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A Plasmid-Borne *Shewanella algae* Gene, *qnrA3*, and Its Possible Transfer In Vivo between *Kluyvera ascorbata* and *Klebsiella pneumoniae*▽

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The plasmid-borne quinolone resistance gene *qnrA1* is prevalent in multidrug-resistant *Enterobacteriaceae*. A chromosomally encoded homologue in *Shewanella algae*, *qnrA3*, has been described. We isolated two *qnrA3*-positive strains, one of *Klebsiella pneumoniae* (He96) and one of *Kluyvera ascorbata* (Kas96), from the feces of an immunocompromised outpatient. The *qnrA3* allele was identical to that of *S. algae* except for 5 nucleotides and differed from *qnrA1* by 29 nucleotides affecting three amino acids. The analysis of the *qnrA3* genetic environment showed that *qnrA3* was inserted downstream from an IS*CR1* element at a recombination crossover site described for other resistance genes, including *qnrA1*, and immediately upstream from IS*26*, a situation not described before. IS*26* preceded an incomplete class 1 integron which contained, among other genes, *aac(6′)-Ib-cr*, another transferable quinolone resistance gene, and the β-lactamase gene $bla_{OXA-1/30}$. The 10-kb fragment encompassing *qnrA3* was compared to previously described *qnrA1*-containing plasmids and multidrug-resistant plasmids; it shares identical sequences with pC15a, pHSH2, pQR1, pQKp311H, and pSAL-1 but with rearrangements, deletions, and mutations. Conjugal transfer of *qnrA3* was highly efficient (10^{-2}) from *K. pneumoniae* He96 or *K. ascorbata* Kas96 to *Escherichia coli* J53 but less so (10^{-5}) from either donor to a clinical strain of *Enterobacter cloacae*. This first description of a plasmid-borne copy and of the in vitro transfer of *qnrA3* is taken to illustrate its likely in vivo transfer from *S. algae* to the *Enterobacteriaceae*.

Two novel mechanisms of resistance to fluoroquinolones in *Enterobacteriaceae* were recently described: quinolone acetylation mediated by AAC-(6′)-Ib-cr, an altered form of the original aminoglycoside 6′-*N*-acetyltransferase, and Qnr-mediated topoisomerase protection (40, 46). They differ markedly from the classical mechanisms, and their genes are plasmid rather than chromosome borne. The classical mechanisms comprise alterations in DNA gyrase and topoisomerase IV (the quinolone targets), enhancement of drug efflux, a decrease in the permeability of the bacterial cell wall, or a combination thereof (14, 36).

The *qnr* genes known so far are *qnrA*, *qnrS*, and *qnrB* (38), with *qnrA* first described in *Klebsiella pneumoniae*, *qnrS* in *Shigella flexneri*, and *qnrB* in *K. pneumoniae* and *Escherichia coli* (20). Subsequent reports showed that these genes are also present in other species of *Enterobacteriaceae*, especially in multidrug-resistant isolates (6, 25, 32, 41). Variants were successively described for each gene, i.e., *qnrA1* to *-A6*, *qnrB1* to *-B19*, and *qnrS1* and *-S2* (2, 6, 20, 30, 37–39, 43). They have recently been reclassified, especially the *qnrB* alleles (17). While the amino acid identity among the proteins encoded by gene variants is between 91 and 99%, it is only 35 to 60% among QnrA, QnrB, and QnrS.

Transferable *qnr* genes are usually carried by large conjugative plasmids (50 to 180 kb) that often encode extended-spectrum β-lactamases (ESBLs) or AmpC-type β-lactamases (18, 30). The *qnr* genes were shown to be located in the vicinity of intact, antibiotic resistance determinant-containing class 1 integrons (20, 22, 26, 50). Transfer of plasmid-borne *qnr* was shown to occur by conjugation (19, 27, 50). Chromosome-borne *qnr*-type genes were discovered in environmental bacteria such as *Photobacterium profundum* (43), *Vibrionaceae* (9, 33, 43), and *Shewanella algae* (30, 34). In *S. algae*, which is hypothesized to be the origin of *qnrA*, the allele is highly homologous (90%) to *qnrA1*.

We recently screened strains of *Enterobacteriaceae* isolated in 2004 from the Hôpital Européen Georges Pompidou in Paris (France) for *qnrA* and found only two *qnrA*-positive isolates (one of *K. pneumoniae* and one of *Kluyvera ascorbata*). They were isolated from the feces of the same immunocompromised patient and possessed an original *qnrA* allele (GenBank accession no DQ435306) different from those in other strains isolated in Paris (6, 26). We found this allele to be homologous to *qnrA3* of *S. algae*, raising the question of how it had been transferred to and among the clinical isolates. It was the purpose of this study to analyze the genomic environment of *qnrA3* in both isolates and to evaluate its in vitro transferability.

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MATERIALS AND METHODS

Bacterial strains. *K. pneumoniae* He96 was isolated in 2004 at Hôpital Européen Georges Pompidou and was screened from 129 nonconsecutive enterobacterial isolates for the coincidence of resistance to third-generation cephalosporins and presence of *qnr*, as previously described (6). This strain was isolated from the feces of a 49-year-old outpatient with Hodgkin's disease. *K. ascorbata* Kas96 was isolated from the same specimen of feces at 10^6 CFU/mg. Both strains were identified using 16S rRNA and *rpoB* gene sequencing, with *K. ascorbata* ATCC 33433 and *Kluyvera cryocrescens* ATCC 33435 as the reference strains.

E. coli J53 Azi^r (50) and *Enterobacter cloacae* Ecl115, a clinical strain isolated from the same ward and during the same period as *K. pneumoniae* He96 and *K. ascorbata* Kas96, served as recipient strains for transfer experiments.

MICs of quinolones were determined using the agar dilution method, and susceptibility to antibiotics other than quinolones was tested using the disk diffusion method (disks from Bio-Rad, Marnes La Coquette, France) on Mueller-Hinton agar as specified elsewhere (http://www.sfm.asso.fr).

Transfer experiments. Conjugal quinolone resistance transfer between *K. pneumoniae* He96 or *K. ascorbata* Kas96 and *E. coli* J53 was tested. Strains were grown to logarithmic phase in brain heart infusion broth, and 2 ml of the donor and the recipient strain suspensions were mixed in a 50-ml flask and incubated at 37°C for 40 min without shaking. Transconjugant selection was performed on Mueller-Hinton plates containing sodium azide (100 μg/ml) and either ampicillin (100 μg/ml) or tetracycline (20 μg/ml). Plates were incubated at 37°C and inspected at 24 and 48 h. Conjugal transfer between *K. pneumoniae* He96 or *K. ascorbata* Kas96 and an in vitro-selected sodium azide-resistant mutant of the clinical strain *E. cloacae* Ecl115 was similarly tested.

