Therefore, comparative genomic analysis of different plasmids carrying $bla_{CTX\text{-}M\text{-}2}$ might be useful to fully understand their evolution, plasticity and spread. While $bla_{CTX\text{-}M\text{-}2}$ has frequently been detected in human pathogens,[1] this is the first report of $bla_{CTX\text{-}M\text{-}2}$-producing *E. coli* isolated from diseased animals and, more specifically, horses. The emergence of $bla_{CTX\text{-}M\text{-}2}$ is therefore a clear cause for concern.

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Transparency declarations

None to declare.

Supplementary data

Table S1 and Figure S1 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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Detection of an international multiresistant clone belonging to sequence type 654 involved in the dissemination of KPC-producing *Pseudomonas aeruginosa* in Argentina

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Sir,

The emergence of *Klebsiella pneumoniae* carbapenemase (KPC) has now become a global concern. KPC producers are mostly Enterobacteriaceae, but *Pseudomonas aeruginosa* have also been reported and mostly identified in the American continent.[1–4] However, it is unknown if this is due to the spread of epidemic strains, since the multilocus sequence type (ST) has not been provided in most of those reports. The aim of this work was to characterize KPC-producing *P. aeruginosa* isolated in Argentina from 2006 to June 2011.

Since 2005, we designed an algorithm to detect carbapenemases (metallo-β-lactamases, KPC, etc.) in *P. aeruginosa* at the level of the clinical microbiology laboratory, which was implemented among 432 hospitals. All *P. aeruginosa* were screened through that algorithm, and KPC production was suspected in isolates with high-level resistance to carbapenems and aztreonam (absence of disc zones) and a negative synergy test result between the carbapenems and EDTA, a phenotype consistent with the reported patterns of KPC-producing *P. aeruginosa*.[1,4]

As a result, 65 isolates were suspected to be KPC producers (Table 1). Strains were isolated from nine hospitals (seven cities, five provinces), six of which were located in the Patagonia region. The first KPC producer was detected in a hospital from Bariloche in 2006. Dissemination to other locations (except

Table 1. Epidemiological and molecular characteristics of KPC-positive *P. aeruginosa* isolates

Year	City, Province	Hospital(s)[a]	No. of isolates suspected of KPC/total no. of *P. aeruginosa* isolates (%)[b]	No. of isolates submitted to the INEI	PFGE pattern (ST)	Susceptibility to non-β-lactam agents[c]
Pre-epidemic period						
2005[d]	Argentina (24 provinces)	WHONET AR	0/8846 (0)	0	NA[e]	NA
Epidemic period						
2006	Bariloche, Rio Negro	SSC	3/28 (11)	2	A (654)	S: CST, AMK, GEN
2008	Bariloche, Rio Negro	SSC, HPR, BAR	16/26 (62)	7	A	S: CST, AMK, GEN
	Lago Puelo, Chubut	HZLP	1[f]/2	1	A	S: CST, AMK, GEN
2009	Bariloche, Rio Negro	BAR, HPR	12/48 (25)	4	A	S: CST, AMK, GEN
	General Roca, Rio Negro	CGR	3[f]/43 (7)	1	A	S: CST, AMK, GEN
	Esquel, Chubut	HZE	2[f]/26 (8)	2	A	S: CST, AMK, GEN
	Córdoba, Córdoba	HPC	1[f]/27 (4)	1	A	S: CST, AMK, GEN
2010	Bariloche, Rio Negro	BAR, HPR	9/29 (31)	4	A	S: CST, AMK, GEN (3) S: CST (1)
	General Roca, Rio Negro	CGR	3/54 (6)	1	A	S: CST, AMK, GEN
	Esquel, Chubut	HZE	1/22 (4)	1	A	S: CST, AMK, GEN
	Ciudad de Buenos Aires	UDA	1/not available	1	A	S: CST, AMK, GEN
	Resistencia, Chaco	HJP	1/167 (0.6)	1	B (162)	S: CST, AMK, GEN, CIP
2011[g]	Bariloche, Rio Negro	SSC	1/6 (17)	1	A	S: CST, AMK, GEN
	General Roca, Rio Negro	CGR	11/36 (31)	3	A	S: CST, AMK, GEN
total	7 cities/5 Provinces	9 hospitals	65/514 (12)	30	A (654), B (162)	S: CST 100%, AMK 97%, GEN 97%, CIP 3%

INEI, Instituto Nacional de Enfermedades Infecciosas.
[a]WHONET AR, WHONET Argentina Network (90 Hospitals, 24 Provinces); SSC, Sanatorio San Carlos; HPR, Hospital Privado Regional del Sur y Sanatorio del Sol; BAR, Hospital Zonal de Bariloche; HZLP, Hospital Zonal de Lago Puelo; CGR, Laboratorio Roca, Clínica Roca; HZE, Hospital Zonal de Esquel; HPC, Hospital Privado Centro Modelo de Córdoba; UDA, Hospital Municipal de Gastroenterologia Udaondo; HJP, Hospital Julio Perrando.
[b]Isolates suspected of producing KPC defined with the phenotypic algorithm indicated in the text. The denominator represents the total *P. aeruginosa* isolates recovered in the indicated hospital(s) of the corresponding row.
[c]As defined by agar dilution according to CLSI. S, susceptible; CST, colistin; AMK, amikacin; GEN, gentamicin; CIP, ciprofloxacin. The number of isolates is shown in parentheses.
[d]Baseline obtained from the WHONET Argentina Network.
[e]NA, not applicable.
[f]The respective index patients of these locations were previously hospitalized in Bariloche.
[g]January–June 2011.

