

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.610.481>

Extended-Spectrum Beta-Lactamases Producing Microorganisms isolated from UTI Patients: an Alarm

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ABSTRACT

UTI is one of the commonest infections encountered by clinicians and Extended—spectrum beta-lactamases in UTI patients have emerged as a major threat worldwide. Due to extensive use of β -lactam antibiotics over the last several decades in the clinical practice various β -lactamases have emerged and despite the worldwide availability of antimicrobial agents. UTI has become difficult to treat because of appearance of pathogens with increasing resistance to antimicrobial agents because of ESBL producers. The objective of this study is to provide a better understanding of ESBL producing organisms in UTI patients and to isolate and identify the bacterial pathogen from clinical samples and to determine their antibiotic susceptibility pattern of isolates in the tertiary care hospital, S.N. Medical College of North India. This study was carried out in S.N. Medical College, Agra (Uttar Pradesh), India from January 2016 to July 2016. Out of the total 1,106 urine samples were tested for the bacterial growth and their antibiotic sensitivity tests was done by the Kirby Bauer disc diffusion method. Out of 1,106 urine samples, 325 (29.38%) samples was positive and included in the final analysis. Among ESBL producing Gram negative uropathogens *Escherichia coli* (65.43%) was the most common isolate followed by *Klebsiella pneumoniae* (66.66%) and *Proteus mirabilis* (66.67%). These were found to be highly susceptible to Carbapenems such as Imipenem, Meropenem (95.06%), and for Amikacin (83.95%) and Nitrofurantoin (85.19%). Among Gram positive cocci *Staphylococcus aureus* and *Enterococcus faecalis* were found to be 100% sensitive for Vancomycin and Teicoplanin and 83.78% for Nitrofurantoin. The most frequently identified ESBL producing uropathogens in the study were *E coli* and *Klebsiella pneumoniae* and *Proteus mirabilis* show increasing resistance pattern to the commonly used or prescribed drugs in health settings that in turn leaves the clinicians with very few alternative options of drugs for the treatment of UTIs. Imipenem, Meropenem, Amikacin and Nitrofurantoin in case of Gram negative ESBL producers and Vancomycin, Teicoplanin and Nitrofurantoin showed a good antimicrobial activity against uropathogens. They may represent good option for the empirical treatment of patients with UTI.

Keywords

Extended-spectrum
Beta-lactamases
(ESBLs), Urinary
tract infection

Article Info

Accepted:

30 August 2017

Available Online:

10 October 2017

Introduction

Globally, Extended-spectrum of β - lactamases
ESBLs are considered to be most problematic,

particularly in hospitalized patients and those
undergoing long term treatment with limited

options. Infections with ESBL producing organisms have been associated with poor outcomes. Extended-spectrum β -lactamases are the enzymes mainly produced by Gram negative bacilli that have the ability to destroy or break down one or more extended spectrum antibiotics belonging to the cephalosporins (beta lactam antibiotics like ceftazidime, ceftriazone, cefotaxime, and cefepime etc), monobactam aztreonam and penicillines, containing an oxyimino group and render them ineffective (1). It is one of the ways in which bacteria develop resistance. With increasing multidrug resistance in uropathogens is an important and emerging public health problem. The prevalence of antimicrobial resistance among uropathogens have been increasing worldwide due to irrational use of antibiotics in practise (2, 3). ESBL infections have occurred in people who are already very sick and in elderly people and also those patients who have been taking antibiotics or previously hospitalized are mainly affected (4). As a result, infections caused by these bacteria can be difficult to treat and infections caused by these bacteria are become more common in both community and health care settings. They are usually plasmid mediated β -lactamases that can be exchanged between bacteria and most commonly found in Gram negative bacteria especially in the member of Enterobacteriaceae such as *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* and *Pseudomonas aeruginosa* (1, 5). The first plasmid mediated β -lactamase in Gram negative bacteria was discovered in Greece in the 1960s and it was named TEM (1). The first derivation of TEM-1 is TEM-2 with a single replacement of amino acid (6). Another common prevalent type of beta-lactamases is SHV-1 that was reported initially in *Klebsiella pneumoniae*. It is estimated that SHV-1 is responsible for plasmid mediated ampicillin resistance in bacteria that harbours it. The replacement of single amino acids

