

First Description of KPC-2-Producing *Klebsiella oxytoca* in Brazil

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The present work reports the detection of the first case of nosocomial *Klebsiella oxytoca* producing class A carbapenemase KPC-2 in Brazil. The isolate KPN106 carried a 65-kb IncW-type plasmid that harbors the *bla*_{KPC} gene and Tn4401b. Moreover, we detected the presence of a class 1 integron containing a new allele, *arr-8*, followed by a 5'-truncated *dhfrIIIc* gene. In view of the recent results, we emphasize the high variability of the bacterial and genetic hosts of this resistance determinant.

Despite the KPC enzymes being frequently associated with several members of the *Enterobacteriaceae* family, few reports have described the presence of KPC in *Klebsiella oxytoca* isolates (1). This work reports the first case of a KPC-2-producing *K. oxytoca* isolate in Brazil and describes its clinical data, susceptibility profile, and molecular analysis.

A 79-year-old female patient with chronic obstructive pulmonary disease and chronic renal failure was readmitted to the intensive care unit (ICU) at the University Hospital Oswaldo Cruz, Recife, Brazil, in December 2008. Due to her prior hospitalization (41 days), the patient received upon admission imipenem (250 mg every 6 h for 2 days) and polymyxin B (500,000 U every 12 h for 8 days). Blood cultures revealed the presence of a carbapenem-resistant *K. oxytoca* isolate (KPN106). The treatment with polymyxin B was maintained in combination with ciprofloxacin (400 mg every 12 h) for 7 days. The blood cultures remained positive after antimicrobial therapy. On the 12th day of her stay, the patient died with a diagnosis of renal and respiratory failure and sepsis. Here, we describe the microbiological and molecular analysis of this isolate.

Broth microdilution showed that the KPN106 isolate was highly resistant to most of the antimicrobial agents tested (Table 1) according to the CLSI standard (2). The isolate was tested for the presence of the class 1 integron, the extended-spectrum β -lactamase (ESBL), and class A and B carbapenemases.

Molecular analysis was carried out as described previously (3). In addition to *bla*_{KPC-2}, KPN106 presented a class 1 integron and additional *bla* genes (Table 1). Moreover, it carried three plasmids (ca. 65 kb, 15 kb, and 12 kb) that were transferable to *E. coli* DH5 α cells by calcium chloride transformation. The sequencing of the variable region of the class 1 integron revealed a new allele of the ADP ribosyltransferase family that confers rifampin resistance,

arr-8 (GenBank accession number KC199968), with 75% protein similarity with the enzymes encoded by *arr-2* and *arr-3* alleles. Furthermore, we found a 5'-truncated form of the *dhfrIIIc* gene that encodes dihydrofolate dehydrogenase, followed by a partial putative insertion sequence. The *Escherichia coli* DH5 α cells (TF106) selected on LB agar plates containing 100 μ g/ml ampicillin acquired the 65-kb plasmid together with the *bla*_{KPC-2} gene and class 1 integron as shown by PCR. The plasmid incompatibility groups were determined as described previously (4), which demonstrated that these plasmids belong to the IncW group in both donor and transformant cells. This acquisition increased MICs of *E. coli* DH5 α cells for extended-spectrum cephalosporins, carbapenems, and rifampin (Table 1). Analysis of the genetic environment (5) of *bla*_{KPC} revealed the presence of the transposon Tn4401b isoform, as observed in a KPC-producing *K. oxytoca* isolate (1) and in other *Enterobacteriaceae* members (5).

Recently, there was reported the presence at the same hospital of a 65-kb IncW-type plasmid carrying *bla*_{KPC} in Tn4401c among *Enterobacteriaceae* species (6). In the present study, we report a 65-kb IncW-type plasmid carrying *bla*_{KPC} in Tn4401b in a *K. oxytoca* isolate, highlighting the diversity of mobile genetic elements

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TABLE 1 Phenotypic and genetic characteristics of the bacterial strains used in this work

Strain ^a	β -Lactamase/class 1 integron cassette array	MIC (μ g/ml) ^b															
		AMK	GEN	CEF	CRO	CAZ	FEP	ATM	IPM	MEM	ETP	CIP	TZP	AMC	RIF	PMB	TGC
KPN106	<i>bla</i> _{KPC-2} , <i>bla</i> _{CTX-M-2} , <i>bla</i> _{SHV-11} , <i>bla</i> _{TEM-1} , <i>arr-8</i> , <i>dhfrIIIc</i>	16	32	≥ 256	≥ 64	≥ 64	8	128	≥ 16	≥ 16	≥ 8	≥ 8	≥ 256	128	≥ 8	0.25	1
TF106	<i>bla</i> _{KPC-2} , <i>arr-8</i> , <i>dhfrIIIc</i>	16	0.25	64	>64	>64	8	128	>16	>16	>8	0.25	>128	32	0.25	0.25	0.03
DH5 α	None	0.12	0.25	8	0.06	0.5	0.5	0.5	0.12	<0.015	0.003	0.004	<2	<1	0.03	<0.12	0.06

^a KPN106, *K. oxytoca* isolate; TF106, *E. coli* transformant KPN106; DH5 α , *E. coli* recipient strain.

^b AMK, amikacin; GEN, gentamicin; CEF, cephalothin; CRO, ceftriaxone; CAZ, ceftazidime; FEP, cefepime; ATM, aztreonam; IPM, imipenem; MEM, meropenem; ETP, ertapenem; CIP, ciprofloxacin; TZP, piperacillin-tazobactam; AMC, amoxicillin-clavulanate; RIF, rifampin; PMB, polymyxin B; TGC, tigecycline.

related to the *bla*_{KPC} gene in this institution and the high variability of the genetic hosts of this gene.

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