10th EURL-AR Workshop, Kgs. Lyngby, April/2016 – minutes

The minutes are listed according to the agenda.

Participants

From the EURL-AR-network, all member states (MS), apart from Malta, were represented at the workshop. Participating non-MS were Albania, Iceland, Kosovo[1], Norway, and Serbia. A representative from Switzerland had to cancel due to health issues. Additionally, representatives from the EU Commission, EFSA, Directorate F (previously FVO), FVE (Federation of Veterinarians of Europe) and ECDC participated.

Thursday, April 14th 2016

Welcome (Christine Nellemann, Director of DTU Food)
Christine emphasized the collaboration across sectors, that is facilitated by DTU National Food Institute as exemplified by:

- COMPARE, a collaborative project across countries worldwide aiming at quantifying antimicrobial resistance genes, among other tasks;
- New building facilities at the DTU campus in which the employees working with food and animal health will be co-located.

Furthermore, Christine mentioned one of the most important topics in antimicrobial resistance that emerged in recent months, i.e. the discovery of the plasmid-mediated colistin resistance gene *mcr-1*. This topic clearly exemplifies the power of Whole Genome Sequencing as within a very short timeframe from the discovery of *mcr-1*, it was possible to mine sequence databases and describe the spread of such gene in *E. coli* and *Salmonella* sp. from different sources, geographical areas and time.

Meet and greet and introduction to the day's agenda (René Hendriksen, EURL-AR)
This year, we have a new contact person at the European Commission; we welcomed Mrs Angela Bolufer de Gea, who was recently appointed as the desk officer responsible for the EURL-AR, together with Mr. Martial Plantady. We are looking forward to continuing our fruitful collaboration with the EC.

[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

The agenda for this meeting included discussion of network tasks; e.g. the EQAS organization and results from 2015 as well as a number of other issues related to our area of responsibility. Also, Directorate F (previously FVO) will give input related to experiences from inspections related to the monitoring legislation.

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

The tasks of the EURL-AR over the last year were presented by examining the workplan 2015. One of the key tasks is scientific advice and support to the EU Commission and also WHO, EFSA, EMA and to some degree ECDC. In 2015, we followed up on the new monitoring legislation EU/652/2013 to ensure that standardized and harmonized data are produced. We participated in meetings on standardization of monitoring of AMR and disseminated relevant knowledge and information to the network.

In addition, we arranged and provided the annual EQAS's within AST, genotyping and the new matrix EQAS.

Update from the EU Commission (Javier Tellachea-Vertiz, European Commission)
See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)

At the EU level, there is a lot of commitment and political involvement within the area of antimicrobial resistance. In 2011, the EU Commission adopted an ambitious strategy for a 5-year period – these were 7 key areas that are still in focus. There are a lot of activities e.g. AMR in the food chain, and we have to make everybody aware of the dimension of the issue.

The seven key areas materialized in 12 actions. Seven of these actions are relevant to the veterinary sector. Examples; at the moment two pieces of legislation are under discussion in the parliament (action 2), and guideline for prudent use in veterinary medicine (action 3). The animal health law has just been adopted (action 5). For Action 10, harmonized surveillance systems have been carried out (ESVAC project, JIACRA report).

Ongoing evaluation and next steps; the EU as a best region – the EU Commission is working to push the AMR agenda at international level, and is striving for more innovation. For this purpose, quality data are necessary and AMR surveillance has to be based on a One-health approach.

Update from EFSA (Pierre-Alexandre Beloeil, European Food Safety Authority)
See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)

Highlights and trends from the 2014 EU summary report on antimicrobial resistance were presented, including conclusions that resistance to fluoroquinolones was observed frequently, but low prevalence of resistance to other critically important antimicrobials was detected. The overview of data provided and analysed by ECDC indicate that *Campylobacter* in humans have high level of resistance to ciprofloxacin. For *Salmonella*, some serovars may show high resistance to ciprofloxacin.

The report received from Asia about the transferable colistin resistance detected in *Enterobacteriaceae* is of public health significance and will have practical importance. In June, this year, we will have data related to colistin consumption.

2015 AMR data expected to be reported was presented. This was the first year that resistance in calves was reported.

All MS are encouraged to validate data before sending it to EFSA. A number of MS have been visited and trained in relation to the AMR Data collection, also web-training is available. For the 2015-data, data must be submitted at the latest on 31 May. On June 3rd, tables will be created for validation.

