboratories across the UK; there appeared to be a variable extent of deletion of *E. coli* sequence on one side of the insertion site in many of these isolates. A representative of OXA-23 clone 1 of *A. baumannii* from 2005 carried an AbaR4 type island containing *bla*<sub>OXA-23</sub> in Tn2006 inserted in *comM*, but there was an additional 8 kb of sequence between two copies of a gene coding for a hypothetical protein that had not been identified from an earlier determination of the sequence using shorter read technology (Roche 454 sequencing) and PCR. An isolate from 2015 carried AbaR4 type islands in both *comM* and on a plasmid.

**Conclusion:** The long reads generated by the minION nanopore sequencing technology have been critical in allowing us to determine the sequence and context of resistance islands and transposons carrying resistance genes in *A. baumannii* and *E. coli*. This work has highlighted that the successful ST38 lineage of *E. coli*, which is the main type carrying *bla*<sub>OXA-48</sub> in the UK, has chromosomally integrated fragments of the pOXA-48a plasmid, and that recent isolates of OXA-23 clone 1 of *A. baumannii* carry *bla*<sub>OXA-23</sub> in both chromosomal and plasmid locations.

## S5 - Poster session I

### PO55 - Metabolic Profiles Of ST131 *Escherichia coli* Subclones Depicted By Fourier-transform Infrared Spectroscopy Unveil Variability Among The H30 Subclone

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**Background:** *Escherichia coli* ST131 has increasingly been reported in the last decade and became the predominant *E. coli* lineage isolated from human extraintestinal infections at global level. Comprehensive analysis of large collections of isolates by molecular techniques including whole-genome sequencing approaches, revealed a polyclonal population structure, some subclones differing in virulence and antibiotic resistance profiles as well as *fimH* alleles exhibiting variable epidemiological features (e.g. H30 subclone, containing allele 30 of *fimH*). The potential of Fourier-Transform Infrared (FTIR) spectroscopy coupled with multivariate data analysis (MDA) to discriminate ST131 subclones is evaluated in this study.

**Materials and Methods:** Sixty-four well characterized ST131 *E. coli* isolates from Portugal and Spain (1996-2014) were tested. These isolates were responsible for community or hospital-acquired infections in different clinical institutions or were identified as colonizers of the gastrointestinal tract of healthy volunteers or household patients and represent main *E. coli* subclones *fimH30* (n=46), *fimH22* (n=6), *fimH21* (n=6) and *fimH41* (n=6). Spectra acquired from individual colonies in FTIR-ATR (attenuated total reflectance) spectroscopy were compared by partial least squares discriminant analysis (PLSDA) using MATLAB 8.3 and the PLS toolbox 7.5 versions, considering the phospholipids/DNA/carbohydrates region (1500-900 cm<sup>-1</sup>). Additional characterization included identification of *gyrA/parC* mutations, *bla<sub>ESBL</sub>* and ExPEC virulence genes by PCR or PCR and sequencing.

**Results:** In the first PLSDA-based model (model 1), we were able to discriminate all strains belonging to the *fimH41* subclone (variable *gyrA/parC* mutations and *bla<sub>CTX-M-1/14</sub>* carriage) and a subset of strains of the *fimH30* subclone (n=12, including strains causing infection or colonizing humans which were identified in Portugal between 2007 and 2014), hereby designated as *fimH30-A*. Most *fimH22* isolates (n=5/6) were subsequently discriminated from *fimH21* (variable *gyrA/parC* alleles and *bla<sub>CTX-M-14/TEM-4/-24</sub>* carriage) and the remaining *fimH30* isolates (n=34, hereby designated as *fimH30-B*) (model 2). Finally, *fimH21* isolates were discriminated from *fimH30-B* (model 3). Both *fimH30-A* and *fimH30-B* isolates show common mutations in GyrA (S83L, D87N) and ParC (S80I, E84V) typical from the H30 lineage, and differ in the content of ESBL (*bla<sub>CTX-M-15</sub>*, *bla<sub>CTX-M-14</sub>* or *bla<sub>CTX-M-27</sub>*) or virulence genes. Interestingly, metabolomics data revealed two *fimH30* subclones (*H30-A*, *H30-B*) not yet depicted by genomic-based approaches.

**Conclusion:** We demonstrate for the first time the high discriminatory power of FTIR spectroscopy coupled with MDA for subclonal typing of ST131 *E. coli*, which constitutes a reliable and cost-efficient alternative for tracking clinically relevant bacteria in clinical or epidemiological contexts. Interestingly, this methodology unveiled metabolically distinct *fimH30* variants that need to be further investigated by complementary approaches (e.g. whole genome sequencing and/or proteomics approaches).

**PO56 - Recovering Full-length Human Papillomavirus Genomes From Cervical Swab Samples Using Benchtop Next Generation Sequencing.**

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**Background:** Cervical cancer is caused by persistent infection of oncogenic high risk human papillomavirus (HPV) genotypes. While detection and genotyping of HPV in cervical swab samples is mostly performed using validated commercial PCR-based methods, benchtop next generation sequencing has the potential to be a cost-efficient alternative means to detect genotypes not covered by commercial assays and to recover full-length viral genomes for monitoring emergence of potential vaccine escape variants.

The objective of our study was to test the feasibility of next generation sequencing for detecting 179 HPV types and for recovering full-length genomes of multiple types present in cervical swabs.

**Materials & Methods:** The study sample consisted of 6 cervical swab samples concordantly positive for high-risk papillomavirus by 2 genotyping assays (Eurolmmune EuroArray, Seegene Anyplex II HPV28) following DNA extraction. By the Anyplex and EuroArray assays, 3 samples were concordantly positive for single infection by type 16 and 3 samples were concordantly positive for multiple HPV infection (types 18-58, 16-18-53-58-66, 16-40-42-43-56, respectively).

Following rolling circle amplification which amplifies circular viral DNA against background linear human DNA, samples were sequenced using the Nextera XT library preparation kit on a Illumina MiSeq benchtop sequencer. Raw reads were mapped using the BWA-SW algorithm to 179 known full length HPV genomes downloaded from the PAVE database and were *de novo* assembled using VELVET. All bioinformatic analysis was performed using SeqSphere+ software (Ridom, Münster, Germany) on a standard Microsoft Windows 7 desktop PC (16GB memory, i5 Core).

**Results:** Five of the 6 samples yielded reads mapping to HPV reference genomes. In these 5 samples, the median proportion of reads mapping to HPV reference genome collection was 5% (range 2.1%-23.7%). NGS detected all the genotypes that were concordantly detected by the 2 commercial assays with a median coverage of 151x (range 2x-2178x). NGS detected additional genotypes 34, 34-90 and 90-91 in the 3 samples with multiple infections. *De novo* assembly recovered 9 full-length HPV genomes of genotypes 16, 18, 34, 53, 58, respectively, from a total of 20 HPV genotypes detected (full length genome recovery rate: 45%).

**Conclusion:** Benchtop next generation sequencing provides a feasible alternative for detecting HPV genotypes that are not detectable by current commercial assays, while simultaneously enabling the recovery of full length HPV genomes. Further wet and dry lab work is required to enhance recovery of viral metagenomic DNA and optimise bioinformatics tools. The significance of full length genome information in relation to cervical disease and vaccine impact remains to be investigated.

## S5 - Poster session I

### PO58 - INNUENDO: A Cross-sectorial Platform For The Integration Of Genomics In Surveillance Of Food-borne Pathogens

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Multinational outbreaks of food-borne pathogens cause considerable threats to European public health. An increased level of cooperation between the local, national and European competent authorities by dedicated training, development of a common pathogen database and validation of new approaches in microbial characterization is warranted. Implementing whole genome sequencing (WGS) in routine surveillance and outbreak investigations is becoming a strategic goal for many public health authorities all over the world. However, small countries with limited resources might not be able to succeed in reaching this goal in the near future, putting several EU member states in a condition of inferior capabilities for outbreak detection and investigation. Therefore, to guarantee the reinforcement of European capacities to ensure protection of citizens against cross border health threats, EU must enable wider access to the new methodologies.

The potential of widespread, routine use of WGS analysis for public health protection is essentially restricted by the absence of accessible IT framework, and the limited skills of public health microbiologists in handling these novel methodologies. With this in mind we developed an initiative aiming to deliver a cross-sectorial framework of bacterial WGS integration in routine surveillance and epidemiological investigations. The aim of this project is aligned with the EFSA mission to promote the development and validation of new approaches in microbial characterization throughout coordinate efforts between all public health and food safety stakeholders. To design an affordable and sustainable diagnostic infrastructure, our consortium includes governmental organisations, authorities and research institutes from the food, veterinary and human sectors, from small countries such as Finland, Estonia, Latvia, Portugal, Basque Autonomous Community in Spain and Austria. Allowing project stakeholder to assess design choices early on in the development cycle, this multinational collaboration in the 'One-health' context will ensure that the planned infrastructure will address the requirement for the integration of WGS in routine analysis in the food chain.

Standardization and calibration of process and simplification of data analysis and interpretation are the two basic conditions allowing the transition from the current diagnostic paradigm to a full WGS consolidation. Moreover, the lack of accessible informatics infrastructure for data processing and integration is still the major obstacle to implementation of WGS. Thus, it is now essential that advances in bioinformatics and bacterial genomics encounter the needs of the public health microbiology. This goal is reachable only throughout an integration of competencies across different disciplines and professions. Therefore, throughout active cooperation between experts in bacterial genomics, evolution, bioinformatics, epidemiology and specialists in validation, food control and public health, our consortium will use a cross-sectorial approach in developing a common framework for maximizing the benefit to use WGS in food safety.

**PO59 - Comparison Of Typing Methods For Hospital-associated *Acinetobacter baumannii* Clones Isolated In Greece And India**

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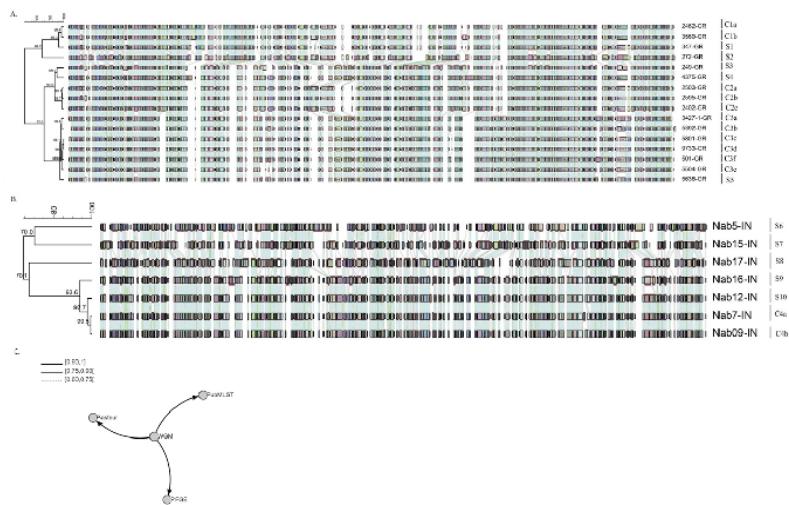
**Introduction:** Hospital outbreaks caused by multidrug-resistant *Acinetobacter baumannii* epidemic global clones such as GC1 and GC2 are a major problem. We utilized three different typing methods pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), and whole genome mapping (WGM) to understand the genetic diversity of hospital-associated *A. baumannii* isolated from two geographically distinct regions (Greece and India) over seven years.

**Methods:** Twenty-three *A. baumannii* were isolated from patients admitted to ICU (n=19), surgery (n=2) and medical (n=2) wards in Greece (Tzaneio Hospital n=13, General hospital Serres n=1, and University hospital Larissa n=2), and in India (Nizam's Institute of Medical Sciences n=7) during 2008-2014. These were screened for resistance to 21 antibiotics ( $\beta$ -lactams with and without  $\beta$ -lactamase inhibitors and carbapenems), by disk diffusion and results were interpreted according to CLSI cut-offs (Table 1). All strains were typed by MLST (PubMLST and Pasteur), PFGE and WGM. Restriction analysis for PFGE was performed using *Apal* using a 7-band and a 3-band difference cut-off to define types and subtypes, respectively. Strains were subjected to WGM (Argus® system, OpGen Inc, Gaithersburg, USA) using *Ncol*. Maps were edited and compared using BioNumerics v7.1 (Applied Maths, Belgium).

**Result:** Antibiotic susceptibility test showed that multi-drug resistance (resistance to  $\beta$ -lactams with and without  $\beta$ -lactamase inhibitors) was detected among all 23 *A. baumannii* strains. Of the 16 Greek strains, *bla*<sub>OXA-23</sub> was detected in 7 and *bla*<sub>OXA-58</sub> in 9 strains. *bla*<sub>NDM-1</sub> was detected in 1 Indian strain, Nab5. MLST (Pasteur) assigned 19 of the 23 strains to ST2 (GC2) and the other 4 isolates to 4 different STs (645, 85, 646 and 82). On the other hand, PubMLST assigned the 23 strains to 13 STs. ST2 (Pasteur) strains were assigned to 10 different PubMLST types. PFGE results showed four different types. Based on a  $\geq$  7-band difference, all Greek and 4 Indian strains (Nab7, Nab9, Nab12 and Nab16) belonged to 1 PFGE type and were divided into 9 subtypes (1a-1i,  $\geq$  3-band difference). The remaining three Indian strains (Nab5, Nab15 and Nab17) were singletons. Based on a 95% similarity co-efficient, WGMs of the 16 Greek strains were divided into 3 clusters and 5 singletons. Overall similarity between the Greek strains was 71.3% (Figure 1a). On the other hand, WGMs of Indian strains isolated over a 5-month period during 2013-14 and from a single hospital were highly diverse. These 6 strains from one cluster and 4 singletons with an overall similarity of 36.2%, (Figure 1b). STs assigned by Pasteur MLST correlated well with PFGE. Application of the adjusted Wallace index showed that WGM clusters were strongly predictive of both MLSTs and PFGE types (Wallace co-efficient of 1.00 for both) (Figure 2). Simpson's diversity index showed that discriminatory power of WGM (1.000, 95% CI: 0.976-0.99) was higher than that of PFGE (0.893, 95% CI: 0.801-0.986) and PubMLST (0.913, 95% CI: 0.857-0.970)

**Conclusion:** Comparison of the high resolution typing technique WGM to other typing methods (MLST and PFGE) showed its superior discriminatory power for analysis of rapidly evolving hospital-associated *A. baumannii* global clones. The high diversity among the Indian strains studied here was noteworthy.

## S5 - Poster session I



Strain ID	Isolation date	Isolation place	Ward	Origin	Presence of OXA-23, OXA-58 and NDM-1	ST (Pasteur)	ST (PUBMLST)	PFGE	WGM
2402	16/07/2008	Athens, Greece	ICU	Blood	OXA-58	2	437	1e	C2c
2462	4/08/2008	Athens, Greece	ICU	Wound	OXA-58	2	452	1d	C1a
2503	14/08/2008	Athens, Greece	ICU	Bronchial	OXA-58	2	437	1e	C2a
249	26/08/2008	Larissa, Greece	ICU	Pus	OXA-58	2	437	1f	S3
2595	5/09/2008	Athens, Greece	ICU	CVC	OXA-58	2	437	1e	C2b
347	30/01/2009	Larissa, Greece	ICU	Bronchial	OXA-58	2	350	1g	S1
3560	25/07/2009	Athens, Greece	ICU	Sputum	OXA-58	2	452	1h	C1b
4375	14/06/2010	Athens, Greece	SS	Drainage	OXA-58	2	452	1i	S4
272	5/1/2011	Serres, Greece	ICU	Bronchial	OXA-58	2	437	1j	S2
501	7/01/2012	Athens, Greece	MW	Blood	OXA-23	645	436	1a	C3f
5504	2/02/2013	Athens, Greece	ICU	Bronchial	OXA-23	2	436	1a	C3e
5636	13/05/2013	Athens, Greece	ICU	Wound	OXA-23	2	851	1a	S5
5801	20/09/2013	Athens, Greece	SS	Wound	OXA-23	2	436	1a	C3e
3427-1	1/11/2013	Athens, Greece	ICU	Pleural	OXA-23	2	425	1a	C3a
5392	5/11/2012	Athens, Greece	MW	Blood	OXA-23	2	208	1a	C3b
9733	11/11/2013	Athens, Greece	ICU	Rectal	OXA-23	2	436	1a	C3d
NAB5	12/09/2013	India	ICU	Blood	NDM1	85	847	2a	S6
NAB9	14/12/2013	India	ICU	Wash	ND	2	848	1b	C4b
NAB7	15/12/2013	India	ICU	ETA	ND	2	848	1b	C4a
NAB16	17/12/2013	India	ICU	Wash	ND	2	195	1c	S9
NAB17	26/12/13	India	ICU	ETA	ND	646	849	4a	S8
NAB15	28/12/2013	India	ICU	Blood	ND	82	447	3a	S7
NAB12	1/01/2014	India	ICU	ETA	ND	2	848	1b	S10

**PO61 - Development And Evaluation Of Double Locus Sequence Typing For Molecular Epidemiological Investigations Of *Clostridium difficile***

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<sup>1</sup>Lausanne University Hospital,<sup>2</sup> Valery Terletsky

Despite the development of novel typing methods based on whole genome sequencing, most laboratories still rely on classical molecular methods for outbreak investigation or surveillance. Reference methods for *Clostridium difficile* include ribotyping and pulse-field gel electrophoresis, which are band-comparing methods often difficult to establish and which require reference strain collections. Here we present the double locus sequence typing (DLST) scheme as a tool to analyse *C. difficile* isolates. Using a collection of clinical *C. difficile* isolates recovered during a one-year period, we evaluated the performance of DLST and compared the results to multilocus sequence typing (MLST), a sequence-based method that has been used to study the structure of bacterial populations and highlight major clones. DLST had a higher discriminatory power compared to MLST (Simpson's index of diversity of 0.979 versus 0.965) and successfully identified all isolates of the study (100% typeability). Previous studies showed that discriminatory power of ribotyping was comparable to that of MLST, thus DLST might be more discriminatory than ribotyping. DLST is easy to establish and provides several advantages, including absence of DNA extraction (PCR is performed on colonies), no specific instrumentation, low cost and unambiguous definition of types. Moreover, implementation of DLST typing scheme on an Internet database, such previously done for *Staphylococcus aureus* and *Pseudomonas aeruginosa* (<http://www.dlst.org>), will allow users to easily obtain the DLST type by submitting directly sequencing files and will avoid problems associated with multiple databases.

## S5 - Poster session I

### PO62 - Mass Spectrometry Proteotyping For Detection, Identification Characterization And Diagnostics Of Infectious Bacteria In Clinical Respiratory Samples

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**Background:** Lower respiratory tract infection (LRTI) is the leading cause of childhood deaths in most developing countries and the world, and are the most common causes of hospital and outpatient visits within the EU, comprising 1 of 3 admissions annually. In general, the over-prescription and use of broad-spectrum antibiotics are common practices that lead to the evolution and development of resistance in infectious bacteria and will lead to loss of time and resources in patient handling and adverse patient outcomes. It is estimated that 25 000 people die every year in Europe from antibiotic-resistant bacteria. Conventional approaches have depended upon cultivation of bacteria with subsequent testing for antibiotic sensitivity. Therefore, reliable and time-effective microbiological diagnostics are essential for more effective treatment of respiratory infections. In this project, we apply state-of-the-art proteomics techniques for identifications of pathogens and antibiotic resistance from clinical samples, without prior cultivation.

**Material and methods:** Nasal and nasopharyngeal swabs samples were collected, in commercial Amies medium supplemented with 5x STGG, as a part of the EU-TAILORED-Treatment project ([www.tailored-treatment.eu/](http://www.tailored-treatment.eu/)). Samples were stored at -80°C until analyses. Different protocols for removal of human cells and mucus were tested, including non-ionic detergents, *i.e.*, Igepal, Saponin, Urea-Chaps, as well as cytolysis. Samples were concentrated and analyzed by 'proteotyping', using a Lipid-based Protein Immobilization (LPI™) technology (WO2006068619), in which intact bacterial cells or cell fractions are bound to a surface. Peptides were generated, using enzymatic digestion, and then separated and analyzed, using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The mass spectra profiles were compared to a database of reference peptide sequences, consisting of all complete genomes of the NCBI Reference Sequence (RefSeq) Database. Results were confirmed by standard microbiology, including cultivation of bacteria in selective media, MALDI-TOF MS analyses and qPCR.

**Results:** Proteotyping applied to clinical samples demonstrated that the number of viable bacteria and detected proteins determined were ten-times higher when nasal and nasopharyngeal swabs were stored in Amies media supplemented with STGG 5X media compared to Amies media without STGG, after 1 and 2 months of storage at -70C. Among the different protocols tested to remove human biomaterial, all treatments proved effective to varying degrees, although the Igepal treatment was able to retain the highest number of discriminatory peptides. Using proteotyping, we were able to identify the pathogenic bacteria directly within clinical samples (nasopharyngeal and nasal swabs) that had been identified to be positive for respiratory infectious bacteria by standard methodologies at clinical bacteriology laboratories at Sahlgrenska University Hospital (Sweden) or at the University Medical Centre Utrecht (Netherlands).

**Conclusions:** Proteotyping of infectious bacteria, using tandem LC-MS/MS enabled the differentiation and identification of infectious bacteria in clinical samples from LRTIs. It has high levels of resolution and highly reproducible detection of protein biomarkers. Proteotyping identified biomarkers for species- and sub-species-level strain discrimination and antibiotic resistance, all from single MS analyses.

**PO63 - Comparison Of MLST, rMLST And wgMLST As Global Typing Schemes For *S. Enterica***

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**Background:** *Salmonella enterica* (*S. enterica*) is a zoonotic, foodborne pathogen responsible for millions of cases of acute gastrointestinal disease worldwide, each year. MultiLocus Sequence Typing (MLST) compares sequence similarity across 7 housekeeping genes; ribosomal MLST (rMLST) compares sequence similarity across a maximum of 53 ribosomal genes, whilst whole genome MLST (wgMLST) compares a dataset against an entire reference genome. These varying levels of gene coverage were compared and the utility of each approach, as a global typing scheme for *S. enterica*, was assessed.

**Materials | Methods:** Pairwise comparisons were used to analyse various sets of isolates, from pubMLST's BIGSdb, at all three levels of genetic discrimination and to generate trees and nets using the built-in Genome Comparator tool. These datasets were chosen for depth and variety: an example of every serovar of *S. enterica* found within BIGSdb was used, to ensure that serovars formed distinct genetic lineages; Enteritidis, Gallinarum and Pullorum isolates were used to analyse closely related serovars; Newport isolates were used to examine antigenically identical isolates; and isolates from the phylogenetic grouping lineage 3 were used to analyse a genetically complex group.

**Results:** Comparisons showed that in general MLST, rMLST and wgMLST all distinguished serovars and previously identified phylogenetic groupings with high congruence. The wgMLST analyses took 24 – 96 hours to perform (compared to 3 – 20 minutes for rMLST) but showed the highest level of resolution. The neighbornets illustrated that possible genetic relationships decreased as number of genes increased. Analyses of serovars within subspecies one, indicated a relatively high level of recombination and horizontal gene transfer.

**Conclusion:** rMLST for *Salmonella enterica* provides greater discrimination and resolution between lineages than MLST, while taking significantly less time to perform than wgMLST. Furthermore, these results show high congruence between the three methodologies, suggesting high compatibility and scalability for future analyses.

## S5 - Poster session I

### PO64 - On The Road Of Transition – From PFGE To NGS Based Strain Typing

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<sup>1</sup>Chr. Hansen A/S

**Background:** Restriction fragment profiling with rare cutting restriction enzymes, commonly called PFGE after the fragment separation methodology Pulsed Field Gel Electrophoresis, has for many years been considered the “gold standard” of strain typing. Multi Locus Sequence Typing (MLST) was in 1998 suggested as an alternative strain typing method based on partial sequences of around seven house keeping genes. In times of Sanger sequencing MLST has been a laborious and expensive method, not offering obvious timely or monetary advantages compared to PFGE. This led to the situation that laboratories, which had built a substantial database of PFGE-fingerprints, would not find it easy to switch to sequence typing. In general it is observed that PFGE has a higher discrimination power, whereas MLST is much less affected by experimental variation and allows for studying the evolutionary relatedness of isolates within a species or a species group. In addition MLST is fully compatible with next generation sequencing (NGS) of whole genomes in the way that the data required for determining the sequence type can be readily extracted from a NG whole genome (WG) sequence. In fact the cost of a NG WG sequence is already below that of traditional MLST. For a laboratory planning to switch from PFGE to sequence based typing the interesting questions, which is addressed here, is, if published MLST schemes provide a resolution power comparable to PFGE or to which extent it will be necessary to increase the number of genes used. MLST can also be seen as a step on the way to whole genome strain typing, where the sequence type can be based on the core genome alleles or the SNP (single nucleotide polymorphism) profile. The latter two require however still a lot of manual reevaluation and are at the moment not readily available for fast automated strain typing.

**Materials & Methods:** Illumina NGS sequencing was performed at BGI (Shenzen, China). MLST typing was accomplished with the help of CLC (Århus, Denmark). PFGE typing was performed with enzyme Smal for both *Lactococcus lactis* and *Streptococcus thermophilus*. The comparison of PFGE and sequence typing was done in BioNumerics (Applied Math).

**Results:** As previously found, our results confirm the lower resolution power of published MLST schemes for *Lactococcus lactis* and *Streptococcus thermophilus* compared to PFGE typing. The combination of two schemes however increases the resolution power to almost PFGE level. Congruence analysis between the two typing approaches shows that they are not fully congruent. Strain differentiation can therefore be further enhanced by combining the two typing methods.

**Conclusion:** MLST of NG whole genome sequences offers fast, robust and easily interpretable strain typing, which can be adjusted to the needed level of differentiation by adjusting the number of genes used.

**PO65 - Whole Genome Sequencing Of Fecal Samples As A Tool For The Diagnosis And Genetic Characterization Of Norovirus**

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**Background:** Norovirus is one of the most frequent causes of gastroenteritis, causing yearly epidemics and hospital outbreaks resulting in a high burden on health care. Advances in molecular diagnostic techniques allow rapid identification of infected patients. Besides, characterization of norovirus by sequence analysis provides useful information for understanding transmission during hospital outbreaks. Next generation sequencing techniques (NGS) are increasingly being used in outbreak management and virus discovery. For norovirus, the use of NGS focused on the most prevalent genotypes, most notably genotype GII.4. A prerequisite for incorporating NGS into routine diagnostics is the ability of these techniques to also detect and characterize novel (recombinant) norovirus genotypes. We describe the detection of different genotypes of norovirus directly from clinical samples using multiplexed whole genome sequencing.

**Materials and methods:** Whole genome sequencing was performed on fecal samples from 10 patients with gastro-enteritis caused by different genotypes of norovirus as confirmed by RT-PCR and Sanger sequencing. The cDNA fractions obtained after rRNA-depletion and reverse transcription were used for whole-transcriptome amplification for the detection of RNA viruses, using the Qiagen Repli-G WTA single cell kit. Approximately 100 ng DNA per sample was used for sequencing on an IonTorrent PGM system with an Ion 318 sequencing chip. Assembly and determination of sequence coverage was done with Bowtie2 using the reference sequence as template. A phylogenetic tree was constructed used a K-mer based Neighbour Joining method, using K-mer length of 15 and a Jukes-Cantor nucleotide distance matrix.

**Results:** Sufficient amounts of RNA were retrieved from all clinical samples to perform whole-transcriptome sequencing for the detection of RNA-viruses. Complete genomic norovirus sequences were obtained from all clinical samples, permitting accurate genotyping by phylogenetic analysis. In addition, a complete coxsackie B1 virus genome was retrieved.

**Conclusion:** Detailed information on viral content can be obtained from fecal samples in a single-step approach, supporting clinical and epidemiological purposes. Next-generation sequencing performed directly on clinical samples can become a powerful tool in patient care and infection control.

## S5 - Poster session I

### PO66 - Plasmablasts After Antigen Challenge - A Diagnostic Tool For Assessing Response To Influenza Vaccination In Patients With Chronic Lymphocytic Leukemia

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**Introduction:** Chronic lymphocytic leukemia (CLL) leads to significant immune system dysfunction. The predominant clinical presentation in 50% of patients involves recurrent, often severe, infections. Infections are also the most common (60-80%) cause of deaths in CLL patients. The scope of infections varies with the clinical stage of the disease. Treatment-naïve patients typically present with respiratory tract infections caused by influenza. Immune system disturbances in CLL are still not well defined. Clinical data indicates that despite normal serum immunoglobulin (Ig) level, treatment-naïve patients may not respond to influenza vaccination.

**Aim of the study:** The aim of the study was to investigate changes in B-cell subpopulations in CLL patients, including plasmablasts, in peripheral blood by flow cytometry after influenza vaccination and to evaluate if plasmablasts may serve as a diagnostic tool for assessing response to vaccination.

**Material and methods:** Forty treatment-naïve CLL patients and twenty healthy volunteers were immunized with influenza vaccine. Specific antibody levels and frequencies of plasmablasts were measured before vaccination and on day 30 by ELISA assay, and day 7 by flow cytometry after vaccination, respectively. Both groups were also evaluated for the levels of IgG and IgG subclasses, and the frequencies of selected peripheral blood lymphocyte subpopulations before and 30 days after immunization.

**Results:** Of the forty CLL patients studied, 100% lacked detectable changes in the serum level of specific anti-influenzae IgG antibodies before and after vaccination (mean:  $122.41 \pm 41.94$  mU/ml vs. mean:  $128.37 \pm 52.13$  mU/ml, respectively;  $p=0.24$ ). In none of patients an increase of the percentages and absolute counts of plasmablasts was noted. In the control group, an increase in circulating plasmablasts on day 7 post immunization corresponded with the appearance of specific antibody levels on day 30 post immunization ( $r=0.823$ ,  $p=0.000001$ ) and was statistically significantly higher than before a dose of influenza vaccine (before vaccination:  $20.12 \pm 14.93\%$ ,  $0.46 \pm 0.36 \times 10^3/\text{mm}^3$ ; after vaccination:  $46.81 \pm 26.87\%$ ,  $1.15 \pm 0.77 \times 10^3/\text{mm}^3$ ;  $p=0.01$ ). In contrast, CLL patients failed to increase plasmablasts significantly in peripheral blood after antigen challenge.

**Conclusions:** Our findings indicate that treatment-naïve CLL patients have a block in terminal B-cell differentiation and that flow cytometry-based assessment of plasmablasts in peripheral blood after vaccination serves as a surrogate diagnostic marker for assessing *in vivo* antibody response in patients with CLL.

**PO67 - Discrimination Of Vancomycin-resistant *Enterococcus faecium* Clones By MLST (eBURST/BAPS) And Spectroscopic Techniques (MALDI-TOF MS And FTIR-ATR)**

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**Background:** The highly recombinogenic population structure of *Enterococcus faecium* (EfM) has been recently updated. Former clonal complexes (CC) delineated by eBURST were split into smaller subgroups by the Bayesian Analysis of Population Structure (BAPS) software, better reflecting their evolutionary history. Still, the application of low-cost high throughput techniques is needed to identify high-risk clones in a fast routine way enabling timely and appropriate infection control measures. Our aim was to exploit the potential of Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR) techniques in the discrimination of vancomycin-resistant *Enterococcus faecium* (VREfm) clones associated with human infections.

**Materials and Methods:** Ninety VREfm isolates obtained from hospitals, healthy humans and swine in 16 countries (1995-2012) were analyzed. They corresponded to 9 sequence types (STs) clustering into 5 BAPS subgroups: BAPS 3.3a1 (18 ST18, 12 ST132), 3.3a2 (10 ST16, 9 ST17) and 2.1a (4 ST78, 12 ST117) greatly linked to hospital outbreaks (former CC17); and BAPS 2.1b (6 ST5, 5 ST185) and 3.2 (14 ST6) mainly linked to healthy pigs/humans and rarely to human infections (former CC5). Bacterial cells grown in Mueller-Hinton agar (18h/37°C) were used for FTIR-ATR (4000-400cm<sup>-1</sup>, resolution of 4cm<sup>-1</sup>/32 spectra co-additions) and to prepare bacterial extracts (according Bruker recommendations) for MALDI-TOF MS (linear positive mode from 2000-20000m/z and alpha-cyano-4-hydroxycinnamic acid matrix) experiments. Infrared (IR) spectra were processed with standard normal variate, Savitzky-Golay filter (9,2,2) prior to analysis. Both sets of spectra after application of mean centring were modelled by partial least square discriminant analysis (PLSDA) with Matlab version 8.3 (MathWorks, Natick, MA, USA) and PLS Toolbox version 7.5 (Eigenvector Research Inc., Wenatchee, WA, USA).

**Results:** Three main clusters were observed in the PLSDA model obtained with the mass spectra (first two latent variables explaining 63.4% of spectral variability): i) BAPS 3.3a1; ii) BAPS 3.3a2 and 2.1a (ST17/ST78 lineages); and iii) BAPS 2.1b and 3.2. BAPS 3.3a2 and 2.1a as well as "CC5" BAPS subgroups include single locus variants (SLVs) as ST17 and ST78, and ST5 and ST6, respectively, and were not separated by MALDI-TOF MS. The PLSDA model (first two latent variables, 75.0% of the spectral variability) obtained with IR spectra (1500–900 cm<sup>-1</sup> enclosing polysaccharide molecules vibrations) exhibited 5 distinct clusters corresponding to the 5 BAPS subgroups. Most STs included a high diversity of isolates as defined by PFGE for ST18 (16 PFGE-types), ST132 (9 PFGE-types), ST16 (7 PFGE-types), ST17 (9 PFGE-types), ST78 (3 PFGE-types) and ST117 (4 PFGE-types). ST5/ST6/ST185 corresponded to one PFGE-type.

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**Conclusions:** MALDI-TOF MS seems to be insufficient to discriminate the main Efm clones. However, FTIR-ATR perfectly corroborates the clustering of main Efm clones as obtained using BAPS. The Efm strains analyzed seem more divergent in their carbohydrates content (evaluated by FTIR-ATR) than in the ribosomal peptides and/or proteins (evaluated by MALDI-TOF MS). The fact that SLVs from different BAPS were discriminated by FTIR-ATR highlights the important contribution of carbohydrates for niche adaption.

**PO68 - Development Of A Conventional Multiplex PCR For Identification Of *Escherichia coli*, *Escherichia fergusonii*, And *Escherichia albertii***

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**Background:** *Escherichia coli*, *Escherichia albertii*, and *Escherichia fergusonii* are closely related organisms with the potential to cause sickness in humans, such as urinary tract infections and diarrheal illnesses. Identification of these species is dependent on phenotypic testing, sequencing, or single PCR methods that target only one species. To facilitate the rapid identification of these potential pathogens, we developed a conventional multiplex PCR assay targeting conserved, species-specific genes.

**Materials | Methods:** Using the Daydreamer™ (Pattern Genomics, USA) software platform, we concurrently analyzed the whole genome sequence for 342 genomes (305 *E.coli*, 22 *E. albertii*, 12 *E. fergusonii* and 3 other *Enterobacteriaceae*) that had been generated in-house or downloaded from NCBI. First, we identified genomic regions that are conserved in, and unique to, each species. We removed sequences that were not specific due to any blast hit to non-target organisms on NCBI and then designed PCR primers for the remaining sequences. Next, the target sequences were annotated using BLAST. For all three species, we selected genes highly conserved at the nucleotide level and primers that had 100% accuracy and 100% coverage to our training dataset as reported by the Daydreamer™ *in silico* PCR tool. We screened the primers in a conventional multiplex PCR assay format with a set of 10 reference strains and selected those that yielded the most efficient amplification. Using a collection of 30 additional *Escherichia* strains, we assessed the ability of the selected primers to amplify their intended target sequences.

**Results:** We identified eight genes that appeared to be conserved in, and unique to, the species of interest: a putative transporter gene and intracellular growth attenuator gene (*igaA*) for *E. coli*; three hypothetical protein genes for *E. albertii*; and a hypothetical protein and two transcriptional regulator genes for *E. fergusonii*. Findings from our screening assay showed there were minimal differences in the performance of the primers identified. We therefore selected primers that had similar annealing conditions and targeting the intracellular growth attenuator protein (*igaA*) for *E. coli*, a hypothetical protein for *E. albertii*, and a transcriptional regulator for *E. fergusonii*. The sensitivity and specificity of the conventional multiplex PCR assay was 100% and 100%, respectively.

**Conclusion:** We report the development of a multiplex PCR assay that can quickly and accurately distinguish between *E. coli*, *E. albertii*, and *E. fergusonii* in a single PCR reaction. This approach should help reference and diagnostic laboratories identify these closely related and easily confused species, thus improving laboratory surveillance.

