Original Article

Determination of Extended-Spectrum β-Lactamases and AmpC Production in Uropathogenic Isolates of *Escherichia coli* and Susceptibility to Fosfomycin

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ABSTRACT

Background: Urinary tract infection due to *Escherichia coli* is one of the common problem in clinical practice. Various drug resistance mechanisms are making the bacteria resistant to higher group of drugs making the treatment options very limited. This study was undertaken to detect ESBLs and AmpC production in uropathogenic *Escherichia coli* isolates and to determine their antimicrobial susceptibility pattern with special reference to fosfomycin.

Materials and Methods: A total number of 150 *E. coli* isolates were studied. ESBL detection was done by double disc synergy and CLSI method. AmpC screening was done using cefoxitin disc and confirmation was done using cefoxitin/cefoxitin-boronic acid discs. In AmpC positive isolates, ESBLs was detected by modifying CLSI method using boronic acid. Antimicrobial susceptibility pattern was determined following CLSI guidelines. Fosfomycin susceptibility was determined by disc diffusion and E-test methods.

Results: ESBLs production was seen in 52.6% of isolates and AmpC production was seen in 8% of isolates. All AmpC producers were also found to be ESBLs positive. ESBLs positive isolates were found to be more drug resistant than ESBLs negative isolates. All the strains were found to be fosfomycin sensitive.

Conclusions: ESBLs and AmpC producing isolates are becoming prevalent in *E. coli* isolates from community setting also. Amongst the oral drugs, no in-vitro resistance has been seen for fosfomycin making it a newer choice of drug (although not new) in future. An integrated approach to contain antimicrobial resistance should be actually the goal of present times.

Key words: AmpC, *E. coli*, extended-spectrum β-lactamases, fosfomycin, urinary tract infection

INTRODUCTION

Urinary tract infection (UTI) is one of the commonest infectious disease presentations in medical practice. The most common cause of UTI in both community and health care settings is *Escherichia coli*.[1] The choice of antibiotic for the treatment of UTI is limited by the rising rates of antibiotic resistance. The production of β-lactamases is the foremost mechanism of antibiotic resistance leading to treatment failure. The β-lactamases which confer resistance to extended-spectrum cephalosporins are extended-spectrum β-lactamases (ESBLs) and AmpC. ESBLs are Ambler class A or D β-lactamases which confer resistance to 3rd and 4th generation cephalosporins and monobactams but are inhibited by cephamycins and β-lactamase inhibitors like clavulanic acid (CA), sulbactam, and tazobactam.[2] AmpC are class C β-lactamases which confer resistance to a variety of β-lactams, including oxyimino-cephalosporins and some cephamycins as well as penicillins and monobactam, when they are produced in large amounts but they are poorly inhibited by β-lactamase inhibitors such as CA and sulbactam.[3] Sometimes, because of the production of both plasmid mediated AmpC and ESBL, we get false negative results in phenotypic confirmatory methods (using CA) for detection of ESBLs.[4] Boronic acid (BA) has been reported to be the inhibitor of AmpC. So, it can be used for the detection of ESBL in isolates harboring both AmpC and ESBL.[4]

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These days, increase in ESBL producing isolates has been observed in outpatient settings especially related to UTI.[1] The production of ESBLs by the isolates narrow down the options for treatment as production of ESBLs is associated with coresistance to other classes of antimicrobial agents like fluoroquinolones, cotrimoxazole, tetracyclines, and aminoglycosides.[5] Coresistance between nitrofurantoin and fluoroquinolones in urinary isolates of *E. coli* has also been noted.[6] The alternative treatment for severe ESBLs producing *E. coli* include carbapenems, tigecycline, β-lactam/β-lactamase inhibitor combinations (BL/BLI) and fosfomycin. But, all these drugs are to be administered parenterally except fosfomycin. Moreover, tigecycline is not a very good option to be used for UTI because of its poor excretion in urine. Fosfomycin is a phosphonic acid bactericidal agent which is known for nearly four decades and is particularly useful for urinary tract pathogens. This is an oral drug and has been found to be effective against ESBLs producing *Enterobacteriaceae* isolates.

So considering in view of all these facts, study was planned with the following objectives:

1. To evaluate ESBLs production amongst *E. coli* isolates from urine samples of patients attending outpatient department and admitted in wards (noncritical care areas).
2. To evaluate AmpC production among these isolates.
3. To determine antimicrobial susceptibility pattern of these isolates.
4. To determine fosfomycin susceptibility for the isolates by disc diffusion and E-test methods.

MATERIALS AND METHODS

This study was conducted on 150 nonduplicate strains of *E. coli* isolated from urine samples of patients with urinary tract infections between July 2009 and December 2009.

