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# A Study of Uropathogenic ESBL Producing Gram Negative Bacilli in a Teaching Hospital

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**A B S T R A C T**

About 150 million people suffer from urinary tract infection each year. In majority of cases, treatment is initiated empirically based on the antimicrobial resistance pattern of the urinary pathogens prevalent in a particular setting. Emerging antibiotic resistance among *Enterobacteriaceae* has posed challenges in choosing empiric regimens. Therefore, the present study was designed to identify etiological agents of urinary tract infections, detect ESBL producing uropathogens and study their antibiotic resistance profile. Around 306 urine (Midstream urine and catheterized) samples were collected and processed by semi-quantitative culture on Cysteine Lactose Electrolyte Deficient media, blood agar, and MacConkey agar by standard loop method. Bacterial colony count more than $10^5$ colony-forming units (CFU)/ml was taken as significant bacteriuria. Antibiotic sensitivity testing was done by Kirby–Bauer disc diffusion method as per Clinical and Laboratory Standards Institute guidelines. ESBL screening and phenotypic confirmation was done by testing the strain against ceftazidime and ceftazidime/clavulanic acid, cefotaxime and cefotaxime/clavulanic acid). Out of 306 urine samples collected, significant bacteriuria was observed in 92.1% (282/306) samples. Out of the culture positive 282 specimens, female patients reported 72.4% growth whereas male patients reported 27.6% growth. Out of 282 isolates, 96.1% isolates were Gram-negative, *E. coli* being predominant isolate whereas 3.9 % were Gram-positive isolates (including Candida spp).The isolates showed least resistance to Imipenem (12%), followed by Nitrofurantoin (24%), Gentamicin (28%) Piperacillin Tazobactam (36%). Higher resistance was reported for Norfloxacin (80%), Cefazolin (76%), and Cotrimoxazole (68%) Ciprofloxcin (64%), Tetracycline (60%). Among the Gram negative isolates, 40.2% (109/271) were found to be ESBL producers.

## Introduction

About 150 million people suffer from urinary tract infection (UTI) each year (Flores-Mireles *et al.*, 2015). Although UTI's occur in all age groups including men and women, clinical studies suggest that the overall prevalence of UTI is higher in women (Salvatore S *et al.*, 2011). *Escherichia coli* is the most common cause of (80–85%) of community-acquired urinary tract infections (Nicolle *et al.*, 2008). Rarely UTI may be due to viral or fungal infections (Amdekar *et al.*, 2011). Healthcare-associated urinary tract infections mainly from urinary catheterization involve a much broader range of pathogens including *E. coli*, *Klebsiella spp*, *Pseudomonas aeruginosa*, *Proteus spp*, *Candida albicans*, and

*Enterococcus faecalis* etc. (Sievert *et al.,* l2013; Bagshaw *et al.,* 2006) In majority of cases, treatment is initiated empirically based on the antimicrobial resistance pattern of the urinary pathogens prevalent in a particular setting. The time required for culture results often exceeds the time to clinical cure with empiric treatment, therefore, in almost all cases of UTI empirical antimicrobial treatment is initiated before the laboratory results of urine culture are available, contributing to increasing antimicrobial resistance due to misuse of antimicrobials (Wilson *et al.,* 2004; Newell *et al.,* 2000). Increasing multidrug resistance in bacterial uropathogens is an important and evolving public health challenge.

The serious increase in the prevalence of extended-spectrum beta lactamases (ESBL's) worldwide creates a need for effective and easy to perform screening methods for detection(Prakash *et al.,* 2013, Yazdi M *et al.,* 2012; Naiemi *et al.,* 2009). ESBL producing organisms are those that hydrolyze the oxyimino beta-lactams and monobactams, but have no effect on the cephamycins and carbapenems. Also, the ESBL producers often also have resistance determinants to other antibiotic groups, leaving an extremely limited range of effective agents (Mukherjee *et al.,* 2013). Detection of ESBL producers from urine specimens is essential because of transfer of drug resistant organisms to other patients. (Aggarwal *et al.,* 2009)