Plasmid analysis. Plasmids were extracted with the High Speed Plasmid Midi kit (Qiagen, Courtaboeuf, France). PCR-based replicon typing was performed on the transconjugants of *E. coli* J53 and of *E. cloacae* Ecl115 after mating with the two donor strains *K. pneumoniae* He96 and *K. ascorbata* Kas96 (strains *E. coli* Tc He96/J53, *E. coli* Tc Kas96/J53, *E. cloacae* Tc He96/Ecl115, and *E. cloacae* Tc Kas96/Ecl115). Primers for PCRs were chosen for the identification of the most frequent replicons, using sets 1 (HI1, HI2, and I1), 2 (X, L/M, and N), 3 (FIA, FIB, and W), and 4 (Y, P, and FIC) in multiplex reactions and primer pairs A/C and OR1/CA1 in simplex reactions. Primer sequences and assay conditions were those described previously (7), except for OR1/CA1 (31).

Amplification and sequencing of *qnrA* and analysis of its genetic environment. The sequence of *qnrA* was determined after amplification with intragenic primers qnrA5s and qnrA6as, using plasmid DNA as the template (6), and that of its environment after amplification with primers specific for genes usually surrounding *qnrA* (PCR sets A, B, and C [Table 1]). PCR was carried out with Long Expand polymerase (Roche Diagnostics, Meylan, France) in an iCycler (Bio-Rad) as follows: 2 min at 94°C; 10 cycles of 10 s at 94°C, 30 s at 55°C, and 2 min at 68°C; 25 cycles of 15 s at 94°C and 30 s at 55°C; and cycle elongation at 68°C starting from 2 min with a further 20 s for each successive cycle. PCR-amplified fragments were sequenced after purification with the Montage PCR Millipore purification kit (Millipore, Saint Quentin-en-Yvelines, France). Sequencing was performed using the ABI Prism BigDye Terminator v3.1 cycle sequencing kit and the ABI Prism 3100 sequencer (Applied Biosystems, Courtaboeuf, France). Nucleotide sequences were analyzed with SeqScape (Applied Biosystems) and compared with each other and with related sequences in the data banks.

Amplification and sequencing of the quinolone resistance-determining regions of *gyrA*, *gyrB*, *parC*, *parE*, and β-lactamase genes. Total DNA was extracted, and the quinolone resistance-determining regions of *gyrA*, *gyrB*, *parC*, and *parE* were amplified as described previously (24). *bla* genes of the TEM, SHV, CTX-M, and OXA types were screened for by PCR as described previously (12, 29).

Nucleotide sequence accession number. The *qnrA* sequence determined in this study was submitted to GenBank under accession number DQ435306. The nucleotide sequences of the encompassing fragment of 10,776 bp from pHe96 and pKas96 were submitted to GenBank/EMBL/DDJB under accession numbers EU495237 and EU495238.

TABLE 1. Oligonucleotides primers used for PCR and sequencing of the ca. 10-kb DNA fragment encompassing the *qnrA3* gene in pHe96, pKas96, and Tc He96/Ecl115

DNA fragment (primer set)	Primer[a]	Primer sequence (5′→3′)
qacΔE1-qnrA3 (A)	**qacΔE1 s**	**GGCTTTTTCTTGTTATCGCA**
	qnrA7 as	**GCGAAAACGGCTGTCACTC**
	orf513 s	CCATGTCGCTGGCAAGGAA
	orf513 s2	TCGCATCGCTCGCTGCATG
	orf513 as	GCTGCCACCAGAACGAGCGCC
	sul1 s	GAGGCGGACTGCAGGCTGGT
qnrA3 (B)	**qnrA5 s**	**GGGTATGGATATTATTGATAAAG**
	qnrA6 as	**CTAATCCGGCAGCACTATTA**
	qnrA9 s	GCCATAAGATGTACTTCTGCT
	qnrA10 as	AGCAGAAGTACATCTTATGGC
qnrA-qacΔE1 (C)	**qnrA8 s**	**GTCAAGATCTGTGCCCTGGCA**
	qacΔE1 as	**CAAGCTTTTGCCCATGAAGC**
	tnpA3 s	AGCTGCATACCGGTTTCTGGG
	51pb s	CGCTAACTTTGCAACAGTGC
	int2 s	GCGAACCACTCATCCGGGGT
	aac(6′)-Ib-cr s	CGATTGGGTATGCCCAGTCG
	blaOXA1/30 s	CGATGCATCCACAACGCT
	blaOXA1/30 s2	CCGCACTTACAGGAAACT
	catB3 s	GGGCATCGGTACGACTGG
	arr-3 s	GCGGCTACATATACATAG
	dfr s	AGTTGTCAGCCGCTCAGG
	ant(3′)-Ij-aac(6′)-Ib as	GCAGCGCAAGGACATTCTTG

[a] Primers used for PCR are in bold; the other primers were used for sequencing. s, sense primer; as, antisense primer.

RESULTS AND DISCUSSION

The plasmid-borne *qnrA3* allele and its genetic environment. *K. pneumoniae* He96 and *K. ascorbata* Kas96 were identified on the basis of their 16S rRNA sequences and particularly their *rpoB* sequences, which are more discriminatory in the identification of *Enterobacteriaceae* (28). Each strain contained a plasmid of ca.70 kb (pHe96 and pKas96, respectively) (data not shown).

Since this is the first observation of *qnrA3* as a plasmid-borne gene, we analyzed in detail the sequences of *qnrA3* and its environment and compared them to published sequences of plasmids containing other *qnrA* genes, such as pHSH2 from *qnrA1*-positive *E. coli* strains isolated in Hong-Kong (50), pQR1 from a *qnrA1*-positive *E. coli* strain isolated in France (Paris) (26), and pQKp311H from a *qnrA1*-positive *K. pneumoniae* strain isolated in Spain (Barcelona) (25).

