Extended-Spectrum β-Lactamase (ESBL) Producing Organism and its Antimicrobial Susceptibility Pattern to Ciprofloxacin, Amikacin and Imipenem

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This study was carried out to determine the susceptibility pattern of ESBL-producing *E. coli, Klebsiella* spp. and *Enterobacter* spp. to ciprofloxacin, amikacin and imipenem. A total of 100 ESBL-producing *E. coli, Klebsiella* spp. and *Enterobacter* spp. were obtained from the tertiary care hospitals of Bangladesh 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-producer by phenotypic confirmatory disc diffusion test (PCDDT). Minimum inhibitory concentrations (MICs) of ciprofloxacin, amikacin and imipenem among ESBL-producing *E. coli, Klebsiella* spp. and *Enterobacter* spp. were determined using agar dilution method. Out of 75 DDST positive ESBL-producing *E. coli,* 71 (94.67%) were also positive by PCDDT. All DDST positive *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 by both agar dilution and disc diffusion method. About 92.95% *E. coli,* 78.95% *Klebsiella* spp. and 100% *Enterobacter* spp. were susceptible to amikacin by both methods. About 14.08% ESBL-producing *E. coli,* 26.32% *Klebsiella* spp. and 66.67% *Enterobacter* spp. were susceptible to ciprofloxacin by agar dilution method but 12.68% *E. coli,* 21.05% *Klebsiella* spp. and 50% *Enterobacter* spp. were susceptible to ciprofloxacin by disc diffusion method. In this study, ESBL-producing *E. coli, Klebsiella* spp. and *Enterobacter* spp. showed high resistance to ciprofloxacin. Imipenem and amikacin were most effective against ESBL positive strains.

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Key words: Extended-Spectrum β-lactamase, *Escherichia coli*, *Enterobacter* spp., *Klebsiella* spp. Minimum inhibitory concentrations

Introduction

Extended spectrum β-lactamases (ESBLs) are enzymes that mediate resistance to extended-spectrum (third generation) cephalosporins (e.g., ceftazidime, cefotaxime and ceftriaxone) and monobactams (e.g., aztreonam) but do not affect cephamycins (e.g., cefoxitin and cefotetan) or carbapenems (e.g., meropenem or imipenem).[1] The majority of ESBL-producing organisms are *Klebsiella* spp. and *E. coli*. Other organisms reported to harbour ESBLs include *Enterobacter* spp., *Proteus mirabilis,Serratia marcescens, Salmonella* sp p.,*Morganella morganii* and *Pseudomonas aeruginosa.*[2]

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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.[4]

ESBL positive isolates show false susceptibility to extended-spectrum cephalosporin in standard disc diffusion method, rendering it difficult to reliably detect ESBL production by the routine DDST.[5] PCDDT is a sensitive procedure for detection of ESBL.[6]

The ESBL-producing organisms are a breed of multidrug-resistant pathogens. Infections caused by these organisms are associated with higher rate of mortality, morbidity as well as health care costs. [7] It is essential to report ESBL production along with the routine sensitivity reporting, which will help the clinicians in prescribing proper antibiotics.[8] Antibiotic options in the treatment of these organisms are extremely limited including carbapenem, fluoroquinolone and aminoglycoside. [9]

The purpose of this study was to determine susceptibility patterns of ESBL producing *E. coli, Klebsiella* spp. *and Enterobacter* spp. to ciprofloxacin, amikacin, and imipenem.

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 (during the period of October, 2010 to December, 2011) were included for the study.

Test for presence of ESBL

Screening for ESBL was carried out by DDST as described by Jarlier *et al*. [10]

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 (10 μg). 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. [11] *E. coli* ATCC 25922 was used as the negative control and in house ESBL-producer *E. coli* was used as the positive control (Fig:I).

Antimicrobial susceptibility test

1. Disc diffusion method

Antimicrobial susceptibility testing of the ESBL producing isolates was done by disc diffusion method using Kirby-Bauer technique[12] and as per recommendations of the Clinical and Laboratory Standards Institute (CLSI).[11] 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.

2. Agar dilution method

Minimum inhibitory concentrations (MICs) of ciprofloxacin, amikacin and imipenem were done by the standard agar dilution method.[11] *E. coli* ATCC 25922 was used as control.

Results

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

Antibiotic susceptibility test results by both agar dilution and disc diffusion method revealed very high susceptibility to imipenem (100%) followed by amikacin (78.95% to 100%). Resistance to ciprofloxacin by both agar dilution and disc diffusion method was very high (Table-I).

Table I: The MIC parameter of ciprofloxacin, amikacin ad imipenem against ESBL-producing *E. coli, Klebsiella* spp. and *Enterobacter* spp.