It is important to emphasize that laboratories should always perform re-testing when observing discrepant results. If some isolates display unusual and/or doubtful antimicrobial resistance patterns also after re-testing, they should be sent to the EURL-AR or to EFSA.

Summary of the plenary discussion:

One topic debated after this presentation concerned what to report as “isolation date” when reporting to EFSA. This is not trivial as different laboratories have different timeframes and procedures to process samples. There were different suggestions/interpretations from the audience:

- Date of confirmation, e.g. the date in which we know it is a *S.* Kentucky
- Date of suspicion, e.g. the date in which we know it is *Salmonella* sp.
- Date of the beginning of the microbiological procedure, as it best reflects the sampling scheme

Furthermore some issues were raised:

- Issue on information available in passive monitoring. A quality assurance document reporting date of sample collection, date of bacterial isolation, etc. should however, always accompany samples. Such information must be available for the laboratory responsible to build-up the database
- Issue on harmonization and comparability of data

Conclusion from EFSA: The intention is to be able to assess the time between the sampling and the testing. For this purpose, it is requested that the 'isolation date' refers to the starting date of the isolation. This will be communicated from EFSA directly to all labs.

Outcomes of the EURL-AR EQAS 2015

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. (Gordan Kompes, NRL Croatia)

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

  - It appears that the performance of *Salmonella* is going better and better over the years.
  - In 2015, and for the first time in 10 years, all labs have less than 5% deviations for *Salmonella* and *E. coli* results!
  - It is puzzling to notice that almost all correctly detect OXA-48 in *Salmonella* but not in *E. coli*.
  - We are happy to obtain strains with known MIC's to use as QC-strain, as *E. coli* 25922 is out of range for QC-testing.

- *Enterococcus* spp. (Jelena Avsejenko, NRL Latvia)

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

  - Benefits of the EQAS for us is to be reassured of quality; to detect weak points or identify problems; and to improve performance
  - In the self-evaluation of the results, the method limitation is +/- one two-fold dilution, i.e. one MIC step deviation from the expected MIC-value is considered OK, if results obtained are R/R or S/S. When one step deviations are connected also with deviation in interpretation of results (S/R), follow-up was done.

- *Staphylococcus* spp. and methicillin-resistant *Staphylococcus aureus* (MRSA) (Mirjam Grobbel, NRL Germany)

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

  - For many antimicrobials, high deviation levels are seen.
  - Internal control has many values close to the breakpoint, and therefore some variation over the years.
  - When troubleshooting is necessary for the laboratory work, consult the CLSI guideline; e.g. for SMX, always the first small spot is your MIC. Some antimicrobials have trailing effect in gram positive cocci which should be considered when reading the MIC (small spots are not regarded).
  - *Staphylococcus* tend to make micro-colonies and therefore thorough vortexing is important to ensure that all wells are inoculated with the same amount of bacteria.
  - Tetracycline – pH or cations are important, therefore, in our laboratory, we shifted to commercial media.

- *Campylobacter* spp. (Luminita Romascu, NRL Romania)

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

  - Of the participating laboratories, 31 performed antimicrobial susceptibility testing by MIC determination and one performed agar dilution
  - Expected interpretation for combination C-10.4/tetracycline was 'S'. 26% of participants found the strain resistant to tetracycline thus this combination was omitted from the report
  - Compared to the previous years, total percentage of the deviation level decreased in 2015.
  - There was one outlier in the *Campylobacter* EQAS 2015

- Genotypic characterization, ESBL-genes (Jannice Schau Slettemeås, NRL Norway)

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

  - Nine laboratories participated.
  - The method used by the participant was PCR except for one lab which used WGS.
  - 2015 was the first year we reported the genotypic results

EQAS on isolation of ESBL/AmpC producing *E.coli* from matrix of caecal and food samples – obtained results and challenges at EURL-AR and at NRL-AR's (Lina Cavaco, EURL-AR)

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

In 2015, the matrix EQAS was performed on meat (beef) and on caecal samples (pigs). For 2016 a repeat of this type of EQAS will be launched, however, it will be poultry samples and will include selective isolation of carbapenemase-producing *E.coli.*

Confirmation of the ESBL/AmpC status is not part of this proficiency test.

