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Emergence of Plasmid-Mediated Quinolone Resistance in *Escherichia coli* in Europe

Hedi Mammeri,[1]† Marc Van De Loo,[1]† Laurent Poirel,[1] Luis Martinez-Martinez,[2] and Patrice Nordmann[1]*

Service de Bactériologie-Virologie, Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine Paris-Sud, Université Paris XI, Le Kremlin-Bicêtre, France,[1] and Servicio de Microbiologia, Hospital Universitario Marquès de Valdecilla, Santander, Spain[2]

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Although quinolone resistance results mostly from chromosomal mutations, it may also be mediated by a plasmid-encoded *qnr* gene in members of the family *Enterobacteriaceae*. Thus, 297 nalidixic-acid resistant strains of 2,700 *Escherichia coli* strains that had been isolated at the Bicêtre Hospital (Le Kremlin-Bicêtre, France) in 2003 were screened for *qnr* by PCR. A single *E. coli* isolate that carried a ca. 180-kb conjugative plasmid encoding a *qnr* determinant was identified. It conferred low-level resistance to quinolones and was associated with a chromosomal mutation in subunit A of the topoisomerase II gene. The *qnr* gene was located on a *sul1*-type class 1 integron just downstream of a conserved region (CR) element (CR1) comprising the Orf513 recombinase. Promoter sequences for *qnr* expression overlapped the extremity of CR1, indicating the role of CR1 in the expression of antibiotic resistance genes. This integron was different from other *qnr*-positive *sul1*-type integrons identified in American and Chinese enterobacterial isolates. In addition, plasmid pQR1 carried another class 1 integron that was identical to In*53* from *E. coli*. The latter integron possessed a series of gene cassettes, including those coding for the extended-spectrum β-lactamase VEB-1, the rifampin ADP ribosyltransferase ARR-2, and several aminoglycoside resistance markers. This is the first report of plasmid-mediated quinolone resistance in Europe associated with an unknown level of plasmid-mediated multidrug resistance in *Enterobacteriaceae*.

Plasmid-mediated resistance to quinolones was first reported in 1998 in a *Klebsiella pneumoniae* clinical strain isolated in 1994 in Birmingham, Ala. (10). Plasmid pMG252 of that isolate codes for a 218-amino-acid protein of the pentapeptide repeat family (11) that protects DNA from quinolone binding (21). This Qnr determinant confers resistance to nalidixic acid and increases the MICs of fluoroquinolones by four- to eightfold (10, 21, 23).

Then, *qnr*-like genes were identified in conjugative plasmids that varied in size from 54 to >180 kb in *Escherichia coli* and *K. pneumoniae* isolates in Shanghai, China, and the United States, respectively (19, 21–24). No *qnr*-like genes have been identified from other parts of the world, including Europe (5, 19). The *qnr* gene has been identified in complex In*4* family class 1 integrons (21, 24), known as complex *sul1*-type integrons. *Sul1*-type integrons possess duplicated *qacEΔ1* and *sul1* genes that surround a sequence (usually *orf513*) that may act as a recombinase for mobilization of the antibiotic resistance genes located nearby (e.g., *qnr*, bla_{CTX-M}, and *ampC*). The *qnr* gene is not associated with the 59-bp element, although common integron-associated resistance genes are (15). The definition of the conserved region (CR) established recently indicates that it consists of an *orf513* gene that encodes a recombinase and a right-hand boundary that may act as a recombination crossover site (15).

Several β-lactamase genes are associated with *qnr*-positive plasmids, such as those coding for the plasmid-mediated cephalosporinase FOX-5, the clavulanic acid-inhibited extended-spectrum class A β-lactamases SHV-7 and CTX-M-9, and the narrow-spectrum penicillinase PSE-1 (1, 10, 21–24).

In the study described here, we have investigated the frequency of the *qnr* gene in nalidixic-acid resistant clinical isolates of *E. coli*, since this species (i) is the most frequent enterobacterial species identified from human specimens and (ii) is involved in nosocomial and community-acquired infections. The transferability of quinolone resistance, the associated chromosomally encoded mechanisms of resistance to quinolones, antimicrobial coresistance, β-lactamase genes, plasmids, integrons, and the promoter sequences for *qnr* expression were determined.