The *qnrA* sequence was the same for *K. pneumoniae* He96 and *K. ascorbata* Kas96 and was 95.6% identical to that of *qnrA1* (46), with 29 nucleotide differences accounting for three amino

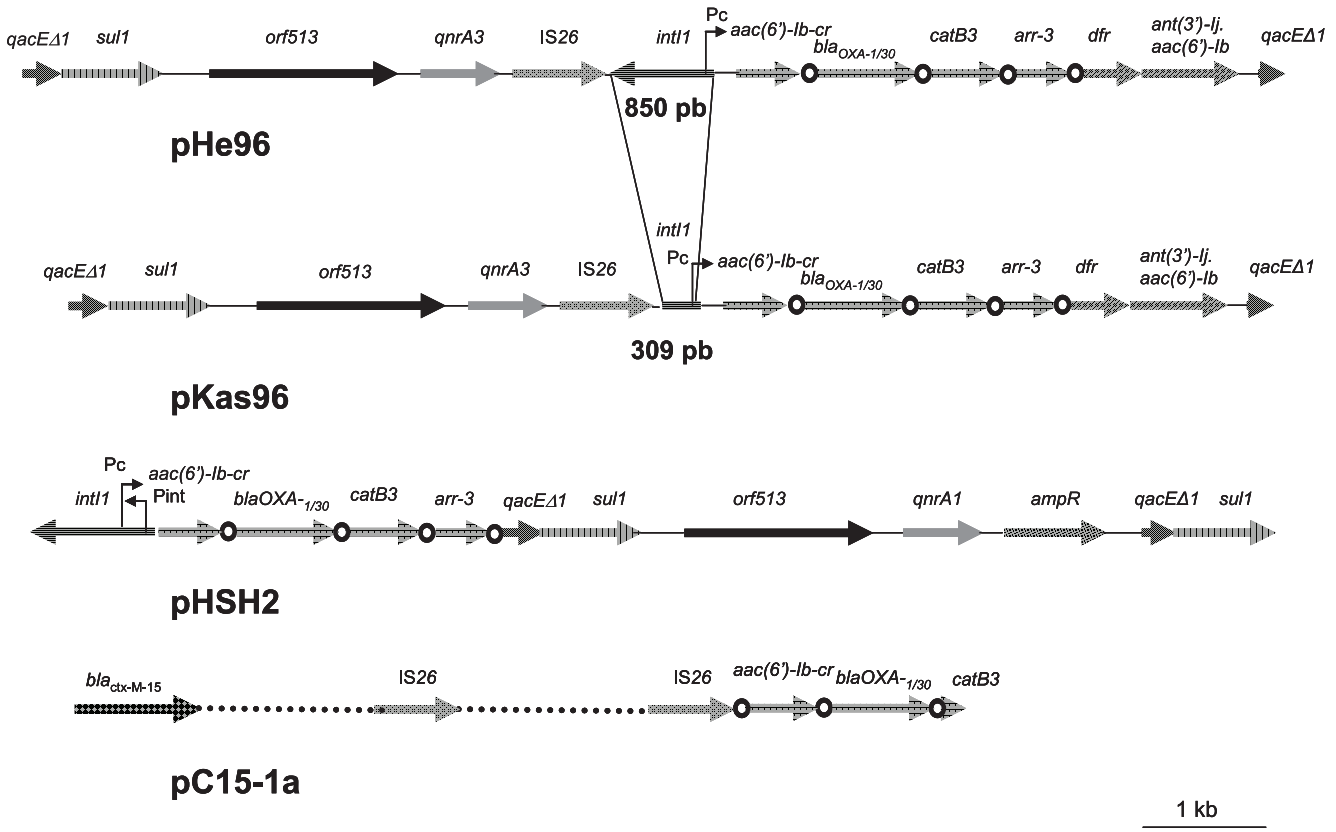

FIG. 1. Genetic environment of *qnrA3* in *K. pneumoniae* He96 (pHe96) and in *K. ascorbata* Kas96 (pKas96). The 59-bp elements are indicated by circles. The sequences of pHe96 are compared with those described for pHSH2 (50) (GenBank accession number AY259086) and pC15-1a (5) (GenBank accession number NC005327).

acid substitutions, i.e., Arg39Gln, Ile108Val, and Ala127Thr. The deduced amino acid sequence was identical to that of the chromosome-borne *qnrA3* gene of *S. algae* (30), but there were five nucleotide differences (99.2% identity). No *qnrB* or *qnrS* gene was detected.

The sequences up- and downstream from *qnrA3* are schematically shown in Fig. 1 and are detailed in Fig. 2. They were obtained from two fragments amplified on one hand with a sense primer in *qacEΔ1* and an antisense primer in *qnrA3* and on the other hand with a sense primer in *qnrA3* and an antisense primer in *qacEΔ1*, with sequences being obtained using primers within the fragments (Table 1). Nucleotide sequences of the encompassing fragment of 10,776 bp from pHe96 and pKas96 were identical in He96 and Kas96, except for 541 bp which were absent in Kas96.

The *qnrA3* gene was observed downstream from an IS*CR1* element similar to that described previously (45). The 233-bp sequence downstream from *orf513* (M233 in Fig. 2) was identical to those in most of the other *qnrA1*-containing plasmids described so far (19, 21, 25, 26, 46, 50) and to that in pSAL-1 upstream from the *ampC-ampR* operon (48) (Fig. 2). In several *qnrA1*-containing plasmids, such as pHSH2 and pQKp311H, *ampR* was observed immediately downstream from *qnrA1*, but this was not the case in our plasmids. This suggests that the insertion of *qnrA3* downstream from IS*CR1* may have followed the same mechanism as that of *qnrA1* and also as that of the insertion of the chromosome-borne region encoding *ampC-ampR* from *Morganella morganii*. Indeed, the insertion probably occurred at the recombination crossover site (ACCC-) at the 3′ end of IS*CR1* (Fig. 2), as described previously (1, 48).

The sequence immediately downstream from *qnrA3* was identical to that found downstream from *qnrA3* in the *S. algae* chromosome (34), which confirms that *qnrA3* has been excised from chromosomal DNA of *S. algae* or similar organisms, and was ended by a 7-bp element (M7) found in the other *qnr*-containing plasmids and also in pSAL-1. After 124 bp (M124) that were 92% identical to pHSH2 and other *qnrA1*-containing plasmids but of unknown origin, there was an unusual insertion of an IS*26* element (11). Proximity of IS*26* and *qnrA1* was recently reported for pQKp311H (25).

