9th EURL-AR Workshop, Kgs. Lyngby, April/2015 – 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, Bosnia-Herzegovina, Iceland, Kosovo[1], Norway, and Switzerland. A representative from Turkey had to cancel due to health issues. Additionally, representatives from the EU Commission, EFSA, EUCAST, FAO, FVO, and US CDC participated.

Thursday, April 23rd 2015

Welcome (Christine Nellemann, Director of DTU Food)
Christine welcomed all and introduced the focus on AMR in Denmark together with focus areas of the National Food Institute. The Institute is part of an EU project called 'COMPARE' in which whole genome sequencing (WGS) is a major focus with the purpose of bringing monitoring into the future. Christine mentioned the ongoing construction of the new 'Life Sciences building' at the DTU Campus which aims to reinforce the collaboration between sectors.

Meet and greet and introduction to the day's agenda (René Hendriksen, EURL-AR)
This is the ninth EURL-AR workshop in the EURL-AR network. The first day of this workshop will focus at network tasks and the implementation of the monitoring implementation. The second day will be joint with the ECDC-FWD network and will focus at the initiatives striving towards harmonization between the public health and the food/veterinary sectors.

Update from the EURL-AR (René Hendriksen, EURL-AR)
In 2014, the EURL-AR participated in an number of scientific meetings, expert meetings or more political meetings related to AMR. A large task has been to draft laboratory protocols related to isolation of ESBL, AmpC and carbapenemase-producing *E. coli* from caecal samples and fresh meat, together with protocols for validation of selective and indicative agar plates and MacConkey agar plates supplemented with 1 mg/L cefotaxime for monitoring of carbapenemase-producing *E. coli*. These are available on www.eurl-ar.eu.

General updates regarding EURL-AR-tasks will be on the agenda for the meeting. Highlights are for example the E-learning modules relating to AMR are now available to participants of the EURL-AR network, also, the EURL-AR participated in the revision of the first joint report on the integrated analysis of consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals (JIACRA report) from EMA /EFSA/ECDC.

Update from the EU Commission (Rosa Peran, European Commission)
The Commission has adopted the proposal regarding veterinary medicines. Discussions will take at least

[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

two years, after which we can have a definitive legislation. This revision on veterinary medicines and medicated feed also includes AMR, introducing a number of issues. EMA is continuing work with antimicrobials that are already on the market (is currently working with colistin). Monitoring consumption is voluntary from now, but will be made compulsory.

If everything is going as foreseen, before summer, legislation on animal health should be in place. Also, a guidance document for prudent use complements the legal tools (http://ec.europa.eu/health/antimicrobial_resistance/docs/2015_prudent_use_guidelines_en.pdf). The purpose of these guidelines is to provide practical guidance for Member States on the development and implementation of strategies to promote the prudent use of antimicrobials. In particular, the strategies focus at antibiotics in veterinary medicine in accordance with Action 3 of the Commission's action plan. These measures may also contribute to and complement the control of AMR in human medicine. These guidelines are addressed to Member States. Some chapters or specific measures are addressed to other relevant parties, including industry, farmers, veterinarians, associations and academia.

Regarding international collaboration, the WHO Global Action Plan is important as well as the work by OECD who, funded by EC/DG SANTE, work with economic impact of AMR calculating the direct and indirect costs of AMR. Also, FAO has actions and resolutions on AMR, and OIE works specifically on consumption at global level. Bilaterally, EU and China are communicating. These are two large partners, and improvements are seen since two years ago in particular related to feed medication. In TATFAR, the first report is published.

In the agricultural division work sponsored by Sweden is ongoing. The output can be put together with work of health division aiming to have a complete picture of the costs of AMR – from both the human and veterinary sectors.

As for Action 10 in the action plan, co-financing of the 2014 has already started and EFSA has opened for data submission. We recommend not waiting till the last deadline!

Announcement: FVO inspections will start October, November 2015. Inspection is not only related to the laboratory work, but also the sampling plan; i.e. everything covered in the 2013/652/EU.

Better-Training-for-Safer-Food: AMR training – call for tender is launched; 2015-16.

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

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

The JIACRA report (joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals) has been issued.

EFSA has opened for data-submission, and MS are advised to submit subsets of data, and not wait until the deadline in May. This way it is easier to provide support and solve any issues.

For the first report, we will apply preliminary ECOFFs for SMX and AZI, for *Salmonella* (SMX: 256 mg/L; AZI: 16 mg/L) and for *E. coli* (SMX: 64 mg/L; AZI: 16 mg/L).

Work is ongoing in EFSA and EMA who have a mandate for a joint scientific opinion with regard to measures to reduce the need to use antimicrobial agents in animal husbandry.

Summary of the plenary discussion:

- Globally, the environment is a large reservoir for antimicrobial resistant bacteria. In EU we do not yet have monitoring of environmental bacteria. Possible impact has been discussed in EFSA. Also, in DG Environment work is being carried out related to residues in the environment. For the time being, this is not linked to the monitoring. It is described in the road map, and the second action plan of the EU Commission would probably take this issue into consideration.

Outcomes of the EURL-AR EQAS 2014 (incl. experiences with the new MIC-panels):

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.

General comments: more skips appears to be seen in these plates compared to the previous ones. The recommendation from TREK is to ignore single skipped wells. Retest if more (see: http://eurl-ar.eu/201-resources.htm). A possible reason for a skip is also that the droplet sits on the side of a well and at a later stage falls into the well to give unexpected growth.

Problems with the testing of aminoglycosides were reported.

