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High Prevalence of PER-1 Extended-Spectrum β-Lactamase-Producing *Acinetobacter* spp. in Korea

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PER-1, an extended-spectrum β-lactamase, has been reported only in Europe. We detected PER-1 in 53 of 97 acinetobacters in Korea, mainly in the sputum of intensive care unit patients. Pulsed-field gel electrophoresis analysis suggested that clonal spread had occurred. Only PCR reliably detected PER-1 producers. PER-1 producers may also exist in other Asian countries.

Multidrug-resistant *Acinetobacter* spp. and *Pseudomonas aeruginosa* frequently cause serious nosocomial infections. β-Lactamase production is the most important mechanism of β-lactam resistance in gram-negative pathogens. PER-1 is an extended-spectrum β-lactamase which was first found in a *P. aeruginosa* strain in France (7) and was then subsequently detected in *Acinetobacter* spp. and *P. aeruginosa* in Turkey and Italy (5, 12). PER-2 was reported in *Salmonella enterica* serovar Typhimurium in Argentina (1). However, to our knowledge, the PER-1 enzyme has not been reported in any other countries.

In a Korean hospital, cefepime-resistant acinetobacters increased significantly from 29 to 47% of isolates, but those of *P. aeruginosa* increased only slightly, from 14 to 17%, between 1991 (2) and 2000 (data not shown), which suggested the presence of PER-1-producing acinetobacters, as cefepime is stable to AmpC but not to PER-1 β-lactamase (5, 11). PER-1 production has been found to be an independent indicator of poor prognosis (13), but its detection by the double-disk synergy (DDS) test is difficult (9).

The aim of this study was to determine the presence of PER-1-producing strains of acinetobacter and *P. aeruginosa* in Korea. The performance of the DDS test for the detection of PER-1-producing isolates was also evaluated.

Strains of acinetobacter and *P. aeruginosa* were isolated in 2001 and 2002 from patients in two tertiary-care Korean hospitals and were identified by conventional tests (3, 10) or by using the ATB 32 GN system (bioMerieux, Marcy l'Etoile, France). Antimicrobial susceptibilities were determined by the disk diffusion and the agar dilution methods (6). DDS was tested with cefotaxime, ceftazidime, aztreonam, cefepime, and amoxicillin-clavulanate disks (Beckton Dickinson, Cockeysville, Md.). Clavulanate (GlaxoSmithKline, Greenford, United Kingdom) and tazobactam (Wyeth, Pearl River, N.Y.) were used to add to cefepime disks.

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bla_{PER-1} and bla_{PER-2} alleles were detected by PCR by using previously reported primers (1, 9), heat-extracted templates, and the mixture containing *Taq* DNA polymerase and dNTP (Premix, Bioneer, Daejeon, Korea). The amplification conditions used were 30 cycles of denaturation at 94°C for 40 s, annealing at 50°C for 30 s, and extension at 72°C for 50 s. The nucleotide sequences of both strands of the bla_{PER-1} allele were analyzed with PCR products by the dideoxy-chain termination method with an ABI 3700 DNA sequencer (Perkin-Elmer, Foster City, Calif.).

The isoelectric points of β-lactamases were determined as described previously (4). The transfer of bla_{PER-1} was tested by plate mating with a rifampin-resistant *Acinetobacter baumannii* recipient (YMC02/8/P534). Pulsed-field gel electrophoresis (PFGE) of the *Sma*I-digested genomic DNA was performed and analyzed as described previously (4). The DNA bands separated by PFGE were transferred to a nylon membrane and hybridized by using a digoxigenin-labeled bla_{PER-1} probe (Roche Diagnostics, Mannheim, Germany).

In a preliminary study, bla_{PER-1} alleles were detected by PCR in five DDS-positive acinetobacters from a hospital in a southern region of Korea. The nucleotide sequences of bla_{PER-1} and the pI of the β-lactamase in our isolates were identical to those reported previously (5, 7, 8, 12).

