ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 2003, p. 2242–2248 Vol. 47, No. 7
0066-4804/03/$08.00+0 DOI: 10.1128/AAC.47.7.2242–2248.2003

Plasmid-Mediated Quinolone Resistance in Clinical Isolates of *Escherichia coli* from Shanghai, China

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Received 27 January 2003/Returned for modification 5 March 2003/Accepted 15 April 2003

Although quinolone resistance usually results from chromosomal mutations, recent studies indicate that quinolone resistance can also be plasmid mediated. The gene responsible, *qnr*, is distinct from the known quinolone resistance genes and in previous studies seemed to be restricted to *Klebsiella pneumoniae* and *Escherichia coli* isolates from the University of Alabama in Birmingham, where this resistance was discovered. In Shanghai, the frequency of ciprofloxacin resistance in *E. coli* has exceeded 50% since 1993. Seventy-eight unique ciprofloxacin-resistant clinical isolates of *E. coli* from Shanghai hospitals were screened for the *qnr* gene by colony blotting and Southern hybridization of plasmid DNA. Conjugation experiments were done with azide-resistant *E. coli* J53 as a recipient with selection for plasmid-encoded antimicrobial resistance (chloramphenicol, gentamicin, or tetracycline) and azide counterselection. *qnr* genes were sequenced, and the structure of the plasmid DNA adjacent to *qnr* was analyzed by primer walking with a sequential series of outward-facing sequencing primers with plasmid DNA templates purified from transconjugants. Six (7.7%) of 78 strains gave a reproducible hybridization signal with a *qnr* gene probe on colony blots and yielded strong signals on plasmid DNA preparations. Quinolone resistance was transferred from all six probe-positive strains. Transconjugants had 16- to 250-fold increases in the MICs of ciprofloxacin relative to that of the recipient. All six strains contained *qnr* with a nucleotide sequence identical to that originally reported, except for a single nucleotide change (CTA→CTG at position 537) encoding the same amino acid. *qnr* was located in complex In4 family class 1 integrons. Two completely sequenced integrons were designated In36 and In37. Transferable plasmid-mediated quinolone resistance associated with *qnr* is thus prevalent in quinolone-resistant clinical strains of *E. coli* from Shanghai and may contribute to the rapid increase in bacterial resistance to quinolones in China.

Quinolones are broad-spectrum antimicrobial agents that have been used widely in clinical medicine. Resistance to quinolones has increased markedly, however, in some areas of the world since their introduction. In China, more than 50% of the clinical strains of *Escherichia coli* are resistant to ciprofloxacin (27, 28).

Previous studies demonstrated that the mechanisms of bacterial resistance to quinolones fall into two principal categories, alterations in target enzymes (DNA gyrase and/or topoisomerase IV) and alterations in drug accumulation, both resulting from chromosomal mutations (9, 10). Recently, low-level quinolone resistance was found to be transferable by a plasmid discovered in a clinical isolate of *Klebsiella pneumoniae* from Birmingham, Ala. (16). The gene responsible, named *qnr*, proved to be distinct from previously identified quinolone resistance genes (25). Its clinical importance, however, seemed questionable, since *qnr* was not detected in a survey of more than 350 multiresistant *K. pneumoniae* and *E. coli* isolates from around the world, except in those from Birmingham, Ala., during a 6-month period in 1994 (13).

In an earlier study, *qnr* was located within an integron-like environment upstream from the *qacEΔ1* and *sul1* genes (25), suggesting the possibility of its presence in a class 1 integron. Many resistance genes, such as those encoding plasmid-mediated β-lactamases, are located within or near mobile elements, such as integrons or transposons, which enhance their dissemination (1). A number of unusual class 1 integrons containing the common region of In6 and In7 that includes orf513 have been described as carrying antibiotic resistance genes such as *catA2*, *dfrA10*, *dfrA19*, $bla_{\text{DHA-1}}$, $bla_{\text{CTX-M-9}}$, $bla_{\text{CTX-M-2}}$, and $bla_{\text{CMY-9}}$ (1, 2, 6, 20, 21, 26). Integrons are common in clinical isolates of gram-negative bacteria. Forty-three percent of gram-negative isolates from different European hospitals were found to contain integrons, and integron-positive isolates were statistically significantly more likely to be resistant to quinolones, aminoglycosides, and β-lactams (15), suggesting the possibility that *qnr* was also present in some of these strains, which were shown to contain the conserved sequences of class 1 integrons.

The frequency of quinolone resistance in clinical isolates of *E. coli* is unusually high in China. The frequency of ciprofloxacin resistance ranged from 53 to 57% between 1993 and 2001 in strains from hospitals in Shanghai (F. Wang, unpublished observation). In this study, we screened for the presence of the *qnr* gene in such strains, determined the transferability of quinolone resistance in strains with plasmids hybridizing with a *qnr* probe, and analyzed the structure of plasmid DNA adjacent to *qnr* in the new quinolone resistance plasmids we discovered.

