Association of Plasmid-Mediated Quinolone Resistance with Extended-Spectrum β -Lactamase VEB-1

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Association of the plasmid-mediated quinolone resistance determinant QnrA and the *bla*_{VEB-1} gene was identified in a single *Enterobacter cloacae* isolate from K.-Bicêtre, France, and in 11 out of 23 *bla*_{VEB-1}-positive enterobacterial isolates from Bangkok, Thailand. This result may explain in part the association between quinolone and extended-spectrum β -lactam resistance.

Quinolone resistance results mostly from chromosomal mutations in genes coding for DNA gyrase and topoisomerase IV and from changes in outer membrane or efflux proteins or their regulatory mechanisms in *Enterobacteriaceae* (28). However, plasmid-mediated resistance to quinolone was first reported in 1998 for a *Klebsiella pneumoniae* isolate from the United States (11). The plasmid-encoded protein responsible for resistance, named Qnr (and recently termed QnrA [A. Robicsek, D. F. Sahm, G. Jacoby, and D. C. Hooper, Abstr. 44th Intersci. Conf. Antimicrob. Agents Chemother., abstr. C2-1898a, 2004]), protects DNA gyrase and topoisomerase IV from the inhibitory activity of quinolones (29; J. H. Tran, G. A. Jacoby, and D. C. Hooper, Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. C1-604, 2003). It confers resistance to nalidixic acid and increases fluoroquinolone MICs up to 32-fold (9, 11, 30, 31). *qnrA*-positive isolates are increasingly identified in the United States (7, 30), and there are scattered reports of their presence in Asia (31) and Europe (8, 9). Several *qnrA*-positive isolates produced clavulanic acid-inhibited extended-spectrum β -lactamases (ESBL) such as SHV-7 and CTX-M-9 (31).

In a previous study, we reported a single *qnrA*-positive *Escherichia coli* isolate that produced the ESBL VEB-1 among nalidixic acid-resistant *E. coli* isolates at the Bicêtre Hospital in 2003 (9). The main goal of the present study was to search for QnrA determinants among nalidixic acid-resistant isolates ($n = 152$) belonging to other enterobacterial species from the same hospital and to search for any association between QnrA and VEB-1 among other characterized isolates. A series of *bla*_{VEB-1}-positive and *bla*_{VEB-1}-negative nonreplicate isolates from Thailand and strains that contained other reference β -lactamases were screened (Table 1). A *qnrA*-like gene was searched for by PCR using previously reported primers (9, 31). A *qnrA*-like gene was detected in a single *Enterobacter cloacae* isolate (GOC) among all nalidixic acid-resistant isolates from Bicêtre Hospital in 2003. The estimated prevalence of this gene was 4% among ESBL-positive enterobacterial isolates ($n = 24$). *E. cloacae* GOC was isolated in January 2003 from the

urine of a 22-year-old man who had multiple urinary tract infections due to cystic diverticulosis and who was treated successively with ciprofloxacin and imipenem. A *qnrA*-like gene was also detected in 11 out of 23 *bla*_{VEB-1}-positive enterobacterial isolates (48%) from Bangkok in 1999 (Table 1). It was identified in *Enterobacter sakazakii* and constituted the first description of the gene in that species (Table 2). Sequence analysis of the PCR-amplified products identified the same *qnrA* gene in all cases (29). No *qnrA*-like gene was identified either in *bla*_{VEB-1}-positive enterobacterial isolates from any country except France and Thailand, in ESBL-positive, *bla*_{VEB-1}-negative enterobacterial isolates from Thailand, in *bla*_{VEB-1}-positive *Pseudomonas aeruginosa* isolates from Thailand, or in other strains that contained reference β -lactamase genes (Table 1). These results showed that the *bla*_{VEB-1} gene was frequently but not systematically associated with a QnrA determinant, and only in *Enterobacteriaceae*.

Conjugations were carried out by a filter mating technique with *E. coli* J53 Az^R as the recipient, as previously described (9). Conjugation experiments yielded *qnrA*-positive transconjugants for *E. cloacae* GOC and for most of the *qnrA*-positive enterobacterial isolates from Thailand except for *E. coli* E2, *E. coli* E10, and *E. sakazakii* E15 (Table 2). Electrophoresis of the plasmid extracts from these latter isolates and from the transconjugants obtained with the other clinical isolates as donors was followed by Southern hybridization, as described elsewhere (9, 21). The probes consisted of a 661-bp fragment for *qnrA* and a 627-bp fragment for *bla*_{VEB-1} (9). Each strain contained a single plasmid that cohybridized with *qnrA*- and *veb-1*-specific probes (Fig. 1). The molecular sizes of the plasmids ranged from 130 to 200 kb (Fig. 1; Table 2).

