Letter to the Editor

Low-virulence phylogenetic background of CTX-M-producing *Escherichia coli* isolated from extraintestinal infections

Andyara L. Paiva[1], Nilton Lincopan[1,2], Ketrin C. Silva[1], Patrícia R. Neves[1], Andrea M. Moreno[3], John A. McCulloch[2,4], Claudete S. Astolfi-Ferreira[5], Antonio J. P. Ferreira[5]

[1]*Department of Microbiology, Institute of Biomedical Sciences, Universidade de São Paulo, São Paulo, Brazil*
[2]*Department of Clinical Analysis, School of Pharmacy, Universidade de São Paulo, São Paulo, Brazil*
[3]*Department of Preventive Medicine and Animal Health, College of Veterinary Medicine, Universidade de São Paulo, São Paulo, Brazil*
[4]*Faculty of Biotechnology, Institute of Biological Sciences, Universidade Federal do Pará, Belém-PA, Brazil*
[5]*Department of Pathology, College of Veterinary Medicine, Universidade de São Paulo, São Paulo, Brazil*

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Background

Escherichia coli can play either a commensal or parasitic relationship with humans, with the latter leading to intestinal or extra-intestinal (EI) infections [1]. Whereas commensal *E. coli* strains represent predominantly (low-virulence) phylogenetic groups A and B1, strains causing EI infections (which are known as ExPEC – Extraintestinal Pathogenic *E. coli*) have been shown to correlate to the high-virulence phylogroups B2 and D [1,2]. However, *E. coli* strains exhibiting low-virulence backgrounds, such as the commensal strains, have been isolated under pathogenic conditions [1,3], indicating that pathogenic commensals can cause EI infections when the bacterium gains access to a normally sterile body site, mainly in patients with susceptibility linked to underlying disease [1,3].

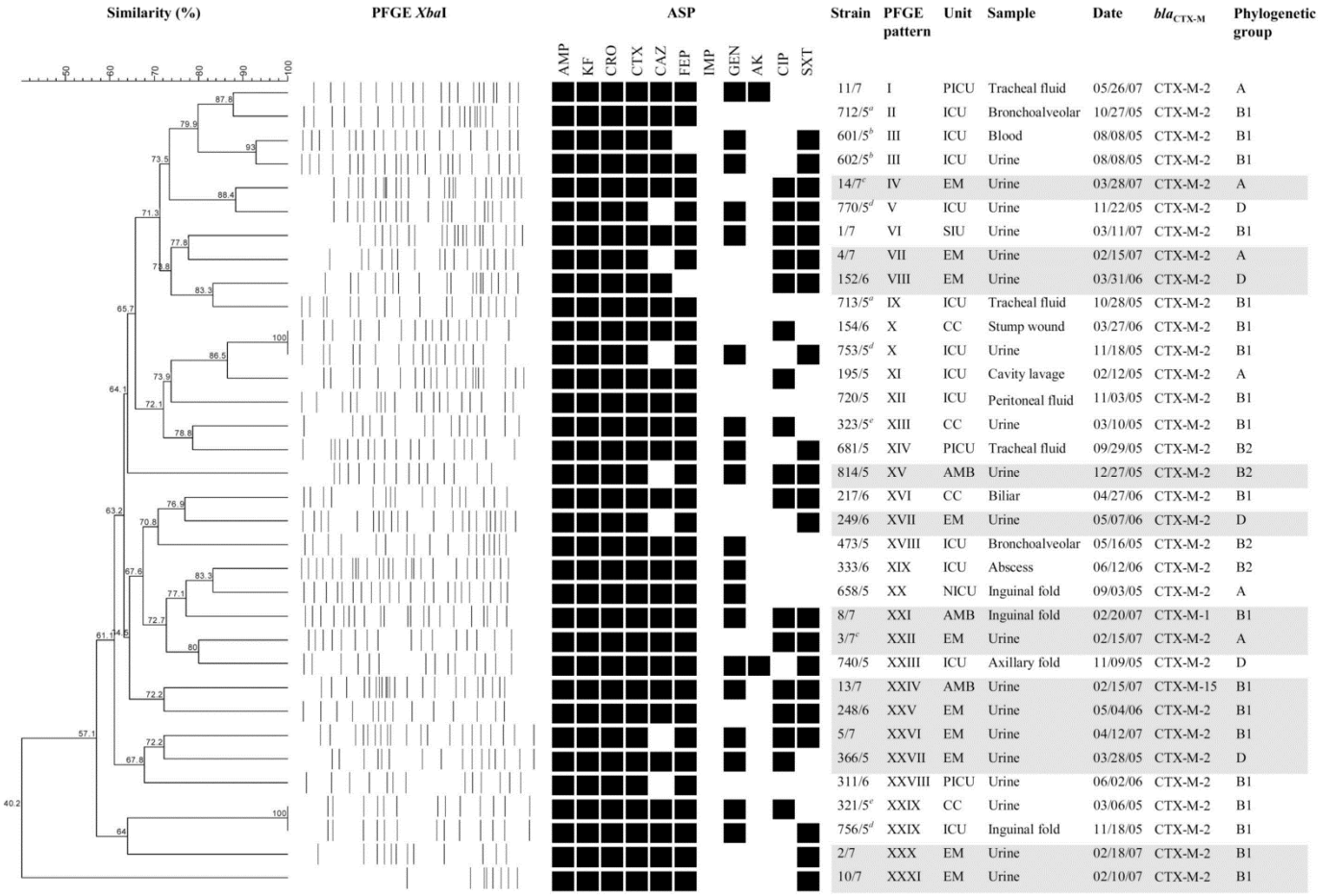
In hospital settings, the treatment of *E. coli* infections has been hindered by the emergence of antibiotic-resistant strains, with extended-spectrum β-lactamase (ESBL) production being of particular concern, because third-generation cephalosporins are the drugs of choice for the treatment of ExPEC [4].

