Journal of Clinical Laboratory Analysis 23 : 404–407 (2009)

Prevalence Characterization of Extended-Spectrum β-Lactamases among *Escherichia coli* Isolates Collected in Zhengzhou

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Objectives: To determine the prevalence and the diversity of extended-spectrum β-lactamases (ESBLs) among *Escherichia coli* isolates in Zhengzhou, China.
Methods: Clinical isolates were collected and investigated from the first affiliated hospital of Zhengzhou University and its associated health-care facilities in Zhengzhou, China, during the period from January 2006 to June 2008. Antibiograms were performed on Mueller–Hinton agar plates with the disc-diffusion method and MICs were determined by the agar-dilution method. Total DNA was extracted with a Qiagen mini kit and screened by PCR.
Results: Of 94 nonduplicate ESBL-positive isolates, TEM-type was encoded in 74 and 79% of the ESBL isolates. Fifty-six isolates were SHV type ESBLs. CTX-M-1, CTX-M-14, CTX-M-25, and CTX-M-38 types were encoded in 30, 54, 6, and 4, respectively. OXA-1-type β-lactamases were encoded in six and OXA-20-type was encoded in two isolates.
Conclusions: We describe a complex ESBL epidemiology. The study revealed a high rate of ESBL-producing *E. coli* isolates. TEM and CTX-M enzymes dominated in ESBL-positive *E. coli* isolates in Zhengzhou, China. J. Clin. Lab. Anal. 23:404–407, 2009. © 2009 Wiley-Liss, Inc.

Key words: prevalence; extended-spectrum β-lactamases (ESBLs); Escherichia coli; Zhengzhou

INTRODUCTION

Multiresistant, extended-spectrum β lactamase (ESBL)-producing pathogens are an increasing problem in daily clinical life. When producing these enzymes, organisms become highly efficient at inactivating the newer third-generation cephaloporins (such as cefotaxime, ceftazidime, and ceftriaxone). In addition, ESBL-producing bacteria are frequently resistant to many classes of non-β-lactam antibiotics, resulting in difficult-to-treat infections. These have been described worldwide and are a major cause of nosocomial infections associated with high mortality (1). In recent years, new non-TEM non-SHV ESBLs (CTX-M, PER-1, and VEB-1) were reported in epidemic clones of Enterobacteriaceae involved in hospital-acquired outbreaks in South America, Asia, and particularly in Turkey and Eastern Europe (2–9). Whereas before 2000 these new types had rarely been identified (6,10).

In this study, nonduplicate clinical isolates of ESBL-positive *Escherichia coli* were collected in the First Affiliated Hospital, Zhengzhou University, China. Our study investigated the occurrence and the diversity of β-lactamase genes among the isolates.

MATERIALS AND METHODS

Bacterial Isolates

A total of 437 clinical *Escherichia* isolates (one isolate per patient) were collected from the Department of Respiratory Medicine, Blood medicine and ICU from three hospitals (first, second, third affiliated hospitals of Zhengzhou University, Zhengzhou, China, during the period from January 2006 to June 2008). The species of the organisms

Grant sponsor: Natural Science of Henan Province; Grant number: 2007310014.

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Received 20 February 2009; Accepted 14 August 2009
DOI 10.1002/jcla.20351
Published online in Wiley InterScience (www.interscience.wiley.com).

were verified by tests with the VITEK120 system (Bios Merieumx, Lyons, France). The presence of ESBLs was confirmed by the E-test (AB Biodisk, Solna, Sweden), where an eightfold reduction of MIC in the presence of clavulanic acid indicated the presence of ESBL.

Antimicrobial Susceptibility and Synergy Testing

Antibiograms were done on Mueller–Hinton agar plates with the disc-diffusion method. MICs of cefotaxime, ceftazidime, and ceftriaxone were determined by the agar-dilution method and interpreted. Antibiotics disks were purchased from Bio-Rad, Marnes la Coquette, France. *E. coli* ATCC 25922 was used as a control strain. The antibiogram of the isolates is shown in Table 1.