Two laboratories have experienced strange growth for temocillin, 4mg/L (EUVSEC2). Sometimes the strain grows in this well but not in the positive control well. One theory is that if the plate is not produced correctly so that the drop of temocillin fell into the positive control well. It would be relevant to know how many in our network experience this problem. EURL-AR will send info to the network to collect info. This info should include batch number, photo for documentation of the issue.

One NRL contacted TREK to add ECOFFS into SWIN software and not R/S, but has not yet received a positive answer. EURL-AR will collect further information from the network, and contact TREK related to this issue.

- *Escherichia coli* and *Salmonella* spp. (Tomáš Černý, Czech Republic)

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

- Even if the method is the same, deviations appear to be seen mostly when testing *Salmonella*.
- Defined two major hazard points: 1) volume of suspension transferred into the CAMHB, and 2) the result obtained when testing the QA reference strains (where in the range is the MIC – top, bottom?).

  Ad 1) The level of CFU's differs when using the different protocols; too low when following ISO 4833-1 (transferring 10uL). This could be related to the lack of detection of meropenem resistance.

  Ad 2) When testing QC-strain (which is susceptible) in EUVSEC plates, it is difficult to get a result for problematic drugs.
- In general, difficult to test for the carbapenemases, even if the gene expression varies. In particular difficult if the strain harbours OXA-48.
- Suggested to add a question to the EQAS database, asking for specifics related to the preparation of the inoculum for each laboratory. The EURL-AR will include this in following EQAS databases.
- Suggested to introduce a second strain, less susceptible, to overcome issues with low QC-range. The EURL-AR will add this to 'action items'.

- *Enterococcus* spp. (Gudrun Overesch, Switzerland)

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

- In Switzerland a new national EQAS is organized (a new task for the NRL).
- Suggested that more species are included, not for AST but to make sure that they can be correctly identified. The EURL-AR will consider how this could be done.
- Results from Ent 8.7/Amp were omitted from further analysis.
- Heterogenous colony morphologies were observed for – Ent 8.1, 8.2 and 8.3. Though, MIC and ID were the same for each colony type.
- One laboratory did not find vancomycin-teicoplanin resistance. It was suggested that this could be due to heteroresistance, and that this might be a relevant research topic. The EURL-AR encourages all NRL's to inform the EURL-AR if detecting strains that exhibit heteroresistance.

- *Staphylococcus* spp. and methicillin-resistant *Staphylococcus aureus* (MRSA) (Irena Zdovc, Slovenia)

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

- Three problem-combinations of strains/antimicrobials were omitted: ST8.1/cip, ST8.5/cip and ST8.8/Q-D.

- *Campylobacter* spp. (Lurdes Clemente, Portugal)

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

- It could be considered to include other species for ID, however, there is an EURL for *Campylobacter.*

- Genotypic characterization, ESBL-genes (Cristina de Frutos Escobar, Spain)

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

- At gene-level all participants performed well.
- Comment that TEM-1 might be detected but not reported because it is does not confer ESBL-production.

Final remarks to the EURL-AR EQASs:

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

It was mentioned that due to budgetary constraints, adjustments need to be made in relation to the number of laboratories included as participants in the proficiency tests. In relation to this, the EU Commission has requested that the EURL-AR work towards inviting one NRL per MS.

***Salmonella/Campylobacter* EQAS results – evaluation of MIC-values (Susanne Karlsmose Pedersen, EURL-AR)**

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

It was considered whether evaluation of the obtained results for the EQAS could be done in a better way; if it could be based on MIC and subsequently be categorized as correct, wrong or inconclusive. Discussions were based on results from last year's EQAS. If allowing a range of +/- 1 dilution step, 50-70% of the obtained results were inconclusive and had to be disregarded. Also, the obtained evaluation exhibited much larger deviation levels compared to the current evaluation method. It was suggested to apply a range of +/- 2 dilution steps to be the correct answer, or to use the mean value/median range of other labs. Conclusion: for now, not to change the evaluation method.

It was considered whether the obtained results when testing the internal control strain (same strain of *Salmonella*/*Campylobacter* included each year) would be a good indicator for the development of the participants' performance. Comparison of 3-year's EQAS results based on the internal control indicated that additional information was necessary and the improvement of performance was not indicated directly by the results obtained when testing the internal control.

General discussion, EQAS, incl. info about the upcoming 'Matrix EQAS'

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

Organizing an EQAS to qualitatively detect ESBL and AmpC producing *E. coli* from a matrix of caecal and food samples (cattle and swine / beef and pork)).

Expected launch is October 2015, at the same time of the *Salmonella*/*Campylobacter* EQAS. When more detailed information is available (e.g. how many samples will be caecal and meat) we will send out information to the network.

Some ECOFFs are missing; how do we define them? (Gunnar Kahlmeter, EUCAST)

ECOFFs are defined as the highest MIC-value of isolates devoid of a phenotypically detectable resistance mechanism.

Datasets submitted for the purpose of defining ECOFFs must follow standards, and are disregarded if methodology is not according to standards, or it has too few MIC-values. Most common reason for disregarding is that MIC-testing was not performed in full scale (truncated) or MIC-distribution is completely skewed.

ECOFFs should only be established on MIC-values determined with methods calibrated to the internationally agreed standard method for broth microdilution. Data should be available in large quantities (>100, preferably >1000), from several geographical locations (preferably >3) and from several providers (>3).

Summary of the plenary discussion:

- Data delivered based on the MIC plates from the new regulation (prepared to cover clinical breakpoints and ECOFFs) for e.g. *Campylobacter jejuni* is truncated data for ciprofloxacin, tetracycline and erythromycin. For these drugs, however, an ECOFF already exists. In EUCAST, two plates are used, going down to 0.008 (usually to 0.002).

