Comp Clin Pathol
DOI 10.1007/s00580-012-1475-9

ORIGINAL ARTICLE

Molecular characteristics of extended-spectrum beta-lactamases among gram-negative isolates collected in Cairo University Hospital

Rasha Hamed El sherif · Hoda Abdel Hamid Maamoun

Received: 28 January 2012 / Accepted: 20 March 2012

Abstract Phenotyping is commonly used for detection of extended-spectrum beta-lactamase (ESBL) production in gram-negative isolates. ESBLs are mainly coded for by four important genes, namely *bla* (TEM), *bla* (SHV), *bla* (CTX-M), and *bla* (OXA). Our aim in this study is to assess use of a multiplex PCR as a rapid method to identify four common genes responsible for ESBL production in different gram-negative isolates. All 793 clinical isolates are subjected to both screen and confirmatory testing for ESBL production using double disc synergy testing (DDST). Two hundred isolates with the ESBL phenotype are subjected to multiplex PCR for detection of the four genes *bla* (TEM, SHV, CTX-M, and OXA). The isolates were obtained from various clinical specimens: 68 (34 %) were isolated from urine cultures, 43 (21.5 %) from sputum, 26 (13 %) from wounds, 34 (17 %) from blood culture, 20 (10 %) from stool of healthy carrier and nine (4.5 %) from bronchoalveolar lavages. In this study, 83 isolates (41.5 %) were from outpatients (urine and stool specimens only), and the remaining 117 isolates (58.5) were from inpatients. By PCR technique, 181 isolates were found to be ESBL producers. blaTEM was the commonest genotype (39.2 %), followed by blaSHV (32.5 %) and blaCTX-M (30.9 %), either alone or in combination. *Acinetobacter baumannii* isolate had none of the ESBL genes. Eighteen (9.9 %) out of 181 isolates carried more than one type of beta-lactamase genes. Our study demonstrated rapid detection of *bla* (TEM, SHV, CTX-M, and OXA) in isolates belonging to Enterobacteriaceae and other nonfermenting clinical isolates using multiplex PCR. This genotypic method provided a rapid and efficient differentiation of ESBLs in the laboratory.

Keywords Extended-spectrum beta-lactamase (ESBL) · Genes · Multiplex PCR

Introduction

Extended-spectrum beta-lactamases (ESBLs) are a heterogeneous group of plasmid-mediated bacterial enzymes that confer significant resistance to oxyimino-cephalosporin and monobactam antimicrobials (Canton et al. 2008; Bradford 2001) Recently, resistance to cephalosporins has become widespread throughout the world, and numerous types of ESBLs have been detected in various bacterial organisms (Shah et al. 2004).

Detection and classification of ESBLs are important in making clinical decisions regarding for appropriate therapy and infection control. However, detection and classification are time-consuming and complicated processes, and some types of ESBLs are frequently not detected during the detection process (Bush 2001).

Various authors have reported the prevalence of ESBLs to be in the range of 6–88 % in various hospitals (Shah et al. 2004; Pai et al. 2004a, b; Lee et al. 2007), especially among *Klebsiella pneumoniae* and *Escherichia coli*.

Most ESBLs are mutants of blaTEM and blaSHV enzymes, but blaCTX-M enzymes are also increasingly becoming important. The blaCTX-M types are diverse with

The study was done in Cairo University, Egypt.

R. H. El sherif (✉)
The Department of Clinical & Chemical Pathology,
Faculty of Medicine, Cairo University,
Cairo, Egypt
e-mail: whiterose_eg@yahoo.com

H. A. H. Maamoun
The Department of Internal Medicine, Faculty of Medicine,
Cairo University,
Giza, Egypt

Published online: 13 April 2012

30 alleles divided into five distinct phylogenetic groups. These blaCTX-M enzymes predominantly hydrolyze cefotaxime 8. In clinical strains, CTX-M encoding genes have commonly been located on plasmids that vary in size from 7 to 160 kb (Bonnet 2004; Baron and Finegold 1990).

In this study, we used a rapid multiplex PCR to identify blaSHV, bla TEM, blaCTX-M, and bla-OXA. By using this method, clinical isolates of ESBL-producing members of the family Enterobacteriaceae, including *Enterobacter* spp., *E. coli*, *Klebsiella* spp., and nonfermenter, could be characterized at molecular level and could evaluate the efficiency and accuracy of this method as compared to the conventional method.