Clinicians have tended to ignore the clinical importance of UTIs despite their significant prevalence, cost, morbidity, and increasing management problems. The reason is largely our opinion that uncomplicated UTIs are common yet not a serious problem, easy to diagnose, and effortless to treat. Antibiotic-resistant organisms causing UTI include Methicillin-resistant Staphylococcus aureus (MRSA), Methicillin-resistant coagulase-negative staphylococci (MRCoNS), vancomycin-resistant enterococci (VRE) and multidrug resistant Gram negative organisms. Candida species are frequently found as a colonizing organism and account for few clinical cases of UTI (Neal *et al.,* 2008).

Recent guidelines from the Infectious Diseases Society of America recommended that empiric antibiotic therapy for UTIs should be based on local resistance data, drug availability, and antibiotic intolerance/allergy history of treated patients.(Gupta *et al.,* 2011; Hooton *et al.,* 2010) For uncomplicated cystitis, nitrofurantoin or trimethoprim-sulfamethoxazole (TMP-SMX, if local resistance $\leq$ 20%) can be used empirically, while fluoroquinolones, ceftriaxone, aminoglycosides, and carbapenems are appropriate for pyelonephritis and complicated UTI. Emerging antibiotic resistance among *Enterobacteriaceae* has posed challenges in choosing empiric regimens, especially in infections due to multidrug-resistant (MDR) *Enterobacteriaceae* (Sanchez *et al.,* 2012) in the past decade, emerging resistance among the *Enterobacteriaceae* due to ESBL has been reported worldwide. (Qi *et al.,* 2010) Therefore, regularly updated surveillance of local microbial prevalence and resistance patterns are needed to guide the empiric therapy for UTIs. Therefore, the present study was designed to identify etiological agents of urinary tract infections, detect ESBL producing uropathogens and study their antibiotic resistance profile.

## Objectives

To isolate and identify uropathogenic gram negative bacilli.

To determine the antibiotic resistance profile of the isolates

To detect ESBL gram negative isolates

As a baseline study to formulate hospital antibiotic policy and empirical treatment.

## Materials and Methods

Type of study: Prospective study

Duration of the study: March 2018 to June 2018

Place of the study: Department of Microbiology, Mahavir Institute of Medical Sciences

### Inclusion criteria

Patients suggestive of symptoms of UTI – fever, burning micturation, frequency or urgency of urination, suprapubic discomfort, gross hematuria

Fever with pyuria and/or imaging evidence of UTI (cystitis, pyelonephritis, etc.)

Patients with urinary catheters and suggestive of UTI

### Exclusion criteria

Patients with sexually transmitted infections, cervicitis, and vulvovaginitis (can present with symptoms similar to cystitis)

Patients with history of antibiotic therapy for UTI before sending specimen for culture

Patients not willing to participate in the study

Around 306 patients with the inclusion criteria were screened in the present study. The details of patient including name, age, gender, ward (for admitted cases) and brief clinical history were noted. Midstream urine (MSU) sample was collected in sterile, wide mouth, leakproof container and transported immediately to Microbiology laboratory. Catherized urine samples were collected as per standard guidelines. Semi-quantitative culture of urine was done on Cysteine Lactose Electrolyte Deficient (CLED) media, blood agar, and MacConkey agar by standard loop method. The culture plates were incubated at 37°C for 18-24 h under aerobic conditions. Identification of bacterial growth was confirmed by standard microbiological techniques (Forbes *et al.,* 2007; Collee *et al.,* 2008). Bacterial colony count more than $10^5$ colony-forming units (CFU)/ml was taken as significant bacteriuria.

