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.

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, a fully synthetic agent with bactericidal effects on most Enterobacteriaceae at clinical concentrations. A pharmacological innovation—addition of a fluoro at the C-6 position and piperazinyl or related ring at position C-7 of the quinolone molecule—yields the fluoroquinolones, first available clinically in the 1980s. 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 exclusively susceptible. A recent survey of enteric bacteria in US intensive care units found that more than 10% of these organisms were resistant to ciprofloxacin. Levels of quinolone resistance in clinical E coli isolates have been reported at 40% in Hong Kong, and about 25% of healthy individuals living in Barcelona were found to be intestinally colonised with quinolone-resistant E coli.

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. When bound to DNA, these enzymes transiently break the closed circular DNA molecule, pass another strand through the break, and then reselate 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. Each of the target enzymes has a quinolone-resistance determining region (QRDR), a portion of the DNA-binding surface of the enzyme 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 either alone or together with decreased expression of outer membrane porins.
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. Accordingly, plasmids encoding mutant gyrasea 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. Although a 1987 report described the identification of PMQR in an outbreak strain of Shigella dysenteriae, the quinolone resistance was later attributed to chromosomal mutation and not a plasmid-encoded gene.

Plasmid-encoded Qnr protein
The discovery of PMQR in the late 1990s was made inadvertently by Luis Martinez-Martinez and colleagues. 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. 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. (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.

Mechanism of Qnr action
The QnrA protein belongs to the pentapeptide-repeat family, which is defined by a tandem five amino acid repeat with the recurrent motif [Ser, Thr, Ala or Val] [Asp or Asn] [Leu or Phe] [Ser, Thr or Arg] [Gly]. 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. Organisms producing B17 also make MbcG, a pentapeptide-repeat protein with 19-6% aminoacid identity with QnrA, that protects DNA gyrase against the effect of the microcin and also some quinolones. 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. 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. 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...
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 to 2·0 μg/mL for other fluoroquinolones. Although for most agents, the increase was between about 16-fold and 125-fold, this increase was less (16-fold to 32-fold) for the developmental fluoroquinolones BAYy3118, premafl oxacin, and sitafloxacin. The agent for which the loss of activity was least pronounced was nalidixic acid (two-fold to eight-fold increases in MIC). Also noteworthy is the finding that qnrA-encoding plasmids from US K pneumoniae yielded transconjugants with very similar quinolone susceptibilities, whereas qnrA-encoding plasmids from Chinese E coli varied in ciprofloxacin susceptibilities by 16-fold. 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 ofl oxacin 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. Another measure of resistance gene effect is a change in the mutant prevention concentration (MPC). The MPC is the lowest

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

<table>
<thead>
<tr>
<th>Agent</th>
<th>E coli J53, wild-type (MIC&lt;sub&gt;90&lt;/sub&gt;)</th>
<th>Transconjugants (n=17)</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C&lt;sub&gt;90&lt;/sub&gt;</td>
<td>C&lt;sub&gt;90&lt;/sub&gt;</td>
</tr>
<tr>
<td>BAYy3118</td>
<td>0·004</td>
<td>0·125</td>
<td>0·06–0·25</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0·008</td>
<td>0·25</td>
<td>0·125–2</td>
<td></td>
</tr>
<tr>
<td>Garenoxacin</td>
<td>0·008</td>
<td>1</td>
<td>0·5–2</td>
<td></td>
</tr>
<tr>
<td>Gatifloxacin</td>
<td>0·008</td>
<td>0·25</td>
<td>0·25–1</td>
<td></td>
</tr>
<tr>
<td>Gemifloxacin</td>
<td>0·004</td>
<td>0·5</td>
<td>0·25–1</td>
<td></td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>0·015</td>
<td>0·5</td>
<td>0·25–1</td>
<td></td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>0·03</td>
<td>0·5</td>
<td>0·5–1</td>
<td></td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>4</td>
<td>16</td>
<td>8–32</td>
<td></td>
</tr>
<tr>
<td>Premafl oxacin</td>
<td>0·03</td>
<td>0·25</td>
<td>0·25–0·5</td>
<td></td>
</tr>
<tr>
<td>Sitafloxacin</td>
<td>0·008</td>
<td>0·125</td>
<td>0·06–0·25</td>
<td></td>
</tr>
<tr>
<td>Sparfloxacin</td>
<td>0·008</td>
<td>0·1</td>
<td>0·25–1</td>
<td></td>
</tr>
</tbody>
</table>

