Minutes – EURL-AR Workshop, Kgs. Lyngby, April/2014

The minutes are listed according to the agenda.

Participants

From the EURL-AR-network, all member states (MS) were represented at the workshop with Luxembourg participating for the first time in the network activities. Austria's representative, however, had to cancel due to health issues. Participating non-MS were Albania, Bosnia-Herzegovina, Kosovo[1], Montenegro, Norway, Serbia, Switzerland and Turkey. Additionally, a representative from the EU Commission and EFSA participated.

Monday, April 7th 2014

Welcome (Henrik Wegener, DTU Provost)

Henrik Wegener welcomed the group to DTU emphasizing the importance of the focus on application and the study of life sciences. Research in surveillance infrastructures is increasing and we know that it is not only about making the right measurements but also about acting on it. As the EURL-AR network, you constitute a big European surveillance infrastructure, and measuring antimicrobial resistance in a meaningful way can lead the right actions. The bad news is that what you are measuring is only getting worse! If we do not turn our measurements into actions it will keep getting worse. Therefore what you do is so incredibly important. Have a great workshop!

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

This year's workshop will focus on the new legislation in relation to the harmonized and standardized AMR monitoring scheme and programme in the field of zoonotic and commensal bacteria together with updates from EFSA and the Commission, the network's proficiency tests and country presentations. In addition, invited speakers will present subjects such as carbapenem-producing bacteria in the public health sector in Europe, aminoglycosides, the theory behind PCR and the new advancement in metagenomic.

Update from the EURL-AR (Rene Hendriksen, EURL-AR)

In 2013, the EURL-AR has been quite involved in the development of the new regulation. We participated in a range of working groups and meetings etc. in relation to the EC, EFSA, ESVAC and WHO. We worked towards extending our network to include more of the candidate countries and potential candidate countries, and many are also present at this workshop.

Follow-up has been done on action items from last year's workshop, activities are still ongoing for some of the activities which will be presented these next days, and for the following: 1) the EURL-AR set up a 'phenomatics'-group aiming to compare genotypes and MICs. We have received strains and cloned genes into a unique system, and 2) the EURL-AR is investigating the mechanisms behind the phenotype displayed in *Campylobacter* by resistance to only CIP or NAL.

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

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

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

Update on the five-year-action plan from November 2011 which is still on-going. Resolutions from the EU Parliament promote and push to speed up the AMR action plan. Activities of the Parliament are broad and diverse questions are coming in and being responded. Actions 2 and 3 relate to the appropriate use of antimicrobials in veterinary medicines. Revision on regulation of veterinary medicine legislation is ongoing, potential measures that the new proposal may include are: incentives for new veterinary antimicrobials, restriction of the cascade, new and better tools for management of antimicrobial resistance, advertisement, decoupling sales and prescription. As part of Action 8, international cooperation is also ongoing with third countries and other organizations: OIE, WHO, China and USA (TATFAR). Exchange of information and visits ongoing to the European organizations (EU, ECDC). A progress report & road map will be published mid-2014. Action 10 is one of the key actions regarding surveillance systems and will lead to a better understanding of the epidemiology. Decision 2013/652/EU was focused on as minimum requirements to be followed in MS, and NRLs are responsible for the testing. The first joint report of EFSA, EMA and ECDC on the relationship between the use of antimicrobials and the occurrence of resistance based on the data of the surveillance systems in both humans and animals is expected by the end of 2014.

Summary of the plenary discussion:

- At this moment, the ESVAC project collects data on sales of veterinary medicine. The revision of the veterinary medicines legislation will provide the legal basis for the collection of data. The work has not yet been concluded and projects are ongoing before the final legislation will be ready. Likewise, the updated SPC-procedure is not yet published.
- Currently, there is legislation on microbiological criteria for food (e.g. absence of *Salmonella* in some foodstuffs, etc.), however, the EU legislation at this moment does not include criteria or norms as regards AMR (e.g absence of ESBL-producing bacteria, etc…). This could be the next step but might still be a little premature.