The prevalence of bla_{PER-1}-carrying acinetobacter was determined in another hospital located approximately 300 km north of the first hospital. Of the 97 consecutive isolates of acinetobacter, 61 were resistant to ceftazidime and 53 (54.6%) were positive for the bla_{PER-1} allele: 51 *A. baumannii*, 1 *Acinetobacter* genomospecies 3, and 1 unidentifiable *Acinetobacter* spp.

bla_{PER-1} was not detected in 101 consecutive or in 181 ceftazidime-nonsusceptible *P. aeruginosa* isolates. bla_{PER-2} was not detected in any of the acinetobacter or *P. aeruginosa* isolates (data not shown).

bla_{PER-1} was detected in 46% of acinetobacter and 11% of *P. aeruginosa* isolates in Turkey (12). It is interesting that bla_{PER-1}-positive acinetobacters were detected in Korea, which is geographically distanced from Europe. The resistance may also exist in other Asian countries.

TABLE 1. Susceptibilities of bla_{PER-1}-positive and -negative isolates of acinetobacter

Antimicrobial agent(s)	bla_{PER-1} test result (no. of isolates tested)	MIC (μg/ml)		Resistance (%)
		MIC range	MIC_{90}[d]	
Ampicillin-sulbactam	Positive (53)	8–64	64	68
	Negative (37)	0.5–64	32	27
Ceftazidime	Positive (53)	>128	>128	100[a]
	Negative (37)	0.5–>128	128	22
Ceftazidime-clavulanate[b]	Positive (53)	≤0.12–>128	>128	68[c]
	Negative (37)	≤0.12–>128	>128	41[c]
Cefepime	Positive (53)	64–>128	>128	100
	Negative (37)	0.12–128	32	30

[a] All of the bla_{PER-1}-positive isolates were also resistant to cefotaxime and aztreonam.
[b] Fixed concentration of 4 μg of clavulanate/ml.
[c] Breakpoint for ceftazidime was applied.
[d] MIC_{90}, MIC at which 90% of the isolates tested are inhibited.

All of the bla_{PER-1}-positive acinetobacters were resistant to ceftazidime, cefepime, cefotaxime, and aztreonam, but the resistance rates of the bla_{PER-1}-negative isolates to these drugs and ampicillin-sulbactam were much lower (Table 1). The resistance rates of bla_{PER-1}-positive and -negative isolates were 64 and 30% to amikacin, 75 and 48% to gentamicin, 75 and 52% to tobramycin, 98 and 41% to trimethoprim-sulfamethoxazole, and 83 and 39% to tetracycline, respectively (data not shown). We anticipate difficulties in treating infections due to PER-1-producing acinetobacters, as they are resistant to all cephalosporins, including cefepime, and frequently to aminoglycosides, trimethoprim-sulfamethoxazole, and tetracycline.

Transfer of a bla_{PER-1}-carrying the 81-MDa plasmid to *Escherichia coli* was reported when plasmid pUZ8 existed in *S. enterica* serovar Typhimurium (11). In our study, bla_{PER-1} was transferred by conjugation to an *A. baumannii* recipient from 2 of 10 isolates tested, but repeated attempts failed to detect a bla_{PER-1}-carrying plasmid. *Sma*I-digested genomic DNA bands of approximately 100 to 350 kb hybridized with the bla_{PER-1} probe, indicating the presence of the gene on the chromosome.

Fifty-three isolates of bla_{PER-1}-positive acinetobacter showed 23 different PFGE patterns, indicating the presence of multiple clones (Fig. 1), but 9 of 16 isolates with pattern A and 7 of 8 isolates with pattern O were from intensive care unit patients, indicating clonal spread. Isolation of 39 (73.6%) strains from sputum samples suggests these were the main source of transmission.

