

## Detection of Extended spectrum beta lactamases in gram negative isolates causing urinary tract infection in Tertiary care centre

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### Abstract

**Introduction:** Urinary Tract infection (UTI's) is the most commonly encountered infectious disease, affecting both the sexes in all age groups. This leads to consumption of large number of antibiotics and development of resistant strains leading to complications.

**Objective:** The study was carried out to know the distribution pattern and to evaluate the sensitivity of ESBL producing urinary isolates from patients with UTI.

**Materials and Methods:** A cross sectional study was conducted at Owaisi Hospital and Research Centre for 6months. A total of 392 samples were obtained from suspected UTI patients. Clean catch mid stream urine samples obtained were subjected to microscopy, culture and antibiotic sensitivity testing as per CLSI guidelines.

**Results:** From the total of 392 samples, 200 samples i.e. 51% samples were culture positive. Among the culture positive cases, 69% were females, 35% were males. 38.8% of Esch.coli and 25% Klebsiella were predominant ESBL producers.

**Conclusion:** This study revealed that majority of Gram negative urinary isolates are ESBL producers, and multidrug resistant. Thus strict policy with guidelines for antibiotic susceptibility testing and prescription should be followed to prevent further emergence of resistant strains and treatment failure.

**Keywords:** Mid stream urine, Extended Spectrum Beta Lactamases, antibiotic susceptibility pattern.

### Introduction

Urinary tract infection (UTI) is one of the most commonly encountered infectious disease.<sup>1</sup> UTIs affect almost all age groups and diagnosed in both hospitalized and outpatients. This infection not only causes a serious burden on the socioeconomic life of individuals but also leads to the consumption of a large proportion of all antibacterial drugs used in the world.<sup>2</sup> Women are more susceptible to UTIs than men due to anatomical structure of their genitourinary tract.<sup>3</sup>

UTI is most commonly caused by bacteria from patients own intestinal flora that enters urinary tract by ascending route via the urethra. It is an illness that can occur from infancy through old age, in otherwise healthy persons and in those who are compromised or debilitated. In fact, UTI are most common group of acute infection observed in nursing home residence and are most frequent cause of bacteraemia in patients with indwelling catheters and in institutional setting. They account for >40% of all institutionally acquired infections.<sup>2</sup>

Gram negative bacilli account for majority of UTI, specifically Esch.coli (upto85%), Klebsiella, Proteus and Pseudomonas aeruginosa.<sup>1</sup> Among gram positive cocci Staphylococcus saprophyticus and Enterococcus species are major etiological agents.<sup>6</sup>

The Gram negative urinary isolates producing ESBLs are those that hydrolyze the oxymino beta-lactams and monobactams, but have no effect on the cephamycins and carbapenems. Problems associated with

ESBL producing isolates are difficult to be detected or treated, thereby causing increased mortality.

Antibiotic susceptibility testing should be performed on isolates from symptomatic patients with bacteriuria and colony counts that are clinically significant. Each laboratory should decide which antibiotics to test and report after consultation with infectious disease practitioners and pharmacy staff. Routine susceptibility testing for most antibiotics should be standardized and interpretation of results should be based on anticipated responses to infection. Thus delay in appropriate treatment leads to complications.<sup>7</sup> Therefore, timely usage of antimicrobial agents discourages the indiscriminate usage of antibiotics and also decreases the hospital stay.

### Objective

The current study is being under taken with the following objectives:

1. To isolate and detect gram negative urinary isolates.
2. To determine antibiotic sensitivity among isolated gram negative bacteria.
3. To detect ESBL producing bacterial isolates among resistant gram negative bacteria.

### Materials and Methods

A cross sectional study, was carried out among patients who attended OP and IP at Owaisi Hospital during August 2016 – January 2017. Patients were referred from departments of Urology, Nephrology and Intensive care units. Patients who presented with

symptoms consistent with cystitis or urethritis underwent a thorough history, physical examination and urine analysis. However, urine culture continues to be important in patients with recurrent UTI or treatment failure.

In view of increasing antimicrobial resistance in urinary pathogens, culture is necessary for performing antimicrobial susceptibility testing. Appropriate collection of microbiology urine specimens, has an important influence on usefulness of culture result. Collection of clean catch mid stream urine specimen, is the most frequently used and preferred method of urine collection because it is non invasive and avoids the risks inherent to catheterization.

### Selection criteria

#### Inclusion criteria:

1. Patient with symptom of urinary tract infection & not started on any antibiotic therapy.
2. Both male and females of age 10 – 60 yrs will be included.

