3rd joint meeting on AMR in *Salmonella* and *Campylobacter*, FWD-Network and EURL-AR Network and 11th EURL-AR Workshop SSI, Copenhagen, April/2017 – minutes

The minutes are listed according to the agenda.

Participants

From the EURL-AR-network, all EU member states (MS), were represented at the workshop. Participating non-MS were Albania, Iceland, Kosovo[1], the former Yugoslav Republic of Macedonia, Norway, Serbia, and Switzerland. Additionally, representatives from the EU Commission, Directorate F, and EFSA participated.

From the FWD-network coordinated by ECDC, 22 EU member states (MS) (i.e. all MS except Austria, Bulgaria, Croatia, Greece Latvia and Luxembourg) were represented at the workshop. In addition, representatives from Albania, Bosnia and Herzegovina, the former Yugoslav Republic of Macedonia, Iceland, Norway, Serbia and Turkey participated.

In total, 37 countries were represented, including all EU member states.

Thursday, April 6th 2017

Welcome to the joint meeting (Kåre Mølbak, SSI, Dominique Monnet, ECDC & Rene Hendriksen, EURL-AR)
Participants were welcomed at the 3rd joint meeting on AMR in *Salmonella* and *Campylobacter*. Kåre emphasised the threat of resistance as one of the most important threats to the health sector as well as to the community. He highlighted the importance of One Health and the need to collaborate to solve problems with zoonoses and AMR.

Introduction to the meeting agenda (Rene Hendriksen, EURL-AR & Dominique Monnet, ECDC)
Meeting agenda was introduced – both related to the joint meeting and the separate meetings. The important thing is that we now have the opportunity to network.

[1] This designation is without prejudice to positions on status, and is in line with UNSCR 1244/99 and the ICJ Opinion on the Kosovo declaration of independence

Update from European Commission (joint meeting) (Ángela Bolufer de Gea, European Commission)

See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)

The European Commission has since long recognized the need for action to tackle antimicrobial resistance. The first Commission strategy was published in 2001 and followed by a comprehensive action plan in 2011. This plan encompassed a series of actions on monitoring, surveillance and antimicrobial consumption in both humans and animals.

In October 2016 an evaluation of the 5-year action plan (2011-2016) was published which highlighted that although the outcome of the actions had been positive and the plan had showed EU added value, continued action was needed, e.g. there is a need to support member states and reinforce the One Health approach. In addition, continued actions should include environment and more research on alternatives to antimicrobials, vaccines and diagnostic tests.

As a response to the evaluation and to the Council Conclusions of June 2016 in which Member States called for new action plan, the European Commission will publish a new European One Health Action plan against AMR in June 2017. The efforts build on three strategic pillars – making the EU a best practice region on AMR, boosting research, development and innovation on AMR and shaping the global agenda on AMR. In the first pillar actions are planned to strengthen One Health surveillance and reporting of AMR and antimicrobial use and to benefit from the best evidence-based analysis and data.

As regards Decision 2013/652/EU, it covers the years till 2020. Currently, audits are being performed by Directorate F. A revision of the Decision is planned under the new One Health action plan.

Update from ECDC FWD (Therese Westrell, ECDC)

See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)

Therese Westrell, ECDC, gave an update on current issues of AMR within the ECDC Food- and Waterborne Diseases and Zoonoses. One topic was the revision of EU case definitions, including the request to make reporting of AMR in e.g. *Salmonella* and *Campylobacter* infections mandatory. The new EU case definitions will most likely published before the end of 2017 and come into force in 2018.

Therese Westrell also informed on the Global Antimicrobial Resistance Surveillance System (GLASS) set up by WHO. GLASS covers clinical resistance in isolates from invasive (hospital-acquired) infections, as well as infections with *Neisseria gonorrhoeae*, *Salmonella* and *Shigella*. WHO and ECDC are currently discussing on the data transfer from EARS-Net and FWD-Net to avoid double reporting for the EU Member States. Surveillance of AMR in *Shigella* will most likely be added to the annual data call in 2018.

Update from the EURL (joint meeting) (Rene Hendriksen, EURL-AR)
See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)
Rene Hendriksen reported from the activities in EURL-AR. They include scientific advice and support to others (GLASS/Global action plan (GAP) and WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR)), co-ordination of National Reference Laboratories and provision of technical support, ring trials (EQAS), comparative testing and quality assurance, evaluation and development of analytic methods, and site visits for specific assistance to individual laboratories. EURL-AR also runs workshops and E-learning (https://www.coursera.org/learn/antimicrobial-resistance).

