Minutes – EURL-AR Workshop, Kgs. Lyngby, April/2013

The minutes are listed according to the agenda.

Participants

From the EURL-AR-network, all member states (MS) with NRL-AR took part in the workshop. Luxembourg has not appointed an NRL-AR and did not take part. Participating non-MS were Croatia, Norway, Switzerland and Turkey, and also, a representative from EFSA participated. The representative from the EU Commission unfortunately had to cancel.

For the joint day between the FWD network and the EURL-AR network (Friday April 5th 2013), additional 26 participants from the public health sector participated, representing 19 EU countries, Iceland and Norway. Also, representatives from EUCAST and ECDC participated.

Thursday, April 4th 2013

Welcome (Rene Hendriksen, EURL-AR)

RH welcomed the EURL-AR-network to the facilities of the Technical University of Denmark (DTU) in Kgs. Lyngby. The meeting objectives and the invited speakers were introduced and the agenda presented. RH informed that Rosa Peran (representative from EU Com) unfortunately had to cancel her participation in the meeting.

Update from the EURL-AR (Frank Aarestrup, EURL-AR)

The issue of antimicrobial resistance is becoming more complicated and activities of the EURL-AR and antimicrobial resistance as such will remain huge and important to public health. Changes such as whole genome sequencing (WGS) will come. EURL-AR activities will be continued and expanded with the gradual inclusion of sequencing into the proficiency tests. We will aim towards the one-health aspect with more involvement from the public health side, for example by including the ECDC-FWD-network in the proficiency tests and in this joint meeting.

This past year, the EURL-AR has participated in a number of working groups addressing joint monitoring of antimicrobial consumption (EMA), ESBL- and carbapenemase-producing organisms and MRSA. We have implemented the ResFinder database that is publically available online. For the Danish monitoring programme, discussions are ongoing on focussing on genotypes instead of phenotypes and making this change within the next five years.

The EURL-AR are open for relevant research collaboration, however, this cannot be funded by the EURL grant but can be conducted in parallel as long as separate funding is available.

Summary of the plenary discussion:

- Concern was expressed as regards the change in monitoring to genotyping, since this would not directly supply an MIC-value. From the EURL-AR, it was stated that microbroth dilution would still be performed for DANMAP in the years to come, and even when genotyping is the first choice, it is foreseen that microbroth dilution will be performed for research purposes and for supplementary information. In addition, the EURL-AR will set up a 'phenomatic'-group aiming to compare genotypes and MIC's.

Update from the EU Commission

Cancelled due to nonattendance of representative from the EU Commission

Update from EFSA (Pierre-Alexandre Beloeil, European Food Safety Authority)

P-AB presented the new EU Summary report 2011 with new features including multi-resistance, co-resistance, analyses at animal population level and serovar level. He also presented the proposals for harmonization and the joint project with ECDC.

The report illustrates high resistance to ciprofloxacin and an increasing trend for cefotaxime resistance in *Salmonella* isolated from poultry. Multi-resistance is seen more often. *E. coli* is found to be more resistant to 3rd generation cephalosporins than *Salmonella*.

Three technical proposals were published from EFSA in 2012, i.e. technical specifications on/for:

- Harmonised monitoring and reporting of AMR in *Salmonella*, *Campylobacter* and indicator *E. coli* and enterococci bacteria transmitted through food.
- Harmonised monitoring and reporting of AMR in MRSA in food-producing animals and food.
- The analysis and reporting of data on AMR in the EU Summary Report.

By the request of the EU Commission, a new project has been launched in which EFSA, ECDC and EMA are collaborating to collect data from humans, animals and food, including microbiological data and consumption data from ESVAC.

Summary of the plenary discussion:

- Legislation on monitoring is pending. Work is ongoing on this issue and the new legislation draft will be discussed in 2013.
- A proposal for a harmonised definition of multi-resistance was made by the WG on AMR data collection, and included criteria from the public health side.
- That *E. coli* is found more resistant than *Salmonella* could be caused by some clonal lineages that are more resistant than others, or it could be caused by the fact that *E. coli* is more prevalent in animal populations than *Salmonella* and therefore is subject to more selective pressure. This issue has been discussed for years and is something that we should consider looking more into.

