Article/Artigo

Nosocomial infection and characterization of extended-spectrum β-lactamases-producing Enterobacteriaceae in Northeast Brazil

Infecção hospitalar e caracterização de enterobactérias produtoras de β-lactamases de espectro ampliado no nordeste do Brasil

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ABSTRACT

Introduction: Extended spectrum β-lactamases (ESBLs) are enzymes that degrade β-lactam antibiotics and have been reported to be an important cause of nosocomial infection in worldwide. **Methods:** During 2009, 659 enterobacteria strains were isolated from different clinical specimens and tested for ESBL production. The disk approximation test, combined disk method and addition of clavulanic acid were used for phenotypic detection of the ESBL-producing strains and PCR for detection of the bla_{TEM} and bla_{CTX-M} genes. **Results:** Among the isolates, 125 were ESBL producers. The bla_{CTX-M} and bla_{TEM} genes were detected in 90.4% and 75% of the strains, respectively. Most strains were isolated from urine. *Klebsiella pneumoniae* was the most prevalent organism. Microorganisms presented high resistance to the antibiotics. **Conclusions:** These results support the need for extending ESBL detection methods to different pathogens of the Enterobacteriaceae family because these methods are only currently standardized by the CLSI for *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Proteus mirabilis*. Carbapenems were the antibiotic class of choice for the treatment of infections caused by ESBL-producing Enterobacteriaceae.

Keywords: Enterobacteriaceae. Antimicrobial resistance. ESBLs. Nosocomial infection.

RESUMO

Introdução: As β-lactamases de espectro ampliado (ESBLs) são enzimas que degradam os antibióticos β-lactâmicos e têm sido reportadas como uma importante causa de infecções hospitalares em todo o mundo. **Métodos:** Em 2009, 659 enterobactérias foram isoladas de diferentes espécimes clínicos e testadas quanto à produção de ESBL. Os testes de aproximação do disco, disco combinado e adição do ácido clavulânico foram utilizados na detecção fenotípica das amostras produtoras de ESBL e PCR para a detecção dos genes bla_{TEM} e bla_{CTX-M}. **Resultados:** Entre os isolados, 125 foram produtores de ESBL. Os genes bla_{CTX-M} e bla_{TEM} foram detectados em 90,4% e 75% das amostras, respectivamente. Com relação ao espécime clínico, a maioria das amostras foi isolada de urina. *Klebsiella pneumoniae* foi a espécie mais prevalente e o teste de susceptibilidade antimicrobiana mostrou uma elevada resistência dos microorganismos aos antibióticos testados. **Conclusões:** Estes resultados suportam a necessidade de se ampliar os métodos de detecção das ESBLs para os diferentes patógenos da família Enterobacteriaceae, uma vez que esses métodos estão padronizados pelo CLSI apenas para *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca* e *Proteus mirabilis*. Os carbapenens foram os antibióticos de escolha para o tratamento de infecções causadas por enterobactérias produtoras de ESBL.

Palavras-chaves: Enterobactérias. Resistência antimicrobiana. ESBLs. Infecção hospitalar.

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INTRODUCTION

β-lactamases are a heterogeneous group of enzymes able to inactivate penicillins, cephalosporins and monobactams. These enzymes, frequently produced by aerobic and anaerobic Gram-negative bacteria, hydrolyze the β-lactam ring by irreversible hydroxylation, consequently inactivating the antibiotic[1-3]. Newly developed β-lactam antibiotics are specifically designed to be resistant to the hydrolytic action of β-lactamases. However, new β-lactamases have emerged because of the indiscriminate use of these antibiotics. A new class of antimicrobial agents, called oxyimino-cephalosporins or third-generation cephalosporins, were used as an alternative to treat severe infections caused by Gram-negative bacteria that produce restricted spectrum β-lactamases, such as TEM (Temoniera), TEM-1 and SHV-1[4].

The resistance mediated by β-lactamases emerged rapidly, and mutations in the bla_{TEM}, bla_{TEM-1} and bla_{SHV} genes led to the emergence of novel enzymes, called extended-spectrum β-lactamases (ESBLs) due to their broad spectrum of activity, especially against oxyimino-cephalosporins[5].

