National Food Institute
Technical University of Denmark

# Antimicrobial Resistance – CRL workshop, May 2007

## Minutes listed according to the agenda

### 1. Formalities, policy, responsibilities and expectations by the Commission (Dr. Kris De Smet)

Comment from participants: The new name for 'CRL for *Salmonella*' that has changed to 'CRL for the analysis and testing of zoonoses' does not give the correct picture of what the CRL are responsible for – or what other CRL's is responsible for.

It was highlighted by both Kris de Smet (EC) and Frank Aarestrup (DK) that the *NETWORK*-part of the CRL work is very important – the CRL as such is the contact point, and wishes to **foster collaboration** between the participants of the network.

### 2. Presentation and discussion of EQAS results – first trial (Mr. Rene Hendriksen, Prof. Frank Aarestrup)

It was discussed whether EFSA/Eucast cut off values or CLSI breakpoints should be used as basis for the EQAS evaluation. It was emphasized that there is a difference between clinical breakpoints and monitoring breakpoints. Eucast do not focus on clinical issues, but use '>' (larger than) and thus do not give a result in between breakpoints. Most breakpoints are the same in CLSI and Eucast, but for flouroquionolones and cephalosporins they are not in agreement – and these are important antimicrobials. The Eucast breakpoints are to be preferred. With the CRL-network we have a possibility to be part of the harmonisation of breakpoints, and the participants at the meeting agreed that this possibility should be used. The aim from the CRL is that five years from now we have harmonised methods, including breakpoints.

**It was agreed that the proposed list of antimicrobials from Eucast will be used in following EQAS's, and that EFSA/Eucast cut off values will be used. Also, in following EQAS's we will not be working with three categories of deviation (minor, major, very major), but will evaluate the interpretation as either correct or incorrect.**

For disc diffusion it is generally more difficult to compare breakpoints since the method could be different thus making the comparison impossible. **The CRL recommends MIC test.** A large number of diagnostic laboratories do not have opportunity to do MIC, and thus use disc diffusion. This is a major issue which it is necessary to focus on.

Regarding disc diffusion, this method needs to be evaluated since the results are difficult to compare. The breakpoints for disc diffusion always have an equivalent in a MIC value since disc diffusion is also about concentration of the antimicrobial in the agar. We lack data on this, but some of the CLSI breakpoints are 'translated' from MIC to an equivalent diameter in disc diffusion.

For *Campylobacter* – disc diffusion is not useful for comparison between laboratories. In the methods guideline M45-A a method for disc diffusion on *Campylobacter* is described, though it only describes detection of resistance to ERY and CIP.

Many deviations in this EQAS were caused by differences in breakpoints. It was generally agreed that this issue disturbs the focus, and to avoid these 'unnecessary' deviations **the CRL will in following EQAS's add a list of breakpoints to use when interpreting results. Additionally the participants will be asked to upload breakpoints that are normally used.**

In this EQAS report there are some details that could be misunderstood, and it is suggested that the report is sent to the NRL's before the final report is sent to the Commission. This will be done in the future.

It was suggested that amoxicillin/clavulanic acid should be omitted from the tests. Also, it was noted that it is necessary to be careful with the conclusions when interpreting the results from the EQAS.

There was a question regarding cephalosporins, considering which are recommended to be used. Frank Aarestrup (DK): It is difficult to give a specific answer, but one thing is clear: with two antimicrobials you detect more. For the sake of harmonisation EFSA's guidelines should be followed.

There were some comments to the adjustments made in the database after participants had already started to upload data. In following EQAS the CRL will make sure to inform all relevant participants when changes are made that have an effect on the evaluation of results.

Database issues:

- Considering CIP/ENRO: In the database for the EQAS 2006 data for CIP and ENRO are not separated. This will be changed in the following EQAS's.
- The concentration of eg. amoxicillin/clavunic acid cannot be uploaded to the database. The CRL will make sure to describe this in the protocol/database.
- When printing the list of MIC's it does not come out very nicely. The CRL will put this on the 'nice-to-have list'.

Question from the CRL: Should we in the following trials give the information about if it is a *C. jejuni* or *C. coli*? Breakpoints differ for these two organisms, and if it is not identified correctly this will lead to a wrong categorization of susceptibility.
The answer from participants differ from countries that would be very happy to have this piece of information, arguing that the focus of the EQAS is on susceptibility testing thus additional differences are not 'necessary', to countries who stress that the problem about giving this information is that in the 'real world' we do not always know which microorganism we are dealing with, thus not knowing whether it is a *C. coli* or a *C. jejuni* resembles the actual problems we are met with in reality. It was suggested to differentiate between the countries. **The CRL will in the following EQAS's add the name of the species as the focus of our EQAS is susceptibility testing.**

One dilution step deviation is regarded as an incorrect result. It was suggested that the laboratories that experience deviation make use of self-evaluation since it forces one to think about what the reason could have been for the non-concordances (Christina Greko, Sweden, uses one). The CRL will look into this to have a self-evaluation report that can be recommended and available.

