

Minutes – EURL Workshop, Berlin, April/2010

The minutes are listed according to the agenda

Participants:

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

Thursday, April 8th 2010

Welcome (Andreas Schroeter, Rene Hendriksen)

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

Andreas Schroeter welcomed the EURL-network to Berlin and introduced the BFR (the German NRL-AR).

Rene Hendriksen welcomed all participants and the invited speakers.

Update from the EURL (Rene Hendriksen)

- 1) Emphasized the importance of collaboration in research and monitoring in MS
- 2) The activities of the EURL in the past year of 2009 were mentioned
 - a) Ring trials on AST and re-tests
 - b) Training courses (individual as well as groups)
 - c) Shipment of reference strains
 - d) Project on quinolones
 - e) Project on epidemiological cut-off values for streptomycin
 - f) Introduced MRSA EQAS (dust samples)
- 3) Stated that the EURL are looking forward to the upcoming drug usage data from FDA and EMA
- 4) Mentioned the WHO-network 'AGISAR' (Advisory Group in Surveillance of Antimicrobial Resistance) meeting hosted in Copenhagen
- 5) Stated the importance of the guideline created during the WHO Codex intergovernmental task force on antimicrobial resistance

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 which are ongoing at the moment, since DG SANCO has given high priority to these issues.

A five-year strategy will be planned within the AMR-area and the aim will be to have a holistic approach

EMA project on monitoring the sales and uses of antimicrobials in animals is ongoing – started 2009.

A mandate on ESC (include real ESBL and AmpC) in food and food-producing animals has been given to EFSA. Deadline is June 2011.

CODEX alimentarius ad hoc Intergovernmental Task Force will be finalised in 2010. The outcome is guidelines on risk analysis on AMR.

TATFAR (transatlantic task force on AMR) has been established by EU-US summit in 2009. This task force aims to identify areas for further cooperation and to suggest them to the summit 2011. Clarification of scope awaits.

Specific workgroup on AMR is scheduled to have a meeting on 23 April 2010 (this meeting was subsequently postponed to May 25th due to air disruptions caused by the volcanic activities in Iceland). This workgroup will exchange of views of future steps on food- and animal-borne AMR, including monitoring, risk assessment and risk management.

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

- ◆ Comment from the audience: Are there directives regarding collection of antimicrobial usage data or sales data, is it mandatory to provide the data?
 - ⇒ Leena Räsänen: The industry must provide data to the national authorities if they require this, but the national authorities are not required to provide them to the EC => the legal basis to collect data is missing and will be discussed in the revision of veterinary pharmaceutical legislation.
- ◆ Comment from the audience: It is not clear what type of off label use is considered illegal. Legislation should be improved to help the interpretation. Especially regarding cephalosporins.
 - ⇒ Leena Räsänen: Off label use of antimicrobials (not illegal) is included in the working paper and it will be considered to propose acts to tackle this issue. In addition, there will be discussions on legal provisions of restriction of off-label use of some antimicrobials in the context of revision of pharmaceutical legislation.

Analysis of the baseline survey on the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in holdings with breeding pigs, in the EU, 2008 (Pierre-Alexandre Beloeil)

(Part A of the EFSA report is accessible: ([link](#) or <http://www.efsa.europa.eu/en/scdocs/scdoc/1376.htm>))

Pierre-Alexandre Beloeil (PAB) presented the data on prevalence of MRSA in breeding holdings and production holdings and described the findings. There is a significant difference between countries regarding pig holding contamination with MRSA. Some countries are negative, while in others the positive-holding prevalence is up to 46%. Also regarding the *spa*-types present there are some differences between countries and a larger diversity of *spa*-types and clonal complexes were found in some countries, while in other only MRSA of ST398 were found. The Part A report published last November included recommendations to perform periodic monitoring of MRSA in animals and better assess the public health importance of MRSA of pig origin.