MIC<sub>90</sub>—minimum concentration of an agent required to inhibit the growth of 90% of organisms tested.
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 \(E\ coli\)30 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
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

<table>
<thead>
<tr>
<th>Reference</th>
<th>Target of screen</th>
<th>Year of isolate collection</th>
<th>Geographic area</th>
<th>Bacterial type</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Martinez-Martinez et al&lt;sup&gt;40&lt;/sup&gt;</td>
<td>-</td>
<td>1994</td>
<td>Alabama, USA</td>
<td><em>K pneumoniae</em></td>
<td>First identification of <em>qnrA</em></td>
</tr>
<tr>
<td>Jacoby et al&lt;sup&gt;34&lt;/sup&gt;</td>
<td><em>qnrA</em></td>
<td>Primarily 1990s</td>
<td>19 countries, three continents</td>
<td>Primarily <em>K pneumoniae</em> and <em>E coli</em> but several other genera represented</td>
<td>Several <em>E coli</em> and <em>K pneumoniae</em> were positive, all from Alabama in 1994; The rest of about 350 isolates were negative</td>
</tr>
<tr>
<td>Rodriguez-Martinez et al&lt;sup&gt;42&lt;/sup&gt;</td>
<td><em>qnrA</em></td>
<td>1990s&lt;sup&gt;7&lt;/sup&gt;</td>
<td>USA, Spain, and unlisted countries</td>
<td><em>E coli</em> (266) and <em>K pneumoniae</em> (159)</td>
<td>None of the <em>E coli</em>, three (2%) of <em>K pneumoniae</em> were positive (collected 1995–1997). Two of these three were fluoroquinolone-sensitive by CLSI breakpoints</td>
</tr>
<tr>
<td>Wang et al&lt;sup&gt;46&lt;/sup&gt;</td>
<td><em>qnrA</em></td>
<td>2000–2001</td>
<td>Shanghai, China</td>
<td><em>E coli</em> (78), all ciprofloxacin-resistant</td>
<td>Six (8%) positive. All from same hospital. Several different plasmids carried <em>qnrA</em></td>
</tr>
<tr>
<td>Wang et al&lt;sup&gt;44&lt;/sup&gt;</td>
<td><em>qnrA</em></td>
<td>1999–2002</td>
<td>USA, nationwide</td>
<td>72 <em>K pneumoniae</em> and 38 <em>E coli</em>, all with MIC of ciprofloxacin ≥0·2 µg/mL and MIC of cefazidime ≥16 µg/mL</td>
<td>None of <em>E coli</em> and eight (11%) of <em>K pneumoniae</em> were positive</td>
</tr>
<tr>
<td>Mammeri et al&lt;sup&gt;43&lt;/sup&gt;, Nordmann &amp; Poirel&lt;sup&gt;46&lt;/sup&gt;</td>
<td><em>qnrA</em></td>
<td>2003</td>
<td>Paris, France</td>
<td>449 nalidixic-acid resistant Enterobacteriaceae</td>
<td>Two (0·5%) were positive, one <em>E coli</em> and one <em>E cloacae</em></td>
</tr>
<tr>
<td>Jonas et al&lt;sup&gt;46&lt;/sup&gt;</td>
<td><em>qnrA</em></td>
<td>2000–2003</td>
<td>Germany, nationwide</td>
<td>326 integron-containing Enterobacteriaceae isolates from 54 German intensive care units</td>
