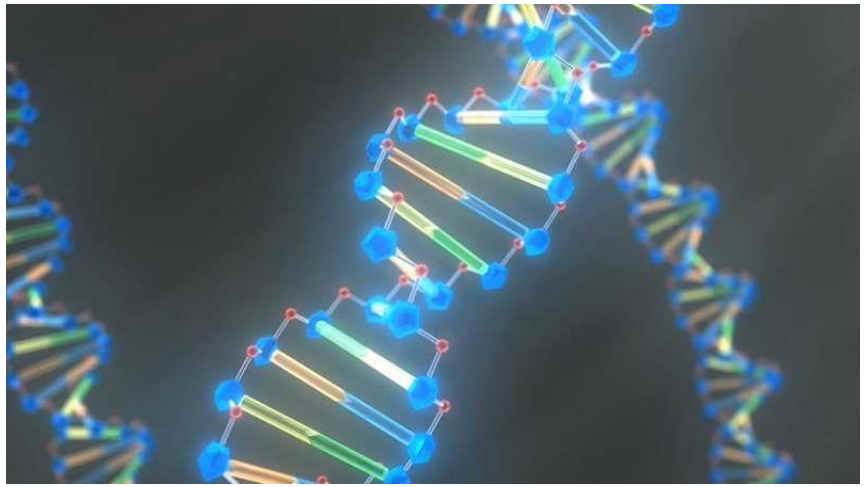
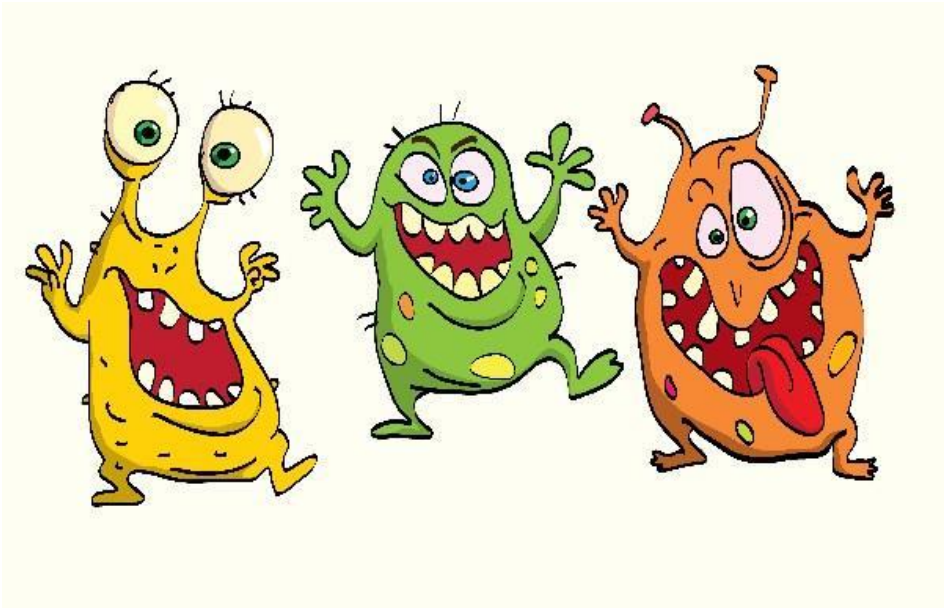
Outbreak investigation and Microbial typing

Yaovi Mahuton Gildas Hounmanou, PhD
Assistant-Professor
APTI-Fellow, National Institutes of Health, MD, USA
https://orcid.org/0000-0003-3991-0864
University of Copenhagen
Faculty of Health and Medical Sciences
Section for Food Safety and Zoonoses
Department of Veterinary and Animal Sciences
Stigbøjlen 4, 1870 Frederiksberg, DK
Cell Phone-WhatsApp: +4552641293 / +1 2402343714
Work phone: +4535326882
Email: gil@sund.ku.dk ; gilmahu@yahoo.fr



What is Microbial typing?

- Differentiation between bacterial isolates belonging to the same species
  - = **any characterization below the species level**
  - After detection it can be useful to distinguish bacteria below species level to determine close relationships or clonality



Group work

- Reflect about these questions
  - Why do we want to type (examples)?
  - What is the difference between genotypic and phenotypic typing methods?
  - What characterizes a good typing method?
  - Which typing methods do you know?

Put your responses on the padlet reachable here:

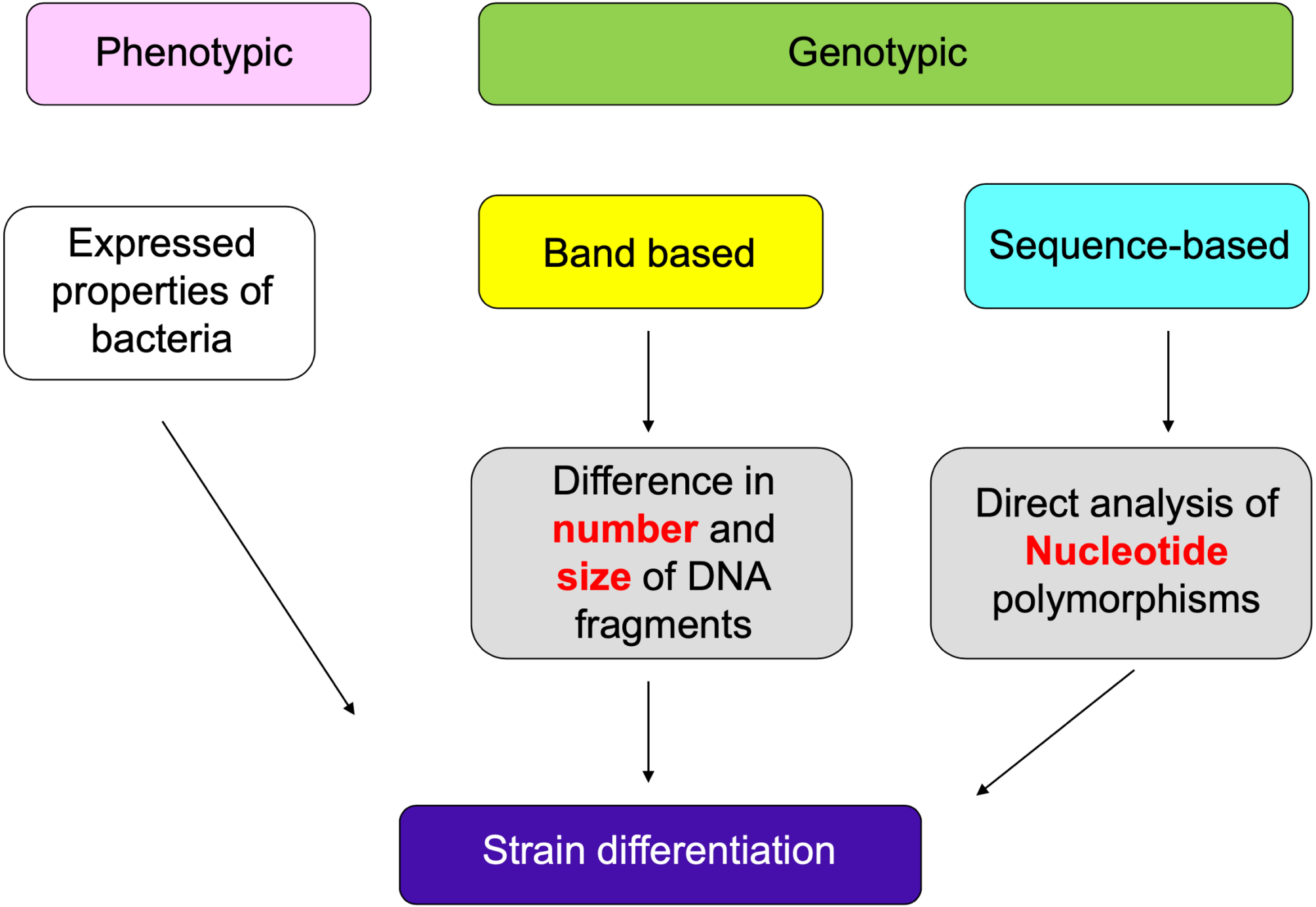
tinyurl.com/sdcgil

Why we want to type

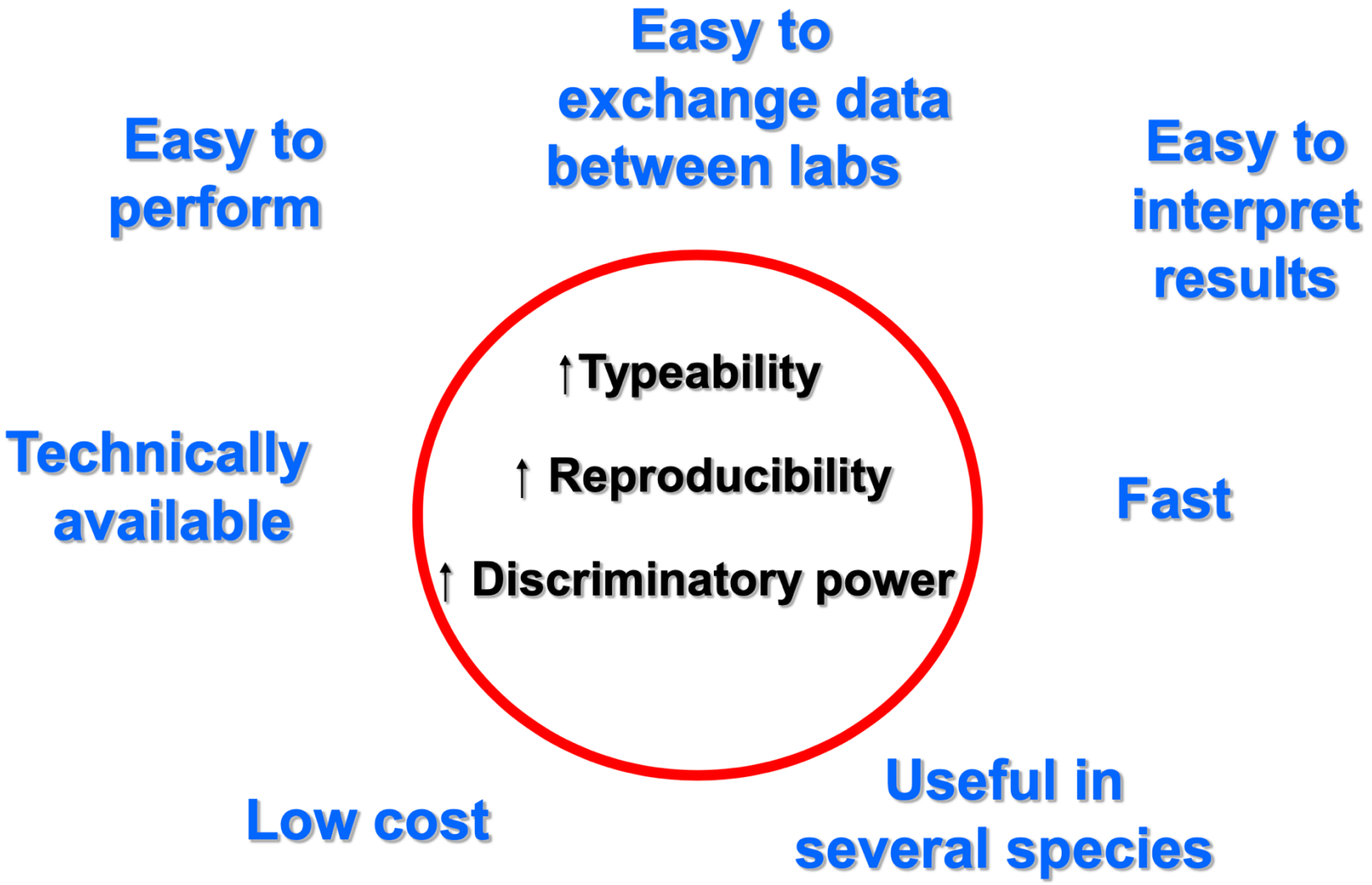
- Outbreak investigations
  – Do multiple isolates derive from the same source or are they all sporadic?
- • Surveillance
- – Are new virulent/resistant strains introduced to our country?
- – What happens over time with resistance levels?
- Evolutionary studies
  - – Where does my strain derive from, is it an ancient strain or newly arisen?
  - – Has my strain acquired virulence/resistance traits? From where?
- Screening individual patients over time
- – Has the infection been cleared or does the same strain persist?