## S5 - Poster session I

### PO69 - A Comparison Of Diversilab rep-PCR And cgMLST For Molecular Typing Of Carbapenem-resistant *Acinetobacter baumannii* Isolates From Greece And Spain

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**Background:** The incidence of carbapenem-resistant *Acinetobacter baumannii* (CRAB) is increasing, especially in Mediterranean countries. Multiple studies have shown that the most common carbapenem-resistance mechanism is OXA-23 and the most prevalent clonal lineage is International Clone 2 (IC2). In this study, we have characterised CRAB isolated from multiple cities in Greece and Spain and compared clustering by rep-PCR (DiversiLab) to an *ad hoc* cgMLST scheme (SeqSphere+) for molecular typing.

**Materials | Methods:** Seventy-six isolates were collected from Greece (n=44) and Spain (n=32). Carbapenem-resistance was investigated by E-test and PCR-based methods. Isolates were typed by rep-PCR (DiversiLab) and clusters were defined as having  $\geq 98\%$  similarity. Rep-PCR fingerprints were compared to our library of IC lineages to determine their clonal background. WGS was performed using Nextera XT chemistry and Illumina MiSeq paired-end read technology. An *ad hoc* cgMLST scheme was set up using SeqSphere+ with *A. baumannii* ACICU as reference strain to define the core genome. Genomes were assembled using Velvet. Carbapenem-resistance genes were identified using ResFinder.

**Results:** Seventy five isolates were identified as CRAB. OXA-23 was the most common resistance mechanism. Rep-PCR divided isolates into seven clusters. The majority of isolates clustered with IC2 control strains (Table 1). Genomes were assembled with a median of 191 contigs and median coverage of 47-fold. cgMLST further subdivided DiversiLab clusters into smaller clusters (Figure 1). Not all DiversiLab clusters correlated well with cgMLST (circled in Figure 1). DiversiLab cluster E isolates from Sevilla were subdivided into two cluster types by cgMLST which matched their carbapenem-resistance mechanism (OXA-58 and OXA-23). Cluster C and cluster J isolates from Athens were identical by cgMLST, as were A Coruña cluster C and cluster D. In the majority of cases, isolates showing  $\leq 10$  allelic differences were from the same city. The one exception was isolate R147 (Athens) with 3 allelic differences to isolates from Larissa. IC1 and unclustered strains were clearly delineated from the main group of IC2 isolates, with  $\geq 1684$  allelic differences.

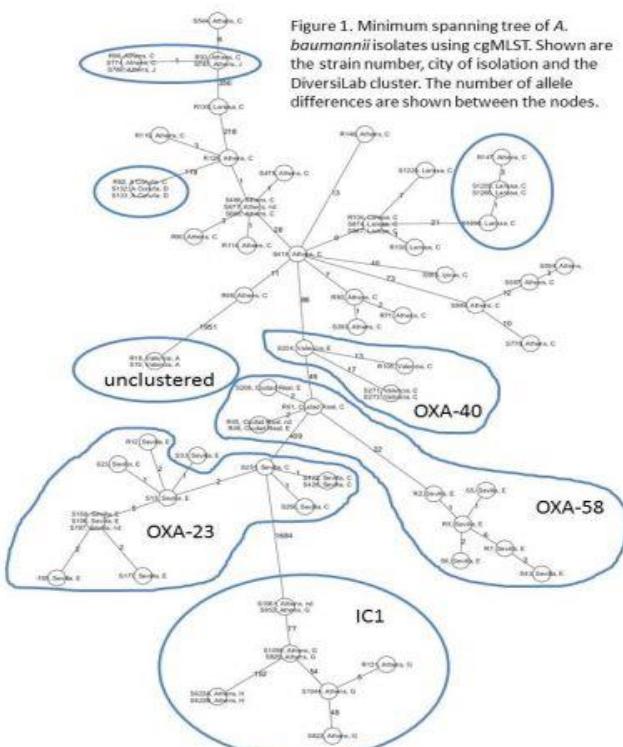
**Conclusion:** The cgMLST *ad hoc* scheme allowed a more detailed delineation than DiversiLab clustering. Isolates that appeared to be closely related by rep-PCR were found to contain upwards of several hundred allelic differences. However, clonal lineages were clearly separated using this scheme. The use of a high-resolution typing scheme will greatly aid in our understanding of *Acinetobacter baumannii* populations.

Table 1. DiversiLab clustering, carbapenem-resistance resistance mechanism and city of isolation

Cluster	City (number of isolates)	Acquired carbapenemases (number of isolates)	International clone
A	Valencia (2)	OXA-40 (2)	Unclustered
C	A Coruna (1), Athens (22), Ciudad Real (1), Ipirus (1), Larissa (9), Sevilla (4), Valencia (3)	OXA-40 (3), OXA-58 (1), OXA-23/58 (2), OXA-23 (35)	IC2
D	A Coruña (2)	OXA-23 (2)	IC2
E	Ciudad Real (2), Sevilla (14) Valencia (1)	OXA-40 (1), OXA-58 (8), OXA-23 (7), *no OXA (1)	IC2
G	Athens (6)	OXA-23 (6)	IC1
H	Athens (2)	OXA-23 (2)	IC1
J	Athens (2)	OXA-23 (2)	IC2
nd	Athens (2), Ciudad Real (1), Sevilla (1)	OXA-23 (3), OXA-58 (1)	nd

nd, not determined; \*no OXA, the isolate was carbapenem-susceptible

Figure 1. Minimum spanning tree of *A. baumannii* isolates using cgMLST. Shown are the strain number, city of isolation and the DiversiLab cluster. The number of allele differences are shown between the nodes.



MST for 79 isolates based on 2239 columns, isolates (ignoring missing values). Distance based on 1 column (n = 1). Taxonomy: MLRT = 2019 targets (KCCV-2019). Date analysis: 10-Nov-2019. A. baumannii reference genome reference. Comparison Table created: Mon 8.10.2018 13:40 AM. Taxk template: A. baumannii/METY\_2018 targets ADGJ.

## S5 - Poster session I

### PO70 - Multilocus Sequencing Typing As A Tool To Investigate Childhood *Haemophilus influenzae* Invasive Disease In Portugal

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**Background:** *Haemophilus influenzae* is an important cause of serious childhood invasive disease despite the use of the vaccine against serotype b strains (Hib). Six capsular types, a-f, have been identified to date, although most of strains are non-capsulated (NC).

Multilocus Sequencing Typing (MLST) is a powerful method that allows a precise and unambiguous characterization of *H. influenzae* genotypes.

A partnership between the National Institute of Health and the Society for Paediatric Infectious Diseases aimed to characterize invasive childhood infection in Portugal.

The objective of this study was to genotype isolates by MLST to follow the epidemiology of disease.

**Material / Methods:** This study was conducted between 1 January 2010 and 31 December 2015. During this period 41 *H. influenzae* strains were analysed, mostly isolated from blood (88%). Antibiotic resistance was assessed by the microdilution assay and β-lactamase production was determined with nitrocefin. Capsular status was characterized by polymerase chain reaction using primers and conditions described in the literature. MLST was performed by amplifying and sequencing internal fragments of the 7 housekeeping genes (*adk*, *atpG*, *frdB*, *fucK*, *mdh*, *pgi* and *recA*). Sequences were analyzed and submitted to the MLST website <http://pubmlst.org/hinfluenzae/> for assignment of the sequence type (ST). To display the allelic distances between the obtained STs, we applied the goeBURST algorithm implemented in the PHYLOViZ platform.

**Results:** Antimicrobial susceptibility testing showed that most strains were susceptible to all beta-lactams studied, with only three strains being ampicillin resistant due to beta-lactamase production.

Most of invasive disease was due to the presence of NC strains (27/41; 66%), while 14 isolates (34%) were capsulated and characterized as follow: two serotype a (5%), 10 b (24%) and two f (5%).

As expected, MLST typing revealed high genetic variability among 27 NC isolates, which had 24 (89%) different sequence types (STs), with four new STs represented by previously unidentified allele combinations. In opposition, capsulated isolates were very clonal: all 10 Hib were assigned to CC6 (eight strains ST6, one ST 1149, one ST 190), the two Hia strains were assigned to CC 23 (ST 23) and the two Hif belonged to CC124 (ST 124 and ST 1188) (Figure 1).

**Conclusions:** Our data indicate that invasive disease among Portuguese children is now due to highly genetically diverse, fully susceptible NC strains, suggesting that no particular virulent clone is responsible for epidemiological change of disease, after vaccine implementation in the year 2000. Nevertheless, we are concerned about Hib disease (24% of the isolates) despite the higher vaccine coverage.

MLST typing continues to show a high genetic diversity among NC strains and clonal relationships among capsulated isolates.

In conclusion, in order to monitor the evolving dynamics of this pathogen and the epidemiology of invasive disease, ongoing surveillance is needed to monitor the true magnitude of this problem.

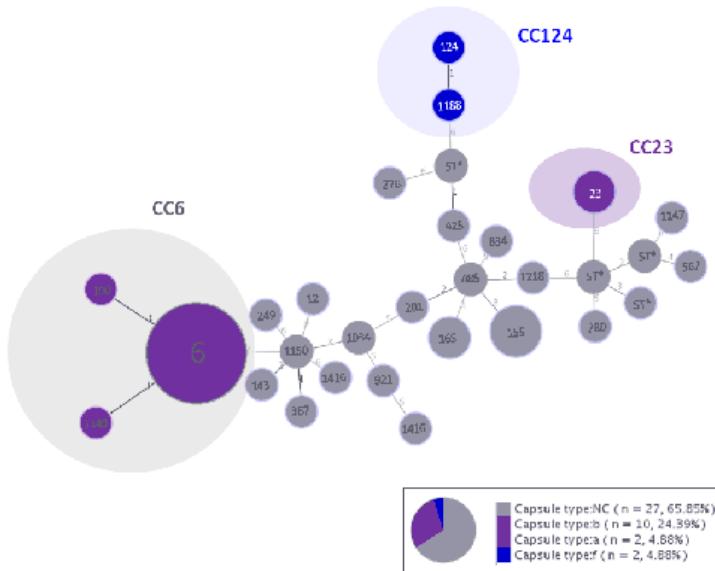


Figure 1. MLST-based representation of genetic diversity among *H. influenzae* invasive disease isolates. MLST sequence types (STs) are indicated by numbers within circles (\* means a novel ST), where the size of the circles is proportional to the number of isolates from each ST and different colors mean different capsular types. Connecting lines are labeled with the number of allelic differences between STs. A clonal relationship is observed in each of capsulated strains, with serotypes a, b and f being assigned to three different clonal complexes (CC), while high genetic diversity is observed among NC isolates.

## S5 - Poster session I

### PO71 - Cost Effective Strategy For Typing *Brucella abortus* In An Endemic Scenario

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**Background:** *Brucella* is a highly clonal microorganism presenting elevated levels of nucleotide identity. For this reason the VNTRs and SNPs are ideal for tracing its molecular evolution. Recently Garofolo et al. (2013) have shown that *Brucella abortus* in Italy is divided in three major clades. Although the VNTRs are extremely valid for typing *B. abortus*, they suffer of a high rate of homoplasy. In a subsequent study we performed WGS SNPs analysis for reconstruct the *B. abortus* phylogeny identifying and characterizing the three genetic clades in a global context (2). SNPs tested on a large number of samples may be prohibitive, in terms of costs and time. To date one cost-effective technique is the Mismatch Amplification Mutation Assay, coupled with melt analysis (Melt-MAMA). Here we present an analytical and epidemiological evaluation of Melt-MAMA assays for the identification of Italian *B. abortus* clades.

#### Material and Methods : Melt-MAMA Design

To identify the three *B. abortus* clades we searched for six SNPs from the six specific branches leading to the Italian strains. The assays satisfied the following five conditions: (i) Two forward primer with in position 3' corresponded to the allelic status for the SNP to be searched; (ii) the presence of a common reverse primer; (iii) an additional mismatch in the forward primer in anti-penultimate and penultimate position alternately; (iv) a GC-clamp in position 5' for one of the forward primer; (v) amplicon of reduced size (60 bp -120 bp).

#### Optimization of MeltMAMA

To determine the optimal condition, for each test, we tested the MeltMAMA in the following manner: use of the pair of forward primer in three different reactions with reciprocal concentration 1:1, 4:1 and 1:4. The optimum ratio was chosen and adjusted with respect to the parameters of amplification. Assays were considered valid when revealed the SNPs under identical amplification.

#### *B. abortus* isolates

A total of 200 *B. abortus* from the Italian epidemiological context were analyzed.

**Results:** Six out of 13 assays developed equal to 46% were efficient, allowing the identification of alleles requested. The optimization of the amplification parameters has allowed to detect SNPs calls through the analysis of melting temperatures. According to the assays, the 200 *B. abortus* were successful typed and demonstrated a specific geographical spread (Fig.1).

**Conclusions:** Using SNPs to test phylogenetic hypotheses according to a hierarchical approach can reduce the analysis of hypervariable markers such as VNTRs. In a scenario of eradication of the disease through the policy of testing and slaughtering of positive animals it is necessary to have tools of molecular epidemiology for tracing outbreaks to monitor the phenomena of reintroduction of the disease in free-areas. The MeltMAMA have proven effective in genotyping isolates using individual PCR. This study is a step forward in the understanding of new strategies for typing *Brucella* as a tool for the study of molecular epidemiology of brucellosis in endemic regions.

**References:** 1-G Garofolo et al. 2013. Investigating genetic diversity of *Brucella abortus* and *Brucella melitensis* in Italy with MLVA-16. Infection, Genetics and Evolution 19, 59-70. 2-G. Garofolo et al. (2014). Insights into the genetic lineages and current spread of *B. abortus* and *B. melitensis* in Italy. Proceedings of Brucellosis 2014 International Research Conference. Berlin 9-12 September.

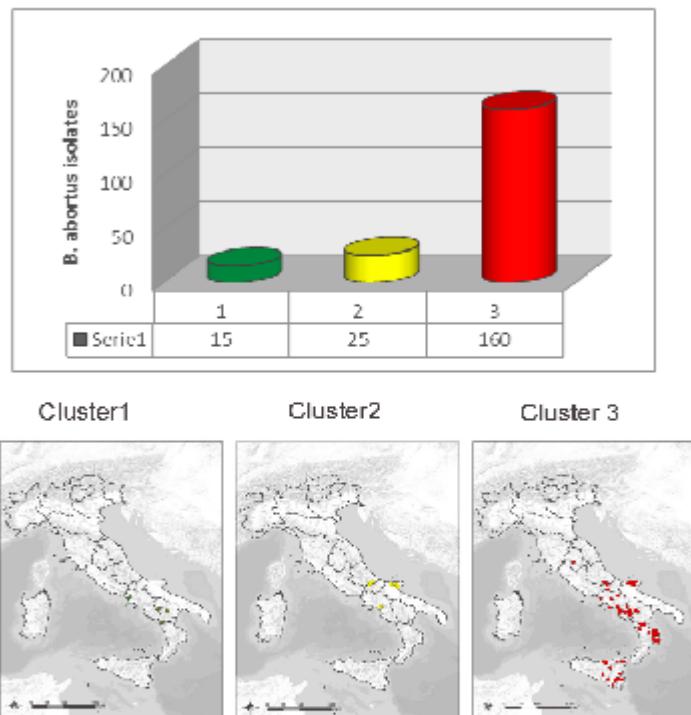


Fig. 1 - Assignation to the Italian *B. abortus* clades for the 200 isolates studied with geographical distribution according to the six Melt-MAMA assays.

## S5 - Poster session I

### PO73 - Mixed *Mycobacterium tuberculosis* Infections In Switzerland Identified With Whole Genome Sequencing

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<sup>1</sup>Swiss Tropical and Public Health Institute, University of Basel,<sup>2</sup> Institute of Social and Preventive Medicine, University of Bern,<sup>3</sup> Clinic for Infectious Diseases, Bern University Hospital, and University of Bern,<sup>4</sup> Institute of Social and Preventive Medicine, University of Bern, and Swiss Tropical and Public Health Institute, University of Basel

**Background:** Infections with multiple *Mycobacterium tuberculosis* genotypes are relevant for tuberculosis diagnosis and treatment, as they can bias drug resistance testing and render treatment of tuberculosis (TB) ineffective. In high TB-incidence settings, their prevalence is up to 20%, however, little is known about the prevalence of mixed *M. tuberculosis* infections in low-incidence settings. Previous studies have used mixed MIRU-VNTR genotyping alleles as indicator for mixed infections, but the sensitivity and specificity of using MIRU-VNTR to detect mixed infections is debated. With the allele frequency data obtained using whole genome sequencing, the presence of multiple genotypes can be studied in detail. In this study, we used sequencing data of 520 *M. tuberculosis* isolates from a nationwide study in Switzerland to identify the presence of mixed infections.

**Methods:** We generated Illumina whole genome sequencing data of 520 single patient *M. tuberculosis* isolates from 2000-2008 from HIV-coinfected and HIV-negative TB patients. We analyzed sequencing reads at 177 genomic positions known to be markers for phylogenetic lineages and sublineages, for the presence of minority variants.

**Results:** We identified at least 2/520 (0.4%) patient isolates which showed clear evidence of a mixed population of two *M. tuberculosis* genotypes. Both isolates consisted of two strains of the L4.5/LAM sublineage of *M. tuberculosis*, however, with different proportions of the minor variant (29% and 16%). Interestingly, one patient was born in South America, where the L4.5/LAM sublineage is known to be highly prevalent. The second patient was born in Switzerland. Both patients were HIV-negative. In the isolate from the Swiss-born patient, a mutation in *rrs* (C491T), which is known to confer streptomycin resistance, was found in the same proportion as the majority variant. Neither of the two isolates had mixed MIRU-VNTR alleles. We did not observe any mixed genotypes in isolates with multiple MIRU-VNTR alleles.

**Conclusion:** We show that infections with multiple *M. tuberculosis* strains do occur in low TB-incidence settings such as Switzerland, but are infrequent compared to high-incidence settings. The concurrent presence of a drug resistant and a susceptible genotype in one isolate emphasizes the need to take mixed infections into account for diagnostic tests and treatment of TB. The different birth origin of the patients indicates that mixed infections do not only occur in particular risk groups such as immigrants or HIV-positive TB patients. Whole genome sequencing data analysis provided a clear advantage to identify mixed infections, however, needs further development, especially for mixed infections of the same phylogenetic sublineage, which were not identified in this study.

## MICROBIAL POPULATION GENOMICS

**Friday, 11 March 2016, 12:00-13:30**

**PO1 - Comparative Genomics Provides Insight In The Evolution Of Antimicrobial Resistance In Canine Methicillin-resistant *Staphylococcus pseudintermedius***

Birgitta Duim<sup>1</sup>; Jaap A. Wagenaar<sup>1</sup>; Mirlin Spaninks<sup>1</sup>; Arjen Timmerman<sup>1</sup>; Els M. Broens<sup>1</sup>; Aldert Zomer<sup>1</sup>

<sup>1</sup>Utrecht University/Faculty of Veterinary Medicine

**PO2 - Comparison Of Fully Sequenced Genomes From Clinical And Non-clinical *Enterococcus faecium* Strains**

Young-Hee Jung<sup>1</sup>; Taesoo Kwon<sup>1</sup>; Won Kim<sup>2</sup>

<sup>1</sup>Korea National Institute of Health,<sup>2</sup> Seoul National University

**PO3 - Phylogeny Of *Mycobacterium leprae* Using Whole Genome Sequencing**

Andrej Benjak<sup>1</sup>; Charlotte Avanzi<sup>1</sup>; Chloé Loiseau<sup>1</sup>; Philippe Busso<sup>1</sup>; Pushpendra Singh<sup>2</sup>; Stewart Cole<sup>1</sup>

<sup>1</sup>Global Health Institute, EPFL,<sup>2</sup> Louisiana State University - School of Veterinary Medicine

**PO4 - *In Silico* Comparison Serotyping And Genotyping Methods For *C.trachomatis*.**

Zhanna Amirkbekova<sup>1</sup>; Dmitriy Babenko<sup>1</sup>

<sup>1</sup>Karaganda State Medical University

**PO5 - Investigation Of The Austrian LA-MRSA Population By Whole Genome Sequence Based Typing**

Sarah Lepuschitz<sup>1</sup>; Burkhard Springer<sup>1</sup>; Alexander Indra<sup>1</sup>; Franz Allerberger<sup>1</sup>; Werner Ruppitsch<sup>1</sup>

<sup>1</sup>Austrian Agency for Health and Food Safety

**PO7 - Water On The Lungs - Characterisation Of Pathogens In Pleural Effusion By The Use Of Metagenomic Sequencing**

Anna Maria Malberg Tetzschner<sup>1</sup>

<sup>1</sup>Department of Systems Biology, The technical university of Denmark

**PO8 - Examination Of Metagenomic Analysis For Typing Of *Campylobacter* In A Cultureless Future Using Present-day Technology**

Katrine Grimstrup Joensen<sup>1</sup>; Søren Persson<sup>1</sup>

<sup>1</sup>Statens Serum Institut

## S6 - Poster session II

**PO9 - The Role Of Surface Adhesins In Epidemic Hypervirulent *Clostridium difficile* BI/NAP1/027 Strain And Non-epidemic Strain Virulence And Bio**

SOZA BABAN<sup>1</sup>

<sup>1</sup>University Of Nottingham

**PO10 - *Bifidobacterium bifidum* YIT 10347 Suppress Gut Microbiota Dysbiosis And Alleviate Severe Diarrhea In *Helicobacter pylori* Eradication Therapy**

Toshifumi Ohkusa<sup>1</sup>; Takao Osaki<sup>2</sup>; Hideo Yonezawa<sup>2</sup>; Shigeru Kamiya<sup>2</sup>

<sup>1</sup>Department of gastroenterology and Hepatology, The Jikei University Kashiwa Hospital,<sup>2</sup> Department of Infectious Diseases, Kyorin University School of Medicine

**PO1 - Comparative Genomics Provides Insight In The Evolution Of Antimicrobial Resistance In Canine Methicillin-resistant *Staphylococcus pseudintermedius***

Birgitta Duim<sup>1</sup>; Jaap A. Wagenaar<sup>1</sup>; Mirlin Spaninks<sup>1</sup>; Arjen Timmerman<sup>1</sup>; Els M. Broens<sup>1</sup>; Aldert Zomer<sup>1</sup>

<sup>1</sup>Utrecht University/Faculty of Veterinary Medicine

**Background:** *Staphylococcus pseudintermedius* is a significant pathogen in dogs and cats and is occasionally associated with infections in humans. Methicillin-resistant strains (MRSP), often resistant to three or more antimicrobial classes (multi-drug resistance; MDR) have emerged since 2004. Recent studies in our lab showed that the majority of MDR MRPS belonged to clonal complex (CC) CC71 and to a lesser extend to CC258 or CC45, and that since 2011 less resistant isolates were obtained from dogs. The aim of this study was to investigate how recombination and mobile genetic elements shape the resistance dynamics in different MRSP lineages.

**Methods:** At the Veterinary Microbiological Diagnostic Centre (VMDC) of Utrecht University, 478 MDR MRSP strains were isolated from canine infections from 2004 to 2014. Fifty MRSP strains, all with a unique MDR profile, with resistances to maximum 8 antimicrobial classes were selected for whole genome sequencing using Illumina MiSeq. Genomes were annotated; orthologous relations between genes were determined using Orthologue. The Harvest suite was used for core-genome alignment and reconstruction single-nucleotide polymorphism (SNP) based phylogeny. Recombination and gene flow was studied using Gubbins.

**Results:** Phylogenetic analysis showed highly conserved genomes of MRSP belonging to CC71 and CC45. Recombination was detected in CC258 and hardly in CC71 and CC45. In all strains mobile genetic elements associated with resistance genes were present. Regions of horizontal transfer were identified and all relevant recombination events will be presented.

**Conclusion:** Whole genome sequencing identifies the evolution of antimicrobial resistance in MRSP and may provide insight in how antimicrobials can co-select for multi-resistant strains.

## S6 - Poster session II

### PO2 - Comparison Of Fully Sequenced Genomes From Clinical And Non-clinical *Enterococcus faecium* Strains

Young-Hee Jung<sup>1</sup>; Taesoo Kwon<sup>1</sup>; Won Kim<sup>2</sup>

<sup>1</sup>Korea National Institute of Health, <sup>2</sup> Seoul National University

Keywords: comparative genomics, candidate of therapy, difference between clinic and nonclinic

**Background:** Enterococci habit in gastrointestinal tract of humans and animals and have been known as an opportunistic pathogen in chronic patients. Most of vancomycin-resistant enterococci (VRE) are *E. faecium* and are resistant to high-level vancomycin. In recent, vaccine candidates have been under development to treat VRE. Therefore, we investigated the difference between clinical and nonclinical strains in genome level to search the candidate genes for VRE treatment.

**Materials and methods:** Four completed genome sequences of *E. faecium* (one clinical VSE, two clinical VRE, one food-born VSE) were download from NCBI and were analyzed using genome sequences of *E. faecium* DO as a reference sequence. Multiple sequence alignment analysis were performed on the proteins under 80% homology.

**Results:** We divided the proteins into six groups according to the homology. Two proteins have 0~10% homology, 10~70% in seven proteins, 70~80% in four proteins, 80~90% in ten proteins, paralog in 97 proteins, over 90% in 267 proteins, and the rest of the proteins have difference among the clinical genomes. Twelve proteins were selected as the candidates by multiple sequence alignment analysis. The proteins under 80% homology are related to ABC transporter, beta-lactamase, cell wall or carbohydrate metabolism.

**Conclusion:** We analyzed four completed genome sequences of one clinical *E. faecium*, two clinical VRE, and one food-born *E. faecium* and we suggested the candidates of therapeutic agents to treat enterococcal infection including VRE using the difference of clinical and nonclinical genomes.

**PO3 - Phylogeny Of *Mycobacterium leprae* Using Whole Genome Sequencing**

Andrej Benjak<sup>1</sup>; Charlotte Avanzi<sup>1</sup>; Chloé Loiseau<sup>1</sup>; Philippe Busso<sup>1</sup>; Pushpendra Singh<sup>2</sup>; Stewart Cole<sup>1</sup>

<sup>1</sup>Global Health Institute, EPFL,<sup>2</sup> Louisiana State University - School of Veterinary Medicine

Keywords: *Mycobacterium leprae*, phylogeny, SNPs

**Background:** *Mycobacterium leprae* strains have remarkably conserved genomes. Our objective is to characterize the *M. leprae*'s genomic variability by whole genome sequencing and to better understand the microevolution of this species. The biggest challenge to this effort is the *in vitro* uncultivability of *M. leprae* and the inadequate amounts of *M. leprae*'s DNA obtained from skin biopsies using standard DNA extraction protocols. As consequence, genomic resources for this species are very scarce.

**Materials / Methods:** Total DNA was extracted from mouse foot-pad samples while a host-depletion step was applied on most biopsy samples prior DNA extraction. Samples with low abundance of *M. leprae* DNA were additionally enriched using whole-genome array capture. Illumina libraries were sequenced either on HiSeq or MiSeq platforms. Reads were adapter-trimmed and quality-trimmed and mapped against the *M. leprae* reference genome sequence. High quality SNPs were inferred from the alignments by applying stringent coverage and quality thresholds.

**Results:** Thanks to our considerably improved DNA extraction and enrichment methods, we have successfully sequenced over 150 *M. leprae* strains deriving from different locations around the world, using high-throughput sequencing. We have identified 2,810 SNP loci, which enabled an accurate phylogenetic analysis and a more refined SNP-based classification of strains. Correlation patterns were observed for geographic locations and drug resistance.

**Conclusion:** We are establishing a genomic database representing the global diversity of *M. leprae* strains. The new genomic resource will facilitate studies on *M. leprae*, with the ultimate goal of eradicating this pathogen.

## S6 - Poster session II

### PO4 - *In Silico* Comparison Serotyping And Genotyping Methods For *C.trachomatis*.

Zhanna Amirbekova<sup>1</sup>; Dmitriy Babenko<sup>1</sup>

<sup>1</sup>Karaganda State Medical University

**Background:** Chlamydia trachomatis is a cause of the most prevalent sexually transmitted bacterial infections with 89 million cases annually worldwide (Gaydos et al., 2004) and is responsible for both asymptomatic and wide spectrum of clinically significant diseases (Manavi , 2006).

Based on serological typing using antibodies against the major outer-membrane protein (MOMP) there are 19 prototypic serovars of *C.trachomatis*. Compared to immunotyping, the genotyping methods have some advances being more precise, sensitive and specific for typing of *C. trachomatis* (Molano et al., 2004). The aims of the present study were to compare phenotypic and genotypic methods for subspecies identification of *C.trachomatis* in term of discriminatory power and concordance.

**Materials | Methods:** The datatable of Chlamydia trachomatis isolates with identified serovars/genovars and sequence types (ST) was used for analysis (<http://pubmlst.org/>). Clonal relationships of ST defined with minimum spanning tree using BURST algorithms (MLVAcompare (Ridom GmbH)). Diversity and concordance between typing methods were calculated using Simpson index and adjusted Rand and Wallace coefficients (<http://www.comparingpartitions.info>).

**Results:** There were 3691 strains of *C.trachomatis* in datatable. For 3242 isolates serovar/genovar were determined. Sequence type based on 7 loci (MLST Chlamydiales scheme) and 5 loci (MLST Uppsala scheme) were identified for 512 and 3277 isolates respectively. Discriminant power of each typing method and concordance are presented in table1.

Table 1. Resolution ability and concordance between serotyping (MOMP)/genotyping (ompA gene) and MLST methods.

Typing method	# Different types	Simpson index (95% CI)	Adjusted Rand's coefficient in comparison with serovar/genovar
Serovar/genovar (n=3242)	27	0.822 [0.814 - 0.831]	1
MLST (Uppsala) (n=3277)	519	0.974 [0.972 - 0.976]	0.171 (n=3104)
MLST (Chlamydiales) (n=512)	58	0.903 [0.893 - 0.914]	0.609 (n=49)

Adjusted Wallace coefficients were 0.096 for W1(serovar/genovar -> MLST (Uppsala)) and 0.793 for W2 (MLST (Uppsala) -> serovar/genovar). In the case with serovar/genovar and MLST (Chlamydiales) the W1 was 0.758, and W2 was 0.508. Clonal complexes (CC) of MLST and their characteristics including pairwise agreement between CC and serovars/genovars are presented in figure 1.

**Conclusion:** Our results showed the higher level of discriminatory power of MLST in comparison with serotyping. The highest resolution ability (97.4%) had Uppsala scheme MLST using 5 loci. In spite of weak/moderate degree of global congruence between serovars/genovars and sequence types (adj.Rand in table 1.) there was greater concordance for clonal complexes with more than 90% successive prediction using serovars/genovars (W1 coefficients in figure1). Interestingly, MLST typing method demonstrated clonal population structure of *C.trachomatis*.

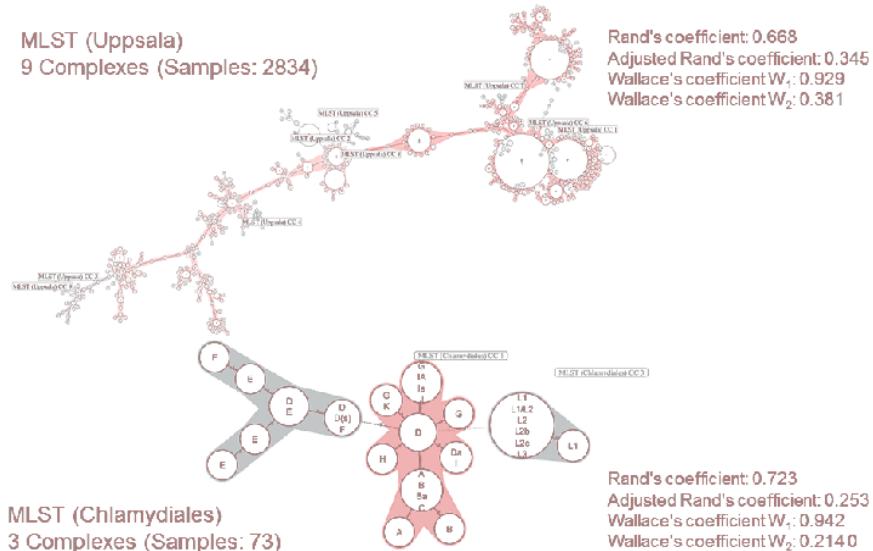


Figure 1 Clonal complexes of sequence types based on MLST (Uppsala – Up, Chlamydiales – down). Colored haloing indicates Clonal Complex. Wallace coefficients –  $W_1$  means prediction CC using serovars/genovars,  $W_2$  is vice versa.

## S6 - Poster session II

### PO5 - Investigation Of The Austrian LA-MRSA Population By Whole Genome Sequence Based Typing

Sarah Lepuschitz<sup>1</sup>; Burkhard Springer<sup>1</sup>; Alexander Indra<sup>1</sup>; Franz Allerberger<sup>1</sup>; Werner Ruppitsch<sup>1</sup>

<sup>1</sup>Austrian Agency for Health and Food Safety

**Background:** In Austria, the National Reference Laboratory for Staphylococci (NRLS) at the Austrian Agency for Health and Food Safety (AGES) observed a steady increase of infections caused by human CC398 LA-MRSA isolates from 0.2% in 2004 to 7.9% in 2015.

Since the majority of Austrian LA-MRSA isolates harbor *spa*-type t011 an arbitrary collection of isolates was analyzed by next generation sequencing in order to evaluate the performance and discriminatory power of NGS.

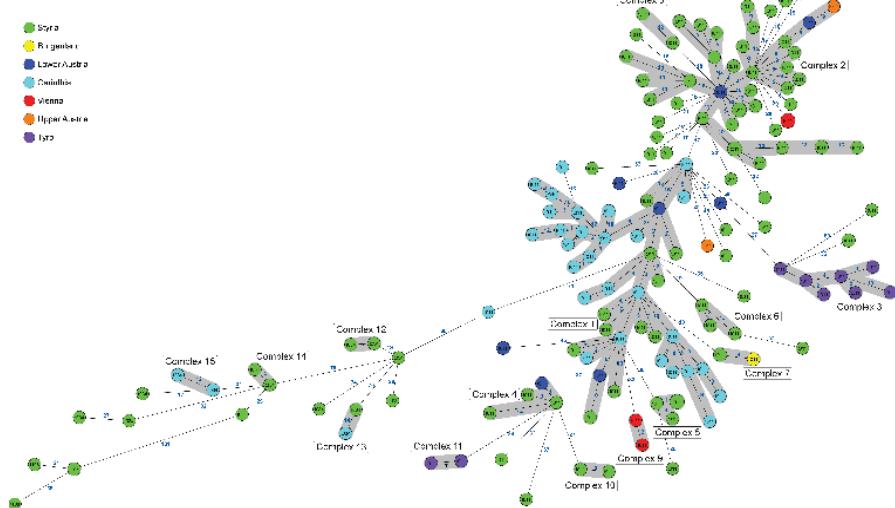
**Material/Methods:** Genomic DNA was isolated using the MagAttract HMW DNA Kit according to the instructions of the manufacturer (Qiagen, Hilden, Germany) from an arbitrary collection of 153 LA-MRSA isolates (14 animal, 20 food, 20 environment, 99 human) collected in Austria from 2004 to 2015 comprising *spa*-types t011 (n=134), t034 (n=14) and t108 (n=5). The fragment library was constructed with the NexteraXT Kit and 2x300 basepair fragments were sequenced on a MiSeq (Illumina Inc., USA). Raw reads were de-novo assembled using Velvet version 1.1.04. Contigs were filtered for a minimum coverage of 5 and a minimum length of 200 bp. A core genome (cg)MLST scheme comprising 1,862 target genes and a cluster threshold (CT) of 15 was used for NGS data interpretation using SeqSphere<sup>+</sup> (Ridom, Germany) (1).

**Results:** Core genome (cg)MLST based typing distinguished 15 different complexes and 41 isolates not assignable to a complex (Figure). Isolates belonging to *spa*-type t011 differed from isolates of *spa*-type t034 by at least 60 alleles and from isolates of *spa*-type t108 by at least 86 alleles. Isolates belonging to *spa*-type t034 showed from *spa*-type t108 an allelic difference of 26. The allelic differences were up to 71 between *spa*-type t011 isolates, 92 between *spa*-type t034 isolates and 36 between *spa*-type t108 isolates. Closely related human-human, pig-human, and environmental-human isolates were identified by cgMLST based NGS.

**Conclusions:** In conclusion NGS based typing allows the differentiation of the highly clonal LA-MRSA population in Austria as determined by *spa*-typing. This improvement in typing will now allow to monitor the evolution of strains, the identification of potential transmission routes, the identification of outbreaks and the identification of resistance and virulence determinants and as a consequence the emergence of more virulent clones.