Update on the 2015 results from AMR monitoring in zoonotic bacteria from animals and humans (Pierre-Alexandre Beloeil, EFSA and Therese Westrell, ECDC)
See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)
Pierre-Alexandre Beloeil, EFSA, presented data on the AMR monitoring in zoonotic bacteria from animals, while Therese Westrell, ECDC, presented the monitoring from humans. Overall, the new legislation on AMR monitoring in animals and food has been successfully implemented by the MSs. To note is that isolates from humans comes from persons who are ill and visit a doctor or a hospitals while in animals, the data are from the main food-producing animals and meat derived thereof. It was stressed that there is a need for more countries to deliver data on both sectors which for 2015 was low. In humans, it is important to split the *Salmonella* data into resistance by serotypes for a better comparison with the data from different animal types and comparisons between countries when the *Salmonella* data are biased to certain serotypes. Doing this means that more isolates are needed to be tested, which is a challenge. Since much resistance is observed in *Campylobacter coli*, it would be good to obtain *C. coli* data from poultry, albeit not mandatory to test, to be able to compare between sectors.

It was highlighted that frequent resistance to fluoroquinolones has been observed in animals, but low resistance to other critically important antimicrobials, except for *C. coli* in a limited number of MSs. There is also a low occurrence of ESBL/AmpC producers. On a voluntary basis, a high number of MS have introduced selective monitoring of carbapenemase-producing *E. coli* (no positive results were reported). Two different MSs each detected one single carbapenem-resistant isolate in other monitoring in 2015 and follow-up has been performed.

Preliminary results from second JIACRA report (Pierre-Alexandre Beloeil, EFSA and Dominique Monnet, ECDC)
See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)
Pierre-Alexandre Beloeil and Dominique Monnet presented the progress of the JIACRA II working group, a joint working group set up at the request of the EC by ECDC, EFSA and EMA. The schedule is very tight, as some 2015 data were not available by mid-February 2017, and the analyses are still on-going. The intention is to cover the years 2013, 2014, and 2015. Multivariate analyses have also been included, thus allowing for the study of the

relationships between multiple factors. The final report is expected to be delivered to the EC by 30 June 2017.

Update from the EURL (separately for EURL-AR network) (Rene Hendriksen, EURL-AR)
See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)

The EURL-AR activities were presented. The ResFinder has been updated including more carbapenemase genes, metal resistance genes, and upregulated AmpC's.

The EURL-AR annually goes on a site visit in one country. The country we approach may have faced issues in one of the EQAS or the visit could be based on comments from the audits performed by Directorate F.

E-learning has been developed to describe the basics about WGS. And a training course is being planned in September 2017 focusing on WGS.

Update from European Commission (separately for EURL-AR network) (Ángela Bolufer de Gea, European Commission)
See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)
Introduction to this year's monitoring which will be of pigs and bovines <1year of age, pig meat and veal. Most MS have responded that they would isolate CP-producing *E. coli* from caecal samples and meat. Based on the reports from the MS, reimbursement ceilings have been defined. It is the impression of the EU Commission that the tests are becoming less and less expensive, therefore the reimbursements ceilings will likely be adjusted.

Note that '300' refers to the number of samples, not the number of isolates.

Summary of the plenary discussion

Directorate F are conducting the audits and one of the main focus areas is to understand where updates to the legislation (2013/652/EU) are necessary. Also, EFSA needs to give advice related to number of isolates and/or microorganisms.

There has been an issue related to samples taken in December. These are analysed the following year, however work in the following year is not covered in the Grant Decision sent to Member States (as the time sheet would belong to the following year). This could be an issue in relation to the reimbursement. Angela mentioned that at the EU Commission they had not heard from MS if this is a problem. If it is, it could be relevant to consider for a revision of the Grant Decisions.

Shipping costs were mentioned as a relevant issue to discuss related to a revision of the Grant Decisions. It was mentioned that, given the timeframe specified in the protocol, it is necessary to use a courier for transporting samples. This implies very high costs that are not reimbursed by the EU.

On the EU-level, no immediate actions are planned based on the monitoring. Monitoring has been done over the last 10 years and no sanctions are foreseen. Directorate F, however, observed in MS that appropriate actions are being taken based on the observations from the monitoring.

Update from EFSA (separately for EURL-AR network) (Pierre-Alexandre Beloeil, Beatriz Guerra Román, European Food Safety Authority)

See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)

Last year at the beginning of the year, two EFSA opinions were published, one is about the risk of transmission of AMR to calves by consumption of wasted milk/colostrum and the other one was about RONAFA (further presented Friday).