- For the testing relating to the 2013/652-regulation, we still need to define ECOFFs for some drug/bug-combinations. The data we already have might be enough to do this. Pierre-Alexandre at EFSA and the EURL-AR will check up on this to see what is available already.

Outcome of 2014 survey on NRL's regarding the participation in EURL-AR network activities; brief presentation by Susanne Karlsmose Pedersen, EURL-AR

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

In 2014, the survey was sent to all laboratories on the EURL-AR contact list, and all replied. The replies obtained gave much information as regards the financial issues and also technically. It was worrying that so few countries are actually providing national proficiency tests. Also, there is a need to have only one contact person per MS and one only participant in each of the EURL-AR activities.

From the upcoming EQAS, only one laboratory per MS will be registered as participant in the EQASs (only one set of strains will be sent), training courses and workshops.

An open question is; if some NRLs are not delivering data to EFSA, who is, and how is follow-up on the performance of those laboratories done?

Introduction to E-learning on antimicrobial resistance (Lina Cavaco, EURL-AR)

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

The E-learning forum is now open, and information has been sent directly to those that have been signed up as course participants. The E-learning consists of a number of lectures and tests relating to antimicrobial resistance, and also a discussion forum is available. Note, however, if you expect a reply from the EURL-AR, send an email directly to the EURL-AR!

Do you have new colleagues that you would like to have added, ask Lina for a new login (email to licav@food.dtu.dk).

AMR monitoring at slaughter for *Campylobacter* and AmpC-, ESBL- and carbapenemase-producing *Salmonella* and *E. coli* – experiences from an NRL in 2014 (Antonio Battisti, Italy)

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

Italy also performed voluntary monitoring of *Salmonella*, ESBL/AmpC-producing *E. coli* and specific monitoring of carbapenemase-producing *E. coli* based on 10 caecal samples per broiler flock and 3 samples per turkey flock. Samples were refrigerated until shipping through an express courier. Transport was at room temperature, with delivery within 24h from sampling. Metadata was collected centrally in an online system. Samples were sent from the Regional Veterinary Services to the NRL-AR.

For an adequate comparability between MS and for the analysis of trends within and across MSs, the importance of standardization in the whole system starts with the study design, the sampling methods and the lab procedures.

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 attached as Appendix 1.

Detection of *E. coli* producing β-lactamases in pork, veal and beef in Belgium: results of a small scale study (Cristina Garcia-Graells, Belgium)

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

40 samples were tested for each method; samples tested were all natural samples (not spiked).

EU legislation protocol takes longer time than the Belgian alternative.

Obtained results were comparable between the methods – *Acinetobacter* and *Citrobacter* were also seen on MacConkey. On TBX, the *E. coli* are green. Only O157 are also white.

Plenary discussion:

- In some countries the observation is that with the experience, laboratory staff have learned to distinguish the colonies on MacConkey.
- This approach was not tested for caecal samples in Belgium. But was done in the Netherlands, where one laboratory did not see any difference between TBX and MacConkey, whereas another laboratory decided to use TBX as a confirmation step to eliminate Acinetobacter.

Country presentation; Activities regarding AMR in Iceland (Thórunn Rafnar Thorsteinsdóttir, Iceland)

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

Activities on AMR at the NRL in Iceland started in 2013, however, the laboratory is not yet a designated NRL-AR. Very low prevalence of *Salmonella* and *Campylobacter* is detected. In 2001-2005, 3.4% resistant *Salmonella* strains were detected (disregarding DT104). Consumption data is only wholesale data for animals and cannot be analyzed per animal species."

Plenary discussion:

- Normally there is a use of antimicrobials in the sector of aquaculture; no testing of AMR is conducted related to the aquaculture.

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

It has been valuable to see excellent presentations and have rewarding discussions working towards obtaining data for action in the field of antimicrobial resistance.

The EURL-AR still offers confirmatory testing, and this year, this task will be higher prioritized and structured relating to isolated ESBL and carbapenemase resistant strains. Further information will be given in an email directly to the network.

We have scheduled a TC in 2015 on request from the EC with a focus on harmonization, assuring that all MS are able to perform the harmonized monitoring according to the new legislation (Decision 2013/652/EU). This will include the relevant parts of identification by selective enrichment, phenotypical testing of ESBL, carbapenem, and AmpC using the new EU plate formats, and interpretation of those result categorizing them according to the legislation, and if possible updates on genomic tools. However, only a handful of laboratories will be invited to participate in this year's training course.

Action items, NRL-AR's:

- Send strains to the EURL-AR for confirmatory testing (i.e. interesting or rare antimicrobial resistance profil, and in particular isolated ESBL and carbapenemase resistant strains); the EURL-AR will send further information directly.
- Send any interesting strains for the network proficiency test. Note, these will always be sent out as coded strains, keeping all details blinded.
- Some laboratories have reported issues related to microbroth panels. NRLs are invited to inform the EURL-AR, when experiencing issues related to microbroth panel. In these cases, please include batch number, photo for documentation of the issue.

Action items, EURL-AR:

- Send further information in an email directly to the network on possibility of confirmatory testing of strains at the EURL-AR.
- The EURL-AR will by email invite the network to report any issues related to microbroth panels. In these cases, it is important to include batch number, photo for documentation of the issue.
- Some NRL's have expressed a wish to have ECOFFS added into SWIN software. The EURL-AR will contact NRL's to have an overview of the extent of this interested, and will subsequently contact TREK related to this issue.
- 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).
- It has been suggested to include more bacterial strains (e.g. enterococcus non-faecium and non-faecalis) for identification, i.e. not for AST but to make sure that they can be correctly identified. The EURL-AR will consider how it might be set up.
- Heteroresistance (vancomycin-teicoplanin) might be a relevant research topic. The EURL-AR encourages all NRL's to inform the EURL-AR if detecting strains that exhibit heteroresistance, and will keep this issue in mind relating to upcoming activities.
- We still need to define ECOFFs for some drug/bug-combinations in relation to EU/652/2013. Together with EFSA, the EURL-AR will follow up, based on what is available already.
- EURL-AR will follow up with the EU Commission in relation to the open question; if some NRLs are not delivering data to EFSA, who is, and how is follow-up on the performance of those laboratories done?

