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Plasmid-Mediated Quinolone Resistance: a Multifaceted Threat

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INTRODUCTION

The first quinolone, nalidixic acid (possessing a naphthyridone core), was introduced into clinical use in 1962 (10). In the mid-1980s, ciprofloxacin, a fluoroquinolone (with a quinolone core) that had a wider spectrum of in vitro antibacterial activity, particularly against gram-negative bacteria, first became available clinically (146). Since then, newer agents with increased antimicrobial activity against gram-positive pathogens have been developed, but the activity of ciprofloxacin against gram-negative pathogens has been largely unsurpassed (80, 177). In the decades that have elapsed since the introduction of

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fluoroquinolones, resistance of the *Enterobacteriaceae* to these agents has become common, widespread, and generally nonclonal. This implies that fluoroquinolone resistance has emerged independently many times (44, 66, 114, 132).

The main mechanism of quinolone resistance is the accumulation of mutations in the bacterial enzymes targeted by fluoroquinolones: DNA gyrase and DNA topoisomerase IV (79). Multiple mutations are generally required for clinically important resistance to result, as wild-type organisms are highly susceptible (79). Because spontaneous double mutations are rare genetic events (occurring at a frequency of 10^{-14} to 10^{-16} for fluoroquinolones) (227), the traditional understanding of quinolone resistance as a mutational phenomenon has not provided a fully satisfying explanation for the frequency with which this resistance has arisen. Such a phenomenon could perhaps be better accounted for if horizontally transferable elements could provide some degree of reduced quinolone susceptibility, enough so that organisms could survive in the face of quinolones, while resistance mutations occurred sequentially rather than simultaneously. The presence of such horizontally transferable elements might also help account for the strong association between resistance to quinolones and resistance to other agents. In the past, the recessive nature of resistant *gyrA* alleles in merodiploids (187) was used to account for the lack of naturally occurring plasmid-mediated quinolone resistance (PMQR) (48).

The discovery in 1998 (123) of *qnr*, a plasmid-mediated horizontally transferable gene encoding quinolone resistance, has shed light on these phenomena. Qnr proteins are capable of protecting DNA gyrase from quinolones and have been in circulation for at least 20 years (90). During this time they have achieved global distribution in a variety of plasmid environments and bacterial genera. Two additional mechanisms of resistance that were predicted to occur were subsequently found (48). AAC(6′)-Ib-cr, a variant aminoglycoside acetyltransferase capable of reducing ciprofloxacin activity, is also carried on plasmids and may be even more prevalent than Qnr proteins (165). Quinolone extrusion, a prevalent chromosomally encoded mechanism of resistance, has also been found to be plasmid borne (70, 143, 215). These mechanisms provide the low-level quinolone resistance shown in vitro to facilitate the emergence of higher-level resistance in the presence of quinolones at therapeutic levels.

Several reviews of the topic of PMQR have been published (22, 121, 133, 163). Research on PMQR is, however, rapidly expanding. Two of the five known *qnr* gene families (*qnrC* and *qnrD*) were first reported within the past year (32, 208). The recent solution of the structure of *aac(6′)-Ib* importantly advanced our understanding of the *aac(6′)-Ib-cr* mechanism of action (124, 204), and various new plasmids carrying PMQR genes have been reported. Ten years have passed since the first report of PMQR, but knowledge is still expanding quickly. Remarkably, a PubMed search using the keywords PMQR, *qnr*, and *aac(6′)-Ib-cr* revealed 212 available original research publications, and 129 (61%) were published since January 2008. In this review we address new information on PMQR in the context of previously established data.

PENTAPEPTIDE REPEAT PROTEINS

Discovery of *qnr* Genes

***qnrA*.** The discovery of PMQR in the late 1990s was made serendipitously by Martínez-Martínez and colleagues (123). That group was studying pMG252, a plasmid from a multiresistant strain of *Klebsiella pneumoniae* that was isolated from a urine specimen from a patient at the University of Alabama at Birmingham in 1994. A quinolone was included as a control in a study of the ability of pMG252 to increase resistance to β-lactam antibiotics in porin-deficient strains of *Klebsiella pneumoniae*. Unexpectedly, an increase in the quinolone MIC was found. The effect of the plasmid was increased 4- to 16-fold in this porin-deficient isolate, but even in an *Escherichia coli* strain with intact porins, pMG252 increased the quinolone MICs between 8- and 64-fold. Although this increase from baseline was not to the level designated to represent clinical resistance (the resistance breakpoint), the plasmid also facilitated the selection of higher-level quinolone resistance. A wild-type *E. coli* strain carrying pMG252 plated onto agar containing nalidixic acid or ciprofloxacin was 100 times more likely to give rise to spontaneous resistant mutants than was a plasmid-free strain (123). The plasmid did not have a general mutator effect since the frequency of other genetically defined mutations was equivalent to that of plasmid-free *E. coli* (123). Subsequent cloning of the gene responsible for this phenotype revealed it to be a 657-bp open reading frame, and the protein which it encoded was named Qnr, for quinolone resistance (GenBank accession number AY070235) (197). (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 QnrA1, since related proteins have been identified.

A *Klebsiella oxytoca* isolate from Anhui Province, China, was reported to carry a variant of *qnrA* differing from the originally detected gene by four amino acids. This variant was designated *qnrA2* (GenBank accession number AY675584) (133). While searching for a chromosomal orthologue of *qnrA* in the genome sequences of environmental organisms, Poirel et al. identified three additional variants (*qnrA3*, *qnrA4*, and *qnrA5*) of this gene in *Shewanella algae*, varying from *qnrA1* in two to four codons (GenBank accession numbers DQ058661, DQ058662, and DQ058663, respectively) (155). At about the same time, *qnrA3* was also detected in clinical salmonella isolates (GenBank accession number AY906856) (38). Subsequently, a group searching for *qnrA* determinants among isolates of the *Enterobacteriaceae* with reduced susceptibility to quinolones found *qnrA6* in a *Proteus mirabilis* isolate (GenBank accession number DQ151889) (17).

***qnrS*.** A subsequent search for plasmids exhibiting transferable resistance to quinolones led to the discovery of four additional similar proteins, QnrS (75), QnrB (91), QnrC (208), and QnrD (32). In October 2003, a single clone of *Shigella flexneri* 2b caused a food-borne outbreak of enterocolitis in Aichi Prefecture, Japan. One of eight strains of this clone was resistant to ciprofloxacin. This strain was found to harbor a unique conjugative plasmid that transferred quinolone resistance. Cloning identified an open reading frame encoding a

218-amino-acid protein of the pentapeptide repeat family. This protein shares only 59% amino acid identity with QnrA1 and was named QnrS (GenBank accession number AB187515) (75).

In the course of searching for *qnr* genes among clinical non-Typhi *Salmonella* isolates from the United States, a *qnrS* variant (*qnrS2*) (GenBank accession number DQ485530) was detected on a plasmid from *Salmonella enterica* serovar Anatum that codes for a protein that is 92.2% identical in amino acid sequence to QnrS1 (70). *qnrS2* was found on a plasmid isolated from the activated sludge basin of a wastewater treatment plant in Germany at about the same time (14, 99). *qnrS* from veterinary clinical *E. coli* isolates in Guangdong, China, that was deposited in GenBank as *qnrS1* (GenBank accession number ABU52984) differed from *qnrS1* in one codon and has thus been renamed *qnrS3*. To avoid such confusion and to bring order into *qnr* numbering, a database of *qnr* allele designations has been established at http://www.lahey.org/qnrStudies (87). A fourth *qnrS* variant in a salmonella isolate from Denmark was recently described (195).

***qnrB*.** While investigating strains of *K. pneumoniae* from India, some of which contained *qnrA*, Jacoby and colleagues found that several could transfer low-level quinolone resistance but were negative by PCR for *qnrA* (91). The PMQR gene responsible for this phenotype coded for a 214- or 226-amino-acid protein (depending on which potential initiation codon was taken as the start) and was termed *qnrB1* (GenBank accession number DQ351241). A recent determination of the transcription start site supports a peptide length of 214 amino acids, and homology between different *qnrB* variants bears this out (23, 209). The QnrB1 protein shares 43% and 44% amino acid identities with QnrA and QnrS, respectively (91).

The repertoire of *qnrB* variants is broader than that of *qnrA* and *qnrS*. The first variant, *qnrB2*, was found in the first survey among several isolates of *Enterobacteriaceae* from the United States. The *qnrB2* gene codes for a 214-amino-acid protein (GenBank accession number DQ351242) that differs from *qnrB1* in five codons (91). Subsequent surveys in the United States have identified *qnrB3* and *qnrB4* (GenBank accession numbers DQ303920 and DQ303921, respectively) among the *Enterobacteriaceae* (166) and *qnrB5* in non-Typhi salmonella isolates (GenBank accession number DQ303919) (70); these differ from *qnrB1* in 2, 14, and 6 codons, respectively. More recently, using the same multiplex PCR method, *qnrB13*, *qnrB14*, and *qnrB15* (GenBank accession numbers ABX72042, ABX72044, and ABX72227, respectively), differing from *qnrB1* in four, five, and five amino acids, respectively, were identified among *Citrobacter freundii* strains from South Korea (190). Using degenerate primers for the detection of *qnrB* in a collection of isolates of *Enterobacteriaceae* from Kuwait City, Kuwait, Cattoir et al. identified *qnrB7* in *Enterobacter cloacae* and *qnrB8* in *C. freundii* (23). These genes differ from *qnrB1* by 4 and 11 amino acids, respectively (GenBank accession numbers EU043311 and EU043312, respectively). *qnrB19*, which differs from *qnrB1* by six amino acids, was also found in an *E. coli* isolate from Colombia by the same group (GenBank accession number EU432277) (23). *qnrB10* (161), discovered in *C. freundii*, differs from *qnrB1* by five amino acids, and *qnrB12* (104), identified in *Citrobacter werkmanii*, differs from *qnrB1* by eight amino acids in addition to the five differences that characterize *qnrB10*. Sequences of six more *qnrB* alleles have been deposited in GenBank. *qnrB6*, which differs from *qnrB1* by two amino acids, was found among several cephalosporin-resistant isolates of *Enterobacteriaceae* in western China (GenBank accession number EF520349). *qnrB9* (GenBank accession number EF526508), *qnrB11* (GenBank accession number EF653270), *qnrB16* (GenBank accession number EU136183), and *qnrB17* and *qnrB18* (GenBank accession numbers AM919398 and AM919399, respectively) were all found among different isolates of *C. freundii*. Before agreement on *qnrB* allele numbering (87), sequences were submitted to GenBank with arbitrary, overlapping, and frequently changing allele numbers. Consequently, *qnrB* allele designations in GenBank files should be accepted with caution.

***qnrC*.** A clinical strain of *Proteus mirabilis* from Shanghai, China, which transferred low-level quinolone resistance, was negative by PCR for the known *qnr* genes. Plasmid pHS9, which, upon conjugation, increased the MIC of ciprofloxacin, carried a 666-bp gene, designated *qnrC1*, coding for a 221-amino-acid protein. QnrC shared 64%, 41%, 59%, and 43% amino acid identities with QnrA1, QnrB1, QnrS1, and QnrD, respectively (208).

***qnrD*.** Four *Salmonella enterica* isolates obtained from humans in the Henan Province of China showed reduced susceptibility to ciprofloxacin that was transferable on a small plasmid of about 4.3 kb, which in *E. coli* conferred a 32-fold increase in the MIC of ciprofloxacin. This plasmid was negative for *qnrA*, *qnrB*, *qnrS*, *aac(6′)-Ib-cr*, and *qepA* (other PMQR genes [see below]) (32). The plasmid encodes a 214-amino-acid pentapeptide repeat protein designated QnrD. *qnrD* (GenBank accession number EU692908) showed 48% similarity to *qnrA1*, 61% similarity to *qnrB1*, and 32% similarity to *qnrS1* (32).

In silico analyses of deposited complete genomes led to the discovery of *qnr*-like genes in the genomes of several gram-positive bacteria including *Enterococcus faecalis*, *Enterococcus faecium*, *Listeria monocytogenes*, *Clostridium perfringens*, *Clostridium difficile*, *Bacillus cereus*, and *Bacillus subtilis* (171) as well as in gram-negative, mainly waterborne, bacteria (153, 178, 179).

Criteria for Definition of *qnr*

A recently proposed consensus for *qnr* nomenclature defined *qnr* as a naturally occurring allele encoding a pentapeptide repeat protein that confers reduced susceptibility to nalidixic acid or a fluoroquinolone. *qnr* families (such as *qnrA*, *qnrB*, or *qnrC*) are defined by a 30% or more difference in nucleotides or derived amino acids. Within each family, *qnr* alleles differ in one or more amino acids. *qnr* genes found on a bacterial chromosome are named after the host organism or assigned to a family if the gene is at least 70% identical to an established *qnr* family (e.g., Sa*qnrA3* from the chromosome of *Shewanella algae*) (87).

What Defines a Qnr?