#### Exclusion criteria:

1. Asymptomatic patients
2. Prior antibiotic therapy
3. Pregnant women.

**Specimen collection and processing:** A single clean voided specimen or a single specimen obtained by catheterization in a patient with specific symptoms was taken, if significant pyuria is demonstrable and culture yields a recognisable uropathogen. For purposes of optimal quantitation, it has been recommended by guidelines that only first morning specimens be processed or, if such specimens are not available, that the urine be allowed to incubate in the bladder for as long as possible (with a minimum of 4 hours) before collection to increase the bacterial density. Quantitation is necessary for the diagnosis of asymptomatic bacteriuria but unnecessary in case of symptomatic patients. Urine specimens from other asymptomatic patient populations, except those demonstrating significant pyuria, could not be reliably interpreted.

**Specimen Transport:** Urine is an excellent supportive medium for the growth of most uropathogens and therefore must be immediately received, refrigerated and processed in the laboratory within 2 hours. Longer delays render examination for significant pyuria unreliable, and the extremes of pH and urea concentration and the presence of antimicrobial agents may adversely affect the recovery of uropathogens.

**Culture:** All of the samples were Mid stream urine specimens and then they were subjected to culture. Generally, routine culture includes plating onto one nonselective medium. Calibrated loops of 0.01ml were used, not 0.001 ml loops, as quantitation is difficult to obtain with low inoculums. Specimens were routinely inoculated on a blood agar plate, a MacConkey agar plate and CLED medium and were incubated for 48 hours.

Specimens with multiple uropathogens (i.e., three or more) indicate probable contamination. Susceptibility test were performed for inpatients. Outpatient cases were subjected to different algorithm that does not routinely calls for susceptibility test, rather it emphasizes empirical selection based on antibiogram. Culture was considered positive if the specimen showed a single organism at concentration of  $\geq 10^5$  CFU/ml or one or two uropathogens present in small number (i.e.  $\geq 10^2$ CFU/ml) or, ( $\geq 10^2$  to  $<10^5$  CFU/ml ) in clinical situation such as in acute urethral syndrome or cases of previous antibiotic therapy<sup>8</sup>.

Microorganisms were identified as gram negative or positive by gram stain and standard biochemical tests.

### Susceptibility Reporting

With the growing number of emerging uropathogens and the simultaneous increase in newer antibiotics, it is mandatory that we use standardized methods and report only appropriate antibiotics for UTIs. Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion test. Antimicrobial agents approved by the Food and Drug administration ( FDA) for routine testing and reporting by clinical microbiology laboratories for urinary tract isolates are listed as Group U supplementary for urine only in the 2006 CLSI Performance standards for Antimicrobial Disc susceptibility test were used.<sup>10</sup>

### ESBL Detection:

#### Phenotypic method

- a. Screening test
- b. Confirmatory method

#### Screening Test (Kirby Bauer Disc Diffusion Test) :

##### Materials:

1. Mueller Hinton agar.
2. Test strain standardized inoculums (0.5 McFarland turbidity)
3. Control strains
4. Antibiotic discs of  
30µg Ceftazidime  
30µg Cefotaxime  
30µg Ceftriaxone  
30µg Aztreonam

##### Method:

1. Lawn culture the standardized inoculums of test isolate (0.5 McFarland turbidity) on Mueller Hinton agar plate.
2. Place 30µg disc each of ceftazidime, cefotaxime, ceftriaxone and aztreonam and 10 µg of cefpodoxime.
3. Incubate for 16-18 hours at 37<sup>0</sup> C.

**Results:** As per the guidelines issued by CLSI, *Klebsiella pneumoniae*, *K. oxytoca*, *E. coli*, and *P. mirabilis* isolates were regarded as positive for screening test under the following conditions.

**Table 1: Screening by Disc diffusion method**

Antibiotic	Zone diameters when testing	
	K.pneumoniae, K.oxytoca, Esch.coli	P.mirabilis
Cefpodoxime 10µg	≤ 17 mm	≤ 22mm
Ceftazidime 30µg	≤ 22 mm	≤ 22mm
Cefotaxime 30µg	≤ 27 mm	≤ 27 mm
Ceftriaxone 30µg	≤ 25 mm	
Aztreonam 30µg	≤ 27 mm	

**ESBL Confirmatory methods****i. Double Disc Synergy Test (DDST)/ Double Disc Diffusion Test (DDDT)****Materials:**

1. Mueller Hinton agar
2. Test strain standardized inoculums (0.5 McFarland turbidity)
3. Control strains
4. Antibiotic discs  
30 µ g ceftazidime,  
30 µ g cefotaxime,  
Amoxicillin/clavulanic acid 30/10 µ g