MATERIALS AND METHODS

Bacterial strains and plasmids. Of 2,700 clinical isolates of *E. coli* collected at Bicêtre Hospital in 2003, 297 nalidixic-acid resistant strains (MICs, greater than or equal to 32 μg/ml) were retained for this study. Each strain was from a unique patient. Identification of the *E. coli* clinical isolates was performed with the API 20E system (bioMérieux, Marcy-l'Etoile, France).

Additional strains tested were *E. coli* J53/pMG252, which was used as a positive control for the *qnr* gene (10); *E. coli* MG-1, which was used as a positive control for bla_{VEB-1} (12); *E. coli* NCTC 50192, which contained 154-, 66-, 48-, and 7-kb reference plasmids (2); *E. coli* J53 Az^r (resistant to azide), which was used as the recipient for conjugation (10); and *E. coli* reference strains, which were used for outer membrane protein (OMP) analysis (17).

Screening for *qnr* gene and conjugation. The strains were screened for the presence of the *qnr* gene by PCR with primers QnrA and QnrB (Table 1). *Qnr*-positive strains produced a 661-bp amplification product when they were resuspended, and boiled colonies of clinical strains and standard PCR techniques were used (20).

* Corresponding author. Mailing address: Service de Bactériologie-Virologie, Hôpital de Bicêtre, 78 rue du Général Leclerc, 94275 Le Kremlin-Bicêtre cedex, France. Phone: 33-1-45-21-36-32. Fax: 33-1-45-21-63-40. E-mail: nordmann.patrice@bct.ap-hop-paris.fr.

† H.M. and M.V.D.L. contributed equally to this work.

TABLE 1. Primers used in this study

Primer	Sequence	Source or reference
ORF513D3	5′-CTCACGCCCTGGCAAGGTTT-3′	This study
ORF513D5	5′-CTTTTGCCCTAGCTGCGGT-3′	This study
CMLA-B	5′-TGGGATTTGATGTACTTTCCG-3′	13
CMLA-F	5′-CAAAAACTTTAGTTGGCGGTAC-3′	13
aadB-B	5′-CGCATATCGCGACCTGAAAGC-3′	13
aadB-F	5′-GATACACAAAATTCTAGCTGCG-3′	13
arr-2F	5′-AATTACAAGCAGGTGCAAGGA-3′	13
arr-2B	5′-TTCAATGACGTGTAAACCACG-3′	13
5′CS	5′-GGCATCCAAGCAGCAAG-3′	13
3′CS	5′-AAGCAGACTTGACCTGA-3′	13
VEB-1A	5′-CGACTTCCATTTCCCGAT GC-3′	13
VEB-1B	5′-GGACTCTGCAACAAATACGC-3′	13
VEB-INV3	5′-GAACAGAATCAGTTCCTCCG-3′	This study
VEB-INV4	5′-ACGAAGAACAAATGCACAAGG-3′	This study
OXA-10CASB	5′-CTTTGTTTT AGCCACCAATGATG-3′	13
OXA-10CASF	5′-TTAGGCCTCGCCGAAGCG-3′	13
OrfG-B	5′-GTCATTTTGAACTGCATTACC-3′	This study
IS26A	5′-CTTACCAGGCGCATTTCGCC-3′	This study
AAC1-F	5′-GTGAATTATTGCGGAATGCAGC	12
Sul1A	5′-CTT CGATGAGAGCCGGCGGC-3′	This study
Sul1B	5′-GCAAGGCGGAAACCCGCGCC-3′	This study
QnrA	5′-GGGTATGGATATTATTGATAAAG-3′	24
QnrB	5′-CTAATCCGGCAGCACTATTA-3′	24
GyrA6	5′-CGACCTTGCGAGAGAAAT-3′	8
gyrA631R	5′-GTTCCATCAGCCCTTCAA-3′	8
ParCF43	5′-AGCGCCTTGCGTACATGAAT-3′	7
ParCF981	5′-GTGGTAGCGAAGAGGTGGTT-3′	7
PreTEM-1	5′-GTATCCGCTCATGAGACAATA-3′	7
PreTEM-2	5′-TCTAAAGTATATATGAGTAAACTTGGT	7
SHV-F	5′-ATGCGTTATWTTCGCCTGTGT-3′	4
SHV-B	5′-TTAGCGTTGCCAGTGCTCG-3′	4
GES1A	5′-ATGCGCTTCATTCACGCAC-3′	4
GES1B	5′-CTATTTGTCCGTGCTCAGG-3′	4
PER-A	5′-ATGAATGTCATTATAAAAGC-3′	4
PER-D	5′-AATTTGGGCTTAGGGCAGAA-3′	4
GSP1	5′-AAGTACATCTTATGGCTGACT-3′	This study
GSP2	5′-ATGAAACTGCAATCCTCGAAACTG-3′	This study
GSP3	5′-TGGCTGAAGTCACACTGATAAAAG-3′	This study

