Journal of Antimicrobial Chemotherapy (2005) **56**, 463–469
doi:10.1093/jac/dki245
Advance Access publication 14 July 2005

JAC

Emergence of plasmid-mediated resistance to quinolones in Enterobacteriaceae

Patrice Nordmann* and Laurent Poirel

Service de Bactériologie-Virologie, Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine Paris-Sud, Université Paris Sud, 78 rue du Général Leclerc, 94275, K.-Bicêtre, France

Although quinolone resistance results mostly from chromosomal mutations in Enterobacteriaceae, it may also be mediated by plasmid-encoded Qnr determinants. Qnr proteins protect DNA from quinolone binding and compromise the efficacy of quinolones such as nalidixic acid. Qnr proteins (QnrA-like, QnrB and QnrS) have been identified worldwide with a quite high prevalence among Asian isolates with a frequent association with clavulanic acid inhibited expanded-spectrum β-lactamases and plasmid-mediated cephalosporinases. The *qnrA* genes are embedded in complex *sul1*-type integrons. A very recent identification of the origin of QnrA determinants in the water-borne species *Shewanella algae* underlines the role of the environment as a reservoir for this emerging threat. It may help to determine the location of *in vivo* transfer of *qnrA* genes. Further analysis of the role (if any) of quinolones for enhancing this gene transfer may be conducted. This could prevent the spread, if still possible, of this novel antibiotic resistance mechanism.

Keywords: nalidixic acid, Qnr, fluoroquinolones

Introduction

Multidrug resistance in Enterobacteriaceae including resistance to quinolones is rising worldwide.[1–5] Quinolone resistance levels increase in human and veterinary enterobacterial isolates.[6,7] The quinolone resistance levels are higher for the narrow-spectrum quinolone nalidixic acid than for the broad-spectrum fluoroquinolones reaching up to 15–20% for nalidixic acid and 10% for fluoroquinolones in several surveys. Until recently, it was considered that plasmid-mediated resistance to quinolones could not be easily developed *in vivo* due to recessivity of a mutant gyrase gene compared with dominance of a wild-type chromosome-encoded allele and to the plasmid curing effect of quinolones.[8,9] Surveys conducted in the late 1970s failed to detect transferable resistance to nalidixic acid.[10] In 1987, plasmid-mediated resistance to quinolones was reported in *Shigella dysenteriae* but was not subsequently confirmed.[11] The first plasmid-mediated quinolone resistance protein Qnr (later termed QnrA) was identified from urine in a *Klebsiella pneumoniae* isolate in Birmingham, AL, USA, in 1994.[12] It opened a novel era in resistance to quinolones.

Mechanism of action of quinolones and of common bacterial resistance

Quinolones enter bacteria through porins or directly through the lipid and cytoplasmic membrane and target DNA topoisomerases.[13] The DNA topoisomerases control the topological state of the chromosomal DNA to facilitate replication, recombination and expression through the breaking and rejoining of DNA strands. Type I topoisomerases cleave one strand of DNA whereas type II topoisomerases cleave both strands in a reaction coupled to ATP binding and hydrolysis.[13–16] Quinolones act by inhibiting the action of type II topoisomerases, DNA gyrase and topoisomerase IV. The primary target for quinolones is DNA gyrase in Gram-negative bacteria and topoisomerase IV in Gram-positive bacteria, although exceptions to this pattern have been seen with some molecules. The DNA gyrase is responsible for introducing negative supercoils into DNA and for relieving topological stress arising from the translocation of transcription and replication complexes along DNA.[13–16] Topoisomerase IV is primary involved in decatenation, the unlinking of replicated daughter chromosome. Quinolones act by binding to gyrase/topoisomerase IV–DNA complex. Complex formation is responsible for the inhibition of DNA replication and the bacteriostatic action of the quinolones. Their lethal action is thought to be a separate event from complex formation, and to arise from the relapse of free DNA ends from quinolone–gyrase–DNA complexes. The blockage of the lethal action of some quinolones by protein synthesis inhibitors implies that a still unknown protein factor is involved.

Resistance to quinolones in Enterobacteriaceae most commonly arises stepwise as a result of mutation usually accumulating in the genes encoding primarily DNA gyrase and also topoisomerase IV. Decreased permeability by changes in the nature and amount of porins (in particular OmpF) or increased efflux by mutations in regulatory genes of chromosomally-encoded multidrug resistance

*Corresponding author. Tel: +33-1-45-21-36-32; Fax: +33-1-45-21-63-40; E-mail: nordmann.patrice@bct.ap-hop-paris.fr

pumps (Acr) or their regulatory systems (MarA, SoxS) may cause increments in quinolone resistance.[14–16]

Qnr family and QnrA-mediated resistance

The plasmid-mediated QnrA determinant first identified from the USA[12] is a 218-amino-acid protein that protects DNA gyrase (and probably also topoisomerase IV) from the inhibitory activity of quinolones.[17,18] QnrA belongs to the protein family with pentapeptide repeats, of which more than 90 members are known.[19] This pentapeptide family of proteins is defined by the presence of repetitions in tandem of the pattern A(D/N)LXX, where X is any amino acid.[19] These proteins have been identified in many bacterial species, but seem particularly common in cyanobacteria, where they are membrane- and cytoplasm-associated.[19] They have an α-helix structure in their external circumference and β parallel leaves in their internal circumference that is an appropriate structure for interaction between proteins.[19] In QnrA, the consensus sequence of the repeat is A/C, D/N, L/F, X, X.[17]

Another QnrA determinant termed QnrA2 has been identified from a *Klebsiella oxytoca* isolate from China (GenBank accession number AY675584). QnrA2 differs from QnrA1 by a few amino acid substitutions (Figure 1).