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MATERIALS AND METHODS

Bacterial strains. Seventy-eight unique quinolone-resistant clinical strains of *E. coli* were collected from five teaching hospitals in Shanghai, China, between March 2000 and March 2001; each isolate was from a separate patient, and most were from hospitalized patients. Additional strains used were *E. coli* V517 (14); *E. coli* J53 containing plasmid R1 (12), p*lac* (12), or R27 (24) as a standard for plasmid size; *E. coli* J53Az^r (resistant to azide) as a recipient for conjugation (16); and *K. pneumoniae* UAB1 (16) as a positive control for *qnr* hybridization. Strains were routinely grown at 37°C in Luria-Bertani (LB) medium except as noted otherwise. Stock cultures were stored at −80°C in 10% glycerol brain heart infusion broth.

Screening for the *qnr* gene in clinical strains of *E. coli*. Clinical strains of *E. coli* were screened for the *qnr* gene by the colony blotting and Southern hybridization methods, which were carried out with the ECL direct nucleic acid labeling and detection system (Amersham Biosciences Corp., Piscataway, N.J.) in accordance with the manufacturer's recommendations. Colony blotting included the following steps: plating of cells and incubation overnight, colony lifts to Hybond-N$^+$ nylon hybridization transfer membrane (Amersham Biosciences Corp.), lysis with 0.5 M NaOH, fixation, rinsing in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), hybridization with the horseradish peroxidase-labeled *qnr* probe, and detection by light generated by the enzyme from luminol. The *qnr* probe was made from plasmid pMG254 (25) by PCR amplification with primers 5′-TCAGCAAGAGGATTTCTCA and 5′-GGCAGCACTATTACTCCCA. Plasmid DNAs were isolated with the QIAGEN plasmid midi kit (QIAGEN, Valencia, Calif.). DNAs were subjected to electrophoresis in 0.5% Certified Megabase Agarose (Bio-Rad Laboratory, Hercules, Calif.) gel without ethidium bromide at 70 V for 3.5 h. After depurination, denaturation, and neutralization of the gel, DNAs were transferred to a Hybond-N$^+$ membrane by capillary blotting overnight. The membrane, which was fixed by UV exposure, was hybridized with the *qnr* probe, and then the signals were detected by exposure of the membrane to Hyperfilm ECL film (Amersham Biosciences Corp.).

Conjugation. In order to determine if quinolone resistance was transferable in the strains with plasmids hybridizing with *qnr*, conjugation experiments were carried out in LB broth or on filters with *E. coli* J53Az^r as the recipient. Cultures of donor and recipient cells in logarithmic phase (0.5 ml of each) were added to 4 ml of fresh LB broth and incubated overnight without shaking. For filter conjugation, cultures of the donor and J53Az^r (alone and together) were collected on sterilized GF/A glass microfiber filters (Whatman International Ltd., Maidstone, England) and the filter was incubated on the surface of an LB agar plate overnight. Transconjugants were selected on Trypticase soy agar (TSA) plates containing sodium azide (100 μg/ml; Sigma Chemical Co., St. Louis, Mo.) for counterselection and chloramphenicol (50 μg/ml), gentamicin (10 μg/ml), or tetracycline (20 μg/ml) to select for plasmid-encoded resistance. To determine if quinolone resistance was cotransferred, colonies were replica plated onto TSA with and without ciprofloxacin (0.06 μg/ml). MICs for the donor, recipient, and transconjugant strains were measured by agar dilution in accordance with the guidelines of the NCCLS (18). The antimicrobials tested were ciprofloxacin (Bayer Corporation Pharmaceutical Division, West Haven, Conn.), ampicillin, cefotaxime, chloramphenicol, gentamicin, sulfamethoxazole, tetracycline, trimethoprim, and trimethoprim-sulfamethoxazole (SXT; Sigma Chemical Co.).

Estimation of plasmid size. Isolated plasmid DNAs from clinical, transconjugant, or reference strains were subjected to electrophoresis on 0.4% Megabase Agarose gel without ethidium bromide. The reference plasmid DNAs were the several plasmids in *E. coli* V517 (sizes, 54, 5.6, 5.1, 3.9, 3.0, 2.7, and 2.1 kb), R1 (92 kb), p*lac* (152 kb), and R27 (182 kb). The migration distances of DNA bands were measured directly from photographs of the gels. Standard polynomial curves were generated with the logarithm of the relative migration of DNAs on the *x* axis and the logarithm of the molecular size of standard plasmids on the *y* axis with the Microsoft Excel program.

