Screening for plasmid-mediated quinolone resistance

The plasmid gene responsible for quinolone resistance (q*nrA, qnrB, qnrC, qnrD qnrS, aac(6´)*Ib-cr and QepA) is carried on class 1 integrons of the In 4 family, an efficient mechanism for rapid horizontal and vertical dissemination og antibiotic resistance determinants among bacteria.
The plasmid mediated mechanisms have led to resistance to almost all clinical important antimicrobials, such as β-lactams, aminoglycosides, macrolides, phenicols, sulphonamides and trimethoprim.

The identification in the US of *qnr* in clinical strains of *K. pneumoniae* isolates besides producing plasmidic β-lactamases and ESBL's (7) and its discovery in strains of *E. coli* from Southeast Asia and Salmonella in Hong Kong indicates the emergence of this new mechanism of quinolones resistance in clinical strains.
It is important to indicate that a significant relation exists between quinolone resistance and resistance to 3. gen. cephalosporins (co-resistance): ESBL and/or plasmid mediated AmpC (6,14).

Poirel et al (10) have shown in vivo selection of fluoroquinolone-resistant E. coli isolates expressing plasmid mediated quinolone resistance and ESBL and physical linkage between ESBL and qnrA-encoding genes in the same integron.

Although *QnrA, QnrB, QnrS* produce low levels of quinolone resistance, it facilitates selection for a high level of quinolone resistance.

QnrB, another plasmid-mediated gene for quinolone resistance has been discovered in plasmids encoding the ESBL: CTA-15 from a K. pneumoniae. These strains show low-level resistance to quinolones and MIC of 16 µg/ml towards nalidixic acid, and show similar multiresistance phenotypes as qnr A containing strains (11).

Lavigne et al (12) screened for qnr genes 112 clinical isolates of ESBL-producing *E. coli* from French hospitals in 2004. 7.7 % of CTX-M-producing *E. coli* presented a plasmid-mediated resistance to quinolones. All strains harboured a qnrA gene located on a class 1 integron.

Poirel et al (13) listed 186 ESBL positive Enterobacteriaceae. From them 2.2 % and 1.6 % carried a QnrA1 and a QnrS1 determinant respectively. The association of the qnrA gene with class 1 integrons was confirmed.

Hyunjoo Pai et al (14) screened *E. coli* and *K. pneumoniae* producing ESBLs or plasmid mediated AmpC beta-lactamases for the presence of qnrA and qnrB genes. QnrB was present in 54 of 54 DHA-1 producing *K. pneumoniae* isolates and 10 of 45 SHV-12 producing isolates. It is possible that qnrB contributes to the widespread sistribution of DHA-1 (plasmid mediated AmpC) in areas, where 3rd generation cephalosporins and fluoroquinolones are widely used.

According to Lavilla (15) and Jones (16): the presence of aac(6´)Ib-cr were associated with quinolone resistance and aminoglycoside resistance (tobramycin is the best indicator).

Pitout et al (18) and Ruiz (20)found that isolates with aac(6´)Ib-cr were often associated with CTX-M-15.

Screening procedure
Perform antibiogram as usual (standard procedure): MH agar, inoculum McF 0.5, incubation at 35-37 °C for 18-24 hours.
Strains of Enterobacteriaceae should be suspected of plasmid-mediated quinolone resistance when showing unusual multiresistance phenotypes such as:

Neo-Sensitabs

Ampicillin	- no zone (HLR)
Sulphonamides	- no zone (HLR)
Trimethroprim	- no zone (HLR)
Trimethroprim+Sulfa	- no zone (HLR)
Streptomycins	- no zone (HLR)
Nalidixan 130 µg	- no zone or zone < 23 mm
Nalidixan 30 µg	- zone < 15 mm (MIC ≥16 µg/ml)
Norfloxacin 10 µg	- zone ≤ 23 mm
Ciprofloxacin 1 µg	- zone < 25 mm (MIC ≥0.125 µg/ml)
Ciprofloxacin 5 µg	- zone < 28 mm (MIC ≥0.125 µg/ml)
Ceftazidime	- zone < 20 mm
Chloramphenicol	- may show resistance
Tetracyclines	- may show resistance
aac(6`)Ib-cr	- Tobra R (20), Kana R
QepA	- Genta R, Tobra R (20), Cipro R, Norflox R (15-17)

Suspected strains can be tested for the presence of the q*nr* gen by PCR.
It should be noted that strains showing the above-mentioned resistance phenotypes are most probably integron-carrying. Enterobacteriaceae and barrier precaution should be established to prevent further spread. A new chromosomal gen,qnrM from S.maltophilia, produce quinolone-resistance in E.coli (21).

