Joint virtual meeting on AMR for the FWD-Network and EURL-AR Network, 14th EURL-AR Workshop, 29 April/2020

Minutes

The minutes are listed according to the agenda.

Participants

Invitation for participation was circulated to the FWD-Network as well as the EURL-AR Network.

The FWD-Network were represented with 36 participants from 19 European countries. Additionally, ECDC was represented.

From the EURL-AR-network, all EU member states (MS) were represented at the workshop except for Malta. Participating non-MS connected to the EURL-AR network were Albania, Iceland, North Macedonia, Norway, Serbia, Switzerland, and Turkey. Additionally, the EU Commission and EFSA were represented.

Wednesday, April 29th 2020

Introduction to virtual meeting (Jette)

Welcome and introduction (Rene Hendriksen, EURL-AR)

EFSA-ECDC AMR report 2017/2018 (Pierre-Alexandre Beloeil, EFSA) See presentation (direct link or https://www.eurl-ar.eu/presentations/workshop-virtual-meeting-april-2020.aspx)

The EFSA/ECDC joint AMR report (The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2017/2018) focuses at indicator bacteria in food-producing animals and derived foods.

It appears that *Salmonella* from human cases decline in resistance to ampicillin and tetracyclines, whereas increasing levels of resistance to fluoroquinolones over time were observed. Combined resistance was generally uncommon. Of note, we observed a high or extremely high level of multi drug resistance in *Salmonella*.

For the corresponding *Salmonella* in food-producing animals, typically, high levels of resistance in commonly used antimicrobials were observed, including also the important resistance to fluoroquinolones in *Salmonella* from poultry. Low resistance levels to 3rd generation cephalosporins were observed. Noticable, the data showed an important variability between the different member states. In particular for the critically important antimicrobials. Still, combined resistance to CIAs (CIP/CTX) were commonly to very low.

As for *Campylobacter* from humans, the level of resistance to erythromycin was low in *C. jejuni* and moderate in *C. coli.* As for resistance to ciprofloxacin and tetracycline, the levels have increased over time. For erythromycin, however, decreasing trends were observed in reporting MS, and also, the level of combined resistance ciprofloxacin/erythromycin was low. For five countries, high to very high proportions of *C. coli* from humans resistant to both ciprofloxacin and erythromycin, leave very few options for treatment.

The observations for *E. coli* in food-producing animals are overall quite similar to the situation for *Salmonella*. High levels of resistance in the commonly used antimicrobials. Combined resistance to CIAs (CIP/CTX) was generally uncommon. For carbapenemase-producing *E. coli* (2018), no presumptive or confirmed carbapenemase-producing *E. coli* were detected from broilers and their derived meat.

For the key outcome indicator in the 2018 report, 'complete susceptibility in indicator *E. coli* from food producing animals', temporal trends interestingly showed a clear association between the complete susceptibility and the total antimicrobial consumption.
Four data points (northern countries) have much higher levels of complete susceptible indicator *E. coli*, and an increasing trend in complete susceptibility is observed in 6 MSs.

For another key indicator, 'prevalence of ESBL/AmpC producing *E. coli*', decreasing trends in 12 countries (40% of MS) have been observed.

Summary of the plenary discussion:

It would be interesting and important to know what drives the decrease in trends related to ESBL/AmpCs' in *E. coli*. 'Reason' is beyond the scope of the report, but the third JIACRA report will address the level of resistance towards cephalosporins in food-producing animals. The scope of the JIACRA report is to relate AMC and AMR data, and into the beneficial impact of reducing the use of antimicrobials. MS have started to implement the action plan or are in process of implementing the action plan. For some countries, it will be difficult to lower the use. Within the framework of the JIACRA analysis, we aim to investigate further whether we see a connection between AMC and AMR.

