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Association of QnrB Determinants and Production of Extended-Spectrum β-Lactamases or Plasmid-Mediated AmpC β-Lactamases in Clinical Isolates of *Klebsiella pneumoniae*▽

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Clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* producing extended-spectrum β-lactamases or plasmid-mediated AmpC β-lactamases were screened for *qnrA* and *qnrB* genes. *QnrB* was present in 54 of 54 DHA-1-producing *K. pneumoniae* isolates and 10 of 45 SHV-12-producing ones, suggesting that the distribution of plasmids conferring resistance to extended-spectrum cephalosporins and quinolones in clinical isolates of *K. pneumoniae* is widespread.

Since the first plasmid-mediated quinolone resistance-conferring gene (*qnr*) was discovered in a *Klebsiella pneumoniae* isolate from Alabama (7), Qnr determinants have been identified worldwide (2, 9, 13, 15). A frequent association of quinolone resistance with the production of extended-spectrum β-lactamases (ESBLs) has been noticed and was explained by the selective pressures of multiple antibiotics and plasmid-mediated *qnr* (3, 9, 12, 15, 17). At present, several *qnrA*-positive isolates have been found to express ESBLs, such as SHV-5, SHV-7, CTX-M-15, and VEB-1, or plasmid-mediated AmpC enzymes, such as DHA-1 (9, 17). The association of *qnrB* and SHV-12 was recently described (3).

In order to study the prevalence of Qnr determinants and to identify ESBLs or plasmid-mediated AmpC β-lactamases associated with *qnr* genes, we screened 239 isolates of *Escherichia coli* and *K. pneumoniae* producing variable ESBLs or plasmid-mediated AmpC β-lactamases for *qnrA* and *qnrB*.

(This study was presented at the 44th IDSA meeting, Toronto, Ontario, Canada, 2006.)

Twenty-seven *E. coli* strains and 62 *K. pneumoniae* strains were obtained from the blood isolate collection of Seoul National University Children's Hospital (SNUCH). These isolates were collected from 1993 to 2005 and were identified as producers of an ESBL or a plasmid-mediated AmpC β-lactamase (part of these data were published previously [6]). Forty-nine isolates of *K. pneumoniae* known to produce ESBLs or to have a plasmid-mediated AmpC enzyme were recruited from the blood isolate collection of Seoul National University Hospital (SNUH) during the 5 years from 1998 to 2002 (10). Fifty-four *E. coli* strains and 23 *K. pneumoniae* strains, selected for resistance to cefoxitin and extended-spectrum cephalosporins (ESCs), were obtained from Hanyang University Hospital (HUH), a tertiary-care teaching hospital in Seoul, from 2003 to 2005. Twenty-four *K. pneumoniae* isolates were collected from Korea University Hospital (KUH), Seoul, in 2005 and were chosen because of their ESC resistance. Only one isolate from each patient was included in all collections. Thus, a total of 81 *E. coli* strains and 158 *K. pneumoniae* strains which produced an ESBL or a plasmid-mediated AmpC were included in this study.

Isoelectric focusing (IEF) was performed by the method of Mathew et al. (8) by using a Mini IEF cell system (Bio-Rad, Hercules, CA). Strains carrying plasmids encoding β-lactamases TEM-1 (R1), TEM-4 (pUD16), SHV-2 (pMG229), SHV-5 (pAFF2), and CMY-1 (pMVP-1) served as the IEF standards (6). The β-lactamase genes from the clinical isolates were amplified and sequenced as described previously (5, 10, 11). The *qnrA* gene was detected by PCR with primers QP1 and QP2 under the PCR conditions described previously (4). For the detection of *qnrB*, PCR was performed with primers FQ1 and FQ2 (3, 13) and primers MFQ1 (5′-GATCGTGAAAGC CAGAAAGG-3′) and MFQ2 (5′-ACGATGCCTGGTAGTT GTCC-3′), as described previously (13). Plasmid pMG252 and the *K. pneumoniae* 35 strain were used as the positive controls for *qnrA* and *qnrB*, respectively (4, 7).

Products were amplified from 54 isolates of *K. pneumoniae* and 2 isolates of *E. coli* by PCR with primers MFQ1 and MFQ2, and the nucleotide sequences of the PCR products from 3 isolates were identical to the nucleotide sequence of *qnrB4*. One *E. coli* isolate and 10 *K. pneumoniae* isolates were positive for amplification with both primers MFQ1 and MFQ2 and primers FQ1 and FQ2. The PCR products obtained from five isolates with primers FQ1 and FQ2 and with primers MFQ1 and MFQ2 were sequenced, which revealed that the nucleotide sequences were identical to that of *qnrB2* in two isolates but to that of *qnrB5* in three isolates. In order to differentiate between *qnrB2* and *qnrB5*, the products amplified with primers FQ1 and FQ2 were restricted with ApaI or HindIII. The results showed that seven and three isolates contained *qnrB2* (restricted into 467- and 95-bp DNA fragments with ApaI) and *qnrB5* (restricted into 333- and 229-bp DNA fragments with HindIII), respectively. One isolate whose PCR product was partially restricted with both ApaI and HindIII but which showed a nucleotide sequence identical to that of *qnrB2* was considered to have both *qnrB2* and *qnrB5* concurrently. The nucleotide sequences of the PCR products from one *E. coli* isolate and three of six *K. pneumoniae* isolates

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TABLE 1. Susceptibilities of wild types and *E. coli* J53 transconjugants to β-lactam and quinolone antibiotics