In a selected group of ciprofloxacin and ceftazidime-resistant Enterobacteriaceae (mainly *K. pneumoniae* and *E. cloacae*), carriage of qnrA gene was 32 % (9). From those 73 % were ESBL-positive.

Cavaco et al (22) found that the combination of ciprofloxacin, norfloxacin and nalidixic acid is the best option for detecting qnr and aac(6`)Ib-cr resistance mechanisms in Enterobacteriaceae.

Detection of qnr and aac(6`)Ib-cr and differentiation from mutations

Screening with nalidixic acid is efficient for the detection of mutants, but it is not efficient for the detection of some isolates carrying qnr and aac(6`)Ib-cr.
Transferable genes will be best detected using ciprofloxacin and norfloxacin, because these fluoroquinolones due to their chemical structures are attacked by strains carrying aac(6`)Ib-cr, reducing their antimicrobial activity.
Combining the use of Nalidixic acid 30 µg, Norfloxacin 10 µg and Ciprofloxacin 1 µg Neo-Sensitabs it is possible to screen for the new resistance mechanisms qnrA, qnrS and aac(6`)Ib-cr in strains of E.coli and Salmonella spp.

Susceptible strains E.coli and Salmonella spp. show zones of inhibition ≤ 25 mm with Nalidixic acid 30 µg Neo-Sensitabs (MIC ≤4-8 µg/ml).
With Ciprofloxacin 1 µg Neo-Sensitabs, susceptible strains show zones of inhibition ≥ 29 mm (MIC ≤0.016-0-03 µg/ml).
With Norfloxacin 10 µg Neo-Sensitabs, susceptible strains show zones of inhibition ≥ 33 mm (MIC ≤0.06 µg/ml).

Strains with **1 or 2 mutations** will show zones of inhibition ≤ 12 mm with Nalidixic acid 30 µg Neo-Sensitabs, while Ciprofloxacin will show zones ≤ 28 mm and Norfloxacin zones ≤ 32 mm.

Strains with **qnr or aac(6`)Ib-cr** will show zones of inhibition ≥ 13 mm with Nalidixic acid 30 µg Neo-Sensitabs, zones of 16-25 mm (MIC 0.125-0.5 µg/ml) with Ciprofloxacin 1 µg Neo-Sensitabs and zones of ≤ 30 mm (MIC 0.25-2 µg/ml) with Norfloxacin 10 µg/ml Neo-Sensitabs.

Conclusion:

Strains with mutations are best detected with Nalidixic acid 30 µg Neo-Sensitabs. Strains with aac(6`)Ib-cr are best detected using Ciprofloxacin 1 µg and Norfloxacin 10 µg Neo-Sensitabs, showing reduzed zones of inhibition compared to fully susceptible strains. Strains possessing aac(6`)Ib-cr, show also resistance to Tobramycin and Kanamycin, because these aminoglycosides are attacked by the mentioned enzyme. This is useful to differentiate them from the qnr enzymes.

