The worldwide emergence of plasmid-mediated quinolone resistance

Ari Robicsek, George A Jacoby, David C Hooper

Fluoroquinolone resistance is emerging in Gram-negative pathogens worldwide. The traditional understanding that quinolone resistance is acquired only through mutation and transmitted only vertically does not entirely account for the relative ease with which resistance develops in exquisitely susceptible organisms, or for the very strong association between resistance to quinolones and to other agents. The recent discovery of plasmid-mediated horizontally transferable genes encoding quinolone resistance might shed light on these phenomena. The Qnr proteins, capable of protecting DNA gyrase from quinolones, have homologues in water-dwelling bacteria, and seem to have been in circulation for some time, having achieved global distribution in a variety of plasmid environments and bacterial genera. AAC(6')-Ib-cr, a variant aminoglycoside acetyltransferase capable of modifying ciprofloxacin and reducing its activity, seems to have emerged more recently, but might be even more prevalent than the Qnr proteins. Both mechanisms provide low-level quinolone resistance that facilitates the emergence of higher-level resistance in the presence of quinolones at therapeutic levels. Much remains to be understood about these genes, but their insidious promotion of substantial resistance, their horizontal spread, and their co-selection with other resistance elements indicate that a more cautious approach to quinolone use and a reconsideration of clinical breakpoints are needed.

Lancet Infect Dis 2006; 6: 629–40

Massachusetts General Hospital, Boston, MA, USA (A Robicsek MD, D C Hooper MD); **Harvard Medical School, Boston** (A Robicsek, D C Hooper, G A Jacoby MD); **Lahey Clinic, Burlington, MA** (G A Jacoby); **and Evanston Northwestern Healthcare, Evanston, IL, USA** (A Robicsek)

Correspondence to:
Dr David C Hooper, Division of Infectious Diseases, Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02114-2696, USA.
Tel +1 617 726 3812; fax +1 617 726 7416;
dhooper@partners.org

Introduction

The development of quinolone resistance by Gram-negative pathogens constitutes a remarkable bacterial success story. Quinolones were introduced into clinical use in 1962 in the form of nalidixic acid,[1] a fully synthetic agent with bactericidal effects on most Enterobacteriaceae at clinical concentrations. A pharmacological innovation—addition of a fluorine at the C-6 position and piperazinyl or related ring at position C-7 of the quinolone molecule—yielded the fluoroquinolones, first available clinically in the 1980s.[2] These agents achieve higher serum levels than those of nalidixic acid and are more potent against Enterobacteriaceae; drug concentrations 1000-fold those required to inhibit growth are routinely achieved. Thus, these agents entered into use endowed with two advantages over the bacteria. First, although organisms could develop mutations that reduced quinolone susceptibility, the potency of these agents was such that a wild-type *Escherichia coli* would need to acquire spontaneously two or more resistance mutations to survive at clinical drug concentrations. Since independent mutations generally arise once per 10^7 cell divisions or less, the likelihood that multiple mutations would occur in a single clone seemed negligible. Second, many resistance genes have co-evolved in nature with the antibiotics that they counteract, especially those that modify or inactivate the drug. Since the quinolones are fully synthetic, it seemed unlikely that resistance genes would be available for recruitment onto mobile elements. Thus, the quinolones seemed to confound resistance; they were a class of agents to which mutational resistance was unlikely to develop and against which resistance genes could not be acquired.

Over the 20 years that have elapsed since the introduction of fluoroquinolones, resistance to these agents by Enterobacteriaceae has become common and widespread, and, remarkably, is generally not clonal. This finding implies that fluoroquinolone resistance has arisen many times in organisms that were once exquisitely susceptible. A recent survey of enteric bacteria in US intensive care units found that more than 10% of these organisms were resistant to ciprofloxacin.[3] Levels of quinolone resistance in clinical *E coli* isolates have been reported at 40% in Hong Kong,[4] and about 25% of healthy individuals living in Barcelona were found to be intestinally colonised with quinolone-resistant *E coli*.[5]

Until recently, two mechanisms of resistance had been found to determine resistance to fluoroquinolones (and quinolones, since in almost all cases organisms resistant to fluoroquinolones are resistant to nalidixic acid as well). The most important of these mechanisms in Enterobacteriaceae is the accumulation of mutations in the bacterial enzymes targeted by fluoroquinolones: DNA gyrase and DNA topoisomerase IV.[6] When bound to DNA, these enzymes transiently break the closed circular DNA molecule, pass another strand through the break, and then reseal the DNA. This process effects changes in DNA topology that are essential in DNA replication, transcription, recombination, and repair. Quinolones bind to these enzymes and stabilise a drug-enzyme-cleaved DNA complex, allowing lethal double-stranded DNA breaks to accumulate unrepaired.[7] Each of the target enzymes has a quinolone-resistance determining region (QRDR), a portion of the DNA-binding surface of the enzyme[8] at which aminoacid substitutions can diminish quinolone binding. Generally, multiple such mutations are required to achieve clinically important resistance in Enterobacteriaceae; when such organisms are quinolone resistant they are nearly always found to have one or more QRDR mutations. The other classically described mechanism of resistance operates by decreasing intracellular drug accumulation by upregulation of native efflux pumps[9] either alone or together with decreased expression of outer membrane porins.[10]

Both mechanisms of resistance are mutational, arising in an individual organism and then passing vertically to surviving progeny. Neither mechanism seems to transfer effectively on mobile genetic elements. A laboratory-generated plasmid overexpressing a quinolone-resistant mutant DNA gyrase gene caused only a modest increase in quinolone minimum inhibitory concentration (MIC), since the host gyrase remained quinolone susceptible.[11] Accordingly, plasmids encoding mutant gyrases have not been found in nature. The development of plasmid-mediated quinolone resistance (PMQR) through decreased drug accumulation has also not been described. The impression that PMQR did not exist was bolstered by surveys in the 1970s that did not uncover any plasmids capable of transferring quinolone resistance.[12] Although a 1987 report described the identification of PMQR in an outbreak strain of *Shigella dysenteriae*,[13] the quinolone resistance was later attributed to chromosomal mutation and not a plasmid-encoded gene.[14]

Plasmid-encoded Qnr protein

The discovery of PMQR in the late 1990s was made inadvertently by Luis Martinez-Martinez and colleagues.[15] A quinolone was included as a control in a study of the ability of a plasmid called pMG252 to increase resistance to multiple antibiotics in a porin-deficient strain of *Klebsiella pneumoniae*. Unexpectedly, a large increase in quinolone MIC was found. The effect of the plasmid was magnified in this porin-deficient isolate, but even in an *E coli* strain with intact porins, pMG252 increased the quinolone MICs between eight-fold and 64-fold. Although this increase from baseline was not to the level designated as representing clinical resistance (the resistance breakpoint), the plasmid also facilitated selection of higher-level quinolone resistance. Wild-type *E coli* carrying pMG252 plated on agar containing nalidixic acid or ciprofloxacin was 100 times more likely to give rise to spontaneous resistant mutants than a plasmid-free strain.[15] Subsequent cloning of the gene responsible for this phenotype showed it to be a 657 basepair open reading frame, and the protein it encoded was named Qnr, for quinolone resistance.[16] (Note that the term "resistance" in the setting of PMQR is used to refer to any increase in MIC—a biological definition—rather than to an increase above a susceptibility breakpoint—a clinical definition.) More recently, this protein has been renamed QnrA, since related proteins have been identified.

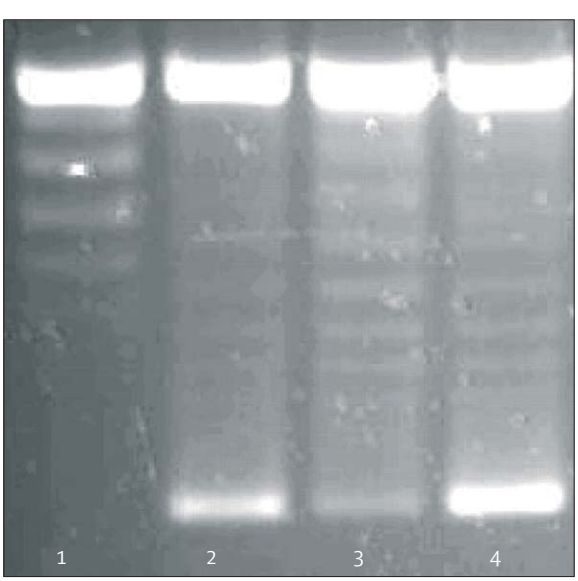

Figure 1: **DNA supercoiling assay showing the protection of DNA gyrase by QnrA[16]**
Relaxed plasmid DNA loaded into the top of each gel migrates more slowly than supercoiled DNA. The presence of DNA gyrase (lane 2) brings about supercoiling and, as a result, more rapid migration. Adding ciprofloxacin (lane 3) inhibits this effect. The further addition of purified QnrA-His_6 fusion protein to relaxed DNA, gyrase and ciprofloxacin (lane 4) restores gyrase-mediated supercoiling.

