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Molecular Characterization of Carbapenemase-producing Gram negative Bacilli from 3 Hospitals in Antioquia, Colombia

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ABSTRACT

Background: Colombia is an endemic country for carbapenemase-producing gram-negative organisms. Genes encoding the carbapenemase enzymes are widely distributed in the country both in *Enterobacteriaceae* and non-fermenting gram-negative bacilli. Colonized patients can readily disseminate these bacteria in hospitals. We aimed to characterize the population structure of carbapenemase-producing gram-negative organisms in colonized and infected patients in order to evaluate the mechanisms of carbapenemase spread in hospitals located in the Antioquia region of Colombia.

Methods: We performed a descriptive study in three hospitals of Antioquia, between November 2013 and October 2015. Patients infected or colonized with carbapenem-resistant gram-negative bacilli were included in the study, according to inclusion criteria. Isolates were characterized by standard microbiological methods and bla_{KPC}, bla_{IMP}, bla_{VIM} and bla_{NDM} were detected by PCR. Genetic relationships was established by molecular typing using rep-PCR.

Results: Seventy five isolates were collected. *Pseudomonas aeruginosa* (28%) and *Klebsiella pneumoniae* (25%) were the most common bacteria. The most frequent carbapenemases in *Enterobacteriaceae* and *P. aeruginosa* were KPC (79%), VIM (16%) and VIM plus KPC in combination (5%) (Table 1). Only one common clonal group of carbapenemase-producing *K.pneumoniae* and *Eschericia coli* was identified in both infected and colonized patients but in the majority of cases a high degree of heterogeneity was observed. The population structure of *Enterobacter cloacae* and *P. aeruginosa* was more homogeneous harboring 4 and 5 clonal groups, respectively, shared between colonized and infected patients and between patients located in similar wards at the time of hospitalization.

Conclusion: The heterogeneity of the population structure of carbapenem-producing *K. pneumoniae* and *E. coli* in Colombia suggests that horizontal gene transfer caused by selective antibiotic pressure is the main mechanism of dissemination. In contrast, in *P. aeruginosa* and *E. cloacae*, transmission of successful clones is likely to play a major role in the spread of the latter two organisms. These information is key to design infection control strategies and antibiotic policies among the hospitals.

BACKGROUND

Colombia is an endemic country for carbapenemase-producing gram-negative organisms. Genes encoding the carbapenemase enzymes are widely distributed in the country both in *Enterobacteriaceae* and non-fermenting gram-negative bacilli.[1]

Colonized patients may serve as reservoirs for transmission and readily disseminate these bacteria in hospitals.[2]

Aim: To characterize the population structure of carbapenemase-producing gram-negative organisms in colonized and infected patients in order to evaluate the mechanisms of carbapenemase spread in hospitals located in the Antioquia region of Colombia.

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METHODS

Between October 2013 and November 2015, 75 carbapenem-resistant gram-negative bacilli isolates were collected from patients infected or colonized in three hospitals in Antioquia.

- **Inclusion criteria**: Isolates of gram-negative bacilli resistant to β-lactams of patients colonized or infected from the three clinics or referred from other hospitals.

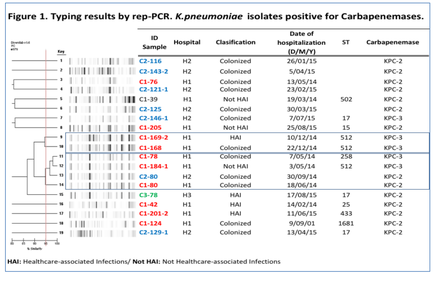
➢ Phenotypic characterization: MHT.
➢ Molecular characterization: Carbapenemases screening by PCR (bla_{KPC}, bla_{IMP}, bla_{VIM} and bla_{NDM}).
➢ Molecular typing: An automated repetitive sequence-based PCR (rep-PCR). Isolates with >95% similarity were considered clonal.

