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First Report of KPC-2-Producing *Klebsiella pneumoniae* Strains in Brazil[▽]

The carbapenems are regarded as the preferential therapeutic option for treatment of serious health care-associated infections with multidrug-resistant gram-negative bacteria. Although carbapenem resistance is rarely described for the *Enterobacteriaceae* (6), this phenotype of resistance has been increasingly reported worldwide, especially due to the emergence and spread of *Klebsiella pneumoniae* carbapenemase (KPC) (1, 5, 8). In this report, we describe the first detection of KPC-2-producing *K. pneumoniae* strains in Brazil. These strains also coproduced an extended-spectrum β-lactamase, CTX-M-2.

Between September and November 2006, four carbapenem-resistant *K. pneumoniae* strains were isolated from four distinct patients hospitalized in an intensive care unit (ICU) of a tertiary hospital located in Recife, a city on the northeastern coast of Brazil. These strains were isolated from blood (two strains) or urine (two strains). The antimicrobial susceptibility profile was determined by the reference agar dilution method according to CLSI guidelines (2). The genetic relatedness of the carbapenem-resistant *K. pneumoniae* strains was evaluated by pulsed-field gel electrophoresis (PFGE) using SpeI (4). The presence of bla_{TEM}, bla_{SHV}, bla_{CTX}, bla_{KPC}, bla_{IMP}, bla_{VIM}, and bla_{SPM} was determined by PCR using specific primers. Amplicons were sequenced and compared to sequences available in the GenBank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Plasmid DNA was extracted from carbapenem-resistant *K. pneumoniae* strains using the QIAprep SpinMiniprep kit (Qiagen, Hilden, Germany). Transformation experiments with *Escherichia coli* DH10B were carried out by electroporation. Transformants were selected on Müeller-Hinton agar containing ampicillin (100 μg/ml), ceftazidime (2 μg/ml), and ertapenem (0.5 μg/ml). The MICs of *E. coli* DH10B and transformants were determined using Etest strips according to the manufacturer's recommendations (AB Biodisk, Solna, Sweden).

All carbapenem-resistant *K. pneumoniae* clinical strains showed resistance to broad-spectrum cephalosporins and carbapenems (Table 1). Three carbapenem-resistant *K. pneumoniae* strains showed a unique PFGE pattern (pattern A), which was distinct from PFGE pattern B, displayed only by a single strain (7). bla_{KPC} and $bla_{CTX\text{-}M\text{-}2}$ were detected in all clinical strains, while bla_{SHV} and bla_{TEM} were carried only by the PFGE pattern A strains. The carbapenem-resistant *K. pneumoniae* clinical isolates and transformants possessed $bla_{KPC\text{-}2}$ (GenBank accession number EU784136) and $bla_{CTX\text{-}M\text{-}2}$, which were carried by a single 60-kb plasmid. This fact could justify the increased MICs of several β-lactams exhibited by the transformant (Table 1). Furthermore, as recently described, our KPC-producing isolates were found to accumulate other β-lactam resistance enzymes (TEM-1, CTX-M-2, and SHV-11) (3).

The report of KPC-2-producing isolates is very worrisome, since these strains are resistant to all β-lactam agents and often to other antimicrobials. This attribute limits the therapeutic options available for treatment of serious infections, which is basically restricted to tigecycline and polymyxins. In addition, the detection of such strains by routine clinical laboratories might be difficult when current standard antimicrobial susceptibility methods are employed. Moreover, $bla_{KPC\text{-}2}$ is plasmid borne, making its dissemination easier, especially when carried by *K. pneumoniae*, an organism notorious for its ability to accumulate and transfer resistance determinants. The clonal spread observed in this Brazilian ICU confirms $bla_{KPC\text{-}2}$ dissemination and points to difficulties with infection control measures for this organism.

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TABLE 1. MICs of selected antimicrobial agents for KPC-producing *K. pneumoniae* strains, the *E. coli* transformant, and the *E. coli* DH10B recipient strain

Isolate	Body site	PFGE pattern	β-Lactamases	MIC (μg/ml) of[a]:												
				IMP	MER	FEP	CAZ	CTX	CRO	ATM	TZP	GEN	CIP	TGC	FOX	PMB
A28006	Blood	A	KPC-2, TEM-1, SHV-11, CTX-M-2	4	32	128	16	>256	>256	64	16	1	4	0.38	32	2
A28008	Blood	A	KPC-2, TEM-1, SHV-11, CTX-M-2	32	4	32	16	>256	>256	64	16	1	4	0.38	16	2
A28009	Urine	A	KPC-2, TEM-1, SHV-11, CTX-M-2	32	>32	64	32	>256	>256	>256	16	1	4	0.38	64	2
A28011	Urine	B	KPC-2, CTX-M-2	16	>32	>256	64	>256	>256	>256	8	1	4	0.50	>256	2
E. coli DH10 B[b]		NT[c]		0.25	0.25	0.25	0.25	<0.25	0.06	0.12	NT	0.12	NT	0.06	4	0.38
E. coli transformant[b]		NT	KPC-2, CTX-M-2	2	0.5	2	4	4	>256	32	NT	0.12	NT	0.06	8	0.38

[a] MICs determined by agar dilution according to CLSI guidelines, except for those of tigecycline, which were determined by Etest. IMP, imipenem; MER, meropenem; ATM, aztreonam; CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime; FOX, cefoxitin; TZP, piperacillin-tazobactam; GEN, gentamicin; CIP, ciprofloxacin; CRO, ceftriaxone; TGC, tigecycline; PMB, polymyxin B.
[b] The MICs of *E. coli* DH10B and transformants were determined using Etest strips according to the manufacturer's recommendations (AB Biodisk, Solna, Sweden).
[c] NT, not tested.

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