Detection of ESBLs

ESBL production was detected by Clinical Laboratory Standard Institute (CLSI) method (using CAZ and CAZ-CA combination discs) and by double disc synergy (DDS) method using ceftazidime (CAZ), cefpodoxime, ceftriaxone, and cefepime discs along with CAZ-CA combination disc (method already used by the authors and work published) [Figure 1].[7] Those strains which were found to be negative for ESBLs were further confirmed to be ESBLs nonproducers by modifying phenotypic confirmatory test for ESBLs detection using BA.[8] For preparation of BA solution, 120 mg of

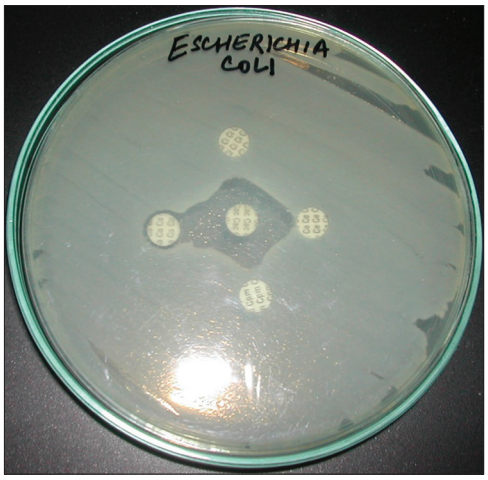

Figure 1: Photograph showing detection of extended-spectrum β-lactamases by using 3rd and 4th generation cephalosporins[7]

3-aminophenyl BA (Sigma) was dissolved in 3 mL of dimethylsulfoxide and 3 mL of distilled water was added to it. Then, 20 μL of this solution was dispensed onto each disk containing CAZ (30 μg) and CAZ/CA (30/10 μg) combination discs. The final amount of BA on the discs was 400 μg.[4] The discs were allowed to dry for 60 minutes and used immediately. A lawn culture of the test strain was made on Mueller-Hinton agar (MHA) and these disks, that is, CAZ/BA and CAZ/CA/BA were placed on it like CLSI phenotypic confirmatory method for ESBL detection. The plate was incubated at 37°C overnight. A ≥3 mm increase in the zone diameter of CAZ/CA/BA disk versus CAZ/BA alone was considered positive for ESBL.

Detection of AmpC

AmpC screening was done using cefoxitin disc. The strains which were found to be cefoxitin-resistant were confirmed by combination disk test using BA (cefoxitin and cefoxitin/BA disk).[9] A total of 20 μL of BA solution (prepared as above) was dispensed onto cefoxitin disks. A lawn culture of the test strain was made on MHA plate according to the Clinical Laboratory Standard Institute (CLSI) guideline. Disks containing cefoxitin (FOX) and cefoxitin plus BA (FOX/BA) were placed on the MHA plate and incubated at 37°C overnight. An increase in the zone size of ≥5 mm for cefoxitin in the presence of BA compared with that of cefoxitin alone was considered as positive result.

Antimicrobial susceptibility

The antimicrobial susceptibility of the following drugs was determined by Kirby-Bauer method following CLSI guidelines: Nitrofurantoin (300 μg), norfloxacin (10 μg),

lomefloxacin (10 μg), gentamicin (10 μg), tetracycline (30 μg), amoxicillin + clavulanic acid (20/10 μg), piperacillin + tazobactam (100/10 μg), ticarcillin + clavulanic acid (75/10 μg), cefoperazone + sulbactam, imipenem (10 μg), meropenem (10 μg), ertapenem (10 μg), fosfomycin (200 μg) containing 50 μg glucose-6-phosphate, amikacin (30 μg). For confirming the results of AmpC production, E-test strips were used.

Fosfomycin minimum inhibitory concentration

Minimum inhibitory concentration (MIC) of fosfomycin was tested by E-test (Biomereiux, India) with fosfomycin gradient concentrations ranging from 0.04 μg/ml to 1,024 μg/mL added along with 50 μg/mL glucose-6-phosphate.

RESULTS

Out of 150 isolates, 98 were derived from female patients and 52 were from male patients. Amongst 150 strains of *E. coli*, the number of ESBLs positive strains was 79 (52.6%) and ESBLs negative strains was 71 (47.3%) by double-disk synergy and/or CLSI modified method as CAZ/BA and CAZ/CA/BA method. The number of strains which was AmpC screening positive (cefoxitin-resistant) were 15 (10%). Out of these 15 strains, 12 were ESBL positive by DDS and/or CLSI methods, while all these 15strains were found to be ESBL positive by CAZ/BA and CAZ/CA/BA method. Out of the 15 AmpC screening positive *E. coli* isolates, 12 were confirmed as AmpC positive by FOX/BA combination disc method. So overall by confirmatory methods, 8% (12/150) of the strains are co-producers of ESBL and AmpC. The antimicrobial susceptibility pattern of these isolates for various drugs is given in Table 1. The MIC of fosfomycin was found to be <64 μg/mL in all the strains.