Conjugation experiments were carried out by a filter mating technique with *E. coli* J53 Az^r as the recipient, as described previously (13, 17). Transconjugants were selected on Trypticase soy agar plates containing sodium azide (100 μg/ml; Sigma-Aldrich, Saint-Quentin-Fallavier, France) for counterselection and ampicillin (100 μg/ml), chloramphenicol (30 μg/ml), tobramycin (8 μg/ml), or ceftazidime (2 μg/ml) for selection of plasmid-encoded determinants. Selected colonies were replica plated onto Trypticase soy agar plates with and without nalidixic acid (16 μg/ml). Transconjugation frequencies were determined by dividing the number of transconjugants by the number of donor cells.

Plasmid analysis and hybridization experiments. Plasmid analyses of the clinical isolates, transconjugants, and reference strains were performed by the technique of Kieser (6), followed by agarose gel electrophoresis analysis (20). Then, reference *qnr*-positive plasmid pMG252, the transconjugant plasmid, and the plasmid of *E. coli* MG-1 were transferred onto a Hybond N$^+$ nylon membrane by using a vacuum blotting system (Amersham Pharmacia Biotech, Orsay, France) and were subsequently cross-linked with a UV Stratalinker cross-linker (Stratagene). Hybridizations were performed with an enhanced chemiluminescence nonradioactive labeling and detection kit (Amersham Pharmacia Biotech), as described by the manufacturer. The probes consisted of the 661-bp fragment for *qnr* and a 627-bp fragment for bla_{VEB-1}, and gene-specific primers were used (Table 1).

Susceptibility testing. Disk diffusion susceptibility testing was performed as described previously (18; http://www.sfm.asso.fr). MICs were determined by an agar dilution technique, as reported previously (14).

PCR and sequencing. Laboratory-designed primers (Table 1) were used for the detection of class A β-lactamase genes bla_{TEM}, bla_{SHV}, bla_{PER-1}, bla_{VEB-1}, and bla_{GES-1}; and whole-cell DNA of *E. coli* Lo was used as the template. A search for additional chromosome-encoded quinolone resistance determinants was performed with primers gyrA6 and gyrA631R for subunit *gyrA* of the topoisomerase II gene and primers ParCF43 and ParCR981 for subunit *parC* of the topoisomerase IV gene (Table 1). The *gyrA* gene-specific primers flanked a 626-bp fragment (base pairs 6 to 631), whereas the *parC* gene-specific primers flanked an 849-bp fragment (base pairs 43 to 890) of the corresponding genes. For PCR mapping of the integrons that contained the bla_{VEB-1} and the *qnr* genes, 500 ng each of whole-cell DNA of *E. coli* Lo, *E. coli* MG-1, and *E. coli* J53/pMG252 was used in standard PCR experiments with a series of PCR primers used in combination, as follows: VEB-1B and aadB-F, 5′CS and aadB-B, OXA-10CASB and aadB-F, 3′CS and OXA-10CASF, VEB-1A and arr-2B, orfG-B and AAC1-F, IS26A and aadB-B, VEB-1A and CMLA-B, ORF513D5 and QnrB, Sul1A and QnrB, 3′CS and QnrA, QnrA and ORF513D3, and 5′CS and 3′CS (Table 1).

After PCR amplification, the DNA was purified with a Qiaquick PCR purification kit (Qiagen, Courtaboeuf, France). Both strands of the amplification products obtained were sequenced with an Applied Biosystems sequencer (ABI 377). The nucleotide and deduced protein sequences were analyzed with software available over the Internet at the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov). The ClustalW program (www.infobiogen.fr) was used to align multiple protein sequences.