The production of ESBLs by enterobacteria is the most common resistance mechanism against β-lactams. These enzymes spread rapidly throughout the world and become the prevalent resistance mechanism once established in a region[4,5]. The prevalence of ESBL-producing strains in Latin American countries is 45%. Data from the SENTRY Antimicrobial Surveillance Program for Brazil show a high incidence of ESBL-producing isolates (*Klebsiella pneumoniae*: 50.3%, *Escherichia coli*: 9.1%)[6]. A high incidence of these strains is also observed in Europe (*Klebsiella* spp.: 32.8%, *E. coli*: 14.4%). A study conducted in Spain detected ESBL-producing strains in 90% of hospitals participating in a surveillance program. These findings demonstrate that the prevalence of ESBL-producing strains varies from country to country[2,7,8].

Because of the increasing incidence of ESBL-producing Gram-negative bacteria and the lack of standardized phenotypic methods for the detection of ESBLs in a larger range of microorganisms, this study aimed to characterize ESBL-producing Enterobacteriaceae isolated at hospitals in northeast Brazil, focusing on the evaluation of their antimicrobial susceptibility profile.

METHODS

Bacterial strains

In two private hospitals and one public hospital in northeast Brazil, 659 enterobacteria strains collected from different clinical specimens between March and August 2009 were analyzed. All isolates were identified using conventional techniques[9] and the Vitek 2 system, an automated assay (BioMérieux®, Marcy l'Etoile, France).

Antimicrobial susceptibility tests

Susceptibility testing was performed and interpreted via disk diffusion method, as recommended by the Clinical and Laboratory Standards Institute (CLSI)[9], and the Vitek 2 system. *Pseudomonas aeruginosa* ATCC 27853, *E. coli* ATCC 25922 and ATCC 35218 were used as quality controls for antimicrobial susceptibility.

Detection of ESBL isolates

The disk approximation method and addition of clavulanic acid were employed for confirmation of ESBL phenotypes. *Klebsiella pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as positive and negative controls, respectively[9].

Multiplex PCR amplification

PCR analysis was performed using sets of primers designed for amplification of genes codifying ESBLs. Primers TEM-164. SE (5'-ATGCGTTATATTCGCCTGTG-3') and TEM-165. AS (5'-TGCTTTGTTATTCGGGCCAA-3') were used for amplification of a 445-bp sequence of the TEM group. CTX-M-U1 (5'-ATGTGCAGYACCAGTAARGTKATGGC-3') and CTX-M-U2 (5'-TGGGTRAARTARGTSACCAGAAYCAGCGG-3') were used for amplification of a 593-bp sequence of the CTX-M group. Detection of the β-lactamase genes was carried out with the following components in a 25μl reaction volume: 1μl of DNA, 10pmol of the specific primers, 0.3μl of Taq DNA polymerase (0.5U/μl) (Invitrogen, Brazil), 1μl of $MgCl_2$ (50mM) (Invitrogen, Brazil), 2.5μl of buffer (10x) (Invitrogen, Brazil), 2.5μl of dNTPs (2mM) (Invitrogen, Brazil) and Milli-Q water. PCR amplification conditions were as follows: initial denaturation step at 95°C for 15min, 30 cycles of denaturation at 94°C for 30s, annealing at 60°C for 30s, extension at 72°C for 2min, followed by a final extension step at 72°C for 10min[10].

Ethical considerations

Ethical approval was obtained from the Ethics Committee of the *Universidade Federal do Maranhão* according to the requirements of the Ministry of Health.

RESULTS

Clinical bacterial strains

Among the 659 enterobacteria strains isolated from the 3 hospitals, 125 (19%) were determined to be as ESBL producers. *K. pneumoniae* was the most frequent species (n = 63, 50.4%), followed by *E. coli* (n = 20, 16%). The largest number of ESBL-producing strains was isolated at hospital 2 (n = 64, 51.2%). In this hospital, *Enterobacter aerogenes* was the most frequent species (n = 14, 21.9%) when compared to hospitals 1 and 3. **Table 1** shows the number of species isolated in each hospital.

Eighty-three (66%) of the isolates originated from ICUs, 27 (22%) from internal medicine units, 8 (6.4%) from surgical units, and 7 (5.6%) from outpatient clinics. In ICUs, *K. pneumoniae* was responsible for 56.6% of infections, followed by *Proteus mirabilis* (13.3%) and *E. aerogenes* (13.3%).