Material and methods

Clinical bacterial isolates

All 793 clinical isolates were obtained from consecutively collected samples in the Department of Clinical Microbiology at Cairo University Hospital from October 2010 to June 2011. Two hundred isolates with the ESBL phenotype, included in this study, were obtained from various clinical specimens: 68 (34 %) were isolated from urine cultures, 43 (21.5 %) from sputum, 26 (13 %) from wounds, 34 (17 %) from blood culture, 20 (10 %) from stool of healthy carrier, and nine (4.5 %) from bronchoalveolar lavages. In this study, 83 isolates (41.5 %) were from outpatients (urine and stool specimens only), and the remaining 117 isolates (58.5 %) were from inpatients.

The selected 200 bacterial isolates include 80 (40 %) *E. coli*, 77 (38.5 %) *Klebsiella* species, 21 (10.5 %) *Citrobacter* species, 13(6.5 %), *Enterobacter* species, 5 (2.5 %) *Pseudomonas aeruginosa*, and four (2 %) *Acinetobacter baumannii* isolates Table 1. The 200 ESBL-positive isolates were subjected to PCR to identify the presence of *blaSHV*, *blaTEM*, *blaCTX-M*, and *blaOXA* genes.

Phenotypic detection for ESBL

All isolates were identified by conventional bacteriological tests. Antibiotic susceptibility testing was performed as recommended by the CLSI using disks containing ceftazidime (CAZ: 30 μg), ceftriaxone (CRO: 30 μg), cefotaxime (CTX: 30 μg), piperacillin (PIP: 100 μg), piperacillin/tazobactam (PT: 110 μg), gentamicin (GM: 10 μg), amikacin (AN: 30 μg), imipenem (IMP: 10 μg), and ciprofloxacin (CIP: 5 μg) (Mast Diagnostics, Merseyside, UK). *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as controls (NCCLS 2002).

Double disc synergy confirmatory test as described by Jarlier et al. (Jarlier et al. 1988). The antibiotic discs used were ceftriaxone (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), and amoxycillin/clavulanic acid (20/10 μg). The three antibiotics were placed at distances of 15 mm (edge to edge) from the amoxycillin/clavulanic acid disc that was placed in the middle of the plate. An enhanced zone of inhibition between either of the cephalosporin antibiotics and the amoxycillin/clavulanic acid disc was indicative of synergistic activity with clavulanic acid and the presence of an ESBL.

PCR amplification for detection of beta-lactamase genes

The isolates were screened for the resistance genes *SHV*, *TEM*, *CTX-M*, and *OXA* by a multiplex PCR assay using universal primers (Table 1) (Fang et al. 2004; Monstein et al. 2007; Boyd et al. 2004; Ouellette et al. 1987).

DNA extraction and multiplex PCR assay

Preparation of DNA template for multiplex PCR

A single colony of each organism was inoculated from MacConkey agar (Mast Diagnostics, Merseyside, UK) into 5 ml of broth (Oxoid, England) and incubated for 24 h at 37 °C. Cells from 1.5 ml of the overnight culture were harvested by centrifugation at 12,000 rpm for 5 min. Plasmid DNA was extracted according to the published method of Johnson and Woodford (Johnson and Woodford 1998). Five microliters of template DNA was added to a 50-μL reaction mixture containing 25 mM Tris/HCl, 50 mM KCl, 1.5 mMMgCl2, 0.2 mM each dNTP (Qiagen, Hilden,

Table 1 The number of ESBL-producing strains among clinical specimens

Site	*E. coli*	*Klebsiella* spp.	*Enterobacter* spp.	*Citrobacter* spp.	*Pseudomonas aeruginosa*	*Acinetobacter baumannii*	Total
Urine	34	26	7	1	0	0	68
Sputum	9	26	3	3	1	1	43
Wound	7	6	5	5	3	0	26
Blood culture	15	9	3	3	1	3	34
BAL	2	5	1	1	0	0	9
Stool	13	5	2	0	0	0	20
Total	80	77	21	13	5	4	200

Germany), all primers at various concentrations, and 2.5 U Taq polymerase Mini kit 12123 (Qiagen, Hilden, Germany).

PCR amplification reactions were performed in a DNA thermal cycler in a volume of 25 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 s, 62 °C for 90 s, and 72 °C for 60 s; and with a final extension at 72 °C for 10 min. The amplified PCR products were subjected to electrophoresis at a 1.5 % agarose gel in 1× TAE buffer. Amplified products were visualized with ultraviolet light after staining with ethidium bromide. The primers and sizes of the expected amplification product for PCR amplification are listed in Table 2.

