The Brazilian Journal of
INFECTIOUS DISEASES
www.elsevier.com/locate/bjid

Original article

Molecular epidemiology of CTX-M producing *Enterobacteriaceae* isolated from bloodstream infections in Rio de Janeiro, Brazil: emergence of CTX-M-15

Liliane Miyuki Seki[a,*], *Polyana Silva Pereira*[a], *Magda de Souza Conceição*[b], *Maria José Souza*[c], *Elizabeth Andrade Marques*[d], *Jupira Miron Carballido*[e], *Maria Elisabeth Serqueira de Carvalho*[f], *Ana Paula D'Alincourt Carvalho Assef*[a], *Marise Dutra Asensi*[a]

[a] *Fundação Oswaldo Cruz (FIOCRUZ), Rio de Janeiro, RJ, Brazil*
[b] *Hospital Federal de Bonsucesso, Rio de Janeiro, RJ, Brazil*
[c] *Hospital dos Servidores do Estado, Rio de Janeiro, RJ, Brazil*
[d] *Hospital Universitário Pedro Ernesto, Universidade Estadual do Rio de Janeiro (UERJ), Rio de Janeiro, RJ, Brazil*
[e] *Hospital Universitário Antônio Pedro, Universidade Federal Fluminense (UFF), Rio de Janeiro, RJ, Brazil*
[f] *Hospital Naval Marcílio Dias, Rio de Janeiro, RJ, Brazil*

ARTICLE INFO

Article history:
Received 8 January 2013
Accepted 21 March 2013
Available online 20 September 2013

Keywords:
Bloodstream infection
Enterobacteriaceae
CTX-M-15
PFGE
KPC
Brazil

ABSTRACT

Objective: The present study was designed to evaluate the molecular epidemiology of CTX-M producing *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Escherichia coli* isolated from bloodstream infections at tertiary care hospitals in the State of Rio de Janeiro, Brazil.

Material and methods: A total of 231 nonduplicate *Enterobacteriaceae* were isolated from five Brazilian hospitals between September 2007 and September 2008. The antimicrobial susceptibility testing was performed by disk diffusion method according to the Clinical Laboratory Standard Institute. Isolates showing resistance to third-generation cephalosporins were screened for ESBL activity by the double-disk synergy test. The presence of $bla_{\text{CTX-M}}$, $bla_{\text{CTX-M-15}}$ and bla_{KPC} genes was determined by Polymerase Chain Reaction (PCR) amplification and DNA sequencing. The molecular typing of CTX-M producing isolates was performed by pulsed-field gel electrophoresis (PFGE).

Results and discussion: Ninety-three isolates were screened as ESBL positive and 85 (91%) were found to carry CTX-M-type, as follows: *K. pneumoniae* 59 (49%), *E. cloacae* 15 (42%), and *E. coli* 11 (15%). Ten isolates resistant for carbapenems in *K. pneumoniae* were $bla_{\text{KPC-2}}$ gene positive. Among CTX-M type isolates, CTX-M-15 was predominant in more than 50% of isolates for *K. pneumoniae*, *E. coli*, and *E. cloacae*. PFGE analysis of CTX-M producing isolates showed the predominance of CTX-M-15 in 10 of 24 pulsotypes in *K. pneumoniae*, 6 of 13 in *E. cloacae* and 3 of 6 in *E. coli*. CTX-M-15 was also predominant among KPC producing isolates.

* *Corresponding author at*: Fundação Oswaldo Cruz (FIOCRUZ), Laboratório de Pesquisa em Infecção Hospitalar, Pavilhão Rocha Lima, 3rd floor, Room 319, Avenida Brasil, 4365, Manguinhos, Rio de Janeiro 21045-900, Brazil.
E-mail addresses: miyuki@ioc.fiocruz.br, lilianemiyuki@gmail.com (L.M. Seki).

http://dx.doi.org/10.1016/j.bjid.2013.03.012

In conclusion, this study showed that CTX-M-15 was circulating in Rio de Janeiro state in 2007–2008. This data reinforce the need for continuing surveillance because this scenario may have changed over the years.

