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Short Communication

Extended-spectrum β-lactamase-producing enterobacteriaceae in community-acquired urinary tract infections in São Luís, Brazil

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Abstract

The number of ESBL-producing Enterobacteriaceae in community-acquired urinary tract infections worldwide is probably underestimated because of the technical difficulties encountered with their detection. In this study, out of 5,672 urine samples analyzed, 916 were positive for uropathogens, 472 of them being enterobacteria of which 7.6% produced β-lactamases. Analysis of the isolated from 36 patients showed a high level of antibiotic resistance, with 52.7% and 80.5% of isolates expressing bla_{TEM} and bla_{CTX-M}, respectively.

Key words: enterobacteriaceae, antimicrobial resistance, ESBL, community-acquired infections.

Beta-lactamases are bacterial enzymes that inactivate β-lactam antibiotics by hydrolysis, which results in ineffective compounds. One group of β-lactamases, extended-spectrum β-lactamases (ESBLs), are able to hydrolyze and cause resistance against a wide variety of novel β-lactams, including third-generation cephalosporins and monobactams, but not against cephamycins and carbapenems (Pitout and Laupland, 2008). ESBLs are common in hospitalized patients (Freitas *et al.*, 2003) and only a few studies have investigated the presence of these bacteria in community patients, especially urine cultures (Minarini *et al.*, 2007). The aim of the present study was to detect bla_{TEM} and bla_{CTX-M} genes by molecular methods in enterobacteria isolated from urine cultures of patients with community-acquired urinary infection and analyze their antimicrobial susceptibility profile.

A total of 5,672 consecutive urines samples were collected from a private diagnostic laboratory located in São Luís, Brazil between March and August, 2009. Of these, 916 were positive uropathogens, including 472 (51.5%) enterobacteria. Susceptibility testing was performed and interpreted by disk diffusion method (CLSI, 2008) and Vitek 2 automated system (BioMérieux®, Marcy l'Etoile, France) for all enterobacteria.

The test was run against the following antibiotics: amoxicillin-clavulanic acid (AMC); aztreonam (AZT); ampicillin (AMP); ampicillin-sulbactam (SAM); amikacin (AMK); gentamicin (GEN); cefepime (CFP); cefotaxime (CFX); ceftazidime (CFZ); ceftriaxone (CTX); ciprofloxacin (CIP); levofloxacin (LVX); nitrofurantoin (NIT); piperacillin-tazobactam (TZP); trimethoprim-sulfamethoxazole (SXT); imipenem (IPM); meropenem (MEM); ertapenem (ERT). *Pseudomonas aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 and ATCC 35218 were used as quality controls for antimicrobial susceptibility.

ESBL production was analyzed phenotypically by the combination disk method (CLSI, 2008). For confirmation of ESBLs production, the isolates were interpreted by the disk approximation method and addition of clavulanic acid using cefotaxime/clavulanic acid and ceftazidime/clavulanic acid disks. *Klebsiella pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as positive and negative controls, respectively. Triplex PCR was used to determine the presence of bla_{TEM} and bla_{CTX-M} genes in ESBLs-

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producing Enterobacteriaceae isolates (Monstein *et al.*, 2007).

The prevalence of ESBL-producing Enterobacteriaceae in community-acquired urinary infections in southern Brazil is 1.5% (Minarini *et al.*, 2007), 3.5% in Italy (Luzzaro *et al.*, 2006) and 4.5% in Saudi Arabia (Khanfar *et al.*, 2009). In this study, 7.6% (36/472) of the enterobacteria isolates were ESBLs-producers according to the phenotypical methods, demonstrating that the frequency of bacteria expressing the ESBL phenotype significantly varies from region to region. Other studies investigating the production of ESBLs in *E. coli* isolates also demonstrated the presence of this microorganism in the community (Calbo *et al.*, 2006; Ho *et al.*, 2007; Pitout *et al.*, 2009; Ruppé *et al.*, 2009).

All isolates ESBLs-producing were resistant to ampicillin, ampicillin-sulbactam, cephalosporins, and aztreonam. With the exception of *E. coli*, all the others microorganisms showed 100% resistance to ciprofloxacin, levofloxacin and nitrofurantoin, in addition to high resistance to gentamicin, trimethoprim-sulfamethoxazole and piperacillin-tazobactam (Table 1). Amikacin, imipenem, meropenem and ertapenem were the most effective antibiotics against the ESBL-producing strains.

