Emergence of carbapenem-resistant *Escherichia coli* producing CMY-2-type AmpC β-lactamase in Brazil

Carbapenem-resistant *Escherichia coli* isolates have not been described to date in South America. However, the emergence of *Shigella flexneri* of South America. However, the emergence isolates have not been described to date in Latin America. In 2007, another two MDR *E. coli* isolates (EC3 and EC4) were recovered from blood and catheter-tip cultures, respectively. Unfortunately, despite receiving parenteral polymyxin B treatment, the patient died due to multiple organ failure as a result of septicemia.

The identification and antimicrobial susceptibility profiles of *E. coli* isolates were determined using the VITEK system (bioMérieux). MICs were subsequently determined using an agar dilution method (CLSI, 2005) and Etest (AB Biodisk). The *E. coli* EC1–EC4 strains were resistant to extended-spectrum cephalosporins, cefoxitin, aztreonam, carbapenems, ciprofloxacin and trimethoprim–sulfamethoxazole, and remained insensitive to clinically available inhibitors, showing susceptibility only to aminoglycosides (Table 1). To elucidate the mechanism involved in carbapenem resistance, firstly the hydrolysis of imipenem was evaluated by bioassay, as described previously (Lincopan et al., 2005). Next, a double-disc synergy test (DDST) using specific β-lactam inhibitors [2-mercaptopyrrol proportions, 2-mercaptoacetic acid, EDTA and aminophenylboronic acid (APB)] was employed to screen for metallo- and plasmid-mediated AmpC β-lactamases (Arakawa et al., 2000; Doi & Paterson, 2007). Additionally, a disc potentiation test (DPT) was performed with APB (Doi & Paterson, 2007). Hydrolysis of imipenem was not detected for any of the isolates. Moreover, imipenemase activity was not inhibited by thiol compounds or EDTA. However, AmpC production was assessed by a positive DPT using piperacillin/tazobactam and cefepime as substrate and APB as inhibitor. Addition of APB to a ceftazidime-containing disc in the DPT resulted in a zone enlargement from 0 to 20 mm, which was taken as a positive result, but, curiously, addition of ABP to ceftazidime-containing discs failed to inhibit AmpC activity (Table 1). DNA amplification by PCR was used to search for *bla* _CTX-M_, *bla* _TEM_, *bla* _SHV_ and *bla* _PER-2_ ESBL genes, *bla* _IMP_, *bla* _IM_(V), *bla* _KPC_ and carbapenemase genes and *bla* _DHA-1_, *bla* _DHA-2_, *bla* _CMY-1_, *bla* _CMY-2_, *bla* _FOX_ and *bla* _MIR/ACT_ plasmid-mediated AmpC genes. PCR screening revealed the presence of both *bla* _KPC_ and *bla* _CMY-2_-like genes in all isolates. Nucleotide sequencing showed that the *bla* _CMY-2_ gene (GenBank accession no. EU531728) had 99% sequence identity with the plasmid-encoded *bla* _CMY-2_ gene recently reported in Argentina (Radice et al., 2007; Rapoport et al., 2008). Here, we report the emergence of carbapenem-resistant *E. coli* producing CMY-2-type AmpC β-lactamase in Brazil, confirming that CMY-2-producing strains have already become established in Latin America.

From June to August 2007, four multidrug-resistant (MDR) *E. coli* strains (EC1–EC4), susceptible only to aminoglycosides, were isolated successively from blood, abdominal drain fluid and catheter-tip cultures from a 46-year-old man hospitalized at the Hospital Beneficência Portuguesa (HBP), São Paulo, southern Brazil. The patient, who had undergone total colectomy, was admitted to the HBP in 2005 for reconstruction of the digestive tract. A few months later, he developed sclerosing cholangitis and liver cirrhosis, requiring liver transplantation, which was performed in May 2007. On the 7th postoperative day after transplantation, the patient developed a catheter-related infection caused by oxacillin-resistant *Staphylococcus aureus*, which was treated with vancomycin and piperacillin/tazobactam. On June 13, an MDR *E. coli* strain (EC1) was isolated from an abdominal drain fluid culture, which was also positive for *Candida albicans*. The patient was then treated with imipenem, vancomycin and fluconazole for 21 days and underwent surgery to wash out the abdominal cavity of blood and pus. On July 16, the patient presented severe gastrointestinal bleeding with acute renal failure, developing a hydroelectrolytic balance disorder. Nine days later, an intrahepatic abscess was identified and a second MDR *E. coli* strain (EC2) was recovered from blood culture. In August 2007, another two MDR *E. coli* isolates (EC3 and EC4) were recovered from blood and catheter-tip cultures, respectively. Unfortunately, despite receiving parenteral polymyxin B treatment, the patient died due to multiple organ failure as a result of septicemia.