Introduction

Since the end of the 1990s, the CTX-M enzymes have spread among the continents, becoming the most prevalent in the world.[1] Among CTX-M-type, specifically CTX-M-15 is the most widely distributed in *Escherichia coli* and *Klebsiella pneumoniae*.[2–4] Particularly, several reports have shown that CTX-M-15-producing *E. coli* isolates are closely associated with a single clone disseminated worldwide, which is represented as sequence type (ST) 131 and serotype O25:H4.[4,5] In *K. pneumoniae*, CTX-M-15 had been found in many countries associated with quinolone resistant strains that belonged to ST11.[2,3,6]

In South American countries (including Brazil), isolates harboring the CTX-M-2 have been the most frequent CTX-M-type detected.[7–9] The occurrence of $bla_{\text{CTX-M-15}}$ was first reported in clinical isolates of *E. coli* and *K. pneumoniae* in the state of São Paulo.[10,11] In the Rio de Janeiro state, the $bla_{\text{CTX-M-15}}$ was found in *E. coli* isolates associated with a dominant clone (sequence type 410).[12]

Despite many reports of CTX-M producing isolates in Brazilian hospitals, few studies have been conducted about the molecular epidemiology of CTX-M-producing *Enterobacteriaceae* isolates. Thus, the main objective of this study was to evaluate the molecular epidemiology of CTX-M-producing *K. pneumoniae*, *E. coli*, and *E. cloacae*, using molecular typing by pulsed-field gel electrophoresis (PFGE) in bloodstream isolates collected in the period of 2007–2008 from patients admitted to five hospitals located in the state of Rio de Janeiro, Brazil.

Material and methods

Bacterial isolates

As part of the Bacterial Nosocomial Infection Resistance Surveillance Network, a total of 231 consecutive non-duplicate *K. pneumoniae*, *E. coli*, and *E. cloacae* isolates originated from bloodstream infections were collected from inpatients during the period from September 2007 to September 2008, in five public tertiary care hospitals located in the state of Rio de Janeiro, Brazil. All species were confirmed by using both conventional techniques and the automated Vitek System (BioMérieux, Marcy, I'Etoile, France).

Antimicrobial susceptibility testing and confirmation of ESBL production

The isolates were tested for antimicrobial susceptibility using the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[13] The following antibiotic disks (Oxoid, Basingstoke, Hants, England) were used: ceftazidime (CAZ), cefotaxime (CTX), cefepime (FEP), aztreonam (ATM), ciprofloxacin (CIP), gentamicin (CN), amikacin (AK), trimethoprim/sulfamethoxazole (SXT), ertapenem (ETP), meropenem (MEM), and imipenem (IPM). Results were interpreted according to the guidelines of the CLSI.[14] Isolates showing resistance to third-generation cephalosporins were screened for ESBL activity by the double-disk synergy test (DDST) using Oxoid disks.[15]

PCR amplification and DNA sequencing

In isolates screened as ESBL producers, we performed Polymerase Chain Reaction (PCR) amplification to determine the presence of $bla_{\text{CTX-M}}$ using primers and conditions previously described.[16] In isolates considered positive, another PCR for $bla_{\text{CTX-M-15}}$ was performed.[16,17] In isolates showing resistance to carbapenems, we performed the PCR methodology for bla_{KPC} gene detection. The PCR products were purified using the GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare, Buckinghamshire, UK) and sequenced.[18] Sequence analysis and alignment results were compared with sequences available from GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Pulsed-field gel electrophoresis

Genomic DNA analysis of *K. pneumoniae*, *E. coli*, and *E. cloacae* isolates were performed by PFGE, after digestion with *Xba*I (Roche Diagnostics, Indianapolis, IN, USA), using the Chef-DR III System (Bio-Rad Laboratories, Richmond, CA, USA). DNA relatedness was computationally analyzed using BioNumerics v.4.0 software (Applied Maths, Sint-Martens-Latem, Belgium). The banding patterns were compared by using the unweighted pair-group method with arithmetic averages (UPGMA), with the Dice similarity coefficient required to be >80% for the pattern to be considered as belonging to the same PFGE type.

Results

Of the 231 bloodstream isolates of *Enterobacteriaceae* from patients admitted to general medical wards or intensive care units, 93 isolates were screened as positive for ESBL by phenotypic test. Among ESBL producers, 85 isolates were found to carry CTX-M-type determinants (91%). The distribution of CTX-M among the species was: 59 of 121 isolates for *K. pneumoniae* (49%), 15 of 36 for *E. cloacae* (42%), and 11 of 74 for *E. coli* (15%). The CTX-M-15-positive isolates represented 61% of CTX-M-producers distributed among the species as follows: 64% (38/59) for *K. pneumoniae*, 53% (8/15) for *E. cloacae*, and 55% (6/11) for *E. coli* (Table 1).

