Minutes – EURL-AR Workshop, Kgs. Lyngby, Denmark, April/2011

The minutes are listed according to the agenda

Participants:

All member states (MS) with NRL-AR, except Cyprus, took part in the meeting. Luxembourg has not appointed an NRL-AR and did not take part.

Participating non-MS were Croatia, Norway and Switzerland.

Monday, April 4th 2011

Welcome (Rene Hendriksen)

Rene Hendriksen welcomed the EURL-network to the new facilities of the EURL-AR located at DTU Campus in Kgs. Lyngby, Denmark.

Rene Hendriksen welcomed all participants and the invited speakers.

Update from the EURL-AR (Frank Aarestrup)

1) Emphasized the importance of collaboration in research and monitoring in MS
2) Stated that monitoring on antimicrobial resistance is dynamic. We are moving towards harmonization in relation to what we are doing. Issues at the moment and ahead of us are selection of the right cut-off values, and challenges regarding disk diffusion.
3) Mentioned that CLSI will be setting a new, lower clinical breakpoint for ciprofloxacin resistance (S=<0.064 ug/mL, I=0.125-0.5 and R=>1 ug/mL; and S=>31 mm; R<=20 mm; unpublished yet) for *Salmonella*, and plan to set breakpoints for resistance to other fluoroquinolones for *Salmonella* and other Enterobacteriaceae.
4) Stated that new types of antimicrobial resistance continue to emerge, e.g. *mecA* has been around for many years, but now a new gene has emerged in the UK (a divergent *mecA* homologue; $mecA_{LGA251}$). This emphasizes that we must constantly struggle to adapt to the future.
5) The activities of the EURL-AR in the 2011 will be a continuation of the 2010-activities:
   a) Proficiency tests on antimicrobial susceptibility tests (AST) and possible re-tests
   b) Proficiency test MRSA (simulated nasal swabs)
   c) Optional proficiency test on genotypic characterization
   d) Training course (on genotypic characterization)
   e) Shipment of reference strains
   f) Project finalization (articles on quinolone resistance and on streptomycin breakpoint for resistance are submitted)
   g) Project continuation (colistin, quinopristin/dalfopristin, ESBLs in Europe)
   h) Project launching will be discussed during this workshop.

Update from the EU Commission (Leena Räsänen)

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

Leena Räsänen described the activities of the EC in relation to antimicrobial resistance and focused on the EC-strategy, review on legislation, EFSA, EMA, other international activities, and the evaluation of the EURL's.

A five-year strategy is being prepared and will be presented on November 18th 2011.

Review on legislation on veterinary medicinal products (VMP) is ongoing at the EC (Public Health impact vs Animal Health).

EMA are in the process of collecting data on consumption of antimicrobials in animals.

EFSA has a mandate on a Scientific Opinion on ESC (include true ESBL and AmpC) in food and food-producing animals which is to be published in July 2011.

CODEX alimentarius guidelines on risk analysis on AMR are ready for adoption in July 2011.

TATFAR (transatlantic task force on AMR) has been established to identify areas for further cooperation between EU-US.

The WHO-EURO office produced a booklet describing strategies on tackling AMR from a food safety perspective which is published on the WHO-EURO website (link).

During the last year's time, an evaluation of 26 EURLs was conducted. The evaluation was based on five categories. The evaluation of the EURL-AR graded all five evaluations with A's (only two other EURL also had five A's). The publication of the report is not yet final, but all labs will be re-nominated.

Subsequent to the presentation by Leena Räsänen, some of the issues brought up for discussion were:

♦ Comment from the audience: What is the timeline for the activities mentioned in the EC strategy?

⟹ Leena Räsänen: This is a long term project which includes the collection of comparable data on the use of antimicrobials in animals. The template has now been harmonized for collection of data based on the experience of 10 countries and aims to have all other EU countries to join. Phase 2 will be to assess data on animal species and to compare to human data.

We are awaiting an opinion on ESBLs from EFSA in July 2011. This opinion will be discussed with MS. For action to be taken, this opinion needs to be put into legislation (it can take up to 2 years) and be prepared for a decision for monitoring of ESBL/MRSA – which in that case would be discussed with MS.

Proposal for legislation on VMP will come out late 2012 for discussion with MS.

In general, guidelines are developed in a relatively short time, but national legislation takes time to implement.

