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Changing epidemiology of KPC-producing *Klebsiella pneumoniae* in Argentina: Emergence of hypermucoviscous ST25 and high-risk clone ST307

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ABSTRACT

Objectives: To assess the epidemiological features of 76 *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae* (KPC-Kp) isolates recovered from three hospitals in Buenos Aires, Argentina, during 2015–2017.

Methods: Antimicrobial susceptibilities were determined according to CLSI Clinical and Laboratoy Standards guidelines. Molecular typing of KPC-Kp was performed by pulsed-field gel electrophoresis (PFGE)-XbaI and multilocus sequence typing. Plasmid encoded genes involved in carbapenem, fosfomycin and colistin resistance were detected by polymerase chain reaction (PCR) and sequencing. Also, *mgrB* inactivation was investigated in those colistin-resistant isolates. Genetic platforms involved in horizontal spread of bla_{KPC} were investigated by PCR mapping.

Results: Besides β-lactams, high resistance rates were observed for gentamycin, quinolones and trimethoprim-sulfamethoxazole. KPC-Kp sequence type (ST)258 corresponded to 26% of the isolates, while 42% corresponded to ST25. The other isolates were distributed in a diversity of lineages such as ST11 (10.5%), ST392 (10.5%), ST307, ST13, ST101, ST15 and ST551. bla_{KPC-2} was detected in 75 of 76 isolates, and one ST307 isolate harboured bla_{KPC-3}. Tn*4401* was identified as the genetic platform for bla_{KPC} in epidemic lineages such as ST258 and ST307. However, in ST25 and ST392, which are usually not related to bla_{KPC}, a bla_{KPC}-bearing non-Tn*4401* element was identified. Alterations in *mgrB* were detected in seven of 11 colistin-resistant isolates.

Conclusions: Despite previous reports in Argentina, ST258 is no longer the absolute clone among KPC-Kp isolates. In the present study, dissemination of more virulent lineages such as the hypermucoviscous ST25 was detected. The emergence of the high-risk clone ST307 and occurrence of bla_{KPC-3} was noticed for the first time in this region.

1. Introduction

Carbapenem-resistant isolates, mainly those producing *Klebsiella pneumoniae* (*K. pneumoniae*) carbapenemase (KPC), are globally spread and associated with high morbidity and mortality rates [1]. KPC-producing *K. pneumoniae* (KPC-Kp) global expansion has been

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associated with the dissemination of epidemic clones, especially the dominant strain *K. pneumoniae* sequence type (ST) 258 and related strains of clonal complex (CC)258 [2]. This ST is a hybrid clone derived from large recombination events between ST11 and ST442 [3–5]. This clone emerged in Argentina in 2010 and became the prevalent ST; it achieved epidemic status in many settings [6,7]. CC258 includes multidrug-resistant microorganisms displaying resistance to all β-lactams, aminoglycosides, quinolones, trimethoprim and sulfonamides [2]. Singularly, ST258 is reported to be mildly virulent. The high mortality rates attributed to this clone may be explained, at least in part, by the low efficacy of the antimicrobials used against KPC-Kp infections and the severity of the underlying conditions of the patients [3]. It is highly susceptible to serum killing and phagocytosis, and lacks typical *K. pneumoniae* virulence factors such as aerobactin, K1, K2 and K5 capsular genes, and the regulator gene of the mucoid phenotype, *rmpA* [5,8]. However, in recent years, new strains of KPC-Kp have emerged internationally, which correspond to hypervirulent clones that have acquired extensively drug resistance markers [9,10].

Transposon Tn*4401* constitutes the most frequent genetic context of bla_{KPC}, and is considered the origin of acquisition and dissemination of this marker. Tn*4401* is approximately 10 kb in size and delimited by two 39-bp imperfect inverted repeat sequences. It harbours two insertion sequences flanking bla_{KPC}, IS*Kpn6* and IS*Kpn7*, in addition to transposase and resolvase genes [5,11]. Moreover, bla_{KPC}-bearing non-Tn*4401* elements have been recognised, including the integration of a Tn*3*-based transposon and a partial Tn*4401* structure (ORF order: Tn*3*-transposase, Tn*3*-resolvase, IS*Kpn8*, bla_{KPC} and IS*Kpn6*). Variants in the upstream bla_{KPC} region, lacking the Tn*3*-transposase and its resolvase and displaying a partial fragment of bla_{TEM}, have been reported [5,12].

