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Brief Original Article

Complex class 1 integrons harboring CTX-M-2-encoding genes in clinical *Enterobacteriaceae* from a hospital in Brazil

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Abstract

Introduction: CTX-M enzymes are the most prevalent extended-spectrum beta-lactamases (ESBLs) in Brazil and around the world. The spread of CTX-M lies in their ability to be mobilized by insertion sequences and integrons. This study aimed to identify the mobile genetic structures associated with bla_{CTX-M} genes from clinical *Enterobacteriaceae* strains.

Methodology: Twenty-eight clinical non-clonal *Enterobacteriaceae* were screened by PCR for the presence of bla_{CTX-M} genes and class 1 integrase (*int1*), and for the association of bla_{CTX-M} with class 1 integrons. Plasmid incompatibility groups were assessed by PBRT. Wild-type plasmids were transformed into electrocompetent *E. coli*, and the S1-PFGE technique was used to verify the presence of high-molecular-weight plasmids in both wild-type strains and *E. coli* transformants.

Results: Sequencing showed that strains carried $bla_{CTX-M-2}$ (n = 25) and $bla_{CTX-M-59}$ (n = 3) genes inserted into the 3'-end of complex class 1 integrons. Thirteen strains also carried bla_{TEM} and bla_{SHV} genes. CTX-M-2/59-containing complex class 1 integrons were also present in *E. coli* transformants. The most frequent Inc groups were IncA/C (n = 10) and IncF (n = 8). Heavy plasmids were observed in both wild-type strains and *E. coli* transformants.

Conclusions: The presence of the same $bla_{CTX-M-2-group}$-containing genetic structure in seven *Enterobacteriaceae* species isolated at seven hospital wards shows the great mobility potential of complex class 1 integrons. Also, this is the first report of TEM-15, SHV-45, and SHV-55 in Latin America. The genetic environment of $bla_{CTX-M-2}$ accounts for their maintenance and spread among Gram-negative bacteria.

Key words: complex class 1 integron; CTX-M-2; CTX-M-59; *Enterobacteriaceae*; Brazil.

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Introduction

According to the last Latin American data reported by the SENTRY Antimicrobial Surveillance Program, extended-spectrum beta-lactamase (ESBL) production rates in *E. coli* and *K. pneumoniae* from Brazil are 12.8% and 49.9%, respectively [1]. Among ESBL families, TEM, SHV, and CTX-M are the most reported around the world, and in Latin America, SHV and CTX-M variants are the most frequent [2-4]. In Brazil, there is no report of TEM enzymes that express an ESBL phenotype, while some SHV ESBLs are frequently reported (SHV-5, SHV-12) [5-6]. CTX-M enzymes are spread worldwide, and in Latin America, they are considered endemic and play an important role in the resistance landscape in Brazil, where several reports are continuously being published [5-14]. There are five main clusters of CTX-M beta-lactamases: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25, and except for the last one, all of them have already been described in strains from Brazil. The most frequent encoding genes reported in our region are $bla_{CTX-M-15}$ [6,12] from the CTX-M-1 cluster, $bla_{CTX-M-2}$ [6-14] and $bla_{CTX-M-59}$ [5,13] from the CTX-M-2 cluster, $bla_{CTX-M-8}$ [8] from the CTX-M-8 cluster, and $bla_{CTX-M-9}$ [10,12-13] from the CTX-M-9 cluster. The reason for the spread of CTX-M lies in their ability to be mobilized by genetic structures, especially insertion sequences and integrons. CTX-M-2 group of enzymes, particularly, are encoded by genes frequently located in complex class 1 integrons, which are the result of the fusion of the insertion sequence IS*CR1* to the 3'CS of class 1 integrons, a combinatorial event that allows the structure to become mobile at high frequency rates, and capable of moving great portions of DNA [15]. Additionally, these structures are frequently mobilized to conjugative plasmids that facilitate their spread. Some resistance-associated plasmid incompatibility (Inc) groups have been considered epidemic due to their presence in bacteria from different sources and

countries, and the most common Inc groups carrying ESBL-encoding genes are IncF, IncA/C, IncL/M, IncI1, and IncH12 [16].

The aim of this study was to characterize and map the mobile genetic structures associated with bla_{CTX-M} genes from clinical *Enterobacteriaceae* strains, and to determine if the genes were located in plasmids or in the bacterial chromosome.

