Iranian J Publ Health, Vol. 38, No.1, 2009, pp.10-17

Original Article

Antibiotic Resistance to Third Generation Cephalosporins Due to CTX-M-Type Extended-Spectrum β-Lactamases in Clinical Isolates of *Escherichia coli*

*M Mirzaee[1], M R Pourmand[2], *M Chitsaz[1], S Mansouri[1]*

[1]*Dept. of Microbiology, Faculty of Medicine, Shahed University, Tehran, Iran*
[2]*Dept. of Pathobiology, School Public Health, Tehran University of Medical Sciences, Iran*

(Received 30 Jul 2008; accepted 11 Jan 2009)

Abstract

Background: Organisms producing CTX-M-β-lactamase are emerging around the world as a source of resistance to oxyiminocephalosporins such as cefotaxime. However, the laboratory detection of these strains is not well defined. The aim of this study was to determine the presence and prevalence of known CTX-M-β-lactamases genes in clinical isolates of *Escherichia coli* from hospitals of Tehran.

Methods: During six months (September to February, 2006), 160 clinical isolates of *Escherichia coli* collected from three university hospitals of Tehran. Phenotypic screening and confirmation tests for ESBL detection was according to CLSI advised. All of the ESBL-producing isolates were examined by PCR for presence of *bla* $_{CTX-M}$ genes.

Results: Primary phenptypic tests revealed that %56.69 (n=89) of *E. coli* isolates produced ESBLs. In confirmatory tests by use of clavulanic acid, ESBL production were confirmed (P+C+) in %96.7 (n=86) of isolates with primary positive test. The presence of an ESBL was not confirmed (P+C-) in 3.3% (n=3) of the screen positive. Of all screen positive isolates, 34 (35.78%) were positive for *bla* $_{CTX-M}$ genes from the CTX-M-I group, indicating CTX-M-1-like β-lactamases and Two (2.1%) were positive for *bla* $_{CTX-M}$ genes from the CTX-M-III group, indicating CTX-M-3-like β-lactamases. The remainder 59 (62.2%) were negative for *bla* $_{CTX-M}$ genes.

Conclusions: The levels of resistance to ceftazidim were remarkably varible among CTX-M producers. This study provides futher evidence of the global dissemination of CTX-M type ESBLs and emphasize the need for their epidemiological monitoring.

Keywords: Escherichia coli, *CTX-M-β-lactamase, ESBL, Iran*

Introduction

During the past decade extended-spectrum β-lactamases (ESBLs) of the CTX-M type emerged in many countries of the world. The first organisms producing β-lactamases of this type were identified both as single and epidemic clinical isolates in very distant geographic regions (Germany, France and Argentina) in the early 1990s (1- 3). More recently, a rapid increase in the proportion of multiple CTX-M variants to the TEM- and SHV-derived ESBLs has been reported in many hospitals in Spain (4), China (5), and Korea (6). Furthermore, CTX-M β-lactamases, mainly types CTX-M-2 and CTXM-3, were found to be widespread or even predominant ESBL types in several countries, including Argentina (7). Based on their amino-acidic sequence diversity, the vast number (more than 50) of CTX-M variants identified thus far have been classified into five major phylogenetic groups: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25 (8, 9). In contrast with TEM- and SHV-type ESBLs, most of the CTX-M enzymes are much more active against cefotaxime and ceftriaxone than against ceftazidime. Thus, most of the CTX-M producers display levels of resistance to cefotaxime significantly higher than those to ceftazidime. However, the classical phenotype of resistance conferred by CTX-M β-lactamases is not universal among all CTX-M producers, since many factors, including production of additional β-lactamases (10) or mutations altering the substrate specificity of CTX-M enzymes (11), can mask their presence. Therefore, the phenotype of resis-

*__Corresponding author__. Tel: +98 21 88964792, Fax: +98 21 88966310, E-mail: Chitsaz@shahed.ac.ir

tance to β-lactams may suggest the presence of CTX-M enzymes, but this is not a completely reliable approach. PCR has been used widely to detect *bla* $_{CTX-M}$ genes, but detection of all the known variants usually required multiple reactions with specific primers for different genes (12).
In the present study we explored the prevalence of various CTX-M-ESBLs among clinical isolates of Escherichia *coli* obtained during 6 months from three university hospitals of Tehran. CTX-M-coding genes were detected by grouping PCR with consensus primers specifically recognizing all the known CTX-M variants. Also we analyzed the genetic relatedness of CTX-M-producing isolates and levels of MICs in these isolates.