<td>One Enterobacter sp isolate and an outbreak strain of <em>Citrobacter freundii</em> were positive</td>
</tr>
<tr>
<td>Paaske et al&lt;sup&gt;44&lt;/sup&gt;</td>
<td><em>qnrA</em></td>
<td>2001–2003</td>
<td>Ulrecht, Netherlands</td>
<td>83 epidemic strains of <em>Enterobacter cloaceae</em></td>
<td>78 (94%) strains carried <em>qnrA</em>. Additionally, 31% of colonising Gram-negative bacteria in patients colonised with epidemic strains also carried <em>qnrA</em></td>
</tr>
<tr>
<td>Nazic et al&lt;sup&gt;47&lt;/sup&gt;</td>
<td><em>qnrA</em></td>
<td>2002–2004</td>
<td>Istanbul, Turkey</td>
<td>49 ESBL-carrying Enterobacteriaceae</td>
<td>Two (4%) isolates were positive</td>
</tr>
<tr>
<td>Wiegand et al&lt;sup&gt;48&lt;/sup&gt;</td>
<td><em>qnrA</em></td>
<td>2001</td>
<td>Cairo, Egypt</td>
<td>30 Enterobacteriaceae with MIC of ciprofloxacin ≥0·25 µg/mL from a burn unit</td>
<td>Three unrelated <em>Providencia stuartii</em> strains carried <em>qnrA</em></td>
</tr>
<tr>
<td>Corkill et al&lt;sup&gt;49&lt;/sup&gt;</td>
<td><em>qnrA</em></td>
<td>2003–2005</td>
<td>Liverpool, UK</td>
<td>47 ciprofloxacin- and cefotaxime-resistant Enterobacteriaceae from blood cultures</td>
<td>15 (32%) carried <em>qnrA</em>. 12 were clonally distinct. Multiple plasmids were involved</td>
</tr>
<tr>
<td>Poirel et al&lt;sup&gt;41&lt;/sup&gt;</td>
<td><em>qnrA</em></td>
<td>1999</td>
<td>Bangkok, Thailand</td>
<td>23 Enterobacteriaceae carrying the bla&lt;sub&gt;TEM&lt;/sub&gt; β-lactamase</td>
<td>11 (48%) isolates were positive</td>
</tr>
<tr>
<td>Joeng et al&lt;sup&gt;42&lt;/sup&gt;, Jun et al&lt;sup&gt;43&lt;/sup&gt;</td>
<td><em>qnrA</em></td>
<td>2001–2003</td>
<td>Seoul, Korea</td>
<td><em>E coli</em> (260)</td>
<td><em>E cloacae</em> (206)</td>
</tr>
<tr>
<td>Cheung et al&lt;sup&gt;44&lt;/sup&gt;</td>
<td><em>qnrA</em></td>
<td>2003</td>
<td>Hong Kong, China</td>
<td>Salmonella enterica serotype Enteritidis, outbreak strains</td>
<td>All four strains tested carried <em>qnrA</em>; four different plasmids</td>
</tr>
<tr>
<td>Poirel et al&lt;sup&gt;45&lt;/sup&gt;</td>
<td><em>qnrA</em></td>
<td>2004</td>
<td>Sydney, Australia</td>
<td>23 ESBL-positive or fluoroquinolone-resistant Enterobacteriaceae</td>
<td>Two (10%) isolates were positive</td>
</tr>
<tr>
<td>Poirel et al&lt;sup&gt;46&lt;/sup&gt;</td>
<td><em>qnrA</em>, <em>qnrS</em></td>
<td>2004–2005, 2000–2002</td>
<td>Calgary, Canada</td>
<td>139 ESBL-negative ciprofloxacin-resistant Enterobacteriaceae; 101 ESBL-positive Enterobacteriaceae</td>
