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First Description of KPC-2-Producing *Pseudomonas putida* in Brazil

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This work reports the identification of the first case of a KPC-2-producing *Pseudomonas putida* isolate (PP36) in Brazil. The PP36 isolate was resistant to all the antimicrobials tested except polymyxin B. In addition to the discovered bla_{KPC-2} gene, genetic analysis showed the presence of a class 1 integron containing the *dhfr*XVb gene and the new allele *arr-6*, which codes for resistance to rifampin. These elements were found in an IncFI 65-kb plasmid.

Since the first detection of a *Klebsiella pneumoniae* isolate harboring the bla_{KPC} gene in a large plasmid in 1996 in North Carolina (15) and until 2005, the geographic distribution of these *K. pneumoniae* carbapenemase (KPC) enzymes in *Enterobacteriaceae*, mainly *K. pneumoniae*, was limited to the eastern part of the United States (6). Nowadays, the worldwide spread of KPC-producing, Gram-negative pathogens represents a potential clinical threat with devastating effects on patient outcomes (13).

Although KPCs are mostly identified in *K. pneumoniae*, they have recently been observed among other Gram-negative pathogens, such as *Pseudomonas* spp. in America (11, 14) and *Acinetobacter baumannii* in Puerto Rico (12). KPC-2-producing *K. pneumoniae* and *Escherichia coli* clinical isolates were also identified in Brazil (8, 9). Besides these, to date, only one report describes the isolation of *Pseudomonas putida* producing KPC-2 in Texas (2). Those few reports show the spreading of the KPC-2 enzyme among potential pathogenic bacteria, an observation which might be of great concern for infection control programs. In the present study, we report the identification of the first case of a KPC-2-producing *P. putida* isolate in Brazil and highlight the importance of this finding in terms of hospital infection control.

An 8-year-old boy was admitted to the Oncology Pediatric Center at the University Hospital Oswaldo Cruz, Recife, Brazil, in July 2008 to initiate a new cycle of chemotherapy for Burkitt's lymphoma. Ten days following admission, he presented fever and diarrhea and was sent to the Pediatric Intensive Care Unit and given a further 10 days of empirical intravenous antibiotic therapy with meropenem (1 g every 8 h) and vancomycin (500 mg every 6 h). The patient evolved with febrile neutropenia and gastrointestinal bleeding. Due to fungal sepsis by *Candida* spp., he received voriconazole (7 mg/kg of body weight every 12 h) for 21 days. Transcatheter bloodstream cultures showed the presence of a carbapenem-resistant *P. putida* isolate (herein named PP36). Since the patient has no history of traveling abroad, we assumed that this infection was acquired at the hospital. The bacterial identification was performed by a mini-Api ID32 GN card (bioMeriéux, Marcy l'Etoile, France). Meropenem therapy (1 g every 8 h) was restarted, and the catheter was removed. After the removal, the patient evolved to negative bloodstream cultures until the 67th day of internment when he was discharged.

Broth microdilution assays (Table 1) showed that the PP36 isolate was resistant to all antimicrobials tested according to Clinical and Laboratory Standards Institute protocols (4, 5), that it was susceptible to polymyxin B according to EUCAST breakpoints (7), and that it showed intermediate resistance to tigecycline according to U.S. Federal Drug Administration breakpoints for *Enterobacteriaceae* that define a MIC of ≤2 as susceptible. *E. coli* ATCC 25922 was used as the control. Due to the resistance to all beta-lactams tested, the isolate was screened for the presence of extended-spectrum beta-lactamases (ESBLs) and class A and B carbapenemases (Table 1). PCR amplifications and plasmid analysis were carried out as previously described (10), followed by sequencing of the amplicon in both strands (ABI 3100 platform; Applied Biosystems) and BLASTn analysis (www.ncbi.nlm.nih.gov/Blast.cgi). PCR-based replicon typing and transposon typing were performed as described (3, 6).

