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First Outbreak of *Klebsiella pneumoniae* Clinical Isolates Producing GES-5 and SHV-12 Extended-Spectrum β-Lactamases in Korea

Klebsiella pneumoniae clinical isolates producing extended-spectrum β-lactamases (ESBLs; TEM and SHV types) are frequently implicated in hospital outbreaks. There are also reports of *K. pneumoniae* isolates producing various non-TEM, non-SHV ESBLs: CTX-M and GES/IBC types (1). Presently, the different GES-type ESBLs are designated by identical names (8). To clarify the misleading nomenclature of GES-type ESBLs, we propose maintaining the current denomination concerning the fully characterized GES-3 and GES-4 ESBLs reported by Wachino et al. (12, 13) and renaming the variants (GES-3 and GES-4) reported by Vourli et al. (11) as GES-5 and GES-6, respectively.

In 2004, six *K. pneumoniae* clinical isolates producing ESBLs were collected from different patients and distributed among several wards (intensive care unit, neurosurgery, pulmonary internal medicine, and general medicine) at Bundang CHA Medical Center, Republic of Korea. These isolates were identified from sputum samples (for three isolates), urine (for one isolate), bile (for one isolate), and pus (for one isolate).

Antibiotic susceptibility testing by disk diffusion tests that were performed according to the recommendations of the Clinical and Laboratory Standards Institute (2) with BBL (Cockeysville, Md.) disks and by the double-disk synergy test of Jarlier et al. (4) suggested the presence of ESBL(s). Double-disk synergies were observed for amoxicillin-clavulanic acid (CLA), ceftazidime, cefotaxime, and aztreonam. All isolates were resistant to penicillins, ceftazidime, cefotaxime, aztreonam, tobramycin, gentamicin, and trimethoprim-sulfamethoxazole. They were susceptible to cefepime and represented reduced susceptibility to imipenem.

The analysis of genomic DNA, digested with SpeI and resolved by pulsed-field gel electrophoresis (PFGE) (5), revealed the same macrorestriction pattern among all isolates, which were therefore classified as indistinguishable.

Screening for ESBL gene(s) was performed by PCR using the primers for bla_{TEM}, bla_{SHV}, bla_{GES}, bla_{PER}, bla_{VEB}, bla_{TOHO}, bla_{SFO}, bla_{BES}, bla_{FEC}, bla_{CME}, bla_{TLA}, bla_{CTX-M}, and bla_{OXA} (1, 5, 7, 10). PCR amplification experiments using primers designed to amplify GES- and SHV-type ESBL genes gave only positive results for all isolates. The nucleotide sequence of the respective amplicon for the bla_{SHV} gene revealed 100% identity with bla_{SHV-12} from *K. pneumoniae* (6). That for the bla_{GES} gene differs by only one silent mutation (G→A) at position 54 from bla_{GES-5}, described elsewhere for *Escherichia coli* (11). On the isoelectric focusing gel, two β-lactamase activities with pIs of 5.8 and 8.2 were detected in all isolates. On the basis of DNA sequencing and pI values, the β-lactamase activity of pI 8.2 corresponds to that of SHV-12 β-lactamase, and the pI value of 5.8 represents GES-5 β-lactamase.

Six isolates were used as donors in transconjugation experiments. Ceftazidime-resistant transconjugants were obtained in all cases at frequencies ranging from 10^{-4} to 10^{-5} per donor cell. All transconjugants produced only GES-5. The SHV-12 gene was not cotransferred, indicating that the *bla* genes for GES-5 and SHV-12 resided on different genetic elements. All GES-5-producing transconjugants were resistant to penicillins and ceftazidime and susceptible to cefoxitin, cefotaxime, and aztreonam. This phenotype is characteristic of GES/IBC-producing *Enterobacteriaceae* (9–13). All transconjugants were susceptible to cefepime and showed reduced susceptibilities to imipenem. MICs of ticarcillin, ceftazidime, and cefotaxime were reduced by clavulanic acid (Table 1). This resistance was due to the acquisition of large (>70-kb) plasmids that exhibited identical HindIII restriction patterns and harbored the GES-5 ESBL gene. Due to the presence of SHV-12 in all isolates, the MIC patterns of clinical isolates were different from those of their transconjugants.

The carbapenemase bioassay (the modified cloverleaf test) (5) showed that extracts derived from all transconjugants producing GES-5 supported growth of the indicator, *E. coli* ATCC 25922, in the presence of an imipenem disk (30 μg), resembling the effect of OXA-23, which was used as a positive control. In contrast, cell extracts of a ceftazidime-sensitive *K. pneumoniae* clinical isolate collected from the Bundang CHA Medical Center did not cause any visible inhibition of carbapenemase activity. These findings indicated that the reduced susceptibilities of all isolates and their transconjugants to imipenem were due to production of GES-5 ESBL with the detectable carbapenemase activity.

Finding the same macrorestriction pattern by PFGE indicated that an endemic *K. pneumoniae* strain producing GES-5 and SHV-12 ESBLs was present in different wards at the Bundang CHA Medical Center in 2004. Six GES-5-producing transconjugants were also resistant to tobramycin, gentamicin, and trimethoprim-sulfamethoxazole. Although the complete nucleotide sequences of integrons from six clinical isolates were not determined, screening for the presence of class 1 through 4 integrase genes from large plasmids by PCR with primer pairs previously described (3) revealed the presence of class 1 integrons. The complete class 1 integron mapping of large plasmids from six clinical isolates and the possible spread of the GES-5 ESBL gene to other Korean hospitals are currently under investigation.

TABLE 1. MICs of β-lactams for ESBL-producing *K. pneumoniae* clinical isolates, their transconjugants, and the azide-resistant *E. coli* J53 recipient

β-Lactam	MIC (μg/ml) for:		
	K. pneumoniae clinical isolates (GES-5, SHV-12)	Trc (GES-5)[b]	Azide-resistant *E. coli* J53
Ticarcillin	>256	128	2
Ticarcillin + CLA[a]	128–256	32	2
Cefoxitin	4–8	4	2
Ceftazidime	128–>256	32	0.125
Ceftazidime + CLA	16–64	1	0.125
Cefotaxime	64–128	1	0.06
Cefotaxime + CLA	8–32	0.25	0.06
Aztreonam	64–256	0.5	0.06
Imipenem	0.5–1	0.5	0.06

[a] CLA was used at a fixed concentration of 2 μg/ml.
[b] Trc, transconjugant.

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Seok Hoon Jeong
Il Kwon Bae
Doelman Kim
Department of Laboratory Medicine
College of Medicine
Kosin University
Busan 602-702
Republic of Korea

Seong Geun Hong
Department of Laboratory Medicine
Pochon CHA University
Pochon
Republic of Korea

Jae Seok Song
Jung Hun Lee
Sang Hee Lee*
Department of Biological Sciences
Myongji University
San 38-2 Namdong
Yongin
Kyunggido 449-728
Republic of Korea

*Phone: 82 31 330 6195
Fax: 82 31 335 8249
E-mail: sangheelee@mju.ac.kr