Thus, the application of NGS based typing is highly recommended for the public health sector to improve MRSA surveillance.

**References:** Leopold S, et al. (2014) J Clin Microbiol 52:2365–2370.



## S6 - Poster session II

### PO7 - Water On The Lungs - Characterisation Of Pathogens In Pleural Effusion By The Use Of Metagenomic Sequencing

Anna Maria Malberg Tetzschnere<sup>1</sup>

<sup>1</sup>Department of Systems Biology, The technical university of Denmark

**Background:** Lung effusion is a common complication for patients hospitalized with pneumonia. Fast and adequate treatment is important for fast recovery and survival, and it relies solely on rapid characterization of the pathogen causing the disease. Unfortunately, the clinical microbiology practice is labor intensive and it can take days or even weeks to determine the pathogen. In this study we aim to develop a new method to improve and accelerate the detection and characterization the infectious pathogen in patients suffering from lung effusion by the use of direct sequencing on clinical sample.

**Methods:** At the hospital we collect pleural fluid by drainage from patients with pleural effusion. DNA is purified directly from the clinical samples, and several steps are used to remove the human contamination prior to sequencing.

After sequencing the raw reads are trimmed and then mapped against predefined databases by the use of the MGMapper software (<https://cge.cbs.dtu.dk/services/MGMapper/>). Firstly the raw reads are mapped against a human genome, thereby removing any human contamination. The remaining unmapped reads are mapped against several databases containing complete and draft bacterial, fungal protozoan and viral genomes. The remaining reads that do not match anything will be mapped against the complete nucleotide database.

In order to obtain detailed characteristics of the pathogenic strain, the raw reads are mapped against databases containing virulence and resistance genes.

Finally a report will be generated containing information for diagnosis to be made. This will include abundance profiles of the strains, virulence factors and resistance markers.

## PO8 - Examination Of Metagenomic Analysis For Typing Of *Campylobacter* In A Cultureless Future Using Present-day Technology

Katrine Grimstrup Joensen<sup>1</sup>; Søren Persson<sup>1</sup>

<sup>1</sup>Statens Serum Institut

**Background:** Hospitals are increasingly using PCR diagnostics directly on clinical samples instead of culturing, due to lower cost as well as higher sensitivity and speed. The lack of culturing presents a problem in the public health surveillance of pathogens that traditionally depends on isolates for molecular typing methods, including WGS. One approach to maintain public health surveillance without culturing would be metagenomics sequencing of patient samples.

**Materials/methods:** Human and chicken fecal samples (negative for *Campylobacter* by culturing and PCR) were spiked with 10-fold dilutions of  $10^5$  to  $10^8$  cfu/g of *C. jejuni* SC181. We sequenced each of the sub-samples, including non-spiked controls, on an Illumina MiSeq, using 250bp paired-end reads and 1/8 of a flowcell for each. The sequences were analysed using Kraken (<https://ccb.jhu.edu/software/kraken/>) and plasmid and phage sequences were filtered out. The sequences matching *Campylobacter* in each of the sub-samples were compared using blastn to estimate the size of the overlap.

**Results:** We detected 446 and 58 *Campylobacter* reads in the human  $10^7$  and  $10^8$  sub-samples, respectively. Of these, we were able to assign 64% and 53%, respectively, of the reads to the SC181 strain. We detected 5118 and 254 *Campylobacter* reads in the  $10^6$  and  $10^5$  chicken sub-samples, respectively, of which 8% and 7% were specific for strain SC181. In the human control sample no *Campylobacter* reads were detected, whereas a single read was detected in the chicken control sample. We compared the SC181 sequences detected in the different sub-samples and found overlaps ranging from 4101bp to 183027bp (see table).

*Table: Number of basepairs overlapping between *Campylobacter* reads in samples spiked with different levels of *Campylobacter*. Probability of observing an individual SNP in parenthesis.*

	Human, $10^8$ cfu/g	Human, $10^7$ cfu/g	Chicken, $10^6$ cfu/g	Chicken, $10^5$ cfu/g
Human, $10^8$ cfu/g	-	-	-	-
Human, $10^7$ cfu/g	4101 (1:365)	-	-	-
Chicken, $10^6$ cfu/g	127598 (1:12)	19278 (1:78)	-	-
Chicken, $10^5$ cfu/g	15039 (1:100)	2615 (1:574)	183027 (1:8)	-

**Conclusion:** This shows that we will be able to identify a known outbreak strain directly in fecal samples containing a load of infection above  $10^5$  cfu/g. Another issue is if we will be able to compare the identified sequences from the metagenomics samples, and estimate how different the strains are. With the loads and sequencing depths we have investigated in this study, it would not be enough for outbreak detection, although with the detection of 1/8 of the genome it is getting close.

## S6 - Poster session II

### PO9 - The Role Of Surface Adhesins In Epidemic Hypervirulent *Clostridium difficile* BI/NAP1/027 Strain And Non-epidemic Strain Virulence And Bio

SOZA BABAN<sup>1</sup>

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*Clostridium difficile* is a major cause of healthcare-associated infection and inflicts a considerable financial burden on healthcare systems worldwide. Disease symptoms range from self-limiting diarrhoea to fatal pseudomembranous colitis. Whilst *C. difficile* has two major virulence factors, toxin A and B, it is generally accepted that other virulence components of the bacterium contribute to disease. *C. difficile* colonises the gut of humans and hence the processes of adherence and colonisation are essential for disease onset. Bacteria within biofilms are protected from multiple stresses, including immune responses and antimicrobial agents. Increased antibiotic resistance and chronic recurrent infections have been attributed to the ability of bacterial pathogens to form biofilms. While biofilms have been well studied for several gut pathogens, little is known about biofilm formation by anaerobic gut species. Numerous cell surface proteins and surface structures such as flagella have been shown to be important for biofilm formation in this Gram-positive pathogen. This study investigated for the first time the role of surface adhesion genes in virulence of two important *C. difficile* isolates; non-epidemic historical *C. difficile* PCR-ribotype012 and newly emerged hypervirulent *C. difficile* BI/NAP1/027 strain which caused large outbreaks of CDAD worldwide. Non-motile, paralyzed-flagellated and non-flagellated mutants were created using ClosTron and Allelic-exchange mutagenesis technologies. Our work demonstrates the ability of epidemic pathogen to form complex biofilms and the involvement of important clostridial pathways in biofilm development. Importantly, the bacterial sensitivity to antibiotics is reduced in clostridial biofilms. Biofilm formation may be a mechanism employed by *C. difficile* to survive in environment such as human gut. We compared the flagellated wild-type to a mutant with a paralyzed flagellum and also to non-flagellated *in vitro* and *in vivo*. This study provides the first strong evidence that by disabling the motor of the flagellum, the structural components of the flagellum rather than active motility, is needed for adherence and colonisation by hypervirulent strain. The hypervirulent epidemic flagellar mutants adhered less than the parental strain *in vitro*, whereas we saw the opposite in 630Δerm. In the later strain we showed that flagella and motility are not needed for successful colonisation *in vivo*. Finally we demonstrated that in the epidemic strain, flagella do play a role in colonisation and adherence and that there are striking differences between strains. The latter emphasises the overriding need to characterize more than just one strain before drawing general conclusions concerning specific mechanisms of pathogenesis. In addition, the hypervirulent strain, form structured biofilms *in vitro*. Biofilm formation by *C. difficile* is a complex multifactorial process and may be a crucial mechanism for clostridial persistence in the host.

**PO10 - *Bifidobacterium bifidum* YIT 10347 Suppress Gut Microbiota Dysbiosis And Alleviate Severe Diarrhea In *Helicobacter pylori* Eradication Therapy**

Toshifumi Ohkusa<sup>1</sup>; Takao Osaki<sup>2</sup>; Hideo Yonezawa<sup>2</sup>; Shigeru Kamiya<sup>2</sup>

<sup>1</sup>Department of gastroenterology and Hepatology, The Jikei University Kashiwa Hospital,<sup>2</sup> Department of Infectious Diseases, Kyorin University School of Medicine

**Background:** An antibiotics-associated diarrhea (AAD) following *Helicobacter pylori* eradication therapy is most side effects and may be due to gut microbiota dysbiosis. However, it has not been a metagenomic analysis study about diarrhea in *Helicobacter pylori* eradication therapy and gut microbiota dysbiosis. We conducted a randomized double-blind placebo-controlled clinical trial to examine the effect of probiotic *Bifidobacterium bifidum* YIT 10347 on *H. pylori* eradication, diarrhea, and gut microbiota dysbiosis.

**Patients and Methods:** 228 Japanese patients 228 patients were divided into active drug and placebo groups at random, and ingested 100 ml of either fermented milk containing *B. bifidum* YIT 10347 (active group) or non-fermented milk (placebo group) once daily for 17 weeks. Triple therapy of lansoprazole 30 mg bid, amoxicillin 750 mg bid, and clarithromycin 200 mg bid (LAC) was treated at week 8 for 1 week to eradicate *H. pylori*. Side effects and rate of eradication were evaluated. Gut microbiota of 105 participants (active group: 49 subjects, placebo group: 56 subjects) selected randomly were analyzed on 8, 9, and 17 weeks by 16S rDNA metagenomics sequencing and the UniFrac index was used to evaluate gut microbiota dysbiosis.

**Results:** *H. pylori* eradication rate and the occurrence of side effects did not significantly differ between the groups, except that the occurrence of severe diarrhea (4 times a day or more of diarrhea) was significantly lower in the active group. Gut microbiota dysbiosis occurred in both the group, but the increase in the UniFrac index on 17 weeks was significantly suppressed in the active group. *B. bifidum* YIT 10347 suppressed decrease of Bifidobacteriaceae and increase of Bacteroidaceae and alleviated decrease of Firmicutes phylum like Lachnospiraceae on 9 weeks. Recovery of gut microbiota was significantly earlier in the active group than in the placebo group on 17 weeks.

**Conclusion:** In the *H. pylori* eradication LAC therapy, ingestion of probiotic *B. bifidum* YIT 10347 alleviated of severe AAD by suppression of gut microbiota dysbiosis.



## ANTIMICROBIAL RESISTANCE AND MOBILE GENETICS ELEMENTS

**Friday, 11 March 2016, 12:00-13:30**

### PO11 - Transfer Of Silenced VanA Gene Cluster By Broad Host-range Plasmid

Audun Sivertsen<sup>1</sup>; Torunn Pedersen<sup>2</sup>; Kjersti Wik Larssen<sup>3</sup>; Kåre Bergh<sup>3</sup>; Torunn Gresdal Rønning<sup>3</sup>; Andreas Radke<sup>3</sup>; Kristin Hegstad<sup>2</sup>

<sup>1</sup>University of Tromsø – The Arctic University of Norway, <sup>2</sup> University hospital of North-Norway, <sup>3</sup> St. Olavs Hospital

### PO12 - High-level Beta-lactam Resistance In A *Staphylococcus aureus* Strain Carrying The MecC Gene Determinant

Catarina Milheiricô<sup>1</sup>; Alexander Tomasz<sup>2</sup>; Hermínia de Lencastre<sup>1,2</sup>

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### PO13 - Resistance To Daptomycin In Vancomycin-resistant *Enterococcus faecium* During Daptomycin Treatment

Christine Elmeskov<sup>1</sup>; Mette Pinholt<sup>1</sup>; Peder Worning<sup>1</sup>; Jesper Boye Nielsen<sup>1</sup>; Heidi Gumpert<sup>1</sup>; Dorte Frees<sup>2</sup>; Henrik Westh<sup>1</sup>

<sup>1</sup>Hvidovre University Hospital, <sup>2</sup> University of Copenhagen

### PO15 - Investigation Of The Mechanism Involved In Tigecycline Resistance In *Enterococcus* Spp.

Stefan Fiedler<sup>1</sup>; Jennifer Bender<sup>1</sup>; Carola Fleige<sup>1</sup>; Uta Geringer<sup>1</sup>; Ingo Klare<sup>1</sup>; Guido Werner<sup>1</sup>

<sup>1</sup>Robert Koch-Institute, Division 13 Nosocomial Pathogens and Antibiotic Resistances

### PO16 - Characteristics Of Publicly Available *Staphylococcus aureus* Genomes

Nir Gilad<sup>1</sup>; Michal Ziv-Ukelson<sup>2</sup>; Vered Chalifa-Caspi<sup>1</sup>; Jacob Moran-Gilad<sup>1</sup>

<sup>1</sup>Ben-Gurion University, <sup>2</sup> Ben Gurion University

## S7 - Poster session II

### PO17 - Comparison Of Extended-Spectrum Beta-Lactamase (ESBL)-producing *Escherichia coli* Isolates From Hospitals, Ambulatory Settings And The Community

Michael Pietsch<sup>1</sup>; Christoph Eller<sup>2</sup>; Linda Falgenhauer<sup>3</sup>; Rasmus Leistner<sup>4</sup>; Giuseppe Valenza<sup>5</sup>; Constanze Wendt<sup>6</sup>; Guido Werner<sup>1</sup>; Yvonne Pfeifer<sup>1</sup>

<sup>1</sup>Robert Koch Institute, FG 13 Nosocomial Pathogens and Antibiotic Resistance,<sup>2</sup> Department of Laboratory Medicine, University Hospital Halle,<sup>3</sup> Institute of Medical Microbiology, Justus Liebig University Giessen and German Center for Infection Research (DZIF), Partner site Giessen-Marburg-Langen, Campus Giessen,<sup>4</sup> Charité Universitätsmedizin, Institut of Hygiene and Environmental Medicine,<sup>5</sup> Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit (LGL),<sup>6</sup> Labor Limbach

### PO18 - Characterization And Molecular Epidemiology Of Plasmid-mediated Quinolone Resistance *Escherichia coli* Isolates From Algeria.

Yanat Betitra<sup>1</sup>; Machuca Jesús<sup>2</sup>; Díaz de Alba Paula<sup>2</sup>; Abdelaziz Touati<sup>1</sup>; Pascual Alvaro<sup>2</sup>; Rodriguez Martinez Jose Manuel<sup>2</sup>

<sup>1</sup>Laboratory of microbial ecology. University of Bejaia,<sup>2</sup> Department of microbiology. Faculty of medicine. University of Seville

### PO19 - Molecular Characterisation Of Carbapenem-resistant Gram-negative Bacteria From A Bulgarian Hospital

Yvonne Pfeifer<sup>1</sup>; Angelina Trifonova<sup>2</sup>; Michael Pietsch<sup>1</sup>; Magdalena Brunner<sup>1</sup>; Iva Todorova<sup>2</sup>; Ivanka Gergova<sup>2</sup>; Gottfried Wilharm<sup>1</sup>; Guido Werner<sup>1</sup>; Encho Savov<sup>2</sup>

<sup>1</sup>Robert Koch Institute,<sup>2</sup> Military Medical Academy

### PO21 - Emergence Of CTX-M-producing Clinical Isolates Of *Klebsiella pneumoniae* In The University Hospital Establishment Of Oran, Algeria.

Zemmour Assia<sup>1</sup>; Dali-Yahia Radia<sup>2</sup>; Saidi Ouahrani Nadja<sup>1</sup>; Rahmani Bouabdallah<sup>1</sup>; Christian G. GISKE<sup>3</sup>

<sup>1</sup>University Of Sciences And Technology,<sup>2</sup> University Hospital Establishment 1st, Novembre 1954,<sup>3</sup> Karolinska University Hospital

### PO22 - Epidemiology Of Carbapenem-resistant *Pseudomonas putida* Group Clinical Isolates Recovered From Screening Cultures

Silke Peter<sup>1</sup>; Philipp Oberhettinger<sup>1</sup>; Ariane Kirste<sup>1</sup>; Wichard Vogel<sup>2</sup>; Daniela Dörfel<sup>2</sup>; Daniela Bezdan<sup>3</sup>; Stephan Ossowski<sup>3</sup>; Matthias Marschal<sup>1</sup>; Jan Liese<sup>1</sup>; Matthias Willmann<sup>1</sup>

<sup>1</sup>Institute of Medical Microbiology and Hygiene, University of Tübingen,<sup>2</sup> Medical Center, Department of Hematology, Oncology, Immunology, Rheumatology & Pulmonology, University of Tübingen,<sup>3</sup> Genomic and Epigenomic Variation in Disease Group, Centre for Genomic Regulation (CRG), Barcelona, Spain  
Universitat Pompeu Fabra (UPF)

**PO23 - First Report Of Macrolide Resistance Among *Shigella* Isolates In Israel Caused By The mphA And Erm(b) Genes**

Analia V. Ezernitchi<sup>1</sup>; Elizabeth Sirotkin<sup>1</sup>; Vered Agmon<sup>1</sup>; Lea Valinsky<sup>1</sup>; Assaf Rokney<sup>1</sup>

<sup>1</sup>Ministry of Health Israel

**PO24 - CTX-M-55 Extended-spectrum Beta-lactamase Among *Enterobacteriaceae* From The Korean Antimicrobial Resistance Monitoring System, 2008 To 20<sup>15</sup>**

Jin Seok Kim, Soo Jin Kim, Jungsun Park, Gyung Tae Chung and Junyoung Kim,

<sup>1</sup>Korea National Institute of Health, Division of Enteric Diseases

**PO25 - Active Screening And Genotyping Successfully Controls Outbreaks Of Multi-drug-resistant *Enterobacteriaceae***

Anneke van der Zee<sup>1</sup>; Arjan Burggraaf<sup>1</sup>; Lieuwe Roorda<sup>1</sup>; Fer Vlaspolder<sup>2</sup>; Jacobus M Ossewaarde<sup>1</sup>

<sup>1</sup>Maastad Hospital,<sup>2</sup> Maastad Hospital

**PO26 - Genetic Diversity And Molecular Analysis Of Quinolone-resistant *Shigella sonnei* Isolates From Humans In Republic Of Korea, 2008-2014**

Junyoung Kim<sup>1</sup>; Soo Jin Kim, Jin Seok Kim, Nan-Ok Kim, Sahyun Hong and Gyung Tae Chung

<sup>1</sup>Korea National Institute of Health, Division of Enteric Diseases

**PO27 - Antimicrobial Resistance And Virulence Profile In Community And Hospital-acquired Urinary Tract Infections Caused By *Klebsiella pneumoniae***

C. Caneiras<sup>1</sup>; L. Lito<sup>2</sup>; J. Melo-Cristino<sup>3</sup>; A. Duarte<sup>1</sup>

<sup>1</sup>iMed.UL - Research Institute for Medicines and Pharmaceutical Sciences, Faculty of Pharmacy, University of Lisbon,<sup>2</sup> Laboratory of Microbiology, Centro Hospitalar Lisboa Norte,<sup>3</sup> Laboratory of Microbiology, Centro Hospitalar Lisboa Norte,<sup>4</sup> Institute of Microbiology, Institute of Molecular Medicine, Faculty of Medicine, University of Lisbon

**PO28 - Increasing Cotrimoxazole Susceptibility Associated With Predominance Of International Clone 2 *Acinetobacter baumannii* Clinical Isolates: A Nationwide**

Spyros Pournaras<sup>1</sup>; Konstantina Dafopoulos<sup>1</sup>; Olympia Zarkotou<sup>2</sup>; Aggeliki Poulopoulos<sup>3</sup>; Stamatina Levidiotou<sup>4</sup>; Achilleas Gikas<sup>5</sup>; Athanasios Tsakris<sup>1</sup>, Greek Study Group on Acinetobacter Antimicrobial Resistance

<sup>1</sup>Medical School, University of Athens,<sup>2</sup> Tzaneio Hospital,<sup>3</sup> Serres Hospital,<sup>4</sup> Ioannina University Hospital,<sup>5</sup> Heraklion University Hospital

## S7 - Poster session II

### **PO29 - Faecal Carriage Of ESBL Producing Bacteria Among Children In Dar Es Salaam, Tanzania**

Marit Gjerde Tellevik<sup>1</sup>; Sabrina J. Moyo<sup>2</sup>; Samuel Y. Maselle<sup>3</sup>; Bjørn Blomberg<sup>1</sup>; Nina Langeland<sup>2</sup>

<sup>1</sup>Haukeland University Hospital, <sup>2</sup>University of Bergen, <sup>3</sup>Muhimbili University of Health and Allied Sciences

### **PO30 - Establishing A Closed Reference Sequence Of A Vancomycin-resistance Plasmid From *Enterococcus faecium***

Heidi Gumpert<sup>1</sup>; Mette Pinholt<sup>1</sup>; Peder Worning<sup>1</sup>; Jesper Boye Nielsen<sup>1</sup>; Henrik Westh<sup>1</sup>

<sup>1</sup>Hvidovre University Hospital

### **PO31 - Ice Carrying Antimicrobial Resistance Genes Among Pig Isolates Of *Streptococcus suis* In Poland**

Ewa Sadowsy<sup>1</sup>; Agnieszka Bojarska<sup>1</sup>; Zygmunt Pejsak<sup>2</sup>; Waleria Hryniwicz<sup>3</sup>

<sup>1</sup>Department of Molecular Microbiology, National Medicines Institute,<sup>2</sup>

Department of Swine Diseases, National Veterinary Research Institute,<sup>3</sup>

Department of Epidemiology and Clinical Microbiology, National Medicines Institute

### **PO32 - Investigations On Bacterial Fitness Of *Escherichia coli* With Plasmid Mediated Beta-lactam Resistance**

Michael Pietsch<sup>1</sup>; Yvonne Pfeifer<sup>1</sup>; Guido Werner<sup>1</sup>

<sup>1</sup>Robert Koch Institute, FG 13 Nosocomial Pathogens and Antibiotic Resistance

### **PO33 - A GI Identified In Clinical And Environmental *Mycobacterium avium* subsp. *hominissuis* Isolates From Germany**

Andrea Sanchini<sup>1</sup>; Torsten Semmler<sup>1</sup>; Lei Mao<sup>1</sup>; Astrid Lewin<sup>1</sup>

<sup>1</sup>Robert Koch Institute

### **PO34 - Magnitude Of Gene Mutations Conferring Drug Resistant In *Mycobacterium tuberculosis* Strains In Southwest Ethiopia**

Mulualem Tadesse<sup>1</sup>; Dossegnew Aragaw<sup>1</sup>; Gemedu Abebe<sup>1</sup>

<sup>1</sup>Jimma University

**PO11 - Transfer Of Silenced VanA Gene Cluster By Broad Host-range Plasmid**

Audun Sivertsen<sup>1</sup>; Torunn Pedersen<sup>2</sup>; Kjersti Wik Larssen<sup>3</sup>; Kåre Bergh<sup>3</sup>; Torunn Gresdal Rønning<sup>3</sup>; Andreas Radke<sup>3</sup>; Kristin Hegstad<sup>2</sup>

<sup>1</sup>University of Tromsø – The Arctic University of Norway, <sup>2</sup> University hospital of North-Norway, <sup>3</sup> St. Olavs Hospital

Keywords: VRE, IS-elements, Outbreak, plasmid, HGT

**Objectives:** The purpose of this study was to explain vancomycin resistance development in a nosocomial outbreak of *vanA*-containing, vancomycin susceptible enterococci within two hospitals in Trøndelag, Norway.

**Materials & methods:** 48 *E. faecium* and one *E. faecalis* linked to a nosocomial outbreak and shown to be *vanA*-containing vancomycin susceptible or resistant enterococci (VSE/VRE) by *vanA* PCR and phenotypic testing, were included in the study. 42 of the *E. faecium* isolates were clonal by PFGE and six were of unique pulsotypes. Four of the clonal *vanA*-containing VSEfm isolates and two VREfm isolated after vancomycin treatment of VSEfm infections were whole-genome sequenced by Illumina MiSeq. The plasmid content of all unique pulsotypes was analyzed with S1-nuclease PFGE and hybridizations. Transfer of *vanA* was assessed by filter mating. The VSE (n=35) were cultivated in BHI broth containing 8 mg/L vancomycin for *in vitro* development of VREfm. The *vanA* cluster composition was determined by contig gap closure with PCR and Sanger sequencing, and transcription profile was assessed with RT-qPCR.

**Results:** The only genetic difference between the *vanA* clusters of VSE and VRE was an *ISL3*-family insertion sequence element found upstream of *vanHAX* in the VSEfm in all pulsotypes. Furthermore, all VSEfm and VREfm isolates had insertions of *IS1216* in the intergenic region between *vanX* and *vanY* as well as *IS1542* between *orf2* and *vanR* as compared to the prototype *vanA* gene cluster.

Growth of all the VSEfm was observed after exposure to vancomycin or teicoplanin for 24 to 72 hours. All the resulting resistant mutants had lost *ISL3*. There was a 60-fold reduction of the essential *vanHAX* gene transcription due to the *ISL3* insertion. *In vitro* produced resistant mutants expressed *vanHAX* constitutively and not through induction during vancomycin exposure, as is the case of wild-type VRE (BM4147). This could be linked a 7-fold reduction in the expression of the *vanHAX* activator, coded by *vanRS*, due to an insertion of *IS1542* in the *vanRS* promoter area.

The *vanA* gene cluster was located on a transferable plasmid of same replicon type and similar size in both PFGE-related and unrelated isolates.

**Conclusions:** *ISL3* insertion upstream of *vanHAX* caused the VanA phenotype to be silenced. Exposure to vancomycin resulted in resistant variants that had lost *ISL3*. *IS1542* insertion upstream of *vanRS* resulted in a constitutive transcription of the VanR-activated *vanHAX* and *vanRS* promoters. The *vanA* gene cluster was located on a transferable broad host-range plasmid also detected in outbreak isolates with different PFGE types as well as an *E. faecalis*. Both genotypic and phenotypic susceptibility testing is necessary to disclose these potentially vancomycin resistant isolates.

### PO12 - High-level Beta-lactam Resistance In A *Staphylococcus aureus* Strain Carrying The MecC Gene Determinant

Catarina Milheiriço<sup>1</sup>; Alexander Tomasz<sup>2</sup>; Hermínia de Lencastre<sup>1,2</sup>

<sup>1</sup>Laboratory of Molecular Genetics, Instituto de Tecnologia Química e Biológica António Xavier (ITQB), Universidade Nova de Lisboa,<sup>2</sup> Laboratory of Microbiology and Infectious Diseases, The Rockefeller University

**Background:** Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important multi-resistant pathogens worldwide. Most MRSA carry the common genetic determinant *mecA* encoding for the low affinity penicillin binding protein PBP2A. A new methicillin resistant determinant – *mecC* – was recently identified in some MRSA isolates: it shares only 69% identity with *mecA* from MRSA strain N315 and is integrated in a new SCC*mec* structure (SCC*mec* XI). Strains carrying *mecC* have typically low MICs to oxacillin; however, no data are available on the impact of extensive beta-lactam selection on the emergence of *mecC* carrying strains with increased levels of resistance.

**Methods:** We exposed the prototype strain LGA251 carrying the *mecC* determinant to a step-selection procedure with increasing concentrations of oxacillin in the growth medium. Three LGA251 derivatives able to grow at elevated oxacillin concentrations were isolated. Efforts to identify the mechanism of increased resistance found no mutations in the determinants of PBP2 and PBP4 or in the *mecC* regulatory genes. However, a point mutation was identified in the *mecC* gene of the most resistant derivative strain of LGA251. Whole genome sequencing (WGS) was used to identify determinants affected by this step-selection procedure.

**Results:** Eleven different single nucleotide polymorphisms (SNPs) affecting eight different open reading frames (*orfs*) were identified in the WGS data analysis. As expected, accumulation of mutations occurred over time: the first derivative strain (LGA251 (50) able to grow in the presence of 50 µg/ml of oxacillin in the growth medium) – was the one with fewer mutations and these were maintained in the more resistant derivative strains (LGA251 (100) and LGA251 (800)). In addition, there were two regions deleted during the selection process, comprising ten different *orfs*. Compromised *orfs* were scattered throughout the core genome of LGA251 affecting several metabolic functions.

**Conclusion:** Detection of mutations in genes without a direct link to the common mechanism of methicillin resistance indicates that determinants located in the genetic background of bacteria can have profound influence on beta-lactam resistance levels. Studies are under way to identify *orfs* that play key roles in defining the oxacillin resistance level of bacterial isolates recovered from the step-selection process.

**Funding:** Project PTDC/BIA-MIC/3195/2012 and SFRH/BPD/63992/2009 (FCT, Portugal), US Public Health Service Award R01-AI457838-15.

## PO13 - Resistance To Daptomycin In Vancomycin-resistant *Enterococcus faecium* During Daptomycin Treatment

Christine Elmeskov<sup>1</sup>; Mette Pinholt<sup>1</sup>; Peder Worning<sup>1</sup>; Jesper Boye Nielsen<sup>1</sup>; Heidi Gumpert<sup>1</sup>; Dorte Frees<sup>2</sup>; Henrik Westh<sup>1</sup>

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Keywords: whole genome sequencing, Enterococcus, VRE

**Background:** Daptomycin is a cyclic lipopeptide used in the treatment of vancomycin-resistant *Enterococcus faecium* (VREfm). However, the finding of daptomycin resistant VREfm has challenged the treatment of nosocomial VREfm infections. VREfm can cause urinary tract infections, intraabdominal infections and catheter related infections usually in older or weaker patients. We report a case where a daptomycin susceptible VREfm isolate became resistant during daptomycin and linezolid therapy. The isolates were identified in a patient with gallstone-induced pancreatitis complicated by pancreatic necrosis and pancreatic abscesses. The patient was treated with a combination of daptomycin and linezolid for 40 days prior to the identification of the daptomycin resistant VREfm.

In the present study we examined the genetic changes in a VREfm strain that became daptomycin resistant during daptomycin treatment.

**Materials | Methods:** The daptomycin susceptible isolate, V1164, was identified in drain fluid from an intraabdominal abscess, and the resistant isolate, V1225, was identified from the patient's bloodstream. Daptomycin MIC was established on both isolates using E-test. Whole genome sequencing of the two isolates was performed to reveal their genetic background and to uncover potential mutations causing daptomycin resistance. Sequencing was performed in an Illumina MiSeq using Nextera XT library preparation Kit running 2x150bp paired end reaction. Reads were assembled using Velvet and VelvetOptimizer. The reads were mapped to a reference genome (AUS004\_NC-017022) using Stampy and SNPs were called using Samtools.

Genes previously identified to be related to daptomycin resistance were investigated in CLC Genomics by using BLAST. The BLASTed genes were cardiolipin synthase (*cls*), cyclopropane fatty acid synthase (*cfa*) and a glycerolphosphoryl diester phosphodiesterase (*gdpD*), all involved in phospholipid metabolism and genes encoding the LiaFSR regulatory system and the YycFGHIJ system, involved in the cell envelope stress response and cell wall homeostasis, respectively. The HD Domain protein believed to be involved in nucleic acid metabolism, signal transduction and possibly other functions, and a hypothetical protein, encoding a putative N-acetylmuramoyl-L-alanine amidase, were also BLASTed.

**Results:** V1164 exhibited a daptomycin MIC of 2 mg/L and V1225 exhibited a daptomycin MIC of 8 mg/L.

The two VRE genomes were ST18 and one SNP apart compared to the reference genome. We identified five mutations in *gdpD*, of which three were non-synonymous mutations and two were synonymous mutations. We also identified a deletion in a hypothetical protein domain of 34 bases, and a 16.000 bp deletion that included the entire genetic region of the HD domain protein.

**Conclusion:** The combination therapy with daptomycin and linezolid did not protect against development of daptomycin resistance. The mutations identified in *gdpD* in V1225 are likely to cause daptomycin resistance, as previous studies have reported that mutations in *gdpD* are associated with daptomycin resistance. The deletion of the HD domain is also interesting, as previous studies only found amino acid changes in a daptomycin resistant strain. Other mutations in genes previously reported to be associated with resistance, such as *cls* or *cfa*, were not identified in this study.

## S7 - Poster session II

### PO15 - Investigation Of The Mechanism Involved In Tigecycline Resistance In *Enterococcus* Spp.

Stefan Fiedler<sup>1</sup>; Jennifer Bender<sup>1</sup>; Carola Fleige<sup>1</sup>; Uta Geringer<sup>1</sup>; Ingo Klare<sup>1</sup>; Guido Werner<sup>1</sup>

<sup>1</sup>Robert Koch-Institute, Division 13 Nosocomial Pathogens and Antibiotic Resistances

**Background:** Tigecycline (TGC) represents one of the last-line therapeutics to combat multi-drug resistant bacterial pathogens including VRE and MRSA. The National Reference Centre for Staphylococci and Enterococci at the RKI received 79 TGC-resistant *E. faecium* and *E. faecalis* isolates in recent years. The precise mechanism of how enterococci become resistant to TGC remains undetermined. As part of an ongoing investigation this abstract is to describe examinations of efflux pumps and their contributions to TGC resistance in clinical isolates of *Enterococcus* spp.

**Methods:** High and low level TGC-resistant strains were analyzed with respect to genome and transcriptome differences by means of whole genome sequencing, whole transcriptome sequencing and RT-qPCR. Genes of interest were cloned and expressed in *Listeria monocytogenes* for functional analyses including determination of the minimum inhibitory concentration (MIC) to TGC.

**Results:** As analyzed by microbroth dilution assays, varying levels of TGC MICs exist for the 27 strains investigated. Comparative genome analyses of three isogenic strains, showing different levels of TGC resistance, revealed the MFS efflux pump TetL and the ribosomal protection protein TetM as possible drug resistance proteins. Subsequent RT-qPCR confirmed the up-regulation of the respective genes compared to the isogenic TGC-sensitive strain. A correlation of gene copy number and level of resistance could be inferred from further analyses. Eventually, expression of both *tet(L)* and *tet(M)* in *L. monocytogenes* unequivocally demonstrated the potential to increase TGC MICs upon acquisition of the loci.

**Conclusion:** Our results indicate that increased expression of two determinants, a *tetL*-encoded MFS-pump and a *tetM*-encoded ribosomal protection protein, is capable of conferring TGC resistance in some enterococcal strains. However, as not all TGC-resistant isolates investigated in this study were tested positive for *tet(L)*, alternative TGC resistance mechanisms are suspected and will be addressed in future experiments.

**PO16 - Characteristics Of Publicly Available *Staphylococcus aureus* Genomes**Nir Gilad<sup>1</sup>; Michal Ziv-Ukelson<sup>2</sup>; Vered Chalifa-Caspi<sup>1</sup>; Jacob Moran-Gilad<sup>1</sup><sup>1</sup>*Ben-Gurion University*, <sup>2</sup>*Ben Gurion University*

**Background:** Genome depositions of important pathogens such as *S. aureus* are rapidly accumulating in public domain databases such as NCBI and could prove useful for development and evaluation of high-throughput bioinformatics tools as well as cross-sectional studies of pathogenomics. However, concerns have been raised regarding the quality of publicly deposited genomic data for many microorganisms. We thus sought to determine the characteristics of publicly available *S. aureus* genomes.

**Methods:** Available *S. aureus* genome assemblies were downloaded from NCBI and checked for quality using several software tools. The genomes were subsequently analysed using an in house *S. aureus* pipeline at the Surveillance and Pathogenomics Israeli Centre of Excellence (SPICE). Genus and species identification (ID) and inferred antimicrobial susceptibility testing (AST) were determined by performing BLAST analyses as well as *in silico* PCR analyses according to published methods and using in house as well as public resistance gene databases such as The Comprehensive Antibiotic Resistance Database (CARD), consisting of over 3,000 genes in total. Additional characteristics such as SCCmec type, PVL and USA300 lineage were determined based on published gene markers.

**Results:** Overall, 4,262 genomes were included in the analysis. All genomes appeared to belong to the *Staphylococcus* genus. The *nuc* gene marker for *S. aureus* was missing in 19 genomes (0.45%). Following additional analysis, 12 samples were identified as species other than *S. aureus* (0.28%). The majority (3,903, 91.6%) of available genomes were methicillin-resistant *S. aureus* (MRSA). Only 122 of those (3.1%) were depositions tagged as MRSA and 2 of those 122 samples (1.6%) were *mecA* and SCCmec negative. SCCmec typing could be determined in 96.3% and types I, II, III and IV accounted for 3.8%, 56.3%, 0.9% and 29.3% of typeable samples, respectively. The pantone valentine leucocidin (PVL) gene was present in 661 samples (15.5%) and 712 samples contained the USA300 lineage markers (16.7% in total, 18.2% of MRSA). Amongst MRSA genome assemblies, major antimicrobial resistance determinants were found in the following proportions: macrolides: *ermA* 60%, *ermC* 6%, *ermB* 1%; beta-lactamases: *blaZ* 53.2%; aminoglycoside modifying enzymes: APH(3')-Illa 25.9%, AAC(6')-Ie-APH(2')-Ia 13.6%, *aad(6)* 3.7%, *ANT(6)*-Ia 0.3%; tetracyclines: *tetK* 8.8%; disinfectants *qacA* 1.6%; chloramphenicol *cat* 0.1%.