causes the change in enzyme structures and affects its activities (1). A substitution is more common among TEM, SHV and OXA enzymes in defined amino acids positions. The combination of altered amino acids produce variety of phenotypes of beta-lactamases enzymes with varying ability to breakdown 3rd generation cephalosporins and increase their level of resistance to beta-lactamases inhibitors (7). They can be inhibited by the action of β -lactamases inhibitors such as clavulanic acid, salbactams and tazobactams (1). This study is important for clinicians in order to facilitate the effective treatment and management of patient with symptoms of urinary tract infections.

Materials and Methods

This study was carried out in a tertiary care centre of North India, in the Department of Microbiology, S.N. Medical College, Agra (Uttar Pradesh), India, during the period of January 2016 to July 2016. The urine samples were collected from different wards of OPD (outpatient department), NICU, PICU, ED pedia, Gynae OPD.

The total of 1,106 urine samples were analysed and patients were advised to collect the clean catch midstream urine into a 50 ml calibrated sterile screw capped container. The specimens were labelled, transported to the laboratory as early as possible. Isolation and identification of bacterial pathogens was done by the microscopy and culture methods. Microscopy was done by Gram's staining and culture method was done with a loopful of the well mixed urine samples were inoculated onto Cystine-Lactose-Electrolyte-Deficient (CLED) agar medium to determine the colony forming unit (CFU). After incubated at 37⁰C aerobically for 24-48 hours and then examined for positive bacterial growth. A specimen was considered positive for UTI

infection, if an organism was cultured at a concentration of $\geq 10^5$ cfu/ml and less than 10^2 cfu/ml was interpreted as negative (8). Bacterial isolates were identified generally by performing conventional biochemical tests.

Detection of ESBL producers

This test was done by using double disc synergy test (DDST) on Muller Hinton agar medium with cephalosporin alone and cephalosporin with combination of β -lactam inhibitors like clavulanic acid, tazobactam and sulbactams. The positive and negative control strains used in this test were *Klebsiella pneumoniae* ATCC 700603 and *E. coli* ATCC 25922, respectively.

Antimicrobial Susceptibility Testing

All isolates were tested for antimicrobial susceptibility testing by the standard Kirby Bauer's disc diffusion method on Muller Hinton Agar medium. After incubation of 24 hours the inhibition zones were measured and interpreted according to clinical and lab standards institute (CLSI) guidelines (9) using the following standard antibiotic discs for the isolates i.e, Ciprofloxacin (05 μ g), Levofloxacin (10 μ g), Ampicillin (10 μ g), Ampicillin-Sulbactam (10/10 μ g), Ceftazidime (30 μ g), Ceftriaxone/Sulbactam (30 μ g), Cefepime (30 μ g), Gentamicin (10 μ g), Doxycycline (30 μ g), Tetracycline (30 μ g), Teicoplanin (30 μ g), Nitrofurantoin (300 μ g), Amikacin (30 μ g), Cefaclor(30 μ g), Cotrimoxazole (1.25/23.75 μ g), High Level Gentamycin (120 μ g), Vancomycin (30 μ g), Piperacillin / Tazobactam (100/10 μ g), Polymyxin-B (300units), Aztreonam (30 μ g), Colistin (10 μ g), Imipenam (10 μ g), Meropenem (10 μ g) etc.

Results and Discussion

Out of the total 1,106 specimens, 325 (29.38%) were positive with identified

pathogens and are subjected to antimicrobial susceptibility testing. Among 325, 173(53.23%) were female patients and 152 (46.77%) were males. The most common isolate in this study have been the Gram negative bacilli.