Two distantly-related Qnr determinants have been identified very recently. QnrB and variants have been identified from *Citrobacter koseri, Escherichia coli, Enterobacter cloacae* and *K. pneumoniae* from the USA and India with several isolates carrying both *qnrA*-like and *qnrB*-like genes[20] (Figure 1). In addition, a QnrS determinant was identified from a *Shigella flexneri* isolate in Japan (Figure 1).[21] QnrB and QnrS that also belong to the pentapeptide repeat family of proteins share 40% and 59% amino acid identity with QnrA, respectively (Figure 1).

The detailed mode of action of Qnr determinants has been studied so far for QnrA only. QnrA binds to both subunits GyrA and GyrB of the gyrase at the early stages of interaction between gyrase and DNA.[18] By lowering gyrase binding to DNA, QnrA reduces the amount of holoenzyme–DNA targets for quinolone inhibition.[17,18]

Among other proteins of the pentapeptide family, there are two members of special interest in quinolone resistance. The first protein is McbG that protects bacteria which synthesize microcin B17 (MccB17) from self-inhibition.[22] MccB17 is a post-transcriptional modified peptide of 3.1 kDa that blocks DNA replication.[22] Like ciprofloxacin, this microcin is able to inhibit the activity of DNA gyrase and to stabilize the DNA–DNA–gyrase complex in the presence of ATP.[23,24] The self-immunity mechanism conferred by McbG requires products of other genes organized as an operon, *mcbE* and *mcbF*, for the expulsion of MccB17 from the cell.[22] The second protein of the pentapeptide family is MfpA of *Mycobacterium smegmatis* that may contribute to quinolone resistance using efflux pumps.[25] QnrA shares 19.6% and 18.9% amino acid identity with McbG and MfpA, respectively.

Qnr-mediated quinolone resistance levels

The QnrA determinant provides resistance to quinolones such as nalidixic acid but not to fluoroquinolones according to the NCCLS breakpoints[26–29] (Table 1). MICs of fluoroquinolones for QnrA-positive transconjugants range from 0.25 to 1 mg/L corresponding up to a 20-fold increase compared with those for a wild-type recipient strain (Table 1). Results of *in vitro* studies and detailed analysis