<td>Two (2%) of the 101 ESBL-positive Enterobacteriaceae carried <em>qnrA</em>, none carried <em>qnrS</em> (same group also reported one of 110 ESBL <em>E coli</em> from Calgary carried <em>qnrA</em>; however, apparently that data set overlaps somewhat)</td>
</tr>
<tr>
<td>Cano et al&lt;sup&gt;47&lt;/sup&gt;</td>
<td><em>qnrA</em></td>
<td>2004–2005</td>
<td>Santander, Spain</td>
<td>100 nalidixic acid-resistant Enterobacteriaceae, 100 multiresistant <em>E coli</em> and 173 ESBL-positive Enterobacteriaceae</td>
<td>Two ESBL-positive isolates (<em>C freundii</em> and <em>E cloacae</em>) from a single patient carried <em>qnrA</em></td>
</tr>
<tr>
<td>Schultza et al&lt;sup&gt;48&lt;/sup&gt;</td>
<td><em>qnrA</em>, <em>qnrS</em></td>
<td>2004</td>
<td>Ho Chi Minh City, Vietnam</td>
<td>28 gentamicin-resistant <em>K pneumoniae</em></td>
<td>24 (86%) of 28 <em>K pneumoniae</em> carried <em>qnrA</em> or <em>qnrS</em>. Eight (25%) of 32 ESBL-positive isolates carried <em>qnrA</em> or <em>qnrS</em></td>
</tr>
<tr>
<td>Poirel et al&lt;sup&gt;49&lt;/sup&gt;</td>
<td><em>qnrA</em>, <em>qnrS</em></td>
<td>2002–2005</td>
<td>Paris, France</td>
<td>185 nalidixic acid-resistant, ESBL-negative Enterobacteriaceae; 187 ESBL-positive Enterobacteriaceae</td>
<td>Among the ESBL-negative isolates, one (0·5%) carried <em>qnrA</em> and five (3%) carried <em>qnrS</em>. Among the ESBL-positive isolates, three (2%) carried <em>qnrA</em> and 3 (2%) carried <em>qnrS</em></td>
</tr>
<tr>
<td>Cano et al&lt;sup&gt;49&lt;/sup&gt;</td>
<td><em>qnrA</em>, <em>qnrS</em></td>
<td>2004–2005</td>
<td>Northern and southern Spain</td>
<td>202 Enterobacter spp</td>
<td>None carried <em>qnrA</em>; 22 (11%) carried <em>qnrS</em>. All <em>qnrS</em>-positive strains were from northern Spain</td>
</tr>
<tr>
<td>Robicsek et al&lt;sup&gt;50&lt;/sup&gt;</td>
<td><em>qnrA</em>, <em>qnrB</em>, <em>qnrS</em></td>
<td>1999–2004</td>
<td>USA, nationwide</td>
<td>313 isolates of <em>E coli</em>, <em>K pneumoniae</em>, and Enterobacter spp, all with MIC of ciprofloxacin ≥0·25 µg/mL and MIC of cefazidime ≥16 µg/mL</td>
<td>Either <em>qnrA</em> or <em>qnrB</em> was present in two (4%) of 47 <em>E coli</em>, 21 (20%) of 106 <em>K pneumoniae</em>, and 50 (31%) of 160 Enterobacter spp. None carried <em>qnrS</em></td>
</tr>
<tr>
<td>Gay et al&lt;sup&gt;51&lt;/sup&gt;</td>
<td><em>qnrA</em>, <em>qnrB</em>, <em>qnrS</em></td>
<td>1996–2003</td>
<td>USA, nationwide</td>
<td>225 non-Typhi salmonella isolates; 223 with ciprofloxacin MIC ≥0·06 µg/mL, 102 with MIC ≤0·03 µg/mL</td>
<td>Ten carried either <em>qnrA</em> or <em>qnrB</em>, all these had ciprofloxacin MIC ≥0·06 µg/mL. None carried <em>qnrA</em></td>
</tr>
</tbody>
</table>

