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Coproduction of KPC-2 and IMP-10 in Carbapenem-Resistant *Serratia marcescens* Isolates from an Outbreak in a Brazilian Teaching Hospital

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We describe an outbreak caused by KPC-2- and IMP-10-producing *Serratia marcescens* isolates in a Brazilian teaching hospital. Tigecycline was the only active antimicrobial agent tested. The bla_{IMP-10} gene was located in a new class 1 integron, named *In*990, carried by a nonconjugative plasmid, in contrast to bla_{KPC-2}.

Serratia marcescens is a common pathogen involved in nosocomial infections affecting several body sites, with a significant impact on morbidity and mortality (1, 2). The spread of carbapenem-resistant *S. marcescens* strains in the nosocomial environment is a matter of concern, since this pathogen is intrinsically resistant to polymyxins (3). To date, a few Brazilian studies have reported the production of KPC-2 in carbapenem-resistant *S. marcescens* isolates from hospitals located in southern and northeastern Brazilian regions (4, 5). We describe herein the microbiological characterization of an outbreak caused by a KPC-2- and IMP-10-producing *S. marcescens* clone in a tertiary teaching hospital located in mid-western Brazil. (This study was presented in part at the 24th European Congress of Clinical Microbiology and Infectious Diseases [ECCMID], in Barcelona, Spain, 10 to 13 May 2014.)

A total of 30 carbapenem-resistant *S. marcescens* isolates, collected on different days and from different body site infections, between September 2011 and February 2013, were recovered from 23 patients hospitalized in intensive care units (ICUs) at a tertiary teaching hospital located in the city of Dourados in Mato Grosso do Sul state. Patients' identification and demographic data were recorded, and all clinical data were entered into a research electronic data capture (Redcap) database. Species identification was performed by Vitek2 (bioMérieux, Hazelwood, MO), and confirmed by matrix-assisted laser desorption ionization−time of flight mass spectrometry (MALDI-TOF MS), using a Microflex LT spectrometer (Bruker Daltonics, MA, USA) (6). The MICs of antimicrobials were determined by broth microdilution according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (7), except for tigecycline, for which they were determined using Etest strips (bioMérieux, Marcy l'Étoile, France), according to the manufacturer's recommendations. All *S. marcescens* isolates were resistant to ertapenem (MIC_{50}, >16 mg/liter), imipenem (MIC_{50}, >8 mg/liter), and meropenem (MIC_{50}, >8 mg/liter). Preliminary screening for the presence of carbapenemase was performed by the modified Hodge test (MHT) (7), and positive results were confirmed by ertapenem hydrolysis using MALDI-TOF MS (8). The presence of β-lactamase genes was evaluated by PCR followed by sequencing, using specific primers (9, 10). Of the 30 isolates, 24 were classified as carbapenemase producers by the MHT and MALDI-TOF MS and shown to codify bla_{KPC-2}. The bla_{IMP-10} gene was also detected in 6/24 isolates (Table 1). The genetic relationships among the KPC-2-producing *S. marcescens* strains were determined by pulsed-field gel electrophoresis (PFGE), using SpeI (New England BioLabs, Ipswich, MA, USA) and analyzed with BioNumerics software v.3.0 (Applied Maths, Sint-Martens-Latem, Belgium). The percentage of similarity between fingerprints was scored by the Dice coefficient (11). All 24 KPC-2-producing strains, including those that coproduced IMP-10, exhibited >94.1% similarity (Fig. 1). The results of the susceptibility tests for the six isolates coproducing KPC-2 and IMP-10 show that tigecycline was the only antimicrobial agent tested with activity against these strains (Table 2). The sequencing analysis of the bla_{IMP-10} genetic context (12, 13) demonstrated that it was inserted in a new class 1 integron cassette arrangement, named *In*990. The bla_{IMP-10} gene was arranged as the first gene cassette of *In*990, immediately downstream of the 5′-conserved sequence (CS), followed by 2 genes (*aacA31* and *aadA1*) encoding aminoglycoside-modifying enzymes (AMEs). The 3′-CS contained the *qacEΔ1-sul1* genes encoding resistance to a disinfectant determinant and sulfonamide, respectively. The plasmid DNA extraction was performed using the Kieser protocol (14) and DNA-DNA hybridization was assessed by Southern blotting with a Hybond-N^+ nylon transfer membrane (GE Healthcare, Little Chalfont, United Kingdom). Digoxigenin (DIG) labeling of the bla_{KPC-2} and bla_{IMP-10} specific probes and signal detection were carried out using the DIG DNA labeling and detection kit (Roche

