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Detection of PER-2-Producing *Enterobacter cloacae* in a Brazilian Liver Transplantation Unit

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High rates of extended-spectrum-β-lactamase (ESBL)-producing *Klebsiella pneumoniae* isolates have been documented in many Brazilian hospitals, with CTX-M-2 being the most frequent ESBL reported (1). Resistance to broad-spectrum cephalosporins among *Enterobacter cloacae* isolates is usually due to hyperproduction of AmpC; however, production of ESBL represents an important cause of resistance to these antimicrobial agents among *E. cloacae* isolates from Brazilian hospitals (2). PER-2 was first identified in 1996 in a *Salmonella enterica* serovar Typhimurium strain isolated from a patient in Argentina who had previously been hospitalized in 1990 (3). Since then, this enzyme has also been described in *Enterobacteriaceae* isolated from Uruguay, Bolivia, and Argentina and in *Acinetobacter* spp. and *P. aeruginosa* isolates from Argentina (4–7). Here, we report the first occurrence of $bla_{\text{PER-2}}$ in Brazil.

In 2006, two cefepime-resistant *E. cloacae* strains were isolated from blood cultures of two distinct patients hospitalized in the Liver Transplant Unit of a tertiary care hospital located in Curitiba, a southern Brazilian city. Antimicrobial susceptibility testing was interpreted using the CLSI broth microdilution (8) method, except for polymyxin B, for which the EUCAST criteria were applied (9). The genetic relatedness of the isolates was evaluated by pulsed-field gel electrophoresis (PFGE) using XbaI (10). Isoelectric focusing analysis was performed with polyacrylamide gel containing ampholines (Amersham Pharmacia Biotech, Sweden; pH range, 3.5 to 9.5). The presence of $bla_{\text{CTX-M-1}}$, $_{\text{-2}}$, $_{\text{-8}}$, $_{\text{-9}}$, and $_{\text{-25}}$, $bla_{\text{OXA-23}}$, $_{\text{-24}}$, $_{\text{-48}}$, $_{\text{-51}}$, and $_{\text{-58}}$, bla_{BES}, bla_{TEM}, bla_{SHV}, bla_{PER}, and bla_{GES} was determined by PCR using specific primers (1, 2). Plasmid DNA (11) and chromosomal DNA (QIAamp DNA minikit; Qiagen, Germany) was extracted, and conjugation and transformation were carried out with *Escherichia coli* J53, *E. coli* DH5∞, and *E. coli* TOP10 recipient strains. Southern blotting and hybridization were performed using the digoxigenin (DIG) DNA labeling and detection kit (Roche Diagnostics, GmbH, Germany).

Furthermore, both isolates showed susceptibility to polymyxin B, imipenem, and meropenem and resistance to broad-spectrum cephalosporins, cefepime, and ertapenem (Table 1). Both isolates showed the ESBL phenotype by disk approximation test (12). $bla_{\text{PER-2}}$ and $bla_{\text{TEM-1}}$ were identified in both clinical isolates, which possessed unique PFGE profiles. Isoelectric focusing analysis showed a pI of 5.4, which may correspond to the PER-2 and/or TEM-1 pI. Multiple attempts to transfer the $bla_{\text{PER-2}}$ gene by electroporation and conjugation failed, and hybridization with $bla_{\text{PER-2}}$- and $bla_{\text{TEM-1}}$-specific probes showed that the $bla_{\text{TEM-1}}$ gene was located on an ~140-kb plasmid, while $bla_{\text{PER-2}}$ was located on the chromosome in both isolates.

Our study constitutes the first report of PER-2 in Brazil. $bla_{\text{PER-2}}$ and other PER variant-encoding genes are usually found on a conjugative plasmid. In contrast, in these *E. cloacae* isolates, $bla_{\text{PER-2}}$ was located on the chromosome. The clonal relatedness demonstrated by the two *E. cloacae* clinical isolates suggests that the patients may have acquired these strains from a common source. Although chromosomal AmpC production may have masked the identification of the ESBL phenotype, both isolates were phenotypically identified as ESBL producers by the disk approximation test. It probably occurred because the chromosomal bla_{AmpC} gene was not derepressed, as shown by the isoelectric focusing results. Although more studies are needed to evaluate the prevalence of PER-2 among Brazilian isolates, a previous study showed that this enzyme was infrequent. It was detected only in these 2 strains out of 205 *Enterobacter* species isolates from bloodstream samples over a 5-year period (2). The reason why PER-2 has not been as frequently detected in Brazil as it is in Argentina, a neighbor country, remains to be further investigated.

TABLE 1 Antimicrobial susceptibility profiles of two *E. cloacae* clinical isolates carrying $bla_{\text{PER-2}}$ and $bla_{\text{TEM-1}}$ in Brazil

Antimicrobial	MIC(s) (mg/liter)[a]	
	ECL532	ECL635
Polymyxin B	0.125	0.125
Imipenem	0.25	0.25
Meropenem	0.5	1
Ertapenem	2	2
Amikacin	8	8
Gentamicin	>64	>64
Cefotaxime	64	128
Ceftazidime	>64	>64
Cefepime	64	128
Ciprofloxacin	>32	>32
Levofloxacin	32	32
Aztreonam	>32	>32
Ticarcillin-clavulanic acid	>256, >2	>256, >2
Piperacillin-tazobactan	>128, >4	>128, >4
Trimethoprim-sulfamethoxazole	>64, >1,216	>64, >1,216

[a] MICs were determined by the CLSI broth microdilution method.

The project was approved by the Research Ethics Committee of the Hospital de Clínicas da Universidade Federal do Paraná (protocol no. 2288.182/2010-07).

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