The Qnr proteins belong to the pentapeptide repeat family, which is defined by a series of tandem 5-amino-acid repeats. In the pentapeptide repeats, no position is completely conserved, but each of the residues of an individual pentapeptide exhibits

A. QnrA1

```
MDIIDKVFQQ
EDFSR QDLSD SRFRR CRFYQ
CDFSH CQLQD ASFED CSFIE
SGAVE G
CHFSY ADLRD ASFKA CRLSL
ANFSG ANCFG IEFRE CDLKG
ANFSR ARFYN QVSHK MYFCS
AYISG CNLAY TNLSG QCLEK
CELFE NNWSN ANLSG ASLMG
SDLSR GTFSR DCWQQ VNLRG
CDLTF ADLDG LDPRR VNLEG
VKI CAWQQ
EQLLEPLGVIVLPD
```

B. QnrB1

```
MALALVG
EKIDR NRFTG EKIEN STFFN
CDFSG ADLSG TEFIG CQFYD
RESQK G
CNFSR AMLKD AIFKS CDLSM
ADFRN SSALG IEIRH CRAQG
ADFRG ASFMN MITTR TWFCS
AYITN TNLSY ANFSK VVLEK
CELWE NRWIG AQVLG ATFSG
SDLSG GEFST FDWRA ANFTH
CDLTN SELGD LDIRG VDLQG
VKLDN
YQASLLMERLGIAVIG
```

C. QnrS1

```
METYNHTYRH
HNFSH KDLSD LTFTA CTFIR
SDFRR ANLRD TTFVN CKFIE
QGDIE G
CHFDV ADLRD ASFQQ CQLAM
ANFSN ANCYG IEFRA CDLKG
ANFSR TNFAH QVSNR MYFCS
AFISG CNLSY ANMER VCLEK
CELFE NRWIG TNLAG ASLKE
SDLSR GVFSE DVWGQ FS
LQGAN LCHAE LDG
LDPRKVDTSGIKIAAWQQELLILEALGIVVYPD
```

D. QnrC

```
MNYSHKTYDQ
IDFSG QDLSD HHFSH CKFFG
CNFNR VNLRD AKFMG CTFIE
SNDFE G
CNFIY ADLRD ASFMN CMLSM
ANFQG ANCFG LELRE CDLKG
ANFSQ ANFVN HVSNK MYFCS
AYITG CNLSY ANFDK QCLEK
CDLFE NKWVG ASLQG ASFKE
SDLSR GSFSD DFWEQ CRIQG
CDLTH SELNG LEPRK VDLTG
VKICS
WQQEQLLEQLGVIVIPDKVF
```

E. QnrD

```
MEKHFIN
EKFSR DQFTG NRVKN IAFSN
CDFSG VDLTD TEFVD CSFYD
RNSLE G
CDFNR AKLKN ASFKS CDLSM
SNFKN ISALG LEISE CLAQG
ADFRG ANFMN MITTR SWFCS
AYITK TNLSY ANFSR VILEK
CELWE NRWNG TVITG AVFRG
SDLSC GEFSS FDWSL ADFTG
CDLTG GALGE LDARR INLDG
VKLDG
EQALQLVESLGVIVHR
```

FIG. 1. Amino acid sequence of Qnr displayed to emphasize the pentapeptide repeating unit with a consensus sequence of S/T/A/V/C-D/N-L/F-S/T/R-G. (A) QnrA1. (B) QnrB1. (C) QnrS1. (D) QnrC. (E) QnrD. No QnrC or QnrD variants have been described yet; yellow highlighting indicates the pentapeptide repeat according to Pfam website platform analysis; boldface type indicates residues that conform to the pentapeptide amino acid motif; boxed areas are amino acid changes in some Qnr variants.

a propensity for a restricted number of amino acids with the recurrent general motif approximately represented by the sequence A(D/N)LXX and more precisely represented by [Ser, Thr, Ala, or Val][Asp or Asn][Leu or Phe][Ser, Thr, or Arg][Gly] (12, 203). Qnr proteins, however, often have a cysteine at position i^{-2} (with position i representing the central amino acid of each repeat) (77). A characteristic feature of the Qnr proteins is that they are formed by two domains of pentapeptide repeats separated by a single amino acid, usually glycine. The primary structures of QnrA, QnrB, and QnrS are similar, with nine pentapeptide repeat units connected by a single glycine, followed by a cysteine, with variable numbers of units (22 in QnrS, 28 in QnrA, and 29 in QnrB, QnrC, and QnrD) (Fig. 1). These differ from MfpA, a pentapeptide repeat protein found in *Mycobacterium smegmatis* that is also capable of gyrase protection from quinolone and for which the three-dimensional structure has also been solved (see below). In that protein, there is a kink in the helical axis midway through the β-helix but no putative glycine "hinge" (77).

As the three-dimensional structure of Qnr has not yet been solved, the contribution of the glycine moiety to function is unknown. Likewise, it is impossible to predict increases in activity from changes in primary structure among the many Qnr variants. For example, when the *Vibrio parahaemolyticus qnr* homologue, VPA0095, was cloned onto a plasmid, it did not confer fluoroquinolone resistance unless cysteine 115 was mutated to a tyrosine (178). When the same amino acid substitution was created in QnrA1 or QnrS1, however, the ability to protect against quinolones declined rather than increased (26). Mutations in amino acids that are conserved among Qnr

proteins had a similar effect of a decrease in quinolone resistance, but the change in the MIC varied among QnrA1, QnrB1, and QnrS1 with the same substitution (167). A phylogenetic analysis of Qnr alleles suggests that recombination has played an important part in their evolution but that a specific role in protection against quinolones is not evident (11).

Chromosomal *qnr* Genes

Genes for pentapeptide repeat proteins with sequence similarity to plasmid-borne Qnr proteins have been found on the chromosomes of both gram-positive and gram-negative bacteria. Some of these proteins also have the primary structure of two pentapeptide strings of variable length connected by a single amino acid. For example, Efs*qnr*, found on the chromosome of *E. faecalis* strains V583 and JH2-2, encodes a 211-amino-acid pentapeptide repeat protein that shares 25% identity and 40% similarity with QnrA. The protein is divided into two pentapeptide repeat domains of 9 and 33 units, each connected by a single asparagine (9). In silico analysis of the pentapeptide domains of *Listeria monocytogenes* (GenBank accession number EAL07413), *Clostridium perfringens* (accession number NP_561876), *Clostridium difficile* (accession number CAJ69589), *Bacillus cereus* (accession number NP_831602), and *Bacillus subtilis* (accession number CAB12929) using the Pfam database of the Wellcome Trust Sanger Institute (64) as a search platform failed to identify two separate pentapeptide domains in any of these sequences (J. Strahilevitz, unpublished data).

***qnrA*-like genes.** Gram-negative species were initially screened for *qnr*-like genes in a search for the reservoir of Qnr determinants (see Origins of *qnr* Genes below) (155). The conclusion that *qnrA3* has a chromosomal location in *Shewanella algae* was based upon whole-genome restriction with the ribosomal endonuclease I-CeuI followed by double hybridization with rRNA and *qnrA* DNA probes to the same high-molecular-weight band. This received further support from the identical G+C content of *qnrA3* and the genome of *S. algae* and the lack of common region 1 that is part of the *sul1*-type integron, which was previously associated with the mobilization of *qnrA1* onto plasmids (155). Subsequent in silico analyses identified *qnr*-like genes in the genomes of other members of the *Shewanellaceae* (153, 179), including *Vibrio vulnificus* (GenBank accession number AA007889), *Vibrio parahaemolyticus* (accession number BAC61438), *Photobacterium profundum* (accession number CAG22829), and other *Vibrio* and *Shewanella* spp. Similarly to *S. algae*, they are not flanked by insertion sequences or genetic structures known to mobilize resistance genes (153). The encoded proteins have at most 67% identity with QnrA1 and are made of two domains of 11 and 32 units connected by a single glycine. Upon expression in a heterologous *E. coli* host system, they conferred an increased MIC of quinolones similar to that obtained with the recombinant plasmid that expressed the *qnrA3* determinant from *S. algae* (153). Similar observations were made for a *qnr*-like sequence in *Vibrio parahaemolyticus* (178). Although a *qnr*-like gene was not identified in *Vibrio cholerae* in the above-mentioned in silico analysis of other *Vibrio* spp. (153), another study examining a ciprofloxacin-resistant *V. cholerae* O1 clone from a cholera epidemic in Brazil identified a *qnr*-like gene encoding a protein with 69% sequence identity to that of the *P. profundum* Qnr-like peptide (65). The gene was designated *qnrVC1*, but according to recommended nomenclature (87), it might better be termed Vc*qnr1*. Vc*qnr1* had a G+C content of 36.8%, which is considerably different from that of the *V. cholerae* genome (47.5%), supporting introduction through horizontal gene transfer. It also has an affiliated *aatC* recombination site, unlike other *qnr* genes, and is incorporated as a cassette into a chromosomal class 1 integron (65).

***qnrB*-like genes.** *qnrB12* was found in three epidemiologically and clonally unrelated *Citrobacter werkmanii* isolates of poultry origin from Germany (104). A chromosomal location of this gene appeared most likely for a number of reasons: repeated plasmid transformation and conjugation experiments failed, Southern blot hybridization studies of I-CeuI-digested genomic DNA gave a signal only with the largest I-CeuI fragment (approximately 800 kb) in each strain, and further S1 nuclease digestion followed by pulsed-field gel electrophoresis did not identify any large *qnrB*-carrying plasmid which might comigrate with this 800-kb I-CeuI fragment (104).

Sm*qnr*, identified in the genome of *Stenotrophomonas maltophilia* strain R551-3, codes for a 219-amino-acid protein that shares about 60% amino acid identity with QnrB. SmQnr has two domains of 5 and 28 pentapeptide repeats separated by a glycine (179, 182). An even closer homologue of *qnrB* has been found in DNA sequences in a marine metagenome, but its location on a bacterial chromosome or on a mobile genetic element is unknown (179).

***qnrS*-like.** In silico analysis of the genome sequence of *Vibrio splendidus* identified an open reading frame encoding a 218-amino-acid protein sharing 84% and 87% amino acid identities with QnrS1 and QnrS2, respectively. When overexpressed in *E. coli* cells, recombinant plasmids with the *V. splendidus qnr* genes conferred an eightfold increase in the MIC of nalidixic acid and 4- to 16-fold increases in the MICs of fluoroquinolones (25).

MECHANISM OF ACTION OF PENTAPEPTIDE REPEAT PROTEIN FAMILIES CONFERRING QUINOLONE RESISTANCE

The vast majority of the pentapeptide repeat-containing proteins currently listed in the Pfam database of the Wellcome Trust Sanger Institute (PF00805) (www.sanger.ac.uk/cgi-bin/Pfam) are found in prokaryotes. However, the function of nearly all of these proteins is unknown. Three pentapeptide repeat proteins are of particular interest because they confer some level of quinolone resistance. The detailed mechanism of action of the PMQR protein Qnr is still unknown. Some lessons have been learned from studies of analogous pentapeptide repeat proteins that also confer fluoroquinolone resistance: McbG and MfpA.

McbG

McbG is a pentapeptide repeat protein sharing 19.6% amino acid identity with Qnr. It protects DNA gyrase against the effect of a microcin (69). Microcins are a class of small inhibitory proteins (less than 10 kDa) that vary in their mechanisms of action. One of these, microcin B17 (MccB17), is a bacterial poison that, like the quinolones, inhibits DNA gyrase (76) (though at a different site than

that of the quinolones [144]). Organisms producing MccB17 also produce McbG, which protects them from the effect of this toxin, and *mcbG* has been found on resistance plasmids in clinical isolates (89). In host *E. coli* J53 cells, plasmid-mediated *mcbG* produced a slight increase in the MIC of sparfloxacin. Additionally, in the presence of *mcbG*, mutants with resistance to sparfloxacin arose at a concentration of sparfloxacin that was twofold higher than that for J53 R^-. Thus, the pentapeptide repeat protein McbG, like Qnr proteins, appears to provide some antiquinolone protection. The effect, however, was much smaller than that of the Qnr proteins (89). Also, McbG did not appear to affect the susceptibility of *E. coli* J53 to ciprofloxacin or nalidixic acid.

MfpA and $MfpA_{Mt}$

MfpA, a pentapeptide repeat protein having 18.9% amino acid similarity to QnrA, has been more thoroughly studied. The *mfpA* gene was first identified on the chromosome of *Mycobacterium smegmatis* (127). When expressed on a multicopy plasmid, this gene resulted in an increase in the MIC of ciprofloxacin for this organism of between four- and eightfold, and the inactivation of the gene on the *M. smegmatis* chromosome resulted in increased ciprofloxacin susceptibility. The three-dimensional structure of this gene variant in *Mycobacterium tuberculosis*, $MfpA_{Mt}$, demonstrated that the pentapeptide repeat sequence encodes a right-handed quadrilateral β-helix (77). $MfpA_{Mt}$ is notable among pentapeptide repeat proteins in that (like *qnrA*) the first residue in the pentapeptide (residue i^{-2}) is often a cysteine (203).

It has been shown that $MfpA_{Mt}$ inhibits ATP-dependent DNA supercoiling and ATP-independent relaxation reactions catalyzed by *E. coli* DNA gyrase. The apparent median inhibitory concentration (IC_{50}) values were calculated to be ~1.2 μM (based on an active dimer) for both reactions in an assay containing 3 units of DNA gyrase.

Surface plasmon resonance is a technique of profiling the binding and dissociation of molecules. In brief, molecules immobilized on a sensor surface alter the refraction of polarized light. When a sample is passed over the sensor surface, and molecules interact with the immobilized molecules, the degree of light emission changes in proportion to the mass of bound material. Experiments employing this methodology indicated that MfpA interacts directly with DNA gyrase. Both the three-dimensional structure of this MfpA homologue and its charge distribution closely resemble those of B-form DNA. Based on these data, it was proposed that MfpA binds DNA gyrase in place of DNA. This displacement may generate some resistance to fluoroquinolones, as DNA gyrase bound to MfpA will not participate in the deleterious quinolone-gyrase-cleaved DNA complex that is the basis for quinolone cell killing (77).

Qnr Proteins

Transconjugants of *E. coli* with *qnrA* do not demonstrate changes in quinolone accumulation, outer membrane porins, or drug inactivation (197); none of these are the mechanism of Qnr activity. Instead, the mechanism by which QnrA protects DNA gyrase from quinolones appears similar, but not identical, to that of MfpA.

The two quinolone targets, the type II topoisomerase enzymes DNA gyrase and topoisomerase IV, regulate conformational changes in DNA topology by catalyzing the breakage and rejoining of DNA strands during normal cellular growth (53, 207). The main function of DNA gyrase is to catalyze the ATP-dependent negative supercoiling of DNA, a unique property of this enzyme that is necessary for the initiation of DNA synthesis, which can be measured in a DNA supercoiling assay (84). In contrast, the main role of topoisomerase IV is to decatenate daughter replicons, measured in the decatenation of kinetoplast DNA (54). Both enzymes are heterodimers; DNA gyrase is composed of two A subunits and two B subunits, and topoisomerase IV is also an A_2B_2 enzyme composed of two ParC and two ParE subunits. The ParC subunit is homologous to GyrA (29% amino acid identity in *E. coli*), and ParE is homologous to GyrB (52).