Methodology

Twenty-eight non-clonal ESBL-producing *Enterobacteriaceae* strains, previously isolated [17] in a hospital set and representing, according to the institution's infection control committee, hospital infections (n = 9), community-acquired infections (n = 4), and colonization strains (n=15), were selected based on their distinct PFGE profiles and the detection of bla_{CTX-M} genes by polymerase chain reaction (PCR) [18]. These non-clonal strains were selected in order to demonstrate the mobility and spread potential of CTX-M-encoding genes even when there is no clonal dissemination. Thirteen of the 28 strains also carried other ESBL genes from TEM and SHV families (Table 1). The methodology described below was performed for all isolates. Minimum inhibitory concentrations (MICs) were determined by Etest strips (AB Biodisk, Solna, Sweden) for cefoxitin, cefotetan, ceftazidime, cefotaxime, aztreonam, cefepime, amoxicillin-clavulanate, piperacillin-tazobactam, meropenem, and ertapenem, according to the manufacturer's instructions. Results were interpreted based on Clinical and Laboratory Standards Institute (CLSI) 2014 guidelines [19]. To search for CTX-M-encoding genes from clusters CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25, PCR for each cluster were separately carried out with a set of primers designed for this study (Table 2). Positive controls were included in each reaction (Table 2), and all PCR products were sequenced in order to validate the results. The PCR mixture was submitted to a denaturation step of 5 minutes at 94°C, followed by 30 cycles of amplification (45 seconds of denaturation at 94°C, 1 minute of annealing at 60°C, 1 minute of elongation at 72°C), and 10 minutes at 72°C for final elongation. A primary screening of class 1 integrases was carried out by PCR using previously described primers for *intI1* gene amplification [20]. The association of bla_{CTX-M} with integrons was assessed using reverse primers for *intI1* genes with reverse primers for bla_{CTX-M} genes. Also, the 3'-ends of complex class 1 integron structures were amplified using a forward primer for *sul1* gene and a reverse primer for *qacEΔ1* gene [21], under the same conditions described above, except for a seven-minute elongation in each cycle. PCR products were purified and directly sequenced, including the previously amplified bla_{TEM} and bla_{SHV} genes [17]. For the incompatibility group determination, plasmids were extracted by alkaline lysis (Wizard *Plus* SV Minipreps, Promega, Fitchburg, USA) followed by alcohol precipitation, and PCR-based replicon typing (PBRT) was carried out according to the methodology described by Carattoli *et al.* [22], which identifies replicons that represent 18 main *Enterobacteriaceae* incompatibility groups. Transformation experiments using One Shot TOP 10 Electrocomp *E. coli* (Invitrogen, Waltham, USA) as recipients were carried out, and transformed cells were selected in Luria agar enriched with 4 µg/mL of cefotaxime. PCR experiments that resulted positive for wild-type strains were also carried out for cefotaxime-resistant transformants. Plasmid sizes were determined by S1-PFGE technique: wild-type strains and transformants were grown overnight in 30 mL Luria broth containing cefotaxime 4 µg/mL. Cells were pelleted and resuspended in 400 µL TE buffer (Tris-HCl 10 mM, pH 7.5; EDTA 1 mM, pH 8.0), and then added to a microtube containing low-melting PFGE agarose (Bio-Rad, Hercules, USA), in a proportion of 1:4. Plug molds were filled with the cell-agarose mix and left at 4°C for solidification. Plugs were then put into a 5 mL lysis solution (EDTA 50 mM, pH 8.0; Tris-HCl 50 mM, pH 7.5; SDS 1%, Sarkosyl 1%, proteinase K 20 mg/mL), and incubated at 55°C for 2 hours. Then, plugs were subjected to two ultrapure water washes at 55°C for 15 minutes, followed by 4 TE buffer washes in the same conditions. Plugs were kept in TE buffer at 4°C. For the linearization of plasmids, plugs were digested with 10 U of S1 nuclease at 37°C for 45 minutes [23]. After that, samples were subjected to pulsed field gel electrophoresis, for 20 hours at 6 V/cm, pulses ranging from 1 second to 40 seconds. PFGE gels were stained with ethidium bromide and visualized at UV light (Epi Chemi II Darkroom, UVP Bioimaging Systems, Upland, USA).