Downstream from IS*26*, 52 bp were identical with those found in pC15-1a, a multiresistance plasmid containing $bla_{CTX\text{-}M\text{-}15}$ and described from an *E. coli* outbreak in Canada (5) (Fig. 3). Sequence identity with pC15-1a ended at the site of insertion of an incomplete *intlI* gene and resumed with the succession of resistance gene cassettes [*aac*(*6′*)*-Ib-cr* to *catB3*] (Fig. 1 and 3). The same sequence organization was also found in In37, present in pHSH2 (Fig. 1) (50). Compared to In37, a 217-bp deletion was observed in pHe96 upstream from *aac*(*6′*)*-Ib-cr*, which included the 83-bp 5′ terminus of *intI1* (Fig. 3). The *intI1* gene also lacked 81 bp at its 3′ end. The 217-bp deletion accounted for the absence of start and stop codons in *intlI* and of the promoter P_{int}, while the promoter P_c was complete (Fig. 3). However, the −10 box of the promoter Pc differed from

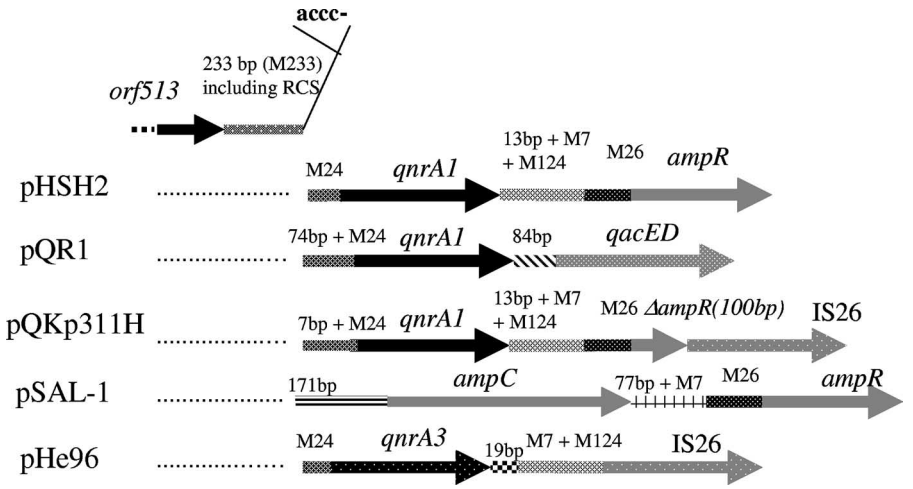

FIG. 2. Schematic map (not to scale) of the regions upstream and downstream from *qnrA3* and comparison with published *qnrA1*-containing plasmids (pHSH2 [50]; pQR1 [26]; pQKp331H [25]) and pSAL-1, containing DHA-1, the AmpC-type β-lactamase gene originating from chromosomal DNA of *Morganella morganii* (48). RCS, recombination crossover site (1). Sequences that were found in at least two plasmids are shown as motifs (M) with the number of base pairs: M233 (sequence under GenBank accession no. EU495237), M7 (5′-ACAAGAG-3′ in all plasmids, except 5′-ACAAGAG-3′ in pHe96), M24 (5′-CCCTCCCTGATTAAAGGAAGCCGT-3′), M26 (5′-CCTAAAGAAAAACTTACAG GTGGATT-3′), and M124 (sequence under GenBank accession no. EU495237). Specific features of pHe96 are the following, from 5′ to 3′: the recombinase gene *orf513*; a 233-bp sequence (M233) common to all plasmids; the 24-bp (M24) sequence common to all *qnrA* plasmids with an additive sequence of 7-bp (GTTAGCA) and 74 bp (GCAAAGGTTGTTGGGAAGGCGCGAACCAACCCCATGTTTGCCTGCCTAGGCAA AGCTCGCCGAAAGAGTTAGCA) upstream in pQKp331H and pQR1, respectively; the *qnrA* gene; 19 bp homologous to the *S. algae* chromosome; M7 (detailed above); M124, common to pHSH2 and pQKp311H; and IS*26*, composed of a complete *tnpA* gene (717 bp) and the specific 51-bp inverted repeat.

In37 by two mutations and was identical to that found in pCTX-M-3, isolated from a multidrug-resistant strain of *Citrobacter freundii* (42). What distinguished the sequence in *K. ascorbata* Kas96 from that in *K. pneumoniae* He96 was a further 541-bp deletion in the integrase gene at the 3′ end (Fig. 1 and 3).

The sequence of the *aac*(*6′*)*-Ib* gene present in pHe96 and pKas96, was identical to that of the alleles found in In37 (pHSH2) and pC15-1a (5, 50), with two substitutions, Thr104Arg and Asp181Tyr with respect to the initially sequenced AAC(6′)-Ib (47), which designated it a *cr1* variant (25, 40). The following gene, $bla_{\text{OXA-30}}$ (44) (recently found to be identical with $bla_{\text{OXA-1}}$ [4]), and the sequence between *aac*(*6′*)*-Ib-cr* and $bla_{\text{OXA-1/30}}$ are identical to those found in In37 and pC15-1a, although in pC15-1a neither gene is present as an integron-borne cassette (5). The chloramphenicol acetyltransferase gene *catB3*, and the rifampin ADP-ribosylating transferase gene *arr-3* are in the same order downstream from $bla_{\text{OXA-1/30}}$ in In37, at variance with the gene organization in pC15-1a (Fig. 1). Since the DNA segments comprising IS*26*–*aac*(*6′*)*-Ib-cr*–$bla_{\text{OXA-1/30}}$–*catB3* are identical in In37, pC15-1a, and pHe96, these elements may be derived from the same ancestral strain or plasmid or have been mobilized similarly. Whether pC15-1a contains a reduced form of In37 with deletion of *intI1* and rearrangements due to IS*26* or whether In37 has gained the antibiotic resistance cassettes from pC15-1a cannot be said. However, the fact that the −35 and −10 promoter boxes of *aac*(*6′*)*-Ib-cr* were found adequately upstream from *aac*(*6′*)*-Ib-cr* in pC15-1a but that the −35 box is absent in In37 favors the second hypothesis. It seems that in pHe96 the integrase gene was inserted between *tnpA* and *aac*(*6′*)*-Ib-cr*, since the −35 and the −10 boxes were separated by 940 bp (mostly the *intI1* gene) in pHe96 (Fig. 3), with the sequence found at the 5′end of *aac*(*6′*)*-Ib-cr* being identical to that in pC15-1a (5) (Fig. 1). Consequently, pHe96 cannot be derived from In37 but may have been derived from pC15-1a.

Characterization of antibiotic resistance determinants. *K. pneumoniae* He96 exhibited higher levels of resistance to quinolones than *K. ascorbata* Kas96 (Table 2). Since *qnrA3* was reported to confer only decreased susceptibility to quinolones (34) and the MICs of ciprofloxacin and of levofloxacin were high in *K. pneumoniae* He96, we sought an additional quinolone resistance mechanism such as topoisomerase mutation. *K. pneumoniae* He96 harbored one *gyrA* mutation (Ser83Phe), with no mutation in *gyrB* or in the topoisomerase IV genes. This *gyrA* mutation added to the quinolone resistance phenotype conferred by the two plasmid-borne quinolone resistance genes, *qnrA3* and *aac*(*6′*)*-Ib-cr*.