Material and Methods

Bacterial strains

Clinical isolates of *Escherichia coli* collected at during six months (Sep 2006 to Feb 2007), from three hospitals (Immam Khomeini, Shahid Mostafa Khomeini and Children Medical Center) of Tehran. These organisms were screened for the presence of ESBLs and then investigated for the presence of CTX-M-β-lactamases. The bacterial isolates kept frozen at -70º C before tested.

Antimicrobial susceptibility testing

MICs of ceftazidime, cefotaxime and cefepime were determined using agar macro dilution on Mueller-Hinton agar (Becton Dickinson, Sparks, Md.). The MICs testing was done according to CLSI methods and interpretive breakpoint criteria (13). *E. coli* ATCC 25922*, Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureuse* (ATCC 29213), and *K. pneumonia* (ATCC 700603) were used as quality controls.

ESBL definitions, screening, and confirmation

The presence of ESBLs was evaluated in both the control strains and the recent clinical isolates. All isolates for which the MICs to either cefotaxime, ceftazidime or cefepim greater than 1 µg/ml were considered to have a positive screening test for an ESBL, and subjected to clavulanate confirmatory testing using methods identical to those recommended by the CLSI (13, 14). Isolates that demonstrated clavulanate enhancement with one or more of the substrates were designated as confirmed while those that did not demonstrate enhancement were designated non-confirmed.

PCR experiments

Selected isolates with positive screen tests were subjected to molecular screening for β-lactamases using polymerase chain reaction for family specific CTX-M. Template for PCR was prepared by the heat lysis method (15) except that bacteria were directly inoculated into 5.0 mL of LB broth in microtubes for overnight culture. For each PCR, 2µL of supernatant containing template DNA was added to a final volume of 50 µL containing: 50 mM KCl, 10 mM Tris-HCl (pH 9), 0.1% Triton X-100, 2 mM $MgCl_2$, a 200 µM concentration of each deoxynucleoside triphosphate, a 0.5 µM concentration of each primer, 32.25 µl distilled water and 1.25 U of Taq DNA polymerase. Amplify 25 cycle programmed CTX-M-1, 2 and 3 are carried out on a DNA Thermolyne programmed as follows: initial denaturation at 94 °C for 3 min and 25 cycles of denaturation at 94 °C for 30 sec, annealing at 55 °C for 30 sec and extension at 72 °C for 60 sec, with a final step of 72 °C for 7 min. Amplify 25 cycle programme CTX-M-4 was carried out on a DNA Thermolyne programmed as follows: initial denaturation at 94 °C for 3 min and 25 cycles of denaturation at 94 °C for 30 sec, annealing at 62 °C for 30 s and extension at 72 °C for 60 sec, with a final step of 72 °C for 7 min. Large scale PCR was carried out on a DNA Multiblock System with the same programmed. PCR products were visualized on horizontal 1.0% agarose gels in 0.5×TBE buffer, loaded with 5 µL of reaction mix and stained with ethidium bromide after electrophoresis (Fig. 1). The primers, sizes of the expected amplification product, and annealing temperatures used for PCR amplification are listed in Table 1.

Results

Clinical bacterial strains

Of the 160 strains included in this study, 115 (71.8%) were isolated from urine cultures, 10

(6.2%) from wounds, 8(5%) from upper airway 5(3.1%) from sputum, 4(2.5%) from blood culture, 3(1.8%) from aspirate, 3(1.8%) from abscess, 3(1.8%) from bipsy, 4(2.5%) from CSF 2(1.2%) from exuda, 2(1.2%) from pleura and 1 (0.6%) from fistula.