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

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

P-AB gave an overview of reports published recently, implementation of new regulation and current activities with ECDC and EMA. Results in new reports will be interpreted with new EUCAST ECOFFs and old results will be re-interpreted for continuity and update.

P-AB explained randomized sampling for monitoring and mentioned that a joint report will be generated with EFSA, ECDC and EMA collaborating on the data analysis looking at consumption data versus occurrence of resistance in animals and humans. This report will be the first of its kind and will be issued by end of 2014, but will not replace the reports of the three agencies separately.

Outcomes of the EURL-AR EQAS 2013

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. (Shona Neal, NRL UK)
  See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)
  - The presentation illustrated that PHE followed up on results and improved them. Follow-up was presented and actions taken described.

- For historical reasons, agar dilution is used, also, ertapenem is also preferred to meropenem even though not in legislation, due to experience and historical use in PHE.
- The presentation showed a good example on how to use a proficiency test for self-evaluation and correction when necessary.

- Genotypic characterization, ESBL-genes (Cindy Dierikx, NRL Netherlands)
  See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)
  - The presentation addressed the following points for discussion: only 8 laboratories were participating; how about the importance of reporting non-ESBL betalactamase-genes; detection of VIM-2 and CTX-M-3 need some attention; false positive results – why?
  - Description of CVI (NRL Netherlands) method with testing with arrays (Checkpoints CT 101 or 102) and evaluation of own procedure.
  - The EURL-AR advice to participate in these tests because it complements and gives more certainty to phenotypic results.
- *Enterococcus* spp. (Björn Bengtsson, NRL Sweden)
  See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)
  - Species ID introduced; generally went well for the EQAS-round
  - AST appears to be becoming better over the years, even if breakpoint issues still occur
  - Challenging strains are not so much seen; all NRL's are very welcome to send us interesting and challenging strains so that they could be candidates for an upcoming EQAS.
- *Staphylococcus* spp. and methicillin-resistant *Staphylococcus aureus* (MRSA) (Suvi Nykäsenoja, NRL Finland)
  See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)
  - Two strain/antimicrobial combinations were omitted due to MIC-value close to cut-off.
  - Incorrect results for MRSA were partly explained by the PCR method used and a strain switch.
  - Information from EURL-AR that for the 2014-iteration, there will be no MRSA PT.
- *Campylobacter* spp. (presentation prepared by Sandra Jelovcan, NRL Austria)
  See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)
  - 30 laboratories submitted data on speciation, one participant did not upload speciation data
  - Discussion of deviations and standardization needed to reduce the issues, e.g. suggestion to have exact MIC values for all antimicrobials tested over a wide test range and to also test antimicrobials not included in the EFSA panel (e.g. AMX, IMP, CHL)
- *Salmonella*/*Campylobacter* EQAS results – evaluation of MIC-values (Susanne Karlsmose, EURL-AR)
  See presentation (link or http://www.eurl-ar.eu/146-presentations.htm)
  - Could evaluation of the obtained results for the EQAS be evaluated in a better way; could it be based on MIC and subsequently be categorized as correct, wrong or inconclusive?
  - Discussion based on results from a three-year-period
  - Conclusion that we will look into further data next year and also that we, for 2014, will include 'new analysis' for both *Salmonella* and *Campylobacter*-results. In addition, for the next workshop, we will into comparison of 3-year's EQAS results based on the internal control.

Final remarks to the EURL-AR EQASs:

All three EQAS-reports were approved for ISBN-registration; i.e. the reports on the EQAS on the *Salmonella*, *Campylobacter* and genotypic characterisation and the EQAS on MRSA were approved

without further comments. The report on the EQAS on E. coli, enterococci/staphylococci was approved for ISBN-registration upon excluding the results from the combination of ENT 7.8/daptomycin.

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. A survey will be sent to the network to obtain and explore relevant input before a final decision in this regard is made. The input from MS at the workshop in this relation will be included as background for setting up the survey.

Monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria; experience with the new legislation (European Commission, European Food Safety Authority and EURL-AR represented in the panel)

1½ months prior to the EURL-AR workshop, participants of the network were encouraged to contribute with questions or concerns in relation to the new monitoring legislation (Decision 2013/652/EU). Representatives from the European Commission, EFSA and the EURL-AR were in the panel responding to the question and concerns.

Summary of panel and plenary discussion:

Sampling and/or randomization

AMR should be measured in representative samples and you should therefore have at least 170 isolates tested. If one MS has five isolates from one flock, only include them all, if you have different serovars. Clearly, in a case like that you should follow more up on resistance in indicator bacteria.

If the same clone appears more than once, even if sampled at different times of the year, it should be tested just once to not have duplication of data.

If encountering different serotypes in one flock the assumption is that one isolate per flock would be representative. If finding extra serotypes and you have specific interest you can include it, but this was not the general idea. Given the situation with low numbers and prevalence, such isolate may be included; this must be adapted to reality of the country in question.

To ensure comparability between countries, the same methodology/procedure should be applied. The monitoring in animals focuses on domestically produced animals (bread in the country). In the case of meat sampled at retail, the meat should not be selected based on the origin. Fresh meat should be included, preferably not vacuum packed or frozen (see Decision 2013/652/EU part 2.3.3).

One pack pr. batch, only, should be included. The definition of fresh meat here is also frozen meat and vacuumpacked meat, but not a meat-product or meat preparation; so no added spices/processed meat.

Imported foods and MRSA are not included in the new legislation but it does not exclude MS from having their own monitoring which is not yet harmonized. Each MS has the possibility of doing it, optionally.

Availability of MIC-plates

Prices for MIC-plates vary from country to country. According to the info from one of the producers, they have no possibility of setting their distributors' price. SVA is thinking of intensifying their production of microbroth plates. The EURL-AR will follow up with a survey collecting info from NRLs on this issue.

The reporting of the 170 isolates must be according to the description in the legislation. Testing by agar dilution is also an option, if using the CLSI standard and following the CLSI guidelines. However, the experience from the network is that getting 15 AB's working perfectly at the same time is seriously challenging.

Technical aspects regarding MIC-testing

The method performed when using a TREK protocol should follow the TREK recommendations. Sheep blood was tested only for agar dilution and is therefore not validated for the microbroth so if wanting to use horse blood for the TREK method, you need to validate the lysed blood in house.

Procedures must follow the legislation to be part of the monitoring. For some antibiotics/species, no interpretative criteria are available. EFSA and EUCAST might set new criteria soon.

Reporting of results

For the 2nd plate for Enterobacteriaceae, check consistency and report the enzyme.

Isolate-based data reporting is mandatory and must be in line with the prerequisites of the legislation. Not all NRL have the possibility of typing and have the possibility of the national competent authority appointing another lab to do the typing.

Co-financing is described in Decision 2013/653/EU. The financing can only be developed year pr. year, so every year we will have new legislation.

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Due to the time schedule, remaining questions would be addressed by email directly to the questioners.

Activities of the NRL in Ireland (Rosemarie Slowey, NRL Ireland)

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

Agriculture in Ireland is important, 11% of export is food products. Imported product also of importance; chicken from Brazil and Thailand are imported.

Laboratory-projects presented, including ESBL-surveillance 2011-2013.

Activities of the NRL in Luxembourg (Joël Mossong, NRL Luxembourg)

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

Large production of adult cattle (16000 tonnes pr. year), but the vast majority of chicken is imported.

In the unit for epidemiology and infectious disease, lab-based surveillance is performed. Prevalence of *Salmonella* was high but was reduced and is very low at the moment. Research is ongoing on sampling from environment and other origins. Same genotypes were observed in bovine, poultry and surface water. The prevalence of *Campylobacter* has been increasing and is much higher than *Salmonella*. Even if there is a large diversity of *Campylobacter*, many clones only occur once sporadically and only few cause small outbreaks.