Typing methods belong to two main categories

- Genotypic methods
  – Studies of bacterial DNA
  – "We don't care if it is expressed or not"
- Phenotypic methods
- – Used to study the expressed properties or traits of bacteria, e.g. morphology, biochemical properties, antibiotic resistance, surface proteins etc.


Phenotypic
Genotypic
Expressed properties of bacteria
Band based
Sequence-based
Difference in number and size of DNA fragments
Direct analysis of Nucleotide polymorphisms
Strain differentiation

What characterises the perfect typing method?

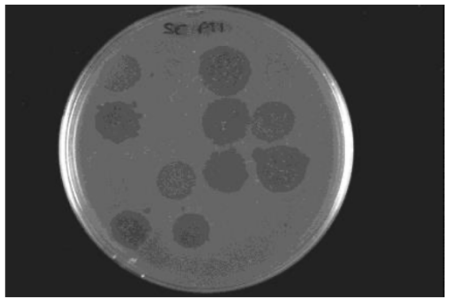


Phenotypic methods

- Biotyping
  - Presence/absence of metabolic reactions
  - E.g. fermentation of sugars

- Serotyping
  - Variability in surface antigens like the cellwall, capsule, fimbriae, flagellae

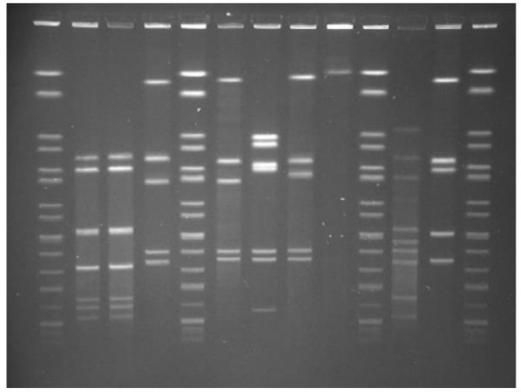
- Phage typing
  - Susceptibility towards different phages

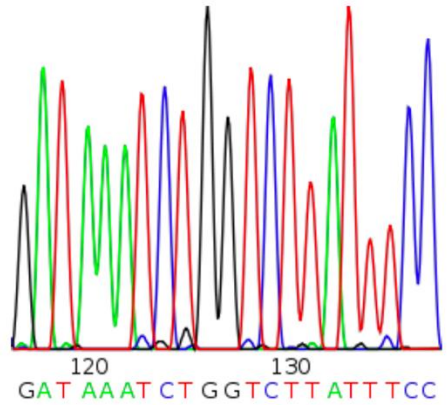


- Antimicrobial susceptibility
  - Susceptibility towards various antibiotics

Genotypic methods

- Band-based
  - RFLP
  - PFGE
  - MLVA
  - RAPD
  - Etc.
- Sequence-based
  - Single-locus methods (e.g. *spa* typing)
  - Multilocus methods (e.g. MLST)
  - Whole-genome sequencing





Sequence-based typing

- Species identification using one gene: **16S rDNA gene**
- The gene is ancient and found in bacteria and Archaea
- Definition of a bacterial species: >98,65% similarity in 16S rDNA gene sequences
- Increasingly other conserved genes are included in a species characterization, known as species-specific genes e.g. opmW in *Vibrio cholerae*
- MLST typing, WGS, Metagenomics