K. pneumoniae He96 exhibited a β-lactam resistance phenotype including resistance to piperacillin as well as combinations of clavulanic acid with amoxicillin or ticarcillin and a decrease in susceptibility to piperacillin combined with tazobactam, cefotaxime, and cefepime, as previously described for OXA-1/30 producers (10), whereas *K. ascorbata* appeared to be susceptible to amoxicillin-clavulanic acid, piperacillin-tazobactam, cefotaxime, and cefepime. However, both strains harbored $bla_{\text{OXA-1}}$ and produced an enzyme compatible with an OXA-type β-lactamase (data not shown). In addition, in He96 we found an SHV-1 variant (Leu35Gln, Thr149Ser) not previously described and different from the chromosomal gene $bla_{\text{LEN-1}}$, but no TEM-type or CTX-M-type genes were found. Both strains were resistant to tetracycline and co-trimoxazole but susceptible to chloramphenicol.

Plasmids of ca. 70 kb containing *qnrA3* were transferred at a

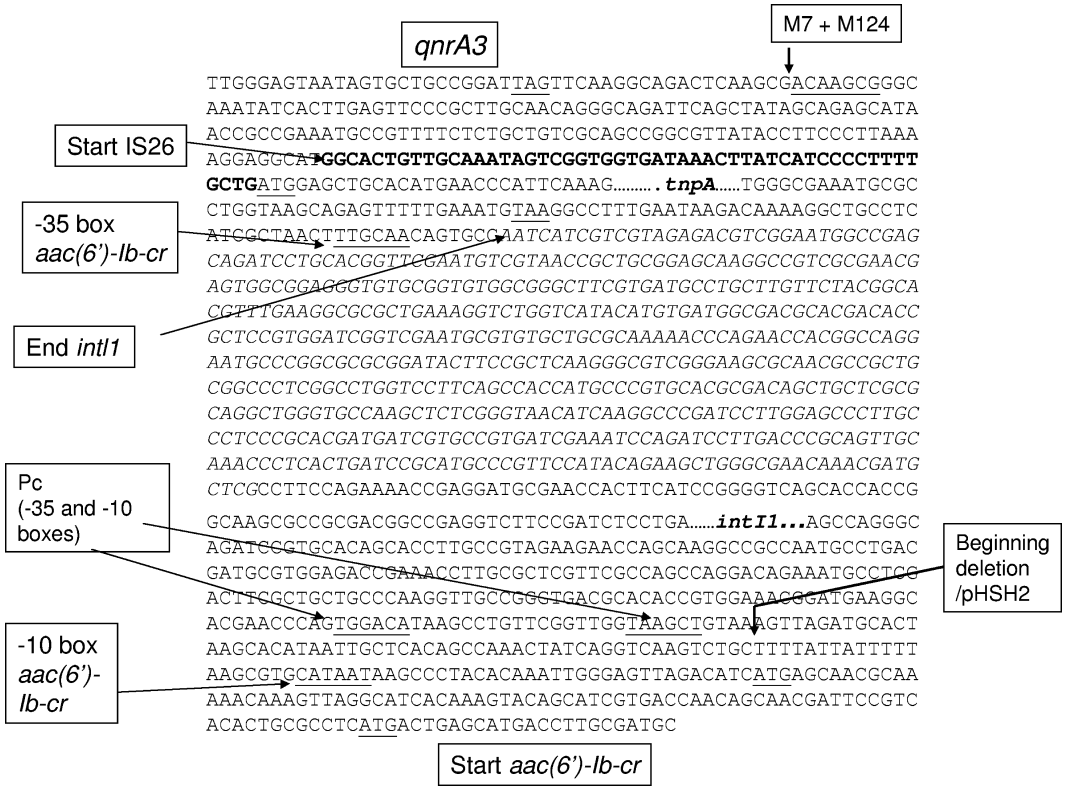

FIG. 3. Detailed sequence downstream from *qnrA3* for pHe96 and pKas96. Italics indicate the part which is deleted in pKas96. M7 and M124 are defined in Fig. 2. Underlined sequences correspond to the following features: stop codon of *qnrA3*, 7-bp element, start codon *tnpA*, stop codon *tnpA*, −35 promoter box of *aac*(*6′*)-*Ib-cr*, −35 and −10 promoter boxes of Pc in *intI1*, −10 promoter box of *aac*(*6′*)-*Ib-cr*, start codon of *aac*(*6′*)-*Ib-cr* as described for pC15-a, and start codon of *aac*(*6′*)-*Ib-cr* as described for pHSH2.

frequency of 10^{-2} from *K. pneumoniae* He96 or *K. ascorbata* Kas96 to *E. coli* J53. This frequency is among the highest reported for other plasmid-borne *qnr* alleles (19, 49). The $bla_{OXA-1/30}$ gene was cotransferred along with tetracycline and trimethoprim resistance determinants. Transfer of *aac*(*6′*)-*Ib-cr* was first assumed on the basis of increased MICs of kanamycin and tobramycin for the *E. coli* transconjugants (Table 2). Since similar MICs were observed in both transconjugants (Table 2), the expression of the *aac*(*6′*)-*Ib-cr* gene from the two plasmids is probably similar, despite the differences in its surrounding se-

TABLE 2. MICs of quinolones and aminoglycosides, and susceptibility phenotypes for other antibiotics, for *K. pneumoniae* He96, *K. ascorbata* Kas96, *E. coli* J53, *E. cloacae* Ecl115, and their respective *qnrA3*-positive transconjugants

Species and strain	MIC (μg/ml)[a]										Susceptibility phenotype[b]			
	Quinolones						Aminoglycosides							
	NAL	NOR	CIP	LVX	MXF	GAT	GEN	KAN	TOB	AMK	TMP	TET	PIP	TZP
K. pneumoniae He96	128	32	8	4	4	2	0.25	48	12	6	R	R	R	I
K. ascorbata Kas96	64	8	2	1	4	2	<0.06	6	2	1.5	R	R	R	S
E. coli														
J53	4	0.03	0.008	0.01	0.06	0.01	<0.06	0.75	0.19	0.75	S	S	S	S
Tc He96/J53	16	1	0.25	0.12	0.5	0.25	0.09	16	4	2	R	R	I	S
Tc Kas96/J53	8	0.5	0.12	0.12	0.5	0.25	0.09	8	3	1.5	R	R	I	S
E. cloacae														
Ecl115	4	0.06	0.01	0.06	0.12	0.06	32	8	4	1.5	S	S	R	S
Tc He96/Ecl115	8	0.5	0.25	0.12	0.5	0.25	24	16	6	2	R	R	R	I
Tc Kas96/Ecl115	8	0.5	0.25	0.12	0.5	0.25	24	24	8	2	R	R	R	I

[a] NAL, nalidixic acid; NOR, norfloxacin; CIP, ciprofloxacin; LVX, levofloxacin; MXF, moxifloxacin; GAT, gatifloxacin; GEN, gentamicin; KAN, kanamycin; TOB, tobramycin; AMK, amikacin.