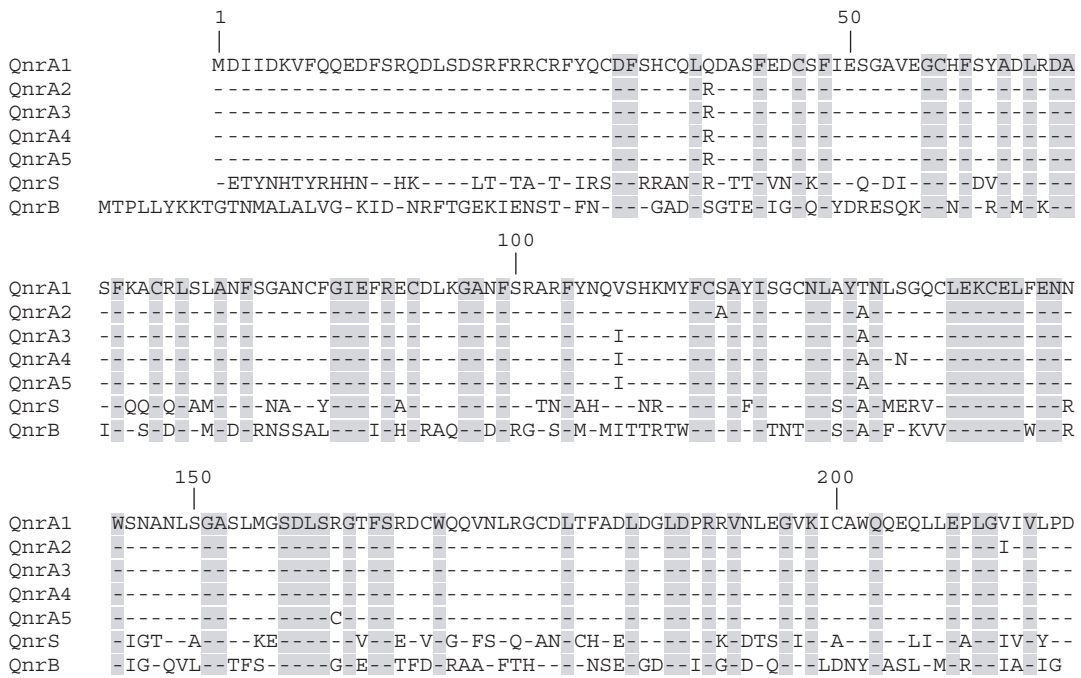

Figure 1. Sequence comparison of the plasmid-mediated QnrA-like, QnrB and QnrS determinants with the chromosome-encoded QnrA-like determinants of *S. algae*.[17,20,21,43] The plasmid-mediated QnrA1 and QnrA2 determinants are from *K. pneumoniae* from the USA[17] and from *Klebsiella oxytoca* from China (GenBank accession number AY675584), respectively. QnrA3 is from *S. algae* reference strain CIP106454T and *S. algae* clinical isolate KB-1 whereas QnrA4 and QnrA5 are from *S. algae* clinical isolates KB-2 and KB-3, respectively.[43] Dots indicate identical amino acid residues compared with QnrA1. Conserved amino acids among the different proteins are shaded in grey.

Table 1. MICs of quinolones for *qnrA*-positive clinical isolates and their transconjugants (adapted from refs 26, 27 and 31)

	MIC_{50} (mg/L)		
Antibiotic	clinical isolates	transconjugants in *E. coli* J53	*E. coli* J53
Ciprofloxacin	16	0.25	0.01
Levofloxacin	32	0.5	0.01
Moxifloxacin	32	0.5	0.03
Nalidixic acid	≥256	16	4
Sparfloxacin	32	1	0.01

of several fluoroquinolone-resistant and QnrA-positive isolates showed that chromosome and plasmid-mediated quinolone resistance determinants have additional effects.[27,29] Indeed, mutations in *gyrA, gyrB,* and in efflux pump and porin genes may increase plasmid-mediated quinolone resistance.[30] This observation explains the higher level of resistance to quinolones in clinical isolates compared with those observed for Qnr-positive transconjugants in several studies (Table 1). This is exemplified by the *E. coli* Lo clinical isolate for which a Ser-83→Leu substitution in the chromosomally-encoded GyrA was identified in addition to a plasmid-mediated QnrA determinant.[27] *In vitro* studies showed that once expressed a porin-deficient strain, the QnrA determinant raised MICs of ciprofloxacin, levofloxacin and moxifloxacin from 8- to 32-fold reaching MIC values of up to 4–8 mg/L.[30] Indeed, the presence of Qnr determinants enhances the selection of quinolone resistance by raising the level of resistance at which they can be selected. In addition, a variability of Qnr expression has been observed in transconjugants whereas QnrA-positive and nalidixic-acid susceptible isolates have been identified recently.[31,32] Thus, such isolates may represent a hidden reservoir enhancing the spread of Qnr determinants. Similarly to QnrA, QnrB and QnrS proteins confer resistance to nalidixic acid and not to fluroquinolones.[20,21]

Worldwide spread of Qnr determinants

Qnr determinants have been identified in a series of enterobacterial species in remotely related areas such as America, Europe and Asia.

In the pioneering study, a QnrA determinant was identified only from *K. pneumoniae* in Alabama[12] and during a 6 month period in 1994 whereas it was not detected among 350 Gram-negative isolates that included strains producing reference plasmid-mediated cephalosporinases and clavulanic-acid expanded-spectrum β-lactamases (ESBLs) and originating in 18 countries and 24 US states.[28] After this initial prevalence survey, another study reported 11% QnrA-positive isolates among ciprofloxacin-resistant *K. pneumoniae* isolates from six US states collected from 1999 to 2002.[33] A QnrA determinant was also identified in seven out of 17 *E. cloacae* isolates of variable susceptibility to ciprofloxacin and in two out of 20 ciprofloxacin-susceptible *K. pneumoniae* isolates from five US states.[34]

QnrA-like determinants in ciprofloxacin-resistant *E. coli* isolates collected from 2000 to 2002 were estimated to be 7.7% in Shanghai, China.[32] A *qnrA*-like gene was detected in 11 out of 23 bla_{VEB-1}-positive enterobacterial isolates (48%) from Bangkok, Thailand, collected in 1999 which were *E. coli*, *K. pneumoniae* and *Enterobacter sakazakii*, adding South East Asia to the list of regions in which QnrA determinants have spread.[31] In addition, QnrA determinants were detected in *E. coli* isolates in South Korea.[35]

The *qnrA* gene was also detected in Europe and first in two out of 449 nalidixic-acid-resistant and non-duplicate enterobacterial isolates (0.5%) collected at the hospital Bicêtre (suburb of Paris, France) in 2003.[27,31] The QnrA-positive isolates were *E. coli* and *E. cloacae*. In another European country, Germany, QnrA-positive *Enterobacter* spp. and *Citrobacter freundii* isolates were detected in four patients in two intensive care units among 703 cephalosporin-resistant or fluoroquinolone-resistant Enterobacteriaceae which were tested from 34 German intensive care units from 2000 to 2003.[36] QnrA determinants have been identified in *C. freundii, E. coli, Enterobacter amnigenus, E. cloacae* and *K. pneumoniae* in the Netherlands.[37]

