



## Original Research Article

### Study of Uropathogens in a Tertiary Care Hospital

A.R.Nirmala \*, Saroj Golia, Asha S Kamath B, and Devendra Kumar Tiwari

Dr B R Ambedkar Medical College, Bangalore, India

\*Corresponding author

#### A B S T R A C T

Urinary tract infections (UTI) are the most important infections in human beings. They are the important cause of nosocomial and community acquired infections. This study was done to isolate the various Uropathogens and also to detect Extended Spectrum  $\beta$ -lactamases (ESBL) in gram negative organisms. A total of 940 urine samples were collected and processed. Antimicrobial sensitivity was done by Kirby-Bauer method. ESBL was detected in Gram negative organisms by using Ceftadizime (30 $\mu$ g) and Ceftadizime in combination with Clavulanic acid (30/10 $\mu$ g) discs according to CLSI guidelines. Significant growth was detected in 282 samples out of 940 urine samples. Among them *E.coli* 122(43.26%), the predominant organism, followed by *Enterococci* 43 (15.24%), *Candida* 30 (10.64%), *Coagulase Negative Staphylococci* 28 (9.93%), *Klebsiella* species 22(7.80%), *Pseudomonas* species 14 (4.96%), *Staphylococcus aureus* 10 (3.55%), *Proteus* species 09 (3.19%) *Enterobacter* species 02(0.71%) and *M.morganii* was detected in 02 (0.71%) samples. Among 122 *E.coli* isolates, 43 (35.24%) were ESBL producers and among 22 *Klebsiella* isolates 05 (22.72%) were ESBL producers. In our study *E. coli* was the predominant uropathogen and in gram negative organisms ESBL (33.33%) producers are also more. *Enterococci* with High level Aminoglycoside Resistant (39.53%) and *Candida* isolates (10.64%) are also more in our study. So it is essential to perform the culture and antibiogram before the start of antibiotics to prevent the emergence of drug resistance strains.

#### Keywords

Urinary tract infections, ESBL, *E.coli*

#### Introduction

Urinary tract infection is defined as a disease caused by microbial invasion of the urinary tract which extends from the renal cortex to the urethral meatus. UTI is relatively more common in females as compared to males due to short urethra and lies in close proximity to perirectal region (Bailey and Scott's, 12<sup>th</sup> edition).

Risk factors for UTI are urinary obstruction due to prostatic enlargement, calculi, pregnancy, diabetes, renal disease, renal transplantation, structural or neurological abnormalities. Infections of the lower urinary tract are usually ascending infections caused by fecal coliforms and upper urinary tract infections-pyelonephritis is due to

hematogenous infections (Ananthanarayan, 9<sup>th</sup> edition)

Nearly 10% of humans will have a UTI at some time in their lives. UTI contributes 35% of nosocomial infections. (Bailey and Scott's, 12<sup>th</sup> edition) *Escherichia coli* is an important uropathogen.

*E. coli* adhere to the umbrella cells of bladder epithelium and also invade and multiply in the epithelium. (Wagenlehner, 2005). *E.coli* multiplication causes inflammation, increased bacterial survival and invasion of the deeper layers of the urothelium. So, these urothelial cells become reservoirs in which pathogens persist in a quiescent state, act as reservoirs and contribute to recurrent UTI (Wagenlehner, 2005).

UTI are usually treated empirically, since antibiotic resistant strains are emerging, it is essential to do the culture and antibiogram for the proper diagnosis and treatment of the cases and also to reduce the morbidity and serious complications. *E.coli* is the predominant isolate both in community acquired and nosocomial UTI, followed by *Klebsiella* species, *Proteus*, *Staphylococci*, *Enterococci*, *Pseudomonas* and *Candida* species. (Mackie, 14<sup>th</sup> edition).