As described in the report, this EQAS has identified 11 laboratories as outliers, these have all been contacted by the CRL before the Workshop. During and after the workshop the CRL will be discussing this subject further with the relevant laboratories. It has not been possible to identify a common cause for the deviations.

Laboratories that perform unsatisfactorily and laboratories that are new on different areas could be included in training courses. Anybody who has own funding is welcome to come for a visit to the CRL to get training in one of our competence areas. Training courses will have to be discussed further and will be set in motion in 2008.

## 3. Status and methods in detection of ESBL producing bacteria (Dr. Henrik Hasman)

## 4. Presentation and discussion of EQAS evaluation and questionnaire (Ms Susanne Karlsmose)

The question of defining multi-resistance was raised. Frank Aarestrup (DK) mentioned that it is very complex to reach a common definition that includes all participants because at the moment the countries use different definitions both in respect to the number of resistance markers and the classes of antimicrobials needed to be present to define multi-resistance. An approach to make the definition more specific is first to define the drugs used and then describe how many drugs the bacteria are resistant to. The EFSA definition is in that respect very useful, it can be found in the EFSA Journal (2007), 96, 1-46, 'Report of the Task Force of Zoonoses Data Collection including a proposal for harmonized monitoring scheme of antimicrobial resistance in *Salmonella* in fowl (*Gallus gallus*), turkeys, and pigs and *Campylobacter jejuni* and *C. coli* in broilers. See on p. 18 under the passage '4 Antimicrobials to be monitored and cut-off values to be used.

The question of hands-on training of the NRL laboratories was raised. Frank Aarestrup (DK) expressed that the CRL is capable of training staff from 5-10 laboratories, either in Copenhagen or locally. **The Commission has proposed a much more comprehensive hands-on training session for all NRL laboratories in 2008. The CRL will work out a proposal.**

It was proposed that the web page and the network should be involved in solving emerging problems as MRSA, ESBLs and transferable fluoroquinolone resistance. In continuation of this, Frank Aarestrup (DK) pointed out, that the CRL is prepared to receive strains from all NRL's for characterization.

It was suggested to include genetic characterization in the EQAS, thus making it possible to upload data on either disc diffusion mecA or a PCR test of microorganisms that are PCR-mecA positive/negative. Frank Aarestrup (DK) pointed out that **the intention of the current EQAS is to harmonise the phenotypic expression, but that genetic characterization could be included in the future if The Commission agree**. This was accepted by the participants, though it was commented that for ESBL which is an emerging problem and therefore guidelines are needed for detecting and characterization of these isolates. **The CRL will submit guidelines for this purpose.**

It was decided that Dariusz Wasyl (PL) will make a draft for common Sensititre MIC panels for monitoring of *Salmonella* and *Campylobacter*. The draft will be sent for comments to all interested participants and includes antimicrobials as well as number of dilutions. If the participants agree it will be arranged that the plates can be ordered from a central distributor.

## 5. Presentation of the work in Eucast / Vetcast /EFSA and harmonisation of breakpoints, antimicrobial ranges and panels (Dr. Dik Mevius, Dr. Stef Bronzwaer)

## 6. Information on fluorquinolone resistance – focus on detection of qnr genes (Prof. Frank Aarestrup)

## 7. Use of nanoarray for detection of antimicrobial resistance genes (Dr. Muna Anjum)

This technology is available from Clondiag Technologies, Germany.
It is fast (50 samples per day) and reasonably cheap.
The high through-put screening is especially useful for screening of ESBL and CipR (fluoroquinolones).

## 8. Status and methods in detection of MRSA – focus on the emerging pig variant (Prof. Frank Aarestrup)

Regarding method for detection of MRSA Dik Mevius (NL) stresses that disc diffusion is better than broth. Also it should be noted that the mecA gene is better expressed at 30° C than at 37° C.

**The CRL is working on a protocol to isolate MRSA from animals.**

Dutch investigations suggest that almost 100% of the human cases of ST398 stem from contact with animals either on the farms or in the slaughterhouses, there has been seen no evidence of spread among humans. MRSA 398 has also been found in food samples but infection from food is rare.

Dik Mevius (NL) commented that in the Netherlands they have experienced problems with isolation of pig farmers in hospitals. ST398 gives serious infections and is now the most prevalent clone.

In Finland they have seen two cases of MRSA in cattle farms, but do not know yet if it is ST398 (Anna Liisa (FIN)).

## 9. Future development, research and training courses (Mr. Rene Hendriksen)

As a priority for the future it was proposed that the network also dealt with harmonisation of sampling strategies.

## 10. Future EQAS trials (Mr. Rene Hendriksen)

## 11. Presentation of the CRL web page and Newsletters (Ms Susanne Karlsmose)

The CRL will generate a list of the participants of the network including a short presentation of each laboratory's competences. This list will be available on the website. Also, the CRL are working towards uploading a list of QC-strains and primers on the website. Both QC-strains and primers are available for distribution upon request from participants.

## 12. General discussion and summary, Topics are raised by NRLs (All, Mr. Rene Hendriksen)

Globalisation / import of food is an important issue for all of our countries. Encouragement to use the network considering these issues as well.