In the three hospitals, most ESBL-producing strains were isolated from urine (n = 45, 36%), followed by tracheal secretions (n = 25, 20%) and blood (n = 15, 12%).

Antimicrobial susceptibility of clinical isolates

Analysis of the antimicrobial susceptibility profile of the ESBL-producing strains showed that 100% of the isolates were resistant to ampicillin, ampicillin-sulbactam, cephalosporins and aztreonam. Most microorganisms had high resistance to ciprofloxacin, gentamicin, levofloxacin and trimethoprim-sulfamethoxazole but were susceptible to amikacin, piperacillin-tazobactam and carbapenems (ertapenem, imipenem and meropenem) **(Table 2)**.

Characterization of ESBL

The presence of the two families of ESBL-coding genes was detected by PCR in 92% (115/125) of the isolates. The bla_{CTX-M} gene was detected in 90.4% (104/115) of the isolates and the bla_{TEM} gene in 75% (86/115). Seventy-five (65.2%) of the isolates carried genes encoding both the CTX-M and TEM-type enzymes **(Table 3)**.

The highest frequency of genes encoding the CTX-M and TEM-type enzymes was observed in *K. pneumoniae* (96.6% [57/59] and 67.8% [40/59], respectively). The frequency of the bla_{CTX-M} gene was detected in 85% (17/20) of *E. coli*, 77.3% (11/15) of *P. mirabilis*, and 93.3% (14/15) of *E. aerogenes* isolates. The bla_{TEM} gene was detected in 70.0% (14/20) for *E. coli*, 93.3% (14/15) for *P. mirabilis*, and 86.7% (13/15) for *E. aerogenes* **(Table 4)**.

Although the susceptibility profile varied little between strains carrying the bla_{TEM} and bla_{CTX-M} genes, analysis showed that bacteria carrying the bla_{CTX-M} gene were more susceptible to the antimicrobial agents tested **(Table 5)**.

TABLE 1 - ESBL-producing enterobacteria strains identified by the phenotypic method.

	H_1[a]		H_2[b]		H_3[c]		Total	
Species	n[d]	%	n	%	n	%	n	%
Klebsiella pneumoniae	17	68.0	31	48.4	15	41.6	63	50.4
Escherichia coli	4	16.0	9	14.1	7	19.4	20	16
Proteus mirabilis	2	8.0	6	9.3	10	27.8	18	14.4
Enterobacter aerogenes	2	8.0	14	21.9	0	0.0	16	12.8
Enterobacter cloacae	0	0.0	3	4.7	1	2.8	4	3.2
Serratia marcescens	0	0.0	1	1.6	1	2.8	2	1.6
Proteus vulgaris	0	0.0	0	0.0	1	2.8	1	0.8
Providencia stuartii	0	0.0	0	0.0	1	2.8	1	0.8
Total	25	100.0	64	100.0	36	100.0	125	100.0

[a,b]private hospitals, [c]public hospital, [d] number of ESBL-producing isolates. **ESBL:** extended spectrum β-lactamases.

TABLE 2 -Antimicrobial resistance profile of ESBL-producing species.

Species	Isolate (n)	Resistance (%)										
		AMP	SAM	AMK	GEN	CIP	LVX	TZP	SXT	IPM	MEM	ERT
Klebsiella pneumoniae	63	100.0	100.0	27.0	85.7	84.1	73.0	76.2	95.2	1.6	0.0	1.6
Escherichia coli	20	100.0	100.0	20.0	60.0	70.0	50.0	30.0	90.0	0.0	0.0	0.0
Proteus *mirabilis*	18	100.0	100.0	33.3	94.5	88.9	66.7	0.0	83.4	0.0	0.0	0.0
Enterobacter aerogenes	16	100.0	100.0	81.3	87.5	93.8	81.3	50.0	93.8	0.0	0.0	0.0
Enterobacter cloacae	4	100.0	100.0	75.0	100.0	75.0	75.0	75.0	75.0	0.0	0.0	25.0
Serratia marcescens	2	100.0	100.0	0.0	0.0	0.0	50.0	0.0	50.0	0.0	0.0	0.0
Proteus vulgaris	1	100.0	100.0	100.0	100.0	100.0	100.0	0.0	100.0	0.0	0.0	0.0
Providenciastuartii	1	100.0	100.0	0.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0

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

TABLE 3 -Distribution of genes encoding the TEM- and CTX-M-type enzymes.