Antibiotic sensitivity testing was done by Kirby–Bauer disc diffusion method on Mueller-Hinton agar, as per Clinical and Laboratory Standards Institute guidelines. (Bauer *et al.,* 1966; Clinical and Laboratory Standards Institute 2017) Antibiotic discs were procured from HiMedia, Mumbai, India. ESBLscreening and phenotypic confirmation was done by testing the strain against ceftazidime and ceftazidime/clavulanic acid, cefotaxime and cefotaxime/clavulanic acid). A difference of >5 mm diameter of the zone of inhibition for combination disc in comparison to the ceftazidime/cefotaxime alone was considered as indicative of ESBL production. *Escherichia coli* ATCC 25922 for ESBL negative and *Klebsiella pneumoniae* 700603 for ESBL positive was used as reference strains. **(**Clinical and Laboratory Standards Institute 2017)

## Results and Discussion

A total of 306 urine samples were collected from 82 male and 224 female patients. Significant bacteriuria was observed in 92.1% (282/306) samples. Out of the culture positive 282 specimens, female patients reported 72.4% growth whereas male patients reported 27.6% growth. Gender wise distribution of specimens and culture positivity is mentioned in Table 1. Out of 282 isolates, 96.1% isolates

were Gram-negative, *E. coli* being predominant isolate whereas 3.9 % were Gram-positive isolates (including Candida spp) as mentioned in Table 2.

The isolates showed least resistance to Imipenem (12%), followed by Nitrofurantoin (24%), Gentamicin (28%) Piperacillin Tazobactam (36%). Higher resistance was reported for Norfloxacin (80%), Cefazolin (76%), and Cotrimoxazole (68%) Ciprofloxcin (64%), Tetracycline (60%). The details of antimicrobial sensitivity are mentioned in Table 3. Among the Gram negative isolates, 40.2% (109/271) were found to be ESBL producers (Table 4).

A total of 282 urine cultures were reported positive, 204 (72.4%) females and 78 (27.6%) male patients. Various research studies also conclude that UTI are more common in females as compared to males (Daniele *et al.,* 2011, Dash *et al.,* 2013).

Most of the uropathogenic bacteria are from the host's own gut flora and enter the bladder via the urethra. Shorter urethra in females as compared to males, with its proximity to anus, facilitates the bacteria to ascend in the urinary tract. (Yamamoto et 1997, Mitsumori *et al.,* 1997) Also, sexually active women have an increased risk of UTI. About 20% of young women with a first UTI will have a recurrent infection. (Scholes *et al.,* 2000)

Of the total 282 uropathogen isolates, *Escherichia coli* was the leading isolate with 70.2% (198/282) specimens reporting the growth, followed by *Klebsiella pneumonia* 19.8% (56/282), *P. aeruginosa* 4.25% (12/282), *Citrobacter spp* 1.06%(03/282), *Proteus spp* 0.7% (02/282). Among gram positive uropathogens, majority were *Coagulase negative Staphylococcus*, followed by *Staphylococcus aureus*, *Enterococcus* and *Candida* spp.

Uropathogenic *Escherichia coli* (UPEC) from the gut are the cause of 80–85% of community-acquired urinary tract infections (Etienne *et al.,* 2014; Schito *et al.,* 2009). In uncomplicated UTIs, *E.coli* is the leading organism, whereas in complicated UTIs the bacterial spectrum is much broader including Gram-negative and Gram-positive and often multidrug resistant organisms.

Research studies suggest that P fimbriae contribute as virulence factors of *E. coli* strains to cause UTI, especially the more clinically severe forms. As per various researches, leading organisms involved in uncomplicated UTIs, after UPEC are *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, group B *Streptococcus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida* spp (Foxman *et al.,* 2014, Nielubowicz *et al.,* 2010, and Kline *et al.,* 2011).

In the present study, gram negative isolates showed least resistance to Imipenem (12%), followed by Nitrofurantoin (24%), Gentamicin (28%) Piperacillin Tazobactam (36%).