Resistance phenotypes of ESBL-producing isolates

Of 160 isolates of *E. coli* primary phenptypic tests revealed that 56.68% (n=89) of *E. coli* isolates produced extended-spectrum beta-lactamases. In confirmatory tests by use of clavulanic acid, ESBL production was confirmed in 96.7% (n=86) of isolates with primary positive phenotype. The presence of an ESBL was not confirmed in 3.3% (n=3) of the screen positive isolates. The data on the activities of various antimicrobial agents ESBL-producing *E. coli* isolates are summarized in Table 2. Cefotaxime MICs were expectedly higher for CTX-M-producing *E. coli* (MIC at which 50% of strains tested were resistant [MIC50], ≥256 μg/ml) than for those expressing other types of ESBLs (MIC50, 16 and 8 μg/ml, respectively). Nevertheless, a high degree of diversity of the levels of resistance to cefotaxime was observed for CTX-M-positive strains, as illustrated by the broad range of MICs (16 to ≥256 μg/ml). Cefepime also demonstrated significantly lower activity against CTX-M producers in term of elevated MIC_{50} and MIC_{90} (Table 2). Yet, the levels of resistance to cefepime varied widely within the group of CTX-M producers (MIC range, 1 to 256 μg/ml) as well as within the group of strains producing other types of ESBLs (MIC range, 0.06 to 256 μg/ml). The cefotaxime MICs for such strains were two to nine two fold dilutions higher than those of ceftazidime (Table 3). Nevertheless, MICs of cefotaxime and ceftazidime were equal or differed by only one two fold dilution in 5 (20.5%) *E. coli* isolates producing CTX-M enzymes. Nevertheless, MICs of cefotaxime and ceftazidime were equal or differed by only one two fold dilution in 9 (26.4%) *E. coli* isolates producing CTX-M enzymes. All isolates equally resistant to cefotaxime and ceftazidime had the highest detectable MICs of these drugs (≥256 μg/ml). In some cases, high-level resistance to ceftazidime was possibly associated with production of mutant CTX-M enzymes. On the other hand, the MICs of cefotaxime for 13 *E. coli* isolates producing non-CTX-M-type ESBLs were one to three two fold dilutions higher than those of ceftazidime. All the above data suggest that a phenotypic approach based on the comparison of cefotaxime and ceftazidime MICs has limited value in predicting the presence of the CTX-M- type ESBLs in clinical.

Table 1: Primers Used For Amplification

Target(s)	Primer Name	Sequence[a]	Product size (bp)	CTX-M Genes
CTX-M group I	CTXM1-F3	GAC GAT GTC ACT GGC TGA GC	499	CTX-M-1, -3, -10 to -12, -15 (UOE-1), -22, -23, -28 to -30
	CTXM1-R2	AGC CGC CGA CGC TAA TAC A		
CTX-M group II	TOHO1-2F	GCG ACC TGG TTA ACT ACA ATC C	351	CTX-M-2, -4 to -7, and -20 and Toho-1
	TOHO1-1R	CGG TAG TAT TGC CCT TAA GCC		
CTX-M group III	CTXM825F	CGC TTT GCC ATG TGC AGC ACC	307	CTX-M-8 and -25
	CTXM825R	GCT CAG TAC GAT CGA GCC		
CTX-M group IV	CTXM914F	GCT GGA GAA AAG CAG CGG AG	474	CTX-M-9, -13, -14, -16 to -19, -21, and -27 and Toho-2
	CTXM914F	GTA AGC TGA CGC AAC GTC TG		

[a] sequence of primer as synthesized 5' to 3'

Table 2: In vitro activities of antimicrobial agents against *E. coli* isolates that produce CTX-M (Total number 36)

Antimicrobial's Name	No. (%) of resistant isolates [a]	MIC (µg/ml)[b]		
		Range	50%	90%
Ceftazidime	36 (100)	1–≥256	8	≥256
Cefotaxime	36 (100)	8–≥256	≥256	≥256
Cefepime	36 (100)	2–≥256	16	128

a Refers to cumulative number (percent) of isolates categorized as intermediate or resistant according to the CLSI's breakpoint criteria. All ESBL producers were considered resistant to ceftazidime, cefotaxime, and cefepime independent of MICs.
b 50% and 90%, MIC50 and MIC90, respectively

Table 3: Frequency distribution of cefotaxime and ceftazidime MIC ratios among *E. coli* isolates producing CTX-M and other types of ESBLs

