Twelve Month Report 2020

JRP14-AMR2.1-FULLFORCE

Responsible Partner: Pieter-Jan Ceyssens

Contributing partners: Muna Anjum, Henrik Hasman, Saria Otani, Benoit Doublet, Stefan Widgren

GENERAL INFORMATION

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Dissemination *Author's suggestion to inform the following possible interested parties.*	OHEJP WP 1 ☐ OHEJP WP 2 ☐ OHEJP WP 3 ☐ OHEJP WP 4 ☐ OHEJP WP 5 ☐ OHEJP WP 6 ☐ OHEJP WP 7 ☐ Project Management Team ☐ Communication Team ☐ Scientific Steering Board ☐ National Stakeholders/Program Owners Committee ☐ EFSA ☐ ECDC ☐ EEA ☐ EMA ☐ FAO ☐ WHO ☐ OIE ☐ Other international stakeholder(s): ..... Social Media: ..... **Other recipient(s):** .....

TITLE

1. Summary of the work carried out

***500 words, 1 page**. Please emphasize the main scientific results and outcomes. Pay special attention to the impact the project may have for the OHEJP and its stakeholders. This part will be published and should therefore be clear on its own. The CommsTeam may use this summary for communication means.*

The goal of the Full Force project is to supply 17 EU partners with a technological toolbox and hands-on training in Single-Molecule Real-Time (SMRT) sequencing, and to apply this knowledge on six study cases and applications in metagenomics and AMR transmission models. Using this state-of-the-art technology, public health and veterinary labs will have the capacity to perform full-length sequencing, and gain detailed insight in mobile genetic elements (MGEs) which carry antimicrobial resistance and virulence genes within and across species.

Unfortunately, this project set-up has been hit hard by the Covid-19 pandemic. The basis of this project was supposed to be an on-site, three day workshop on SMRT sequencing, held at the State Serum Institute (SSI, DK), followed by a proficiency test to analyse each partner's capacity to perform SMRT sequencing. Due to restrictions imposed by all EU governments, we had to **postpone and reoriented this workshop to an online course held from September 7-8**. Moreover, all research activities were suspended for more than two months during lockdown, and many consortium members were reoriented towards Covid-19 surveillance. Likewise, the physical kick-off meeting, planned during ECCMID 2020 in Paris, was cancelled and replaced by a meeting in Brussels on October 8. It goes without saying that all this is causing significant delays in deliverables and milestones, as elaborated more in detail in the sections below. However, we are still confident that all goals of the Full Force project are still within reach.

- Applying intensive workload during the SMRT course, we still hope that our consortium partners with limited SMRT skills will be able to reach a sufficient technical level in plasmid sequencing. To maximize the output from this project, SSI scientists involved in WP1 are developing an easy-to-use software package (tentatively named Full Force Plasmid Assembler – FuFoPA), which will automatically perform hybrid assemblies through the build-in UniCycler program from a combination of short and long sequence reads.
- A proficiency test, to assess each institute's capacity for SMRT sequencing, has been organised by SSI, using reference material from BfR. Samples are sent out in November 2020, and results are expected by the end of February 2021.
- Although WP2 (five cases studies implementing long-read sequencing on existing datasets) has suffered some delays, substantial progress has been made. The focus will be hypothesis generation based on short-read sequence data for the five defined case studies (T2.1-2.5). Short-read Illumina data will allow comparison of both AMR profile and total plasmid content, as well as relatedness of isolates . Based on these results a subset of isolates will be choosen for MinION runs.
- WP5 has not been impacted by the pandemic, as no lab work is required. The design of a transmission spread model of pAMR in the simulation framework SimInf has been initiated by collaboration between consortium partners.

2. Work carried out in the JRP/JIP, scientific results and integrative outcomes

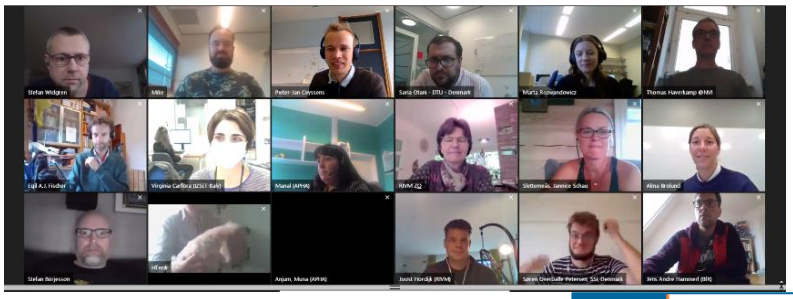
5000 words, 10 pages. *Please provide enough detail to understand the work done per task, without additional information. All aspects should be discussed, scientific and others (integrative activities, etc.).*

WP 0: PROJECT MANAGEMENT (M25-M54)

This overarching WP ensures proper coordination at both the overall project and the individual WPs, as well as timely reporting of results and budgets according to the formal EU requirements.

Task 0.1*: MEETINGS AND TELCALLS (M25-M54)*

The two major consortium meetings, planned during ECCMID (Paris) and the SMRT sequencing workshop (SSI), were **postponed** due to Covid-19 measures. These were replaced by one-to-one teleconferences held by the PI with each individual partners, and by specific calls organized by task and WP leaders. An online workshop on SMRT sequencing (See WP1) was held is planned on September 7-8, and the online kick-off/progress meeting was organized on October 8 in Brussels.