IEF and OMP analyses. The β-lactamase extracts from cultures of *E. coli* Lo and the transconjugants were subjected to analytical isoelectric focusing (IEF), as described previously (18). The OMP profile of *E. coli* Lo was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (17, 20) and was compared to the profiles of the *E. coli* reference strains expressing either OmpC or OmpF, as described previously (17).

Mapping of the *qnr* transcription start site. Reverse transcription and rapid amplification of cDNA ends (RACE) were performed with the 5′RACE system

(version 2.0), according to the instructions of the manufacturer (Invitrogen, Life Technologies, Cergy-Pontoise, France) (3). Briefly, 5 μg of total RNA extracted from an ampicillin-containing culture of *E. coli* Lo with an RNeasy Maxi kit (Qiagen) was used to determine the transcription initiation site of the *qnr* gene. After a reverse transcription step with gene-specific primer GSP1 (Table 1) and reverse transcriptase, the cDNA was tailed with terminal deoxynucleotidyl transferase and was subsequently amplified with another gene-specific primer, GSP2 (Table 1) combined with an oligo(dT) adapter primer provided with the kit. This PCR product was used as a template for a nested PCR with another adapter primer and primer GSP3 (Table 1). The PCR product obtained was cloned into pCR-BluntII-Topo (Invitrogen), and the corresponding clones possessing the larger insert were sequenced. Analysis of the cloned sequence allowed determination of the transcription initiation site, defined as the first nucleotide following the sequence of the adapter primer.

Nucleotide sequence accession numbers. The nucleotide sequences of the *qnr*-positive integrons of pQR-1 and pMG252 have been submitted to the GenBank nucleotide sequence database and have been assigned accession numbers AY655485 and AY655486, respectively.

RESULTS

Screening for the *qnr* gene and clinical cases. The *qnr* gene was detected in 1 of 297 nalidixic-acid resistant *E. coli* strains (0.3%). This *qnr*-positive strain, *E. coli* Lo, was isolated from a 39-year-old man in December 2003. This human immunodeficiency virus-positive patient had been hospitalized in intensive care units of several Paris hospitals for pneumonia. One month after the case of pneumonia, the patient had a cholecystitis episode caused by an antibiotic-susceptible *E. coli* isolate that was treated by endoscopic sphincterotomy and with ciprofloxacin. Then, *E. coli* Lo was isolated from stool and urine samples and was considered a colonizing agent. Preliminary antibiotic susceptibility testing by disk diffusion revealed that *E. coli* Lo was resistant to most β-lactams, including cefotaxime, ceftazidime, and aztreonam. Synergies between extended-spectrum cephalosporins and clavulanate were detected, consistent with the presence of extended-spectrum class A β-lactamases. *E. coli* Lo was also resistant to chloramphenicol, nalidixic acid, rifampin, sulfamethoxazole, tetracycline, trimethoprim, and a series of aminoglycosides and had reduced susceptibilities to fluoroquinolones (Table 2).

TABLE 2. MICs of antibiotics for *E. coli* Lo, transconjugant *E. coli* J53/pQR1, and *E. coli* J53 Az

Antibiotic(s)	MIC (μg/ml)		
	E. coli Lo[a]	*E. coli* J53/pQR1	*E. coli* J53
Nalidixic acid	>256	32	4
Ofloxacin	4	1	0.12
Ciprofloxacin	1	0.25	0.01
Moxifloxacin	2	0.5	0.03
Sparfloxacin	4	1	0.01
Rifampin	>256	32	8
Chloramphenicol	32	32	4
Gentamicin	16	8	0.12
Tobramycin	128	64	0.12
Streptomycin	>256	>256	2
Amikacin	16	8	0.25
Sulfamethoxazole	>512	>512	0.12
Trimethoprim	>512	>512	0.12
Tetracycline	>64	1	1
Amoxicillin	>512	>512	4
Amoxicillin-CLA[b]	128	32	4
Ticarcillin	>512	>512	4
Ticarcillin-CLA	256	4	4
Piperacillin	128	16	2
Piperacillin-TZB[c]	4	2	1
Cephalothin	128	128	8
Cefoxitin	4	2	2
Cefotaxime	4	4	0.06
Ceftazidime	256	256	0.06
Ceftazidime + CLA	0.25	0.25	0.06
Aztreonam	32	32	0.06
Cefepime	1	1	0.01
Imipenem	0.25	0.25	0.06

[a] *E. coli* Lo and *E. coli* J53/pQR1 contained the *qnr*-positive plasmid that also harbored the bla_{VEB-1} gene.
[b] CLA, clavulanic acid at 2 μg/ml.
[c] TZB, tazobactam at 4 μg/ml.