In Brazil, there is a paucity of studies on phylogenetic analysis of commensal and pathogenic ESBL-producing *E. coli*. The aim of this study, therefore, was to characterize the extended-spectrum β-lactamase (ESBL) production, phylogenetic backgrounds, and the clonal relationship among *E. coli* strains isolated from EI infections in inpatients and outpatients admitted in a university hospital, in São Paulo, Southeastern Brazil.

The Study

From January 2005 to July 2007, a total of 34 *E. coli* strains exhibiting an ESBL phenotype were isolated from clinical samples obtained from 28 patients with EI in a single secondary care teaching hospital in São Paulo, Brazil. Antimicrobial susceptibility profiles were determined by Kirby-Bauer disk diffusion method [5], and ESBL production was investigated by using a double-disc synergy test and Etest ESBL strips (bioMeriéux, Marcy-l'Etoile, France). DNA amplification by PCR was used to search for $bla_{\text{CTX-M}}$-, bla_{TEM}-, bla_{SHV}-, bla_{GES}- and bla_{PER}-type ESBL genes [6], whereas phylogenetic typing (A, B1, B2 and D) was performed by means of a multiplex PCR reaction with phylogenetic markers, *chuA*, *yjaA*, and TspE4.C2 [2]. The genetic relatedness of the isolates was determined by PFGE of *Xba*I-digested genomic DNA using the protocol proposed by the National Molecular Subtyping Network (http://www.cdc.gov/pulsenet/protocols/ecoli_salmonella_shigella_protocols.pdf), and multilocus sequence typing (MLST) analysis was performed to characterize the genotype of CTX-M-15-producing *E. coli* (http://mlst.ucc.ie/mlst/dbs/Ecoli). PFGE patterns were

Figure 1. Dendrographic analysis of PFGE (*Xba*I-digested DNA) data for ESBL-producing *E. coli* isolates from extra-intestinal infections. Abbreviations: ASP, antibacterial susceptibility profile (black and white squares represent resistant and susceptible isolates, respectively); AMP, ampicillin; KF, cephalothin; CRO, ceftriaxone; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; IMP, imipenem; GEN, gentamicin; AK, amikacin; CIP, ciprofloxacin; SXT, sulfamethoxazole/trimethoprim. Units: PICU, Pediatric Intensive Care Unit; ICU, Intensive Care Unit; EM, Emergency room; SIU; Semi-Intensive Care Unit; CC, Surgical Clinic; AMB, Ambulatory Care; NICU, Neonatal Intensive Care Unit. Gray boxes identify isolates recovered from outpatients. Strains obtained from a same patient were identified by using superscript lowercase letters "*a*" to "*e*".