Table 1. Summary of strain characteristics and most relevant results

Strain ID	Bacterial species	Wards, samples	MIC (µg/mL)										ESBL	Inc groups	Plasmids (kb)
HOSPITAL INFECTIONS															
			FOX	**CTT**	**CAZ**	**CTX**	**ATM**	**FEP**	**AMC**	**PIP**	**MEM**	**ERT**			
209/05	*Morganella morganii*	AMW Urine	>256 R	8 S	2 S	>256 R	>256 R	>256 R	>256 R	1.5 S	0.125 S	0.047 S	CTX-M-2	A/C	179
211/05	*Klebsiella pneumoniae*	AMW Blood	6 S	0.25 S	6 R	64 R	12 R	32 R	3 S	2 S	0.125 S	0.19 S	CTX-M-2 SHV-28	A/C	112/194
237/05	*Klebsiella pneumoniae*	IMC Urine	3 S	0.125 S	24 R	>256 R	>256 R	64 R	12 R	4 S	0.032 S	0.25 S	CTX-M-59 SHV-110	Not typeable	No
246/05	*Escherichia coli*	ER Urine	6 S	0.19 S	6 R	>256 R	>256 R	>256 R	6 R	2 S	0.023 S	0.047 S	CTX-M-2 SHV-5	I1 F FIB P	82/145.5
262/05	*Klebsiella pneumoniae*	NW urine	3 S	0.38 S	256 R	24 R	>256 R	2 S	4 S	32 R	0.023 S	0.19 S	CTX-M-2 SHV-5	F	48.5/97/194
306/05	*Escherichia coli*	SW Urine	4 S	0.25 S	48 R	6 R	64 R	0.75 S	8 R	2 S	0.023 S	0.023 S	CTX-M-2 SHV-5	H11	130.5/209
318/05	*Providencia stuartii*	ICU Bronchoal veolar lavage	24 R	0.38 S	2 S	48 R	4 S	64 R	48 R	6 S	0.094 S	0.094 S	CTX-M-2	A/C W	160.5/339.5
326/05	*Escherichia coli*	ICU Bronchoal veolar lavage	6 S	0.38 S	2 S	>256 R	12 R	24 R	6 R	3 S	0.023 S	0.047 S	CTX-M-2	F FIB	145.5
328/05	*Escherichia coli*	ICU Bronchoal veolar lavage	6 S	0.19 S	4 S	>256 R	32 R	192 R	6 R	4 S	0.016 S	0.023 S	CTX-M-2	F FIB	145.5
COMMUNITY-ACQUIRED INFECTIONS															
			FOX	**CTT**	**CAZ**	**CTX**	**ATM**	**FEP**	**AMC**	**PIP**	**MEM**	**ERT**			
314/05	*Escherichia coli*	ER Urine	8 S	0.38 S	8 R	>256 R	>256 R	>256 R	8 R	12 S	0.047 S	0.19 S	CTX-M-2	H11 P	97/242.5
317/05	*Klebsiella pneumoniae*	ER Blood	24 R	1.5 S	>256 R	>256 R	>256 R	>256 R	12 R	>256 R	0.125 S	0.5 S	CTX-M-2 SHV-28 SHV-55	A/C	63.5/112/209
324/05	*Klebsiella oxytoca*	ICU Ascitic fluid	48 R	2 S	32 R	>256 R	192 R	>256 R	12 R	>256 R	0.064 S	0.38 S	CTX-M-2 SHV-28	FIIs H11	145.5/242.5/388
325/05	*Morganella morganii*	ER Urine	96 R	3 S	0.5 S	256 R	4 S	0.75 S	64 R	0.75 S	0.064 S	0.012 S	CTX-M-2	Not typeable	No

Table 1 *(continued)*. Summary of strain characteristics and most relevant results