Since  $\beta$ -lactam antibiotics are still widely used, emergence of  $\beta$ -lactamase producers has become a matter of serious concern. The various mechanisms of drug resistance in gram-negative bacilli include production of  $\beta$ -lactamases (Jarlier et al., 1988), Amp C lactamases (Phillippon et al., 2002), ef-flux mechanisms (Fukuda and Hiramatsu, 1997) and porin deficiency (Ananthan and Subha, 2005). ESBL producers may exhibit more than one such resistance mechanism, further complicating the situation. (K.Aruna, 2012).

Emergence of drug resistance in uropathogens is an important public health problem especially in developing countries. The production of ESBLs by the isolates lower down the options for treatment since ESBL production is associated with coresistance to other classes of antimicrobial agents like fluoroquinolones, cotrimoxazole, tetracyclines, and aminoglycosides. (Varsha Gupta, 2013)

This study was aimed to isolate and identify the various Uropathogens and to detect the ESBL production in Gram negative organisms.

## Materials and Methods

This study was done in a tertiary care hospital over a period of 6 months from January 2014 to June 2014. 940 urine samples were collected from both male and female patients including children and culture was done by semiquantitative method by inoculating 0.001ml of urine by using calibrated loop on Cysteine Lactose Electrolyte Deficient (CLED) agar. The plates were incubated at 37°C overnight. Cultures were examined for growth and colony count was done. A colony count of 100000 CFU/ml (Kass, 1957) of urine is considered as significant bacteriuria and the isolates were identified by standard biochemical tests. (Koneman, 6<sup>th</sup> edition)

Antibiotic sensitivity testing was done on Mueller Hinton Agar according to (CLSI guidelines) by Kirby-Bauer Disc Diffusion Method. The gram negative isolates were tested for their susceptibility to the third generation cephalosporins e.g. ceftazidime (30  $\mu$ g), cefotaxime (30  $\mu$ g) and ceftriaxone (30  $\mu$ g) by using the standard disc diffusion method, according to CLSI guidelines.

If a zone diameter of < 22 mm for ceftazidime, < 27 mm for cefotaxime and < 24mm for ceftriaxone, the strain was considered as “suspicious for ESBL production” . Only those isolates which were resistant to one of the above 3 drugs were selected and processed for the ESBL production.

Confirmation of ESBL production was done by using the Phenotypic confirmatory disc diffusion test ( PCDDT) according to CLSI guidelines. . In this test, the test strain was inoculated on Mueller Hinton Agar and ceftazidime (30 µg) discs alone and in combination with clavulanic acid (ceftazidime + Clavulanic acid ,30/10 µg) discs were applied.A 5mm increase in the inhibition zone diameter of the Ceftazidime and in combination with Ceftazidime+ Clavulanic acid was considered as ESBL production (CLSI guidelines).

In *Enterococci* High Level Gentamicin Resistance (HLGR) was detected by using 120 µg high content Gentamicin discs. In *Staphylococci* Methicillin resistance was detected by using Cefoxitin 30 µg discs. In *Candida* isolates , germ tube test was done to identify the *Candida albicans*.

## Results and Discussion

Of the 940 samples, significant growth was detected in 282 samples. Among 282 isolates, *E.coli* in 122 (43.26%) samples, *Enterococci* in 43 (15.24%), *Candida* in 30 (10.64%), *Coagulase Negative Staphalococci* in 28 (9.93%), *Klebsiella* species in 22 (7.80%), *Pseudomonas* species in 14 (4.96%), *Proteus* species in 9 samples (3.19%), *Staphylococci aureus* in 10 (3.55%), *Enterobacter species* in 2 (0.71%) and *Morganella morganii* in 2 (0.71%) samples.

Among 122 *E.coli* isolates, 43 isolates (35.24%) showed ESBL production . Among 22 *Klebsiella* species, ESBL was detected in 5 (22.72%) of the isolates.

Of the 43 *Enterococci* isolates ,HLGR was detected in 17 (39.53%) isolates .In *Coagulase negative Staphylococci* , Methicillin resistance was detected in 09 (32.14%) of the isolates.