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

Table 2: Epidemiology of *qnr*
Review

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 Photobacterium profundum, a water-dwelling environmental organism, than with QnrB. BAC61438 is found in Vibrio parahaemolyticus, AAO07889 is found in Vibrio vulnificus, MTMipA is found in Mycobacterium tuberculosis, and McBG is found in Enterobacteriaceae. QnrA and chromomonomally encoded proteins from Shewanella spp are more than 98% related. 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, 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.

**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). Subsequently, a K oxytoca isolate from Anhui Province, China (where the rate of ciprofloxacin resistance in E coli is 70%) was reported to carry a variant of qnrA differing in four codons from the originally detected gene. This variant was designated qnrA2, 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. At about the same time, qnrA3 was also detected in clinical salmonella isolates. 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. 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.

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. 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-His₆ protein was found to protect DNA gyrase, much like QnrA, although with greater in-vitro potency. 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). 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. 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 cefazidime.
(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. This survey also identified two new variants of qnrB, 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. Recent European surveys have suggested that qnrS could be more prevalent than qnrA in clinical Enterobacteriaceae.

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. 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. 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.

The linkage between resistance to the latest cephalosporin antibiotics and to quinolones has been noted by several investigators. One mechanism for this association is incorporation on the same plasmid of genes resembling those found on the chromosome of marine bacteria. 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.

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 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. 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. Another group showed that the *V parahaemolyticus* Qnr analogue exhibited higher levels of quinolone resistance when it had acquired a single aminoacid change. 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. Several such proteins are known—eg, microcin B17, CcdB, and ParE (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 in postsegregational killing, a mechanism through which 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, and quinolones. 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.

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, 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), 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. 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, 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).
Although apparent differences in levels of expression of 
qnrA in transconjugants have been associated recently with different levels of resistance,\(^{71}\) 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.\(^{72}\) 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 additional North American Enterobacteriaceae.\(^{76}\) Our group has subsequently identified this variant with different levels of resistance,\(^{73}\) we found that this aac(6')-Ib-cr variant was widespread\(^{74}\) and of these 28% we found to be necessary and 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.\(^{75}\) 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.\(^{76}\)

Little is known as yet about the epidemiology of this gene variant. Although only first reported in 2004,\(^{40,75}\) 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\(^{40}\)). Nine (11%) of the 78 strains carried non-cr aac(6')-Ib, and 40 (51%) harboured the cr-variant aminoglycoside acetyltransferase.\(^{77}\) Thus, in some strain sets aac(6')-Ib-cr is highly prevalent (more so than qnrA) and circulates 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.\(^{78}\) 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

### Table 3: Effect of AAC(6')-Ib-cr on fluoroquinolone MIC

<table>
<thead>
<tr>
<th>Strain</th>
<th>Ciprofloxacin</th>
<th>N-acetyl ciprofloxacin</th>
<th>Enrofloxacin</th>
<th>Norfloxacin</th>
<th>Pefloxacin</th>
<th>Levofloxacin</th>
<th>Gemifloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type E coli</td>
<td>0.02</td>
<td>0.08</td>
<td>0.02</td>
<td>0.156</td>
<td>0.08</td>
<td>0.08</td>
<td>0.005</td>
</tr>
<tr>
<td>E coli expressing aac(6')-Ib-cr</td>
<td>0.04–0.08</td>
<td>0.08</td>
<td>0.02</td>
<td>0.625</td>
<td>0.08</td>
<td>0.08</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Units are µg/mL. Note that this enzyme only reduces the activity of fluoroquinolones with an unsubbound 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.
by the finding that a DNA gyrase mutation known to increase susceptibility to ciprofloxacin was present in nine of ten qnr-positive Salmonella spp in our recent survey.63 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.64 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.65 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-lactamases66-67 and aminoglycosides.68 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 blaTEM, but in none of 22 that did not.69 Likewise, in our survey of US Enterobacteriaceae, qnr genes were very strongly associated with aminoglycoside resistance.70 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.

**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, whenever 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 antimicrobial resistance, these plasmids were the abstracts of recent conferences. 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.
Still, the news might not all be bad. We are aware of at least one instance in which 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.

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