Update from the EURL-AR (Rene Hendriksen, EURL-AR)
See presentation (direct link or https://www.eurl-ar.eu/presentations/workshop-virtual-meeting-april-2020.aspx)

At the EURL-AR, a number of tasks described in the workplan 2019/2020 have been carried out or is ongoing. Some of them are:

- The EURL-AR investigated how many laboratories performed MRSA-detection and ESBL-quantification in caecal and meat samples. Out of 24 respondents, 11 reported that they used the samples for MRSA-detection and/or ESBL-quantification.
- A MIC-survey was conducted, based on 45 images of MIC panels for visual assessment. This was based on our observations at training session in our own laboratory which showed that reading posed quite some variability.
- The EURL-AR have been working on bioinformatic tools for detection and interpretation of AMR genes and chromosomal point mutations in sequences. ResFinder 4.1 is now available (https://cge.cbs.dtu.dk/services/ResFinder/), and also includes as a result, the resistance phenotype connected to a detected AMR gene.
- The EURL-AR have worked with harmonization of WGS in order to obtain comparable data across the EU. Material obtained in various projects (CGE tools, EFSA ENGAGE, COMPARE) have been collected on the EURL-AR website (https://www.eurl-ar.eu/wgs.aspx). On that website, find also the 'Protocol for whole genome sequencing and bioinformatics analysis of bacterial isolates related to the EU monitoring of antimicrobial resistance' (endorsed by the EURL WG for NGS) presenting useful information when starting up WGS.
- The EURL-AR have been working with developing and introducing new results submission tools for the EQAS which have now been introduced for the AMR-EQAS. Work is ongoing in relation to setting up a PT for WGS (materials planned to be sent in September 2020).

Summary of the plenary discussion:

The number of caeca included in the testing when looking into detecting ESBL/AmpC-producing E. coli was discussed. For pigs and cattle, only one is required to have enough material, but for broilers, there is a difference in relation to how many are pooled. Some countries test the caecum content from just one broiler caecum, other pool five or ten (or another number) of caeca. There are arguments to take only one (less prone to contamination) and there are arguments to pool (small volumes of caecal content). It has not yet been investigated what difference pooling makes to the obtained result. The EURL-AR will aim at including studies in relation to this issue for the upcoming workplan (2021).

In relation to storage time of the caecal samples, the EURL-AR do not encourage to store and test the samples at the latest possible time. The possibility of long storage refers to samples collected on Thursdays/Fridays. The preference is that caecal samples are tested immediately after collection.

Update from ECDC FWD (Therese Westrell and Erik Alm, ECDC)
See presentation (direct link (Therese Westrell's slides), direct link (Erik Alm's slides) or https://www.eurl-ar.eu/presentations/workshop-virtual-meeting-april-2020.aspx)

Input from Therese Westrell (updates from ECDC FWD):
Activities that are planned or ongoing in the ECDC FWD Network relates to 1) the legal mandate

for zoonotic AMR collection from public health, 2) data collection for 2019 and surveillance reports, 3) EQA for AST, 4) development of ECDC Surveillance Atlas for Infectious Diseases to include also FWD AMR data.

For the collection of data this year, due to difficulties (e.g. COVID-19) there have been delays in data collection. Last year: 24 countries collected *Salmonella* data and 20 on *Campylobacter* AMR.

The EFSA-ECDC AMR report 2019 (or 2018/2019) has a new report format – summarizing data and highlighting trends.

The JIACRA III report is expected to be delivered by the end of 2020. [Due to the pandemic, the delivery of the report had to be extended to June 2021]

AST EQA (6th round in 2020) was organised by SSI, Denmark. For *Salmonella*, the EQA includes phenotypic AST, where 5 priority antimicrobials are mandatory. For *Campylobacter,* the EQA includes phenotypic AST, where 3 priority antimicrobials are mandatory. It is also possible to report results on resistance predicted by genotypic methods.

Input from Erik Alm (ECDC WGS system update):
For the ECDC WGS system, we now have improved data upload options. For the user interface: Isolate views show uploaded data, Cluster views show genetic clusters. Dataset view for example per year. Visualization in charts, tables etc. are generated on the fly.

AMR genotyping software has not been decided. ResFinder has been used ad-hoc, but other softwares and databases might also be interesting.