The antimicrobial susceptibility testing for CTX-M-producing *Enterobacteriaceae* isolates showed that all isolates were resistant to cefotaxime (100%), while 76%, 71%, and

Table 1 – Characteristics of the CTX-M-producing *Enterobacteriaceae* isolates.

Species	Antimicrobial resistance, *n* (%)											β-Lactamases genes, *n* (%)	
	CAZ	CTX	FEP	ATM	CN	AK	CIP	SXT	IP	MER	ERT	$bla_{CTXM-15}$	bla_{KPC}
K. pneumoniae	48	59	45	27	26	15	46	51	10	10	10	38	10
(*n* = 59)	(81)	(100)	(76)	(46)	(44)	(25)	(78)	(86)	(17)	(17)	(17)	(64)	(17)
E. cloacae	10	15	9	6	9	4	8	13	–	–	9	8	–
(*n* = 15)	(66)	(100)	(60)	(40)	(60)	(26)	(53)	(87)			(60)	(53)	
E. coli	7	11	6	3	3	4	10	9	–	–	–	6	–
(*n* = 11)	(64)	(100)	(55)	(27)	(27)	(36)	(91)	(82)				(55)	
Total	65	85	60	36	38	23	64	73	10	10	19	52	10
(*n* = 85)	(76)	(100)	(71)	(42)	(45)	(27)	(75)	(86)	(12)	(12)	(22)	(61)	(12)

CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime; ATM, aztreonam; CN, gentamicin; AK, amikacin; CIP, ciprofloxacin; SXT, trimethoprim/sulfamethoxazol; IP, imipenem; MER, meropenem; ERT, ertapenem; *n*, number of isolates; (%), percentage.

Table 2 – Distribution of the pulsotypes of CTX-M-producing *K. pneumoniae*, *E. cloacae* and *E. coli* isolates in five hospitals in Rio de Janeiro.

Hospitals	Pulsotypes		
	K. pneumoniae	*E. cloacae*	*E. coli*
H1	A, B, C, E, M, N, Q, S, T, V, X	C, J	B, C
H2	A, C, D, E, F, H, I, J, L, P, U, Y, Z	B, C, E, F, H, J, K, L, M	B, C, D
H3	A, B, C, F, H, I, O	–	A, B, C
H4	B, C, D, J, K	–	–
H5	F, G	A, D, G	A, B

42% were resistant for ceftazidme, cefepime, and aztreonam, respectively. In addition, co-resistance for amikacin (27%), gentamicin (45%), ciprofloxacin (75%), and trimethoprim/sulfamethoxazole (86%) was observed. We found 10 isolates of *K. pneumoniae* and nine of *E. cloacae* showing resistance to the tested carbapenems. PCR for bla_{KPC} gene showed that 17% (10/59) of CTX-M-producing *K. pneumoniae* isolates were positive and this gene was not detected in nine isolates of *E. cloacae* resistant to ertapenem (Table 1). In our isolates of *K. pneumoniae* KPC-2 type were found in four hospitals studied in 2007 and 2008. Among the KPC isolates, CTX-M-15 was observed in 80% of isolates (8/10).

Genetic polymorphism analyses showed 24 different pulsotypes in *K. pneumoniae*, designated from A–Z, displaying >80% similarity within each type. Of the 59 *K. pneumoniae* isolates, 30 (51%) belonged to four dominant pulsotypes: A (*n* = 7), B (*n* = 8), C (*n* = 9), and D (*n* = 6). The $bla_{CTX-M-15}$ was found in 10 pulsotypes (A, B, C, D, F, J, T, U, Y, and Z). All isolates of pulsotype A (*n* = 7) and one isolate of each pulsotypes D, F, and H were KPC producers. The CTX-M-producing *K. pneumoniae* pulsotypes were distributed among the hospitals as showed in Table 2. Other groups of CTX-M were also found in *K. pneumoniae* (Fig. 1).

