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Original Article

Extended-spectrum β-lactamase (ESBL): Phenotypic detection and antimicrobial susceptibility pattern to ciprofloxacin, amikacin and imipenem

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Abstract :

Background: Extended-spectrum β-lactamases (ESBLs) are enzymes capable of hydrolyzing extended-spectrum cephalosporins, penicillins and monobactams but inactive against cephamycins and carbapenems.

Objectives : This study was aimed to detect ESBL by phenotypic confirmatory disc diffusion test (PCDDT) and to determine the susceptibility pattern of ESBL-producing *E. coli*, *Klebsiella* spp. and *Enterobacter* spp. to ciprofloxacin, amikacin and imipenem.

Methods : A total of 100 ESBL-producing *E. coli*, *Klebsiella* spp. and *Enterobacter* spp. were obtained from Bangabandhu Sheikh Mujib Medical University, Dhaka and were studied for susceptibility pattern from October 2010 to December 2011. These isolates were identified by double disc synergy test (DDST) and were confirmed phenotypically as ESBL by PCDDT.

Results : Out of 75 DDST positive ESBL-producing *E. coli*, 71 (94.67%) were also positive by PCDDT. All DDST positive ESBL-producing *Klebsiella* spp. and *Enterobacter* spp. were also positive by PCDDT. All ESBL-producing *E. coli*, *Klebsiella* spp. and *Enterobacter* spp. were 100% susceptible to imipenem. About 95.78% ESBL-producing *E. coli*, 78.95% *Klebsiella* spp. and 100% *Enterobacter* spp. were susceptible to amikacin. About 87.32% ESBL-producing *E. coli*, 73.69% *Klebsiella* spp. and 33.33% *Enterobacter* spp. were resistant to ciprofloxacin.

Conclusion : ESBL-producing *E. coli*, *Klebsiella* spp. and *Enterobacter* spp. showed high resistance to ciprofloxacin. Imipenem and amikacin were most effective against ESBL-producing organisms.

Key words : ESBL, *E. coli*, *Klebsiella* spp., *Enterobacter* spp., Disc diffusion method

Introduction :

Bacterial antibiotic resistance has become a major clinical concern worldwide. The use of second and third generation cephalosporins has led to the selection of Gram-negative organisms resistant to β-lactamase stable cephalosporins. This resistance is attributed to the production of extended-spectrum β-lactamases (ESBL). These enzymes are plasmid mediated and they confer resistance to oxyimino-cephalosporins (cefotaxime, ceftriaxone, ceftazidime etc) and to monobactams (aztreonam), but they are not active against cephamycins (e.g., cefoxitin and cefotetan) and carbapenems (e.g., meropenem or carbapenem). ESBLs are most commonly found in Klebsiella spp and Escherichia coli, but they have also been detected in other members of the Enterobacteriaceae family[1,2].

Several phenotypic methods for detection of ESBLs have been proposed including; Screening for ESBL, Double disc synergy test (DDST), Phenotypic confirmatory disc diffusion

test (PCDDT), E-test ESBL strips, Three dimensional test, Vitek system, The Cica Beta Test 1. Phenotypic methods are based upon the resistance that ESBLs confer to oxyimino-beta-lactams (*e.g.* ceftriaxone, cefotaxime, ceftazidime and aztreonam) and the ability of a beta-lactamase inhibitor, usually clavulanate, to block this resistance[3]. Till now there is no gold standard test for detection of ESBLs[1].

PCDDT is a sensitive procedure for detection of ESBL. A study in Sher-i-Kashmir Institute of Medical sciences, Kashmir, India[4] showed that while DDST was able to detect 19.8% ESBL-producers, PCDDT detected 99.2% ESBL producers among 118 screen positive ESBL producers. Umadevi *et al*.,[5] showed that PCDDT was able to detect 81.06% *E. coli* and 74.07% Klebsiella pneumoniae as ESBL producers, DDST detected only 43.9% *E. coli* and 40.7% *Klebsiella pneumoniae* as ESBL producers.

The ESBL-producing organisms are a breed of multidrug-resistant pathogens. Infections caused by these ESBL producing organisms are associated with higher rates of mortality, morbidity as well as health care costs[6]. It is essential to report ESBL production along with the routine sensitivity reporting, which will help the clinicians in prescribing proper antibiotics[5]. Antibiotic options in the treatment of these organisms are extremely limited including carbapenem, fluoroquinolone and aminoglycoside[7].