**Method:**

1. Lawn culture the standardized inoculums of test isolate (0.5 McFarland turbidity) on Mueller Hinton agar plate
2. An amoxicillin/clavulanic acid 30 µ /10 µ g is placed at the center of the plate.
3. Discs containing 30 µ g ceftazidime, cefotaxime, are placed 20-30 mm away from the central disc.
4. Incubate for 16-18 hours at 37°C

**Result:** An extension in the zone of inhibition around the peripheral discs towards the centrally placed Amoxicillin/clavulanic acid 30/10 µ g disc indicates ESBL production.

**ii CLSI Phenotypic Confirmatory Test (PCT)/ Combined Disc Method****Materials:**

1. Mueller Hinton agar

2. Test strain standardized inoculums (0.5 McFarland turbidity)
3. Control strains
4. Antibiotics discs  
Ceftazidime (30 µg),  
ceftazidime/clavulanic acid (30/10 µg),  
cefotaxime (30 µg),  
cefotaxime/clavulanic acid (30/10µg).

**Method:**

1. Lawn culture the standardized inoculums of test isolate (0.5 McFarland turbidity) on Mueller Hinton agar plate
2. Ceftazidime (30 µg), ceftazidime/clavulanic acid (30/10 µg), cefotaxime (30 µg), cefotaxime/clavulanic acid (30/10µg) disks are placed on the plate 20mm apart
3. Incubate for 16-18 hours at 37°C

**Result:** An increase in the zone diameter by ≥ 5 mm around the discs with clavulanic acid over the discs with cephalosporin alone confirms ESBL production.

**Results**

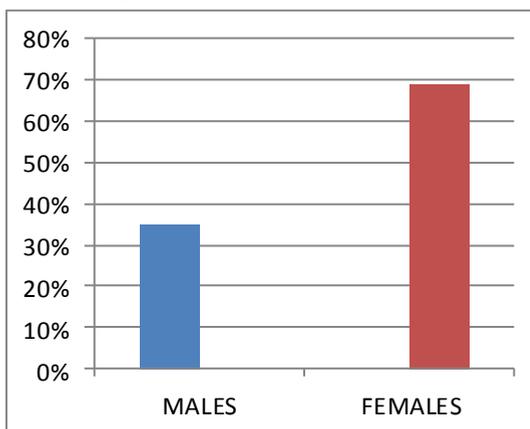
During the study, 210(53.6%) males and 182(46.4%) female samples were obtained from the total of 392 urine samples. Out of 392 samples, total 200(51%) samples were found to be positive for uropathogen in culture.

Out of **200 positive** urine samples for uropathogen, **74(35%) and 126(69%)** samples were from **Male and Female** patients, respectively.

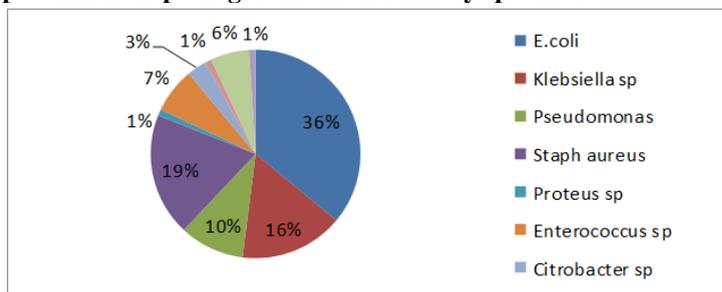
**Table 2: Age & Gender wise distribution of UTI patients with POSITIVE cultures**

Age(Yrs)	Females	Culture positive	%	Males	Culture positive	%
12-20	12	9	75	8	0	0
21-30	18	15	83	36	8	22
31-40	34	30	88	32	8	25
41-50	42	34	81	42	18	43
51-60	38	30	78	34	14	41
>60	38	8	22	58	26	45
Total	182	126	69	210	74	35

**Graph 1: Gender Distribution of culture positive UTI cases**



**Graph 2: Distribution pattern of uropathogens from the urinary specimens**



**Table 3: Antibiotic susceptibility of Gram Negative isolates**

Antibiotic	Sensitive (%)	Resistance (%)
Ciprofloxacin	43	57
Amikacin	70	30
Cotrimoxazole	40	60
Amoxiclav	32	68
Imipenem + cilastin	88	12
Colistin	78	22
Piperacillin + Tazobactam	79	21
Levoflox	59	41
Nitrofurantoin	50	50
Cefotaxime	41	59
Cefotaxime + clavulnic acid	69	31
Aztreonam	57	43