Summary of the plenary discussion:

ARIBA and CARD were suggested as alternatives/supplements to applying ResFinder.

Beatriz (EFSA) mentioned that she could share directly with Erik some experience that EFSA have with benchmarking AMR tools, and also which were the biggest problems and differences that were observed (has been presented previously at EURL-AR meetings).

Update from the European Commission: progress report on implementation of the 2017 European AMR Action Plan and AMR monitoring in food-producing animals and food as from 2021 (Martial Plantady and Aurelién Perez, European Commission)
See presentation (direct link or https://www.eurl-ar.eu/presentations/workshop-virtual-meeting-april-2020.aspx)
Input from Aurelién Perez:
Related to the Progress Report on implementation of the One Health Action Plan against AMR, there are four progress reports (https://ec.europa.eu/health/amr/action_eu_en) ensuring that all actions are on track. For each action, there are timelines and deliverables for concrete activities, some of which are:

- The Regulation of 2019/6 on veterinary medicinal product is ongoing.
- Revision of the 2013/652/EU.
- European Antibiotic Awaredness Day on 18 November 2019.
- Network meetings in 2019.
- One-health visits to MS continued (Directorate F),
- G7, G20 renders an international push in the AMR area. Promoting AMR policies to fight against AMR. EU and the EU-MS are seen as good examples which is why we are trying to explain and present what we do.

For further inspiration, see : https://ec.europa.eu/health/antimicrobial-resistance/eu-action-on-antimicrobial-resistance_en

EU action plan:
https://ec.europa.eu/health/sites/health/files/antimicrobial_resistance/docs/amr_2017_action-plan.pdf

Latest progress report:
https://ec.europa.eu/newsroom/sante/item-detail.cfm?item_id=697424

Commissioner Kyriakides' mission letter:
https://ec.europa.eu/commission/commissioners/sites/comm-cwt2019/files/commissioner_mission_letters/mission-letter-stella-kyriakides_en.pdf

Input from Martial Plantady:
The revision of the legislation related to AMR monitoring in food-producing animals and food is ongoing, and will be implemented as from 2021. New legislation is necessary as we need to keep in mind that AMR is an evolving threat and new scientific developments occurred since 2014. The legislation will be based on EFSA scientific opinion of June 2019 and field experience. Discussions have been ongoing with representatives of MS's Competent Authorities within working group on AMR in food.

The upcoming Decision is envisaged future monitoring largely in line with current one: Consistency must be possible to continue to follow the trends of the current system. As for **animal species**, they will be the same and food concerned: poultry, pigs, bovines and fresh meat derived thereof. **Bacterial species** will be the same as today (*Salmonella*, *Campylobacter jejuni*, *Campylobacter coli*, commensal *E. coli*, enzyme-producer *E. coli*) and there discussions to include *Enterococcus*. **Stages of the food chain**: Primary production, slaughter houses and retail for fresh meat. As a novelty is included to sample for Border Control Posts (BCP), as it will be an obligation for MS to sample from BCP's (fresh meat imported from non-EU-countries). As for **sample design**, the current text is largely unchanged, but new definitions of epidemiological units for broilers and turkeys and for pigs and bovines have been clarified. In relation to **sample size**, the target of 170 isolates is maintained but more flexibility is offered in order to take into account situations in which there is a low prevalence (e.g for very low prevalence of *Salmonella*) or insufficient number of epidemiological units available. To prevent recurrent sampling in the same epidemiologica units there is an opening for MS to be able to adapt sampling size to their situations. **Analytical methods** will remain based on the EURL guidance. For panels, there will be some alterations also,

there is a possibility to use WGS as an alternative method to phenotypic methods for enzyme-producer *E. coli*.
Next steps are that a vote is scheduled for June/July 2020 and the Decision is envisaged entering into force: 1/1/2021

Summary of the plenary discussion:

For financial estimation of the cost of the new Decision, we have to wait for the final vote. As soon as that is in place, the EU Commission will send the templates for reporting financial estimates.