The meeting outline was as follows, giving an overview of all ongoing work packages:

A full meeting report was published online at 10.5281/zenodo.4275887

Task 0.2*: REPORTING (M25-M54)*

Five deliverables from WP0 and WP1 were uploaded to Zenodo. The nine month report was submitted in due time.

Task 0.3*: CENTRAL DATA REPOSITORY (M25-M36)*

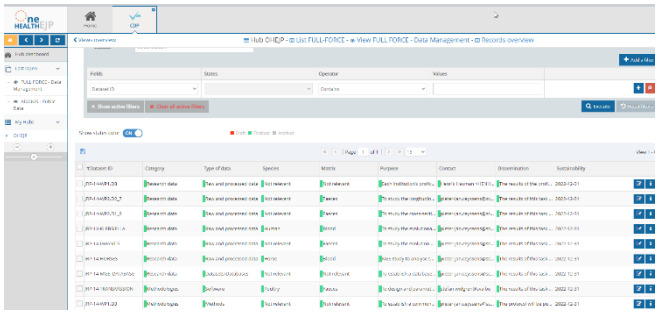
FULL_FORCE will use a centralized data repository to upload sequence- and metadata which are generated during WP1 and WP2. Originally, we planned to use the AMR data hub of the European COMPARE Consortium and the European Nucleotide Archive (ENA). However, we were not successful in reaching an agreement with ENA, who is still negotiating single-subcontractor model for hubs created during COMPARE. Therefore, we have decided to use a commercial cloud tool (OwnCloud) for temporal data storage during Full Force.

The platform was presented during the online workshop with a vitual tour of Owncloud, and an overview of the various groups and possibilities. This presentation was recorded, and shared online at 10.5281/zenodo.4277545.

Interestingly, other EJP projects (e.g., ADONIS, FARMED) were inspired by Full Force, and chose to use the same platform for data sharing.

***Task 0.4**: DATA MANAGEMENT PLAN (M25-M54)*

In September 2020, a new Data Management Platform based on the CDP software was embraced by OHEJP. Full Force's PI followed the training coordinated by the EJP WP4 responsible, Géraldine Boseret. In September 2020, a complete DMP of Full Force was created and uploaded to https://apps.lisam.com/app/#Apps/CDP.



This plan will be updated annualy. As important part of the DMP, a framework agreement on Material Transfer was drafted, circulated and signed among all 18 participating institutions. This agreement covers all transfer of data and strains during Full Force.

WP 1: SMRT IMPLEMENTATION (M25-M36)

In the pre-pandemic planning of Full Force, we scheduled three-day workshop on practical implementation of long-read sequencing in Copenhagen. The main goals was to get less advanced users of SMRT sequencing up to speed, and to use the technological know-how in WP2-4. However, as explained below, we were forced to postpone this workshop and suffer from delays in deliverables and milestones.

***Task 1.1** METHODOLOGY FOR MGE SEQUENCING (M25-M27)*

In two rounds of teleconferences (January and March 2020) led by RIVM, task participants shared experiences in SMRT sequencing. In short, SSI results are between N50 of 15-20k, RIVM results N50 of 35k. Regarding DNA extraction methodologies, there was a choice between faster (semi-)automated protocols using magnetic beads (as used by Sciensano, APHA and SSI), and more elaborate protocols based on DNA precipitation as used by RIVM. As N50 of the RIVM protocol is clearly higher, it was decided to go for the longer procedure to produce highest-quality data. Both protocols can be compared during the proficiency testing of Task 1.3.

A final consensus protocol for SMRT sequencing was elaborated by RIVM, based on the rapid library generation protocol from Nanopore. It is published under embargo at 10.5281/zenodo.4277521 and contains the following parts:

1. DNA isolation from strains cultured in liquid medium, using DNA/RNA shield and QuickExtract Bacterial DNA Extraction Solution
2. A classical DNA precipitation step, using 3M sodium acetate pH 5.2 for ethanol precipitation
3. SMRT run set-up using the Rapid Barcoding Sequencing system, using the manufacturers' recommendations.

This protocol (JRP-WP1.D3) was shared among all participating institutes alongside the instructions of the proficiency test (Task 1.2)

Task 1.2 *SMRT SEQUENCING WORKSHOP (M28-M30)*

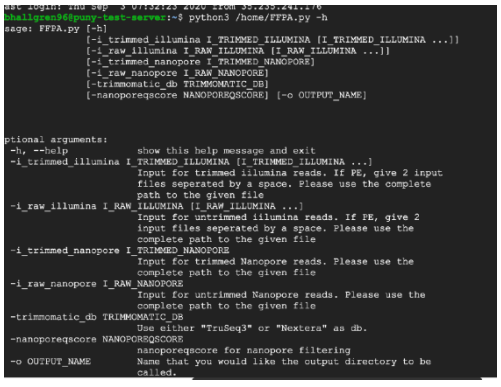
The basis of this project was supposed to be an on-site, three day workshop on SMRT sequencing, held at the Statens Serum Institut (SSI, DK) in Q2 of 2020, followed by a proficiency test to analyse each partner's capacity to perform SMRT sequencing. Due to restrictions imposed by all EU governments, we had to postpone and reorient this workshop to an online course to be held on September 7-9, 2020.

