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Research Article

Extended spectrum β-lactamases in urinary isolates of *Escherichia coli* - prevalence and susceptibility pattern at a tertiary care hospital

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ABSTRACT

Introduction: Urinary Tract infections caused by *Escherichia coli* have become a significant global public health problem. The resistance to β- lactam antibiotics in these clinically important gram negative bacteria further adds to the problem. Knowledge about their prevalence is essential to guide towards appropriate antibiotic treatment. The study was thus undertaken to know prevalence and susceptibility pattern of Extended spectrum β - lactamase (ESBL) producing *Escherichia coli* isolates among urinary samples.
Methods: A total of 216 *E. coli* isolates in urinary samples were studied for ESBL production by using CLSI Phenotypic screening and confirmatory tests.
Results: 53% of *E.coli* isolates were ESBL producers. The susceptibility of ESBL producers to Imipenem, Tigecycline and Nitrofurantion was found to be 100%, 100% and 88% respectively. A high degree of associated resistance to Amikacin, Co-trimoxazole and Quinolones was found in ESBL producers. Majority of ESBL producers were detected among patients admitted in various ICUs and surgery ward.
Conclusion: Our study shows presence of ESBL producer *E.coli* in large number of urinary isolates.

Keywords: ESBL, *Escherichia coli*, Double-disk approximation test, Multi-drug resistance, Prevalence

INTRODUCTION

Urinary tract infections (UTIs) having *Escherichia coli* as etiological agent are common infections with an estimated annual global incidence of at least 250 million cases.[1]

This important pathogen has shown an increasing antimicrobial resistance to most antibiotics particularly Extended-Spectrum-Cephalosporins.[2,3] Resistance to these antibiotics can occur via production of Extended Spectrum β lactamases (ESBLs). ESBLs are defined as β-lactamases capable of hydrolyzing oxyimino- cephalosporins and are inhibited by beta-lactamase inhibitors.[4]

The incidence of ESBL producing *E.coli* has been increasing worldwide. It varies according to geographic location and is directly linked to use and misuse of antibiotics.[5] These enzymes are plasmid borne and confer multiple drug resistance.[6] Co-resistance to non β-lactam antibiotics is either by the co-transfer of resistance determinants in the same genetic elements (such as aminoglycoside resistance) or simply by the co-relation of both resistance mechanisms, as occur with fluroquinolones.[7] Since there are limited therapeutic options urinary tract infections become difficult to treat. Hence, the present study was undertaken to estimate the burden of ESBL producing *E.coli* isolates and also to find out their susceptibility to non-β lactam antibiotics. Moreover the aim of the study was also to compare the antibiotic sensitivity pattern of ESBL producers with that of Non ESBL producers and to delineate the magnitude of the problem and to define appropriate therapeutic options.

METHODS

A prospective study was conducted over a period of six months (January to July 2012) at a tertiary care teaching hospital. Total of 1136 urine samples were processed for significant bacteuria in the department of microbiology from patients clinically suspected to have UTI. The samples received were inoculated on CLED (Cysteine lactose electrolyte deficient media) and after 24 h of aerobic incubation at 37º C, isolates were identified to the species level using standard biochemical tests. All *E.coli* (216) isolates were included in the study. Clinico-demographic data of study patients was noted. Chi-square test was used to analyze the susceptibility pattern of non β-lactam antibiotics in ESBL producers and non-producers.

1.1 Antibiotic susceptibility testing

Susceptibility to various antimicrobial agents was determined by Disc diffusion method of Kirby Bauer on Mueller Hinton Agar (Hi-media) as described by Clinical Laboratory and Standard Institute (CLSI) guidelines.[8] The following antibiotic discs (drug concentration in µg) were used: ampicillin (30) gamikacin (30), ceftazidime (30), cefotaxime (30), ceftriaxone (30), co-trimoxazole (25), imipenem (10), ciprofloxacin (5), cefoperozone-sulbactam (30), piperacillin-tazobactam (30) and nitrofurantoin (300).

1.2 Test for ESBL production

1.2.1 Screening test

All E.coli isolates were subjected to Screening tests by using cefotaxime (30µg) and ceftriaxone (30µg) discs. Those isolates with cefotaxime zone <=27mm and ceftriaxone zone <=25mm were then subjected to confirmatory tests.

1.2.2 Confirmatory test

Double disc approximation test.[9] The organism was swabbed on to a Mueller-Hinton agar plate. Antibiotic discs of amoxicillin/clavulanic acid (20/10 µg) and cefotaxime (30 µg) were placed at a distance of 15 mm apart and incubated. Organism that showed a clear extension of cefotaxime inhibition zone towards the disc containing clavulanate was considered as ESBL producer.

