



FWD AMR. RefLabCap

Wrap up of the exercises/group work 3rd Multidisciplinary training workshop June 2024

Susanne Schjørring
Foodborne Infections, SSI, Denmark

Agenda (Day 5)

Day 5: 28 June, Online meeting		Presenter
09:00 - 09:50	Summary of the Table-top exercises	Project team FWD + Susanne Schjørring
09:50 - 10:00	<i>Coffee break</i>	
10:00 - 10:30	WGS-based surveillance as a paradigm shift in outbreak detection, AMR monitoring and source attribution in <i>Campylobacter</i> spp. in Portugal	Mónica Oleastro, Portugal
10:30 - 11:00	Salmonella Strathcona outbreak investigation and antimicrobial resistance profiles of <i>Salmonella</i> spp. isolates from 2000-2022 in Austria	Sabine Maritschnik, Austria
11:00 - 11:50	Data sharing, events, clusters and cut-offs	Cecilia Jernberg (ECDC)
11:50-12:00	Final remarks	Susanne Schjørring (SSI)

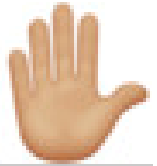
Data downloads

Evaluation survey

Wrap up exercise "House Keeping rules"



Microphones off



- Raise the "hand" or use the chat to ask questions



When the word is yours

- Please say your name, country - before the question



Key for a successful workshop is participation and you ask questions

The goal of this workshop

Each country

- Facilitate a stronger collaboration between microbiologists and epidemiologists
- Enhance knowledge of opposite expertise
 - Exercise

Between countries

- Enhance collaboration across Europe (everyone is an expert of their own country)
 - New acquaintances
- Knowledge exchange
 - Group meetings

Exercises in general

- Designed for teaching purposes
 - Both exercises are fictive
 - Do have elements from real outbreaks mixed
 - UK had an Salmonella outbreak of pork scrapings in 2021 – the data is not related!
 - <https://www.bbc.com/news/uk-england-manchester-58329447>
 - Campy ST464 is an ongoing outbreak in Denmark and EU (Cecilia), but the story is different
- During an investigation, a lot of things happen at the same time
 - In an Exercise – things need to be added stepwise
- Download the Illumina data for training purposes
 - Link in email after this meeting

Highlights Exercise *Salmonella*

Cut-offs

- FWD AMR-RefLabCap “proposed protocol for WGS..... “
 - – **A GUIDELINES** – NOT FIXED cut-offs!
 - Approach, analysis tool, number of loci, sequence quality, type of outbreak, time
 - clonal type (STm, SEnt, very few alleles) other serotypes allow more differences
 - Cross border – joint analysis!

Proposed protocol for whole genome sequencing-based analysis for detection and tracing of epidemic clones of antimicrobial resistant *Salmonella* and *Campylobacter* to be used for national surveillance and integrated outbreak investigations by NRLs for public health

<https://www.fwdamr-reflabcap.eu/-/media/arkiv/projekt-sites/fwdamrreflabcap/resources/protocols-and-guidelines/fwd-amr-reflabcap-wgs-protocol-8-july-2022.pdf>

Highlights Exercise *Salmonella*

Cut-offs

- Look at the Typing EQAs

<https://www.fwdamr-reflabcap.eu/eqas-and-ring-trails/links-to-other-egas>

Table 7. Reported results of allele-based cluster analysis

Laboratory	Approach	Allele-based analysis					
		Allelic calling method	Assembler	Scheme	Number of loci	Difference within cluster	Difference outside cluster
Provider	BioNumerics	Assembly- and mapping-based	SPAdes	Applied Maths (cgMLST/Enterobase)	3002	0-1	10-32
19	BioNumerics	Assembly based and mapping based	SPAdes	Applied Math (cgMLST/Enterobase)	3002	0-1	10-32
36	SeqPHERE	Only assembly based	SKESA 2.3.0	Enterobase (cgMLST)	3002	0-1	10-32
49	BioNumerics	Assembly based and mapping based	SPAdes	Applied Math (cgMLST/Enterobase)	3002	0	2-33
49*	BioNumerics	Assembly based and mapping based	SPAdes	Applied Math (wgMLST)	15.847	0-2	14-51
100	SeqPHERE	Only assembly based	Velvet	Enterobase (cgMLST)	3002	0-1	10-42
106	SeqPHERE	Only assembly based	SPAdes	Enterobase (cgMLST)	3002 [§]	0-1	9-39
127	Enterobase	Assembly based and mapping based	SPAdes	Enterobase (cgMLST)	3002	0-1	12-51
129	SeqPHERE	Only assembly based	Velvet	[§] Ad hoc scheme ("wgMLST")	3696	0-1	9-34
134	SeqPHERE	Assembly based and mapping based	SKESA 2.3.0	Enterobase (cgMLST)	3002	0-1	10-32
135	SeqPHERE	Only assembly based	SPAdes	Enterobase (cgMLST)	3002	0-2	11-32
142	Galaxy (In house)	Only assembly based	SPAdes	Enterobase (cgMLST)	3002	0-1	11-34
147	SeqPHERE	Only assembly based	SPAdes 3.11.1	Enterobase (cgMLST)	3003	0-1	11-33
148	Enterobase	Only assembly based	SPAdes	Enterobase (cgMLST)	3003	0-3	13-52
149	SeqPHERE	Only assembly based	Velvet	Enterobase (cgMLST)	3002	0-1	10-32