Disk diffusion susceptibility testing and MIC determinations, performed as described elsewhere (9), showed that transconjugants were resistant to nalidixic acid, chloramphenicol, tobramycin, sulfonamides, and most β -lactams, including ampicillin, cefotaxime, and ceftazidime. Resistance to trimethoprim was cotransferred only in transconjugant TcE3 (Table 2). Whereas transconjugants were always resistant to nalidixic acid, clinical isolates 7, 10, and 18 were susceptible (Table 2). These results are reminiscent of a previous study indicating that the QnrA determinant may be expressed at variable levels, possibly depending on host functions (10). As shown previ-

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TABLE 1. Strains screened for *qnrA*-like gene

Strain (acquired β -lactamase[s])	No. of strains			Origin	Reference(s)
	Total	<i>bla</i> _{VEB}	<i>qnrA</i>		
Nalidixic acid-resistant <i>Enterobacteriaceae</i> (<i>E. coli</i> excluded) including 24 ESBL-positive strains	152	1	1	Bicêtre, France	This study
<i>Enterobacteriaceae</i> (ESBL, VEB-1)	23	23	11	Bangkok, Thailand	5
<i>Enterobacteriaceae</i> (ESBL, non-VEB-1)	22	0	0	Bangkok, Thailand	5
<i>P. aeruginosa</i> (VEB-1)	33	33	0	Bangkok, Thailand	4
<i>P. aeruginosa</i> KU-1 (VEB-1a)	1	1	0	Kuwait City, Kuwait	26
<i>P. aeruginosa</i> 10.2 (VEB-1a)	1	1	0	New Delhi, India	1
<i>P. aeruginosa</i> KU-2 (VEB-1b)	1	1	0	Kuwait City, Kuwait	26
<i>Proteus mirabilis</i> Lil-1 (VEB-1)	1	1	0	Paris, France	12
<i>E. coli</i> MG-1 (VEB-1)	1	1	0	Vietnam	23
<i>P. aeruginosa</i> RP-1 (SHV-2a)	1	0	0	Garches, France	13
<i>P. aeruginosa</i> RNL-1 (PER-1)	1	0	0	Garches, France	15
<i>K. pneumoniae</i> ORI-1 (GES-1)	1	0	0	Bicêtre, France	21
<i>P. aeruginosa</i> GW-1 (GES-2)	1	0	0	Pretoria, South Africa	27
<i>K. pneumoniae</i> ILT-3 (CTX-M-19)	1	0	0	Paris, France	24
<i>Enterobacter asburiae</i> MS-7 (IMI-2)	1	0	0	Minneapolis, MN	2
<i>P. aeruginosa</i> COL-1 (VIM-2)	1	0	0	Marseilles, France	25
<i>E. coli</i> BL1 (ACC-1)	1	0	0	Bicêtre, France	Personal data
<i>K. pneumoniae</i> 45956 (DHA-2)	1	0	0	Paris, France	3
<i>P. aeruginosa</i> Mus (OXA-18, OXA-20)	1	0	0	Paris, France	14, 17
<i>P. aeruginosa</i> ED-1 (OXA-28)	1	0	0	Bicêtre, France	19
<i>P. aeruginosa</i> CY-1 (OXA-32)	1	0	0	Clamart, France	18
<i>Acinetobacter baumannii</i> CLA-1 (OXA-40)	1	0	0	Bicêtre, France	6
<i>K. pneumoniae</i> 11978 (OXA-48)	1	0	0	Istanbul, Turkey	20

ously, combined mechanisms of resistance might explain resistance to fluoroquinolones in the *qnrA*-positive clinical strains (9–11).

Using a series of specific primers (3, 5, 12, 13, 14, 18, 20), the

*bla*_{VEB-1} and *bla*_{OXA-10} genes were identified in all *qnrA*-positive isolates. Both of these β -lactamase genes were located in a class 1 integron that has been characterized in detail previously (5, 9, 26). Using a PCR-based strategy, the structures of

TABLE 2. MICs of quinolones for clinical isolates carrying the *qnrA* gene, their transconjugants (Tc strains), and the *E. coli* J53 recipient strain^a