Related to WGS, EFSA supports more WGS, by thematic grants (i.e. ENGAGE, working on *E. coli* and *Salmonella* and INNUENDO, working on *Campylobacter*, VTEC, *Salmonella* and *Yersinia*). The reference testing also includes WGS, and published a scientific opinion on *Listeria*. EFSA will be asked to expand the PFGE database.

A questionnaire was circulated to give an overview of the MS application of WGS. Response from all NRLs was received and data will be presented more officially on May 11th 2017.

Summary of the plenary discussion

If growth is seen on a carbapenem-agar plate, but the isolate cannot be confirmed as carbapenemase-producer, it should be reported as negative (could be related to the short shelf life of the selective agar plates).

Outcomes of the EURL-AR EQAS 2016

Small changes will be introduced to the EURL-AR EQAS *E. coli*, enterococci, staphylococci 2016 report and the EURL-AR EQAS *Salmonella*/*Campylobacter*/genotypic characterization 2016 report. After that, all three reports were approved without further comments:

- EURL-AR EQAS *E. coli*, enterococci, staphylococci 2016 report
- EURL-AR EQAS Matrix 2016 report
- EURL-AR EQAS *Salmonella*/*Campylobacter*/genotypic characterization 2016 report

Additionally, summary on the outcome from discussion in groups is collected in Appendix 1.

EFSA/EURL Reference testing (Beatriz Guerra Román, EFSA and Valeria Bortolaia, EURL-AR)

See presentation (link and link or http://www.eurl-ar.eu/146-presentations.htm)

The procedure has been that EFSA sent a list of the strains selected for confirmatory testing to the EURL-AR, and the EURL-AR contacted the Member States to retrieve these strains.

Different criteria for inclusion were applied with a special emphasis on relevant phenotypes such as carbapenem, tigecycline and azithromycin resistance, among others, as well as to confirm the status of putative ESBL/AMPC-producer. The EURL-AR re-test the isolates for their antimicrobial susceptibility (AST) and perform whole genome sequencing (WGS) to identify the underlying resistance mechanisms and the presence of emerging clones. In general, if discrepancies among results obtained by the MSs and the EURL-ARs are found, the discrepant data are clarified not included in the EUSR-AMR report, unless the MS confirm their own results.

Regarding the AST, in comparison to the reference testing exercise 2014 (isolates from 2014 monitoring included in the ESUR-AMR 2014), in the Reference testing exercise 2015 (isolates collected in 2015 and included in the EUSR-AMR 2015) less discrepancies where found regarding colistin and azithromycin resistance confirmation, whereas for tigecycline there are still issues when performing AST. i.e, in 2015, 72% of the reported colistin resistant strains were confirmed at the EURL-AR.

At the EURL-AR, MIC-determination, WGS and comparison of the genotypes and phenotypes are performed. Subsequently, communication/troubleshooting is carried out with the NRLs. Not all strains requested were tested as some were never received and some were in heavily contaminated cultures. Analysis of data regarding the EFSA/EURL reference testing 2015 (WGS vs. AST) will be finalized by summer.

All NRLs are welcome to contact the EURL-AR in case of clarifications.

Summary of the plenary discussion

It has been discussed what could be the reason for the colistin resistance phenotypes not confirmed at genotypic level. It could be a new resistance gene and it would be interesting to study this further.

Joint reports on AMR in humans, animals and food (moderator: Therese Westrell, ECDC)
Therese Westrell, ECDC, moderated a panel discussion with members from Denmark (Eva Møller Nielsen and Helle Korsgaard), Ireland (Rosemary Slowey), the Netherlands (Kees Veldman), Norway (Jannice Schau Slettemeås and Umaer Naseer) and Sweden (Märit Pringle and Thomas Åkerlund). The panel discussed their experience with joint reports and the process of getting there. Ireland is the newest country to engage in the project of joint reports, starting in November, while Denmark has published joint reports since 1996. Austria and Iceland also have joint reports. All panel participants had brought a copy of their joint reports, which could be browsed. All reports are also available in electronic form.

There is an overall agreement that joint reports strengthen the collaboration between the sectors and also fit nicely into the one-health perspective. The biggest challenge for all countries is time and the reports are often ready for publishing close to or after summer the year after the data was collected.

There was a large difference in the frequency of meetings needed to organise these joint reports. In some countries, they have monthly meetings while others have annual meetings supplemented by Skype meetings. Most of the reports are published in English, as researchers all over the world, as well as industry and politicians use them.

