Characterization of outer-membrane proteins and carbapenemase in carbapenem-resistant *Enterobacter aerogenes* and *Enterobacter cloacae*.

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The emergence and rapid spread of multidrug-resistant *Acinetobacter baumannii* isolates have become a serious health threat worldwide. Carbapenem resistance in *Enterobacter* spp. has increased significantly in recent years. Few studies, however, assessed the mechanisms involved with carbapenem resistance in this pathogen. The objective of this study was to evaluate the presence of carbapenemase, alteration of outer membrane proteins, and clonality of clinical isolates of *E. aerogenes* and *E. cloacae* isolated in 03 Brazilian hospitals from 2005 to 2012.

METHODS

The inhibitory concentration of the following antimicrobials: imipenem, meropenem, ertapenem, fosfomycin, tigecycline, and polymyxin B was performed by microdilution according to the CLSI. PCR of genes of carbapenemase (KPC, VIM, SPM, IMP, NDM, NMC/SME, Oxa-48), outer membrane proteins (OMP35-36) was achieved for all the isolates. The clonality of the isolates was evaluated by PFGE and isolates with different clonal profiles were selected for SDS-PAGE analysis. Hydrolysis of imipenem was evaluated for the isolates that did not show any of carbapenemase searched.

RESULTS

• 105 isolates *E. aerogenes* and 25 isolates of *E. cloacae* totaling 130 isolates were evaluated, of which 44 and 9 were respectively resistant to carbapenem. Of the 44 isolates of *E. aerogenes* carbapenem- resistant 45 (100%) showed resistance to imipenem, 36 (65.5%) to ertapenem and 35 (79.5%) to meropenem. The 9 isolates of *E. cloacae* were resistant to all carbapenem. (table I)

•Surgical site infection was the most frequent site of infection (23 of 54) followed by blood (14 of 54).

• KPC-2 was the only carbapenemase identified and present in both *E. aerogenes* and *E. cloacae* (table II)

•29 Isolates were selected to SDS-page, 10 showed diminished and 8 lost of OMP35-OMP36 and 16 diminished and 1 lost of 42KDA.

• Eleven isolates with imipenem MIC that ranged from 8 ug/mL to 32 ug/mL and meropenem from 2 ug/mL to 16 ug/mL exhibited more than a mechanism of resistance as KPC, the absence of genes of Porin (35-36KDa) and decrease and or absence of outer membrane proteins (35-36 Kda and 39 and 42 Kda) by SDS-PAGE.

•Seventeen isolates did not present any of carbapenemases evaluated, of which 5 showed hydrolysis of imipenem, and 2 were screening test of metallo-beta-lactamase with EDTA positive.

•There is a predominant clone among *E. aerogenes isolates* and one among *E. clocae*. isolates

	E. aerogenes (45)		*E. cloacae (9)*	
Antibióticos	CIM 50 (µg/mL)	CIM 90 (µg/mL)	CIM 50 (µg/mL)	CIM 90 (µg/mL)
Imipenem	16	32	16	32
Meropenem	4	16	8	16
Ertapenem	0,25	16	32	64
Tigecycline	0,12	0,25	0,5	2
Fosfomycin	4	16	8	32
Polymyxin B	2	2	0,5	2
Cefepime	0,25	>128	>128	>128

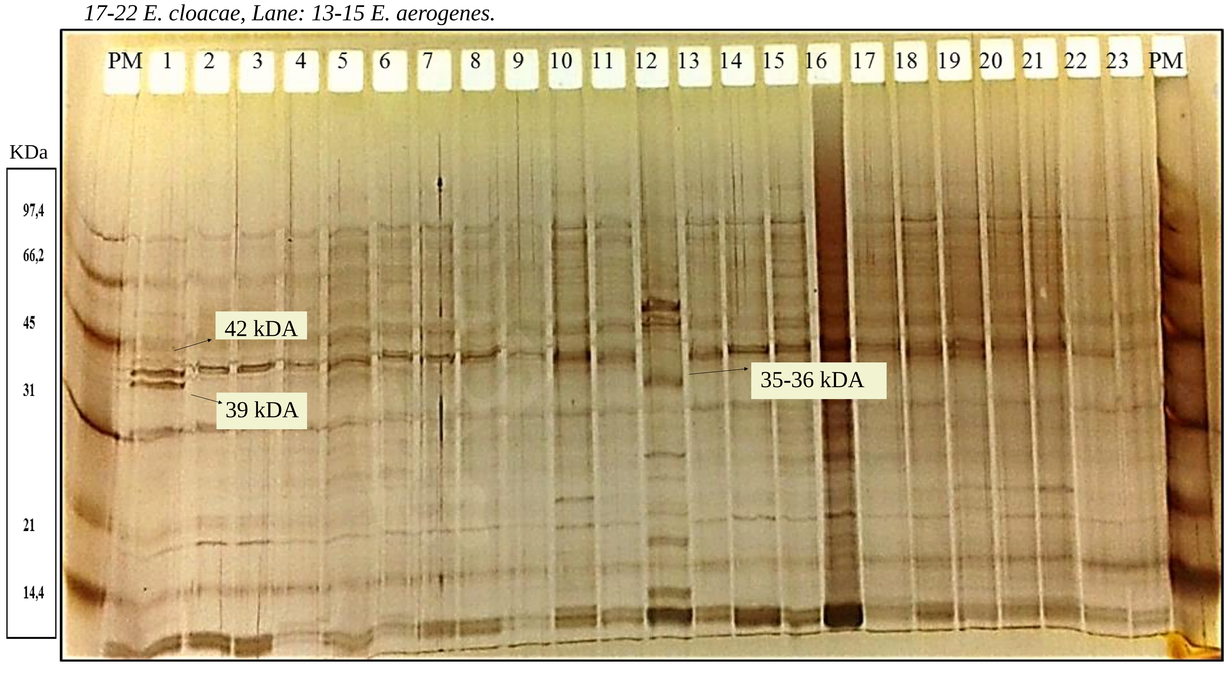
Table I: MIC 50 and 90 of *Enterobacter cloacae* and *E. aerogenes* carbapenem-resistant .

Gene	*E. aerogenes* (45)		*E. cloacae* (9)	
	Positivo	%	Positivo	%
KPC- 2	30	66,6%	6	66,6%

Table II: Percentage of gene bla_{KPC} found in *Enterobacter cloacae* and *Enterobacter aerogenes* carbapenem-resistant.

•Lost of protein of 39KDa was associated with high imipenem MIC, lost of protein of 42KDa and diminished of OMP35 and OMP36 with imipenem resistant and meropenem susceptible.

Fig. I: Detection of porins OMP 35-36-39-42 kDa components *in E. aerogenes* e *E. cloacae* carbapenem-resistant in SDS-PAGE. Lane PM: proteins of known weight, Lane 12: *Pseudomonas aeroginosa, Lane: 1-11 E. aerogenes, Lane: 17-22 E. cloacae, Lane: 13-15 E. aerogenes.*



CONCLUSION

• KPC was the most frequent mechanism of resistance identified, which alerts to the potential rapid spread of resistance in this genus. However, lost ou dismisnhed of outer-membrane protein were very frequent .

• High imipenem MIC and difference in carbapenem susceptibility profile (imipenem resistance and meropenem susceptibility) was associated with alteration of outer-membrane protein.

•All isolates were susceptible to polymyxin B and tigecycline; fosfomycin showed excellent activity against both *E. cloacae* e *E. aerogenes*.

• Presence of other carbepenemases, EBSL and the role of eflux pump on carbapenem resiatnce will be futher evaluate.

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