CLSI confirmatory test[8]

While performing antibiotic testing, ceftazidime (30 µg) and ceftazidime plus clavulanic acid (30/10 µg) were placed on Mueller-Hinton agar and incubated. Organism was considered as ESBL producer if there was a >= 5mm increase in zone diameter of ceftazidime/clavulanate disc and that of ceftazidime disc alone. *Escherichia coli* ATCC 25922 and *Klebsiella pneumonia* ATCC 700603 were used as negative and positive controls respectively.

RESULTS

Of the 1136 urine samples processed, 427 samples yielded various bacterial isolates. There were 216 *E.coli* isolates among them. While 59% (128/216) *E.coli* were Screening test positive, 53% of *E.coli* (117/216) were confirmed ESBL by CLSI confirmatory test.

The double disk approximation test failed to detect ESBLs in two isolates. The ESBL positive isolates were obtained from 49 male and 68 female patients with a male to female ratio of 1:1.3. Almost equal percent of ESBL producers were obtained from OPD and IPD. Among IPD maximum ESBL were isolated from ICUs and Surgery wards.

The antimicrobial susceptibility results of ESBL producers are shown in Figure 1. Susceptibility of ESBL producers to imipenem, tigecycline and nitrofurantoin was found to be 100%, 100% and 88% respectively. Sensitivity to non-b lactam antibiotics varied from 0 to 45%.Statisticallysignificant (p<0.01) co-resistance to non-b lactam antibiotics was observed with ESBL producers Table 1.

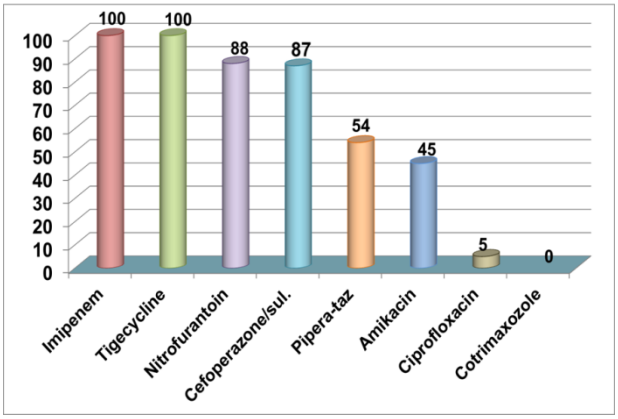

Figure 1: Percentage susceptibility of ESBL producers to various antibiotics (n=117).

Table 1: Antibiotic susceptibility of ESBL producers and non producers to various non β lactam antibiotics.

Antibiotics	ESBL producers (n=117) Susceptible No.	%	Non-producers (n=99) Susceptible No.	%
Tigecycline	117	100	99	100
Nitrofurantoin	103	88	98	99
Amikacin	53	45*	74	75
Ciprofloxacin	6	5*	33	33
Cotrimaxozole	0	0*	9	9

*p<=0.01

DISCUSSION

ESBLs are now a problem worldwide. Prevalence of ESBL varies across continents, countries and hospitals as demonstrated by large scale studies like SENTRY, SMART, MYSTIC. As per the SMART study conducted in Asian-Pacific in 2007, the prevalence of ESBL production in Enterobacteriaceae was reported to be highest from India. ESBL production among *E. coli* was 79.0%.[10]

Table 2 shows different Indian studies conducted over the last 10 years, which demonstrate regional differences in prevalence rates ranging from 24.80% to 87.1% in *E. coli.*

In our study the overall ESBL production in urinary isolates of *E. coli* was found to be 53%.

Table 2: ESBL producing *E. coli* detection rates in different Indian studies.

Author	Year & Place	ESBL Positive *E. coli* %
Trupti Bajpai *et al.* [11]	2014 Indore	41.6%
Meeta Sharma *et al.*[12]	2013Jaipur	52.49%
Mohamudha *et al.* [13]	2012 Pondicherry	87.1
Manoharan *et al.*[14]	2011Mumbai	78
Shoorashetty *et al.* [15]	2011Bangalore	41
Rao *et al.* [16]	2010 Davangere	62.9
Varaiya *et al.* [17]	2010 Mumbai	27.77
Wani *et al.* [18]	2009 Srinagar	52.94
Goyal *et al.* [19]	2009 Lucknow	63.6
Tsering *et al.* [20]	2009 Sikkim	26.15
Agarwal *et al.* [21]	2008 Pune	30
Varsh gupta [22]	2007 Chandigarh	63.8
SMART study[10]	2007Asia Pacific	79
Kumar *et al.* [23]	2006 Hyderabad	24.8
Babypadmini *et al.*[24]	2004 Coimbatore	41
Manchanda & Singh [25]	2003 New Delhi	55

Among the ESBL positive isolates, maximum numbers of samples were received from surgery ward (29.4%) and ICUs (18.21%). Like our study Babypadmini *et al.*, and Wani *et al.*, have also detected majority of ESBL producers from ICU and surgical ward.[18,24]

In vitro, the Carbapenems (including Imipenem, Meropenem, and Ertapenem) have the most consistent activity against ESBL-producing organisms, given their stability to hydrolysis by ESBLs.[26] In our study also carbapenems are the most effective with 98.2% ESBL producers sensitive to Meropenem.