The content and set up of the reports from different countries vary and most are not actually including an integrated data analysis, but is instead a combined report with data analysis done for the sectors side by side. A few countries had completely integrated data from both sectors in their analysis. Naturally, there has been a development in the joint reports over time. The design has become more uniform, and they have also been made more reader friendly. In Sweden the figures in the report are downloadable and interactive. The reports also contain articles and highlights on focus areas. There is no doubt that the reports will be needed in the future, but that the form will probably change from printed reports to online versions. It was highlighted that the data available from both sectors is fairly limited and in most countries the EU legislation nowadays determines which data that are collected from food and animals. After the panel discussion, there was a short discussion in break-out groups. The session inspired many of the participants to launch an initiative to start collaborations in their countries. This also highlighted some challenges including collaboration and comparability between sectors and the political and financial support in countries.

Public Health Perspectives on antimicrobial resistance plasmids in *Enterobacteriaceae* (Alessandra Carattoli, Istituto Superiore di Sanità)
See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)
Alessandra Carattoli gave a presentation on plasmids and their role in transmission of resistance.

Horizontal transmission causes the spread of plasmids, some plasmids are very successful and can be regarded as epidemic plasmids. Plasmids evolved acquiring multiple, important, critical resistance genes (ESBL, carbapenemases, plasmid-mediated colistin resistance genes) and plasmid typing contributed to understand the epidemiology of transmission and the worldwide dissemination of such clinically relevant resistance traits in bacteria of human, animal and environmental origin. The epidemiology of plasmids can be studied using PCR-Based Replicon Typing and for genomic analysis by the PlasmidFinder and pMLST tools.

Summary of the plenary discussion

To find the origin of a strain, it is important to look at both plasmid and the bacterial host as they co-evolve. If looking for a bacterial clone but not finding one, the next step is to look for a “plasmid-clone”.

To know what is new it is important to disseminate knowledge of the new discovered plasmids. There was no obvious solution for this as researchers are depending on publications and if not sharing data this might not be possible.

Carbapenemases in a one-health-perspective (Yvonne Pfeifer, Robert Koch Institute)
See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)
Yvonne Pfeifer gave an overview of the carbapenem consumption and the resistance to carbapenems in Europe. Carbapenems are being used more and more for treatment in humans. The level of complexity is really amazing, and transfer of carbapenem resistance involves clones, plasmids, transposons and different species. There were examples of transfer of carbapenemase between animals and humans. OXA-48 are common in various clones of *K. pneumoniae* and *E. coli* from human patients and some clones were found in pets. VIM-1 producing *Salmonella* Infantis and *E. coli* were repeatedly found in poultry/swine farms (faeces, manure, flies).

The involved clones/plasmids appear to be persistent in livestock animals. KPC-2 has been found from a multi-species outbreak, and the gene seems to be located on a very successful plasmid. Another multi-species outbreak indicated spread of clones and different plasmids with NDM-1 encoding gene on a similar transposon. Two studies had found carbapenemases in travelers, and there is no doubt that we will see more of this in the future.

Summary of the plenary discussion

Currently, sequencing of sewage (sampled at inlet to treatment facilities) is ongoing from sewage treatment plants globally. This gives a good indication of the contents of AMR-genes in the global environment.

Yvonne mentioned that they will look more into the possible connection between human and veterinary isolates and into how this plasmid has evolved.

Friday, April 7th 2017

Ongoing inspections related to (2013/652/EU) (Javier Tellechea-Vertiz, DG Health and Food Safety, European Commission)
See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)
Directorate F is performing audits to evaluate Member state compliance with Decision 2013/652/EC which details and harmonise sampling and testing procedures. The audits aim to identify the good practices and implementation difficulties encountered by Member States, and based on the information gathered consider how we can review and improve the Decision. We are now producing an interim report giving an overview of the outcome of the audits.

The EURL-AR-network were encouraged to make efforts to get *Salmonella* isolates from Food Business Operators own checks and randomise sample and isolate selection.

For the testing of ESBL-producing organisms, it is important that the media is used within the shelf-life and the isolates obtained are storage adequately.

We always bring a national expert (i.e. colleagues from the EURL-AR network) that allows for a lot of important exchange of information.

The laboratory visit focuses in the laboratory methods used and the standards applied including the use of reference strains and handling of the unusual results. The overall description of the AMR monitoring reported to EFSA is also important – e.g. if all the months of the year are covered in the sampling should be described in this section.