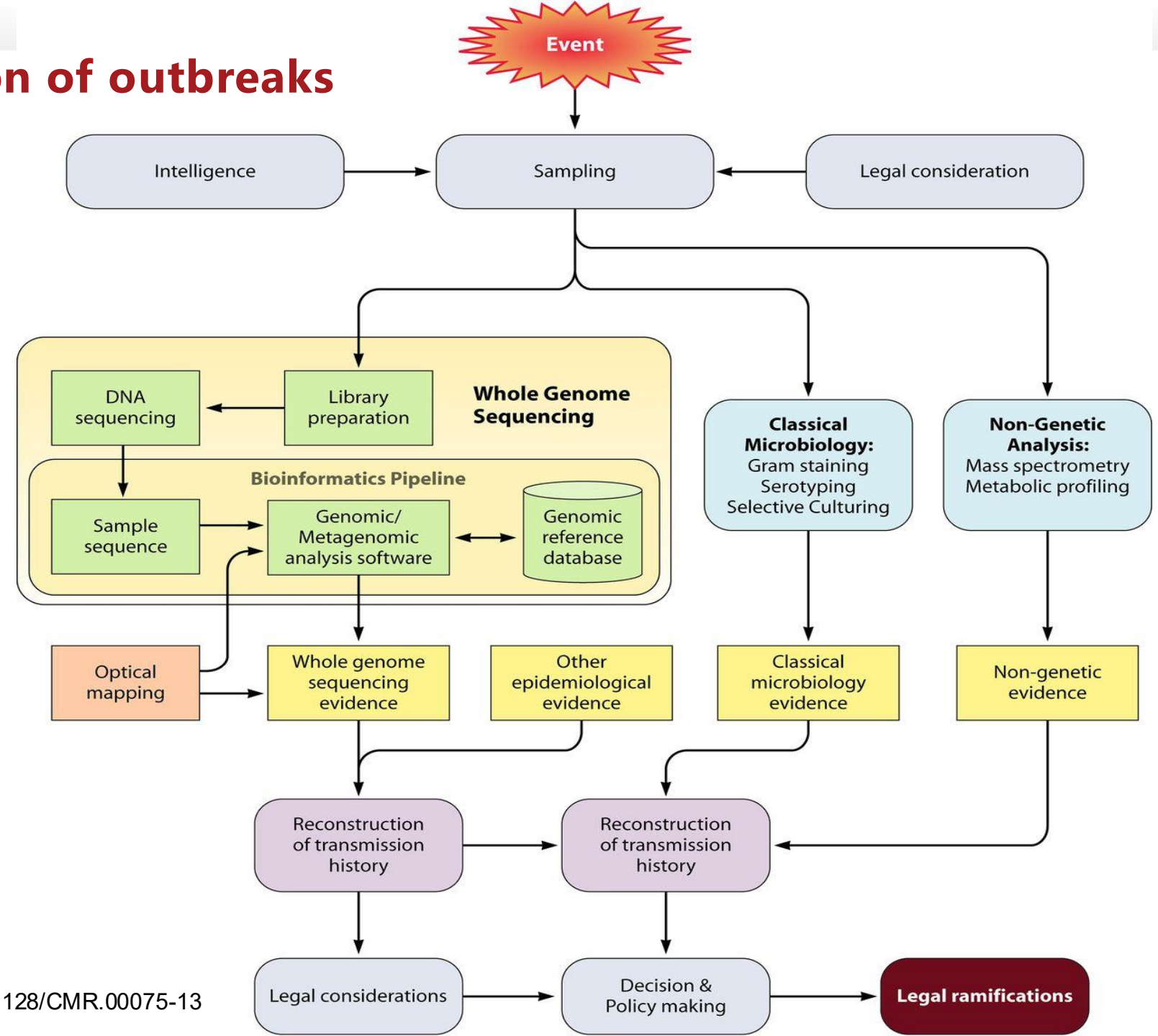
Foodborne disease outbreak

- A foodborne disease outbreak occurs when two or more people get the same illness from the same contaminated food or drink.
- Foods Associated with Foodborne Illness are various origins
  - raw meat and poultry,
  - raw eggs,
  - unpasteurized milk,
  - raw shellfish etc.
  - vegetables/fruits

Steps in the investigation (CDC)

1. Determine existence of an outbreak – assemble team (multidisciplinary)
2. Confirm diagnosis
3. Develop case definition
4. Perform case finding
5. Describe time, place, person data (= descriptive epidemiology)
6. Interview cases and generate hypothesis
7. Test hypothesis (= analytical epidemiology)
8. Perform further studies (microbiological, **Genomics** and trace-back)
9. Inform risk managers, advise on control measures
10. Disseminate findings, conduct evaluation

Microbiological investigation of outbreaks



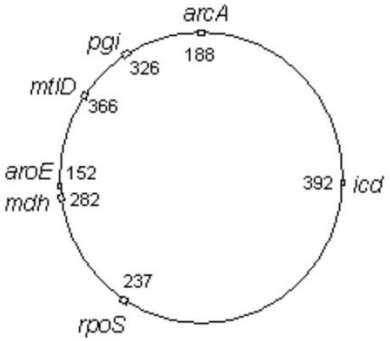
Clin Microbiol Rev. 2015 Jul;28(3):541-63. doi: 10.1128/CMR.00075-13

MLST as a tool in foodbordne outbreak investigation

- The most widely used sequence-based method for bacterial typing
- Has been developed for more than 70 bacterial species – Also for several plasmid types in recent years
- Relies on diversity in housekeeping genes – Slowly evolving – very conserved
  – Usually 7 genes are analysed