Update from EFSA (Pierre-Alexandre Beloeil)

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

Pierre-Alexandre Beloeil (PAB) presented the main findings in The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food for 2009. For human isolates, one of the issues observed was the lack of harmonization on human data which makes assessment difficult at EU level; many MS do not report data, and many use disk diffusion for AST of *Campylobacter*. For data from animal and food, more MIC-data were reported. Antimicrobial resistance profiles commonly found in animals show important variations between animal species, and also large differences between MS.

New chapters have been included in the Summary Report, i.e. farm-to-fork analysis compared to human and animal data.

Data on extended-spectrum beta-lactamases will be used for an opinion to respond to the EC-mandate. In addition, MS are encouraged to monitor and report data on MRSA.

As regards to the data from 2010, a similar report is being planned and a pilot is being prepared to report data in a harmonized way (included 11 MS in 2010).

In the future, ECDC, EMA and EFSA will collect data in a report on antimicrobial resistance and consumption, including data at the isolate level and monitoring of indicator bacteria (mandatory).

♦ Comment from the audience: what are plans for having data on human side more comparable and harmonized?

⟹ Pierre-Alexandre Beloeil: The ECDC would be thinking of ring trials for these labs.

⟹ Leena Räsänen: New requirements need to be laid down in legislations. Baseline-studies can be co-financed by the EC but not routine monitoring which is on MS expenses. Voluntary reporting is increasing, therefore it could be justified to include this in mandatory reporting.

Presentation and discussion of EQAS results, *Salmonella, Campylobacter* and optional genotypic characterization (Susanne Karlsmose)

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

For both microorganisms, the majority of NRLs performed with deviation levels below the acceptance level. No outliers were identified, but challenges for *Salmonella* were met for ciprofloxacin and for detection and confirmation of ESBL-producers. For the optional genotypic characterization of the two test strains, results from the few participants were in good agreement with the expected.

It was discussed whether or not the results from the strain/antimicrobial combinations with less than 75% correct results should be evaluated or disregarded. Several tests in a number of laboratories rendered MIC's below the cut off (both expected results were at the cut off (R)). With the precision of the microbroth method allowing +/- one MIC step, the interpretation of the streptomycin MIC in these two borderline strains – even when tested with a quality assured method – was found to be susceptible in more cases than resistant. Based on the limitations of the technique it was decided to exclude results that have a high deviation level due to a one step deviation in MIC.

Genotypic characterization was included as an optional part of this EQAS as the focus on molecular typing is increasing. Four laboratories participated, with two and four laboratories uploading results on the Gram-positive and the Gram-negative strain, respectively. It was stressed out by the EURL-AR that participation in this optional part does not require upload of a full list of genes. Suggestion from the audience to include a *Salmonella* and a *Staph. aureus* as test strains.

The training course in week 45 will be on molecular typing methods and will include theoretical lectures as well as labwork.

General comment for the proficiency tests: The organizers must take reasonable precautions to prevent collusion between participants or falsification of results, therefore instant individual evaluation of results before deadline cannot be offered in future EQAS's. Instead, individual evaluation report will be made available upon deadline.

Acceptance of the report: Draft report was approved, after excluding from the evaluation the results of the strain/antimicrobial combinations with less than 75% correct results.

Presentation and discussion of EQAS results, *E. coli,* enterococci and staphylococci (Lourdes Migura)

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

For all three microorganisms, the majority of the participants obtained a deviation level nicely placed below the acceptance criteria (at 5%). Two outliers were indentified, one for the enterococci trial, and one for the *E. coli* trial.

Four strain/antimicrobial combinations resulted in less than 75% correct results, in parallel to the *Salmonella* trial, these will also be disregarded in the evaluation. The discussion also concluded in agreements that the EURL-AR will consider carefully the inclusion of strains which have MIC's on the borderline.

♦ Comment from the audience: Could the MIC and not the interpretation be evaluated?
   ⇒ EURL-AR: There are pro's and con's to that approach, until now the objective has been to evaluate the monitoring data for EFSA, and in this context, the interpretation is most relevant and the MIC is background information.

Acceptance of the report: Draft report was approved, after excluding from the evaluation the results of the strain/antimicrobial combinations with less than 75% correct results.

Presentation and discussion of MRSA EQAS results (Lina Cavaco)

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

Results on this year's simulated nasal swabs were excellent – no deviations were observed regarding detection of MRSA. *Spa* typing was performed by 12 out of 21 labs, and one deviation was observed.

The draft report of the MRSA EQAS 2010 was approved.

MRSA EQAS 2011 on simulated nasal swabs is in preparation, and will include swabs with differing levels of CFU. The stability testing of the swab samples were carried out over 2½ months at room temperature and at 5ºC.

An acceptance threshold has not been set.