Joint day, FWD- and EURL-AR network, Kgs. Lyngby, April/2015

The minutes are listed according to the agenda.

Participants

From the FWD-network coordinated by ECDC, all member states (MS) apart from Croatia and Germany, were represented at the workshop. In addition, a representative from Norway participated.

From the EURL-AR-network, all member states (MS), apart from Malta, were represented at the workshop. Participating non-MS were Albania, Bosnia-Herzegovina, Iceland, Kosovo[2], Norway, and Switzerland. A representative from Turkey had to cancel due to health issues.

Additionally, representatives from the EU Commission, EFSA, FAO, FVO, US CDC and WHO EURO participated.

Friday, April 24th 2015

Welcome to this meeting which is the second meeting together in these networks.

Update from the EU Commission (Rosa Peran, European Commission)

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

Rosa gave a general update to the human and veterinary actions in the EU action plan and indicated that revision of the regulations for veterinary and medicated feed is ongoing. One of the actions of the EU action plan refers to the consumption of antimicrobials; in relation to this, the ESVAC report reports 15% decrease in veterinary antibiotic sales in the period 2010-2012.

Better Training for Safer Food (BTSF) will start in November 2015 and FVO (Food and Veterinary Office) will start audits later this year with reference to the EU/652/2013.

Update from the ECDC-FWD (Therese Westrell, ECDC)

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

Therese introduced the EURL network to the activities of the FWD network referring to harmonization of AMR data collection and the joint analysis of AMR in zoonotic bacteria.

There were initially many differences between the public health and the veterinary sector regarding the objectives for and the set-up of the AMR data collection. Several of these problems have, however, been overcome as an agreement has been reached within the FWD network on the objectives, test methods, antimicrobial panel etc. to be applied for EU-level reporting, which is also in line with the data collection in the veterinary sector. The harmonization is further supported by an EQA scheme for the FWD-network with the first scheme being arranged in November last year. The next one will probably start in January 2016.

Isolate-based quantitative reporting was introduced in TESSy last year with a few countries then able to report. After summer 2015, we will know how many countries that have reported isolate-based data for 2014. The quantitative data are interpreted with EUCAST ECOFFs for the joint report with EFSA and the

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data provided to ECDC already interpreted with clinical breakpoints (from the case-based reporting) is adapted so that it aligns as close as possible with the ECOFFs.

EUCAST have concluded in the joint ECDC-EUCAST project on setting disk diffusion ECOFFs that it will not be possible to set an ECOFF for sulfamethoxazole since the disks from different manufacturers do not give comparable results and there is no market for producing better disks.

The standardized EUCAST disk diffusion method was not strictly applied in the laboratories for *Campylobacter* AST, resulting in not fully comparable results.

Introduction to the day's agenda (Rene Hendriksen, EURL-AR)

Summarized the content of the EURL-AR meeting, where the focus was at the missing ECOFFS.

In the EURL-AR right now, we are working with setting up and EQAS for detecting ESBL, AmpC and carbapenemases in caecal and/or meat samples. We have set up an E-learning training component for antimicrobial susceptibility testing. Access to this is currently by invitation only, please contact Lina (licav@food.dtu.dk) to receive an invitation.

The agenda was introduced, also mentioning that there has been much debate about genome sequencing and the use of this method for monitoring. In the US and in the NARMS programme they also work with these issues.

AST of *Salmonella* spp. and *Campylobacter* - summary of NRL-AR- EQAS (Susanne Karlsmose Pedersen, EURL-AR) and ECDC-FWD-EQAS (Mia Torpdahl, SSI, Denmark)

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

The two networks work with different methods for AST, therefore comparison of results is difficult. In the FWD EQA, the disk diffusion method generally performed better than dilution method due to the fact that several countries were using automated AST systems set up for medical purposes. These focus the testing around the clinical breakpoints and are not capturing the lower concentrations where the epidemiological cut-off is to be found.

Antimicrobial resistance genes in monitoring programmes, a review (Frank Aarestrup, EURL-AR, Denmark)

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

The world needs surveillance and the medical doctor needs diagnostics, however, traditional phenotypic tests are not always reliable. The workflow in a clinical laboratory is complicated, whereas for sequencing this can be performed more easily and within a shorter timeline.

There is a high concordance between sequencing results and phenotypic results when analyzing; Zankari et al. presents 99.8% concordance.

Monitoring can be performed by testing isolates and performing gene detection, however, metagenomics by sampling farms or sewage and sequencing the samples directly and analyzing them using

metagenomics tools is also currently being explored (in the EU-setting in the COMPARE-project). This is a new technology but we already have an encouraging result.

The advantage with doing the metagenomics is that we do not have to subculture and test each single bacterium. The gut flora changes extremely rapidly when staying in a different country. We have also been looking into this by collecting samples from different places of the world – by sampling plane toilets.

Plenum discussion:

- As for the results of the sequencing when performed in different labs, we are doing a proficiency test now within the GMI – Global Microbial Identifier – for this, both bacteria and DNA are sent for sequencing and analysis. Late 2015, a proficiency test report should be available. For metagenomics, we are not there yet. It is important to extract DNA in a way where you get an equal distribution of the bacteria in the sample.