Results

CLSI ESBL screen method

The793 clinical isolates were obtained from consecutively collected samples in the Department of Clinical Microbiology at Cairo University Hospital from October 2010 to June 2011. The three antibiotics used in the ESBL screen were ceftazidime (30 μg), cefotaxime (10 μg), and ceftriaxone (30 μg). The zones were measured using the CLSI breakpoints for ESBL screening. The quality control isolates used in this test were within the control limits, for all indicator cephalosporins.

ESBL confirmatory test double disc synergy test (DDST)

The CLSI ESBL screen was followed by ESBL confirmatory test on the793 clinical isolates, using double disc synergy test (DDST). The results from the test are summarized in Table 3. Table 3 demonstrates the overall percentage of positive, negative, and indeterminate isolates obtained in the DDST. Only 30 %, 27 %, 20 %, 12 %, 33.3 %, and 28.5 of *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., *P. aeruginosa*, and *A. baumannii* isolates, respectively, were found positive using the DDST.

Antimicrobial susceptibility test

The results of the antimicrobial susceptibility test for the 200 positive clinical isolates are summarized in Table 4. The table demonstrates the percentage of resistant isolates to a range of common groups of antibiotics for the 80 isolates of *E. coli*, 77 isolates of *Klebsiella* spp., 21 isolates of *Enterobacter* spp., 13 isolates of *Citrobacter* spp., five isolates of *P. aeruginosa*, and four isolates of *A. baumannii* tested. It showed that *Klebsiella* spp. are intrinsically resistant to ampicillin. Similarly, *P. aeruginosa* and *A. baumannii* strains as well as common *Enterobacter* and some *Citrobacter* species are intrinsically resistant to ampicillin, amo/cla, and many of the earlier cephalosporins. This is mostly because of their chromosomal beta-lacatamase genes, and it has nothing to do with the acquired ESBL genes. The breakpoints used were the interpretive standards recommended by the CLSI. The quality control isolates used in this test were within the allowed control limits.

DNA amplification for detection of TEM-, SHV- , CTX-M- and OXA-type beta-lactamases

All ESBL-producing isolates were screened by PCR using *bla*TEM, *bla*SHV, *bla*CTX-M, and *bla*OXA specific primers (Fig. 1). ESBLs were found in 181 isolates (90.5 %): 80 *E. coli*, 77 *Klebsiella* spp., 20 *Enterobacter* spp., two *Citrobacter* spp., and two *P. aeruginosa* isolates. Some of the isolates harbored two or more ESBL genes, e.g., three clinical isolates of *E. coli* demonstrating both TEM- and OXA-specific products in multiplex PCR. However, the *A. baumannii* isolate had none of the ESBL genes.

TEM-type ESBLs were found in 68.75 % of *E. coli*, 7.8 % of *Klebsiella* spp., 42.8 % of *Enterobacter* spp., and 7.7 % of *Citrobacter* spp., and none of *P. aeruginosa* isolates had the gene (Table 5). Eighteen (9.9 %) out of 181

Table 2 Primers used for detection of different B-lactamase genes in the multiplex PCR

Amplicon	Temperature (°C)	Primer sequence (5_ to 3_)	Size (bp)	Reference
*bla*SHV-F	60	CTT TAT CGG CCC TCA CTC AA	237	Fang et al. (2004)
*bla*SHV-R	62	AGG TGC TCA TCA TGG GAA AG		
*bla*TEM-F	60	CGC CGC ATA CAC TAT TCT CAG AAT GA	445	Monstein et al. (2007)
*bla*TEM-F	62	ACG CTC ACC GGC TCC AGA TTT AT		
*bla*CTX-M-F	60	ATG TGC AGY ACC AGT AAR GTK ATG GC	593	Boyd et al. (2004)
*bla*CTX-M_R	62	TGG GTR AAR TAR GTS ACC AGA AYC AGC GG		
*bla*OXA-F	64	ACA CAA TAC ATA TCA ACT TCG C	813	Oullette et al. (1987)
*bla*OXA-R	62	AGT GTG TTT AGA ATG GTG ATC		

Table 3 Double disc synergy test. Percentage of positive, negative, and indeterminate