Genetic polymorphism analyses of *E. cloacae* showed 13 pulsotypes. The $bla_{CTX-M-15}$ was found in six pulsotypes (C, F, G, J, K, and M), in the hospitals designated H1, H2, and H5. The group 9 of bla_{CTX-M} gene was found in seven isolates of *E. cloacae* and in seven pulsotypes (A, B, D, E, H, I, and L) (Fig. 2). Of the four *E. coli* pulsotypes (A, B, C, and D) observed, eight isolates (73%) belonged to pulsotypes B (four isolates; four hospitals), and C (four isolates; three hospitals). The $bla_{CTX-M-15}$ was found in six isolates, three pulsotypes (A, B, and C) and four hospitals (H1, H2, H3, and H5). The groups 2 and 8 of bla_{CTX-M} gene also were found in *E. coli* (Fig. 3). The distribution of pulsotypes of *E. cloacae* and *E. coli* isolates among the hospitals was demonstrated in Table 2.

Discussion

CTX-M-producing organisms are a clinical problem worldwide,[1] particularly in Latin American countries and especially in Brazil, where high endemic rates in *Enterobacteriaceae* isolates have been reported.[8,9] In this study, we observed that the majority of the ESBL-producing isolates (91%) were characterized as CTX-M-producers, supporting the recognition of CTX-M as the most prevalent type of ESBL in the world.[1] *K. pneumoniae* and *E. cloacae* were the most prevalent CTX-M-producing species.

In this study, CTX-M-producing *Enterobacteriaceae* isolates had high rates of resistance to sulfamethoxazole/trimethoprim, ciprofloxacin and gentamicin. Co-resistance to antimicrobial agents other than beta-lactams among ESBL and KPC producing isolates has been very common, imposing severe restriction on therapeutic choices for patients with such infections. Resistance to other classes of antimicrobial agents may be due to simultaneous transference of the resistance genes via plasmids and integrons.[19]

In this report, we analyzed the molecular epidemiology of CTX-M in five hospitals from Rio de Janeiro (Brazil). We observed the emergence of CTX-M-15 among bloodstream isolates in 2007–2008.

The predominance of the $bla_{CTX-M-15}$ gene involved in different pulsotypes of *K. pneumoniae, E. cloacae*, and *E. coli* is important because it is representative of the epidemiology at