In the part B report on the MRSA baseline survey the effects of certain factors associated with MRSA positive holdings were studied. The results were briefly described by PAB, but results have not yet been published (report of part B expected by mid May 2010). Subsequent to the presentation by Pierre-Alexandre Beloeil, some of the issues brought up for discussion were:

- ◆ Comment from the audience: Have actions regarding contaminated holdings been decided?
 - ⇒ Pierre-Alexandre Beloeil: What has been done is a prevalence survey including an analysis of associated factors. Risk management is pending and must be considered by the European Commission working group on zoonoses where risk managers will discuss possible introduction of monitoring of MRSA and possible risk management options available.
 - ⇒ Leena Räsänen: EFSA states that MRSA at the moment is not a big food safety issue. Normal slaughter should destroy MRSA, and it is therefore not considered an important hazard.
- ◆ Comment from the audience: Is data on human infection available? And is it a health problem? The main focus should be the human problem.
 - ⇒ Leena Räsänen: CC398 human cases are one of the data gaps. There are data from Netherlands but there is a gap of data from other MS.
 - ⇒ Dik Mevius: Infections in the Netherlands are endemic and cases are related to farming. Infection control methods are carried out, but CC398 does not spread from human to human, as human clones do. However, it is understandable that in some MSs further measures are taken.
- ◆ Comment from the audience: What about other animal species?
 - ⇒ Pierre-Alexandre Beloeil: Monitoring of MRSA in pigs is of interest, but also in all other relevant food producing animals, as well as horses and pets. The survey targeted pigs as the opportunity of the *Salmonella* baseline survey in breeding pigs was seized to perform in parallel an EU-wide MRSA baseline survey in pigs.
 - ⇒ Leena Räsänen: A meeting is planned for April, 23rd 2010 (the meeting has been postponed to the 25th of May because of air disruptions due to volcanic activities in Iceland), where proposals for other monitoring of MRSA will be discussed, as will management in production systems,

trade, potential occupational hazards to farmers, and human health effects. It will be the start of the discussion of possible management options, to start the process to take some action.

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>)

Performance has improved for all three microorganisms. No outliers have been identified, however, laboratories with high percentage of deviations will be contacted for directly regarding possible follow-up.

The selection of antimicrobials used for testing the *E. coli*, enterococci and staphylococci were discussed. We aim to keep the list simple. For the enterococci panel of antimicrobials, daptomycin, tigecycline and avilamicin will be omitted. Ciprofloxacin will be kept on the list. The EURL-AR will discuss this further and will contact Andreas Schroeter to discuss including some of the antimicrobials for enterococci/staphylococci.

MRSA identification has improved.

ESBL-producing *E. coli* is still considered a priority area. When new EUCAST-guidelines on detection and confirmation of ESBLs are published, the protocol regarding ESBLs will be reviewed and modified according to these.

The terms 'resistant' and 'susceptible' were discussed as opposed to 'wild-type' and 'non-wild-type'. There is a difference in reporting between AST for clinical purposes and AST for monitoring purposes. It is a question of terms, but in the EQAS are both EUCAST cut off values and clinical breakpoints are used as reference, and we need to stick to one set of terms. Consequently, in the EQAS-reports, the terms 'resistant' and 'susceptible' will continuingly be used, however, a paragraph will be inserted to describe the used terms.

We will continue assessing the categorizations S or R, and not the MIC value.

Acceptance of the report: Draft report was approved, with a small paragraph explaining the terms S and R, wild-type and non-wild type.

Suggested projects for consideration:

- Testing enterococci for Synacid susceptibility (methodological problem)
- Florfenicol in *E.coli*
- Colistine in *Salmonella* (The EURL-AR already has an oncoming project about this)

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

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

For both microorganisms, the vast majority performed with deviation levels less the acceptance level. For *Salmonella* there were no outliers, but challenges were met for ciprofloxacin and for detection and confirmation of ESBL-producers.