Species	Isolates (n)	Only TEM		Only CTX-M		TEM and CTX-M		TEM		CTX-M	
		n	%	n	%	n	%	n	%	n	%
Klebsiella pneumoniae	59	2	3.4	19	32.2	38	64.4	40	67.8	57	96.6
Escherichia coli	20	3	15.0	6	30.0	11	55.0	14	70.0	17	85.0
Proteus mirabilis	15	4	26.7	1	26.7	10	66.7	14	93.3	11	77.3
Enterobacter aerogenes	15	1	6.7	2	13.3	12	80.0	13	86.7	14	93.3
Enterobacter cloacae	4	1	25.0	1	25.0	2	50.0	3	75.0	3	75.0
Serratia marcescens	0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Proteus vulgaris	1	0	0.0	0	0.0	1	100.0	1	100.0	1	100.0
Providencia stuartii	1	0	0.0	0	0.0	1	100.0	1	100.0	1	100.0
Total	**115**	**11**	**9.6**	**29**	**25.2**	**75**	**65.2**	**86**	**75.0**	**104**	**90.4**

TEM: Temoniera, **CTX-M:** cefotaxime hydrolysis.

TABLE 4 - Detection of ESBL-coding genes by polymerase chain reaction according to species and source of infection.

Species	Source of infection	Isolates(n)	Multiplex PCR		Species	Source of infection	Isolates(n)	Multiplex PCR	
			bla_{TEM}	bla_{CTX-M}				bla_{TEM}	bla_{CTX-M}
Klebsiella pneumoniae	Bile	2	+	+	*Escherichia coli*	Urine	6	+	+
Klebsiella pneumoniae	Bile	1		+	*Escherichia coli*	Urine	2	+	
Klebsiella pneumoniae	Eschar	4	+	+	*Escherichia coli*	Urine	3		+
Klebsiella pneumoniae	surgical wound	1	+	+	*Proteus mirabilis*	Eschar	1	+	+
Klebsiella pneumoniae	surgical wound	1		+	*Proteus mirabilis*	surgical wound	1		
Klebsiella pneumoniae	Feces	1	+	+	*Proteus mirabilis*	lesion fragment	1	+	+
Klebsiella pneumoniae	pleural fluid	1	+	+	*Proteus mirabilis*	bone fragment	1		+
Klebsiella pneumoniae	catheter tip	1	+	+	*Proteus mirabilis*	catheter tip	2	+	+
Klebsiella pneumoniae	catheter tip	3		+	*Proteus mirabilis*	catheter tip	1	+	
Klebsiella pneumoniae	catheter tip	1			*Proteus mirabilis*	catheter tip	1		
Klebsiella pneumoniae	Blood	6	+	+	*Proteus mirabilis*	Blood	1	+	
Klebsiella pneumoniae	Blood	3		+	*Proteus mirabilis*	tracheal secretion	3	+	+
Klebsiella pneumoniae	Blood	1			*Proteus mirabilis*	anal swab	1	+	
Klebsiella pneumoniae	tracheal secretion	7	+	+	*Proteus mirabilis*	Urine	3	+	+
Klebsiella pneumoniae	tracheal secretion	1	+		*Proteus mirabilis*	Urine	1	+	
Klebsiella pneumoniae	tracheal secretion	5		+	*Proteus mirabilis*	Urine	1		
Klebsiella pneumoniae	vaginal secretion	1	+	+	*Proteus vulgaris*	lesion fragment	1	+	+
Klebsiella pneumoniae	anal swab	1	+	+	*Enterobacter aerogenes*	Urine	5	+	+
Klebsiella pneumoniae	anal swab	1		+	*Enterobacter aerogenes*	Urine	1	+	
Klebsiella pneumoniae	Urine	13	+	+	*Enterobacter aerogenes*	Urine	1		
Klebsiella pneumoniae	Urine	1	+		*Enterobacter aerogenes*	tracheal secretion	5	+	+
Klebsiella pneumoniae	Urine	5		+	*Enterobacter aerogenes*	tracheal secretion	2		+
Klebsiella pneumoniae	Urine	2			*Enterobacter aerogenes*	Blood	1	+	+
Escherichia coli	Eschar	1		+	*Enterobacter aerogenes*	catheter tip	1	+	+
Escherichia coli	surgical wound	2	+	+	*Enterobacter cloacae*	surgical wound	1	+	
Escherichia coli	surgical wound	1	+		*Enterobacter cloacae*	tracheal secretion	1		+
Escherichia coli	surgical wound	1		+	*Enterobacter cloacae*	anal swab	1	+	+
Escherichia coli	lesion fragment	1	+	+	*Enterobacter cloacae*	Urine	1	+	+
Escherichia coli	Blood	1	+	+	*Serratia marcescens*	Blood	1		
Escherichia coli	Blood	1		+	*Serratia marcescens*	tracheal secretion	1		
Escherichia coli	vaginal secretion	1	+	+	*Providencia stuartii*	surgical wound	1	+	+