Tuesday, April 5th 2011

Implementation of a system for monitoring antimicrobial susceptibility in indicator *E. coli* - the experience of Poland (Dariusz Wasyl)

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

The background, the setup and the preliminary conclusions of the current system for monitoring antimicrobial susceptibility in indicator *E. coli* was presented.

Overall, the monitoring of antibiotic resistance in indicator *E. coli* was successfully implemented. It has been harmonized according to the EFSA guidelines, sampling is randomized and country-wide, and baseline data on AMR in surveyed populations is collected.

Different antimicrobial usage polices and husbandry practices were observed. Trend analyses were performed, resistance phenotypes were detected and analyzed (quinolones, ESC)

Comments/questions from the audience:

♦ Were the cattle divided into age-groups? – and swine?

⇒ D. Wasyl: Division by age-groups would give too few samples pr. group. Cattle were in one group, but no calves were sampled. Swine were registered by age, but the data has not yet been analysed

♦ We see high level of antimicrobial resistance in bacteria from broilers, but not so high in layers.

⇒ D. Wasyl: The life length of the layer is much higher than the broilers, so this is a possible reason why the broilers present higher levels of antimicrobial resistant bacteria.

♦ Very nice quality assessment and design divided into imported/domestic poultry – is there the same division for swine and cattle?

⇒ D. Wasyl: It was not the intention to sample from imported animals, but it happens occasionally in the random sampling.

♦ Are you working with the human side also in this project? And what about consumption data?

⇒ D. Wasyl: Unfortunately this is a veterinary project only, but antimicrobial consumption data are included as a part in the project.

Surveillance of veterinary antimicrobial agens in Europe - the ESVAC project (Kari Grave, EMA)

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

Kari Grave described the mandate of the ESVAC project in which EMA has the lead in collecting data on the use of antimicrobial agents in animals. Data collection is an integrated approach in which ECDC, EFSA and the EURL-AR are consulted.

The purpose is among others to obtain reliable data for risk profiling and risk assessment, to assess the impact of measures taken in relation to prudent use of antimicrobials and to aid comparison of usage of antibacterial drugs between time periods, countries, etc. The collection of antimicrobial usage data should

ultimately help to optimize antimicrobial usage. The idea is to collect data as close to the end-user as possible.

Of note, it is not possible to use DDD to report aggregated data on sales of antimicrobials since we deal with different animal species.

A possibility of collaboration within the EURL-AR network could be to start using terms in a harmonized way, e.g. fluoroquinolones and not ciprofloxacin.

Protocol, form and instructions are available on EMA's webpage (www.ema.europa.eu)

Comments/questions from the audience:

♦ Any time a new usage pattern shows, we look into what is used for human/veterinary treatment. The usage-pattern can be very useful when considering which drugs to test for and in what range.

♦ For the next time, maybe include data on species level also.

European surveillance of antimicrobial consumption in humans (Samuel Coenen)

See poster presenting the subject (link or http://www.eurl-ar.eu/146-presentations.htm)

The European Surveillance of Antimicrobial Consumption (ESAC) project is currently funded by ECDC to continue the collection of antimicrobial use data in Europe. A proper measurement of antimicrobial consumption is complicated. Though not a perfect outcome measure, the most common method to evaluate the antimicrobial use in humans is the DID (DDD per 1000 inhabitants per day). An alternative would be the number of packages sold (per 1000 inhabitants per day) or the number of persons treated. Antimicrobial use in Europe expressed in DID seems to be increasing. More and more countries have implemented or continue to implement actions to control antimicrobial resistance through rational use of antibiotics. The impact of these actions is monitored by using DID and other indicators of antibiotic use.

Some references:

- Adriaenssens N. et al. JAC 2010, 65:769-774 and BMJ Qual Saf 2011, Mar 21 (Epub ahead of print)
- Ansari F. et al. CID 2009, 49:1496-1504 and JAC 2010, 65:2685-2691
- Davey P. et al. JAC 2008, 62:1441-1447

Comments/questions from the audience:

♦ You can do something without even measuring – if you know the epidemiology and the practitioners' use of antimicrobial. We did this in my country and could see a 40% decrease in the use. The decision was taken before the measurements were done.

♦ More than one outcome measure is good. Looking at more than one outcome measure and still seeing the same trend gives a good indication as to which direction the trend goes.

Antimicrobial resistance phenotypes of *Salmonella* strains isolated from animals and food of animal origin in Portugal (2008-2010) (Lurdes Clemente)

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

Isolates were obtained from *Salmonella* National Control Programmes for poultry breeders, layers, broilers and food of animal origin. In addition, data from poultry farmers' own control programmes were included, as well as studies of prevalence in reproduction swine farms and other routine samples.