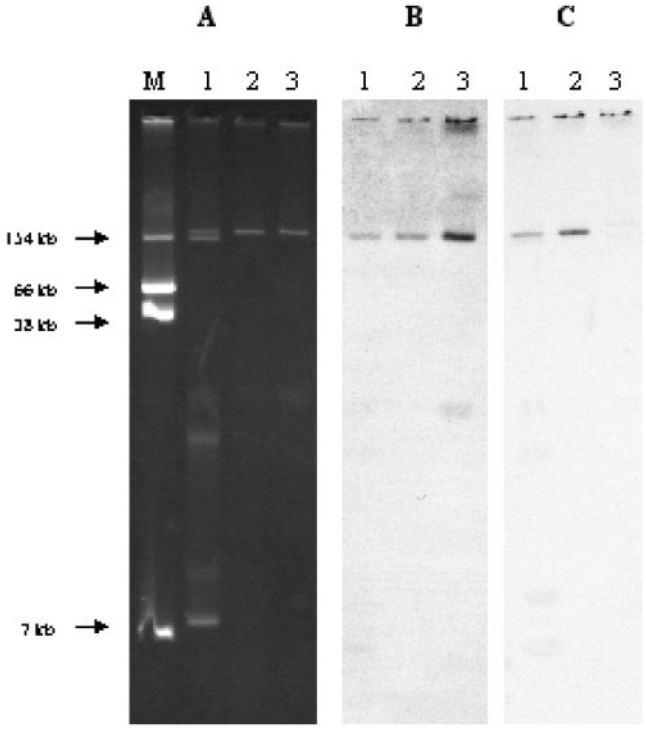

FIG. 1. Plasmid DNAs from clinical isolate *E. coli* Lo and *E. coli* transconjugant strains (A) and Southern hybridization of plasmid DNAs with the *qnr*-specific probe (B) and the bla_{VEB-1}-specific probe (C). Lanes: 1, *E. coli* Lo; 2, *E. coli* J53/pQR1; 3, *E. coli* J53/pMG252 (used as a positive control); M, *E. coli* NCTC50192 (used as a negative control and a reference for plasmid sizes).

Transfer of quinolone resistance and plasmid characterization. Quinolone resistance was transferred by conjugation after selection with a series of antibiotic resistance markers but not with quinolones to avoid the selection of spontaneous gyrase mutations in the recipient *E. coli* strain. The conjugation frequencies (the number of transconjugants divided by the number of donor cells) ranged from 1×10^{-5} to 2×10^{-6}. The frequencies of coresistance (the percentage of selected colonies that were resistant to the antibiotic used as the selector and to nalidixic acid) were 40 and 70% for amoxicillin and tobramycin, respectively, whereas the *qnr* gene was identified in 24 of 25 nalidixic acid-susceptible transconjugants tested. In most of the transconjugants, the antibiotic resistance markers cotransferred were those for resistance to ampicillin, ceftazidime, chloramphenicol, rifampin, sulfamethoxazole, tobramycin, and trimethoprim but not tetracycline.