Analysis of plasmid structures. The *qnr* genes from *qnr* hybridization-positive strains were sequenced directly from PCR-amplified DNA, and the sequences were compared with the sequence of the *qnr* gene from *K. pneumoniae* UAB1 (GenBank accession no. AY070235). The primers used were 5′-GGGTATGG ATATTATTGATAAAG and 5′-CTAATCCGGCAGCACTATTA. From selected plasmids, the sequences of DNA adjacent to *qnr* were determined with a series of outward-facing primers starting from both sides of the *qnr* gene on plasmid DNA purified from transconjugants. An ABI Prism 3100 DNA Sequencer (Applied Biosystems, Foster City, Calif.) was used for sequencing. Sequence analyses and comparison with known sequences were performed with the BLAST programs at the National Center for Biotechnology Information.

Stability of *qnr* in transconjugants. To determine the stability of *qnr*-mediated quinolone resistance, six transconjugants were grown separately in LB broth without antibiotics. After overnight growth, cells were diluted into LB broth to a concentration of approximately 10^3 CFU/ml and incubated at 37°C with shaking. Cultures were similarly diluted into fresh broth and incubated daily for 3 successive days. On days 2, 3, and 4, 0.1-ml samples of the cultures were diluted in 0.9% sodium chloride, spread on TSA plates, and incubated overnight. Ten random colonies were picked from plates from each culture on each day (a total of 30 for each transconjugant) and tested for susceptibility to ciprofloxacin. For colonies with loss of ciprofloxacin resistance (determined by disk diffusion), MICs of ciprofloxacin and other antimicrobials were tested. Plasmid DNA was isolated and used for Southern blotting with a *qnr* probe to confirm the loss of *qnr* with or without its associated plasmid.

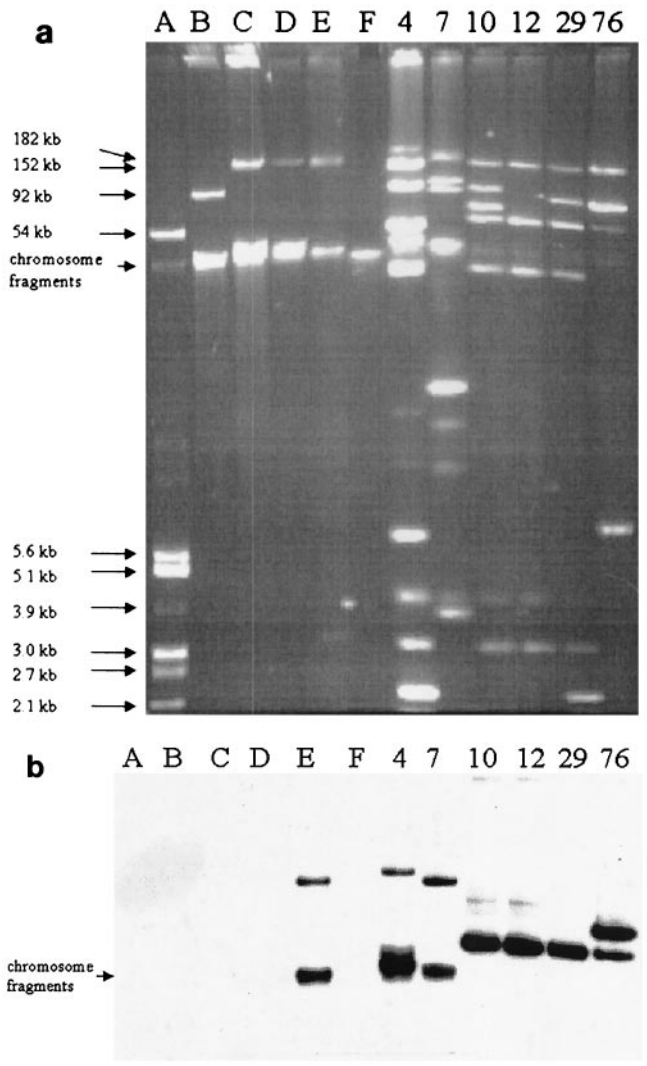

FIG. 1. Plasmid DNAs from clinical and reference strains of *E. coli* (a) and Southern hybridization of plasmid DNAs from clinical strains of *E. coli* with a *qnr* probe (b). Lanes: A, *E. coli* V517; B, *E. coli* J53 R1; C, *E. coli* J53 p*lac*; D, *E. coli* J53 R27; E, *K. pneumoniae* UAB1 (used as a positive control); F, *E. coli* J53 (used as a negative control); 4, 7, 10, 12, 29, and 76, corresponding designations of *E. coli* clinical strains.

Nucleotide sequence accession numbers. The complete sequences of integrons In36 and In37 have been submitted to the GenBank database and assigned accession numbers AY259085 and AY259086, respectively.