MIC ratios	No. (%) of *E. coli* isolates Producing:		No. (%) of *E. coli* isolates Producing:	
	CTX-M ESBLs *(n = 34)*	Non- CTX-M ESBLs *(n = 56)*	CTX-M ESBLs *(n = 34)*	Non- CTX-M ESBLs *(n = 56)*
	(ceftazidime)		(cefotaxime)	
1/64	0	0	0	0
1/32	0	0	0	0
1/16	0	1 (1.7)	0	1(1.7)
1/8	0	2 (3.5)	0	1(1.7)
1/4	1 (5.8)	1 (1.7)	0	2 (3.5)
1/2	2 (8.8)	3 (5.3)	0	3 (5.3)
1	1 (2.9)	2 (3.5)	0	5 (8.9)
2	3 (8.8)	4 (7.1)	2 (8.8)	2 (3.5)
4	3 (8.8)	6 (10.7)	2 (8.8)	2 (3.5)
8	7 (20.5)	7 (12.5)	1(2.9)	7 (12.5)
16	6 (17.6)	8 (14.7)	2 (8.8)	1 (1.7)
32	4 (11.7)	12 (21.4)	4 (11.7)	13 (23.2)
64	4 (11.7)	6 (10.7)	10 (29.4)	10 (17.8)
128	3 (8.8)	5 (8.9)	13 (38.2)	9 (16)

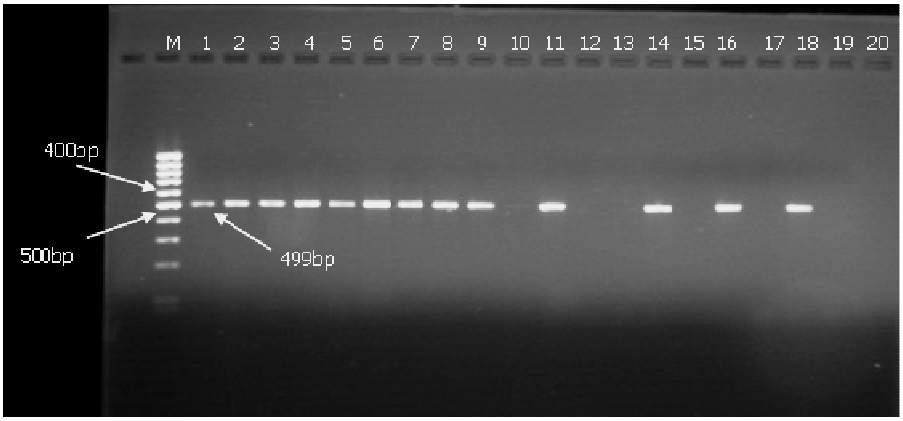

Fig. 1: 1% agarose gel of *E. coli* PCR products with primer CTXM1-F3& CTXM1-R2. M: DNA molecular weight standard 100 bp. Lane 1-20: Lanes 1 to 9, 7, 11, 14, 16 and 18 were positive (499bp) & Lanes 10,12, 13, 15, 17, 19 and 20 were negative

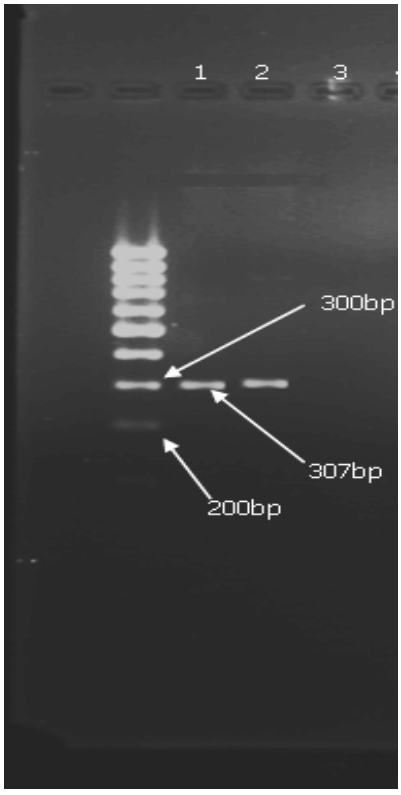

Fig. 2: 1% agarose gel of *E. coli* PCR products with primer CTXM825F & CTXM825R. M: DNA molecular weight standard 100 bp.
Lane 1-3: Lanes 1 and 2 were positive (307bp) & Lanes 3 Was negative control