The ciprofloxacin issue regarding the detection of plasmid-mediated quinolone resistance (PMQR), is similar for *E. coli* and *Salmonella*; for both microorganisms the cut-off value for microbroth is considerably lower than the breakpoint for zone diameters and therefore is a possible cause of deviations for laboratories performing disk diffusion assays for AST of these microorganisms. The EQAS-protocol and the paper by Cavaco LM and Aarestrup FM (J Clin Microbiol. 2009 Sep;47(9):2751-8) give information on interpretation of ciprofloxacin results in the EURL EQAS. Also, EUCAST is working on setting up a cut-off value for ciprofloxacin for interpretation of zone diameters.

For *Campylobacter*, one laboratory was found to be an outlier. This laboratory has recently introduced MIC-methods for AST of *Campylobacter*, and validation is ongoing.

ISO-standards are preferred to CLSI guidelines/standards, when possible. However, ISO standards do not set criteria of interpretation. For interpretation purposes, EUCAST are preferred.

There is already an ISO standard for performing AST by MIC-determination. An ISO standard for DD is being worked on. A EUCAST document is also on the way (will also be presented at the ECCMID 2010).

Chloramphenicol in *Salmonella* was suggested as a project. We will look if we have funding to start a new project

The panel of strains appears to be of a good balance for accreditation purposes and for covering the emerging resistance profiles

Several items discussed regarding the EC/Staph/Ent EQAS are also relevant for the Salm/Camp EQAS

Genotypic characterisation was included as an optional part of this EQAS as the focus on molecular typing is increasing. Five laboratories participated, and there was interest in continuing this optional part of the EQAS to test the in-house methods. When reporting the obtained results, background of the isolate(s) will be given.

The workshop agreed that the laboratories participating in the test should be made public in the report. This will be done as of next year.

Comments for the proficiency test on genotypic characterisation:

- It should be possible to distinguish between negative results and genes not tested in the database.
- Suggestions for additional genes to be included in the list, are welcome
- Request to include a Gram-positive microorganism, also (e.g. MRSA)
- It could be relevant to arrange a training course on genetic characterization of important genes to ensure relevant trained and quality assurance. Will be considered for the Workplan 2011
- Information on methods should be included - primers

Acceptance of the report: Draft report was approved, with a small paragraph explaining the terms S and R, wild-type and non-wild type.

Presentation and discussion of MRSA EQAS results (Lina Cavaco)

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

The overall results regarding detection of MRSA were good. Confirmatory testing of MRSA showed that labs obtained reliable results of identification and *mecA* detection. Differences regarding morphology might have induced false negative results.

Spa typing was performed in 10 out of 23 labs, but only few uploaded results for the eight samples. The results were reproducible and comparable, and deviations were caused by lack of detection, and either contamination or switch between samples.

Report of the MRSA EQAS 2009 was approved and concluded ultimo 2009 (<http://www.crl-ar.eu/203-reports.htm>)

MRSA EQAS 2010 is in preparation. This EQAS will also follow the baseline study guidelines (dust samples with background). The EURL are working towards including a quantitative test by spiking the dust samples with different CFU/g.

The stability testing of the dust samples were carried out over four weeks at room temperature and at 5°C. This year the stability test will be run over a longer period of time.

The deadline for submission of data will be extended.

The network was asked to comment on the panel of strains, and it was suggested that a *S. pseudintermedius* could also be included, and to continue with eight samples. In addition it was suggested to add different matrices in parallel to the dust, e.g. nasal swabs. Including a second matrix will mean a set of extra eight samples and will not be feasible for 2010 but likely in 2011.

An acceptance threshold will not be set.

Evaluation of the *Salmonella* microbroth panel (Dariusz Wasyl)

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

EUMVS2-panel used for harmonized *Salmonella* monitoring (2007/407/EC decision). Colistin used to be 8 and 16, but has been changed to 2 and 4.