β-Lactam/β-lactamase inhibitor combinations are usually active against organisms possessing a single ESBL. As has been noted previously, many organisms now produce multiple ESBLs, which may reduce the effectiveness of β -lactam/ β-lactamase inhibitor combinations.[26] In vitro resistance of ESBL-producing isolates to such combinations is increasing; in our study out of 117 ESBL positive isolates, 54.0% were sensitive to Piperacillin-tazobactam, and 87.0 % to Cefoperazone-sulbactam. Tsering *et al.* in a similar study have reported that 97.53% and 48.11% of isolates were sensitive to Imipenem and Piperacillin-tazobactam respectively.[20] In another study by Mohanty *et al.* sensitivity of ESBL positive isolates was reported to be 81.37% to Piperacillin-tazobactam, 76.06% for Cefoperazone-sulbactam and 45.48% for Ticarcillin-clavulanic.[27] Manoharan *et al.* reported 89.7% sensitivity to Amikacin and 85.3% to Piperacillin-tazobactam.[14] Sharma *et al.* reported sensitivities to Piperacillin-tazobactam 89%, Amikacin 22%, Gentamicin 56% and Tobramycin 78% (28). Similar kind of resistance pattern was also reported in SMART study (Asia-Pacific) and MYSTIC study group (Europe) in Table 2.[10,29]

Table 3: Resistance pattern in international studies.

Organism	Antimicrobial	% of Sensitive isolates		
		Our Study	Smart Study	Mystic Study Group (Europe)
E. coli	Imipenem	100	98	98.9
	Piperacillin-tazobactam	54	98.5	94.2

ESBL producers have shown good sensitivity to Tigecycline and Polymyxin B. Nitrofurantoin has also shown good sensitivity among urinary isolates and is a good choice for urinary tract infection, being available orally and cheaper than its alternatives.

Many workers (Table 4) have found that resistance to third generation cephalosporins coexists with resistance to other antibiotics like, Cotrimoxazole, Tetracycline, Ciprofloxacin, Amikacin etc. indicating multidrug resistance pattern. We found such associated resistance with co-trimoxazole -100%, amikacin - 55% and fluroquinolones - 95% ($p<0.01$).

Similar findings have been noted by other workers.

CONCLUSION

In the current study a high prevalence of ESBL producing *E. coli* was detected.

Table 4: Sensitivity profile for various antibiotics across India.

Study	AN	GM	NET	TE	C	CIP	OFX	F/M	SXT
Mohanty *et al.*[*27]	52.8	15.7	52.8	-	-	27.1	-	-	-
Agarwal *et al.* [*21]	44	31	50	44	60	54	-	-	70
Wani *et al.*[*18]	78.2	34.8	-	-	-	6.9	3.8	91.5	69.1
Babypadmini *et al.* [*24]	86	25	-	-	-	9	-	89	26
Tsering *et al.*[*20]	-	45.5	21.5	25.3	-	48.1	-	-	-
Khanduri *et al.* [*30]	83.3	27.4	73.0	17.2	50.5	8.1	8.1	89.4	5.6

*ESBL producers.

Most of these isolates were from hospitalized patients indicating that these were important nosocomial pathogens. The outpatient presence of ESBL is of concern as it is now come to the alert of the physician that ESBL is spreading fast in the community and responsible for community-acquired ESBLs. Delayed recognition and inappropriate treatment of severe infections caused by ESBL producers with cephalosporin has been associated with increased morbidity.

Significant difference was observed between ESBL producers and non-producers regarding antibiotic sensitivity. Many ESBL producers were found resistant to non β-lactam antibiotics like Quinolones and to lesser extent to Aminoglycosides. Tigecycline, Meropenem, Polymyxin B were found highly effective. Nitrofurantoin was the only oral drug to which uniform susceptibility was found. Though ESBLs were found susceptible to Piperacllin-tazobactam and Cefoperazone-sulbactam, they should not be given for empirical therapy since co production of Amp C renders these combinations clinically ineffective.

It is important therefore that patients report must state the isolate is a suspected or a proven ESBL producer and should include a note stating that production may predict therapeutic failure with β- lactam antibiotics, irrespective of their in vitro susceptibility.

To reduce the prevalence of antimicrobial resistant pathogens, including ESBL producing *E.coli*, effective infection control measures like hand washing and barrier precautions are required. Monitoring the judicious use of extended spectrum cephalosporins, periodic surveillance of antibiotic resistance patterns and efforts to decrease empirical antibiotic therapy would go a long way in addressing some of the problems associated with these pathogens.

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