Mechanism of Qnr action

The QnrA protein belongs to the pentapeptide-repeat family, which is defined by a tandem five aminoacid repeat with the recurrent motif [Ser, Thr, Ala or Val] [Asp or Asn] [Leu or Phe] [Ser, Thr or Arg] [Gly].[17] To date, more than 500 proteins are known to contain such pentapeptide-repeat motifs, but the function of nearly all of these proteins is unknown. Two pentapeptide-repeat proteins are of particular interest. A naturally occurring peptide, microcin B17, is a bacterial poison with a mechanism of action much the same as that of the quinolones; it inhibits DNA gyrase.[18] Organisms producing B17 also make McbG, a pentapeptide-repeat protein with 19·6% aminoacid identity with QnrA, that protects DNA gyrase against the effect of the microcin[19] and also some quinolones.[20] MfpA, another pentapeptide-repeat protein having 18·9% aminoacid similarity to QnrA, has been more thoroughly studied. The *mfpA* gene was first identified on the chromosome of *Mycobacterium smegmatis*.[21] When expressed on a multicopy plasmid, this gene resulted in an increase of between four-fold and eight-fold in the MIC of ciprofloxacin of this organism, and inactivation of the gene on the *M smegmatis* chromosome resulted in increased ciprofloxacin susceptibility. Subsequently, a variant of this gene found in *Mycobacterium tuberculosis* was shown to inhibit the activity of DNA gyrase by directly interacting with the enzyme. Both the three-dimensional structure of this MfpA variant and its charge distribution closely resemble those of DNA.[22] Thus, this protein is thought to inhibit gyrase through competition with DNA for binding. This interaction has also been proposed to underlie the fluoroquinolone resistance that the gene confers: DNA gyrase bound to MfpA will not participate in the quinolone-gyrase-cleaved DNA complex that is deleterious for cells.

The mechanism by which QnrA protects DNA gyrase has also been studied. As expected from its pentapeptide-repeat structure, QnrA did not seem to effect a change in

intracellular quinolone accumulation nor did it cause drug inactivation. The direct effect of QnrA on quinolone inhibition of DNA gyrase activity has been studied using a DNA supercoiling assay (figure 1). In this assay, the inhibition of gyrase-mediated DNA supercoiling caused by ciprofloxacin was reversed in a dose-dependent manner by purified QnrA protein (tagged with histidine residues to facilitate purification). QnrA-His_6 alone, however, did not itself effect DNA supercoiling,[16] nor did it, by contrast with MfpA, inhibit gyrase-mediated DNA supercoiling,[23] although a Qnr variant can do so at very high concentrations.[24] QnrA did, however, reduce gyrase binding to DNA as would be predicted if it formed, like MfpA, a DNA-like structure.[23] In addition, QnrA has now been shown to bind gyrase (both subunits and holoenzyme) directly, as well as *E coli* topoisomerase IV, which it likewise protects from quinolone inhibition.[25] QnrA binding to gyrase or topoisomerase IV, which inhibits the gyrase-DNA interaction, could account, at least in part, for the protection against quinolones by minimising opportunities for these agents to stabilise the lethal gyrase-DNA-quinolone cleavage complex. How QnrA might compete with DNA for gyrase binding without substantially inhibiting gyrase activity in vitro is not yet known.

Resistance activity of Qnr

The extent to which QnrA protects Enterobacteriaceae against fluoroquinolones has usually been examined by measuring the difference in quinolone MIC for an *E coli* strain with and without a *qnrA*-bearing plasmid. The first report of a *qnrA* plasmid found that the MIC of ciprofloxacin increased from 0·008 µg/mL to 0.25 µg/mL in an *E coli* J53 transconjugant, with a range from 0·125 µg/mL[26] to 2·0 µg/mL[27] for other *qnr* plasmid transconjugants of this strain. One study assessed the quinolone resistance conferred by 17 clinical *qnrA*-bearing plasmids (table 1). Donor bacteria originally harbouring these plasmids all had exhibited higher levels of resistance to quinolones than the transconjugants, suggesting that additional mechanisms of quinolone resistance frequently coexist with that of *qnrA*. There were also differences among transconjugants in the *qnr* plasmids' effects on the MICs of fluoroquinolones. Although for most agents, the presence of a *qnr* plasmid increased their MIC by between about 16-fold and 125-fold, this increase was less (16-fold to 32-fold) for the developmental fluoroquinolones BAYy3118, premafloxacin, and sitafloxacin. The agent for which the loss of activity was least pronounced was nalidixic acid (two-fold to eight-fold increases in MIC).[27] Also noteworthy is the finding that *qnrA*-encoding plasmids from US *K pneumoniae* yielded transconjugants with very similar quinolone susceptibilities,[27] whereas *qnrA*-encoding plasmids from Chinese *E coli* varied in ciprofloxacin susceptibilities by 16-fold.[26] In some cases these differences probably resulted from the presence on some plasmids of a resistance determinant in addition to *qnrA* that affected some quinolones but not others.

MIC studies assess the effect of a resistance gene on growth inhibition by an antimicrobial agent. There are other indices by which the effect of a resistance gene can be assessed. A time-kill study has examined the bactericidal activity of ciprofloxacin and ofloxacin in the presence of QnrA. Despite the fact that QnrA protects against quinolone growth inhibition, it did not block the bactericidal activity of these quinolones at concentrations of twice MIC or greater.[28] Another measure of resistance gene effect is a change in the mutant prevention concentration (MPC). The MPC is the lowest

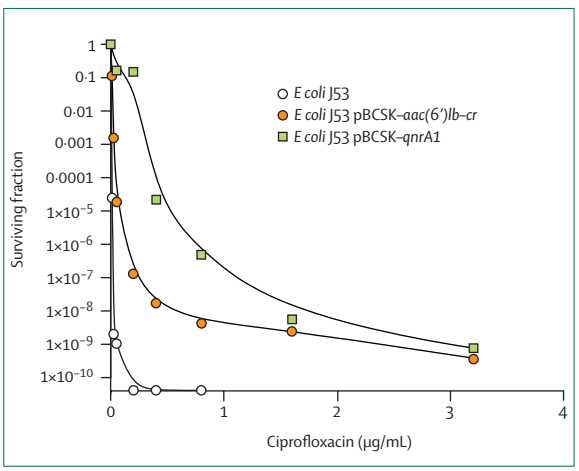

Figure 2: **Mutant prevention concentration (MPC) assay**
About 10^{10} organisms and appropriate dilutions were applied to Mueller-Hinton agar plates containing the indicated concentrations of ciprofloxacin. Surviving colonies were counted after incubation for 72 h at 37°C. The lowest concentration of ciprofloxacin at which no mutant colonies were seen was 0·2 µg/mL for J53 and 3·2 µg/mL for J53 pBCSK-*aac(6′)Ib-cr* or J53 pBCSK-*qnrA1*.

Agent	*E coli* J53, wild-type (MIC_{90})	Transconjugants (n=17)	
		MIC_{90}	MIC_{range}
BAYy3118	0·004	0·125	0·06–0·25
Ciprofloxacin	0·008	0·25	0·125–2
Garenoxacin	0·008	1	0·5–2
Gatifloxacin	0·008	0·25	0·25–1
Gemifloxacin	0·004	0·5	0·25–1
Levofloxacin	0·015	0·5	0·25–1
Moxifloxacin	0·03	0·5	0·5–1
Nalidixic acid	4	16	8–32
Premafloxacin	0·03	0·25	0·25–0·5
Sitafloxacin	0·008	0·125	0·06–0·25
Sparfloxacin	0·008	1	0·25–1

MIC_{90}=minimum concentration of an agent required to inhibit the growth of 90% of organisms tested.

Table 1: **In-vitro activity of quinolones against wild-type *E coli* J53 and *E coli* J53 carrying 17 clinically derived *qnrA* plasmids[27]**

concentration of quinolone required to prevent the growth of quinolone-resistant mutants from a starting inoculum of about 10^{10} bacteria (a large inoculum is used to ensure the detection of mutants present in very small numbers). So long as the quinolone concentration remains above the MPC, resistant mutants should not arise. The early finding that QnrA facilitated recovery of mutants with higher levels of quinolone resistance prompted an assessment of its effect on the MPC of ciprofloxacin. The MPC for wild-type *E coli* J53 is 0·125 μg/mL; *E coli* J53 carrying a *qnrA* plasmid has an MPC more than ten-fold greater (figure 2).[29] Thus, as with chromosomal quinolone resistance mutations, although low-level resistance conferred by these mechanisms might not allow a population of bacteria to survive in the presence of a quinolone, it substantially enhances the number of resistant mutants that can be selected from the population. In the case of QnrA, this phenomenon has been experimentally shown both for *E coli*[15] and for *Enterobacter* spp,[30] and probably holds true with other genera as well. Indeed, a pharmacodynamic model has recently shown that *Providencia stuartii* with *qnrA* (but not without it) is insufficiently killed by a large single ciprofloxacin dose, and rapidly acquires resistance.[31]

Another means by which QnrA has been shown to contribute to clinically important levels of resistance is by acting additively with other resistance mechanisms present in a cell. *qnrA* has frequently been observed in the company of other resistance mechanisms in clinical strains. The interaction of chromosomal resistance with QnrA has been assessed. pMG252, the plasmid on which *qnrA* was originally identified, was introduced into *E coli* strains containing a variety of chromosomal mutations that enhanced or diminished resistance through alterations in DNA gyrase, topoisomerase IV, efflux, or outer membrane porin channels.[32] The presence of *qnrA* was found to supplement both types of mutation-based resistance. Whether particular chromosomal mutations are favoured by the presence of a Qnr protein is not yet known.