ISO and CLSI standardization of resistance genes (Jean Patel, CDC, Atlanta, US)

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

JP introduced her activities and her role at CDC; setting standards and defining method standards. This work also includes adding important tests, e.g. tests for carbapenemase-production in *Enterobacteriaceae*, in the standards and in this way promote these.

Plenary discussion:

- MIC is still considered the golden standard, but is actually not always right. Applying genome sequencing data instead might not work, as genes might be missed if they are unknown. Also, the phenotype is still needed for therapeutic decision as PK/PD data are still necessary.

Introduction to the WHO Global Action Plan (GAP) (Danilo Lo Fo Wong, WHO EURO)

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

WHO have worked for many years within the field of antimicrobial resistance and have defined a WHO Global AMR Task Force. The GAP came from a resolution which was adopted last year on the World Health Assembly.

The European strategic plan was also introduced, as we are all connected and there should be data sharing and communication. Many institutions and agencies are involved in the effort. An early draft was made public and opened for comments. Was on the agenda for high-level ministerial meetings to obtain political support. Submitted for adoption at WHA 2015.

Europe as a region also introduced a strategic action plan on antibiotic resistance – WHO EURO covers 55 countries. When the GAP is adopted, we will not need to change anything as such in the WHO EURO as we already have a regional action plan.

FAO-activities in relation to AMR and in relation to the WHO Global Action Plan (GAP) (Henk Jan Ormel, FAO)

See presentation (part1, part2, part3, part4 or http://www.eurl-ar.eu/146-presentations.htm)

FAO is a UN organization, and one of the strategic objectives is to make livestock production more sustainable. The world population is rising drastically in sub-Saharan Africa and in south-east Asia. Also the climate changes and urbanization need to be taken into account. It is estimated that the rise in the demand of animal protein till 2050 will be 70%.

At FAO we talk about sustainable livestock production. There are many different views of this, which vary from part of the world to part of the world. 70% of human infectious diseases come from animals, and the knowledge about the environment is the gap in our knowledge!

Prudent use of antimicrobials is necessary. The problem is that countries do not want to hear about antimicrobial resistance since this can affect their trade negatively. There are activities in some countries, but much work is still needed. A very good course was held in Kenya, where three programmes of a soap opera told the story about a guy who is a janitor-type and does little jobs at houses and farms. This was a tremendous success in relation to presenting the message to the public.

Take-home-message: Challenge to invent a test where you can see directly which antimicrobial to use. The test must be safe, simple, fast and accurate. This will help prevent in the rest of the world that the same happens that happened the last decades in Europe. There is a need for innovation!

A good example is APHCA (Animal Production and Health Commission for Asia and the Pacific) – a group of experts discussing how to cooperate in the field of AMR and have a website on AMR in 6 languages.

The 'tripartite' (WHO – OIE – FAO) work closely together. However, we really need a 'global stage manager'. From FAO, the message is: 'Let's cooperate on the field of AMR and the Global Action Plan!*'

Plenary discussion:

- In the end, the economy drives the process and makes sure we move forward. We can say a lot about the health importance ethics and other, but down to earth the economy makes the difference.
- Also, for veterinarians, we need a system for selling antibiotics that is advantageous and clever.
- The mechanisms to ensure that laboratories around the world can detect and test priority organisms include that 40 countries are involved in a global health security plan, responding to pathogens. One of the targets included, is AMR.

A comprehensive study on ESBL-producing *E. coli* in Sweden from food producing animals, food, and humans (Sara Byfors, Sweden)

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

Sara described her experience with the collaborative study performed with a one health approach; sampling was done from blood stream infections, food, healthy humans, food producing animals, sewage, raw surface water, and gulls.

Risk factors were found to be: Travel abroad, contact with health care abroad (although few participants in this category). No difference was seen for: Age, sex, previous antimicrobial treatment.

Gene level analysis and plasmid studies were performed, as was MLST and susceptibility testing.

It appeared that sampling community carriers and sewage gives a comparable picture as regards the detected ESBL genes. Food is a possible transmission matrix, however, it is not likely to be a major factor.

ESBL-bacteria are everywhere – even in a country like Sweden where not much antimicrobial is used, neither in humans, nor animals.

A report is available (in Swedish) and was aimed at the industry. Scientific publications are planned.

Plenary discussion:

- Towards the food industry we concluded that ESBL-producing *E. coli* are not likely to be a major contributor to the problem of ESBL in the healthcare system at the moment. For now, what we see is that imported food contains more than Swedish domestic foods (with chicken as an exception)
- As for the carriers, we invited them to contact a doctor, since it could cause concerns. We did not have much negative communication. Very few were worried.
- The poultry association has been part in this all along and putting pressure on the grandparent production. They have been taking this seriously.
- As for plasmids, we know already that IncI1 is the type that we find in all sectors. Plasmid sequencing and WGS is ongoing and will be presented in a scientific publication.

Outcomes of the work with the JIACRA report (Pierre-Alexandre Beloeil, EFSA; Dominique Monnet, ECDC)

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

For the JIACRA report, the first step was to discuss and agree between the three EU agencies: ECDC, EFSA, and EMA, on how the report would be produced. The report was published on 30 January 2016 and can be found on the websites of each of the three EU agencies.

The technical part of the JIACRA report was described by PAB and DM focusing on antimicrobial resistance and antimicrobial consumption in both sectors alone and across the sectors. Many possible relationships between antimicrobial consumption and resistance were investigated, including the relationship between antimicrobial consumption in animals and the resistance level in bacteria from humans. The report also includes a comparison of antimicrobial consumption in humans and in food-producing animals as a rate per kg biomass; this is the first time that such a comparison is made.