Summary of the plenary discussion:

Some of the issues that have been identified include a) the 48 hours between sampling and testing mentioned in the protocol which will be re-considered by the EURL-AR, b) the sampling of *Campylobacter* (jejuni/coli) EFSA will give a scientific opinion and based on this, changes might be introduced in the Decision and c) some carcass *Salmonella* isolates obtained in the MS cannot currently be used for AMR testing because they do not fulfil the Decision requirements while we cannot achieve the AMR testing of the *Salmonella* isolate number required.

Linezolid resistance (*optrA*) in *Enterococcus* (Lina Cavaco, EURL-AR)
See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)
Linezolid (an oxazolidone) is important for treatment of multi resistant strains in humans. The *optrA*-gene confers resistance by an efflux pump and was recently described in isolates from human and animal origin in China. We have in 2016 observed it for the first time in three *Enterococcus* isolated from poultry meat in Colombia. Furthermore, we followed up by looking back in MIC-results in the Danish monitoring and detected two more strains harbouring *optrA* which were isolated from imported turkey and Danish produced veal. Additional *in silico* screenings in the WGS-sequences saved were found negative for *optrA*. Should any of the NRLs discover linezolid-resistant Enterococci (or Staphylococci), the EURL-AR can be contacted to do reference-testing to confirm.

The *optrA*-gene has also been found in methicillin resistant Staphylococci and it is problematic if it spreads in MRSA as it might affect the treatment of patients that have few treatment possibilities left. It is not know what to expect with regards to future spread, but it is an emerging resistance and we should keep an eye on it.

Country presentation: Change of protocol for detection of AmpC-, ESBL- and carbapenemase-producing *E. coli* (Birgitte Borck Høg, DTU Food)
See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)
The methodology for detection of AmpC-, ESBL- and carbapenemase-producing *E. coli* was changed from 2014 to 2015 aiming to improve the sensitivity of the method.

From 2014 to 2015, a drastic increase was observed in the number of broiler meat samples (Danish as well as imported broiler meat) with ceftriaxone-resistant *E. coli* (note: cephalosporins are not used for treatment in the Danish poultry production). This change could be due to the change of method, but this cannot be confirmed, since there has been no parallel testing using both the previous and the new method. Furthermore, the obtained isolates from broiler meat were not investigated further. Whole genome sequencing of the isolates could add important information concerning the obtained *E. coli* isolates.

The new method has an increased sensitivity and with this, more upregulated ampC's are detected.

QC of antimicrobial panels (Valeria Bortolaia, EURL-AR)
See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)
Candidate strains for QC of MIC plates were sent to all NRLs in October 2016 and we can now conclude on the analysis of the obtained MIC-values submitted to the EURL-AR to identify new recommended QC-strains. These new strains do not represent a perfect QC for all antimicrobials but they add information compared to the currently used ATCC reference strains. If other laboratories have alternative strains providing a more comprehensive QC compared to the strains proposed by the EURL-AR, we are eager to hear from them and we will be willing to test and distribute such strains.

These QC strains could be included regularly in the QC-testing to check the MIC-panels and the AST procedures. However, they currently represent only an addition and not an alternative to the ATCC strains recommended by CLSI. We suggest to continue testing the ATCC strains according to CLSI recommendation and add testing of the EURL strains at regular intervals. In case of deviations, follow-up should be performed according to the criteria described by CLSI for the ATCC reference strains.

It is important to notice that this would increase the costs and EU funding currently does not cover these expenses.

The NRL-AR who did not submit data for these strains were invited to consider performing the tests as the more observations we have the more precise the definition of accepted MIC intervals. After data collection will be finalized, the network will receive conclusive information on expected MIC intervals by email.

RONAFA Opinion; EMA and EFSA Joint Scientific Opinion on measures to reduce the need to use antimicrobial agents in animal husbandry in the European Union, and the resulting impacts on food safety (Beatriz Guerra Román, EFSA)
See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)
The RONAFA opinion was a joint Opinion within EMA (CVMP) and EFSA (lead by the BIOHAZ panel, in collaboration with the AHAW and FEEDAP panels) and it included expert input from

additional stakeholders. The different measures existing were exhaustively reviewed. The impact on reducing antimicrobial resistance normally is linked to the combination of different measures. The recommendations of the Scientfic Opinion are to reduce (the use of antimicrobials), replace (i.e. antimicrobials with alternative treatments) and rethink (i.e. the livestock production system).