**Conclusion:** Analysis of over 4,000 *S. aureus* genome assemblies available through the public domain showed a very low rate of erroneous genus, species and MRSA classification, suggesting publicly available *S. aureus* datasets could be safely used for developing and validating Staph bioinformatics pipelines for clinical and public health usage. Systematic analysis of important microbiological characteristics of *S. aureus* revealed that publicly available datasets are relatively enriched with samples of MRSA and PVL-positive samples compared to natural epidemiology, most probably reflecting the medical and research focus in this field. High throughput screening of *S. aureus* genomes for inferring antimicrobial resistance was feasible and its application on publicly available samples may be helpful for generating relevant cross-sectional data on the global prevalence of resistance determinants.

## S7 - Poster session II

### PO17 - Comparison Of Extended-Spectrum Beta-Lactamase (ESBL)-producing *Escherichia coli* Isolates From Hospitals, Ambulatory Settings And The Community

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**Background:** The increase of *Escherichia coli* producing extended-spectrum beta-lactamases (ESBL) in hospitals and their emergence as colonisers of healthy humans is of concern. Here we report the results of the molecular characterisation of ESBL-producing *E. coli* isolates from German hospitals and medical practices. The isolates were collected in different studies in the scope of the interdisciplinary research project "RESET" ([www.reset-verbund.de](http://www.reset-verbund.de)).

**Materials/Methods:** We analysed 527 *E. coli* with ESBL phenotype from three studies: 128 nosocomial *E. coli* and 105 "ambulant" *E. coli* isolates from outpatient departments, 211 *E. coli* from healthy participants (community study) and 83 community-acquired *E. coli* from a case-control study. The presence of beta-lactamase genes was tested by PCR and sequencing. Phylogenetic grouping and screening for the sequence type *E. coli* ST131 was performed by PCR. Furthermore, we randomly selected 264 isolates (50%) for multilocus sequence typing (MLST) analyses.

**Results:** The ESBL-*E. coli* from hospital patients and outpatients mainly harboured CTX-M-15 (ambulant 51%; nosocomial 49%), CTX-M-1 (ambulant 26%; nosocomial 30%) and CTX-M-14 (ambulant 7%; nosocomial 5%). *E. coli* from healthy persons (community study) showed a similar high proportion of CTX-M-15 (46%) and CTX-M-1 (24%) but *E. coli* from the case-control study harboured more CTX-M-1 (42%) and less CTX-M-15 (29%). Sequence type ST131 was present in 40% of the ambulant and 32% of the nosocomial *E. coli*, and the ST131 proportion among CTX-M-15 producing *E. coli* was particularly high (92% ambulant and 86% nosocomial). Analyses of ESBL-*E. coli* of the case-control-study and the community study revealed a lower proportion of ST131 which was 26% and 12%, respectively. MLST analyses revealed a high variety of STs (n=62) but several STs (including 2-12 isolates) were present in all three study collections: ST410, ST405, ST10 and ST38.

**Conclusion:** We confirmed CTX-M-15 and CTX-M-1 as the most frequent ESBL-types in *E. coli* from humans. Furthermore, we found the worldwide successful clonal lineage *E. coli*-ST131 to be the dominant ST among isolates causing different infections (*E. coli* from hospitalised patients and outpatients). Apart from ST131 we detected *E. coli* ST410, ST405, ST10 and ST38 in all three different study populations. These four STs were also detected in previous studies involving *E. coli* from livestock, whole genome comparisons of isolates from different settings are ongoing as part of this project. The great variety of *E. coli*-STs in our analyses indicated the importance of horizontal transfer of ESBL genes, a hypothesis which needs more detailed plasmid analyses in future.

## PO18 - Characterization And Molecular Epidemiology Of Plasmid-mediated Quinolone Resistance *Escherichia coli* Isolates From Algeria.

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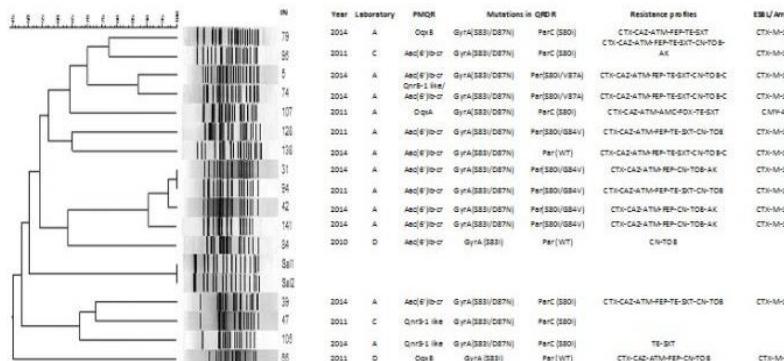
Keywords: *E. coli*, PMQR, PFGE

**Background:** Fluoroquinolone resistance in *Escherichia coli* is an important issue all over the world. The aim of this study is to evaluate the genetic relatedness of *Escherichia coli* isolates coding for Plasmid-Mediated Quinolone Resistance mechanisms (PMQR) and recovered from urine of outpatients in Algeria.

**Methods:** Ninety six nalidixic acid resistant *E. coli* isolates were collected from four different laboratories in Northern Algeria between 2010 and 2014. Antibiotic susceptibility was determined according to the CLSI guidelines. Genes coding for PMQR and beta-lactamases were identified by PCR and their identities were confirmed by DNA sequencing. Quinolone Resistance-Determining Region (QRDR) of *gyrA* and *parC* genes was also analyzed. Clonal relatedness was studied by pulsed-field gel electrophoresis (PFGE).

**Results:** The rate of PMQR genes in *E. coli* was 16,6% (16/96): 11 contained *aac(6')*-*lb-cr* (one of which was also positive for *qnrB1*-like), 2 *qnrS1*-like and 3 *oqxA/B* genes. Either, two mutations in *gyrA* (Ser83→Leu and Asp87→Asn) or one or two substitutions in *parC* (Ser80→Ile and Glu84→Val or Val 87→Ala) were the most common associated mechanisms in those isolates with MICs of ciprofloxacin ranging from 8 to >256 mg/L. PMQR was associated with ESBL and plasmidic AmpC-type β-lactamase in 75% and 6,25% respectively. The most prevalent betalactamases were CTX-M-15 (n:11), CTX-M-3 (n:1) and one CMY-4 (n:1). Most of PMQR-positive *E. coli* isolates were clonally unrelated with the exception of two small clonal groups comprising two and four isolates and producing both AAC(6')-*lb-cr* and CTX-M-15.

**Conclusion:** A high prevalence of PMQR in *E. coli* isolates has been detected in Northern Algeria. These determinants were frequently associated to other resistance determinants (ESBL and plasmidic AmpC). Most of the isolates were genetically unrelated.



**Figure 1.** Dendrogram of patterns generated by pulsed-field gel electrophoresis (PFGE) of *E. coli* isolates containing transferable quinolone resistance determinants. IN, isolate number; AK, amikacin; AMC, amoxicillin/clavulanic acid; ATB, Aztreonam; CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime; FOX, cefoxitin; C, chloramphenicol; CN, gentamicin; TE, tetracycline; TOB, toramycin; SXT, cotrimoxazole; S, Ser; I, Ile; D, Asp; N, Asn; G, Glu; L, Leu; A, Ala; V, Val.

## S7 - Poster session II

### PO19 - Molecular Characterisation Of Carbapenem-resistant Gram-negative Bacteria From A Bulgarian Hospital

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<sup>1</sup>Robert Koch Institute,<sup>2</sup> Military Medical Academy

**Background:** Gram-negative bacteria with resistance to carbapenems are an emerging threat in hospitals worldwide. From September 2014 to January 2015, 71 carbapenem-resistant isolates (50 *Acinetobacter* spp., 14 *Proteus mirabilis*, five *Escherichia coli*, one *Providencia rettgeri* and one *Pseudomonas aeruginosa*) were identified in a hospital in Sofia, Bulgaria. The *Acinetobacter* spp. were isolated mainly from respiratory specimen, the Enterobacteriaceae were mainly from urine samples. In January 2015, all isolates were sent to the Robert Koch Institute for molecular characterisation.

**Materials/Methods:** *Acinetobacter* species identification was performed by PCR and sequencing of the *rpoB* gene. Antimicrobial susceptibility testing for all 71 isolates was performed by microbroth dilution and Etest. Beta-lactamase genes were identified by PCR and sequencing. Bacterial strain typing was performed by enzymatic macrorestriction and subsequent pulsed field gel electrophoresis (PFGE). Selected strains of *A. baumannii* and *E. coli* were analysed by multilocus sequence typing (MLST).

**Results:** Species identification of the 50 *Acinetobacter* isolates revealed the presence of 47 *Acinetobacter baumannii*, one *Acinetobacter seifertii*, one *Acinetobacter radioresistens*, and one *Acinetobacter pittii*. The *A. pittii* strain and 44 *A. baumannii* isolates produced either carbapenemases OXA-23 and/or OXA-72. These isolates were resistant to imipenem, meropenem, amikacin and ciprofloxacin but remained susceptible to colistin. The *A. radioresistens* strain harboured the *bla*<sub>OXA-23</sub> gene but was only ertapenem-resistant similar to three *A. baumannii* and the *A. seifertii* strain without any carbapenemase genes. PFGE typing of the 47 *A. baumannii* showed the presence of at least seven different strains, and five of these were assigned to the international clonal lineage 2 (IC 2). MLST analyses confirmed the dominance of sequence type ST2 (Pasteur scheme) which included ST350, ST208, ST436 and ST437 (Oxford scheme), respectively. Furthermore, eight *A. baumannii*-ST231 (IC 1) and one *A. baumannii*-ST502 (IC 3) were identified.

Analyses of the Enterobacteriaceae isolates revealed VIM-2 presence in the *P. aeruginosa* isolate and NDM-1 production in one *P. rettgeri*, one *P. mirabilis* and in the five *E. coli* isolates, respectively. These NDM-1 *E. coli* co-harboured beta-lactamases CMY-4 and CTX-M-15, showed identical macrorestriction patterns (PFGE) and were assigned to sequence type ST101. Furthermore, 13 *P. mirabilis* isolates from 13 patients were positive for VIM-1, CM-99 and SHV-12 and their identical PFGE patterns indicated the clonal transfer.

**Conclusion:** Our study confirmed the presence of different carbapenemase producing bacteria in Bulgarian hospitals. For OXA carbapenemase producing *A. baumannii* the occurrence of different strains over the full period of time may indicate a permanent presence in the hospital environment or an import of distinct strains from outside and subsequent clonal transfer within the hospital. Furthermore, the repeated occurrence of one VIM-1 producing *P. mirabilis* and one NDM-1 producing *E. coli*-ST101 clone is of concern since only few therapeutic options are available in case of infections. Intensified hygiene measurements and surveillance are necessary to control the further spread of these bacteria.

**PO21 - Emergence Of CTX-M-producing Clinical Isolates Of *Klebsiella pneumoniae* In The University Hospital Establishment Of Oran, Algeria.**

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Keywords: *Klebsiella pneumoniae*, ESBL, CTX-M-producing, SYBR Green PCR, 16S rRNA methylases, AmpC, Algeria.

**Background:** *Klebsiella pneumoniae* (*KP*) is a gram-negative bacterium with rapidly emerging resistance compared to most other gram-negative species. Infections with *KP* are usually hospital-acquired and occur primarily in patients with impaired host defenses.

**Materials / Methods:** The aim of study was to characterize the molecular epidemiology of 198 Extended-Spectrum Beta-lactamase (ESBL)-producing *KP* from the University Hospital Establishment of Oran, Algeria. Species identification was done with API®20E and ESBL-production was confirmed according to The Clinical and Laboratory Standards Institute 2015 recommendations. Strains were derived from blood (n=28), protected specimen brush (n=27), urine (n=45), wound secretions (n=50), central venous catheters (n=27), cerebrospinal fluid (n=9), vaginal discharge (n=4), abdominal, or dialysis fluid (n=7) and (n=1) liquid pleural.

A SYBR Green PCR assay was carried out for detection of the following genotypes CTX-M-1 group, CTX-M-2 group, and CTX-M-9 group. To cover other potential CTX-M genotypes, a set of CTX-M universal primers was also used. Strains with resistance to amikacin were also tested for the presence of the 16S rRNA methylases *armA*, *rmtA*, *rmtB*, *rmtC*, and *rmtD*. Strains which were negative for CTX-M and/or cefoxitin resistant were tested with the microarray Check MDR CT101 (Checkpoints®, The Netherlands).

**Results:** The phenotypical tests and analyses with WHONET 5.6 software, almost strains (99%) was resistant to Cefotaxim, 40 % of strains were resistant to ciprofloxacin and only 6/198 were also resistant to cefoxitin (3 %), indicating that they could have concomitant production of an acquired AmpC enzyme. Resistance to ceftazidime was also very common (84 %). Gentamicin resistance was seen in 92.5 % of isolates whereas amikacin resistance was seen in 26/198 of isolates (13 %). Two strains were resistant to ertapenem and only one strain was resistant to imipenem (See the table).

The molecular analyses showed that 190 /198 strains (95.95 %) were positive with primers for the CTX-M-1 group, 1/198 was positive with primers for the CTX-M-9 group, and 7/198 strains (3.5%) were negative with CTX-M universal primers. For strains that were resistant to amikacin we found 20/26 isolates (76.2%) positive for *armA* and 6/26 isolates were negative for all 16S rRNA methylases. Microarray analysis showed that 5/7 were negative for all ESBLs in the assay, one isolate was positive for CTX-M-1 group, and one isolate had an SHV-ESBL Among cefoxitin resistant isolates 5/6 isolates were CMY II, and one isolate was negative.

**Conclusion:** The study showed that the CTX-M is still the predominant genotype of ESBL, and that the ESBL positive strains have high resistance levels. The strains are mainly producing CTX-M group-1 and concurrent occurrence in some isolates of CTX-M producing and AmpC (CMY II) or 16S rRNA methylases raises clinical concern and may become a major therapeutic challenge in the future.

## S7 - Poster session II

TOTAL=198 (100%)	S+I (No)	S+I (%)	R (No)	R (%)
Gentamicin (10µg)	15	7.5 %	183	92.5 %
Amikacin (30µg)	172	87 %	26	13 %
Cefoxitin (30µg)	192	97 %	6	3 %
Imipenem (10µg)	197	99.5 %	1	0.5 %
Ertapenem (10µg)	196	99 %	2	1 %
Amoxicillin + clavulanic acid (20µg)	170	86 %	28	14 %
Ceftazidim (30µg)	31	16 %	167	84 %
Ciprofloxacin (5µg)	119	60 %	79	40 %
Cefotaxim (30µg)	2	1%	196	99 %

Table: Susceptibility interpretations of zone diameters against different antibiotics according to The Clinical and Laboratory Standards Institute 2015 recommendations.

**PO22 - Epidemiology Of Carbapenem-resistant *Pseudomonas putida* Group Clinical Isolates Recovered From Screening Cultures**

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Universitat Pompeu Fabra (UPF)

*Pseudomonas putida* is a gram negative, rode-shaped, non-fermenting bacterium that is ubiquitously encountered in the environment. *P. putida* is a rather rare cause of human disease but is frequently found in water systems. Antimicrobial resistance patterns of isolates reported varied between the different studies and multidrug resistant *P. putida* isolates harbouring metallo-beta-lactamases (MBLs) have been reported from all over the world. *P. putida* was suggested to be a platform for the exchange of genetic resistance elements between *P. putida* and *P. aeruginosa*. However, the detailed mechanisms and frequency of antimicrobial resistance exchange and their relevance within the hospital setting remain poorly understood.

In total, 101 *P. putida* group strains were recovered from rectal swabs, throat swabs or stool samples taken within the scope of a weekly routine screening program for a period of 28 month. Antimicrobial susceptibility testing, genomic fingerprinting and whole genome sequencing (WGS) was performed.

The following resistant rates were detected in the *P. putida* isolates: resistance to piperacillin (63%), piperacillin-tazobactam (60%), meropenem (57%), ciprofloxacin (45%) and gentamycin (14%). Meropenem non-susceptible strains (n=64) were further subjected to molecular detection of the MBL genes *bla<sub>VIM</sub>* and *bla<sub>IMP</sub>*. VIM was detected in 42 isolates (41.6%). The VIM positive isolates displayed a similar antimicrobial susceptibility patterns with the exception of gentamycin, which was susceptible in 28 of 42 (66.7%) isolates, suggesting the presence of at least two different VIM positive *P. putida* group clusters. Genomic fingerprinting of the isolates applying the DiversiLab Pseudomonas Kit (bioMérieux, France) revealed 15 different clusters. The cluster 8 and cluster 15 comprised 10 isolates each, followed by cluster 1 containing 3 isolates.

During the study period five *P. aeruginosa* VIM positive isolate were recovered from the same patient cohort. WGS was performed for all VIM positive *P. putida* group and *P. aeruginosa* isolates. The phylogenetic analysis and the comparison of the resistance gene content are still on-going at present.

## S7 - Poster session II

### PO23 - First Report Of Macrolide Resistance Among *Shigella* Isolates In Israel Caused By The mphA And erm(b) Genes

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<sup>1</sup>Ministry of Health Israel

*Shigella* species remain an infection agent of public concern, affecting mostly children, with high incidence in developing areas as well as in industrialized countries. Although azithromycin is recommended for treatment of shigellosis, there are currently no CLSI susceptibility breakpoints. Recently, cases of *Shigella flexneri* with decreased susceptibility to azithromycin (DSA) have been reported globally. We aimed to assess the prevalence of macrolide resistance genes among *Shigella flexneri* in Israel.

All *Shigella flexneri* isolates (162), received at the National Reference Center during 2014, were serotyped, and screened by PCR for the presence of macrolide resistance genes *erm(B)* and *mphA* that are commonly plasmid-encoded. Azithromycin minimum inhibitory concentration (MIC) values were assessed by the Broth Microdilution (BMD) method. Antimicrobial susceptibility to additional drugs (ampicillin, trimethoprim-sulfamethoxazole, ceftriaxone, tetracycline, nalidixic acid and chloroamphenicol) was tested by the disk diffusion method according to the CLSI guidelines. Clonal relatedness was assessed by Pulsed-Field Gel Electrophoresis (PFGE).

The *mphA* gene was detected in 11 isolates (7%), 4 of which harbored *erm(B)* as well. The presence of *mphA* or *erm(B)* correlated with high azithromycin MIC values (>256 µg/ml). Among the 11 isolates, 3 were MDR and 2 were XDR, and all were sensitive to ciprofloxacin. The strains displaying high azithromycin MIC values included different *Sh. flexneri* serotypes (1b, 1a, 3a, and 6) and different PFGE patterns, indicating different lineages. Among *Sh. flexneri*, 34 isolates (20%) were resistant to nalidixic acid.

Tracing antimicrobial resistance trends has direct impact on the outcome of shigellosis. The emergence of DSA *Shigella* reinforces the necessity of the establishment of clinical breakpoints that would warrant routine testing, reporting and surveillance for this drug of choice.

**PO24 - CTX-M-55 Extended-spectrum Beta-lactamase Among Enterobacteriaceae From The Korean Antimicrobial Resistance Monitoring System, 2008 To 2015**

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Keywords: ESBL, CTX-M-55, IS*Ecp1*, IncI1, IncA/C

**Background:** The emergence and rapid dissemination of CTX-M-type extended-spectrum beta-lactamases (ESBLs) in *Enterobacteriaceae* are a serious global concern for the public health. Recently, CTX-M-55, a variant of the most common CTX-M-15, has been increasingly reported in several countries. This study characterized the CTX-M-55 beta-lactamase in *Enterobacteriaceae* from clinical isolates in Republic of Korea.

**Materials and Methods:** Among cefotaxime-resistant strains collected, the *bla*<sub>CTX-M-55</sub> gene and their genetic environment were determined by using PCR and DNA sequencing. PCR-based replicon typing (PBRT) of plasmids encoding CTX-M-55 was also carried out. Two IncI1 plasmids (pSEN101134 and pSal111105) were sequenced using an Ion Torrent PGM sequencer and the initial reads were de novo assembled using Newbler and CLC Genomics Workbench.

**Results:** From the national surveillance of antimicrobial resistance among *Enterobacteriaceae*, we screened five CTX-M-55-producing *Salmonella* and one *Shigella* strains. By PBRT, the *bla*<sub>CTX-M-55</sub> genes were placed on the IncI1 (*n*=2), IncA/C (*n*=3) and IncB/O (*n*=1) plasmids. IncI1 plasmids possessed a known IS*Ecp1*-*bla*<sub>CTX-M-55</sub>-*orf477* transposable unit, whereas the *bla*<sub>CTX-M-55</sub> genes in IncA/C and IncB/O plasmids were preceded by IS26 or IS5 sequences, respectively. Plasmid pSEN111105 is 86179 bp in size with a GC content of 49.7% and contains 114 putative ORFs, and pSEN101134 has quite similar structure (86194 bp; 117 ORFs, >99.9% nucleotide identities). When compared with other sequenced IncI1 plasmids encoding *bla*<sub>CTX-M-55</sub> identified from *Shigella sonnei* (p1081-CTXM) and *Klebsiella pneumoniae* (p628-CTXM), they were closely related to each other with the exceptions of IS elements in the IncI1 shufflon.

**Conclusions:** With regard to various plasmid replicon types and the genetic environment surrounding the *bla*<sub>CTX-M-55</sub> gene, CTX-M-55 ESBL has emerged independently and disseminated in Korea. In addition, these results indicate that the IncI1 plasmid may be used as a successful genetic vehicle, favoring the spread of various *bla*<sub>CTX-M</sub> genes among different members of *Enterobacteriaceae*.

## S7 - Poster session II

### PO25 - Active Screening And Genotyping Successfully Controls Outbreaks Of Multi-drug-resistant *Enterobacteriaceae*

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**Abstract:** Nosocomial infections pose an increasing threat to vulnerable patients by the rapid emergence and dissemination of multi-drug-resistant Enterobacteriaceae. Enterobacteriaceae can exhibit resistance to multiple antibiotics such as extended-spectrum beta-lactamases (ESBL), including carbapenemases, cephalosporins, quinolones and aminoglycosides. Treatment options for infected patients can therefore be severely limited.

**Methods:** In our hospital we implemented a modular screening protocol for detection of relevant resistance genes, ie: KPC / VIM / IMP / NDM / OXA-48, ESBL (CTX-M), and AmpC. Rectal swabs of critical patients (intensive care, neonatal unit, burn wound unit), patients at risk or hospitalized for >7 days were cultured overnight in different selective broths containing: ertapenem, cefotaxime, ceftazidime, gentamicine/ciprofloxacin, tobramycin/ciprofloxacin, ceftazidime/ciprofloxacin. Fully automated screening PCRs were carried out, and if positive, samples were subcultured on selective or chromogenic agars for isolate identification, antibiotic susceptibility testing, and PCR confirmation. Multi-drug-resistant isolates are defined by the presence of gentamicin/tobramycin and ciprofloxacin, or ESBL, AmpC, or carbapenemase positive. Possible transmission or source tracing was investigated by genotyping of MDR strains with amplified fragment length polymorphism (AFLP). Genotype banding patterns were analysed using Bionumerics.

**Results:** Five outbreaks of ESBL positive *Klebsiella pneumoniae* and *Escherichia coli* were identified by clusters of identical genotypes within a timespan of 7 months. The largest outbreak included 5 neonates who were colonized with ESBK *K.pneumoniae*. Two clusters of OXA-48 producing *Enterobacter cloacae* were identified. Appropriate measurements and precautions were taken for containment of the outbreaks.

**Conclusion:** Active screening for, and genotyping of MDR Enterobacteriaceae has proven to be an effective method for reducing the frequency of nosocomial infections. It provides increased safety for patients, many clusters will be detected in an early phase still without clinical infections. Furthermore, the method is patient-friendly as usually only one swab is required for screening. Thus, routine identification and monitoring of clusters of MDR strains can limit further dissemination, and keep our hospitals safe.

**PO26 - Genetic Diversity And Molecular Analysis Of Quinolone-resistant *Shigella sonnei* Isolates From Humans In Republic Of Korea, 2008-2014**

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Keywords: *Shigella sonnei*, Quinolone, QRDR, PFGE, MLVA

**Background:** *Shigella sonnei*, a causative agent of bacterial dysentery, has become prevalent in the developing countries as well as developed countries. However, the increase of drug-resistant *S. sonnei* is becoming more difficult to treat these infections, posing a serious public health problem. We characterized quinolone resistance determinants and genetic relatedness of *S. sonnei* isolates in Republic of Korea.

**Materials and Results:** PCR-based sequence analysis was carried out for screening the determinants of quinolone resistance as follows; for the quinolone resistance-determining regions (QRDRs) in DNA topoisomerase II and IV, and for the presence of plasmid-mediated quinolone resistance (PMQR) genes. Quinolone-resistant isolates were typed by pulsed-field gel electrophoresis (PFGE) and multiple-locus variable number tandem repeat analysis (MLVA).

**Results:** Between 2008 and 2014, we collected 157 quinolone-resistant *S. sonnei* isolates from the stool samples of patients with diarrhea including 31 domestic and 126 travel-related cases. All quinolone resistant isolates contain one or more mutations in QRDRs in GyrA (Ser83Leu and Asp87Gly) and ParC (Ser80Ile and Gln91Arg). The *aac(6')-lb-cr* gene was identified in five isolates of *S. sonnei*, whereas none of PMQR genes was detected. Based on the genetic relatedness among resistant isolates using PFGE and MLVA, domestic clones were clearly distinct from isolates linked to foreign travel that were further discriminated from each country of location.

**Conclusions:** These results indicate that with an increase of international travel to shigellosis endemic area, importation and subsequent regional spread of drug-resistant *S. sonnei* has increased in Korea. Therefore, continued monitoring is necessary for preventing the spread of drug-resistance *S. sonnei* strains.

## S7 - Poster session II

### PO27 - Antimicrobial Resistance And Virulence Profile In Community And Hospital-acquired Urinary Tract Infections Caused By *Klebsiella pneumoniae*

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**Background:** Urinary tract infections (UTIs) are the most common bacterial infection. Emergence of antimicrobial resistance and production of extended spectrum β-lactamases (ESBLs) are responsible for empirical therapy failures with significative medical and financial impact. The aim of this study was to evaluate the relationship between the resistance and virulence determinants in *Klebsiella pneumoniae* clinical isolates recovered in hospital and community environment.

**Materials | Methods:** This study included 50 UTI clinical isolates of *K.pneumoniae* collected in 10 community laboratories. Susceptibilities to antimicrobial agents were determined by disk diffusion: imipenem, ciprofloxacin, gentamicin, cefoxitine, cefotaxime, ceftazidime, fosfomycin, levofloxacin, nitrofurantoin, trimethoprim/sulfamethoxazole and amoxicillin/clavulanic acid. The results were interpreted according to CLSI and EUCAST guidelines. The ESBLs were identified for *bla*<sub>CTX-M</sub>, *bla*<sub>TEM</sub> and *bla*<sub>KPC</sub> gene and screened for gene markers of 8 virulence factors (VF) genes: *K2A*, *fimH*, *mrkD<sub>V1</sub>*, *mrkD<sub>V2-4</sub>*, *khe*, *rmpA*, *magA*, and *iucC* by PCR amplification. The virulence results were compared with 31 urinary clinical isolates from one tertiary care hospital.

**Results:** Six β-lactamases (12%) were identified: TEM-156 (n=2), SHV-11 (n=1) and the ESBL TEM-24 (n=1), SHV-33 (n=1) and CTX-M-15 (n=1). Significative antimicrobial resistance (22%) was found to nitrofurantoin and trimethoprim/sulfamethoxazole. In community-acquired UTI isolates the absence of VF (16% Vs 0%, p<0,005) and the presence of type 3 fimbrial adhesins - variant 2-4 (44% Vs 0%, p<0,0001) was significant compared with hospital isolates characterized by haemolysin (84% Vs 46%, p=0,0009), fimbrial adhesins type 1 (97% Vs 40%, p<0,0001), fimbrial adhesins type 3 – variant 1 (94% Vs 20%, p<0,0001) and K2 serotype (29% Vs 6%, p=0,0081). Community (C-VP) and hospital-virulence profiles (H-VP) were numbered according the predominance. 18 different C-VP were identified. 10% of the isolates presented the C-VP1 (*khe*), the C-VP2 (*khe*, *mrkD<sub>V2-4</sub>*) and C-VP3 (*fimH*, *mrkD<sub>V2-4</sub>*). 8% had the C-VP4(*fimH*, *iucC*, *mrkD<sub>V2-4</sub>*). None of this C-VP was detected in hospital isolates which presented 9 H-VP. The most frequent profiles were H-VP1 (*fimH*, *khe*, *mrkD<sub>V1</sub>*) in 41% and H-VP2 (*K2*, *fimH*, *khe*, *mrkD<sub>V1</sub>*) in 22% of the isolates, not identified in community isolates. The profile of community β-lactamases producers was also studied: TEM-156 (*mrkD<sub>V1</sub>* and *khe*, *mrkD<sub>V1</sub>*), SHV-11 (*mrkD<sub>V2-4</sub>*) TEM-24 (absence of VF), SHV-33 (*fimH*, *mrkD<sub>V2-4</sub>*) and CTX-M-15 (*khe*). Different profiles were identified for hospital β-lactamases: TEM-24 (H-VP1 and *fimH*, *khe*) and for CTX-M-15 ESBL alone (H-VP1 and *fimH*, *mrkD<sub>V1</sub>*) or in co-expression with KPC-3 carbapenemase: H-PV 4 (*fimH*, *khe*, *mrkD<sub>V1</sub>*, *iucC*) and H-PV6 (*K2*, *fimH*, *mrkD<sub>V1</sub>*) and H-PV9 (*K2*, *fimH*, *khe*, *mrkD<sub>V1</sub>*, *iucC*).

**Conclusions:** Our results indicate that the hospital or community source significantly impacts on the virulence of *K. pneumoniae* isolates, with abilities to simultaneously express several virulence factors, organized in specific and successful virulence patterns. The presence of ESBL enzymes itself does not suggest an association to virulence but the convergence of virulence and resistance genes is more significant at hospital-adquired UTI and can potentially lead to the emergence of untreatable *K. pneumoniae* infections.

**PO28 - Increasing Cotrimoxazole Susceptibility Associated With Predominance Of International Clone 2 *Acinetobacter baumannii* Clinical Isolates: A Nationwide**

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Keywords: Clonal complex; molecular epidemiology; carbapenem-hydrolyzing oxacillinase

**Background:** *Acinetobacter baumannii* clinical isolates are very common in Greek hospitals and currently exhibit almost universal resistance to carbapenems and most antimicrobials except colistin. Lately, cotrimoxazole (trimethoprim/sulfamethoxazole, SXT)-susceptible isolates are increasingly recovered. We report herein that the enhanced SXT susceptibility is associated with the predominance of international clone (IC) 2 *A. baumannii* isolates in multiple hospitals from all over Greece.

**Methods:** A collection of 142 single-patient carbapenem-resistant *A. baumannii* invasive isolates consecutively recovered during 2015 from nine hospitals located throughout Greece were tested. Antimicrobial susceptibility testing was performed by automated systems and/or Etest. The 3LST multiplex PCR and the single-locus *bla*<sub>OXA-51-like</sub> sequencing for clonal assignment and PCR/sequencing for carbapenemase-encoding genes were performed. PCR mapping for the possible presence of ISAb1 upstream of *bla*<sub>OXA-23</sub> gene was also undergone.

**Results:** Of the 142 isolates tested, 23 (16.2%) belonged to IC1, carrying *bla*<sub>OXA-69</sub>, and 119 (83.8%) to IC2, carrying *bla*<sub>OXA-66</sub>. In the 9 participating hospitals overall, 34.5% of isolates showed SXT susceptibility; all 23 IC1 isolates were SXT-resistant, while 49 of the IC2 isolates (41.2%) were SXT-susceptible. Among the other antibiotics tested, isolates belonging to IC1 exhibited considerably higher activity to tetracycline (35.3%), minocycline (100%), tobramycin (64.7%) and ampicillin/sulbactam (18.2%) compared with IC2 isolates (1.7%, 21.1%, 12.5% and 4.3%, respectively), while 8.8% of the isolates overall exhibited resistance to colistin, without considerable differences between lineages. Within IC2, SXT-susceptible and SXT-resistant isolates had similar antibiotic resistance phenotypes. All isolates carried the gene *bla*<sub>OXA-51-like</sub>, 140/142 isolates (98.6%) carried *bla*<sub>OXA-23-like</sub> and 2/142 (1.4%) had both *bla*<sub>OXA-23-like</sub> and *bla*<sub>OXA-58-like</sub> genes. The ISAb1 insertion was found upstream of *bla*<sub>OXA-23-like</sub> in all isolates.

**Conclusion:** Almost all *A. baumannii* isolates tested produced OXA-23 carbapenemase; the majority of isolates belonged to IC2 and fewer to IC1. Of the limited antimicrobials that retained susceptibility, SXT exhibited a considerable degree of activity, which was observed exclusively among IC2 isolates. Given the ongoing expansion of IC2 *A. baumannii* isolates, this finding may indicate a potential for further increase in SXT activity. It should be noted that IC2 isolates exhibited higher resistance rates to several antimicrobials, except SXT, and this might have contributed to its predominance in Greek hospitals. The clinical efficacy of SXT against *A. baumannii* infections warrants active investigation.

## S7 - Poster session II

### PO29 - Faecal Carriage Of ESBL Producing Bacteria Among Children In Dar Es Salaam, Tanzania

Marit Gjerde Tellevik<sup>1</sup>; Sabrina J. Moyo<sup>2</sup>; Samuel Y. Maselle<sup>3</sup>; Bjørn Blomberg<sup>1</sup>; Nina Langeland<sup>2</sup>

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Keywords: ESBL, Tanzania, faecal carriage

**Background:** Faecal carriage of Extended-spectrum beta-lactamase (ESBL)-producing bacteria is a potential risk for transmission and infection, and of particular concern in healthcare settings, especially in developing countries where infection control is limited. Infections caused by resistant organisms pose an important challenge for treatment of both common and life-threatening infections. Little is known about faecal carriage of antibiotic resistance in Tanzania. This study aimed to investigate the prevalence, etiology and risk factors of faecal carriage of ESBL-type antibiotic resistance among young children in Tanzania.

**Materials/Methods:** This project was part of a larger study assessing causes of diarrhoea among children. Study population was children in Dar es Salaam < 2 years of age, recruited in the period August 2010 to July 2011. Cases were children admitted due to diarrhoea at three study hospitals. Controls were children with no history of diarrhoea for one month prior to the study enrollment. Stool specimens were collected on inclusion. Samples were screened for ESBL-producing bacteria using ChromID ESBL agar (ESBL<sub>A</sub> and ESBL<sub>M</sub>) and ChromID CARBA SMART (ESBL<sub>CARBA</sub>) (BioMérieux, Marcy l'Etoile, France). Isolates were identified with MALDI-TOF MS using the Microflex LT instrument and MALDI Biotyper 3.1 software (Bruker Daltonics, Bremen, Germany). Confirmation of ESBL phenotype was performed using the BBL Sensi-Disc ESBL Confirmatory Test Disks (Becton Dickinson, Sparks, MD, USA). Molecular methods will be applied for further characterization.

**Results:** 12% (30/250) of the controls and 48.4% (120/248) of the cases were ESBL screening-positive. The prevalence of ESBL-producing isolates was significantly higher in children aged >12 months compared to children <12 months, 39.2% vs. 18.1% ( $P < 0.001$ ; OR = 2.91; 95% CI: 1.91 – 4.44). HIV-positive children had higher risk of ESBL carriage compared to HIV-negative children, 89.3% vs. 17.1% ( $P < 0.001$ ; OR = 40.43; 95% CI: 11.78 – 138.72). Use of antibiotics was a risk factor for faecal carriage of ESBL in the total study population ( $P < 0.001$ ; OR = 2.65; 95% CI: 1.78 – 3.93), but when analyzing cases and controls separately, use of antibiotics was not associated with ESBL carriage. The majority of isolates were identified as *Klebsiella pneumoniae* or *Escherichia coli*.

**Conclusion:** We report high prevalence of faecal carriage of ESBL-positive bacteria on hospital admission among young children in Tanzania. These children may pose a risk of transmission in healthcare settings. Age > 12 months and HIV infection increased the risk of carrying ESBL in this study population. Information on use of antibiotics was only collected for the last 2 weeks prior to study enrollment. Colonization with ESBL-producing bacteria can last for several weeks. Hence, the collected data on antibiotic use are possibly not comprehensive enough to evaluate the impact of prior use of antibiotics in this study. ESBL<sub>CARBA</sub> phenotype was not prevalent in this study.