Organism	Total	Positive/%	Negative/%	Indeterminate/%
E. coli	266	80/30	130/49	56/21
Klebsiella spp.	285	77/27	165/58	43/15
Enterobacter spp.	105	21/20	62/59	22/21
Citrobacter spp.	108	13/12	81/75	13/13
Pseudomonas aeruginosa	15	5/33.3	10/66.6	0
Acinetobacter baumannii	14	4/28.5	10/71.5	0
Total	793			

isolates carried more than one type of beta-lactamase genes, with ten isolates carrying *bla*TEM and *bla*SHV genes, three isolates harboring *bla*TEM and *bla*OXA genes, and five isolates harboring *bla*SHV and *bla*CTX-M genes. No isolates with *bla*TEM, *bla*SHV, *bla*CTXM, and *bla*OXA genes together were detected. *bla*TEM genes were the most common ESBLs detected in *E. coli* (68.75 %).

Discussion

The correct identification of the genes involved in ESBL-mediated resistance is necessary for the surveillance and epidemiological studies of their transmission in hospitals. In recent years, the problem of gradually increasing resistance to antibiotics has threatened the entire world. Production of beta-lactamase, which hydrolyses and inactivates beta-lactam antibiotics, has been one of the most important resistance mechanisms of many bacterial species, mainly in the family Enterobacteriace (Akcam et al. 2004). Resistance to extended-spectrum beta-lactams among gram-negative pathogens is increasingly associated with ESBLs (Kimura et al. 2007). ESBL-positive enterobacterial species are becoming widespread throughout the world (Timko 2004).

In this study, ESBL-positive isolates were detected by DDST and PCR. Two hundred isolates were positive in the disk diffusion test and 181 isolates were positive using PCR. Nineteen of the positive isolates with DDST had none of ESBL genes with PCR; these isolates may have different ESBL genes than the ESBL tested in this study. Eighteen (9.9 %) out of 181 isolates carried more than one type of beta-lactamase genes, with ten isolates carrying blaTEM and blaSHV genes, three isolates harboring blaTEM and blaOXA genes, and five isolates harboring blaSHV and blaCTX-M genes.

TEM gene was found in 60 % of *E. coli*, 1.2 % of *Klebsiella* spp., 28.5 % of *Enterobacter* spp., and 7.7 % of *Citrobacter* spp. In our work, we used universal primer of TEM and we didn't separate TEM-1 and TEM-2 that may be the cause of high percentage of TEM gene in *E. coli* which is very common among *E. coli* strains. While SHV gene was found in 12.5 % of *E. coli* and 45.5 % of *Klebsiella* spp. and CTX-M was found in 11.2 % of *E. coli*, 44 % of *Klebsiella* spp., 47.6 % of *Enterobacter* spp., and 40 % of *P. aeruginosa*.

E. coli isolate ESBL-producing strains are significantly more frequently found to be resistant to other antibiotics, in particular fluroquinolones. In our study, 35 of 80 ESBL strains (43.7 %) were also resistant to ciprofloxacin. In a study conducted by Edelstein, 39.7 % of *E. coli* isolates and 21.8 % of *K. pneumoniae* isolate ESBL-producing strains were resistant to ciprofloxacin. Alternatively, some other studies have demonstrated better ciprofloxacin activity against ESBL-positive *E. coli* (Sinha et al. 2007).

Most investigators in Egypt have used phenotypic methods and have reported prevalence ranging from 11.6 to 68.8 % (Eiman and Rasha 2011). There are a few reports of molecular identification of these beta-lactamases. In comparison with the rest of the world, there is generally a lack of comprehensive data regarding ESBL-producing Enterobacteriaceae in African countries. However, there is sufficient evidence to highlight the prevalence of ESBLs in Africa. It is recognized that Egypt has an extremely high rate of ESBL producers, with up to 70 % of isolates producing the enzyme (Borg et al. 2006). One survey found that Egypt, Lebanon, Saudi Arabia, South Africa, and Egypt had the highest rates of ESBLs (Bouchillon et al. 2004).