A significant amount of resistance was documented to antibiotics like Norfloxacin (80%), Cefazolin (76%), and Cotrimoxazole (68%) Ciprofloxcin (64%), Tetracycline (60%). In the current study, overall 40.2% (109/271) gram negative isolates were detected positive for ESBL production. *E. coli* was the predominant 77.06% (84/109) ESBL producing isolate. ESBL production has been reported ranging from 38.9% (Rishabh *et al.,* 2018), 39.5% (Vasumathi *et al.,* 2016), 40% (Babypadmini *et al.,* 2004), 42% (Babek *et al.,* 2012), 44.5% (Saeide *et al.,* 2014) which is similar to our findings. However, even higher incidence of 58% and 84.6% has been reported by Mathur *et al.,* (2002) & Rejitha *et al.,* (2014) respectively (Table 5).

**Table.1** Gender wise and department wise distribution of urine specimens

| | No. of specimens | Culture positive (n=282) |
|---|---|---|
| Male | 82 | 78 (27.6%) |
| Female | 224 | 204 (72.4%) |
| Total | 306 | 282 |
| OPD and IPD distribution of urine specimens | | |
| | No. of specimens | Culture positive (n=282) |
| OPD | 232 | 196 (69.5%) |
| IPD | 74 | 86 (30.5%) |
| Total | 306 | 282 |

**Table.2** Distribution of uropathogens (Total isolates n= 282)

| S. no | Organism isolated | No. of isolates | ESBL positive** |
|---|---|---|---|
| Gram negative (n=271) | | | |
| 1. | *Escherichia coli* | 198 | 84 |
| 2. | *Klebsiella spp* | 56 | 22 |
| 3. | *Pseudomonas aeruginosa* | 12 | 02 |
| 4. | *Citrobacter spp* | 03 | 01 |
| 5. | *Proteus spp* | 02 | ----- |
| Total isolates | | 271 | 109 |
| Gram positive uropathogenic isolates (n=11) | | | |
| 1. | *Staphylococcus aureus* | 03 | |
| 2. | *Coagulase negative Staphylococcus (CoNS)* | 05 | |
| 3. | *Candida spp* | 02 | |
| 4. | *Enterococcus* | 01 | |
| Total urinary isolates | | 282 | |

** ESBL not tested in Gram positive isolates

**Table.3** Antimicrobial susceptibility pattern of gram negative urinary pathogens (n=271)

| S. no | Antimicrobial | Sensitive (%) | Resistant (%) |
|---|---|---|---|
| 1. | Norfloxacin 10 µg | 20% | 80% |
| 2. | Cefazolin 30µg | 24% | 76% |
| 3. | Cotrimaxazole 25 µg | 32% | 68% |
| 4. | Ciproflaxacin 5µg | 36% | 64% |
| 5. | Tetracycline 30ug | 40% | 60% |
| 6. | Ceftriaxone 30 µg | 54% | 46% |
| 7. | Ceftazidime 30µg | 58% | 42% |
| 8. | Piperacillin-Tazobactam 100/10µg | 64% | 36% |
| 9. | Gentamicin 10 µg | 72% | 28% |
| 10. | Nitrofurantoin 300 µg | 76 % | 24 % |
| 11. | Imipenem 10µg | 88% | 12% |