ESBL producing isolates / Antimicrobial agents	MIC (µg/ml)			% Susceptibility
	Range	MIC_{50}	MIC_{90}	
E. coli (n=71)				
Ciprofloxacin	0.004-8	16	128	14.08
Amikacin	0.0625-128	0.5	8	95.78
Imipenem	0.125-32	0.25	0.25	100
Klebsiella spp. (n=19)				
Ciprofloxacin	0.004-8	16	128	26.32
Amikacin	0.0625-128	1	8	78.95
Imipenem	0.125-32	0.25	0.25	100
Enterobacter spp. (n=06)				
Ciprofloxacin	0.004-8	0.5	32	66.67
Amikacin	0.0625-128	0.25	2	100
Imipenem	0.125-32	0.25	0.25	100

Note: Based on susceptibility breakpoints defined by CLSI: ciprofloxacin ≤ 1µg/ml, amikacin ≤ 16µg/ml and imipenem ≤ 1µg/ml.

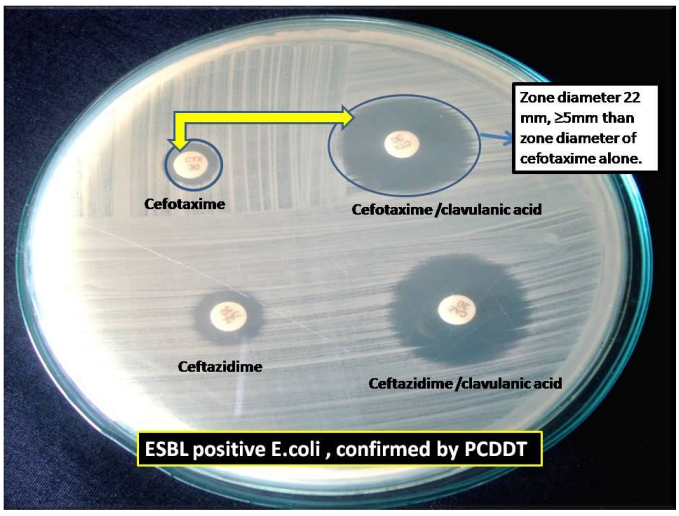

Figure 1. Phenotypic confirmatory disc diffusion test (ESBL positive strain)

Discussion

Extended-spectrum β-lactamases (ESBLs) constitute a class of plasmid-mediated β-lactamases which confer resistance to broad spectrum β-lactam antibiotics. The prevalence of ESBL-producing organism 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. (19) and *Enterobacter* spp. (06) were confirmed as ESBL-producer by PCDDT. The result of the present study was consistent with the study by Ingviya *et al*., (2003)[5] 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, 83.10% ESBL-producing *E. coli*, 68.42% *Klebsiella* spp. and 33.33% *Enterobacter* spp. showed high MIC value against ciprofloxacin (4 μg/ml to 128 μg/ml) indicating high level resistance to ciprofloxacin. This less susceptibility may be due to widespread indiscriminate use, their oral route of administration, easy availability and affordability of ciprofloxacin over the country (Hassan *et al*., 2011). [13] Inviya *et al*., (2003) [5] reported 47% ESBL-producing *E. coli* and 12% *K. pneumoniae* to be resistant to ciprofloxacin. These findings suggest that sensitivity of ESBL-producing bacteria to ciprofloxacin is gradually decreasing.

About 95.78% ESBL-producing *E. coli* and 78.95% *Klebsiella* spp. in this study were sensitive to amikacin. Similar findings were described by Soriozano *et al*., (2007)[14] and Liao *et al*., (2006),[15] who found 100% ESBL-producing *E. coli* and 72.3% *Klebsiella* spp. to be sensitive to amikacin. All ESBL-producing *Enterobacter* spp. in this study were sensitive to amikacin and MIC value of amikacin against these isolates were low (0.0625 μg/ml to 4 μg/ml). This result indicates that amikacin can be considered as drug of choice in the treatment of infections caused by ESBL-producing organisms.

In this study, 100% ESBL-producing *E. coli*, *Klebsiella* spp. and *Enterobacter* spp. were sensitive to imipenem (MIC 0.125 μg/ml to 0.25 μg/ml) by both agar dilution and disc diffusion method. Similar findings were observed by Liao *et al*., (2006),[15] Soriozano *et al*., (2007),[14] Ingviya *et al*., (2003),[5] who found 100% sensitivity to imipenem against ESBL-producing *E. coli*, *Klebsiella* spp. and *Enterobacter* spp. Carbapenems (e.g., imipenem) are known to be stable against ESBL enzymes and effective in the treatment caused by ESBL-producing bacteria.[16]

Minor difference observed between the sensitivity result of disc diffusion and agar dilution method for ciprofloxacin in this study. About 14.08% ESBL-producing *E. coli*, 26.32% *Klebsiella* spp. and 66.67% *Enterobacter* spp. were susceptible to ciprofloxacin in agar dilution method but 12.68% *E. coli*, 21.05% *Klebsiella* spp. and 50% *Enterobacter* spp. susceptible to ciprofloxacin in disc diffusion method. This difference may be due to several factors affecting disc diffusion method; medium formulation and P^H, disc content, its storage and drug diffusion, inoculums size, incubation time and temperature.[17]

In conclusion, 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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