Table 4 Resistance pattern of positive clinical isolates for ESBL

Organism	No	Percentage (%) of resistance to antimicrobial agent								
		A	AC	C^2	C^3	C^4	C	AK	T	FQ
E. coli	80	91.9	66.7	62.2	35.6	32.6	0	13.3	70.4	43.7
Klebsiella spp.	77	100	71.8	67.6	53.5	50.7	0	21.1	81.7	36.6
Enterobacter spp.	21	100	87.5	83.3	41.7	37.5	0	12.5	70.8	60
Citrobacter spp.	13	95	87	100	71	40.4	0	12.8	75.2	35.6
Pseudomonas aeruginosa	5	100	100	100	100	75	20	40	100	29
Acinetobacter baumannii	4	100	100	100	100	100	76	97	96	60

A ampicillin, *AC* amoxicillin–clavulanic, C^2 second generation cephalosporins, C^3 third generation cephalosporins, C^4 fourth generation cephalosporins, *C* carbapenems, *AK* amikacin, *T* trimethoprim–sulfamethoxazole, *FQ* fluoroquinolones

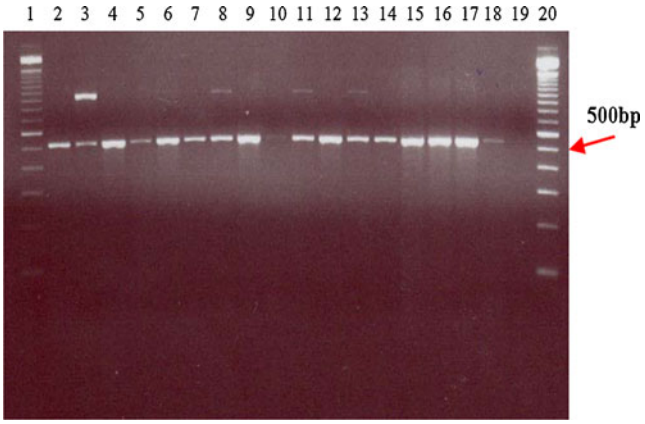

Fig. 1 Ethidium bromide-stained agarose gels showing amplicons obtained when DNA was amplified with SHV, TEM, CTX-M, and OXA primers using multiplex PCR

A recent outbreak was reported in a neonatal intensive care unit in Cairo, Egypt, in which 80 % of the isolates were *K. pneumoniae*, of which 58 % were ESBL producers (Moore et al. 2005). Therefore, it is important not to limit extended-spectrum cephalosporin susceptibility screening in Egypt to *E. coli* but to include *K. pneumoniae* as well as other Enterobacteriaceae such as *Enterobacter cloacae*.

The prevalence of confirmed ESBL-positive isolates in the USA, Europe, Latin America, the Middle East, and Asia Pacific was 3, 5, 10, 13, and 17 % for *E. coli* and 7, 11, 14, 20, and 18 % for *Klebsiella* spp. (Paterson et al. 2003). ESBL production in *Acinetobacter* strains in India was 28 % (Sinha et al. 2007). Bülüç et al. (2003) found that ESBL frequencies were 48 % for *K. pneumoniae*, 40 % for *K. oxytoca*, and 14 % for *E. coli* isolates. However, Delialioglu et al. (2005) stated that ESBL frequencies of *K. pneumoniae*, *K. oxytoca*, and *E. coli* isolates were 29.7, 4.2, and 18.3 %, respectively.

TEM and SHV beta-lactamases are mainly found in *E. coli* and *K. pneumoniae*, but can occur in other members of the family Enterobacteriaceae and in nonenteric organisms such as *Acinetobacter* species (Turner 2005). SHV-2- (*K. pneumoniae*, *E. coli*), SHV-5- (*K. pneumoniae*, *Enterobacter aerogenes*, and *E. cloacae*), SHV-12- (*K. pneumoniae* and *E. coli*), OXA-16- (*Pseudomonas aeruginosa*), PER-1- (*Acinetobacter baumannii* and *P. aeruginosa*), CTX-M-2-, CTX-M-15-, and TEM-1 (*K. pneumoniae*)-type ESBLs were reported (Gupta 2007; Velasco et al. 2007).

TEM-, SHV-, and other class A-type β-lactamases have been described in *P. aeruginosa*, but very rarely. Although OXA β-lactamases have also been reported in other gram-negative isolates, they occur predominantly in *P. aeruginosa* and *Acinetobacter* species (Chanawong et al. 2001).

Most strains simultaneously produced CTX-M-15, OXA-1, and TEM-1. When transferred by conjugation (achieved in one *K. pneumoniae* isolate and four *E. coli* isolates), blaCTX-M-15 was accompanied by blaOXA-1, blaTEM-1, and genes coding for decreased susceptibility to tetracycline, kanamycin, gentamicin, tobramycin, amikacin, and ciprofloxacin (Boyd et al. 2004).

