

# Minutes – CRL Workshop Copenhagen June/2008

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The minutes are listed according to the agenda

## Participants:

All member states (MS) with NRL-AR took part in the meeting, apart from Malta (Malta's NRL-AR activities are carried out by HPA, UK). Luxembourg has not appointed an NRL-AR.

## **Thursday, June 19th 2008**

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### Welcome (Mr. Rene Hendriksen)

- Emphasized the importance that all participants actively contribute to the meeting
- Looking forward to having presentations from participants this year – which we plan on having as a continuing item on the CRL-workshop agenda
- News regarding the former network, ARBAO: Two articles based on the ARBAO-data (pig/cattle) have been finally accepted by AVS

### Update from the EU Commission (Dr. Kris De Smet)

**See powerpoint presentation** (<http://www.crl-ar.eu/146-presentations.htm>)

- The importance of awareness of antimicrobial resistance and monitoring in MS was stressed
- Mandatory monitoring of enterococci/*E. coli* in food animals as well as MRSA was discussed
- The cooperation between EFSA experts, the task forces and the EU Commission was presented

### Update from the CRL (Prof. Frank Aarestrup)

- Future monitoring:
  - Highlighted the severeness of *E. coli* in UTI
  - Discussed the mandatory monitoring of *Campylobacter*
  - Considered a mandatory monitoring of newer zoonotic bacteria e.g. streptococci, staphylococci and *E. coli*
- Emphasized the importance of collaboration in research and monitoring in MS
- Emphasized the importance of standardisation (as opposed to harmonisation) in AST worldwide
  - Mentioned the new WHO-network 'AGISAR' (Advisory Group in Surveillance of Antimicrobial Resistance)
- Underlined the EQAS/networking as the main task of the CRL
- Tasks undertaken in 2007 by the CRL
  - Website (data and information from participants was requested)
  - Newsletters (the CRL intend to keep them concise)
  - Evaluation of different cephalosporins aiming to find the best substance for differentiating between ESBL/non-ESBL (in collaboration with the Netherlands (NL)), to find the best flouroquinolone for detecting FQ-resistance and the best marker for detecting MRSA
  - Participating in advisory groups with/for the EU Commission, with special emphasis on Codex
  - Shipment of reference strains
- Aiming towards the launch of MRSA proficiency test in Spring 2009 (quantitative or qualitative)
- EFSA is working on monitoring schemes of biocides in poultry

**See powerpoint presentation** (<http://www.crl-ar.eu/146-presentations.htm>)

- *E. coli*
    - Very good performance by participants
- Discussion in plenum regarding *E. coli*:
- Consider testing of *E. coli* every second year due to the excellent performance, or annually include four ESBL-positive *E. coli*. However, this might conflict with the discussion regarding mandatory monitoring of *E. coli* in food animal
  - ESBL results should be evaluated separately for MIC and DD – report will be updated
  - Interested in including *aac(6<sup>i</sup>)/b-cr* variants among the test strains
- Enterococci
    - A few outliers with high deviation percentages
    - ~ 50% had deviation percentages above the goal
  - Staphylococci
    - Method for detection of positive/negative *mecA* is not defined – all betalactam antibiotics should be regarded resistant when *mecA*-positive
- Discussion in plenum regarding staphylococci:
- Include oxacillin (with and without clavulanic acid) with a defined method and temperature
  - Include cefoxitin
  - Cutoff values for tetracycline were discussed – future trials will only include *S. aureus*.
- General considerations and discussion (in plenum):
    - Choice of strains should be standardised (same of proportion of difficult strains)
    - Including more strains, and more internal controls will be considered. Possibility of including strains with emerging resistance mechanisms, and excluding them in the overall evaluation
    - Results should be further analysed (possibly ignored) if 75% are incorrect (test strain/ antimicrobial combination). The final report will be issued after assessing this matter
    - The possibility of the optional identification of test strains was discussed. This issue will be discussed further internally by CRL
    - Discussion of lowering the acceptance threshold. Agreement that the threshold should be the same for all microorganisms, and will be consistent at 7% in 2008. The item will be assessed next year.
    - Deviations in disk diffusion (reference strains) might be caused by different methods (e.g. inoculum). Until further notice, participants should only report result when CLSI guidelines are used
    - For future AST's, the ISO standard 20776-1 will be used as reference for the Broth Microdilution test
    - If data is changed in the database, the relevant participants will be notified
    - Suggested to visualise MIC-distributions and DD-zonediameter distributions (however, the latter is only useful if all use exactly the same method)
    - Suggested to collect reference strain data in distribution graphs (with a 95% confidence interval). Consider leaving out the data of outlier participants, as a reference dataset to be used for QC purposes
    - The procedure for follow-up on unacceptable performance was discussed:
      - Direct contact
      - Re-test
      - Site visit and/or training course

**See powerpoint presentation** (<http://www.crl-ar.eu/146-presentations.htm>)

- *Salmonella*

- One outlier received retest

Discussion in plenum regarding *Salmonella*:

- ESBL results should be evaluated separately for MIC and DD – report will be updated
- Accepted QC-range of *E. coli* ATCC 25922 for ciprofloxacin will be set to 0.004-0.016 mg/L (slightly extended range compared to the CLSI-guideline and ISO standard in which 0,015 is the upper limit)

- *Campylobacter*

- Three outliers received retest

Discussion in plenum regarding *Campylobacter*:

- Results for test strain C2.1 for streptomycin look odd (86% deviation for this test strain/antimicrobial), no explanation was found. The results for this test strain/antimicrobial will be left out in the final report
- The high deviation percent for test strain C2.5 towards tetracycline (77%) was analysed – plausible reasons are the fact that isolate is borderline, in combination with the pH-dependent antimicrobial. No changes will be made in the final report