Sample M1.8 gave rise to a large number of deviations. Stability was tested in our lab up to two weeks after shipment and it was fine. Maybe it could be caused by a homogeneity issue as this was a natural contaminant in the caecal sample (see also follow-up under the plenary discussion)

The majority of deviations were obtained when testing susceptibility to ertapenem as the MIC was close to the breakpoint.

Summary of the plenary discussion:

Some strains could not be identified (1.1 and 1.4) when the identification was performed by testing on TBX agar plates, as the strain (1.1 and 1.4 were the same strain sent with two different codes) became white on TBX and additional identification tests were needed to confirm it as *E. coli*.

The deviations observed with sample 1.8 were likely to be due to issues with stability but also with homogenization of this particular caecal sample. The issue related to homogeneity has been added to the report before publishing.

Lina will send to the NRLs the background behind preparation of meat and caecal samples to enable the NRLs to implement the method in their labs if needed.

General discussion, EQAS

Both EQAS reports on the 'EQAS on the *Salmonella*, *Campylobacter* and genotypic characterisation' and the 'EQAS on *E. coli*, enterococci, staphylococci' were approved for ISBN-registration without further comments.

The EQAS report on the 'EQAS on isolation of ESBL/AmpC-producing *E. coli* from matrix of caecal and food samples has been adjusted as discussed, i.e. the issue related to homogeneity has been added to the report before publishing.

Ongoing FVO inspections related to (2013/652/EU) (Javier Tellechea-Vertiz, European Commission)
See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)

FVO is now called Directorate F: Health and Food Audit and Analysis under DG Health and Food Safety, EC

The objective of the project is the evaluation of the implementation of the harmonised AMR monitoring, as required by Decision 2013/652/EU, in audited Member States.

The audits include an evaluation of the sampling procedures. Checks for inconsistencies are done, e.g. repetition of epidemiological units, issues with isolates, who reports depends on the MS. We have identified some challenges, e.g. randomization, collection date, 48 sampling-testing, laboratory availability (weekend-open at laboratories?)

The challenges highlighted by the audits include the gathering of *Salmonella* isolates obtained by Food Business Operators for subsequent antimicrobial susceptibility testing and also the sampling delivery to the laboratory within the 48 hours rule: probably something to discuss. Indeed, why have the 48 hours limit for retail samples if the shelf life of the product is, for example, two weeks.

At the end of the audit series an overview report will be published. After this, more audits might be planned and we will explore synergies with other areas such as ECDC, prudent use of antimicrobials and collaboration between the veterinary and human sectors.

Summary of the plenary discussion:

Some countries have experienced difficulties in obtaining information from private laboratories regarding the samples (related to epidemiological unit) as different national rules are introduced. Dir 2003/99/EC describes that results should be communicated to national authorities. The approach may be different between Member States with some having provisions that go beyond the Directive.

Experience with the FVO inspection related to (2013/652/EU) (Annette Nygaard, NRL Denmark)
See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)

One of the FVO-audits in 2015 was in Denmark. FVO asked the laboratory to provide a lot of information in advance and a couple of months before the visit, a pre-audit questionnaire was received.

There was a very thorough check of the points listed under "general lab supporting activities". Regarding SOP for specific AMR methods, validation reports were requested for any deviation from the standard.

The FVO audit emphasized the importance of collecting, analyzing and reporting data in full compliance with 2013/652/EU

Break-out groups focused at specific monitoring of ESBL- or AmpC- or cabapenemase-producing *E. coli*; experience with Decision 2013/652/EU

Summary on the outcome from discussion in groups is collected in Appendix 1.

Experiences related to improving procedures based on comments from an external laboratory visit (Vasiliki Christodoulou, NRL Cyprus)
See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)

The outcome of an external laboratory visit was presented, including how the recommendations were considered and actions decided and introduced, e.g. a list of unusual AMR phenotypes has been defined to induce action (re-testing or additional investigations) if necessary, frequent checks including weighing of plates (colored liquid) has been introduced and the biosafety cabinet has been investigated and a malfunctioning sensor repaired.

Friday, April 15th 2016

Summary of day 1 (Rene Hendriksen, EURL-AR)

Pierre-Alexandre (EFSA) stated that regarding the isolation date, the intention is to be able to assess the time between the sampling and the testing. For this purpose, the starting date of the isolation is relevant. This will be communicated from EFSA to all relevant laboratories (EFSA originally had the information that the starting date of isolation would not be available).