Of the isolates, 97.2% (35/36) carried at least one of the genes investigated by PCR (Table 2). The frequency of the bla_{TEM} gene was 52.7% (19/36), whereas frequencies of 95% and 1% have been reported in studies conducted in southern Brazil (Minarini *et al.*, 2007) and Spain (Rodrígues-Baño *et al.*, 2010). The $bla_{CTX\text{-}M}$ gene was detected in 80.5% (29/36) of the isolates and the simultaneous presence of the bla_{TEM} and $bla_{CTX\text{-}M}$ genes was demonstrated in 36.1% (13/36) (Table 2). Although TEM and SHV type ESBLs are mainly identified in hospitalized patients, a growing number of community-acquired infections caused by CTX-M-producing species have been recently reported, especially the ones caused by *E. coli* (Ho *et al.*, 2007; Pitout *et al.*, 2005). In addition to this specie, the present results show that the $bla_{CTX\text{-}M}$ gene had high frequency in all isolates, except for *Enterobacter cloacae*. A significant presence of CTX-M-producing species among strains isolated from community patients has been reported in other studies conducted in Brazil (Minarini *et al.*, 2007), Cambodia (Ruppé *et al.*, 2009), Canada (Pitout *et al.*, 2004), France (Arpin *et al.*, 2005) and Italy (Luzzaro *et al.*, 2006).

Table 2 - Detection of ESBL-coding genes by PCR according to species.

Species	No. isolates	bla_{TEM}	$bla_{CTX\text{-}M}$
E. coli	3	+	-
	13	-	+
	3	+	+
	1	-	-
K. pneumoniae	1	+	-
	3	-	+
	6	+	+
E. cloacae	2	+	-
	1	+	+
E. aerogenes	1	+	+
P. mirabilis	1	+	+
M. morganii	1	+	+
Total	36	19 (52.7%)	29 (80.5%)

This is the first study to detect the presence of ESBL-producing Enterobacteriaceae in community-acquired urinary infections in São Luis, Brazil. The results demonstrate that other enterobacteria species produce ESBLs, such as *Enterobacter aerogenes*, *E. cloacae* and *Morganella morganii* (Table 1). This indicates the need to expand the phenotypic methods for these pathogens. Currently, these methods are standardized by the CLSI only for *E. coli*, *K. pneumoniae*, *K. oxytoca* and *P. mirabilis*. Given this lack of standardized phenotypic methods for detection of ESBLs for all enterobacteria, this study will contribute to a better understanding of the susceptibility of these pathogens.

Our data confirm that carbapenems remain active against these microorganisms. *E. coli* was the prevalent specie in the ESBL-producing strains isolated from urine

Table 1 - Antimicrobial resistance profile of the different ESBL-producing species.

Species	No. of isolates	% resistance											
		AMP	SAM	AMK	GEN	CIP	LVX	NIT	PTZ	SXT	IPM	MEM	ERT
E. coli	20	100.0	100.0	6.6	16.7	72.3	80.0	35.0	11.2	89.9	0.0	0.0	0.0
K. pneumoniae	10	100.0	100.0	10.0	70.0	100.0	100.0	100.0	60.0	90.0	10.0	0.0	0.0
E. cloacae	3	100.0	100.0	25.0	75.0	100.0	100.0	100.0	75.0	75.0	0.0	0.0	0.0
E. aerogenes	1	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	100.0	0.0	0.0	0.0
P. mirabilis	1	100.0	100.0	0.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	0.0	0.0
M. morganii	1	100.0	100.0	0.0	100.0	100.0	100.0	100.0	0.0	100.0	0.0	0.0	0.0

AMP: ampicillin; SAM: ampicillin-sulbactam; AMK: amikacin; GEN: gentamicin; CIP: ciprofloxacin; LVX: levofloxacin.
NIT: nitrofurantoin; TZP: piperacillin-tazobactam; SXT: trimethoprim-sulfamethoxazole; IPM: imipenem; MEM: meropenem.
ERT: ertapenem.

culture of community patients and CTX-M the most frequent enzyme.

In summary, although clinicians need to be aware of the presence of ESBLs-producing Enterobacteriaceae in the community, public health control measures are needed to prevent the further spread of these pathogens.

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