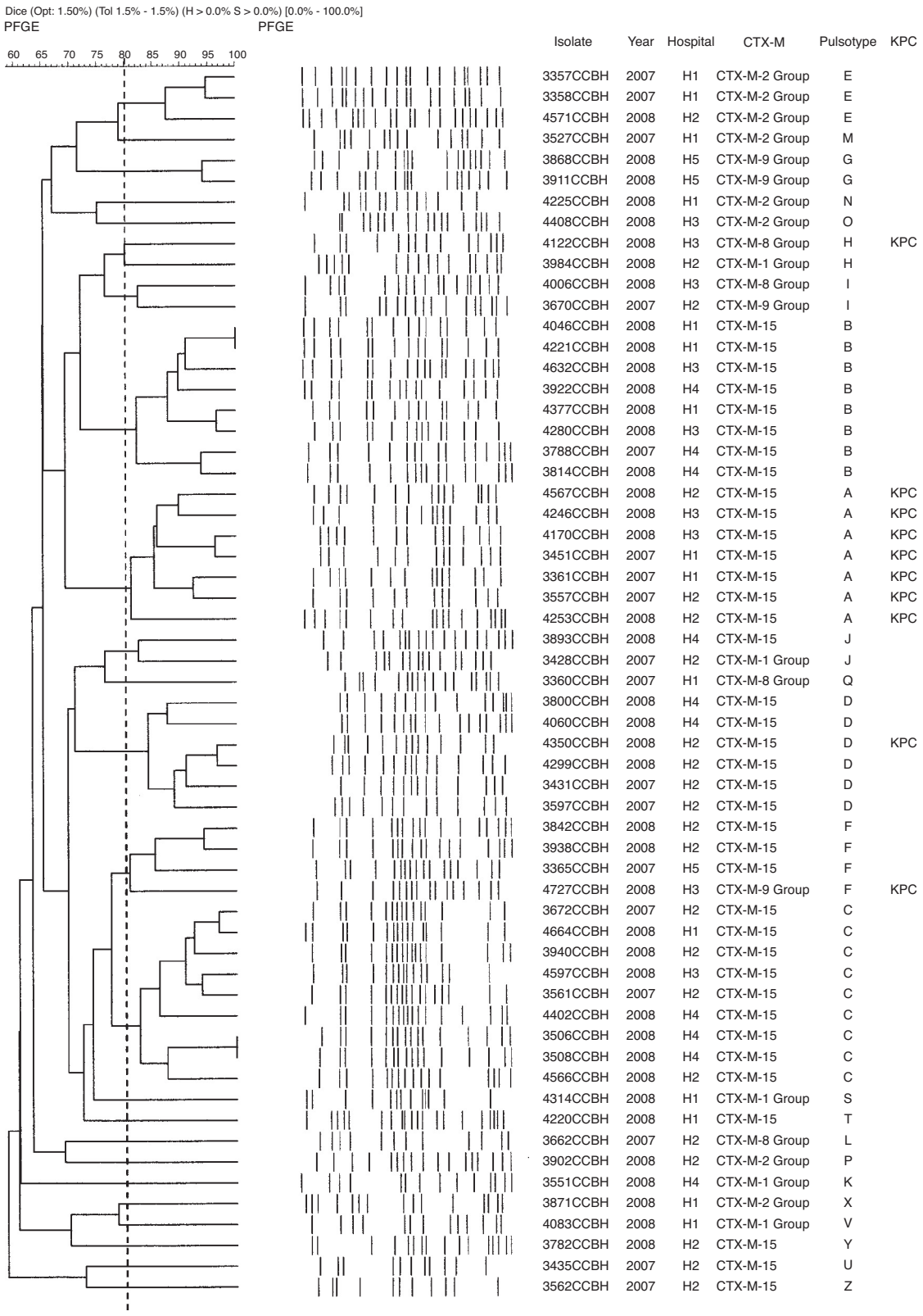
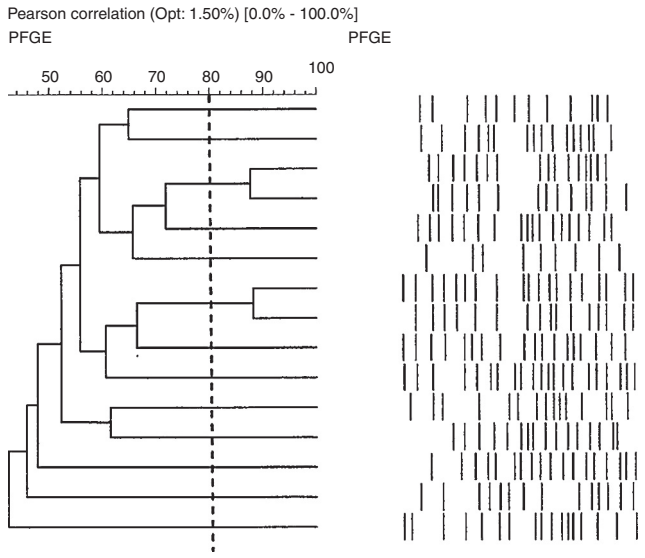


Fig. 1 – Clinical data and molecular typing of CTX-M-producing *K. pneumoniae*.



Isolate	Year	Hospital	CTX-M	Pulsotype
4127CCBH	2008	H5	CTX-M-9 Group	A
4022CCBH	2008	H2	CTX-M-9 Group	B
4378CCBH	2008	H1	CTX-M-15	C
4647CCBH	2008	H2	CTX-M-15	C
3499CCBH	2007	H5	CTX-M-9 Group	D
4575CCBH	2008	H2	CTX-M-9 Group	E
3834CCBH	2007	H2	CTX-M-15	F
3824CCBH	2008	H2	CTX-M-15	F
3626CCBH	2007	H5	CTX-M-15	G
3558CCBH	2007	H2	CTX-M-9 Group	H
4259CCBH	2008	H2	CTX-M-9 Group	I
3943CCBH	2008	H1	CTX-M-15	J
3906CCBH	2008	H2	CTX-M-15	K
3654CCBH	2007	H2	CTX-M-9 Group	L
3433CCBH	2007	H2	CTX-M-15	M