## PO30 - Establishing A Closed Reference Sequence Of A Vancomycin-resistance Plasmid From *Enterococcus faecium*

Heidi Gumpert<sup>1</sup>; Mette Pinholt<sup>1</sup>; Peder Worning<sup>1</sup>; Jesper Boye Nielsen<sup>1</sup>; Henrik Westh<sup>1</sup>

<sup>1</sup>Hvidovre University Hospital

**Background:** The increase in hospital-associated vancomycin-resistant *Enterococcus faecium* (VREfm) isolates is a worrisome trend. Indeed, in Copenhagen a ten-fold increase in the number of vanA VREfm isolates was observed between 2012 and 2014. All isolates are whole genome sequenced and genomic epidemiology reveals the dissemination of the vanA Tn1546 transposon amongst a polyclonal VREfm population (see M. Pinholt). However, the plasmid that is believed to have disseminated the Tn1546 transposon has proven to be difficult to assemble. Performing a hybrid assembly using high-quality Illumina reads coupled with long PacBio reads can overcome the difficulty in assembling *E. faecium* isolates due to their propensity to acquire mobile genetic elements, resulting in high-quality assemblies including closed plasmids.

**Methods:** One VREfm isolate belonging to the largest clonal group (ST80) of *E. faecium* isolates from Copenhagen between 2012 and 2014 was selected for whole genome sequencing on Illumina MiSeq and Pacific Biosciences PacBio sequencing platforms. Illumina sequencing produced 150 base-pair (bp) paired-end (PE) reads and PacBio sequencing produced an average sub-read length of 5.8 kbp. Coverage of 30X and 441X was obtained from Illumina and PacBio sequencing, respectively, based on an *E. faecium* genome size of 3.2MB. A hybrid assembly using both sequencing platforms was performed using SPAdes, and annotated using RAST. Plasmid replicons and resistance genes were identified via PlasmidFinder and ResFinder online tools, respectively. Closed plasmids were identified by overlapping contig ends and enumerating aligned PE reads that bridged the plasmid contig ends. Specifically, PE reads were aligned using Bowtie2, while bridging reads were determined using a developed BioPython script with the PySam library. Additionally, BLAST and the NCBI database was used to find plasmids sharing homology.

**Results:** Incorporating the PacBio reads into the assembly reduced the number of contigs from 203 to 34 and increased the N50 nearly 10-fold from 45,373 to 424,944, when compared to assembly using PE reads alone.

A total of 5 plasmid replicons were detected in the VREfm assembly using PlasmidFinder, with only a rep2 replicon not on a closed plasmid. A further 4 closed plasmids were identified based on difference in coverage compared to the chromosome, identification of closed circular sequences, replication and mobilization genes, and homology to previously sequenced plasmids. Plasmid sizes range from 4.5kbp to 183kbp.

A closed 32kbp plasmid bearing a rep17 replicon was found to harbour the Tn1546 vanA transposon, in addition to genes involved in macrolide (*ermB*), and aminoglycoside (*aph-3*, *sat4*, *ant-6*) resistance. This plasmid is highly similar to the multi-drug resistance pS177 plasmid, except that it does not contain the Axe-Txe toxin-antitoxin system. Interestingly, a 61kbp plasmid was found to be highly similar to the Tn1546 harbouring pZB18 plasmid, except that Tn1546 is not harboured on this plasmid.

**Conclusions:** The inclusion of PacBio sequencing reads greatly improved the quality of the assembly of the VREfm isolate resulting in closed plasmid sequences for 8/9 identified plasmids. The ability to reduce the vancomycin-resistance plasmid from 11 contigs into one closed plasmid will prove to be a valuable reference for further study of the dissemination of this resistance plasmid amongst polyclonal isolates from the Copenhagen region.

### PO31 - Ice Carrying Antimicrobial Resistance Genes Among Pig Isolates Of *Streptococcus suis* In Poland

Ewa Sadowsy<sup>1</sup>; Agnieszka Bojarska<sup>1</sup>; Zygmunt Pejsak<sup>2</sup>; Waleria Hryniewicz<sup>3</sup>

<sup>1</sup>Department of Molecular Microbiology, National Medicines Institute,<sup>2</sup>

Department of Swine Diseases, National Veterinary Research Institute,<sup>3</sup>

Department of Epidemiology and Clinical Microbiology, National Medicines Institute

**Background:** *Streptococcus suis* represents an important factor of morbidity and mortality in pigs, and an emerging zoonosis in humans. The aim of this work was to perform molecular and microbiological analyses of *S. suis* isolated from pigs in Poland and to characterize integrative and conjugative elements (ICEs) involved in acquisition of antimicrobial resistance.

**Materials & methods:** The studied group comprised 96 *S. suis* isolates (42 from brain and 54 from lungs), collected during 2003-2012. Antimicrobial susceptibility of isolates was evaluated by microdilution method, their clonality by multilocus sequence typing (MLST) and serotypes were determined using available PCR-based schemes. ICEs in selected six isolates were analyzed by the whole genome sequencing (WGS).

**Results:** Among 96 *S. suis* isolates, 14 serotypes were observed, with most common serotype 2 (29 isolates); 6 isolates could not be serotyped using the current PCR-based serotyping schemes. MLST revealed 52 different sequence types (STs), with 39 novel STs; the most common ST1 (13 isolates) was characteristic for isolates from brain. Seven isolates (7.6%) showed intermediate penicillin nonsusceptibility (MIC, 0.25-2.0 mg/L); 54%, 62%, 35%, 43%, 84% of isolates were resistant to erythromycin, lincomycin, tiamulin, streptomycin, tetracycline, respectively; one isolate was resistant to gentamicin and full susceptibility to florfenicol, linezolid, and vancomycin was observed. Major determinant of tetracycline resistance was *tet(O)* (55 isolates), followed by *tet(M)* and *tet(W)* (17 and 11 isolates, respectively); *tetS*, *tetQ*, *tetT*, *tetL*, *tetK*, *tet40* were not found. Macrolide-resistance was mediated by *erm(B)* and *mef(A)* genes (61 and 5 isolates, respectively). All streptomycin-resistant isolates carried *aad(E)*. Among six isolates analyzed by WGS, the acquisition of resistance determinants was due to the presence of ~50 - ~70 kb ICEs in various genomic localizations and showing different structures, especially in their central part, containing "cargo" antimicrobial resistance genes. Genomic localization and structures of these ICEs, and their comparisons with other streptococcal MGEs will be presented.

**Conclusions:** Pig isolates of *S. suis* are remarkably diverse in the respect of their STs, serotypes and antimicrobial resistance profiles. ICEs play a key role in the acquisition of resistance determinants under the selective pressure of high use of antimicrobials in pig farms, leading to formation of a significant reservoir of resistance genes.

**PO32 - Investigations On Bacterial Fitness Of *Escherichia coli* With Plasmid Mediated Beta-lactam Resistance**Michael Pietsch<sup>1</sup>; Yvonne Pfeifer<sup>1</sup>; Guido Werner<sup>1</sup><sup>1</sup>*Robert Koch Institute, FG 13 Nosocomial Pathogens and Antibiotic Resistance*

**Background:** An important factor for the dissemination of antibiotic resistance are the fitness costs. Conjugative plasmids that carry antibiotic resistance genes are beneficial for the bacterial host, but may cause fitness costs in absence of antibiotic selective pressure. Successful plasmids, however, often show low fitness costs or even beneficial fitness effects caused by better adaption to the host. In this study we investigated the adaption mechanisms of different conjugative plasmids *in vitro* onto an isogenic *Escherichia coli* strain.

**Material/Methods:** Plasmids carrying different genes encoding carbapenemases, AmpC or ESBL were transferred by broth mate conjugation experiments into an isogenic strain, *E. coli* K12J53. Using S1 nuclease-restriction followed by pulsed field gel electrophoresis (PFGE), transconjugants harbouring only one plasmid were selected and plasmid sizes and replicon types were determined. Furthermore, the antimicrobial susceptibilities were tested. To characterise fitness loss or gain through plasmid acquisition, growth comparisons of plasmid-carrying *E. coli* K12J53 vs. plasmid-free *E. coli* K12J53 were conducted including parallel Bioscreen® assays in rich medium (lysogeny broth (LB)), and artificial urine medium (AUM). Six transconjugants (carrying: *bla*<sub>C<sub>M</sub>Y-2</sub> (n=2), *bla*<sub>CTX-M-15</sub>, *bla*<sub>CTX-M-14</sub>, *bla*<sub>VIM-1</sub>, *bla*<sub>KPC-2</sub>, *bla*<sub>OXA-48</sub>) were selected for long-term transfers (4000 generations) in LB and checked weekly for possible changes in the relative fitness and antimicrobial susceptibility.

**Results:** For the growth comparison experiments we selected 14 transconjugants carrying one of the following resistance genes on a plasmid: *bla*<sub>SHV-12</sub>, *bla*<sub>CTX-M-1</sub>, *bla*<sub>CTX-M-14</sub>, *bla*<sub>CTX-M-15</sub> (n=2), *bla*<sub>C<sub>M</sub>Y-2</sub> (n=3), *bla*<sub>KPC-2</sub>, *bla*<sub>VIM-1</sub> (n=2), *bla*<sub>NDM</sub>, *bla*<sub>OXA-48</sub> (n=2). The plasmid sizes varied between 60 - 260 kb and plasmid incompatibility groups IncF, IncA/C, IncL/M, IncN, IncR and IncI1 could be identified. Investigation of relative fitness in rich medium showed no significant fitness loss in the majority of the isolates. Analyses revealed a fitness loss for three isolates (isolate 1: *bla*<sub>OXA-48</sub>, IncL/M, 60 kb; isolate 2: *bla*<sub>C<sub>M</sub>Y-2</sub> + *bla*<sub>TEM-1</sub>, IncA/C, 150 kb; isolate 3: *bla*<sub>VIM-1</sub>, IncN/R, 100 kb), and for one isolate a fitness gain (isolate 4: *bla*<sub>C<sub>M</sub>Y-2</sub> + *bla*<sub>TEM-1</sub>, IncA/C, 150 kb) comparing to the plasmid-free *E. coli* K12J53. Comparative results were observed in AUM: Most of the isolates showed no significant change in growth rate and the above named three isolates indicated a fitness loss. However, no fitness gain could be observed.

**Conclusion:** A spectrum of *E. coli* with various plasmid mediated beta-lactam resistance genes was created to investigate genetic adaptive changes to acquired plasmids. The results of our growth experiments are in accordance with previous studies, reporting the fitness cost of resistance caused by the acquisition of conjugative plasmids as highly variable. This may result in fitter plasmid harbouring hosts than their plasmid-free ancestors (isolate 4). Long-term growth experiments and competitive growth experiments are initiated at the moment to evaluate the observed fitness costs and investigate compensation of initial fitness costs over time in detail.

## S7 - Poster session II

### PO33 - A GI Identified In Clinical And Environmental *Mycobacterium avium* subsp. *hominissuis* Isolates From Germany

Andrea Sanchini<sup>1</sup>; Torsten Semmler<sup>1</sup>; Lei Mao<sup>1</sup>; Astrid Lewin<sup>1</sup>

<sup>1</sup>Robert Koch Institute

**Background:** Among more than 150 species belonging to the group of non-tuberculous mycobacteria, *Mycobacterium avium* subsp. *hominissuis* (MAH) belongs to the clinically relevant species. MAH is an opportunistic pathogen and also widespread in the environment. Genomic islands (GIs) contribute to the accessory genome of microorganisms. GIs influence virulence, drug-resistance or fitness and trigger bacterial evolution. Research on GIs has only been started in MAH. We previously identified a GI in four MAH genomes. In extension to our previous study, here we further characterized this GI in a collection of MAH isolates from Germany. We aimed to explore the MAH genomic diversity and its potential impact on pathogenicity.

**Results:** We investigated the whole genome sequencing data of 41 MAH isolates from clinical (n=20) and environmental sources (n=21), including the two reference strains *M. avium* 104 and MAH TH135. We identified the GI in 39/41 isolates (95.1 %). All GIs integrated in the same genomic locus that we termed as insertion hotspot. We found high diversity in the genetic structure of the GI: a total of eight highly divergent types of GI have been identified, designated A-H (sizes 6.2 - 73.3 kb). In the insertion hotspot, these GIs arranged in single GI (23/41, 56.1 %), combination of two different GIs (14/41, 34.1 %) or three different GIs (2/41, 4.9 %). Remarkably two GI types shared more than 80% DNA sequence identity with the highly pathogenic *M. canetti*, responsible for Tuberculosis. We identified 253 different genes in all GIs, among which the previously documented virulence-related genes *mmpL* and *mce*. We performed homology protein modeling on 47 proteins of the GIs annotated as hypothetical protein, in order to decipher their putative function. Five proteins were predicted to have a function in drug resistance.

**Conclusions:** Our study expands the knowledge on MAH genome plasticity. The diversity of the GIs in the insertion hotspot and the sequence similarities with other mycobacteria suggests cross-species transfer, involving also highly pathogenic species. The presence of virulence-related genes on the GIs and their shuffling may generate new species that can cause new outbreaks of public health relevance.

**PO34 - Magnitude Of Gene Mutations Conferring Drug Resistant In *Mycobacterium tuberculosis* Strains In Southwest Ethiopia**

Mulualem Tadesse<sup>1</sup>; Dossegnew Aragaw<sup>1</sup>; Gemedu Abebe<sup>1</sup>

<sup>1</sup>Jimma University

Keywords: drug resistance, gene mutation, *Mycobacterium tuberculosis*

**Background:** The nature and frequency of mutations in rifampicin (RIF) and isoniazid (INH) resistance *M. tuberculosis* isolates vary considerably according to the geographic locations. However, information regarding specific mutational patterns in Ethiopia remains limited.

**Methods:** Mutations associated with RIF and INH resistance were studied by GenoType MTBDRplus line probe assay in 112 *M. tuberculosis* isolates. Culture (MGIT960) and identification tests were performed at Mycobacteriology Research Center of Jimma University, Ethiopia.

**Results:** Mutations conferring resistance to INH, RIF and MDR were detected in 36.6% (41/112), 30.4% (34/112) and 27.7% (31/112) of *M. tuberculosis* isolates respectively. The retreatment category 'treatment failure' is associated with a high rate of mutations associated with drug resistance (*p*-value <0.05). Among 34 rifampicin resistant isolates, 82.4% (28/34) had *rpoB* gene mutations at codon 531, 2.9% (1/34) at codon 526 and 5 had mutations only at wild type probes. The later isolates were depicted as unknown. Of 41 INH resistant strains, 87.8% (36/41) had mutations in the *katG* gene at Ser315Thr1 and 9.8% (4/41) of strains had mutation in the *inhA* gene at C15T. One INH resistant strain had mutation only at *KatG* wild type probe. Mutations in *inhA* promoter region were strongly associated with INH monoresistance. Monoresistance to INH (10 isolates) was frequently observed as compared to RIF monoresistance (3 isolates).

**Conclusions:** High rate of drug resistance, including MDR, was commonly observed among failure cases. The most frequent gene mutations associated with the resistance to INH and RIF were observed in the codon 315 of the *katG* gene and codon 531 of the *rpoB* gene, respectively. Further studies on mutations in different geographic regions using DNA sequencing techniques are warranted to improve the kit by including more specific mutation probes in the kits.



## EPIDEMIOLOGY AND PUBLIC HEALTH - SURVEILLANCE

**Friday, 11 March 2016, 12:00-13:30**

### PO35 - Transforming Public Health Microbiology In The United States With Whole Genome Sequencing (WGS) - PulseNET And Beyond

Peter Gerner-Smidt for PulseNet USA<sup>1</sup>

<sup>1</sup>Centers for Disease Control and Prevention, Atlanta, United States

### PO36 - Oxa-48-producing *Enterobacteriaceae* In Poland, 2013-2015

Izdebski Radosław<sup>1</sup>; Baraniak Anna<sup>1</sup>; Urbanowicz Paweł<sup>1</sup>; Fiett Janusz<sup>1</sup>; Żabicka Dorota<sup>1</sup>; Bojarska Katarzyna<sup>1</sup>; Literacka Elżbieta<sup>1</sup>; Hryniwicz Waleria<sup>1</sup>; Gniadkowski Marek<sup>1</sup>

<sup>1</sup>National Medicines Institute

### PO37 - Report Of The First Worldwide Diffusion Of A Multiresistant Coagulase Negative Staphylococci And Comparative Genomics With Other *S. capitis*

M Butin<sup>1</sup>; P Martins Simões<sup>2</sup>; L Hoden<sup>3</sup>; Y Dumont<sup>3</sup>; H Lemriss<sup>4</sup>; A Ibrahim<sup>4</sup>; J-C Picaud<sup>1</sup>; A. Kearns<sup>5</sup>; M A Deighton<sup>6</sup>; O Denis<sup>7</sup>; J-P Rasigade<sup>2</sup>; F Laurent<sup>2</sup>

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### PO39 - Monitoring Clonal Diversity Of Extended-spectrum Cephalosporin-resistant *Enterobacteriaceae* Colonizing The Human Gut 3 Months After Traveling To India

João Pires<sup>1</sup>; Esther Kuenzli<sup>2</sup>; Sara Kasraian<sup>1</sup>; Regula Tinguely<sup>1</sup>; Hansjakob Furrer<sup>3</sup>; Markus Hilty<sup>1</sup>; Christoph Hatz<sup>2</sup>; Andrea Endimiani<sup>1</sup>

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### PO40 - Ongoing Evolution In The Hypervirulent CC17 Clone Of *Streptococcus agalactiae*

Elisabete Martins<sup>1</sup>; Cristiano Rousado<sup>1</sup>; José Melo-Cristino<sup>1</sup>; Mário Ramirez<sup>1</sup>

<sup>1</sup>Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa

## S8 - Poster session II

### PO41 - Integrated Analysis Pipelines For Whole Genome MLST And Whole Genome SNP In Bionumerics® 7.6 Applied To Publicly Available *Listeria monocytogenes* Isolates

Katleen Vranckx<sup>1</sup>; Katrien De Bruyne<sup>1</sup>; Bruno Pot<sup>1</sup>; Hannes Pouseele<sup>1</sup>

<sup>1</sup>Applied Maths NV

### PO42 - From Typened To Regiotype To Eurotype: Moving Towards A Comprehensive Surveillance Strategy For Emerging Viruses

R. Poelman<sup>1</sup>; D. Luijt<sup>2</sup>; R. van Rhee-Luderer<sup>3</sup>; W. Niessen<sup>4</sup>; M. van Genne<sup>1</sup>; C. Van Leer-Buter<sup>1</sup>; B. Niesters<sup>1</sup>

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### PO43 - Detection Of An Expanding *Streptococcus pneumoniae* Serotype 12F Clone With Acquired Antibiotic Resistance, Circulating In Africa And Asia

Rebecca A. Gladstone<sup>1</sup>; Brenda Kwambana-Adams<sup>2</sup>; Mignon Du Plessis<sup>3</sup>; Maaike Alaerts<sup>4</sup>; Paulina Hawkins<sup>5</sup>; Rama Kandasamy<sup>6</sup>; Ben J. Metcalf<sup>7</sup>; Somporn Srifuengfung<sup>8</sup>; Stuart C. Clarke<sup>9</sup>; Abdullah W. Brooks<sup>10</sup>; Veeraraghavan Balaji<sup>11</sup>; Diederik van de Beek<sup>12</sup>; Sarah E. Burr<sup>2</sup>; Bernard Beall<sup>7</sup>; Andrew J. Pollard<sup>6</sup>; Paul Turner<sup>13</sup>; Dean B. Everett<sup>4</sup>; Martin Antonio<sup>2</sup>; Anne von Gottberg<sup>14</sup>; Keith P. Klugman<sup>5</sup>; Lesley McGee<sup>7</sup>; Robert F. Breiman<sup>15</sup>; Stephen D. Bentley<sup>1</sup>

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**PO44 - Two Decades Of Epidemiological Surveillance Of *Burkholderia cepacia* Complex Bacteria In Respiratory Infections At A Portuguese Cystic Fibrosis Center**

Carla P. Coutinho<sup>1</sup>; Ana S. Moreira<sup>1</sup>; A. Amir Hassan<sup>1</sup>; Sandra C. dos Santos<sup>1</sup>; Celeste Barreto<sup>2</sup>; Luísa Pereira<sup>2</sup>; Pilar Azevedo<sup>3</sup>; Luís Lito<sup>4</sup>; José Melo-Cristino<sup>4</sup>; Isabel Sá-Correia<sup>1</sup>

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**PO45 - Transforming Public Health Microbiology For *Campylobacter* With Whole Genome Sequencing: PulseNET And Beyond**

Collette Fitzgerald<sup>1</sup>; Vikrant Dutta<sup>1</sup>; Jan Pruckler<sup>1</sup>; Darlene Wagner<sup>1</sup>; Grant Williams<sup>1</sup>; Heather Carleton<sup>1</sup>; Christy Bennett<sup>1</sup>; Lavin Joseph<sup>1</sup>; Eija Trees<sup>1</sup>; Andrew Huang<sup>1</sup>; Lee S. Katz<sup>1</sup>; Lori Gladney<sup>1</sup>; Martin C. J. Maiden<sup>2</sup>; William Miller<sup>3</sup>; Y. Chen<sup>4</sup>; S. Zhao<sup>4</sup>; Pat McDermott<sup>4</sup>; J. Whichard<sup>1</sup>; E. M. Ribot<sup>1</sup>; H. Pouseele<sup>5</sup>; P. Gerner-Smidt<sup>1</sup>

<sup>1</sup>CDC,<sup>2</sup> Department of Zoology, University of Oxford,<sup>3</sup> Agricultural Research Service, United States Department of Agriculture,<sup>4</sup> Center for Veterinary Medicine, United States Food and Drug Administration,<sup>5</sup> Applied Maths, Inc.

**PO46 - Carbapenemase Genes, Oxy Types And MLST Types Of Four Danish Meropenem Non-susceptible Clinical *Klebsiella oxytoca* Strains**

Hansen DS<sup>1</sup>; Littauer P<sup>2</sup>; Justesen US<sup>3</sup>; Hammerum AM<sup>4</sup>; Hasman H<sup>4</sup>

<sup>1</sup>Herlev Hospital,<sup>2</sup> Hvidovre Hospital,<sup>3</sup> Rigshospitalet,<sup>4</sup> Statens Serum Institut

**PO47 - *Staphylococcus epidermidis* Nosocomial Strain In Breast Milk Of Mothers Of Preterm Neonates**

Hiie Soeorg<sup>1</sup>; Tuuli Metsvaht<sup>2</sup>; Imbi Eelmäe<sup>2</sup>; Sirli Treumuth<sup>1</sup>; Mirjam Merila<sup>3</sup>; Mari-Liis Ilmoja<sup>4</sup>; Irja Lutsar<sup>1</sup>

<sup>1</sup>Department of Microbiology, University of Tartu,<sup>2</sup> Pediatric Intensive Care Unit, Tartu University Hospital,<sup>3</sup> Department of Neonatology, Children's Clinic, Tartu University Hospital,<sup>4</sup> Pediatric Intensive Care Unit, Tallinn Children's Hospital

## S8 - Poster session II

### **PO48 - Implementation Of Whole Genome Sequencing (WGS) For Surveillance And Outbreak Detection Of Shiga Toxin-producing *Escherichia coli* (STEC) In The United States**

Rebecca L. Lindsey<sup>1</sup>; Heather Carleton<sup>1</sup>; Sung Im<sup>1</sup>; Morgan Schroeder<sup>1</sup>; Katrine Joensen<sup>2</sup>; Flemming Scheutz<sup>2</sup>; Lisley Garcia-Toledo<sup>1</sup>; Nancy Strockbine<sup>1</sup>; Efrain Ribot<sup>1</sup>; Eija Trees<sup>1</sup>; Kelley Hise<sup>1</sup>; Hannes Pouseele<sup>3</sup>; Peter Gerner-Smidt<sup>1</sup>

<sup>1</sup>*Centers for Disease Control and Prevention*, <sup>2</sup>*Statens Serum Institut*, <sup>3</sup>*Applied Maths*

### **PO49 - Molecular Characterization Of Vancomycin-resistant Enterococci In Hong Kong.**

Margaret Ip<sup>1</sup>; Christopher Lai<sup>2</sup>; CY Chiu<sup>1</sup>; Kitty Fung<sup>3</sup>; Raymond Lai<sup>4</sup>; Dominic Tsang<sup>2</sup>

<sup>1</sup>*Chinese University of Hong Kong*, <sup>2</sup>*Queen Elizabeth Hospital*, <sup>3</sup>*United Christian Hospital*, <sup>4</sup>*Prince of Wales Hospital*

### **PO50 - Biocide Resistance Genes In *Staphylococcus aureus* From Portuguese Ex-colonies In Africa: Very High Prevalence And Antimicrobial Cross-resistance**

Teresa Conceição<sup>1</sup>; Céline Coelho<sup>1</sup>; Hermínia de Lencastre<sup>2</sup>; Marta Aires-de-Sousa<sup>3</sup>

<sup>1</sup>*Instituto de Tecnologia Química e Biológica António Xavier*, <sup>2</sup>*Laboratory of Microbiology and Infectious Diseases, The Rockefeller University*, <sup>3</sup>*Escola Superior de Saúde da Cruz Vermelha Portuguesa*

### **PO51 - Emergence Of Pan-resistance In Kpc-2 Carbapenemase-producing *Klebsiella pneumoniae* In Crete, Greece**

E Bathoorn<sup>1</sup>; C Tsoutis<sup>2</sup>; JM da Silva Voorham<sup>1</sup>; EV Scoulica<sup>3</sup>; E. Ioannidou<sup>4</sup>; K. Zhou<sup>1</sup>; JW Rossen<sup>1</sup>; A Gikas<sup>2</sup>; AW Friedrich<sup>1</sup>; H Grundmann<sup>1</sup>

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**PO52 - Characterization Of Uncommon Clones Of Carbapenemase-producing *Acinetobacter baumannii* From Nosocomial Settings In Tunis (Tunisia)**

Aymen Mabrouk<sup>1</sup>; Filipa Grosso<sup>2</sup>; Liliana Silva<sup>3</sup>; Clara Sousa<sup>4</sup>; Wafa Achour<sup>1</sup>; Assia Ben Hassen<sup>1</sup>; Luísa Peixe<sup>2</sup>

<sup>1</sup>Laboratories UR12ES02-The National Bone Marrow Transplant Centre,<sup>2</sup> University of Carthage, Faculty of Sciences of Bizerte,<sup>3</sup> UCIBIO-REQUIMTE-Laboratório de Microbiologia, Faculdade de Farmácia, Universidade do Porto,<sup>4</sup> UCIBIO-REQUIMTE, Laboratório de Microbiologia, Faculdade de Farmácia, Universidade do Porto,<sup>5</sup> ESALD, Instituto Politécnico de Castelo Branco,<sup>6</sup> CEB-Centro de Engenharia Biológica, Universidade do Minho

**PO53 - Phylogenetic Analysis Of Severe Fever With Thrombocytopenia Syndrome Virus And Migratory Bird Routes Between China, South Korea, And Japan**

Ji-Hoon Kang<sup>1</sup>; Sang Taek Heo<sup>1</sup>

<sup>1</sup>Jeju National University

**PO54 - Burden Evaluation Of Hand Soap's Contamination By *Pseudomonas aeruginosa* In A Tertiary Care Hospital Using Whole Genome Sequencing**

Bárbara Magalhães<sup>1</sup>; Mohamed M. H. Abdelbary<sup>1</sup>; Guy Prod'Hom<sup>2</sup>; Gilbert Greub<sup>2</sup>; Jean-Blaise Wasserfallen<sup>3</sup>; Patrick Genoud<sup>4</sup>; Giorgio Zanetti<sup>1</sup>; Laurence Senn<sup>1</sup>; Dominique S. Blanc<sup>1</sup>

<sup>1</sup>Service of Hospital Preventive Medicine, Lausanne University Hospital,<sup>2</sup> Institut of Microbiology, Lausanne University Hospital,<sup>3</sup> Medical directorate,<sup>4</sup> Nurse directorate

**PO55 - Molecular Surveillance And Emergence Of Carbapenemases At A University Hospital In Germany Over A Five Year-period**

Andreas F. Wendel<sup>1</sup>; Colin R. MacKenzie<sup>1</sup>

<sup>1</sup>Institute of Medical Microbiology and Hospital Hygiene

**PO57 - A Molecular Survey Of *Campylobacter jejuni* And *Campylobacter coli* Virulence And Diversity**

Mahdi Ghorbanalizdgan<sup>1</sup>; BitaBakhshi<sup>2</sup>; ShahinNajar-Peerayeh<sup>2</sup>

<sup>1</sup>Department of Bacteriology, Faculty of Medical Sciences, TarbiatModares University, Tehran, IR Iran,<sup>2</sup> Department of Bacteriology, Faculty of Medical Sciences, Baqiyatallah University, Tehran, IR Iran

## S8 - Poster session II

### PO58 - Emergence And Dissemination Of A Linezolid-resistant *Staphylococcus capitnis* Clone In Europe

P Martins Simoes<sup>1</sup>; M. Butin<sup>1</sup>; C. Dupieux<sup>1</sup>; L. d'Anthouard<sup>1</sup>; D. Leyssene<sup>2</sup>; S. Bordes-Couecou<sup>3</sup>; B. Pichon<sup>4</sup>; H. Meugnier<sup>1</sup>; N. Lemaitre<sup>5</sup>; F. Schramm<sup>5</sup>; F. Vandenesch<sup>1</sup>; I. Spiliopoulou<sup>6</sup>; H-L Hyryläinen<sup>7</sup>; A. Kearns<sup>4</sup>; O. Dumitrescu<sup>1</sup>; F. Laurent<sup>1</sup>

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### PO59 - Whole Genome Sequencing Data Confirm A Transmission Of *Salmonella enterica* Serovar Typhimurium Phage Type Dt41 In Danish Poultry Production

Ann-Sofie Hintzmann<sup>1</sup>; Gitte Sørensen<sup>1</sup>; Dorte Lau Baggesen<sup>1</sup>

<sup>1</sup>National Food Institute

### PO60 - Dominance Of *Escherichia coli* ST131 H30 Subclone In A Portuguese Long Term Care Facility

Ana Constança Mendes<sup>1</sup>; Carla Rodrigues<sup>1</sup>; Helena Ramos<sup>2</sup>; Luísa Peixe<sup>1</sup>; Ângela Novais<sup>1</sup>

<sup>1</sup>UCIBIO @REQUIMTE, Laboratório Microbiologia, Faculdade de Farmácia, Universidade do Porto,<sup>2</sup> Centro Hospitalar do Porto

### PO61 - Salmonella Surveillance By Whole Genome Sequencing: WG-MLST And WG-SNP Approaches Using The Bionumerics Platform.

Hannes Pouseele<sup>1</sup>; Katrien De Bruyne<sup>1</sup>; Johan Goris<sup>1</sup>; Bruno Pot<sup>1</sup>; Koen Janssens<sup>1</sup>

<sup>1</sup>Applied Maths NV

### PO62 - Molecular Epidemiology Of *Klebsiella pneumoniae*: Multiclonal Dissemination Of CTX-M-15 Extended Spectrum B-lactamase

C. Caneiras<sup>1</sup>; L. Lito<sup>2</sup>; J. Melo-Cristino<sup>3</sup>; A. Duarte<sup>1</sup>

<sup>1</sup>iMed.UL - Research Institute for Medicines and Pharmaceutical Sciences, Faculty of Pharmacy, University of Lisbon,<sup>2</sup> Laboratory of Microbiology, Centro Hospitalar Lisboa Norte,<sup>3</sup> Laboratory of Microbiology, Centro Hospitalar Lisboa Norte,<sup>4</sup> Institute of Microbiology, Institute of Molecular Medicine, Faculty of Medicine, University of Lisbon

**PO63 - Molecular Characterization Of The *Staphylococcus aureus* CC398 Population In Italy**

Fabiola Feltrin<sup>1</sup>; Patricia Alba<sup>1</sup>; Roberta Amoruso<sup>1</sup>; Maria de los Angeles Argudín<sup>2</sup>; Birgit Lauzat<sup>3</sup>; Manuela Iurescia<sup>1</sup>; Luigi Sorbara<sup>1</sup>; Samuele Dottarelli<sup>1</sup>; Virginia Carfora<sup>1</sup>; Erika Menichini<sup>1</sup>; Fiorentino Stravino<sup>1</sup>; Beatriz Guerra<sup>3</sup>; Antonio Battisti<sup>1</sup>

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**PO64 - *Staphylococcus aureus* USA300 In Sub-saharan Africa**

Lena Strauß<sup>1</sup>; Marc Stegger<sup>2</sup>; Anders Rhod Larsen<sup>2</sup>; Geoffrey Coombs<sup>3</sup>; Sébastien Breurec<sup>4</sup>; Alexander Mellmann<sup>1</sup>; Frieder Schaumburg<sup>1</sup>

<sup>1</sup>University of Münster,<sup>2</sup> Statens Serum Institut,<sup>3</sup> Murdoch University,<sup>4</sup> University of Antilles

**PO65 - Evergreen; A Webtool For Surveillance Of Bacterial Outbreaks**

Johanne Ahrenfeldt<sup>1</sup>; Martin Christen Frølund Thomsen<sup>1</sup>; Jose Bellod Cisneros<sup>1</sup>; Ole Lund<sup>1</sup>

<sup>1</sup>Technical University of Denmark

**PO66 - Demography And Intercontinental Spread Of The USA300 CA-MRSA Lineage**

P Martins Simões<sup>1</sup>; P Glaser<sup>2</sup>; A. Villain<sup>2</sup>; M Barbier<sup>3</sup>; A Tristan<sup>1</sup>; C Bouchier<sup>2</sup>; L Ma<sup>2</sup>; M Bes<sup>1</sup>; F Laurent<sup>1</sup>; D Guillemot<sup>4</sup>; T Wirth<sup>3</sup>; F Vandenesch<sup>1</sup>

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**PO67 - In Vivo Characterisation Of Human Cytomegalovirus Genome Diversity Directly From Clinical Specimens Sampled Over Time Or From Different Compartments**

Elias Hage<sup>1</sup>; Tina Ganzenmueller<sup>1</sup>; Gavin Wilkie<sup>2</sup>; Silvia Linnenweber-Held<sup>3</sup>; Julius Schmidt<sup>3</sup>; Eva Mischak-Weissinger<sup>4</sup>; Anke Schwarz<sup>3</sup>; Albert Heim<sup>1</sup>; Thomas F. Schulz<sup>1</sup>; Andrew J. Davison<sup>2</sup>

<sup>1</sup>Institute of Virology, Hannover Medical School,<sup>2</sup> Centre for Virus Research, University of Glasgow,<sup>3</sup> Department of Nephrology, Hannover Medical School,<sup>4</sup> Department of Haematology, Haemostasis and Oncology, Hannover Medical School

## S8 - Poster session II

### PO68 - Frequency And Predisposing Factors Of Upper Respiratory Tract Colonization By Gram-negative Rods In Untreated Chronic Lymphocytic Leukaemia Patients

Ewelina Grywalska<sup>1</sup>; Izabela Korona-Głowniak<sup>1</sup>; Anna Malm<sup>1</sup>; Jacek Rolinski<sup>1</sup>

<sup>1</sup>Medical University of Lublin

### PO69 - Re-evaluation Of *Streptococcus pneumoniae* Carriage By Real-time PCR In Adults Over 60 Years Of Age

Sónia T. Almeida<sup>1</sup>; Tânia Pedro<sup>1</sup>; Hermínia de Lencastre<sup>2</sup>; Raquel Sá-Leão<sup>1</sup>

<sup>1</sup>Laboratory of Molecular Microbiology of Human Pathogens, ITQB/UNL,<sup>2</sup>

Laboratory of Microbiology and Infectious Diseases, The Rockefeller University

### PO70 - Whole Genome Sequencing Of Methicillin-resistant And Sensitive *Staphylococcus aureus* Strains Isolated In The Cardiosurgical Area Of The Italian L. Sac

Gentile Bernardino<sup>1</sup>; Rimoldi Sara Giordana<sup>2</sup>; Pagani Cristina<sup>2</sup>; Anselmo Anna<sup>1</sup>;

Palozzi Anna Maria<sup>1</sup>; Fortunato Antonella<sup>1</sup>; Di Gregorio Aannamaria<sup>2</sup>; Lista

Florigio<sup>1</sup>; Gismondo Maria Rita<sup>2</sup>

<sup>1</sup>Histology and Molecular Biology Section, Army Medical Research Center,<sup>2</sup>

Microbiology Unit, L.Sacco University Hospital

### PO71 - DNA Structural Properties At Origin-of-transfer Regions Determine Hosts Of Mobile Plasmids

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<sup>1</sup>Faculty of Health Sciences, University of Primorska,<sup>2</sup> Institute of Metagenomics

and Microbial Technologies,<sup>3</sup> Institute of Metagenomics and Microbial

Technologies,<sup>4</sup> Saratov state university

### PO35 - Transforming Public Health Microbiology In The United States With Whole Genome Sequencing (WGS) - PulseNET And Beyond

Peter Gerner-Smidt for PulseNet USA<sup>1</sup>

<sup>1</sup>Centers for Disease Control and Prevention, Atlanta, United States

**Background:** Multiple different phenotypic and molecular methods and technologies are used in public health laboratories (PHLs) to characterize and subtype foodborne bacterial pathogens. This traditional strain characterization is labor and resource intensive and has a turn-around-time (TAT) of up to several months. Most of this information may be extracted from the genome sequence of any organism. With the introduction of next generation sequencing technologies and advances in bioinformatics it is now possible to characterize foodborne pathogens in a single workflow with a TAT of ≤ four working days in a cost-efficient manner.