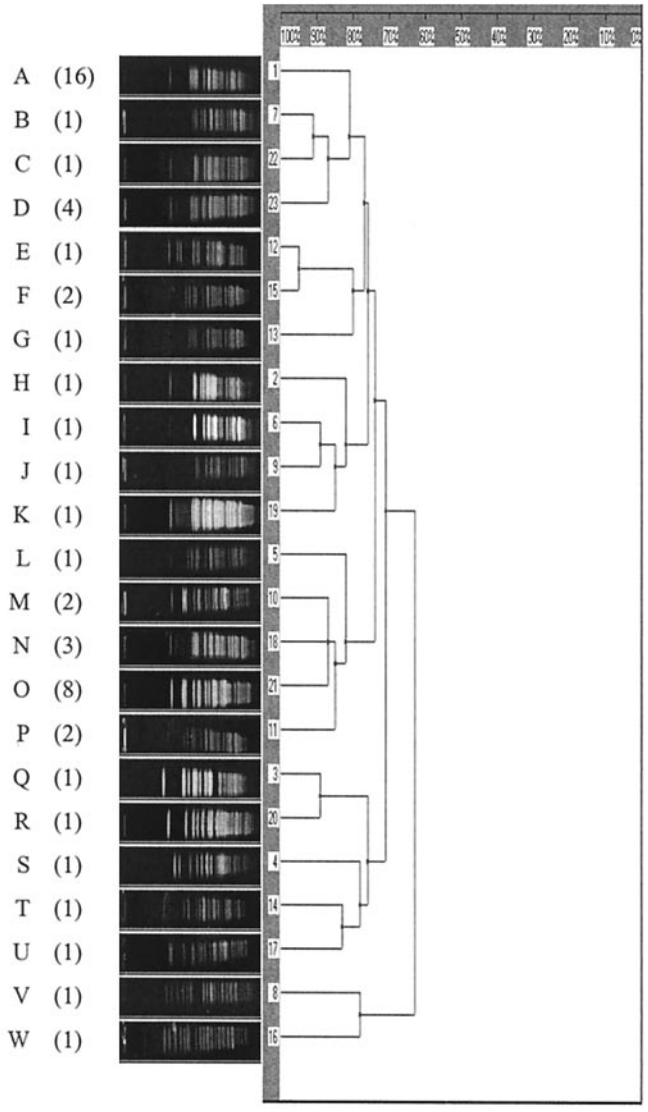

FIG. 1. Dendrogram of PFGE bands based on the coefficient of Dice. Numbers of multiple isolates with the same band patterns are shown in parentheses. Sixteen isolates had pattern A, and eight had pattern O.

TABLE 2. Phenotypic differentiation of 51 PER-1-producing isolates by cefepime disk and β-lactamase inhibitor-supplemented cefepime disk

Inhibitor added to a cefepime disk	No. (%) of isolates with zone diam difference of[a]:	
	≥5 mm	<5 mm
Clavulanate, 10 μg	42 (82.4)	9 (17.6)
Clavulanate, 20 μg	50 (98.0)[b]	1 (2.0)
Tazobactam, 10 μg	39 (76.5)	12 (23.5)
Tazobactam, 20 μg	51 (100)	0 (0)

[a] Difference in zone diameter between a cefepime disk and a cefepime disk plus inhibitor.
[b] Mean inhibition zone diameter difference between cefepime and cefepime plus 20 μg of clavulanate was only 6.7 mm.

DDS testing using ceftazidime, cefotaxime, or aztreonam disks detected only 17 of the 53 bla_{PER-1} allele-positive isolates when amoxicillin-clavulanate disks were placed 10 mm distant, edge to edge. The use of 5-mm-distanced cefepime disks detected 52 positive isolates, but the synergistic zones were small and difficult to interpret. Slightly larger synergistic zones were obtained when a cefepime disk and a 20-μg clavulanate disk were used (data not shown). These results indicated unreliability of the DDS tests for the screening of PER-1-producing acinetobacters.

Inhibition zone diameters by disks with cefepime alone and cefepime plus clavulanate or tazobactam were compared, and

a difference of ≥5 mm was arbitrarily defined as positive (Table 2). More positive isolates were detected with a cefepime disk containing 20-μg than 10-μg inhibitors, but the zone size differences remained small for both inhibitors. Therefore, PCR is considered the more reliable method for detecting bla_{PER-1}-producing *Acinetobacter* spp. This is the first report of high prevalence of bla_{PER-1}-positive acinetobacters outside Europe and indicates possible presence of the resistance in other countries.

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