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TABLE 1 Clinical characteristics of the 23 patients involved in the ICU outbreaks caused by an epidemic of a KPC-2-producing *S. marcescens* strain

Patient no.	Age (yr), sex[a]	Clinical isolate(s)	Strain[b]	Date of admission (mo/day/yr)	Date of isolation (mo/day/yr)	Hospital unit[c]	Length of stay (days)	Place prior to admission	Outcome	Antibiotic exposure	Carbapenemase gene(s)	Treatment (dosage)/days of therapy[d]
1	60, F	Tracheal aspirates	P	9/26/2011	10/3/2011	ICU	65	Another hospital	Death	Carbapenems	bla_{KPC-2}	AMI (500 mg) + CST-PMB (500 mg)/13
2	63, F	Tracheal aspirates	P	9/25/2011	10/12/2011	ICU	15	Home	RH[e]	Cephalosporins	bla_{KPC-2}	AMI (250 mg) + CST-PMB (500 mg)/14
2	63, F	Tracheal aspirates	P	9/25/2011	11/12/2011	ICU	15	Home	Recovery	Cephalosporins	bla_{KPC-2}	AMI (250 mg) + CST-PMB (500 mg)/17
3[f]	82, M	Urine culture	P	10/24/2011	10/28/2011	ICU	180	Another hospital	RH	Carbapenems/cephalosporins	bla_{KPC-2}/bla_{IMP-10}	AMP-SAM (4 g) + CST-PMB (500 mg)/20
3	82, M	Urine culture	P	10/24/2011	4/3/2012	ICU	180	Another hospital	Death	Carbapenems/cephalosporins	bla_{KPC-2}/bla_{IMP-10}	AMP-SAM (4 g) + CST-PMB (500 mg)/14
4	1, M	Rectal swab	C	11/20/2011	11/25/2011	ICUneo	29	Home	Recovery	Cephalosporins	bla_{KPC-2}	AMI (50 mg) + CST-PMB (500 mg)/17
5	75, F	Scar	C	9/26/2011	11/29/2011	ICU	24	Another hospital	RH	Carbapenems/cephalosporins	bla_{KPC-2}	AMI (250 mg) + CST-PMB (500 mg)/7
5	75, F	Nasal swab	C	09/26/2011	12/27/2011	ICU	24	Another hospital	RH	Carbapenems/cephalosporins	bla_{KPC-2}	AMI (250 mg) + CST-PMB (500 mg)/10
5[f]	75, F	Tracheal aspirates	P	09/26/2011	12/28/2011	ICU	24	Another hospital	Death	Carbapenems/cephalosporins	bla_{KPC-2}/bla_{IMP-10}	AMP-SAM (4 g) + CST-PMB (500 mg)/10
6	76, F	Tracheal aspirates	P	12/6/2011	12/21/2011	ICU	28	Home	RH	Aminoglycosides/cephalosporins	bla_{KPC-2}	AMP-SAM (4 g) + CST-PMB (500 mg)/10
6	76, F	Blood culture	P	12/6/2011	01/5/2012	ICU	28	Home	Death	Aminoglycosides/cephalosporins	bla_{KPC-2}	FLU (750 mg) + CAR (500 mg)/18
7	2, M	Catheter	C	12/10/2011	12/26/2011	ICUped	90	Home	Recovery	Penicillins	ND[g]	AMI (250 mg) + CAR (500 mg)/22
8	25, M	Scar	C	1/2/2012	1/7/2012	ICU	30	Another hospital	Recovery	Fluoroquinolone	bla_{KPC-2}	AMI (250 mg) + CST-PMB (500 mg)/20
9	1, M	Urine culture	P	1/10/2012	1/14/2012	ICUneo	15	Home	Recovery	Cephalosporins	ND	CEPH (50 mg) + CAR (500 mg)/10