**Methods:** The Enteric Diseases Laboratory Branch at CDC is working with partners in federal agencies, PHLs in the states and internationally to build applications to extract information for strain characterization and subtyping using the gene by gene approach (MLST) from the genome sequences. Average nucleotide identity (ANI) is used for species identification, tools for serotyping, virulence, plasmid, and antimicrobial resistance characterization developed by others are used, as are established schemes for 7-gene MLST and core genome (cg)MLST. Whole genome (wg)MLST schemes are developed as needed. The PulseNet infrastructure, analytical platform and databases are used because of their comprehensiveness and versatility, and because the PHLs are familiar with them after working with them for 20 years.

**Results:** WGS has successfully been used for national surveillance of listeriosis and in outbreak investigations of all enteric foodborne bacterial pathogens since 2013. WGS adds phylogenetic relevance, increased resolution and precision compared to current methods thereby enhancing case definition and source tracking in outbreak investigations. By the end of 2015, a WGS database and application will be implemented for routine identification, lineage determination, 7-gene MLST and subtyping by cg- and wgMLST of *Listeria monocytogenes* at CDC and in states with sequencing capacity. At the beginning of 2016, similar applications for identification of *Campylobacteraceae*, 7-gene MLST and subtyping of *C. jejuni/coli*, identification, serotyping, virulence and antimicrobial resistance characterization of Shiga toxin-producing *E. coli* (STEC) will be undergoing external validation; a pilot-application for the identification, serotyping, plasmid profiling, antimicrobial resistance characterization and MLST of *Salmonella* and identification, toxin-gene subtyping and MLST for *Clostridium botulinum* will be undergoing internal validation; databases for *Vibrio* spp., other diarrhoeagenic *E. coli* (enterotoxigenic *E. coli* [ETEC], enteropathogenic *E. coli* [EPEC], enteroinvasive *E. coli* [EIEC] including *Shigella*), *Yersinia* and *Cronobacter* to follow within two years.

**Conclusion:** WGS will gradually replace and enhance current laboratory methods for foodborne pathogens in the United States within the next three- five years.

## S8 - Poster session II

### PO36 - Oxa-48-producing Enterobacteriaceae In Poland, 2013-2015

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**Background:** Carbapenemase-producing *Enterobacteriaceae* (CPE) create a major threat for public health. Three main types of carbapenemases are observed in CPE, namely the KPC-, MBL- and OXA-48-like enzymes. OXA-48 was identified first in 2001 in Turkey, and with years most of the east and south Mediterranean region has become endemic for its producers. Another large reservoir is India where a related enzyme, OXA-181, has been routinely identified. Extensive travelling and migrations are at the origin of the increasing presence of organisms with OXA-48-like enzymes in Europe, especially in France, Germany or Spain. The dissemination of OXA-48 producers is attributed to high mobility of *bla*<sub>OXA-48</sub>-like genes due to their location in Tn 1999-like transposons and pOXA-48-like plasmids but also to spread of some bacterial clone variants, like *Klebsiella pneumoniae* ST11 or ST395, or *Escherichia coli* ST38. So far Poland has recorded a relatively small number of OXA-48 clinical cases (n=24 by November 2015). The aim of this study was to analyze all but two cases identified by the mid-2015.

**Materials and Methods:** Twenty non-duplicate *Enterobacteriaceae* isolates (11 *E. coli*, 8 *K. pneumoniae* and 1 *Enterobacter aerogenes*) from 18 patients in 8 cities were analyzed. The species were identified by Vitek2. OXA-48 detection was done by Carba NP test, temocillin disc, and by PCR and sequencing of *bla*<sub>OXA-48</sub> genes. Location of the genes in Tn 1999-like elements was checked by PCR mapping. Identification of *bla*<sub>OXA-48</sub>-carrying plasmids was performed by the S1 analysis, followed by hybridization with *bla*<sub>OXA-48</sub>. The isolates were subjected to mating; plasmid DNAs of transconjugants was used in the PBRT analysis. Chromosomal localization of *bla*<sub>OXA-48</sub> genes in some isolates was confirmed by the I-CeuI analysis. Other β-lactamase genes were identified by PCR and sequencing. *E. coli* & *K. pneumoniae* isolates were typed by PFGE and MLST.

**Results:** Of the 18 cases, 14 have been associated with arrivals from different countries. Eight PFGE patterns were distinguished among each *E. coli* and *K. pneumoniae* isolates, classified then by MLST into 4 and 5 STs, respectively. The most prevalent were *E. coli* ST38 (n=6), *E. coli* ST410 (n=3) and *K. pneumoniae* ST395 (n=3). All of the isolates carried the *bla*<sub>OXA-48</sub> gene which resided in the Tn 1999.1 (n=8), Tn 1999.2 (n=10) or ΔTn 1999 (n=2) transposon variants. The S1 analysis showed high diversity of plasmid profiles (1-6 plasmids per isolate), including *bla*<sub>OXA-48</sub>-carrying plasmids of ~50kb—260kb in 12 isolates; of which 11 isolates (6 *K. pneumoniae*, 4 *E. coli* and 1 *E. aerogenes*) produced transconjugants with high efficiency ( $10^{-3}$ - $10^{-5}$  per donor cell). The PBRT revealed replicons L (n=7; plasmids of ~60kb), M (n=1; ~80kb) and X3 (n=3; ~50kb). The only *bla*<sub>OXA-48</sub> plasmid of ~260kb was non-transferable either by conjugation or electroporation. The remaining 8 isolates had *bla*<sub>OXA-48</sub> inserted into the chromosome. In all *E. coli* ST38 isolates *bla*<sub>OXA-48</sub> was located in the chromosome. Fifteen isolates had ESBLs, including CTX-M-15 (n=9), -24 (n=4), -3 (n=1) and -55 (n=1); three isolates produced also the CMY-2 cephalosporinase.

**Conclusions:** This is the first broad analysis of OXA-48 CPE in Poland, encompassing most of the cases recorded so far. It showed high diversity of OXA-48 producers, attributed to multiple independent imports. Only the 3 *E. coli* ST410 isolates represented a small outbreak in one of the hospitals.

**PO37 - Report Of The First Worldwide Diffusion Of A Multiresistant Coagulase Negative Staphylococci And Comparative Genomics With Other *S. capitis***

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**Background:** Cases and series of neonatal sepsis involving methicillin-resistant *Staphylococcus capitis* (MRSC) have been previously reported from different countries, and were initially considered as unrelated epidemic bursts. However, we recently found that a single multidrug-resistant clone of SC, named NRCS-A and defined by a specific pulsed-field gel electrophoresis (PFGE) banding pattern was involved in neonatal sepsis in several neonatal ICUs (NICUS) from France, Belgium, the UK and Australia. All NRCS-A isolates exhibited a decreased susceptibility to all antimicrobial agents frequently used in NICUs. This led us to suspect that the initial report of NRCS-A in four distant countries was only the tip of the iceberg. Here, we investigated the diffusion of this clone at a larger geographical scale and we analyzed the WGS of 4 NRCS-A strains from distinct countries to determine the clone's genomic characteristics.

**Materials and Methods:** In collaboration with laboratories of hospitals worldwide, clinical MRSC isolates from blood cultures of neonates have been collected and subjected to PFGE typing using *Sma*I restriction enzyme. Four of these SC strains, isolated in France, Belgium, Australia and United Kingdom (n=1 each), were whole genome sequenced. A closed reference genome and methylome was obtained (SMRT-BacBio) for the prototype strain CR01. NRCS-A genomes were searched for virulence factors, resistance genes, mobile genetic elements (MGEs) and clone specific genes. Six publicly available WGS of non-NRCS-A strains were used for comparison.

**Results:** The NRCS-A PFGE profile was identified in 154 SC isolates collected between 1994 and 2015 from 17 distant countries (Australia, Belgium, Brazil, Canada, Czech Republic, Denmark, Finland, France, Germany, Netherlands, New Zealand, Norway, South Korea, Switzerland, Taiwan, UK and USA). The WGS analysis of 4 of these NRCS-A strains showed that antimicrobial resistance genes were found in MGEs and that all SC genomes (NRCS-A and non-NRCS-A) shared virulence factors including phenol soluble modulins (PSMs), hemolysins, cell wall associated fibronectin, fibrinogen binding proteins and the *icaRADBC* operon. Of importance, when comparing *S. capitis* genomes, we found exclusively within the NRCS-A clone a gene coding for nisin resistance (*nsr*), a "broad-spectrum" bacteriocin, that could play a pivotal role and give a selective advantage to NRCS-A clone in the initial step of gut colonization in neonates.

**Conclusions:** Our results highlight the endemic distribution of the clone *S. capitis* NRCS-A in NICUs worldwide, that constitutes the first report of a single coagulase-negative staphylococci clone dissemination on such a large geographical scale. Furthermore, the genomic characteristics of the NRCS-A clone emphasize the contribution of MGEs to the emergence of its multidrug-resistance profile. Comparative genomic analysis with non-NRCS-A genomes revealed differences in the core genome, MGEs content but not in virulence factors profiles. The identification of NRCS-A lineage specific genes suggests that it might be well adapted to the NICU environment both by its high adaptation to antibiotic pressure but also by specific survival advantages, contributing to a better fitness in the very early steps of colonization. These observations raise important questions

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regarding pathogen reservoirs and ways of transmission within and between NICUs of different hospitals.

## PO39 - Monitoring Clonal Diversity Of Extended-spectrum Cephalosporin-resistant *Enterobacteriaceae* Colonizing The Human Gut 3 Months After Traveling To India

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**Background:** The role of international travel is being increasingly recognized as a very important factor in the spread of extended-spectrum cephalosporin-resistant *Enterobacteriaceae* (ESC-R-Ent). In particular, colonization by ESC-R-Ent in the human gut is frequently reported when people return from high prevalence areas. This phenomenon increases the risk of importation of new epidemic clones which can spread in new geographical regions. Silent carriage of these pathogens in the gut is of extreme importance as being colonized by ESC-R-Ent represents a risk factor for developing future extra-intestinal infections. However, factors modulating the acquisition and persistence of ESC-R-Ent in the gut are unknown. The aim of this work was to monitor this condition in volunteers travelling to a high ESC-R-Ent prevalence geographic area.

**Methods:** Thirty-six Swiss volunteers travelling to India underwent screening before and after their trip. For a subset of 17 volunteers, a screening after 3 months was also performed. An aliquot of negative stools were enriched in LB broth with a cefuroxime disc. Enrichments were plated in BLSE, ChromID ESBL, and a modified Supercarba plate containing imipenem. Species identification was obtained using the MALDI-TOF. At least 5 *Enterobacteriaceae* colonies were analyzed. Double-disk synergism test was performed and clonal relatedness in *E. coli* was assessed using rep-PCR, Phylogenetic group (PhG) analysis, and MLST. The Check Points CT103 XL microarray and PCR/DNA sequencing were implemented to detect and characterize ESBL, plasmid-mediated AmpC, and carbapenemase-producing *Enterobacteriaceae*.

**Results:** For the 36 volunteers, 5 of them were colonized before travelling and 29 after travelling. In the subgroup of 17 volunteers, only 2 were found colonized before travelling while all of them were colonized after their trip; 4 of them still remained colonized three months later. Only one volunteer was colonized at the three time points. For this subgroup, we detected 57 different isolates (56 *E. coli* and one *K. pneumoniae*) of which 50 were ESBL and 7 plasmid-mediated AmpC producers. A low β-lactamase diversity was identified, with isolates mostly CTX-M-types, mainly group 1 (n=47, including CTX-M-15), and to a lesser extent DHA (n=5), CTX-M-group 4 (n=2), CMY-2 (n=2), and VEB (n=1). Surprisingly, rep-PCR revealed that the clonal *E. coli* diversity found in each volunteer can vary from 1 to 5 clones when returning to Switzerland. These clones were mostly from PhG A (60.7%) and D (21.7%) and diverse non-epidemic STs. Remarkably, when multiple clones were colonizing the gut, they can also produce different β-lactamase types. Interestingly, for the volunteers still colonized after 3 months, only one clone was persisting in the gut.

**Conclusion:** Our data indicate that the initial stages of colonization when traveling to high prevalence areas involve a polyclonal acquisition of ESC-R-Ent. This might be linked to non-human sources (e.g., food-chain, environment), since the isolates belong to a multiplicity of genetic backgrounds that are usually linked with these settings. Moreover, colonization of the gastrointestinal tract by ESC-R-Ent also decreases over time but when persistent, only one clone is then identified. A further characterization of these isolates will elucidate the bacterial features that might be associated with enhanced colonization capabilities and persistence in the gut.

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### PO40 - Ongoing Evolution In The Hypervirulent CC17 Clone Of *Streptococcus agalactiae*

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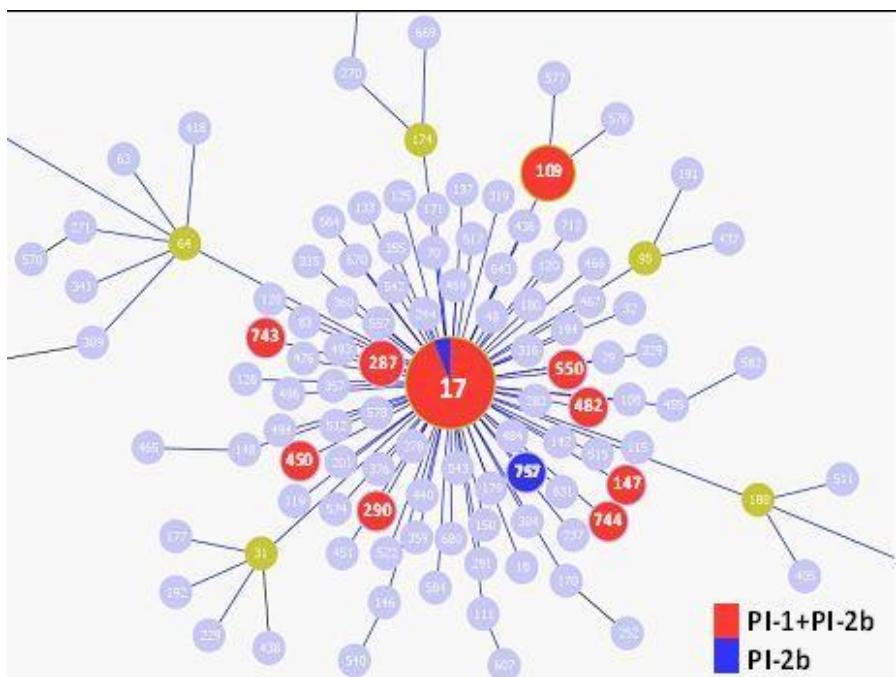
**Background:** Group B streptococci (GBS) is a leading cause of neonatal invasive disease (NID) in most countries. CC17 is recognized as a worldwide disseminated hypervirulent clone due to its increased ability to cause neonatal infections, particularly late-onset disease (LOD) and meningitis. This clone has been shown to be highly stable and to have persisted for decades as a leading cause of NID.

**Material/methods:** We have analysed a collection of 97 isolates representing CC17 recovered from NID in newborns in Portugal (2005-2014). These had been previously characterized by serotyping, multilocus sequence typing, presence of genes encoding alpha-like proteins and resistance to macrolides and tetracycline, and evaluated for susceptibility to chloramphenicol, clindamycin, erythromycin, levofloxacin, penicillin, tetracycline and vancomycin. High-level resistance to gentamicin and streptomycin was tested by disk diffusion according to the CLSI guidelines for enterococci. Presence of the pilus islands (PI) 1, 2a and 2b was tested by PCR.

**Results:** The collection was mostly composed of representatives of ST17 and its SLVs, carrying the surface protein encoding gene *rib* and the combination of PI-1+PI-2b. Five new STs were identified among the 15 SLVs of ST17, indicating an ongoing diversification within CC17. Additionally, five isolates carried only PI-2b (Figure 1). The lack of PI-1 had not been previously observed in CC17 isolates from Portugal.

All isolates were susceptible to penicillin, vancomycin and levofloxacin, and one isolate was resistant to chloramphenicol. Most isolates (n=87) were resistant to tetracycline and carried the *tetM* gene (n=83). One isolate presented high-level resistance to gentamicin and carried the *aac(6')-Ie-aph(2')*-*la* gene, encoding a bifunctional aminoglycoside-modifying enzyme, but no other resistance determinant. Macrolide resistance was low (n=7), in agreement with previous reports, as this clone is frequently susceptible. The isolates carrying exclusively PI-2b also carried the *ermB* gene and presented the cMLS<sub>B</sub> phenotype. Most carried the unusual *tetO* gene (n=3) and presented high-level resistance to aminoglycosides (n=4), harbouring the *aph(3)-IIIa* and *aadE* genetic determinants, both encoding streptomycin-modifying enzymes.

**Conclusions:** While the dominance of serotype III/CC17 among neonatal invasive disease has been described worldwide, our data suggest that diversification within this very stable genetic lineage is ongoing, as illustrated by the possible emergence of a highly resistant sublineage within CC17. Antimicrobial resistance has a significant impact in clinical practice, including preventive strategies such as intrapartum antibiotic prophylaxis but also in the treatment of GBS infections. The increasing burden of GBS disease and dynamic nature of this pathogen substantiate the need for continued monitoring and detailed characterization of the genetic lineages circulating at both regional and global contexts.



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### PO41 - Integrated Analysis Pipelines For Whole Genome MLST And Whole Genome SNP In BioNumerics® 7.6 Applied To Publicly Available *Listeria monocytogenes* Isolates

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<sup>1</sup>Applied Maths NV

**Background:** *Listeria monocytogenes* (Lmo) is a ubiquitous organism in the environment and a rare cause of human disease. Though listeriosis occurs infrequently, it is characterized by a high case-fatality rate which can exceed 30%. Following a considerable costs reduction, complete Lmo genome sequencing dramatically increased the number of publically available genomes on the Sequence Read Archive (SRA) of NCBI. A key challenge is to rapidly compute and interpret the relevant information from this vast amount of data. Rapid and automated processing of whole genome sequencing (WGS) data ensures a reliable and easy to follow workflow in routine molecular surveillance, reducing the time needed to detect and contain an outbreak. Moreover, WGS has the potential to provide information on traditional typing technologies such as MLST, MLVA, virulence and resistance determination.

**Materials and Methods:** In this study, we compare two subsequent pipelines for high resolution WGS-based molecular typing.

First, whole genome multilocus sequence typing (wgMLST) is applied to WGS data from around 6000 isolates available on NCBI, with the purpose to detect clusters of highly related strains. A wgMLST scheme was created from a limited set of reference sequences, from which all coding regions were extracted and used to create a set of discernible loci. An assembly-free and a BLAST-based allele calling algorithm were used to independently determine locus presence and allelic variants.

A cluster defined by wgMLST can then be further characterized by whole genome single-nucleotide polymorphism analysis (wgSNP). As a working example we here focus on clusters containing isolates originating from avocado or guacamole. SNP variants are detected by mapping the WGS reads to a reference chosen from within the cluster to maximize the resolution.

Both analysis pipelines run on the BioNumerics® Calculation Engine, which is fully integrated with the BioNumerics® 7.6 software.

**Results:** BioNumerics® 7.6 software offers a powerful platform where both wgMLST and wgSNP analysis can be performed at high-throughput rates. Using this platform, WGS analysis results can be validated against traditional data such as MLST or PFGE, rapidly providing a robust, portable and high resolution picture of molecular typing data. We demonstrate that wgMLST is suitable for the analysis of very large (and growing) datasets, making it a suitable technique for outbreak surveillance. The added resolution of wgSNP against an internal reference sequence increases the confidence in the detected clusters and supports epidemiologist in their source tracking efforts.

**Conclusion:** The combination of two complementary approaches, wgMLST and wgSNP, on a virtually unlimited number of samples, managed by a single software platform that also stores historical data as well as metadata, opens many perspectives for food safety and public health monitoring programs. The BioNumerics® solution combines the power of a cluster or cloud implementation with the ease of use of a local database and management software. The possibility to extract 'historical' typing data, resistance and virulence data from wgMLST schemes, moreover reduces the total analysis time and cost, and may lead to more efficient outbreak detection.

### PO42 - From Typened To Regiotype To Eurotype: Moving Towards A Comprehensive Surveillance Strategy For Emerging Viruses

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**Background:** Patients with respiratory illness are routinely screened for viral infections. This screening was expanded by routinely sequencing enteroviruses and rhinoviruses to identify outbreaks and transmission patterns. Additionally, we included noroviruses and parechoviruses in patients with gastro-intestinal complaints. For surveillance purposes, a national structure called TYPENED is available through the Dutch National Institute of Health (RIVM). Our main objective is to provide a rapid regional sequencing service that create a source of clinical information and to correlate this with epidemiological patterns.

**Methods:** A regional sequencing and epidemiological strategy for surveillance, called REGIOTYPE, was implemented by including GP's, regional hospitals and regional Public Health Services which covers the Northern part of the Netherlands, for a rapid flow of information within our region. Sequencing is performed locally and results are available within one week. Clinical data are exchanged and phylogenetic analysis is performed.

**Results:** Phylogenetic analysis has contributed to the knowledge of circulating viral strains and the relatedness within and between multiple institutions. This rapid sequencing strategy in combination with the clinical data enables the application of infection control measures in the participating (local) health institutions. Enterovirus D68 and recently the 'new' C-group enteroviruses were identified. This not only indicated a possible outbreak, but it also improved detection methodologies in our region. Besides, a vaccine derived poliovirus strain was recently identified in a refugee, using this local strategy.

**Conclusion:** REGIOTYPE has proven to be an adequate strategy for European surveillance on local circulating viruses and not only for the hospitalized population with severe (underlying) illnesses. This underlines the importance for regional surveillance by sequencing, of which several networks are available within Europe but still acting independently from each other.

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### PO43 - Detection Of An Expanding *Streptococcus pneumoniae* Serotype 12F Clone With Acquired Antibiotic Resistance, Circulating In Africa And Asia

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**Background:** The Global Pneumococcal Sequencing project (GPS) aims to study evolution in 20,000 pneumococcal genomes. Isolates were collected from around the world, focusing on resource limited nations over the introduction of pneumococcal conjugate vaccines (PCVs). Current PCVs target serotypes associated with disease and antibiotic resistance. Over 40 globally-disseminated clones have been designated by the Pneumococcal Molecular Epidemiology Network (PMEN). We describe an expanding clone (clonal complex CC989) with acquired antibiotic resistance expressing non-vaccine serotype (NVT) 12F. Serotype 12F is found more frequently in disease than carriage and has been increasing in the PCV era. The CC989 clone represents a lineage distinct from the 12F PMEN clone, Denmark12F-34.

**Methods:** Whole genome data for ~10,000 pneumococcal isolates were screened for known acquired antibiotic resistance determinants using ARIBA. A phylogenetic tree of all taxa was produced using RAxML from a concatenated MLST alignment. Metadata were visualised on the tree using Phylocanvas.org and Microreact.org. Phenotypic data, where available, were used to confirm the inferred resistant phenotype.

**Results:** A cluster of isolates with a shared antibiotic resistance profile was identified in the phylogeny; >90% were of sequence type 989, serotype 12F. CC989 isolates (172) represent the largest 12F clone in GPS and were predominantly from The Gambia (69) and South Africa (61) but also from Malawi (12), Thailand (10), Ghana (4), Bangladesh (4), the USA (4), Senegal (2), India

(2), The Netherlands (2), Niger (1) and Nepal (1). The earliest isolate in the GPS collection was from The Gambia in 2002 (95% CI -2.7% to 8.4%), in South Africa the earliest isolates (n=2) were from 2005 (95% CI -0.3% to 1.6%). In 2014, 11 (95% CI 6.5% to 22.1%) and 19 (95% CI 3.7% to 9.5%) were isolated in The Gambia ( $p=0.09$ ) and South Africa ( $p=0.0001$ ) respectively. In 2015 CC989 cases were observed in the USA Active bacterial core surveillance for the first time. CC989 isolates were more often from disease than expected (95% CI 75.5% to 87.5%) when compared to GPS collection overall (95% CI 57.2% to 58.8%).

Almost all isolates had the tet(M) gene and a chloramphenicol acetyltransferase gene (cat pC194). Phenotypic tetracycline minimum inhibitory concentrations (MICs) were available for 56 isolates, 95% had a MIC of  $\geq 2\mu\text{g/ml}$  (resistant) with a mode MIC of  $8\mu\text{g/ml}$ . Phenotypic chloramphenicol MICs were available for 38 isolates, 92% had a MIC of  $\geq 8\mu\text{g/ml}$  (resistant) with a mode MIC of  $8\mu\text{g/ml}$ . Pbp1a, 2b and 2x allele profiles and phenotypic data suggest that the isolates are fully susceptible to penicillin.

**Conclusions:** Isolates of ST989 usually expressing serotype 12F have previously been reported in small numbers; the MLST database includes sporadic CC989 isolates (n=55) from African, Asian and European countries with the oldest isolate that we know of from 1998 in Kenya. The global perspective of the GPS collection, combined with genomics and resistance profiles, highlighted the intercontinental dissemination of an expanding NVT clone with acquired resistance. The clone's prevalence in Africa and 12F's association with disease make it a potential threat that may become important in vaccine serotype replacement. Global genomic surveillance allows early detection of expanding lineages expressing serotypes not currently targeted by vaccines, providing vital information for future control of pneumococcal disease.

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### PO44 - Two Decades Of Epidemiological Surveillance Of *Burkholderia cepacia* Complex Bacteria In Respiratory Infections At A Portuguese Cystic Fibrosis Center

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**Background:** The *Burkholderia cepacia* complex (Bcc) comprises 20 species of Gram-negative opportunistic bacterial pathogens that generally lead to long term respiratory infections and a more rapid decline in cystic fibrosis (CF) patients lung function and, in some cases, to a fatal necrotizing pneumonia (cepacia syndrome). Bcc bacteria can be transmitted between patients with or without CF but the environment, including contaminated medications, may act as reservoir for novel infections.

**Materials & Methods:** The epidemiological surveillance of Bcc respiratory infections at the major Portuguese CF Center at Hospital de Santa Maria (HSM) has been carried out over the past 2 decades [1]. More than 800 clinical isolates were retrieved from sputum samples collected from 52 CF patients (32 paediatric and 20 adult), as part of routine monitoring at this Center [1]. The identification and strain discrimination was performed using PCR amplification with specific primers, recA, hisA, MLST and RFLP-PFGE analyses [1-3]. Extensive phenotypic analyses of clonal variants obtained during long-term lung infection were performed [1-3].

**Results:** This systematic and longitudinal survey of a CF population for Bcc-associated respiratory infections (in 5% of the patients) covered different Bcc species and disease outcomes, including chronically colonized patients (56%) between 7 months and 16 years. The infecting bacteria belongs not only to the most frequent species worldwide, *B. cenocepacia* (36% of Bcc-infected patients) and *B. multivorans*, but also species that are rarely isolated in CF and therefore poorly studied, in spite of their damaging effects, as it is the case of *B. dolosa* and *B. stabilis* [1,3]. Remarkably, the CF population under analysis showed an abnormal prevalence of *B. cepacia* [4]. Recently, after re-examination of the *B. cepacia* isolates belonging to recA RFLP type K, they were reclassified as *B. contaminans* [2]. The current data indicates that *B. cepacia* and *B. contaminans* were isolated from 28% and 17% of the Bcc-infected patients, respectively. The systematic phenotypic characterization (e.g. susceptibility to antimicrobials, cell motility and hydrophobicity, colony morphology, fatty acid composition, growth under iron limitation/load conditions, exopolysaccharide production, size of biofilms) of several clonal variants obtained during long-term infections suggested the occurrence of clonal expansion which ultimately proves beneficial by providing an adaptive evolution leverage [1,5].

**Conclusion:** Although rarely isolated from CF patients, *B. cepacia*, *B. contaminans* and *B. dolosa* were highly represented during this retrospective study. The importance of the surveillance of unexplained outbreaks involving poorly represented species [4] is highlighted. The collection of isolates gathered is essential to get clues on the adaptive strategies adopted by Bcc species during chronic infection and how these may affect clinical outcome and disease progression.

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**References:** [1]. Coutinho, *et al.* Front. Cell. Infect. Microbiol. 1(12), 2011. [2]. Coutinho, *et al.* J. Med. Microbiol. 64: 927-35, 2015. [3]. Moreira, *et al.* J. Med. Microbiol. 63: 594–601, 2014. [4]. Cunha, *et al.* J. Clin. Microbiol. 45: 1628-33, 2007. [5] Coutinho, *et al.* Infect. Immun. 79: 2950–60, 2011

### PO45 - Transforming Public Health Microbiology For *Campylobacter* With Whole Genome Sequencing: PulseNET And Beyond

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**Background:** Conventional phenotypic and genotypic methods employed for identification and subtyping of *Campylobacter* are labor intensive, expensive, and imprecise. We have begun development of Enteric Reference Identification and *Campylobacter* subtype characterization whole genome sequence (WGS) databases. The PulseNet infrastructure (BioNumerics) will be used in conjunction with the existing BIGSdb platform to build the databases, with the goal of characterizing *Campylobacter* in a single workflow using WGS.

**Methods:** Reference genomes (n=103) provided by FDA and USDA and strains (n=100) sequenced at CDC were used to develop the database. Assemblies and annotations were performed using the Computational Genomics Pipeline v0.4. These genomes cover the known members of the species and genera within *Campylobacteraceae* and the known genetic diversity of *C. jejuni*. The data will be used to set criteria for the current PubMLST.org/campylobacter locus definitions. Multiple subschema are being set up within the databases to perform identification and scalable, hierarchical subtyping that will include seven locus, ribosomal, core genome and whole genome (MLST, rMLST, cgMLST and wgMLST).

**Results:** To date 203 reference genomes have been sequenced, annotated and used for development of the BioNumerics databases. The *Campylobacter* allele database 2.0 contains approximately 25,000 loci. Overall the average number of bases covered was 87% and the average number of bases in coding sequences covered was 93%. An additional 600 isolates are in the process of being used to validate the prototype databases.

**Conclusions:** These WGS BioNumerics databases will provide a single, unified, cost-effective approach for accurate species identification and subtyping to aid the surveillance of sporadic and outbreak related *Campylobacter* infections. Through continued collaboration with domestic and international partners, we will test and refine the nomenclature databases and validate the reference identification subschema for diagnostic accreditation within the next year.

## PO46 - Carbapenemase Genes, Oxy Types And MLST Types Of Four Danish Meropenem Non-susceptible Clinical *Klebsiella oxytoca* Strains

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**Background:** *K. oxytoca* is amongst the top ten enterobacteria causing infections, particularly UTI, RTI and sepsis. Due to mutations in the regulator sequence of the class A broad spectrum chromosomal betalactamase (OXY) *K. oxytoca* frequently becomes cephalosporin resistant and does in some clinical settings appear quite common. In recent years a number of OXY-types have been described. Institut Pasteur hosts a webpage that keep track of the presently known 37 OXY alleles (<http://bigsdb.web.pasteur.fr/klebsiella/>). In 2014 a MLST typing scheme for *K. oxytoca* was suggested by Herzog et al, which already comprise 170 sequence types (ST) (<http://pubmlst.org/koxytoca/>). August 2015 a collection of 68 expanded-spectrum cephalosporin resistant *K. oxytoca* isolates were characterized with regard to betalactamase, OXY type and MLST type. A number of (also new) ST and OXY-types were described. Beside a number of ESBL and AmpC enzymes two isolates with the VIM-1 carbapenemase were found. Denmark have an active surveillance of clinical meropenem non-susceptible enterobacteria isolates; we here present data on four OXA-48 *K. oxytoca* strains characterized with regard to other acquired resistance genes, OXY types and MLST types.

**Material and Methods:** The four isolates were from four patients in four hospitals and with no known epidemiological link. Isolates were from urine (N=3) and wound (N=1). Primary identification was by MALDI-TOF and susceptibility testing by disc-diffusion (EUCAST). Due to non-susceptibility to meropenem isolates were submitted to Statens Serum Institut. Whole-genome sequencing (WGS) was performed by Illumina 251-bp paired-end sequencing. From WGS data, acquired antimicrobial resistance genes were retrieved using ResFinder 2.1 (<https://cge.cbs.dtu.dk/services/ResFinder/>) with a threshold of 100% identity for betalactamase genes and 98% identity for other genes. OXY-types and MLST types were found using the above web pages. Mutations and other events in the blaOXY gene and in the MLST alleles were checked using CLCbio's Genomics Workbench. Identification of single nucleotide polymorphisms (SNPs) was performed using CSI Phylogeny 1.1 (<https://cge.cbs.dtu.dk/services/CSIPhylogeny>) with *K. oxytoca* KCTC 1686 (CP003218.1) as the reference.

**Results:** The four strains had in addition to the OXA-48 carbapenemase also from 1-11 of the following acquired antimicrobial resistance genes: blaCTX-M-15, blaSHV-2, blaSHV-12, blaTEM-1B, aadA2, aadB, aph(3')-Ia, aac(6')-lb-cr, aacA4, strA, strB, QnrA1, QnrS1, mph(A), sul1, dfrA12, dfrA18. MLST showed that one isolate was ST-85, one ST-9-like (1-7-2-1-9mut-1-2, one mutation in phoE) and two strains each showed new allele combinations (3-5-21-19mut-24-6-20 and 1-7-2-15-16mut-1-14) and additional mutation in an allele. OXY-types were OXY-1-1 (N=2), OXY-2-4 and OXY-2-11. By SNP phylogeny isolate were grouped in two pairs, corresponding to OXY-1 or OXY-2, and with a pairwise distance of 15.414 and 11.941 SNPs, respectively.

**Conclusion:** The four OXA-48 *K. oxytoca* strains were quite diverse by all parameters looked at, as number of acquired resistance genes, OXY types, MLST types and SNP phylogeny. One isolate (ST-9) belonged to the dominating and international spread, clonal complex CC2. When it is possible in four isolates to find two new STs, a much larger diversity of STs (and OXY-types) can be expected.

## S8 - Poster session II

### PO47 - *Staphylococcus epidermidis* Nosocomial Strain In Breast Milk Of Mothers Of Preterm Neonates

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**Background:** *Staphylococcus epidermidis* is the commonest causative agent of late-onset sepsis (LOS) in preterm neonates. Mothers of preterm neonates visit neonatal intensive care unit (NICU) and often require hospitalization or antimicrobial therapy. Therefore, breast milk (BM) may become colonized with nosocomial strains rendering BM as a potential source of infecting strains. We aimed to describe the prevalence of nosocomial *S. epidermidis* strains in BM of mothers of preterm neonates.

**Material and Methods:** From January 2014 to March 2015 preterm neonates (gestational age (GA) <37 w, n=30) hospitalized to nicu of tartu university hospital within the first week of life and their mothers were studied healthy term neonates ga>37 w (n=20) and their mothers were included as controls. Stool samples and skin swabs from neonates and BM from mothers were collected weekly in the first month of life and cultured onto mannitol salt agar. Five colonies typical to staphylococci were identified to the species level by MALDI-TOF MS. All *S. epidermidis* isolates were typed by multilocus variable-number tandem-repeats analysis (MLVA), each distinct MLVA-profile was considered as a separate MLVA-type (MT) and was arbitrarily assigned an integer. The presence of the *mecA* gene, the *ica*-operon, IS256 was determined. A randomly chosen representative isolate of each MT was typed by multilocus sequence typing. Maternal and neonatal characteristics potentially influencing colonization of mother were assessed in logistic regression model.