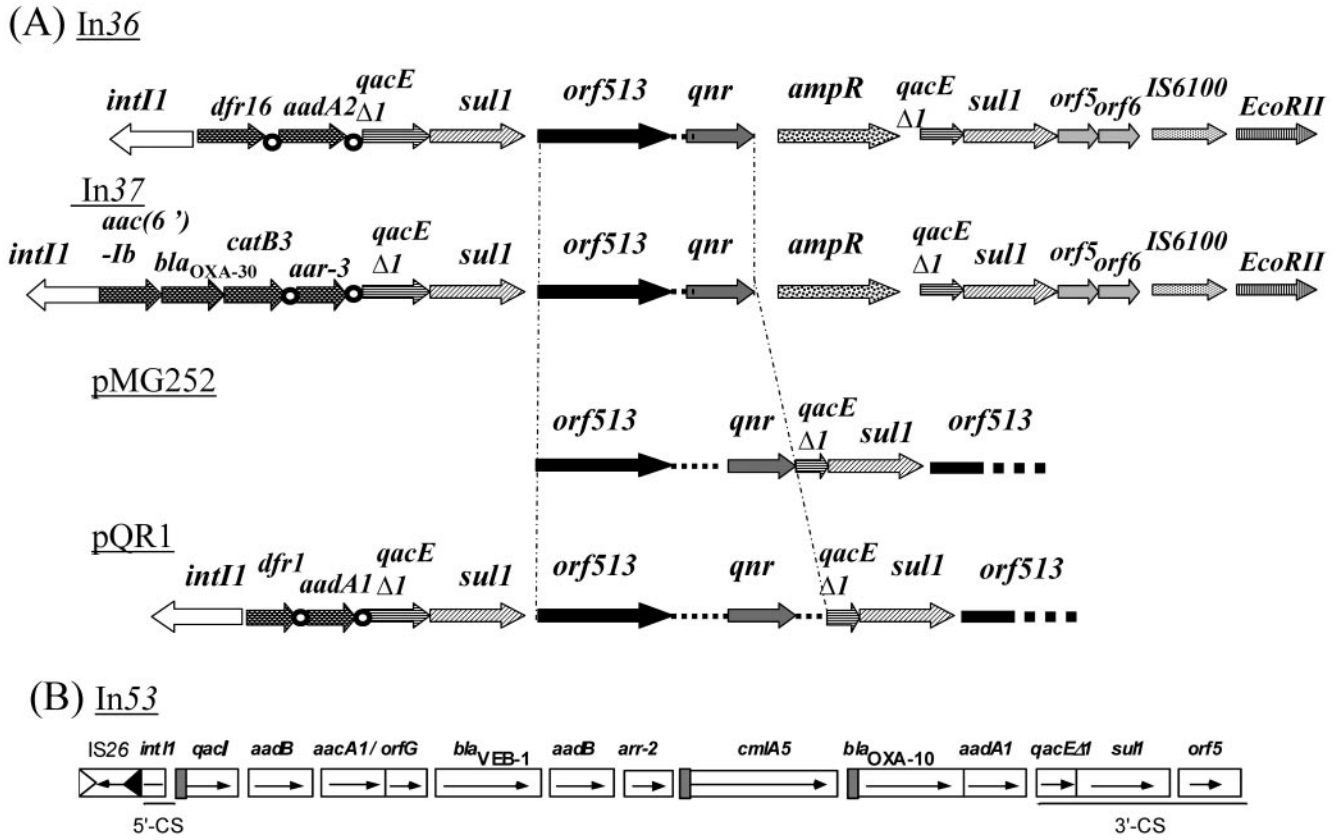

FIG. 2. Comparison of *sul1*-type integrons that contain a *qnr* gene (A) and the bla_{VEB-1}-positive class 1 integron In*53* (B). Shaded boxes in In*53* indicate gene cassettes possessing their own promoter sequences.

E. coli Lo contained three plasmids with estimated sizes of ca. 180, 150, and 10 kb, respectively, whereas transconjugants contained a single ca. 180-kb plasmid that hybridized with the *qnr*-specific probe (Fig. 1). The *qnr*-positive plasmid was designated pQR1. Plasmid pQR1 also hybridized with the *veb-1*-specific probe (Fig. 1).

Antibiotic susceptibility testing. Plasmid pQR1 conferred increased quinolone MICs (from 8- to 100-fold) for the transconjugants (Table 2). The patterns of susceptibility to β-lactams of *E. coli* Lo and the transconjugants corresponded to the expression of a clavulanic acid-inhibited expanded-spectrum β-lactamase (Table 2). In addition, plasmid pQR1 conferred resistance or decreased susceptibility to amikacin, gentamicin, streptomycin, tobramycin, chloramphenicol, rifampin, sulfamethoxazole, and trimethoprim (Table 2).