In the months leading to the workshop, and in an effort lead by Søren Overballe-Petersen, Henrik Hasman (SSI) and involving multiple members of their team, the Full Force Plasmid Assembler (FFPA v1.0) was created. This python script joins best-in-field tools to trim and QC short en long sequence reads (qcat, Trimmometric), enables species identification through Kraken en performs either Nanopore and hybrid assemblies through Unicycler. Currently, further refinement of the FFPA software is planned by WP1 members.

The package can be locally installed by members of the Full Force consortium, but was installed on a Google cloud to enable training during the workshop. The outline of this two-day event is shown above, and resulted in basic training of non-expert bioinformaticians for plasmid assembly.

	PART I	
Time	Monday	Tuesday
9.15 - 9.30	Logging in and Welcome	Welcome back
9.30. - 10.00	Introduction to FULL-FORCE	Finding the relevant data the FFPA results
10.00 - 11.00	Short presentation by all groups	Tips'n tricks for improving assembly quality + hand poslishing
11.00 - 11.45	Presentation of wet-lab protocols (RIVM)	The profiency test + Datasharing
11.45 - 12.30	Presentation of ONT - news and technical questions	The way forward in the Full Force project - Are we ready?
12.30 - 13.30	Lunch	Lunch
13.30 - 15.30	Introduction to FFPA and running the pipeline by yourself	Setting up Google cloud and installing FFPA (optional)

	PART II
Time	TBD
9.15 - 9.30	Welcome back
9.30. - 10.00	Introduction to the individual programs in FFPA
10.00 - 11.00	Digging into the individual programs of FFPA part 1
11.00 - 11.45	Digging into the individual programs of FFPA part 2
11.45 - 12.30	Programs for handling minION data
12.30 - 13.30	Lunch
13.30 - 14.30	Drylab -Running manual assembly in UniCycler
14.30 - 14.45	Coffee
14.45 - 16.00	Discussing optimization of FFPA to v2.0



Task 1.3 *PROFICIENCY TEST FOR MGE SEQUENCING (M31-M42)*

Each institution's proficiency in SMRT sequencing will be assessed afterwards using a proficiency test, organised and coordinated by SSI. Given the delay in the workshop, this EQA is being organised in M37-42. Upon discussion during and after the workshop, it was decided to include 5 *Escherichia coli* strains from BfR (GER) as reference strains, since they have been sequenced using Illumina, MinION and PacBIO technologies.

A	B	
Post-sequencing reporting	strain1	str
Institution: *Please insert your institution here!!*		
DNA purification method (RIVM/other)	RIVM	RIV
Flow cell total output		
Total raw output from entire flow cell in gigabasepair?	15,2	
Number of barcodes/samples in flow cell?	5	
raw ONT output before filtering (NanoPlot on sequencing_summary.txt)		
Guppy version used for basecalling?	v4.0.14	
basecalling configuration? (fast, high-accuracy, high-accuracy methylation-aware)	hac_m	
number of reads?	200.000	
number of bases?	3.000.000.000	
median fragment length?	6.200	
median quality?	10,1	
read length N50?	15.000	
longest read?	150.000	
ONT after filtering to q≥8 (NanoPlot on filtered barcodeXX.fastq.gz)		
tools used for filtering? (program, version, used options)	NanoFilt	
number of reads?	160.000	
number of bases?	270.000.000	
median fragment length?	6.200	
median quality?	10,1	
read length N50?	15.000	
longest read?	150.000	
Short read technology used? (Illumina/Ion Torrent)	Illumina	
raw short reads before filtering		
number of reads?	1.500.000	
number of bases?	225.000.000	
short reads after filtering		
tools used for filtering? (program, version, used options)	rimmomatic_v3.6	
adaptor removal? (yes/no)	yes	
minimum quality for filtering?	q20 end-trim	
minimum length for filtering?	140	
number of reads?	800.000	
number of bases?	120.000.000	
hybrid assembly		
tools used for assembly? (program, version, used options)	FFPA_v1.py	
number of contigs? (circular)	4	
number of contigs? (linear >10 kb)	1	
number of contigs? (linear <10 kb)	3	
total length of contigs? (sum of contig lengths)	5,3	
identified ESBL/pAmpC gene	CTX-M-15	
identified replicon of plasmid ESBL/pAmpC gene	incI1	
exact length of contig carrying ESBL/pAmpC gene	112.456	
name, which you choose to give the contig carrying ESBL/pAmpC gene to submit for OwnC	p1_SSI_RIVM_R	
ONT coverage calculation	50.943.396	
Short reads coverage calculation	42.452.830	
Did you handpolish the plasmid sequence?	yes	
Did you run other polishing tools on the plasmid sequence?	no	
If yes, which polishing tools and how?	n/a	

In the PT instructions, it is highlighted that the Full Force SMRT protocol by RIVM (current version 2) should be followed to produce the ONT sequencing data, so all data can be aggregated into one common method paper. However, as many partners have already established in-house/homemade sequencing protocol (e.g. for DNA purification or hybrid assembly), the option to benchmark these against the Full Force results is also foreseen.

The strains have been send out mid-November, and results are expected to be shared with SSI by March 2021.

Figure. Some post-sequencing QC parameters, which will be assessed during the EQA.