Standardization of AST method for *Campylobacter* MIC testing (Concha Porrero Calonge, VISAVET, Spain)

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

The laboratory experienced problems with the MIC-testing of *Campylobacter* and observed a peak of deviations observed in EQAS 2010 which continued in 2011. Strains were re-checked, but problem remained. The protocol applied in the laboratory was adjusted in different parameters, and the laboratory therefore followed-up by testing three protocols in parallel, and selecting a new protocol following international guidelines for use in the laboratory. Applying this, EQAS 2012 and 2013 were performed without deviations.

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

General discussion:

- For the MRSA-isolation, one point in the protocol where you can stall the process would be when you have the isolate on the plate. Some countries store samples, e.g. environmental samples can be stored over the weekend for processing Monday.
- Zink is now being used massively in more than one EU-country. We know that it is connected to MRSA but we know very little about zink and commensal bacteria. In Belgium a research project is on the way to be started out at the end of the year. It could be a possibility to collect overview data in a survey.

Tuesday, April 8th 2014

Carbapenemase-producing bacteria in Europe (Hajo Grundmann, the Netherlands)

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

To get the message on the importance of antimicrobial resistance through to politicians, this must be said in burden of disease estimates. Probably as many people die from resistant infections as those who die from road traffic accidents, but resistant bacteria do not affect DALY's so much since they often occur late in life whereas for road traffic accidents, the number of expected life years after the incident are probably higher than for resistant bacteria.

But, concerning carbapenems; they have been giving some buffer to the clinician, but we are losing it, and this leaves practically no alternative (but polymyxin). In Poland, a KPC-producing *K. pneumonia*, spread over two years and was found as 160 non-repetitive isolates.

The EuSCAPE-project involves all EU countries and a number of associated countries and have detected carbapenemases in the majority of countries (regional spread) and in other countries the situation is endemic. The question is how we knock on the politicians' doors; how do we prevent that these bacteria are introduced into livestock productions; approaches could be to 1) make sure that the hospitals know what to do at national level, and 2) at international level, the WHO national assembly has heard the message and there is a lot of work done in introducing a global monitoring. However, as for the interface between animals-humans, we do not know much about it yet.

Summary of the plenary discussion:

As for the referral patterns for hospitals very illustratively presented visually; this could look like the trade with pigs and MRSA and it could be interesting to see the same model and situation on animal level. The data can be introduced and easily modelled. A couple of assumptions would also allow for predictions and could become a wake-up-call.

Evaluation of methods for detection of ESBL/pAmpC and carbapenemase producing *E. coli* and *Salmonella* in meat and faecal samples - a progress report (Henrik Hasman, EURL-AR and Beatriz Guerra-Roman, Germany)

See Henrik's presentation (link or http://www.eurl-ar.eu/146-presentations.htm)
See Bea's presentation (http://www.eurl-ar.eu/146-presentations.htm (note: divided in parts 2A-2E))

Henrik Hasman:

Danish ground beef is known to have a low prevalence of ESBL-producing bacteria, and was used as a matrix and spiked with bacteria. Method for testing described (six variants presented in overview and details), based on variants of the DANMAP-method and on variants of the method used for isolation of *Salmonella* (McConkey broth +/- a cephalosporin and pre-enrichment with peptone +/- a cephalosporin, respectively). Incubating at 44°C meant getting rid of much of the background flora.

Based on the results, method #1 (with peptone water) is suggested for the meat samples. Method for caecum samples is being investigated at the moment.

Beatriz Guerra-Roman:

Challenges at detecting carbapenemases and results of evaluation of different methods to detect different carbapenemase-producers in a food matrix were presented. From the results obtained at the BfR (NRL-AR and the National German project RESET) with spiked beef minced meat, pre-enrichment without carbapenems resulted better (otherwise advantage is given to non-fermenters). Several commercial and "in-house" made media (different carbapenems, concentrations and agar media) were compared.

Recommendations were made. Testing on caecum samples not performed yet.

Note: When you perform the analyses, remember that carbapenems are not very stable! This is very important for the "life" of the agar plates. If you use commercial plates, do not use them if the date is over, and if you prepare them themselves (i.e. MacConkey Agar with meropenem), try to prepare them fresh or use them very close to the "preparation date".