Strain	PFGE pattern	Unit	Sample	Date	bla_{CTX-M}	Phylogenetic group
11/7	I	PICU	Tracheal fluid	05/26/07	CTX-M-2	A
712/5[a]	II	ICU	Bronchoalveolar	10/27/05	CTX-M-2	B1
601/5[b]	III	ICU	Blood	08/08/05	CTX-M-2	B1
602/5[b]	III	ICU	Urine	08/08/05	CTX-M-2	B1
14/7[c]	IV	EM	Urine	03/28/07	CTX-M-2	A
770/5[d]	V	ICU	Urine	11/22/05	CTX-M-2	D
1/7	VI	SIU	Urine	03/11/07	CTX-M-2	B1
4/7	VII	EM	Urine	02/15/07	CTX-M-2	A
152/6	VIII	EM	Urine	03/31/06	CTX-M-2	D
713/5[a]	IX	ICU	Tracheal fluid	10/28/05	CTX-M-2	B1
154/6	X	CC	Stump wound	03/27/06	CTX-M-2	B1
753/5[d]	X	ICU	Urine	11/18/05	CTX-M-2	B1
195/5	XI	ICU	Cavity lavage	02/12/05	CTX-M-2	A
720/5	XII	ICU	Peritoneal fluid	11/03/05	CTX-M-2	B1
323/5[e]	XIII	CC	Urine	03/10/05	CTX-M-2	B1
681/5	XIV	PICU	Tracheal fluid	09/29/05	CTX-M-2	B2
814/5	XV	AMB	Urine	12/27/05	CTX-M-2	B2
217/6	XVI	CC	Biliar	04/27/06	CTX-M-2	B1
249/6	XVII	EM	Urine	05/07/06	CTX-M-2	D
473/5	XVIII	ICU	Bronchoalveolar	05/16/05	CTX-M-2	B2
333/6	XIX	ICU	Abscess	06/12/06	CTX-M-2	B2
658/5	XX	NICU	Inguinal fold	09/03/05	CTX-M-2	A
8/7	XXI	AMB	Inguinal fold	02/20/07	CTX-M-1	B1
3/7[c]	XXII	EM	Urine	02/15/07	CTX-M-2	A
740/5	XXIII	ICU	Axillary fold	11/09/05	CTX-M-2	D
13/7	XXIV	AMB	Urine	02/15/07	CTX-M-15	B1
248/6	XXV	EM	Urine	05/04/06	CTX-M-2	B1
5/7	XXVI	EM	Urine	04/12/07	CTX-M-2	B1
366/5	XXVII	EM	Urine	03/28/05	CTX-M-2	D
311/6	XXVIII	PICU	Urine	06/02/06	CTX-M-2	B1
321/5[e]	XXIX	CC	Urine	03/06/05	CTX-M-2	B1
756/5[d]	XXIX	ICU	Inguinal fold	11/18/05	CTX-M-2	B1
2/7	XXX	EM	Urine	02/18/07	CTX-M-2	B1
10/7	XXXI	EM	Urine	02/10/07	CTX-M-2	B1

analyzed using the Dice similarity coefficient and the unweighted-pair group method using average linkages cluster method (BioNumerics software, Applied Maths, Kortrijk, Belgium). PFGE clusters I to XXXI were assigned based on less than 90% similarity of banding patterns.

ESBL-positive *E. coli* strains, isolated mainly from urinary tract infections (56%), exhibited resistance to ampicillin (100%), cephalothin (100%), ceftriaxone (100%), cefotaxime (100%), ceftazidime (79%), cefepime (94%), ciprofloxacin (50%), sulfamethoxazole/trimethoprim (62%), gentamicin (56%) and amikacin (6%), remaining susceptible to imipenem (100%). All strains carried bla_{CTX-M}-type genes, with $bla_{CTX-M-2}$ being the most prevalent variant (n = 32), whereas the genes $bla_{CTX-M-15}$ and $bla_{CTX-M-1}$ were only found in one strain of *E. coli* (Table 1). *Xba*I PFGE analysis revealed the presence of 31 PFGE types among CTX-M producers (named clusters I to XXXI) (Figure 1), and phylogenetic investigation showed that low-virulence phylogenetic groups A (18%) and B1 (52%) were predominant over high-virulence phylogenetic groups B2 (12%) and D (18%). Correlation between ESBL genotype and phylogenetic group revealed that of 18 *E. coli* isolates belonging to phylogenetic group B1, 16 isolates carried the $bla_{CTX-M-2}$ gene, whereas another two strains harbored the $bla_{CTX-M-1}$ and $bla_{CTX-M-15}$ genes, respectively (Table 1, Figure 1). The CTX-M-15-producing ExPEC belonged to the international sequence type ST648. In urinary tract infections, CTX-M-producing *E. coli* represented predominantly low-virulence phylogenetic group B1 (57.9%).