PCR Amplification for Detection of β-Lactamase Genes

All isolates with ESBL phenotypes were subjected to DNA extraction with a Qiagen mini kit (Qiagen, Courtaboeuf, France) and screened for the resistance genes SHV, TEM, CTX-M, and OXA by a PCR assay using universal primers and specific primers for diverse CTXM groups (CTX-M-1, CTX-M-14, CTX-M-25, and CTX-M-31) and OXA groups (OXA-1, OXA-2, OXA-3, OXA-5, OXA-7, OXA-18, OXA-20, OXA-23, and OXA-25) (Table 2). For their little homology, all the types were difficult to detect if only a kind of primer was amplificated. We classified all the subgroups according to the cladogram method. Several paired of selected primers were amplificated to obtain all the known epidemic types. PCR amplification reactions were performed in a volume of 25 μl containing 12.5 μl of 2 × Qiagen Multiplex PCR Master Mix (Qiagen GmbH, Hilden, Germany), 0.2 μM concentrations of each primer, and 2 μl of DNA template. The cycling parameters were as follows: an initial denaturation at 95°C for 15 min; followed by 30 cycles of 94°C for 30 sec, 55°C for 90 sec, and 72°C for 60 sec; and with a final extension at 72°C for 10 min. The amplified PCR products were subjected to electrophoresis on a 1.5% agarose gel in 1 × TAE buffer. Isolates detected to be CTX-M and OXA gene positive in the PCR were further identified by using in-house designed primers for the groups. The size of products was about 900 bp.

TABLE 1. The Antibiogram of the Isolates

Antibacterials	MIC (μg/ml)	Result
Ampicil	256*	R
TMP-SMZ	256*	R
Cefotaxim	256*	R
Imipenem	0.25	S
Cefepime	32	R
Piperacillin	256*	R
Cefuroxime	256*	R
Amikacin	3	S
Ceftazidime	4	S
Alfatil	256*	R
Cefatrizine	256*	R

The results were assessed according to international standard by NCCLs.

RESULTS

A total of 437 clinical isolates collected were studied. Ninety-four (21.5%) isolates were defined as ESBLs producers according to the results of the disc-diffusion method showing a resistance to ESBL marker antibiotics. PCR was discriminatory to genes encoding SHV, TEM, CTX-M, and OXA enzymes (Fig. 2). ESBL-positive *E. coli* isolates were found in lane TEM,

TABLE 2. Primers Used for Detection of Different β-Lactamase Genes in the Multiplex PCR

Primers	Sequences up: (5′→3′)	Sequences down: (5′→3′)	Annealing temperature (°C)
TEM	gat,cca,tga,gta,ttc,aac,a	tcg,agt,tac,caa,tgc,tta,a	51
SHV	gat,cca,tgc,gtt,ata,ttc,gcc	tcg,agt,tag,cgt,tgc,cag,tgc	55
CTXM1	atg,gtt,aaa,aaa,tca,ctg,c	tcc,gtt,tcc,gct,att,aca,a	55
CTXM14	cct,ttg,aga,tgg,tga,caa	gtt,aca,gcc,ctt,cgg,cga	55
CTXM25	atg,atg,aga,aaa,agc,gt	tta,ata,acc,gtc,ggt,gac	55
CTXM31	atg,atg,act,cag,agc,a	agt,cag,aaa,ccg,tgg,g	55
OXA1	atg,aaa,aac,aca,ata,cat,atc	gag,tta,taa,att,tag,tgt,gtt,tag	51
OXA2	atg,tta,tgg,agc,agc,a	tta,tcg,cgc,agc,gtc,c	51
OXA3	atg,gca,atc,cga,atc	tta,tcg,cgc,tgc,gtc	51
OXA5	atg,cgg,cct,aac,aat,tcg,tc	tta,gcc,acc,aat,gat,gat,gc	51
OXA7	atg,aaa,aca,ttt,gcc,gca	tta,gcc,acc,aat,gat,gcc	51
OXA18	atg,cat,cac,aac,tgg,gca,aa	tca,gaa,gtt,ttc,cga,cag,gg	51
OXA20	atg,ata,atc,cga,ttt,cta	cta,gtt,ggg,tgg,caa,agc	51
OXA23	aaa,atg,aat,aaa,ta	ctc,gag,ctc,tta,aa	51
OXA25	atc,cgt,aat,gaa,aaa,a	tta,aat,gat,tcc,aag,a	51