The CTX-M genotype appears to be the most common type in North Africa (Khalaf et al. 2009). There have also been reports of CTX-M *K. pneumoniae* in Kenya (Kariuki et al. 2001) and SHV- and TEM-types in South Africa (Essack et al. 2001).

The prevalence of blaCTX-M was reported by Shahid et al. (2006). Seventy-two (77.4 %) of the 93 *E. coli* isolates were found to be CTX-M group 1-positive by PCR in north Indian isolates.

Amplification of blaCTX-M produced a band at 593 bp, blaTEM at 445 bp, and blaSHV at 237 bp. and both reactions could be combined together because annealing temperatures were more or less similar. Multiplex was done at on annealing temperature of 60 °C.

In our work, 21 %, 15 %, 21 %, and 13 % ESBL screen-positive isolates of *E. coli*, *Klebsiella* spp., *Enterobacter* spp., and *Citrobacter* spp., respectively, were negative by confirmatory test and considered as indeterminate those strains could be AmpC-positive. Robberts et al. (2009) stated that *E. coli*, *K. pneumoniae*, *K. oxytoca*, and *Proteus mirabilis* may be AmpC-positive, and ESBL confirmatory test were negative by CLSI methodology.

Table 5 Correlation between the beta-lactamase genotypes and their phenotype

Phenotype isolates	No. of negative strains by PCR	No. of positive strains by PCR	Genotypes of beta-lactamase genes in positive strains						
			TEM	SHV	CTX-M	OXA	TEM and SHV	TEM and OXA	SHV and CTX-M
E. coli	0	80	48	10	9	7	2	3	1
Klebsiella spp.	0	77	1	35	34	0	5	0	2
Enterobacter spp.	1	20	6	0	10	0	3	0	1
Citrobacter spp.	11	2	1	0	0	0	0	0	1
Pseudomonas aeruginosa	3	2	0	0	2	0	0	0	0
Acinetobacter baumannii	4	0	0	0	0	0	0	0	0

Current plasmid-mediated AmpC beta-lactamase detection methods include an insusceptibility screen using a cephamycin (usually cefoxitin) disc. This is not 100 % accurate as reduced outer membrane permeability can also cause insusceptibility. Also, the Amp-C Class (ACC)-like plasmid-mediated AmpC beta-lactamases are susceptible or weakly insusceptible to cefoxitin and will not be detected by the screen (Moland et al. 2008).

Detection of SBLs in AmpC-producing Enterobacteriaceae is problematic. A modification of the double-disk test (MDDT) has been developed for successful detection of ESBLs in gram-negative bacilli producing well-characterized beta-lactamases (Pitout et al. 2003).

ESBL-positive strains of *Klebsiella* spp., *E. coli*, *A. baumannii*, and *P. aeruginosa* are increasingly found in hospital isolates. These strains are usually multidrug resistant. Because these strains become resistant to available antibiotics and they can pass the gene to other clinical strains, the quick detection of these strains in microbiology laboratories is very important. Molecular typing would determine which types of ESBL are present in each isolate. Molecular detection and identification of beta lactamases would be essential for a reliable epidemiological investigation of antimicrobial resistance. These enzymes can be chromosomal or plasmid-mediated. The gene code for the enzymes may be carried on integrons. Integrons help in the dissemination of antimicrobial drug resistance in health care settings. Therefore, ESBL proproducing organisms should be identified quickly so that appropriate antibiotic usage and infection control measures can be implemented (Robberts et al. 2009).

Conclusion

To conclude, phenotypic methods are only screening methods for detection of ESBLs in a routine laboratory. The genotypic methods help us to confirm the genes responsible for ESBL production. Sometimes multiple genes are responsible for production of ESBLs in single isolates. We used a multiplex PCR for the detection of *bla*TEM, *bla*SHV, *bla*OXA, and *bla*CTX-M genes in ESBL-producing bacteria. This method provided an efficient, rapid differentiation of ESBLs in selected species of Enterobacteriaceae and nonfermenting gram-negative bacilli and could be used as a rapid tool for epidemiological studies among ESBL isolates.