Summary of the plenary discussions:

The problem of zinc-oxide was highlighted, as it is included as an alternative. In February 2016 the Netherlands and France submitted a referral to the EMA (art. 35) for all veterinary medicinal products containing zinc oxide for oral route to food producing animals. The grounds for the referral were on the potential risk that zinc oxide poses to the environment and the risk for co-selection of antimicrobial resistance (e. g. SCCmec cassette type Vc containing the zinc resistance gene). In December 2016 the EMA Committee for Veterinary Medicinal products (CVMP) concluded that the treatment benefits of zinc oxide for the prevention of diarrhea in pigs did not outweigh the environmental risk associated with their use.

Based on its scientific conclusions, the CVMP recommended withdrawal of the marketing authorisations for these products. The committee's conclusion was challenged by the marketing authorisation holders of the affected products. The CVMP undertook a re-examination and concluded that the benefit/risk balance for veterinary medicines containing zinc oxide (at therapeutic dosage, e. g. 2000-3000 ppm) is still to be considered negative.

The information in the RONAFA opinion is based on an exhaustive review including also the input from experts and is relevant for decision makers to be used for action. It is in the hands of the EU Commission to pass it on to the decision makers. EFSA and EMA are promoting the information on different for fora, and for this, the use of the interactive infographic tool at the EFSA website is recommended (https://www.efsa.europa.eu/en/interactive_pages/Antimicrobial_Resistance)

Project on fluoroquinolone-resistant *C. jejuni* (Rene Hendriksen, EURL-AR)
See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)
This project on 'genomic epidemiology of fluoroquinolone resistant *Campylobacter jejuni* from poultry (GENCAMP) is an example of one of the small ongoing projects at the EURL-AR.

To determine the genomic diversity of FQ-resistant *C. jejuni* across the largest poultry producing countries in EU, 400 *C. jejuni* have been collected from the member states that produce the largest amount of poultry in EU. These sequences will be investigated to study whether the diversity observed may be related to e.g. country specific use of quinolones and/or trade connections.

Later this year, data will be wrapped up and collected in report for EFSA

Summary of the plenary discussion:

For *Campylobacter*, the topology of phylogenetic trees might differ based on the different methods. Any SNP-based tool should give a good overview, though, of the relation between the strains.

The current version of ResFinder (2.1) finds 'acquired AMR'. Version 3.0 includes the point-mutations also. Those interested are welcome to receive a link to 'ResFinder 3.0', only, know that this is still a work in process.

Currently, human *Campylobacter* isolates are not included in the analysis and we cannot offer to WGS any human strains but if sequencing data is already available, we could include it in the analysis.

AOB, general discussion and future perspectives (Rene Hendriksen, EURL-AR)
Antonio is running a small project on *S.* Infantis – looking into phylogeny of strains from the veterinary sector and connected to the public health sector. Any who would like to join in the project by forwarding strains/sequences of *S.* Infantis. We look at the core genome and also plasmids (the Italian clone includes a plasmid also).

Antonia Ricci runs a small project on S. Derby and if you would like to join, you are welcome to contact Rene (rshe@food.dtu.dk).

Methods for colistin testing – what works and what does not? (Erika Matuschek, EUCAST)
See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)
Erika Matuschek presented various methods for colistin testing – dilution and diffusion tests. There is no question that colistin resistance is a growing problem. There is therefore a need for being able to test for colistin resistance. Options are: Broth microdilution (ISO 20776-1), agar dilution, gradient tests (E-test, MIC-strips), disk diffusion.

EUCAST and CLSI started a joint working group and agreed on a standard method (a guidance document is available on the EUCAST and the CLSI websites). The conclusion was that the correlation with reference MICs was good for all broth microdilution methods. DD methods had poor correlation and gradient tests generally underestimated colistin MICs, resulting in very major errors (false susceptibility). Micronaut strips might be a good choice for performing MIC determination of colistin. EUCAST will soon evaluate two methods for colistin susceptibility testing: Colistin sensitest (Liochefilm) and UMIC (Biocentric). All details are reported on EUCAST website that is constantly updated. Agar dilution might be possible to evaluate but this work has not been done. The EUCAST recommendation for colistin quality control is to both use a susceptible strain (*E. coli* ATCC 25922 or *P. aeruginosa* ATCC 27853) AND the colistin resistant *E. coli* NCTC 13846 (mcr-1 positive). Related to the ECOFF for *Salmonella*, it has to be decided whether it should be divided into serotypes. It was discussed to start up a working group for testing the VITEK and Phoenix platforms.

Summary of the plenary discussion:

These tests are laborious for the laboratories. It was argued that it is still necessary to test all strains because colistin resistance is increasing and as we are running out of treatment options, it is important to have a reliable colistin test.