Among *Staphylococci* isolates , Methicillin Resistant Staphylococci was detected in 03(30%) of the isolates.

Around 80% of the UTI is caused by gram negative organisms like *E.coli*, and other Enterobacteriaceae and the rest is by gram positive organisms and candida species. Fungal infections are now increasing due to emergence of HIV and AIDS.

*E. coli* is the most common isolates in the clinical laboratories and also one of the most common organism involved in gram negative sepsis and endotoxin induced shock. (Koneman,6<sup>th</sup> edition)

In this study *E.coli* was the predominant organism and isolated 122 *E.coli*, (44.20%), it is comparable to the study done by Mallikarjuna Reddy et al., 2014. and Dnyaneshwari Purushottam Ghadage, 2014.

*E. coli* to be one of the most frequently encountered drug-resistant uropathogen.(Gupta et al., 1999, 2007; Tambekar et al.,2006);It is also true in this study. Extended spectrum β-lactamase (ESBL) are β –lactamases which are capable of conferring bacterial resistance to the Penicillins, first, second, and third- generation cephalosporins, and Aztreonam (but not the Cephamycins or Carbapenems) by hydrolysis of these antibiotics, and which are inhibited by β-lactamase inhibitors such as clavulanic

acid. ESBLs belong to group 2be of Bush's functional classification. (Bradford PA, 2001)

In India, the prevalence of ESBL has been reported from 1990. Among 122 *E.coli* isolates, 43 (35.24%) were ESBL producers. It is in concordance with the study done by Aruna et.al 2012. Among 22 *Klebsiella* isolates 05 (22.72%) were ESBL producers.

The production of  $\beta$ -lactamase may be of chromosomal or plasmid origin 21. Plasmid mediated production is often acquired by transfer of genetic information from one organism to another. Such transferable plasmid also codes for resistant determinants to other antimicrobial agents. Hence multidrug resistance is expected to be more common in ESBL producing organisms. (Supriya S, 2004). In this study ESBL producing organisms are more resistance to routinely used antibiotics compared to non-ESBL isolates. It is in concordance with the study done by B.Sasirekha,2013. It is essential to do the continuous monitoring and good hospital infection measures should be initiated to prevent the emergence of drug resistance strains.

ESBL producers are sensitive to Imipenem and Amikacin. Similar results are shown in studies done by K. Aruna, 2012. Among gram positive organisms, *Enterococcus spp* 43 (4.57%) were predominant isolates. It is comparable to the study done by Dnyaneshwari Purushottam Ghadage et al , 2014. Among 43 *Enterococci* isolates, 17 isolates (39.53%) showed HLGR. Serious infections due to *Enterococci* are usually treated with combination of penicillins and aminoglycosides. *Enterococci* are intrinsically resistant to cephalosporins and also produce low level resistance to aminoglycosides. In penicillin sensitive strains , synergism occurs with combination of treatment with penicillin and aminoglycosides. But if the strain develops

high level aminoglycoside resistance , this synergism does not occur and complicating the treatment (Ananthanarayan, 9<sup>th</sup> edition)

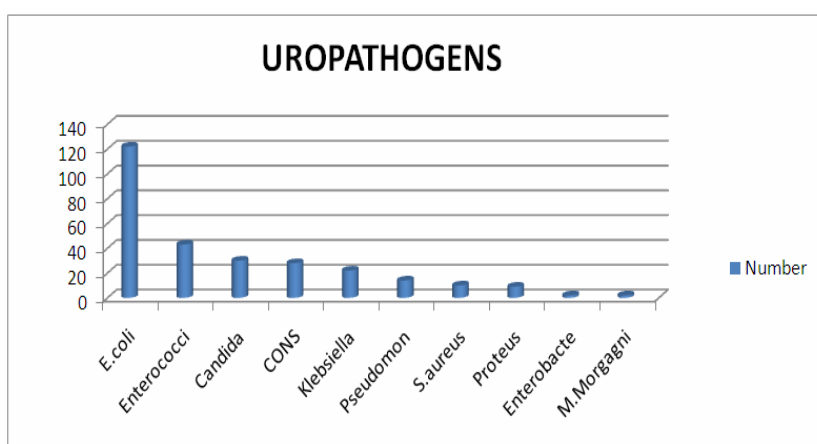
In this study isolated more *Candida* species, it may be due to samples are from a tertiary care centre who often have multiple predisposing factors, including diabetes mellitus, indwelling urinary catheters, ICU patients who may be critically ill, and exposure to broad spectrum antibiotics.