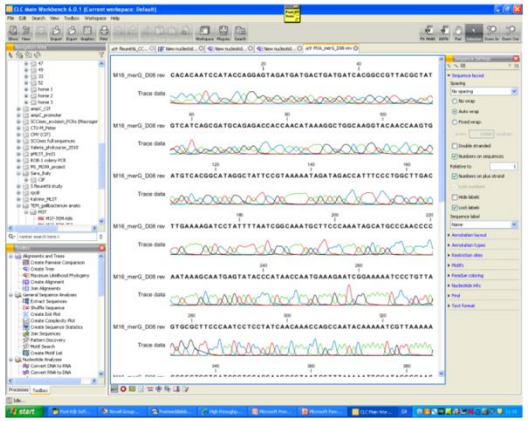
MLST flow

1. PCR of housekeeping genes (~500bp)

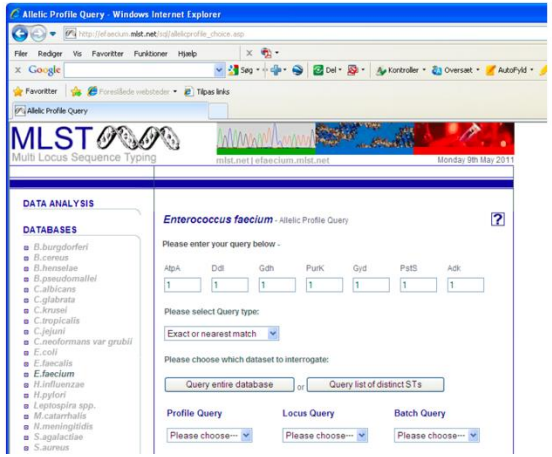


2. Sequencing of PCR products

3. Sequence analysis



4. Assign allele for each gene



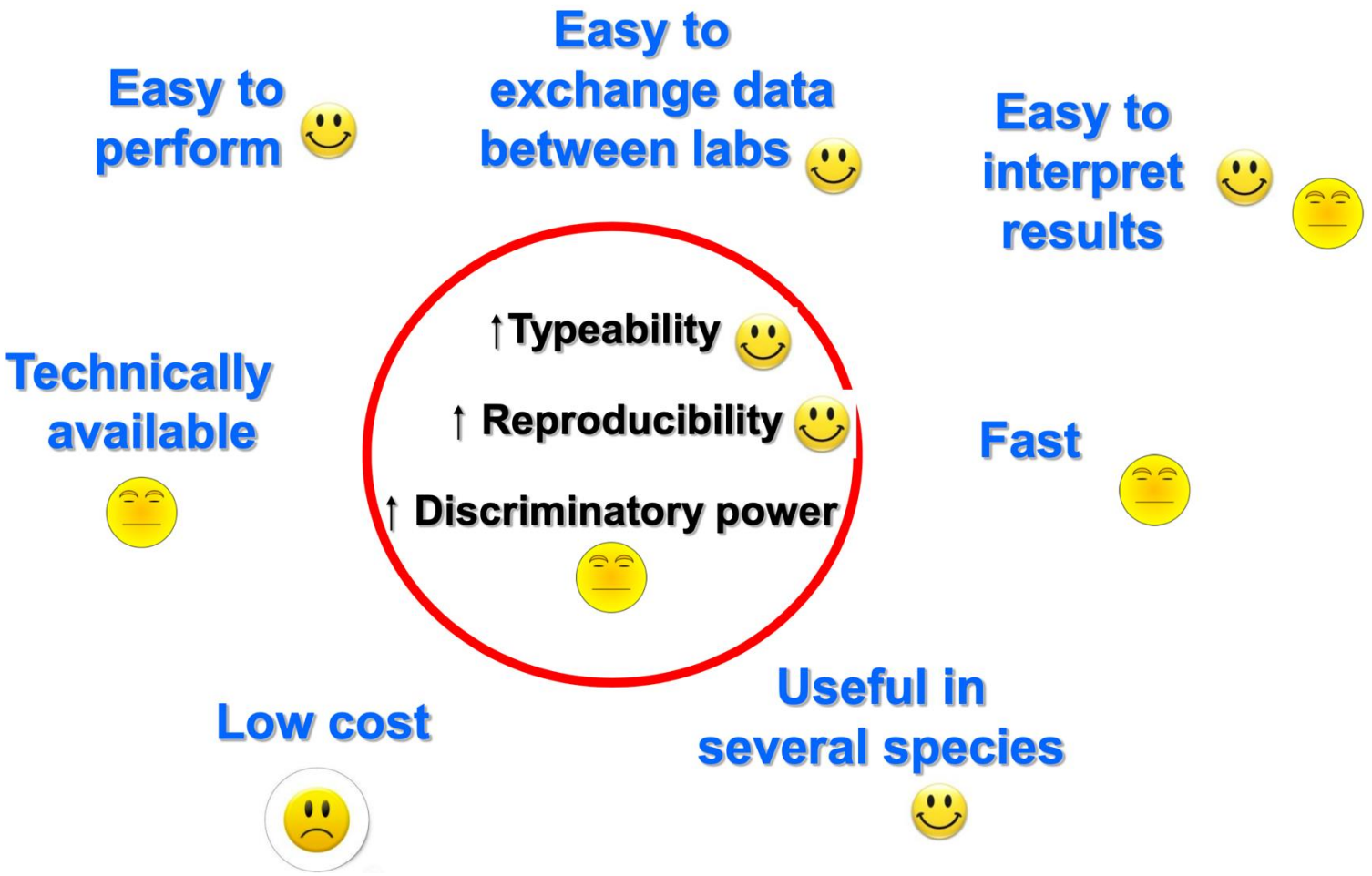
MLST DEMO

- Demonstration: MLST analysis using the Lm database
  - https://bigsdb.pasteur.fr/listeria/

Group EXERCISE MLST

- A 12-year old girl suffers from diarrhoea. A fecal sample is submitted to a diagnostic laboratory. A few days later the lab reports *Listeria monocytogenes* in the fecal sample.
- Make one slide
  - Mention possible sources of infection
  - Samples are taken from various potential sources – *Listeria monocytogenes* is detected in all suspected sources
  - What will you do to find which of these is the source of infection?
  - Use the MLST typing scheme and the seven gene sequences in the .doc file to answer the question above
    - The Files are here: https://figshare.com/s/332416449e2b09d5fbf9

Pros and cons of MLST



WGS-based tying methods

Whole Genome Sequencing in outbreak investigation

- Like people, microorganisms also have DNA
- WGS allows to study these microbes by looking at their DNA fingerprints with extreme precision.
- How precise?
  - Imagine someone giving you two books to compare, and you could use a tool that showed whether every letter of every word in both books is the same. That's how precise WGS is.
- By comparing the WGS of bacteria, we can determine if they are closely related to one another and thus link cases (outbreak or not), identify origins of cases, indicate if strains likely came from the same source etc.
- WGS data also help shape government food safety policies and food industry practices that help to make food safer and save lives.