The aim of this study was to assess the epidemiological features of KPC-Kp in order to understand the ongoing evolution of carbapenem resistance in the current region.

2. Material and methods

2.1. Bacterial isolates and antimicrobial susceptibility testing

This study included 76 non-repetitive *K. pneumoniae* isolates that were positive in the double-disk synergy test using phenyl boronic acid (300 μg) and carbapenem containing disks. These isolates were recovered from inpatients at three hospitals in Buenos Aires during 2015–2017. A total of 374 *K. pneumoniae* isolates were recovered from inpatients during the studied period: 81 (22%) were carbapenem resistant and 76 (20%) were KPC producers. These resistant isolates were delivered to the Laboratorio de Resistencia Bacteriana in an encrypted way (to preserve the identity of the patients).

Susceptibilities to ampicillin, ampicillin-sulbactam, piperacillin-tazobactam, ceftazime, ceftriaxone, imipenem, meropenem, gentamycin, amikacin, ciprofloxacin, levofloxacin and trimethoprim-sulfamethoxazole were assessed by automated systems. Colistin and tigecycline minimum inhibitory concentrations (MICs) were determined by broth microdilution method, and the agar dilution method was performed to determine fosfomycin susceptibility. Antimicrobial susceptibilities were interpreted using the Clinical and Laboratory Standards Institute (CLSI) breakpoints, except for colistin and tigecycline where European Committee on Antimicrobial Susceptibility testing (EUCAST) and U.S. Food and Drug Administration (FDA) breakpoints were used, respectively [13].

2.2. Molecular detection of antimicrobial resistance genes

The presence of the most prevalent carbapenemase genes (bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{NDM} and bla_{OXA-48}-like) was investigated by multiplex-polymerase chain reaction (PCR) using specific primers [14]. Plasmidic *mcr-1* and *mcr-2*, and *fosA3*, *fosA4* and *fosC2* were screened by PCR in those isolates, which displayed resistance to colistin and fosfomycin, respectively [15,16]. Colistin resistance mediated by *mgrB* inactivation was studied according to Cannatelli et al. [17]. Complete bla_{KPC} was amplified as previously described [18]. All amplicons were sequenced at external facilities (Macrogen Inc., Seoul, Korea) and analysed using the BLAST program at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST).

2.3. Molecular typing of K. pneumoniae isolates harbouring bla_{KPC}

Clonal relationship was analysed, for the 76 isolates, by pulsed-field gel electrophoresis (PFGE) after digestion of genomic DNA with XbaI and interpreted according to van Belkum et al. [19,20]. Multilocus sequence typing analysis was performed in representative isolates of each different pulsotype. Sequence types were assigned amplifying and sequencing the following seven housekeeping genes: *gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB*, *tonB* in accordance with http://bigsdb.web.pasteur.fr/klebsiella/.

2.4. Analysis of genetic platforms carrying bla_{KPC-2}

Natural plasmids were extracted using the Kado and Liu extraction method [21]. The genetic context of bla_{KPC} was studied by a PCR mapping approach in order to detect the Tn*4401* structure and bla_{KPC}-bearing non- Tn*4401* elements, as previously described [11,12].

3. Results

Clinical features of KPC-Kp isolates and main demographic data are shown in Table 1. The incidence rate of KPC-Kp was ca. 7.3 per 1000 discharges in these hospitals. Most of the 76 isolates were recovered from urine and blood samples. Patients' ages ranged from 20–94 years (median age: 72 years and 70 years for females and males, respectively). The male to female ratio was 1.76. Although all patients presented underlying conditions, cancer and

Table 1
Sites of isolation of KPC-*Klebsiella pneumoniae* isolates and clinical features of patients.