Discussion

There has been a dramatic increase in the number of organisms reported in the literature that produce CTX-M-β-lactamases (16). This class of β-lactamases has been recognized worldwide as an important mechanism of resistance to oxyiminocephalosporins used by gram-negative pathogens (16). In most cases, organisms producing these enzymes display higher levels of resistance to cefotaxime and ceftriaxone than ceftazidime (16). However, organisms producing some CTX-M variants, including CTX-M-16 and -19 and UOE-1, are resistant to ceftazidime which harboring the Asp 240Gly subsituation (17, 18). Phenotypic differentiation of organisms producing CTX-M-β-lactamases from organisms producing other types of ESBLs can be difficult. The difficulty is due to overlapping phenotypes resulting in interference from other β-lactamases produced by the organism capable of hydrolyzing ceftazidime (19). Therefore, susceptibility testing which relies on identifying organisms that are resistant to cefotaxime and/or ceftriaxone but susceptible to ceftazidime is not a reliable approach (16). The data on the activities of cefotaxime, ceftazidime and cefepim against ESBL-producing *E. coli* isolates indicate that 60% of ESBLs-producer strains will not be detected if ceftazidime is used as initial screen. This data for cefotaxime and cefepim were 30% and 43% respectively.
Cefotaxime MICs were expectedly higher for CTX-M producing *E.coli* than for expressing other type of ESBLs. Nevertheless, a high degree of diversity of the levels of resistance to cefotaxime was observed for CTX-M-positive strains, as illustrated by the broad range of MICs (8 to ≥256 μg/ml). Similar to our study, a very high rate of resistance to cefotaxime (67.8%) has been reported by Mobaiyen et al., who analyzed a relatively 786 of ESBL-producing *E.coli* strains isolated in Tabriz hospitals the period from 2006 to 2007 (20). More surprisingly, no statistically significant difference was observed in ceftazidime MICs between the groups of *E. coli* isolates producing CTX-M and non-CTX-M type ESBLs. The resistance phenotype of the majority of CTX-M-positive isolates was consistent with production of CTX-M ESBL. In the study of Edelstein et al. similar to our study, no significant difference was observed in ceftazidime MICs between the groups of *E. coli* and *K. pneumoniae* isolates producing CTX-M and non-CTX-M type ESBLs (21). Cefepime also demonstrated significantly lower activity against CTX-M producers. Yet, the levels of resistance to cefepime varied widely within the group of CTX-M producers as well as within the group of strains producing other types of ESBLs. It is interesting that the three isolates of *E. coli* which were found to produce the same type of CTX-M β-lactamase differed considerably in the levels of resistance to cefotaxime (MICs, 8, 64, and ≥256 μg/ml, respectively) and

cefepime (MICs, 4, 16, and ≥256 μg/ml, respectively). This observation supports the findings of Baraniak et al., who found that resistance phenotype conferred by the CTX-M-3 enzyme may be variable, possibly reflecting the fluctuations in the level of its expression (22). Although in the study of Yu et al. high-level resistance to cefepime was strongly associated with the CTX-M-type ESBLs (23), our data indicate that the MICs of cefepim alone are not always predictive of a particular ESBL type. All the above data suggest that a phenotypic approach based on the comparison of cefotaxime, ceftazidime and cefepim MICs has limited value in predicting the presence of the CTX-M-type ESBLs in clinical isolates. The presence of an ESBL was not confirmed in over 4.21% of the screen positive presumably because these strains had additional mutations affecting porin channels for antibiotic uptake (24) or because they concomitantly expressed AmpC β-lactamase. Detecting $AmpC^+$ isolates may be clinically important not only because of their broader cephalosporin resistance but also because carbapenem resistance can arise in such strains by further mutations, resulting in reduced porin expression (24). A number of mechanisms have been described that could account for the lack of clavulanate enhancement in the screen-positive nonconfirming *E. coli* isolates that possessed only TEM genes. These include TEM-1 hyper production, modification of outer membrane proteins, or the presence of inhibitor-resistant TEM enzymes (25).

All of the ESBL-producing isolates were examined by PCR for presence of *bla* $_{CTX-M}$ genes. Of all screen positive isolates, 34 (35.78%) were positive for *bla* $_{CTX-M}$ genes from the CTX-M-I group, indicating CTX-M-1-like β-lactamases (Fig. 1), and two (2.1%) were positive for *bla* CTX-M genes from the CTX-M-III group, indicating CTX-M-3-like β-lactamases (Fig. 2). The remainder 59 (62.2%) of the ESBL-producing isolates in this report were negative by PCR for *bla* CTX-M. In the study of Mobaiyen et al. similar to our study, β-lactamases of the CTX-M-1 cluster were the predominant CTX-M enzymes (20). These data could indicate that the ESBL phenotype is due to production of ESBLs other than CTX-Ms. However, the negative PCR results in this report do not negate the possibility that modified *bla* $_{CTX-M}$ were present in these isolates. Due to the increased complexity of β-lactam resistance in gram-negative organisms, the key to effective surveillance is the use of both phenotypic and genotypic analyses in concert (26).

