



FWD AMR. RefLabCap

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Wrap up of the exercises/group work 2nd Multidisciplinary training workshop October 2023

Susanne Schjørring
Foodborne Infections, SSI, Denmark

Agenda (Day 5)

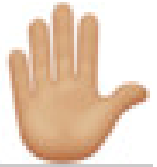
Day 5: 27 October, Online meeting		Presenter
9:00 - 10:30	Wrap up of the group work and results	Susanne Schjørring and volunteers
10:30 - 10:40	<i>Coffee break</i>	
10:40 - 11:00	One Health status on Campylobacter in Denmark	Katrine G. Joensen (SSI)
11:00 - 11:30	EU level surveillance using EpiPulse molecular typing tool	Cecilia Jernberg (ECDC)
11:30 - 11:45	Wrap up	Susanne Schjørring (SSI)

Evaluation survey

Wrap up exercise "House Keeping rules"



Microphones off



After each presentation there will be time for questions

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



When the word is yours

- Please say your name, country - before the question



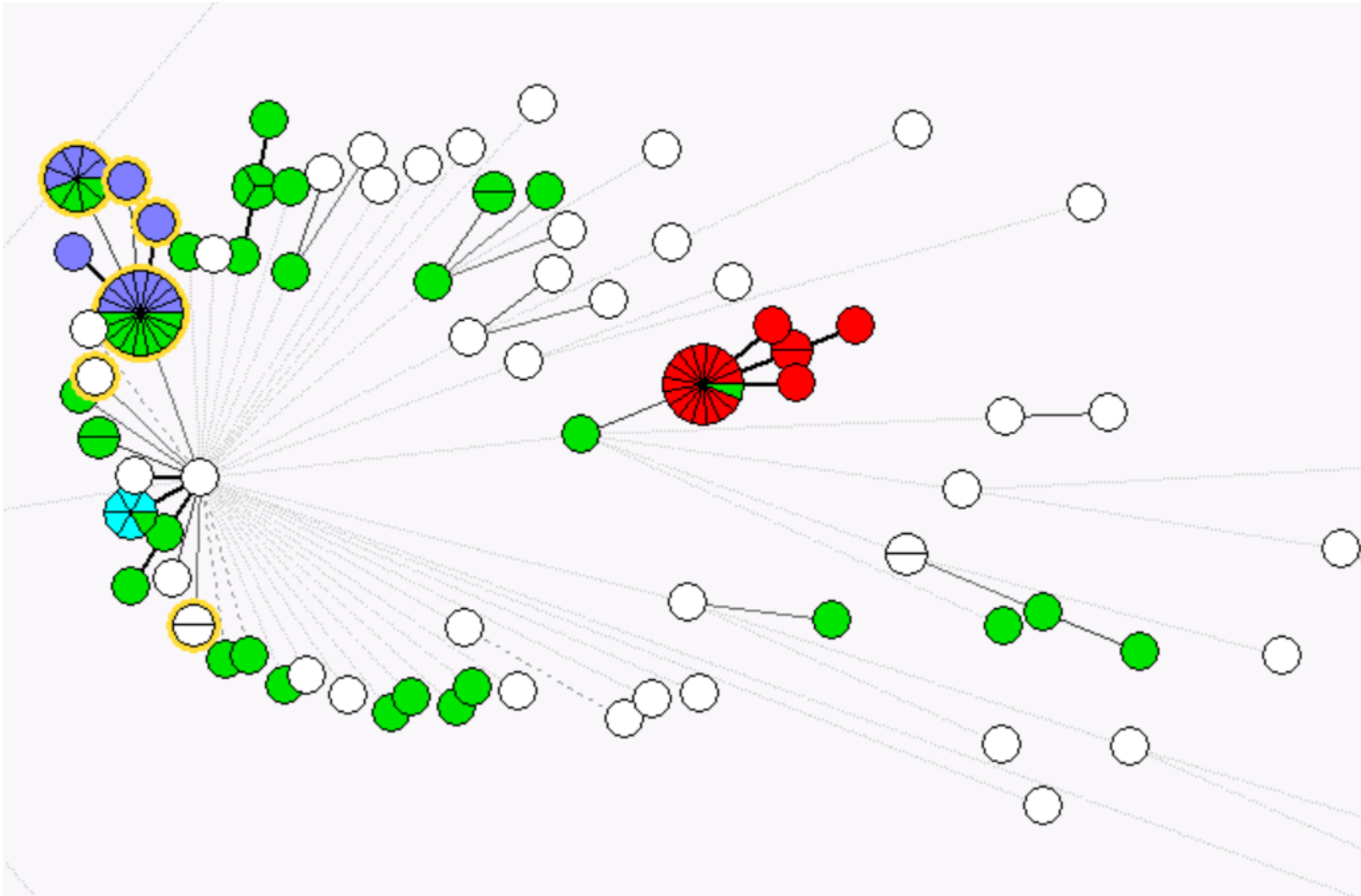
Key for a successful workshop is participation and you ask questions

Exercises in general

- Designed for teaching purposes
 - Do have elements from real outbreaks mixed together
- During an investigation, a lot of things happen at the same time
 - In an Exercise – things need to be added stepwise
- All the part about the involvement of the COMG is true, we meet every Thursday at 9 and discuss all signals and prioritization and plan the investigation, have ad hoc meetings
- 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)