Epidemiology of *qnrA*

After the initial discovery of *qnrA* in a *K pneumoniae* isolate obtained in 1994 from the urine of a patient in Alabama, USA, efforts were made to find this gene elsewhere. A survey for *qnrA* by PCR of more than 350 Gram-negative isolates collected mainly in the 1990s and chosen to include a broad geographic range and a variety of genera of Gram-negative bacteria found *qnrA* in only six isolates (four *E coli* and two *Klebsiella* spp), all from the same centre in Alabama where the original strain had been detected, and all collected between July and December, 2004.[20] All six isolates transferred nalidixic acid resistance together with a gene encoding FOX-5 β-lactamase, which was also present on pMG252. Strikingly, while FOX-5 β-lactamase-carrying isolates were still present in surveys of 1995 and 2001 organisms from the same centre, *qnrA* was no longer found.[20]

Since this early study, about 20 more epidemiological surveys have been reported (figure 3 and table 2). Most

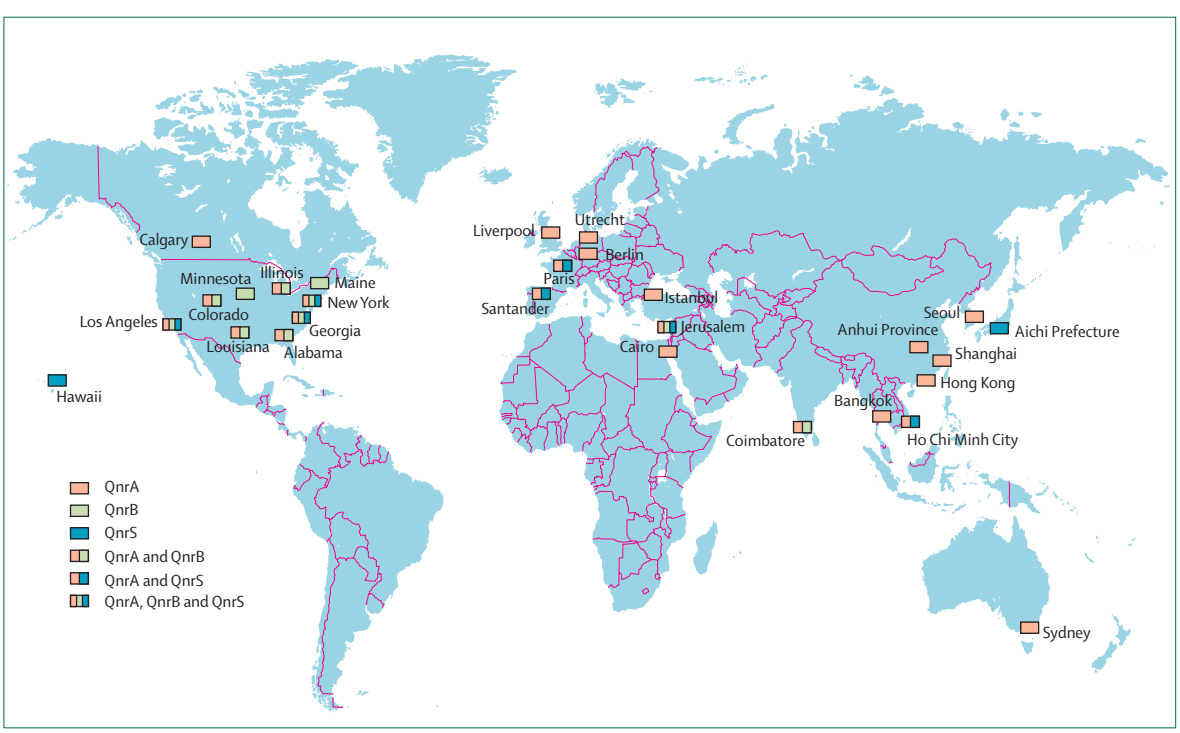

Figure 3: **Global distribution of Qnr**
Geographical names are representative of the region and may not be the site of origin of the strains.

have used PCR methodologies to examine clinical Enterobacteriaceae collected in the late 1990s or early 2000s for *qnrA*. Although the gene seems to be uncommon in general populations of Gram-negative isolates, prevalence in certain organisms carrying extended spectrum β-lactamases has exceeded 20%. In these studies, QnrA was found in all populated continents except South America, and in most clinically common Enterobacteriaceae. These species include *E coli*, *Klebsiella* spp (*K pneumoniae* and *Klebsiella oxytoca*), *Enterobacter* spp (*Enterobacter cloacae*, *Enterobacter amnigenus*, and *Enterobacter sakazakii*), *Citrobacter freundii*, and

Reference	Target of screen	Year of isolate collection	Geographic area	Bacterial type	Findings
Martinez-Martinez et al[15]	..	1994	Alabama, USA	*K pneumoniae*	First identification of *qnrA*
Jacoby et al[20]	*qnrA*	Primarily 1990s	19 countries, three continents	Primarily *K pneumoniae* and *E coli* but several other genera represented	Several *E coli* and *K pneumoniae* were positive, all from Alabama in 1994; The rest of about 350 isolates were negative
Rodriquez-Martinez et al[33]	*qnrA*	1990s?	USA, Spain, and unlisted countries	*E coli* (266) and *K pneumoniae* (159)	None of the *E coli*, three (2%) of *K pneumoniae* were positive (collected 1995–1997). Two of these three were fluoroquinolone-sensitive by CLSI breakpoints
Wang et al[26]	*qnrA*	2000–2001	Shanghai, China	*E coli* (78), all ciprofloxacin-resistant	Six (8%) positive. All from same hospital. Several different plasmids carried *qnrA*
Wang et al[34]	*qnrA*	1999–2002	USA, nationwide	72 *K pneumoniae* and 38 *E coli*, all with MIC of ciprofloxacin ≥2 μg/mL and MIC of ceftazidime ≥16 μg/mL	None of *E coli* and eight (11%) of *K pneumoniae* were positive
Mammeri et al;[35] Nordmann & Poirel[36]	*qnrA*	2003	Paris, France	449 nalidixic-acid resistant Enterobacteriaceae	Two (0·5%) were positive; one *E coli* and one *E cloacae*
Jonas et al[37]	*qnrA*	2000–2003	Germany, nationwide	136 integron-containing Enterobacteriaceae isolates from 34 German intensive care units	One *Enterobacter* sp isolate and an outbreak strain of *Citrobacter freundii* were positive
Paauw et al[38]	*qnrA*	2001–2003	Utrecht, Netherlands	83 epidemic strains of *Enterobacter cloacae*	78 (94%) strains carried *qnrA*. Additionally, 31% of co-colonising Gram-negative bacteria in patients colonised with epidemic strains also carried *qnrA*
Nazic et al[39]	*qnrA*	2002–2004	Istanbul, Turkey	49 ESBL-carrying Enterobacteriaceae	Two (4%) isolates were positive
Wiegand et al[40]	*qnrA*	2001	Cairo, Egypt	30 Enterobacteriaceae with MIC of ciprofloxacin ≥0·25 μg/mL from a burn unit	Three unrelated *Providencia stuartii* strains carried *qnrA*
Corkill et al[41]	*qnrA*	2003–2005	Liverpool, UK	47 ciprofloxacin- and cefotaxime-resistant Enterobacteriaceae from blood cultures	15 (32%) carried *qnrA*. 12 were clonally distinct. Multiple plasmids were involved
Poirel et al[42]	*qnrA*	1999	Bangkok, Thailand	23 Enterobacteriaceae carrying the bla_{VEB-1} β-lactamase	11 (48%) isolates were positive
Joeng et al;[43] Jun et al[44]	*qnrA*	2001–2003	Seoul, Korea	*E coli* (260) *E cloacae* (206)	Two (0·7%) *E coli* were positive; 11 (5%) *E cloacae* were positive
Cheung et al[45]	*qnrA*	2003	Hong Kong, China	*Salmonella enterica* serotype Enteritidis, outbreak strains	All four strains tested carried *qnrA*; four different plasmids
Poirel et al[46]	*qnrA*	2004	Sydney, Australia	23 ESBL-positive or fluoroquinolone resistant Enterobacteriaceae	Two (10%) isolates were positive
Poirel et al[47,48]	*qnrA*, *qnrS*	2004–2005; 2000–2002	Calgary, Canada	139 ESBL-negative ciprofloxacin-resistant Enterobacteriaceae; 101 ESBL-positive Enterobacteriaceae	Two (2%) of the 101 ESBL-positive Enterobacteriaceae carried *qnrA*, none carried *qnrS* (same group also reported one of 110 ESBL *E coli* from Calgary carried *qnrA*; however, apparently that data set overlaps somewhat)
Cano et al[49]	*qnrA*	2004–2005	Santander, Spain	100 nalidixic acid-resistant Enterobacteriaceae, 100 multiresistant *E coli* and 173 ESBL-positive Enterobacteriaceae	Two ESBL-positive isolates (*C freundii* and *E cloacae*) from a single patient carried *qnrA*
Schultsz et al[50]	*qnrA*, *qnrS*	2004	Ho Chi Minh City, Vietnam	28 gentamicin-resistant *K pneumoniae* 32 ESBL-positive *E coli* or *Citrobacter* spp	24 (86%) of 28 *K pneumoniae* carried *qnrA* or *qnrS*. Eight (25%) of 32 ESBL-positive isolates carried *qnrA* or *qnrS*
Poirel et al[51]	*qnrA*, *qnrS*	2002–2005	Paris, France	185 nalidixic acid-resistant, ESBL-negative Enterobacteriaceae; 187 ESBL-positive Enterobacteriaceae	Among the ESBL-negative isolates, one (0·5%) carried *qnrA* and five (3%) carried *qnrS*. Among the ESBL-positive isolates, three (2%) carried *qnrA* and 3 (2%) carried *qnrS*
Cano et al[52]	*qnrA*. *qnrS*	2004–2005	Northern and southern Spain	202 *Enterobacter* spp	None carried *qnrA*; 22 (11%) carried *qnrS*. All *qnrS*-positive strains were from northern Spain
Robicsek et al[54]	*qnrA*, *qnrB*, *qnrS*	1999–2004	USA, nationwide	313 isolates of *E coli*, *K pneumoniae*, and *Enterobacter* spp, all with MIC of ciprofloxacin ≥0·25 μg/mL and MIC of ceftazidime ≥16 μg/mL	Either *qnrA* or *qnrB* was present in two (4%) of 47 *E coli*, 21 (20%) of 106 *K pneumoniae*, and 50 (31%) of 160 *Enterobacter* spp. None carried *qnrS*
Gay et al[53]	*qnrA*, *qnrB*, *qnrS*	1996–2003	USA, nationwide	335 non-Typhi salmonella isolates. 233 with ciprofloxacin MIC ≥0·06 μg/mL, 102 with MIC ≤0·03 μg/mL	Ten carried either *qnrB* or *qnrS*; all these had ciprofloxacin MIC ≥0·06 μg/mL. None carried *qnrA*