QnrA was also identified from *C. freundii* and *E. cloacae* in Turkey[38] and from *Providencia stuartii* in Egypt.[39]

As indicated above whereas QnrS was identified from Japan only, the QnrB determinant was from India and the USA.[20,21]

None of the plasmid-mediated Qnr determinants have been identified so far in non-enterobacterial Gram-negative species (*Pseudomonas aeruginosa, Acinetobacter baumannii*, etc.), whereas several Qnr screening surveys included those types of isolates.[28,31] However, it was shown that a plasmid-mediated QnrA determinant was able to be transferred to a *P. aeruginosa* reference strain by conjugation.[12]

Association with expanded-spectrum β-lactamases

Several *qnrA*-like-positive isolates expressed clavulanic acid-inhibited extended-spectrum β-lactamases (ESBLs) such as SHV-5,[32,38] SHV-7,[32] CTX-M-15[20] and VEB-1.[27,31] The QnrA-positive *C. freundii* isolate from Turkey harboured a bla_{VEB-1} gene on the same *qnrA*-positive plasmid whereas a carbapenem-hydrolysing oxacillinase bla_{OXA-48} gene was located on another conjugative plasmid in the same isolate.[38] Whereas a limited number of ESBL-positive strains has been studied, the estimated prevalence of QnrA-positive and ESBL-positive strains was 4% at the hospital Bicêtre in 2003.[27,31] However, QnrA determinants were found in up to 48% of VEB-1-positive enterobacterial isolates from Bangkok, Thailand.[31] Plasmid-mediated resistance to quinolones was also estimated to be up to 24% of ciprofloxacin- and ceftazidime-resistant *Enterobacter* spp. isolates in the USA.[34]

QnrA determinants were also reported with plasmid-mediated cephalosporinases such as a bla_{FOX-5} gene in *K. pneumoniae* isolates from the USA.[12,40]

QnrB determinants were associated with the ESBL SHV-12 in several isolates.[20]

Association of antibiotic resistance genes may explain in part the frequent association between fluoroquinolone and expanded-spectrum cephalosporin resistance in Enterobacteriaceae.[41] In addition, it raises the issue of the nature of antibiotic molecules that may select this co-resistance. We do not know if there is a special link between the two emerging mechanisms of resistance in Enterobacteriaceae, i.e. plasmid-mediated quinolone resistance and ESBL in community-acquired pathogens.

Genetic vehicles

The very first identification of a QnrA determinant corresponded to an unexpected result of mating-out experiments for identification of a cephalosporin-resistant determinant in a *K. pneumoniae* isolate.[12] Plasmids that carry a QnrA determinant vary in structure and size ranging from 54 to >180 kb.[12,27,28,31–33,38,40] These plasmids carry other antibiotic resistance genes conferring resistance to expanded-spectrum cephalosporins (see above), aminoglycosides, chloramphenicol, rifampicin, sulphonamides, tetracycline and trimethoprim. Co-localization of antibiotic resistance genes on the same plasmids explains the frequent multidrug resistance of Qnr-positive enterobacterial isolates. In most of the cases, the QnrA determinant was easily transferable by conjugation whereas in rare cases it was not.[12,27,28,31–33,38,40] The expression of the QnrA determinant may vary after its transfer in reference strains suggesting heterogeneous expression of quinolone resistance determinants.[31–33]

Another degree of mobility of *qnrA*-like genes has been identified since these genes are embedded in In4 family class 1 integrons, also known as complex *sul1*-type integrons.[17,27,31–33] These genetic structures possess duplicated *qacEΔ1* and *sul1* genes that surround a sequence encoding Orf513 (Figure 2).[42] This protein may act as a recombinase for mobilization of downstream-located antibiotic resistance genes. The *qnrA* gene was not associated with a 59-be element as a form of a gene cassette as found in common class 1 integrons. The definition of the CR1 conserved region (CR) established recently indicates that it consists of an *orf513* gene that encodes a recombinase and a right-hand boundary that may act as a recombination cross-over site. It was shown that promoter sequences for expression of plasmid-encoded QnrA determinants overlap this CR1 element.[27] Structural comparison of *qnrA*-positive integrons showed variability both in the upstream- and downstream-*qnrA* located DNA sequences (Figure 2). This suggests that the process that had led to *qnrA* gene insertion in the *sul1*-type integron may vary. An *ampR* gene involved in regulation of expression of the naturally-encoded cephalosporinase of *Morganella morganii* was located next to the *qnrA* gene in a *sul1*-type integron (Figure 2). However, no expanded-spectrum β-lactamase gene was located inside any *qnrA*-positive *sul1*-type integrons (Figure 2). This observation indicates that co-localization of *qnrA* and expanded-spectrum β-lactamase genes on the same plasmids probably results from unrelated genetic events.

In a *qnrA*-positive *sul1*-type integron from China, inverted repeats of 25 bp were identified at the outer end of the 5′-CS and inner and outer next to the second copy of the 3′-CS downstream of the *qnrA* gene.[32] These inverted repeats were bracketed by 5 bp duplication suggesting that *qnrA* gene plasmid integration may result from a transposition process (although no transposase gene was identified in its immediate vicinity).