Strain ID	Bacterial species	Wards, samples	MIC (µg/mL)										ESBL	Inc groups	Plasmids (kb)
COLONIZATIONS															
			FOX	**CTT**	**CAZ**	**CTX**	**ATM**	**FEP**	**AMC**	**PIP**	**MEM**	**ERT**			
201/05	*Proteus mirabilis*	IMC Inguinal fold	4 S	0.25 S	1.5 S	>256 R	8 R	>256 R	4 S	0.38 S	0.032 S	0.032 S	CTX-M2	A/C FIC	179
202/05	*Klebsiella pneumoniae*	IMC Inguinal fold	4 S	0.125 S	3 S	32 R	12 R	0.094 S	6 R	8 S	0.023 S	0.016 S	CTX-M-2 SHV-28	Not typeable	48.5/145.5/<291
205/05	*Escherichia coli*	ICU Eschar	16 R	2 S	24 R	>256 R	96 R	>256 R	6 R	>256 R	0.023 S	0.38 S	CTX-M-2	F FIB H12 P Y	48.5/82/97/242.5/291
208/05	*Klebsiella pneumoniae*	ICU Tracheal fluid	3 S	0.19 S	8 R	128 R	32 R	64 R	8 R	6 S	0.047 S	0.19 S	CTX-M-2 SHV-27 SHV-45	A/C	97/145.5
220/05	*Klebsiella pneumoniae*	ER Urine	12 R	3 S	8 R	>256 R	96 R	256 R	12 R	>256 R	0.25 S	3 R	CTX-M-2 SHV-12	A/C	48.5/97/145.5/209
248/05	*Klebsiella pneumoniae*	NW Stool	8 S	2 S	16 R	>256 R	>256 R	32 R	32 R	>256 R	0.19 S	1 R	CTX-M-59 SHV-27	A/C	160.5/209
276/05	*Klebsiella pneumoniae*	P.ICU Tracheal fluid	12 R	0.75 S	4 S	>256 R	48 R	128 R	16 R	>256 R	0.094 S	0.25 S	CTX-M-59 SHV-12	A/C	82/145.5/160.5
253/05	*Klebsiella pneumoniae*	ICU Urine	32 R	1.5 S	24 R	>256 R	96 R	32 R	12 R	>256 R	0.094 S	1.5 R	CTX-M-2	Not typeable	>339.5
259/05	*Klebsiella pneumoniae*	ICU Eschar	32 R	1.5 S	6 R	128 R	>256 R	48 R	6 R	>256 R	0.047 S	0.5 S	CTX-M-2	Not typeable	>339.5
264/05	*Klebsiella pneumoniae*	ICU Axillary fold	12 R	0.75 S	64 R	>256 R	>256 R	256 R	12 R	256 R	0.64 S	5 R	CTX-M-2 SHV-110 TEM-15	Not typeable	130.5/291
265/05	*Escherichia coli*	IMC Bone fragment	16 R	3 S	6 R	>256 R	96 R	48 R	12 R	>256 R	0.64 S	0.25 S	CTX-M-2	A/C FIA FIB	<97/160.5/339.5
269/05	*Klebsiella pneumoniae*	ICU Urine	3 S	0.25 S	8 R	128 R	24 R	16 R	8 R	4 S	0.064 S	0.125 S	CTX-M-2	K	194/436.5
308/05	*Morganella morganii*	ER Urine	>256 R	16 S	64 R	96 R	16 R	6 SDD	>256 R	24 R	0.25 S	0.094 S	CTX-M-2	Not typeable	No
316/05	*Klebsiella pneumoniae*	AMW Eschar	8 S	1 S	128 R	>256 R	>256 R	64 R	16 R	>256 R	0.047 S	0.25 S	CTX-M-2	Not typeable	242.5
332/05	*Morganella morganii*	ER Urine	>256 R	16 S	3 S	>256 R	24 R	192 R	>256	2 S	0.19 S	0.125 S	CTX-M-2	Not typeable	No

Table 1 ***(continued)*****.** Summary of strain characteristics and most relevant results