CLSI=Clinical and Laboratory Standards Institute; ESBL=extended spectrum β-lactamase

Table 2: Epidemiology of *qnr*

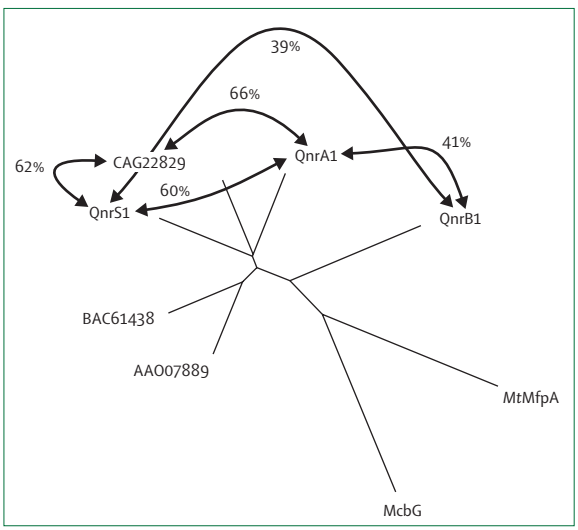

Figure 4: **The aminoacid relation of pentapeptide repeat proteins known to affect DNA gyrase**
QnrA and QnrS seem to share more common ancestry with each other and with CAG22829, a chromosomal protein from *Photobacterim profundum*, a water-dwelling environmental organism, than with QnrB. BAC61438 is found in *Vibrio parahaemolyticus*, AAO07889 is found in *Vibrio vulnificus*, MtMfpA is found in *Mycobacterium tuberculosis*, and McbG is found in Enterobacteriaceae. QnrA and chromomomally encoded proteins from *Shewanella* spp are more than 98% related.[58] Unrooted dendrogram was generated using ClustalW (http://align.genome.jp/).

Providencia stuartii. Notably absent from this list are *Proteus* spp and clinically important non-enteric Gram-negative bacteria (eg, *Pseudomonas aeruginosa* and *Acinetobacter* spp). All three have been included in small surveys,[20,42,55,56] but whether the lack of detection reflects a true absence or the small number of strains tested is not clear.

In addition to surveys of baseline *qnr* epidemiology, one report from the Netherlands details a very large hospital outbreak (>80 patients) of an *E cloacae* strain. Isolates of this clonal strain from most patients carried *qnrA*. To assess the promiscuity of the *qnrA* plasmid in this clone, the investigators examined additional Gram-negative bacteria co-colonising patients who carried the outbreak strain. Remarkably, 31% of these other organisms also carried *qnrA*.[38]

Newly identified *qnr* genes

Until recently, the sequence of *qnrA* was believed to be highly conserved. Initial reports of *qnrA* from the USA, Europe, and China reported sequences that varied in a single silent polymorphism (CTA→CTG at position 537).[16,26,30,34,35] Subsequently, a *K oxytoca* isolate from Anhui Province, China (where the rate of ciprofloxacin resistance in *E coli* is 70%[57]) was reported to carry a variant of *qnrA* differing in four codons from the originally detected gene. This variant was designated *qnrA2*,[36] and the original *qnrA* was retrospectively renamed *qnrA1*. While searching for a chromosomal analogue of *qnrA* in the genome sequences of environmental organisms, a French group identified three additional variants (*qnrA3*, *qnrA4*, and *qnrA5*) of this gene in *Shewanella algae*, varying in two to four codons from *qnrA1*.[58] At about the same time, *qnrA3* was also detected in clinical salmonella isolates.[45] Subsequently, another *qnrA* variant has been deposited in GenBank (DQ151889); we are designating it *qnrA6*.

In October, 2003, a single clone of *Shigella flexneri* 2b caused a foodborne outbreak in Aichi Prefecture, Japan. One of eight strains of this clone was resistant to ciprofloxacin, unlike the rest. This strain was found to harbour a unique conjugative plasmid that transferred quinolone resistance at a level much the same as that conferred by *qnrA*. Cloning identified an open reading frame encoding a 218 aminoacid protein of the pentapeptide-repeat family. This protein shares 59% aminoacid identity with QnrA, and was named QnrS.[59] A variant of QnrS has now been identified in a US isolate of *Salmonella anatum*. This variant shares 91% aminoacid identity with QnrS (now QnrS1), and has been designated QnrS2.[53]

Another *qnr* gene has been recently described. While studying the properties of β-lactamase-carrying plasmids from clinical *K pneumoniae* isolates obtained from Coimbatore, India, several of these plasmids were noted to transfer quinolone resistance.[24] The gene responsible for this property was found to encode a 226 aminoacid protein belonging to the pentapeptide repeat family. The encoded protein shared 40% and 37% aminoacid identity with QnrA1 and QnrS1, respectively. The effect of this gene, designated *qnrB1*, on quinolone activity was found to be comparable to that of the other known *qnr* genes, and purified QnrB-His_6 protein was found to protect DNA gyrase, much like QnrA, although with greater in-vitro potency.[24] A variant of *qnrB* (*qnrB2*) also found in the course of this work encodes a 215 aminoacid protein differing from QnrB1 by five aminoacid residues. Other studies have now uncovered *qnrB3* through *qnrB5* (GenBank AJ971344).[54] The diversity of Qnr genes adds credence to the notion that overall structure is the key to their function; if catalytic activity were important we might expect more conservation of aminoacid sequences.

Several surveys for *qnrB* and *qnrS* have been reported. The first description of *qnrB* included a survey of a convenience sample of plasmids from more than 100 Enterobacteriaceae. Eight plasmids (including from *Citrobacter koseri*, *K pneumoniae*, *E coli*, and *E cloacae*) carried *qnrB1* or *qnrB2*.[24] More recently, our group has used a multiplex PCR method to screen simultaneously for all three *qnr* genes in clinically important bacterial populations. The TRUST study maintains a database of non-duplicate clinical isolates from clinical laboratories in all nine census regions of the continental USA. Using multiplex PCR, we surveyed 313 isolates of *E coli*, *K pneumoniae* and *Enterobacter* spp collected from 1999 to 2004 that showed reduced susceptibility to ceftazidime

(a marker for broad-spectrum β-lactamases) and low-level resistance to ciprofloxacin. The prevalence of any *qnr* gene was two of 47 (4%) in *E coli*, 21 of 106 (20%) in *K pneumoniae*, and 50 of 160 (31%) in *Enterobacter* spp. *qnrA* and *qnrB* were present in similar proportions; *qnrS* was not found. Over the period of the survey, the only change in prevalence was an increase in *qnrB* among *Enterobacter* spp; otherwise *qnr* prevalence was stable.[54] This survey also identified two new variants of *qnr*B, designated *qnrB3* and *qnrB4*. The other multiplex survey looked at 107 non-typhi salmonella isolates collected from clinical laboratories around the USA from 1996 to 2003 by a CDC-affiliated health agency. *qnrS1* was detected in *Salmonella bovismorbificans*, *qnrS2* was carried by a strain of *S anatum*, *qnrB2* was detected in one *Salmonella mbandaka* isolate, and seven *Salmonella berta* isolates were found to carry a new variant, *qnrB5* (figure 4). Another group reporting on the epidemiology of multiple *qnr* genes found either *qnrA* or *qnrS* in about 25% of β-lactam-resistant *E coli* and 85% of gentamicin-resistant *K pneumoniae* in a Vietnamese intensive care unit.[50] Recent European surveys have suggested that *qnrS* could be more prevalent than *qnrA* in clinical Enterobacteriaceae.[51,52]

Qnr plasmids

Plasmids carrying *qnr* genes vary widely in size and associated resistances but almost all carry multiple resistance determinants. Genes for *qnrA* and sometimes *qnrB* are found as part of complex *sul1*-type integrons containing a presumed recombinase, Orf513 (figure 5). Typically, resistance genes within an integron are associated with 59-base element recombination sites and are situated immediately 3′ to an integrase.[61] The absence of these features for *qnrA* suggests that the mechanism through which this gene was mobilised and integrated into a plasmid is unusual. The conservation of this atypical arrangement in plasmids from strains isolated over almost 10 years in widely separated parts of the world suggests dissemination from a common source with subsequent modification locally. The *qnrB* gene is also associated with Orf513 as well as with another presumed recombinase, Orf1005, and with non-resistance genes resembling those found on the chromosome of marine bacteria.[24] In the few *qnrS* plasmids sequenced to date, *qnrS* was not part of an integron, but in one plasmid it was bracketed by inverted repeats with insertion sequence-like structure that could have been responsible for its mobilisation.[53]