Outcomes of the EURL-AR EQAS 2012

Participants in the EURL-AR EQAS's presented summaries of results and commented on the outcome of the EQAS in their opinion. The audience participated in discussions about data and suggestions.

- *Escherichia coli* and *Salmonella* spp. (Dariusz Wasyl, Poland)
    - Provider is accredited to perform proficiency tests and the participation in the EQAS is an important tool for accreditation and management of the laboratory function.
    - Reporting via web-database works well.

- Agree with the current form of the EQAS; e.g. number of strains, availability of results, analysis and reporting, and with the exclusion of results with less that 75% concordance between obtained and expected results for a certain strain/antimicrobial combination.
- Overall, deviation level for *E. coli* and *Salmonella* as a group was okay.
- Suggestion: Protocol for identification of acc-1 gene could have been sent out, not only by request, but to all participants.
- Regarding the ESBLs, the EURL-AR has until now included strains that had a number of different ESBL-genes. It was discussed whether the selection should include 'normal' ESBL-producing strains rather than 'tricky' ones. The EURL-AR has based the selection of the strains on trying to mimic what is found in the daily routine with an effort to balance the challenges. Please note that the results obtained in the ESBL issues are not included in the deviation percent.
- For the ESBL-producing strains, it was suggested that differentiation should be made between chromosomal ampC and plasmidic ampC, also, it was discussed that the reporting of ESBL-producing organisms should be re-considered, e.g. to include a possibility to characterize isolates as ESBL-/AmpC-suspected.
- The EURL-AR will form a group to discuss the ESBL-component of the *E. coli* and the *Salmonella* EQAS.

- *Enterococcus* spp. (Gudrun Overesch, Switzerland)
  - Results obtained when testing streptomycin, erythromycin and ampicillin might indicate more general problems.
  - One laboratory performing DD exhibited a very high level of deviations.
- *Staphylococcus* spp. (Andreas Schroeter, Germany)
  - It appears that there is a need for laboratories performing DD for AST to move towards MIC-determination for more quantitative results.
  - It was suggested that the EURL-AR try to cover a larger range as regards MIC's of chloramphenicol, florphenicol, sulfamethoxazole and trimethoprim.
  - It was discussed to lower the acceptance threshold to 4% but the network agreed to maintain the threshold at 5%.
  - It was suggested to prepare cumulative graphs indicating three years' results for each laboratory.
  - In the current approach for evaluation of the EQAS results, the interpretation is evaluated; it was proposed to change this to an evaluation of the MIC values. This would demand large changes to the current system and would challenge the comparability with historical data. I will, however, be considered by the EURL as a future approach.
- *Campylobacter* spp. (Isabelle Kempf, France)
  - Especially regarding the accreditation, the NRL benefits from participating. Also, the EQAS is free and it is possible to have several technicians perform the test.
  - For routine AST, chloramphenicol resistance is rare; it would therefore be appreciated if CHL^R *Campylobacter* could be included from time to time.
- Genotypic characterization, ESBL-genes (Cristina Garcia, Belgium)
  - Stated that the EQAS was useful since it gives confirmation of the phenotypes, and checks the laboratory's methods for genotyping. The obtained results were satisfactory at gene level but not at variant level.
- Methicillin-resistant *Staphylococcus aureus* (MRSA) (Antonio Battisti, Italy)
  - Overall good results, with the challenge at the NRL to either run the EQAS-samples in the same system as routine samples or to let them undergo special handling. With the first approach, the performance would indicate any relevant issues.
  - Some results were false-positives due to the fact that one MSSA was *spa*-typed but no result was expected from this strain. MRSP was included due to a request from a

former workshop; these may be underreported because laboratories usually look at coagulase-positive staphylococci.