Update from EFSA (Pierre-Alexandre Beloeil, European Food Safety Authority)
See presentation (direct link or https://www.eurl-ar.eu/presentations/workshop-virtual-meeting-april-2020.aspx)

In 2019, EFSA conducted a satisfaction survey on the EU Summary report on AMR (EUSR). Stakeholders were generally positive and considered the EUSR on AMR useful, adequate and relevant for the decision-making needs. The new format for the EUSR on AMR still covers all food-producing animals and derived meat and thereby we have a complete overview of the findings each year. It addresses the variability among the MSs though maps and graphs. The expected added value of the new format is improved awareness and improved comparability that we are working on with EMA.

Additionally, EFSA has set up a working group that issued a scientific report on Technical Specifications for AMR monitoring in June 2019, and we are working on the JIACRA III project (joint project for ECDC, EMA and EFSA). The European court of auditors has underlined the importance of using the key outcome indicators for assessing the progress made by the MS in relation to AMR.

EFSA received several mandates, one is to work with AMR residues (24 substances). A WG has been established, led by EFSA (BIOHAZ etc) and EMA with the TOR to assess the specific concentrations of antimicrobials resulting in cross contamination in non-target feed for food-producing animals, below which there would not be an effect on the emergence of and/or selection of resistance in microbial agents relevant for human and animal health.

EFSA has a self-tasking mandate for a scientific opinion on the role played by the environment in the emergence and spread of AMR through the food chain.

NDM-4 carbapenemase gene harboured by a novel IncFII plasmid in *E. coli* of pig origin, Italy (Virginia Carfora and Elena Diaconu, IZSLT)
See presentation (direct link or https://www.eurl-ar.eu/presentations/workshop-virtual-meeting-april-2020.aspx)
As part of the specific monitoring of carbapenems, in an *E. coli* of pig origin, a bla_{NDM-4} gene was detected. The *E. coli* was MDR, including resistant to carbapenems (MERO, IMI, ERTA) and therefore was selected for in-depth WGS analysis.

Bioinformatic analysis was performed based on Illumina short-reads and Oxford Nanopore (ONT) long-reads allowing for closing of the gaps and verification of the correct assembly of repetitive regions of the *bla*$_{NDM-4}$ harbouring plasmid. A 53kb plasmid (pMOL412_FII) was identified to harbour the carbapenease gene, *bla*$_{NDM-4}$. The comparative analysis based showed that the detected plasmid was most closely related to a plasmid (pM109_FII) previously isolated from a human patient in Myanmar.
In conclusion, this study proved how the combined use of ONT long-reads and Illumina short-reads is useful for bioinformatic analysis of an IncFII plasmid.

Summary of the plenum discussion:

In Italy, it is the first time we detect a carbapenemase producer as part of the monitoring, so we are in contact with the local veterinary services and our follow up will be set in motion. In other regions of Italy, carbapenemase-producers have been detected in humans. We will be looking into this and make comparisons.

We applied the CARBA SMART (divided plate) for the analysis. The NDM-producing *E. coli* was not detected on the selective agar MC+CTX, on which it may have been overgrown by other ESBL/AmpC-producing *E. coli*. Indeed by using this latter method we only cultured an ESBL-producing *E. coli* (CTX-M-32 type) from the same sample. These results underline the importance of continuous and specific monitoring of carbapenem-producing Enterobacteriales in food-producing animals and along the food chain in the EU.

Beatriz Roman-Guerra, EFSA mentioned that the NDMs usually have very high frequencies of transference and stated that for anything resistant to meropenem, it is suggested that the NRL confirms the finding.

Overall outcomes of the EURL-AR EQAS 2019 for E. coli, Staph and Enterococcus (Valeria Bortolaia, EURL-AR)
See presentation (direct link or https://www.eurl-ar.eu/presentations/workshop-virtual-meeting-april-2020.aspx)

Deviations presented in the participant evaluation report may sometimes be caused by an MIC obtained in the acceptable range but interpreted erroneously, it may indicate a technical problem, or it may be due to limitation of the method (a so-called 'on-fold dilution issue')

For all three organisms, an excellent performance was observed, with no outliers. For enterococci as well as staphylococci, the overall deviation level related to AST results is at around 1%, whereas for *E. coli* it is at around 0.5%.