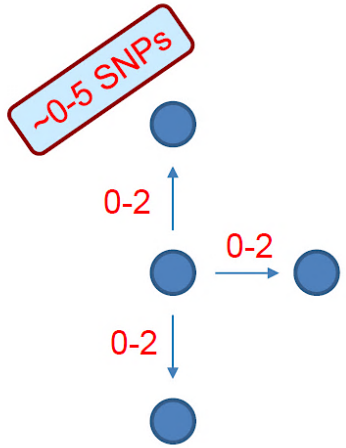
Beyond foodborne outbreaks

- WGS is regularly used to analyze samples collected from sick people to better understand how diseases are spread around the world, to discover trends in disease transmission, and to uncover new or unknown sources of infections. e.g. COVID-19 outbreak, cholera outbreaks etc.

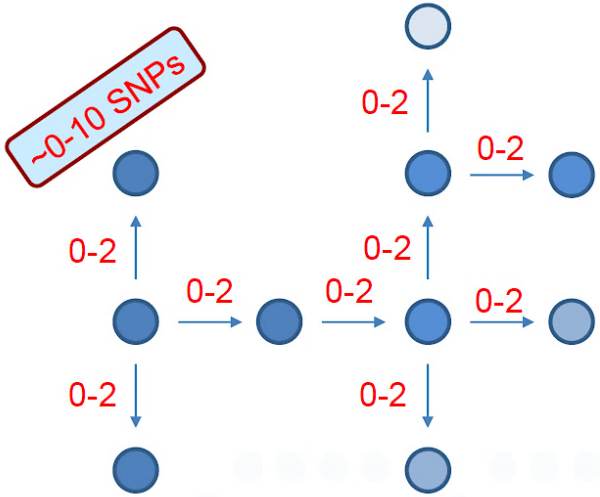
- WGS can tell if bacteria and fungi have genes that make them resistant to antibiotics.
- WGS can elucidate how germs become resistant and how resistance spreads.
- WGS can also serve as tool to assess intervention measures in disease or resistance control

Phylogenetic thresholds in outbreaks (WGS)

SINGLE SOURCE OUTBREAKS

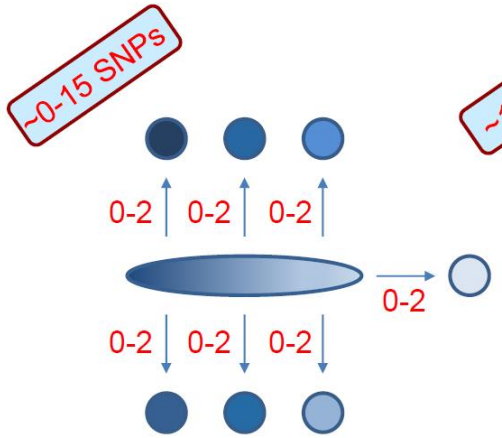


Single source
Short time span
"Contaminated dish"
"Single infected patient"

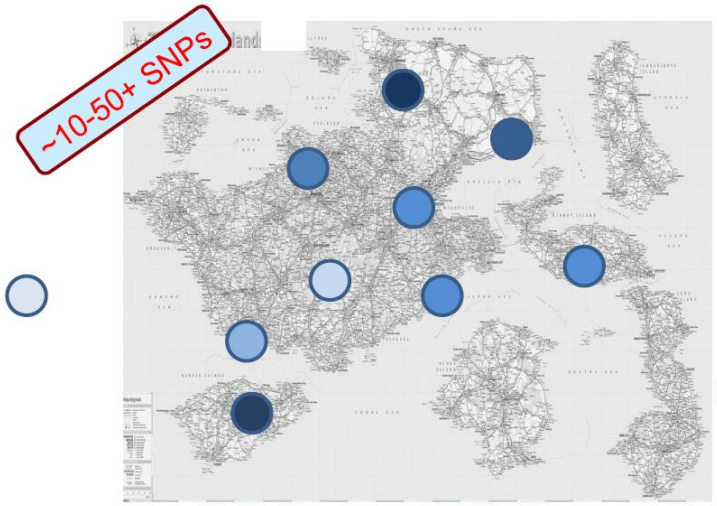


Single source – local spread
Long time span
"Hospital or regional outbreak"

COMPLICATED OUTBREAKS



Single source
Long time span
"Contaminated processing plant / industry"
"Long-term colonized patient / healthcare worker"



International spread
Long time span
"Imported food source"
"Travel related outbreak"

Thresholds variations by species

Table 1
Examples of relatedness criteria for wg/cgMLST and SNP typing schemes of representative clinically relevant bacteria