Isolate	MIC (μ g/ml) of ^b :						Plasmid size (kb)	Associated non- β -lactam antibiotic resistance markers ^c
	NAL	NOR	OFX	MXF	SPX	CIP		
<i>E. coli</i> E1	>256	>256	>256	>256	>256	>256	160	CHL CIP KAN NAL SUL SXT TET TOB
TcE1	32	1	1	1	1	0.25	160	CHL KAN NAL SUL TOB
<i>E. coli</i> E2	>256	>256	>256	>256	>256	>256	150	CHL CIP KAN NAL SUL SXT TET TOB
<i>E. coli</i> E3	>256	>256	>256	>256	>256	>256	200	CHL CIP KAN NAL SUL SXT TET TOB
TcE3	32	1	0.5	0.5	1	0.12	200	CHL KAN NAL SUL SXT TOB
<i>E. coli</i> E4	>256	2	4	2	4	1	150	CHL KAN NAL SUL SXT TOB
TcE4	32	1	1	1	2	0.25	150	CHL KAN NAL SUL TOB
<i>E. coli</i> E5	>256	>256	>256	>256	>256	>256	150	CHL CIP KAN NAL SUL SXT TET TOB
TcE5	32	1	1	0.5	1	0.25	150	CHL KAN NAL SUL TOB
<i>E. coli</i> E7	8	0.5	0.5	0.25	0.25	0.12	150	CHL KAN SUL SXT TET TOB
TcE7	32	1	1	1	2	0.25	150	CHL KAN NAL TOB SUL
<i>E. coli</i> E8	>256	1	1	0.5	0.25	0.25	150	CHL KAN NAL SUL SXT TET TOB
TcE8	32	1	1	0.5	1	0.25	150	CHL KAN SUL SXT TET TOB
<i>E. coli</i> E10	2	0.12	0.06	0.01	0.01	0.01	130	CHL KAN SUL SXT TET TOB
<i>E. sakazakii</i> E15	32	1	1	0.5	1	0.25	200	CHL KAN NAL SUL SXT TET TOB
<i>K. pneumoniae</i> E16	32	2	2	1	1	1	140	CHL KAN NAL TOB SUL
TcE16	32	2	2	1	1	1	140	CHL KAN NAL TOB SUL
<i>K. pneumoniae</i> E18	8	2	1	0.5	1	0.5	180	CHL KAN NAL SUL SXT TET TOB
TcE18	32	1	1	0.5	0.5	0.25	180	CHL KAN NAL TOB SUL
<i>E. cloacae</i> GOC	>256	16	8	2	4	4	190	CHL KAN NAL SUL SXT TET TOB
TcGOC	32	1	1	1	2	0.5	190	CHL KAN NAL TOB SUL
<i>E. coli</i> J53	4	0.06	0.06	0.06	0.01	0.01		

^a Sizes of the plasmids, and associated non- β -lactam resistance markers in isolates also harboring the *bla*_{VEB-1} gene and in their transconjugants, are also shown.

^b NAL, nalidixic acid; NOR, norfloxacin; OFX, ofloxacin; MXF, moxifloxacin; SPX, sparfloxacin; CIP, ciprofloxacin.

^c Antibiotic resistance markers: CHL, chloramphenicol; KAN, kanamycin; SUL, sulfonamides; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline; TOB, tobramycin.

FIG. 1. Plasmid DNAs from clinical isolates and transconjugants (A) and Southern hybridizations with the *bla*_{VEB-1} (B) and the *qnrA*-like (C) probe. Lanes: 1, *E. coli* J53/pQR1 (used as a control); 2, *E. coli* J53/pMG252 (used as a control); 3, *E. coli* TcE1; 4, *E. coli* E2; 5, *E. coli* TcE3; 6, *E. coli* TcE4; 7, *E. coli* TcE5; 8, *E. coli* TcE7; 9, *E. coli* TcE8; 10, *E. coli* E10; 11, *E. sakazakii* E15; 12, *E. coli* TcE16; 13, *E. coli* TcE18; 14, *E. cloacae* GOC; M, *E. coli* 50192 (used as a molecular size marker [9] and negative control).

the *qnrA*-type-containing integrons identified from the resistance plasmids of the 12 enterobacterial isolates reported in this study were determined. The *qnrA*-like gene was located in *sulI*-type class 1 integrons. It was bracketed by a duplication of the 3' conserved sequence region of the class 1 integron and was not associated with a 59-bp element as reported previously (9). These *sulI*-type integrons were almost identical, at least in their central regions, to the *sulI*-type integron of pQR1 from

the *E. coli* Lo strain previously found at the Bicêtre Hospital (Fig. 2), (9). A 2-bp deletion was found at the right boundary of the CR1 element that contained *orf513* compared to the structure identified in pMG252 (Fig. 2) (31). This result indicated that the mobilization mechanism of the *qnrA* gene mediated by CR1 may vary. Further PCR amplifications revealed that strains 1, 4, 5, and GOC contained another copy of the *orf513* gene immediately downstream of the *sulI*-type integron,

FIG. 2. Schematic comparison of the different *sulI*-type integrons that contain the *qnrA* gene. Shown are the structures of *sulI*-type integrons identified in pMG252 from *K. pneumoniae* (A) (29), in pQR1 from *E. coli* Lo (B) (9), in enterobacterial isolates from Thailand and *E. cloacae* isolate GOC from Bicêtre (C), and in other enterobacterial isolates from Thailand (D). The vertical rectangle indicates the right-hand boundary of the CR1 element as determined previously (9), and the vertical arrow stands for a 2-bp deletion. Question mark indicates DNA sequences that are unknown but different from those reported in panels A, B, and C. Dashed vertical lines indicate the absence of the DNA fragment between *qnrA* and *qacE*ΔI in pMG252.

whereas this *orf513* duplication was not found for strains 2, 3, 7, 8, 10, 15, 16, and 18 (Fig. 2).

This study further underlines the spread of the QnrA determinant in Europe (being the second report from France [9]) and in Southeast Asia. Association of the *qnrA* and *bla*_{VEB-1} genes may account in part for the association between expanded-spectrum cephalosporins and fluoroquinolone resistance (16). Finally, identification of QnrA-positive and nalidixic acid-susceptible clinical isolates raised concern about a hidden spread of this resistance determinant.

Nucleotide sequence accession numbers. The nucleotide sequences reported here have been assigned accession numbers AY931017 and AY931018.

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