Final remarks to the EURL-AR EQASs:

All three EQAS-reports were approved for ISBN-registration without further comments; i.e. the reports on the EQAS on the *Salmonella*, *Campylobacter* and genotypic characterisation, the EQAS on E. coli, enterococci/staphylococci and the EQAS on MRSA.

For the 2013, *Enterococcus* and *Campylobacte*r species will not be given, but a protocol for speciation will be available. Also, in 2013, the MRSA EQAS will be together with the staphylococcus AST EQAS, including confirmatory testing and typing. I.e., no isolation component will be included.

EFSA scientific opinion on monitoring (Pierre-Alexandre Beloeil, EFSA)

Combined reports on monitoring incl. *Salmonella*, *Campylobacter* and enterococci were presented. The monitoring proposal focused on animal populations and also includes review of the microbroth panels.

Summary of the plenary discussion:

- It was stated that the EC is working to derive funding for co-financing of monitoring of resistance since some countries may not have capacity to execute the monitoring requirements without financial support. The EC will communicate more on this when the agreements are final.
- The veal calves mentioned as a specific animal population cover fattening veal calves under 1 year of age.

Comments from a national point of view to the EFSA scientific opinion on monitoring (Yvonne Agersø, Denmark)

YA gave an introduction to the general monitoring priorities in Denmark and the less focused areas. Special focus the latest years was given to ESBL-producing organisms and MRSA, and the recent results and conclusions so far.

The national monitoring programme in Denmark and the EFSA requirements correlate, however, for *Campylobacter* in pork or beef it is not feasible due to our very low prevalence. Due to this low prevalence in pork and because most *Campylobacter* human infections are *C. jejuni*, *Campylobacter* (which is mainly *C. coli*) will not be monitored in pigs in 2013. Also, in addition to the monitoring of nationally produced meat, we monitor the imported meat. On the human side we divide *Salmonella* and *Campylobacter* cases into travel-acquired infections and nationally acquired. We have less focus on enterococci than previously. But *E. faecalis* will still be monitored in pigs and enterococci will still be monitored in Danish and imported pork, beef and broiler meat.

Summary of the plenary discussion:

- Regarding the monitoring of MRSA if the intention is to monitor the diversity of MRSA strains or detect emerging MRSA monitoring at the slaughterhouse can be done. However real prevalence studies should be done at farm level. Denmark has done work with comparing the prevalence at farm levels with the prevalence at the slaughterhouses which clearly shows an increase over time at slaughter.
- As for comparison of resistance between domestic and imported products into Denmark; actually, EFSA had recommended to focus at domestic food, and imported food from 3rd countries, but this was not retained by the EC and is therefore voluntary.
- It might not be necessary to monitor each source each year. Monitoring for carbapenemase-producers should be introduced with selective isolation, for example.
- The number of 170 is a compromise. Note that it is stated in the guidelines that in case of low prevalence, the possible number should be tested for susceptibility.

Review of designs for microbroth panels

Based on the latest EFSA and EC proposals, presentations and discussion were made to the extent possible on the microbroth panel design. For all panel designs, we need to await a (hopefully relatively quick) decision from the EU Commission to be able to order the new plates. When we know the final legislation we would go on and see what flexibility is offered and instigate work on the actual panel design.

Comments on the specific proposals for panel designs:

- *Enterococcus* spp. (Suvi Nykasenoja, Finland)
  - For the moment, Q/D is not available for the production of microbroth panels (subsequent to the meeting, we received a message from Thermo Fisher Scientific stating that they have *been supplied Synercid by Pfizer and currently have stock. As to the future this is really in Pfizer's hands, however they have told us they have solved their problems. So as far as we are concerned we can continue supplying plates with this compound).*
- *Staphylococcus* spp. (Andreas Schroeter, Germany)
  - The ranges on the antimicrobials are long; maybe we could include more different antimicrobials instead.
- Gram negative microorganisms (Sophie Granier, France)
  - In the new panel, kanamycin was removed; ceftazidime and meropenem were added. Other antimicrobial (ex: colistin) concentration ranges were updated according to EFSA and EC proposals.
- ESBL-panel (Dariusz Wasyl, Poland)
  - Two ESBL-panels are presented which should fit into one microbroth panel (96 wells). Isolates from the selective isolation must be further tested with the second and third panel of antimicrobial substances.