The linkage between resistance to the latest cephalosporin antibiotics and to quinolones has been noted by several investigators.[62,63] One mechanism for this association is incorporation on the same plasmid of *qnr* and genes for an extended-spectrum or AmpC-type β-lactamase. *qnrA* has been found with a gene for β-lactamases CTX-M-9,[34] CTX-M-14,[45] FOX-5,[15,34] SHV-5,[39] SHV-7,[34] SHV-12,[41] or VEB-1,[35,39,42] whereas *qnrB* has been located on plasmids carrying genes for CTX-M-15,[24] SHV-12,[24] or SHV-30.[53] Although the *qnr* and β-lactamase genes have generally been found on different integrons, suggesting that they are acquired independently, the coexistence of *qnrA* and the gene encoding β-lactamase VEB-1 on a single integron has recently been reported.[47]

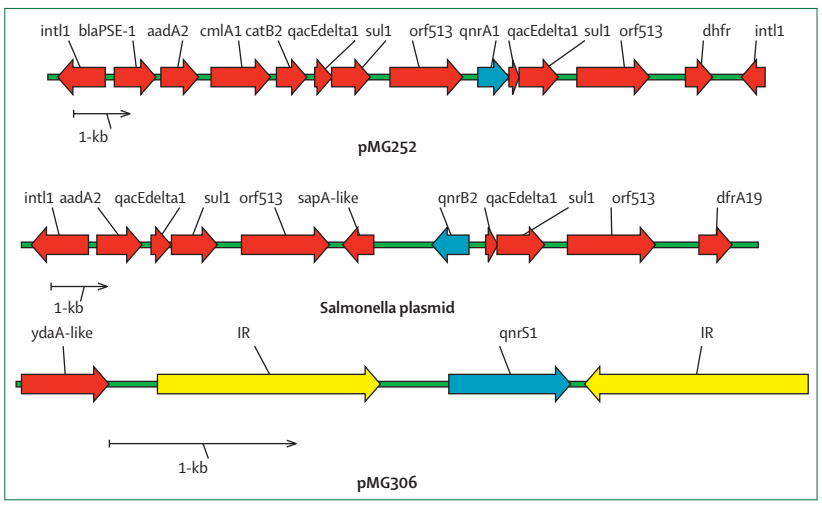

Figure 5: **Genetic environment of *qnr* genes**
sul1-type integron from the original QnrA1 plasmid pMG252 (GAJ, unpublished data), sul1-type integron from a QnrB2 plasmid found in *Salmonella enterica* serotype Keurmassar (Garnier et al[60]], GenBank AM234698), and portion of QnrS1 plasmid pMG306 found in *Salmonella enterica* serotype Bovismorbificans.[53] Gene abbreviations: *aadA2*=aminoglycoside 3″-adenylyltransferase; blaPSE-1=PSE-1 β-lactamase; *catB2*=chloramphenicol acetyltransferase; *cmlA1*=non-enzymatic chloramphenicol resistance protein; *dfrA* or *dhfr*=dihydrofolate reductase; *intI1*=integrase; IR=inverted repeat; *Orf*=open reading frame, generally coding for a protein of unknown function; *Orf513*=a putative recombinase; *qacEΔ1*=quaternary ammonium compound resistance protein; *qnr*=quinolone resistance protein; *sapA*=peptide transport system permease component; *sul1*=dihydropteroate synthase; *ydaA*=a putative resolvase. The scale for the different plasmids varies and is indicated by the 1-kb arrow.

Origins of *qnr* genes

The recent profusion of *qnr* variants, along with the evidently extensive penetration of these genes into populations of Enterobacteriaceae worldwide strongly suggests that these genes have considerably predated our knowledge of them. This finding raises the interesting questions of where these genes came from, and what they were doing there before clinical use of quinolones selected for their dissemination. Provisional answers to the first question are provided by a number of recent reports. Postulating that *qnr* genes originated on the chromosome of an organism occupying a human, veterinary, or environmental reservoir, Poirel and colleagues[58] screened the genome sequences of 48 Gram-negative species from a wide range of genera for *qnrA*. Four variants of *qnrA* (*qnrA2–qnrA5*) were found in three strains of *S algae*. The quinolone MIC levels of this organism were four-fold to eight-fold higher than those of *Shewanella putrefaciens*, a closely related organism lacking a chromosomal *qnrA* gene. Importantly, these genes seem to have a chromosomal location in *Shewanella* spp.[58] These data suggest *S algae* as a reservoir of *qnrA*. *Shewanella* spp are water-dwellers, present in both marine and freshwater environments. Subsequent work

noted that pentapeptide-repeat proteins showing 40–67% aminoacid identity to *qnr* genes were present in other water-borne species, including *Vibrio vulnificus*, *Vibrio parahaemolyticus*, and *Photobacterim profundum*. Recombinant plasmids expressing these proteins increased the MIC of ciprofloxacin for transformed *E coli* from 0·003 µg/mL to up to 0·25 µg/mL.[64] Another group showed that the *V parahaemolyticus* Qnr analogue exhibited higher levels of quinolone resistance when it had acquired a single aminoacid change.[65] Collectively, these findings suggest that the *qnr* genes in circulation could have originated in the chromosomes of water-dwelling or other environmental organisms. In the face of intense quinolone pressure, such genes have entered circulation on mobile genetic elements.

As for what these genes had been doing before being recruited by antibiotic-pressured bacteria, little is known. One of the functions of these genes could possibly have been to protect against naturally occurring DNA gyrase inhibitors.[66] Several such proteins are known—eg, microcin B17, CcdB,[67] and ParE[68] (located on the broad host-range RK2 plasmid, confusingly sharing a name with a topoisomerase IV subunit). This last protein has a role in postsegregational killing, a mechanism through which a plasmid kills daughter cells that have not received a copy of the plasmid during cell division. Like proteins of the Qnr group, McbG and MfpA both protect DNA gyrase against inhibitors, as does GyrI, an 18-kDa protein (not in the pentapeptide-repeat family) encoded on the chromosome of *E coli* that has been shown to protect against microcin B17, CcdB,[69] and quinolones.[70] Interestingly, both GyrI and MfpA are known to inhibit the supercoiling activity of DNA gyrase. The benefits of these genes, realised in the form of protection against gyrase inhibitors and perhaps other functions yet to be learned, could warrant their costs. If genes of this family do indeed exact a fitness cost, it might be expected that they would be maintained in a population only if selective pressures are strong.

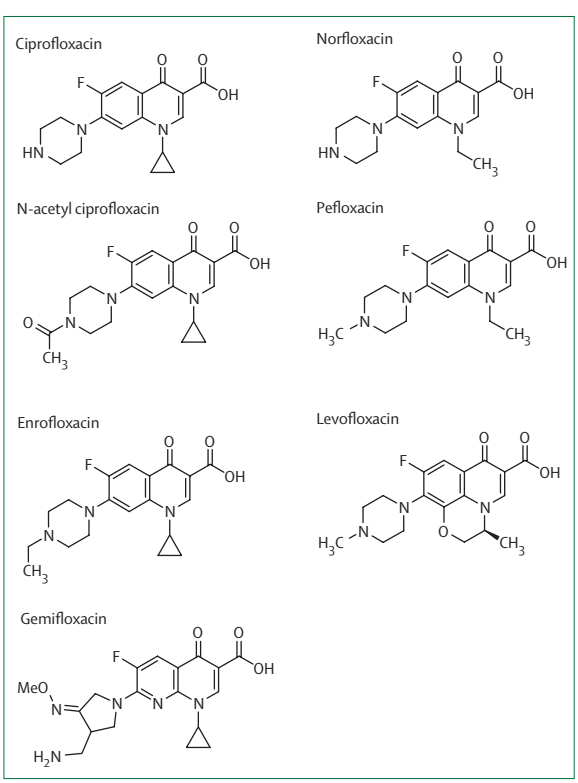

Figure 6: **Chemical structures of fluoroquinolones**

One of the remarkable aspects of the Qnr story is its modernity. 36 years elapsed between the introduction of nalidixic acid into use and the first validated report of PMQR. Although thorough surveys of historic strains for *qnr* genes have not yet been reported in human pathogens, these genes could possibly have not been found earlier simply because they did not exist in the early days of quinolone use. Intense quinolone pressure in clinical settings,[3,71,72] or the accumulation of these agents in the environment and veterinary reservoirs, could have driven these genes into circulation only more recently, once the benefits of these genes outweighed their costs. In addition to the demonstrated inhibition of DNA gyrase by a number of pentapeptide-repeat proteins (including QnrB at high concentrations[24]), several observations support the notion that these genes are not easily maintained. A striking finding in the first survey for QnrA was its disappearance from an Alabama hospital between 1994 and 1995 despite the fact that the FOX-5 β-lactamase also present on the original *qnr* plasmid could still be found in 1995 and again in 2001. Indeed, quinolone resistance has been unstable on experimental transfer. In one study, 12% of pMG252 transconjugants lost resistance to nalidixic acid.[20] In the survey of all three *qnr* genes in ceftazidime-resistant Enterobacteriaceae, *qnr* genes were substantially more likely to be present when the organisms were collected from inpatients,[54] perhaps suggesting that ongoing selective pressure helps to keep these genes on their plasmids. In the same survey, although 73 (23%) of 313 strains carried a single class of *qnr* gene, none carried two. Studies are necessary to determine whether plasmid-borne *qnr* genes inhibit the growth of the bacteria that host them. Whatever the costs they impose, the kaleidoscopic variety displayed by the plasmid-borne *qnr* genes suggests that conditions must have favoured their entry into pathogenic bacteria on many occasions.

