

Original Research Article

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A Study of Extended Spectrum Beta-Lactamase (ESBL) Producing Gram Negative Uropathogens and their Antimicrobial Susceptibility Pattern in a Tertiary Care Hospital

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ABSTRACT

Urinary tract infection (UTI) includes the infection of urethra, bladder, ureters and kidneys which comprise the urinary tract. UTI is an important cause of morbidity and mortality in both developing and developed countries of the world. Urine samples were collected from In-patients and Out-patients attending hospital with the history suggestive of Urinary Tract Infection. Clean catch mid-stream urine samples were collected in sterile universal containers after proper cleansing of urethra and perineum. 1200 samples were collected from IP/OP patients. The sample size was higher in number because we got more samples than what are expected approximately for two months while starting the study. These samples were processed within 2 hours of collection without delay. A total no of 1,200 samples were collected from in-patient and Out-patient departments of the tertiary care hospital. Out of these 1,200 samples significant bacteriuria (30%) was observed in 354 samples. Out of these 354 isolates, 63(10.1%) isolates were found to be Gram positive cocci, 290(81.9%) were Gram negative bacilli and 1(0.2%) fungi-Candida spp. Among total 290 GNB isolates *E. coli* 146(41.2%), *Klebsiella pneumoniae* 81(22.8%), *Klebsiella oxytoca* 15 (4.2%), *Pseudomonas aeruginosa* 20(5.6%), *Proteus spp* 10(2.8%), *Acinetobacter spp* 8(2.2%), *Citrobacter spp* 6(1.6%), *Morganella spp* 3(1%), and *Providencia spp* 1(0.2%) were isolated. Among the total GPC isolates *Staphylococcus aureus* 8(2.2%), CONS 54(15.2%) and *Enterococcus spp* 1(0.2%) were isolated. ESBLs are now causing the major threatening infections in hospitalized patients throughout the world. The prevalence of ESBLs among clinical isolates varies greatly in worldwide. Major risk factors for colonization of ESBLs are long term antibiotic therapy, prolonged hospital stay and H/O instrumentation or catheterization. In India the prevalence of ESBL production varies from 10% to 84%. Cefoperazone/Sulbactam 98.5% and Piperacillin/Tazobactam 97% are very effective in treatment of ESBL producing organism associated urinary tract infection. Amikacin, Nitrofurantoin followed by Gentamicin are showed higher susceptibility rate when compared to other drugs and these can be used as I line empirical drugs in UTI according to the patient's renal condition.

Keywords

Extended Spectrum Beta-Lactamase (ESBL), Uropathogens

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Introduction

Urinary tract infection (UTI) includes the infection of urethra, bladder, ureters and kidneys which comprise the urinary tract. UTI

is an important cause of morbidity and mortality in both developing and developed countries of the world. UTI affects all age

groups and both sexes and most UTI occur by the common route of infection i.e. ascending route after entry via the urethral meatus (Ahmed *et al.*, 2014). Gram negative bacilli are the most common organism to cause UTI about 80-85% in both out-patients and in-patients. In the Gram Negative pathogens, β -lactamase production remains the most important contributing factor to β -lactam antibiotic resistance (Lakshminarayanan and Sangeetha, 2015). The major group of Beta lactamase enzymes that conferring resistance to oxyimino-cephalosporins are Extended spectrum Beta-lactamases (ESBL) (Alan Philpppon *et al.*, 1989). These ESBL enzymes are plasmid mediated and belongs to Ambler class A and Bush- Jacoby-Medieros functional class 2be. By common definition, ESBLs are capable of conferring bacterial resistance to the Penicillins, Cephalosporins (I, II and III generation) and Aztreonam (but not to the Cephameycin and Carbapenem) by hydrolysis of these antibiotics, and which are inhibited by β -lactamase inhibitors such as Clavulanic acid, Sulbactam and Tazobactam (Shaikh *et al.*, 2015; Chauhan *et al.*, 2014).

ESBLs are the important factor for emerging infections nowadays because they are plasmid borne enzymes and have a potential role in transferring multiple drug resistance among different organisms and also from one patient to another (Supriya *et al.*, 2004). Since it represents the epidemiological marker of colonization, detection and regular surveillance of ESBL producers from the sample such as urine is of utmost important (Paterson *et al.*, 2004). The prevalence of ESBL producing organisms among clinical isolates vary greatly in worldwide and is steadily increasing over the various parts and it results in severe complications because of limited and ineffective antibiotic treatment. This will lead to major clinical crisis such as prolonged hospital stay, increased morbidity, mortality and health care costs to patients (Tripti Bajpai *et al.*, 2014). Therefore to

control the outbreak of ESBL producing organisms are considered as a clinically important and major concern now.