+: positive, **ESBL:** extended spectrum β-lactamases, **PCR:** polymerase chain reaction.

TABLE 5 - Antimicrobial resistance profile of ESBL-producing strains according to type of gene.

Gene	Isolates (n)	Resistance (%)										
		AMP	SAM	AMK	GEN	CIP	LVX	TZP	SXT	IPM	MEM	ERT
bla_{TEM}	11	100.0	100.0	27.3	72.7	90.9	63.6	18.2	72.7	0.0	0.0	9.1
bla_{CTXM}	29	100.0	100.0	10.3	62.1	65.5	55.2	48.3	93.1	0.0	0.0	0.0
bla_{TEM} and bla_{CTXM}	75	100.0	100.0	50.7	93.3	88.0	76.0	58.7	93.3	1.3	0.0	1.3

ESBL: extended spectrum β-lactamases, **AMP:** ampicillin, **SAM:** ampicillin-sulbactam, **AMK:** amikacin, **GEN:** gentamicin, **CIP:** ciprofloxacin, **LVX:** levofloxacin, **TZP:** piperacillin-tazobactam, **SXT:** trimethoprim-sulfamethoxazole, **IPM:** imipenem, **MEM:** meropenem, **ERT:** ertapenem.

DISCUSSION

ESBL-producing bacteria have rapidly spread worldwide, indicating the need for continuous monitoring systems and effective control measures of infection. In addition, the therapeutic options for infections caused by ESBL-producing microorganisms are becoming increasingly more limited. The use of antibiotics, particularly oxyimino-cephalosporins, and hospital transfer are well-defined risk factors for the acquisition of ESBL-producing bacteria[11].

The frequency of ESBL-producing Enterobacteriaceae detected was 19% (125/659), although rates of 29%[12] and 24%[13] have been reported in two other Brazilian studies. The rate of ESBL-producing microorganisms within the Enterobacteriaceae family is 11.1% in Poland[14], 7.4% in Italy[15], 6% in Saudi Arabia[1], 1.7% in France[16], and 30 to 60% in Latin America[17]. These data indicate that the prevalence of bacteria expressing the ESBL phenotype varies significantly in different regions and hospitals within the same region.

In this study, 37.5% (63/168) of the *K. pneumoniae* strains were producers of ESBLs, which supports similar results in other studies[18]. A frequency of approximately 50% is observed in Brazil, versus 5% in the United States and Japan, 15-20% in Europe, and 20-50% in Asian countries[6,13,19,20].

Klebsiella pneumoniae was the most frequently observed ESBL-producing microorganism in this study. Similar results have been reported in members of the family Enterobacteriaceae[7,13,18,19] and in a multicenter study investigating bacterial resistance in Brazilian hospitals[6]. In contrast, *E. coli* was found to be the most prevalent ESBL-producing microorganism in Saudi Arabia[1], France[16] and Italy[15].

The occurrence of ESBL-producing *E. aerogenes* was similar to that of *E. coli* and *P. mirabilis*. In addition, the occurrence of this microorganism was higher than that of the other species in one of the hospitals (H2). The prevalence of *E. aerogenes* exceeded those found in Italy[15]. In contrast, other Brazilian studies have reported a higher prevalence of *E. cloacae*[13].

Studies conducted in the United States indicate that standard ESBL testing in non-*E. coli* or non-*Klebsiella* spp. is not required because of the low phenotypic incidence[21]. However, our results demonstrated a high occurrence of non-*E. coli*, non-*Klebsiella* spp. and non-*Proteus* spp. Because of these findings and those of other studies[3,13,21], standard detection techniques for ESBL enzymes in pathogens of the Enterobacteriaceae family are needed.