The genetic environment of the plasmid-encoded *qnrB* gene is unknown. However, the *qnrS* gene reported recently from Japan was not part of a *sul1*-type integron and not as a form of a gene cassette in a common class 1 integron.[21] It was adjacent to a Tn*3* transposon structure containing the β-lactamase bla_{TEM-1} gene.[21]

Origin of Qnr determinants

A series of Gram-negative species were screened by PCR to search for the reservoir of QnrA determinants. It included clinically-significant bacterial species and environmental species such as Enterobacteriaceae, Aeromonadaceae, Pseudomonadaceae, Xanthomonadaceae, Moraxellaceae and Shewanellaceae.[43]

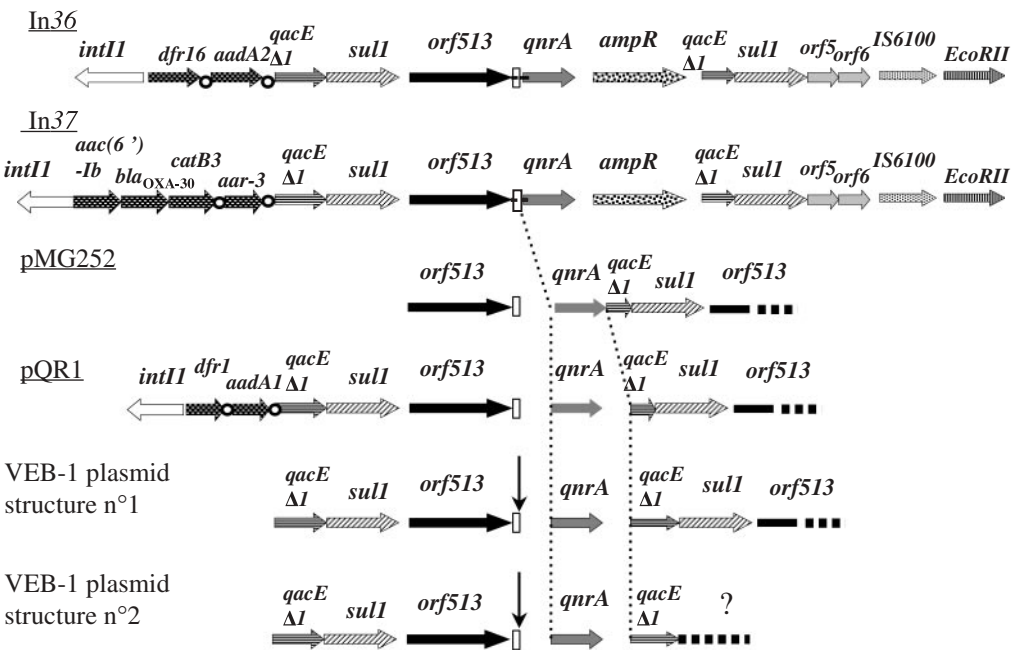

Figure 2. Schematic comparison of *sul1*-type integrons that contain *qnrA* genes. The compared structures are those of *E. coli* isolates from China (In36 and In37),[32] a *K. pneumoniae* isolate from the USA (pMG252),[17] an *E. coli* isolate from France (pQR1),[27] enterobacterial isolates from Thailand and France (VEB-1 plasmid structure no. 1)[27] and other enterobacterial isolates from Thailand (VEB-1 plasmid structure no. 2).[31] The vertical rectangle indicates the right-hand boundary of the CR1 element and the vertical arrow indicates a 2 bp deletion. The question mark indicates unknown DNA sequences but different from those reported above. Dotted vertical lines indicate the absence of a DNA fragment between the *qnrA* gene, *qacEΔ1* in pMG252.

Positive results were obtained for *Shewanella algae* with identification of novel chromosome-encoded QnrA determinants termed QnrA3 to QnrA5 that differed by a few amino acid substitutions from the plasmid-mediated QnrA determinants (Figure 1).[43] The G + C content (52%) of the *qnrA*-like genes of *S. algae* matches exactly that of the genome of *S. algae*.[43] *S. algae* is a Gram-negative species belonging to the Shewanellaceae family that is widely distributed in marine and freshwater environments.[44] *S. algae* is rarely involved in human infections, most being related to seawater exposure.[45,46] The MIC of nalidixic acid was 2 mg/L and the MICs of the fluoroquinolones ciprofloxacin, ofloxacin, sparfloxacin and norfloxacin were 0.12, 0.5, 0.5 and 0.5 mg/L for *S. algae* strains, remaining in the susceptibility range according to NCCLS breakpoints.[43] The CR1 element that provides promoter sequences for high-level expression of the plasmid-mediated *qnrA* gene in Enterobacteriaceae was not identified in *S. algae*.[43] Since quinolones are also extensively used in animals and aquaculture,[6,47] it is possible that subinhibitory concentrations of quinolones that are stable molecules in the environment[48,49] may select for water-borne *S. algae* strains and enhance transfer of naturally occurring quinolone resistance determinants to Enterobacteriaceae. The aquatic environment has been shown to be a reservoir for antibiotic resistance genes and their transfer.[50,51] In addition, whereas quinolones used in therapy are synthetic molecules, naturally-produced quinolones have been discovered recently[52] that may also play a role in this horizontal transfer. In addition, it has been shown that quinolones induce an SOS repair system and antibiotic resistance gene transfer.[53]