Another clarification: Related to the discussion about the FVO inspections, batch control was discussed. Batch control is necessary for produced media. However a stability test might also be needed, depending on the volume produced. In the CLSI guideline there are schemes that can be implemented for batch control.

EFSA reference testing; confirmatory testing (Rene Hendriksen, EURL-AR)

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

The reference testing 2014, i.e. verification of agreement between phenotype and genotype, has been delayed mainly due to administrative issues and in particular the need of different Material Transfer Agreements (MTAs) to be signed before shipping the strains. To avoid delays for the reference testing 2015, we will therefore work towards requiring signed MTAs/MoU's before selecting strains for verification.

EURL and EFSA established criteria for selection of strains. The results indicated issues, e.g. carbapenemase-production that could not be confirmed (25 isolates), and discrepancies between panel 1 and panel 2.

For the reference testing 2015, it is necessary that the MS self-evaluate their tests and perform re-testing if necessary. For example, discrepancies between panel 1 and panel 2 should induce the laboratory should perform re-testing right away.

If you experience unexpected phenotypes, please inform the EURL-AR, and we will perform confirmatory testing. In total for the reference testing 2014, we sequenced around 200 strains, and aim at around the same number for the reference testing 2015.

Preparatory communication about the Workflow and Criteria for ESBL/AmpC/Carbapenemase –Phenotypes to be applied for the next EFSA 2015 EUSR on AMR report (Beatriz Guerra, European Food Safety Authority)
See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)

The workflow and the criteria that will be applied for the EUSR-AMR 2016 report to identify presumptive phenotypes of ESBL, AmpC and/or carbapenemase producers were presented.

The background documents used to set these criteria were the EUCAST guidelines for resistance mechanisms V1.0 (2013; link), the 2013/652/EU legislation (link) and the EFSA Scientific report "Technical specifications on the harmonised monitoring and reporting of antimicrobial resistance in *Salmonella*, *Campylobacter* and indicator *Escherichia coli* and *Enterococcus* spp. bacteria transmitted through food" (2012; link).

These criteria were reviewed by/agreed with the EURL-AR (V. Bortolaia and L. Cavaco, and the former member H. Hasman) and expert members of the Working Group who drafted the EFSA Scientific Report (S. Granier and C. Teale). Molecular data provided by the MSs supported the appropriateness/rightness of the criteria selected.

The isolates will be ascribed to "presumptive" ESBL/AmpC/carbapenemase-phenotypes based on their beta-lactam resistance phenotypes (Panel 2; only isolates that exhibit resistance to cefotaxime, ceftazidime or meropenem in the first panel should be tested for the second one) following the workflow presented. It shall be always taken into account that the categorization done following this workflow leads always to "presumptive" status, as only by molecular analyses the genotypes can be confirmed.

In this workflow, five main "presumptive" categorizations were made: ESBL-phenotype, AmpC-phenotype, ESBL/AmpC-phenotype, CP-phenotype and other phenotypes.

Some remarks were made:

- The workflow does not distinguish between chromosomal and plasmid-mediated AmpC beta-lactamases.
- A difference with previous recommendations is not to consider cefepime resistance to infer the presumptive phenotypes. Isolates producing AmpC or ESBLs can present microbiological resistance to cefepime (ECCOF >0.125 for *E. coli*, no ECOFF for *Salmonella*). High resistance to cefepime (MIC >=4 mg/ml) could be expected from ESBLs producers. In the case of isolates with co-existence of an ESBL and an AmpC beta-lactamase, to phenotypically confirm the presence of the ESBL, it would be necessary to test cefepime + clavulanic acid synergy (recommended by EUCAST). This combination of antimicrobials is not in the panel and therefore it is difficult to ascertain the molecular bases of resistance based solely on phenotype. In case of high cefepime resistance in presence of an AmpC phenotype, NRLs are invited to consider contacting the EURL-AR.
- Any presumptive carbapenemase phenotype should be confirmed by molecular methods. For other phenotypes, molecular analyses are recommended.

When applying this workflow, in the case of doubts (different combinations of genes can exist, strange results can appear, susceptibility testing issues can also happen), re-test, be critical and ask the EURL-AR.

Please always keep in mind that this data are based on extrapolations.