[b] TMP, trimethoprim; TET, tetracycline; PIP, piperacillin; TZP, piperacillin-tazobactam; R, resistant; I, intermediate; S, susceptible.

quences mentioned above. Furthermore, the presence of this gene, which has the peculiar property of conferring selective resistance to quinolones with a nonsubstituted piperazinyl group at C-7, may explain why the transconjugants of *E. coli* J53 showed a greater increase in the MICs of ciprofloxacin and norfloxacin (16- and 32-fold increases) than in those of levofloxacin, moxifloxacin, and gatifloxacin (2- to 16-fold) in comparison to parental strains. The second aminoglycoside resistance gene in the 10-kb fragment, *ant*(*3′*)-*Ij*-*aac*(*6′*)-*Ib*, was truncated at its 3′ end for the last 585 bp and thus was not assumed to confer additional aminoglycoside resistance.

For the *E. coli* J53 transconjugants obtained from the parental strains He96 and Kas96, PCR-based replicon typing was positive for N-type replicons and negative for the other types, which suggests that *qnrA3* is harbored on an IncN plasmid. *qnrA1* genes have been associated so far with the IncA/C-type plasmids but not with IncN-type plasmids (35). Conversely, IncN-type plasmids were previously associated with the β-lactamase genes $bla_{\text{VIM-1}}$ (8) and $bla_{\text{CTX-M-3}}$ (15) but not with *qnr* genes, although replicon typing was seldom done in *qnr*-positive strains.

Interspecies transfer of *qnrA3*. In light of the observation of the *qnrA3* variant, so far described only for *Shewanella* (30), on two close-to-identical ca. 10-kb plasmid-borne fragments in isolates of different bacterial species from the same patient, we suspected the possibility of an in vivo interspecies transfer of *qnrA3*. We therefore tried to reproduce the presumptive in vivo transfer from *K. ascorbata* Kas96 or *K. pneumoniae* He96 to other clinical isolates of *Enterobacteriaceae*. A *qnrA*-negative *E. cloacae* strain that was susceptible to tetracycline and trimethoprim, thereby allowing for the selection of the *qnrA3* plasmid-containing transconjugants, was successfully conjugated. However, the transfer was observed at a frequency of 10^{-5}, i.e., 1,000-fold lower than that between *K. ascorbata* and *E. coli* J53. The low frequency of transfer may be due to inefficient conjugation or to the occurrence of recombination within the host plasmid (the strain was an ESBL producer) mediated by integron-like structure (3).

To check this hypothesis, we first compared the plasmid contents in *E. cloacae* Ecl115 and in the two transconjugants *E. cloacae* Tc He96/Ecl115 and *E. cloacae* Tc Kas96/Ecl115. In *E. cloacae* Ecl115, the presence of two plasmids of the IncHI2 and IncL/M types, but none of the IncN type, was suspected on the basis of replicon typing results. The IncHI2 type has previously been associated with $bla_{CTX\text{-}M\text{-}9}$-containing plasmids (15), while to our knowledge an association of the L/M group with antibiotic resistance genes has not been reported. The *E. cloacae* transconjugants were indeed positive for both HI2 and L/M replicons and also for the N-type replicon corresponding to the plasmid from strains He96 and Kas96.

We also studied *E. cloacae* Ecl115 for genes similar to those included in pHe96 and the two transconjugants for additional genes in the same order as in the 10-kb fragment containing *qnrA3*. In *E. cloacae* Ecl115, we detected an IS*26* element, similar to that of pHe96 and pKas96, with a partial *aac*(*3*)-*II* gene upstream from IS*26*, in the same genetic context as described for pC15-1a (5) but downstream from an IS*CR1* element in the case of *E. cloacae* Ecl115. The ESBL gene was $bla_{\text{CTX-M-3}}$, a gene shown to originate from the *K. ascorbata* chromosome and from which $bla_{\text{CTX-M-15}}$, contained in pC15-1a, was derived by a point mutation leading to Asp240Gly (42). Sequencing of the 10-kb fragment amplified from the transconjugant Tc He96/Ecl115 revealed sequence identity with pHe96, including *qnrA3*. Overall, this favors the hypothesis of a conjugative transfer between two strains of clinical origin, strains which usually are more difficult to conjugate than laboratory strains (50).

K. ascorbata is an environmental, waterborne bacterium that may cause food-borne infections in humans (13). Infections due to *K. ascorbata* have rarely been described but may occur in immunocompromised patients such as the patient with Hodgkin's disease from whom strain Kas96 was isolated. In this patient, the strain did not cause infection; however, it was found to be dominant in his gut flora, together with *K. pneumoniae*. The patient had received β-lactams and vancomycin but not quinolones during the month before the isolation of *K. pneumoniae* He96 and *K. ascorbata* Kas96. The role of *K. ascorbata* as a reservoir of resistance genes has been recognized with the discovery, in their chromosomes, of a variety of CTX-M genes (16, 23, 42). Since *K. ascorbata* may live for long periods as a commensal in the human gut, it may well contribute to resistance gene transfer by conjugation to other inhabitants of this ecosystem, transient or not, such as *K. pneumoniae* and *E. cloacae* (13).

