A novel plasmid-mediated quinolone resistance gene, \textit{qnrB}, has been discovered in a plasmid encoding the CTX-M-15 \beta-lactamase from a \textit{Klebsiella pneumoniae} strain isolated in South India. It has less than 40\% amino acid identity with the original \textit{qnr} (now \textit{qnrA}) gene or with the recently described \textit{qnrS} but, like them, codes for a protein belonging to the pentapeptide repeat family. Strains with \textit{qnrB} demonstrated low-level resistance to all quinolones tested. The gene has been cloned in an expression vector attaching a polyhistidine tag, which facilitated purification to 
\textit{\geq}95\% homogeneity. As little as 5 pM of \textit{QnrB-His6} protected purified DNA gyrase against inhibition by 2 \mu g/ml (6 \mu M) ciprofloxacin. With a PCR assay \textit{qnrB} has been detected in \textit{Citrobacter koseri}, \textit{Enterobacter cloacae}, and \textit{Escherichia coli} isolates from the United States, linked to SHV-12 \beta-lactamase and coding for a product differing in five amino acids from the Indian (now \textit{QnrB1}) variety. The \textit{qnrB} gene has been found near \textit{Orf1005} in some, but not all, plasmids and in association with open reading frames matching known chromosomal genes, suggesting that it too was acquired by plasmids from an as-yet-unknown bacterial source.

The first plasmid-mediated quinolone resistance gene (\textit{qnr}) was discovered in a \textit{Klebsiella pneumoniae} isolate from Birmingham, Alabama, collected in 1994 (10). It occurred in a multiresistance plasmid, pMG252, in an integron-like structure near \textit{Orf513} (17). \textit{Qnr}, the gene product, is a member of the pentapeptide repeat family of proteins and has been shown to block the action of ciprofloxacin on purified DNA gyrase and topoisomerase IV (17, 19). In \textit{Escherichia coli} pMG252 determines low-level quinolone resistance but facilitates the selection of higher-level resistance mutations (10). \textit{qnr} plasmids have been found in clinical isolates of \textit{Citrobacter freundii}, \textit{Enterobacter spp.}, \textit{E. coli}, \textit{K. pneumoniae}, \textit{Providencia stuartii}, and \textit{Salmonella} spp. from the United States, Europe, and the Near and Far East (1, 13). Another \textit{qnr} gene, \textit{qnrS}, has also recently been found in a plasmid from a strain of \textit{Shigella flexneri} isolated in Japan (2).

While investigating strains of \textit{K. pneumoniae} from India, some of which contained \textit{qnr}, it was realized that several could transfer low-level quinolone resistance but were negative for PCR for \textit{qnr}. The new plasmid-mediated quinolone resistance gene has been termed \textit{qnrB}, and the original gene is now designated \textit{qnrA}. \textit{qnrB} has been cloned and sequenced. Purified \textit{QnrB} protects DNA gyrase from quinolone action like \textit{QnrA} does. A PCR assay for \textit{qnrB} indicates that it is as common as \textit{qnrA} in samples from the United States and has greater amino acid variability.

(The results of this study were presented in part at the 44th Interscience Conference on Antimicrobial Agents and Chemotherapy, 30 October to 2 November 2004, Washington, D.C.)
ucleotide accession numbers. The qnrB1 and qnrB2 sequences have been submitted to GenBank with accession numbers DQ351241 and DQ351242.

RESULTS

Discovery of qnrB. The K. pneumoniae strains from India were originally studied to determine the basis of their ESBL phenotype. Transconjugants of strains 17, 19, 24, and 25, selected for cefotaxime resistance, were noted to have low-level quinolone resistance, but when tested by PCR with primers QP1 and QP2, only K. pneumoniae strains 19 and 24 were positive for the known qnr gene.

Accordingly, DNA of plasmid pMG298 from strain 17 was digested with PstI endonuclease and ligated into vector pBC SK (determining chloramphenicol resistance), selecting for simultaneous resistance to ciprofloxacin and chloramphenicol. A recombinant plasmid containing a 15.3-kb insert was obtained. Quinolone resistance was also expressed from pBC SK containing a 4.8-kb BamHI fragment from plasmid pMG299 originating in strain 24 and from a 5.9-kb PstI fragment from plasmid pMG300 transferred from strain 25.