Since 1980s there has been a marked increase in opportunistic fungal infections involving the urinary tract, of which *Candida* species are the most prevalent, candiduria represents colonization or contamination of the specimen cultured rather than infection and most patients are asymptomatic. Rarely antifungal therapy required. However, occasionally the presence of yeast in the urine is a sign of a disseminated infection or candidemia and may serve as a marker for increased mortality, especially in critically ill surgical patients with comorbidities. In contrast to that which occurs in adults in the medical and surgical ICU settings, candiduria in the critically ill newborn very often reflects candidemia or disseminated candidiasis and in addition may be accompanied by obstructing, urinary tract fungus ball formation. (Jack D, Oxford journal). MRSA has been associated with higher hospital cost and possesses a greater treatment challenge to physicians. MRSA strains generally are now resistant to other antimicrobial classes including aminoglycosides, beta-lactams, carbapenems, cephalosporins, fluoroquinolones and macrolides. Most of the resistance was secondary to production of beta -lactamase enzymes or intrinsic resistance with alterations in penicillin - binding proteins. The current prevalence for MRSA in hospitals and other facilities ranges from <10% to 65%.

**Table.1** Shows the various uropathogens isolated and their percentage.

Organisms isolated	Number	Percentage(%)
Escherichia coli	122	43.26
Enterococci	43	15.24
Candida species	30	10.64
Coagulase negative staphylococci	28	9.93
Klebsiella species	22	7.80
Pseudomonas species	14	4.96
Staphylococcus aureus	10	3.55
Proteus species	09	3.19
Enterobacter species	02	0.71
Morganella morganii	02	0.71
Total	282	

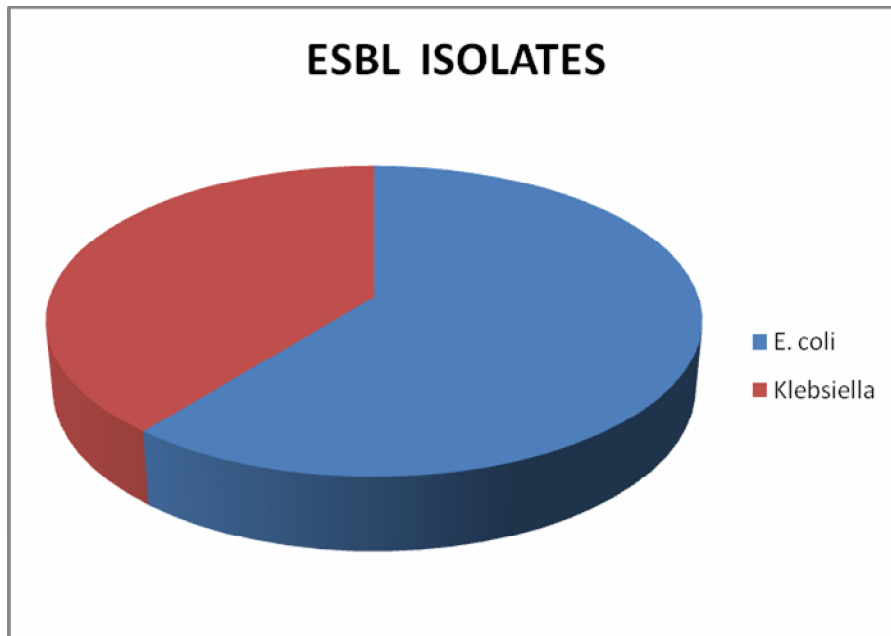
**Chart.1** Shows the various uropathogens