WP 2: GENOME STUDIES (M25-M54)

In WP2, the acquired SMRT toolbox will be applied in the (re-)sequencing of AMR strains from various research and surveillance projects in WP2 including EU projects such as EFFORT, COMPARE, ENGAGE and ARDIG, as well as national and EU surveillance activities for which short read sequences are available. Given the postponed SMRT workshop, focus in WP2 in the first months of Full Force was lead on **hypothesis generation based on short-read sequence data** for the five defined studies cases (T2.1-2.5). Short-read Illumina data will allow comparison of total plasmid content and phylogenetic relatedness of isolates and based on those results choose a subset of isolates for MinION runs.

Task 2.1 *MGE evolution in longitudinal sample sets (ARDIG, ABRES) (M25-M54)*

During a teleconference coordinated by Muna Anjum (APHA, UK), task participants decided to focus on plasmid evolution in longitudinal datasets from livestock and human samples. It was agreed to focus on plasmid evolution within the IncI1 plasmids encoding $bla_{CTX\text{-}M\text{-}1}$, with main research question being:

- What is the European diversity in the complete sequences of these plasmids?
- Can specific factors be recognized in the most successful plasmids?
- How have these plasmids evolved over the past decade?

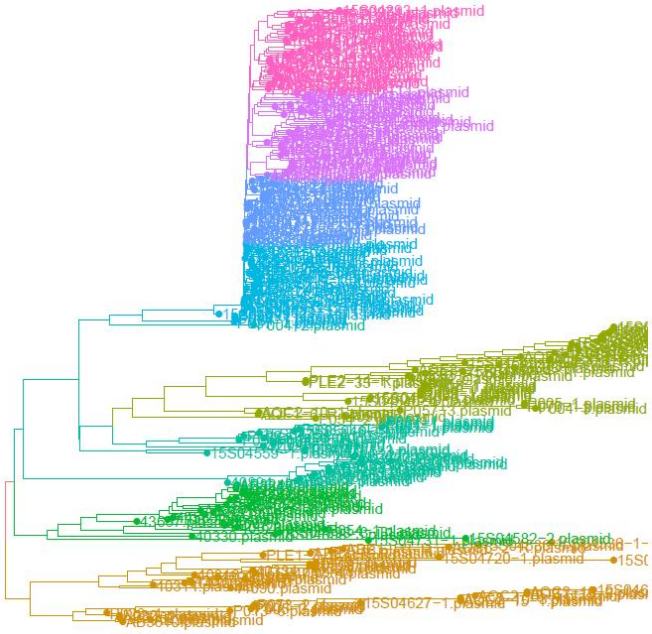
All partners will examine short read sequence data from their databases to enable selection of isolates harbouring IncI1 plasmids encoding $bla_{CTX\text{-}M\text{-}1}$, for long-read sequencing. Based on the available data, it will be decided if isolates before 2010, other ESBLs and non-ESBLs will be included in the final dataset.

Task 2.2 *MGE evolution in cross-sectional data sets (EFFORT, ENGAGE & National Surveillance) (M28-M54)*

Jens-Andre Hammerl (Bfr, GER) coordinates the group studying cross-sectional datasets. In a series of teleconferences, it was decided to focus on IncK plasmids (with/without CMY-2). The main issue was the preclassification as IncB/O/K/Z-positive by PlasmidFinder, while this task would focus only on IncK plasmids. Therefore, more discussion is needed on the identification of reliable detection markers for IncK classification, like RNAI or phylogeny.

Task 2.3 *Klebsiella pneumoniae: the canary in the coalmine (M28-M54)*

Alma Brolund (PHAS) organised teleconferences with task participants, in which it was decided to focus on *K. pneumoniae* isolates with reduced susceptibility to carbapemens. Participants from Norway, Denmark, Sweden, The Netherlands and Portugal agreed that isolates with proposed high variation in genetic context were seen as most interesting to study. A separate work group will be initiated where *Klebsiella* isolates from the animal (and environmental?) sector can be further discussed. A first analysis of diversity/overlap between these is performed by RIVM. The task leader sent around a metadata sheet serving as basis for isolate inclusion.



Task 2.4 *ESBL-producing Enterobacteriaceae in horses – A separated epidemiology of plasmids? (M28-M54)*

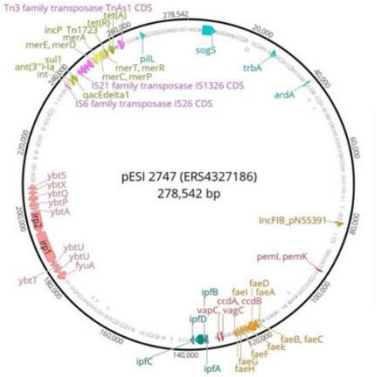
SVA (Stefan Borjesson) coordinated a teleconference with task participants, in which it was decided to focus on *E. coli* isolates from horses encoding $bla_{CTX\text{-}M\text{-}1}$ and $bla_{SHV\text{-}12}$ genes. All short-read sequencing has been performed, and data has been shared on OwnCloud.

A first rough analysis has been performed by Aldert Zomer (Utrecht University), which will serve to select representative isolates of each cluster for long read sequencing (see figure on the left).

It is also worth noting that task leader (Stefan Borjesson) has been replaced by Joost Hendrickx (RIVM, NL) due to changes in job positions.