Characterization of quinolone resistance determinants. The clinical strains and transconjugant *E. coli* J53/pQR1 harbored the same *qnr* gene that was originally identified from a *K. pneumoniae* isolate in the United States (10, 21). In addition, a single Ser83Leu substitution was identified in the quinolone resistance-determining motif of subunit A of topoisomerase II, whereas a wild-type topoisomerase IV gene was found. The OMP profile of *E. coli* Lo was not modified compared to that of an *E. coli* reference strain (data not shown).

Characterization of β-lactamases. IEF analysis of culture extracts of *E. coli* Lo and *E. coli* J53/pQR1 gave three β-lactamase bands with pIs of 7.4, 6.3, and 5.4, respectively (data not shown). The genes of these β-lactamases were identified as bla_{VEB-1}, bla_{OXA-10}, and bla_{TEM-1}, according to the sequencing results.

PCR mapping and sequencing of the integrons. The *qnr* gene was located on a *sul1*-type class 1 integron (Fig. 2). It was bracketed by a duplication of the 3′ conserved sequence (3′-CS) region of the class 1 integron and was not associated with a 59-bp element. This *sul1*-type integron did not contain an *ampR* sequence coding for a LysR-type regulatory element. *Orf513* was found immediately upstream of the *qnr* gene, and a common structure of a class 1 integron that contained the *dfr1* and *aadA1* genes was identified further upstream (Fig. 2). The *dfr1* and *aadA1* genes explained resistance to trimethoprim and to streptomycin and spectinomycin, respectively. A duplication of part of *orf513* was identified downstream of the second copy of the 3′-CS element (*qacEΔ1-sul1*) (Fig. 2).

This *sul1*-type class 1 integron was different from the In*36* and In*37* integrons identified in *E. coli* isolates from Shanghai, since (i) the gene cassettes upstream of *orf513* were different, (ii) the downstream region of *orf513* in pQR1 did not contain an *ampR*-like sequence, and (iii) the DNA sequence between the right-hand boundary of CR1 and the *qnr* gene was 32 bp longer in the *sul1*-type integron of pQR1 than in In*36* and In*37* (Fig. 2 and 3). The *sul1*-type integron of pQR1 was also different from that of pMG252, since (i) in pQR1 the gene cassettes identified upstream of *orf513* were absent from

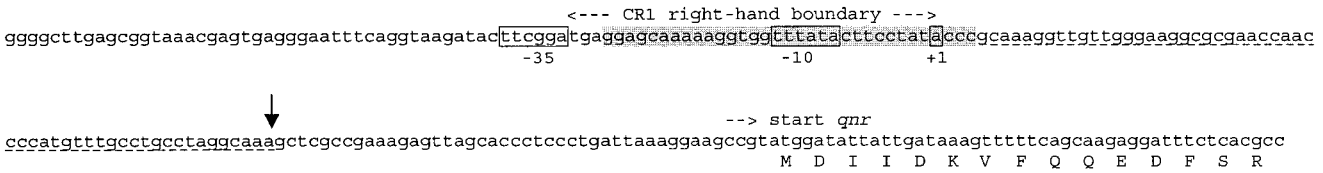

FIG. 3. Nucleotide sequence of the promoter structure for *qnr* expression, as determined by the 5′ RACE experiment. The deduced amino acid sequence of Qnr is designated in single-letter code below the nucleotide sequence. The transcription orientation of the *qnr* gene is indicated by a horizontal arrow. The right-hand boundary of CR1 is shaded in gray. The −35 and −10 promoter sequences are boxed, as is the +1 transcription initiation site; all of these are part of the CR1 element. The vertical arrow indicates the position of the extremity of the CR1 right-hand boundary of In*36* (24), in which the 67-bp sequence with a dotted underlined is lacking.

pMG252, and (ii) the downstream region of the *qnr* gene in the *sul1*-type integron of pQR1 contained an additional 265-bp sequence compared to the sequence of pMG252. This additional 265-bp fragment contained 25 bp belonging to the *qacEΔ1* gene in pQR1 (Fig. 2).