Our results agree with other studies that also found a high frequency of microorganisms isolated from urine[1,22]. However, in a study of various regions of Brazil, most ESBL-producing bacteria were isolated from blood and the respiratory tract[6].

The main risk factors for colonization or infection with ESBL-producing bacteria are previous antibiotic use[2,5], presence of invasive devices such as catheters[23,24], prolonged hospital stay[25,26], previous hospitalization[17], delay in appropriate treatment, presence of ulcers[5,23] and ICU stay[2,17]. The highest incidence of ESBL-producing bacteria was observed in ICUs, which can be explained by the emergent character of this unit. In addition, multiresistant bacteria dissemination occurs frequently because of the peculiar characteristics of this unit. These characteristics include the following: restricted unit, high frequency of healthcare worker contact with patients, increased possibility of pathogen cross-transmission, high selective pressure for broad-spectrum antibiotics, increased probability of environmental contamination (e.g., surgeries), use of medications that interfere with the natural chemical barrier or alter the immune response, and use of tubes and catheters that impair physiologic microorganism elimination[27].

In general, the isolates presented high rates of antibiotic resistance, including resistance to other classes and cross-resistance. Some Brazilian studies have indicated fluoroquinolones as alternative drugs for the treatment of infections caused by ESBL-producing bacteria[6]. However, ESBL-producing bacteria were found to be highly resistant to these drugs in our results. Carbapenems were the most active drugs against ESBL-producing strains. These antibiotics can easily enter the bacteria and are more stable against hydrolysis mediated by ESBLs[7]. However, the administration of these drugs should be based on antimicrobial susceptibility testing. In the present study, two isolates (*K. pneumoniae* and *E. cloacae*) were found to be resistant to ertapenem and one (*K. pneumoniae*) was resistant to imipenem. These results were confirmed by the E-test method.

Most ESBLs evolved from gene mutations in classical ß-lactamases (TEM-1, TEM-2 and SHV-1), giving origin to ESBL varieties of mostly the TEM and SHV types[5]. A new family of ESBLs, CTX-M, has emerged over recent years, especially in *E. coli*. This family has become one of the most important families of ESBL enzymes in many countries[28-30]. CTX-M ß-lactamases are the predominant type of ESBLs in Europe and South America[29,31], including Brazil[32].

High frequencies of the bla_{CTX-M} and bla_{TEM} genes were discovered in this study. The frequency of the bla_{CTX-M} gene was 90.4% (104/115). Other researchers also reported high prevalence rates of 92%[33], 82%[14], 72%[34] and 70%[10]. The frequency of the bla_{TEM} was 75% (86/115), which resembles the results of the studies conducted in Sweden and Brazil[10,33].

A high occurrence of CTX-M-type ESBLs in *E. coli* has also been reported in recent studies[16,30,34,35]. However, the same was not observed for *K. pneumoniae*, as prevalence rates of 14.8% and 15.8% were reported in studies conducted in France[16] and Norway[35], respectively. The frequency of genes encoding CTX-M-type ESBLs is not restricted to *E. coli*. These genes are also observed in other species such as *K. pneumonia*, *E. aerogenes* and *P. mirabilis*.

Ten isolates (*K. pneumoniae*, n = 4; *P. mirabilis*, n = 3; *Serratia marcescens*, n = 2, and *E. aerogenes*, n = 1) identified as ESBL producers by the phenotypic methods did not produce the CTX-M or TEM enzyme. These strains probably produce other types of enzymes that were not investigated in this study.