Strain ID	Bacterial species	Wards, samples	MIC (µg/mL)										ESBL	Inc groups	Plasmids (kb)
***E. coli* TRANSFORMANTS**															
Strain ID	**Wild-type strain of origin**		**MIC (µg/mL)**										**ESBL**	**Inc Groups**	**Plasmids (kb)**
			FOX	**CTT**	**CAZ**	**CTX**	**ATM**	**FEP**	**AMC**	**PIP**	**MEM**	**ERT**			
EC201	FSP201/05		4 S	0.25 S	6 R	>256 R	>256 R	>256 R	6 R	3 S	0.047 S	0.032 S	CTX-M-2	A/C	179
EC202	FSP202/05		0.38 S	0.125 S	4 S	>256 R	16 R	6 SDD	8 R	2 S	0.023 S	0.016 S	CTX-M-2	Not typeable	48.5
EC205	FSP205/05		6 S	1 S	32 R	>256 R	>256 R	>256 R	12 R	6 S	0.064 S	0.125 S	CTX-M-2	FIB	48.5/97/242.5/291
EC209	FSP209/05		6 S	2 S	16 R	>256 R	>256 R	>256 R	12 R	>256 R	0.047 S	0.094 S	CTX-M-2	A/C	179
EC246	FSP246/05		0.38 S	0.125 S	3 S	>256 R	12 R	4 SDD	4 S	2 S	0.016 S	0.016 S	CTX-M-2	Not typeable	82
EC248	FSP248/05		6 S	0.75	4 S	>256 R	12 R	6 SDD	12 R	>256 R	0.032 S	0.016 S	CTX-M-59	A/C	160.5
EC276	FSP276/05		6 S	0.38 S	96 R	>256 R	>256 R	>256 R	12 R	>256 R	0.094 S	0.125 S	CTX-M-59	A/C	160.5
EC317	FSP317/05		6 S	0.19 S	16 R	2 R	48 R	0.5 S	4 S	2 S	0.012 S	0.012 S	CTX-M-2	Not typeable	64
EC326	FSP326/05		6 S	0.38 S	4 S	>256 R	24 R	48 R	12 R	>256 R	0.125 S	0.19 S	CTX-M-2	FIB	160.5

MIC: minimum inhibitory concentration; ER: emergency room; SW: surgical ward; AMW: adult medical ward; PW: pediatric ward; AICU/NICU/PICU: adult/neonatal/pediatric intensive care units; IMC: intermediate care unit; NW: neonatal ward; FOX: cefoxitin; CTT: cefotetan; CAZ: ceftazidime; CTX: cefotaxime; ATM: aztreonam; FEP: cefepime; AMC: amoxicillin/clavulanate; PIP: piperacillin/tazobactam; MEM: meropenem; ERT: ertapenem; R: resistant or non-susceptible; S: susceptible; SDD: susceptible dose-dependent [15]

Table 2. Primers designed for this study

Primer	5'-3' Sequence	Target	Product (bp)	Positive controls[a]
CTX-M-1 F CTX-M-1 R	AAATCACTGCGYCAGTTCA GGTGACGATTTTAGCCGCCG	$bla_{\text{CTX-M-1}}$	854	FSP1271/09
CTX-M-2 F CTX-M-2 R	GACTCAGAGCATTCGCCGC TCAGAAACCGYGGGTTACGA	$bla_{\text{CTX-M-2}}$	870	FSP309/05
CTX-M-8 F CTX-M-8 R	GATGAGACATCGCGTTAAG GGTGACGATTTTCGCGGCA	$bla_{\text{CTX-M-8}}$	861	FSP1312/09
CTX-M-9 F CTX-M-9 R	TGACAAAGAGARTGCAACGG CGATGATTCTCGCCGCTGAA	$bla_{\text{CTX-M-9}}$	857	FSP339/05
CTX-M-25 F CTX-M-25 R	ATGAGAAAAAGCGTAAGGCGGG CCGTCGGTGACWATTCTG	$bla_{\text{CTX-M-25}}$ and other $bla_{\text{CTX-M-}}$	865	FSP164/14

[a]Positive control strains belong to the Public Health School Laboratory Culture Collection from the Public Health School, University of São Paulo.