10	62, M	Tracheal aspirates	P	1/05/2012	1/16/2012	ICU	80	Home	Recovery	Carbapenems/cephalosporins	bla_{KPC-2}	AMI (500 mg) + CST-PMB (500 mg)/17
11	1, F	Blood culture	P	12/29/2011	2/9/2012	ICUped	90	Home	Death	Cephalosporins	bla_{KPC-2}	AMI (250 mg) + CST-PMB (500 mg)/17
12[f]	52, M	Urine culture	P	1/31/2012	2/13/2012	ICU	90	Another hospital	Death	Aminoglycosides/carbapenems	bla_{KPC-2}/bla_{IMP-10}	AMI (500 mg) + CST-PMB (500 mg)/20
13	62, M	Urine culture	P	2/6/2012	2/16/2012	ICU	23	Another hospital	RH	Aminoglycosides/carbapenems	ND	AMI (250 mg) + CAR (500 mg)/8
13	62, M	Tracheal aspirates	P	2/6/2012	3/18/2012	ICU	23	Another hospital	Recovery	Aminoglycosides/carbapenems	bla_{KPC-2}	AMP-SAM (4 g) + CST-PMB (500 mg)/10
14	56, M	Tracheal aspirates	C	2/8/2012	2/16/2012	ICU	31	Home	RH	Aminoglycosides/carbapenems/ fluoroquinolone	bla_{KPC-2}	AMP-SAM (4 g) + CST-PMB (500 mg)/10
14	56, M	Urine culture	P	2/8/2012	3/19/2012	ICU	31	Home	Recovery	Aminoglycosides/carbapenems/ fluoroquinolones	bla_{KPC-2}	AMI (500 mg) + CST-PMB (500 mg)/14
15	82, M	Nasal swab	C	2/8/2012	2/17/2012	ICU	33	Another hospital	Recovery	Carbapenems/cephalosporins	bla_{KPC-2}	AMI (250 mg) + CST-PMB (500 mg)/17
16	62, M	Tracheal aspirates	P	2/6/2012	2/27/2012	ICU	47	Another hospital	Recovery	Aminoglycosides/carbapenems	bla_{KPC-2}	AMI (500 mg) + CST-PMB (500 mg)/12
17[f]	67, M	Tracheal aspirates	P	3/31/2012	4/16/2012	ICU	90	Another hospital	Death	Aminoglycosides/carbapenems	bla_{KPC-2}/bla_{IMP-10}	AMP-SAM (4 g) + CST-PMB (500 mg)/10
18	45, M	Rectal swab	C	3/15/2012	4/20/2012	ICU	28	Another hospital	Death	Carbapenems/cephalosporins	bla_{KPC-2}	AMI (250 mg) + CST-PMB (500 mg)/12
19[f]	85, F	Tracheal aspirates	P	4/17/2012	4/23/2012	ICU	43	Another hospital	Death	Aminoglycosides/carbapenems	bla_{KPC-2}/bla_{IMP-10}	AMP-SAM (4 g + CST-PMB (500 mg)/10
20	75, F	Blood culture	P	6/8/2012	6/13/2012	ICU	30	Home	Recovery	Penicillins/carbapenems	ND	AMI (250 mg) + CAR (500 mg)/14
21	83, F	Tracheal aspirates	P	6/30/2012	7/4/2012	ICU	27	Home	Recovery	Cephalosporins	bla_{KPC-2}	AMI (500 mg) + CAR (500 mg)/16
22	2, F	Catheter	C	10/09/2012	11/4/2012	ICU	90	Home	Recovery	Cephalosporins	ND	AMI (250 mg) + CAR (500 mg)/15
23	1, M	Catheter	C	1/29/2013	2/9/2013	ICUneo	30	Home	Recovery	Cephalosporins	ND	AMI (250 mg) + CAR (250 mg)/20

