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Interspecies Dissemination of the *bla* Gene Encoding PER-1 Extended-Spectrum β-Lactamase▽

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PER-1 extended-spectrum β-lactamase-producing Gram-negative bacilli are resistant to oxyimino-cephalosporins. However, the bla_{PER-1} gene has never been reported in *Klebsiella pneumoniae*. Here, we studied interspecies dissemination of the bla_{PER-1} gene by horizontal transfer of Tn*1213* among *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *K. pneumoniae*. In a *K. pneumoniae* clinical isolate, the bla_{PER-1} gene was located on a 150-kbp incompatibility group A/C plasmid.

The *bla* gene encoding PER-1 extended-spectrum β-lactamase (ESBL), which can hydrolyze penicillins, oxyimino-cephalosporins, and aztreonam but not oxacillins, cephamycins, and carbapenems, was first detected on a plasmid of *Pseudomonas aeruginosa* RNL-1 from France in 1991 (9). The widespread dissemination of the gene in *Acinetobacter* spp. (46%) and *P. aeruginosa* (11%) in Turkey was reported in 1997, and further dissemination of the gene into European countries, such as Italy, Belgium, and Russia, has been noted (5, 7, 8, 10, 19). In 2003, a high prevalence of the bla_{PER-1} gene in *Acinetobacter* spp. (55%) isolated from patients hospitalized in an intensive care unit (ICU) in Korea was reported, and further dissemination of the gene into Asian countries, such as China, Japan, and India, has also been detected (6, 20–22). The bla_{PER-1} gene has been detected mainly in glucose-nonfermenting Gram-negative bacilli, such as *P. aeruginosa*, *Acinetobacter* spp., and *Alcaligenes faecalis*; however, it has also recently been found in *Enterobacteriaceae*, such as *Providencia* spp., *Proteus* spp., *Salmonella* spp., and *Aeromonas media* (2, 9, 11–13, 18, 19).

A 68-year-old female who presented with dyspnea and facial paralysis after a bamboo stick injury on the left leg was admitted to the ICU of a tertiary-care hospital in Gwangju, Republic of Korea, on 16 May 2006. She showed symptoms of pneumonia, and *Acinetobacter baumannii* and *Staphylococcus aureus* were repeatedly recovered from sputum specimens. Ceftazidime and vancomycin were administered for the treatment of pneumonia; however, she expired on 16 June due to the occurrence of disseminated intravascular coagulation. *Klebsiella pneumoniae* CS1711 isolate was recovered from the blood specimen obtained 1 day before she died.

Strain CS1711 exhibited resistance to ampicillin, piperacillin, ceftazidime, cefotaxime, cefepime, gentamicin, amikacin, and tetracycline and was susceptible to cefoxitin and imipenem by a disk diffusion assay (5). Synergy was observed between the amoxicillin-clavulanic acid (20 and 10 μg) disk and the ceftazidime (30 μg), cefotaxime (30 μg), cefepime (30 μg), and aztreonam (30 μg) disks (Becton Dickinson, Sparks, MD) in double-disk synergy tests, indicating the production of ESBL (17). Agar dilution MIC testing on Mueller-Hinton agar (Difco Laboratories, Detroit, MI) with an inoculum of 10^4 CFU per spot confirmed MICs of ceftazidime (MIC, 16 μg/ml), cefotaxime (MIC, 64 μg/ml), cefepime (MIC, 64 μg/ml), aztreonam (MIC, 16 μg/ml), cefoxitin (MIC, 4 μg/ml), amikacin (MIC, >256 μg/ml), and ciprofloxacin (MIC, 1 μg/ml) for strain CS1711 (17). Clavulanic acid (Sigma, St. Louis, MO) at a fixed concentration of 4 μg/ml lowered the MICs of ceftazidime, cefotaxime, and cefepime to 1 μg/ml, 0.12 μg/ml, and 0.5 μg/ml, respectively (Table 1).

The strain transferred an ~150-kbp plasmid (pCS1711) to the *Escherichia coli* J53 azide^R recipient in mating experiments in which transconjugants were selected on MacConkey agar (Difco Laboratories, Detroit, MI) plates supplemented with cefotaxime (2 μg/ml) and sodium azide (100 μg/ml) (3). MICs of ceftazidime, cefotaxime, cefepime, amikacin, and ciprofloxacin for the transconjugant (trcCS1711) were 4 μg/ml, 32 μg/ml, 8 μg/ml, 0.25 μg/ml, and 0.025 μg/ml, respectively (Table 1).

PCR and sequencing experiments for the detection of genes encoding TEM-, SHV-, CTX-M-, GES-, VEB-, and PER-type ESBLs were performed as described previously (1) (Table 2). Strain CS1711 carried two β-lactamase genes, bla_{PER-1} and $bla_{CTX-M-9}$. The location of antimicrobial resistance genes was identified by hybridization of I-CeuI-digested genomic DNA or S1 nuclease-treated linearized plasmids with probes specific for the β-lactamase genes, various replicons of plasmids, and 16S rRNA genes as described previously (16). Clinical isolates of *P. aeruginosa* ($n = 8$) and *A. baumannii* ($n = 14$), which were recovered from clinical samples of patients hospitalized at the same hospital during May and June 2006, carrying the bla_{PER-1} gene were included in this study for comparison. The bla_{PER-1} and the $bla_{CTX-M-9}$ genes in *K. pneumoniae* strain CS1711 were located on the ~150-kbp IncA/C plasmid (pCS1711 in transconjugant *E. coli* trcCS1711). However, the probe specific for the bla_{PER-1} gene did not hybridize with any plasmids in 8 *P. aeruginosa* isolates and 14 *A. baumannii* isolates. The probe hybridized with I-CeuI macrorestriction fragments of ~500