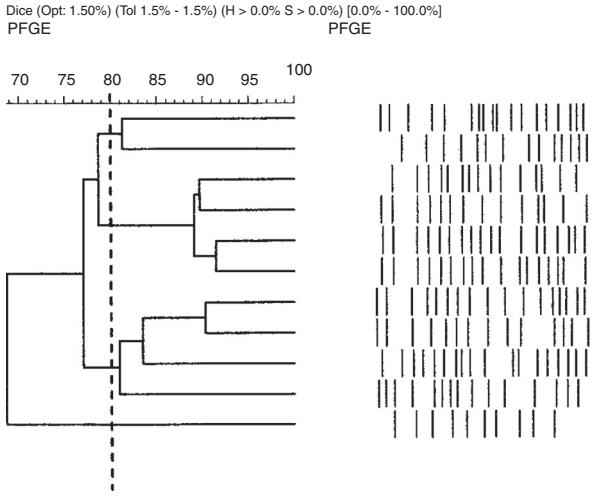
Fig. 2 – Clinical data and molecular typing of CTX-M-producing *E. cloacae*.

that time. Usually, in *K. pneumoniae* and *E. coli*, species, the CTX-M-15 has been reported as the dominant type of CTX-M in many countries causing nosocomial and community acquired infections by both clonal and polyclonal spread.[2–4,20] In *E. cloacae*, CTX-M-15 genes have been described since 2004,[21] but information about the molecular epidemiology of CTX-M-15 producing isolates in this specie is scarce.

Although in South America the CTX-M-2 has been the most prevalent type of ESBL detected,[9] CTX-M-15 was reported in 2004 among fecal *E. coli* isolates from Peru and Bolivia,[22] later Colombia,[23] and recently in Argentina.[24] In Brazil, the occurrence of CTX-M-15 was first reported in one *E. coli* clinical isolate (in 2006) from a private hospital of São Paulo State,[10] and later in *K. pneumoniae* isolates in a teaching hospital also in the state of São Paulo with high coefficient of similarity (>86.5) among isolates, indicating a close genetic relationship.[11] In Rio de Janeiro state, the $bla_{\text{CTX-M-15}}$ gene was found in *E. coli* isolates associated both to a dominant clone (ST 410), and ST131 (two isolates), which is spread worldwide.[12]

The presence of KPC has already been described worldwide,[25] including reports from Brazil.[16,19,26,27] In our isolates, the majority of KPC producing isolates carried also the $bla_{\text{CTX-M-15}}$ gene. The frequent association of CTX-M with KPC in *K. pneumoniae* in this and other studies suggests the acquisition of transmissible plasmids carrying $bla_{\text{KPC-2}}$ gene by local endemic strains harboring the $bla_{\text{CTX-M-15}}$ gene. Among the KPC producing isolates the dominant pulsotype (pulsotype A) was found, belonging to ST437, which is disseminated in five Brazilian States.[19,28,29] This ST437 belonged to clonal complex (CC) 11, which is an important complex because it also includes two STs (ST258 and ST11) that have a prominent role in the spread of the bla_{KPC} gene. The ST11 has been reported in Hungary, Spain and China[2,3,30] and has also been extensively associated with CTX-M-15 and CTX-M-14.[6]

The bla_{KPC} gene was not detected in the nine isolates of *E. cloacae* resistant to ertapenem, perhaps due to other possible resistant mechanisms, such as an association between CTX-M production and porin loss that are also



Isolate	Year	Hospital	CTX-M	Pulsotype
4345CCBH	2008	H3	CTX-M-15	A
3694CCBH	2007	H5	CTX-M-8 Group	A
4244CCBH	2008	H3	CTX-M-15	B
4155CCBH	2008	H2	CTX-M-15	B
4226CCBH	2008	H1	CTX-M-15	B
3912CCBH	2008	H5	CTX-M-15	B
4631CCBH	2008	H3	CTX-M-15	C
3822CCBH	2007	H2	CTX-M-8 Group	C
4630CCBH	2008	H3	CTX-M-2 Group	C
3995CCBH	2008	H1	CTX-M-8 Group	C
4158CCBH	2008	H2	CTX-M-8 Group	D

Fig. 3 – Clinical data and molecular typing of CTX-M-producing *E. coli*.

responsible for decreased susceptibility to carbapenem, as previously reported.[31]

Although more studies need to be conducted to clarify some questions, this study shows that the increased frequency of CTX-M-15 producing isolates may have been influenced by both multiple clones and/or several mobile genetic elements.