[a] F, female; M, male.

[b] P, pathogen; C, colonizer.

[c] ICU, general intensive care unit; ICUneo, neonatal intensive care unit; ICUped, pediatric intensive care unit.

[d] AMI, aminoglycosides; CST, colistin; PMB, polymyxin B; AMP-SAM, ampicillin-sulbactam; FLU, fluoroquinolones; CAR, carbapenems; CEPH, cephalosporins.

[e] RH, remained hospitalized.

[f] The patient was admitted after previously hospitalization in the same hospital.

[g] ND, not detected.

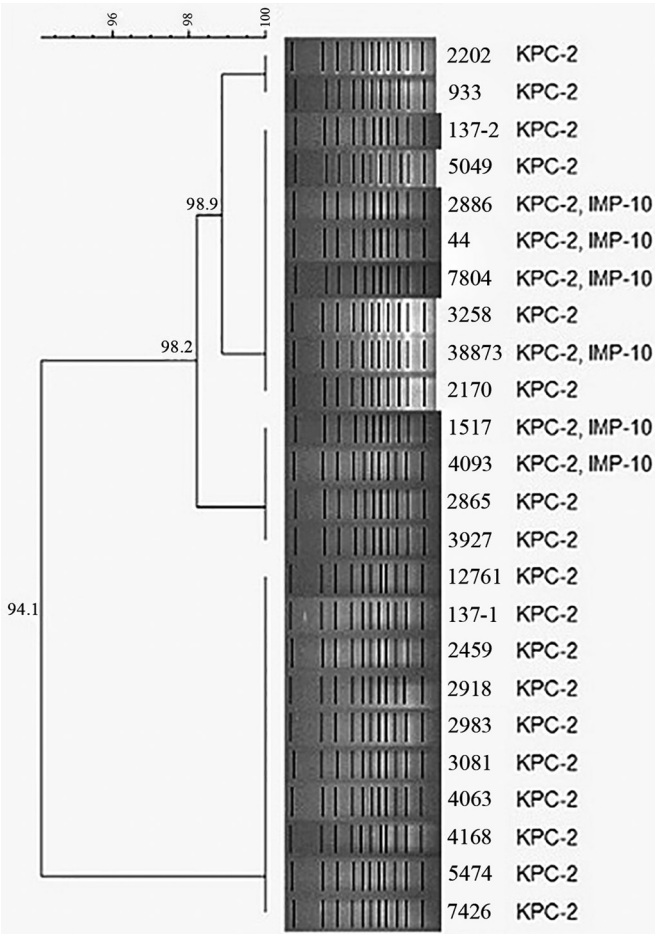

FIG 1 Dendrogram displaying the genetic relatedness of 24 carbapenem-resistant *S. marcescens* isolates recovered during an outbreak in the ICUs of a Brazilian teaching hospital according to the PFGE data and carbapenemase content.

Diagnostics GmbH, Penzberg, Germany). Conjugation assays were performed as described previously (15). The bla_{KPC-2} gene was inserted into a 100-kb conjugative plasmid in all 24 carbapenem-resistant *S. marcescens* isolates and was successfully transferred to the *Escherichia coli* J53 strain by conjugation, while the bla_{IMP-10} gene was located in a nonconjugative plasmid of approximately 150 kb in all six isolates (Table 2). Numerous attempts to transfer the plasmid harboring bla_{IMP-10} failed.

This study was conducted with the approval of the research ethics committee from the Universidade Federal da Grande Dourados (no. 039439/2012).

Since May 2011, the hospital has observed the dissemination of KPC-2-producing isolates, mainly in *Klebsiella pneumoniae* and *Enterobacter* spp. As part of the control measures, surveillance cultures were obtained weekly from nasal and rectal swabs from ICU patients for detection of methicillin-resistant *Staphylococcus aureus* and carbapenem-resistant *Enterobacteriaceae*, respectively. The carbapenem-resistant *S. marcescens* index strain was isolated from the tracheal aspirate of an ICU patient on 25 September 2011. The following 29 carbapenem-resistant *S. marcescens* isolates were recovered from 23 patients hospitalized in different ICUs during the study period. Among them, 60.9% were from male patients ($n = 14$). The patients' ages ranged from 1 to 85 years, and the median length of their hospital stay was 66 days (range, 15 to 180 days). Prior to the isolation of carbapenem-resistant *S. marcescens*, all patients had received antimicrobial therapy, including penicillins, fluoroquinolones, aminoglycosides, and/or mainly broad-spectrum cephalosporins and carbapenems ($n = 21$; 91.3%), except for a single patient who had received monotherapy with a fluoroquinolone. After the first positive culture, most patients received combination therapy of colistin or polymyxin B combined with an aminoglycoside or ampicillin-sulbactam, based on susceptibility testing. Although tigecycline was the only antimicrobial agent tested with activity against KPC-2 and IMP-10-coproducing strains, the patients infected did not receive this therapy because it was not available in the hospital during the study time. Among the 30 isolates studied, 20 were considered true pathogens and 10 were considered colonizers (Table 1). Colonization was defined as the isolation of strains without clinical manifestation of infection. Clinical infection was defined by a medical diagnosis according to clinical criteria (sepsis, fever, changes in frequency or color of the secretion, or new radiological findings) associated with the decision to initiate antibiotic therapy and the isolation of one strain (16). The patients who were infected by KPC-2 and IMP-10-coproducing *S. marcescens* showed a higher mortality rate (100%) than those infected by isolates that produced KPC-2 alone (22% mortality rate; $P \leq 0.01$).