Table 1. Distribution of resistance profiles and CTX-M extended-spectrum β-lactamases types among *Escherichia coli* strains isolated from extra-intestinal infection according to phylogenetic group

Phylogenetic group	Clinical samples (%)		Resistance profile (%)[b]										CTX-M ESBL gene (%)		
	urine	Others[a]	AMP	KF	CRO	CTX	CAZ	FEP	CIP	SXT	GEN	AK	CTX-M-1	CTX-M-2	CTX-M-15
A	3 (9)	3 (9)	6 (18)	6 (18)	6 (18)	6 (18)	5 (15)	6 (18)	4 (12)	3 (9)	2 (6)	1 (3)	-	6 (18)	-
B1	11 (32)	7 (20)	18 (53)	18 (53)	18 (53)	18 (53)	15 (44)	17 (50)	9 (26)	11 (32)	9 (26)	-	1 (3)	16 (47)	1 (3)
B2	1 (3)	3 (9)	4 (12)	4 (12)	4 (12)	4 (12)	3 (9)	4 (12)	1 (3)	2 (6)	4 (12)	-	-	4 (12)	-
D	4 (12)	2 (6)	6 (18)	6 (18)	6 (18)	6 (18)	4 (12)	5 (15)	3 (9)	5 (15)	4 (12)	1 (3)	-	6 (18)	-
TOTAL	19 (56)	15 (44)	34 (100)	34 (100)	34 (100)	34 (100)	27 (79)	32 (94)	17 (50)	21 (62)	19 (56)	2 (6)	1 (3)	32 (94)	1 (3)

[a]blood, tracheal fluid, bronchoalveolar, stump wound, cavity lavage, peritoneal fluid, biliar secretion, abscess, inguinal fold, axillary fold

[b]AMP, ampicillin; KF, cephalothin; CRO, ceftriaxone; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; CIP, ciprofloxacin; SXT, sulfamethoxazole/trimethoprim; GEN, gentamicin; AK, amikacin

The results of the present study show that dissemination of bla_{CTX-M} genes (mainly $bla_{CTX-M-2}$) is the leading cause of resistance to β-lactam antibiotics in *E. coli* strains isolated in the university hospital studied, whereas PFGE analysis revealed that the strains encompass a great genetic diversity, with the 34 strains presenting 31 different profiles (Figure 1), which is indicative of probable infection by *E. coli* strains of commensal origin. In fact, in this study, low-virulence phylogenetic groups B1 and A were predominant over high-virulence phylogenetic groups B2 and D (Table 1). Phylogenetic analyses have shown that *E. coli* strains fall into four main phylogenetic groups (A, B1, B2, and D), and that virulent extra-intestinal strains belong mainly to group B2 and, to a lesser extent, to group D, whereas most commensal strains belong to A and B1 groups [1,2]. Therefore, most likely the widespread use of antibiotics in Brazil may be contributing to the selection of silent carriers of resistance genes among commensal strains. In fact, a study conducted in remote communities from the Brazilian Amazon region revealed that commensal *E. coli* from healthy children showed high levels of multidrug resistance [7]. Similar results were reported from Bolivian and Peruvian communities [8], where the identification of CTX-M ESBLs in *E. coli* from healthy children have been identified as a serious emerging threat [9]. CTX-M beta-lactamases had been widely distributed in South America at least since 1989, and possibly before appearing in Europe. After a period of CTX-M-2 prevalence, new CTX-M variants started to be progressively reported worldwide, with the international CTX-M-15-producing *E. coli* clone O25-ST131 representing a major public health problem [10]. In Brazil, only a few MLST studies of ESBL-producing *E. coli* have been published [11,12]. In these studies, while predominance of ST410 (CC23) was observed among CTX-M-15-producing *E. coli* strains belonging to phylogroup A, MLST analysis indicated high genetic diversity among CTX-M-2-producing *E. coli* strains in the Southeast region of Brazil [11,12]. In this study, we report further data on the identification of CTX-M-15-producing ExPEC belonging to the international sequence type ST648. ST648 with CTX-M-15 has previously been described in clinical isolates from USA [13], China [14], Netherlands [15], Canada [16], Korea [17] and Tanzania [18].

Conclusion

The spread of bla_{CTX-M} genes is the main problem associated with resistance to cephalosporins in clinical isolates of *E. coli* at the university hospital studied. One finding of interest is the identification of CTX-M-15-producing *E. coli* belonging to the international sequence type ST648, which had not previously been reported in this region. The predominance of low-virulence phylogenetic groups A and B1 among CTX-M-producing ExPEC strains suggests that, in this study, most EI infections caused by ESBL-producing *E. coli* are endogenous, probably resulting from infection with strains of commensal origin that can play key roles as reservoirs of antibiotic resistance genes and as drug-resistant pathogens, which is a worrisome prospect, given that *E. coli* is ubiquitous among humans and other animals that can serve as hosts.

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Corresponding author

Professor Nilton Lincopan, PhD
Department of Microbiology
Institute of Biomedical Sciences
Universidade de São Paulo, Brazil
Email: lincopan@usp.br
Telephone: +55-11-30917296

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