RESULTS

Screening for the *qnr* gene. Of the 78 ciprofloxacin-resistant *E. coli* strains studied, 65 (83.3%) were highly resistant to

TABLE 1. Conjugation with J53Az^r and selection of transconjugants

Donor	Mating condition	Antimicrobials used for selection[a]	Conjugation frequency[b]	Coresistance rate[c]	Transferred plasmid size (kb)
4	Liquid	AZD + CHL	1.6×10^{-4}	96.4	>180 (4-3), 85 (4-59, pHSH1), 54 (4-62)
7	Liquid	AZD + CHL	1×10^{-4}	100	>180
10	Liquid	AZD + TET	4×10^{-3}	96.3	68
12	Liquid	AZD + TET	6×10^{-7}	83.3	68 (pHSH2)
29	Liquid	AZD + TET	5×10^{-4}	100	68
76	Filter	AZD + GEN	1×10^{-2}	1.3	>180

[a] AZD, azide; CHL, chloramphenicol; GEN, gentamicin; TET, tetracycline.
[b] Conjugation frequency = number of transconjugants/number of donor cells.
[c] The coresistance rate is the percentage of selected colonies for which the MIC of ciprofloxacin increased.

ciprofloxacin (MIC, ≥32 μg/ml), with a range of ciprofloxacin MICs of 8 to ≥256 μg/ml. Thirty-one (39.7%) of the strains were resistant to cefotaxime, 56 (71.8%) were resistant to gentamicin, 69 (88.5%) were resistant to sulfamethoxazole-trimethoprim, and 28 (35.9%) were resistant to all four antimicrobials. Six (7.7%) of the 78 strains gave a reproducible signal for hybridization with a *qnr* gene probe on colony blots. These six strains came from a single hospital but were isolated from separate patients. Plasmid DNA from these strains also yielded strong hybridization signals with the *qnr* gene probe. Each of the six positive strains contained two to four large plasmids and one to five small plasmids (Fig. 1a). Each strain had a unique plasmid profile, although strains 10, 12, and 29 had a number of plasmid bands in common. The *qnr* probe hybridized with plasmids with a range of sizes in these six strains and with the known *qnr* plasmid from *K. pneumoniae* UAB1 but not with plasmids from other strains. In three strains (10, 12, and 29), the *qnr* probe hybridized to plasmids of 68 kb; in strain 4 and 7, it hybridized to large plasmids of >180 kb (plasmid sizes of >180 kb could not be estimated accurately with the reference plasmids and electrophoretic techniques used); and in strain 76, it hybridized to two plasmids of 68 and 93 kb (Fig. 1a and b).

Transfer of quinolone resistance. Transconjugants were selected with several antimicrobial agents but not with quinolones to avoid spontaneous quinolone resistance mutations in the recipient. Quinolone resistance was cotransferred with different resistance determinants from different donors (Table 1). For donor strains 4, 7, 10, 12, and 29, transconjugants were easily selected with tetracycline or chloramphenicol, and 83 to 100% of the colonies that grew on selection plates had decreases in susceptibility to ciprofloxacin; for donor 76, transconjugants with reduced susceptibility to ciprofloxacin could be selected with gentamicin but with a low coselection rate. Transconjugants with decreased susceptibility to ciprofloxacin contained plasmids hybridizing with the *qnr* probe. In transconjugants from donors 4, 7, 10, 12, and 29, the *qnr*-positive plasmids were the same sizes as those in the respective donors. For donor 4, transconjugants were selected in which *qnr* was also located on smaller plasmids of 54 and 85 kb. For transconjugant 76-65, the plasmid hybridizing with *qnr* was larger (>180 kb) than the two *qnr*-positive plasmids in donor 76 that themselves were not observed to transfer, suggesting a possible cointegration event or mobilization of the integron structure to the third large (initially *qnr*-negative) plasmid in the donor transfer process (Fig. 2a and b).