AAC(6′)-Ib-cr, another PMQR protein

Shortly after the discovery of QnrA, it was observed that not all *qnr*-bearing plasmids transferred the same level of quinolone resistance. Wild-type *E coli* have an MIC of ciprofloxacin of about 0·008 µg/mL. Most *qnr* plasmids determine an MIC of ciprofloxacin of 0·25 µg/mL in *E coli*. We saw, however, that certain plasmids from clinical *E coli* collected in Shanghai provided about four-fold higher levels of ciprofloxacin resistance (1·0 µg/mL).

Strain	Ciprofloxacin	*N*-acetyl ciprofloxacin	Enrofloxacin	Norfloxacin	Pefloxacin	Levofloxacin	Gemifloxacin
Wild-type *E coli*	0·02	0·08	0·02	0·156	0·08	0·08	0·005
E coli expressing AAC(6′)-Ib-cr	0·04–0·08	0·08	0·02	0·625	0·08	0·08	0·005

Units are µmol/L. Note that this enzyme only reduces the activity of fluoroquinolones with an unbound amino nitrogen on the piperazine ring (ie, ciprofloxacin and norfloxacin). In the presence of AAC(6′)-Ib-cr, the MIC of ciprofloxacin rises to that of synthetically *N*-acetylated ciprofloxacin.

Table 3: Effect of AAC(6′)-Ib-cr on fluoroquinolone MIC[74]

Although apparent differences in levels of expression of *qnrA* in transconjugants have been associated recently with different levels of resistance,[73] we found that this high-level resistance was not owing to increased expression of *qnrA*. We then used random transposon insertion to knock out the plasmid gene responsible for the added resistance.[74] Remarkably the gene proved to be an aminoglycoside acetyltransferase, *aac(6′)-Ib*, which confers resistance to tobramycin, amikacin, and kanamycin. Sequencing showed this allele to be unique among the approximately 30 variants of *aac(6′)-Ib* described since 1986 in (among other polymorphisms) two codon changes that we found to be necessary and sufficient for the ciprofloxacin resistance phenotype. An acetylation assay showed the capacity of this AAC(6′)-Ib variant (which we designated AAC(6′)-Ib-cr, for ciprofloxacin resistance) to *N*-acetylate ciprofloxacin at the amino nitrogen on its piperazinyl substituent.[74] As predicted by its chemical structure, norfloxacin was similarly susceptible. Other quinolones lacking an unsubstituted piperazinyl nitrogen were unaffected (figure 6 and table 3). Although the increase in MIC of ciprofloxacin and norfloxacin was modest (three-fold to four-fold), the effect on MPC was marked (figure 2). In the presence of *aac(6′)-Ib-cr*, resistant clones of wild-type J53 *E coli* could still be recovered at 1·6 µg/mL, a level approximating the peak serum concentration of free ciprofloxacin during therapy.[74]

Little is known as yet about the epidemiology of this gene variant. Although only first reported in 2004,[26,75] the gene has been detected in Asia and in widely separated regions of North America. Our group surveyed the 78 Chinese *E coli* from one of which *aac(6′)-Ib-cr* had been isolated (and among which we had previously reported an 8% prevalence of *qnrA*[26]). Nine (11%) of the 78 strains carried non-cr *aac(6′)-Ib*, and 40 (51%) harboured the cr-variant aminoglycoside acetyltransferase.[74] Thus, in some strain sets *aac(6′)-Ib-cr* is highly prevalent (more so than *qnrA*) and circulates both together with, and independently of, *qnrA*. Our group has subsequently identified this variant among additional North American Enterobacteriaceae.[76] Among 313 isolates with MICs of ciprofloxacin of 0·25 µg/mL or more and reduced susceptibility to ceftazidime, 50% carried *aac(6′)-Ib* and of these 28% carried the cr variant. *aac(6′)-Ib-cr* was widespread geographically and stable over time.

Clinical importance of PMQR

The discovery of PMQR could help explain how numerous independent clones of initially exquisitely susceptible wild-type Enterobacteriaceae have managed to develop high-level quinolone resistance. In the face of clinical concentrations of quinolone, organisms with even a single resistance mutation can survive—and give rise to increasingly resistant mutants. In our recent survey of 313 ceftazidime-resistant US Enterobacteriaceae, *qnr*-bearing strains were as likely to be susceptible by the criteria of the Clinical and Laboratory Standards Institute (CLSI) as not susceptible.[54] These susceptible organisms—carrying a *qnr* gene but lacking sufficient chromosomal mutations to qualify as resistant—are clinically worrisome. Such organisms, in vitro, readily develop resistance. CLSI breakpoints were developed based on data that most non-resistant Enterobacteriaceae strains had very low MICs of quinolones. If *qnr*-bearing plasmids widely infiltrate a population of pathogens, the practice of exposing such organisms to quinolones could fuel the rapid development of resistance. This phenomenon has already been documented in a clinical setting; a sensitive strain of *E coli* harbouring *qnrA* but no classic quinolone resistance mutations was found to develop chromosomal mutations and subsequent high-level resistance after 5 days of norfloxacin therapy.[47] More work is needed to define further the proportion of clinical Enterobacteriaceae harbouring such low-level resistance and the effect of these genes on clinical outcomes. Currently, whether it is safe to use fluoroquinolone to treat *qnr*-bearing organisms with fluoroquinolone MICs lower than currently accepted breakpoints is not known; this is a key issue to be resolved by further investigations.

Interestingly, screening by MIC might not be sufficient. *aac(6′)-Ib-cr* alone confers a degree of resistance sufficiently low that MIC might not distinguish organisms that carry it from those that do not, and *qnr* genes, although capable of transferring resistance, might not always cause a change in quinolone MIC when present. For instance, Poirel and colleagues[42] found that for several clinical Enterobacteriaceae from Thailand that carried *qnrA*, quinolone resistance could be transferred to transconjugants but was present at very low levels or not at all in the donors. Although the explanation for this observation is not yet clear, one possibility is suggested

by the finding that a DNA gyrase mutation known to increase susceptibility to ciprofloxacin was present in nine of ten *qnr*-positive *Salmonellae* spp in our recent survey.[53] Perhaps gyrase mutations acquired to protect against the inhibitory effects of Qnr increase quinolone susceptibility, thereby masking the presence of the resistance gene. A greater understanding of the best way to screen for these genes and address them at the bedside is needed.

This concern extends to non-typhi salmonella which, in cases of invasive disease, are often treated with fluoroquinolones. Currently, the nalidixic acid breakpoint is used to determine fluoroquinolone susceptibility because the ciprofloxacin breakpoint (4 µg/mL) is not sensitive enough to detect single resistance mutations capable of causing clinical failure.[77] A concerning finding is that the current nalidixic acid breakpoint (32 µg/mL) serves poorly as a screen for *qnr* genes, which tend to affect this quinolone relatively little. Among our ten *qnr*-bearing salmonella isolates, two had an MIC of nalidixic acid of 16 µg/mL.[53] A better picture of the epidemiology of these genes in salmonella is much needed.

A long-standing question about the occurrence of quinolone resistance has been its close association with resistance to other agents, particularly to β-lactamases[61,62] and aminoglycosides.[3] Both types of PMQR might have a role in this phenomenon. There seems to be a strong association between broad-spectrum β-lactamases and *qnr* genes; indeed most *qnr*-bearing plasmids for which sequencing is available carry such a β-lactamase gene. A survey of Thai organisms detected *qnrA* in 11 (48%) of 23 organisms carrying a bla_{VEB-1}, but in none of 22 that did not.[42] Likewise, in our survey of US Enterobacteriaceae, *qnr* genes were very strongly associated with aminoglycoside resistance.[54] The existence of at least one aminoglycoside resistance gene that itself facilitates the emergence of high-level quinolone resistance could also add to the association of these two types of resistance. Unfortunately, the biological linking of these types of resistance creates a situation ripe for the dissemination of multidrug-resistant Enterobacteriaceae, a class of organisms against which the number of effective agents is limited. Clinicians must realise that when they prescribe a quinolone, they could be selecting not only for quinolone resistance, but for resistance to cephalosporins, aminoglycosides, and any other forms of resistance borne on plasmids carrying PMQR genes. The discovery of PMQR adds further substance to concerns about the injudicious use of the fluoroquinolones.

Not all fluoroquinolones are created equal with respect to PMQR. This distinction is particularly true for *aac(6')-Ib-cr*, which acts only against fluoroquinolones with an unprotected amino nitrogen on the piperazine ring (table 3). Ciprofloxacin, one such agent, may be the most widely prescribed fluoroquinolone in the world, especially since it is now available in a generic formulation in the USA. Although an infection caused by a Gram-negative bacterium lacking this resistance gene might be equally well treated with ciprofloxacin as with another fluoroquinolone, an infection caused by an organism carrying this gene may not. Again, more work is needed to clarify the effect of this gene on clinical outcomes and resistance development, but it would seem that ciprofloxacin will emerge as a suboptimal fluoroquinolone where *aac(6')-Ib-cr* prevalence is high.

Search strategy and selection criteria

An English language literature search without time restrictions was done using the PubMed database for studies examining PMQR. The keywords used were "qnr", "plasmid", "quinolone", "fluoroquinolone", "resistance", "pentapeptide repeat", and "extended spectrum beta lactamase". Reference lists of related articles were searched for relevant studies, as were the abstracts of recent conferences.