S. marcescens shows intrinsic resistance to several antimicrobial agents (3, 17) and is capable of acquiring multiple drug resis-

TABLE 2 Antimicrobial susceptibility patterns, genetic similarity, and carbapenemase content of the 6 KPC-2- and IMP-10-producing *S. marcescens* isolates

Strain	PFGE[c]	MIC (mg/liter)[a]														Gene location (kb)[b]	
		CAZ	CTX	CRO	FEP	ATM	IPM	MEM	ETP	AMK	GEN	CIP	LVX	TGC	PMB	bla_{KPC-2}	bla_{IMP-10}
44	A	>256	>256	>256	>256	>32	>8	>16	>32	64	>64	16	8	0.5	>64	P^+ (100)	P^- (150)
1517	A	>256	>256	>256	128	>32	>8	>16	>32	64	32	4	8	0.5	>64	P^+ (100)	P^- (150)
2886	A	>256	>256	>256	>256	>32	>8	>16	>32	64	>64	4	4	0.5	>64	P^+ (100)	P^- (150)
4093	A	>256	>256	>256	>256	>32	>8	>16	>32	64	>64	4	4	0.5	>64	P^+ (100)	P^- (150)
7804	A	128	>256	128	>256	>32	>8	>16	>32	32	2	2	4	0.5	>64	P^+ (100)	P^- (150)
37510	A	>256	>256	>256	>256	>32	>8	>16	>32	64	2	4	4	0.5	>64	P^+ (100)	P^- (150)

[a] CAZ, ceftazidime; CTX, cefotaxime; CRO, ceftriaxone; FEP, cefepime; ATM, aztreonam; IMP, imipenem; MEM, meropenem; ETP, ertapenem; AMK, amikacin; GEN, gentamicin; CIP, ciprofloxacin; LVX, levofloxacin; TGC, tigecycline; PMB, polymyxin B.
[b] P^+, conjugative plasmid; P^-, nonconjugative plasmid.
[c] A, genetic profiles.

tance mechanisms during antimicrobial therapy (1, 2). In Brazil, resistance to carbapenems in *S. marcescens* strains has rarely been reported and is associated exclusively with KPC-2 production (4, 5, 18). Outbreaks of *S. marcescens* infections in ICUs are frequently associated with considerable mortality rates, ranging from 14% to 60% (18, 19). This observation is in accordance with our findings, as the overall mortality rate was 39%. Interestingly, six KPC-2-producing *S. marcescens* isolates described in the present study also produced the metallo-β-lactamase (MBL) IMP-10 and were recovered from five patients over the course of 6 months. These patients were ≥50 years old, were previously hospitalized in the ICU for 30 to 90 days, and displayed several comorbidities. During hospitalization, they were subjected to central venous catheterization, mechanical ventilation, or surgical procedures and received more than five different antibiotics prior to the isolation of *S. marcescens* strains. Therefore, the high mortality rate (100%) among this group of patients could not be attributed solely to the presence of KPC-2 and IMP-10-coproducing *S. marcescens* isolates but could also be related to unfavorable clinical conditions. All patients infected by an *S. marcescens* strain producing both KPC-2 and IMP-10 were previously hospitalized in another hospital. The first patient was admitted to our hospital and remained colonized by IMP-10- and KPC-2-producing *S. marcescens* for 6 months, demonstrating the ability of this strain to persist. The clinical evidence suggests that this clonal strain was introduced into our hospital by colonized patients who previously had been hospitalized elsewhere. This observation is highly indicative of former dissemination and ineffective detection and isolation of such strains. Following the initial detection and characterization of this clone, infection control measures were reinforced and rectal surveillance swabs were collected from all patients in the standard care wards and ICUs. No new KPC-2 and/or IMP-10-coproducing strains have been found so far.

Both carbapenemase-encoding genes were found to be located in two different plasmids. Although the bla_{KPC-2} gene was transferred to the recipient *E. coli* J53 strain, the bla_{IMP-10} gene was not, a finding that differed from that observed by Hu and Zhao (20). This finding may explain why the bla_{IMP-10} gene was identified in only 6 out of 24 KPC-2-producing *S. marcescens* isolates.

In conclusion, to our knowledge, we report for the first time the production of IMP-10 in Brazil as well as the coproduction of KPC-2 and IMP-10 in *S. marcescens* isolates causing an outbreak associated with high mortality in ICUs at a Brazilian teaching hospital. The production of both carbapenemases by the same strain is a matter of great concern, since this pathogen exhibits intrinsic resistance to polymyxins, and carbapenems are often the drugs of the last resort for treatment. Our findings highlight the urgent need for development of efficacious strategies for the prevention and control of multidrug-resistant Gram-negative bacilli.

Nucleotide sequence accession number. The sequencing analysis of a new class 1 integron cassette arrangement, named In990, was submitted to GenBank under accession number KP177456.

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