This present study was aimed to detect ESBL by PCDDT and to determine susceptibility pattern of ESBL producing *Escherichia coli*, *Klebsiella* spp. *and Enterobacter* spp. to ciprofloxacin, amikacin, and imipenem.

Materials and Methods:

Bacterial isolates: A total of 100 ESBL-producing *E. coli* (75), *Klebsiella* spp. (19) and *Enterobacter* spp. (06) obtained from urine, pus, wound swab, blood, sputum, bile samples that were received in the department of Microbiology & Immunology, Bangabandhu Sheikh Mujib Medical University, Dhaka during the period of October 2010 to December 2011.

Test for presence of ESBL:

Screening for ESBL was carried out by DDST as described by Jarlier *et al*[8]. The test is based on the synergy between a cephalosporin and clavulanic acid. The synergy effect is detected when a disc of amoxicillin/clavulanic acid (20/10 µg) is placed 30 mm apart (center to center) from a disc containing a third generation cephalosporin. Extension of the edge of the cephalosporin zone on the side exposed to the disc containing clavulanic acid caused by synergy, indicate the presence of an ESBL.

Phenotypic confirmatory disc diffusion test (PCDDT) for ESBL production:

ESBL detection was performed as recommended by CLSI confirmatory procedure PCDDT using cefotaxime (30 µg) and ceftazidime (30 µg) discs alone and in combination with clavulanic acid (10µg). A ≥ 5 mm increase in zone diameter for cefotaxime and ceftazidime in combination with clavulanic acid versus its zone when tested alone, confirmed an ESBL-producing organism[9]. *E. coli* ATCC 25922 was used as the negative control and in house ESBL-producer was used as the positive control.

Antimicrobial susceptibility test:

Antimicrobial susceptibility testing of the ESBL producing isolates were done by disc diffusion method using Kirby-Bauer technique[10] and as per recommendations of the Clinical and Laboratory Standards Institute (CLSI)[9]. All discs were obtained from Oxoid Ltd., Basingstoke, Hampshire, UK. Antibiotic potency of the discs were standardized against the reference strain, *E. coli* ATCC 25922.

Results :

Out of 75 DDST positive *E. coli*, 71 (94.67%) were also found positive by PCDDT. All 19 DDST positive *Klebsiella* spp. and 06 DDST positive *Enterobacter* spp. were also positive by PCDDT (Figure-1).

Antibiotic susceptibility test results revealed very high susceptibility to imipenem (100%) followed by amikacin (78.95% to 100%). Resistance to ciprofloxacin was very high (74% to 87%) (Table-1).

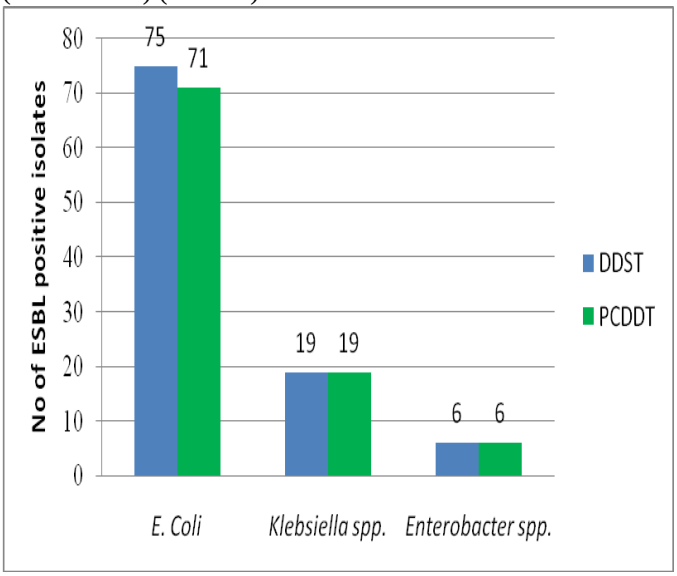

Figure-1: Confirmation of ESBL positive isolates by PCDDT (Phenotypic confirmatory disc diffusion test) among DDST (Double disc synergy test) positive ESBL-producing isolates (n=100 for DDST and n=96 for PCDDT).