RESULTS

TABLE 1. Distribution by type of microorganism and carbapenemase

Microorganism	CARBAPENEMASE			
	KPC-2	VIM-2	VIM-4	VIM-2+KPC-2
K.pneumoniae (25%)	17	0	0	2
E.cloacae (20%)	15	0	0	0
E.coli (15%)	10	0	1	0
S.marcescens(7%)	5	0	0	0
C.freundii (5%)	4	0	0	0
P.aeruginosa (28%)	13	7	0	1

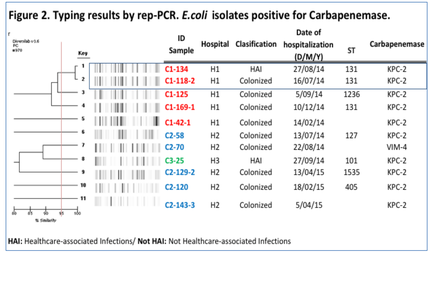
Figure 1. Typing results by rep-PCR. *K.pneumoniae* isolates positive for Carbapenemases.



Key	ID Sample	Hospital	Clasification	Date of hospitalization (D/M/Y)	ST	Carbapenemase
1	C2-116	H2	Colonized	26/01/15		KPC-2
2	C2-143-2	H2	Colonized	5/04/15		KPC-2
3	C1-76	H1	Colonized	13/05/14		KPC-2
4	C2-121-1	H2	Colonized	23/02/15		KPC-2
5	C1-39	H1	Not HAI	19/03/14	502	KPC-2
6	C2-125	H2	Colonized	30/03/15		KPC-2
7	C2-146-1	H2	Colonized	7/07/15	17	KPC-3
8	C1-205	H1	Not HAI	25/08/15	15	KPC-2
9	C1-169-2	H1	HAI	10/12/14	512	KPC-3
10	C1-168	H1	Colonized	22/12/14	512	KPC-3
11	C1-78	H1	Colonized	7/05/14	258	KPC-3
12	C1-184-1	H1	Not HAI	3/05/14	512	KPC-3
13	C2-80	H2	Colonized	30/09/14		KPC-2
14	C1-80	H1	Colonized	18/06/14		KPC-2
15	C3-79	H3	HAI	17/08/15	17	KPC-2
16	C1-42	H1	HAI	14/02/14	25	KPC-2
17	C1-201-2	H1	HAI	11/06/15	433	KPC-2
18	C1-124	H1	Colonized	9/09/01	1681	KPC-2
19	C2-129-1	H2	Colonized	13/04/15	17	KPC-2

HAI: Healthcare-associated Infections/ **Not HAI:** Not Healthcare-associated Infections

Figure 2. Typing results by rep-PCR. *E.coli* isolates positive for Carbapenemase.



Key	ID Sample	Hospital	Clasification	Date of hospitalization (D/M/Y)	ST	Carbapenemase
1	C1-134	H1	HAI	27/08/14	131	KPC-2
2	C1-118-2	H1	Colonized	16/07/14	131	KPC-2
3	C1-125	H1	Colonized	5/09/14	1236	KPC-2
4	C1-169-1	H1	Colonized	10/12/14	131	KPC-2
5	C1-42-1	H1	Colonized	14/02/14		KPC-2
6	C2-58	H2	Colonized	13/07/14	127	KPC-2
7	C2-70	H2	Colonized	22/08/14		VIM-4
8	C3-25	H3	HAI	27/09/14	101	KPC-2
9	C2-129-2	H2	Colonized	13/04/15	1535	KPC-2
10	C2-120	H2	Colonized	18/02/15	405	KPC-2
11	C2-143-3	H2	Colonized	5/04/15		KPC-2

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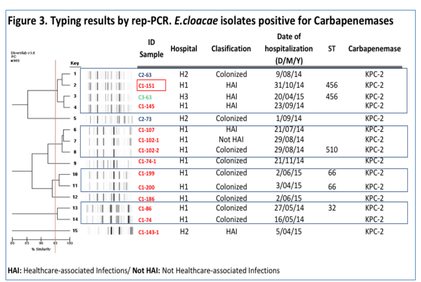
Figure 3. Typing results by rep-PCR. *E.cloacae* isolates positive for Carbapenemases