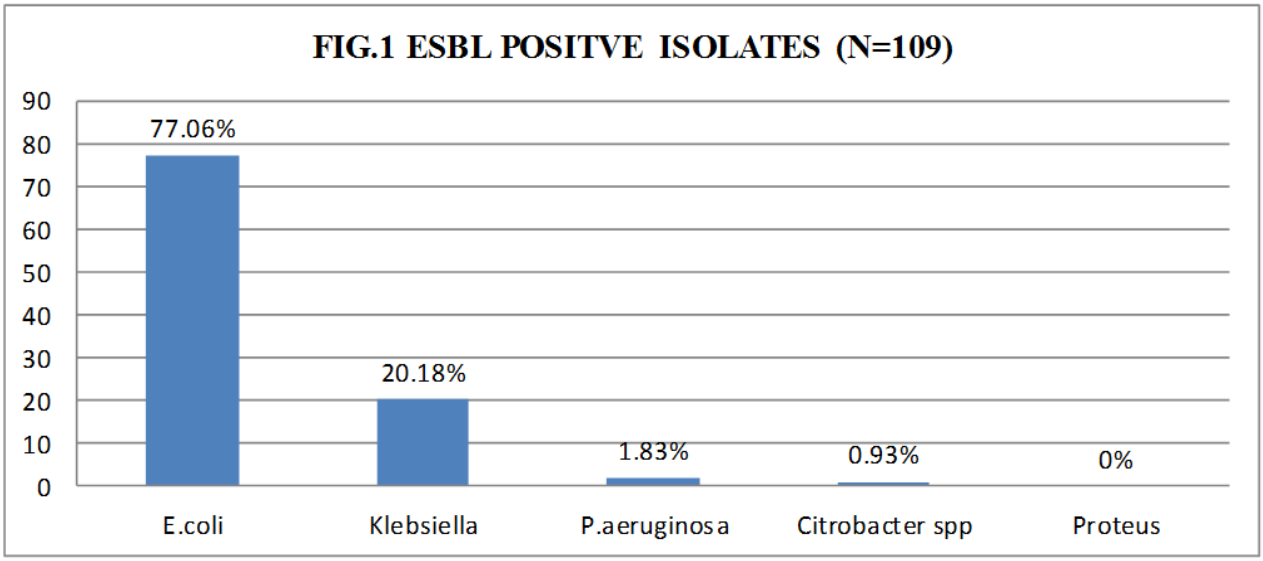
**Table.4** Antimicrobial Sensitivity of ESBL isolates (n=109)

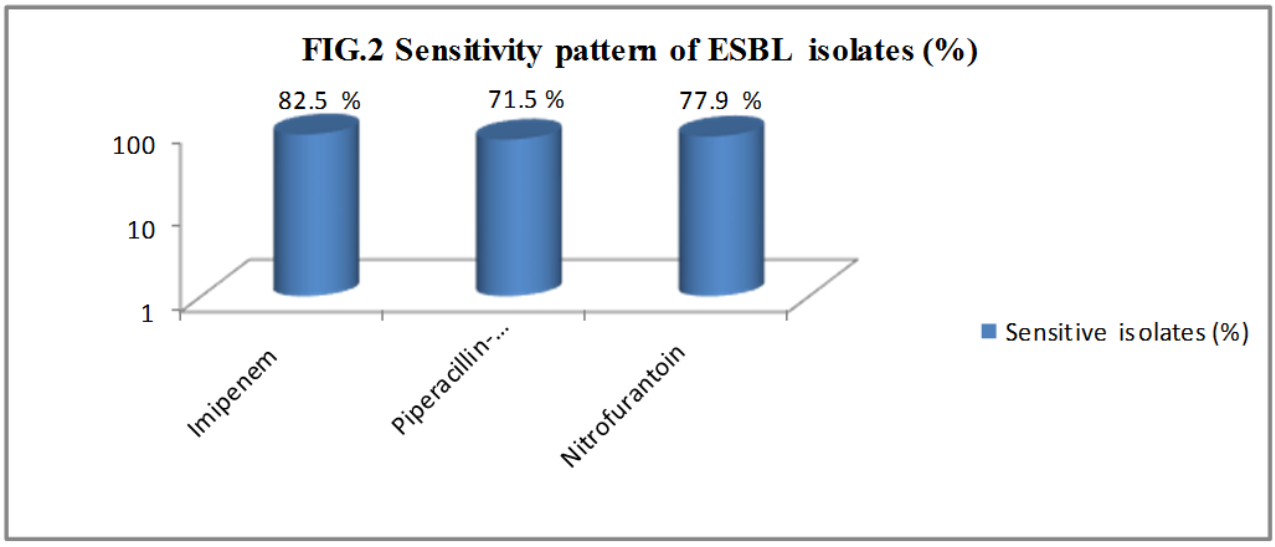
| S. no | Antimicrobial | Sensitive ESBL isolates (%) |
|---|---|---|
| 1. | Imipenem 10µg | 90 (82.5) |
| 2. | Nitrofurantoin 300 µg | 85 (77.9%) |
| 3. | Piperacillin-Tazobactam 100/10µg | 78 (71.5%) |