Colistin resistance in *Salmonella* is very rare. The EURL has commenced a project in which the prevalence of colistin resistant *Salmonella* will be studied.

The situation regarding ESBL's in the Netherlands (Dik Mevius)

An increase in cefotaxime-resistance in *E. coli* in broilers has been seen. In a not yet published study, 50% of the *E. coli* from broiler randomly picked were resistant. In other animal species it was not that common. Study on the genes showed a striking variation of genes CTX-M1, TEM-52 and also CMY-2 on plasmids (typically incl1).

The majority of the farms were positive without enrichment, indicating a very high prevalence. The farmers were also examined, 30% were positive. Four out of six of the farmers had similar types as the farm. Usage of XNL is associated, and may be one of the factors but not the only one. Fluoroquinolones stimulate plasmid transfer and may also play a role. An unwanted type of off-label use may cause this resistance, which in the US it is related to use of ceftiofur in day-old chicks and in Europe it might as well be used in ovo or sprayed in hatcheries (quantities are unknown). The animal sources of the meat samples in the study were in 92 cases (37.0%) chicken; 75 (30.1%) beef; 53 (21.9%) pork and 29 (11.6%) were from other animals. The ESBL was most frequently recovered from chicken (ESBL resistant isolates was found in 88% of the meat samples), and this problem is also growing in many other countries (Poster presented at ECCMID 2010; Extend spectrum beta-lactamase producing Enterobacteriaceae (ESBL) in retail meat, I.T.M.A. Overdeest *et al.*)

To analyse further it is important to look into the plasmids by determining the genetic relation, incompatibility groups, RLFP or pMLST system. Research is ongoing to try to determine why Incl1 is such a successful plasmid.

Friday, April 9th 2010

Project status: Qnr genes in *Salmonella* and *E. coli* (Kees Veldman)

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

A collection of data on quinolone resistance in *Salmonella* and *E. coli* from NRL's in Europe was analysed through establishment of a database on quinolone resistance. Retrospectively, screening for transferable quinolone resistance mechanisms of isolates with a plasmid-mediated quinolone resistant (PMQR) phenotype was performed. Results from this screening were presented.

Qnr genes (A, B, D, and S) have been identified in both *Salmonella* and *E. coli* from numerous countries in Europe in isolates of different origin but mostly in poultry/turkey isolates and human isolates and related to different *Salmonella* serotypes. Variants of *qnrB* and *qnrS1* were predominant, and *aac(6')Ib-cr* was only found associated to *qnr* and in few isolates. Resistance was not strongly associated to ESC resistance in these strain collections.

The prevalence cannot be calculated because the data has been biased by the selection, however a spread of PMQR genes in Europe is indicated.

Collaborators agreed on data publication in a manuscript which will be written by Kees as first author and included in his PhD.

Project status: Establishing streptomycin epidemiological cut-off values for *Salmonella* and *E. coli* (Lourdes Migura)

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

Twelve institutes provided data: Germany, Denmark, Norway, Sweden, Spain, Portugal, the Netherlands, France, Italy, Finland, UK and Canada, and examined by PCR for the presence of the most common

streptomycin resistance genes in *Enterobacteriaceae* (*aadA*, *strA* and *strB*) in 217 *Salmonella* and 208 *E. coli* exhibiting MICs between 4 and 32 mg/L.

In addition, each country provided information on the streptomycin MIC distributions for both species during a year period.

These studies have been complex due to a large proportion of isolates exhibiting high MICs despite the lack of a known mechanism of resistance. The establishment of a common cut-off value based on evaluation of both MIC distribution of the population and genetic characterization of resistance genes is vital to facilitate a global harmonisation of surveillance programmes.