Key	ID Sample	Hospital	Clasification	Date of hospitalization (D/M/Y)	ST	Carbapenemase
1	C2-63	H2	Colonized	9/08/14		KPC-2
2	C1-151	H1	HAI	31/10/14	456	KPC-2
3	C1-61	H3	HAI	20/04/15	456	KPC-2
4	C1-145	H1	HAI	23/09/14		KPC-2
5	C2-73	H2	Colonized	1/09/14		KPC-2
6	C1-107	H1	HAI	21/07/14		KPC-2
7	C1-102-1	H1	Not HAI	29/08/14		KPC-2
8	C1-102-2	H1	Colonized	29/08/14	510	KPC-2
9	C1-74-1	H1	Colonized	21/11/14		KPC-2
10	C1-199	H1	Colonized	2/06/15	66	KPC-2
11	C1-200	H1	Colonized	3/04/15	66	KPC-2
12	C1-195	H1	Colonized	2/06/15		KPC-2
13	C1-86	H1	Colonized	27/05/14	32	KPC-2
14	C1-74	H1	Colonized	16/05/14		KPC-2
15	C1-143-1	H2	HAI	5/04/15		KPC-2

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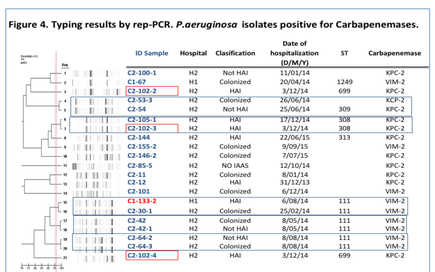
Figure 4. Typing results by rep-PCR. *P.aeruginosa* isolates positive for Carbapenemases.



Key	ID Sample	Hospital	Clasification	Date of hospitalization (D/M/Y)	ST	Carbapenemase
1	C2-100-1	H2	Not HAI	11/01/14		KPC-2
2	C1-67	H1	Colonized	20/04/14	1249	VIM-2
3	C2-102-2	H2	HAI	3/12/14	699	KPC-2
4	C2-53-3	H2	Colonized	26/06/14		KPC-2
5	C2-54	H2	Not HAI	25/06/14	309	KPC-2
6	C2-105-1	H2	HAI	17/12/14	308	KPC-2
7	C2-102-3	H2	HAI	3/12/14	308	KPC-2
8	C2-144	H2	HAI	22/06/15	313	KPC-2
9	C2-155-2	H2	Colonized	9/09/15		VIM-2
10	C2-146-2	H2	Colonized	7/07/15		KPC-2
11	C2-85-5	H2	NO IAAS	12/10/14		KPC-2
12	C2-11	H2	Colonized	8/01/14		KPC-2
13	C2-12	H2	HAI	31/12/13		KPC-2
14	C2-101	H2	Colonized	6/12/14		VIM-2
15	C1-133-2	H1	HAI	6/08/14	111	VIM-2
16	C2-30-1	H2	Colonized	25/02/14	111	VIM-2
17	C2-42	H2	Colonized	8/05/14	111	VIM-2
18	C2-42-1	H2	Not HAI	8/05/14	111	VIM-2
19	C2-64-2	H2	Not HAI	8/08/14	111	VIM-2
20	C2-64-3	H2	Colonized	8/08/14	111	VIM-2
21	C2-102-4	H2	HAI	3/12/14	699	KPC-2

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CONCLUSIONS

- Heterogeneous KPC-2-producing *K. pneumoniae* non-ST-258 lineages was detected in colonized and infected patients, presumably due to the horizontal transfer of bla_{KPC}-harboring plasmids. KPC-3-producing *K. pneumoniae* ST512 was less predominant with clonal dissemination. Also, heterogeneity of the population structure of carbapenem-producing *E. coli* was detected (KPC2 and VIM4).
- In contrast, different groups of clones of *P. aeruginosa* and *E. cloacae* (few polyclonal) would be related to the transmission of successful clones between infected and colonized patients given the time, place of stay and epidemiological characteristics of patients. It is also noteworthy that several patients were colonized and subsequently infected with the same organism.
- This suggests follow-up and monitoring of colonized patients as a strategy to limit the subsequent infection and dissemination of these bacteria.

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