Regarding interpretation of MIC values, remember for the EQAS to apply the values presented in the EQAS protocol. As for ECOFFs changing over time, please see this important link: https://www.eucast.org/mic_and_zone_distributions_and_ecoffs/new_and_revised_ecoffs/

For the ESBL/AmpC categorization, minor issues mainly related to definitions were observed. Molecular methods highlight genetic background that was overlooked by using phenotype only.

For the MRSA detection, the molecular and/or latex agglutination methods complement phenotypic test results.

The EURL-AR recommends to be alert regarding carbapenemase detection: as they are infrequent at present, they might be difficult to detect. Re-test any isolate that looks suspicious to you.

The EFSA/EURL-AR confirmatory testing for 2018 data (Valeria Bortolaia, EURL-AR)
See presentation (direct link or https://www.eurl-ar.eu/presentations/workshop-virtual-meeting-april-2020.aspx)
This year, 384 strains were requested from 31 countries. Some issues of contaminations were observed. In some cases, isolates were not found or not viable. Some isolates were not tested due to re-testing at the NRL => 368 strains from 30 countries. Still some strains were contaminated or not found/not viable => 349 strains for MIC.

The request to revise data is indeed a kind request (not compulsory), and we always ask to retest at the NRL. Actually, only half the countries reported back what is actually the action following the request. It would be good to have a defined procedure to ensure that the communication does not get stuck somewhere but in fact reaches the relevant person at the MS that can take action.

For this year, the laboratory work related to the phenotypic testing is finished and the work related to genotypic testing is ongoing.

Summary of the plenary discussion:

You may find in the report from ResFinder that different *bla*$_{TEM}$ genes have been obtained simultaneously. ResFinder outputs the best match, and if it does not find a match, it outputs all the genes that are potential matches (could be one-nucleotide differences from a known *bla*$_{TEM}$ gene). So more than one *bla*$_{TEM}$ gene in the report does not necessarily mean that there are more genes, only, it has to be analysed manually.

Overall outcomes of the EURL-AR EQAS 2019 for Matrix (Jette Sejer Kjeldgaard, EURL-AR)
See presentation (direct link or https://www.eurl-ar.eu/presentations/workshop-virtual-meeting-april-2020.aspx)
Five pork meat samples and three cattle caecal samples were analysed by in total 35 laboratories.

Overall, 94 % results correctly classified the samples as ESBL/AmpC. There were 7 qualitative deviations.

Challenges observed included:

- For M-5.4: 5 of 31 participants did not isolate the carbapenemase producing strain from the meat sample
- For M-5.5: 9 of 31 participants isolated a presumptive carbapenemase (n=7), ESBL (n=1) or other phenotype (n=1) isolate from a meat sample spiked with a susceptible *E. coli*.

- For M-5.7: Phenotypically the isolate would be characterized as ESBL + AmpC, whereas genotypically it would be characterized as an ESBL.
- Some difficulties in prediction of ESBL or ESBL+AmpC or AmpC phenotypes.

Plenum follow-up related to EURL-AR EQAS

Approval of the three EURL-AR EQAS reports pending.

- EURL-AR EQAS *Salmonella*/*Campylobacter* 2019 report
    - Approval pending
- EURL-AR EQAS *E. coli*, enterococci, staphylococci 2019 report
    - Approval pending
- EURL-AR EQAS Matrix 2019 report
    - Approval pending

The EURL-AR will get back with the reports for approval.
And also, further details will be circulated related to the upcoming EQAS this autumn.

AOB and general discussions (René Hendriksen, EURL-AR)

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

Further details related to upcoming workshop in 2021 will be circulated – hopefully a face to face joint meeting with the FWD-network.

The next EURL-AR training course will be online and will he held – on 19-23 October 2020 (Practical use of the knowledge obtained and WGS tools demonstrated in the past TC's)