Challenges with tigecycline and temocillin (Lina Cavaco, EURL-AR)
See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)

Temocillin was included in the test panel as an indicator for the detection of OXA-48, and a study is now ongoing to define an ECOFF for *E. coli* and *Salmonella* based on 112 *E. coli* strain and 104 *Salmonella* strains. Some differences were observed between the three laboratories performing AST on these strains. Higher MIC-values were observed in the *Salmonella* collection compared to the *E. coli* collection. The obtained data has been sent to EUCAST for the purpose of defining an ECOFF.

During the EQAS for *Enterococcus* 2015, issues with the testing of tigecycline were observed. None of the test strains were expected to be resistant, however, 23 deviations were observed, all with higher MIC values than expected and categorized as resistant. Follow-up done on the batches of panels indicated that the problem was not due to the panels as the results at EURL were satisfactory, but that more follow-up was necessary locally, including also other possible factors. NRLs indicated the following sources of error: light sensitivity and oxidation (contact with air) of tigecycline, long incubation time, media batches (age of medium).

As a general consideration, tigecycline resistance is rare at present, therefore always follow-up when observing it.

The potency of tigecycline in the Sensititre plates (Michel Rapallini, NVWA, the Netherlands)
See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)

Tigecycline is tested using EUVENC. 1015 strains were tested in 2014 with 25% tigecycline resistance results. All resistant isolates were retested at CVI (Kees Veldman) and found susceptible.

NVWA then re-tested 20 susceptible and 30 resistant strains, this time opening the panel right before testing. All thirty strains previously scored as resistant were found susceptible!

As a practical consideration: tigecycline is sensitive to light and temperature, therefore plates should be kept in dark and incubated for the right time!

Plenum discussion:

We did not see the same in the gram-negative plate – maybe due to the different breakpoint.

Note that incubating in an incubator with a window might also cause degradation of tigecycline.

The emergence of *mcr-1* in *Salmonella* and *E. coli* (Sophie Granier, NRL-AR France)
See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)

Some background:
Many bacteria such as Gram positives, *Proteus* sp., *Morganella morganii*, *Serratia marcescens*, *Yersinia enterocolitica* are intrinsically resistant to colistin. Before 18th November 2015 (when the discovery of *mcr-1* was first published by Liu et al. on Lancet Infect. Dis), colistin was mainly a tool added to antimicrobial susceptibility test panels to comfirm species identification and/or absence of culture contamination as it is well know which bacterial species possess intrinsic resistance to colistin and, in addition, some bacterial species such as *Serratia marcescens* yield a very characteristic inhibition zone. In addition, it was known that colistin binds to the plastic of the trays and that it diffuses badly in the agar (which explains why the inhibition zone diameter is not perfectly round), which implied that colistin susceptibility test results were not given much consideration.

In human medicine, colistin use was initiated in the 1960's but stopped in the 1970's because of toxicity issues. The drug was re-discovered in the 1990's to treat infections by multidrug-resistant Gram negatives, also in view of the fact that toxicity is reversible as soon as treatment is discontinued. Acquired resistance to colistin appeared around 2010, which prompted inclusion of colistin among the critically important antimicrobials.
In veterinary medicine, colistin is often a first line treatment to administer orally to treat colibacillosis and salmonellosis.

mcr-1
It is striking that this gene is highly conserved and that no mutations either at nucleotide or at amino acid level have been described to date. However, the genetic platforms contributing to spread of this gene are diverse, which increases the chances for horizontal transmission.
New articles on detection of *mcr-1* in different countries and host species appear almost on a weekly basis. A suggested reading is the editorial by Skov et al. in Euro Surveillance 2016, 21(9). In addition, Poirel and Nordman published a culture medium for screening for polymyxin resistance in Gram negatives on J. Clin. Microbiol. in March 2016.

Development and standardization of reliable methodologies to assess colisitin resistance are urgently needed.
Plenum discussion:

- Banning colistin use in animals may give rise to different problems as it would force veterinarians to use other antimicrobials such as fluoroquinolones which are even more important than colistin in human medicine

- Since colistin in animals however, is mainly used to treat diarrhea which is often related to poor management, there is room for actions for lowering colistin use without increasing use of alternative antimicrobials

ENGAGE – an EFSA project that strives to build NGS capacity in EU (Rene Hendriksen)
See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)

ENGAGE is an EFSA co-financed project which is further presented on http://www.engage-europe.eu/

We invite the EURL-AR network to participate as so-called 'outside users' to open for collaboration in sharing and analysis of whole genome sequence data.