Hence the present study was designed to detect the ESBL production among Uropathogens in UTI patients by phenotypic methods and to assess the changes in antimicrobial susceptibility pattern for preparing antibiotic policy in our hospital.

Extended Spectrum Beta-lactamase (ESBL) isolates were first detected in Western Europe in mid-1980s. Since then their incidence have been increasing sharply and have become recognized as a major worldwide problem. ESBL producing organisms have been detected in every inhabited continent. Several workers from foreign and India including Rugved Lulkarni *et al.*, (2013) of BJ Govt. Medical college (Pune), Tripti Bajpai *et al.*, (2014) of IGNOU (New Delhi), Supriya *et al.*, (2004) (Nagpur), Syed Muataq Ahmed *et al.*, (2014) (Kerala), Chauhan *et al.*, (2014) (Dehradun) and Lakshminarayana *et al.*, (2015) (Bengaluru) had done researches based on ESBL producers and noted their sensitivity pattern in UTI.

David Paterson *et al.*, (Pennsylvania), Amita Jain *et al.*, (Luknow), Modi Dhara *et al.*, (Ahmedabad) and Mita D Wadekar *et al.*, (Mysore) were done the studies regarding detection of ESBL among gram negative bacilli from various samples. Similarly this study highlights the prevalence of ESBL producers and their antibiotic susceptibility pattern in a tertiary care hospital.

The main aim and objectives of this study includes to determine the Bacterial profile and their Anti-microbial susceptibility pattern of microbes in UTI patients and to detect and evaluate the prevalence of Extended Spectrum Beta Lactamase (ESBL) producing Gram Negative Uropathogens among the isolates. Also to determine the Antimicrobial

susceptibility pattern for ESBL producers and ESBL non producers.

Materials and Methods

Type of study : Prospective study.

Place of study : Govt. Mohan
Kumaramangalam Medical
College Hospital, Salem.

Study period : 6 months (July to
december2016)

Nature of sample: Urine

Sample size : 1200 samples

Sample collection

The study was carried out after getting the ethical committee approval and informed consent was obtained from each patient before sample collection. Urine samples were collected from In-patients and Out-patients attending hospital with the history suggestive of Urinary Tract Infection. Standard guidelines were followed for sample collection and processing. Clean catch mid-stream urine samples were collected in sterile universal containers after proper cleansing of urethra and perineum. 1200 samples were collected from IP/OP patients. The sample size was higher in number because we got more samples than what are expected approximately for two months while starting the study. These samples were processed within 2 hours of collection without delay.

Sample processing method

After receiving the samples, Gram staining (Picture 1) was done for all samples and then inoculated in Nutrient agar, MacConkey agar and 5% sheep Blood agar. For inoculation, semi-quantitative plating method was adapted by using a calibrated loop delivering 0.001ml of urine. The plates were incubated at 37°C for 24 – 48 hours. After incubation, the bacterial isolates were identified based on the

colony characteristics. Only the isolates correlate with Direct Gram staining and with colony count $>10^5$ CFU/ml (significant bacteriuria) and 10^4 CFU/ml (according to patient's condition) were considered for further processing. All confirmed isolates were further speciated by standard biochemical test protocols (Bailey and Scott's Diagnostic Microbiology, 2007. XII edn).

The isolates were tested for Anti-microbial susceptibility by Modified Kirby Bauer Disc-diffusion method with commercially available discs (Hi-media) on Mueller Hinton agar plates and the results were interpreted according to CLSI guidelines using *Escherichia coli* ATCC 25922 as a control strain (Kulkarni et al., 2013).

The panel of antibiotic discs like Ampicillin (10µg), Amikacin (30µg), Gentamicin (15µg), Cefotaxime (30µg), Ceftazidime (30µg), Cotrimoxazole (25µg), Ciprofloxacin (5µg), Norfloxacin(10µg) and Nitrofurantoin(300µg) were used as first line drugs and Cefoperazone/Sulbactam (30/10mcg), Piperacillin/Tazobactam (100/10mcg) and Imipenem (10mcg) were used as second line drugs.

An isolate has been considered as multi drug resistant (picture 2) if it is resistant to 3 or more than 3 Antimicrobial agents. Relevant demographic data is also collected.