Genome-based epidemiology of *mcr-1*-positive *E. coli* (Linda Falgenhauer, Justus-Liebig University)

Linda Falgenhauer started with a presentation of the history and pharmadynamics of colistin. Colistin was discovered in 1949 and was first used clinically 1959. Banned in 1970 and is now in use again. Then she continued her lecture with the spreading of *mcr-1*-positive *E. coli*. *mcr-1* is present in different sources with higher prevalence in livestock, and the spreading is driven by plasmids. Different plasmids seem prevalent depending on the region. Colistin-resistance has already been found together with carbapenemase resistance and there is probably an undiscovered silent reservoir as phenotypic testing is not done routinely and resistance genes only found after WGS. The prevalence in humans is unknown. Colistin-resistance should be tested on regular basis to avoid spread of mcr-1 in human population. However, the methods to identify colistin-resistant isolates have to be improved as the present methods are time-consuming.

Tools/databases for analysis of sequences with regard to AMR-genes (Henrik Hasman, SSI)

See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)

Henrik Hasman gave an overview of WGS tools and databases. His conclusion was that WGS is a wonderful tool to study AMR but you have to take several points into consideration. It is easier to detect “known knowns” than “Known unknowns” and completely impossible to detect “Unknowns unknowns”. The “Unknowns unknowns” are the undiscovered resistance and undiscovered mechanisms and genes and it is impossible to find anything we are not looking for. It is therefore extremely important to still perform some phenotypic testing to find new genes and mechanisms responsible for resistance.

If you decide on using WGS for AMR it is necessary to define your need first and then find the best tool(s) to get there; read the paper describing the tool you select to use it properly and consider hiring a bioinformatician as many tools are difficult to use by non-bioinformaticians.

Summary of the plenary discussion:

To implement and trust this method, we need to define cutoffs. Upcoming generations of sequencing come with longer reads and it would be easier to map.

For AMR monitoring purposes this will work fine. For treatment purposes, to prove that a resistance mechanism is not present is difficult. Very good data is needed to correlate the genotype with the phenotype and to implement this tool as a routine diagnostic tool will take some years.

AOB, general discussions, future perspective and closing remarks for the joint meeting (Dominique Monnet, ECDC & Rene Hendriksen, EURL-AR)

Dominique and Rene closed the joint meeting and thanked the participants for their important input to the subjects addressed at the meeting. All the results of the fruitful discussions in the various groups are very rewarding and will be used in future efforts. The organizers were also thanked and SSI for hosting the meeting.

Any suggestions for issues to address in future EURL-AR workshops are welcome. Please send them by email to us (rshe@food.dtu.dk).

Appendix 1
Summary from group discussions at the EURL-AR workshop 2017

Experiences and challenges in relation to the 2016 EURL-AR EQAS

In break-out groups, experiences and challenges encountered following the 2016 proficiency tests were discussed. The workshop participants had the opportunity to bring up any important observations with regard to the 2016 EQAS's. This could be challenges faced or other observations made during the testing, or it could be remarks to the draft reports.

Discussion items were been drafted for the groups to consider. All NRL participants were encouraged to read and discuss the following topics/questions locally prior to attending the workshop, and also to bring additional observations, challenges or questions into the discussions.

Following the breakout group discussions, Rene Hendriksen (EURL-AR) headed the plenum summary and discussions, and with this appendix, the main issues addressed by the groups are reported.

Regarding EURL-AR EQAS *E. coli*, enterococci, staphylococci 2016:

1. In the *E. coli* trial, interpretation of panel 2 results: quite a few participants were confused. Is it because the criteria are not clear? Is it because it was not clear that the protocol changed compared to previous years? Other reasons?

   Response and comments:
   It was mentioned that the criteria for phenotype were clear, but one laboratory used the old EQAS protocol.

   Note from the EURL-AR: For each EQAS, an updated protocol must be downloaded from the website and applied.

2. In all components: it was not infrequent that laboratories report MIC values in agreement with those expected but then report wrong interpretation. What are the causes of these errors?

   Response and comments:
   It was generally agreed that this was a matter of clerical errors or a matter of applying the correct ECOFF for interpretation.
   It was suggested that to overcome this problem, two persons could be assigned to submitting results or submission could be done on two different days, to confirm the value submitted to the database.

