POSTER
A-1010

Study of the genetic platform of *bla*$_{KPC}$ genes in clinical *Enterobacteriaceae* isolated in different countries

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INTRODUCTION

Klebsiella pneumoniae carbapenemase-1 (KPC-1) was first detected in a *K. pneumoniae* strain isolated in North Carolina in 2001[1]. Since that time, several reports of KPCs worldwide have been made, especially in the North East United States such as New York State. KPC ß-lactamases confer resistance to all ß-lactams, reducing therapeutic options to quinolones, aminoglycosides, polymyxins, or combinations of agents for which there are few data on efficacy or for which susceptibility testing is not routinely performed. The emergence and spread of these carbapenem resistant bacteria present challenges with antibiotic therapy as well as infection prevention and control.

AIM

The goal of this study was to compare the genetic platforms of 10 KPC-producing *Enterobacteriaceae* isolated in New York, USA (n=2)[‡]; Ontario, Canada (n=4), and Buenos Aires, Argentina (n=4).

MATERIALS & METHODS

Strains and antimicrobial susceptibility testing. Strains included in this work were isolated in Sanatorio Trinidad Mitre, Buenos Aires, Argentina (1 *K. pneumoniae*, 1 *Citrobacter freundii*, 1 *Serratia marcescens*, and 1 *Enterobacter cloacae*); Shared Hospital Labs, Mount Sinai Hospital and St. Joseph's Health Centre, Toronto, and Ottawa Hospital General Campus, Ottawa, Ontario, Canada (4 *K. pneumoniae*); and in the New York Presbyterian Hospital, Weill Cornell Medical Center, NY, USA (2 *K. pneumoniae* kindly provided by Dr. Stephen G. Jenkins) (Table 1). Susceptibility testing was performed by Etest and interpreted according to the CLSI guidelines[2].

Molecular assays. Transconjugant clones were obtained by carrying out conjugation experiment followed by antibiotic plate selection. Plasmids were extracted from all 10 samples using the method described by Nakamura et al.[3] Southern blot was performed by using standard procedures. The genetic platforms of *bla*$_{KPC}$ genes were mapped by PCR cartography using Tn*4401*-based primers and standard methods. *Eco*RI DNA libraries from 2 of the Argentinian strains were constructed in *Escherichia coli*. Cloned fragments containing *bla*$_{KPC}$ were sequenced. PCR cartography of the other 2 strains was performed using laboratory-designed primers. Sequences were obtained using the BigDye Terminators method and a 3130xl Genetic Analyzer. The genetic relatedness of all *K. pneumoniae* isolates was analyzed by pulse-field gel electrophoresis (PFGE) according with Tenover et al. criteria[4]. All the strains were digested with *Xba*I.

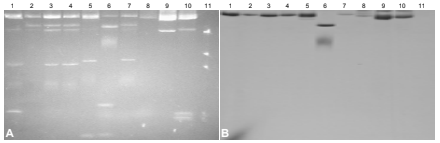
RESULTS

Table 1. MICs of donor and transconjugant strains.

Strains	MIC (µg/ml)															
	Aminoglycosides			β-lactams										Others		
	AMK	TOB	GEN	AMP	PIP	FOX	CTX	CAZ	FEP	IPM	ERT	MEM	CIP	TET	CHL
Donor																
Arg-Cfr-9169*	1.5	1.5	1.5	≥256	≥256	96	128	48	≥256	1	1	0.5	0.25	1	4
Arg-Kpn-9171*	8	12	6	≥256	≥256	96	48	≥256	64	≥32	≥32	≥32	0.75	4	≥256
Arg-Sma-11181*	2	3	0.75	≥256	≥256	256	12	2	6	12	6	3	0.064	8	3
US-Kpn-68523*	24	32	8	≥256	≥256	12	24	48	12	2	3	1	0.094	1	12
Arg-Ecl-11180	8	≥96	128	≥256	≥256	≥256	≥256	256	256	6	12	8	≥32	16	≥256
US-Kpn-105938	64	≥256	32	≥256	≥256	16	96	256	32	6	12	3	≥32	1.5	≥256
Can-Kpn-H0273173	32	32	1.5	≥256	≥256	192	256	≥256	256	32	32	32	≥32	3	12
Can-Kpn-01552	32	24	2	≥256	≥256	96	256	≥256	48	16	32	32	≥32	2	≥256
Can-Kpn-3549	24	24	12	≥256	≥256	128	192	256	256	24	≥32	32	≥32	4	24
Can-Kpn-4363	48	24	12	≥256	≥256	32	128	256	192	32	16	3	≥32	3	≥256
*** Transconjugant**																
Sal-9169	2	2	0.75	≥256	≥256	12	256	24	16	2	1.5	1	0.012	0.75	1.5
Sal-9171	1.5	1.5	0.5	≥256	≥256	8	24	12	16	0.75	0.75	0.38	0.016	0.75	1.5
ER-11181	0.125	0.064	0.064	≥256	96	8	1.5	2	1.5	0.5	0.19	0.125	0.094	0.5	1.5
ER-68523	1.5	4	3	≥256	≥256	12	16	32	8	0.5	0.5	0.38	0.094	0.5	3
Acceptor																
Salmonella spp. 1744	2	1.5	1	1	2	2	0.047	0.125	0.064	0.19	0.008	0.016	0.016	1	2
E. coli ER1793	0.5	0.094	0.125	6	1	8	0.094	0.125	0.047	0.25	0.016	0.023	0.125	0.5	3

References: Arg, clinical strains from Argentina; Can, from Canada; US, from United States of America; Cfr, *Citrobacter freundii*; Kpn, *K. pneumoniae*; Sma, *Serratia marcescens*; Ecl, *Enterobacter cloacae*; AMK, amikacin; TOB, tobramycin; GEN, gentamicin; AMP, ampicillin; PIP, piperacillin; FOX, cefoxitin; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; IMP, imipenem; ERT, ertapenem; MEM, meropenem; CIP, ciprofloxacin; TET, tetracycline; CHL, chloramphenicol.