Producing this first report has been a challenge and EU agencies are aware that it could be improved. EU agencies welcome comments from the audience on how to improve the JIACRA report in the future. The intention is that a JIACRA report should be published regularly, and the next report should not be available before 2017. For this, there should be discussions, in each of the involved surveillance networks of each EU agency, to define areas where improvement is needed.

Plenary discussion:

- Different measures of resistance are applied, e.g. ECOFFs >< CLSI breakpoints (example: fluoroquinolone-resistant Salmonella)

- Reporting of consumption in animals is not yet mandatory in EU. It will, however, be part of the new legislation for medicines in animals. This new legislation still needs to be approved and adopted by the Council of the EU and the European Parliament, and then to be implemented in each EU Member State.

Two-by-two discussion on your take-home message (all participants) (Therese Westrell, ECDC)

- No minutes taken.

Plenary discussion and AOB

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

Future perspective and closing remarks (René Hendriksen, EURL-AR; Dominique Monnet, ECDC)

We are working towards getting more harmonized and comparable results, and during this meeting we discussed a number of challenges and technical issues for this process. One key issue that was discussed was sampling and uniformization of the sample.

When testing for significant associations, we must keep in mind that we must also consider the biological connection. We should explain what is plausible and what is not (e.g. for Campylobacter there is no inter-human transmission, so for consumption of antimicrobials in humans and the resistance in Campylobacter, there is no biological connection).

For some of the antimicrobials listed in the regulation 2013/652/EU the ECOFFs are missing which is unfortunate as both the MSs and EFSA needs them. To provide these, EFSA will acquire permission from the MSs to utilize the submitted susceptibility data to set preliminary ECOFFs. The EURL will look into possibilities for facilitating this with the help of also EC. ECDC and EUCAST has together with the FWD-Net created ECOFFs for Disk Diffusion for *Salmonella* and the project will probably continue for *Campylobacter*.

In the future, the EURL will collect some common EQA strains , in particular we aim at sending strains that are resistant to carbapenems to assess the capacity of laboratory to correct detect and report resistane to carbapenems. Rosa Peran mentioned how complicated these issues are, and that the Commission notices the work that is being done. The Commission thanks MS and the networks for their efforts and all the work that has been accomplished so far.

There is, however, room for improvement; especially for obtaining information about antimicrobial resistance in bacteria from imported food products and from the environment. The challenge is to find resources to set these additional activities in motion.

We hope to arrange a joint meeting with the FWD-network for the upcoming EURL-AR workshop in two years' time, i.e. in spring 2017.

GROUP SESSION

Initial experiences and challenges

relating to

The specific monitoring of ESBL, AmpC and carbapenemase-producing *E. coli* in food and caecal samples of porcine and bovine origin

Most NRLs should by now have 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 this 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.

During the annual EURL-AMR workshop, the participants representing the different NRLs were grouped in 8 groups to discuss the initial experiences and challenges in relation to the specific monitoring program. The 8 groups received 8 different topics, where each group focused on 2 topics. Unfortunately, the time allocated for this activity was not sufficient and the later discussion and summary was unsatisfactory as time was too short for further plenum discussions and clarification.

Subsequently, the EURL-AMR has after workshop re-visit all notes and comments raised by the groups and tried to bundle those and provide the missing oral feedback.

Topic 1. Sample collection, shipment including temperature issues, storage and handling of the samples.

Questions:

Several questions have been raised about sampling handling such as difficulties to perform the test within the required 48 hours / delays due to transportation infrastructure or weekends but also how important refrigerating of the samples during transport and storage is prior to tests as well as the way the temperatures are described e.g. between 2°C and 5°C in contrast to 2-5°C. In addition, it was questioned if meat samples should be tested if collected within the expiry date but tested after this date. It was also suggested to record and forward deviation in relation to the described handling procedures to EFSA but uncertain how to record this and if this would be in the interest of EFSA.

In addition to the more handle associated questions, there were a few questions raised about sampling size i.e. what to do if the "magic" number of 170 samples couldn't be reached and more specifically; "how many samples; 2, 5, or 10 from one batch is necessary for one representative pooled sample". In addition, some quested if meat should be fresh and or the most popular in the country and if imported meat should be included.

Answers:

EURL-AMR Laboratory Protocol; "Isolation of ESBL, AmpC and carbapenemase producing *E. coli* from fresh meat." Issued the December 2014 Version 2 describes that "*Samples arriving after the expiring date should be discarded. Also, samples which have not been stored appropriately (between + 2 °C and + 8 °C) under transportation or storage, should be discarded. Samples with*

damaged packaging should be discarded as well. Samples should arrive at the laboratory within 36 hours after sampling. The stored samples shall be kept at a constant temperature between + 2 °C and + 5 °C until bacteriological examination at the laboratory. This should be initiated as soon as possible after receipt at laboratory, preferably within 24 hours. It is recommended as a rule that the analysis is started within 48 hours after collecting the sample." This refers to the EU Guidance Document "on official controls, under Regulation (EC) No 882/2004, concerning microbiological sampling and testing of foodstuffs". Document No 882/2004 states under item 5.7 - Transport of samples, storage and starting of the analysis; "*Standardized procedures for the transport of samples to the laboratory, the storage and the starting of the analysis are presented in ISO/DIS 7218: Microbiology of food and animal feeding stuffs – General rules for microbiological examinations. ISO/DIS 7218 does not set a maximum limit for the time of transportation of products not stable at ambient temperature. However, given the potential for change in the levels of the target organisms, it is recommended that this type of sample should arrive at the laboratory within 36 hours after sampling. According to the above-mentioned ISO/DIS document the microbiological analysis should be started as soon as possible after receipt at laboratory, preferably within 24 hours. It is recommended that analysis is started, as a rule, within 48 hours of taking the sample, unless the testing protocol specifically states otherwise."* The EURL-AMR is not in a position to grant NRLs permission to exceed time limits or testing expired samples but acknowledge that it rightfully could be a challenge. However, the EURL-AMR urge the NRLs to comply with the regulation and protocol as exceeding the time limit and temperatures as well as testing expired meat samples might have an impact on the results. It might be that NRLs has to change sampling workflows such as requesting samples taken early in the mornings or other habits such as working in weekends, and requesting new and better sampling boxes to replace older and damaged ones etc. to fulfil the requirements. The EURL-AMR recommend in case of irregularities confer to the protocol and regulation that these are recorded and reported to EFSA along with the results.