Finding OXA-48 *Shewanella* spp. during the monitoring (Daniela Ceccarelli, NRL the Netherlands)
See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)

During 2013-2015 carbapenemase surveillance in the Netherlands, all samples were negative for bla_{KPC}, bla_{NDM}, bla_{IMP} and bla_{VIM}. Only OXA48-like producing *Shewanella* spp. and no *Enterobacteriaceae* were identified, showing variable MICs to imipenem and meropenem. Since *Shewanella* are ubiquitous aquatic organisms proven to be the environmental reservoir of the OXA-48, these isolates are not considered to be a public health risk.

Plenum discussion:

- Interesting to hear of the environment as a reservoir of OXA-48.

Spread of an emerging clone of MDR, ESBL-producing *Salmonella* Infantis harbouring a conjugative megaplasmid in Italy (Antonio Battisti, NRL Italy)
See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)

S. Infantis has been causing disease in Europe. A rise in the level of resistance is seen in poultry in Italy, mainly due to the rise in the number of *S.* Infantis.

Plenary discussion:

- Authorities and stakeholders, incl. NRL *Salmonella*, are involved in discussing the way forward to limit the spread of this clone.
- CTX-M-1 is the most prevalent ESBL. The plasmid found in *S.* Infantis may have acquired the CTX-M-1-encoding gene from the intestine of chickens, for example. The

plasmid is seen in a few clusters of holdings which is different from the rest of production in Italy.

- This specific clone (ESBL-producing *S.* Infantis) has not been found in other countries

Overview of different online tools and databases for antimicrobial resistance genes (Ea Zankari, EURL-AR)

Presentation cancelled due to illness.

Introduction to EFFORT against antimicrobial resistance (Jaap Wagenaar, the Netherlands)
See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)

EFFORT (Ecology from Farm to Fork Of microbial drug Resistance and Transmission; http://www.effort-against-amr.eu/) is set up to provide scientific evidence and high quality data to enable decision makers, the scientific community and other stakeholders to know about the consequences of AMR in the food chain. EFFORT collects data on the consequences of AMR to animal health and welfare, food safety and to economic aspects.

It has been observed that the comparable animal production varies strongly between countries. In NL, there was a 65% reduction of antimicrobial consumption in farm animals from 2007 to 2014, and we have looked at how consumption and AMR are connected to the farm management.

Issues related to shipment of samples, freezing, thawing have been complicated, and Dik Mevius has produced a protocol to overcome these.

Sequencing is currently ongoing, and data will soon be available.

AOB and plenum discussion

A number of NRLs have been experiencing issues with ordering the CARBA-plates from Biomerieux. From the EURL-AR we have contacted Biomerieux. They work in clusters, not centralized; the problem appears to be smaller now, and we do not expect further response from Biomerieux.

The EURL-AR still offers confirmatory testing. Further information will be given by email directly to the network.

Action items, NRL-AR's:

- Send strains to the EURL-AR for confirmatory testing as requested by direct email from the EURL-AR.
- Send any interesting strains for the network proficiency test. Note, these will always be sent out as coded strains, keeping all details blinded.

Action items, EURL-AR:

- Send further information by email directly to the network on possibility of confirmatory testing of strains at the EURL-AR.
- The EURL-AR will look into suggesting supplementary bacterial strains (less susceptible), which will be useful for the QC-testing of the currently used microbroth panels (to overcome issues with low QC-range).

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

Future perspective and closing remarks (René Hendriksen, EURL-AR)

- A new Coursera platform is being finalized and members of the EURL-AR network will receive instructions via email on how to access such course.
- Monitoring of antimicrobial resistance in Europe is a unique, well-functioning model. There are discussions regarding the possibility to implement this model and use the same Antimicrobial Susceptibility Testing plates in the NARMS programme in the US and in some countries in Africa
- EQAS organization: as usual, we continue our effort to try to capture all different antimicrobial resistance mechanisms with the test strains selected for ring trials. One strain per species is pan-susceptible, as being able to correctly identify susceptibility is also very important
- Issue regarding quality control strains to use for the panels currently recommended for antimicrobial susceptibility testing: it is not easy to identify strains exhibiting MIC values within the range included in the panel for all different antimicrobials. The process to identify and validate strains is ongoing at the EURL-AR, and information/strains will be send to NRLs as soon as available
- During the meeting a quiz allowed participants to respond to various questions in relation to some of the presentations. A MIC-reading exercise was also included in the quiz, i.e. antimicrobial susceptibility test panels were shown and participants were asked to read the minimum inhibitory concentration (MIC). This exercise exemplified that there may be quite a few cases in which deviations from expected results in ring trials are due to reading problems and not to wrong performance of the MIC determination procedure. The EURL-

AR will prepare a survey to analyze the extent of reading problems and to look into possible solutions for that.