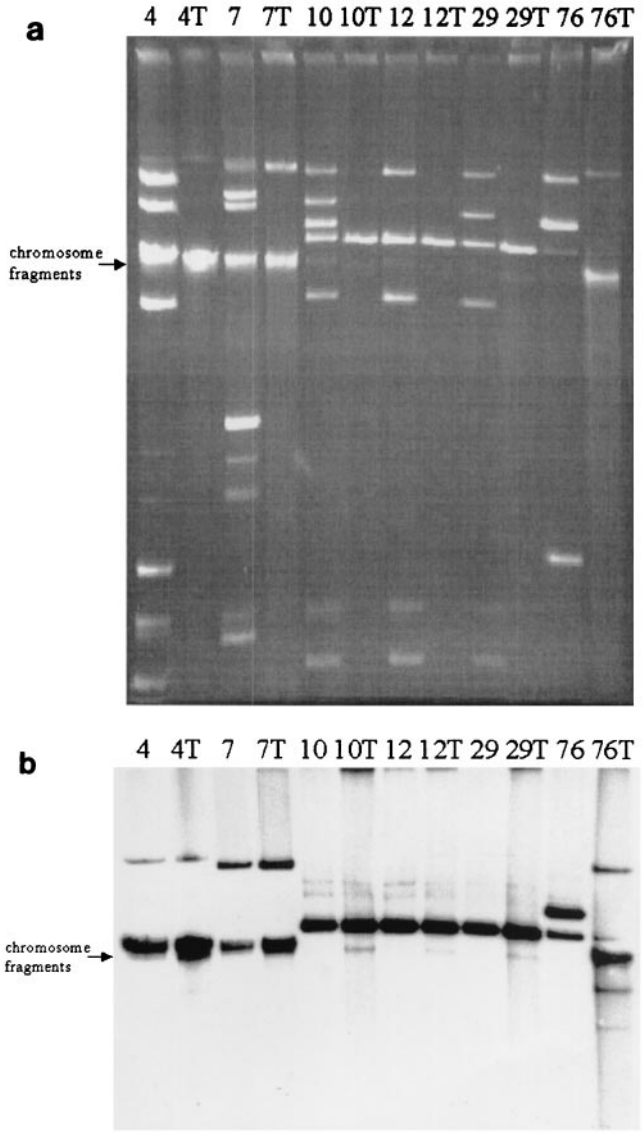

FIG. 2. Plasmid DNAs of donors and transconjugants (a) and Southern hybridization of plasmid DNAs from donors and transconjugants with the *qnr* probe (b). Strain designations are above the lanes, and those of the respective transconjugants end in the letter T.

TABLE 2. Resistance profiles of donor strains and transconjugants

Group and strain	MIC (μg/ml)								
	CIP[a]	AMP	CTX	GEN	SXT	SMZ	TMP	CHL	TET
Clinical strains (donors)									
4	64	≥512	≥512	≥512	≥512	≥512	≥128	≥512	≥512
7	128	≥512	≥512	≥512	≥512	≥512	≥128	≥512	128
10	128	≥512	≥512	≥512	≥512	≥512	≥128	64	256
12	128	≥512	≥512	≤1	≥512	≥512	≥128	64	256
29	64	≥512	256	≤1	≥512	≥512	≥128	64	256
76	≥256	≥512	64	≥512	≥512	≥512	≥128	256	256
Recipient, J53Az	0.008	16	0.03	0.125	0.5	16	0.125	4	1
Transconjugants[b]									
4-3	0.25	≥512	32	8	≥512	≥512	≥128	512	32
4-59	0.25	≥512	2	0.125	≥512	≥512	≥128	≥512	64
7-24.1	0.125	≥512	8	2	≥512	≥512	≥128	256	1
7-24.2	0.125	32	≤0.03	1	0.5	≥512	0.125	4	1
10-5	0.25	32	≤0.03	0.125	≥512	≥512	≥128	4	64
10-2	1	≥512	0.06	0.125	1	≥512	0.125	4	64
12-4	1	≥512	0.06	0.125	1	≥512	0.125	4	64
29-11	1	≥512	0.06	0.125	0.5	≥512	0.125	4	64
76-65	2	≥512	8	128	≥512	≥512	≥128	256	128

[a] CIP, ciprofloxacin; AMP, ampicillin; CHL, chloramphenicol; CTX, cefotaxime; GEN, gentamicin; SMZ, sulfamethoxazole; TET, tetracycline; TMP, trimethoprim.
[b] *qnr* is located on different-size plasmids for transconjugants 4-3 and 4-59, as well as 7-24.1 and 7-24.2, whereas *qnr* is located on the same-size plasmids for 10-5 and 10-2. For another transconjugant, 12-5, the MICs were the same as those for 12-4.

Antimicrobial resistance of transconjugants. The MICs of ciprofloxacin for transconjugants were 0.125 to 2 μg/ml, representing an increase of 16- to 250-fold relative to the recipient, *E. coli* J53Az^r (Table 2). The MICs of antimicrobials for the transconjugants produced from a given donor were uniform, but for donor 10, two transconjugants, 10-2 and 10-5, had different phenotypes, with ciprofloxacin MICs of 1 and 0.25 μg/ml, ampicillin MICs of ≥512 and 32 μg/ml, and SXT MICs of 1 and ≥512 μg/ml, respectively. Another tested transconjugant of donor 10 had the same phenotype as transconjugant 10-2. The sizes of the transferred plasmids in transconjugants 10-2 and 10-5 were similar, however.

Transconjugant 7-24.1 resulted from an experiment in which donor 7 and J53Azi^r were conjugated with chloramphenicol selection (Table 2). Purification on drug-free agar yielded a variant that was unexpectedly susceptible to chloramphenicol. This variant, 7-24.2, was susceptible to trimethoprim, as well as chloramphenicol. Both 7-24.1 and 7-24.2 contained single plasmids that hybridized with *qnr*. The *qnr*-hybridizing plasmid in 7-24.2 (Fig. 2b) was, however, smaller than that in 7-24.1(data not shown) but similar in size to the *qnr*-hybridizing plasmid in the donor. Thus, we speculate that *qnr* was mobilized upon conjugation from the smaller donor plasmid to a second chloramphenicol resistance plasmid in donor 7 (which was slightly larger than the *qnr*-hybridizing plasmid, Fig. 1a), thereby generating the larger plasmid in 7-24.1. We postulate further that the smaller plasmid in 7-24.2 resulted from the loss of the chloramphenicol and trimethoprim resistance determinants from the larger plasmid with growth in the absence of chloramphenicol selection.