The structure of the class 1 integron that contained the *veb-1* cassette was also determined (Fig. 2). It was identical to that reported in In*53* (4, 13). The *veb-1* cassette in pQR1 was not part of a Tn*2000* composite transposon, since the right copy of IS*26* of Tn*2000* was missing (Fig. 2) (13). A series of PCR-based experiments failed to identify the bla_{VEB-1}-positive integron in the immediate vicinity of the *qnr*-positive *sul1*-type integron.

Mapping of *qnr* transcription site. By using 5′ RACE PCR experiments, the site of initiation of transcription of the *qnr* gene was mapped in *E. coli* Lo to be 104 bp upstream of the start codon of this gene (Fig. 3). Upstream of this transcriptional start site, a −35 promoter sequence (TTCGGA) was found, and this was separated by 18 bp from a −10 promoter sequence (TTTATA) (Fig. 3). These promoter sequences overlapped the CR1 element.

DISCUSSION

This study reports on the first detection of plasmid-mediated quinolone resistance in Europe. Twenty-five *qnr*-positive *K. pneumoniae* and *E. coli* isolates have been identified from U.S. and Chinese isolates among a total of 967 gram-negative isolates of worldwide origin tested (5, 19, 21–24). The variability of the criteria for the strains tested makes the precise determination of the prevalence of this plasmid-borne mechanism of resistance impossible. In the present study, the Qnr determinant was very rare, since only a single *E. coli* isolate with this determinant was identified. The prevalence of this gene in nalidixic-acid resistant *E. coli* isolates (0.3%) seems to be much lower than that reported in ciprofloxacin-resistant *E. coli* isolates from Shanghai (7.7%) (24).

However, one cannot rule out the possibility that the failure of detection of *qnr*-positive strains may have resulted from weak expression of the Qnr determinant. Indeed, we, like others (5, 21–24), have found that several transconjugants could be nalidixic acid susceptible and truly *qnr* positive, which raises the question of its expression. In addition, nucleotide changes in the extremities of the *qnr*-like genes corresponding to the location for hybridization of the PCR primers designed for the study may have limited further detection of *qnr*-positive strains.

As reported previously (9, 10, 21, 23), the Qnr determinant alone did not provide resistance to fluoroquinolone, according to the NCCLS guidelines. In *E. coli* Lo, it was associated with an Ser83Leu substitution in the chromosomally encoded subunit A of topoisomerase II. Combined mechanisms of resistance to quinolones in *E. coli* Lo corresponded to what had been predicted in in vitro studies (9), and explained the lower levels of quinolone resistance for the transconjugants than for the clinical strain.

The sequence of the *qnr* gene was identical to that first reported for a *K. pneumoniae* strain isolated in Alabama in 1994 (21). The variability of the *qnr*-like genes identified worldwide seems to be very much limited, with only a single nucleotide change (without an amino acid change) identified among the *qnr*-like genes from American and Chinese isolates that have been sequenced (19, 21–24), thus suggesting a common source. The *qnr* gene G+C content of 60% argues for a non-enterobacterial origin.

Structure analysis of the *qnr*-positive integrons indicated sequence variability, with the integron of pQR1 being more related to that of pMG252 of *K. pneumoniae* UAB1 from Alabama (21) than to those of *E. coli* isolates from Shanghai (24). Whereas the *qnr* gene itself remains quite invariable, it is likely that different recombination events that resulted in the acquisition of the *qnr* gene in integrons had occurred.

The expression of *qnr* depended on the −35 and −10 promoter sequences located in the CR1 element. This CR1 element and the Orf513 recombinase have been associated with a series of antibiotic resistance genes, such as those coding for CTX-M-type β-lactamase; plasmid-mediated cephalosporinase; and sulfonamide, chloramphenicol, macrolide, and tetracycline resistance determinants (15). Thus, these structures may be involved not only in mobilization of the antibiotic resistance genes located downstream, as suggested previously (15), but also in their expression.

The *qnr* determinant was associated with the bla_{VEB-1} gene, which may further explain a tight association between resistance to expanded-spectrum cephalosporins and resistance to quinolones (16).

Finally, we showed that a single conjugative plasmid may carry two types of class 1 integrons that may confer resistance to quinolones, most β-lactams except carbapenems, most aminoglycosides, sulfonamides, rifampin, trimethoprim, and chloramphenicol. This is the outmost evolution of coresistance to broad-spectrum antibiotics located on a single genetic vehicle.

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