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Correspondence

Table 1. Susceptibility profiles, AmpC screening and genotyping analyses of porin-deficient E. coli producing CMY-2-type AmpC \( \beta \)-lactamase

<table>
<thead>
<tr>
<th>Strain</th>
<th>Antimicrobial susceptibility profile MIC (mg ( \Gamma ))(^{*} )</th>
<th>AmpC screening DPT (mm)( ^{\dagger} )</th>
<th>PCR ( \text{bla} ) genes</th>
<th>ERIC profile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AZT</td>
<td>CAZ</td>
<td>FOX</td>
<td>CEP</td>
</tr>
<tr>
<td>EC1</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>32</td>
</tr>
<tr>
<td>EC2</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>32</td>
</tr>
<tr>
<td>EC3</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>64</td>
</tr>
<tr>
<td>EC4</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>64</td>
</tr>
</tbody>
</table>

\(*\) AZT, Aztreonam; CAZ, ceftazidine; FOX, cefoxitin; CEP, cepemide; CT/ + CLA, cefotaxime/cefotaxime–clavulanic acid; IMP/ + EDTA, imipenem/imipenem–EDTA; ERT, ertapenem; MER, meropenem; CIP, ciprofloxacin; AK, amikacin.

\( ^{\dagger} \) DPT, Disc potentiation test (diameter inhibition, mm); CAZ/ + APB, ceftazidine/cefazidime–aminophenylboronic acid (400 \( \mu \)g per disc); FOX/ + APB, cefoxitin/cefoxitin–aminophenylboronic acid (400 \( \mu \)g per disc).

of a 36 kDa OMP conferred a high level of resistance to carbapenems (mainly ertapenem), extended-spectrum cephalosporins and cefoxitin upon the E. coli isolates, contributing to treatment failure and death of the patient. This interplay between absence of porin and CMY-2-type AmpC expression in carbapenem-resistant E. coli has been previously reported in the literature (Liu et al., 2008; Mammeri et al., 2008; Poirel et al., 2004). However, in Latin America this appears to be an emerging phenomenon, since carbapenem resistance in members of the Enterobacteriaceae has only been associated with the production of IMP-1 and KPC-2 enzymes (Lincopan et al., 2006; Villegas et al., 2006; Pasteran et al., 2008). Regarding CMY-type cephapycinases, since the first description of CMY-1 in 1989, 36 CMY-variant enzymes have been reported worldwide (http://www.lahey.org/studies/), with the CMY-2 variant being the most prevalent and most widely distributed so far (Liu et al., 2008; Poirel et al., 2004; Rapoport et al., 2008).

We underline the need for continuous surveillance of the prevalence and evolution of carbapenem-resistant isolates producing AmpC \( \beta \)-lactamase in Brazil. Dissemination of plasmid-mediated AmpC enzymes may become an important public health issue in South America.

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\(^{1} \) Laboratory of Clinical Microbiology, School of Pharmacy, University of São Paulo, SP 06083, São Paulo, SP, Brazil

\(^{2} \) School of Public Health, University of São Paulo, Avenida Doutor Arnaldo 715, São Paulo, SP, Brazil

\(^{4} \) Institute of Biomedical Sciences, Department of Microbiology, University of São Paulo, São Paulo, Brazil

Correspondence: Nilton Lincopan (lincopan@usp.br)


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