Next year, in April 2017, we will meet at a joint meeting with the FWD-network.

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Appendix 1
Summary from group discussions at the EURL-AR workshop 2016

Experiences and challenges in relation to the specific monitoring of ESBL, AmpC and carbapenemase-producing *E. coli* in food and caecal samples of porcine and bovine origin

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All NRLs have by now gained their first experiences with the specific monitoring of ESBL-, AmpC- and carbapenemase-producing *E. coli* in food and caecal samples of porcine and bovine origin, which was initiated last year. This is most likely to have led to (unforeseen) challenges locally, which might be of relevance to other NRLs. Also, suggestions for possible solutions to such local challenges might be available from NRLs, who have encountered and solved similar problems.

It was therefore the intention this year at the EURL-AR workshop 2016, to discuss the experiences and challenges in relation to the specific monitoring program. Javier Tellechea-Vertiz (FVO) and Rene Hendriksen (EURL-AR) headed the following plenum summary and discussions, and with this appendix, the main issues addressed by the groups are reported.

Question 1: According to the legislation *Salmonella* isolates obtained from food business operators own-checks can be used provided that they have been obtained in compliance with EU legislation (*Salmonella* National Control Programme in primary production and process hygiene criteria at slaughterhouses).
Please elaborate on the major difficulties encountered in ensuring availability of *Salmonella* isolates from the private laboratory network. Give examples of solutions found in your country to overcome these difficulties. (e.g. preservation of isolates, all necessary information on the epidemiological unit, coordination with foreign laboratories, etc.)

Response and comments:

NRLs observed that official laboratories perform monitoring only, and in most countries most own-checks are done at private laboratories (e.g. at the slaughterhouse – every one or two weeks tests are performed on samples taken from 5 carcasses). Most of the private laboratories are accredited. Most of the samples are negative.

Caecal isolates or isolates from flocks are considered to represent exposure to treatment while isolates from carcasses and retail products are indication of the exposure of consumers. Official sampling might be done for verifying FBO compliance; these samples may be used for AMR. In some cases MSs are not sampling. It is necessary to randomly select the samples to take and this type of samples must be used to supplement what you already have to achieve the minimum number.

In fact, from the EU (i.e. from the MS), the intention was to have the samples from three domains, domestic animals, the carcass and from meat at retail. For the carcasses, some MS said that we

should take advantage of the samples that are available. The Commission accepted. From an epidemiologist point of view, these own-checks should also be random to agree with the criteria. These isolates should be representative. There are examples where the MS is not sampling, but where the FBO's are sampling and the isolates obtained are used for AMR monitoring and reported to EFSA.

Question 2: In order to follow ISO procedures and the EU guideline, analysis should start within 48 hours after collecting the samples. Therefore, to respect the 48 hours window (when laboratories do not work during the weekend), samples are generally not taken on Thursday/Friday. It is common practice, for hygiene purposes, in the poultry production chain, that the slaughter of *Salmonella* positive flocks takes place at the end of the week. It is reasonable to think that these flocks, that could be subjected to more treatments, are often excluded from sampling for AMR.
Do you think that this could have any influence on the recorded instances of AMR, since it is reasonable to consider? Is sampling taking place at the end of the week (Fridays) in your country? Please elaborate on possible solutions to overcome this practical limitation.

Response and comments:

Typically, *Salmonella* positive flocks are slaughtered at the end of a week. A number of NRLs therefore work at weekends. If not handling samples at weekends, a validation of samples collected on Thursdays and Fridays would be necessary.

The justification for the 48 hours is not clear, and as the ISO standard is being revised at the moment, it might be possible to address the issue.