To facilitate DNA sequencing, a Tn7-based transposon carrying a kanamycin resistance gene was inserted into the recombinant plasmids, and colonies were screened for loss of nalidixic acid resistance. Using primers that matched sequence at the ends of the inserted transposon, sequencing was initiated and continued by primer walking with the original unmodified recombinant plasmid. A new quinolone resistance gene, qnrB1, was discovered. It had 49.5% nucleotide identity and 39.5% amino acid identity with qnrA and 49.3% nucleotide identity and 37.4% amino acid identity with qnrS (Fig. 1) and had the potential to code for a 226-amino-acid protein, which belonged to the pentapeptide repeat family.

Distribution of qnrB. Using PCR with primers derived from the qnrB1 sequence, the gene was found also in K. pneumoniae strains 21, 24, and 25 from southern India, but the plasmids involved differed in resistance properties (Table 1). Plasmid pMG299 from strain 24, like plasmid pMG298 from strain 17, carried blaCTX-M-15, but the qnrB1 plasmid pMG300 from strain 25 encoded SHV-12 and had no CTX-M gene by PCR. The blaSHV-12 gene was also present in strain 21, but neither β-
lactam nor other antibiotic resistances could be transferred from it by conjugation, implying the presence of a Trp plasmid.

Over 100 other plasmid-carrying strains were screened for qnrB. A strain with another CTX-M-15 plasmid was negative, but 4 of about 20 independently derived plasmids encoding SHV-12 were positive, including plasmids found in C. koseri, E. cloacae, and E. coli (Table 1). All had identical sequences which differed from that of qnrB1 by 26 of 680 nucleotides, including an additional nucleotide prior to a second potential ATG start codon at position 36. The extra nucleotide would cause a frameshift if translation began at the first site, so that the qnrB2 gene codes for a 215-amino-acid protein that in addition differs from QnrB1 in 5 amino acids (Fig. 1). The qnrB2 plasmids all encode SHV-12 but differ in other associated resistances (Table 1). Attempts to clone qnrB2 with BamHI or PstI failed, but the gene was cloned into pBC SK as a 2.4-kb EcoRI fragment.

Genetic environment of qnrB. The immediate genetic environment of qnrB1 in plasmids pMG298, pMG299, and pMG300 were similar (Fig. 2). qnrB1 was found downstream from Orf1005, which encodes a putative transposase and is brack-

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**TABLE 2. Susceptibility of E. coli J53 transconjugants to various quinolones**

<table>
<thead>
<tr>
<th>Plasmid in E. coli J53</th>
<th>Qnr protein</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nalidixic acid</td>
</tr>
<tr>
<td>R</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>pMG252</td>
<td>QnrA</td>
<td>32</td>
</tr>
<tr>
<td>pHSH4-3</td>
<td>QnrA</td>
<td>16</td>
</tr>
<tr>
<td>pMG298</td>
<td>QnrB1</td>
<td>16</td>
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<td>pMG299</td>
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<td>pMG300</td>
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etected by imperfect 83-bp inverted repeat segments (16). In pMG298 a 
*traA* gene was found downstream from Orf1005, but this gene was absent in pMG300. Between Orf1005 and 
*qnrB1* was a 383-bp open reading frame, Orf1, which is >70% identical to a truncated *pspF* gene coding for the transcriptional activator of the stress-inducible *psp* operon (7). Orf1 contains an EcoRI site, and a shorter segment of Orf1 was found upstream from the *qnrB2* gene, which was cloned on an EcoRI fragment (Fig. 2). Downstream from *qnrB2* was an open reading frame (Orf2), with >60% identity to hypothetical proteins of several gram-negative species, and Orf3, with 83% identity to the *sapA* gene, which encodes a peptide transport periplasmic protein in gram-negative bacteria. By a PCR assay 
Orf1005 was present in *K. pneumoniae* strains 17, 24, and 25 but not in any of the strains containing *qnrB2*. Orf512 was not detected in any of the strains.