Transition to newly adapted Hungarian AMR monitoring system (Szilárd Jánosi, Hungary)

The Hungarian NRL performs a large number of diagnostic tests. Until 2010, AST were performed by DD for *Salmonella*, *E. coli*, *Enterococcus* and by MIC determination for *Campylobacter*. Since 2011, AST by DD has been discontinued and MIC determination, only, is performed. Challenges in the transition period were presented.

Comment from the EURL-AR:

- NRLs which are setting up new methods and experience difficulties are welcome to contact the EURL-AR for assistance. The EURL-AR has funding that can be used for site-visit for this purpose.

ESBL in poultry meat in Slovenia (Irena Zdovc, Slovenia)

In the work with pet animals the largest problem appears to be MRSP. For livestock the largest problem appears to be regarding MRSA/ESBL. MRSA screenings are done with special focus on therapeutic animals that are in contact with patients. In MRSA screenings in cattle, CNS has also been detected. In humans, MRSP might be underestimated, as human colleagues isolate coagulase positive staphylococci and do not identify *S. pseudintermedius* strains.

ESBL studies showed good collaboration between the veterinarian and public health sector and ESBL were mostly CTX-M type 1 (in human, animals and meat). The plans for the future include finding critical points for contamination.

Activities of the NRL in Norway (Marianne Sunde, Norway)

In Norway, most livestock production is small scale with little export, except for salmon. Several NRL-functions and diagnostics for pets, production animals and fish are based at the Norwegian Veterinary

Institute. The NORMVET-programme includes data on resistance and antimicrobial usage and indicates a low and stable resistance level, however, many new resistance types have been detected lately: MRSA, MRSP, ESBL in broilers, and PMQR in *E. coli*.

Several studies have been made on MRSA in swine, documenting low prevalence. In 2011, however, several samples were positive in one slaughterhouse. An attempt to identify the positive herds did not lead to MRSA-detection. Another approach was taken, in which the environment inside the slaughterhouse was sampled and MRSA detected. All were same *spa*-type. More sampling is upcoming.

ESBLs were first found in broilers in 2006. In 2011, CMY-2 were found in 43% of flocks. New research projects are starting out, one of them a collaborative project between Norway, Iceland and Sweden.

For fish, infections used to be controlled with massive consumptions of antimicrobials. Today, due to vaccination, the problem with infectious diseases is almost absent. Currently, we have almost no knowledge of resistance in fish pathogens.

<u>General discussion incl. suggestions for future network projects</u> (René Hendriksen, EURL-AR)

Action items, NRLs:

- The network was encouraged to pass on ideas for training courses for the EURL-AR to arrange relevant courses, possibly including differential teaching, i.e. parallel tracks.
- EUCAST is interested in receiving data on veterinary pathogens and specific serovars. Templates and further information for sending data is available upon request from the EURL-AR.

Action items, EURL-AR:

- The EURL-AR will work towards setting up a 'phenomatic'-group aiming to compare genotypes and MIC's.
- For the microbroth panels, the EURL-AR will contact EU Com with relevant input from this meeting.
- When the final legislation is issued, the EURL-AR will assess what flexibility is offered and instigate work on the actual panel design for broth microdilution panels.
- The fact that *E. coli* is found more resistant than *Salmonella* could be caused by some clonal lineages that are more resistant than others or by the fact that *E. coli* is more prevalent in animal populations than *Salmonella* and therefore is subject to more selective pressure. This issue has been discussed for years and is something that we should consider looking more into.
- For ESBL- and carbapenemase-component of the *Salmonella* and *E. coli* EQASs, the EURL-AR will suggest an approach that can be applied in future EQAS's.
- The EURL-AR will discuss the evaluation of EQAS results could be based on obtained values instead of the interpretations.
- For the EQAS reports, the EURL-AR will consider including cumulative graphs indicating three years' results for each laboratory.