Salmonella Exercise

- The outbreak sequences are from a true monophasic *Salmonella* Typhimurium outbreak
- We do detect almost all Salmonella outbreaks after WGS analysis (*S. Ent*, *S. Typ* and *S. mono Typ*)
 - cgMLST as routine, however local labs are alert
- The ASSuT profile of the cluster is most common here in DK
 - The AMR profile is used in an outbreak if special enough
- Interview data – specific for each country (like the cucumber for DK)
- Purchase cards/loyalty cards are a very valuable tool that we often use
 - A lot of information - more work, restaurants/ schools /nursing homes etc.
 - Joint effort by DVFA / SSI –DVFA during traceback detects connections between brands/production sites
- Microbiological evidence: Withdrawal (pulling the product back) / recall (“Calling”)
 - DK: Analytical studies also have an important impact



Pernille Gympoese et al, 2017: Investigation of Outbreaks of *Salmonella enterica* Serovar Typhimurium and Its Monophasic Variants Using Whole-Genome Sequencing, Denmark

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5621559/pdf/16-1248.pdf>

Campylobacter Exercise

- We have had a milk outbreak from a local producer
 - Different metadata, place and ST50, but a different cluster
- Signal was from the hospital during a national holiday – much higher number of cases
- We did sequences as many isolates as possible
- We did perform a case-control study (Some countries do case-case)
- Enterobacteriaceae was detected in the reference samples from days prior
 - Error in pasteurization or contamination of the product hereafter
- Negative *Campylobacter* results
 - In general only 25g of a food sample is tested, difficult food matrix, Campy is “delicat”
- Discussions about “water”

The goal of this workshop

- **Each country**
 - Facilitate a strong collaboration between microbiologists and epidemiologists
 - Enhance knowledge of opposite expertise

- **Between countries**
 - Enhance collaboration
 - Knowledge exchange

Cluster identification

- Different cut-offs in general
 - **There are NO fixed cut-offs (we only provide guidance)**
 - Salm: 6 sequences very closely related – 2 sequences with some additional distance
 - Prioritization – “strict vs loose cut-off”
 - **Pernille’s last Question**
 - Is it better to include too many or too few?
 - What works best for you, your data and your epidemiologist
 - DK: we prefer a tight cut-off, small outbreaks, too many “false” interviews will influence the investigation negatively (ALWAYS TALK TO THE EPI about borderline cases)
- Difficult cut-offs for clonal serovars
 - Additional tools (wgMLST, SNP) or hierarchical clustering (UPGMA / single linkage)
 - Epi data
 - Sharing the knowledge during the outbreak investigation – Campy Exercise
 - 2 clusters – the reason for not being a multistrain outbreak was the epi data (for the small cluster 2 cases)

Additional indicators for clustering

- AMR profile
 - Discrepancies between ResFinder vs AMRFinder+
 - What is best when reporting an outbreak ?
 - Listing specific genes/ variants or non-specific (antibiotic group)
 - Some also used CARD
 - check your blaOXA-genes (and all other bla-genes) in the Beta-lacam database (bldb.eu)
- Plasmid profile
 - <https://cge.food.dtu.dk/services/PlasmidFinder/>
- Loss/acquisition of the plasmids/new markers: unstable

International outbreak of ST34 in chocolate in 2022

Resistance profile

According to the analyses in the UKHSA, the outbreak strain is **susceptible** to azithromycin (MIC = 4-8 mg/L), ciprofloxacin (MIC = <0.06 mg/L), meropenem (MIC = 0.03-0.06 mg/L), cephalosporins including cefotaxime (MIC = 0.25 mg/L) and ceftazidime (MIC = 0.25 mg/L).

The outbreak strain is **resistant** to six families of antibiotics:

- penicillins (*bla*_{TEM-1});
- aminoglycosides (streptomycin, spectinomycin, kanamycin, and gentamycin (gene combination varies); *strA-strB*, *aac(6')*-Ia, *aac(3)*-IIId, *aph(6)*-Id, *aadA*-2, *aadA*-8b, *aadA*-12, *aadA*-15 and *aadA*-17;
- phenicols (*cmiA1*, *floR*);
- sulfonamides (*sul2* with some strains having an additional *sul3* gene);
- trimethoprim (*dhfrA12*); and
- tetracyclines (*tetA* and *tetM*).

In addition, some strains contained the *Inu(F)* gene encoding resistance to **lincosamide**, but this could not be confirmed phenotypically.

The resistance to aminoglycosides, phenicols, and trimethoprim is rare in monophasic *S. Typhimurium* and could therefore be used for screening of probable cases. However, France has noted that a minor proportion of their isolates matching the outbreak sequence have lost a DNA block containing several genes, including those encoding for this rare resistance.

Other questions

- Campylobacter survival when sent from clinical labs
- Questionnaire for Salmonella and Campylobacter available in Danish
- UPGMA: SSI only knows about command line
 - TreeIQ?
 - <http://genomes.urv.cat/UPGMA/>
 - [https://bioinformatics.com.cn/plot basic upgma clustering plot 102 en](https://bioinformatics.com.cn/plot_basic_upgma_clustering_plot_102_en)
 - “R”
- CSI Phylogeny – online but is it also command line? – not yet
 - NASP and SNIPPY as options for SNP analysis
 - MTBseq is good for wgSNP analysis, but it’s designed for TB, you can add your own reference strain for mapping
- Tools for visualization of phylogenetic trees
 - Fandango, GrapeTree, FigTree ... other suggestions –
 - iTOL: iTOL is a tool for visualising a tree and you can use an excel file (saved only on your computer) as a metadata file to populate on tree
- Approximate distance in SNPs in the phylogenetic tree
 - Use the distance matrix

EpiPulse events / When do we share sequences with ECDC

- The use of Events is escalating
 - Time-consuming for all countries
 - Some countries prioritize already
- DK: As a small country we find the information from our colleagues very valuable
- If everyone uploads all the sequences, then we don't need to download and compare ourselves – as the Molecular tool does that for us
- Wait for Cecilia (ECDC) to join



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COFFEE BREAK BACK AT 10:40