Quinolones inhibit gyrase-mediated DNA supercoiling and topoisomerase IV-mediated DNA decatenation (71, 106). Purified N- or C-terminal histidine-tagged QnrA reversed this quinolone inhibition of DNA gyrase and topoisomerase IV activities in a dose-dependent manner. The median concentration of QnrA required to protect against the DNA gyrase-inhibitory action of 1.5 μM (0.5 μg/ml) ciprofloxacin was ~0.32 μM (197–199) (0.93 nM DNA gyrase, which corresponded to ~2 units). $QnrA\text{-}His_6$ alone did not itself effect DNA supercoiling (197), nor did it inhibit DNA supercoiling even at a high concentration, unlike MfpA (77). $QnrB1\text{-}His_6$ or $QnrB4\text{-}His_6$ could also protect DNA gyrase in vitro, although at high concentrations, an inhibitory effect was seen (91, 125).

The mechanism of the Qnr protective effect is not completely understood. It has been shown through gel retardation assays that QnrA can bind to the DNA gyrase holoenzyme as well as to its respective subunits, GyrA and GyrB. This binding occurred in the absence of relaxed DNA, ciprofloxacin, or ATP, indicating that the binding of QnrA to gyrase did not require the presence of the ternary complex of enzyme, DNA, and quinolone (198). Similar findings were also reported for QnrA and *E. coli* topoisomerase IV (199). DNA filter binding assays have shown that DNA binding of gyrase decreased when the gyrase was exposed to Qnr (198). Thus, it was proposed that QnrA protection from quinolones is the result of QnrA binding to gyrase or topoisomerase IV at a site overlapping the DNA binding site. However, it is not clear how, in contrast to MfpA, QnrA might compete with DNA for gyrase binding without functionally inhibiting gyrase activity in vitro.

Recent data cast further doubt on the functional similarity between $MfpA_{Mt}$ and Qnr proteins. The effect of purified $MfpA_{Mt}$ and QnrB4 was investigated using various catalytic and noncatalytic type II topoisomerase enzyme assays (125). Histidine-tagged $MfpA_{Mt}$ inhibited the catalytic activity of *M. tuberculosis* gyrase; IC_{50}s for supercoiling, relaxation, and decatenation were 1.75 μM, 2 μM, and 2 μM, respectively, similar to previously reported results (77). $MfpA_{Mt}$ also inhibited DNA supercoiling mediated by the *E. coli* gyrase, with an IC_{50} of 3 μM. In contrast, histidine-tagged QnrB4 did not inhibit the catalytic activity of *E. coli* or *M. tuberculosis* gyrase (unless concentrations were over 30 μM). In addition to suggesting that $MfpA_{Mt}$ and QnrB4 interact with DNA gyrases in a dif-

TABLE 1. In vitro activity of quinolones against wild-type *E. coli* strains carrying PMQR genes

Agent	MIC susceptibility breakpoint[a] (μg/ml)	MIC (μg/ml)									
		E. coli J53 (wild type)	*E. coli* DH10B (wild type)	*E. coli* KF130 (*gyrA*[r] mutant)[b]	*qnrA1*[c,d]	*qnrS1*[c,e]	*qnrB1*[c,f]	*qnrC*[c,g]	*qepA2*[h]	*qnrD*[i,j]	*aac(6′)-Ib-cr*[i,k]
BAYy31180		0.004			0.125						
Ciprofloxacin	≤1	0.008	0.002	0.25	0.25	0.25	0.25	0.25	0.12	0.06	0.004–0.008
Garenoxacin		0.008			1						
Gatifloxacin	≤2	0.008			0.25		0.5		0.12		
Gemifloxacin	≤0.25	0.004			0.5						No change
Ofloxacin	≤2		0.015							0.125	
Levofloxacin	≤2	0.015		0.5	0.5	0.38	0.5	0.25	No change		No change
Moxifloxacin		0.03			0.5		1		0.12		
Nalidixic acid	≤16	4	2	≥256	16	16	16	16	No change	4	
Norfloxacin		0.06	0.16						1		0.64
Premafloxacin		0.03		2	0.25						
Sitafloxacin		0.008			0.125						
Sparfloxacin		0.008		0.25	1						

[a] According to CLSI standards (43).
[b] See reference 122. KF130 is a KL16 derivative (81). Information added for comparison of effects on MIC.
[c] *E. coli* J53 transconjugant.
[d] See reference 211.
[e] MICs for *qnrS1* were determined using pAH0376 (75) and pHS8 (85) transferred into HB101 and J53, respectively.
[f] See reference 91.
[g] See reference 208.
[h] *E. coli* J53 transformant. See reference 28.
[i] *E. coli* DH10B transformant.
[j] See reference 32.
[k] See reference 165.

ferent manner, this work also indicates that the mechanism of these proteins may be gyrase specific: QnrB4 protected *E. coli* but not *M. tuberculosis* gyrase from the inhibitory effect of fluoroquinolones; $MfpA_{Mt}$ protected *M. tuberculosis* but not *E. coli* gyrase. Similarly, QnrA does not protect against the inhibition of gyrase by the GyrB-targeting agent novobiocin or MccB17 (G. A. Jacoby and D. C. Hooper, unpublished data).

RESISTANCE ACTIVITY OF Qnr

Effect on MIC

In general, the acquisition of a *qnrA*-bearing plasmid will not render a wild-type organism fluoroquinolone insusceptible according to CLSI clinical breakpoints. The extent to which QnrA protects isolates of *Enterobacteriaceae* against fluoroquinolones has usually been examined by measuring the difference in quinolone MICs 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 (212) to 2.0 μg/ml (211) 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 harboring these plasmids all had exhibited higher levels of resistance to quinolones than the transconjugants, suggesting that additional mechanisms of quinolone resistance frequently coexist with *qnrA*. There were also differences among transconjugants in the *qnrA* effect on fluoroquinolone MICs. Although for most agents, the presence of a *qnr* plasmid increased their MIC by between 16-fold and 125-fold, this increase was less (16-fold to 32-fold) for sitafloxacin. The agent for which the loss of activity was least pronounced was nalidixic acid (twofold to eightfold increases in MIC) (211). Illustrating this phenomenon, Hopkins et al. found that in non-Typhi *Salmonella* isolates, a phenotype of reduced susceptibility to ciprofloxacin (MIC > 0.06 μg/ml) but preserved susceptibility to nalidixic acid (MIC ≤ 16 μg/ml) identified *qnr*-positive strains (82, 83). Also noteworthy is the finding that some *qnrA*-carrying plasmids from U.S. *K. pneumoniae* isolates yielded transconjugants with very similar quinolone susceptibilities (211), whereas other *qnrA*-carrying plasmids from U.S. and Chinese isolates of *Enterobacteriaceae* varied in ciprofloxacin susceptibilities by 16-fold (212). There are several reasons for this phenomenon. In some cases these differences resulted from the presence of an additional resistance determinant, *aac(6′)-Ib-cr*, on some plasmids (165, 220). For other strains, the copy number and especially the transcriptional level of the *qnr* genes affected quinolone resistance (174, 220).

qnrS- and *qnrB*-carrying plasmids confer quinolone resistance that is similar to that conferred by *qnrA1* (Table 1). When cloned into a derivative of *E. coli* DH10B, *qnrS1* increased the MICs of nalidixic acid, ciprofloxacin, and ofloxacin 8-, 83-, and 24-fold, respectively. These changes led only to nalidixic acid resistance by CLSI breakpoints (26). The impact of some *qnr* allele variants on quinolone MICs has additionally been examined. Overall, the patterns of resistance have been similar, with 2- to 8-fold and 8- to 32-fold increases in MICs of nalidixic acid and ciprofloxacin, respectively (17, 23, 70, 91, 126, 161, 166).

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 activities of ciprofloxacin and ofloxacin in the presence of QnrA. Despite the fact

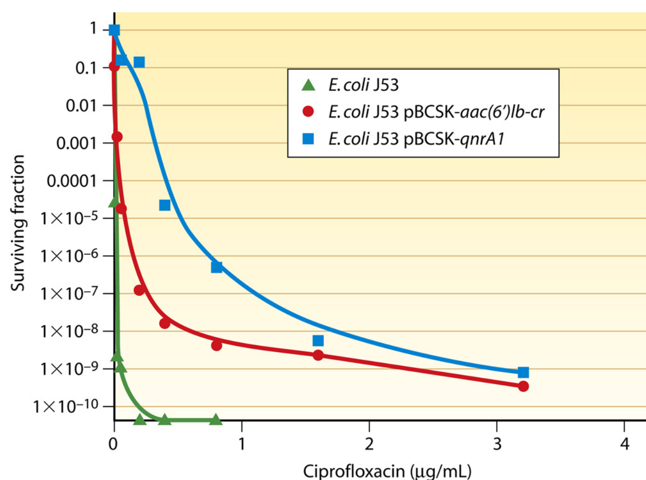

FIG. 2. MPC assay. About 10^{10} organisms and appropriate dilutions were applied onto Mueller-Hinton agar plates containing the indicated concentrations of ciprofloxacin. Surviving colonies were counted after incubation for 72 h at 37°C. The lowest concentration of ciprofloxacin at which no mutant colonies were seen was 0.2 μg/ml for J53 and 3.2 μg/ml for J53 pBC SK-*aac(6′)Ib-cr* or J53 pBC SK-*qnrA1.* (Reprinted from reference 163 with permission from Elsevier.)

that QnrA protects against quinolone growth inhibition, it did not block the bactericidal activity of these quinolones at concentrations of twice the MIC or greater (119, 169).

Effect on Mutant Prevention Concentration

Another measure of resistance gene effect is a change in the mutant prevention concentration (MPC). The MPC is the lowest 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 presence of single-step mutants occurring at a low frequency). When the quinolone concentration remains above the MPC, single-step resistant mutants are unlikely to arise. The early finding that QnrA facilitated the 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 that is more than 10-fold greater (88) (Fig. 2). Thus, the low-level resistance conferred by these mechanisms might not allow a population of bacteria to survive in the presence of a quinolone. However, it substantially enhances the number of strains with resistance mutations that can be selected from the population, as also occurs with chromosomal quinolone resistance mutations. In the case of QnrA, this phenomenon has been experimentally shown both for *E. coli* (123, 173) and for *Enterobacter* spp. (164) and probably holds true for other genera as well. Indeed, data from a pharmacodynamic model suggested that *Providencia stuartii* with *qnrA* (but not without it) is insufficiently killed by a large single ciprofloxacin dose and rapidly acquires resistance (215).

Effect on In Vivo Activity of Quinolones

By increasing the MIC and widening the mutant selection window (55), Qnr may thus lead to decreased therapeutic efficacy. Rodriguez-Martinez et al. recently demonstrated this effect in a murine model of *K. pneumoniae* pneumonia (169). The *K. pneumoniae* strain used in that study lacked the porins OmpK35 and OmpK36, had an active efflux system for quinolones, and carried a resistance mutation in *gyrA* and therefore magnified the protective effect of *qnrA1* to the maximum. All 30 animals infected with the *qnrA* mutant organism and treated with a fluoroquinolone survived. In contrast, the survival rate among 30 animals infected by a pMG252 (*qnrA*$^+$) transconjugant of that strain was 53% ($P < 0.03$). Similarly, the clearance of bacteria from the lungs was affected by *qnrA*; the $\log_{10}$ CFU/g of lung in untreated, *qnrA*$^+$ strain-infected, and *qnrA*-negative strain-infected mice were ca 9.16, 7.74, and 3.53, respectively ($P < 0.001$) (169). Whether *qnr* would have a similar effect on a strain without enabling mutations is not yet known.

Interaction between Qnr and Other Quinolone Resistance Mechanisms

qnrA has frequently been observed in the company of other resistance mechanisms in clinical strains and is capable of acting additively with these mechanisms. pMG252, the plasmid on which *qnrA1* 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 (92, 122, 173). The presence of *qnrA* was found to supplement all types of mutation-based resistance. Interestingly, the presence of a *qnr* gene may affect the distribution of chromosomal resistance mutations that are selected in the presence of fluoroquinolones. In a recent study, quinolone-resistant mutants were selected from wild-type *E. coli* strains and corresponding transconjugants harboring *qnrA1*, *qnrA3*, *qnrB2*, or *qnrS1* (34). Although the proportion of resistant mutants selected was the same, fewer *qnr*-positive transconjugants yielded clones with a quinolone resistance-determining region mutation. The cause of this change in mutant distribution is not known but might be explained if Qnr binding results in alterations in the positioning of quinolones in relation to the gyrase-DNA complex (34).

Resistance Activity of a Combination of Qnr Proteins

A few studies have found bacteria harboring more than one *qnr* gene. This occurrence has been usually but not exclusively *qnrS* with either *qnrB* or *qnrA* (27, 85, 110, 217). Whether multiple Qnr proteins have an additive effect on the MIC is unclear. In one example of the cooccurrence of *qnrB4* and *qnrS1*, transconjugation experiments showed that the effect of both genes on ciprofloxacin MIC was the same as that of one alone, suggesting that the two gene products may compete for binding to gyrase (27, 85).