*:additional analysis

§: modified from submitted information

For detailed data, see Annex 11.



ECDC ROAs

Multi-country outbreak of Salmonella Senftenberg ST14 infections, possibly linked to cherry-like tomatoes

European outbreak case definition

The European outbreak case definition is as follows:

A confirmed outbreak case

- A laboratory-confirmed *Salmonella* Senftenberg ST14 case with disease onset on or after 1 June 2022 (date of sampling or date of receipt by the laboratory if date of onset is not available).

AND

- Fulfilling at least one of the following laboratory criteria:
 - within five cg-allelic differences (AD) from at least one of the representative German outbreak strains in the national cgMLST pipeline, OR
 - clustering within six cg-allelic differences in a single linkage analysis in a centralised whole genome sequencing (WGS) analysis, OR
 - belonging to the cgMLST HC5_325504 hierarchical cluster by EnteroBase scheme, OR
 - clustering according to a national single-nucleotide polymorphism (SNP) pipeline within five SNPs of the German outbreak strain, OR
 - belonging to a 5-SNP single linkage cluster with SNP designation 1.1.4.204.227.230.% according to the pipeline in the United Kingdom Health Security Agency (UKHSA) [3].

A possible outbreak case

- A laboratory-confirmed *S.* Senftenberg case with symptom onset on or after 1 June 2022 (date of sampling or date of receipt by the reference laboratory if date of onset was not available) without molecular typing data.

AND

- No or unknown history of travel outside of the EU/EEA seven days prior to onset of symptoms.

https://www.ecdc.europa.eu/sites/default/files/documents/ROA_S_Senftenberg-ST15_2023-FWD-00009.pdf

Multi-country outbreak of monophasic Salmonella Typhimurium sequence type (ST) 34 linked to chocolate products

European outbreak case definition

The European outbreak case definition is as follows:

A confirmed outbreak case

- A laboratory-confirmed monophasic *Salmonella* Typhimurium case with symptom onset on or after 1 October 2021 (date of sampling or date of receipt by the reference laboratory if date of onset is not available).

AND

- Fulfilling the following laboratory criteria: a monophasic *S.* Typhimurium ST34 isolate clustering with any of the two representative outbreak strains by:
 - the national cgMLST pipeline within five allelic differences (AD); OR
 - clustering in a centralised whole genome sequencing (WGS) analysis within five ADs in a single linkage analysis; OR
 - belonging to the same cgMLST HC5_296366 cluster according to the EnteroBase scheme; OR
 - falling into the 5-SNP (single nucleotide polymorphism) single linkage cluster 1.1.1.124.6096.7575.% (t5.7575) (eburst group 1), according to the UK Health Security Agency (UKHSA) pipeline; OR
 - the national SNP pipeline within five SNPs.

A probable outbreak case

- A laboratory-confirmed monophasic *S.* Typhimurium case with symptom onset on or after 1 October 2021 (date of sampling or date of receipt by the reference laboratory if date of onset is not available).

AND

- By phenotypical testing expressing resistance to ampicillin/amoxicillin, kanamycin/gentamicin, trimethoprim/co-trimoxazole (trimethoprim-sulfamethoxazole) and chloramphenicol.

OR

- MLVA (multilocus variable number tandem repeat analysis) profile 3-11-14-NA-0211 (determined by Belgium).

https://www.ecdc.europa.eu/sites/default/files/documents/ROA_monophasic-S-Typhimurium-ST34-linked-to-chocolate_2022-00014-final_UK.pdf

Highlights Exercise *Salmonella*

Cut-offs

- FWD AMR-RefLabCap “proposed protocol for WGS..... “
 - – **A GUIDELINES** – NOT FIXED cut-offs!
 - Approach, analysis tool, number of loci, sequence quality
 - clonal type (STm, SEnt, very few alleles) other serotypes allow more difference
 - Cross border – joint analysis!

Quick test

- Phenotypic serotyping
- AMR phenotypic
- Nanopore (DK: used for Listeria, other countries?)

EpiPulse events / When do we upload sequences to ECDC

- The use of Events is escalating
 - Time-consuming for all countries
 - Some countries prioritize already
 - Small countries find the information from our colleagues very valuable
- The more sequences in the joint DB - Molecular tool does the work !!!