References:

1) Minggui Wang et al: Emerging plasmid-mediated quinolone resistance associated with the *qnr* gene in *K. pneumoniae* clinicial isolates in the U.S. Antimicr. Ag. Chemother., **48**, 1295-9, 2004.
2) Rodriguez-Martinez J.M.: Mechanisms of plasmid-mediated resistance to quinolones. Enferm. Infecc. Microbiol. Clin., **23**, 25-31, 2005.
3) Hedi Mammeri et al: Emergence of plasmid-mediated quinolone resistance in *E. coli* in Europe. Antimicr. Ag. Chemother., **49**, 71-6, 2005.
4) Xian-Zhi Li: Quinolone resistance in bacteria: emphasis on plasmid-mediated mechanisms. Intl. J. Antimicrob. Ag., **25**, 453-63, 2005.
5) Jin-Yong Jeong et al: Detection of *qnr* in clinical isolates of *E. coli* from Korea. Antimicr. Ag. Chemother., **49**, 2522-4, 2005.
6) Poirel L. et al: Association of plasmid-mediated quinolone resistance with ESBL: VEB-1. Antimicr. Ag. Chemother., **49**, 3091-4, 2005.
7) Robicsek A. et al: Broda distribution of plasmid-mediated quinolone resistance in the U.S. Antimicr. Ag. Chemother., **49**, 3001-3, 2005.
8) Nordmann P, Poirel L: Emergence of plasmid-mediated resistance to quinolones in Enterobacteriaceae. J. Antimicr. Chemother., **56**, 463-9.
9) Corkill J.E. et al: High prevalence of the plasmid mediated quinolone resistance determinant anrA in multidrug-resistant Enterobacter from blood cultures in Liverpool, UK. JAC, **56**, 1115-7, 2005.
10) Poirel L. et al: In vivo selection of fluoroquinolone-resistant E. coli isolates expressing plasmid-mediated quinolone resistance and ESBL. Antimicr. Ag. Chemother., **50**, 1525-27, 2006.

11) Jacoby g.A. et al: QnrB, another plasmid-mediated gene for quinolone resistance. Antimicr. Ag. Chemother., **50**, 1178-82, 2006.
12) Lavignes P. et al: qnrA in CTX-M producing E. coli isolates from France. Antimicr. Agents Chemother., **50**, 4224-28, 2006.
13) Poirel L. et al: Prevalence and genetic analysis of plasmid mediated quinolone resistance determining 5 QnrA and QnrS in Enterobacteriaceae from a French University Hospital. Antimicr. Ag. Chemother., **50**, 3992-97, 2006.
14) Hyundoo Pai et al: Association of QnrB determinants and production of ESBL's or plasmid-mediated AmpC beta-lactamases in clinical isolates of K. pneumoniae. Antimicr. Ag. Chemother., 51, 366-68, 2007.
15) Lavilla S. et al: Prevalence of the quinolone modifying enzyme aac(6´)Ib-cr in ESBL-producing Enterobacterial isolates in Barcelona. Poster P-1523 ECCMID, april 2008.
16) Jones G. L. et al:The prevalence and distribution of plasmid-mediated quinolone resistance in clinical isolates of E.coli. Poster P-1530, ECCMID, Barcelona, april 2008.
17) Yamane K. et al: Plasmid-mediated qepA gene among E.coli clinical isolates from Japan. Antimicr. Ag. Chemother **52**, 1564-66, 2008.
18) Pitout J. D.D et al: Surveillance of plasmid-mediated quinolone resistance determinants in Enterobacteriaceae with the Calgary region Canada: the emergence of aac(6´)Ib-cr. JAC **61**, 999-1002, 2008.
19) Fabregas A. et al: Quinolone resistance in the food chain. Int.J. Antimicr. Agents, **31**, 307-315, 2008.
20) Ruiz E. et al: Detection of genes qnr and aac(6´)Ib-cr in strains of E.coli and K. pneumoniae producing ESBLs. Poster 39P XIII Congress SEIMC, Madrid, may 2008.
21) Sanchez M. B. et al: A new chromosomal qnr gene, qnrM from S.maltophilia produces quinolone-resistance in E.coli. Presentation 3, XIII Congress SEIMC, may 2008, Madrid.
22) Cavaco L.M. et al: 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 E.coli and Salmonella enterica and determinations of wild type distributions. J. Clin. Microbiol. **47**, 2751-58, 2009.
23) Cavaco L.M. et al: qnrD, a novel gene conferring transferable quinolone resistance in Salmonella enterica serovar Kentucky and bovismorbificans strains of human origin. Antimicr. Ag. Chemother **53**, 603-608, 2009.