After isolation and identification, the *Salmonella* isolates were serotyped and tested for antimicrobial susceptibility.

For isolates from *Gallus gallus,* broilers showed the highest percentage of antimicrobial resistance, followed by breeders and layers. Across most serotypes, the antimicrobial class with the highest percentage of resistant strains was represented by the quinolones.

For isolates from swine farms and in food of animal origin, high level of resistance to chloramphenicol (not allowed in production animals) is notorious, which might be due to cross-resistance to florfenicol and to genes transported on mobile genetic elements. In addition, resistance to third generation cephalosporins was observed in strains of swine origin only and with a low frequency.

Comments/questions from the audience:

♦ Is it correct that your data show the detection of *Salmonella* isolates resistant to florfenicol and susceptible to chloramphenicol?

⇒ L. Clemente: This is indeed the case (will send to the EURL-AR for reference testing)

A nationwide survey on MRSA in pigs in Finland (Lasse Nuotio)

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

In the EU baseline study in 2008, 207 Finnish pig farms were sampled. One farm was found positive for MRSA, spa type t034 (CC398). Five months after the original sampling, an additional,more thorough sampling was performed (all pigs in this farm and further dust samples were examined; none were positive). When setting up the MRSA diagnostics we came by chance across three MRSA positive farrowing farms.

The sensitivity of the approach to detect farms with a minority of animals colonized by MRSA is unknown. The true prevalence of MRSA positive pig farms may have been 15-20% in 2009 - 2010. The following questions are still open: Are there common sources? What is the typical within-farm prevalence of MRSA in different types of farms and in different age groups? How long does MRSA persist in different types of farms?

Comments/questions from the audience:

♦ Are pigs imported into Finland?

⇒ L. Nuotio: In very low numbers and normally from Scandinavia.

♦ What type of samples do you propose to use for the follow-up study?

⇒ L. Nuotio: Nasal swabs

♦ Should we as a network do more to be pro-active as regards to MRSA? This could be achieved by screening for virulence genes in the separate labs. Resources for this should be found at the NRL.

Validation of current interpretative criteria for colistin susceptibility in *Salmonella* spp. and for quinupristin/dalfopristin susceptibility in *Enterococcus faecium* (Yvonne Agersø)

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

Quinupristin/dalfopristin susceptibility in *Enterococcus faecium*, was evaluated and described in 'Evaluation of the quinupristin/dalfopristin breakpoints for *Enterococcus faecium* (Hammerum AM, Agersø Y, Garcia-Migura L, Seyfarth AM, Porsbo LJ, Emborg HD, Bogø Jensen L in Int J Antimicrob Agents, 2009)

CLSI has suggested a breakpoint for resistance of ≥ 4 mg/L for Q/D in *Enterococcus faecium.* EUCAST has suggested > 4mg/L as the breakpoint for resistance to Q/D *Enterococcus faecium.*

Our findings support the use of an MIC value > 4 mg/L as the resistance breakpoint for Q/D, whereas a MIC of 4 mg/L should be reported as intermediate-resistant. Intermediate-resistant isolates should be tested for the presence of streptogramin resistance genes.

Comments/questions from the audience:

♦ A comparison of Virginiamycin and Q/D showed that Virginiamycin works much better than Q/D.

♦ EUCAST now recommends an epidemiological cut-off value at >1 mg/L.

As regards to colistin susceptibility in *Salmonella* and *E. coli*, a manuscript is in preparation by Agersø Y, Seyfarth AM, Hammerum AM and Møller Nielsen E.

Colistin resistance in *E. coli* and *Salmonella* can be used in the laboratory setting to identify an incorrect ID or MIC.

CLSI and EUCAST recommend a resistance breakpoint > 2 mg/L, but the question is whether *E. coli* and *Salmonella* with MIC > 2 mg/L are truly resistant?

The EUCAST value for *Salmonella* is not divided into serovars. In addition, only a low number of isolates is included. It appears that the merging of all *Salmonella* spp. into the same MIC distribution lead to an epidemiological cut-off value set too low. For *S.* Dublin, the cut off is set in the middle of the MIC distribution, therefore we recommend to increas it from > 2 to > 8 mg/L.