In this study we have shown the high prevalence of CTX-M-producing *E. coli* isolates in Tehran hispitals. β-lactamases of the CTX-M-1 cluster were the predominant CTX-M enzymes in these isolates. Out of this group only CTX-M-3-like enzymes were identified among selected isolates. In addition to the clonal spread of the strains, plasmid transfer played an important role in the dissemination of the CTX-M-1-cluster enzymes among clinical isolates of *E. coli*. The alarming situation with global dissemination of CTX-M-producing isolates highlights the need for their epidemiological monitoring and prudent use of antimicrobial agents.

Acknowledgements

The authors kindly thank the research deputy of Shahed University for its financial support. We acknowledge Dr. Zohre Maleki from Imam Khomeini Hospital, Dr. Maryam Aghdaee, Dr. Safoora Salehi and Dr. Haleh Ghezelbash graduates of Medicine from Shahed University, Ms. Mina Abedini from Children Medical Center of Tehran and Mr. Hassan Shahin from Shaheed Mostafa Khomeini Hospital for their support in sample collection.

The authors declare that they have no conflict of interests.

References

1. Bauernfeind A, Grimm H, Schweighart S (1990). A new plasmidic cefotaximase in a clinical isolate of *Escherichia coli*. *Infection*, 18: 294-48.

2. Bauernfeind A, Casellas JM, Goldberg M (1992). A new plasmidic cefotaximase from patients infected with *Salmonella typhimurium*. *Infection,* 20: 158-63.
3. Bernard H, Tancrede C, Livrelli V, Morand A, Barthe´le´my M, Labia R (1992). A novel plasmid-mediated extended-spectrum β-lactamase not derived from TEM- or SHV-type enzymes. *Antimicrob Agents Chemother*, 29: 590–92.
4. Bou G, Cartelle M, Tomas M, Canle D, Molina F, Moure R, Eiros JM, Guerrero A (2002). Identification and broad dissemination of the CTXM-14 β-lactamase in different *Escherichia coli* strains in the northwest area of Spain. *J Clin Microbiol,* 40: 4030-36.
5. Chanawong A, M'Zali FH, Heritage J, Xiong JH, Hawkey PM (2002). Three cefotaximases, CTX-M-9, CTX-M-13 and CTX-M-14 among *Enterobacteriaceae* in the People's Republic of China. *Antimicrob Agents Chemother*, 46: 630-37.
6. Pai H, Choi EH, Lee HJ, Hong JY, Jacoby GA (2001). Identification of CTX-M-14 extended-spectrum beta-lactamase in clinical isolates of *Shigella sonnei*, *Escherichia coli* and *Klebsiella pneumoniae* in Korea. *J Clin Microbiol*, 39: 3747–49.
7. Petroni A, Corso A, Melano R, Cacace ML, Bru AM, Rossi A, Galas M (2002). Plasmidic extended-spectrum β-lactamases in *Vibrio cholerae* O1 El-Tor isolates in Argentina. *Antimicrob Agents Chemother*, 46: 1462–6 8.
8. Brinas L, Moreno MA, Zarazaga M, Porrero C, Saenz Y, Garcia M, Dominguez L, Torres C (2003). Detection of CMY-2, CTX-M-14, and SHV-12 β-lactamases in *Escherichia coli* fecal-sample isolates from healthy chickens. *Antimicrob Agents Chemother*, 47: 2056–8.
9. Lucia P, Bartoloni A, Fiorelli C, Mantella A, Maggio TD et al. (2007). Rapid dissemination and diversity of CTX-M extended-spectrum β-lactamase genes in commensal *Escherichia coli* isolates from healthy children from low-resource settings in Latin America. *Antimicrob Agents Chemother* 51: 2720–25.
10. Baraniak A, Fiett J, Hryniewicz W, Nordmann P, Gniadkowski M (2002). Ceftazidime-hydrolyzing CTX-M-15 extended-spectrum β-lactamase (ESBL) in Poland. *Antimicrob Agents Chemother*, 50: 393-96.
11. Bradford PA, Urban C, Jaiswal A, Mariano N, Rasmussen BA, Projan SJ, Rahal JJ, Bush K, (1995). SHV-7, a novel cefotaxime-hydrolyzing β-lactamase, identified in *Escherichia coli* isolates from hospitalized nursing home patients. *Antimicrob Agents Chemother*, 39: 899-905.
12. Canton R, Oliver A, Coque TM, del Carman Varela M, P'erez-Diaz JC, Baquero F (2002). Epidemiology of extended-spectrum β-lactamase producing *Enterobacter* isolates in Spanish hospital during a 12-year period. *J Clin Microbiol*, 40: 1237-43.
13. Clinical and Laboratory Standards Institute (2006). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard. 7th ed. M7–A7. *Clinical and Laboratory Standards Institute*, Wayne, PA.
14. Clinical and Laboratory Standards Institute (2006). Performance standards for antimicrobial susceptibility testing: 16th informational supplement M100–S16. *Clinical and Laboratory Standards Institute*, Wayne, PA.
15. Pitout JD, Thomson DKS, Hanson ND, Ehrhardt AF, Coudron F, Sanders CC (1998). Plasmid-mediated resistance to expanded-spectrum cephalosporins among *Enterobacter aerogenes* strains. *Animicrob Agent Chemoter,* 42: 596-600.
16. Bonnet R (2004). Growing group of expanded-spectrum β-lactamase: the CTX-M enzymes. *Animicrob Agent Chemoter,* 48: 1-14.