References

Akcam FZ, Gönen I, Kaya O, Yaylı G (2004) The determination of susceptibility of beta-lactam antibiotics and extended spectrum beta-lactamase production in Enterobactericeae which responsible from nosocomial infections. Med J Sdu 11:6–9

Baron EJ, Finegold SM (1990) Bailey and Scott's diagnostic microbiology, 8th edn. CV Mosby Company, St. Louis

Bonnet R (2004) Growing group of extended-spectrum beta-lactamases: the CTX-M enzymes. Antimicrob Agents Chemother 48:1–14

Borg MA, Scicluna E, De Kraker M et al (2006) Antibiotic resistance in the southeastern Mediterranean preliminary results from the ARMed project. Euro Surveill 11(7):164–167

Bouchillon SK, Johnson BM, Hoban DJ et al (2004) Determining incidence of extended spectrum β-lactamase producing Enterobacteriaceae, vancomycin-resistant *Enterococcus faecium* and methicillin-resistant *Staphylococcus aureus* in 38 centres from 17 countries: the PEARLS study 2001–2002. Int J Antimicrob Agents 24(2):119–124

Boyd DA, Tyler S, Christianson S, McGeer A, Muller MP, Willey BM, Bryce E et al (2004) Complete nucleotide sequence of a 92-kilobase plasmid harboring the CTX-M-15 extended spectrum beta-lactamase involved in an outbreak in long-term-care facilities in Toronto, Canada. Antimicrob Agents Chemother 48:3758–3764

Bradford PA (2001) Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev 14:933–951

Bülüç M, Gürol Y, Bal Ç (2003) Rates of extended spectrum beta lactamases: 2000–2002. Turk Microbiol Soc 33:31–34

Bush K (2001) New beta-lactamases in gram-negative bacteria: diversity and impact on the selection of antimicrobial therapy. Clin Infect Dis 32:1085–1089

Canton R, Novais A, Valverde A et al (2008) Prevalence and spread of extended-spectrum beta-lactamase-producing Enterobacteriaceae in Europe. Clin Microbiol Infect 14:144–153

Chanawong A, M'Zali FH, Heritage J, Lulitanond A, Hawkey PM (2001) SHV-12, SHV-5, SHV-2a, and VEB-1 extended-spectrum β-lactamases in gram-negative bacteria isolated in a university hospital in Thailand. J Antimicrob Chemother 48:839–852

Delialioğlu N, Öcal ND, Emekdaş G (2005) Rates of extended-spectrum beta-lactamases in *Escherichia coli* and *Klebsiella* species isolated from various clinical specimens. Ankem 19:84–87

Eiman MA, Rasha HE (2011) High rates of intestinal colonization with extended-spectrum β-lactamase-producing Enterobacteriaceae among healthy individuals. J Investig Med 59:1284–1286

Essack SY, Hall LM, Pillay DJ, McFadyen ML, Livermore DM (2001) Complexity and diversity of *Klebsiella pneumoniae* strains with extended-spectrum β-lactamases isolated in 1994 and 1996 at a teaching hospital in Durban, South Africa, Antimicrob Agents Chemother 45(1):88–95

Fang H, Lundberg C, Olsson-Liljequist B, Hedin G, Lindback E, Rosenberg A, Struwe J (2004) Molecular epidemiological analysis of *Escherichia coli* isolates producing extended-spectrum beta-lactamases for identification of nosocomial outbreaks in Stockholm, Sweden. J Clin Microbiol 42:5917–5920

Gupta V (2007) An update on newer beta-lactamases. Indian J Med Res 126:417–427

Jarlier V, Nicolas MH, Fournier G, Philippon A (1988) Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. Rev Infect Dis 10:867–877

Johnson A, Woodford N (1998) Plasmid analysis. In: Johnson A, Woodford P (eds) Molecular bacteriology protocols and clinical application. Human Press, London, pp 24–28

Kariuki S, Corkill JE, Revathi G, Musoke R, Hart CA (2001) Molecular characterization of a novel plasmiden coded cefotaximase (CTX-M-12) found in clinical *Klebsiella pneumoniae* isolates from Kenya. Antimicrob Agents Chemother 45(7):2141–2143

Khalaf NG, Eletreby MM, Hanson ND (2009) Characterization of CTX-M ESBLs in *Enterobacter cloacae*, *Escherichia coli* and

Klebsiella pneumoniae clinical isolates from Cairo, Egypt. BMC Infect Dis 9:84

Kimura S, Ishii Y, Tateda K, Yamaguchi K (2007) Predictive analysis of ceftazidime hydrolysis in CTX-M-type beta-lactamase family members with a mutational substitution at position 167. Int J Antimicrob