Screening for ESBL

After Antimicrobial susceptibility test, isolates which have zone of inhibition ≤ 27 mm for cefotaxime and ≤ 22 mm for Ceftazidime are considered as presumptive ESBL producers and these isolates were subjected to phenotypic tests such as Combination Disc Test (CDT) and Double Disc Synergy Test (DDST) (Kulkarni et al., 2013).

Phenotypic confirmation methods for ESBL

All ESBL detection and confirmation was done according to CLSI (2014) guidelines using *E. coli* ATCC 25922 as negative control and *Klebsiella pneumoniae* ATCC 700603 as positive control.

Combination Disc Test (CDT) [Combined Disc Test]

For each test, inoculum was prepared and the turbidity was adjusted to 0.5 McFarland standard. The organism was swabbed on the Mueller Hinton agar plate.

Discs containing Ceftazidime (30µg) alone and combined with clavulanic acid (10µg) were placed at a distance (edge to edge) of 20mm in the centre of plate. After incubation, the positive result was indicated when the difference in zone of inhibition (picture 3) around the Ceftazidime/clavulanic acid disc and Ceftazidime disc alone was ≥ 5 mm (Jain et al., 2003; Modi Dhara et al., 2012).

Double Disc Synergy Test (DDST)

For each test, inoculum was prepared and the turbidity was adjusted to 0.5 McFarland standard and the inoculum was swabbed on the Mueller Hinton agar plate. The disc containing Amoxicillin (20µg)/Clavulanic acid (10µg) was placed in the centre of the agar plate.

Third generation Cephalosporins like disc containing Cefotaxime (30µg), Ceftazidime (30µg) and Ceftriaxone (30µg) were applied to the periphery of Amox/Clav disc (centre to centre in position). The distances between the discs (edge to edge) were adjusted to be 15-20mm. Positive result was indicated when the inhibition zones around any of the

cephalosporin discs are augmented towards the direction of disc containing clavulanic acid (Modi Dhara et al., 2012; Liofilchem, 2014).

Results and Discussion

A total no of 1,200 samples were collected from in-patient and Out-patient departments of the tertiary care hospital. Out of these 1,200 samples significant bacteriuria (30%) was observed in 354 samples. Out of these 354 isolates, 63(10.1%) isolates were found to be Gram positive cocci, 290(81.9%) were Gram negative bacilli and 1(0.2%) fungi-*Candida* spp. Among total 290 GNB isolates *E. coli* 146(41.2%), *Klebsiella pneumoniae* 81(22.8 %), *Klebsiella oxytoca* 15 (4.2%), *Pseudomonas aeruginosa* 20(5.6%), *Proteus* spp 10(2.8%), *Acinetobacter* spp 8(2.2%), *Citrobacter* spp 6(1.6%) *Morganella* spp 3(1%), and *Providencia* spp 1(0.2%) were isolated. Among the total GPC isolates *Staphylococcus aureus* 8(2.2%), CONS 54(15.2%) and *Enterococcus* spp 1(0.2%) were isolated.

Among 290 GNB isolates 160(55%) isolates were found to be Multi drug resistant. 153(52.7%) were resistant to Cefotaxime, 162(56%) were resistant to Cefotaxime and Ceftazidime and 162(56%) were found to be presumptive ESBL producers. Those were subjected to confirmation test by Combination Disc Test (CDT) and Double Disc Synergy Test (DDST).

After the confirmation test, Combination Disc test yield positive results for 134(46%) isolates and 115(40%) by Double Disc Synergy test and isolates confirmed as ESBL producers by both phenotypic methods were 115(40%) [Table-1].

Of those ESBL (phenotypic confirmed) producers 79% were susceptible to Amikacin, 52% to Gentamicin, 73% to Nitrofurantoin,

32% to Ciprofloxacin, 18% to Cotrimoxazole, 15% to Norfloxacin, 9 % to Ampicillin, 98.5% to Cefoperazone/Sulbactam and 97% to Piperacillin/Tazobactam.

While the non-ESBL producers were 86% susceptible to Amikacin, 74% to Nitrofurantoin, 51% to Gentamicin, 45% to Cotrimoxazole 44% to Ciprofloxacin, and 36% to Norfloxacin, 11 % to Ampicillin, 100% Cefoperazone/Sulbactam, 100% to Piperacillin/Tazobactam. All GNB isolates were showed 100% sensitivity to Imipenem [Chart-2].