Organism	Relatedness threshold[a]		References
	wg/cgMLST (allele)	SNPs	
Acinetobacter baumannii	≤8	≤3	[25,26]
Brucella spp.	Epidemiologic validation in progress[b]		http://www.applied-maths.com/applications/wgmlst
Campylobacter coli, C. jejuni	≤14	≤15	[27,28]
Cronobacter spp.	Epidemiologic validation in progress[b]		http://www.applied-maths.com/applications/wgmlst
Clostridium difficile	Epidemiologic validation in progress[b]	≤4	[29], http://www.cgmlst.org/ncs, http://www.applied-maths.com/applications/wgmlst
Enterococcus faecium	≤20	≤16	[30]
Enterococcus raffinosus	Epidemiologic validation in progress[b]		http://www.applied-maths.com/applications/wgmlst
Escherichia coli	≤10	≤10	[31,32], https://enterobase.warwick.ac.uk/
Francisella tularensis	≤1	≤2	[33,34]
Klebsiella oxytoca	Epidemiologic validation in progress[b]		http://www.applied-maths.com/applications/wgmlst
Klebsiella pneumonia	≤10	≤18	[35,36]
Legionella pneumophila	≤4	≤15	[37]
Listeria monocytogenes	≤10	≤3	[38,39]
Mycobacterium abscessus		≤30	[40]
Mycobacterium tuberculosis	≤12	≤12	[41]
Neisseria gonorrhoeae	Epidemiologic validation in progress[b]	≤14	[42], http://www.applied-maths.com/applications/wgmlst
Neisseria meningitidis	Epidemiologic validation in progress[b]		http://www.cgmlst.org/ncs
Pseudomonas aeruginosa	≤14	≤37	[31,43]
Salmonella dublin	Epidemiologic validation in progress[b]	≤13	[44], https://enterobase.warwick.ac.uk/
Salmonella enterica	Epidemiologic validation in progress[b]	≤4	[45], http://www.cgmlst.org/ncs, http://www.applied-maths.com/applications/wgmlst, https://enterobase.warwick.ac.uk/
Salmonella typhimurium	Epidemiologic validation in progress[b]	≤2	[46], https://enterobase.warwick.ac.uk/
Staphylococcus aureus	≤24	≤15	[47,48]
Streptococcus suis		≤21	[49]
Vibrio parahaemolyticus	≤10		[50]
Yersinia spp.	0		[51]

cg, core genome; MLST, multilocus sequence typing; SNP, single nucleotide polymorphism; wg, whole genome.

[a] Data often represent single studies that can be used to begin formulation of species-specific interpretation criteria. Thus, these data should be coupled with newly published similar studies to ensure that resulting values are not atypical and can be generally applied.

[b] Proposed wg/cgMLST schemes are available online (http://www.cgmlst.org/ncs, http://www.applied-maths.com/applications/wgmlst, https://enterobase.warwick.ac.uk/) but as yet have not been epidemiologically validated.

Case study

RAPID COMMUNICATIONS

Cross-border outbreak of listeriosis caused by cold-smoked salmon, revealed by integrated surveillance and whole genome sequencing (WGS), Denmark and France, 2015 to 2017

Susanne Schjørring[1], Sofie Gillesberg Lassen[2], Tenna Jensen[3], Alexandra Moura[4], Jette S Kjeldgaard[5], Luise Müller[2], Stine Thielke[3], Alexandre Leclercq[4], Mylene M Maury[4], Mathieu Tourdjman[6], Marie-Pierre Donguy[7], Marc Lecuit[4,8], Steen Ethelberg[2], Eva M Nielsen[1]

1. Department of Bacteria, Parasites and Fungi, Statens Serum Institut, Copenhagen, Denmark
2. Department of Infectious Disease Epidemiology and Prevention, Statens Serum Institut, Copenhagen, Denmark
3. The Danish Veterinary and Food Administration, Copenhagen, Denmark
4. Institut Pasteur, National Reference Centre and WHO collaborating Center for Listeria, Biology of Infection Unit, Paris, France
5. National Food Institute, Technical University of Denmark, Kgs. Lyngby, Denmark
6. Santé Publique France, the French Public Health Agency, Saint-Maurice, France
7. Ministry of Agriculture, and Forestry, Paris, France
8. Paris Descartes University, Sorbonne Paris Cité, Division of Infectious Diseases, Necker-Enfants Malades University Hospital, Institut Imagine, F-75743 Paris, France

Correspondence: Susanne Schjørring (SSC@ssi.dk)