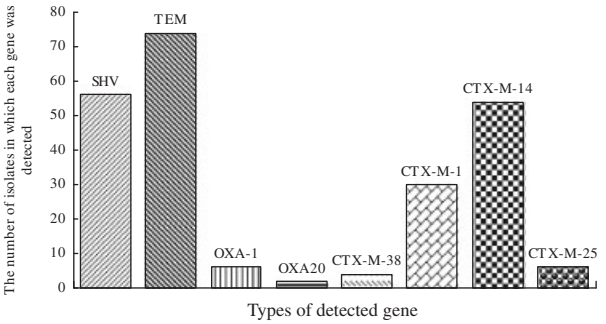

Fig. 1. Distribution of different β-lactamase genes among ESBL-positive *E. coli*. The figure shows among the 94 ESBL-positive isolates, 56 isolates were SHV-type. CTX-M-1, CTX-M-14, CTX-M-25, CTX-M-38 types were respectively, encoded in 30, 54, 6, and 4. Seventy-four isolates were TEM-type; OXA-1-type β-lactamases were encoded in six and OXA-20-type were encoded in two.

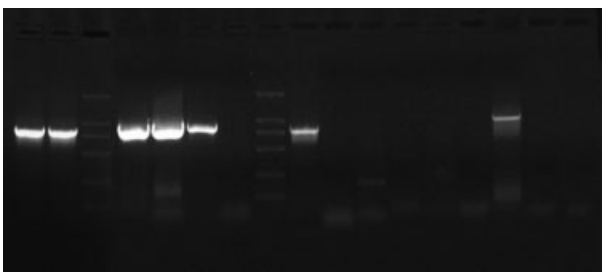

Fig. 2. ESBLs plasmid PCR production agar electrophoresis. ESBL-positive *E. coli* isolates were found in lane TEM, SHV, CTX-M-1, CTX-M-14, CTX-M-25, OXA-1 and OXA-20.

SHV, CTX-M-1, CTX-M-14, CTX-M-25, OXA-1 and OXA-20. The dissemination of the isolates is illustrated in Figure 1. PCR amplification showed that among the 94 ESBL-positive isolates, TEM-type isolates were encoded in 74 and 79% of the ESBL isolates. Fifty-six isolates were SHV type ESBLs. CTX-M-1, CTX-M-14, CTX-M-25, and CTX-M-38 types were encoded in 30, 54, 6, and 4, respectively. OXA-1-type β-lactamases were encoded in six and OXA-20-type β-lactamases were encoded in two isolates.

DISCUSSION

The results of this study provide insights into the prevalence and the diversity of ESBLs among *E. coli* isolates at a general hospital. The ESBL-positive *E. coli* isolates, investigated here, encoded mainly in TEM-type (74 and 79% of the ESBL isolates) and CTMs types (94 isolates). Fifty-six isolates were SHV-type ESBLs. OXAs-type β-lactamases were seldom detected in the isolates. The incidence of ESBL-producing isolates varies according to countries, regions, or even hospitals. The ESBLs prevalence rate in this study is much higher than those found in the United States (11) and Canada, and illustrates the increase of the resistance to extended-spectrum cephalosporins in China. The high percentage of ESBL-producing isolates is indicative of a selection pressure. The new β lactams, such as cefotaxime, are extensively used in hospital practice. ESBLs producers presented a wide resistance spectra, including resistance to aminoglycosides and sulfonamides. A close association of ESBL production with aminoglycosides and sulfonamides resistance has been previously reported (12). Some studies reported that the widespread overuse of aminoglycosides in hospitals could be the driving-selection pressure for ESBLs emergence, because plasmids-encoding ESBLs frequently carry determinants of aminoglycosides resistance (13). But we consider the main cause as the overuse of β-lactam antibiotics. Aminoglycosides antibiotics only act as cooperation and the mechanism is in nubibus.

CTX-M-3 and CTX-M-15 enzymes are commonly detected worldwide in hospitals as well as in community isolates (14–18). They are the most described ESBL types in Algeria (19–23) and have also been detected in Tunisia (23). In this study, TEM CTX-M enzymes are mostly detected. The absolute predominance of the TEM ESBLs in our isolates probably results from both selection pressure and dissemination of organisms or resistant genes. Currently, the emerging fact is that CTX-M enzymes have recently and sharply accumulated in *E. coli* and Klebsiella species (18). The presence of the bla TEM, SHV, and blaCTX-M-14 indicates a spreading of the β-lactamases all over the country. This situation is more serious than those reported in numerous other countries. This is a public-health concern, which requires an increased monitoring and an implementation of a policy of antibiotics use.

In conclusion, TEM and CTX-M enzymes dominated in ESBL-positive *E. coli* isolates in a China general hospital and its associated health-care facilities. CTX-M and SHV-type β-lactamase genes were also present in more than half of the ESBL isolates and OXA-type in a few strains.

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