Total number of isolates: 76		n
Isolation sites	Urine	31
	Blood	20
	Lower respiratory tract	8
	Rectal swab	8
	Skin and soft tissue	3
	Abdominal	3
	Cerebrospinal	2
	Stool	1
Total number of inpatients: 69		n
Underlying diseases[a]	Gastrointestinal	3
	Oncology	15
	Cardiovascular	11
	Kidney transplant	11
	Lung	8
	Urinary tract	15
	Traumatism	3
	Diabetes	4
	Other	6
Wards	Intensive care unit	30
	Medical wards	39
Prior use of antibiotics	Yes	60
	No	9
Female	(n)/Age median (range)	25/72 (40–94)
Male		44/70 (20–93)

[a] Some patients presented more than one underlying disease.

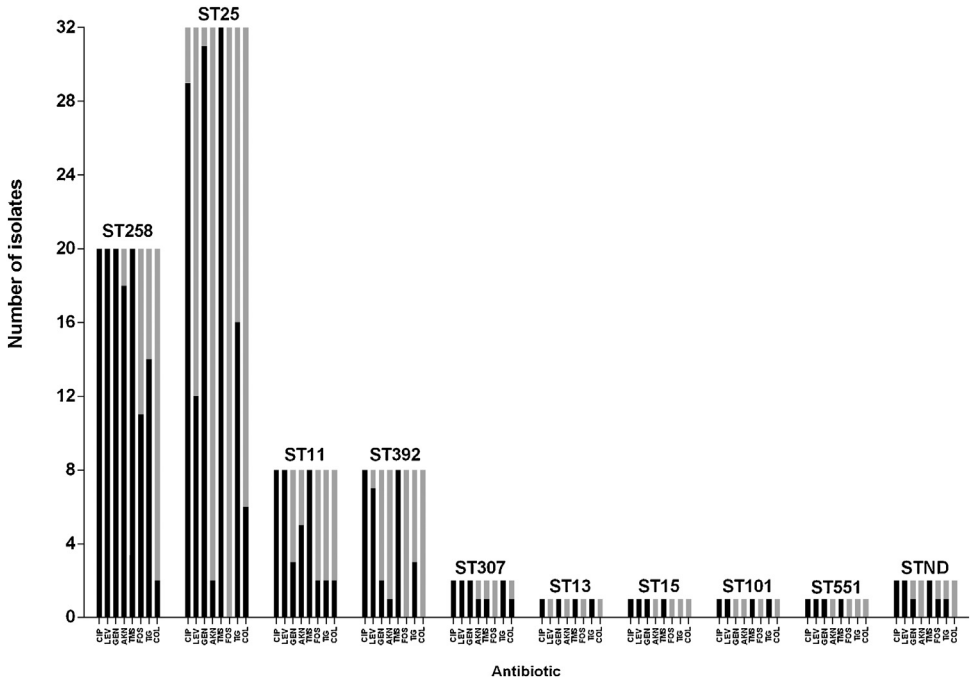

Fig. 1. Accompanying resistance in KPC-producing *K. pneumoniae* isolates.
In each column, resistant isolates are shown in black and susceptible ones in grey.
Abbreviations: ND, sequence type not determined; CIP, ciprofloxacin; LEV, levofloxacin; GEN, gentamycin; AKN, amikacin; TMS, trimethoprim-sulfamethoxazole; FOS, fosfomycin; TIG, tigecycline; COL, colistin.

urinary tract diseases were the most common primary cause; 43.5% of them were hospitalised in intensive care units and 87% had received previous antibiotic treatment (Table 1).