Detection of extended spectrum Betalactamase producing Uropathogens are an important marker of endemicity. In our study 290 (81.9%) negative bacilli were isolated among the total isolates. *E. coli* was the most common isolate (41.2%) followed by *Klebsiella* spp(27.1%). This is similar to Rugved Kulkarni *et al.*, (2013) (*E. coli*-29.1%, *Klebsiella* spp-7.9%), Lakshminarayana *et al.*, (2015) (*E. coli*-65.9%, *Klebsiella* spp-15.4%) and Trupti Bajpai *et al.*, (2014) (*E. coli*-42.7%, *Klebsiella* spp, 17.7%) studies.

Our study culture positivity is higher in IPD (87%) samples as compared to OPD (12.9%) samples. This is supported by Trupti Bajpai (2014) *et al.*, (IPD-42.1%, OPD-30%) and Rugved Kulkarni *et al.*, (2013) (IPD-89.7%, OPD-10.2%) and Lakshminarayana (2015) *et al.*, (IPD-53.1%, OPD-46.8%) studies.

In our study, ESBL producers were more prevalent in female (55.08%) than male (44.9%) patients. This supported by Lakshminarayana *et al.*, (2015) (female-59.9%, male-40.09%) study. In contrast Trupti Bajpai *et al.*, (2014) study showed more isolates from male (39.8%) than female (36.6%) patients. This may be due to number of samples and other co-morbid conditions were high in female patients in our study.

ESBLs are more prevalent in *Escherichia coli* 21.7% followed by *Klebsiella* spp 13.4% in our study. This report is correlated with Babypadmini *et al.*, (2004) study in which ESBL production was found to be 41% in *E. coli* and 40% in *Klebsiella* spp. In contrast Supriya *et al.*, (2004) study showed *Klebsiella* spp (25.6%) followed by *E. coli* (18.5%), Lakshminarayana *et al.*, (2015) study *E. coli* 35.1% followed by *Citrobacter* spp 30%, Trupti Bajpai *et al.*, (2014) study *E. coli* 41.6% followed by *Pseudomonas aeruginosa* 36.1% and in Rugved kukarni *et al.*, (2013) study showed *E. coli* 40.7% followed by *Proteus* spp 25.7%.

The present study showed that the Double disk synergy test has less sensitivity than Combination disk test (DDST-40%, CDT-46%) which is similar to Mita *et al.*, study and it may be due to storage temperature of discs and problem of optimal disc space (Wadekar *et al.*, 2013).

In our study ESBL producers were showed high susceptible rate to first line drugs like Amikacin 79%, Nitrofurantoin 73% followed by Gentamicin 52% and showed more than 95% susceptibility to second line drugs like Cefoperazone/Sulbactam and Piperacillin/Tazobactam. All ESBL producers were showed 100% susceptibility to Imipenem. This first line drug susceptibility pattern is supported by Lakshminarayana *et al.*, (2015) (Amikacin 88.3%, Nitrofurantoin 84.6%) and Rugved kukarni *et al.*, (2013) (Amikacin 70.4%, Nitrofurantoin 75%) studies.

In our study non ESBL producers were showed high susceptible rate to first line drugs like Amikacin 86%, Gentamicin 74% followed by Nitrofurantoin 51%. In contrast Rugved kukarni *et al.*, (2013) study showed Amikacin 47.8%, Gentamicin 27.4% followed by Nitrofurantoin 26.8%.

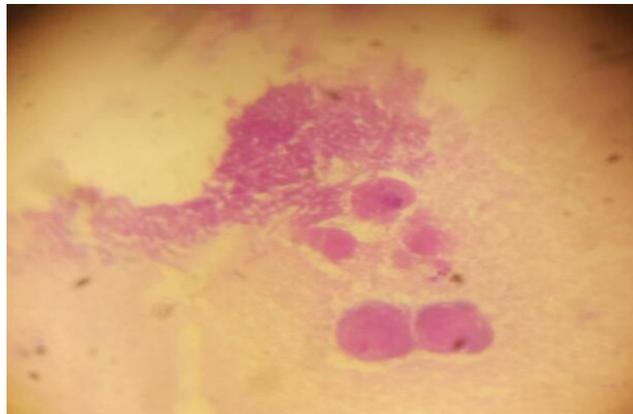
ESBL producers showed susceptible rate to Cotrimoxazole 18% and Norfloxacin 15%. This is similar to Trupti Bajpai *et al.*, (2014) (Cotrimoxazole 12% and Norfloxacin 17.4%). Our study showed high degree of susceptible rate to Aminoglycosides (Amikacin 79%,

86% and Gentamicin 52%, 74%) for ESBL and non ESBL producers respectively. This is supported by Trupti Bajpai *et al.*, (2014) (Amikacin 87.5%, 63.2% and Gentamicin 68.7%, 50.5%) study.