Lee J, Pai H, Kim YK, Kim NH, Eun BW, Kang HJ et al (2007) Control of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumonia* in a children's hospital by changing antimicrobial agent usage policy. J Antimicrob Chemother 60:629–637

Moland ES, Kim SY, Hong SG, Thompson KS (2008) Newer beta lactamases: clinical and laboratory implications, part I and II. Clin Microbiol Newsl 30(10):71–85

Monstein HJ, Stholm-Balkhedm AO, Nilsson MV, Nilsson M, Dornbusch K, Nilsson LE (2007) Multiplex PCR amplification assay for the detection of blaSHV, blaTEM, and blaCTX-M genes in Enterobacteriaceae. APMIS 115:1400–1408

Moore KL, Kainer MA, Badrawi N, Afifi S, Wasfy M, Bashir M et al (2005) Neonatal sepsis in Egypt associated with bacterial contamination of glucose-containing intravenous fluids. Pediatr Infect Dis J 24(7):590–594

NCCLS (2002) Performance standards for antimicrobial susceptibility testing. 12th informational supplement. NCCLS document M100-S12. National Committee for Clinical Laboratory Standards, Wayne

Ouellette M, Bissonnette L, Roy PH (1987) Precise insertion of antibiotic resistance determinants into Tn21-like transposons: nucleotide sequence of the OXA-1 beta-lactamase gene. Proc Natl Acad Sci USA 84:7378–7382

Pai H, Kang CI, Byeon JH, Lee KD, Park WB, Kim HB et al (2004a) Epidemiology and clinical features of bloodstream infections caused by AmpC-type-beta-lactamases-producing *Klebsiella pneumoniae*. Antimicrob Agents Chemother 48:3720–3728

Pai H, Hong JY, Byeon JH, Kim YK, Lee HJ (2004b) High prevalence of extended-spectrum β-lactamase-producing strains among blood isolates of *Enterobacter* spp. collected in a tertiary hospital during an 8-year period and their antimicrobial susceptibility patterns. Antimicrob Agents Chemother 48:3159–3161

Paterson DL, Hujer KM, Hujer AM, Yeiser B, Bonomo MD, Rice LB, Bonomo RA (2003) The International *Klebsiella* Study Group extended-spectrum beta lactamases in *Klebsiella pneumonia* bloodstream isolates from seven countries: dominance and widespread prevalence of SHV- and CTX-M-type β-lactamases. Antimicrob Agents Chemother 47:3554–3560

Pitout JD, Reisbig MD, Venter EC, Church DL, Hanson ND (2003) Modification of the double-disk test for detection of Enterobacteriaceae producing extended-spectrum and AmpC β-lactamases. J Clin Microbiol 41(8):3933–3935

Robberts FJ, Kohner PC, Patel R (2009) Unreliable extended spectrum beta-lactamase detection in the presence of plasmid-mediated AmpC in *Escherichia coli* clinical isolates. J Clin Microbiol 47:358–361

Shah AA, Hasan F, Ahmed S, Hameed A (2004) Characteristics, epidemiology and clinical importance of emerging strains of gram-negative bacilli producing extended-spectrum beta-lactamases. Res Microbiol 155:409–421

Shahid M, Singhal M, Malik A, Shukla I, Khan HM (2006) ESBL phenotypes and prevalent genotype of CTX-M type beta lactamases in clinical isolates of *E. coli* in a North Indian tertiary care hospital. 46:70

Sinha M, Srinivasa H, Macaden R (2007) Antibiotic resistance profile and extended spectrum beta-lactamase (ESBL) production in *Acinetobacter* species. Indian J Med Res 12(6):63–67

Timko J (2004) Changes of antimicrobial resistance and extended-spectrum beta-lactamase production in *Klebsiella* spp. strains. J Infect Chemother 10:212–215, Agents. 29: 326-331

Turner PJ (2005) Extended spectrum beta-lactamases. Clin Infect Dis 41:273–275

Velasco C, Romeroa L, Martinez MR, Rodríguez-Baño J, Pascual A (2007) Analysis of plasmids encoding extended-spectrum beta lactamases (ESBLs) from *Escherichia coli* isolated from nonhospitalised patients in Sevile. Int J Antimicrob Agents 29:89–92