**Table 4: Antibiotic susceptibility of Gram positive isolates**

Antibiotic	Sensitive (%)	Resistance (%)
Ciprofloxacin	54	46
Azithromycin	50	50
Cotrimoxazole	70	30
Amoxiclav	46	54
Vancomycin	77	23
Colistin	65	35
Piperacillin + Tazobactam	65	35

Linezolid	85	15
Nitrofurantoin	54	46
Cefoxitin	70	30
Ceftazidime + clavulanic acid	50	50
Ceftazidime	30	70
Ceftriaxone	70	30

**Table 5: Prevalence of ESBL Producers**

Isolate	Total no. of Isolates	No. of ESBL'S	% of ESBL
Escherichia coli	72	28	38.88%
Klebsiella species	32	8	25%
Pseudomonas aeruginosa	20	6	30%
Citrobacter species	6	2	33%
Proteus species	2	0	0%
Acinetobacter species	2	0	0%

## Discussion

In the present study, it was found that there is a prevalence rate of 51% culture positivity as compared to 28% reported by Md. Yusuf et al,<sup>12</sup> 34% by Singh et al<sup>13</sup> and 38% by Ritu.<sup>14</sup> It goes along parallel with study done by Prakash et al<sup>15</sup> which showed 53% of culture positivity. Differences in positivity rate could be due to media selection, technique of growth and local prevalence rate.

The UTI in Females (69%) vastly outnumbered to those in Males (35%). These discrepancies could be related to factors such as the length of urethra, distance of urogenital meatus from anus and antibacterial properties of prostatic fluid.<sup>18</sup>

The young patients are at low risk for occult genitourinary tract abnormalities and are less likely to have co-morbid conditions. Certain patient sub groups, however have complicating conditions that increase the risk for acquiring invasive or systemic infection that include occurrence in men, children and pregnant women. But complications are particularly common in Elderly (61 years or more) particularly in Males (45%) than Females (22%), immunocompromised patients and in individuals with neurological disorders, underlying structural abnormalities (e.g. prostate enlargement) and infection due to antimicrobial resistant organisms.<sup>20</sup>

The study demonstrates that among urinary isolates *E. coli* (36%) was predominant uropathogen. Followed by *Staphylococcus aureus* (19%) and *Klebsiella* species (16%) in consistent with study done by Singh et al.<sup>13</sup>

We also found that among the gram negative urinary isolates ESBL producers were 38.8% *Esch. coli* and 25% *Klebsiella* as compared to other studies with the total ESBL rate being 32.8%. ESBLs are product of overuse of the 3<sup>rd</sup> generation Cephalosporins, making it difficult for valid comparison of the prevalence of ESBLs because of variation from hospital to hospital.

These types of discrepancies between the susceptibility data and the disc diffusion results have increased the need for an improved and advanced method of ESBL detection and incorporate it into routine susceptibility procedure.

Amoxiclav (68%), Co-trimoxazole (60%) and Cefotaxime (59%) revealed resistance on higher side to gram negative isolates as correlated with previous studies done. Aminoglycosides, most of them being in injectable form and nephrotoxic are preferred less as the drug of choice and therefore showed less resistance to *Esch. coli*. Retrospectively, our study should resistance of 57% to Fluoroquinolones.<sup>24,25</sup>

This study also revealed high resistance to 3<sup>rd</sup> generation Cephalosporins, predominantly to Ceftazidime (70%) and cefotaxime (59%). Over the time there are significant changes in antimicrobial resistance patterns and increase in antimicrobial resistance among uropathogens. Keeping this in view we should have drugs as reserve for last line of treatment which go in combination with inhibitors (like Augmentin).<sup>27</sup>

But most probably in near future if this irrational use is not stopped, infection with gram negative organisms will increase the rate of resistance to drugs that are now sensitive resulting in increase morbidity and mortality.<sup>27</sup>

## Conclusion

This study revealed that majority of the urinary isolates obtained from UTI patients are Gram negative. The two principal urinary pathogens are *Escherichia coli* (gram negative) and *Staphylococcus aureus* (gram positive). This study indicates that it is essential for prompt recognition of antimicrobial resistant organism. Infection control practitioners and clinicians requires the clinical lab to rapidly identify and characterize

different types of resistant bacteria especially ESBLs to efficiently minimize the spread of these bacteria and help to select more appropriate antibiotics. Thus strict policy for antibiotic susceptibility testing and prescription should be followed to prevent treatment failure and further emergence of resistant strains.

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