Friday, April 5th 2013

Welcome (Rene Hendriksen, EURL-AR)

This is the first time we have a joint meeting between the ECDC-FWD-network and the EURL-AR network. The agenda was drafted in collaboration with EFSA and ECDC with the aim to illustrate and understand the difference between the networks. Unfortunately there was a cancellation from Rosa Peran from the EU Commission for today's meeting.

Proposal for harmonisation of AMR in human *Salmonella* and *Campylobacter* isolates (Therese Westrell, ECDC)

The FWD-network only recently started focusing on AMR and when collecting data for the joint EFSA-ECDC report, harmonisation activities were instigated. The FWD-network evaluated the panel of antimicrobials used for the AST of *Salmonella* and *Campylobacter* and added rationales to the inclusion of each antimicrobial. It is proposed that the FWD-laboratories would report MIC values or zone diameters to ECDC so that the data can be interpreted with either clinical breakpoints or epidemiological cut-off values, depending on the situation.

Harmonisation of AMR in veterinary *Salmonella* and *Campylobacter* isolates (Pierre-Alexandre Beloeil, EFSA)

The background for the current monitoring of AMR in the food/vet-side was presented together with the reports intended for the drafting of the new legislation. Proposed panels were presented with rationales.

Panel discussion (EFSA (Pierre-Alexandre Beloeil), ECDC (Dominique Monnet, Therese Westrell), EURL-AR (Frank M. Aarestrup), EUCAST (Robert Skov), *representative from the EU Commission unfortunately had to cancel*) Each of the participants of the panel briefly introduced their concerns and expectations as regards harmonisation initiatives between the human and food/vet sectors) Chair: Jean-Yves Madec, NRL-AR, France

Summary of the panel presentation and discussion:

- Monitoring of AST and antimicrobial consumption in the food and veterinary sector is performed for the purpose of protecting public health.
- Data comparability is important for the epidemiological overview so the antimicrobial panels should be in agreement for the most important data. Preferably data should be quantitative from both the veterinary and the public health side.

- EUCAST encourages the application of susceptibility testing methods for obtaining quantitative data. The most important thing when obtaining data for monitoring is, however, to apply well-calibrated methods for AST.
- The EU Com requests joint reports including data on AMR and antimicrobial consumption. It is a process to introduce this in a comparable manner across sectors. The FWD-network has been discussing whether it is feasible to report zone diameters or MIC values and report this data to ECDC for the interpretation.
- Discussions on cephalosporins and carbapenems are ongoing, also regarding sampling and enrichment strategies for ESBL- and carbapenemase producing organisms. In this context, the preferred approach may be different in the food/vet-sector compared to the public health sector.
- It will be continually important to have an ongoing proficiency test programme. The financial issue in this context needs consideration.
- Instead of accepting quantitative DD-results, it was suggested to select a subset of the samples and test them by microbroth dilution and in this way obtain comparable data (maybe at the end of the year). An opposing argument to this was that if following the protocol, AST by DD supplies good data for most drug combinations, and also, selecting a subset would mean disregarding data on many samples.
- In general, results should be reliable, reproducible and comparable. As long as data is validated, well-evaluated and quantitative, allowing the change of breakpoints for potential re-analysis, both inhibition zones and MIC-values are fit for use. However, the EURL-AR has found it easier to harmonize the broth microdilution method and has experienced that AST by DD is less reproducible than AST by microbroth dilution.
- The 170 isolates mentioned in the draft monitoring pr. country for each bacterial species/animal population is a statistical measure decided by statisticians and is a minimum requirement.
- There is a very large interest in colistin, and when testing *Salmonella* in the food/veterinary sector, colistin should be included with a higher range than just a few steps. It might be reasonable to act differently in the sectors regarding this issue so that the food/veterinary sector monitors this drug and can detect if it is becoming a problem. ECDC will ask their network whether colistin can be included in the panel for human isolates. EUCAST states that colistin should not be tested by DD but only by dilution methods. (The latter is because of its chemical properties which also cause problems when doing microbroth dilution as it binds to the plastic. These problems will of course affect the reliability of the results.)
- There is a continuous need to update the legislation on the food/veterinary sector when the guidelines change. The list of antimicrobials, ranges, and/or interpretative criteria may need to be reviewed every 3-4 years.