Sequencing of *qnr* and analysis of plasmid structures. DNA sequencing showed that all six strains contained *qnr* with a nucleotide sequence identical to that of the originally reported *qnr* sequence (25), except for a single nucleotide change (CTA→CTG at position 537) which did not encode a change in amino acid.

Two transconjugants, 4-59 and 12-4, were chosen for sequencing of the *qnr*-containing region of the plasmids. For transconjugant 12-4, the *qnr*-positive plasmid (68 kb) was the same size as that in donor 12. For transconjugant 4-59, *qnr* was located on an 85-kb plasmid, whereas *qnr* was in a >180-kb plasmid in the original donor, strain 4. Transconjugant 4-59 was chosen for sequencing to elucidate the possible genetic mechanism by which *qnr* could have been transferred from one plasmid to another during conjugation. The plasmids in transconjugants 4-59 and 12-4 were designated pHSH1 and pHSH2, respectively.

Analysis of the gene structures indicated that *qnr* was located in large, complex In4 family class 1 integrons, with sizes of 11.6 and 12.8 kb in plasmids pHSH1 (85 kb) and pHSH2 (68 kb), respectively. Both integrons had the backbone of In4 (19, 20) with additional structures, including orf513, *qnr*, *ampR*, and the second copy of 3′ conserved segments (3′-CS). These two new integrons in transconjugants 4-59 and 12-4 have been designated In36 and In37, respectively (R. Hall, personal communication). In both integrons, *qnr* was located between duplicate 3′-CS, downstream from orf513, the region common to In6 and In7, and directly upstream from *ampR*, a regulator of the expression of *ampC*, although *ampC* itself was missing in the *qnr* integrons. Between the 5′-CS and the first copy of the 3′-CS, two gene cassettes were inserted into In36 and four gene cassettes were inserted into In37. In both integrons, the outer end of the 5′-CS was bounded by a 25-bp inverted repeat (IR) sequence called IRi. Additional 25-bp sequences, an inner IRt and an outer IRt, flanked IS*6100*, which was located next to the second copy of the 3′-CS. There was also a 5-bp (CTGTT) direct duplication directly before IRi and after the outer IRt sequence, indicating the possibility of transposition.