E. coli was the most frequently identified Gram negative bacteria accounted for 162 (49.84%) followed by other uropathogens like *Enterococcus faecalis* (11.38%), *Klebsiella pneumoniae* (6.46%), *Proteus mirabilis* (2.76%), *Staphylococcus aureus/sp* (2.46%), *Pseudomonas aeruginosa* (2.15%) among the major isolates. (Table No. 1)

ESBL producers

Among ESBL producers the predominant isolate was *E. coli* 106(65.43%) followed by *Klebsiella pneumoniae* 14(66.66%) and *Proteus mirabilis* 6(66.67%). (Figure-1)

Antibiotic susceptibility test

Among the tested antibiotics the highest susceptibility for Gram negative bacteria was shown by carbapenems such as Imipenem, Meropenem, Nitrofurantoin and Amikacin. In our study the resistance rate of isolated uropathogens to fluoroquinolones, second and third generation cephalosporins was high ranging from (70% to 90%).

In ESBL producer, *E. coli* was the predominant isolate were susceptible to Imipenem (95.06%), Meropenem (95.06%), Nitrofurantoin (85.19%) Amikacin (83.95%). Cefepime and Ceftazidime (74.07%) resistance indicates ESBL production. (Table no.3). *Ps. aeruginosa* were found 100% susceptible for Vancomycin and Teicoplanin and 98% for Imipenem and 71.43% for Ceftazidime and Aztreonam. Similarly *E. faecalis* and *S. aureus* were showed 100% susceptibility for Vancomycin and Teicoplanin and 83.78% for Nitroflurantoin.

Table.1 Distribution of uropathogens among both gender

S. no	Isolated Uropathogen	Female (%) n-173 (53.23)	Male (%) n-152 (46.77)	Total no of patients- n-325
1	<i>Escherichia coli</i>	84 (51.85)	78 (48.15)	162 (49.84)
2	<i>Enterococcus faecalis</i>	18 (48.65)	19 (51.35)	37 (11.38)
3	<i>Klebsiella pneumoniae</i>	13 (61.90)	8 (38.09)	21 (6.46)
4	<i>Proteus mirabilis</i>	3 (33.33)	6 (66.66)	9 (2.76)
5	<i>Staphylococcus aureus</i>	6 (75)	2 (25)	8 (2.46)
6	<i>Pseudomonas aeruginosa</i>	1 (14.29)	6 (85.71)	7 (2.15)
7	<i>Candida sp.</i>	48 (59.26)	33 (40.74)	81 (24.92)

n = Total no. of patients

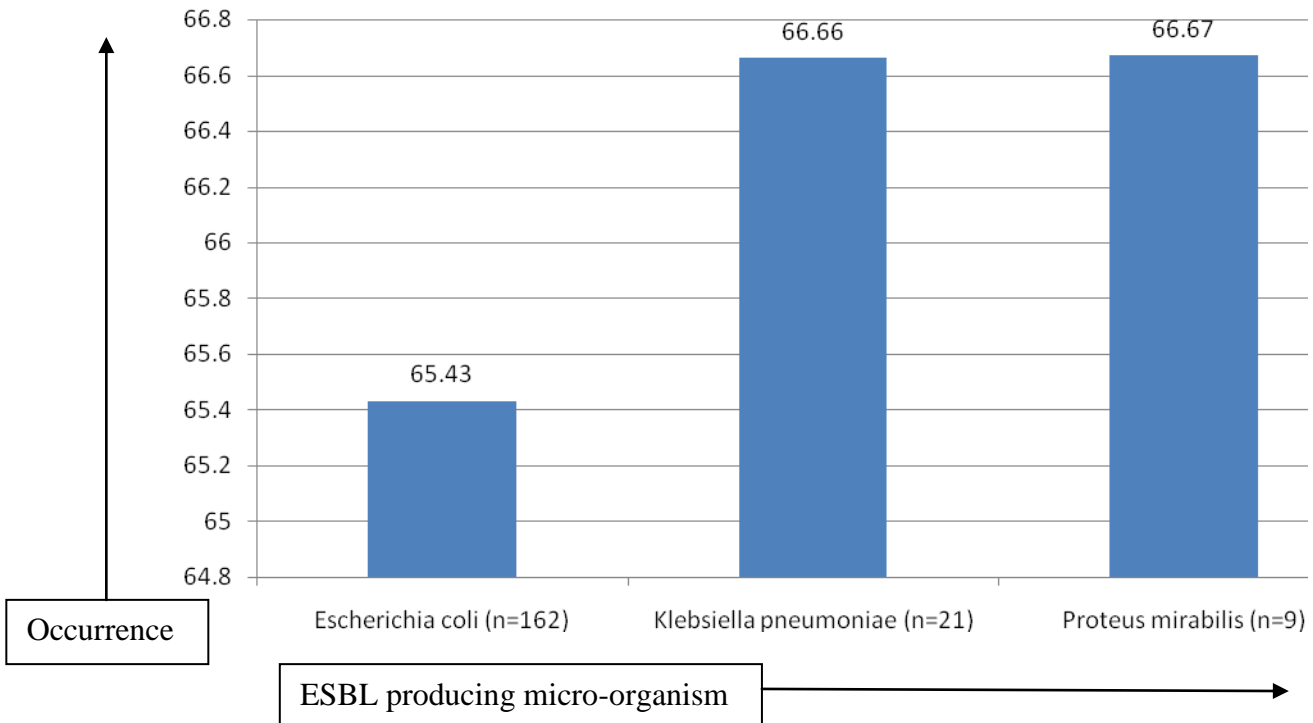
Table.2 Susceptibility rates (%) for isolated Gram negative uropathogens