♦ It could be reasonable to include even more data on different serovars in the analysis at the basis of this conclusion– NRL Poland and NRL Netherlands both mentioned that they have several isolates already characterized which could be merged with the existing data

Interpretative criteria for florfenicol susceptibility in *Escherichia coli* (Valeria Bortolaia)

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

Genes encoding florfenicol resistance in *E. coli* are located on chromosome and/or mobile genetic elements. EUCAST lists a cut-off value at 16 mg/L.

Method for routine testing of beta-lactamase production in staphylococci (Kees Veldman)

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

Three things must be tested: susceptibility to penicillin (*blaZ*), production of beta-lactamases (*blaZ*), susceptibility to cefoxitin (*mecA*), which gives the following three possibilities:

Penicillin S, β-lactamase negative and cefoxitin-S:

- Bacteria susceptible to all beta-lactam antibiotics
- *blaZ*- and *mecA*-negative

Penicillin R and or β-lactamase positive and cefoxitin-S:

- Bacteria resistant to penicillins, but susceptible to semi-synthetic penicillins, inhibitor combinations and all cephalosporins
- *blaZ*-positive and *mecA*-negative

Cefoxitin-R (regardless of in vitro penicillin resistance):

- Bacteria resistant to all beta-lactam antibiotics, except for anti-MRSA cephalosporins.
- *mecA*-positive (and *blaZ*-positive or -negative)

Comments/questions from the audience:

♦ A scientific paper describes staphylococci having other beta-lactamases, e.g. an enzyme detected by the use of nitrocephin and a profile different in *mecA*-negative strains

Future developments, training courses, EQAS, research – general discussion and summary (Rene Hendriksen)

Suggestions for topics to include in the workplan 2012 are welcome.

- It was mentioned the idea to design a common ESBL-microbroth plate. A standard ESBL panel is available from TREK (no specific ESBL-plates from VetMIC). The EURL-AR is satisfied with the use of the TREK-panel. The network decided not to work towards a customized ESBL-panel.

- The EURL-AR will take the lead on a survey of microbroth panels used, including producer, antimicrobials and ranges used. This could possibly lead to joint orders of plates and thereby cheaper prices. Included in this, there is also the idea to contact TREK to possibly have the

network's Enterobacteriaceae-panel registered as a standard plate – this would ease the purchasing of smaller quantities.

- Discussion about ESBL-phenotypes and metallo-beta-lactamase producers (MBLs) with no genetic verification. We should consider looking for MBLs, but they have not been found in animals so far. An additional question is whether florfenicol resistance could be the reason for the selection of ESBLs, for example when used for cattle. NRL France (Lyon) would be interested in being part of a project involving these issues. EURL-AR will arrange an email discussion or conference call to further discuss this.

- Suggested project: *Staphylococcus* strains from EURL-EQAS exhibiting resistance to ciprofloxacin – we need to set up methodology to investigate the resistance mechanism behind the phenotype

- Suggested project: AmpC-*E. coli*: Difficulties in determination of resistance mechanisms present in *E. coli* containing either plasmid mediated or chromosomal AmpC overexpression. Strains resistant to cefoxitin might "hide" a concurrent ESBL production – we need to improve detection of resistance background in those strains

- Suggested project: Colistin resistance in *Salmonella* isolates, and divide into serotypes. We need coordination of follow-up.

- During the EQAS's, it should be reported if different methods involving different QC-ranges are used to give an indication of the actual distribution according to the methods.
- The manuscript on the streptomycin-project was accepted by Microbial Drug Resistance (MDR) after the EURL-AR workshop.
- In future EQAS-reports, in addition to the evaluation of results from the EU-designated NRL's, the results from the national reference laboratory in affiliated non-MS will be included.

Summary:

The EQAS reports on *Salmonella/Campylobacter* and genotypic characterization and on *E. coli*/enterococci/staphylococci are approved after extraction of the strain/antimicrobial-combinations with less than 75% of the results according to the expected.

The upcoming MRSA EQAS will include simulated nasal swabs with different CFU-levels.

Survey of antimicrobials and ranges used in microbroth panels will be conducted. EURL-AR will take the lead. This could possibly lead to joint orders of plates.

The data uploaded from seven countries to the ESBL-database are currently being evaluated (occurrence of ESBL-encoding genes in *Salmonella* and *E. coli* in Europe), and a scientific paper is in progress.

Conference calls and/or email-groups will be arranged for three new project ideas.

A survey will be arranged to collect data on Metallo-beta-lactamases.

A training course on molecular typing methods is planned to take place in week 45, 2011. This training course will include theoretical lectures and lab-work on molecular methods as well as hands–on on analysis of results. Participants should be people working in the lab with these methods routinely – one participant from each designated NRL is invited.