Conclusions

Faced with the challenge of potent fluoroquinolones, bacteria have not devised a high-level defense mechanism, such as the *mecA* gene that protects staphylococci from β-lactam antibiotics. Instead, Enterobacteriaceae have improvised multiple mechanisms for low-level resistance, assembling them together to chisel away at quinolone effectiveness. Chromosomal mutations accrue, progressively barring quinolone entry, diminishing quinolone accumulation in the cytoplasm, and discouraging quinolone binding to its target. Plasmid-mediated mechanisms are also enlisted in this effort. *qnr* genes, apparently the objects of transnational bacterial exchange beneath our notice for some time, encode proteins that block the formation of a molecular complex vulnerable to quinolone activity. And an old gene, evolved to inactivate an ancient class of antimicrobials, has been refitted to undermine a modern agent. High-level resistance has emerged, not once, but scores of times, independently, wherever the quinolones have been used.

In this persistent way, Gram-negative pathogens have pulled together a quinolone resistance suite, genes that are not only additive in effect, but that facilitate the emergence of one another; a resistance plasmid allows low-level resistant mutants to survive, and this success will allow for the further promulgation of these plasmids when selective pressure demands. PMQR is not good news. Although their discovery at a time of burgeoning quinolone resistance could be coincidental, these genes pose a real threat. Cotransmission of PMQR with aminoglycoside-modifying enzymes, broad spectrum β-lactamases, and even carbapenemases[39] can accelerate the pace at which we drive dangerous multidrug resistance. At the same time, the spread of these plasmids into apparently susceptible organisms raises concerns about the appropriateness of current quinolone breakpoints.

Still, the news might not all be bad. We are aware of at least once instance in which QnrA disappeared from a population, and our survey of Gram-negative pathogens in the USA suggests that *qnr* prevalence might have been relatively stable in recent years. Perhaps we will learn that these genes, exacting a fitness cost, require strong selective pressure to be maintained. In such a case, rational antimicrobial stewardship in the clinic and on the farm might well be rewarded.

Conflicts of interest

DCH has received research grant support from Daiichi Pharmaceuticals and has been a consultant for Daiichi Pharmaceuticals, Ortho McNeil, and Oscient Pharmaceuticals. GAJ has received grant support from AstraZeneca. AR has no conflicts of interest to declare.

Acknowledgments

This work was supported in part by grants AI57576 (DCH) and AI43312 (GAJ) from the National Institutes of Health, US Public Health Service.

References

1 Ball P. Quinolone generations: natural history or natural selection? *J Antimicrob Chemother* 2000; **46** (suppl T1): 17–24.

2 Paton JH, Reeves DS. Fluoroquinolone antibiotics. Microbiology, pharmacokinetics and clinical use. *Drugs* 1988; **36:** 193–228.

3 Neuhauser MM, Weinstein RA, Rydman R, Danziger LH, Karam G, Quinn JP. Antibiotic resistance among gram-negative bacilli in US intensive care units: implications for fluoroquinolone use. *JAMA* 2003; **289:** 885–88.

4 Ling TK, Xiong J, Yu Y, Lee CC, Ye H, Hawkey PM. Multicenter antimicrobial susceptibility survey of gram-negative bacteria isolated from patients with community-acquired infections in the People's Republic of China. *Antimicrob Agents Chemother* 2006; **50:** 374–78.

5 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.

6 Hooper DC. Mechanisms of quinolone resistance. In: Hooper DC, Rubenstein E, eds. Quinolone antimicrobial agents. 3rd edn. Washington, DC: American Society for Microbiology Press, 2003: 41–67.

7 Froelich-Ammon SJ, Osheroff N. Topoisomerase poisons: harnessing the dark side of enzyme mechanism. *J Biol Chem* 1995; **270:** 21429–32.

8 Morais Cabral JH, Jackson AP, Smith CV, Shikotra N, Maxwell A, Liddington RC. Crystal structure of the breakage-reunion domain of DNA gyrase. *Nature* 1997; **388:** 903–06.

9 Poole K. Efflux-mediated antimicrobial resistance. *J Antimicrob Chemother* 2005; **56:** 20–51.

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

11 Gomez-Gomez JM, Blazquez J, Espinosa De Los Monteros LE, Baquero MR, Baquero F, Martinez JL. In vitro plasmid-encoded resistance to quinolones. *FEMS Microbiol Lett* 1997; **154:** 271–76.

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

13 Munshi MH, Sack DA, Haider K, Ahmed ZU, Rahaman MM, Morshed MG. Plasmid-mediated resistance to nalidixic acid in *Shigella dysenteriae* type 1. *Lancet* 1987; **2:** 419–21.

14 Rahman M, Mauff G, Levy J, et al. Detection of 4-quinolone resistance mutation in *gyrA* gene of *Shigella dysenteriae* type 1 by PCR. *Antimicrob Agents Chemother* 1994; **38:** 2488–91.

15 Martinez-Martinez L, Pascual A, Jacoby GA. Quinolone resistance from a transferable plasmid. *Lancet* 1998; **351:** 797–99.

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

17 Vetting MW, Hegde SS, Fajardo JE, et al. Pentapeptide repeat proteins. *Biochemistry* 2006; **45:** 1–10.

18 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.

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

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

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

22 Hegde SS, Vetting MW, Roderick SL, et al. A fluoroquinolone resistance protein from *Mycobacterium tuberculosis* that mimics DNA. *Science* 2005; **308:** 1480–83.

23 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.

24 Jacoby GA, Walsh KE, Mills DM, et al. *qnrB*, another plasmid-mediated gene for quinolone resistance. *Antimicrob Agents Chemother* 2006; **50:** 1178–82.

25 Tran JH, Jacoby GA, Hooper DC. Interaction of the plasmid-encoded quinolone resistance protein QnrA with *Escherichia coli* topoisomerase IV. *Antimicrob Agents Chemother* 2005; **49:** 3050–52.

26 Wang M, Tran JH, Jacoby GA, Zhang Y, Wang F, Hooper DC. Plasmid-mediated quinolone resistance in clinical isolates of *Escherichia coli* from Shanghai, China. *Antimicrob Agents Chemother* 2003; **47:** 2242–48.

27 Wang M, Sahm DF, Jacoby GA, Zhang Y, Hooper DC. Activities of newer quinolones against *Escherichia coli* and *Klebsiella pneumoniae* containing the plasmid-mediated quinolone resistance determinant *qnr*. *Antimicrob Agents Chemother* 2004; **48:** 1400–01.

28 Mammeri H, Poirel L, Nordmann P. Bactericidal activity of fluoroquinolones against plasmid-mediated QnrA-producing *Escherichia coli*. *Clin Microbiol Infect* 2005; **11:** 1048–49.

29 Jacoby GA. Mechanisms of resistance to quinolones. *Clin Infect Dis* 2005; **41** (suppl 2): S120–26.

30 Robicsek A, Sahm DF, Strahilevitz J, Jacoby GA, Hooper DC. Broader distribution of plasmid-mediated quinolone resistance in the United States. *Antimicrob Agents Chemother* 2005; **49:** 3001–03.

31 Wiegand I, Luhmer-Becker I, Wiedemann B. In vitro studies of oral ciprofloxacin (CIP) doses: influence of plasmid encoded fluoroquinolone resistance determinant *qnr*. 45th Interscience Conference on Antimicrobial Agents and Chemotherapy; Washington, DC, USA; Dec 16–19 2005. Abstract A-448.

32 Martinez-Martinez L, Pascual A, Garcia I, Tran J, Jacoby GA. Interaction of plasmid and host quinolone resistance. *J Antimicrob Chemother* 2003; **51:** 1037–39.

33 Rodriguez-Martinez JM, Pascual A, Garcia I, Martinez-Martinez L. Detection of the plasmid-mediated quinolone resistance determinant *qnr* among clinical isolates of *Klebsiella pneumoniae* producing AmpC-type beta-lactamase. *J Antimicrob Chemother* 2003; **52:** 703–06.

34 Wang M, Sahm DF, Jacoby GA, Hooper DC. Emerging plasmid-mediated quinolone resistance associated with the *qnr* gene in *Klebsiella pneumoniae* clinical isolates in the United States. *Antimicrob Agents Chemother* 2004; **48:** 1295–99.

35 Mammeri H, Van De LM, Poirel L, Martinez-Martinez L, Nordmann P. Emergence of plasmid-mediated quinolone resistance in *Escherichia coli* in Europe. *Antimicrob Agents Chemother* 2005; **49:** 71–76.

36 Nordmann P, Poirel L. Emergence of plasmid-mediated resistance to quinolones in Enterobacteriaceae. *J Antimicrob Chemother* 2005; **56:** 463–69.

37 Jonas D, Biehler K, Hartung D, Spitzmuller B, Daschner FD. Plasmid-mediated quinolone resistance in isolates obtained in German intensive care units. *Antimicrob Agents Chemother* 2005; **49:** 773–75.

38 Paauw A, Fluit AC, Verhoef J, Leverstein-van Hall MA. *Enterobacter cloacae* outbreak and emergence of quinolone resistance gene in Dutch hospital. *Emerg Infect Dis* 2006; **12:** 807–12.

39 Nazic H, Poirel L, Nordmann P. Further identification of plasmid-mediated quinolone resistance determinant in Enterobacteriaceae in Turkey. *Antimicrob Agents Chemother* 2005; **49:** 2146–47.

40 Wiegand I, Khalaf N, Al-Agamy MHM, Wiedemann B. First detection of the transferable quinolone resistance in clinical *Providencia stuartii* strains in Egypt. *Clin Microbiol Infect* 2004; **10** (suppl 3): 64.