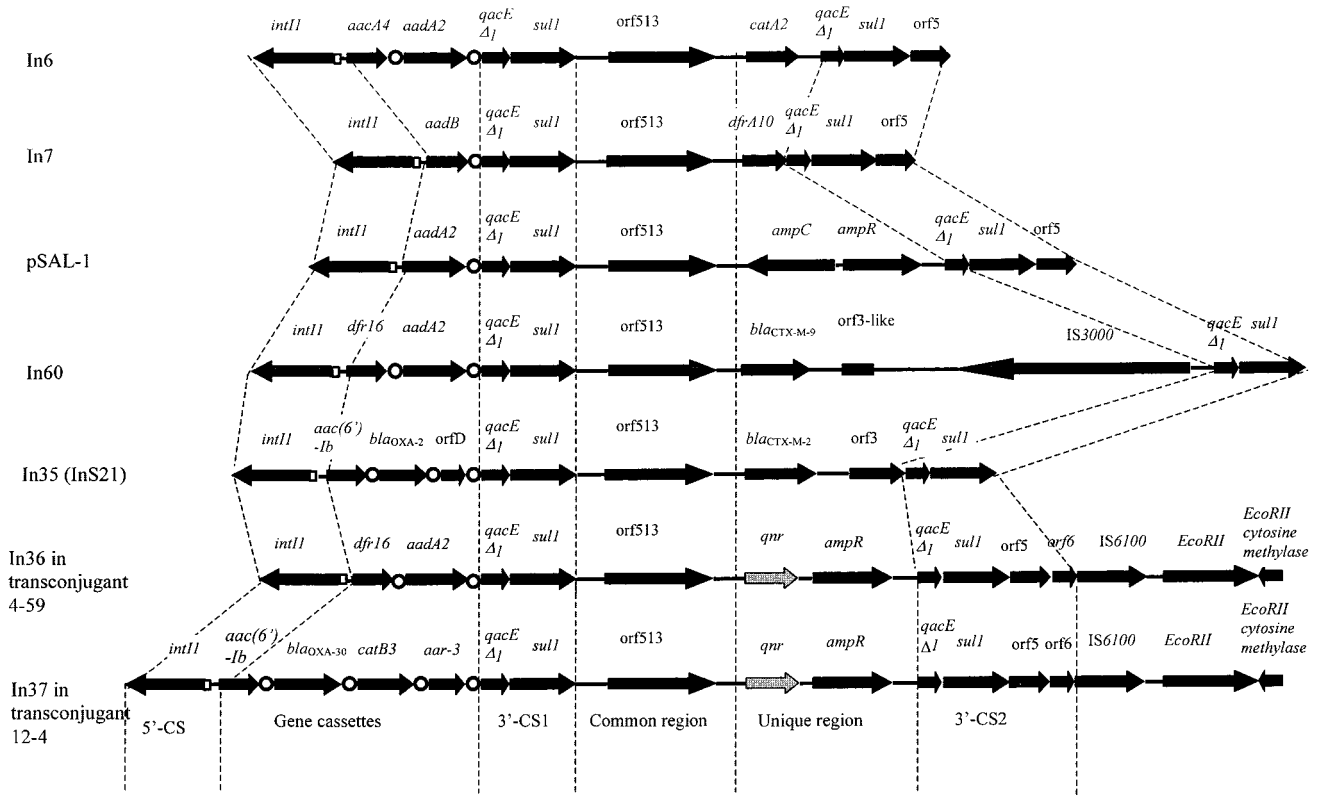

FIG. 3. Comparison of the sequences of In36 and In37 in transconjugants 4-59 and 12-4 with In6, In7, In60, the integron in pSAL-1, and In35 (InS21).

These two integrons were similar to a number of class 1 integrons reported to date, i.e., In6 (8, 22); In7 (8, 22), the integron in pSAL-1 carrying *ampC* (the bla_{DHA-1} gene) and *ampR* (26); In60 (21), carrying $bla_{CTX-M-9}$; In35 (1) or InS21 (6) (which appear to be identical), carrying $bla_{CTX-M-2}$ (Fig. 3); and integrons in pIncF1/97 and pCMR1 carrying *dfrA19* and bla_{CMY-9}, respectively. For the four plasmids in transconjugants 7-24, 10-5, 12-5, and 76-63, DNA sequences adjacent to *qnr* were determined, and the structures were similar to those of 4-59 and 12-4, with orf513 upstream and *ampR* downstream of *qnr*.

Stability of *qnr* in transconjugants. Only 1 colony (of 30 tested) of transconjugant 4-3 lost ciprofloxacin resistance and the *qnr*-hybridization signal after 2 days of incubation (approximately 40 generations). Resistance to SXT, chloramphenicol, and tetracycline was also lost in this colony. A slightly smaller plasmid was identified in the plasmid preparation, suggesting that at least parts of the integron were lost from this plasmid (data not shown). No other transconjugants lost ciprofloxacin resistance during the 3 days of incubation, indicating that *qnr* was relatively stable in these transconjugants.

DISCUSSION

Plasmid-mediated resistance to nalidixic acid was originally reported in *Shigella dysenteriae* in 1987 (17), but the original findings could not be confirmed and were likely due to selection of chromosomal mutations in transconjugants selected on nalidixic acid-containing agar (4, 5). The first valid quinolone resistance plasmid (pMG252) was discovered in a clinical isolate of *K. pneumoniae* from Birmingham, Ala., that could serially transfer quinolone resistance to *E. coli* and other gram-negative organisms when ceftazidime was used for selection (15). The plasmid-mediated quinolone resistance gene, *qnr*, was subsequently cloned from pMG252 and sequenced (25). It encodes a 218-amino-acid protein that belongs to the pentapeptide repeat family and that was shown to block the inhibitory activity of ciprofloxacin against DNA gyrase in a cell-free system (25).

Previous studies suggested that *qnr* was rare (13), but we found that 6 of 78 ciprofloxacin-resistant *E. coli* strains from a hospital in Shanghai contained *qnr* by colony blotting and Southern hybridization, indicating that in this setting, plasmid-mediated quinolone resistance is not uncommon. *qnr* was located on plasmids encoding different resistance phenotypes and with sizes of 68 to >180 kb. The large plasmids were easily broken during plasmid purification, so that positive hybridization with the *qnr* probe in the region of chromosomal DNA for strains 4 and 7 and control *K. pneumoniae* strain UAB1 on Southern transfers likely represents sheared plasmid DNA.

Extended analysis of the DNA sequences of plasmids pHSH1 and pHSH2 from two transconjugants showed that *qnr* was located in large, complex In4 family class 1 integrons adjacent to orf513 and upstream from *ampR*, *qacEΔ1*, and *sul1* (Fig. 3) (2). The original *qnr* gene in plasmid pMG252 from *K. pneumoniae* UAB1 has a somewhat similar location, but in the

Shanghai plasmids, an *ampR* gene is immediately downstream from *qnr*, while in pMG252, *qacEΔ1* and *sul1* are directly downstream. In comparison with related integrons reported to date, the two *qnr*-containing integrons, In36 and In37, from Shanghai were most similar to the integron in pSAL-1, with the replacement of *ampC* with *qnr*. In all six of the integrons analyzed in the present study, *ampR* was intact next to orf513 but *ampC* was replaced with *qnr*.