In the case of difficulties to reach 170 isolates, it is stated in the Commission Implementing Decision (2013/652/EU) of 12 November 2013 on "the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria" it is indicated in item 2.2 Sampling Size that "*In those Member States where, due to a low bacterial prevalence or low number of epidemiological units, in any given year, the number of isolates required in accordance with the first paragraph for some of the combinations of bacterial species and type of sample of animal population or food category listed in point 1(a), (b), (c), (e) and (f), cannot be achieved, all available isolates at the end of the monitoring period shall be included in the antimicrobial susceptibility testing".* The EURL-AMR also here acknowledge that reaching the "magic" number of 170 isolates for some NRLs might be difficult due to infrastructure and organization. In this case, the EURL-AMR can only encourage the NRLs to consider how to change the plans in order to take more samples to increase the chance of identifying more isolates.
In relation to the question about pooled samples, EFSA replied to this question during the workshop indicating that one caecal sample per epidemiological unit should be collected but the chance to isolate a pathogen will increase by pooling several caeca (optional and no specific numbers mentioned or recommended) from the sample epidemiological unit. However, it should be mentioned that a pooled sample still counts for only one sample.
In the Commission Implementing Decision (2013/652/EU) of 12 November 2013 on "the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria" and in the EURL-AMR Laboratory Protocol; "Isolation of ESBL, AmpC and carbapenemase producing *E.*

coli from fresh meat." Issued the December 2014 Version 2, it have been indicated that fresh meat should be collected and in specific in item 2.3.3. Collection of samples at retail of the Commission Implementing Decision; "Member States shall collect at retail random samples of fresh meat of broilers, pig meat and bovine meat without pre-selecting samples based on the origin of the food" meaning that this also include imported meat but also without pre-selecting e.g. not pre-selecting the most popular food item of the country.

Topic 2. Assessing plate quality and reference strains for ESBL/AmpC as well as carbapenemase detection

Questions:

It was indicated that NRLs appreciated the received reference strains and found them useful.

Answers:

Comment well-received!

Topic 3. Selective pre-enrichment

Questions:

One group indicated being satisfied with the protocol not including a selective pre-enrichment but utilizing none selective buffered peptone water (BPW) whereas another group indicated no experience with BPW.

Answers:

The EURL-AMR along with a NRL strived to implement the methodologies in existing national workflows to avoid additional expenses for EU and new parallel workflows for the NRLs. However, the EURL-AMR also acknowledge that the new EU monitoring workflows also mean that all NRLs in different degrees have to modify and make changes to the existing national workflows e.g. some were satisfied with using none-selective BPW whereas other had no experience. This is just an example showing that harmonization is difficult and some will face changes but overall all will benefit from having comparable data.

Topic 4. Selective detection of presumptive ESBL/AmpC-producing *E. coli*

Questions:

It was indicated that other selective media such as Eosin Methylen Blue agar or TBX were better for isolation and selection for indicator *E.coli*.In contrast, one group indicate that they already work with MacConkey agar containing 1 mg/L cefotaxime (CTX) as described in the EURL-AMR Laboratory Protocols. Said that, some countries indicated that there might be differences by providers and that some of the countries produce the plates themselves and this raise the question about QC and shelf life. In addition, some countries chose themselves to streak plates differently compared to the protocol. Other indicated that sometimes the differences in media inoculation might lead to growth of a "smear" and not recognizable *E. coli* colonies. Furthermore, one group mentioned that technicians were confused by the different incubation temperatures of MacConkey agar containing 1 mg/L cefotaxime i.e. 44°C for selective isolation and 37°C for Sub-cultivation.

Answers:

This might be true that EMB and TBX perform as good as the media chosen for the EURL-AMR Laboratory Protocols but these were not tested in relation to implementation of existing methodologies in national workflows as mentioned above.
The EURL-AMR acknowledges that there might be differences in the media depending on providers and in-housed produced plates. However, the EURL-AMR has supplied each NRL with a set of reference strains to ensure quality control i.e. the isolate of the target organism and testing the validity of the plates (activity of the antimicrobial). The EURL-AMR has for some brands verified the stability of the MacConkey agar containing 1 mg/L cefotaxime and found that the stability last for up to 6 months depending on the quality (brand) of the antimicrobial but would recommend using the reference strains to monitor this.
The way the MacConkey agar containing 1 mg/L cefotaxime plates are streaked is not vital if the NRLs ensure to provide single colonies. The important factor is that the streaking is well extended in several streak lines on the plates using new loops so that it is possible to observe single colonies and see their typical morphology. To avoid smear it might be relevant to check that plates have a dry surface when streaking. Also the incubation temperature control will influence the growth of background flora and hopefully improve the isolation of recognizable *E. coli* colonies.
MacConkey agar containing 1 mg/L cefotaxime are incubated at 44 ± 0.5 °C to minimize the influence of natural background flora. This temperature is shown to be permissive for growth of most *E. coli* strains, and both the EURL-AMR and one of the NRLs of the network did comparisons testing different E. coli collections, confirming this fact.

