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Molecular Characterization of Enterobacteria from Argentina Producing blakec. not associated with Tn4401

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ABSTRACT

Background: The surge of Klebsiella pneumoniae Carbapenema (KPC) producers in Argentina was leaded by K. pneumoniae (KPN ST258 harboring bla_{ser}, in Tn4401a However, sporadic isolates of colates of the same specie) without bla_{kPC2} in Tn4401a were found different regions.

Aim: to characterize blaKPC-2 strains non-associated to Tn4401. Methods: We studied 16 bla.... dinical isolates: 6 KPN, 5 Enternhanter cloanae 3 Citrohanter freundii 1 Serratia marnescens cherichia coli MI ST was done according to the MI ST Databas PCR mapping, sequencing, southern blot (bla,... probe) and onjugation were done by standard procedures. Plasmid content analysis was performed by phenol-chloroform extraction and PCR ased replicon typing (RepT) as reported by Carattoli et al. Results: KPN blager, belonged to 3 STs (n): ST11 (4); ST476 (1) ST526 (1). The new environment named Variants 1a.b., was found to bla_{sPC-2} in all isolates: flanked by Tn801-like element (upstream) and (CVonG-like (downstream). The blaTEM-1 of ToRO1-like use E'-delete ov /SKpn8 and its tripA was disrupted by a composite transposon rund in the plasmid nERACTS of Aeromonas nunctata Plasmid size anned between 7,80 kb and some strains had more than 1 plasmid The bla... probe hybridized with plasmids of different sizes. A single no group was detected in 11/16 isolates (n): IncHI2 (3), IncL/M (3). cA/C (5). 3 Inc groups were found in 2 strains (E. coli, IncFIA-IncFIE IncFrenB KPN ST11 Incl MulncA/CulncFIIST and 3 strains were egative for all Inc groups. RepT allowed us to associate blager, with ncL/M transferable plasmids in 2/3 transconjugants while the 3rd one was negative for all the Inc groups tested as the parental strain. enclusions: Our results suggest that not only conjugation but also mobilization among different plasmids could have had a majo

role in the Argentinean KPC emergence, leading to the dissemination

Epidemiological data.

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of Variants 1a/h in diverse clones and ETB species

SPECIE ISOLATE

SMA M1118

M11421

M9171

M11245

M11354

M984

M11808

ECO M9884

CFR M9988

INTRODUCTION

♦The first Klebsiella pneumoniae carbapenemase (KPC) was discovered in North Carolina in 1996. Since then, KPCs have been reported from China, Israel, Europe, South America and Canada. The worldwide spread of KPC-producing K. pneumoniae strains revealed the successful dissemination of a major clone defined as sequence type (ST)258.

♦ blame has been commonly associated with Tn 4401, possibly responsible for its acquisition A different blagge-environment was described in plasmids from Chinese enterobacteria (ETB) where blagge, was associated with

a Tn801-like transposon. STII was demonstrated to be the dominant clone of KPC-producing K. pneumoniae in China where ST258

♦ In Argenting, the surge of KPC was leaded by K. pneumoniae (KPN) \$7258 harboring blayer. in Tn4401a. However, sporadic isolates of diverse enterobacterial (ETB) species (non-clonal relation among isolates of the same specie) without blayer, in Tn 4401a were found in different regions (Table 1 and Figure 1).

OBJECTIVES

To characterize ETB strains from Argentina harboring blaype., not associated with Tn 4401

DATE

26/02/2009

21/07/2008

15/12/2006

09/10/2008

10/10/2008

15/12/2004

11/10/2008

26/03/2009

20/07/2009

20/07/2009

07/01/2010

26/02/2009

13/01/2010

10/06/2008

28/05/2010

SAMPLE SITE

Blood

Pleural fluid

Skin

Urine

Prostate

Skin

Blood

Urine

Urine

BAL

Urine

Blood CSF

Urine

Pleural fluid

REGION²

BAC

NEUQUEN

BAC

NEUQUEN

BAC

BAP

MENDOZA

MENDOZA

MENDOZA

BAP

BAC

BAC

MENDOZA

BAC

Materials & Methods

. We studied 16 clinical isolates that harbour blacer., in a platform different form Tn4401 Table 1 and Figure 1

• Multilocus sequence typing (MLST) was performed according to the MLST Database (http://www.pasteur.fr/recherche/genopole/PF8/mlst/).

♦ PCR and DNA sequencing of 6-lactamase genes: The screening for bla_{spc}, bla_{per} and bla_{CTX}, was performed in all isolates and their transconjugants by PCR. PCR products were sequence.