Further work may also identify the reservoir of the distantly related QnrB and QnrS determinants that might also be psychrophilic bacterial species. Interestingly, several isolates from the US were found to produce both the QnrA and QnrB determinants[20] suggesting that their progenitors may share an identical niche. Analysis of a *qnrA*-positive *sul1*-type integron from a Shanghai isolate that also contained an *ampR* gene (from *M. morganii*) indicated that construction of those *sul1*-type integrons may result from successive recombination events involving genes of unrelated bacterial origin. The role, if any, of those Qnr determinants in their natural hosts remains to be determined.

Concluding remarks

The emergence of plasmid-mediated quinolone resistance determinants in Enterobacteriaceae may compromise further the efficacy of quinolones that are, together with β-lactams and macrolides, the most commonly prescribed antibiotics for treating human infections. This novel mechanism of resistance may be important for the treatment not only of nosocomial but also of community-acquired infections. However, it remains to be determined if plasmid- mediated Qnr determinants in Enterobacteriaceae really compromise the clinical efficacy of fluoroquinolones in the absence of additional chromosomally-encoded quinolone resistance determinants.

By comparison with known flux of antibiotic resistance genes (such as narrow-spectrum penicillinase genes), it is possible that plasmid-mediated quinolone resistance determinants may be transferred to community-acquired Gram-negative bacterial species such as *Neisseria* spp. and *Haemophilus* spp. Current knowledge on Qnr determinants indicates that they are more diverse than previously expected. Their prevalence and the prevalence of their association with ESBL-encoding genes remain to be determined whereas Asian isolates seem already to be an important reservoir of Qnr determinants. Identification of *qnrA* genes embedded in integrons argues for their recent emergence in clinical isolates (rather than for their recent identification) since an increase in integron prevalence in multidrug-resistance in Enterobacteriaceae has been reported recently.[54]

The identification of the natural host, *S. algae*, as the source of plasmid-mediated QnrA determinants is an important step in discovering the location of this gene exchange (water-related environment, animals, etc.) and their enhancing factors. This may represent a unique opportunity for limiting the spread of these emerging antibiotic resistance determinants.

Acknowledgements

This paper reflects knowledge gained in work with former and current collaborators and colleagues: H. Mammeri, L. Martinez-Martinez, J. M. Rodriguez-Martinez and M. Van De Loo. We thank G. A. Jacoby for providing us with the unpublished QnrB sequence. This work was funded by a grant from the Ministère de l'Education Nationale et de la Recherche (UPRES-EA3539), Université Paris XI, France and by the European Community (6th PCRD, LSHM-CT-2003–503–335). L. P. is a researcher from INSERM (Paris, France).

References

1. Garau J, Xercavins M, Rodriguez-Carballeira M *et al.* Emergence and dissemination of quinolone-resistant *Escherichia coli* in the community. *Antimicrob Agents Chemother* 1999; **43**: 2736–41.

2. Hooper DC. Emerging mechanisms of fluoroquinolone resistance. *Emerg Infect Dis* 2001; **7**: 337–41.

3. Lautenbach E, Strom BL, Nachamkin I *et al.* Longitudinal trends in fluoroquinolone resistance among *Enterobacteriaceae* isolates from inpatients and outpatients, 1989–2000; differences in the emergence and epidemiology of resistance across organisms. *Clin Infect Dis* 2004; **38**: 655–62.

4. Chaniotaki S, Giakouppi P, Tzouvelekis LS *et al.* Quinolone resistance among *Escherichia coli* strains from community-acquired urinary tract infections in Greece. *Clin Microbiol Infect* 2004; **10**: 75–8.

5. Clifford McDonnald L, Chen FJ, Lo HJ *et al.* Emergence of reduced susceptibility and resistance to fluoroquinolones in *Escherichia coli* in Taiwan and contributions of distinct selective pressures. *Antimicrob Agents Chemother* 2001; **45**: 3084–91.

6. Webber M, Piddock LVJ. Quinolone resistance in *Escherichia coli. Vet Res* 2001; **32**: 275–84.

7. Blanco JE, Blanco M, Mora A *et al.* Prevalence of bacterial resistance to quinolones and other antimicrobials among avian *Escherichia coli* strains isolated from septicemic and healthy chickens in Spain. *J Clin Microbiol* 1997; **35**: 2184–5.

8. Courvalin P. Plasmid-mediated 4-quinolone resistance: a real or apparent absence? *Antimicrob Agents Chemother* 1990; **34**: 681–4.

9. Gomez-Gomez J, Blasquez J, Espinosa LE *et al.* In vitro plasmid-encoded resistance to quinolones. *FEMS Microbiol Lett* 1997; **154**: 271–6.

