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Outbreak of GES-1 β-Lactamase-Producing Multidrug-Resistant *Klebsiella pneumoniae* in a University Hospital in Lisbon, Portugal

Plasmid-located extended-spectrum β-lactamase genes are mostly found in *Klebsiella pneumoniae* (4), which is an important cause of nosocomial infections (1). In this study, we report the existence of *K. pneumoniae* clinical isolates producing the Ambler class A enzyme GES-1. This enzyme has been reported in Europe from *K. pneumoniae* (4) and *Pseudomonas aeruginosa* (2).

Between February 1999 and 2001, 30 *K. pneumoniae* clinical isolates were collected from different patients, distributed among several wards (surgery and medical services and in different intensive care units) at the Hospital de Santa Maria, Lisbon, Portugal.

Twenty-four isolates were identified from urine, four were identified from respiratory tract samples (three from sputum and one from bronchial exudate), and the remaining two isolates were found in blood and pus.

Antibiotic susceptibility testing by disk diffusion (3) suggested the presence of an extended-spectrum β-lactamase. Synergies were observed among clavulanic acid-amoxicillin, cefotaxime, aztreonam, and cefepime. All isolates were resistant to clavulanic acid, ceftazidime, cefuroxime, gentamicin, kanamycin, netilmicin, nalidixic acid, and norfloxacin. They were susceptible to imipinem and cefepime and presented reduced susceptibilities to amikacin, cefotaxime, and aztreonam.

The analysis of genomic DNA, digested with *Xba*I and resolved by pulsed-field gel electrophoresis (1), revealed the same macrorestriction pattern among all isolates, classified as indistinguishable according to the work of Tenover et al. (6).

On the isoelectric focusing gel two β-lactamase activities with pIs of 5.9 and 7.6 were detected. The β-lactamase activity of pI 7.6 corresponds to chromosomal SHV penicillinase, and the pI value of 5.9 represented the GES-1 β-lactamase.

Plasmid extraction, performed according to the alkaline lysis method (5), revealed plasmids with molecular sizes ranging from 3 to 23 kb. From five selected *K. pneumoniae* strains we obtained *Escherichia coli* DH5α transformants more resistant to ceftazidime than to cefotaxime and aztreonam and harboring a plasmid with a molecular size of ca. 9 kb. Under standard PCR conditions, plasmid DNA preparations from *K. pneumoniae* and *E. coli* DH5α transformants were used as templates for amplification of the bla_{GES-1} gene with primers GES-1A and GES-1B (4). All isolates revealed an 864-bp PCR product. The resulting amplicon was cloned into the *Sma*I site of pBK-CMV. The *E. coli* TOP10 harboring pMFA-62 was selected for subsequent analysis and sequencing. MICs of β-lactam antibiotics were determined with E-test strips (AB Biodisk, Solna, Sweden). For the *K. pneumoniae* clinical isolates, *E. coli* DH5α transformants, and *E. coli* TOP10 harboring recombinant plasmid pMFA-62, the cefuroxime and ceftazidime MICs were >256 μg/ml, and the MIC ranges (in micrograms per milliliter) were 3 to 0.75 for aztreonam and 6 to 8 for cefotaxime (Table 1). The nucleotide sequence of the cloned fragment revealed 100% identity with bla_{GES-1} from *P. aeruginosa* Pa695 (2) and differs by a single silent mutation at position 591 from bla_{GES-1} described elsewhere for *K. pneumoniae* ORI-1 (4).

The same macrorestriction pattern by pulsed-field gel electrophoresis indicated that an endemic *K. pneumoniae* strain producing GES-1 β-lactamase was presenting in different wards in the Hospital de Santa Maria. The persistence of these multiresistant microorganisms in the hospital may be associated with the existence of other resistance genes, inserted in multidrug-resistant integrons and/or plasmids.

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TABLE 1. MICs of β-lactams for *K. pneumoniae* clinical isolates, *E. coli* DH5α transformants, *E. coli* TOP10 harboring recombinant plasmid pMFA-62, and reference strains *E. coli* DH5α and *E. coli* TOP10 with pBK-CMV

β-Lactam	MIC (μg/ml)				
	K. pneumoniae clinical isolates	*E. coli* DH5α transformants[a]	*E. coli* DH5α	*E. coli* TOP10(pMFA-62)[b]	*E. coli* TOP10(pBK-CMV)
Amoxicillin	>256	>256	4	>256	4
Amoxicillin + CLA[c]	12	12	4	16	2
Cefuroxime	>256	>256	3	>256	4
Cefotaxime	6–8	2	0.064	8	0.125
Ceftazidime	256	16–256	0.25	>256	0.75
Aztreonam	3–0.75	0.75	0.125	1.5	0.094
Cefepime	2	0.5	0.047	ND[d]	0.047
Imipenem	0.25–0.387	0.125	0.19	0.5	0.25

[a] *E. coli* DH5α harboring natural plasmids expressed GES-1 β-lactamase.
[b] *E. coli* TOP10 harboring multicopy plasmid pMFA-62 produced GES-1 β-lactamase.
[c] CLA, clavulanic acid.
[d] ND, not determined.

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