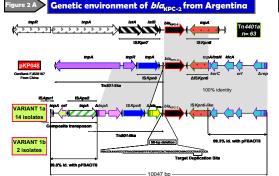
 Analysis of the Genetic environment of the bla_{kpc} gene: The genetic surroundings of bla_{kpc} were studied by PCR cartography and sequencing. FCRI DNA libraries from M9189 and M1181 were generated digesting total DNA and the resulting fragments were cloned by standard

 Plasmid content analysis was performed by phenol-chloroform extraction and PCR-based replicon typing as reported by Carattoli et al.

. Biparental conjugation: E. coli J53 cells (sodium azide resistant) were used as the recipient strain. Selection of transconjugants (TC) was achieved in sodium azide (200 mg/ml) and ceftazidime (25 mg/ml) plates by standard procedures.



RESULTS



Plasmid profile and Replicon type

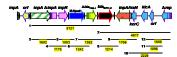
ISOLATE	PFGE type ²	PLATFORM ³	ST ⁴	R determinants ⁵	plasmid size (Kb)	REPLICON TYPE ⁶
M11181		Variant 1a		neg	76	L/M
M9884		Variant 1b		neg	76, 60*, 7*	FIA, FIB, FrepB
M9169	C1	Variant 1a		neg	130	L/M
M9988	C2	Variant 1b		RmtD+	130	NT
M11421	C3	Variant 1a		neg	130	L/M
M9171	КЗ	Variant 1a	476	PER	130	HI2
M11245	K4	Variant 1a	526	PER	130	NT
M11213	K2	Variant 1a	11	neg	ND	A/C
M11353	K2	Variant 1a	11	neg	ND	A/C
M11354	K2	Variant 1a	11	neg	ND	A/C
M11598	K5	Variant 1a	-11	CTX-M-2	76	L/M, A/C, FIIS
M11180	E1	Variant 1a		CTX-M-15	130	NT
M11609	E1	Variant 1a		CTX-M-2	76, 54*	A/C
M9847	E2	Variant 1a		neg	ND	A/C
M11808	E3	Variant 1a		PER	76, 51*	HI2
M11851	E4	Variant 1a		CTX-M-2, PER	51	HI2
TC-sma 11181		Variant 1a		neg	76	L/M
						L/M NT
	M11181 M9884 M9169 M9988 M11421 M9171 M11245 M1123 M11353 M11354 M11598 M11180 M11609 M9847 M11808	M1181 M9884 M9169 C1 M9189 C2 M11421 C3 M9171 K3 M9171 K3 M11213 K2 M11213 K2 M11984 K5 M11808 E1 M11808 E3	SOLATE type2 PLATFORM'	SOLATE SPLATFORM ST		No. No.

¹CFR. Citrobacter freundii: SMA. Serratia marcescens: KPN. Klebsiella pneumoniae: ECL. Enterobacter cloacae: ECO. Escherichia coli. *Xba digested DNA-pulse field gel electrophoresis, *See Fig. 2, *ST Seguence Type: ⁶R. resistance: ⁶PCR based replicon typing applied to KPC-2 positive ETB.

neg, negative PCR for all genes tested. * Not associated with KPC. TC: transconjugant; NT non typeable

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PCR cartography



(A) pKP048, isolate from China, depicted for comparison. Variant 1a represents the sequence obtained from C. freundii M9169 and E. cloacae M11180. ΔΔ/α_{res,1} indicates a deletion of 10 by upstream to the blame, start plus the first 291 nucleotides of the encoding gene of the Tn3 equence. Only the 58-bp deleted fragment in Variant 1b is shown indicating the Tn3-based IRR (underlined text). Plasmid pFBAOT6 from A. punctata. (B) PCR cartography used to characterize Variant 1a/b-harboring isolates. Amplicons 7 and 8 were sequenced in all isolates to discriminate hebween Veriente 1e and h

Plasmid Profile and Southern blot

with several isolates harboring more than one plasmid. Southern blot analysis with blo_{kDC} probe showed that i associated with plasmids of different sizes.

CONCLUDING REMARKS

- + Here we report the occurrence of blagge, in diverse species of ETR obtained from distant locations in Argentina.
- + blayoca, was detected in an unusual genetic environment (Variants 1a/b), transferable by conjugation in plasmids of different sizes.
- * Replicon Typing analysis revealed that blame. was associated to INC-L/M plasmids in TC strains. Further analysis is needed to determine the specific characteristics (size, identity) of the plasmids that harbor blame in these strains.
- transposition) could have played a major role in the dissemination of Variants 1a/b in diverse clones

Htal Madre v el Niño Formosa **FORMOSA** 23/06/2010 *CFR. Citrobacter freundit: SMA. Serratia marcescens: KPN. Kiebsiella pneumoniae: ECL. Enterobacter cloacae: ECO. Escherichia col DAC City of Dunner Aires DAD Dunner Aires Doubles 2005 Cember spiral fluid DAI bronche shapely business