1. In the staphylococci trial, one laboratory had percentages of deviations above the threshold for acceptable laboratory performance. This was mainly due to deviations in two strains. The laboratory sent the two strains they tested to the EURL-AR which obtained the same values as the participant and therefore deviations from expected results. This problem was not experienced by any other participant. WGS is ongoing to determine if this problem

might be linked to loss of resistance plasmid(s). Does anybody foresee other possible explanations? How could that happen that only one laboratory experienced the problem?
Response and comments:
It could have been due to loss of a plasmid.

2. In the enterococci trial, the antimicrobial resulting in the majority of deviations was quinupristin/dalfopristin. Analysis of the results showed that this was mainly due to reporting interpretation of MIC of quinupristin/dalfopristin for *E. faecalis* which is considered intrinsically resistant to such antibiotic. Do all agree that these should be considered deviations? Any other suggestion on how to deal with this issue?
Response and comments:
It was agreed that it is important to identify *E. faecalis* and *E. faecium*. For this purpose, a validated procedure is needed (e.g. PCR). Quinupristin/dalfopristin should be tested and reported. In case of intrinsic resistance as in *E. faecalis*, it should be considered as a kind of control. It should be reported resistant and not considered a deviation.

Regarding EURL-AR EQAS Matrix 2016:

3. The qualitative results for the ESBL/AmpC-detection have improved in 2016. Consider why this could be? Could it be related to better understanding of the protocol or the laboratories having more routine in running the method? Could it be that the laboratories have been stricter regarding the laboratory procedures? Could it be related to the species or type of samples?

   Response and comments:
   The interpretation criteria from EFSA have been very helpful.
   Also, more routine in the groups and quality of samples could have had an influence.

4. The sensitivity for the carbapenemase plates seems not to be optimal. Specify and discuss the issues observed with the plates or in the ID procedures?

   Response and comments:
   Problems with the CARBA-SMART from Oxoid were reported related to M2.2 [spiked sample, VIM-1] where isolates were detected on McConkey but not CARBA-SMART – even if the VIM-1 was detected by PCR in the isolate from McConkey). It could be that in relation to the CARBA-SMART, this was maybe a batch-issue.
   It had been observed that in general, labs appear to have problems with *Pseudomonas* and *Aeromonas* growing on the plates, especially when plating meat samples.

5. Why do the AST results for the matrix have more deviations than for the *E. coli* susceptibility testing trial (*E. coli* EQAS)? Is this related to the retrieving of the strains from the samples, to the strain characteristics (difficult phenotypes) or potentially to different people in charge of procedures in the laboratory?

   Response and comments:
   Potentially, it could be related to the influence of the flora of the meat.

It could also be related to the fact that the matrix results are ESBL-producers, which means that two panels of antimicrobials are tested, also in some cases close to the breakpoint. In addition, there is the risk of MIC-testing one of the background flora strains.

6. Issues with "tricky interpretations" especially with FOX results. Did you experience difficulties on interpreting results with the EFSA criteria? Which?

   Response and comments:
   This could be due to breakpoint issues.
   It was mentioned by that one NRL that had these issues with FOX that they had to do genotypic testing to confirm.

Regarding EURL-AR EQAS *Salmonella*/*Campylobacter*/genotypic characterization 2016:

7. For the categorizations related to the AmpC, ESBL- and carbapenemase-producing *Salmonella*, almost 100% of the participating laboratories delivered results according to the expected categorization. Is it relevant that we act on this to attempt to make it more complicated? If so, please suggest any genotypes that could be relevant to look for and include.

   Response and comments:
   No, it is not necessary. As long as for example upregulated AmpC and carbapenemase-producing strains are included, the EQAS is demanding enough.

8. In the *Campylobacter* trial, no particular issues with the AST or with the speciation of the test cultures have been identified. The only issue was related to C11.4/streptomycin combination (a drug that in the legislation is included at a voluntary basis). Where do you see the challenges in the *Campylobacter* EQAS and where would you 'expect' issues related to the culturing/testing/reading of *Campylobacter* AST.

   Response and comments:
   Breakpoint issues are well known, also, when culturing, testing and reading the *Campylobacter*, the fading end-points could be an issue (must be read in MH+blood).

9. This year, 11 of 31 laboratories submitted results for the genotypic characterization of ESBL, AmpC, and carbapenemase genes. Should something be done to encourage more laboratories to participate in this component? If so, what?

   Response and comments:
   It was mentioned that some laboratories perform the genotypic characterization but do not report the genes.
   One thing that could encourage submission of results is when it is used in the monitoring program and the training course that is already being prepared. Also, with this proficiency test, we can have our method accredited.
   Potentially a questionnaire to all NRLs could be a possibility to ask why or why the laboratory does not participate in this component of the EQAS.