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# NEWSLETTER

**to the**
**National Reference Laboratories for Antimicrobial Resistance**

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Community Reference Laboratory Antimicrobial Resistance

## Content



## Detection of quinolone resistance in *Enterobacteriaceae* – the challenge of detecting low-level resistance when performing susceptibility testing by MIC testing or disk diffusion

Lina Cavaco, Frank M. Aarestrup

Fluoroquinolone resistance in *Enterobacteriaceae* has until recently been attributed to point mutations. However, in the late 1990, a new plasmid mediated mechanism, encoded by the *qnrA* gene was found. Later, *qnrB*, *qnrS*, *qnrC and qnrD* and several variants as well as other genes such as *aac(6')Ib-cr* and *qepA* have been described.

The current phenotypic methods for susceptibility testing are designed for detection of strains with mutations and are not efficient in detecting the new resistance determinants which confer low-level resistance. The detection of resistance caused by mutations is simple, as a first mutation causes full resistance to nalidixic acid, however, that is not the case with the transferrable quinolone resistance genes. We have recently conducted a study at the CRL on a collection of 69 *Escherichia coli* and 62 *Salmonella* strains including strains with different resistance backgrounds including mutations and *qnr* or *aac(6')Ib-cr* genes which aimed at optimization of the detection of the different resistance mechanisms by studying the distribution of the results of susceptibility testing against a large panel of quinolone drugs.
The results of this study confirm that low-level resistance to fluoroquinolones would be best detected by testing MIC of both nalidixic acid (for detection of mutants) and of a fluoroquinolone, (preferably ciprofloxacin or norfloxacin) to improve detection of isolates carrying both *qnr* and *aac(6')Ib-cr*.

MIC testing is preferable, allowing better detection using quantitative criteria (Table 1), however, if disk diffusion is performed, low level breakpoints might be used to select suspected strains which can then be tested further by molecular methods. Furthermore, a low concentration of ciprofloxacin (1μg) in the disks increases the sensitivity of the disk diffusion assay and should also be used to improve the detection (Table 2).

**Table 1:** Mechanisms of resistance and expected phenotypes for *Escherichia coli* and *Salmonella enterica* isolates using MIC testing

| **Mechanism of resistance** | **MIC (mg/L)[a)]** | | **Interpretation [b)]** | **OBS[c)]** |
|---|---|---|---|---|
| | **NAL** | **CIP** | | |
| **Susceptible strain** | <8 | <0.125 (*Salmonella*)<br><0.06 (*E. coli*) | Susceptible | **Wild type phenotype** |
| **Single mutations** | >16 | 0.125-1 (*Salmonella*)<br>0.06-1 (*E. coli*) | NAL resistant CIP reduced susceptibility | **Reduced susceptibility** |
| **Multiple mutations** | >16 | >1 | NAL resistant CIP resistant | **High level resistance** |
| **Transferable res genes** | **<64 (8-32)** | **0.125-1** (*Salmonella*)<br>**0.06-1** (*E. coli*) | **NAL susceptible CIP reduced susceptibility** | **Transferable resistance genes** |

Legend: NAL (nalidixic acid); CIP (ciprofloxacin)
a) Most probable MIC values in brackets.
b) Interpretation of the MIC values was performed by using the EUCAST cut-off values (http//:www.eucast.org).
c) High level resistance might be due to a combination of several resistance mechanisms

**Table 2:** Mechanisms of resistance and expected phenotypes for *Escherichia coli* and *Salmonella enterica* isolates using disk diffusion testing

| **Mechanism of resistance** | **Diameter of inhibition zone (mm)[a)]** | | | **Interpretation[b)]** | **OBS[c)]** |
|---|---|---|---|---|---|
| | **NAL** | **CIP5** | **CIP1** | | |
| **Susceptible strain** | >18 | >30 | >23 | Susceptible | **Wild type phenotype** |
| **Single mutations** | 6 | 26-30 | 17-23 | NAL R<br>CIP S | **Reduced susceptibility** |
| **Multiple mutations** | 6 | 6-9 | 6 | NAL R<br>CIP R | **High level resistance** |
| **Transferable res genes** | **<21 (6-19)** | **<31** | **<24** | **NAL S/I/R**<br>**CIP S/I/R** | **Suspect of transferable resistance genes** |

Legend: NAL (nalidixic acid); CIP (ciprofloxacin)
a) Most probable diameter zone measurements in brackets
b) Interpretation of the disk diffusion values was performed by using the CLSI guidelines (M100 S19)
c) High level resistance might be due to a combination of several resistance mechanisms

## Suggested further reading


Cavaco LM, Aarestrup FM. Evaluation of quinolones for use in detection of determinants of acquired quinolone resistance, including the new transmissible resistance mechanisms qnrA, qnrB, qnrS, and aac(6')Ib-cr, in Escherichia coli and Salmonella enterica and determinations of wild-type distributions. J Clin Microbiol. 2009 Sep;47(9):2751-8.

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Yamane, K., Wachino, J. I., Suzuki, S., Kimura, K., Shibata, N., Kato, H., Shibayama, K., Konda, T., and Arakawa, Y., "New Plasmid-Mediated Fluoroquinolone Efflux Pump, QepA, Found in an Escherichia coli Clinical Isolate," Antimicrobial Agents and Chemotherapy, Vol. 51, 2007, pp. 3354-3360.

## WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (WHO-AGISAR)

Awa Aidara-Kane

A WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (WHO-AGISAR) was established in December 2008 to support WHO's effort to minimize the public health impact of antimicrobial resistance associated with the use of antimicrobials in food animals. In particular, the Advisory Group will assist WHO on matters related to the integrated surveillance of antimicrobial resistance and the containment of food-related antimicrobial resistance. The Terms of reference of WHO-AGISAR are as follows:

- Develop harmonized schemes for monitoring antimicrobial resistance (AMR) in zoonotic and enteric bacteria. This should include appropriate sampling
- Support WHO capacity-building activities in Member Countries for antimicrobial resistance monitoring (AMR training modules for training courses within the Global Foodborne Infections Network (GFN), formerly Global Salm-Surv)
- Promote information sharing on AMR
- Provide expert advice to WHO on containment of antimicrobial resistance with a particular focus on Human Critically Important Antimicrobials
- Support and advise WHO on the selection of sentinel sites and the design of pilot projects for conducting integrated surveillance of antimicrobial resistance
- Support WHO capacity-building activities in Member Countries for antimicrobial usage monitoring

The WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance comprises over 20 internationally-renowned experts in a broad range of disciplines relevant to antimicrobial resistance, appointed following a web-published call for advisers, and a transparent selection process. WHO-AGISAR holds quarterly telephone conferences and annual face-to-face.

The first meeting of WHO-AGISAR was held in June 2009 in Copenhagen Denmark.

Participants of the 1st Meeting of the WHO Advisory Group of Integrated Surveillance, 15-19 June 2009, Copenhagen, Denmark

### Reference material

On the CRL-website (www.crl-ar.eu), information on different issues can be found. For example, under FAQ there is a list of reference material which may be of relevance if you are looking for microorganisms with specific pheno- or genotypes.

Moreover, the CRL recommend that the NRL's store the EQAS test strains in their strain collection for possible later reference, as the aim is that the EQAS test strains cover as many as possible of the relevant resistance profiles and mechanisms.

## ESBL producing *Salmonella* Concord in a pig isolate from the Czech Republic

Rene Hendriksen, Tomas Černy

During the CRL workshop on antimicrobial resistance in 2009, Tomas Černy from the State Veterinary Institute, Prague, Czech Republic, gave a presentation of the data gained from the baseline survey on the prevalence of *Salmonella* in breeding pigs in the Czech Republic 2008.

He explained that his laboratory found an ESBL producing Salmonella isolate among the breeding pigs. The isolate was interesting to him as it was of a rare serovar. Rene Hendriksen commented the finding of the *S.* Concord isolate and referred to two recently published studies about Ethiopian adoptees infected with this serovar (1, 2). The data in the publications revealed that all the isolates were harbouring up to two genes conferring resistance to 3rd generation cephalosporins. The CRL offered to test the pig isolate and compare the pheno- and genotype with those of the isolates from the Ethiopian adoptees.

At the CRL, the isolate was screened by Microarray (Identibac Amr-ve Array tubes http://www.identibac.com/, New Haw, Addlestore, Surrey, UK), and MIC determination was performed as well as PFGE. PCR's against the SHV and CTX genes were run, followed by sequencing.

The pig isolate had an identical susceptibility pattern with the isolates of the adoptees and was resistant to chloramphenicol, florfenicol, trimethoprim, sulphamethoxazole, ampicillin, amoxicillin+clavulanic acid, ceftiofur, cefotaxime, ceftriaxone, ceftazidime, cefepime, ciprofloxacin, streptomycin, tetracycline, and gentamicin. The micro-array revealed that the isolate harboured the following genes $bla_{\text{CTX-M-15}}$, $bla_{\text{SHV-12}}$, $bla_{\text{TEM-1}}$, *qnr*B, *sul*1, *tet*D, *str*A, and *str*B and the PFGE clustered the isolate among isolates from Ethiopian adoptees.

Based on the laboratory data, Tomas initiated an epidemiological investigation which concluded that nobody from the farm has adopted a child from Ethiopia nor had any of the employees recently been in Ethiopia or Africa on vacation. The owner and the employees have not been showing symptoms of salmonellosis.

The pig was bred on the farm with semen imported from the Netherlands and no other pigs were found infected with *S.* Concord. The feeds used on the farm were partly handmade, domestically prepared or imported from England and none of the investigated samples contained *Salmonella*. The source of the infection is still unknown and no further investigation will be initiated.

### References

1. Hendriksen RS, Mikoleit M, Kornschober C, Rickert RL, Duyne SV, Kjelsø C, Hasman H, Cormican M, Mevius D, Threlfall J, Angulo FJ, Aarestrup FM. Emergence of Multidrug-Resistant *Salmonella* Concord Infections in Europe and the United States in Children Adopted From Ethiopia, 2003-2007. Pediatr Infect Dis J. 2009 Sep; 28 (9): 814-818

2. Fabre L, Delauné A, Espié E, Nygard K, Pardos M, Polomack L, Guesnier F, Galimand M, Lassen J, Weill FX. Chromosomal integration of the extended-spectrum beta-lactamase gene *bla*CTX-M-15 in *Salmonella* enterica serotype Concord isolates from internationally adopted children. Antimicrob Agents Chemother. 2009 May; 53 (5): 1808-16

### Future Newsletters

In future newsletters from the CRL-AR, we aim to include further scientifically related topics within the area of antimicrobial resistance. Therefore, we encourage you as an NRL to contribute to the newsletters by sending us materials for the future newsletters. This could be abstracts of recently published papers, case stories or other kinds of descriptions of topics related to our field.

We hope you support this idea and forward material for future newsletters to Rene Hendriksen (rshe@food.dtu.dk) or Susanne Karlsmose (suska@food.dtu.dk)