**Effect of QnrB on quinolone susceptibility.** Like QnrA, QnrB provided low-level resistance to all quinolones tested (Table 2).

**Effect of QnrB on quinolone inhibition of gyrase.** To investigate the mechanism of quinolone protection, *qnrB* was cloned into an expression vector that attached a C-terminal polyhistidine tag, which facilitated purification of QnrB-His$_6$ protein by Ni affinity chromatography. His-tagged QnrB appeared to be >95% homogenous by gel assay.

QnrB-His$_6$ demonstrated a concentration-dependent protection of purified gyrase from ciprofloxacin inhibition of DNA supercoiling (Fig. 3). With 2 µg/ml (6 µM) ciprofloxacin, the concentration of QnrB-His$_6$ required for half protection was about 0.5 nM, and a protective effect was seen with as little as 5 pM. The highest concentration of QnrB-His$_6$ tested (25 µM) inhibited gyrase-mediated DNA supercoiling, but inhibition was not seen with 5 µM QnrB-His$_6$, a concentration still 750 times higher than that of DNA gyrase (Fig. 3, compare lanes 4 and 5).

**DISCUSSION**

QnrB, like QnrA and QnrS (2), provides low-level resistance to quinolones and belongs to the pentapeptide repeat family of proteins, one member of which has recently been shown to have a DNA-like structure which would allow it to mimic DNA as a substrate for DNA gyrase (3). For a protein in which overall structure is important rather than catalytic activity, considerable amino acid variability may be permissible. Thus, QnrB and QnrS have only 39.5% of their amino acids in common, while QnrB and QnrS share 37.4%, but all are pentapeptide repeat proteins with two domains joined by a glycine residue (17).

Purified QnrB, like QnrA, protected DNA gyrase from quinolone action. It seems to be even more potent than QnrA in blocking the action of ciprofloxacin (17). At a concentration almost 4,000 times that of DNA gyrase, QnrB inhibited the enzyme, but this effect disappeared at a fivefold lower concentration of QnrB. Thus, in contrast to MfpA, another pentapeptide repeat protein that blocks ciprofloxacin action (3), QnrB did not inhibit gyrase-mediated DNA supercoiling over a wide range of quinolone-protective concentrations. Accordingly, models of Qnr action that do not require direct gyrase inhibition must be considered.

A close relative and likely progenitor of QnrA, differing in only 1 to 2% amino acids, has recently been found in the commensal water organism *Shewanella algae* (15). The origin of QnrB is not yet known, since the closest relative currently disclosed by a BLAST search is a hypothetical protein from *Photobacterium profundum* with only 44.5% amino acid identity. The *qnrA* gene has been found in plasmids with a variety of other resistance determinants but always as part of a *sul1*-type integron (13). *qnrB1* is located near a putative transposase, Orf1005, in plasmids pMG298 and pMG299, but Orf1005 was absent from QnrB plasmids from the United States. The linkage of *qnrB* to Orf1, Orf2, and Orf3, which resemble known chromosomal genes, suggests that all were acquired from a chromosomal source. The homologues of Orf1 and Orf3 are adjacent to each other on the *P. profundum* chromosome, but the homologues of *qnrB* and Orf2 occupy separate and distant locations. The mechanism of *qnrB* acquisition, like that of *qnrS*, remains to be elucidated.

In both India and the United States *qnrB* has been found on plasmids also encoding ESBLs: CTX-M-15 and SHV-12 in India and SHV-12 in the United States. The frequent association of quinolone resistance with ESBL production has been noted in several studies (8, 14). The presence of *qnr* and *bla*<sub>ESBL</sub> genes on the same plasmid is one of several possible explanations for this association.

*qnrB2* has been found on plasmids collected in 1996, and the
association of qnrB with SHV-12 on plasmids found in both India and the United States suggests that this resistance mechanism has been present long enough to disseminate widely. Preliminary surveys indicate that in ceftazidime-resistant *Enterobacter* and *Klebsiella* isolates from the United States qnrB is as common as qnrA (Robicsek et al., unpublished observations). It would not be surprising if even more members of the qnr family were discovered.

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