Qnr PLASMIDS

Genes for quinolone resistance have been found on plasmids varying in size and incompatibility specificity (Table 2), indicating that the spread of multiple plasmids has been responsible for the dissemination of this resistance around the world. The immediate genetic environment of each gene type, how-

TABLE 2. Representative PMQR plasmids

Plasmid	PMQR gene	Host	Yr of isolation	Size (kb)	Inc group	Country	Linked *bla* gene[a]	Reference(s) or source
pMG252	*qnrA1*	*K. pneumoniae*	1994	~180		United States	FOX-5	123
pHSH2	*qnrA1*	*E. coli*	2000–2001	85		China		212
pQR1	*qnrA1*	*E. coli*	2003	180	A/C_2	France	VEB-1	120, 157
	qnrA1	*E. cloacae*	2002–2005	75	H12	France	SHV-12	152, 157
	qnrA1	*E. aerogenes*	2002–2005	150	FII	France	SHV-12	152, 157
pQC	*qnrA1*	*E. cloacae*	2002	180		The Netherlands	CTX-M-9	136, 137
pKO97	*qnrA1*	*E. coli*	2001–2003			South Korea		93
	qnrA3	*S. enterica* serovar Enteritidis	2003			Hong Kong	CTX-M-14	38, 42
	qnrA6	*P. mirabilis*	2002–2005			France	PER-1	17
pMG298	*qnrB1*	*K. pneumoniae*	2002–2003	340		India	CTX-M-15	91
	qnrB2	*E. coli*	2004	55		Brazil		126
pJIBE401	*qnrB2*	*K. pneumoniae*	2003	>150	L/M	Australia	IMP-4	59, 60
	qnrB2	*S. enterica* serovar Keurmasser	2000			Senegal	SHV-12	68
pMG317	*qnrB3*	*E. coli*	1999–2004	75		United States		166
pMG319	*qnrB4*	*E. cloacae*	1999–2004	200		United States	DHA-1	166
pPMDHA	*qnrB4*	*K. oxytoca*	2002	(Tra^-)		France	DHA-1	202
	qnrB4	*E. cloacae*	—[b]	119		China	DHA-1	223
pMG305	*qnrB5*	*S. enterica* serovar Berta	1997	80		United States		70
pHND2	*qnrB6*	*K. pneumoniae*	2006			China	CTX-M-9G[c]	116
	qnrB7	*E. cloacae*	2002–2004			Kuwait	SHV-12	29
	qnrB8	*C. freundii*	2002–2004			Kuwait	VEB-1b	29
pCF41	*qnrB9*	*C. freundii*	—[b]			China		GenBank accession no. EF526508
pARCF702	*qnrB10*	*C. freundii*	2005			Argentina		161
pR4525	*qnrB19*	*E. coli*	2002	40		Columbia	SHV-12 CTX-M-12	23
pLRM24	*qnrB19*	*K. pneumoniae*	2007	80		United States	KPC-3	58, 162
	qnrB19	*S. enterica*	1999–2006		N	The Netherlands		67
pAH0376	*qnrS1*	*S. flexneri*	2003	~50		Japan		75
pINF5	*qnrS1*	*S. enterica*	2004	58		Europe		103
TP*qnrS*-2a	*qnrS1*	*S. enterica*	2004	44	N	United Kingdom		83
pK245	*qnrS1*	*K. pneumoniae*	2002	98		Taiwan	SHV-2	37
	qnrS1	*K. pneumoniae*	2004–2006		L/M	South Korea		190
	qnrS1	*S. enterica*	1999–2006	>250	HI2	The Netherlands	LAP-2	67
	qnrS1	*S. enterica*	1999–2006		R	The Netherlands		67
pGNB2	*qnrS2*	—[d]	2004	8.5	Q	Germany		14
p37	*qnrS2*	*A. punctata*	2006	55 (Tra^-)	U	France		24
pGD007	*qnrS3*	*E. coli*						226
	qnrS4	*S. enterica*	2007			Denmark		195
pHS10	*qnrC1*	*P. mirabilis*	2006	~120		China		208
p2007057	*qnrD1*	*S. enterica*	2006–2007	4.3		China		32
pHSH10-2	*aac(6')-Ib-cr*	*E. coli*	2000–2001			China		165
	aac(6')-Ib-cr	*E. coli*	2003–2006	150	FII	United Kingdom	CTX-M-15	102
pC15-1a	*aac(6')-Ib-cr*	*E. coli*	2000–2002	92	FII	Canada	CTX-M-15	15
p34	*aac(6')-Ib-cr*	*A. allosaccharophila*	2005	80		Switzerland		149
pHPA	*qepA1*	*E. coli*	2002		FII	Japan	CTX-M-12	222
pIP1206	*qepA1*	*E. coli*	2000–2005	168	FI	Belgium		148
pQep	*qepA2*	*E. coli*	2007	90 (Tra^-)	FI	France		28
pOLA52	*oqxAB*	*E. coli*	—[b]	52	X1	Denmark		134, 186

[a] Only unusual *bla* genes are shown.
[b] Not specified.
[c] CTX-M-9 group. See reference 116 for details.
[d] Unidentified bacteria in activated sludge.

ever, is similar enough to suggest a limited number of acquisition events followed by transposition, recombination, replicon fusion and resolution, and deletion and insertion of DNA to generate the diversity of plasmid structures seen today. Sometimes the plasmids are so similar that a broad dissemination of the same plasmid does appear likely.

For example, *qnrA1* plasmids can vary in size from 20 to 320 kb (17, 112) and belong to at least three plasmid incompatibility (Inc) groups (157). *qnrA1* is usually associated with IS*CR1* (formerly *orf513*) (193), although 6.2% of *qnrA1*-positive strains in a study from South Korea were negative for IS*CR1* by PCR (142). Usually, a single copy of IS*CR1* is found downstream from *qnrA1*, but in pMG252 and related plasmids, the *qnrA1* gene is bracketed by two copies of IS*CR1* (163, 172). The *qnrA1* IS*CR1* complex is inserted in turn into a *sul1*-type integron containing several other resistance gene cassettes. Some examples are shown in Fig. 3. Genes for extended-spectrum and AmpC β-lactamases are often found on the same

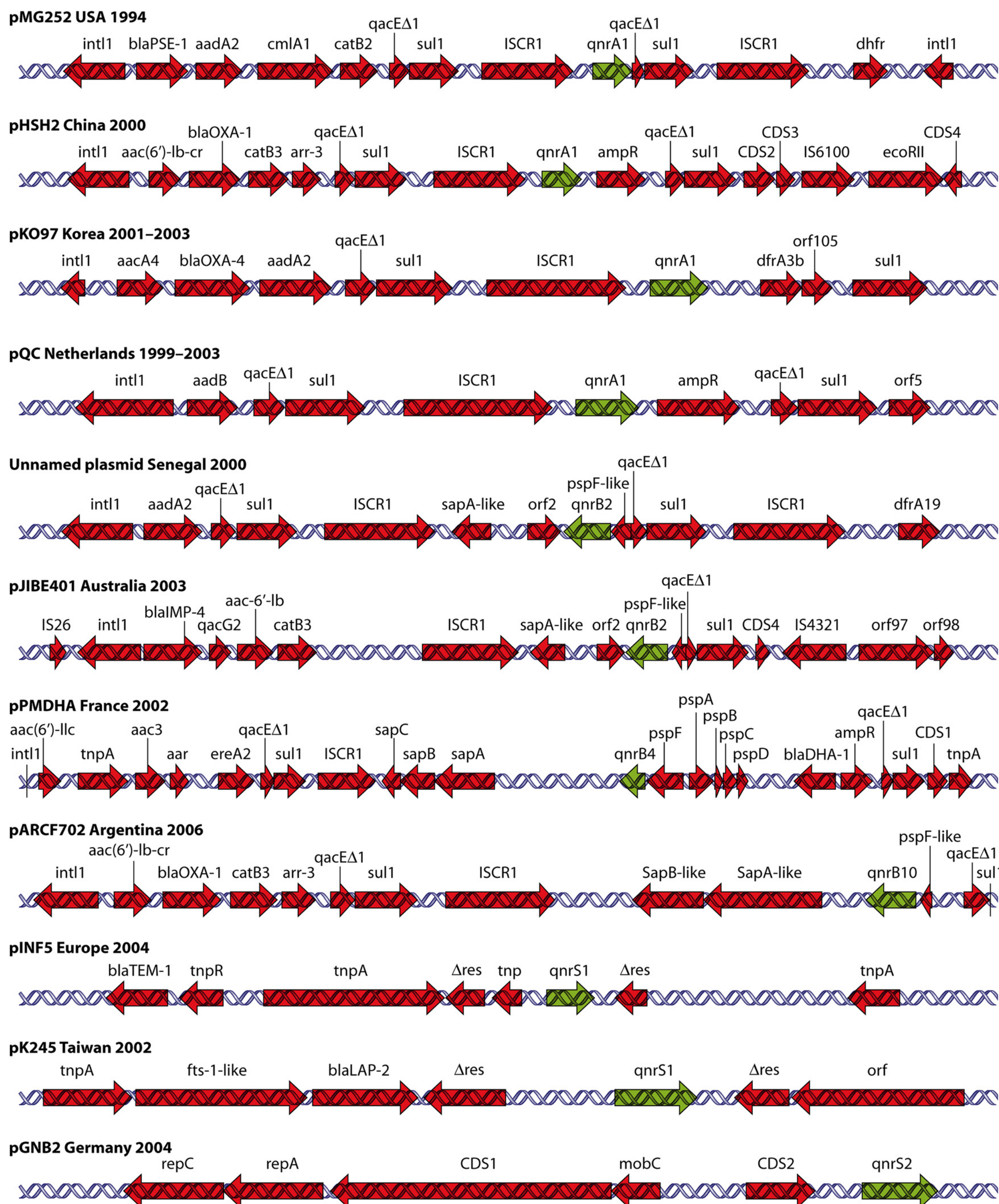

FIG. 3. Genetic environment of plasmid-determined *qnrA*, *qnrB*, and *qnrS* alleles. See Table 2 for references.

plasmid. *qnrA1* was discovered in an isolate from Alabama on plasmid pMG252 also expressing the uncommon FOX-5 β-lactamase. Plasmids carrying *qnrA1* and FOX-5 have subsequently been found in specimens from Delaware, Kentucky, New York, North Carolina, and Tennessee (168, 210) and from as far away as Brazil (21). In isolates from Canada, France, Thailand, and Turkey, *qnrA1* is associated with VEB-1 β-lactamase (120, 131, 154, 156), while in samples from other countries, *qnrA1* and extended-spectrum β-lactamase (ESBL) SHV-12 and various CTX-M enzymes are linked (17, 30, 47, 56, 95, 111, 112, 136, 138, 152, 170, 189, 190, 212, 217, 223).

qnrB1 was discovered on a 340-kb multiresistance plasmid from India (91) and was subsequently reported for isolates from Algeria (86), Denmark (195), South Korea (142), Nigeria (185), and Scotland (130). It is associated not with IS*CR1* but with another putative transposase, Orf1005 (91, 185). The *qnrB2* allele has an even wider distribution, having been found originally in isolates from the United States (91) and subse-

quently from China (223), Brazil (126), Hungary (189), Israel (40), South Korea (142), The Netherlands (200), Portugal (158), Scotland (130), Senegal (68), Switzerland (113), Taiwan (218), and the United Kingdom (82). *qnrB2* is linked to a single copy of IS*CR1* in some plasmids (60), while in other plasmids, duplicate copies of IS*CR1* surround the gene (68) (Fig. 3). As with *qnrA1* plasmids, the IS*CR1* complex is inserted into a *sul1*-type integron. β-Lactamase genes associated with *qnrB2* include CMY-1 (138), CTX-M-14 (223), CTX-M-15 (113, 158), IMP-4 (60), IMP-8 (218), KPC-2 (40), and SHV-12 (91); *qnrB3* has been reported only from the United States (166), but *qnrB4* has a worldwide distribution and a strong linkage with the gene for the AmpC-type β-lactamase DHA-1, with plasmids in strains from China (223), France (202), South Korea (138, 190), Switzerland (113), Taiwan (218), and the United States (G. A. Jacoby, unpublished data) showing this association. The *qnrB4* allele has also been associated with, or linked to, the ESBLs SHV-12 (27, 138), TEM-52 (138), CTX-M-3 (95, 113), CTX-M-9 (113), CTX-M-14 (95, 138), and CTX-M-15 (113). In addition, *qnrB4* alleles are closely linked to eight genes or pseudogenes unrelated to antibiotic resistance: *sapA* and *sapB* (both peptide transport system permeases); *sapC*, *pspA*, *pspB*, *pspC*, and *pspD* (all phage shock proteins); and *pspF* (a transcriptional activator for the *psp* operon) (Fig. 3). *sapA*-like and *pspF*-like genes surround *qnrB1* (91), *qnrB2* (60, 68, 91), *qnrB10* (161), and probably other *qnrB* alleles, while the other six *sap* and *psp* genes appear to be missing.

qnrB5 has been found in *K. pneumoniae* (20, 138) but seems especially common in serovars of *S. enterica* (70, 82, 195). With the exception of *qnrB19*, the remaining *qnrB* alleles have been too recently described for much to be known about their plasmid locations. The *qnrB19* allele was found on a 40-kb plasmid from an *E. coli* strain isolated in Colombia, South America, in a transposon comprising IS*Ecp1* and *qnrB19* inserted into another transposon carrying a gene for tetracycline resistance (23). Within the year of its publication, *qnrB19* was reported for an *S. enterica* serovar Typhimurium strain from The Netherlands (67), commensal enterobacteria from children living in Bolivia and Peru (140), and a *K. pneumoniae* isolate on an 80-kb plasmid from the United States (58). In the U.S. isolate, a transposon composed of IS*Ecp1* and *qnrB19* and another one containing $bla_{\mathrm{KPC\text{-}3}}$ were inserted into a third transposon related to Tn*3*, forming what has been termed the KQ element (162).

Unlike *qnrA* and *qnrB*, *qnrS* genes are not associated with IS*CR1*, nor are they part of complex integrons. More *qnrS* plasmids are small and nonconjugative (14, 67, 105, 216, 217) than are *qnrA* or *qnrB* plasmids, and they are also more likely to be found in *S. enterica* serovars and not to carry ESBL or AmpC β-lactamase genes. In several *qnrS1* plasmids (103), including pAH0376, in which *qnrS* was discovered (75), the gene is found near Tn*3* encoding TEM-1 β-lactamase. In other plasmids of similar structure, the neighboring gene is $bla_{\mathrm{LAP\text{-}1}}$ (151), $bla_{\mathrm{LAP\text{-}2}}$ (67), or *mobABC* (105). An insertion sequence variably termed ΔIS*2* (105) or IS*Ec12* (151) lies downstream from *qnrS1*, and elements of IS*26* also surround it in plasmid pINF5 (105). The *qnrS2* allele has been found in two different genetic environments. In small plasmids in isolates from the United States (70) and Germany (14), open reading frames related to replication genes are located immediately downstream from *qnrS2*, while in plasmids from *Aeromonas* spp., *qnrS2* is part of a transposon-like structure inserted into a putative metalloprotease gene (24, 149).