Proposed to increase specificity by using a cut off value at 16 for *Salmonella* and at 8 for *E. coli*. The EURL will consult Gunnar Kahlmeter (EUCAST) to assess our data and will continue with the collection of this data in a letter

Project status: ESBL project (Lourdes Migura)

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

The project intends to investigate the presence and geographical dissemination of the different types of ESBLs found in *Salmonella* spp and *E. coli* from animal origin and food for human consumption across Europe, aiming to obtain information about the trends of ESBLs circulating in the community and to be able to control or minimise the spread of this resistance.

The EURL is available to verify ESBL-production if this is needed.

We aim at making the database publicly accessible for all NRL-AR's.

Data from seven countries have already been collected. Discussion of the project database revealed the following suggestions for improvement which will be introduced to the database a.s.a.p.:

- To report CTX-M within the five groups
- To include companion animals and 'others' as categories
- To include phenotypic data
- To divide laying hens and broilers into two categories
- To divide into clinical and monitoring
- To define the denominator (total no. of isolates) more clearly
- An appropriate field for combinations of genes (CTX-M and TEM for example) could be relevant (info about this should be mentioned as a comment)
- Inc-data could be included as an optional part to alert for epidemiological links and strengthen the database (info about this could be mentioned as a comment)

Research results, projects in past and at the moment (Liidia Häkkinen)

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

A number of monitoring schemes are running at the Estonian University of Life Sciences of antimicrobial resistance in bacteria from food chain. Results from the last years were presented of a number of microorganisms including *Salmonella*, *Campylobacter*, *E. coli*, *Staphylococcus* and indicator bacteria from different animals or food sources.

A linezolid-resistant *E. faecium* was mentioned as was a vancomycin resistant *E. faecalis*. This is interesting, and the EURL-AR have asked for more information on these findings.

AMR resistance monitoring in Italy: Emerging issues in *Enterobacteriaceae* and *S. aureus* (Antonio Battisti)

Monitoring activities from the NRL-AR Italy, beside testing and reporting in agreement with EU guidelines and legislation, include also work on Extended Spectrum Cephalosporins and plasmid mediated quinolone resistance (PMQR) in *Salmonella* and *E. coli*. In addition, work on clonally spread resistance determinants is carried out (e. g. on *Campylobacter*, some *Salmonella* serotypes, and MRSA).

Studies in animals show that 13% of all available *Salmonella* isolates in broilers from 2008 were cefotaxime resistant (11% due to ESBLs, CTX group I and II and SHV-12), and was 7% of all *E. coli* from turkeys, 2007.

In 2007 and 2008, qnr's were rarely found associated with ESBLs determinants. It was seen only in indicator *E. coli*.

In *S. Typhimurim* and *S. Kottbus* from humans, qnrB was found associated with SHV-12(IncI1). One *S. Typhimurium* harboured CTX-M 15 (IncNT), and one harboured SHV-12.

Studies on MRSA in Italy show that the most common ST398 MRSA is *spa*-type t899, and that MRSA CC1 (t127, ST1) and CC97 are very common among Italian pig holdings (both breeding and fattening), where a wide diversity of *spa*-types and STs is evident. This picture is different from that in other countries with high MRSA prevalence among pig holdings. In this respect, beside CC398 MRSA, pigs could represent an additional source of human exposure for non-CC398 MRSA, especially for *spa*-type t127, ST1, which is considered a human-associated lineage.

Results of the 2008 survey in finishing pig holdings are published by Battisti *et al.* in Veterinary Microbiology.

The EU Reference Laboratory for *Escherichia coli*, including Verotoxigenic *E. coli* (VTEC) (Rosangela Tozzoli)

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

VTEC is an important foodborne pathogen in the developed countries. VTEC infections can cause the life-threatening haemolytic uremic syndrome. VTEC has a very low infectious dose and caused very large community outbreaks. VTEC is a zoonotic pathogen and cattle have been recognised as the main animal reservoir. Antimicrobials can induce VT-production therefore treatment with such drugs is not recommendable. Transmission of infection occurs via consumption of contaminated food of animal origin, as well as contacts with infected animals and environment.