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TABLE 1. MICs of *K. pneumoniae* wild strain (CS1711), its transconjugant, and the recipient *E. coli* J53

Antimicrobial agent[a]	MIC (μg/ml) of strains		
	Wild strain, *K. pneumoniae* CS1711	Transconjugant, *E. coli* trcCS1711	Recipient, *E. coli* J53
Ceftazidime	16	4	0.25
Ceftazidime-clavulanic acid	1	0.12	0.12
Cefotaxime	64	32	0.06
Cefotaxime-clavulanic acid	0.12	0.06	0.06
Cefepime	64	8	0.06
Cefepime-clavulanic acid	0.5	0.06	0.06
Aztreonam	16	8	0.25
Cefoxitin	4	4	4
Amikacin	>256	0.25	0.25
Ciprofloxacin	1	0.25	0.015

[a] Clavulanic acid was added at a fixed concentration of 4 μg/ml.

kbp and ~800 kbp in *P. aeruginosa* and *A. baumannii* isolates, respectively. The probe specific for 16S rRNA genes also hybridized with the I-CeuI macrorestriction fragments, indicating chromosomal location of the bla_{PER-1} gene in those *P. aeruginosa* and *A. baumannii* isolates.

To investigate genetic environments surrounding the bla_{PER-1} gene, sequencing experiments of several overlapping PCR fragments obtained from whole DNA of the *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa* isolates with primers corresponding to internal region of Tn*1213* were performed as previously described (15). Identically to the results for the bla_{PER-1} gene located on the chromosome in *P. aeruginosa* RNL-1 (GenBank accession no. AY779042), IS*Pa12* and IS*Pa13* elements were present upstream and downstream of the bla_{PER-1} gene, respectively, in all the isolates studied. The bla_{PER-1} gene located on a plasmid in two *Salmonella enterica* serovar Typhimurium isolates and in strain *A. baumannii* C.A. has been reported to be preceded by IS*Pa12* but not followed by IS*Pa13*, while the bla_{PER-1} gene located on the plasmid pCS1711 was surrounded by both IS*Pa12* and IS*Pa13* elements (4, 14, 15) (Fig. 1). Our results suggest that the bla_{PER-1} gene in *K. pneumoniae* strain CS1711 might be mobilized from bla_{PER-1} gene-carrying *A. baumannii* or *P. aeruginosa*, since the genetic environments of the gene in those strains were identical.

This report shows further dissemination of the bla_{PER-1} gene into *Enterobacteriaceae* and is the first report of *K. pneumoniae* carrying the bla_{PER-1} gene.

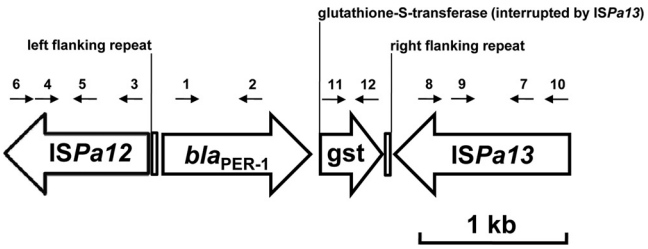

FIG. 1. Genetic environment of the bla_{PER-1} gene in *K. pneumonia* CS1711. Numbered arrows indicate positions and directions of the primers used in this study as listed in Table 2.

TABLE 2. Primers used in PCR and sequencing studies for antimicrobial resistance genes

Target gene(s)	Primer name	Sequence (5′ to 3′)	Position in Fig. 1
bla_{TEM} cluster	TEM-F	TCCGCTCATGAGACAATAACC	
	TEM-R	ACGCTCAGTGGAACGAAAAC	
bla_{SHV} cluster	SHV-F	CGCCGGGTTATTCTTATTTG	
	SHV-R	CCACGTTTATGGCGTTACCT	
bla_{VEB} cluster	VEB-F	AAAATGCCAGAATAGGAGTAGCA	
	VEB-R	TCCACGTTATTTTTGCAATGTC	
bla_{GES} cluster	GES-F	CGCTTCATTCACGCACTATT	
	GES-R	GTCCGTGCTCAGGATGAGTT	
$bla_{CTX-M-1}$ cluster	CTX-M-1F	CCGTCACGCTGTTGTTAGG	
	CTX-M-1R	ACGGCTTTCTGCCTTAGGTT	
$bla_{CTX-M-9}$ cluster	CTX-M9-F	CAAAGAGAGTGCAACGGATG	
	CTX-M9-R	CCTTCGGCGATGATTCTC	
bla_{PER-1} cluster	PER-F	CCTGACGATCTGGAACCTTT	1
	PER-R	TGGTCCTGTGGTGGTTTC	2
IS*Pa12* gene	ISPa12-F	AAGCCCTGTTTTCAGAGCAA	3
	ISPa12-R	AATCAACGTTTCGGCTATCG	4
	ISPa12-mF	GCCGATGCAGGTTATTTTTC	5
	ISPa12-wR	TCATGATTCATATGTGATTTCCAA	6
IS*Pa13* gene	ISPa13-F	TTTTCAGCAGCAGAGCTTGA	7
	ISPa13-R	CGTTGATTAGCCAGCGTTTT	8
	ISPa13-mF	TGATAAAGAGGCGGGTGAAG	9
	ISPa13-wR	TTTACGCCTCATAGGTATGATCTTTAG	10
gst gene	GST-F	CCCCTTTTGTTCGTCGTTTA	11
	GST-R	AAGGAGTCTGTGCAGGCATT	12

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