Letter to the Editor

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

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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 (bioMerieux, Marcy-l’Etoile, France). DNA amplification by PCR was used to search for *bla*<sub>CTX-M</sub>*, *bla*<sub>TEM</sub>*, *bla*<sub>SHV</sub>*, *bla*<sub>GES</sub>* and *bla*<sub>PER</sub>-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 *XbaI*-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
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%), cephalexin (100%), ceftaxone (100%), ceftriaxone (100%), cefotaxime (100%), cefazidime (79%), cefepime (94%), ciprofloxacin (50%), sulfamethoxazole/trimethoprim (62%), gentamicin (56%) and amikacin (6%), remaining susceptible to imipenem (100%). All strains carried *bla*<sub>CTX-M-2</sub>-type genes, with *bla*<sub>CTX-M-2</sub> being the most prevalent variant (*n* = 32), whereas the genes *bla*<sub>CTX-M-15</sub> and *bla*<sub>CTX-M-1</sub> were only found in one strain of *E. coli* (Table 1). *XbaI* 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*<sub>CTX-M-2</sub> gene, whereas another two strains harbored the *bla*<sub>CTX-M-15</sub> and *bla*<sub>CTX-M-1</sub> 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>
<thead>
<tr>
<th>Phylogenetic group</th>
<th>Clinical samples (%)</th>
<th>Resistance profile (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>CTX-M ESBL gene (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>urine</td>
<td>Others&lt;sup&gt;a&lt;/sup&gt;</td>
<td>AMP</td>
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<tr>
<td>A</td>
<td>3 (9)</td>
<td>3 (9)</td>
<td>6 (18)</td>
</tr>
<tr>
<td>B1</td>
<td>11 (32)</td>
<td>7 (20)</td>
<td>18 (53)</td>
</tr>
<tr>
<td>B2</td>
<td>1 (3)</td>
<td>3 (9)</td>
<td>4 (12)</td>
</tr>
<tr>
<td>D</td>
<td>4 (12)</td>
<td>2 (6)</td>
<td>6 (18)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>19 (56)</td>
<td>15 (44)</td>
<td>34 (100)</td>
</tr>
</tbody>
</table>

<sup>a</sup>blood, tracheal fluid, bronchoalveolar, stump wound, cavity lavage, peritoneal fluid, biliar secretion, abscess, inguinal fold, axillary fold

<sup>b</sup>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<sub>CTX-M</sub> genes (mainly bla<sub>CTX-M,2</sub>) 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-lactamasases 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<sub>CTX-M</sub> 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 E1 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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**Conflict of interests:** No conflict of interests is declared.