Table.1 Percentage of ESBL producers among GNB

No of ESBL producers by screening test (Isolates resistant to Ceftazidime and Cefotaxime)	No of ESBL producers by phenotypic confirmation tests		
	CDT	DDST	Both test
162(56%)	134(46%)	115(40%)	115(40%)

Picture.1 Gram staining showing pus cells with Gram negative bacilli



Picture.2 Antibiotic susceptibility test shows Multi drug resistant isolate



Picture.3 Combined Disc test showing difference in zone of inhibition



Chart.1 Distribution of pathogens in UTI patients

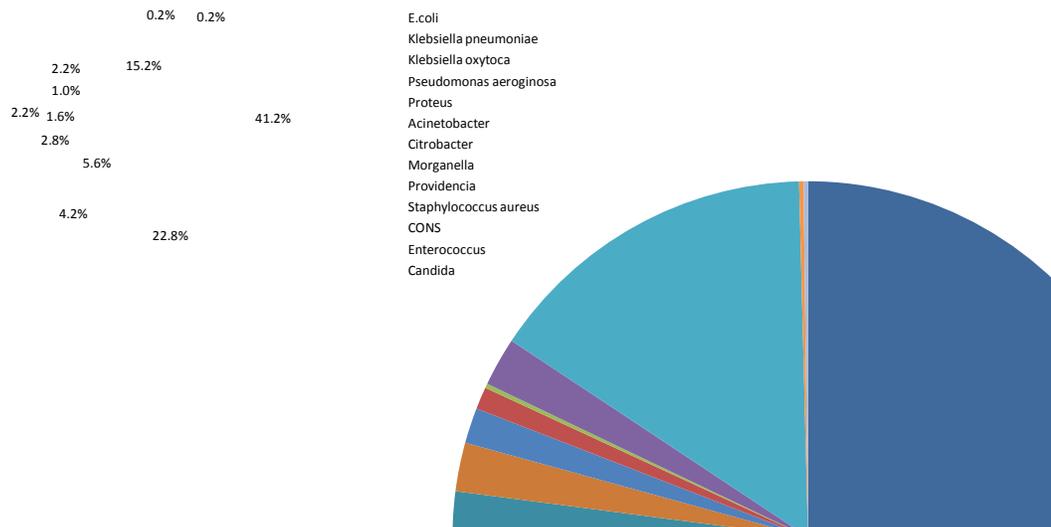
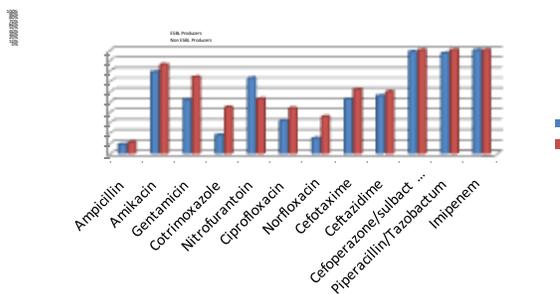


Chart.2 Antibiotic Susceptibility pattern for ESBL and non ESBL producers



ESBLs are now causing the major threatening infections in hospitalized patients throughout the world. The prevalence of ESBLs among clinical isolates varies greatly in worldwide. Major risk factors for colonization of ESBLs are long term antibiotic therapy, prolonged hospital stay and H/O instrumentation or catheterization. In India the prevalence of ESBL production varies from 10% to 84%. Cefoperazone/Sulbactam 98.5 % and Piperacillin/Tazobactam 97% are very effective in treatment of ESBL producing organism associated urinary tract infection.

Amikacin, Nitrofurantoin followed by Gentamicin are showed higher susceptibility rate when compared to other drugs and these can be used as I line empirical drugs in UTI according to the patient's renal condition.

The present study concludes that ESBL production was high among Uropathogens (50to 60%) and most of the ESBL producers were multi drug resistant (50to 60%). UTI caused by this multi drug resistance organisms are not able to treat by routine oral antibiotics like norfloxacin and ofloxacin and injectable cefotaxime like antibiotics.

Due to delay in diagnosis and delay in treatment with appropriate antibiotics in UTI which lead to septicaemia and pylonephritis like complication can occur. Phenotypic detection of ESBLs in routine antibiotic susceptibility testing and monitoring the continuous surveillance of ESBL detection and their susceptibility patterns are helpful to prevent treatment failure and formulation of appropriate antibiotic policy for UTI.

Early diagnosis of ESBL and treatment with appropriate antibiotics in urinary tract infection can control the spread of antibacterial resistance enzymes among patients and also we can prevent the hospital acquired infections.

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