Chaco) was associated with patients previously hospitalized in Bariloche (Table 1).

A subset ($n=30$) of suspected isolates was submitted to the National Reference Laboratory (Malbrán Institute), where bla_{KPC-2} was confirmed in all strains by PCR/sequencing. XbaI PFGE analysis, using previously described criteria,[5] revealed that all but one of the isolates belonged to a single pulsotype (Table 1). In Argentina, these PFGE patterns had not previously been observed. Multilocus sequence typing of one strain of each PFGE type revealed that the dominant clone (PFGE type A) belonged to ST654, while the unique strain of PFGE type B (Chaco), belonged to ST162 (http://pubmlst.org/paeruginosa) (Table 1).

To investigate the genetic organization of bla_{KPC-2} in *P. aeruginosa*, we used PCR primer pairs located in the Tn*4401* structure and in the flanking sequences found in Enterobacteriaceae with KPC in our country, called Variant 1a (IS*Kpn8* and IS*Kpn6-like*).[6] All isolates belonging to ST654 harboured bla_{KPC-2} in Tn*4401b*, which was confirmed by sequencing. The ST162 isolate harboured bla_{KPC-2} in Variant 1a.[6] By plasmid content analysis and Southern blotting with a bla_{KPC-2} probe,[6] we found that ST654 contains bla_{KPC} in a plasmid of 50 kb, while the strain ST162 contains several plasmids but only one of 47 kb hybridized with KPC. Plasmid incompatibility (Inc) groups were further analysed using the PCR-based replicon-typing protocol described for Enterobacteriaceae.[7] Both ST162 and ST654 gave negative results for all the Inc groups tested.

The strains were highly resistant (MICs >128 mg/L) to aztreonam, cefepime, imipenem, meropenem and piperacillin/tazobactam by agar dilution. Colistin (MICs 1 mg/L), gentamicin (MIC_{90}s 2 mg/L) and amikacin (MIC_{90}s 4 mg/L) were the most active drugs. ST162 was susceptible to ciprofloxacin (MIC 0.5 mg/L) (Table 1).

The origin of bla_{KPC-2}-possessing ST654 remains uncertain. The mobilization of bla_{KPC} from Enterobacteriaceae was ruled

out: first, these strains were not detected in Bariloche until 2010 and, second, they harboured bla_{KPC-2} in the Variant 1a.[6] ST162 was isolated from a patient with no recent history of travel. Interestingly, this patient shared the same ward simultaneously with two other patients undergoing infections due to KPC-producing *Enterobacter cloacae* and *K. pneumoniae*. In these isolates, the bla_{KPC-2} genetic environment matched that of ST162. However, bla_{KPC-2} in *E. cloacae* and *K. pneumoniae* was associated with plasmids of different sizes (40 and >150 kb, respectively) and different Inc groups (N and FIIS, respectively)[6] from that detected in ST162. Thus, we speculated that the surge of ST162 could be due to bla_{KPC-2} mobilization among different plasmids (i.e. transposition).

Clonal complexes CC111 and CC235 have been reported as the major clones involved in the global dissemination of extended-spectrum and metallo-β-lactamases in *P. aeruginosa*.[8] However, we observed that the main dissemination of KPC was mediated by a new clone (ST654) not related to both CC111 and CC235. ST654 is endemic in Singapore,[9] where it has been associated with IMP-1 and IMP-26; it has also been reported in Sweden as a VIM-2 producer, although the isolate was imported from Tunisia.[10] Unlike other Latin American regions, such as Puerto Rico and Colombia, where different PFGE types and STs have been identified,[1,4] the dissemination of KPC-producing *P. aeruginosa* in Argentina is mainly associated with a single clone. These findings confirm that ST654 plays an important role in the global spread of carbapenemases, either metallo-β-lactamases or KPC. Thus, the worldwide dissemination of KPC-producing *P. aeruginosa* of ST654 might be expected and should be monitored.

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Transparency declarations

None to declare.

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In vitro activity of tigecycline against multidrug-resistant Gram-negative blood culture isolates from critically ill patients

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Sir,

With increasing resistance to currently available antibiotics and decreasing numbers of newer antimicrobial agents, tigecycline