Results

Table 1 summarizes most of the present study's results. The highest MIC values expressed by both wild-type strains and transformants were for cefotaxime, cefepime, aztreonam, and piperacillin-tazobatam. PCR results showed that all strains carried CTX-M-2-group encoding genes, which were identified by sequencing as $bla_{\text{CTX-M-2}}$ (n = 25) and $bla_{\text{CTX-M-59}}$ (n = 3). All strains possessed class 1 integrases, and positive *sul1-qacEΔ1* amplicons (4.5 kb) showed the association of $bla_{\text{CTX-M-2-group}}$ genes with complex class 1 integrons, which was confirmed by sequencing analysis. The genetic structure found was the insertion of $bla_{\text{CTX-M-2}}$ or $bla_{\text{CTX-M-59}}$ into the 3'-end of a complex class 1 integron, flanked upstream by *sul1* and IS*CR1* and downstream by a sequence having high homology to the *Kluyvera* spp. chromosome and the *orf3::qacEΔ1* fusion gene (Figure 1). Regarding the incompatibility groups, the most common was IncA/C, which was present in ten (36%) strains, followed by IncF-like groups, found in eight (29%) strains. Nine isolates (32%) were not typeable by PBRT due to the limitations presented by this technique, which detects the 18 most common replicons that represent the main incompatibility groups in *Enterobacteriaceae* species, therefore not detecting less frequent or non-characterized groups [22]. Transformation assays resulted in nine *E. coli* transformants that received plasmids extracted from *E. coli*, *K. pneumoniae*, *M. morganii*, and *P. mirabilis*. PCR and sequencing confirmed the presence of the CTX-M-2-group-containing complex class 1 integrons structures in all transformants. TEM and SHV-encoding genes were not co-transferred with $bla_{\text{CTX-M-2-group}}$. S1-PFGE results (Figures 2 and 3, Table 1) showed that all but two wild-type strains possessed high-molecular-weight plasmids, ranging from 48.5 kb to 436.5 kb. This technique also showed that, except for strain 205/05, which transferred four plasmids to its respective transformant EC205, only one plasmid was transferred to each *E. coli* transformant, which harbored plasmids sizing from 48.5 kb to 179 kb that carried the $bla_{\text{CTX-M-2-group}}$-containing complex class 1 integrons.

Discussion

CTX-M enzymes are spread worldwide, and the CTX-M-2 group is frequently reported in Brazil and other Latin American countries [2-4]. In the present study, $bla_{\text{CTX-M-2-group}}$ genes were found disseminated through 28 non-clonal *Enterobacteriaceae* strains from seven different species, isolated from patients at

Figure 1. Complex class 1 integron containing $bla_{\text{CTX-M-2-group}}$ genes

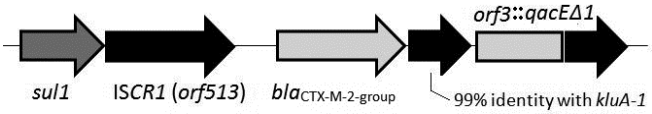
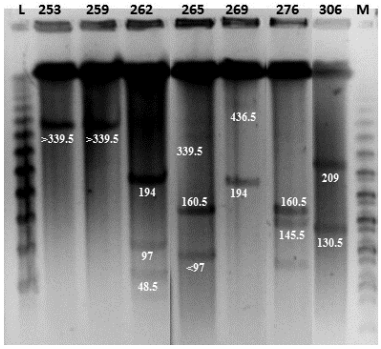
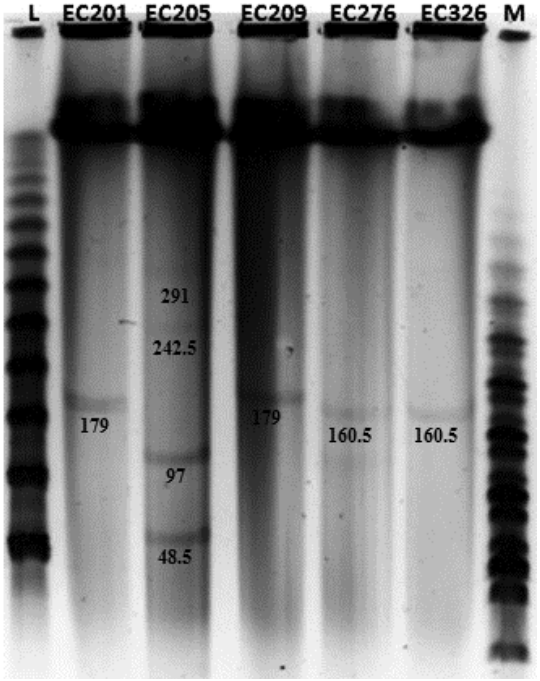


Figure 2. PFGE agarose gel containing seven from the 28 strains selected for the study after total DNA extraction followed by S1 digestion for plasmid linearization.



L: Lambda PFG Marker (New England BioLabs [NEB], Ipswich, USA), bands ranging from 48.5 kb to 727.5 kb; **253**, **259**, **262**, **265**, **269**, **276**, and **306:** wild-type strains after S1 nuclease digestion; **M:** MidRange PFG Marker I (NEB), bands ranging from 15 kb to 242.5 kb.

Figure 3. PFGE agarose gel containing five of the nine CTX-M-2-group-producing *E. coli* transformants after total DNA extraction followed by S1 digestion for plasmid linearization.