10. Burman LG. Apparent absence of transferable resistance to nalidixic acid in pathogenic gram-negative bacteria. *J Antimicrob Chemother* 1977; **3**: 509–16.

11. Munshi MH, Sack DA, Haider K *et al.* Plasmid-mediated resistance to nalidixic acid in *Shigella dysenteriae* type 1. *Lancet* 1987; **ii**: 4419–21.

12. Martinez-Martinez L, Pascual A, Jacoby GA. Quinolone resistance from a transferable plasmid. *Lancet* 1998; **351**: 797–9.

13. Drlica K, Zhao XL. DNA gyrase, topoisomerase IV, and the 4-quinolones. *Microbiol Mol Biol Rev* 1997; **61**: 377–92.

14. Ruiz J. Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA gyrase protection. *J Antimicrob Chemother* 2003; **51**: 1109–17.

15. Hooper DC. Mechanisms of action and resistance to older and newer fluoroquinolones. *Clin Infect Dis* 2000; **31** Suppl S2: S24–8.

16. Hawkey PM. Mechanisms of quinolone action and microbial response. *J Antimicrob Chemother* 2003; **51** Suppl S1: S29–35.

17. Tran JH, Jacoby GA. Mechanism of plasmid-mediated quinolone resistance. *Proc Natl Acad Sci USA* 2002; **99**: 5638–42.

18. Tran JH, Jacoby GA, Hooper DC. Interaction of the plasmid-encoded quinolone resistance protein Qnr with *Escherichia coli* DNA gyrase. *Antimicrob Agents Chemother* 2005; **49**: 118–25.

19. Bateman A, Murzin AG, Teichmann SA. Structure and distribution of pentapeptide repeats in bacteria. *Protein Sci* 1998; **7**: 1477–80.

20. Jacoby GA, Walsh K, Mills D *et al*. A new plasmid-mediated gene for quinolone resistance. In: *Abstracts of the Forty-fourth Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, 2004.* Abstract C2-1898a. American Society for Microbiology, Washington, DC, USA.

21. Hata M, Suzuki M, Matsumoto M *et al*. Cloning of a novel gene for quinolone resistance from a transferable plasmid in *Shigella flexneri* 2b. *Antimicrob Agents Chemother* 2005; **49**: 801–3.

22. Garrido MC, Herrero M, Kolter R *et al*. The export of the DNA replication inhibitor microcin B17 provides immunity for the host cell. *EMBO J* 1988; **7**: 1853–62.

23. Zamble DB, Milier DA, Heddle JG *et al*. *In vitro* characterization of DNA gyrase inhibition by microcin B17 analogs with altered bisheterocyclic site. *Proc Natl Acad Sci USA* 2001; **98**: 7712–7.

24. Heddle JG, Blance SJ, Zamble DB *et al*. The antibiotic microcin B17 is a DNA gyrase poison: characterisation of the mode of inhibition. *J Mol Biol* 2001; **307**: 1223–34.

25. Montero C, Mateu G, Rodriguez R *et al*. Intrinsic resistance of *Mycobacterium smegmatis* to fluoroquinolones may be influenced by new pentapeptide protein MfpA. *Antimicrob Agents Chemother* 2001; **45**: 3387–92.

26. Wang M, Sahm DF, Jacoby GA *et al*. Activities of newer quinolones against *Escherichia coli* and *Klebsiella pneumoniae* containing the plasmid-mediated quinolone determinant Qnr. *Antimicrob Agents Chemother* 2004; **48**: 1400–1.

27. Mammeri H, Van De Loo M, Poirel L *et al*. Emergence of plasmid-mediated quinolone resistance in *Escherichia coli* in Europe. *Antimicrob Agents Chemother* 2005; **49**: 71–6.

28. Jacoby GA, Chow N, Waites KB. Prevalence of plasmid-mediated quinolone resistance. *Antimicrob Agents Chemother* 2003; **47**: 559–62.

29. National Committee for Clinical Laboratory Standards. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically—Fourth Edition: Approved Standard M100-S14*. NCCLS, Wayne, PA, USA, 2004.

30. Martinez-Martinez L, Pascual A, Garcia I *et al*. Interaction of plasmid and host quinolone resistance. *J Antimicrob Chemother* 2003; **51**: 1037–9.

31. Poirel L, Van De Loo M, Mammeri H *et al*. Association of plasmid-mediated quinolone resistance with extended-spectrum β-lactamase VEB-1. *Antimicrob Agents Chemother* 2005; **49**: 3091–4.

32. Wang M, Tran JH, Jacoby GA *et al*. Plasmid-mediated quinolone resistance in clinical isolates of *Escherichia coli* from Shanghai, China. *Antimicrob Agents Chemother* 2003; **47**: 2242–8.

33. Wang M, Sahm DF, Jacoby GA *et al*. Emerging plasmid-mediated quinolone resistance associated with the *qnr* gene in *Klebsiella pneumoniae* clinical isolates in the United States. *Antimicrob Agents Chemother* 2004; **48**: 1295–9.