A single ~120-kb *qnrC1* plasmid is currently known (208), while *qnrD1* is encoded by a 4.3-kb plasmid (32). Both were found in China.

ORIGINS OF *qnr* GENES

It would seem likely from the impressive number, variety, and geographical and bacteriological penetration of *qnr* variants that these or similar genes existed for a considerable time before the report of their discovery in 1998 (105, 123). This raises questions about from where these genes originated and what purpose they served before being recruited to protect bacteria from antimicrobial agents.

Postulating that *qnr* genes originated on the chromosome of an organism occupying a human, veterinary, or environmental reservoir, Poirel and colleagues (155) screened the genome sequences of 48 gram-negative species from a wide range of genera for *qnrA*. Variants of *qnrA* (*qnrA3* to *qnrA5*) were located on the chromosome of *Shewanella algae*. The quinolone MICs of this organism were four- to eightfold higher than those of *Shewanella putrefaciens*, a closely related organism lacking a chromosomal *qnrA* gene (155). These data suggest that *S. algae* is a possible reservoir of *qnrA*. *Shewanella* spp. are water dwellers present in both marine and freshwater environments. Recently, *qnrA3*-positive *Klebsiella pneumoniae* and *Kluyvera ascorbata* strains isolated from the feces of an immunocompromised outpatient in Paris were analyzed (109). The sequence immediately downstream from *qnrA3* was identical to that found downstream from *qnrA3* in the *S. algae* chromosome, supporting the notion that *qnrA3* has been excised from chromosomal DNA of *S. algae* or similar organisms (109).

As noted previously, pentapeptide repeat proteins showing 40 to 67% amino acid identity to *qnrA* were present in other waterborne *Shewanella* spp. (153, 178). Additional studies found *qnrB*-like and *qnrS2* genes in water and other environmental isolates.

Sánchez et al. and Venter et al. found a gene encoding a pentapeptide repeat in a sequence database of microbial populations collected en masse from seawater samples collected from the Sargasso Sea (179, 201). The protein, designated marine metagene Qnr (GenBank accession number AACY020347520), was 88% similar to QnrB5 and QnrB19, but its functionality was not examined. We note that sequence analysis shows that metagene *qnr* is 98% similar to *qnrB8*, and the first 214 amino acids are 99% identical to QnrB8. In addition, BLAST analysis revealed the same *sapA* sequence that is closely linked to *qnrB* genes on plasmids (see above) downstream of metagene *qnr*, further supporting the aquatic origin of this PMQR determinant.

qnrS2 genes have also been found on plasmids carried by environmental organisms. In 2006, Cattoir et al. sampled water from urban sites in the Seine river and found *Aeromonas punctata* subsp. *punctata* and *Aeromonas media* strains carrying plasmids that transferred quinolone resistance (24). These plasmids were shown to carry *qnrS2* (24). The same gene has also been found in plasmids from *Aeromonas allosaccharophila*

found in Lake Lugano (a Swiss-Italian vacation area) in 2005 (149) and, together with a Tn*1721*-like transposon, on plasmid pGNB2 in sludge basin bacteria (from a German wastewater treatment plant) in 2004 (14). This plasmid also conferred decreased susceptibility only to quinolones (14).

Collectively, these findings suggest that some *qnr* genes in circulation likely originated in the chromosomes of water-dwelling environmental organisms. The recent detection of *qnr*-bearing plasmids in water organisms suggests that freshwater in inhabited areas may be a reservoir in which pathogens acquire these elements. Quinolones are excreted unmetabolized by mammals into wastewater. The carboxylic acids of quinolones are degradable by sunlight in aqueous solution (194), but when these compounds enter the aquatic environment via sewage water, photodegradation may be of only minor importance. Elimination can also occur via adsorption to sediments, degradation by terrestrial fungus (145), or environmental *Mycobacterium* spp. (3), but a substantial quantity of active drug may remain in the environment (5). For example, ciprofloxacin and other fluoroquinolones have been found at concentrations of up to 0.005 μg/ml in water sources, including water downstream from a wastewater treatment plant in the United States and in the Seine River in France (13, 191). Thus, it is possible that environmental quinolone accumulation has contributed to the success of these genes, perhaps by helping to maintain a reservoir of aquatic organisms for which a low-level quinolone resistance gene provided a survival advantage.

BIOLOGICAL FUNCTIONS OF *qnr*

The native function of *qnr* genes is unknown. Ellington and Woodford postulated that Qnr could be an antitoxin, protecting DNA gyrase and topoisomerase IV from some naturally occurring toxins (57). There are known natural DNA gyrase toxins. These include CcdB, a toxin encoded on the F plasmid (49), ParE (distinct from the ParE subunit of topoisomerase IV), located on the broad-host-range RK2 plasmid (94), and MccB17, a posttranslationally modified peptide produced from the plasmid-borne MccB17 operon (76, 144). Each of these toxins is paired with an inhibitor (antitoxin) that protects cells from death and is encoded in the same operon: CcdA (49), ParD (94), and McbG (which is in fact a pentapeptide repeat protein) (76, 175). Qnr may thus be analogous to one of these antitoxins. However, the hypothetical toxin against which Qnr protects has not been demonstrated. Qnr may alternatively serve a function similar to that of the chromosomally encoded non-pentapeptide-repeat protein GyrI, a DNA gyrase regulator that also is capable of some antitoxin and antiquinolone effect (35, 36).

Notably, plasmid-carried *qnrB* alleles have been shown to have upstream LexA binding sites, leading to increased *qnrB* expression levels upon exposure to quinolones, mitomycin, and possibly other DNA-damaging agents as part of the SOS response (118, 209, 225). Thus, if such induction is also seen in an organism with a chromosomal *qnrB* progenitor, it suggests that QnrB may have a native function in protection from naturally occurring DNA-damaging agents.

AAC(6′)-Ib-cr

Discovery of *aac(6′)-Ib-cr*

Several years after the discovery of QnrA, our group was investigating the phenomenon of inequality in the level of quinolone resistance transferred with different *qnr* plasmids. Wild-type *E. coli* strains have an MIC of ciprofloxacin of about 0.008 μg/ml, and most *qnr* plasmids determine an MIC of ciprofloxacin of 0.25 μg/ml for *E. coli*. We noted that certain plasmids from clinical *E. coli* strains collected in Shanghai provided about fourfold-higher levels of ciprofloxacin resistance (1.0 μg/ml). We found that this high-level resistance was not caused by an increased level of expression of *qnrA*, as it has been with other plasmids (174, 220). By random transposon mutagenesis of plasmid DNA, we found that the gene responsible for the incremental resistance was an aminoglycoside acetyltransferase, *aac(6′)-Ib*, which confers resistance to tobramycin, amikacin, and kanamycin (165). Sequencing showed this allele to be unique among the approximately 30 known variants of *aac(6′)-Ib* in two codon changes, Trp102Arg and Asp179Tyr, which 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 acetylate ciprofloxacin at the amino nitrogen on its piperazinyl substituent (165).

Resistance Activity of AAC(6′)-Ib-cr

The increase in MIC conferred by AAC(6′)-Ib-cr is smaller than that conferred by Qnr proteins, and as predicted by its specific quinolone target, it was selective only for ciprofloxacin and norfloxacin, which both have piperzinyl secondary amines. Other quinolones lacking an unsubstituted piperazinyl nitrogen were unaffected. Although the increase in the MICs of ciprofloxacin and norfloxacin was modest (threefold to fourfold), the effect on the MPC was marked (Fig. 2). In the presence of *aac(6′)-Ib-cr*, resistant clones of wild-type *E. coli* strain J53 could still be recovered at concentrations of 1.6 μg ciprofloxacin per ml, a level approximating the peak serum concentration of free ciprofloxacin during therapy (165).

AAC(6′)-Ib-cr Protein

Kinetic studies of purified AAC(6′)-Ib and its cr variant indicated that the mutant enzyme had only slightly reduced efficiency (relative to that of the wild-type enzyme) for the acetylation of kanamycin (204). The acetylation of ciprofloxacin, although less efficient than that of kanamycin, was sufficient in bacterial cells to produce a reduced-susceptibility phenotype equivalent to that of cells exposed to chemically synthesized *N*-acetyl ciprofloxacin (165), suggesting that complete ciprofloxacin acetylation had occurred under conditions of bacterial growth. Dead-end quinolone (pefloxacin) and aminoglycoside (lividomycin) substrates, for which the target sites of drug acetylation are either blocked (pefloxacin) or absent (lividomycin), both produced a competitive inhibition of acetylation of the true substrates kanamycin and ciprofloxacin, indicating a functional overlap in the binding sites for both classes of substrate of AAC(6′)-Ib-cr (204).

The X-ray crystallographic structure of the wild-type enzyme with kanamycin and acetyl coenzyme A has been solved and allowed the construction of a molecular model of ciprofloxacin binding to the cr variant (204). In the model, the Asp179Tyr mutation had the greatest effect, resulting in a π stacking interaction with the quinolone rings to enhance drug binding. The Trp102Arg mutation was more distantly positioned, serving to stabilize the positioning of Tyr179. This model is consistent with the magnitude of the effects of the individual mutations, with Asp179Tyr having a partial resistance phenotype, Trp102Arg having little detectable resistance phenotype, and the two mutations together having the full resistance phenotype (165). An alternative model of positioning of ciprofloxacin that emphasizes plasticity in the enzyme active site proposes that Arg92 (equivalent to Arg102) has a direct interaction with the carboxyl group of ciprofloxacin (124) but does not account for the relative effects of the two individual mutations. A direct structural analysis of AAC(6′)-Ib-cr with both acetyl coenzyme A and ciprofloxacin substrates is needed.

Genetic Environment of *aac(6′)-Ib-cr* Plasmids

aac(6′)-Ib-cr, like its parent *aac(6′)-Ib*, is in an integron cassette with an associated *attC* site. It is hence found in various integrons, some of which are shown in Fig. 3, but especially on IncF11 plasmids expressing CTX-M-15 that have spread rapidly so that CTX-M-15 has become the predominant ESBL in many countries around the world (15, 46, 63, 102, 117, 139, 150, 158, 176). *aac(6′)-Ib-cr* has been associated with other PMQR genes including *qnrA1* (95, 165, 220), *qnrB2* (158, 223), *qnrB4* (95, 116), *qnrB6* (95, 116), *qnrB10* (161), *qnrS1* (95, 116), *qnrS2* (149), and *qepA* (116) and with other β-lactamases including CTX-M-1 (185), CTX-M-14 (95), CTX-M-24 (95), DHA-1 (116), SHV-12 (116), and KPC-2 (39).

Plasmid-Mediated Quinolone Efflux

Antimicrobial efflux mechanisms can act on single or multiple agents and can be carried on both plasmids and chromosomes. Numerous such mechanisms have been described, and they are increasingly being recognized as important determinants of antimicrobial resistance in a variety of organisms (160). Efflux determinants of quinolone resistance in gram-negative bacteria are largely multidrug transporters of the resistance-nodulation-cell division (RND) family encoded by endogenous chromosomal genes (159). This family of traditionally chromosomal efflux pumps is ubiquitous in gram-negative bacteria, serving to remove unwanted compounds from the cytoplasm and membrane. Two plasmid-mediated quinolone transporters have now been found: OqxAB and, more recently, QepA.

OqxAB

A conjugative plasmid, pOLA52, conferring resistance to the antibiotic olaquindox (a quinoxaline derivative that is used in agriculture as a veterinary growth promoter) was found in *E. coli* strains isolated from swine manure (186). The resistance mechanism was identified to be a multidrug efflux pump, OqxAB, which belongs to the RND family (73). It confers resistance to other agents as well, including chloramphenicol. Upon expression in an *E. coli* strain lacking a native *acrA* gene, pOLA52 conferred 8- and 16-fold increases in the MICs of nalidixic acid and ciprofloxacin, respectively (72). In the single prevalence study performed, OqxAB was rare. Ten of 556 (1.8%) *E. coli* strains isolated between 1995 and 1998 in Denmark and Sweden were shown to have an MIC of olaquindox of ≥64 μg/ml; in 9 of the 10 strains, the *oqxA* gene was detected (74). Plasmid-mediated OqxAB was recently detected in a human clinical *E. coli* isolate from South Korea. *oqxAB* genes are also present on the chromosome of *K. pneumoniae*, with different levels of expression being correlated with differences in susceptibility to olaquindox (108).

QepA

The novel efflux pump QepA was found to be encoded on plasmid pHPA, discovered in an *E. coli* strain from a urine specimen from an inpatient in Hyogo Prefecture, Japan, in 2002 (222). This plasmid displayed a multiple-resistance profile for aminoglycosides, fluoroquinolones, and broad-spectrum β-lactams. *qepA* encoded a 511-amino-acid protein putatively belonging to the 14-transmembrane-segment major facilitator superfamily of transporters. The G+C content of QepA is 72%, higher than that of the chromosomes of *Enterobacteriaceae* (50%). Interestingly, according to phylogenetic analysis, QepA belongs to the 14-transmembrane-segment family transporters of gram-positive *Actinomycetales* but not those of gram-negative bacteria (222). Yamane and coworkers found that *qepA* cloned into pSTV28 increased the MICs of several compounds in an *E. coli* transconjugant (222). The MICs of nalidixic acid, ciprofloxacin, and norfloxacin increased 2-, 32-, and 64-fold, respectively; a twofold increase was observed for erythromycin, acriflavine, and ethidium bromide, whereas the MICs of other antibiotic classes and common transporter substrates did not change (222). Since its discovery, a variant of *qepA* possessing two amino acid substitutions has been found. This variant (named QepA2) conferred a phenotype similar to that of the QepA determinant (now renamed QepA1) (28).