In conclusion, the high percentage of ESBL-producing isolates detected in the three hospitals studied supports the need for extending the ESBL detection methods to different pathogens of the Enterobacteriaceae family. Currently, these methods are only standardized by the CLSI for *E. coli*, *K. pneumoniae*, *K. oxytoca* and *P. mirabilis*. CTX-M was the most prevalent enzyme in the ESBL-producing strains. Carbapenems remain the treatment of choice for infections caused by these pathogens.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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REFERENCES

1. Khanfar HS, Bindayna KM, Senok AC, Botta GA. Extended-spectrum beta-lactamases (ESBLs) in *Escherichia coli* and *Klebsiella pneumoniae*: trends in the hospital and community settings. J Infect Dev Ctries 2009; 3:295-299.
2. Mendelson G, Hait V, Ben-Israel J, Gronish D, Granot E, Raz R. Prevalence and risk factors of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in an Israeli long-term care facility. Eur J Clin Microbiol Infect Dis 2005; 24:17-22.
3. Minarini LAR, Gales AC, Palazzo ICV, Darini ALC. Prevalence of Community-Occurring Extended Spectrum β-Lactamase-Producing Enterobacteriaceae in Brazil. Curr Microbiol 2007; 54:335-341.
4. Bradford PA. Extended-spectrum-β-lactamases in the 21st century: caracterização, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev 2001; 14:933-951.
5. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: a clinical update. Clin Microbiol Rev 2005; 18:657-686.
6. Sader HS, Gales AC, Pfaller MA, Mendes RE, Zoccoli C, Barth A, et al. Pathogen frequency and resistance patterns in brazilian hospitals: summary of results from three years of the SENTRY Antimicrobial Surveillance Program. Braz J Infect Dis 2001; 5:200-214.
7. Freitas ALP, Machado DP, Soares FS, Barth AL. Extended-Spectrum β-lactamases in *Klebsiella* spp. and *Escherichia coli* obtained in a Brazilian teaching Hospital: detection, prevalence and molecular typing. Braz J Microbiol 2003; 34:344-348.
8. Jones RN, Pfaller MA. Antimicrobial activity against strains of *Escherichia coli* and *Klebsiella* spp. with resistance phenotypes consistent with extended-spectrum β-lactamases in Europe. Clin Microbiol Inf 2003; 9:708-712.
9. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing: eighteenth informational supplement. CLSI document M100-S18. Wayne (PA), USA: CLSI; 2008.
10. Monstein HJ, Östholm-Balkhed A, Nilsson NV, Nilsson M, Dornbusch K, Nilsson LE. Multiplex PCR amplification assay for rapid detection of ^{bla}SHV, ^{bla}TEM and ^{bla}CTX-M genes in Enterobacteriaceae. APMIS 2007; 115:1400-1408.
11. Kiratisin P, Apisarnthanarak A, Laesripa C, Saifon P. Molecular Characterization and Epidemiology of Extended-Spectrum β-Lactamase-Producing Escherichia coli and *Klebsiella pneumoniae* Isolates Causing Health Care-Associated Infection in Thailand, Where the CTX-M Family is Endemic. Antimicrob Agents Chemother 2008; 52:2818-2824.
12. Mendes C, Hsiung A, Kiffer C. Evaluation of the *in vivo* Activity of Antimicrobials Against Bacterial Strains Isolated from Patients in Intensive Care Units in Brazil: MYSTIC Antimicrobial Surveillance Program. Braz J Infect Dis 2000; 4:236-244.
13. Nogueira KS, Higuti IH, Nascimento AJ, Terasawa LB, Oliveira S, Matos AP, et al. Occurrence of Extended-Spectrum Beta-lactamases in Enterobacteriaceae Isolated from Hospitalized Patients in Curitiba, Southern Brazil. Braz J Infect Dis 2006; 10:390-395.
14. Empel J, Baraniak A, Literacka E, Mrówka A, Fiett J, Sadowy E, et al. Molecular Survey of β-Lactamases Conferring Resistance to Newer β-Lactams in Enterobacteriaceae Isolates from Polish Hospitals. Antimicrob Agents Chemother 2008; 52:2449-2454.
15. Luzzaro F, Mezzatesta M, Mugnaioli C, Perilli M, Stefani S, Amicosante G, et al. Trends in Production of Extended-Spectrum β-Lactamases among Enterobacteria of Medical Interest: Report of the Second Italian Nationwide Survey. J Clin Microbiol 2006; 44:1659-1664.
16. Galas M, Decousser J, Breton N, Godard T, Allouch PY, Pina P. Nationwide Study of the Prevalence, Characteristics, and Molecular Epidemiology of Extended-Spectrum β-Lactamase-Producing Enterobacterieceae in France. Antimicrob Agents Chemother 2008; 52:786-789.
17. Menashe G, Borer A, Yagupsky P, Peled N, Gilad J, Fraser D, et al. Clinical significance and impact on mortality of extended-spectrum beta lactamase-producing Enterobacteriaceae isolates in nosocomial bloodstream infections. Scand J Infect Dis 2001; 33:188-193.
18. Superti SV, Augusti G, Zavascki AP. Risk factors for and mortality of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* and *Escherichia coli* nosocomial bloodstream infections. Rev Inst Med Trop São Paulo 2009; 51:211-216.
19. Turner PJ. Extended-spectrum β-lactamase. Clin Infect Dis 2005; 41:273-275.
20. Gales AC, Sader HS, Jones RN. Respiratory tract pathogens isolated from patients hospitalized with suspected pneumonia in Latin American: frequency of occurrence and antimicrobial susceptibility profile: results from the SENTRY Antimicrobial Surveillance Program (1997-2000). Diagn Micr Infect Dis 2002; 44:301-311.
21. Schwaber MJ, Raney PM, Rasheed JK, Biddle JW, Williams P, McGowan JE, et al. Utility of NCCLS Guidelines for Identifying Extended-Spectrum β-Lactamases in Non-*Escherichia coli* and Non-*Klebsiella* spp. of Enterobacteriaceae. J Clin Microbiol 2004; 42:294-298.
22. Akpaka PE, Swanston WH. Phenotypic detection and occurrence of extended-spectrum beta-lactamases in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli* at a tertiary hospital in Trinidad & Tobago. J Infect Dis 2008; 12:516-520.
23. Pfaller MA, Segreti J. Overview of the epidemiological profile and laboratory detection of extended-spectrum beta-lactamases. Clin Infect Dis 2006; 42:153-163.
24. Silva N, Oliveira M, Bandeira AC, Brites C. Risk factors for infection by extended-spectrum beta-lactamases producing *Klebsiella pneumoniae* in Salvador, Brazil. Braz J Infect Dis 2006; 10:191-193.
25. Denton M. Enterobacteriaceae. Int J Antimicrob Ag 2007; 3:9-22.
26. McGowan AP. Clinical implications of antimicrobial resistance for therapy. J Antimicrob Chemother 2008; 62:105-114.
27. Murthy R. Implementation of strategies to control antimicrobial resistance. Chest 2003; 119:405-411.
28. Bonnet R, Dutour C, Sampaio JLM, Chanal C, Sirot D, Labia R, et al. Novel cefotaximase (CTX-M-16) with increased catalytic efficiency due to substitution Asp-2403Gly. Antimicrob Agents Chemother 2001; 45:2269-2275.
29. Livermore DM, Brown DFJ. Detection of β-lactamases-mediated resistance. J Antimicrob Chemother 2001; 48:59-64.

30. Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey MR, et al. Molecular epidemiology of CTX-M-producing *Escherichia coli* in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob Agents Chemother 2007; 51:1281-1286.

31. Canton R, Coque TM. The CTX-M β-lactamase pandemic. Curr Opin Microbiol 2006; 9:466-475.

32. Bonnet R, Sampaio JLM, Labia R, Champs C, Sirot D, Chanal C, et al. A novel CTX-M b-lactamase (CTX-M-8) in cefotaxime resistant Enterobacteriaceae isolated in Brazil. Antimicrob Agents Chemother 2000; 44:1936-1942.

33. Fang H, Ataker F, Hedin G, Dornbusch K. Molecular epidemiology of extended-spectrum beta-lactamases among *Escherichia coli* isolates collected in a Swedish hospital and its associated health care facilities from 2001 to 2006. J Clin Microbiol 2008; 46:707-712.

34. Lewis JS, Herrera M, Wickes B, Patterson JE, Jorgensen JH. First Report of the Emergence of CTX-M-Type Extended-Spectrum β-Lactamases (ESBLs) as the Predominant ESBL Isolated in a U.S. Health Care System. Antimicrob Agents Chemother 2007; 51:4015-4021.

35. Tofteland S, Haldorsen B, Dahl KH, Simonsen GS, Steinbakk M, Walsh TR, et al. Effects of Phenotype and Genotype on Methods for Detection of Extended-Spectrum-β-Lactamase-Producing Clinical Isolates of *Escherichia coli* and *Klebsiella pneumoniae* in Norway. J Clin Microbiol 2007; 45:199-205.