

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* and *Klebsiella pneumoniae* isolates producing CMY-2-type plasmid-mediated AmpC was 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, a 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 septicæmia.

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-mercaptopropionic acid, 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 DDST 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 APB to cefoxitin-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*_{VIM}, *bla*_{SPM} and *bla*_{KPC} 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*_{TEM} 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 first described in *K. pneumoniae* in Greece (Bauernfeind *et al.*, 1996). Although the presence of plasmid was verified in all MDR *E. coli* strains, transformation of plasmid DNA into *E. coli* DH5 α , and conjugation experiments between the clinical isolates EC1–EC4 and *E. coli* K-12, were unsuccessful. Epidemiological typing of *E. coli* isolates was performed by ERIC-PCR. Genotyping revealed that the four *E. coli* isolates were clonally related, showing identical band profiles. Finally, the outer-membrane proteins (OMPs) of carbapenem-resistant *E. coli* isolates were extracted and analysed by SDS-PAGE, and compared with profiles of carbapenem-susceptible *E. coli* control strains K-12 and ATCC 25922. Carbapenem-resistant isolates lacked a 36 kDa OMP that was present in the carbapenem-susceptible isolates. In fact, OMP profiles of *E. coli* K-12 and *E. coli* ATCC 25922 strains showed expression of 35 and 36 kDa porins, as described previously (Liu *et al.*, 2008; Poirel *et al.*, 2004). In this respect, two major porins, OmpF and OmpC (homologous to the OmpK35 and OmpK36 porins, respectively, from *K. pneumoniae*), have been described in *E. coli*, and the loss or diminished expression of either of these two porins has been related to resistance to imipenem, meropenem, ertapenem and cefoxitin (Clarke *et al.*, 2003; Lartigue *et al.*, 2007; Liu *et al.*, 2008).

In summary, our preliminary results show that CMY-2 production coupled with loss

Table 1. Susceptibility profiles, AmpC screening and genotyping analyses of porin-deficient *E. coli* producing CMY-2-type AmpC β -lactamase

Strain	Antimicrobial susceptibility profile MIC (mg l ⁻¹)*										AmpC screening DPT (mm)†		PCR <i>bla</i> genes	ERIC profile
	AZT	CAZ	FOX	CEP	CT/ +CLA	IMP/ +EDTA	ERT	MER	CIP	AK	CAZ/ +APB	FOX/ +APB		
EC1	>256	>256	>256	32	>16/>1	24/8	128	16	>32	4	0/20	0/0	<i>bla</i> _{TEM} -like, <i>bla</i> _{CMY-2}	A
EC2	>256	>256	>256	32	>16/>1	24/24	128	16	>32	4	0/20	0/0	<i>bla</i> _{TEM} -like, <i>bla</i> _{CMY-2}	A
EC3	>256	>256	>256	64	>16/>1	32/24	256	32	>32	6	0/20	0/0	<i>bla</i> _{TEM} -like, <i>bla</i> _{CMY-2}	A
EC4	>256	>256	>256	64	>16/>1	24/8	128	16	>32	6	0/20	0/0	<i>bla</i> _{TEM} -like, <i>bla</i> _{CMY-2}	A

*AZT, Aztreonam; CAZ, ceftazidime; FOX, ceftaxitin; CEP, cefepime; CT/+CLA, cefotaxime/cefotaxime-clavulanic acid; IMP/+EDTA, imipenem/imipenem-EDTA; ERT, ertapenem; MER, meropenem; CIP, ciprofloxacin; AK, amikacin.

†DPT, Disc potentiation test (diameter inhibition, mm); CAZ/+APB, ceftazidime/ceftazidime-aminophenylboronic acid (400 μ g per disc); FOX/+APB, ceftaxitin/ceftaxitin-aminophenylboronic acid (400 μ g per disc).

of a 36 kDa OMP conferred a high level of resistance to carbapenems (mainly ertapenem), extended-spectrum cephalosporins and ceftaxitin 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 cephalosporins, 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 β -lactamase in Brazil. Dissemination of plasmid-mediated AmpC enzymes may become an important public health issue in South America.

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