41 Corkill JE, Anson JJ, Hart CA. High prevalence of the plasmid-mediated quinolone resistance determinant *qnrA* in multidrug-resistant Enterobacteriaceae from blood cultures in Liverpool, UK. *J Antimicrob Chemother* 2005; **56:** 1115–17.

42 Poirel L, Van De LM, Mammeri H, Nordmann P. Association of plasmid-mediated quinolone resistance with extended-spectrum beta-lactamase VEB-1. *Antimicrob Agents Chemother* 2005; **49:** 3091–94.

43 Jeong JY, Yoon HJ, Kim ES, et al. Detection of *qnr* in clinical isolates of *Escherichia coli* from Korea. *Antimicrob Agents Chemother* 2005; **49:** 2522–24.

44 Jun J, Kwak Y, Kim S, et al. Prevalence of plasmid-mediated quinolone resistance in clinical isolates of *Enterobacter cloacae* from Korea. 45th Interscience Conference on Antimicrobial Agents and Chemotherapy; Washington, DC, USA; Dec 16–19 2005. Abstract C2-787.

45 Cheung TK, Chu YW, Chu MY, Ma CH, Yung RW, Kam KM. Plasmid-mediated resistance to ciprofloxacin and cefotaxime in clinical isolates of *Salmonella enterica* serotype Enteritidis in Hong Kong. *J Antimicrob Chemother* 2005; **56:** 586–89.

46 Rodriguez-Martinez JM, Poirel L, Pascual A, Nordmann P. Plasmid-mediated quinolone resistance in Australia. *Microb Drug Resist* 2006; **12:** 99–102.

47 Poirel L, Pitout JD, Calvo L, Rodriguez-Martinez JM, Church D, Nordmann P. In vivo selection of fluoroquinolone-resistant *Escherichia coli* isolates expressing plasmid-mediated quinolone resistance and expanded-spectrum beta-lactamase. *Antimicrob Agents Chemother* 2006; **50:** 1525–27.

48 Poirel L, Pitout JD, Calvo AL, Church DL, Nordmann P. Plasmid-mediated quinolone resistance in Canada. 45th Interscience Conference on Antimicrobial Agents and Chemotherapy; Washington, DC, USA; Dec 16–19 2005. Abstract C2-789.

49 Cano ME, Martinez-Martinez L, Garcia-Lobo JM, Calvo J, Aguero J. Detection of *orf513* and *qnrA* among multiresistant Gram-negative clinical isolates in Spain. 45th Interscience Conference on Antimicrobial Agents and Chemotherapy; Washington, DC, USA; Dec 16–19 2005. Abstract C1-1043.

50 Schultsz C, Yen LM, Linh LD, et al. High prevalence of *qnrS* and *qnrA* genes among Enterobacteriaceae on an ICU in Ho Chi Minh City, Viet Nam. 45th Interscience Conference on Antimicrobial Agents and Chemotherapy; Washington, DC, USA; Dec 16–19 2005. Abstract LB-22.

51 Poirel L, Leviandier C, Nordmann P. Emergence of plasmid-mediated quinolone resistance QnrS determinant in enterobacterial isolates in Europe. 16th European Congress of Clinical Microbiology and Infectious Diseases; Nice, France; April 1–4 2006. Abstract O51.

52 Cano ME, Rodriguez-Martinez JM, Aguero J, et al. Detection of *qnrS* in clinical isolates of *Enterobacter cloacae* in Spain, 16th European Congress of Clinical Microbiology and Infectious Diseases; Nice, France; April 1–4 2006. Abstract O52.

53 Gay K, Robicsek A, Strahilevitz J, et al. Plasmid mediated quinolone resistance in non-Typhi *Salmonella*. *Clin Infect Dis* 2006; **43:** 297–304.

54 Robicsek A, Strahilevitz J, Sahm DF, Jacoby GA, Hooper DC. *qnr* prevalence in ceftazidime-resistant Enterobacteriaceae isolates from the United States. *Antimicrob Agents Chemother* 2006; **50:** 2872–74.

55 Henrichfreise B, Pfister W, Wiegand I, Wiedemann B. Resistance mechanisms and epidemiology of multiresistant *Pseudomonas aeruginosa* strains from Germany. 45th Interscience Conference on Antimicrobial Agents and Chemotherapy; Washington, DC, USA; Dec 16–19 2005. Abstract C2-1423.

56 Wang M, Ye X, Wu S, Liu Y, Zhang Y. Prevalence of the plasmid-mediated quinolone resistance determinant *qnrA* among consecutive clinical strains isolated from a teaching hospital in Shanghai, China. 45th Interscience Conference on Antimicrobial Agents and Chemotherapy; Washington, DC, USA; Dec 16–19 2005 2005. Abstract C2-785.

57 Li JB, Yu YS, Ma YL, Zhou WL, Yu XZ. Prevalence and analysis of risk factors for infections caused by resistant *Escherichia coli* strains in Anhui, China. *Infection* 2001; **29:** 228–31.

58 Poirel L, Rodriguez-Martinez JM, Mammeri H, Liard A, Nordmann P. Origin of plasmid-mediated quinolone resistance determinant QnrA. *Antimicrob Agents Chemother* 2005; **49:** 3523–25.

59 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:** 801–03.

60 Garnier F, Raked N, Gassama A, Denis F, Ploy MC. Genetic environment of quinolone resistance gene *qnrB2* in a complex sul1-type integron in the newly described *Salmonella enterica* serovar Keurmassar. *Antimicrob Agents Chemother* 2006; **50:** 3200–02.

61 Collis CM, Hall RM. Expression of antibiotic resistance genes in the integrated cassettes of integrons. *Antimicrob Agents Chemother* 1995; **39:** 155–62.

62 Lautenbach E, Strom BL, Bilker WB, Patel JB, Edelstein PH, Fishman NO. Epidemiological investigation of fluoroquinolone resistance in infections due to extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*. *Clin Infect Dis* 2001; **33:** 1288–94.

63 Paterson DL, Mulazimoglu L, Casellas JM, et al. Epidemiology of ciprofloxacin resistance and its relationship to extended-spectrum beta-lactamase production in *Klebsiella pneumoniae* isolates causing bacteremia. *Clin Infect Dis* 2000; **30:** 473–78.

64 Poirel L, Liard A, Rodriguez-Martinez JM, Nordmann P. Vibrionaceae as a possible source of Qnr-like quinolone resistance determinants. *J Antimicrob Chemother* 2005; **56:** 1118–21.

65 Saga T, Kaku M, Onodera Y, Yamachika S, Sato K, Takase H. *Vibrio parahaemolyticus* chromosomal *qnr* homologue VPA0095: demonstration by transformation with a mutated gene of its potential to reduce quinolone susceptibility in *Escherichia coli*. *Antimicrob Agents Chemother* 2005; **49:** 2144–45.

66 Ellington MJ, Woodford N. Fluoroquinolone resistance and plasmid addiction systems: self-imposed selection pressure? *J Antimicrob Chemother* 2006; **57:** 1026–29.

67 Kampranis SC, Howells AJ, Maxwell A. The interaction of DNA gyrase with the bacterial toxin CcdB: evidence for the existence of two gyrase-CcdB complexes. *J Mol Biol* 1999; **293:** 733–44.

68 Jiang Y, Pogliano J, Helinski DR, Konieczny I. ParE toxin encoded by the broad-host-range plasmid RK2 is an inhibitor of *Escherichia coli* gyrase. *Mol Microbiol* 2002; **44:** 971–79.

69 Chatterji M, Nagaraja V. GyrI: a counter-defensive strategy against proteinaceous inhibitors of DNA gyrase. *EMBO Rep* 2002; **3:** 261–67.

70 Chatterji M, Sengupta S, Nagaraja V. Chromosomally encoded gyrase inhibitor GyrI protects *Escherichia coli* against DNA-damaging agents. *Arch Microbiol* 2003; **180:** 339–46.

71 Chen DK, McGeer A, de Azavedo JC, Low DE. Decreased susceptibility of *Streptococcus pneumoniae* to fluoroquinolones in Canada. Canadian Bacterial Surveillance Network 1. *N Engl J Med* 1999; **341:** 233–39.

72 Doern GV. Antimicrobial use and the emergence of antimicrobial resistance with *Streptococcus pneumoniae* in the United States. *Clin Infect Dis* 2001; **33** (suppl 3): S187–92.

73 Rodriguez-Martinez JM, Velasco C, Pascual A, Garcia I, Martinez-Martinez L. Correlation of quinolone resistance levels and differences in basal and quinolone-induced expression from three *qnrA*-containing plasmids. *Clin Microbiol Infect* 2006; **12:** 440–45.

74 Robicsek A, Strahilevitz J, Jacoby GA, et al. Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. *Nat Med* 2006; **12:** 83–88.

75 Boyd DA, Tyler S, Christianson S, et al. Complete nucleotide sequence of a 92-kilobase plasmid harboring the CTX-M-15 extended-spectrum beta-lactamase involved in an outbreak in long-term-care facilities in Toronto, Canada. *Antimicrob Agents Chemother* 2004; **48:** 3758–64.

76 Park CH, Robicsek A, Jacoby GA, Sahm D, Hooper DC. Prevalence of *aac(6')Ib-cr* encoding a ciprofloxacin-modifying enzyme in the United States. *Antimicrob Agents Chemother* (in press).

77 Crump JA, Barrett TJ, Nelson JT, Angulo FJ. Reevaluating fluoroquinolone breakpoints for *Salmonella enterica* serotype Typhi and for non-Typhi salmonellae. *Clin Infect Dis* 2003; **37:** 75–81.