Task 2.5 *Salmonella Infantis and S. Kentucky across reservoirs: role of MGEs (M28-M54)*

ISS (Laura Villa) coordinated a teleconference among task participants, in which it was decided to focus on the pESI virulence plasmid of *Salmonella infantis* from animal and human origin. All task participants completed a metadata sheet, and samples were selected for short-read sequencing which should be completed by M36. It was decided that each partner selects 20-30 *S. Infantis* isolates from their collection, with maximal diversity in selection



(year/source), and focusing on the pESI markers: SMX-TET-SUL resistance (NAL/CIP), and/or the presence of IncFIB(pN55391), tet(A), sul1 and dfrA14.

Task 2.6 *Evaluation of publicly available and in-house tools for MGE typing (M30-M54)*
This task has not yet been initiated at the time of writing.

WP 3: CULTURE-INDEPENDENT TYPING AND METAGENOMICS (M30-M54)

The development of culture-independent methods to detect, quantify and identify bacterial plasmids carrying antimicrobial resistance genes is greatly encouraged for future surveillance efforts. This WP will focus on (i) enhanced mining of existing metagenomics data, including those from the EFFORT and COMPARE projects, and correlate this to AMR-gene abundance, and (ii) development of diagnostic tools for direct identification of MGE/plasmid identifications from various sample types.

Task 3.1 *MGE ANALYSES IN METAGENOMIC DATASETS (M30-M54)*
In the first annual year, it was planned to develop, evaluate and make available a database of MGEs detection from single isolates and metagenomics datasets. In research performed by Markus H K Johansson (DTU, DK), this databases was established and consists of ~4450 MGE sequences that originate from ~1050 different species. They contain several types of mobile elements:

- Insertion sequences (ISs) are among the smallest types of iMGEs. They are often composed of a transposase gene flanked by two inverted repeats (IRs). They are notable for their ability to modulate gene expression and promote mobility by forming composite transposons (ComTns), translocatable units (TUs) and in the case of elements from the IS26 family pseudo-composite transposons (PCTs).
- Unit transposons (Tns) are generally flanked by IRs and carry a transposase gene. They usually carry a resolvase gene, accessory genes and/or additional iMGEs.Miniature Inverted Repeats (MITEs) are non-autonomous ISs or Tns that have undergone deletions in their core genes but have retained the IR and can form ComTn-like structures.
- Integrative Conjugative Elements (ICEs), Cis-Mobilizable Elements (CIMEs) and Integrative Mobilizable Elements (IMEs) are larger iMGEs capable of conjugation. They can either conjugate independently or be co-mobilized by conjugation of other elements. These elements carry many accessory genes and other MGEs.

In the following years, this database will be updated and implemented in the evaluation of bioinformatics pipelines for quantification of MGE in metagenomics datasets, and in determining the presence and abundance of MGEs in public and de novo generated metagenomics datasets.

Task 3.2 *CULTURE INDEPENDENT METHODS FOR PLASMID IDENTIFICATION (M30-M54)*
Genomic Epidemiology, DTU, DK (Saria Otani *et. al.*,) has developed a plasmidome-DNA extraction protocol from complex biomes (*e.g.*, sewage and faeces). The protocol allows plasmid DNA isolations, degrades linear gDNA and enriches circular elements in metagenomics samples. In short, plasmid DNA isolation was performed on individual sewage pellets (420 mg) using Plasmid Purification Mini Kit (Qiagen, Cat No./ID: 12123) following the manufacturer's instruction with the following modifications: protein precipitation with P3 buffer mixture was incubated on ice for 20 minutes, elution buffer QF and EB buffer were preheated at 65°C prior applications, and the DNA pellet washing step was done

using ice-cold 70% ethanol after isopropanol precipitation. LyseBlue dye for cell lysis indication was added, and all buffer volumes were adjusted to sewage pellet weight. The plasmid DNA pellet was dissolved in 25 µl EB buffer for 1 hour at room temperature. Linear chromosomal DNA was reduced by Plasmid-Safe ATP-Dependent DNase (Epicentre, USA) treatment for 24 hours at 37°C. The DNase was inactivated at 70°C for 30 minutes. Circular DNA was enriched using phi29 DNA polymerase (New England Biolabs, USA) following the manufacturer's instructions, similar to as previously described.This can be combined with an assembly workflow, utilizing the long-read length of Oxford Nanopore sequencer. The pipeline was already tested at DTU using sewage samples from 22 countries (5 continents) as part of DTU global sewage surveillance project. 105 Gpb Oxford Nanopore data were obtained and 159.322 circular contigs were assembled. Data annotation is in progress to further validate the pipeline.

WP 4: FUNCTIONAL CHARACTERIZATION OF AMR MOBILE GENETIC ELEMENTS (MGE)-CARRYING AMR GENES AND BACTERIAL HOST ASSOCIATIONS (M25-M54)

The overall goals of this WP are to (i) gain knowledge on molecular mechanisms of spread and persistence of main MGEs carrying critically/highly important antimicrobial resistances, (ii) identify key molecular interactions between AMR-MGEs and bacterial host important for dissemination and maintenance.