Sequence analysis revealed that the *qepA* gene is located in a ~10-kb region, with a flanking sequence that is in large part identical in different plasmids (28, 147, 222). However, *qepA1*-positive isolates from Japan (221, 222), Belgium (148), and South Korea (41, 143) were flanked by two copies of IS*26* and associated with the *rmtB* gene, encoding an aminoglycoside ribosomal methylase, whereas *qepA2* was flanked by a novel insertion sequence element (IS*CR3C*), and *rmtB* was not found (28).

Genetic Environment of Efflux-Encoding Plasmids

The only *oqxAB* plasmid analyzed to date is a 52-kb IncX1 plasmid from an *E. coli* isolate from Denmark (186) that has been completely sequenced (134). *qepA1* has been found on 113- and 168-kb IncF1 plasmids from Belgium (147, 148), while *qepA2* was discovered on a 90-kb IncF1 plasmid from France.

EPIDEMIOLOGY OF PMQR

Methods of Detection of PMQR

As a rule, the resistance phenotype does not distinguish between PMQR and other resistance mechanisms. PMQR genes confer low-level quinolone resistance that is below the CLSI breakpoint for nonsusceptibility, similar to that conferred by first-step DNA gyrase mutations, transporters that extrude quinolones, and decreased levels of expression of porins. The phenotype of low-level nalidixic acid resistance and reduced ciprofloxacin susceptibility sometimes observed among *qnr*-positive strains is neither sensitive nor specific. Thus, screening for *qnr* genes is generally done by PCR amplification of the target genes. To facilitate a higher throughput, a number of groups have employed multiplex PCR for the detection of *qnrA*, *qnrB*, and *qnrS* (29, 166). Later, additional sets of primers were used, including multiplex PCR with degenerate primers for *qnrB*, to overcome the wider variability within this group (29). No method is perfect. For example, primers which we have used did not fully match all *qnrB* genes, and the reverse primer mismatched at the 3′ end with *qnrB5*, *qnrB10*, and *qnrB19*. *qnrB5* was, however, detected using these primers (70). Similarly, the reverse degenerate primer for *qnrB* (29) did not completely align with *qnrB17*. Also, false-positive amplicons have been reported for multiplex PCR procedures that are not seen with monoplex PCR using the same individual primer pairs within the multiplex primer mixture, emphasizing the importance of extensive DNA sequence confirmation (107). Because a comparison of the detection methods has not been performed, the prevalence studies were potentially subject to detection bias.

Because the difference between *aac(6′)-Ib-cr* and *aac(6′)-Ib* is in only two nucleotides, screening for *aac(6′)-Ib-cr* has traditionally involved *aac(6′)-Ib* amplification followed by sequencing (141) or restriction analysis (150). To overcome this cumbersome and costly method, we recently employed the gap-ligase chain reaction for the G-to-T change in the cr variant at nucleotide 535, one of the two defining mutations of *aac(6′)-Ib-cr*, to screen a large database for this gene (213). In this technique, two same-directional primers, separated by a gap of several nucleotides, are chosen. These primers hybridize to complementary strands of target DNA and will be extended by a DNA polymerase and subsequently ligated into a single long oligonucleotide when the mutation of interest, which corresponds to the 3′ end of the first primer, is present. This oligonucleotide can then be amplified (1). All *aac(6′)-Ib-cr*-positive control strains were identified, and by optimizing the assay conditions, we were able to use unquantified extracts of whole-cell DNA and maintain specificity (213).

Epidemiology of *qnr* and *aac(6′)-Ib-cr* Genes

After the initial discovery of *qnrA* in a *K. pneumoniae* isolate obtained from a urine sample from a patient in Alabama in 1994, 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* sp. isolates), all from the same center in Alabama where the original strain had been detected and all collected between July and December 2004 (89). Since that early study, more epidemiological surveys have been reported (Table 3). The earliest known *qnr* alleles are a *qnrB8*-like gene in a *C. freundii* isolate from Brooklyn, NY, and a *qnrB9*-like gene in a *K. pneumoniae* isolate from Cordoba, Argentina, both collected in 1988 (90). In general, studies have been localized to a narrow geographical region and a limited range of genera. Most studies used PCR methodologies to examine clinical isolates of *Enterobacteriaceae* collected in the 1990s or early 2000s, spanning periods ranging from a few months to more than a decade. Through 24 November 2008, more than 70 publications in peer-reviewed journals and conference abstracts reported over 20,960 isolates that were tested for PMQR. The average prevalences of *qnrA*, *qnrB*, *qnrS*, and *aac(6′)-Ib-cr* in this compiled database were 1.5%, 4.6%, 2.4%, and 10.8%, respectively. Early surveys looked for *qnrA*, and in recent studies, *qnrS*, *qnrB*, *aac(6′)-Ib-cr*, and *qepA* were also included. *E. coli* has been the most common species screened for PMQR. However, in the vast majority of surveys, *qnr* was more prevalent among *Enterobacter* spp. and *Klebsiella* spp. than in *E. coli* strains (17, 27, 93, 95, 98, 112, 120, 126, 141, 142, 152, 156, 166, 206). On the other hand, from surveys that included a balanced distribution of isolates of *Enterobacteriaceae*, it was evident that *aac(6′)-Ib-cr* is most common among *E. coli* strains (95, 141, 176, 223).

The selection criteria for isolates included in surveys could potentially bias prevalence data. Surveys generally have been performed with isolates collected over a short period or isolates that are resistant to various drugs, most commonly quinolones or ESBLs. In some studies, strains were collected only in the context of an outbreak. Hence, prevalence data in general must be interpreted with caution. It does appear to be the case, however, that the incidence of PMQR genes has increased in recent years. Several studies were conducted over sufficiently long periods to assess trends (6, 17, 27, 107, 120, 150, 152, 156, 188, 218). For example, in a survey in Paris, France, no *qnr* genes were found in 2002, and 10 were found in 2005 (120, 152, 156) (27). In addition, 41 of 1,147 *K. pneumoniae* bloodstream isolates collected in Taiwan from 1999 through 2005 were *qnrB4* positive; no *qnrB4* genes were found in 1999 to 2000, but in 2005, 14 isolates (7.6%) were positive (218). In a cohort of clinical *Enterobacter* sp. isolates from Jerusalem, Israel, that were collected from 1990 through 1993, none of 94 isolates had *qnr*; in isolates from 1994 through 2005, 33 out of 485 (6.8%) isolates had *qnr* ($P < 0.01$). Findings were similar for *K. pneumoniae* (188).

A similar picture is seen with *aac(6′)-Ib-cr*. The cr variant was not found among 150 *aac(6′)-Ib*-positive strains collected between 1981 and 1991 (90). In a study from Ljubljana, Slovenia, from 2000 through 2002, *aac(6′)-Ib-cr* was detected in 1 of 17 *Klebsiella* isolates, whereas in 2003 to 2005, the prevalence increased significantly to 24 of 57 isolates ($P = 0.02$) (6). In Calgary, Canada, the prevalence of *aac(6′)-Ib-cr* significantly increased from 5 of 121 isolates (4.1%) in 2004 to 52 of 346 *E. coli* strains (15%) isolated in 2007 ($P = 0.001$) (150). In Jerusalem, *E. coli* isolates bearing *aac(6′)-Ib-cr* emerged in 1998, and since then, *aac(6′)-Ib-cr* has progressively penetrated into multiple clinical *E. coli* clones (213). A recent survey of the *qnr*, *aac(6′)-Ib-cr*, and *qepA* genes among 461 unselected,

TABLE 3. Studies reporting prevalences of *qnr* and *aac(6')-Ib-cr*[a]

Reference(s)	No. of targets of screen (%)				Yr of isolate collection	Geographic area of isolation	Total no. of strains included	Bacterial type (no. of isolates)	Selection criterion or resistance profile	Description
	qnrA	*qnrB*	*qnrS*	*aac(6')-Ib-cr*						
118					1994	Alabama		*K. pneumoniae*		First identification of *qnrA*
89	6 (18)	—	—	—	1994–1995	Alabama	33	*E. coli* and *Klebsiella* spp. (33)	ESBL positive	Of 33 isolates, 1 *E. coli*, 4 *K. pneumoniae*, and 1 *Klebsiella* sp. isolate were *qnrA*$^{+}$; 26 isolates from 2000–2001 were *qnr* negative
89	0	—	—	—	Primarily 1990s	19 countries, three continents	338	*K. pneumoniae* (191), *E. coli* (91), other genera (56)		All *qnr* negative
168	3 (0.7)	—	—	—	1990s?	United States, Spain, and unlisted countries	425	*E. coli* (266), *K. pneumoniae* (159)	AmpC-type β-lactamase phenotype	None of the *E. coli* and 3 (2%) of *K. pneumoniae* strains were positive (collected 1995–1997); 2 of 3 *qnr*$^{+}$ fluoroquinolone susceptible by CLSI breakpoints
70, 184	1 (0.4)	13 (4.8)	13 (4.8)	1 (0.4)	1996–2006	United States, nationwide	273	Non-Typhi salmonella isolates	Cip MIC ≥ 0.06 μg/ml	*qnr* was not found in any of the fully Cip-resistant isolates or in control strains with Cip MIC ≤ 0.03 μg/ml
210	8 (7.3)	—	—	—	1999–2002	United States, nationwide	110	*K. pneumoniae* (72), *E. coli* (38)	MIC of Cip ≥ 2 μg/ml and MIC of ceftazidime ≥ 16 μg/ml	None of the *E. coli* and 8 (11%) of the *K. pneumoniae* isolates were positive
141, 166	34 (10.9)	39 (12.5)	0	44 (14)	1999–2004	United States, nationwide	313	*E coli* (47), *K pneumoniae* (106), *Enterobacter* spp. (160)	Nonduplicate isolates, MIC of Cip ≥ 0.25 μg/ml and MIC of ceftazidime ≥ 16 μg/ml	*qnr* was most prevalent in *Enterobacter* spp. (31%) and least prevalent in *E. coli* (4%), whereas *aac(6')-Ib-cr* was most prevalent in *E. coli*
128	0	—	—	~25%	1999–2004	Texas	78	*E. coli*	Clinical isolates with varied susceptibilities to quinolones	
91	—	4 (~4)	—	—		United States, India	~100	Over 100 strains	Undefined plasmid-carrying strains	First identification of *qnrB*; in screening, *qnrB* was found only among plasmids cocarrying SHV-12
20	2 (1.5)	6 (4.5)	1 (0.7)	1 (0.7)	2007	United States, nationwide	134	*Enterobacteriaceae*	Nonduplicate β-lactamase-producing isolates, part of a larger cohort	
58	1 (2.4)	1 (2.4)	0	0	2006–2007	United States	42	*K. pneumoniae*	Randomly collected, nonduplicate isolates positive for bla_{KPC}	bla_{KPC-3} and *qnrB19* carried on the same plasmid
154	3 (2.9)	—	0	—	2000–2002	Calgary, Canada	102	*Enterobacteriaceae*	ESBL positive	83% of strains were also resistant to nalidixic acid
150	0	0	0	6 (4.1)	2004	Calgary, Canada	148	*E. coli* (139), *Klebsiella* spp. (5), *P. mirabilis* (3), *M. morganii* (1)	Consecutive nonduplicate isolates, Cip- and/or tobramycin-resistant *Enterobacteriaceae*	*aac(6')-Ib-cr* identified only in *E. coli*; all ESBL (CTX-M-15)- or AmpC β-lactamase-positive isolates from 2004 and 2007 also carried *aac(6')-Ib-cr*
150	0	1 (0.2)	3 (0.7)	55 (13)	2007	Calgary, Canada	416	*E. coli* (398), *Klebsiella* spp. (7), *P. mirabilis* (10), *M. morganii* (1)	Same criteria as in 2004	All isolates had *qnrS* and 53 of 55 had *aac(6')-Ib-cr* found for *E. coli*; *qnrB* was found in *Klebsiella* isolates
126	0	6 (2.3)	0	0	2002–2005	Minas Gerais State, Brazil	257	*E. coli* (194), *K. pneumoniae* (32), *K. oxytoca* (11), *P. mirabilis* (6), *E. cloacae* (5), *S. marcescens* (2), *E. aerogenes* (2), *P. stuartii* (2), *C. freundii* (1), *M. morganii* (1), *P. vulgaris* (1)	Nonduplicate, nalidixic acid resistant	*qnrB* found in 3 *E. coli*, 2 *K. pneumoniae*, and 1 *C. freundii* isolate; *qepA* not found but would probably be screened out by selection criteria
140	0	9 (4.9)	0	10 (5.4)	2002, 2005	Bolivia, Peru	183	*E. coli*	Pediatric population; random isolates	Increased prevalence of PMQR between two periods
161	0	8 (44.4)	0	—	Not specified	Argentina	18	*Enterobacteriaceae*	Resistant to Cip and to at least two different families of antibiotics	Selection criteria of isolates from data set are unclear
46	—	—	—	2 (2.9)	2006	Intensive care unit, Montevideo, Uruguay	68	*E. coli* (22), *K. pneumoniae* (13), *Enterobacter* spp. (27), other *Enterobacteriaceae* (6)	Cip and/or ceftazidime	*aac(6')-Ib-cr* was detected among *E. coli* isolates