17. Bonnet R, Dutour C, Sampaio JL, Chanal C, Sirot D, Labia R, De Champs C, Sirot J (2001). Novel cefotaximase (CTX-M-16) with increased catalytic efficiency due to substitution Asp-240→Gly. *Animicrob Agent Chemoter*, 45: 2269-75.
18. Poirel L, Kampfer P, Nordmann P (2002). Biochemical analysis of the ceftazidime-hydrolysing expanded-spectrum β-lactamase CTX-M-15 and of its structurally related β-lactamase CTX-M-3. *Animicrob Agent Chemoter*, 46: 4038-40.
19. Baraniak A, Fiett F, Sulikowska A, Hryniewicz W, Gniadkowski M (2002). Countrywide spread of CTX-M-3 expanded-spectrum β-lactamase producing microorganisms of family Enterobacteriaceae in Poland. *Animicrob Agent Chemoter*, 46: 151-9.
20. Mobaiyen H, Nahaei MR, Amirmozafari N, Moaddab SR, Hoseni F, Biaytmacoo R (2007). First report of CTX-M type β-lactamase producing clinical Escherichia coli isolates from intensive care units in Tabriz hospitals. Abstr. 1st *Iranian Cong of clinical Microbio,*Shiraz, Iran, Abstracts' Book, 152.
21. Edelstein M, Pimkin M, Palagin I, Edelstein I, Stratchounski L (2003). Prevalence and molecular epidemiology of CTX-M extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Russian hospitals. *Animicrob Agent Chemoter*, 47: 3724-32.
22. Bradford PA, Yang Y, Sahm D, George I, Gardovska D, Storch G (1998). CTX-M-5, a novel cefotaxime-hydrolyzing beta-lactamase from an outbreak of *Salmonella typhimurium* in Latvia. *Antimicrob Agents Chemother*, 42: 1980–84.
23. Yu WL, Pfaller MA, Winokur PL, Jones RN (2002). Cefepime MIC as a predictor of the extended-spectrum β-lactamase type in *Klebsiella pneumoniae*, Taiwan Emerg *Infec Dis* 8: 522–24.
24. Alvarez M, Tran JH, Chow N, Jacoby GA (2004). Epidemiology of conjugative plasmid-mediated AmpC β-lactamases in United States. *Antimicrob Agents Chemother* 48: 533-7.
25. Bell JM, Chitsaz M, Turnidge JD, Barton MD, Walters LJ, Jones RN (2007). Prevalence and significance of a negative extended-spectrum β-lactamase (ESBL) confirmation test result after a positive ESBL screening test result for isolates of *Escherichia coli* and *Klebsiella pneumoniae*: results from the SENTRY Asia-Pacific surveillance program. *J Clin Microbiol*, 45: 1478-82.
26. Pitout JD, Hossain DA, Hanson ND (2004). Phenotypic and molecular detection of CTX-M-β-lactamases produced by *Escherichia coli* and *Klebsiella* species. *J Clin Microbiol*, 42: 5715-21.