High resistance rates were detected for β-lactams, gentamycin, quinolones and trimethoprim-sulfamethoxazole (TMS) (Fig. 1). All isolates were resistant to ampicillin, ampicillin-sulbactam, ceftazidime, ceftriaxone, eight of 76 were intermediate and 68 of 76 were resistant to imipenem, while four of 76 and 72 of 76 isolates were intermediate and resistant to meropenem, respectively. The bla_{KPC-2} carbapenemase gene was detected in 75 isolates, while bla_{KPC-3} was harboured in the remaining. Eleven of 76 (15%) isolates were resistant to colistin, while 14 (18%) were resistant to fosfomycin. The plasmidic resistance genes for colistin and fosfomycin were not detected. Alterations in *mgrB* locus were identified in seven of 11 colistin-resistant isolates: four displayed Δ*mgrB* and three exhibited mutations that were not previously described. Two isolates presented g85t mutation (D29Y), and the remaining presented g59a, rendering a premature stop (Table 2).

The PFGE-XbaI analysis showed the presence of 18 different pulsotypes that were related to different STs, corresponding to: ST25 (n:32), ST258 (n:20), ST11 (n:8), ST392 (n:8), ST307 (n:2), ST13 (n:1), ST101 (n:1), ST15 (n:1) and ST551 (n:1). Those isolates that belonged to ST258, ST307, ST101 and four of eight ST11 presented the complete Tn4401, while those isolates that corresponded to ST25, ST392, ST13, ST15, ST551, and one of eight ST11 presented IS*Kpn8* upstream bla_{KPC}, as part of Tn3 (Tn3-IS*Kpn8*), and IS*Kpn6* downstream (Fig. 2). Presence of Δbla_{TEM} upstream to bla_{KPC} was not detected in any isolate.

4. Discussion

Even if previous studies performed in Argentina showed that KPC-Kp dissemination corresponded to the propagation of a unique clone, ST258 [6,7], CC258 currently accounts for 37% of the isolates: ST258 (26%), ST11 (11%). New lineages of KPC-Kp were detected, and among them, a high proportion of isolates (42%) belonged to the emerging ST25, which is a virulent and hypermucoviscous clone that presents the capsular serotype K2. Interestingly, almost all ST25 isolates displayed susceptibility to amikacin. Reports on this ST from clinical samples are scarce: in China, Yao et al. reported one clinical isolate of *K. pneumoniae* ST25 recovered from a tracheal secretion, but this one was susceptible to carbapenems [10]. Also, carbapenem-susceptible *K. pneumoniae* ST25 has been described as a cause of septicaemia in neonate pigs (in England and Australia) being responsible for a total of 19 outbreaks in 16 piggeries and two outbreaks in two pig farms, respectively [22]. One NDM-1 producing *K. pneumoniae* ST25 isolate was recovered in Serbia in 2011 and five KPC-2-producing *K. pneumoniae* ST25 isolates, causing invasive human infections, were previously described in Ecuador in 2016 [23,24]. Furthermore, 10.5% of the isolates corresponded to ST392, a lineage related to the dissemination of OXA-48 in Europe. Even though the emergence and spread of KPC-2-Kp ST392 was described in 2013 (in China), this strain has been sporadically reported [25]. More recently, in 2017, KPC-3-Kp ST392 was reported in Italy [26].

The remaining isolates were distributed in a diversity of lineages as ST13, ST15, ST101, ST551 and ST307. Singularly KPC-Kp ST307 is recognised as a high-risk clone, which is increasingly being documented in several countries and considered as a candidate to become the worldwide epidemic clone [27–31]. Compared with the low virulent ST258, capsulated ST307 isolates show higher resistance to complement-mediated killing. Furthermore, the ST307 clone harbours a cluster for glycogen synthesis, which could provide an advantage, allowing long-term survival and growth in environments outside the host [31]. This high-risk clone has not been previously recognised in Argentina. The two isolates that belong to this ST were recovered from different hospitals in 2017 and presented different non-β-lactam susceptibility profiles. One of them presented bla_{KPC-2}, and was susceptible to colistin and resistant to TMS. The other one harboured bla_{KPC-3}, and was resistant to colistin and susceptible to TMS. This bla_{KPC} allele has not been previously described in Argentina, it is worth highlighting that mutations in

Table 2
Molecular characterisation of KPC-producing *K. pneumoniae* isolates.