<u>The Odyssey of multi-drug resistant *Salmonella* Kentucky ST198 strain</u> (Simon Le Hello, Institut Pasteur, France and Dariusz Wasyl, NRL-AR Poland)

Recently, an emerging *Salmonella* Kentucky ST198-X1 clone has been described (Le Hello, JID 2011). It has accumulated various chromosomal resistance determinants since the mid 1990s with the integration of the *Salmonella* genomic island 1, followed by cumulative mutations in the *gyrA* and *parC* genes leading to resistance to nalidixic acid then to ciprofloxacin in 2002. This population has now rapidly spread throughout Africa and the Middle East and now India and Southeast Asia. Another matter of concern is the widening livestock reservoir of this *Salmonella* Kentucky CIP-R strain: initially identified in autochthonous poultry but then found in various animals and food (contaminated spices in France and the US, turkey flocks in Germany and Poland). Recently, an outbreak of human-born infection of turkey flocks was noted in France, whereas in Poland the clone was found in companion carnivorous reptiles. Of great concern, these isolates resistant to ciprofloxacin are now producers of various carbapenemases and/or cephamycinase and/or ESBLs and the countries of acquisition are widening

Summary of the plenary discussion:

- When something is emerging and is a threat to human health, ECDC will do a risk assessment. Microbiologists therefore need to link with epidemiologists to collect sufficient data to act on from the political perspective.

Who looks... can find them. Carbapenemases are also present in animal/food isolates (Beatriz Guerra Román, Federal Institute for Risk Assessment (BfR), Germany)

Within the German RESET project (www.reset-verbund.de) several longitudinal and cross-sectional studies, collecting potential ESBL-carrier organisms from German farms, were performed (using MacConckey with 1mg/L cefotaxime as selective medium). Three of 14 farms sampled (no kknown epidemiological link) resulted positive for carbapenemase-producing *Escherichia coli* and *Salmonella* isolates. The isolates, *S.* Infantis and *E. coli* phylogroup A, were isolated from one broiler and two swine farms from faeces, environmental (in or outside farm), manure, mice and fly samples. The isolates showed resistance to third generation cephalosporins, and carried the ACC-1 AmpC-encoding gene. In the carbapenem susceptibility tests, these isolates showed values equal/above the EUCAST cut off values, but did not show resistance according to the CLSI or EUCAST breakpoints (so they were detected by chance). In the presence of high concentration of carbapenems, the isolates grow. The isolates carried produced the blaVIM-1 carbapenemases. Both *bla*ACC-1 and *bla*VIM-1 genes form part of integron/transposons located on large HI2 plasmids. Although carbapenamase occurrence is primarily related to human community and hospital settings, carbapenemase producing isolates are also present in livestock, probably more frequently than we thought.

Summary of the plenary discussion:

- Frustrating that this type of bacteria can be found around or in farms even though carbapenems are not used for treatment of animals. The scientists do not know if the farmers had been hospitalized or travelled, no common veterinarian. There was no apparent link.
- We should be alert and make sure that we have methods that ensure that we can detect these carbapenem non-susceptible phenotypes.

Guidelines for detection and confirmation of ESBL-, ampC- and carbapenemase-producing *Enterobacteriaceae* (Pierre-Alexandre Beloeil, EFSA and Robert Skov, EUCAST)

Pierre-Alexandre presented the methodology for the detection and isolation of ESBL and carbapenemases from EFSAs technical specifications and Robert Skov presented the guidelines based on EUCAST recommendations.

Summary of the plenary discussion:

- Document from EUCAST is on consultation, and is hopefully finalized for publication in July.
- Genotypic confirmation has not been included in the EUCAST guidelines, for the time being, as a routine method. Many of these enzymes may be present without having an effect on the clinical outcome, so without reason, the only option for the medical doctor could be colistin. At one point of time, genotyping may be the first choice and phenotype next, but for the time being, the EUCAST recommendations do not include genotypic confirmation.