Proposed protocol for whole genome sequencing-based analysis for detection and tracing of epidemic clones of antimicrobial resistant *Salmonella* and *Campylobacter* - to be used for national surveillance and integrated outbreak investigations by NRLs for public health

<https://www.fwdamr-reflabcap.eu/-/media/arkiv/projekt-sites/fwdamrreflabcap/resources/protocols-and-guidelines/fwd-amr-reflabcap-wgs-protocol-8-july-2022.pdf>

Highlights Exercise *Salmonella*

Multiple outbreaks of the same species at the same time

- Keep them separated as individual outbreaks, overlapping team members
- ?

Outliers

- ?

Communication

- Microbiologist – Epidemiologist
 - Suggestions for Improvements?

Highlights Exercise *Salmonella*

- Plasmid (mobile elements), phages
 - Include or exclude?

- Targeted plasmid analysis during an outbreak investigation
 - how/when do you know the outbreak is potentially plasmid mediated

Quistions?

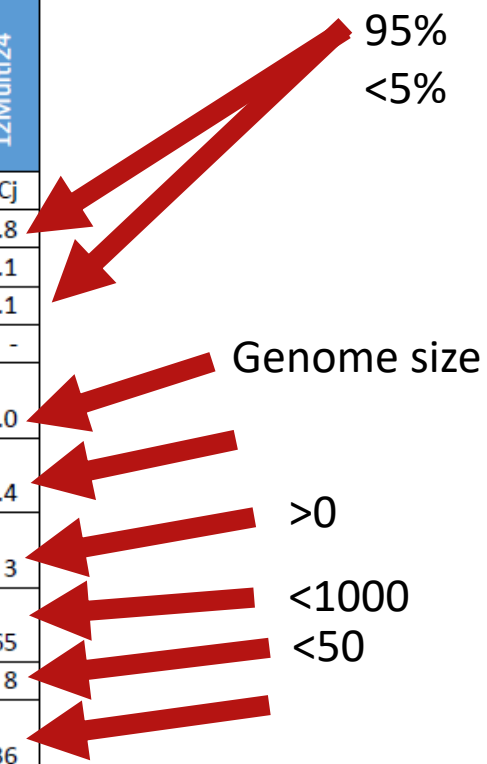
Highlights Exercise *Campylobacter*

- Surveillance of campylobacter is improving across Europe
 - Case-based, limited number of isolates -> Sentinel - > full surveillance
- Importance of Quality Control of the sequences
 - or conclusions might be wrong

QC Bifrost (SSI)

Table 3: Overview of QC output (Bifrost pipeline <https://github.com/ssi-dk/bifrost>)

Sample	1Multi24	2Multi24	3Multi24	4Multi24	5Multi24	6Multi24	7Multi24	8Multi24	9Multi24	10Multi24	11Multi24	12Multi24
Detected species	Cj	Cj	Cj	Cj	Cj	Cj	Cj	Cj	Cj	Cj	Cj	Cj
% Species 1	95.7	91.7	90.8	93.8	91.8	92.3	95.1	86.7	91.3	94.4	91.1	95.8
% Species 2	0.1	0.8	0.8	0.8	0.9	0.8	0.8	0.8	0.8	0.8	0.8	0.1
% unclassified	4.1	7.4	8.3	5.2	7.2	6.8	4.0	12.5	7.8	4.6	7.9	4.1
Sequence type	50	464	464	464	464	464	464	464	464	464	464	-
Length at >25X coverage (in Mbp)	1.7	1.7	1.7	1.7	1.7	1.7	1.8	1.7	1.7	1.7	1.7	0.0
Length at [1,25]X coverage (in Kbp)	0.0	0.0	0.9	0.0	0.4	0.0	0.0	0.0	0.4	0.0	2.5	379.4
Contigs at 25X coverage	52	55	77	47	64	57	47	68	62	68	143	3
Contigs at [1,25]X coverage	0	0	2	0	1	0	0	0	1	0	8	865
Average coverage	288	298	214	208	226	406	129	395	212	417	202	8
# Reads (in thousands)	3577	3941	2922	2651	2926	5316	1617	5831	2871	5302	2816	36
Average read length	138	134	129	138	137	134	143	122	129	138	126	138
Average insert size	213	195	195	242	211	218	280	153	199	199	166	203
N50 (in Kbp)	63	156	69	116	82	143	156	128	110	77	25	1
Bifrost	OK	OK	OK	OK	OK	OK	OK	OK	OK	OK	OK	warning



Cj: *Campylobacter jejuni*, -: missing data

Warning: **Test** 10xgenomesize: fail, Value (45057) below or above expected (1530000, 1840000). **Test** 1x10xsize: fail, Value (339602) above expected (250000). **Test** 1xgenomesize: fail, Value (384659) below or above expected (1530000, 1840000). Test avg coverage: fail, Lack of reads (7.674862670573157 < 10)