**Results:** Of 30 preterm neonates (median GA 29 w (IQR 27-33 w), median birth weight 1306 g (IQR 906-1889 g) LOS caused by *S. epidermidis* (2 by MT1, 1 by MT2) developed in 3 neonates. All preterm neonates and their mothers were colonized with *S. epidermidis*, yielding a total of 1239 isolates representing 174 different MTs. MT1 was isolated from 22 neonates (skin of 21, gut of 15) and MT2 from 9 neonates (skin of 8, gut of 6). Median age at the first isolation of MT1 was 6 d (IQR 3-13 d) and MT2 10 d (IQR 8-19 d). MT1 was isolated from BM of 8 of 30 (26.7%) mothers of preterm neonates at median of 14.5 d (IQR 11-19.5 d) postpartum. Neonates of all 8 mothers were colonized with MT1 (7 in gut, 7 on skin) median of 7.5 d (IQR 6.5-14 d) prior to isolation from BM. Of neonatal and maternal characteristics potentially influencing colonization with bacteria, only neonatal gut colonization with MT1 was a significant factor ( $p=0.0375$ ) increasing the odds of colonization of BM with MT1 according to multivariate logistic regression model (Table). BM from mothers of the neonates with LOS caused by MT1 did not contain MT1. MT2 was not isolated from any BM sample. All term neonates and their mothers were colonized with *S. epidermidis*, yielding a total of 981 isolates representing 190 different MTs. None of the term neonates or their mothers were colonized with MT1 or MT2. Representative isolates of MT1 and MT2 were of multilocus sequence type 5, carried the *mecA* gene and IS256, but not *ica*-operon.

**Conclusion:** Our results indicate that BM of mothers of preterm neonates can become colonized with nosocomial *S. epidermidis* strains. Preceding neonatal gut colonization as the only factor associated with the occurrence of the nosocomial strain in BM suggests that neonate could be a source of maternal colonization. Therefore, reducing transmission of nosocomial strains to microbiota of preterm neonates could prevent transfer of the strains to mother's BM.

**Table.** Neonatal and maternal characteristics associated with the presence of MT1 in breast milk according to logistic regression model.

	MT1 in mother's BM		Univariate model		Multivariate model <sup>1</sup>
	Yes (n=8)	No (n=22)	OR (95% CI)	OR (95% CI)	
GA – w; median (IQR)	31.5 (29-34.5)	28 (26-32)	1.34 (1.01-1.77) <sup>2</sup> #	1.25 (0.82-1.88)	
Length of NICU stay (d); median (IQR)	7.5 (3.5-13.5)	15.5 (9-24)	0.88 (0.77-1) *	0.89 (0.76-1.03)	
Neonatal colonization with MT1 of gut – n (%)	7 (87.5%)	8 (36.4%)	12.3 (1.27-118.4) <sup>2</sup> #	1.92 (1.19-311.5) <sup>2</sup> #	
Neonatal colonization with MT1 of skin – n (%)	7 (87.5%)	14 (63.6%)	4 (0.41-38.7)		
Maternal					
antibiotic therapy in the first week postpartum – n (%)	6 (75%)	16 (72.7%)	1.13 (0.18-7.19)		
length of postpartum hospitalization (d); median (IQR)	3 (2.5-4)	3 (1-4)	0.97 (0.66-1.43)		

GA – gestational age; NICU – neonatal intensive care unit.

<sup>1</sup>Characteristics associated with colonization in univariate model at a p-value of <0.1 were entered into multivariate logistic regression model.

<sup>2</sup>To avoid confounding by highly correlated variables, GA rather than birth weight was used ( $r=0.900$ ) in the analyses.

\* p-value <0.1; # p-value <0.05

## S8 - Poster session II

### PO48 - Implementation Of Whole Genome Sequencing (WGS) For Surveillance And Outbreak Detection Of Shiga Toxin-producing *Escherichia coli* (STEC) In The United States

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**Background:** Shiga toxin-producing *Escherichia coli* (STEC) is an important foodborne pathogen capable of causing mild to severe disease in humans. Current methods for characterization of STEC are expensive and time-consuming. Work has begun to replace traditional methods with those using whole genome sequence (WGS) data by developing an allele database of individual *Escherichia* genes in BioNumerics 7.5 (Applied Maths, Austin, TX). This will allow characterization of *Escherichia* in a single workflow using a multi-locus sequence typing (MLST) approach.

**Materials and Methods:** The *Escherichia* allele database version 2.0 was built with 20 annotated reference genomes from a geographically diverse collection of human, animal and environmental strains. Included in BN 7.5 is a genotyper tool that uses genes encoding virulence factors, antimicrobial resistance and O and H antigens from databases at the Center for Genomic Epidemiology (<http://genomicepidemiology.org>, DTU, Lyngby, Denmark). The reference genomes represent eleven *Escherichia coli* (*E. coli*) serogroups and two *Shigella* species. Multiple subschema will be built within the database to perform identification, characterization and subtyping, including MLST capabilities. To test the ability of the BioNumerics-based whole genome MLST approach to correctly identify, characterize and cluster strains, we analyzed 50 *Escherichia* isolates from sporadic and outbreak-related infections and compared the findings to those obtained previously with phenotypic and molecular subtyping methods.

**Results:** The *Escherichia* allele database 2.0 contains 9,494 loci. Overall the average number of bases covered was 77% and the average number of bases in coding sequences covered was 90.8%. Identification by serotype, virulence profile characterization and wgMLST analysis was completed for 50 *E coli* isolates.

**Conclusion:** The BioNumerics-based wgMLST approach provides a single, cost effective strategy to identify and characterize isolates for surveillance and outbreak investigations. The analysis tools in BioNumerics will enable end-users in public health laboratories to analyze WGS data they generate with little bioinformatics expertise, making the system equally efficient for local and central investigations. The system will be refined through continued collaboration with domestic and international partners.

**PO49 - Molecular Characterization Of Vancomycin-resistant Enterococci In Hong Kong.**

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Vancomycin-resistant enterococcus (VRE), particularly *Enterococcus faecalis* and *E. faecium*, causes nosocomial infections and raises infection control concerns because of their prolonged survival in the environment and from asymptomatic carriers. In Hong Kong, VRE was first detected in 1997 and sporadically reported and small clusters of cases have been noted at various acute care hospitals between 2009 to 2014. A VRE screening program was thus commenced in all public hospitals and some private hospitals.

**Methods:** 240 strains of VRE collected from six hospitals across three of 7 clusters of public hospitals in Hong Kong were examined. They were isolated from clinical specimens of infected patients or in the surveillance screening program during the periods from 2013 - 2014. Colonization screen were performed on inpatients who had a history of hospital admission in the previous 3 months and from renal patients on hemodialysis in 2013 to early 2014. All VRE were identified as *E. faecium*, either by biochemical methods or by MALDI-TOF MS. All strains were analyzed by pulse field gel electrophoresis (PFGE) of *Sma*I-digested DNA. The similarity of the PFGE fingerprints were analysed using the BioNumerics software (Applied Maths, Belgium). A PFGE dendrogram was drawn by the Dice method and by clustering by UPGMA with 1% position and 2% band tolerance. MLST was performed on representative strains by obtaining the allelic profiles through sequences of internal fragment of seven housekeeping genes – *atpA*, *ddl*, *gdh*, *purK*, *gyd*, *pstS* and *adk*. Van genes were performed by PCRs.

**Results:** A dendrogram was drawn and two main clusters were obtained using a cut-off at 90% similarity. The major Cluster A consists of 177 strains and another 21 strains belong to cluster B. Each cluster of isolates consists of strains from various hospitals, and the predominant cluster A were identified ST761 and that of cluster B was ST555. Both were members of clonal complex-17 of *E. faecium*. An additional 42 strains did not belong to these two clusters but were not further typed. All strains carried Van A genes.

**Conclusion:** Two predominant clones of VREs were present in these hospitals though a number of various heterogenous strain types were also identified among asymptomatic carriers. An active surveillance program effectively identified these carriers and with extensive infection control practice, prevented nosocomial transmission of VRE.

## S8 - Poster session II

### PO50 - Biocide Resistance Genes In *Staphylococcus aureus* From Portuguese Ex-colonies In Africa: Very High Prevalence And Antimicrobial Cross-resistance

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**Background:** Biocide resistance in *Staphylococcus aureus* is mediated by multidrug efflux pumps, which frequently promote cross-resistance to antibiotics. The overuse of biocides in infection control in hospitals led to the emergence of *S. aureus* strains with decreased antiseptic susceptibility, which became a problem in different regions of the world. Geographic distribution of biocide resistance genes has been poorly studied and, to date, there are no data from the African continent where MRSA prevalence is not negligible. In the present work, we assessed for the first time the prevalence of six biocide resistance genes and its association with antimicrobial resistance, among a collection of *S. aureus* isolates from three former Portuguese African colonies.

**Materials and Methods:** A total of 301 *S. aureus* isolates [including 82 methicillin resistant *S. aureus* (MRSA) and 219 methicillin susceptible *S. aureus* (MSSA)] were selected from a collection of 519 *S. aureus* nasal carriage isolates recovered from patients and healthcare workers in Angola, São Tomé and Príncipe and Cape Verde. Isolates were previously characterized by different molecular typing techniques, including PFGE, spa typing, MLST, and SCCmec typing. Antimicrobial susceptibility was determined by disk diffusion and the presence of biocide resistance genes was detected by PCR amplification of internal fragments of six efflux pumps genes (*qacAB*, *smr*, *norA*, *ImrS*, *mepA* and *sepA*) in all isolates.

**Results:** The global prevalence of antiseptic resistance genes observed in the three African countries was very high for *sepA* (95.3%), *mepA* (89.4%) and *norA* (86.4%), intermediate for *ImrS* (60.8%) and *qacAB* (40.5%) and low for *smr* (3.7%). A similar distribution was observed when comparing MRSA and MSSA except for *qacAB* and *sepA* genes that were more prevalent among MRSA ( $p=0.0137$  and  $p<0.0001$ , respectively). The genes *norA*, *ImrS* and *mepA* were widespread in São Tomé and Príncipe (97.4%, 87% and 98.7%, respectively) and Cape Verde (83.8%, 54.3% and 96.2%, respectively), while *qacAB* was mainly detected in Angola (56.3%,  $p<0.0001$ ). Except for *smr*, the remaining other five biocide resistance genes were very common (range: 41-100%) among the major MRSA clonal lineages circulating in these countries: A-ST5-IVa, B-ST88-IVa and C-ST8-IV/V. Moreover, the presence of biocide resistance genes was significantly associated to antibiotic resistance: (i) *qacAB* was associated to cefoxitin ( $p<0.0001$ ), gentamicin ( $p=0.0013$ ), rifampicin ( $p<0.0001$ ), trimethoprim-sulfamethoxazole (SXT) ( $p<0.0001$ ) and cloramphenicol ( $p=0.0244$ ); (ii) *mepA* was highly associated to tetracycline ( $p=0.0024$ ) and rifampicin ( $p=0.0007$ ); (iii) and *smr* and *sepA* were associated to gentamicin ( $p=0.0236$ ) and SXT ( $p=0.0021$ ), respectively.

**Conclusion:** Biocide resistance genes are highly prevalent in Angola, São Tomé and Príncipe and Cape Verde. The use of antiseptics might be selecting for antibiotic-resistant strains in Africa and assisting their survival in the healthcare environment, which is of major concern for future infection control programs.

**PO51 - Emergence Of Pan-resistance In Kpc-2 Carbapenemase-producing Klebsiella pneumoniae In Crete, Greece**

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**Background:** KPC-2-producing *Klebsiella pneumoniae* (KPC-KP) sequence type (ST) 258 has been rapidly expanding and is often associated with serious nosocomial infections. Last-line antibiotics such as colistin and tigecycline often remain the only treatment option. We here describe the characteristics of KPC-KP isolates from Crete and present the results of molecular fingerprinting by core genome multilocus sequence typing (cgMLST).

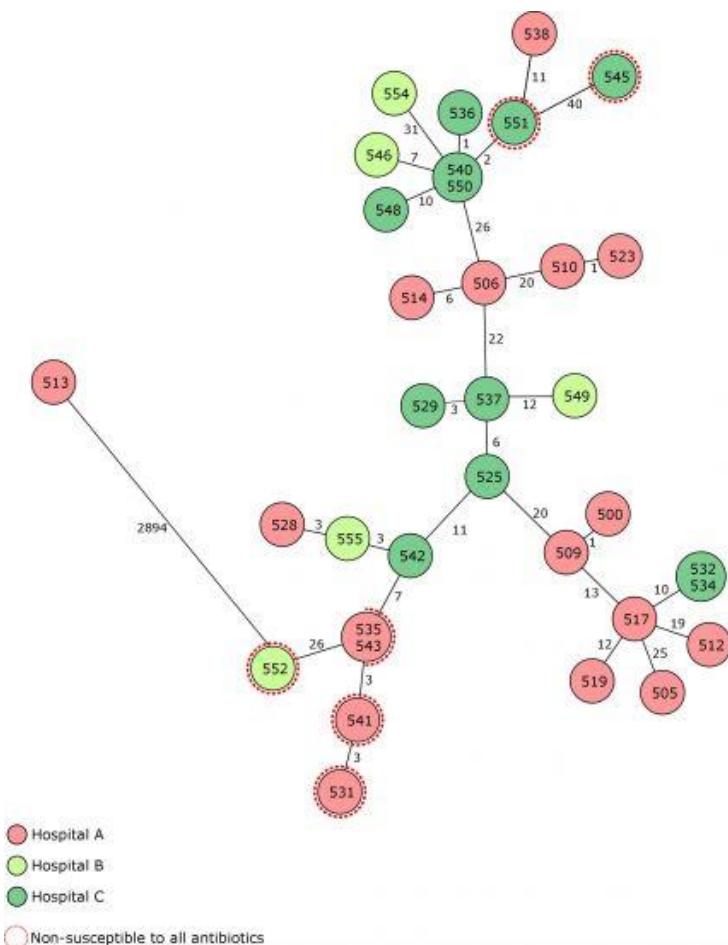
**Materials/Methods:** Clinical isolates from sputum, urine, blood, ascetic fluid or pus samples had randomly been collected from 34 unique patients hospitalised in three hospitals on Crete in 2010 and 2013/2014. The antibiotic susceptibility of these isolates was tested and their whole genome sequences were analysed for acquired resistance genes and gene mutations. CgMLST analysis was performed using an *ad hoc* scheme including 3664 genes. The DNA sequence of *mrbB* (GI:695277517), and *phoQ* (GI:6938724) were used as reference for detecting gene mutations associated with colistin resistance. Insertion sequences were identified using *Isfinder*.

**Results:** All KPC-KP isolates belonged to ST258 with exception of one ST147 isolate. From 2014, 6 out of 23 isolates were pan-resistant to all antibiotics, compared to none out of 11 isolates from 2010. The characteristics and antibiotic susceptibility of pan-resistant isolates are presented in Table 1. Colistin-resistance was associated with mutations in *mrbB*, which were present in all pan-resistant isolates. CgMLST analysis showed that isolates in general clustered by the hospital in to which patients were admitted (see Figure 1). Pan-resistant isolates were closely related and appeared in a cluster of four isolates, and a separate set of 2. Identical insertion sequences ISL3 inserted in nucleotide position 12 in *mrbB* were detected in the isolates of the cluster of four.

**Conclusion:** KPC-KP is rapidly evolving to pan-resistance in Crete. We identified molecular resistance markers for pan-resistant isolates, and showed that cgMLST is a promising tool for molecular fingerprinting of KPC-KP ST258.

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Isolate	Hospital	Isolation date	MIC MER(mg/L)	MIC IMP(mg/L)	Susceptibility GN/SXT/CPX/FOS	MIC COL(mg/L)	MIC TIG(mg/L)	Bla-genes	Mutations in mgrB or phoQ
545	C	31-08-13	>16 R	>16 R	I/R/R/R	16 R	1.5 I	KPC-2 OXA-9 SHV-12	IS5 91
551	C	17-03-14	>16 R	>16 R	I/R/R/R	12 R	1.5 I	KPC-2 OXA-9 SHV-11	ISL3 11
541	A	14-04-14	>16 R	4 I	I/R/R/R	6 R	2 I	KPC-2 OXA-9 SHV-12	ISL3 12
543	A	14-04-14	>16 R	8 I	I/R/R/R	4 R	2 I	KPC-2 OXA-9 SHV-12	ISL3 12
531	A	16-04-14	>16 R	8 I	I/R/R/R	32 R	2 I	KPC-2 OXA-9 SHV-12	ISL3 12
552	B	03-07-14	>16 R	>16 R	I/R/R/R	12 R	2 I	KPC-2 OXA-9 SHV-12	ISL3 12



**PO52 - Characterization Of Uncommon Clones Of Carbapenemase-producing *Acinetobacter baumannii* From Nosocomial Settings In Tunis (Tunisia)**

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Keywords: CHDL, FTIR-ATR, MLST

**Background:** *Acinetobacter baumannii* is commonly associated with nosocomial infections and severe outbreaks. Worldwide dissemination of carbapenem resistant *A. baumannii*, has been mainly associated with carbapenem-hydrolyzing class D-beta lactamases (CHDL) limiting the therapeutic options. In Northern Africa, and in particular in Tunisia, the occurrence of carbapenem-resistant *A. baumannii* (CRAB) has also been associated with CHDLs, mainly OXA-23 and OXA-58-like enzymes. In addition, GES-11 and GES-14 have also been reported. However, little is known about the clones driving the carbapenemases dissemination, with the exception of GES-14/ST499 (personal communication, Abstr. 25<sup>th</sup> ECCMID, 2015).

In this study we assessed the occurrence of CHDL and associated clones on a CRAB collection from nosocomial settings in Tunis, Tunisia. The suitability of FTIR and chemometric analysis for *A. baumannii* clonal characterization in a barely studied region was also evaluated.

**Methods:** This study included 192 CRAB clinical isolates obtained from different biological samples from inpatients attending the Neonatal Intensive Care Unit of the Rabta's Hospital or The National Bone Marrow Transplant Centre in Tunis, Tunisia (2004-2013). Antimicrobial susceptibility was determined by disc diffusion method. CHDLs search was performed by PCR and confirmed by sequencing. Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR) spectra were acquired and modeled by partial least squares discriminant analysis (PLSDA) for all isolates, a methodology previously validated for depicting *A. baumannii* clones common in our region. Representative isolates from the resulted PLSDA clusters were selected for MLST analysis, according with the Oxford MLST database.

**Results:** Isolates were resistant to all b-lactams, presenting variable susceptibility to quinolones and aminoglycosides. OXA-23 was detected in 88 isolates (45,8%, 2007-2013) and OXA-58 was detected in 104 isolates (54,2%, 2004-2010). PLSDA regression model using the 1500–900 cm<sup>-1</sup> spectral region revealed two main clusters, explained by the first latent variable (LV), which represents the higher spectral diversity. MLST analysis of representative isolates from each cluster revealed the association of OXA-23 producing isolates with ST945 while OXA-58 were associated with ST441. These are unusual STs that have been described in Saudi Arabia (ST441) and Iraq (ST945), according with MLST database. They are single locus variant (SLV), differing only on *gpi* allele. The remaining LVs allowed the description of small sub-cluster that included both OXA-23 and OXA-58 producing isolates and associated also with an infrequent clone, ST282.

**Conclusion:** This is the first study characterizing CHDL *A. baumannii* clones in Tunisia. FTIR-ATR spectroscopy coupled with chemometric tools allowed a preliminary, quick, and low cost, discrimination of the different clones. These results which were further supported by MLST confirm this methodology as an important tool for epidemiology and outbreak monitoring. Despite the long period of time analyzed, it was observed the persistence of very few clones. Interestingly, they are not associated with the most disseminated STs worldwide, being previously identified only in the Middle East, which unveils a regional ecology that worth to be explored.

## S8 - Poster session II

### PO53 - Phylogenetic Analysis Of Severe Fever With Thrombocytopenia Syndrome Virus And Migratory Bird Routes Between China, South Korea, And Japan

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Severe fever with thrombocytopenia syndrome (SFTS) is a tick-borne viral disease. The SFTS virus (SFTSV) has been detected in the *Haemaphysalis longicornis*, which acts as a transmission host between animals and humans. SFTSV was first confirmed in China in 2009 and has also been circulating in Japan and South Korea. However, it is not known if a genetic connection exists between the viruses in these regions and, if so, how SFTSV is transmitted across China, South Korea, and Japan. We therefore hypothesize that the SFTSV in South Korea share common phylogenetic origins with samples from China and Japan. Further, we postulate that migratory birds, well-known carriers of the tick *H. longicornis*, are a potential source of SFTSV transmission across countries. Our phylogenetic analysis results show that the SFTSV isolates in South Korea were similar to isolates from Japan and China. We connect this with previous work showing that SFTSV-infected *H. longicornis* were found in China, South Korea, and Japan. In addition, *H. longicornis* were found on migratory birds. The migratory bird routes and the distribution of *H. longicornis* are concurrent with the occurrence of SFTSV. Therefore, we suggest that migratory birds play an important role in dispersing *H. longicornis*-borne SFTSV.

**PO54 - Burden Evaluation Of Hand Soap's Contamination By *Pseudomonas aeruginosa* In A Tertiary Care Hospital Using Whole Genome Sequencing**

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**Background:** An investigation of potential sources of *Pseudomonas aeruginosa* in the intensive-care unit (ICU) showed that the liquid hand soap was highly contaminated with this bacterium. From August 2012 to January 2013, all isolates retrieved from clinical specimens and from contaminated soap containers were analysed by Double Locus Sequence Typing (DLST). This molecular typing method showed that three patients harboured the same DLST type, 13-31, as the one observed in the majority of the tested contaminated soap containers (17/20). Whole Genome Sequencing (WGS) was used to confirm or rule out the burden of hand soap's contamination on patients' infection.

**Material and Methods:** A set of ten *P. aeruginosa* isolates belonging to DLST type 13-31 was analyzed with WGS. Among these, two were retrieved from two different soap batches, three from the patients suspected to be contaminated by the soap, and 5 from unrelated strains retrieved by routine surveillance of *P. aeruginosa* in the hospital. The isolates' sequence type (ST) was assigned from the short reads data. With Snippy software, the core genome alignment of all ten isolates was acquired, which in turn was used to construct the maximum likelihood tree. Subsequently, regions of high SNPs density (suggestive of recombination) were excluded using the Gubbins software.

**Results:** All ten sequenced isolates belonged to ST 155, which was fully concordant with DLST results. The phylogenetic tree showed the occurrence of three major clades (A, B, and C). Clade A comprised half of the analysed isolates, which were retrieved from 2003 to 2014. Two isolates that were retrieved from infected patients clustered in clade A, while the third patient's isolate was part of clade B. In contrast, both soap isolates were clustered in clade C. A high number of core SNPs differences was detected between the three patients of the study, and the two soap isolates. No close genetic relation was observed between contaminated soap isolates and the patients included in the investigation.

**Conclusion:** The three patients suspected to be contaminated by the soap, clustered in different clades from the one harbouring the soap isolates. Additionally, a high number of SNPs differences was observed between patient and soap isolates, hence, it was possible to exclude patients' infection due to contamination of the soap. WGS enabled the exclusion of a possible burden on our hospital due to the soap's contamination, which would not be achieved using other classical molecular typing methods.

## S8 - Poster session II

### PO55 - Molecular Surveillance And Emergence Of Carbapenemases At A University Hospital In Germany Over A Five Year-period

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**Introduction:** Resistance to the clinically relevant broad-spectrum antibiotic group of carbapenems driven by carbapenemase enzymes is emerging in Gram-negative bacteria. Germany is a low-prevalence country, though there is some regional spread (e.g. of KPC enzymes) and numbers and diversity of carbapenemase genes detected are increasing. Little surveillance data is available at the local level. A molecular surveillance of Gram-negative multidrug-resistant carbapenem-non-susceptible organisms was established at the Institute of Medical Microbiology and Hospital Hygiene in Düsseldorf (Germany) in 2010.

**Materials | Methods:** We analyzed prospectively all Gram-negative isolates (*Enterobacteriaceae*, *Acinetobacter* spp. and *Pseudomonas* spp.) non-susceptible to imipenem and/or meropenem (*Pseudomonas* spp., only if additionally non-susceptible to ceftazidime) from clinical and screening specimens. Real-time and conventional PCRs targeting relevant carbapenemase genes (*bla*<sub>IMP-1</sub>, *bla*<sub>VIM-1-like</sub>, *bla*<sub>VIM-2-like</sub>, *bla*<sub>GIM-1</sub>, *bla*<sub>NDM</sub>, *bla*<sub>KPC</sub>, *bla*<sub>OXA-48</sub> and *bla*<sub>GES</sub>; for *Acinetobacter* spp. (since 2012) additionally: *bla*<sub>OXA-23</sub>, *bla*<sub>OXA-24</sub>, *bla*<sub>OXA-51</sub>, *bla*<sub>OXA-58</sub>, *bla*<sub>OXA-143</sub> and *bla*<sub>OXA-235</sub>) were used. Epidemiologically related isolates were further genotyped by several methods (RAPD, DiversiLab, PFGE and MLST) and subjected to S1-nuclease for plasmid analysis.

**Results:** Approximately 500 isolates were analyzed, of which 112 were positive as follows: *Pseudomonas* spp., *bla*<sub>GIM-1</sub> (n=32), *bla*<sub>VIM-2</sub> (n=14), *bla*<sub>VIM-1</sub> (n=1); *Enterobacteriaceae*, *bla*<sub>GIM-1</sub> (n=23), *bla*<sub>OXA-48</sub> (n=9), *bla*<sub>VIM</sub> (n=4), *bla*<sub>NDM-1</sub> (n=3), *bla*<sub>KPC</sub> (n=3), *bla*<sub>KPC-1</sub>/*bla*<sub>VIM</sub> (n=1); *Acinetobacter* spp., *bla*<sub>OXA-23</sub>/*bla*<sub>OXA-51</sub> (n=17), *bla*<sub>OXA-24</sub>/*bla*<sub>OXA-51</sub> (n=2), *bla*<sub>NDM-1</sub>/*bla*<sub>OXA-51</sub> (n=3). This data reveals an endemic situation involving GIM-1-producing organisms and local acquisition of at least three different clusters of VIM-2-producing *P. aeruginosa*. Detection of OXA-23, OXA-24, OXA-48, KPC or NDM-1 was clearly associated with a previous hospital stay abroad. Two patients were colonized/infected with several species carrying the same carbapenemase gene (*bla*<sub>VIM-1</sub>-resp. *bla*<sub>NDM-1</sub>) proven to be plasmid mediated. Two patients with a hospital stay abroad (Morocco and Greece) carried three different carbapenemase-producing organisms. The first emergence of NDM-1 was in 2012 (interestingly community-acquired) and of KPC in 2013 (patient transferred from Greece). We observed a shift in multi-drug resistant *A. baumanii* from *bla*<sub>OXA-58</sub> to *bla*<sub>OXA-23</sub> compared to strains collected before 2010 and analysed retrospectively..

**Conclusion:** This data gives insight to the changing epidemiology of carbapenemases in a tertiary care center in Germany over the last 5 years. Carbapenemase-mediated carbapenem resistance on the whole is still uncommon, but is increasing and becoming more diverse. We were able to demonstrate a regional spread of *bla*<sub>GIM-1</sub> and *bla*<sub>VIM-2</sub> and the "genetic import" of several other carbapenemase genes (*bla*<sub>VIM</sub>, *bla*<sub>OXA-48</sub>, *bla*<sub>NDM-1</sub> and *bla*<sub>KPC</sub>) from abroad. The results support the need for a local molecular surveillance system.

**PO57 - A Molecular Survey Of *Campylobacter jejuni* And *Campylobacter coli* Virulence And Diversity**

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Keywords: *C. jejuni*,*C.coli*, genotyping, virulence genes

**Background:** The aim of this study was to determinethe prevalence of virulence-associated genesandenterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) analysis of *Campylobacter* spp. isolated from children with diarrhea in Iran.

**Methods:** A total of 200 stool specimens were obtained from children under 5 years duringJuly 2012 to July 2013. Detection of *C. jejuni* and *C.coli* wasperformed by standard biochemical and molecular methods. The presence of virulence-associatedgenes and genetic diversity of isolateswas examined with PCR andERIC-PCR analysis.

**Results:** A total of 12 (6%) *Campylobacter* spp. were isolated frompatients including10 (4.5%) *C. jejuni*and 2 (1.5%)*C.coli*.The *flaA*, *cadF* and *ciaB*genes were present in 100% of isolates while no Plasmid *virB11* gene was present their genome. The prevalence of *iam* was 100% among*C.coli* and was not detected in*C.jejuni*isolates. The distribution of both*pldA*and the genes associated with CDTwas 58.3% in*C. jejuni* isolates. Seven distinct ERIC-PCR profiles in three clusters were distinguished by ERIC-PCR analysis. Genotyping analysis showed a relative correlation with geographic location of patients and virulence gene content of isolates.

**Conclusion:** To our knowledge, this is the first molecular survey of *Campylobacter* spp. in Iran which concerns genotyping and virulence gene content of both *C. jejuni* and *C. coli*. ERIC-PCR revealed appropriate discriminatory power for clustering of *C. jejuni* isolates with identical virulence gene content. However, more studies are needed to clearlyunderstand thepathogenesis properties of specific genotypes.

## S8 - Poster session II

### PO58 - Emergence And Dissemination Of A Linezolid-resistant *Staphylococcus capitis* Clone In Europe

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**Background:** *Staphylococcus capitis* is a low virulence staphylococcus coagulase negative (SCoN) seldom involved in human diseases and classically considered as a contaminant when isolated from clinical samples. We investigated clinical, microbiological and genetic characteristics of linezolid-resistant (LNZ-R) *S. capitis* isolates from France, Greece and Finland.

**Material/methods:** All LZD-R resistant *S. capitis* isolates available in the collection of the French National Reference Center for Staphylococci collection were included in this study (n=9, Bayonne, Strasbourg, Lille). These isolates were compared with international LZD-R *S. capitis* strains collected from the Staphylococcal Research Laboratories of Greece (n=10, Patras) and Finland (n=1, Turku). Antimicrobial susceptibility tests were performed using the standard agar diffusion method and Etest (for daptomycin, LZD, teicoplanin, vancomycin) for all isolates. Presence of the *cfr* gene and mutations in the 23S rRNA loci were investigated by PCR and sequencing. Genetic relationships of isolates were determined by PFGE using *Sma*I restriction enzyme. Finally, for the French (n=9) and Greek isolates (n=1), whole genome sequencing (Illumina HiSeq) and genomic comparison were performed.

**Results:** The 9 french isolates were isolated between June 2013 and December 2014 from 9 adult patients hospitalized in 3 distant ICUs (2 to 5 strains per center). Only 5 of the 9 patients had previously received LZD therapy. These 9 French isolates as well as the 10 Greek ones and the isolate from Finland were all resistant to methicillin, aminoglycosides and linezolid (MIC >24mg/L). Additional resistances to vancomycin, teicoplanin and daptomycin were observed in 1, 3 and 4 isolates, respectively. The *cfr* gene was absent in all isolates while two mutations (T2319C and G2576T) in the 23S rRNA were found in all isolates. Moreover, they belong to a same clone as defined by their shared PFGE profile. Additionally, for the 9 French and 1 Greek isolates, their high genetic relationship was further confirmed by WGS: these isolates show at most 216 SNPs (over 1,484,801 shared positions) between the 10 isolates, versus a mean of 17,851 SNPs when compared with 7 publicly available, unrelated *S. capitis* genomes.

**Conclusions:** In this study we highlight for the first time the European dissemination of a LZD-R *S. capitis* clone, harboring 2 mutations in the 23s rRNA, and present in at least 3 French, 1 Greek and 1 Finnish hospitals. The G2576T mutation, observed in all isolates, is classically found in coagulase negative staphylococci. Although the role of mutation T2319C (not previously described in the literature) in the LZD-R phenotype remains to be definitely established. The emergence and inter-hospital diffusion of this LZD-R clone involved in infections in several European hospitals is both worrisome and unexpected as *S. capitis* is usually known as a very low-virulent species. The absence of linezolid prior therapy in 4 of the French patients suggests a nosocomial transmission within hospital. However, the inter-hospital dissemination pathways of this clone at such a large geographical scale is still unexplained and requires further investigations. Finally, we actively continue to collect LZD-R *S. capitis* isolates from other European countries, to better determine the european distribution of this clone.

**PO59 - Whole Genome Sequencing Data Confirm A Transmission Of *Salmonella enterica* Serovar Typhimurium Phage Type Dt41 In Danish Poultry Production**Ann-Sofie Hintzmann<sup>1</sup>; Gitte Sørensen<sup>1</sup>; Dorte Lau Baggesen<sup>1</sup><sup>1</sup>National Food Institute

**Introduction:** *Salmonella* is the cause of numerous foodborne outbreaks every year in both developing and developed countries. It spreads through the entire food production chain and thus can be difficult to manage. One specific serotype *Salmonella enterica* serovar Typhimurium phage type DT41 (S. Typhimurium DT41) has previously been isolated from broiler breeder flocks in Denmark, particularly in older poultry.

**Materials | Methods:** Danish S. Typhimurium DT41 isolates from 1999 to 2014 covering poultry (N=50), other birds (N=17), other animals (N=6) and other sources (N=3) were whole genome sequenced on an Illumina MiSeq platform (Illumina) and compared to Multi Locus Variable number tandem repeats Analysis (MLVA) results. Isolates were either epidemiologically linked or considered as "background" to increase the resolution of the analysis. The Multi Locus Sequence Type (MLST) and Single Nucleotide Polymorphism (SNP) phylogeny was constructed using tools available at Center for Genomic Epidemiology ([www.genomicepidemiology.org](http://www.genomicepidemiology.org)) and BioNumerics 7.1 (Applied Maths).

**Results:** Based on epidemiological data it is possible that a transmission had occurred in 2001 from layers to hatchery and broiler breeding farm. In the SNP tree a cluster of 9 poultry isolates were identified, originating from 2001. All isolated were MLST ST19 and had a minimum and maximum distance of 0 and 6 SNP, respectively, and a maximum distance of 123 SNPs over the entire tree. The isolates shared a MLVA profile that was otherwise not seen in the entire dataset, confirming a link between the isolates. Within the cluster there are two identical isolates, where one is from a hatchery and the other is from a broiler breeding flock, suggesting that a direct transmission event has occurred.

**Conclusion:** A natural source to S. Typhimurium DT41 has still not been found; however, just outside the 2001 cluster is an isolate from a Swedish herring gull. It would therefore be interesting to study this link further to see if there is a link to reservoir of wild birds. More research is planned in an attempt to identify a source to this specific *Salmonella* phage type.

## S8 - Poster session II

### P060 - Dominance Of *Escherichia coli* ST131 H30 Subclone In A Portuguese Long Term Care Facility

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**Background:** The *Escherichia coli* B2-ST131 clone is nowadays one of the predominant lineages causing extraintestinal infections in healthcare associated settings, and long-term care facilities (LTCF) have been established as important reservoirs of ST131 in some countries. Genome wide studies revealed a subclonal structure within *E. coli* ST131, but the prevalence and distribution of the different subclones (being the *fimH30* or the more extensively resistant *fimH30-Rx* the predominant ones) in different settings and hosts is still poorly understood. We aim to assess the faecal carriage rate of ESBL- and/or carbapenemase-producing *E. coli* clones and subclones among LTCF residents in Portugal, where different ST131 *E. coli* subclones have been associated with hospital-acquired infections but situation in LTCF is still underexplored.

**Materials | Methods:** A pilot study was designed in which faecal samples from all residents (n=24, 17 females and 7 males) of a Portuguese LTCF were collected in July 2015. Eighty-three percent of the residents were >70 years old, 46% were previously hospitalized and 42% received recent (<3 months="" antibiotic="" treatment="" samples="" were="" suspended="" in="" 2ml="" of="" saline="" solution="" and="" 200="" l="" seeded="" chromid="" sup="">>® ESBL directly or ChromID® Carba SMART plates after enrichment of 100µl of faecal suspension in APP supplemented with a meropenem disk (37°C/18h) and further incubated (37°C/24-48h). Thirty presumptive ESBL-producing *E. coli* isolates presenting different morphotypes/ESBL chromID plate (n=1-6) were selected, and its identification assessed by MALDI-TOF MS or PCR. ESBL production was confirmed in all isolates by PCR (*bla<sub>CTX-M</sub>*) and sequencing, and *E. coli* ST131 clone and subclones (H30, H30 Rx) were identified by PCR.

**Results:** ESBL-producing *E. coli* were detected in 63% (n=15/24) of the samples, whereas carbapenemase producers were not detected. Isolates produced almost exclusively CTX-M-15 (n=29/30; 97%) and rarely CTX-M-1 (n=1/30; 3%). All CTX-M-producing *E. coli* belonged to the H30 lineage of the pandemic B2-ST131 O25b:H4. The *E. coli* ST131 clade H30-Rx represented 87% (n=26/30) of the ST131 population, all of them being CTX-M-15 producers, while variability (H30 Rx and H30 non-Rx) was observed in two samples. ESBL carriers were previously hospitalized in different hospitals or had previously been admitted to different long-term care facilities, whereas only 22% (n=2/9) of non-carriers had been previously hospitalized. Recent antibiotic treatment existed in 33% (n=5/15) and 56% (n=5/9) of carriers and non-carriers, respectively.