34. Robicsek A, Sahm DF, Jacoby GA *et al*. Broader distribution of plasmid-mediated quinolone resistance in the United States. In: *Abstracts of the Forty-fourth Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, 2004.* Abstract C2-1898b. American Society for Microbiology, Washington, DC, USA.

35. Kim SH, Kwak Y, Lee M *et al*. Plasmid-mediated quinolone resistance in clinical isolates of *Escherichia coli* from Korea. In: *Abstracts of the Forty-fourth Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, 2004.* Abstract C2-1711, p. 119. American Society for Microbiology, Washington, DC, USA.

36. Jonas D, Biehler K, Hartung D *et al*. Plasmid-mediated quinolone resistance in isolates obtained in German intensive care units. *Antimicrob Agents Chemother* 2005; **49**: 773–5.

37. Paauw A, Fluit AC, Verhoef MA *et al*. A major outbreak with plasmid-mediated, *qnr* encoded, quinolone resistance. In: *Abstracts of the Forty-fourth Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, 2004.* Abstract C2-1898, p. 128. American Society for Microbiology, Washington, DC, USA.

38. Nazik A, Poirel L, Nordmann P. Further identification of plasmid-mediated quinolone resistance determinant in *Enterobacteriaceae*, in Turkey. *Antimicrob Agents Chemother* 2005; **49**: 2146–7.

39. Wiegand I, Khalaf N, Al-Agamy MHM *et al*. First detection of the transferable quinolone resistance in clinical *Providencia stuartii* strains in Egypt. In: *Abstracts of the Fourteenth European Congress of Clinical Microbiology and Infectious Diseases, Prague, 2004*. Abstract O347, p. 164. European Society of Clinical Microbiology and Infectious Diseases, Basel, Switzerland.

40. Rodríguez-Martínez JM, Pascual A, García I *et al*. Detection of the plasmid-mediated quinolone resistance determinant *qnr* among clinical isolates of *Klebsiella pneumoniae* producing AmpC-type β-lactamase. *J Antimicrob Chemother* 2003; **52**: 703–6.

41. Paterson DL, Mulazimoglu L, Casellas JM *et al*. Epidemiology of ciprofloxacin resistance and its relationship to extended-spectrum β-lactamase production in *Klebsiella pneumoniae* isolates causing bacteremia. *Clin Infect Dis* 2000; **30**: 473–8.

42. Partridge SR, Hall RM. In34, a complex In5 family class 1 integron containing orf513 and *dfrA10*. *Antimicrob Agents Chemother* 2003; **47**: 342–9.

43. Poirel L, Rodriguez-Martinez JM, Mammeri H *et al*. Origin of plasmid-mediated quinolone resistance determinant QnrA. *Antimicrob Agents Chemother* 2005; in press.

44. Nozue H, Hayashi T, Hashimoto Y *et al*. Isolation and characterization of *Shewanella alga* from human clinical specimens and emendation of the description of *S. alga* Simidu et al., 1990, 335. *Int J Syst Bacteriol* 1992; **42**: 628–34.

45. Fonnesbech-Vogel B, Holt HM, Gerner-Smidt P *et al*. Homogeneity of Danish environmental and clinical isolates of *Shewanella algae*. *Appl Environ Microbiol* 2000; **66**: 443–8.

46. Fonnesbech-Vogel B, Jorgensen K, Christensen H *et al*. Differentiation of *Shewanella putrefaciens* and *Shewanella alga* on the basis of whole-cell protein profiles, ribotyping, phenotypic characterization, and 16S rRNA gene sequence analysis. *Appl Environ Microbiol* 1997; **63**: 2189–99.

47. NORM/NORM-VET2002. *Consumption of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway*. ISSN 1502-2307. Oslo: Tromso, 2003; 13.

48. Turiel E, Martin-Esteban A, Bordin G *et al*. Stability of fluoroquinolone antibiotics in river water samples and in octadecyl silica solid-phase extraction cartridges. *Anal Bioanal Chem* 2004; **380**: 123–8.

49. Nedoluha PC, Owens S, Russek-Cohen E. Effect of sampling method on the representative recovery of microorganisms from the surfaces of aquaculture finfish. *J Food Protect* 2001; **10**: 1515–20.

50. Young HK. Antimicrobial resistance spread in aquatic environments. *J Antimicrob Chemother* 1993; **31**: 627–35.

51. Kümmerer K. Resistance in the environment. *J Antimicrob Chemother* 2004; **54**: 311–20.

52. Pesci EC, Milbank JB, Pearson JP *et al*. Quinolone signaling in the cell-to-cell communication system of *Pseudomonas aeruginosa*. *Proc Natl Acad Sci USA* 1999; **96**: 11229–33.

53. Beaber JW, Hochhut B, Waldor MK. SOS response promotes horizontal dissemination of antibiotic resistance genes. *Nature* 2004; **427**: 72–4.

54. Schmitz FJ, Hafner D, Geisel R *et al*. Increased prevalence of class 1 integrons in *Escherichia coli*, *Klebsiella* species, and *Enterobacter* species isolates over a 7-year period in a German university hospital. *J Clin Microbiol* 2001; **39**: 3724–6.