



Short Communication

In vivo horizontal dissemination of the *bla*_{KPC-2} gene carried on diverse genetic platforms among clinical isolates of Enterobacteriaceae

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ABSTRACT

This study investigated the molecular characteristics of six *bla*_{KPC-2}-positive Enterobacteriaceae recovered from three patients in Argentina. Antimicrobial susceptibility testing was performed following Clinical and Laboratory Standards Institute (CLSI) 2014 recommendations. Molecular characterisation of the isolates was performed by biparental conjugation, PCR, sequencing, S1 nuclease restriction, and Southern blot hybridisation with a *bla*_{KPC} probe using standard protocols and conditions. The isolates studied were as follows. Case 1: *Escherichia coli* (ECO-P1) and *Klebsiella pneumoniae* (KPN-P1) isolated from a rectal swab harboured *bla*_{KPC-2} in transposon Tn4401a on non-typeable and non-conjugative plasmids. Case 2: *Enterobacter cloacae* (ECL-P2) and *K. pneumoniae* (KPN-P2) were isolated from two blood cultures. *bla*_{KPC-2} was found in a novel genetic variant of ISKpn8-*bla*_{KPC-2}-ISKpn6-like on conjugative plasmids of IncL/M type. Case 3, *Citrobacter freundii* (CFR-P3) and *Klebsiella oxytoca* (KOX-P3) were isolated from skin and skin-structure infection. The *bla*_{KPC} gene was detected on ISKpn8- Δ *bla*_{TEM}-*bla*_{KPC-2}-ISKpn6-like located on an IncA/C conjugative plasmid. CFR-P3 and KOX-P3 harboured *bla*_{PER-2} in addition to the *bla*_{KPC} gene. In conclusion, we document the horizontal dissemination of *bla*_{KPC-2} from diverse Enterobacteriaceae clinical isolates with different genetic backgrounds. This is the first report of *E. coli* harbouring *bla*_{KPC} associated with Tn4401a in Argentina.

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1. Introduction

Genes encoding KPC enzymes are predominantly identified among *Klebsiella pneumoniae* isolates and increasingly among other Enterobacteriaceae and non-fermenters. The global predominance of KPC-producing bacteria has been mainly associated with the successful and hyperepidemic clone of *K. pneumoniae* sequence type (ST) 258 and single and double-locus variants such as ST11 [1]. *bla*_{KPC} genes are typically transposon-encoded (Tn4401 and its variants) and therefore have the potential to disseminate between plasmids and across bacterial species [2]. The spread of *bla*_{KPC} by horizontal genetic transfer has been previously documented in a

simultaneous blood infection [3], in colonised patients possessing KPC-3, Tn4401a and pKpQIL-IT elements [4] and through the transmission of a promiscuous plasmid carrying the KPC gene in Tn4401a [5].

In Argentina, health institutions throughout the country refer clinical strains for characterisation to the National Reference Laboratory in Antimicrobial Resistance (LNRAR) following local guidelines. In this context, six Enterobacteriaceae isolates recovered from three patients with phenotypes indicative of KPC production were sent to LNRAR for confirmation and molecular characterisation.

2. Methods

2.1. Patients and clinical isolates

The epidemiological data and clinical characteristics of the patients are shown in Table 1. Patients 1, 2 and 3 were hospitalised

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Table 1Epidemiological and clinical characteristics of patients infected or colonised with *bla*_{KPC}-positive isolates.

Patient	Isolate	Isolation date	Age (years)/sex	Hospital (city)	Reason for admission	Underlying conditions	KPC-positive specimen	LOS (days)	Antibiotic treatment (during hospital stay)	Outcome
1	ECO-P1 KPN-P1	24 Oct. 2010 25 Oct. 2010	94/F	Clínica y Maternidad Suizo Argentina (Buenos Aires)	Colostomy	Colon cancer	Rectal swab	45	UKN	UKN
2	ECL-P2 KPN-P2	14 March 2011 21 March 2011	87/M	Sanatorio Dupuytren (Buenos Aires)	UKN	Neurological patient with MV	Blood	>12	COL, VAN ^a	UKN
3	CFR-P3 KOX-P3	4 April 2012	52/M	Hospital Municipal de Urgencias (Córdoba)	Wound	DM, DVT, CD, bypass, supracondylar amputation	Wound	26	FLZ ^a , CAZ, VAN ^a , CLI ^a , CIP, SXT+TZP	Died

LOS, length of stay; ECO, *Escherichia coli*; KPN, *Klebsiella pneumoniae*; ECL, *Enterobacter cloacae*; CFR, *Citrobacter freundii*; KOX, *Klebsiella oxytoca*; UKN, unknown; MV, mechanical ventilation; DM, diabetes mellitus; DVT, deep vein thrombosis; CD, cardiovascular disease; COL, colistin; VAN, vancomycin; FLZ, fluconazole; CAZ, ceftazidime; CLI, clindamycin; CIP, ciprofloxacin; SXT, trimethoprim/sulfamethoxazole; TZP, piperacillin/tazobactam.