Afterwards, genetic analysis resulted in the detection of the bla_{KPC-2} gene. The detection of the *intI1* gene by specific PCR showed the presence of a class 1 integron. Furthermore, its variable region of ca. 1.5 kb was submitted to DNA sequencing that revealed the presence of the *dhfr*XVb gene, encoding the dihydrofolate reductase involved in resistance to trimethoprim, and the new allele *arr-6*, encoding the rifampin ADP-ribosylating transferase involved in resistance to rifampin. This new allele differs from *arr-2* by an A-to-G mutation at the position +293 (Lys98Arg) and from *arr-3* by a silent G-to-A mutation at the position +411. The PP36 isolate displayed two distinct plasmids with sizes of ca. 65 kb and 147 kb (Table 1) that were extracted and introduced into *Escherichia coli* DH5α (10). The transformed cells, designated TF36, that were selected on Mueller-Hinton agar containing 100 μg/ml ampicillin showed the presence of only the 65-kb plasmid that carried the bla_{KPC-2} gene as attested by specific PCR. Recently, Andrade et al. (1) described the presence of the plasmid types IncFII, IncN, and IncL/M carrying bla_{KPC} among *Enterobacteriaceae* species in Brazil. In the present work, we identified a 65-kb IncFI-type plasmid carrying bla_{KPC}. Acquisition of this plasmid yielded resistance to aztreonam, cephalosporins, carbapenems, and combinations of beta-lactam/beta-lactamase inhibitors to the transformed cells (Table 1). It was noteworthy

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TABLE 1 Antimicrobial drug susceptibility and molecular analysis of the bacterial strains used in this work

Strain[a]	MIC (μg/ml) for[b]:														Presence of bla_{KPC-2}/class 1 integron[c]	Plasmid size(s) (kb)
	GEN	CEF	CTX	CRO	CAZ	FEP	ATM	IPM	MEM	CIP	TZP	AMC	PMB	TGC		
PP36	64	>256	256	128	16	256	128	64	>32	0.25	256	≥128	0.12	4	+/+	65, 147
TF36	64	>256	256	64	8	32	128	16	2	0.25	64	64	0.12	4	+/+	65
DH5α	0.25	8	<0.12	0.06	0.5	0.5	0.5	0.12	<0.015	0.004	<2	<1	<0.12	0.06	−/−	
ATCC 25922	0.06	4	0.12	0.06	0.12	<0.12	0.12	0.12	0.015	0.008	<2	2	0.25	0.03	−/−	

[a] PP36, *P. putida* isolate; TF36, *E. coli* transformant of PP36; DH5α, *E. coli* recipient strain; ATCC 25922, *E. coli* control strain.
[b] GEN, gentamicin; CEF, cephalothin; CTX, cefotaxime; CRO, ceftriaxone; CAZ, ceftazidime; FEP, cefepime; ATM, aztreonam; IPM, imipenem; MEM, meropenem; CIP, ciprofloxacin; TZP, piperacillin-tazobactam; AMC, amoxicillin-clavulanic acid; PMB, polymyxin B; TGC, tigecycline.
[c] +, present; −, absent.

that MICs for ceftriaxone and imipenem increased 64- and 134-fold, respectively, in the TF36 strain compared to those of the DH5α strain.

The analysis of the genetic environment of bla_{KPC} by amplification of the region between IS*Kpn7* and the bla_{KPC} gene revealed a 200-bp deletion upstream of the bla_{KPC} gene. It indicates the presence of a Tn*4401*c isoform located in the 65-kb IncFI plasmid. Since KPC-producing *K. pneumoniae* isolates are frequent in the hospital of study, it is possible that *P. putida* acquired a bla_{KPC}-containing plasmid from these isolates. This report confirmed for the first time in Brazil the presence of a plasmid-based bla_{KPC-2} gene in a hospital isolate of *P. putida*. Moreover, a new allele for rifampin resistance was identified. Despite careful and conservative use of antibiotics and good control practices, the present work and the recent identifications of the bla_{KPC} genes in distinct Gram-negative pathogens emphasize the quick spreading of this gene in long-term facilities, limiting therapeutic options for infected hospitalized patients.

Nucleotide sequence accession numbers. The sequences for the newly detected bla_{KPC-2} gene, *dhfr*XVb gene, and *arr-6* allele have been deposited in GenBank under accession numbers JF922884, JF922882, and JF922883, respectively.

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