Task 4.1 *SELECTION of MGEs and HOST STRAINS FOR DETAILED CHARACTERIZATION (M25-M42)*

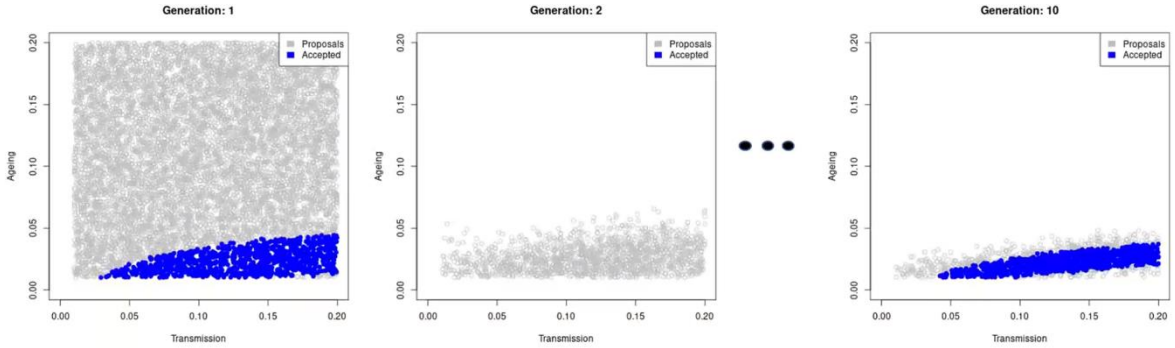
Given the delay caused by postponing both the kickoff meeting as well as the workshop on SMRT sequencing, it was decided that the selection of MGEs will be performed within tasks 2.1-2.5. Therefore, a current focus lies on pESI of S. Infantis, pKpQIL of KPC-producing Klebsiella pneumoniae, IncX3-SHV and IncHI1-CTX-M plasmids from horses, IncI1-AmpC/ESBL plasmids of E. coli. To allow smooth exchange of reference strains and/or donor-acceptor strains for conjugation experiments, a Material Transfer Agreement has been approved by all WP4 partners. A meeting on available reference strains and protocol is planned in early 2021. Drafting a common conjugation protocol is in progress that will be available on January 2021 for discussion/improvement and then to be shared among involved partners.

Task 4.2 *FUNCTIONAL CHARACTERIZATION of MGEs (M31-M54)*

This task has not yet been initiated at the time of writing.

WP 5: MODELLING (M25-M54)

The objectives of WP5 are to address: i) gaps in quantitative knowledge on the spread of pAMR which will be essential to direct future focused research, ii) insight in the uncertainty around the effect of measures reducing pAMR prevalence in the food production chains, and iii) identification of key elements in the production chains to mitigate the risk of human exposure.



Task 5.1 *MODEL DESIGN for AMR TRANSMISSION (M25-M42)*

The design of a transmission spread model of pAMR in the simulation framework SimInf has been initiated. SVA (Stefan Widgren) has coordinated two teleconferences with task participants. The first teleconference was a startup meeting and the second teleconference was a meeting to discuss horizontal vs. vertical AMR transmission.

A necessary but challenging step in stochastic modelling is to determine parameters such that the model generates data that are consistent with observations. Parameterization is preferably conducted within a Bayesian framework and in WP5 we are focusing on using Approximate Bayesian computation (ABC), a recent computational approach for simulation-based inference. In the first annual year, development is underway to add ABC functionality to the open-source SimInf modelling R package (https://github.com/stewid/SimInf). The figure below illustrates using ABC in SimInf to fit parameters from data of infected chicken broilers published in Dame-Korevar et al. (2017), and a model with susceptible (S) and infected (I) chicken, showing (for example) that the ageing parameter has a tighter posterior distribution compared to the transmission parameter. Work is ongoing to identify and include other sources of broiler data for parameterisation of more complex models.

Task 5.2 *EXPOSURE ASSESSMENT of HORIZONTAL and VERTICAL TRANSMITTED AMR (M25-M54)*

This task has not yet been initiated at the time of writing.

3. Progress of the project: milestones and deliverables

JRP /JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted: Forecast delivery date	Is this delay due to COVID-19?	Comments *(Please mention: public or confidential, the Zenodo reference and other comments)*	Proposed category* *(1 to 8) (several categories may be applicable)*
14	D-JRP14-WP0.D1	Start-up meeting report	M27	M34		YES	Confidential due to research updates; 10.5281/zenodo.4275887	8
14	D-JRP14-WP0.D2	Financial and activity report Y3	M36	M36			The annual 12M will be published on time.	
14	D-JRP14-WP0.D3	Recorded webinar tutorial ENA AMR data hub	M27	M33		YES	Public; 10.5281/zenodo.4277545	3
14	D-JRP14-WP1.D1	Teleconference to assess required protocols and infrastructure	M25	M26			Public; 10.5281/zenodo.3733393	8
14	D-JRP14-WP1.D2	Teleconference to discuss proposed protocols and infrastructure (follow-up)	M26	M26			Public; 10.5281/zenodo.3759335	8
14	D-JRP14-WP1.D3	Completion of final protocol for SMRT sequencing	M27	M34		YES	CONFIDENTIAL UNTIL PUBLICATION; 10.5281/zenodo.4277521	2
14	D-JRP14-WP1.D4	Invitation to workshop delivered to all participating institutions	M28	M27			Public; 10.5281/zenodo.3693741	8
14	D-JRP14-WP1.D5	Completion of workshop organization plan including selection of course material	M29	M35		YES	CONFIDENTIAL UNTIL PUBLICATION; 10.5281/zenodo.4290698	8