The EURL-VTEC is working with discriminating VTEC from commensal *E. coli* and organises annual inter-laboratory studies on the identification and typing of VTEC isolates by using different harmonised methods including conventional and Real Time PCR. The last PT was on animal sample (simulated carcass swabs) in order to prepare NRLs for the forthcoming EFSA monitoring plan for VTEC.

VTEC are class 3 micro-organisms in several EU member states and thus handling and shipment of proficiency samples must be performed accordingly.

The WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) (Rene Hendriksen on behalf of Awa Aidara-Kane)

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

Health consequences of AMR are increased number of infections, increased frequency of treatment failures, increased severity of infections, and increased costs to society.

More than 50% of all antimicrobials are used non-therapeutically in animal husbandry, and this use of antimicrobials in food animals can lead to Antimicrobial Resistance (AMR) in human pathogens. The same classes of antimicrobials are used both in humans and animals and few new antibiotics are being developed to replace those becoming ineffective through resistance

Food is generally considered to be the most important vector for spread of resistance between humans and animals and due to the globalization of food trade there is a need for international action.

WHO's approach to minimise antimicrobial resistance in humans due to use of antimicrobial resistance in food animal production is 1) The Concept of Critically Important Antimicrobials (quinolones, 3rd and 4th generation cephalosporins, and macrolides), and 2) The Integrated Approach (WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance; AGISAR).

The AGISAR has formed subcommittees working on Usage Monitoring, Antimicrobial Resistance Surveillance, Capacity Building & Pilot studies, and Data Management.

Comment from the audience: Monitoring as carried out in the EU is not good enough, we must take action. Legislation must be made, and surveillance must be carried out – the monitoring data must be acted on!

Leena Räsänen: On the WG-meeting planned for April 23rd, discussions on risk management and actions will be carried out. There has to be an agreement by the member states to start the actions.

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

Reading and interpretation of SMX by MIC and by DD was mentioned and also a few slides were shown to describe the problem (see [link](#), or <http://www.crl-ar.eu/146-presentations.htm>). The MIC is determined at 80% reduction of growth equal to 20% of the lawn of growth for disk diffusion. From the Netherlands it was mentioned that a small reduction in the size of the pellet compared to the positive control would be determined as the MIC. Also, with the new sensititre system it is possible to take photos of the wells which makes it easy to compare growth patterns.

A standard panel for testing of ESBL-producers can be bought from TREK (ESB1F). These can be purchased for standard price and down to low numbers (packages of 10 panels). If using ESBL brilliance agar, be aware that this does not pick up ampC.

In future newsletters from the EURL-AR, we aim to include further scientifically related topics within the area of antimicrobial resistance. Therefore, we encourage NRL's to contribute by sending us material in the form of for example abstracts of recently published papers, case stories or other kinds of descriptions of topics related to our field.

Summary:

Trends regarding performance are still positive.

In the EQAS-reports, the terms 'susceptible' and 'resistant' will continually be used, however, a paragraph will be inserted to describe that these terms in some instances refer to 'wild-type' and 'non-wild-type' strains.

It was agreed that in future EQASs, the threshold for the performance will remain at 5%.

With the EQAS in October the EURL will again distribute an *Enterobacteriaceae* and probably also a Gram-positive strain for optional testing of resistance genes.

The MRSA EQAS will be following the EU Commission's requirements for monitoring if/when it comes.

In 2010, no training courses have been planned. Possibly a training course on molecular typing methods will be arranged in 2011.

ESBL project is ongoing (occurrence of ESBL genes in *Salmonella* and *E. coli* in Europe)

It was suggested that future development of MIC panels should be an EURL task. The EURL are, however, not for all microorganisms using the same panel as other NRLs (e.g. for staphylococci)

Next year's venue for the EURL workshop has not yet been decided.