**Table.5** Comparison of community vs hospital ESBL isolates

| ESBL production | Community acquired UTI | Hospital acquired UTI | Total |
|---|---|---|---|
| ESBL positive | 31 | 78 | 109 |
| ESBL negative | 165 | 08 | 173 |
| Total | 196 | 86 | 282 |

The p value is <0.00001.The result is significant at p <0.05 by Chi square test



The local epidemiology, study population, patterns and protocols of antibiotic usage have a remarkable influence on differences in the ESBL pattern documented in the above mentioned studies.

In the present study, hospital ESBL isolates i.e 71.5% (78/109) were significantly higher than community ESBL isolates 28.5% (31/109). Higher incidences of ESBL uropathogens in hospitalized patients as compared to community settings are reported by Babypadmini *et al.,* (2004) and Babek *et al.,* (2012). Factors predisposing to emergence and transmission of ESBL organisms include the duration of exposure to broad spectrum antibiotics, length of stay in hospital, severity of underlying illness, use of invasive devices such as urinary catheters, or surgery. (Flaherty *et al.,* 1996) It is well known that ESBL producing bacteria cause infections in hospitalized patients but now-days ESBL isolates are reported from community infections also (Nesher *et al.,* 2007). Mahesh *et al.,* (2010) and Taneja *et al.,* (2008) reported higher ESBL isolates around 56.2% and 36.5% from community setting as compared to our study.

ESBL producing organisms causing community UTI can lead to treatment failure or delayed clinical response. Furthermore, these isolates being multidrug resistant, antimicrobials like amino glycosides, quinolones and cotrimoxazole are often ineffective clinically (Auer *et al.,* 2010) Carbapenems are generally considered the drug of choice for the treatment of ESBL infections (Pitout *et al.,* 2008; Rodriguez *et al.,* 2012). Therefore, on basis of above considerations, in the present study we have analyzed sensitivity of ESBL producers for imipenem, nitrofurantion and piperacillin tazobactam which can retain their activity even against ESBL pathogens. In the present study 82.5% (90/109) ESBL producing isolates were sensitive to Imipenem. Also, as per the results in the current study, nitrofurantoin can also be considered as a good treatment option, the resistance being less documented. The findings are in consensus with studies done by Bajpai *et al.,* (2014), Sasirekha *et al.,* (2013), and Khameneh *et al.,* (2009) which have reported nitrofurantion as a suitable agent for the first line treatment of the community acquired UTI.

Piperacillin-tazobactam (PTZ) is one of the most frequently utilized agents for empiric gram negative bacterial coverage and retains activity against ESBL producers.

Furthermore, antimicrobial stewardship practices encourage the use of carbapenem sparing treatment regimens for infections due to ESBL gram negative infections. Results from this study suggest that PTZ is effective in the treatment of urinary tract infection caused by ESBL when the in vitro test indicates susceptibility. Randomized controlled trial by Seo *et al.,* (2017) also confirms effectiveness of PTZ in ESBL producing uroparthogenic *E. coli*.

ESBL uropathogens are isolated from community as well as hospital settings. ESBL producers generally do not respond to the usually prescribed empirical therapy. Presently, alternative antimicrobial therapy to treat ESBL positive UTI on outpatient basis is limited. Imipenem was the most effective drug; however, it should not be administered as empirical drug unless infection is life threatening, as carbapenems are considered the drug of last resort. But alternatives for the treatment of ESBL-producing bacteria are urgently needed to suppress the emergence of carbapenem resistance. Results from this study suggest that Piperacillin-tazobactam, nitrofurantoin, are also effective in the treatment of UTI caused by ESBL pathogens.

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