Question 3: Do you use cultures "over 24h" for antimicrobial susceptibility testing? What are, in your view, the necessary steps to validate changes in the reference method in order to allow the use of cultures older that 24h? (e.g. minimal set of data, antimicrobials used, etc.)

Response and comments:

From the NRLs this is typically not recommended, however there were some additional comments, as CLSI, EUCAST describe a certain CFU in the inoculum i.e. the 'fitness of the organism' as well as growth rate and growth rate in the presence of antibiotics are important. Maybe programmable incubators are a possible solution. In any case, validation is necessary when the followed procedures are outside specifications. One NRL indicated that they validated three days to be fine.

Question 4: Do you think the methodology for identification of species of *Campylobacter* and *Enterococcus* should be harmonised? What are the main difficulties? Which are the best methodologies (cost benefit)?

Response and comments:

For *Campylobacter* and *Enterococcus* no difficulties were identified. No need for further harmonization in this context.

For *E. coli*, however, it was mentioned that a MALDI TOF provides a very exact ID which would be way more certain compared to using chromogenic agars.

Question 5: How do you implement in your laboratory the "Validation of selective MacConkey agar plates supplemented for monitoring of ESBL- and AmpC-producing *E. coli*"? At which stages and how often do you validate the plates?

Response and comments:

NRLs indicated that QC is performed with the strains supplied by the EURL-AR by the person that manufactures the batch of agar plates. Each batch is tested. The batch is discarded after the expiry date.

Question 6: Is your laboratory aware of the situation as regards to AMR in public health in your country? Are results from veterinary field and human health fields discussed between laboratory experts from both disciplines? Please share with the group your experience, main obstacles and main achievements in this field.

Response and comments:

NRLs indicate that it is getting better as time goes. Much coordination between the two systems is necessary, and it is very expensive to run these systems. Loss of knowledge is an issue. Good networks can easily be lost. Legislation has been introduced in the Food/Vet-sector, however, this is not possible in the public health area – as it is one of the fundamentals in the EU that it is not allowed to set up regulations in the Public Health area.

Question 7: How does your country ensure that the monitoring results on animals and food supplement the monitoring programmes from the human health area (art 7(3) of Directive 2003/99/EC?

Response and comments:

The results supplement the monitoring. Examples are available of successful combined reporting; Netherlands, Denmark, Sweden, Austria. Also Ireland is starting up a process.

Question 8: Is the information on resistance patterns gathered at the laboratory further investigated with genotyping? Are there any further epidemiological investigations? In which cases? What are the main results of these investigations in your country?

Response and comments:

NRLs observed very different approaches in the different countries. Genotyping is not only relevant for the field of AMR. Especially genotyping is performed retrospectively – it is good to apply these results for comparison – between sectors as well as between countries.

Question 9: Decision 2013/652/EU has been in force for two years. Could you reflect on the following:

a. The main challenges for your laboratory (e.g. resources, workload, technical limitations, etc.)
b. Main benefits (e.g. better equipment, expertise gained, etc.)
c. Aspects for which you would like to have more external support (Commission, EURL or other)

Response and comments:

Last year was the first year, all MS had to report the isolate- based data. Help desk was fortunately available.

NRLs indicated that the important thing was the introduction of commercially prepared panels. Simplification of reports would be important, and EFSA is aware that this is quite an exercise to deal with data.

Question 10: Based on your experience (projects you are involved with) or your knowledge, what other areas could give added value to the current AMR monitoring system?

Response and comments:

Suggestions and reflections from the participants:

- Animals at farm level, not only at slaughterhouses,
- Other species could be included, e.g. farmed fish and other water animals,
- Wild animals,
- Sewage and sludge is ongoing,
- Maybe there are some intermediate we could look at for example some NRLs are looking at seals – injured seals – isolating ESBLs etc.
- Imported products,
- Pets (probably non-sustainable financially),
- The importance of monitoring of carbapenemase occurrence should be further emphasized.

Overall, it is challenging to add additional sectors/areas to the monitoring. It would be relevant to monitor fish or other food items from sectors in which antimicrobials are used, but it should also be considered what would be the purpose of adding, for example, monitoring of 'ready-to-eat-salads' in which antimicrobial resistance would mainly reflect the environmental contamination. In any case, studies with no immediate link to the food chain (e.g. detection of ESBLs in seals) may be useful to have a complete overview of possible pathways of transfer of antimicrobial resistance which go beyond the food to fork process.