45	—	—	—	41 (95.3)	2000–2006	France, Portugal, Spain, Switzerland, Canada, Kuwait	43	*E. coli*	CTX-M-15 positive	Highly selected possibly related strains that spread worldwide
136	1 (0.2)	—	—	—	1997–1998	23 European hospitals	514	*Enterobacteriaceae*		One *E. cloacae* isolate was positive
96	2 (1.5)	—	—	—	2000–2003	34 intensive care units, Germany, nationwide	136	*Enterobacteriaceae*	Integron containing; resistant to Cip and/or cephalosporins	One *Enterobacter* sp. isolate and an outbreak strain of *Citrobacter freundii* were positive
51	1 (0.1)	4 (0.5)	3 (0.4)	—	1998–2007	Abattoirs in Belgium, Germany	729	Non-Typhi *Salmonella*	Isolates from poultry, with MIC of Cip ≥ 0.12 μg/ml	None of the ESBL-producing isolates carried *qnr* genes
104	0/35	0	0	0	2001–2005	Germany	35	Non-Typhi *Salmonella*	Isolates from chickens	
100	5 (2.8)	2 (1.1)	1 (0.6)	92 (52)	2006–2007	Germany	177	*E. coli* (102), *K. pneumoniae* (75)	Nonduplicate, ESBL positive	*aac(6′)-Ib-cr* equally distributed among species; associated predominantly with CTX-M-15
63	—	—	—	3 (4)	1999–2001	Paris, France	75	*E. coli* (23), *Klebsiella* spp. (27), *Enterobacter* spp. (18), *Citrobacter* spp. (3), *P. mirabilis* (3), *S. marcescens* (1)	Nonduplicate nonclonal, ESBL positive	*aac(6′)-Ib-cr* was detected with CTX-M-15 among *E. coli* isolates
30	1 (0.2)	0	0	—	2002	France, nationwide	516	Non-Typhi salmonella isolates	499 representative strains, 17 ESBL positive	*qnrA* found in an ESBL-positive strain; probably originated from East Africa
27, 120, 152, 156	5 (1.3)	1/186 (0.5)	8 (2.2)	—	2002–2005	Paris, France	371	*E. coli* (210), *Klebsiella* spp. (56), *Enterobacter* spp.(52), *Citrobacter* spp. (17), *Proteus* spp.(22), *P. stuartii* (9), *S. marcescens* (3), *M. morganii* (1), *S. enterica* serovar Typhimurium (1)	186 ESBL positive, 185 ESBL negative, nalidixic acid resistant	*qnr* genes were found in 9 *Enterobacter* sp., 2 *E. coli*, 1 *K. pneumoniae*, and 1 *S. marcescens* strain; no *qnr* was found in 2002, and *qnr* was found in 10 in 2005; the screen for *qnrB* was limited to ESBL-positive strains; *qnrB* coproduced with *qnrS* in the same *E. cloacae* strain
17	28 (1.9)	—	—	—	2002–2005	France, nationwide	1,466	*E. coli* (857), *Klebsiella* spp. (297), *E. cloacae* (135), *E. aerogenes* (63), *C. freundii* (40), *C. koseri* (24), *Proteus* spp. (27), *Serratia* spp. (18), *M. morganii* (5)	Nonduplicate isolates with ESBL- or AmpC-type-positive phenotype and/or reduced susceptibility to nalidixic acid and Cip	All *qnrA*$^+$ isolates were also ESBL positive, but non-ESBL strains were collected only in 2002–2003; 22 *qnrA*$^+$ isolates were *E. cloacae*, and there were none found in *E. coli* strains; within ESBL-positive isolates, more Cip susceptible among *qnr*$^+$ than *qnr*-negative isolates
149	0	0	1 (2)	1	2002–2005	Switzerland	50	*Aeromonas* spp.	Isolates with nalidixic acid or Cip MIC ≥ 1 μg/ml from lakes and rivers	Same isolate contained *qnrS* and *aac(6′)-Ib-cr*
6	0	0	0	25 (33.8)	2000–2005	Slovenia	74	*Klebsiella* spp.	Nonduplicate, ESBL-positive isolates with Cip MIC ≥ 2 μg/ml or nalidixic acid MIC ≥ 16 μg/ml	Looked for PMQR genes in transconjugants; *aac(6′)-Ib-cr*$^+$ strains were Cip resistant; more common in recent yr
176	0	7 (4.3)	0	52 (31.9)	2000–2005	Sofia, Bulgaria	163	*Enterobacteriaceae*	ESBL positive	*aac(6′)-Ib-cr* was found predominantly in *E. coli*, and strains coproduced CTX-M-15
189	7 (3)	2 (0.8)	1 (0.4)	19 (8)	2002–2006	Budapest, Hungary	237	*E. coli* (70), *K. pneumoniae* (101), *Enterobacter* spp. (61), *C. freundii* (5),	ESBL positive	
47	15 (31.9)	—	—	—	2003–2005	Liverpool, United Kingdom	47	*E. coli* (13), *K. pneumoniae* (15), *E. cloacae* (16), *C. freundii* (1), *M. morganii* (2)	Nonrepetitive, bloodstream isolates, Cip and cefotaxime resistant	Out of *qnrA*$^+$ strains, 9 were *E. cloacae* and 4 were *K. pneumoniae* strains; 12 were clonally distinct; multiple plasmids were involved
83	0	0	6 (5.1)	—	1993–2005	United Kingdom	118	*Salmonella enterica*	102/118 with MICs of cefotaxime >1 μg/ml, and for 54/118 Cip MICs >0.125 μg/ml	Plasmid analysis suggests that *qnrS1* is situated in different genetic environments but on plasmids that share a common ancestor
97	0	0	1 (0.4)	13 (5.7)	2000–2005	United Kingdom	227	*E. coli*	Quinolone resistant, non-ESBL-producing strains	Highly selected screened population
7	0	0	4 (4.8)	50 (60.2)	?	London, United Kingdom	83	*Enterobacteriaceae*	Nonduplicate, resistant to Cip and/or gentamicin	*qepA* present in 3 isolates (3.6%), none of which carried *rmtB*

Continued on following page

TABLE 3—*Continued*

Reference(s)	No. of targets of screen (%)				Yr of isolate collection	Geographic area of isolation	Total no. of strains included	Bacterial type (no. of isolates)	Selection criterion or resistance profile	Description
	qnrA	*qnrB*	*qnrS*	*aac(6')-Ib-cr*						
31	1 (1.2)	0	1 (1.2)	11 (12.9)	1996–2006	Denmark	85	*E. coli*	Reduced susceptibility to Cip or nalidixic acid	*qnr* and *aac(6')-Ib-cr* were not found among 39 *E. coli* isolates from pigs
67, 200	0	3 (7.7)	31 (79.5)	0	1999–2006	The Netherlands, nationwide	39	*Salmonella* spp.	Isolates from human, animals, and other sources with Cip MIC ≥ 0.25–1 μg/ml and nalidixic acid MIC ≥ 8–16 μg/ml (susceptible)	*qnrS1* genes were located on four different plasmid types
136	78 (94)	—	—	—	2001–2003	Utrecht, The Netherlands	83	*Enterobacter cloacae*	Epidemic strain	78 (94%) strains carried *qnrA*; additionally, 21% of cocolonizing gram-negative bacteria in patients colonized with epidemic strains also carried *qnrA*
62	0	8	4	—	1999–2007	Stockholm, Sweden	301	*Enterobacteriaceae*	β-Lactamase positive	234 isolates from 2007; *qnr*B was predominant
112	14 (4.6)	0	1 (0.3)	—	2003–2004	Barcelona, Spain	305	*E. coli* (247), *Klebsiella* spp. (42), *E. cloacae* (8), *Proteus* spp. (2), non-Typhi *Salmonella* (3), *Hafnia alvei* (1), *Raoultella ornithinolytica* (1), *M. morganii* (1)	Nonduplicate, ESBL positive	7 *qnr* genes found in *K. pneumoniae*, 6 in *E. cloacae*, 2 in *E. coli*
19	0	2 (1)	22 (11)	3 (1.5)	2004–2005	Santander and Seville, Spain	200	*E. cloacae* (153), *E. aerogenes* (47)	ESBL-, AmpC-, quinolone-, or aminoglycoside-resistant phenotype	*qnrS* was found only in *E. cloacae* strains from northern Spain; 20/22 belonged to 3 REP-PCR profiles
18	2 (1.2)	—	—	—	2004–2005	Santander, Spain	173	*Enterobacteriaceae*	ESBL positive	*C. freundii* and *E. cloacae* from a single patient carried *qnrA*
158	0	1 (1.6)	0	—	2004–2006	Lisbon, Portugal	61	*E. coli*	Nonduplicate isolates from animals at a veterinary hospital	The same strain also carried *aac(6')-Ib-cr* and CTX-M-15
131	2 (4.1)	—	—	—	2002–2004	Istanbul, Turkey	49	*E. coli* (36), *K. pneumoniae* (7), *E. cloacae* (4), *C. freundii* (2)	Nalidixic acid resistant, ESBL carrying	One strain of each, *E. cloacae* and *C. freundii*, were positive
135	5 (6.4)	0	0	—	2004	Turkey, nationwide	78	*E. coli* (34), *K. pneumoniae* (44)	Patient unique, ESBL positive	Some strains were clonally related
29	0	3 (4.7)	0	—	2002–2004	Kuwait City, Kuwait	64	*E. coli* (29), *K. pneumoniae* (19), *Proteus mirabilis* (6), *E. cloacae* (4), *E. aerogenes* (3), *C. freundii* (2), *S. marcescens* (1)	Nonrepetitive, ESBL positive	*qnrB* found in 2 *E. cloacae* and 1 *C. freundii* isolate
95	5 (1.4)	4 (1.1)	5 (1.4)	36 (9.9)	1998–2002	Six provinces or districts, China	362	*E. coli* (263), *K. pneumoniae* (99)	ESBL positive	5.3% of *E. coli* and 16.2% of *K. pneumoniae* strains were *qnr*$^+$, and 8% of *E. coli* and 5.7% of *K. pneumoniae* strains were *aac(6')-Ib-cr*$^+$; *aac(6')-Ib-cr* was more common among *qnr*$^+$ isolates
165, 212	6 (7.7)	—	—	40 (51.3)	2000–2001	Shanghai, China	78	*E. coli*	Cip resistant	6 (8%) positive strains, all from the same hospital; several different plasmids carried *qnrA*
38	4 (100)	—	—	—	2003	Hong Kong, China	4	*Salmonella enterica* serotype Enteritidis	Outbreak strains	All four strains tested carried *qnrA*; 4 different plasmids
220	7 (1.3)	—	—	—	2005	Shanghai, China	541	*K. pneumoniae* (169), *E. coli* (98), *Enterobacter* spp. (13), *Citrobacter* spp. (9), other *Enterobacteriaceae* (20), nonfermenting bacilli (232)	Consecutive isolates with Cip MIC ≥ 2 μg/ml	
219	5 (6.2)	5 (6.2)	7 (8.6)	3 (3.7)	2005	Anhui Province, China	81	*E. cloacae*	Nonduplicate strains	

223	18 (6.8)	49 (18.5)	35 (13.2)	45 (17)	2006	China, nationwide	265	*Enterobacteriaceae* (*C. freundii*, *E. cloacae*, *E. coli*, *K. pneumoniae*) (265)	Nonduplicate isolates, Cip MICs ≥ 0.25 μg/ml	Prevalence of *qnr*, but not of *aac(6′)-Ib-cr*, was significantly higher among isolates with higher MICs of cefotaxime or ceftriaxone; *qnr* and *aac(6′)-Ib-cr* least common in *E. coli* and *E. cloacae* isolates, respectively
226	0	14 (6)	13 (5.6)	2	2003–2005	Animal farms, Guangdong, China	232	*E. coli*	Nonduplicate isolates from diseased animals	2 *qnr*$^+$ isolates also carried *aac(6′)-Ib-cr*
115	0	1 (2.1)	9 (18.8)	6 (12.5)	2005–2006	Pig farms, China	48	*E. coli*	*rmtB* (16S rRNA methyltransferase)-positive strains	Screened highly selected bacterial population; *qepA* found in 28 (58.3%) of isolates
116	0	5 (5)	3 (3)	19 (18.8)	2003–2007	Guangzhou, China	101	*E. coli* (89), *K. pneumoniae* (9), *E. cloacae* (2), *C. freundii* (1)	Strains isolated from diseased animals, MIC of ceftiofur ≥ 8 μg/ml	*qepA* found in 16 (15.8%) isolates
205	10 (2.4)	25 (6.1)	61 (14.9)	—	2005–2006	Children's hospitals, China	410	*K. pneumoniae*	Patient unique, ESBL-, Amp-C- resistant phenotype	Pediatric population, about one-third neonates
206	8 (3.8)	10 (4.7)	8 (3.8)	—	2005–2006	Children's hospitals, China	213	*E. coli* (146), *K. pneumoniae* (67)	Patient unique, Cip resistant	7.5% of *E. coli* and 11.9% of *K. pneumoniae* isolates were *qnr*$^+$; 6 of 19 strains carried more than one *qnr* gene; because all *qnr*$^+$ *K. pneumoniae* isolates were also ESBL positive, that data set apparently overlaps with that reported in reference 205
50	2 (0.9)	1 (0.5)	1 (0.5)	18 (8.1)	2006	Wuhan, China	221	Non-Typhi salmonella isolates	Pediatric population	All *aac(6′)-Ib-cr*$^+$ strains were Cip susceptible
218	0	101 (3.2)	40 (1.3)	—	1999–2005	Tainan, Taiwan	3,182	*E. coli* (2,035), *K. pneumoniae* (1,147)	Patient-unique bloodstream isolates	0.6% of *E. coli* and 7.8% of *K. pneumoniae* strains were *qnr*$^+$; prevalence of *qnrB4* in *K. pneumoniae* increased from 0% to 7.6% over the study period
216	0	2 (0.4)	2 (0.4)	—	2003–2006	Tainan, Taiwan	446	Non-Typhi *Salmonella*	Patient unique	Each pair of *qnrB2*- and *qnrS1*-positive isolates had the same PFGE pattern
217	3 (0.6)	53 (10.1)	34 (6.5)	—	2004	Tainan, Taiwan	526	*E. cloacae*	Patient unique	*qnr* more prevalent among ESBL producers; *qnr* cotransferred with IMP-8 β-lactamase
156	11 (24.4)	—	—	—	1999	Bangkok, Thailand	45	*Enterobacteriaceae*	ESBL positive	11 isolates were positive; all nonreplicative bla_{VEB-1}-positive β-lactamase
107	6 (1.3)	39 (8.4)	13 (2.8)	10 (2.1)	1998–2001, 2005–2006	Seoul, South Korea	461	*E. coli* (264), *K. pneumoniae* (133), *E. cloacae* (64)	Nonduplicate bloodstream isolates	Prevalence of PMQR genes increased significantly over time; 10 *qepA*, first detected in 1998
93, 98	13 (2.8)	—	—	—	2001–2003	Seoul, South Korea	466	*E coli* (260), *E cloacae* (206)		2 (0.7%) *E coli* strains were positive; 11 (5%) *E cloacae* strains were positive
190	4 (1.1)	135 (36.7)	2 (0.5)	—	2004–2006	Daegu, South Korea	368	*Enterobacteriaceae*	Consecutive nonduplicate isolates, resistant to nalidixic acid and ESBL positive	*qnrB* probably disseminated in a restricted no. of plasmids
142	32 (5)	81 (12.6)	2 (0.3)	—	2005	South Korea	644	*E. cloacae* (186), *E. aerogenes* (154), *C. freundii* (138), *S. marcescens* (166)	Consecutive, nonduplicate isolates	*qnr* more common among *E. cloacae* (28.5%) and *C. freundii* (38.4%) isolates than among *E. aerogenes* (3.2%) and *S. marcescens* (2.4%) isolates; *qnr* associated with ESBL phenotype
183	0	41 (20.3)	0	—	2005–2006	Busan, South Korea	202	*E. coli* (143), *K. pneumoniae* (59)	Nonduplicate	Multiple clones; *qnrB* 10-fold more common in *K. pneumoniae* than in *E. coli*
180	5 (8.3)	—	28 (46.7)	—	2004	Tetanus intensive care unit, Ho Chi Minh City, Vietnam	60	*K pneumoniae* (28), *E. coli* (29), *Citrobacter* spp. (3)	Gentamicin-resistant *K. pneumoniae*, ESBL-positive *E. coli* or *Citrobacter* spp.	23 (82%) *K. pneumoniae* strains carried *qnrS*