Summary of the plenary discussion:

The method will be defined in legislation and therefore must be used.

This is not only a scientific process but a legal process. MS must follow the last version of the protocol for the EURL-AR.

Reference strains as well as a proficiency test with ESBL and carbapenemase-producing bacteria in a matrix would be helpful. Type strains are available and have been used in the public health sector, only, in our field we need to be able to detect strains with low MICs which are not so easy to find.

A reduced scheme will be run on ceacal samples and aim to have a protocol ready before summer. For 2015 we need a protocol for cattle and pigs, so we will not consider chicken meat specifically right now.

Aminoglycosides (Bruno Gonzalez Zorn, Spain)

Overview of different aminoglycoside families. They have different modes of action, have a broad-spectrum and low cost.

Many different enzymes cause resistance to aminoglycosides – ACC, APH, ANT… Even to a drug that is not yet on the market (ACHN-490), resistance can be detected.

Fitness cost of having methyl transferases is not great, and many bacteria have been found to have more than one. Also, of 140 isolates with a methyl transferase, 64% were found to also have NDM-gene

Summary of the plenary discussion:

A study is ongoing in MedVetNet to look for ArmA, it could also be interesting to look into our strain collections for high-level resistance in Europe; there were only few originally, probably there are many more now.

Integrons – what and where (Benoit Doublet, France)

Cancelled.

PCR for beginners (Valeria Bortolaia, Denmark)

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

The theory behind PCR was presented and the method introduced in detail, including primer design and PCR in the laboratory. Questions relative to QC presented; the use of internal controls as well as sequencing based on PCR.

Metagenomics (Frank M. Aarestrup, EURL-AR)

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

As of now, 23 days is the median for a result from an outbreak – we need to speed up this time of and create frontline diagnostics tools to have real-time data.

We looked into Next-Generation-Sequencing (NGS) directly on samples and tested urine samples, and in the EFFORT project we will see if we can take samples directly from manure and to detect an 'antimicrobial profile' of the farm. With the *metagenomics mapper* we will map the reads towards all the bacterial genomes listed in NCBI.

Summary of the plenary discussion:

- Many laboratories perform sequencing; the analysis of the data will be the issue and using it as a basis for good predictions. For having the right analytic tools available, a way forward is that we make data available in real time; the tools are already available on the web, for example from NCBI and EBI.
- NGS on urine and metagenomics on sewage – what about faeces? In the future it will be faecal swabs. We need a sufficient amount of DNA (1ug) to do PCR-free high scale sequencing. We have been able to detect pathogens in faeces but not to obtain their resistance profile. Handling of the samples including purification are important issues to focus on the relevant DNA in the sample.

Future perspectives and closing remarks (Rene 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 scientific presentations highlighted the need for global surveillance and the emerging problems in relation to the spread of ESBL-producing *Enterobacteriaceae*. With the new legislation we will have harmonized follow-up in relation to the food and veterinary sector in EU.

The EURL-AR is in a dialogue with the ECDC, hoping to arrange a joint meeting with the FWD-network for the upcoming EURL-AR workshop in 2015.

Action items, NRL-AR's:

- Send any interesting strains for the network proficiency test. Note, these will always be sent out as coded strains, keeping all details blinded.
- Remember that isolates may be sent to the EURL-AR for verification of interesting or rare antimicrobial resistance profile.

Action items, EURL-AR:

- Will look into comparison of 3-year's EQAS results based on the internal control
- There are some considerations to take into account in relation to accepting just one set of EQAS results per MS per microorganism. The EURL-AR will send out a questionnaire to capture feedback from the NRL's on this issue.
- Many questions were asked in the survey sent out before the workshop, the EURL-AR will send answers to those that did not get replies during the meeting.
- Will follow up with Hajo Grundmann regarding interesting strains
- Will work with Bea from BfR on the methodology in relation to the new legislation for the testing of carbapenemases. We hope to have a concrete protocol before summer.
- Will explore the possibilities of arranging a proficiency test on a food or ceacal sample matrix.

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