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Isolation and Identification of Uropathogenic *E. coli* and its Antimicrobial Susceptibility Pattern with Special Reference to Extended Spectrum Beta-Lactamases (Esbl)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Urinary tract infections (UTI) are the most common bacterial infection among the humans. One of the most important factors impacting the management of UTI over the past decade is emergence of anti-microbial resistance among uropathogens. ESBL production is one of the most common mechanisms of anti-microbial resistance, the other being Amp C β-lactamases in gram negative bacteria. This study was undertaken to establish the prevalence of ESBL producing strains and their anti-microbial susceptibility pattern to newer agents to guide therapy for urinary tract infection. The present studies isolates and identify Uropathogenic Escherichia coli and its antibiotic susceptibility pattern with special reference to ESBL. The emergence of β-lactamase is a Metter of serious concern .The drug resistance in gram negative bacilli is due to production of β-lactamases, AmpC lactamases, Efflux mechanisms and Porin deficiency. Out of the total 3580 urine samples, 987 samples (27.56%) showed No growth, 1786 (49.88%) showed the presence of Gram negative bacteria. Totally, 1081 *E. coli* species were isolated and < 30% of them were found to be ESBL positive. Uropathogenic *E. coli* isolates are highly susceptible towards Meropenem and Imipenem

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antibiotics and highly resistant towards β-lactam and Cephalosporins antibiotics. Hence, the present study urged to implement the management plan for using those antibiotics in patients for preventing the antibiotic resistance.

Keywords: Urinary tract infections; β-lactamases; Amp C β-lactamases; uropathogens; ESBL.

1. INTRODUCTION

Urinary tract infection (UTI) is defined as the microbial colonization, inflammation and infection of the genitourinary tract. It is a bacterial infection that affects any part of the urinary tract. UTI is the most prevalent infections worldwide with a high global burden [1] .UTI affects 150 million people each year and is responsible for around seven million hospital visits per year [2,3] Gram negative bacteria like *Escherichia coli, Enterobacter species Proteus species, Klebsiella species and* Gram positive bacteria *Staphylococcus saprophyticus and enterococcus* are predominant causative organisms. Epidemiologically, 12% men and 10-20% women experience an acute symptomatic UTI and even greater numbers develop asymptomatic bacteriuria [4,5] without any presenting symptoms. The common ascending urinary tract infection is caused by human faecal coliforms and may cause Urethritis, cystitis and Prostatitis. Upper UTI involves the Kidney (Pyelitis & Pyelonephritis). Pyelonephritis is through hematogenous spread of infection. Incidence of UTI is more in females as compared to males due to shorter urethra and proximity to anus. In elderly males,UTI due to enlarged prostate is very common. Other cause of UTI includes pregnancy, prostatic hypertrophy, reflux of urine from bladder up to the ureters and into renal pelvis, neurogenic bladder dysfunction, multiple sclerosis etc [6].

E. coli cause about 90% of first episode of UTI in children [7]. Uropathogenic Esherichia coli (UPEC) have several virulence factors that enables it to colonize bladder mucosa and injure it causing inflammation and subsequent infection. Extended spectrum β-Lactamase (ESBL) producing bacilli are the chief pathogens that have become endemic in many hospital and health care settings. The prevalence of ESBL widely depends upon the clinical setting and epidemiological pattern. The increasing incidence of ESBL producing organisms are mainly seen in old age population, patients who are severely ill and urinary tract infections, patients with indwelling urinary catheter, malignancy, patients who have a long stay in hospital wards and intensive care units (ICU), patients who are functionally dependent. Treatment of ESBL producing organisms increases the mortality and morbidity. In the current scenario, drug resistant pathogens carry higher morbidity and mortality and also they are difficult to identify by routine laboratory methods and hence the diagnosis is delayed and finally there is delay in administration of appropriate anti-microbial therapy. The major concern is the lack of new antibiotics for multi-drug resistant strains of uropathogenic *E. coli* that produces ESBL.