Strain[a]	MIC (μg/ml)[b]							*qnrB* allele	β-Lactamase
	NA	CIP	LEVO	MOXI	FOX	CTX	CAZ		
E. coli J53 Azi^r	8	0.03	0.06	0.06	4	<0.25	1		
12-1183	>64	>16	>16	>8	>128	16	128	*B4*	DHA-1
T12-1183	32	0.25	0.5	1	32	4	128		
35	>64	16	4	>8	32	>128	>128	*B5*	SHV-12
T35	32	0.5	0.25	0.5	4	16	128		
07-006	>64	16	8	8	16	64	128	*B2*	SHV-12
T07-006	32	0.5	0.25	1	4	16	128		
03-222	>64	16	16	8	16	4	128	*B2*	SHV-12
T03-222	32	1	0.25	0.5	4	2	64		
12-1502	16	0.5	0.25	0.5	>128	64	128	*B2*	CMY-1
T12-1502	32	1	0.25	0.5	>128	64	64		

[a] T, an *E. coli* J53 Azi^r transconjugant of each isolate. Isolate 12-1502 was *E. coli*, and all other clinical isolates were *K. pneumoniae*.
[b] NA, nalidixic acid; CIP, ciprofloxacin; LEV, levofloxacin; MOX, moxifloxacin; FOX, cefoxitin; CTX, cefotaxime; CAZ, ceftazidime.

obtained by *qnrA*-specific PCR were identical to the nucleotide sequence of *qnrA1*.

A conjugation experiment was performed for 10 of 54 *K. pneumoniae* isolates containing *qnrB4*, 10 of 10 *K. pneumoniae* isolates with *qnrB2* or *qnrB5*, and 1 *E. coli* isolate with *qnrB2* by using *E. coli* J53 Azi^r as a recipient, as described previously (3). The selective medium used contained ceftazidime (10 μg/ml), sodium azide (100 μg/ml), and nalidixic acid (12 μg/ml) or ciprofloxacin (0.125 μg/ml). One of 10 DHA-1-producing *K. pneumoniae* isolates, 3 of 10 SHV-producing *K. pneumoniae* isolates, and a CMY-1-producing *E. coli* isolate transferred resistance to ESCs and quinolones. Table 1 shows the susceptibilities of the wild types and *E. coli* J53 Azi^r transconjugants to several β-lactam and quinolone antibiotics.

Table 2 shows the distribution of *qnrB* among the clinical isolates of *E. coli* and *K. pneumoniae* according to their β-lactamase subtypes.

The distribution of *qnrB* was closely related to the presence of certain β-lactamases; all 54 *K. pneumoniae* isolates and 2 *E. coli* isolates producing DHA-1 with or without other enzymes contained *qnrB4* (100%). Among 45 *K. pneumoniae* isolates that produced SHV-12 with or without other enzymes, excluding 12 isolates that coproduced SHV-12 and DHA-1, *qnrB2* was present in 6 strains (13.3%), *qnrB5* was present in 3 strains (6.7%), and both *qnrB2* and *qnrB5* were present in 1 strain (2.2%). No isolate of *E. coli* or *K. pneumoniae* harbored *qnrA* and *qnrB* concurrently.

TABLE 2. Distribution of Qnr determinants according to ESBL or plasmid-mediated AmpC β-lactamases among clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli*[a]

Strain and ESBL(s) or plasmid-mediated AmpC β-lactamase(s)	No. of isolates with:					
	Total	*qnrA1*	*qnrB4*	*qnrB2*	*qnrB5*	*qnrB2* and *qnrB5*
Klebsiella pneumoniae (n = 158)						
CTX-M-14	7	2				
DHA-1	39		39			
DHA-1, CTX-M-14	1		1			
DHA-1, TEM-52	2		2			
SHV-12	41	4		5	3	1
SHV-12, DHA-1	12		12			
SHV-12, pI 7.8	1			1		
Total		6	54	6	3	1
Escherichia coli (n = 81)						
CMY-1	3			1		
CMY-2, CTX-M-14	2	1				
DHA-1	1		1			
DHA-1, TEM-52	1		1			
Total		1	2	1		

[a] The TEM-1 β-lactamase is not described in this table.

Blood isolates from SNUH and SNUCH were proved to be epidemiologically not related by pulsed-field gel electrophoresis in previous studies (6, 10). Enterobacterial repetitive intergenic consensus sequence (ERIC) PCR was performed with the DHA-1-producing *K. pneumoniae* isolates (23 and 16 isolates from HUH and KUH, respectively), as described previously (1). Isolates from HUH and KUH showed 11 and 6 distinct ERIC patterns, respectively. However, six isolates from KUH revealed indistinguishable ERIC patterns.

This study shows the absolute association between *qnrB4* and DHA-1 determinants in isolates of the family *Enterobacteriaceae*, which was explained by the partial nucleotide sequences of pTN60013 from *K. pneumoniae* containing $bla_{\text{DHA-1}}$ (16). The nucleotide sequences of pTN60013 from nucleotides 1225 to 1586 completely match the partial nucleotide sequences of *qnrB4* (14). The nucleotide sequences of our purified products obtained from three clinical isolates by PCR with primers MFQ1 and MFQ2 were identical to those of pTN60013 from nucleotides 1196 to 1609. Thus, we considered them to be *qnrB4* (G. A. Jacoby, personal communication).

Although the association of SHV-12 and *qnrB* was not as close as that of DHA-1 and *qnrB*, 20% of the SHV-12-producing *K. pneumoniae* isolates evaluated in this study were positive for *qnrB*. Further spread of the resistance plasmid encoding *qnrB* as well as $bla_{\text{SHV-12}}$ may occur in the near future, and strains should be carefully observed for this.

The increase in the prevalence of the DHA-1 β-lactamase in Korea was recently reported (10). We suspect that *qnrB* possibly contributes to the widespread distribution of DHA-1 in areas where ESCs and fluoroquinolone are widely used.

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