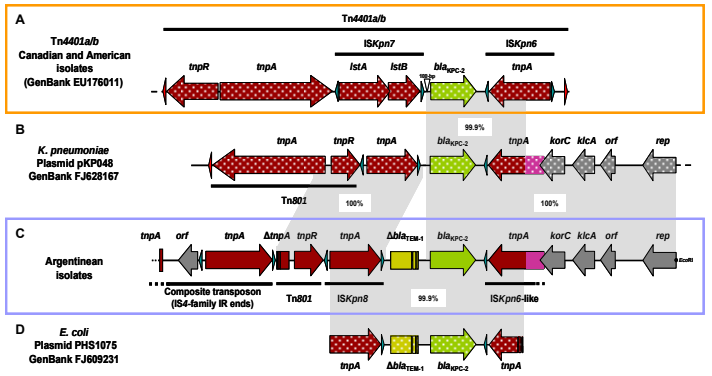
Plasmid profiles from the strains studied, and Southern blot using a probe specific for *bla*$_{KPC}$.



(A) Plasmids extracted from 10 KPC-producing isolates **(B)** hybridization using a specific probe for *bla*KPC genes

References: 1, US-Kpn-68523; 2, Can-Kpn-01552; 3, Can-Kpn-4363; 4, US-Kpn-105938; 5, Can-Kpn-3549; 6, Arg-Ecl-11180; 7, Can-Kpn-H0273173; 8, Arg-Sma-11181; 9, Arg-Cfr-9169; 10, Arg-Kpn-9171; 11, λ-phage DNA/HindIII.

Schematic representation of *bla*$_{KPC}$-genetic environments.



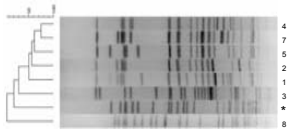
(A) The 10 kb-Tn*4401* platform harboring the *bla*$_{KPC}$ gene sequenced from clinical strain Can-Kpn-4363. Tn*4401* was mapped by PCR cartography in all isolates from USA and Canada. Two isoforms (Tn*4401a* and Tn*4401*b, differing by 100-bp deletion upstream of *bla*$_{KPC}$ genes) were detected.

(B) Genetic platform of *bla*$_{KPC}$-harboring plasmid previously described in *Enterobacteriaceae* from China[6].

(C) Schematic representation of *bla*$_{KPC}$-genetic environment (partial *Eco*RI-*Eco*RI sequence) obtained from *C. freundii* Arg-Cfr-9169 and *E. cloacae* Arg-Ecl-11180. The same platform was mapped by PCR cartography in the remaining two Argentinean strains.

(A) Genetic platform of *bla*$_{KPC}$-harboring plasmid, unpublished results.

PFGE confirmed a non-clonal relationship between *K. pneumoniae* strains included in this study.



References: 1, Can-Kpn-01552; 2, Can-Kpn-3549; 3, US-Kpn-68523; 4, US-Kpn-105938; 5, Can-Kpn-H0273173; 7, Can-Kpn-4363; 8, Arg-Kpn-9171; *, Arg-Kpn-1823 (non-related, non-KPC).

CONCLUSIONS

- Tn*4401* isoforms a and b were detected in unrelated *K. pneumoniae* isolates from Canada and USA.
- Genetic platforms of Argentinean strains harboring *bla*$_{KPC}$ genes were found to be similar to the one previously described in China[6]. However, the disruption of Tn*801* in these strains indicate an alternative transposition way for these genetic structures.
- The Argentinean strains showed the presence of *bla*$_{KPC}$ on plasmids of different sizes, spread in 4 different species. These results suggest that plasmids and transposons carrying *bla*$_{KPC}$ genes are playing a main role in spreading KPC ß-lactamases between bacterial species.

REFERENCES

[1] **Yigit, H. et al.** 2001. *Antimicrob. Agents Chemother.* **45**:1151-1161. [2] **Clinical and Laboratory Standards Institute.** 2009. Performance standards for antimicrobial susceptibility testing; 19th informational supplement. CLSI document M100–S19. Clinical and Laboratory Standards Institute, Wayne, PA. [3] **Nakamura, M. et al.** 1998. *J. Clin. Microbiol.* **33**:360-365. [4] **Tenover, F. C. et al.** 1995. *J. Clin. Microbiol.* **33**:2233-2239. [5] **Hunter, S. B. et al.** 2005. *J. Clin. Microbiol.* **43**:1045-1050.; [6] **Shen, P. et al.** 2009. *Antimicrob. Agents Chemother.* **53**:4333-4338.