**Conclusion:** Despite the reduced sample size, this pilot study suggests a high colonization rate by ESBL-producing *E. coli* among Portuguese LTCF residents, dominated by the CTX-M-15-producing B2-ST131 H30 lineage. Our data further support the role of LTCFs as reservoirs of the ST131 *E. coli* in Portugal and suggest a high potential for transmissibility and persistence of the H30 lineage in this population, anticipating the need for further studies.

## PO61 - **Salmonella Surveillance By Whole Genome Sequencing: WG-MLST And WG-SNP Approaches Using The BioNumerics Platform.**

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<sup>1</sup>Applied Maths NV

**Introduction:** The standard for molecular surveillance of bacterial foodborne diseases has been PFGE for many years. With the advent of high-throughput Whole Genome Sequencing (WGS), the key challenge remains the rapid and automated processing of WGS data, obtaining higher resolution compared to traditional methods. Whole genome Multi Locus Sequence Typing (wgMLST) and the availability of reliable and easy to use workflows allow for outbreak cluster detection in WGS-based surveillance. Similar to MLST, the wgMLST method requires a schema describing the loci and alleles. In this study, we report on the development and validation of a species-wide wgMLST schema for *Salmonella enterica*.

**Material and methods:** The *Salmonella enterica* wgMLST schema was created based on 163 genome and plasmid sequences, representing the major serotypes. An in-house developed schema creation procedure used a sampling-based multi-reciprocal BLAST search to determine those sets of alleles that make up the stable loci in the accessory, i.e. pan genome. Next, a per-locus allele assessment procedure determined the central prototype allele and thus the locus definition. The final schema contained 11636 loci, covering 94% on average of the CDS defined on the reference genomes.

Allele calls for each of the loci in the schema were a consensus of two approaches: (1) an assembly-based allele calling, done through a BLAST based procedure on de novo assembled genomes and (2) an assembly-free allele calling, using a k-mer approach directly on the reads.

Validation of the wgMLST schema was performed on publicly available whole genome sequences, described in outbreak reports or WGS studies. Since in the latter studies mainly whole genome single nucleotide polymorphism (wgSNP) based approaches were used, we re-analyzed the data with an in-house developed wgSNP tool as additional reference.

**Results:** The *Salmonella enterica* schema validation includes analysis of CDS and base recovery rates, and within and between locus diversity statistics. After validation, wgMLST analysis is performed on data from public studies and outbreak reports. Without exception, this analysis reliably identified isolates belonging to documented *Salmonella* outbreaks. wgMLST overall exhibited a very high resolution, obviously much higher than PFGE but also surpassing core SNP approaches. Moreover, the resolution of wgMLST can be tuned by switching to (local) typing schemes e.g. a core MLST schema or even the traditional 7-gene MLST schema. Using a set of quality criteria, problematic WGS runs could be clearly identified. The allele calling procedure proved to be robust, since the number of called alleles remains relatively unaffected by noticeable drops in sequence coverage or N50. For a few samples, the analysis provided clear evidence for contamination (mixed cultures).

A good correlation was observed between wgMLST and wgSNP results, with the resolution of wgSNP only surpassing that of wgMLST when a cluster-specific reference sequence was chosen.

**Conclusion:** The developed wgMLST schema for *Salmonella* offers ample resolution for outbreak cluster detection and, in combination with BioNumerics' analysis tools, enables a species-wide sample comparison in routine surveillance. While wgMLST and wgSNP essentially capture the same signal, wgMLST has the advantage of being reference-independent and hence can form the basis for fast screening and more important, for a stable nomenclature to be used internationally.

## S8 - Poster session II

### PO62 - Molecular Epidemiology Of *Klebsiella pneumoniae*: Multiclonal Dissemination Of CTX-M-15 Extended Spectrum $\beta$ -lactamase

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Keywords: CTX-M-15, *Klebsiella pneumoniae*, epidemiology

**Background:** *Klebsiella pneumoniae* is now recognized as an urgent threat to public health because of the emergence of multidrug-resistant strains associated with hospital outbreaks and severe community-acquired infections. However, little is known about the emergence of clinically important clones. The aim of this study was to verify the clonal relationship among *Klebsiella pneumoniae* isolates producing CTX-M-15 extended spectrum  $\beta$ -lactamase (ESBL) since 2003, in Portugal.

**Materials | Methods:** This retrospective surveillance study included 740 *K. pneumoniae* clinical isolates collected from 5 different hospitals and 1 community laboratory in Portugal, between 2003 and 2013. Antimicrobial susceptibility testing was performed by disk diffusion and the results were interpreted according to EUCAST guidelines. Genes encoding  $\beta$ -lactamases including CTX-M, TEM, SHV, DHA, FOX and CMY were screened by PCR and confirmed by sequencing. The common elements involved in the mobilization and expression of *bla*CTX-M-15 gene, ISEcp1 and IS903, were analyzed by PCR with specific primers. Plasmid replicon was used to identify the plasmids incompatibility groups. The clonal relationship was evaluated by M13 fingerprinting and multilocus sequence typing (MLST).

**Results:** All isolates had resistance to gentamicin, ciprofloxacin, cefepime, ceftazidime, cefotaxime and showed synergic effect among clavulanic acid and cephalosporins indicative of CTX-M-15 extended spectrum  $\beta$ -lactamase. In three isolates were also found the carbapenemase KPC-3 which confer resistant to carbapenems. The gene *bla*CTX-M-15 was associated with an upstream-inserted insertion sequence ISEcp1. The isolates CTX-M-15 producers belonged to the IncF, IncN and IncA/C groups. The relationship between isolates by M13 fingerprinting showed different profiles and MLST was performed for representative isolates from each cluster. 6 different clones were found: ST-15; ST-76; ST-133; ST-147; ST-276 and ST-307. The ST15-CTX-M-15 accounted for 69% of the isolates analysed by MLST and was identified in community and in all five hospitals studied.

**Conclusions:** Our data indicate a widespread of ST15-CTX-M-15 *K. pneumoniae* which should be considered as clinical relevant for public health. The dissemination of ESBL CTX-M-15 was due both to carriage of related plasmids and to multiclonal spread of *K. pneumoniae*. The tracking of multidrug resistant organisms is one of the four core actions proposed in European Center for Disease Prevention and Control action plan to limit the emergence and spread of this increasingly important pathogen.

## PO63 - Molecular Characterization Of The *Staphylococcus aureus* CC398 Population In Italy

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**Background:** Methicillin-resistant *Staphylococcus aureus* (MRSA) Complex Clonal (CC) 398 have been reported as the most prevalent LA-MRSA lineage in primary productions in Italy. The aim of this study was to provide molecular characterisation of MRSA and methicillin-susceptible *S. aureus* (MSSA) CC 398, isolated in primary productions in Italy in the last five years, in order to describe the CC398 population structure in Italy.

**Materials and Methods:** A total of 60 CC398 isolates were characterized by using spa-typing, Multilocus Sequence Typing (MLST), SCCmec typing and a microarray genotyping kit (Alere GmbH, Germany) that includes the main pathogenicity, virulence-, host-, clone-associated and antimicrobial resistance genes. The majority (n=59) were from different food producing animal holdings: 31 from swine (6 MSSA), 27 from bovine (1 MSSA), 1 from broiler chicken, and one (MSSA) from wild boar. Thirty-six representative isolates were further characterized using Pulsed-Field Gel Electrophoresis (PFGE).

**Results:** All CC398 isolates were Sequence type (ST)398 except one ST2485 (Single Locus Variant, SLV, of ST398), distributed in 16 different spa-types, with spa-type t899 and related spa-types as the most frequent, followed by spa-type t034 and t011. Twenty-seven MRSA isolates carried SCCmec cassette type V while the remainders were type IV. No significant differences in host distribution of the SCCmec type or spa-type were observed. While the genetic pattern was almost the same in all the isolates, the antimicrobial-resistance genes were found in different proportions among the isolates except for the *tem(M)* gene encoding tetracycline resistance, that was presented in all but the MSSA wild boar isolate (fig.1). None of the isolates presented the human-specific virulence PVL or immune evasion cluster genes. The macro-restriction profiles observed showed a high heterogeneity (fig.1) in agreement with previous observations.

**Conclusion:** These observations confirm that *S. aureus* CC398 circulating in livestock in Italy have undergone adaptation to animal host(s), with loss of phage-carried human virulence genes, and that the population is well established. However, as observed in other studies, the selection pressure by the use of tetracyclines, beta-lactams and other classes of antimicrobials, contributed to the emergence and radiation worldwide of the methicillin-resistant, multi-drug resistant CC398 lineage in food producing animals.

**Acknowledgments:** This work was supported by grants from the EMIDA ERA-NET (FP7-KBBE) first transnational research call, Project "LA-MRSA" (acronym title) ID 68.

## S8 - Poster session II

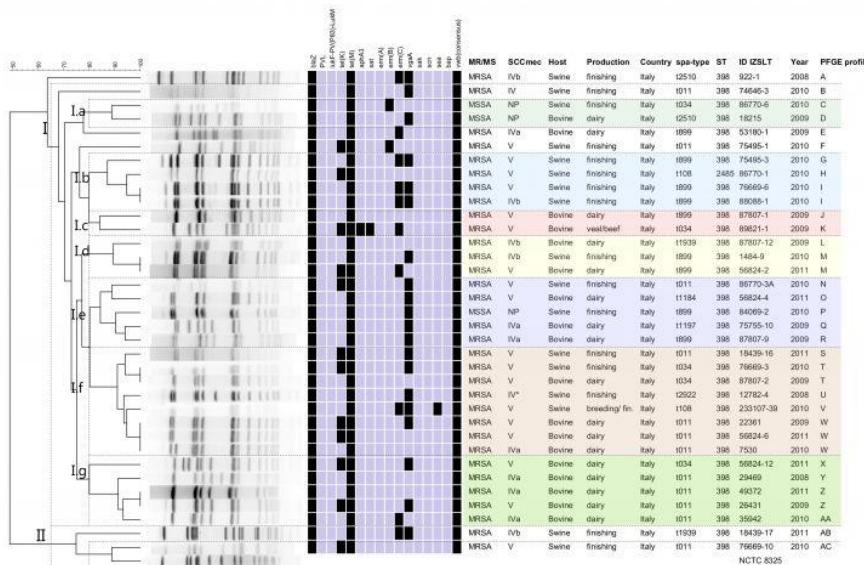


Fig1:Dendrogram of Crf91 PFGE macro-restriction patterns, with selected virulence and antimicrobial resistance marker genes and elements of Clonal Complex 398 MRSA and MSSA from Italian livestock.

**PO64 - *Staphylococcus aureus* USA300 In Sub-saharan Africa**

Lena Strauß<sup>1</sup>; Marc Stegger<sup>2</sup>; Anders Rhod Larsen<sup>2</sup>; Geoffrey Coombs<sup>3</sup>; Sébastien Breurec<sup>4</sup>; Alexander Mellmann<sup>1</sup>; Frieder Schaumburg<sup>1</sup>

<sup>1</sup>University of Münster, <sup>2</sup>Statens Serum Institut, <sup>3</sup>Murdoch University, <sup>4</sup>University of Antilles

**Background:** USA300 is a hypervirulent community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) lineage. The classic USA300 clone is the dominant CA-MRSA in North America. It is characterized by specific molecular features, which include the affiliation to multilocus sequence type (ST) 8 and spa type t008, the possession of the arginine catabolic mobile element (ACME) and a SCCmec IV element, and the production of Panton-Valentine leukocidin (PVL). In South America, a distinct lineage of USA300 is dominant, which lacks ACME and harbors a different SCCmec IV element. Since a few years, both lineages are also increasingly reported in all other continents - except for Africa. However, there have been recent reports of MRSA with USA300-like molecular characteristics in sub-Saharan Africa; but due to differing spa types, they were not regarded as true USA300.

**Materials | Methods:** Our objective was to clarify the phylogenetic relationship of 52 ST8 MRSA and MSSA (methicillin-susceptible *S. aureus*) from different countries of sub-Saharan Africa and various published ST8 isolates, including two NCBI reference genomes of USA300 and its assumed ancestor, USA500. The African isolates were whole genome sequenced using Illumina technology and analysed with regard to both their core genome and USA300-specific molecular characteristics using the software SeqSphere<sup>+</sup> (Ridom GmbH, Münster, Germany).

**Results:** Most African MSSA isolates were found to cluster together in one separate branch of the phylogenetic tree. Seven MSSA and MRSA isolates from Nigeria and three MRSA from Gabon were found to form a different group, which is related to the USA500 reference genome. Moreover, twelve African isolates were identified that cluster in the same branch like the "classic" USA300 isolates; despite their differing spa types (t112 or t121), which display single spa repeat locus variants of t008. Only these African isolates possess all USA300-specific accessory molecular features; including PVL, ACME and SCCmec IV. Interestingly, they display a monophyletic clade within the group of classic USA300 isolates, which suggests an independent evolution of the African USA300 lineage.

**Conclusion:** In conclusion, the data shows that the USA300-related MRSA isolates detected in West and Central Africa are "true" USA300 clones. This suggests that USA300 is more dispersed than previously assumed and raises the question about the origin of this pandemic clone.

## S8 - Poster session II

### PO65 - Evergreen; A Webtool For Surveillance Of Bacterial Outbreaks

Johanne Ahrenfeldt<sup>1</sup>; Martin Christen Frølund Thomsen<sup>1</sup>; Jose Bellod Cisneros<sup>1</sup>; Ole Lund<sup>1</sup>

<sup>1</sup>Technical University of Denmark

**Background:** The cost of whole genome sequencing is falling, and therefore its use in diagnostics is increasing. We wish to make a platform for surveillance of pathogenic bacteria, where everything that is uploaded to our servers automatically will be compared to everything else in our databases, so that in case of an outbreak or an epidemic we will be able to see this pattern very fast.

**Methods:** When an isolate is uploaded to our servers, first it will be compared to a homology-reduced database of reference sequences, where the closest reference sequence will be found. Afterwards the isolate will be mapped to the found reference. Then the mapped sequence will be compared to all other uploaded sequences that previously have been found to match to the same reference. The phylogenetic distances will then be reported, together with the number of isolates, which are within the following distances 0, 1, 5, 10, 100 and 1000 SNPs.

Once a day a tree will be calculated for each of the references. And whenever a new isolate is uploaded, it will be placed in the tree.

## PO66 - Demography And Intercontinental Spread Of The USA300 CA-MRSA Lineage

P Martins Simoes<sup>1</sup>; P Glaser<sup>2</sup>; A. Villain<sup>2</sup>; M Barbier<sup>3</sup>; A Tristan<sup>1</sup>; C Bouchier<sup>2</sup>; L Ma<sup>2</sup>; M Bes<sup>1</sup>; F Laurent<sup>1</sup>; D Guillemot<sup>4</sup>; T Wirth<sup>3</sup>; F Vandenesch<sup>1</sup>

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**Background:** Since the end of 1990's, several genetically distinct community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) lineages have emerged worldwide. In the US, the ST8-IV clone USA300, has become highly prevalent, outcompeting MSSA and other MRSA both in the community and hospital settings. In Europe, we observe the co-existence of the predominant ST80-IV European CA-MRSA clone with the USA300 clone as well as other lineages. The presence of USA300 CA-MRSA in Europe rises the question of this lineage capacity to successfully expand in Europe, similarly to the USA's worrisome scenario, by replacing the current European ST80 CA-MRSA.

**Material/Methods:** We performed whole-genome sequencing to a collection of French USA300 CA-MRSA strains responsible for sporadic cases and micro-outbreaks (n=67) over the past decade and US ST8 MSSA and MRSA isolates (n=431). Genome-wide phylogenetic relationships and coalescence-based analyses were performed to trace the origin, evolution, and dissemination pattern of the USA300 CA-MRSA clone in France.

**Results:** Genome-wide phylogenetic analysis demonstrated that the population structure of the French isolates is the product of multiple introductions dating back to the onset of the USA300 CA-MRSA clone in North America. Coalescent-based demography of the USA300 lineage shows that a strong expansion occurred during the 90s concomitant to the acquisition of the ACME element and antibiotics resistance, followed by a sharp decline initiated around 2008. This scenario is reminiscent of the rise and fall pattern previously observed in the ST80 lineage.

**Conclusions:** Here we show that in most instances, the USA300 CA-MRSA sporadic and micro-outbreaks cases identified in France, in the last decade, corresponded to sporadic and independent imports from the USA (USA300-NA MRSA clone) without further indication of spreading on the French territory. The reasons behind the apparent lack of success of these USA300-NA CA-MRSA isolates to diffuse within the French community are unknown but they argue against the hypothesis that the USA300-NA MRSA clone is intrinsically more successful than the European CA-MRSA ST80 and, thus, that it could be a potential threat in Europe.

At a global level, in the transition from a MSSA lineage to a successful CA-MRSA clone, the USA300 lineage first became resistant to multiple antibiotics and it acquired the ACME element and, subsequently, it acquired a resistance to fluoroquinolones. These two steps seem to be associated with a dramatic demographic expansion. This expansion was followed by the current stabilization and expected decline of this lineage. Our findings highlight the significance of horizontal gene acquisitions and point mutations in the success of such disseminated clones and illustrate their cyclic and sporadic life cycle.

## S8 - Poster session II

### PO67 - In Vivo Characterisation Of Human Cytomegalovirus Genome Diversity Directly From Clinical Specimens Sampled Over Time Or From Different Compartments

Elias Hage<sup>1</sup>; Tina Ganzenmueller<sup>1</sup>; Gavin Wilkie<sup>2</sup>; Silvia Linnenweber-Held<sup>3</sup>; Julius Schmidt<sup>3</sup>; Eva Mischak-Weissinger<sup>4</sup>; Anke Schwarz<sup>3</sup>; Albert Heim<sup>1</sup>; Thomas F. Schulz<sup>1</sup>; Andrew J. Davison<sup>2</sup>

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**Background:** Human cytomegalovirus (HCMV) belongs to the subfamily *Betaherpesvirinae* and is an important pathogen causing a wide spectrum of disease in immunocompromised individuals or congenitally infected children. Introduction of high-throughput next-generation sequencing (NGS) has opened up opportunities to study the intrahost structure of HCMV populations. To gain insights into HCMV diversity among different anatomical compartments or longitudinally (i.e. over time), we performed NGS of complete HCMV genomes directly from clinical samples.

**Materials & Methods:** We sequenced 37 HCMV-positive diagnostic specimens such as urine, whole blood, plasma, vitreous body fluid or respiratory material obtained from three children with congenital HCMV disease, six renal transplant recipients (RTR-1 to RTR-6) and two haematopoietic stem cell recipients (SCTR-1 and SCTR-2). For six out of these patients, blood samples obtained longitudinally at different time-points were available (2-6 time-points per patient). The median HCMV load determined by quantitative HCMV PCR was  $3.0 \times 10^5$  copies/ml (range  $7.5 \times 10^3$  -  $4.2 \times 10^6$ ) for whole blood and  $1.4 \times 10^6$  copies/ml (range  $1.3 \times 10^5$  -  $1.9 \times 10^6$ ) for other specimens. NGS was performed on an Illumina MiSeq platform after preparation of target-enriched libraries. Consensus viral genomes ("major genome types") were created by *de novo* assembly of sequence reads.

**Results:** We were able to assemble HCMV genomes from 34 out of 37 samples with an average read depth ranging from 23 to 5,450 (median 218) for whole blood and from 159 to 4,677 (median 1,391) for other specimens. Samples with  $\leq 1.0 \times 10^4$  HCMV copies/ml did not yield enough HCMV reads for sufficient assembly. Analysis of HCMV diversity between longitudinally collected blood samples showed different patterns: while no major differences between different time-points were detected in three patients, we observed a complete change of the HCMV genome type in SCTR-1 (six time-points within 154 days), with the second genome type being already detectable at a low frequency at time-point 1 and becoming dominant at time-point 4. Similarly, longitudinal samples (five time-points within one year) from RTR-1 showed low HCMV diversity among the first three samples, but marked differences located especially in the RL11 and US6 gene families between the populations present at t1 and t4/t5. Major HCMV genome types from different anatomical compartments were highly similar in blood and urine of congenitally infected children, a corresponding breast milk sample from one mother, and in blood, bronchioalveolar lavage fluid and a gut biopsy from RTR-5. In contrast, high diversity was observed between vitreous body fluid and blood from RTR-6 who suffered from HCMV retinitis. Furthermore, low-frequency variants (i.e. present in 2-49% of the reads at a nucleotide position) were observed particularly in blood from several patients (e.g. SCTR-1, -2 or RTR-6). These might reflect mixed infections or a small fraction of the dominant HCMV type being present in another body compartment.

**Conclusion:** Our data show that application of NGS to sequence HCMV directly from a variety of diagnostic specimens is technically feasible and gives promising insights into the viral diversity and evolution of HCMV *in vivo*. Detailed analysis of different variability patterns observed in clinical samples will further elucidate the pathogenic role of HCMV diversity *in vivo*.

**PO68 - Frequency And Predisposing Factors Of Upper Respiratory Tract Colonization By Gram-negative Rods In Untreated Chronic Lymphocytic Leukaemia Patients**Ewelina Grywalska<sup>1</sup>; Izabela Korona-Głowniak<sup>1</sup>; Anna Malm<sup>1</sup>; Jacek Rolinski<sup>1</sup><sup>1</sup>*Medical University of Lublin*

**Background:** Infectious complications are still one of the major causes of morbidity and mortality in patients with chronic lymphocytic leukaemia (CLL). Infections affect mainly the respiratory tract, skin, or urinary tract. The most common respiratory infections are acute and chronic sinusitis and pneumonia. In the past pneumonia was caused mainly by *Streptococcus pneumoniae*, but with current chemotherapeutic regimens, the spectrum of pathogens includes Gram-negative rods (GNR), *Nocardia* species, *Legionella* species, and *Pneumocystis carinii*. An increase in infections caused by GNR, particularly bacteraemia and pneumonia, may reflect more advanced disease and profound myelosuppression in these patients.

**Aim of the study:** The aim of this study was to assess the frequency and predisposing factors of colonization of upper respiratory tract by GNR in previously untreated CLL patients. Antimicrobial susceptibility of the isolated strains was determined.

**Material and methods:** This prospective study included 30 previously untreated patients with CLL and 24 healthy volunteers. Throat and nasal specimens were taken using sterile alginate-tipped swabs. Swabs were placed directly into Stuart's trans-medium, and samples were delivered to the laboratory, where specimens were streaked onto nonselective medium (blood agar) and selective medium (MacConkey agar). Plates were incubated for 24–48 h at 35°C under aerobic conditions. Presence of GNR in sample from nostrils and/or throat was called colonization. The identification of isolates was determined and antimicrobial susceptibility of the isolates was tested by the disc diffusion method according to the European Committee on Antimicrobial Susceptibility Testing recommendations. ESBL production screening was detected by double-disk synergy test. Peripheral blood (PB) specimens for flow cytometric studies were obtained from 30 CLL patients and 24 healthy donors.

**Results:** A significantly higher frequency of GNR colonization in CLL patients (36.7%) was observed in comparison to healthy volunteers (8.3%). This difference was statistically significant ( $P=0.02$ ; RR 4.4; 95%CI 1.1–18.0). The overall 16 isolates of GNR (from 3 patients, 2 different species of GNR were isolated) were cultured: 8 (50%) isolates were obtained from throat, 6 (37.5%) from nostrils, and 2 (12.5%) isolates colonized both throat and nostrils. GNR isolates mainly belonged to the Enterobacteriaceae family, and only 1 isolate, *Pseudomonas aeruginosa*, belonged to nonfermentative Gram-negative rod-Pseudomonadaceae. ESBL-producing isolate, *Proteus vulgaris*, was obtained from one CLL patient. Four isolates, *Citrobacter koseri*, *Serratia marcescens*, and two isolates of *Hafnia alvei*, demonstrated the presence of AmpC  $\beta$ -lactamases. Colonization rate was higher among CLL patients with lower level of IgG in serum ( $P=0.017$ ) and patients with higher number of lymphocytes ( $P=0.005$ ). It was shown that the longer the time elapsed since diagnosis of CLL, the higher frequency of GNR colonization was observed ( $P=0.025$ ).

**Conclusions:** Awareness of risk factors predisposing to pathogens colonization, allows to identify group of patients which should be considered for immunoglobulin or antibiotic prophylaxis. Moreover, knowledge about antibiotic resistance of the colonizing pathogens is important to propose not only optimal antibiotic prophylaxis scheme but also empiric and targeted therapy with greater likelihood of clinical success.

## S8 - Poster session II

### PO69 - Re-evaluation Of *Streptococcus pneumoniae* Carriage By Real-time PCR In Adults Over 60 Years Of Age

Sónia T. Almeida<sup>1</sup>; Tânia Pedro<sup>1</sup>; Hermínia de Lencastre<sup>2</sup>; Raquel Sá-Leão<sup>1</sup>

<sup>1</sup>Laboratory of Molecular Microbiology of Human Pathogens, ITQB/UNL,<sup>2</sup>

Laboratory of Microbiology and Infectious Diseases, The Rockefeller University

**Background:** *Streptococcus pneumoniae* (or pneumococcus) is a human commensal that frequently colonizes the nasopharynx. It is also a major cause of infectious diseases worldwide, especially at the extremes of age. Currently, most studies on pneumococcal carriage among the elderly are based on culture-based methods followed by identification of presumptive colonies. More recently, new strategies to detect pneumococcal carriage, based on real-time PCR, have been proposed. The aim of this study was to evaluate the prevalence of nasopharyngeal and oropharyngeal pneumococcal carriage in adults over 60 years of age, using real-time PCR.

**Materials and Methods:** Two collections of samples previously characterized by routine procedures based on culture-based methods were studied: one corresponding to samples of 78 individuals living in a retirement home and other corresponding to samples of 200 individuals living in their family homes. Pneumococcal carriage was re-evaluated by real-time PCR, targeting two pneumococcal genes: *lytA* (major pneumococcal autolysin) and *piaA* (iron uptake ABC transporter lipoprotein PiaA). *S. pneumoniae* capsular types present in positive samples were determined by multiplex PCR and DNA sequencing.

**Results:** By real-time PCR *S. pneumoniae* was more frequently detected in oropharyngeal samples than in nasopharyngeal samples: 6.0% vs 4.0% ( $p=0.49$ ), respectively, in the family home collection and 16.7% vs 15.4% ( $p=1.0$ ), respectively, in the retirement home collection. Use of real-time PCR when compared with culture-based methods increased pneumococcal carriage in oropharyngeal samples from 0% to 6.0% ( $p=0.001$ ) in the family home collection, and from 3.8% to 16.7% ( $p=0.01$ ) in the retirement home collection. By contrast, no significant differences were observed in carriage detection in nasopharyngeal samples when both methods were compared. In the retirement home collection 10 of 18 isolates were of serotype 19A. Overall, the use of real-time PCR increased significantly the detection of *S. pneumoniae* from 3.5% to 9.0% ( $p=0.05$ ) in the family home collection and non-significantly from 16.7% to 20.5% ( $p=0.38$ ) in the retirement home collection.

**Conclusion:** The results of this study indicate that the use of real-time PCR increases the detection of *S. pneumoniae* carriage among adults over 60 years of age where colonization density is often low. The use of molecular methods is particularly valuable when sampling the oropharynx.

**PO70 - Whole Genome Sequencing Of Methicillin-resistant And Sensitive *Staphylococcus aureus* Strains Isolated In The Cardiosurgical Area Of The Italian L. Sacco**

Gentile Bernardino<sup>1</sup>; Rimoldi Sara Giordana<sup>2</sup>; Pagani Cristina<sup>2</sup>; Anselmo Anna<sup>1</sup>; Palozzi Anna Maria<sup>1</sup>; Fortunato Antonella<sup>1</sup>; Di Gregorio Aannamaria<sup>2</sup>; Lista Florigio<sup>1</sup>; Gismondo Maria Rita<sup>2</sup>

<sup>1</sup>Histology and Molecular Biology Section, Army Medical Research Center,<sup>2</sup> Microbiology Unit, L.Sacco University Hospital

**Background:** Methicillin-resistant *Staphylococcus aureus* (MRSA) in a intensive care unit (ICU) may be acquired from colonized or infected patients and healthcare workers (HCWs). Community reservoirs have been implicated in MRSA introduction in health care settings by increasing the colonization prevalence among patients, visitors and workers. However, identifying reservoirs and tracking the source of implicated strains has proven to be difficult due to the limitation of genotyping methods for clinical practice. The study was designed to retrospectively evaluate the molecular characterization of *Staphylococcus aureus* strains collected in the Cardiosurgical Area at the "Luigi Sacco" Hospital, Milan (Italy).

**Material/methods:** During April 2015 a total of 4 patients with deep sternal wound infection positive for *S. aureus* were identified. Moreover, during the previous months other 3 cases were reported. To evaluate putative transmission events between patients and operators, 98 nasal swabs were collected from HCWs. Microorganisms identification and susceptibility test were performed using Vitek2 (Biomerieux, Marcy l'Etoile, France). *S. aureus* isolates were analyzed by real time PCR (RealCycler SAMAPV, Progenie Molecular, Valencia, Spain) to detect the presence of the *mecA* gene, coding for methicillin resistance, and the Panton-Valentine leucocidine toxin (PVL) gene. The whole-genome sequencing (WGS) approach was used to perform MLST (MultiLocus Sequence Typing) and SNPs (Single Nucleotide Polymorphisms) *in silico* analysis of the *S. aureus* isolates.

**Results:** Among seven patients, 2 out of 7 *S. aureus* strains collected were MSSA (Methicillin-sensitive *Staphylococcus aureus*, *mecA* negative), 5 were MRSA (*mecA* positive). The epidemiological surveillance carried out on the staff showed that 12 out of 98 operators (12%) were positive to *S. aureus*: 7 resulted as MSSA and 5 as MRSA. The PVL toxin gene was not detected. *In silico* extrapolation of MLST profiles from the 12 operators and the 7 patients positive to *S. aureus* (n=19), revealed the presence of 10 different sequence types (STs): the most represented was the ST22 (7 strains), followed by ST121, ST30 and ST8 (2 strains). ST5, ST10, ST15, ST45 were each representative of one single strain. Two new STs, differing from the ST22 and the ST5 for a single MLST gene, were also identified. The SNPs analysis outcome was in agreement with the MLST results but allowed us to discriminate between samples with the same ST, as in the case of the most predominant ST22 group (Fig.1).

**Conclusion:** Discrimination among genetically related strains is indispensable for clinical and epidemiological investigation and WGS is emerging as the gold standard in bacterial genotyping. The diversity of isolates detected during the epidemiological evaluation in the Cardiosurgical Area of the "Luigi Sacco" Hospital may reflect a larger epidemiology within the hospital and/or the community. Further molecular investigations are requested considering that the ST5, 8, 15, 22, 30 are reported in other Italian hospital settings while the ST121 and ST45 have been identified also in animals.

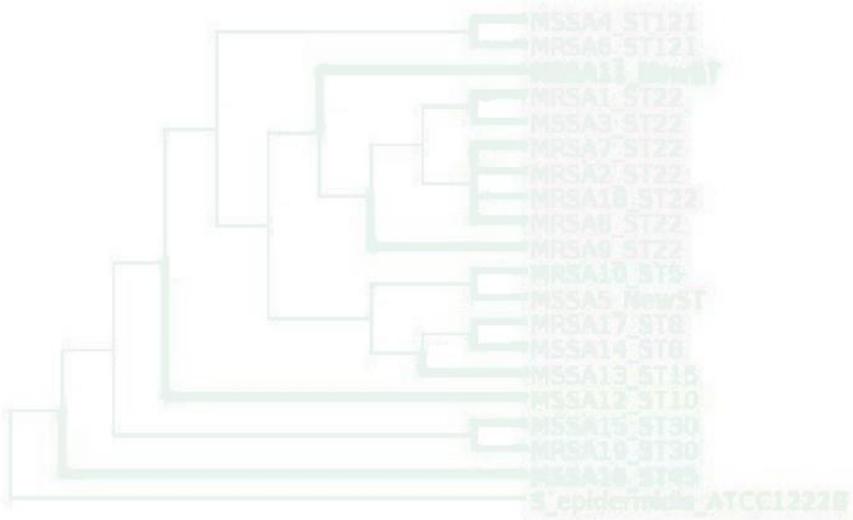


Fig. 1: CoreSNPs tree of nineteen *Staphylococcus aureus* strains. The maximum likelihood coreSNPs tree was constructed using the software *KSNPv2.1.2*. The sample *S. Epidermidis* ATCC12228 was used as outgroup in order to root the tree. The green lines indicate the samples isolated from healthcare workers; blue lines indicate samples isolated from hospital staff; MRSAs: Methicillin-resistant *Staphylococcus aureus*; MSSA: Methicillin-sensitive *Staphylococcus aureus*.

**PO71 - DNA Structural Properties At Origin-of-transfer Regions Determine Hosts Of Mobile Plasmids**Jan Zrimec<sup>1</sup>; Aleš Lapanje<sup>2</sup><sup>1</sup>*Faculty of Health Sciences, University of Primorska, <sup>2</sup>Institute of Metagenomics and Microbial Technologies,<sup>3</sup> Institute of Metagenomics and Microbial Technologies,<sup>4</sup> Saratov state university*

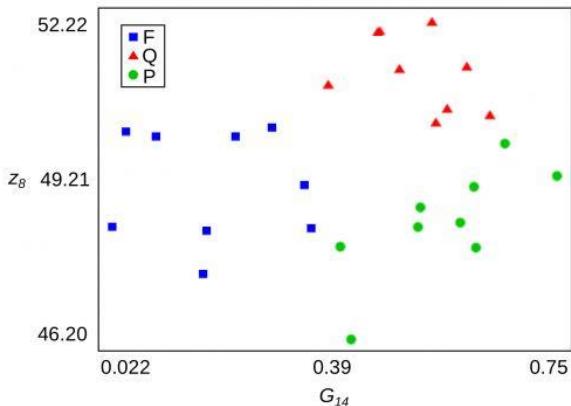
**Background:** Horizontal gene transfer of conjugative plasmids that carry antimicrobial resistance (AMR) genes is a major health concern, as they spread AMR to infected patients from the large environmental pool. Since the repertoire of bacterial hosts of such plasmids is characterized by their MOB group, it is essential to analyze and enable fast prediction of MOB systems. MOB systems are defined by relaxases, essential enzymes that nick plasmid DNA and initiate transfer. Since each relaxase is specific to one type of origin-of-transfer (oriT), DNA in each oriT region must have specific conformational and physicochemical properties that most efficiently attract and enable activity of the particular relaxase. We therefore analysed the conservation of oriT structural properties according to their relaxase defined MOB groups.

**Materials | Methods:** DNA structural properties of oriT regions from conjugative elements from 4 MOB groups were predicted with parametric models and included nucleotide sequence, conformational and physicochemical properties. Conformational and physicochemical properties were predicted using parametric models as well as new models based on Nearest Neighbor and Peyrard-Bishop-Dauxois models. The bioinformatic framework incorporated prediction of oriT structural properties, analysis of oriT nucleotide sequences and machine learning algorithms.

**Results:** Our computational analysis of oriT regions showed that oriT structural properties enable accurate characterization of plasmids, as we were able to successfully sort conjugative elements into their corresponding MOB groups (classification accuracy over 99%, Figure 1). This was possible, because predicted properties distinguished known experimentally determined structural features of the oriT regions. Conventional classification based on oriT nucleotide sequences was inaccurate (estimated classification accuracy was approx. 58%).

**Conclusion:** Mobile plasmids can be correctly classified into MOB groups not only according to conservation of the amino acid sequence of relaxase, but also as a function of DNA structural properties of the relaxase substrate oriT. Our results show that within particular MOB groups oriT regions have relatively conserved structural features (Figure 1). We propose that especially structural properties of oriT regions are connected to and coevolve with the proteins involved in DNA recognition, nicking and transfer reactions within their particular MOB group. Since our method can help to determine the most probable hosts and AMR pathways based on merely 240 bp of DNA, it can help clinicians apply promising approaches to treat AMR infections by using antibiotic combinations that inhibit the transfer of AMR genes, or to develop new ways to inhibit AMR gene transfer within polymicrobial infections.

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**Figure 1.** A scatter plot of predicted *oriT* structural properties  $G_{14}$  (thermodynamic stability of binding sites for auxiliary proteins in MOB F) versus  $z_8$  (persistence length in vicinity of nic site) showing that there exist clear separation boundaries between the particular 27 mobile plasmids that enable classification of the plasmids into their respective MOB groups.

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### **Special thanks to**

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Catarina Mendes  
Mickael Silva



### **Conference Dinner, 10 March 2016 – 19.30h**

The conference Dinner will be held at Pousada de Cascais.

Bus transfers will be organized from the Estoril Conference Centre to the restaurant. Return buses will also be provided.

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