DISCUSSION

ESBLs-producing *E. coli* are the significant cause of increased morbidity in patients with UTI. In our study, more than half (52.6%) of *E. coli* isolates were ESBL producing and this was detected better with BA methodology as the 15 cefoxitin-resistant strains, 15 were also ESBL producers by BA method, but only 12 were positive by CLSI method. As reported earlier, BA is a reversible inhibitor of AmpC.[8] Out of 15 cefoxitin-resistant *E. coli*, only 12 were AmpC producer by using BA method and this was confirmed by using E-test method also. So in three of these, *E. coli* mechanism of cefoxitin resistance could be other than AmpC production. A total of 8% (12/150) of the isolates were coproducers of ESBL and AmpC enzymes using BA method; these isolates are still difficult to treat because of very limited treatment options left as has been reported earlier also.[10]

Regarding the antibiotic susceptibility *E. coli* was found to be susceptible to BL/BLI combinations like piperacillin-tazobactam, cefoperazone-sulbactam, aminoglycosides, and also carbapenems more so the ESBL negative strains; but all these are parenteral antibiotics [Table 1] for use in indoor patients. In the outpatient setting, oral antibiotics are preferred for administration but we are left with very limited options of oral drugs for the treatment of UTI except to some extent cotrimoxazole and nitrofurantoin, which shows good sensitivity pattern. Surprisingly, cotrimoxazole showed good sensitivity in ESBL positive strains also (70%). The combination oral antibiotic amoxicillin-clavulanic acid showed high percentage of resistance in both ESBL positive and negative strains. Quinolones like norfloxacin can be used in ESBL-negative isolates though in ESBL-positive isolates, it showed high percentage of resistance. We at

Table 1: Antimicrobial susceptibility testing pattern

Antibiotic	ESBL positive (79)			ESBL negative (71)		
	Sensitive (%)	Resistant (%)	Intermediate (%)	Sensitive (%)	Resistant (%)	Intermediate (%)
Amoxyclav	0 (0)	79 (100)	0 (0)	14 (19.7)	57 (80.3)	0 (0)
Ticarcillin+clavulanic acid	0 (0)	74 (93.7)	5 (6.3)	35 (49.3)	29 (40.8)	7 (9.9)
Piperacillin+tazobactam	64 (81)	8 (10.1)	7 (8.9)	69 (97.2)	2 (2.8)	0 (0)
Cefoperazone+sulbactam	69 (87.3)	3 (3.8)	7 (8.9)	69 (97.2)	2 (2.8)	0 (0)
Imipenem	79 (100)	0 (0)	0 (0)	71 (100)	0 (0)	0 (0)
Meropenem	77 (97.5)	2 (2.5)	0 (0)	69 (97.2)	2 (2.8)	0 (0)
Ertapenem	76 (96.2)	3 (3.8)	0 (0)	69 (97.2)	2 (2.8)	0 (0)
Norfloxacin	4 (5.1)	75 (94.9)	0 (0)	44 (62)	25 (35.2)	2 (2.8)
Lomefloxacin	3 (3.8)	76 (96.2)	0 (0)	46 (64.8)	25 (35.2)	0 (0)
Gentamicin	54 (68.4)	8 (10.1)	17 (21.5)	56 (78.9)	10 (14.1)	5 (7)
Amikacin	74 (93.7)	5 (6.3)	0 (0)	69 (97.2)	2 (2.8)	0 (0)
Cotrimoxazole	56 (70.9)	23 (29.1)	0 (0)	30 (42.3)	41 (57.7)	0 (0)
Nitrofurantoin	73 (92.4)	0 (0)	6 (7.6)	69 (97.2)	2 (2.8)	0 (0)
Fosfomycin	79 (100)	0 (0)	0 (0)	71 (100)	0 (0)	0 (0)

ESBL: Extended-spectrum β-lactamase

our centre do not suggest using ciprofloxacin as first-line drug for uncomplicated UTI due to reasons like overuse and misuse of this drug and emergence of other resistance pathogens due to its overuse. Usually, it is kept as a reserve drug for more complicated infections. Our study also corroborates the finding that ESBL producing isolates are much more multidrug-resistant than ESBLs-negative isolates thereby, narrowing down the choice of antibiotics for treatment.[11] Considering above findings, there is a dire need of introducing some new antimicrobial drug for UTIs. Although, fosfomycin is an oral antibiotic with low-resistance rates and is commonly used for treatment of community-acquired UTI in Europe, but not yet marketed in India.[12] In our study, the resistance rate of fosfomycin for both ESBL-positive and -negative isolates was found to be nil by both disk diffusion and E-test methods. Maraki *et al*., in their study on susceptibility of various urinary tract bacteria to fosfomycin have also found no resistance to fosfomycin in *E. coli*.[13] The other benefits of use of fosfomycin are its less cost, dosage friendly, nontoxic, nonallergic, and tendency to display little cross-resistance to other antibiotics.[14,15] Fosfomycin is an age-old drug and the reason for the emergence of use of this drug is the lack of newer drugs for the treatment of multidrug-resistant organisms. There are no Indian studies as yet available on this antibiotic so that we know the baseline levels of sensitivity before this drug is put to use in the country.

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