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REFERENCES

1. **Bae, I. K., Y. N. Lee, W. G. Lee, S. H. Lee, and S. H. Jeong.** 2007. Novel complex class 1 integron bearing an IS*CR1* element in an *Escherichia coli* isolate carrying the $bla_{\text{CTX-M-14}}$ gene. Antimicrob. Agents Chemother. **51:** 3017–3019.
2. **Bonemann, G., M. Stiens, A. Puhler, and A. Schluter.** 2006. Mobilizable IncQ-related plasmid carrying a new quinolone resistance gene, *qnrS2*, isolated from the bacterial community of a wastewater treatment plant. Antimicrob. Agents Chemother. **50:**3075–3080.
3. **Bouvier, M., G. Demarre, and D. Mazel.** 2005. Integron cassette insertion: a recombination process involving a folded single strand substrate. EMBO J. **24:**4356–4367.
4. **Boyd, D. A., and M. R. Mulvey.** 2006. OXA-1 is OXA-30 is OXA-1. J. Antimicrob. Chemother. **58:**224–225.
5. **Boyd, D. A., S. Tyler, S. Christianson, A. McGeer, M. P. Muller, B. M. Willey, E. Bryce, M. Gardam, P. Nordmann, and M. R. Mulvey.** 2004. Complete nucleotide sequence of a 92-kilobase plasmid harboring the CTX-M-15 extended-spectrum beta-lactamase involved in an outbreak in long-term-care facilities in Toronto, Canada. Antimicrob. Agents Chemother. **48:**3758–3764.
6. **Cambau, E., C. Lascols, W. Sougakoff, C. Bebear, R. Bonnet, J. D. Cavallo, L. Gutmann, M. C. Ploy, V. Jarlier, C. J. Soussy, and J. Robert.** 2006. Occurrence of *qnr*A-positive clinical isolates in French teaching hospitals during 2002–2005. Clin. Microbiol. Infect. **12:**1013–1020.
7. **Carattoli, A., A. Bertini, L. Villa, V. Falbo, K. L. Hopkins, and E. J. Threlfall.** 2005. Identification of plasmids by PCR-based replicon typing. J. Microbiol. Methods **63:**219–228.
8. **Carattoli, A., V. Miriagou, A. Bertini, A. Loli, C. Colinon, L. Villa, J. M. Whichard, and G. M. Rossolini.** 2006. Replicon typing of plasmids encoding resistance to newer beta-lactams. Emerg. Infect. Dis. **12:**1145–1148.
9. **Cattoir, V., L. Poirel, D. Mazel, C. J. Soussy, and P. Nordmann.** 2007. *Vibrio splendidus* as the source of plasmid-mediated QnrS-like quinolone resistance determinants. Antimicrob. Agents Chemother. **51:**2650–2651.
10. **Dubois, V., C. Arpin, C. Quentin, J. Texier-Maugein, L. Poirel, and P. Nordmann.** 2003. Decreased susceptibility to cefepime in a clinical strain of *Escherichia coli* related to plasmid- and integron-encoded OXA-30 beta-lactamase. Antimicrob. Agents Chemother. **47:**2380–2381.
11. **Eckert, C., V. Gautier, and G. Arlet.** 2006. DNA sequence analysis of the genetic environment of various blaCTX-M genes. J. Antimicrob. Chemother. **57:**14–23.