SNP stats

Table 2: Statistical analysis of the NASP analysis (sample_stats.tsv output)

Sample::Analysis Sample/Aligner/SNP Caller combination; was_called: Number of positions called A/C/G/T; was_called (%): Percentage of previous; passed_coverage_filter: Number of positions that passed the coverage filter[1], passed_coverage_filter(%): Percentage of above; passed_proportion_filter: Number of positions that passed the proportion filter[1]; passed_proportion_filter(%): Percentage of above; quality_breadth: Number of positions called A/C/G/T and passed all filters[1]; quality_breadth (%): Percentage of above; called_reference: Number of positions that matched the reference; called_reference (%): Percentage of above; called_snp: Number of positions that differed from the reference; called_snp(%): Percentage of above; called_dgen: Number of positions not called A/C/G/T[2]; called_dgen (%): Percentage of above.
<https://tqennorth.github.io/NASP/usage.html#id5>

Sample	Sample::Analysis	was_called	was_called (%)	failed_coverage_filter	failed_coverage_filter (%)	failed_proportion_filter	failed_proportion_filter (%)	reference_length	quality_breadth	quality_breadth (%)	called_reference	called_reference (%)	called_snp	called_snp (%)	called_dgen	called_dgen (%)
[any]		1713793	98.36%	70	0.00%	0	0.00%	1742330	1713723	98.36%	1713722	98.36%	18726	1.07%	920671	52.84%
[all]		795061	45.63%	711241	89.46%	58	0.01%	1742330	83815	4.81%	790331	45.36%	0	0.00%	2009	0.12%

Highlights Exercise *Campylobacter*

- Surveillance of campylobacter is improving across Europe
 - Case-based, limited number of isolates -> Sentinel - > full surveillance
- Importance of Quality control of the sequences
 - or conclusions might be wrong
- Cut-offs
 - FWD AMR-RefLabCap
 - – A GUIDELINES – NOT FIXED !
 - Approach, analysis tool, number of loci, sequence quality, type of outbreak, time
 - The species clonal
 - Cross border – same analysis
- AMR
 - ResFinder, AMRFinder+ or CARD
 - Use what you like and find useful
 - Participate in the FWD AMR EQA and Ringtrail
 - ECDC AST

Proposed protocol for whole genome sequencing-based analysis for detection and tracing of epidemic clones of antimicrobial resistant *Salmonella* and *Campylobacter*
- to be used for national surveillance and integrated outbreak investigations by NRLs for public health

Highlights Exercise *Campylobacter*

- Human–food side collaboration
 - Limited for most countries
- Communication to the public
 - Homepages ... SoMe?

Quistions?

General impression during the group work

- We saw good collaboration all around
 - Some were physically sitting together
 - Learning the complexity/specific knowledge of each other's daily work
- You were interested to hear how other countries are working
- Different setup of surveillance and outbreak management
- Interviews of all cases for a species vs. only looking for trends -> outbreak
 - Resources: focus only on other species or resistances (e.g. clusters with unwanted resistances)
- Collaboration with food-animal sector still often lacking (some NRL have food/animal isolates)
 - **Use the exercise of engaging in a training session with the food side**
- Different setup: if the signal of an outbreak comes from the lab side or epi side
 - National level – primarily “only local”



Denmark

Comparison of sequences from human and food/animal/environment

- national **S**equence based surveillance **O**f **F**oodborne **I**nfections (SOFI)
 - Presented at the 12th FWD network meeting in Helsinki (Sept 2023)
 - When working real-time surveillance and joint analysis

Outbreak group:

- We meet every Thursday at 9-10:30 and discuss all signals and prioritization and plan the investigation, have ad hoc meetings
- **Know each other from peacetime**
- permanent members /alternates (2 on each position)
 - SSI: Epi /Lab (comparison human-food sequences)
 - DVFA: Management /Lab (physical) /Lab surveillance
 - DTU Food: Epi – risk assessors + (comparisons human-food sequences)

Denmark joint Exercise in 2022

– 1½ day out of the office

- Human – food/vet side
- The focus was on cross-sector cooperation, communication, and information sharing
 - Enhance communication cross-sectoral daily work
 - Enhance public health impact – using the media
- Despite different sectors, we want to be seen as a United team
 - During an interview – we need to allow the “opposite” part to explain the entire story of the outbreak
- Want to include the communication departments early in the process
- More active on the website and SoMe
- Conclusion power Table
 - “unknown”, possible, likely and confirmed
 - List of criteria for each category





FWD AMR. RefLabCap

3rd Multidisciplinary training workshop
June 2024

COFFEE BREAK BACK AT 10:05