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JRP /JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted: Forecast delivery date	Is this delay due to COVID-19?	Comments *(Please mention: public or confidential, the Zenodo reference and other comments)*	Proposed category* *(1 to 8) (several categories may be applicable)*
14	D-JRP14-WP1.D6	Selection of proficiency test data	M32	M35		YES	CONFIDENTIAL UNTIL PUBLICATION; 10.5281/zenodo.4290707	8
14	D-JRP14-WP1.D7	Delivery of analysis results of proficiency test by partners to SSI	M33		M39	YES	CONFIDENTIAL UNTIL PUBLICATION; Proficiency test will be organised as follow-up of the postponed online course. Therefore, this delivery dates shifts backwards.	8
14	D-JRP14-WP1.D8	Completion of final report of proficiency tests	M36		M46	YES	Proficiency test is ongoing, but suffered from some delays due to COVID-19	
14	D-JRP14-WP2.D1	Submission of sequence- and metadata of longitudinal samples at ENA hub	M33		M38		CONFIDENTIAL UNTIL PUBLICATION; Given the lab closures in M28-30, we expect all short-read sequencing now to be done by M38.	3
14	D-JRP14-WP2.D2	Submission of sequence- and metadata of cross-sectional samples at ENA hub	M33		M38	YES	CONFIDENTIAL UNTIL PUBLICATION; Given the lab closures in M28-30, we expect all short-read sequencing now to be done by M38.	3
14	D-JRP14-WP2.D3	Submission of sequence- and metadata of K. pneumoniae samples at ENA hub	M36		M38	YES	CONFIDENTIAL UNTIL PUBLICATION; Given the lab closures in M28-30, we expect all short-read sequencing now to be done by M38.	3
14	D-JRP14-WP2.D4	Submission of sequence- and metadata of S. enterica samples at ENA hub	M36		M38	YES	CONFIDENTIAL UNTIL PUBLICATION; Given the lab closures in M28-30, we expect all short-read sequencing now	3

JRP /JIP code	Project deliverable number *(Original number, if different from the actual one)*	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWP 2020	Date delivered on Project Group website	If deliverable not submitted: Forecast delivery date	Is this delay due to COVID-19?	Comments *(Please mention: public or confidential, the Zenodo reference and other comments)*	Proposed category* *(1 to 8) (several categories may be applicable)*
							to be done by M38.	
14	D-JRP14-WP2.D5	Submission of sequence- and metadata of horse-related samples at ENA hub	M36		M38	YES	CONFIDENTIAL UNTIL PUBLICATION; Given the lab closures in M28-30, we expect all short-read sequencing now to be done by M38.	3
14	D-JRP14-WP2.D6	List of relevant publicly available and in-house developed tools.	M36		M42		Taskgroup will assembly first time at the beginning of 2021	2
14	JRP14-WP3.D1	Database construction tailored at MGEs	M36	M36			Public; 10.5281/zenodo.4305711	3
14	D-JRP14-WP3.D2	Protocol for plasmid DNA extraction from environmental samples	M34		M42	YES	Delay caused by lab closure, due to COVID-19	2
14	D-JRP14-WP4.D1	First collection of type-materials (MGEs and strains) to be shared between involved partners	M36		M42		Taskgroup will assembly first time at the beginning of 2021	3
14	D-JRP14-W5.D1	Source code of the implementation of a SimInf model designed for pAMR transmission.	M34	M36			Public; 10.5281/zenodo.4305750	1

** Categories of Integrative activities : 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance*

data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities ; 7. Prevention: aligned use of facilities and models; 8. Other (please specify);

Milestones

JRP/JIPCode	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
14	M-JRP14-M1	Creation of specific data hubs in ENA AMR hub	M27	Yes		Password-protected data hubs for each individual task were created at OwnCloud (not ENA)
14	M-JRP14-M2	A teleconference or physical meeting on horizontal vs. vertical AMR transmission	M27	Yes		
14	M-JRP14-M3	Selection of MGEs and host strains to be studied T4.2	M28	Yes		
14	M-JRP14-M4	A teleconference or physical meeting on input/output relationship between SimInf and sQMRA	M29	Yes		
14	M-JRP14-M5	Publication of first version of data management plan	M30	Yes		
14	M-JRP14-M6	3-days workshop on SMRT sequencing event	M30	Yes		
14	M-JRP14-M7	Shipment of proficiency test data and strains	M32	Yes		
	M-JRP14-M8	Sharing of protocols, recipient- and host-strains, molecular tools	M33	No	M42	Due to delays in WP2