A/B	Isolated Uropathogen			
	<i>E coli</i> (n-162)	<i>K. pneumoniae</i> (n-21)	<i>Proteus mirabilis</i> (n-9)	<i>Ps. aeruginosa</i> (n-7)
	Sensitive (%)	Sensitive (%)	Sensitive (%)	Sensitive (%)
Imipenem	95.06	95.24	88.89	85.72
Meropenem	95.06	90.48	88.89	86.41
Nitrofurantoin	85.19	52.38	00.00	00.00
Amikacin	83.95	61.90	77.78	71.42
Doxycycline	39.51	23.80	44.45	42.85
Tetracycline	38.27	23.80	44.45	42.85
Gentamicin	40.74	42.86	66.67	14.29
Cefepime	25.93	28.57	33.33	28.57
Ceftazidime	24.69	23.80	44.44	71.43
Ceftriaxone/sulbactam	66.04	66.66	66.67	57.14
Levofloxacin	12.35	19.05	22.23	28.57
Ciprofloxacin	11.73	28.57	22.23	28.57
Moxifloxacin	12.35	23.80	33.34	14.28
Ampicillin	7.41	00.00	11.12	14.28
Amoxyclovanic acid	30.86	23.80	22.23	–
Cotrimoxazole	–	9.52	77.78	–
Polymycin-B	–	–	–	100
Colistin	–	–	–	100
Aztreonam	–	–	–	71.43
Piperacillin/tazobactam	–	–	–	42.86

Table.3 Susceptibility rates (%) for Gram positive uropathogen

Antibiotic	Isolated Uropathogen	
	<i>E. faecalis</i> (n-37)	<i>S. aureus</i> (n-8)
	Sensitive (%)	Sensitive (%)
Vancomycin	100	100
Teicoplanin	100	100
Nitrofurantion	83.78	—
Doxycycline	45.95	62.5
Tetracycline	51.35	62.5
Ampicillin	13.51	—
Ampicillin/salbactum	13.51	37.5
Amoxyclavunic acid	18.92	—
Levofloxacin	16.22	12.5
Cotrimoxazole	13.51	25
High-level gentamycin	37.84	—
Cefaclor	—	25
Piperacillin/tazobactum	—	37.5
Cefepime	—	25
Penicillin-G	0	0
Clindamycin	—	62.5