^a Chemotherapy used to treat fungal infections or Gram-positive bacteria.

in 2010, 2011 and 2012, respectively, in different institutions in two cities in Argentina. The isolates belonged to five species, including *Escherichia coli* ($n = 1$), *K. pneumoniae* ($n = 2$), *Enterobacter cloacae* ($n = 1$), *Klebsiella oxytoca* ($n = 1$) and *Citrobacter freundii* ($n = 1$) (Table 1).

2.2. Susceptibility testing and detection of resistance genes

Minimum inhibitory concentrations (MICs) to imipenem, meropenem, ertapenem, cefepime, cefotaxime, ceftazidime, gentamicin, amikacin, ciprofloxacin, minocycline, colistin, fosfomycin and tigecycline were determined by agar dilution using standard methods according to Clinical and Laboratory Standards Institute (CLSI) criteria [6]. MIC interpretation was according to CLSI guidelines (Table 2A in CLSI document M100-S24 [7]) for all antimicrobials except for fosfomycin (endovenous), colistin and tigecycline, which were interpreted following European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations. KPC production was detected phenotypically considering imipenem inhibition zones ≤ 22 mm and positive synergy between carbapenem disks and 3-aminophenyl-boronic acid (APB) and negative synergy between carbapenem disks and ethylene diamine tetra-acetic acid/sodium mercaptoacetic acid (EDTA/SMA) [8,9]. Extended-spectrum β -lactamase (ESBL) production was suspected phenotypically by positive synergy with clavulanic acid. Locally prevalent ESBL genes were confirmed by specific PCRs using standard conditions (CTX-MU1, 5'-ATGTGC AGYACCAGTAARGT-3'; CTX-MU2, 5'-TGGGTRAARTARGTSACCAGA-3'; PER-2F, 5'-GTAG-TATCAGCCCAATCCCC-3'; PER-2R, 5'-CCAATAAAGGCCGTCCAT CA-3'). All resistance genes and genetic contexts were sequenced using the above primers as well as others previously reported using BigDyeTM Terminator methodology [10].

2.3. Conjugation experiments

Transfer of KPC-harboring elements was tested by biparental conjugation with *E. coli* J53 (sodium azide-resistant) on Luria–Bertani (LB) agar plates in a 1:3 donor/recipient ratio. Transconjugants were selected on LB agar plates with sodium azide (150 μ g/mL) and ampicillin (50 μ g/mL) [10].

2.4. Genetic typing

For pulsed-field gel electrophoresis (PFGE), total DNA was digested with *Xba*I and the fragments were separated using 2.2 s and 54.2 s as initial and final pulse times over 20 h. The running

was performed using a CHEF-DR[®] III Apparatus (Bio-Rad, Hercules, CA) and the gel was recorded and DNA patterns were analysed according to the criteria of Tenover et al. [10,11].

2.5. Plasmid analysis

Plasmid extraction was performed using a QIAGEN Midi Kit (QIAGEN, Hilden, Germany). Plasmids were classified by PCR-based replicon typing (PBRT) and S1 nuclease digestion of total DNA [12,13] and were analysed by Southern blot and hybridisation of the PFGE gels using a *bla*_{KPC-2}-specific probe (PCR DIG Probe Labeling Mix; Roche Applied Science, Barcelona, Spain).

2.6. Nucleotide sequence accession number

A 1554-bp sequence including the *bla*_{KPC-2} gene corresponding to isolate ECL-13354 (ECL-P2) and KPN-13355 (KPN-P2) has been submitted to the GenBank nucleotide sequence database under accession nos. KR108242 and KR108243, respectively.

3. Results and discussion

Epidemiological data for the patients is shown in Table 1. The three patients had prolonged hospitalisation and serious underlying conditions. Patients 2 and 3 had had previous antibiotic treatment due to multiple nosocomial bacterial and fungal infections. Isolate recovery was 1 day and 7 days apart for Cases 1 and 2, respectively, and simultaneously in Case 3.

The susceptibility patterns of all isolates are shown in Table 2. Susceptibility to carbapenems was variable, from fully susceptible to resistant, whilst the isolates were highly resistant to expanded-spectrum β -lactams. Resistance to aminoglycosides and fluoroquinolones was also variable, whereas colistin and fosfomycin remained susceptible. All studied isolates harboured *bla*_{KPC-2}, and the only ESBL detected was *bla*_{PER-2} in isolates from Patient 3 (Table 2).

The *K. pneumoniae* isolates from Patients 1 and 2 were not clonally related (>6 band difference); however, the isolate recovered from Patient 1 was genetically related to *K. pneumoniae* ST258 (data not shown).

Transconjugants were obtained for the isolates from Patient 2 (TC-ECL-P2 and TC-KPN-P2) and Patient 3 (TC-CFR-P3 and TC-KOX-P3). All transconjugants were shown by PCR to contain *bla*_{KPC-2}. Carbapenem MICs of transconjugants obtained from Patient 2 were at least three times higher than those of the recipient cells (*E. coli* J53) (Table 2). Moreover, *bla*_{PER-2} was

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