Topic 5. Selective detection of presumptive carbapenemase-producing *E. coli*
Questions:
Some countries indicated that they have no or limited experience with detection of carbapenemase producers and that commercial Oxa-48 plates are still to be ordered. In addition, the EURL-AMR protocol does not specify what selective and indicative media to use for specific isolation of carbapenemase producing *E. coli*. Furthermore, the NRLs ask about the sensitivity of the carbapenemase isolation method.

Answers:
Monitoring of also carbapenemase producing *E.coli* is optional. However, the EURL-AMR can only urge this being implemented if possible to nuance the data of this phenotype. Should training be needed then please request this to the EURL-AMR.
The EURL-AMR do not recommend a specific manufacture of suitable selective agars to isolation of carbapenemase producing *E. coli* to avoid favoring specific providers. Furthermore the EURL-AMR cannot ensure the media will have similar performance as the providers might introduce slight changes to the media composition without changing the brand designation. Regarding the sensitivity of the method, it is of course quite dependent on the commercial plates used, therefore the EURL-AMR can only provide the NRL's that request the information about the results which were obtained media that have been tested in the validation studies (however this information has to be interpreted with caution as the manufacturer might introduced changes in the composition of these products, since this testing was performed). Experiences obtained by the EURL-AMR and one of the NRLs during the validation of the isolation methods were shared in different presentations during the 2014 and 2015 EURL-AMR Workshops.

Topic 6. Confirmatory ID of *E. coli*
Questions:

No major problems as different approaches such as PCR, MALDI and biochemicals are employed by the NRLs.

Answers:
Great.

Topic 7. Susceptibility testing on panel 1 (EUVSEC) and panel 2 (EUVSEC2)
Questions:
It was mentioned that the second panel plates, EUVSEC2, are expensive. In addition these plates are hard to manually read due to the orientation of the antimicrobials in the format.

Answers:
Correct, some NRLs will face higher prices than others due to local suppliers adding additional costs to the basic negotiated Trek prices. Correct, the second panel; EUVSEC2 is hard to manually read but a compromise as it was impossible to fit all ranges of the antimicrobials in a more logic way.

Topic 8. Evaluation of results from panel 1 (EUVSEC) and panel 2 (EUVSEC2)
Questions:
Some NRLs indicate that they have observed lack of growth in one of the control wells and it has been hypothesized that this issue could be eventually related to "carry over" for temocillin to the growth control. Another observation was described for meropenem where the value often differs with one dilution step between EUVSEC and plate EUVSEC2. Similarly, some strains are interpreted resistant to cefotaxime and ceftazidime on the first plate, EUVSEC, but susceptible on the second confirmatory plate EUVSEC2. Also in relation to isolates it has been mentioned that there were observed "unexpectedly high results" for presumptive AmpC and higher MIC's for true ESBLs.
In addition, it was mentioned that the QC values for tetracycline and tigecycline testing *Enterococcus* were too high and would be investigated by the NRL. Anyway, it was suggested to report batch numbers of the plates showing abnormalities.
Some NRLs report an unusual high number of colistin resistant *Salmonella* isolates. It would also be ideal to jointly agree on new additional more resistant "reference strains" than those mentioned in CLSI.

Answers:
It is correct that in some cases for meropenem the value differ with one dilution step and diverging interpretation for cefotaxime and ceftazidime between plates. This has also been observed by EFSA when assessing the EU monitoring data. This is a problem if affecting the interpretation i.e. resistant vs susceptible. EURL-AMR will collaborate with EFSA to reference test a number of isolates exhibiting deviating results / interpretation by genotypic characterization to determine the magnitude of this issue but also how to act. To ensure the most uniform results the EURL-AMR would recommend to re-test the EUVSEC and EUVSEC2 panel in parallel using the same inoculum to avoid any difference in inoculum levels, however this might still lead to differences either resulting from differences in the panels, or due to the normal expected variation in MIC determination.
In regards to classification of the isolates and the expected phenotypes a lot of data has been collected by EFSA at isolate level and at the moment the EURL-AMR and EFSA are discussing the interpretation criteria for ESBL and AmpC as well as carbapenemase classification schemes, taking

in account the data and all the criteria published, including the EUCAST criteria published after the technical specifications from EFSA were released, which might reduce the doubtful situations and the "unusual phenotype" classifications. As specifically for AmpC it has been observed that sometimes the occurrence of resistance for cefepime might be higher than expected for the classification, and this can be related to the interpretation of resistance using the ECOFFs. As indicated for the EURL-AMR ring trials, FOX resistance should be considered the major indicator of AmpC activity while as the synergy factor should be considered primarily in the distinction from ESBL. Regarding the occurrence of resistance for different compounds the classification in the "EFSA boxes" is in any case a simplification of the reality since there are many genes and variants involved and the different enzymes have differences in the affinity for the subtracts, so that the TAZ and FOT occurrence of resistance will be distinct if we are dealing with a TEM or CTX variant causing higher resistance to CTX than TAZ (but not others) or another variant might case higher level of resistance to TAZ or relatively low levels of resistance close to the breakpoint, as referred above.

The problem related to a higher frequency of *Salmonella* resistant to colistin is not surprising. It has been described that the ECOFF of colistin is depended on the serovar tested why these data should be interpreted with care and preferable be genotyped for confirmation.

It would be appreciated and ideal if any abnormalities e.g. meropenem, temocillin, azitromycin etc. observed in certain plate batches are disseminated to the network via the EURL-AMR for consideration and action.

The EURL-AMR support the idea of selecting new QC reference strains than are more resistant than those from ATCC. The EURL-AMR will seek to select and disseminate relevant candidates.