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JRP/JIPCode	Milestone number	Milestone name	Delivery date from AWP 2020	Achieved (Yes/No)	If not achieved: Forecast achievement date	Comments
14	M-JRP14-M9	An implementation of a SimInf model designed for pAMR transmission to be studied in T5.1	M33	Yes		
14	M-JRP14-M10	Final selection of longitudinal samples for SMRT sequencing	M34	No	M39	Will be based on phylogenetic analyses of short-read data, foreseen for M39
14	M-JRP14-M11	Final selection of cross-sectional samples for SMRT sequencing	M34	No	M39	Will be based on phylogenetic analyses of short-read data, foreseen for M39
14	M-JRP14-M12	Final selection of *K. pneumoniae* samples for SMRT sequencing	M34	No	M39	Will be based on phylogenetic analyses of short-read data, foreseen for M39
14	M-JRP14-M13	Final selection of *S. enterica* samples for SMRT sequencing	M34	No	M39	Will be based on phylogenetic analyses of short-read data, foreseen for M39
14	M-JRP14-M14	Final selection of horse-related samples for SMRT sequencing	M34	No	M39	Will be based on phylogenetic analyses of short-read data, foreseen for M39
14	M-JRP14-M15	Analysis of proficiency test data by all partners	M35	No	M38	New deadline for proficiency test
14	M-JRP14-M16	Individual reports of proficiency test sent to partners	M36	No	M40	Update reporting date
14	M-JRP14-M17	Successful adaptation of plasmid purification protocol to field samples	M36	No	M42	Delay due to lab closure (COVID-19)

4. Publications and additional outputs

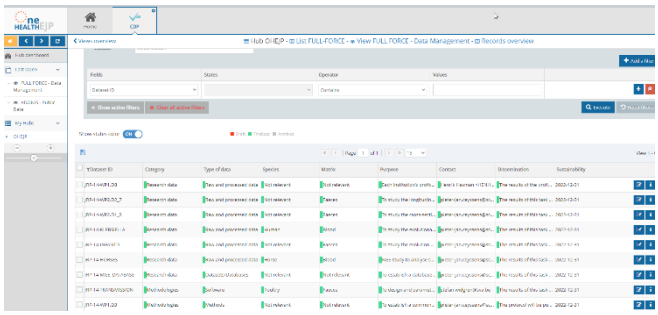
No publications & additional output thus far.

5. On-going and planned collaborations with national or European projects or networks

- The SOLIDNESS network (JPIAMR, 2019-2020) which grouped experts in sequencing, plasmid biology and bioinformatics, and aims to streamline procedures for MGE sequencing. We build on their expertise to organise the PT.
- Cross-sectional and longitudinal bacterial samples of ENGAGE, EFFORT (Horizon 2020, 2013-2018) and ARDIG (OHEJP, JRP2, 2018-2020) projects will be selected for long-read sequencing during WP2.
- The KENTUCKY PhD project (OHEJP, 2020-2022) will use fully sequenced S. Kentucky strains (T2.4) to focus on the cell biology behind MGE transfer.
- Potential collaborations with ECDC and EFSA might be envisioned, for sustainable implementation of long-read sequencing technology in surveillance of AMR in Europe.

6. Data Management Plan

1. Have you uploaded a first version of the project´s DMP to the DMP group on the OHEJP website?



In September 2020, a new Data Management Platform based on the CDP software was embraced by OHEJP. Full Force's PI followed the training coordinated by the EJP WP4 responsible, Géraldine Boseret. In September 2020, a complete DMP of Full Force was created and uploaded to https://apps.lisam.com/app/#Apps/CDP. This plan will be updated annualy. As important part of the DMP, a framework agreement on Material Transfer was drafted, circulated and signed among all 18 participating institutions. This agreement covers all transfer of data and strains during Full Force.

2. Have you encountered any problems or difficulties when setting up and updating the DMP? If yes, please specify.

No, thanks to great assistance of Ms. Boseret.

7. Follow-up of the recommendations and comments in previous review(s) by the Ethics Advisors

WP3/WP4 Team pre-filled the table below with available information. Please clearly explain the actions and measures that have been taken to comply with the recommendations you received from the Ethics Advisors

Requirements of ethical reviewers in 2020	What measures and actions do you propose?	Comments of Ethics Advisors, December 2020	Comments Project Leaders, January 2021	Comments of Ethics Advisors, October 2021	Comments Project Leaders, January 2022
Human biological samples. As 'spread of AMR will be investigated in people living in proximity to farms' (p14/26), the beneficiaries must confirm that, if relevant, appropriate authorizations will be sought to collect human samples.	No human samples will be collected in the context of this study. We will only re-analyse DNA extracted from bacterial strains in our stocks.	Satisfactory reply. Please update your ethical submission if this should change in any way	No change so far.		

8. List of critical risks

Please indicate possible risk within your JRP/JIP

Description of risk	Yes/No
Loss of key-persons (staff and / or leaders)	No
Delay in work plan execution	Yes
Conflicts within the consortium	No
Lack of commitment of partners	No
Delay in duties, tasks or reporting	Yes
Poor intra-project relationship	No
Potential entry/exit of partners	No
Other risks (please describe)	No

Additional information

9. List of dissemination and communication activities

Please fill in one table per event you attended/organised in 2020. You should also register these activities online on the OneHealth EJP webpage : https://onehealthejp.eu/internal-events-survey/

Name of the activity:			
Date:			
Place:			
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		Yes / No
Organisation of a Conference		Participation to a Conference	
Organisation of a Workshop		Participation to a Workshop	
Press release		Participation to an Event other than a Conference or a Workshop	
Non-scientific and non-peer-reviewed publication (popularised publication)		Video/Film	
Exhibition		Brokerage Event	
Flyer		Pitch Event	
Training		Trade Fair	
Social Media		Participation in activities organized jointly with other H2020 projects	
Website		Other	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		Number
Scientific Community (Higher Education, Research)		Media	
Industry		Investors	
Civil Society		Customers	
General Public		Other	
Policy Makers			