- General considerations and discussion (in plenum):

- Several items discussed regarding the EC/Staph/Ent EQAS are also relevant for the Salm/Camp EQAS
- Collection method-related data e.g. incubation time, inoculum, atmosphere, temperature, media, plate seals was discussed
- Agreed upon that phenotypical ESBL testing of *Salmonella* and *E. coli* will be mandatory from 2008 (Metallo-betalactamases not included)

Summing up the ASM-conference (Prof. Frank Aarestrup)

**See powerpoint presentation** (<http://www.crl-ar.eu/146-presentations.htm>)

MRSA baseline survey, status and discussion (Prof. Frank Aarestrup)

- Problem that enterococci grow on the oxoid plates
- Remember correct NaCl%
- Problem with wet sodibox
- The need for common panel for AST was discussed
- There seems to be a need for a training course and a ring trial

## **Friday, June 20th 2008**

Choosing cephalosporins for detecting ESBL-producing microorganisms (Prof. Frank Aarestrup)

ESBL detection:

- Cefoperazone is not a good marker
- Ceftriaxone seems to be the best substance

MRSA detection:

- Ceftiofur and cefquinome resistance are not good markers
- Cefoxitin (disk diffusion) is a good marker

Detection of quinolone resistance

- Screening with nalidixic acid is efficient for detection of mutants, however for detection of transferable resistance genes it needs to be complemented with screening with a fluoroquinolone

- Detection of transferable genes would be maximized by adding a quantitative screening with either ciprofloxacin or norfloxacin. Other quinolones were useful for detection of mutants and *qnr* genes, but not *aac(6')Ib-cr*
- The use of ciprofloxacin-disks: 5 µg does not distinguish as well as 1 µg disks

ESBL in *E. coli* (Dr. Jean-Yves Madec, France)

**See powerpoint presentation** (<http://www.crl-ar.eu/146-presentations.htm>)

- Presentation of a well-functioning network since 1985
- Many local laboratories involved in cooperation regarding research in ESBL in *E. coli*

Streptomycin cut-off values in *E. coli* and *Salmonella* (Prof. Dik Mevius, The Netherlands)

**See powerpoint presentation** (<http://www.crl-ar.eu/146-presentations.htm>)

- Streptomycin resistance is regulated by *strA*, *strB* and *aadA*. More data is needed to decide which cut-off value (R>8 or R>16) should be used for streptomycin for *E. coli* and *Salmonella*.
- The CRL, in collaboration with the NRL in NL, will be the facilitator of collecting and evaluating data
- The matter will be discussed with the Commission and in EFSA working groups

The occurrence of *qnr* genes in *Salmonella* (Mr. Kees Veldman, The Netherlands)

**See powerpoint presentation** (<http://www.crl-ar.eu/146-presentations.htm>)

- Kees (NL) and Lina Cavaco (DK) will initiate a collaboration regarding *qnr* genes in *Salmonella*

Antimicrobial resistance in bioterrorism agents (Dr. Stina Englund, Sweden) - CANCELLED

Antimicrobial resistance monitoring – building up capacities and key observations (Dr. Dariusz Wasyl, Poland)

**See powerpoint presentation** (<http://www.crl-ar.eu/146-presentations.htm>)

- Resistance data from *Salmonella* was thoroughly analysed, including resistance profiles and resistance mechanisms over the last five years
- *Salmonella* antimicrobial resistance data was re-evaluated (CLSI >> EUCAST)
- Very interesting data on resistance in *E. coli* isolates was analysed – trends were followed in data from four years and from five animal species

CRL webpage and EQAS database (Ms. Susanne Karlsmose)

See [www.crl-ar.eu](http://www.crl-ar.eu)

- List of reference strains is available. Some reference strain cannot be distributed from the CRL, however it is possible to send DNA or to provide information regarding who can provide NRL's with the strain in question
- Links to relevant legislation will be updated
- Relevant publications will be listed
- Mandatory monitoring of *E. coli*/enterococci in food animals
- The reference regarding methods used for AST will be to either CLSI or ISO

EQAS database

- The EQAS database instantly evaluates the uploaded results when approved. All participants should make sure to have the evaluation report and should use this for self-evaluation
- The systems developer will look into improving the setup of the print of the evaluation reports
- 'Intermediate' is not accepted. It is recommended to regard an intermediate result as resistant. Any uploaded data will be evaluated towards the expected results
- The database evaluation of ESBL results was presented

- Two sets of data can be uploaded to the database (ask for an extra set of username and login), however, only one set will be evaluated

Future developments, training courses, EQAS, research – general discussion and summary (All, Prof. Frank Aarestrup)

EQAS:

- The CRL will consider working with two kinds of strains in future EQAS: 'Core strains', which would be test strains as known from EQAS's until now, and 'variable strains', which would include more demanding strains dependent on the present development in AR. Additionally, the CRL will consider to include identification of *Campylobacter* and enterococci as an option for the EQAS.
- Planning a proficiency test on MRSA, including isolation, identification and classification.

Advice for the Commission

- The CRL is participating in EFSA working groups

Newsletters – coming up:

- Description of international activities relevant for antimicrobial resistance and MRSA phenotypic identification

Research – coming up:

- Survey of Cip-R and Nal-R in Europe (Kees Veldman and Lina Cavaco)
- Studies on streptomycin breakpoints (CRL + NL)

Training courses

- On basic AST
- On MRSA: isolation, identification, typing
- Considering to include new member states
- Considering to begin more advanced courses

Collaboration between laboratories is highly encouraged

Common panel for AST of staphylococci (Dik Mevius and Andreas Schröter will be coordinating)