L: Lambda PFG Marker (NEB), bands ranging from 48.5 kb to 727.5 kb; **EC201**, **EC205**, **EC209**, **EC276**, and **EC326:** *E. coli* transformants after S1 nuclease digestion; **M:** MidRange PFG Marker I (NEB), bands ranging from 15 kb to 242.5 kb.

seven distinct wards of a healthcare institution. Also, $bla_{\text{CTX-M-2-group}}$ genes were found to be associated with TEM and SHV-encoding genes, and this is the first report of TEM-15, SHV-45, and SHV-55 in Latin America. The high mobility rate of $bla_{\text{CTX-M-2}}$ genes among Gram-negative bacteria, as well as the exposure of strains to a severe antimicrobial selective pressure in the hospital, are the probable reasons for the spread of these genes. Besides $bla_{\text{CTX-M-2}}$, another CTX-M-2-group variant, $bla_{\text{CTX-M-59}}$, was also detected herein, in association with $bla_{\text{SHV-12}}$, $bla_{\text{SHV-27}}$, and $bla_{\text{SHV-110}}$. CTX-M-59 was first described in a *K. pneumoniae* outbreak in Brazil [5], and its spread in our country has been recently associated with IncA/C plasmids [13], which were detected in two of three CTX-M-59-producing strains in this study, and had also been transferred to CTX-M-59-producing *E. coli* transformants EC248 and EC276 (Table 1). The successful mobilization of complex class 1 integrons is mainly due to the presence of IS*CR1*, which can move big portions of DNA by its rolling circle mechanism [15,24-25]. This structure has already been reported in Brazil and other Latin American countries, and it is seriously implicated in the spread of CTX-M-2 enzymes in this region [26-27]. Brazilian strains carrying complex class 1 integrons that contain $bla_{\text{CTX-M-2}}$ genes inserted in their second variable region date from 2000, as described by Clímaco *et al*. [10], so these structures have been present among Brazilian isolates for at least 15 years. The CTX-M-2-producing strains selected for this study were isolated in 2004 and 2005, from patients hospitalized in seven different wards of an institution [17], and the presence of the same $bla_{\text{CTX-M-2-group}}$-containing genetic structure in 28 non-clonal *Enterobacteriaceae* isolates shows its spread potential. In addition, the CTX-M-2-containing complex class 1 integrons were not exclusively located in plasmids, so the integration properties of the structure into the bacterial chromosome also account for its maintenance among the strains within the environment. Also, the dissemination of these integrons was not due to a particular plasmid, as different plasmid sizes and PBRT patterns were found among wild-type strains and *E. coli* transformants. Therefore, it is possible that the spread of CTX-M-2 and CTX-M-59 through the hospital isolates had occurred mostly due to the mobilization of class 1 integrons to high-molecular-weight plasmids, which were frequent among the studied strains and may have been spread by conjugation events (Figures 2 and 3). Transformation experiments were carried out using cefotaxime (4 μg/mL) selection, so the probable location of $bla_{\text{CTX-M-2}}$ and $bla_{\text{CTX-M-59}}$ in those strains that did not yield any *E. coli* transformant (n = 19) would be the bacteria chromosome, which therefore could result in clonal dissemination of these genes. Regarding EC205, which received four high-molecular-weight plasmids from its parental strain FSP205/05, the high number of plasmids harbored by FSP205/05 (five plasmids) likely favored this transference.

As shown in Table 1, CTX-M-2-group-containing strains were present in nosocomial and community-acquired infections, as well as in colonizing bacteria. Therefore, the genetic environment of CTX-M-2-cluster encoding genes accounts for their maintenance and spread among Gram-negative bacteria present in healthcare environments.

Conclusions

CTX-M-2 cluster of enzymes are encoded by *bla* genes, which are commonly part of complex class 1 integrons, conferring these enzymes' success in both vertical and horizontal dissemination. The present work shows that these structures can be spread among several Enterobacteria species through high-molecular-weight plasmids; they can be located in the bacterial chromosome and have clonal spread potential, thus posing a challenge to the control of resistant infections in the hospital environment. Finally, the finding of the same genetic structure in bacteria isolated from several wards of an institution alerts for the need for reinforcement in hospital infection control measures to avoid the spread of these easily transferable resistance genes.

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