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TABLE 3—*Continued*

Reference(s)	No. of targets of screen (%)				Yr of isolate collection	Geographic area of isolation	Total no. of strains included	Bacterial type (no. of isolates)	Selection criterion or resistance profile	Description
	qnrA	*qnrB*	*qnrS*	*aac(6′)-Ib-cr*						
4	0	6 (2.6)	4 (1.7)	1 (0.4)	2006	Asa Zoological Park, Hiroshima Prefecture, Japan	232	*E. coli* (122), *Klebsiella* spp. (30), *Proteus* spp. (21). *Enterobacter* spp.(25), *Citrobacter* spp. (9), *M. morganii* (4), *Salmonella* spp. (3), *S. marcescens* (2), *Aeromonas* spp. (1), *P. aeruginosa* (12), *A. baumannii* (1), *P. fluorescens* (1), *Edwardsiella tarda* (1)	Random	Samples originated from mammals, reptiles, birds, and water sources at the park; unusual sources of *qnrB* were *Proteus mirabilis* and *P. fluorescens*, and unusual sources of *aac(6′)-Ib-cr* were *Aeromonas* spp.
192	0	6 (5.2)	0	—	2008	Singapore	116	*K. pneumoniae*	Cip resistant	
170	1 (4.3)	—	0	—	2002	Sydney, Australia	23	*Enterobacteriaceae*	Cip resistant	The qnr^+ plasmid from *E. cloacae* could not be mobilized
129	3 (18.7)	10 (62.5)	0	8 (50)	2001–2007	Brisbane, Australia	16	*E. cloacae* (8), *Enterobacter hormaechei* (8)	Sample from dogs	First report of PMQR genes in isolates from companion animals
214	3 (10)	—	—	—	2001	Burn unit, Cairo, Egypt	30	*E. coli* (4), *K. pneumoniae* (4), *E. cloacae* (1), *S. marcescens* (2), *Proteus rettgeri* (4), *P. stuartii* (4), *M. morganii* (1), *P. mirabilis* (10)	MIC of Cip ≥ 0.25 μg/ml	3 (10%) unrelated *Providencia stuartii* strains carried *qnrA*
101	0	0	0	10 (62.5)	1997–2002	Beirut, Lebanon	16	*E. coli* (8), *K. pneumoniae* (8)	ESBL positive	*aac(6′)-Ib-cr*$^+$ strains also carried CTX-M-15
188	11 (0.9)	25 (2)	7 (0.6)	—	1990–2005	Jerusalem, Israel	1,258	*K. pneumoniae* (679), *E. cloacae* (462), *E. aerogenes* (117)	Patient-unique bloodstream isolates	For each genus, emergence of *qnr* coincided with increase in Cip resistance; Cip-susceptible qnr^+ strains were 3.4 times more likely to be ceftazidime resistant than were *qnr*-negative strains
213	1 (0.1)	0	0	32 (4.5)	1991–2005	Jerusalem, Israel	718	*E. coli*	Patient-unique bloodstream isolates	*aac(6′)-Ib-cr* detected by gap-LCR; *aac(6′)-Ib-cr*$^+$ strains first appeared in 1998, coinciding with an increase in Cip resistance; *aac(6′)-Ib-cr*$^+$ strains were multiclonal and associated with ESBL; 22 strains were CTX-M-15 positive
39	0	0	0	6 (12.7)	2000–2006	Tel-Aviv, Israel	47	*K. pneumoniae*	Carbapenem resistant	*aac(6′)-Ib-cr* was the most common PMQR gene among KPC-positive strains

[a] Publications are generally grouped according to geographic areas, years of study, and type of population studied. Cip, ciprofloxacin; REP-PCR, repetitive extragenic palindromic PCR; KPC, *K. pneumoniae* carbapenemase; gap-LCR, gap-ligase chain reaction; —, not done; PFGE, pulsed-field gel electrophoresis; *qnrA*$^+$, *qnrA* positive.

consecutive bloodstream isolates collected in Seoul, South Korea, in two periods, 1998 to 2001 and 2005 to 2006, corroborate the findings from prior studies. There was a significant increase in the rate of ciprofloxacin resistance over time ($P < 0.001$), and the overall prevalence of PMQR genes increased significantly over time as well ($P = 0.02$). Although *qnrB* was the most prevalent PMQR gene, there was an overall increase in the prevalence of PMQR genes, representing an increasing diversity of PMQR genes rather than a dominance of a single gene (107). Also noteworthy in that study was the finding that in strains with PMQR genes, there was a significant increase in *gyrA* and/or *parC* resistance mutations over time, but in strains without PMQR genes, these mutations remained stable over time. This epidemiological association supports the role of PMQR genes in promoting higher levels of resistance by mutation in clinical settings, as has been demonstrated in the laboratory.

qnr genes have already been found in all populated continents (as well as the waters in between them) and in most clinically common *Enterobacteriaceae*. These species include *E. coli*, *Klebsiella* spp. (*K. pneumoniae* and *Klebsiella oxytoca*), *Enterobacter* spp. (*Enterobacter cloacae*, *Enterobacter aerogenes*, *Enterobacter amnigenus*, and *Enterobacter sakazakii*), *Citrobacter freundii*, and *Providencia stuartii*. Among these, *qnr* has been more commonly identified in *Enterobacter* spp. followed by *K. pneumoniae* and less so in *E. coli*, where *aac(6')-Ib-cr* seems to be more prevalent. Until recently, absent from this list were *Proteus* spp. and clinically important nonenteric gram-negative bacteria (e.g., *Pseudomonas aeruginosa* and *Acinetobacter* spp.). All three have been included in small surveys of isolates of human origin (78, 89, 156, 181, 208), without PMQR genes being detected. *qnr* was also not found among multidrug-resistant *Acinetobacter baumannii* isolates (2, 224). Interestingly, in a recent survey of isolates from zoo animals, *qnrB* was detected in *Pseudomonas fluorescens* from a turtle and in *Proteus mirabilis* from feces of Bengalese finches (4). QnrA has also recently been found in an isolate of *A. baumannii* (196).

Epidemiological studies have been useful in supporting genetic data indicating a linkage of PMQR with other resistance genes, particularly ESBLs. Various investigators have demonstrated higher *qnr* prevalences among ESBL-positive strains (142, 188, 217, 223). For example, Strahilevitz and coworkers found that among clinical isolates of *Enterobacter* spp. and *Klebsiella pneumoniae*, the relative risk for ceftazidime resistance (a surrogate for ESBL presence) in *qnr*-positive *K. pneumoniae* isolates was 1.8 (95% confidence interval, 1.3 to 2.5), and in *Enterobacter* isolates, it was 3.5 (95% confidence interval, 2.7 to 4.5) (188). Further work demonstrated that the ceftazidime resistance in *qnr*-positive *Enterobacter* strains was associated with a true ESBL-mediated mechanism (188). Because fluoroquinolones remain one of the few options for treating infections caused by such organisms, it is concerning that a substantial fraction of the ceftazidime-resistant *qnr*-carrying isolates in this study were susceptible to ciprofloxacin according to CLSI criteria (188). Similarly, in a French survey of ESBL-positive isolates of *Enterobacteriaceae*, 43% of *qnrA*-positive isolates tested as ciprofloxacin susceptible. Whether such *qnrA*-positive, fluoroquinolone-"susceptible" isolates can be effectively treated with fluoroquinolones requires further investigation.

A further concern arising from epidemiological studies is the close association of *aac(6')-Ib-cr* with CTX-M-15, an ESBL that has emerged worldwide in recent years, including in community settings (45, 150).

Despite continued efforts to control its spread, non-Typhi *Salmonella enterica* persists as the most common food-borne pathogen in the United States (33). Unlike most *Enterobacteriaceae* species in which *qnr* genes have been detected, non-Typhi serotypes of *Salmonella enterica* are carried largely in the intestinal tract of food animals and are transmitted to humans through the food chain (8). Therefore, quinolone use in agriculture may drive the dissemination of *qnr*-mediated resistance in these pathogens, and mapping of *qnr* in non-Typhi serotypes of *S. enterica* could serve as a marker of the route of infiltration of antibiotic resistance from the food animal industry to humans (16, 61). Over 2,300 non-Typhi *S. enterica* isolates from human cases and animals in North America, Europe, and Asia have been tested (30, 38, 50, 67, 70, 83, 104, 184, 200, 216). *qnrA*, *qnrB*, *qnrS*, and *aac(6')-Ib-cr* were found in the majority of the surveys, at overall prevalences of 0.2%, 1.0%, 2.4%, and 6.4%, respectively, among several salmonella serotypes, thus suggesting broad host and geographic distribution. Similar to other isolates of *Enterobacteriaceae*, some of the PMQR-positive isolates had a relatively small increase in MICs of nalidixic acid and ciprofloxacin and were associated with an ESBL or AmpC β-lactamase phenotype.

Epidemiology of *qepA*

Less information is available about the epidemiology of the newly discovered PMQR pump QepA. A survey performed in Japan found *qepA* in 2 (0.3%) of 751 *E. coli* isolates (cutoff of MIC of norfloxacin, ≥0.025 μg/ml) collected from 140 hospitals between 2002 and 2006 (221). A second large survey was done by PCR in France. A single *E. coli* isolate among 121 (0.8%) ESBL-positive *Enterobacteriaceae* strains isolated in 2007 was positive for a variant named *qepA2* (28). In a study of pig farms in China, *qepA* was found in 28 of 48 (58.3%) *rmtB*-positive *E. coli* isolates (115). A follow-up study from the same region in China tested for *qepA* among ceftiofur-resistant isolates of *Enterobacteriaceae*. *qepA* was found in 16 of 101 (15.8%) isolates, including, for the first time, *K. pneumoniae* and *E. cloacae* (116). Few recently published studies indicated a broad distribution of the gene. A survey of 461 isolates of *Enterobacteriaceae* in South Korea found *qepA* in one isolate from 2005 (107). *qepA* has also been found in the United Kingdom. Three of 83 (3.6%) human *Enterobacteriaceae* clinical isolates in London were *qepA* positive (7). Two additional studies screened isolates from Seoul, South Korea, for *qepA*. Four clonally unrelated strains of 621 (0.6%) *E. coli* bloodstream isolates were found to be positive in one study (41), and two *E. aerogenes* isolates of 223 (0.9%) *E. cloacae*, *E. aerogenes*, *C. freundii*, and *Serratia marcescens* isolates with reduced susceptibility to quinolones were *qepA* positive in the second survey (143). *qepA* was not found in a large survey of non-Typhi *Salmonella enterica* isolates collected in the United States from 1996 to 2006 (184).

CONCLUSIONS AND PERSPECTIVE

In the decade since the discovery of *qnrA1*, there has been an explosion of knowledge about a phenomenon previously thought not to exist. We have challenged bacteria with a class of synthetic antimicrobial agents against which mutational resistance was unlikely to develop in a clinical setting. Despite this, resistance has emerged independently, countless times, worldwide. We now understand that, concomitant with the expansion in quinolone use, gram-negative bacteria assembled an arsenal of horizontally transmissible genetic elements that facilitated the emergence of mutational quinolone resistance. These elements are preexisting tools refitted for a novel purpose. Chromosomal genes not native to the *Enterobacteriaceae* have been recruited to these genera to effect topoisomerase protection and quinolone efflux, and a naturally occurring resistance gene has been outfitted with a new target. These elements may have played a leading role in the drama of emerging quinolone resistance, or theirs may have been only a supporting part; we have not yet fully taken stock of their importance. However, it is clear that PMQR has made extensive inroads among organisms of clinical importance, for humans and animals, everywhere that quinolones are used. Whatever these organisms have contributed to the global rise of resistance, their current prevalence is a problem. Cooccurrence with other resistance elements allows mutual resistance promotion and the spread of organisms that are difficult to treat. Additionally, clinical breakpoints have not yet been assessed in the context of PMQR, a form of reduced susceptibility that can contribute to therapeutic failure despite going undetected by traditional phenotypic methods. With the discovery of *qnr* in 1998, we took an important step in the battle against resistance, but it is clear that the bacteria have had a head start.

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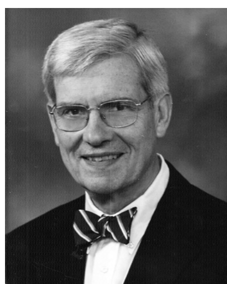

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