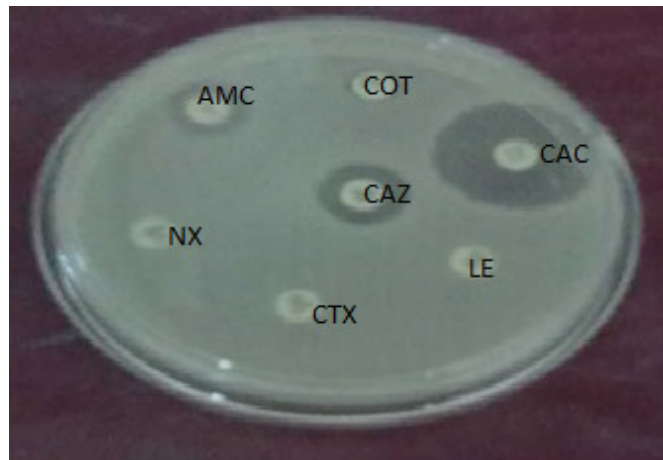
**Table.2** Shows ESBL isolates among Gram negative organisms with percentage

<i>E.coli</i> (n=122)	ESBL- 43	35.24%
<i>Klebsiella</i> (n=22) Species	ESBL-05	22.72%

**Chart.2** Shows the ESBL isolates among Gram negative organisms.



**Figure.1** Photograph showing multidrug resistant ESBL strain.

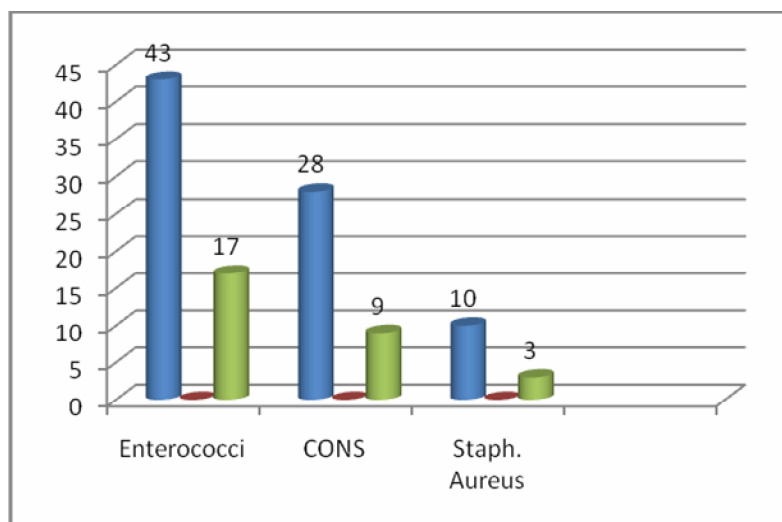


CAZ-Ceftazidime, CAC- Ceftazidime+ Clavulanic acid, N-Norfloxacin, COT-Co-trimoxazole, AMC- Amoxicillin/clavulanic acid, LE-Levofloxacin, CTX-Cefotaxime

**Table.3** Shows the various Gram positive organisms with antibiotic resistance.

Organisms	Total isolates	Resistance	Percentage(%)
Enterococci	43	HLGR-17	39.53
CONS	28	MR-CONS-09	32.14
Staphylococcus aureus	10	MRSA-03	30.00

**Chart.3** Shows various Gram positives organisms with resistance



In this study among the isolated Uropathogens, ESBL producers are high and multidrug resistance is also more in ESBL producers than in non ESBL producers. So it is essential to perform the culture and antibiogram with routine ESBL testing for proper management of the cases.

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