In conclusion, this study provides additional information of the epidemiological scenario of CTX-M-types among *Enterobacteriaceae* isolates circulating in Brazilian hospitals in 2007–2008. This data reinforce the need for continuing surveillance because this scenario may have changed over the years.

Financial support

We thank by financial support for Fundação de Amparo à Pesquisa do Rio de Janeiro (FAPERJ), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Organização Pan-Americana de Saúde (OPAS), Agência Nacional de Vigilância Sanitária (ANVISA).

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

We thank the platform Genomic for DNA sequencing do Programa de Desenvolvimento Tecnológico em Insumos para Saúde (PDTIS) do Instituto Oswaldo Cruz and all the contributing laboratories that provided isolates for this study.

REFERENCES

1. Canton R, Coque TM. The CTX-M beta-lactamase pandemic. Curr Opin Microbiol. 2006;9:466–75.
2. Damjanova I, Tóth A, Pászti J, et al. Expansion and countrywide dissemination of ST11, ST15 and ST147 ciprofloxacin-resistant CTX-M-15-type β-lactamase-producing *Klebsiella pneumoniae* epidemic clones in Hungary in 2005-the new 'MRSAs'? J Antimicrob Chemother. 2008;62:978–85.
3. Oteo J, Cuevas O, López-Rodriguez I, et al. Emergence of CTX-M-15-producing *Klebsiella pneumoniae* of multilocus sequence types 1,11, 14, 17, 20, 35 and 36 as pathogens and colonizers in newborns and adults. J Antimicrob Chemother. 2009;64:524–8.
4. Peirano G, Pitout JD. Molecular epidemiology of *Escherichia coli* producing CTX-M β-lactamases: the worldwide emergence of clone ST131 O25:H4. Int J Antimicrob Agents. 2010;35:316–21.
5. Nicolas-Chanoine MH, Blanco J, Leflon-Guibout V, et al. Intercontinental emergence of *Escherichia coli* clone O:25 H4-ST131 producing CTX-M-15. J Antimicrob Chemother. 2008;6:273–81.
6. Ko KS, Lee JY, Baek JY, et al. Predominance of an ST11 ESBL-producing *Klebsiella pneumoniae* clone causing bacteraemia and urinary tract infections in Korea. J Med Microbiol. 2010;59:822–8.
7. Clímaco EC, Minarini LAR, Darini ALC. CTX-M-producing *Klebsiella* spp. in a Brazilian hospital: what has changed in 6 years? Diagn Microbiol Infect Dis. 2010;68:186–9.
8. Minarini LAR, Poirel L, Trevisani NAC, Darini ALC, Nordmann P. Predominance of CTX-M-type extended-spectrum β-lactamase genes among enterobacterial isolates from outpatients in Brazil. Diagn Microbiol Infect Dis. 2009;65:202–6.
9. Villegas MV, Kattan JN, Quinteros MG, Cassellas JM. Prevalence of extended-spectrum beta-lactamases in South America. Clin Microbiol Infect. 2008;14:154–8.
10. Cergole-Novella MC, Guth BEC, Catanheira M, Carmo MS, Pignatari CC. First description of $bla_{CTX-M-14}$- and $bla_{CTX-M-15}$-producing *Escherichia coli* isolates in Brazil. Microb Drug Resist. 2010;16:177–84.
11. Tollentino FM, Polotto M, Nogueira ML, et al. High prevalence of bla_{CTX-M} extended-spectrum beta-lactamase genes in *Klebsiella pneumoniae* isolates from a Terciary Care Hospital: first report of bla_{SHV-12}, bla_{SHV-31}, bla_{SHV-38}, and $bla_{CTX-M-15}$ in Brazil. Microb Drug Resist. 2011;17:7–16.
12. Peirano G, Asensi MD, Pitondo-Silva A, Pitout JD. Molecular characteristics of extended-spectrum β-lactamase-producing *Escherichia coli* from Rio de Janeiro, Brazil. Clin Microbiol Infect. 2011;17:1039–43.
13. [CLSI] Clinical and Laboratory Standards Institute. M02-A11 performance standards for antimicrobial disk susceptibility tests. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.
14. [CLSI] Clinical and Laboratory Standards Institute. M100-S21 performance standards for antimicrobial susceptibility testing, 21st informational supplement. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.
15. Jarlier V, Nicolas MH, Fournier G, Philippon A. Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in *Enterobacteriaceae*: Hospital prevalence and susceptibility patterns. Rev Infec Dis. 1988;10:867–78.
16. Peirano G, Seki LM, Val Passos VL, Pinto MC, Guerra LR, Asensi MD. Carbapenem-hydrolysing β-lactamase KPC-2 in *Klebsiella pneumoniae* isolated in Rio de Janeiro, Brazil. J Antimicrob Chemother. 2009;63:265–8.
17. Mendonça N, Leitão J, Manageiro V, Ferreira E, Caniça M. Spread of extended-spectrum β-lactamase CTX-M-producing *Escherichia coli* clinical isolates in community and nosocomial environments in Portugal. Antimicrob Agents Chemother. 2007;51:1946–55.
18. Otto TD, Vasconcellos EA, Gomes LH, et al. ChromaPipe: a pipeline for analysis, quality control and management for a DNA sequencing facility. Genet Mol Res. 2008;7: 861–71.
19. Seki LM, Pereira PS, De Souza MPAH, et al. Molecular epidemiology of KPC-2-producing *Klebsiella pneumoniae* isolates in Brazil: the predominance of sequence type 437. Diagn Microbiol Infect Dis. 2011;70:274–7.
20. Oteo J, Pérez-Vasquez M, Campos J. Extended-spectrum β-lactamase producing *Escherichia coli*: changing epidemiology and clinical impact. Curr Opinion Infec Dis. 2010;23: 320–6.
21. Paterson DL, Bonomo RL. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev. 2005;18:657–86.
22. Pallecchi I, Malossi M, Mantella A, et al. Detection of CTX-M-type β-lactamase genes in fecal *Escherichia coli* isolates from healthy children in Bolivia and Peru. Antimicrob Agents Chemother. 2004;48:4551–6.
23. Valenzuela de Silva EM, Mantilla Anaya JR, Reguero Reza MT, et al. Detection of CTX-M-1, CTX-M-15 and CTX-M-2 in clinical isolates of Enterobacteriaceae in Bogota, Colombia. J Clin Microbiol. 2006;44:1919–20.