Table-1: Susceptibility pattern of ESBL-producing *E. coli, Klebsiella* spp. and *Enterobacter* spp. against ciprofloxacin, amikacin and imipenem

ESBL producing bacteria isolated (n = 96)	Susceptibility pattern of ESBL-producing isolates								
	Ciprofloxacin			Amikacin			Imipenem		
	S	IS	R	S	IS	R	S	IS	R
E. coli (n = 71)	9 (12.6)	-	62 (87.32)	66 (92.95)	2 (2.82)	3 (4.23)	71 (100)	-	-
Klebsiella spp. (n = 19)	4 (21.0)	1 (5.26)	14 (73.69)	15 (78.95)	-	4 (21.05)	19 (100)	-	-
Enterobacter spp. (n = 6)	3 (50)	1 (16.67)	2 (33.33)	6 (100)	-	-	6 (100)	-	-

Note: Figures in the parentheses indicate percentage.

S= Sensitive, IS = Intermediate sensitive, R =Resistant.

Discussion :

The prevalence of ESBL-producing organisms is increasing worldwide. In addition resistance to cephalosporins, ESBL producing organisms are also exhibiting resistance to fluoroquinolones group of drugs limiting further therapeutic options[3].

In this study, out of 75 DDST positive *E. coli*, 71 (94.67%) were confirmed as ESBL-producer when tested by PCDDT. All the DDST positive *Klebsiella* spp. (n=19) and *Enterobacter* spp. (n=06) were confirmed as ESBL-producer by PCDDT (Figure-1). The result of the present study was consistent with the study by Ingviya *et al*., (2003)[11] in Thailand, who showed that among 100 DDST positive *E. coli* and 137 DDST positive *K. pneumoniae*, 96 (96.0%) *E. coli* and 129 (94.2%) *K. pneumoniae* were proved as ESBL-producer by PCDDT.

In the present study, about 87.32% ESBL-producing *E. coli*, 73.69% *Klebsiella* spp. and 33.33% *Enterobacter* spp. were resistant to ciprofloxacin (Table-1). Increased resistance to ciprofloxacin might be due to widespread indiscriminate use, their oral route of administration, easy availability and affordability of ciprofloxacin over the country[12]. This result was consistent with the study by Datta *et al*., (2004)[13] in India, who showed 90.8% ESBL-producing *E. coli*, 74.7% ESBL-producing *Klebsiella pneumoniae* and 50% ESBL-producing *Enterobacter* spp. were resistant to ciprofloxacin. Chaikittisuk and Munscrichoom (2007)[7] in Thailand, showed that 89% ESBL-producing *E. coli* and 72% ESBL-producing *Klebsiella* spp. were resistant to ciprofloxacin. These findings suggest that sensitivity of ESBL-producing bacteria to ciprofloxacin is gradually decreasing.

About 95.78% ESBL-producing *E. coli*, 78.95% *Klebsiella* spp. and 100% *Enterobacter* spp. were sensitive to amikacin in this study (Table-1). This result was consistent with the study by Datta *et al*., (2004)[13], who showed 84% ESBL-producing *E. coli*, 71.5% *Klebsiella pneumoniae* & 80% *Enterobacter* spp. were sensitive to amikacin. Sashirekha *et al*., (2010)[14] in Karnataka, India, showed that 95% ESBL-producing *E. coli* and 90% ESBL-producing *Klebsiella* spp. were sensitive to amikacin. This result indicates that amikacin can be considered as drug of choice in the treatment of infections caused by ESBL-producing organisms.

100% ESBL-producing *E. coli*, *Klebsiella* spp. and *Enterobacter* spp. in this study were sensitive to imipenem. The results were in consistent with the study of Chaikittisuk and Munsrichoom, (2007)[7], Sasirekha *et al*., (2010)[14], who showed that ESBL-producing *E. coli*, *Klebsiella* spp. and *Enterobacte*r spp. were 100% sensitive to imipenem. Carbapenems (e.g., imipenem) are known to be stable against ESBL enzymes and effective in the treatment caused by ESBL-producing bacteria[15].

Conclusion:

So, the treatment of choice for infections caused by ESBL-producing organism can be the imipenem and amikacin, as ESBL-producing organism are highly sensitive to these two drugs. ESBL-producing organisms in this study exhibited high resistance to ciprofloxacin. It should be given if they show in vitro susceptibility.

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