ST (%)	PFGE type	Total No. of isolates	bla_{KPC}	bla_{KPC} genetic context	Colistin resistance	
					No. of resistant isolates	*mgrB* status
258 (26%)	A	20	bla_{KPC-2}	Tn*4401*	2	Δ*mgrB* locus (n:2)
25 (42%)	B	32	bla_{KPC-2}	Tn*3*-IS*Kpn8*	6	g85t (D29Y) (n:2) WT (n:3) Δ*mgrB* locus (n:1)
11 (10.5%)	C_1 C_2 C_3 C_4 C_5	1 1 1 4 1	bla_{KPC-2}	Tn*4401*, Tn*3*-IS*Kpn8* and unknown	2	Δ*mgrB* locus (n:1) WT (n:1)
392 (10.5%)	D_1 D_2 D_3 D_4	5 1 1 1	bla_{KPC-2}	Tn*3*-IS*Kpn8*	0	–
307 (2.6%)	E	2	bla_{KPC-2} bla_{KPC-3}	Tn*4401*	1	g59a (nonsense, premature termination) (n:1)
13 (1.3%)	F	1	bla_{KPC-2}	Tn*3*-IS*Kpn8*	0	–
15 (1.3%)	G	1	bla_{KPC-2}	Tn*3*-IS*Kpn8*	0	–
101 (1.3%)	H	1	bla_{KPC-2}	Tn*4401*	0	–
551 (1.3%)	I	1	bla_{KPC-2}	Tn*3*-IS*Kpn8*	0	–
ND (2.6%)	J K	1 1	bla_{KPC-2}	Tn*3*-IS*Kpn8* Tn*4401*	0	–

Abbreviations: WT, Wild type; ND, not determined; PFGE, pulsed-field gel electrophoresis; ST, sequence type.

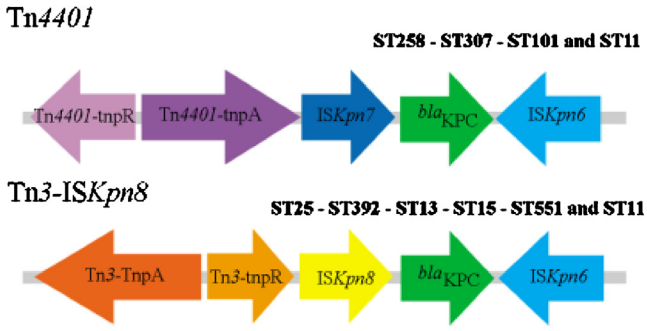

Fig. 2. Genetic contexts of bla_{KPC}.

bla_{KPC-3} can conduce to KPC-3 variants with significantly reduced ceftazidime-avibactam susceptibility [32].

Two main bla_{KPC} genetic platforms were identified. Except for ST11, each context could be associated with a particular ST. In accordance with the literature, the well-established Tn*4401* was identified in those lineages recognised as epidemics (ST258 and ST307), while Tn*3*-IS*Kpn8* was detected in those STs not usually related to bla_{KPC}, as ST25 and ST392 [4].

The colistin plasmid-encoded mechanisms do not seem to have an epidemiological impact in *K. pneumoniae*. Although, in this study, mutations in *mgrB* locus were detected in many of the colistin-resistant isolates, changes in other loci involved in upregulation of the Pmr system, which is responsible for modification of the lipopolysaccharide polymyxin target, could be also present [17].

5. Conclusions

This study describes the changing epidemiology of KPC-Kp lineages in clinical settings. The almost absolute prevalence of ST258 in the current region is being replaced by the dissemination of more virulent lineages such as ST25 and ST11, and the worrisome emergence of the high-risk clone ST307. It reports, for the first time in Argentina, the detection of ST307-bla_{KPC-3}-*K. pneumoniae*. The dichotomy between carbapenem-resistant *K. pneumoniae* and hypervirulent *K. pneumoniae* has been blurred by the emergence of carbapenem-resistant hypervirulent clones; their dissemination may mark an evolutionary step toward their establishment as major nosocomial pathogens.

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Competing interests

None.

Ethical approval

Not required.

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