12. **Eckert, C., V. Gautier, M. Saladin-Allard, N. Hidri, C. Verdet, Z. Ould-Hocine, G. Barnaud, F. Delisle, A. Rossier, T. Lambert, A. Philippon, and G. Arlet.** 2004. Dissemination of CTX-M-type beta-lactamases among clinical isolates of *Enterobacteriaceae* in Paris, France. Antimicrob. Agents Chemother. **48:**1249–1255.
13. **Farmer, J. J., III, G. R. Fanning, G. P. Huntley-Carter, B. Holmes, F. W. Hickman, C. Richard, and D. J. Brenner.** 1981. *Kluyvera*, a new (redefined) genus in the family *Enterobacteriaceae*: identification of *Kluyvera ascorbata* sp. nov. and *Kluyvera cryocrescens* sp. nov. in clinical specimens. J. Clin. Microbiol. **13:**919–933.
14. **Hooper, D.** 2003. Mechanisms of quinolone resistance, p. 41–67. *In* D. Hooper and E. Rubinstein (ed.), Quinolone antimicrobial agents. ASM Press, Washington, DC.
15. **Hopkins, K. L., E. Liebana, L. Villa, M. Batchelor, E. J. Threlfall, and A. Carattoli.** 2006. Replicon typing of plasmids carrying CTX-M or CMY beta-lactamases circulating among *Salmonella* and *Escherichia coli* isolates. Antimicrob. Agents Chemother. **50:**3203–3206.
16. **Humeniuk, C., G. Arlet, V. Gautier, P. Grimont, R. Labia, and A. Philippon.** 2002. Beta-lactamases of *Kluyvera ascorbata*, probable progenitors of some plasmid-encoded CTX-M types. Antimicrob. Agents Chemother. **46:**3045–3049.
17. **Jacoby, G., V. Cattoir, D. Hooper, L. Martinez-Martinez, P. Nordmann, A. Pascual, L. Poirel, and M. Wang.** 2008. *qnr* gene nomenclature. Antimicrob. Agents Chemother. **52:**2297–2299.
18. **Jacoby, G. A.** 2005. Mechanisms of resistance to quinolones. Clin. Infect. Dis. **41**(Suppl. 2)**:**S120–S126.
19. **Jacoby, G. A., N. Chow, and K. B. Waites.** 2003. Prevalence of plasmid-mediated quinolone resistance. Antimicrob. Agents Chemother. **47:**559–562.
20. **Jacoby, G. A., K. E. Walsh, D. M. Mills, V. J. Walker, H. Oh, A. Robicsek, and D. C. Hooper.** 2006. *qnrB*, another plasmid-mediated gene for quinolone resistance. Antimicrob. Agents Chemother. **50:**1178–1182.
21. **Jeong, J. Y., H. J. Yoon, E. S. Kim, Y. Lee, S. H. Choi, N. J. Kim, J. H. Woo, and Y. S. Kim.** 2005. Detection of *qnr* in clinical isolates of *Escherichia coli* from Korea. Antimicrob. Agents Chemother. **49:**2522–2524.
22. **Kehrenberg, C., S. Friederichs, A. de Jong, G. B. Michael, and S. Schwarz.** 2006. Identification of the plasmid-borne quinolone resistance gene *qnr*S in Salmonella enterica serovar Infantis. J. Antimicrob. Chemother. **58:**18–22.
23. **Lartigue, M. F., L. Poirel, D. Aubert, and P. Nordmann.** 2006. In vitro analysis of IS*Ecp1B*-mediated mobilization of naturally occurring beta-lactamase gene $bla_{\text{CTX-M}}$ of *Kluyvera ascorbata*. Antimicrob. Agents Chemother. **50:**1282–1286.
24. **Lascols, C., J. Robert, V. Cattoir, C. Bebear, J. D. Cavallo, I. Podglajen, M. C. Ploy, R. Bonnet, C. J. Soussy, and E. Cambau.** 2007. Type II topoisomerase mutations in clinical isolates of *Enterobacter cloacae* and other enterobacterial species harbouring the *qnr*A gene. Int J. Antimicrob Agents **29:**402–409.
25. **Lavilla, S., J. J. Gonzalez-Lopez, M. Sabate, A. Garcia-Fernandez, M. N. Larrosa, R. M. Bartolome, A. Carattoli, and G. Prats.** 2008. Prevalence of *qnr* genes among extended-spectrum beta-lactamase-producing enterobacterial isolates in Barcelona, Spain. J. Antimicrob. Chemother. **61:**291–295.
26. **Mammeri, H., M. Van De Loo, L. Poirel, L. Martinez-Martinez, and P. Nordmann.** 2005. Emergence of plasmid-mediated quinolone resistance in *Escherichia coli* in Europe. Antimicrob. Agents Chemother. **49:**71–76.
27. **Martinez-Martinez, L., A. Pascual, and G. A. Jacoby.** 1998. Quinolone resistance from a transferable plasmid. Lancet **351:**797–799.
28. **Mollet, C., M. Drancourt, and D. Raoult.** 1997. *rpoB* sequence analysis as a novel basis for bacterial identification. Mol. Microbiol. **26:**1005–1011.
29. **Mugnier, P., I. Casin, A. T. Bouthors, and E. Collatz.** 1998. Novel OXA-10-derived extended-spectrum beta-lactamases selected in vivo or in vitro. Antimicrob. Agents Chemother. **42:**3113–3116.
30. **Nordmann, P., and L. Poirel.** 2005. Emergence of plasmid-mediated resistance to quinolones in Enterobacteriaceae. J. Antimicrob. Chemother. **56:**463–469.
31. **Osborn, A. M., R. W. Pickup, and J. R. Saunders.** 2000. Development and application of molecular tools in the study of IncN-related plasmids from lakewater sediments. FEMS Microbiol. Lett. **186:**203–208.
32. **Park, C. H., A. Robicsek, G. A. Jacoby, D. Sahm, and D. C. Hooper.** 2006. Prevalence in the United States of *aac*(*6′*)*-Ib-cr* encoding a ciprofloxacin-modifying enzyme. Antimicrob. Agents Chemother. **50:**3953–3955.
33. **Poirel, L., A. Liard, J. M. Rodriguez-Martinez, and P. Nordmann.** 2005. Vibrionaceae as a possible source of Qnr-like quinolone resistance determinants. J. Antimicrob. Chemother. **56:**1118–1121.
34. **Poirel, L., J. M. Rodriguez-Martinez, H. Mammeri, A. Liard, and P. Nordmann.** 2005. Origin of plasmid-mediated quinolone resistance determinant QnrA. Antimicrob. Agents Chemother. **49:**3523–3525.
35. **Poirel, L., L. Villa, A. Bertini, J. D. Pitout, P. Nordmann, and A. Carattoli.** 2007. Expanded-spectrum beta-lactamase and plasmid-mediated quinolone resistance. Emerg. Infect. Dis. **13:**803–805.
36. **Poole, K.** 2005. Efflux-mediated antimicrobial resistance. J. Antimicrob. Chemother. **56:**20–51.
37. **Quiroga, M. P., P. Andres, A. Petroni, A. J. Soler Bistue, L. Guerriero, L. J. Vargas, A. Zorreguieta, M. Tokumoto, C. Quiroga, M. E. Tolmasky, M. Galas, and D. Centron.** 2007. Complex class 1 integrons with diverse variable regions, including *aac*(*6′*)*-Ib-cr*, and a novel allele, *qnrB10*, associated with IS*CR1* in clinical enterobacterial isolates from Argentina. Antimicrob. Agents Chemother. **51:**4466–4470.
38. **Robicsek, A., G. A. Jacoby, and D. C. Hooper.** 2006. The worldwide emergence of plasmid-mediated quinolone resistance. Lancet Infect. Dis. **6:**629–640.
39. **Robicsek, A., D. F. Sahm, J. Strahilevitz, G. A. Jacoby, and D. C. Hooper.** 2005. Broader distribution of plasmid-mediated quinolone resistance in the United States. Antimicrob. Agents Chemother. **49:**3001–3003.
40. **Robicsek, A., J. Strahilevitz, G. A. Jacoby, M. Macielag, D. Abbanat, C. H. Park, K. Bush, and D. C. Hooper.** 2006. Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. Nat. Med. **12:**83–88.
41. **Robicsek, A., J. Strahilevitz, D. F. Sahm, G. A. Jacoby, and D. C. Hooper.** 2006. *qnr* prevalence in ceftazidime-resistant *Enterobacteriaceae* isolates from the United States. Antimicrob. Agents Chemother. **50:**2872–2874.
42. **Rodriguez, M. M., P. Power, M. Radice, C. Vay, A. Famiglietti, M. Galleni, J. A. Ayala, and G. Gutkind.** 2004. Chromosome-encoded CTX-M-3 from *Kluyvera ascorbata*: a possible origin of plasmid-borne CTX-M-1-derived cefotaximases. Antimicrob. Agents Chemother. **48:**4895–4897.
43. **Saga, T., M. Kaku, Y. Onodera, S. Yamachika, K. Sato, and H. Takase.** 2005. *Vibrio parahaemolyticus* chromosomal *qnr* homologue VPA0095: demonstration by transformation with a mutated gene of its potential to reduce quinolone susceptibility in *Escherichia coli*. Antimicrob. Agents Chemother. **49:**2144–2145.
44. **Siu, L. K., J. Y. Lo, K. Y. Yuen, P. Y. Chau, M. H. Ng, and P. L. Ho.** 2000. Beta-lactamases in *Shigella flexneri* isolates from Hong Kong and Shanghai and a novel OXA-1-like beta-lactamase, OXA-30. Antimicrob. Agents Chemother. **44:**2034–2038.
45. **Toleman, M. A., P. M. Bennett, and T. R. Walsh.** 2006. IS*CR* elements: novel gene-capturing systems of the 21st century? Microbiol. Mol. Biol. Rev. **70:**296–316.
46. **Tran, J. H., and G. A. Jacoby.** 2002. Mechanism of plasmid-mediated quinolone resistance. Proc. Natl. Acad. Sci. USA **99:**5638–5642.
47. **Tran van Nhieu, G., and E. Collatz.** 1987. Primary structure of an aminoglycoside 6′-*N*-acetyltransferase AAC(6′)-4, fused in vivo with the signal peptide of the Tn*3*-encoded beta-lactamase. J. Bacteriol. **169:**5708–5714.
48. **Verdet, C., G. Arlet, G. Barnaud, P. H. Lagrange, and A. Philippon.** 2000. A novel integron in *Salmonella enterica* serovar Enteritidis, carrying the *bla*(DHA-1) gene and its regulator gene *ampR*, originated from *Morganella morganii*. Antimicrob. Agents Chemother. **44:**222–225.
49. **Wang, M., D. F. Sahm, G. A. Jacoby, and D. C. Hooper.** 2004. Emerging plasmid-mediated quinolone resistance associated with the *qnr* gene in *Klebsiella pneumoniae* clinical isolates in the United States. Antimicrob. Agents Chemother. **48:**1295–1299.
50. **Wang, M., J. H. Tran, G. A. Jacoby, Y. Zhang, F. Wang, and D. C. Hooper.** 2003. Plasmid-mediated quinolone resistance in clinical isolates of *Escherichia coli* from Shanghai, China. Antimicrob. Agents Chemother. **47:**2242–2248.