24. Sennati S, Santella G, Di Conza J, et al. Changing epidemiology of extended-spectrum β-lactamases in Argentina: emergence of CTX-M-15. Antimicrob Agents Chemother. 2012, doi: 10.1128/AAC. 00745-12.
25. Cuzon G, Naas T, Truong H, et al. Worldwide diversity of *Klebsiella pneumoniae* that produces β-lactamase bla_{KPC-2} gene. Emerg Infect Dis. 2010;16:1349–56.
26. Andrade LN, Curiao M, Clímaco EC, et al. Dissemination of bla_{KPC-2} by the spread of CC258-*Klebsiella pneumoniae* clones (ST258, ST11, ST437) and plasmids (IncFII, IncN, IncL/M) among *Enterobacteriaceae* in Brazil. Antimicrob Agents Chemother. 2011;55:3579–83.
27. Monteiro J, Santos AF, Asensi MD, Peirano G, Gales AC. First report of KPC-2-producing *Klebsiella pneumoniae* strains in Brazil. Antimicrob Agents Chemother. 2009;53:333–4.
28. Nicolet AG, Fehberg LCC, Picao RC, Machado AO, Gales AC. Clonal complex 258, the most frequently found multilocus sequence type complex in KPC-2-producing *Klebsiella pneumoniae* isolate in Brazilian Hospitals. Antimicrob Agents Chemother. 2012;56:4563.
29. Pereira PS, de Araujo CF, Seki LM, Zahner V, Carvalho-Assef AP, Asensi MD. Update of the molecular epidemiology of KPC-2-producing *Klebsiella pneumoniae* in Brazil: spread of clonal complex 11 (ST11, ST437 and ST 340). J Antimicrob Chemother. 2012, doi:10.193/jac/dks396.
30. Qi Y, Wei Z, Ji S, Du X, Shen P, Yu Y. ST11, the dominant clone of KPC-producing *Klebsiella pneumoniae* in China. J Antimicrob Chemother. 2011;66:307–12.
31. Yang D, Guo Y, Zhang Z. Combined porin loss and extended spectrum beta-lactamase production is associated with an increasing imipenem minimal inhibitory concentration in clinical *Klebsiella pneumoniae* strains. Curr Microbiol. 2009;58:366–70.