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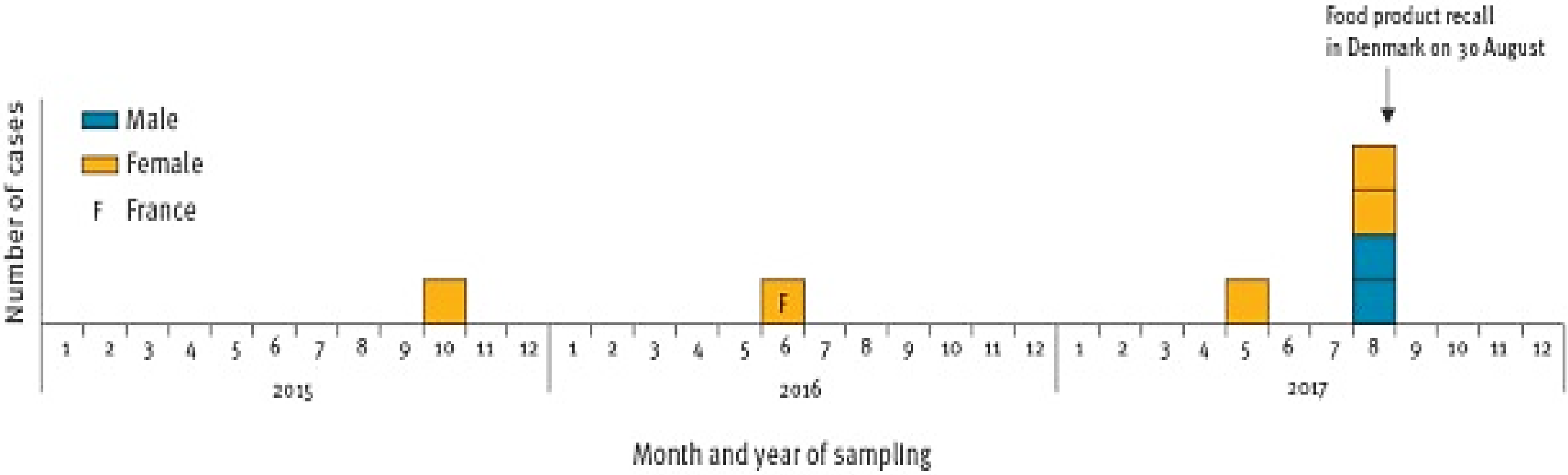
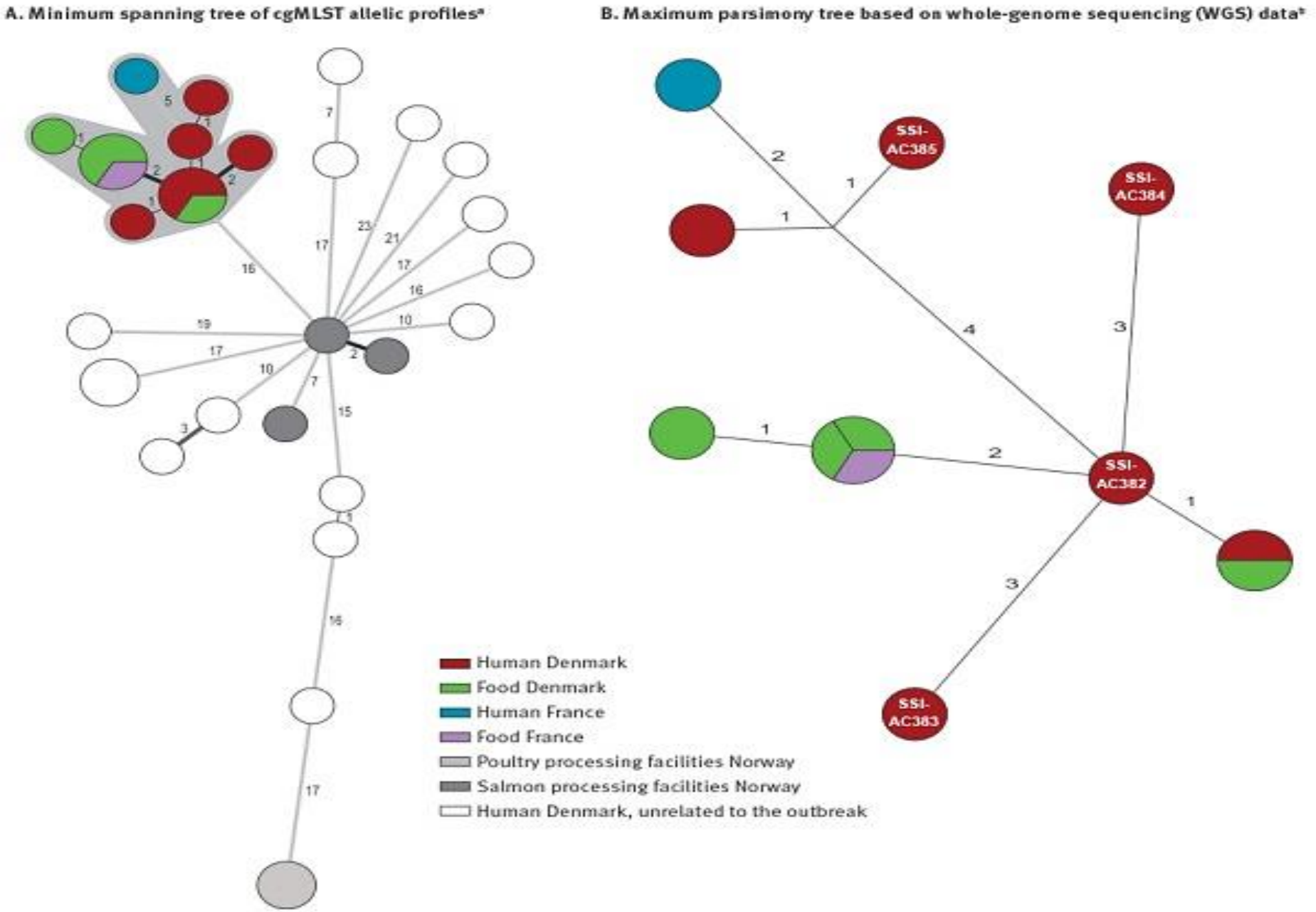
Description of the outbreak

- Read the paper and summarize the outbreak investigation steps
- How identical are the outbreak strains
- Which food was identified as source of the outbreak?
- How is the French case related to the Danish cases?



- cgMLST and SNP tree



Conclusion

- WGS has a higher discriminatory power and the ability to determine genetic distance between isolates.
- The introduction of WGS for surveillance of food-borne infections has shown that it improves outbreak detection and facilitates outbreak investigations and likely helps reduce the number of infections
- The studied case work highlights that by the application of cross-disciplinary and real-time cross-border comparison of WGS data, L. monocytogenes infections can be prevented and thereby providing safer food for at-risk groups such as the elderly, immunodeficient individuals and pregnant women.

Collaboration is key in foodborne outbreak investigation

Epidemiologist

Socio-anthropologist

Microbiologist