ESBL‟s are capable of hydrolyzing oxyimino cephalosporins. They are chromosomal or plasmid mediated, mutated from the exisiting broad spectrum β lactamases (TEM-1,TEM-2,SHV-1) due to widespread use of 3rd generation Cephalosporins as well as Aztreonam [8,9]. ESBL‟s are inhibited by β lactamase inhibitors such as Clavulanic acid or Sulbactam. Many resistant ESBL producers are encountered, by virtue of Amp-C β-Lactamase excess production or loss of porin [10]. Production of ESBL enzymes confers multiple drug resistance, hence making infections difficult to treat and increasing the mortality and morbidity among the patients. Patients admitted to hospitals are more likely to serve as reservoirs for these resistant organisms and eventually, the patients in the community acquire ESBL-producing strains [11]. A variety of drugs [12]. ESBL producingorganisms are resistant to all drugs except Carbapenams and Cephamycins. This present study is doneto study the prevalence of Uropathogenic *E. coli* among various urine samples isolated in the central lab of Sree Balaji Medical College and Hospital and its antibiotic susceptibility pattern with special reference to ESBL.

2. MATERIALS AND METHODS

The following microbiological media were used for the study. Cysteine lactose electrolyte deficient agar (CLED), MacConkey agar, Blood agar plate, 0.5 Mc Farland‟s standard medium and Mueller-Hinton agar (MHA). The bacteria were identified using biochemical parameters

such as indole, citrate, methyl red (MR) test, voges-proskauer (VP) test, citrate utilization test, triple sugar iron test, urease test, sugar fermentation test (glucose, lactose, sucrose, maltose, mannitol, arabinose), nitrate reduction test, tube and slide catalase and oxidase test. The following antibiotics were used for the study (Table 1).

The patients of all age groups presenting with complaints of Fever, increased frequency of urination, urgency, dysuria, lower abdominal and flank pain and supra pubic tenderness that are suggestive of lower or upper urinary tract infection were considered and included in this study. The Inclusion criteria were Fresh case of urinary tract infection, No history of antibiotic intake, No history of instrumentation, No immunosuppressive illness, Non pregnant women, No history of recent delivery and No history of liver or renal dysfunction. The exclusion criteria were Patient with urinary tract surgery, Patient on antibiotics in last 48 hours, History of trauma involving urinary tract and Patient of HIV or any other immunological disorder.

Urine sample was examined macroscopically for presence of hematuria and turbidity After centrifugation, urine sample was examined under high power objective (40X) for presence of pus cells and bacteria. A drop of well mixed centrifuged urine was placed over the microscope slide, air dried, heat fixed and gram staining was done and examined under oil immersion. Presence of > 1 -5 bacteria/field was taken as significant bacteriuria and >105CFU/ml, presence of pus cell taken as definite indication of urinary tract infection 35.Uncentrifuged urine was mixed well by gently rotating the container by keeping it over the table. Using a calibrated loop 0.01 ml of urine sample was inoculated in the following media by standard loop technique. The plates were incubated at 37° C overnight in an incubator. Cultures grown on the media was counted using hand lens. The number of colonies was multiplied by 1000 to determine the number of microorganisms/ml in the original specimen. The organism thus isolated was subjected to characterizing using with gram staining, motility and standard biochemical reactions such as Catalase test, Indole test,Methyl Red test,Voges Proskeur test, Citrate utilization test, Urease test, Nitrate reduction, oxidase test,triple sugar iron test(TSI), Carbohydrate fermentation tests.

3. RESULTS AND DISCUSSION

The study was carried out in the Central laboratory Department of Microbiology Sree Balaji Medical College and Hospital, Chrompet, Chennai for a period of one year from August 2017 to August 2018. A total of 3580 urine samples were received in Central lab during the study period and the samples were analyzed for the prevalence of Escherichia coli and its antimicrobial susceptibility pattern.

Out of a total of 3580 urine samples received, majority of the samples were from inpatient departments (2800) while 780 samples were from outpatient.