Fig.1 Distribution of ESBL producers of isolated Uropathogen



Urinary tract infections are the common clinical condition worldwide. A variety of enteropathogenic bacteria are known to cause UTI. In our study we described the relationship between sex, isolated uropathogens and their antibiotic susceptibility. This study showed a higher prevalence of UTI in females (53.23%) than in males (46.77%) which is analogous with those of other findings revealing that the frequency of UTI is higher in females as compared to males (10,11). Our study showed that *E coli* (49.84%) was the commonest Gram negative uropathogen identified in both gender as described previously (12, 13). Other Gram negative uropathogens known to cause UTI including *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and Gram positive *Staphylococcus aureus* and *Enterococcus faecalis* etc., these also correspond to the data obtained by other groups (14,15). Second common isolated uropathogen in our study was *Enterococcus faecalis* showed higher prevalence in men than in women, similar to other findings (16) and differ from other studies as explained *Klebsiella pneumoniae* was second lead uropathogen (17). The similarities and differences are based on the type and distribution of uropathogens and they may result from different environmental conditions and host factors. On the basis of our study the resistance rate of isolated uropathogens mainly in *E coli* and *Klebsiella pneumoniae* for flouroquinolones includes ciprofloxacin, Levofloxacin, Moxifloxacin etc and to second and third generation cephalosporines was showed higher resistance. The resistance rate of upto 70-90% against flouroquinolones and upto 80-90% against cephalosporines. This is similar to the rate of antibiotics resistance previously reported studies (18, 19). The clinical trials conducted worldwide have reported that these antibiotics are the most common used ones because of their easier

administration and cheaper availability. And also a hisgher proportion of uropathogens were resistance to ampicillin, Cotrimaxazole and amoxicillin. The emergence of resistance for flouroquinolones is multifactorial due to an important mechanism of antibiotic resistance among uropathogens is through ESBL production. Among the Gram negative uropathogens, the emergence of resistance to extended spectrum cephalosporins has been a major concern (19). Mainly Gram negative bacteria are the common host of ESBL producers such as *E coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* of these *E coli* is the one of main ESBL producing pathogen. Production of ESBL is plasmid mediated and this plasmid carries multiple drug resistance (MDR) genes against aminoglycosides, quinolones and sulfamethoxazole at the same time (20). MDR increases the number of difficulties to the clinical treatments due to limited therapeutic options (21, 22). It has been reported that Amikacin, Imipenem. Meropenem and Nitrofurantoin are the most affective antibiotics (in case of ESBL producers) against *E coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* (23). Our study further supported by another study where the susceptibility rate of *E coli* and *Klebsiella pneumoniae* to Amikacin remained 80-90% (24). Carbepenem such as Meropenem, Imipenem were found to be most sensitive drugs against all isolates. The sensitivity rate of carbepenems among uropathogens was as follow *E coli* Imipenem (95.06%) and Meropenem (95.06%), for *Klebsiella pneumoniae* Imipenem (95.24%), Meropenem (90.48%), for *Ps aeruginosa* Imipenem (87.72%), and for *Proteus mirabilis* Imipenem(88.89%) and Meropenem (88.89%). These antibiotic susceptibility results similar to other previously reported studies (25). In case of Gram positive uropathogens such as *S. aureus* and *Enterococcus faecalis* were showed high

susceptibility frequency towards Vancomycin, Teicoplanin. These two antibiotics were reported 60-100% sensitive for both Gram positive bacteria. Other sensitive antibiotics were Tetracycline and Doxycycline which accounted for 62.50%.

This study highlights the need for the development of few generic drugs; otherwise the resistant to fluoroquinolones, the cheapest of the drugs that remains highly efficient will increase rapidly in the future.

The study concluded that ESBL producing Gram negative bacilli such as *E coli*, *K. pneumonia*, and *Proteus mirabilis* are the main uropathogen responsible for causing UTIs. Of concern is that the resistance rate of these pathogens against commonly used (fluoroquinolones and cephalosporins) antibiotics has increased. Nitrofurantoin should be recommended for the first line empirical oral treatment of UTI and also Carbapenems (Imipenem and Meropenem) and Amikacin showed a good antimicrobial activity against ESBL producing isolates. Vancomycin and Teicoplanin are effective in treating UTI due to Gram positive cocci. However, the mechanism on bacterial resistance is complex and diverse, and the phenomenon of multidrug-resistance (MDR) bacteria has become a global burden. Susceptibility testing is a valuable tool to help in the selection of antibiotic treatment.

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How to cite this article:

Arti Agrawal, Namita Srivastava, Vikas Kumar, Neha Bhati, Rashmi and Ankur Goyal. 2017. Extended-Spectrum Beta – Lactamases Producing Micro Organisms Isolated from UTI Patients: an Alarm. *Int.J.Curr.Microbiol.App.Sci*. 6(10): 5071-5078. doi: <https://doi.org/10.20546/ijcmas.2017.610.481>