Methods for AST; pro's and con's of disk diffusion and microbroth dilution including discussion (Kees Veldman, NRL-AR, the Netherlands and Robert Skov, EUCAST)

Kees gave an overview of both methodologies and of variants of MIC-determination mentioning pro's and con's for microbroth dilution: pro's; Internationally standardized, hard (quantitative) figures (MIC's), reproducible and robust, automated systems available, con's; expensive, requires more lab facilities, and mentioning pro's and con's for disk diffusion: pro's; cheap, easy to perform con's: results

derived from MICs, many methods available (e.g. ISO, CLSI etc), maybe less robust and reproducible.

Robert followed up with additional comments to the methods and highlighted that the microbroth dilution method masks a lot of the variation since it is based on 2-fold dilutions.

Summary of the plenary discussion:

- In the EURL-AR network we have observed that AST performed by DD exhibits a higher deviation level. Strict QC-control is crucial, and there are many factors that need to be standardized when performing AST by DD compared to microbroth dilution, so possibly microbroth dilution is easier QC-assured. However, it should be noted that a large part of the deviation is due to problems with the breakpoint for disk diffusion for ciprofloxacin and not an error of the DD method in itself. EUCAST will look into the problems with the DD breakpoint for salmonellae.
- The acceptable deviation for a normal susceptible strain is five dilution steps or 10 mm in zone diameter.
- It is great to be able to use EUCAST; it is free and available.

AST EQAS of *Salmonella* spp. and *Campylobacter* - summary of NRL-AR- and ECDC-FWD-network-results (Susanne Karlsmose, DTU Food, Denmark)

Results from the networks' participation in the same EQAS rendered acceptable results from both networks on both the *Salmonella* and the *Campylobacter* spp. The low-level quinolone resistance appeared to cause some problems when disk diffusion was applied for AST. For *Campylobacter*, some public health laboratories applied E-test for AST which does not have international references for quality assurance.

Summary of the plenary discussion:

- For this type of joint EQAS with strict reference to EUCAST ECOFFs, the applied interpretative criteria for all possible methods should be clearly specified in the protocol. It was considered an unfair comparison to interpret all results with ECOFFs when FWD laboratories using DD had been advised to use their normal interpretation, which is based on clinical breakpoints.
- It was discussed whether the EQAS instead of the interpretations could evaluate the obtained values. The EURL-AR will discuss if this should be pursued and in that case how.

(Subsequent to the meeting, the comparison report has been discussed further between ECDC and the EURL-AR).

Genotyping using whole-genome sequencing – a realistic alternative to surveillance based on phenotypic AST (Henrik Hasman, DTU Food, Denmark)

Henrik gave a description of the NGS system, assembly and structure of tools and databases available. Furthermore, the study comparing phenotypic monitoring with WGS was presented and detailed; out of 200 bacteria (50 *Salmonella*, 50 *E. coli*, 50 *E. faecium* and 50 *E. faecalis*), ID of three could not be confirmed in the genotyping, therefore, in total, 197 isolates were included in the analysis. The study concludes that it is a possible solution to apply genotyping in the surveillance as an alternative to phenotypic AST.

Summary of the plenary discussion:

- For the time being, we do not directly get the information on the location of genes (plasmids or co-location), however, sometimes if the contigs are very big it might be possible to find.
- A specific MIC is not predicted directly by the genotype, the variables we are working with are 'resistant/susceptible'.

Future perspectives and closing remarks (René Hendriksen, EURL-AR / Dominique Monnet, ECDC)

The joint day has exhibited differences and similarities between the two networks. It has been valuable to see that both networks are on the same page in many areas. The scientific presentations on *S.* Kentucky and on carbapenemase-producing organisms indicate that we have emerging problems. For studies such as these, the one-health perspective is important and epidemiological data should be included in future studies.

For the time being, future joint workshops have not been agreed, but will be discussed.

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