Quinolone resistance was transferred from all six *qnr*-positive strains. The transconjugants exhibited a 16-fold range of MICs of ciprofloxacin, from 0.125 to 2 μg/ml, possibly reflecting different numbers of copies of the *qnr* gene associated with the general inverse relationship of plasmid size and copy number or reflecting different levels of expression of *qnr* due to variations in promoter strength depending on its relative position in the integron structures (3). For the three transconjugants from donors 10, 12, and 29, which each had the same-size *qnr*-hybridizing plasmids, the MIC of ciprofloxacin was the same, 1 μg/ml. The sequence of the P_c promoter located within *intI1* from transconjugants 4-59 and 12-4 was the same (TGG ACA-17 bp-TAAACT) and was likely too distant to influence downstream *qnr* expression. Several putative promoter sequences near the end of the common region, a region that is upstream of *qnr*, were also identical in the two integrons (data not shown). Whether other plasmid factors affect the expression of *qnr* or whether other plasmid-encoded genes contribute to the differences in quinolone resistance independently of *qnr* in the these two transconjugants remains to be determined.

The integrons carrying *qnr* appear to be mobile among plasmids. This interpretation is supported by several observations. First, *qnr* is located on two different-size plasmids (68 and 93 kb) simultaneously in clinical strain 76. Second, *qnr* was transferred to three different-size plasmids (54, 85, and >182 kb) in three different transconjugants from donor 4. Third, *qnr* in donor 10 or 12 could be transferred to two different-size plasmids simultaneously in one transconjugant (data not shown). Fourth, the *qnr* hybridization signal was lost from transconjugant 76-91 (without loss of the plasmid itself) during growth at elevated temperature (data not shown) and was similarly lost from transconjugant 4-3 upon growth in the absence of antibiotic selection pressure. Furthermore, *qnr* may have been mobilized to a larger chloramphenicol resistance plasmid upon conjugation between donor 7 and the recipient. The mechanism by which integrons are mobilized is unclear, but their mobility is supported by their many locations, indicating past movement, and by the presence in some of them of 5-bp direct repeats, consistent with movement by a transpositional mechanism (19). It seems reasonable to predict that class 1 integrons can be mobilized as long as IRi and IRt are present and *tni* genes encoding transposition enzymes are supplied in *trans* (19). The two integrons in this study, In36 and In37, both contain IRi, IRt, and 5-bp direct duplications, indicating that movement by transposition was possible. Although *tni* genes have not yet been identified, mobility might have been supported by *tni* genes in another transposon present in the clinical strain.

In conclusion, transferable, plasmid-mediated quinolone resistance associated with *qnr* has been found in 8% of the quinolone-resistant clinical strains of *E. coli* from Shanghai, China. Although quinolone resistance conferred by *qnr*-containing plasmids may be low in level, the level of ciprofloxacin resistance is similar to or higher than that conferred by common, single *gyrA* mutations (0.32 μg/ml) (11). Furthermore, the resistance phenotype appears to be stable and *qnr* facilitates selection of additional chromosomal mutations upon exposure to quinolones (16). In this regard, all of the *qnr*-containing donor strains had levels of quinolone resistance 64- to 1,024-fold higher than those of their respective transconjugants, with ciprofloxacin MICs of 64 to ≥256 μg/ml. We have not yet fully investigated the causes of this increment, but we did observe in many of our strains a solvent tolerance phenotype (data not shown) that has been associated with increased efflux mechanisms in similar multidrug-resistant strains of *E. coli* (28). It is also likely that these donor strains had additional mutations in the genes encoding the subunits of DNA gyrase and topoisomerase IV that have been found commonly in resistant clinical isolates of *E. coli* (7, 23, 28). *qnr*-mediated quinolone resistance associated with integrons and multidrug resistance has the additional effect of genetically linking low-level quinolone resistance with other antibiotic resistances and thus promoting coselection upon exposure to other antimicrobials with resistance also encoded on the integron. The emergence of plasmid-determined quinolone resistance thus may contribute by several means to the rapid increase in bacterial resistance to quinolones in China.

ACKNOWLEDGMENTS

We thank Zijian Hu, Institute of Antibiotics, Huashan Hospital, for collection of the clinical strains used in this study and the original determinations of MICs.

This work was supported by grants AI43312 (to G.A.J.) and AI23988 (to D.C.H.) from the National Institutes of Health, U.S. Public Health Service, a grant from Kyorin Pharmaceuticals (to D.C.H.), and a grant from Project 211, Ministry of Education, People's Republic of China (to M.W.).

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