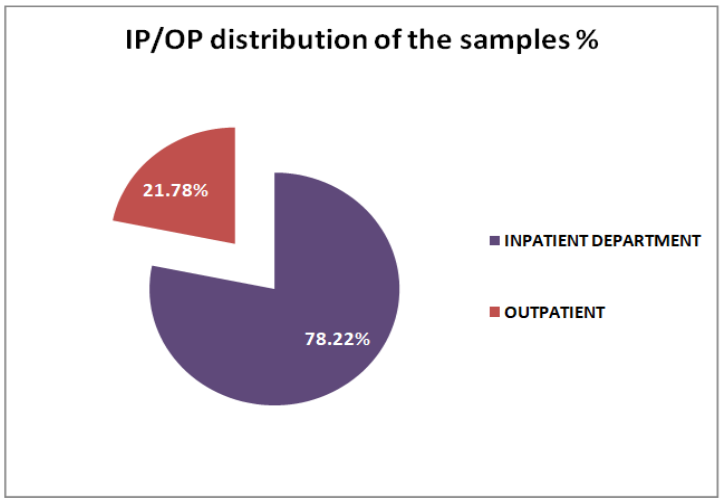

Fig. 1. IP/OP distribution of the samples %

Table 1. Antibiotics used

Antibiotic	Concentration
Ampicillin	10 mcg
Amoxicillin Clavulanic acid	20/10 mcg
Amikacin	30 mcg
Cefazolin	30 mcg
Ceftazidime	30 mcg
Cefotaxime	30 mcg
Ciprofloxacin	05 mcg
Cotrimoxazole	1.25/23.75 mcg
Gentamycin	10 mcg
Imipenem	10 mcg
Meropenem	10 mcg
Piperacillin-Tazobactum	100/10 mcg
Nitrofurantoin	300 mcg

Out of the total 3580 urine samples received in the Central lab, 987 samples (27.56%) showed No growth, 1786 (49.88%) showed growth of Gram negative bacilli and 807 (22.54%) showed Gram positive cocci.

A total of 1081 *E. coli* was isolated from the urine samples of both inpatient and outpatient. In the current study out of 1081 *E. coli* isolated, 360 (33.28%) were from Department of Medicine, 190 (17.61%) isolated from Department of Obstretics & Gynaecology, 190 *E. coli* (17.33%) isolated from Department of Paediatrics, 119 (11.09%) from Department of Surgery, 116 (10.81 %) from Department of Urology and 106 (9.84%) isolated from Intensive care unit of the Hospital. 150 random Escherichia coli samples isolated from urine were tested further for ESBL and antibiotic sensitivity pattern.

The study mainly focuses on the prevalence of ESBL among Escherichia coli isolated from urine samples with their anti microbial susceptibility pattern. All the isolates in the study was screened for ESBL production according to the CLSI guidelines described criteria. Urinary tract infection (UTI) are the most common bacterial infection among the humans. One of the most important factors impacting the management of UTI over the past decade is emergence of anti microbial resistance among uropathogens 48. ESBL production is one of the most common mechanism of anti microbial resistance, the other being Amp C β-lactamases in gram negative bacteria. This study was undertaken to establish the prevalence of ESBL producing strains and their anti microbial susceptibility pattern to newer agents to guide therapy for urinary tract infection.

In the present study 150 Mid stream urine samples were collected for identification and isolation of Escherichia coli.The clean –catch mid stream urine collection is primarily aimed at avoiding contamination of voided urine by urethral and perineal flora, which might confuse the interpretation of culture results. The normal urethral flora consists of primarily diphtheroids, streptococci and staphylococci 24. In contrast,50 concluded that in ambulant adult perineal cleansing before voided urine sample is taken does not influence the bacteriological profile. In a study, 51 on pregnant women, demonstrated that vulval cleansing did not decrease the contamination rate of mid stream urine specimen. It is also probable that in some instance, the use of disinfectant and antiseptics in the cleaning procedure might alter and decrease the true bacterial count.

3.1 Age and Sex wise Prevalence of UTI

Various studies noted that uropathogenic *E. coli* is the predominant organism in females than males .In the present study too, out of 150 *E. coli* isolated, 30% were males and 70 % were females with the male to female ratio of 1:2.3.Similar findings was recorded by 52 in Tamil nadu. The higher incidence of urinary tract infection in females is due to unique anatomical features of the female genitourinary tract, which includes a shorter urethra and the more proximal location of the urethral meatus to the anus makes it easy for bacteria to ascend to the urinary tract 26.Fecal-perineal-urethral contamination is the most probable explanation for infections caused by *E. coli* strains causing UTIs in women [5,10]. About 50% of women will experience at least one UTI in their lifetime, and of those 25% will suffer from one or more recurrent or relapsing infections54. UPEC isolates can also be transmitted via sexual activities.

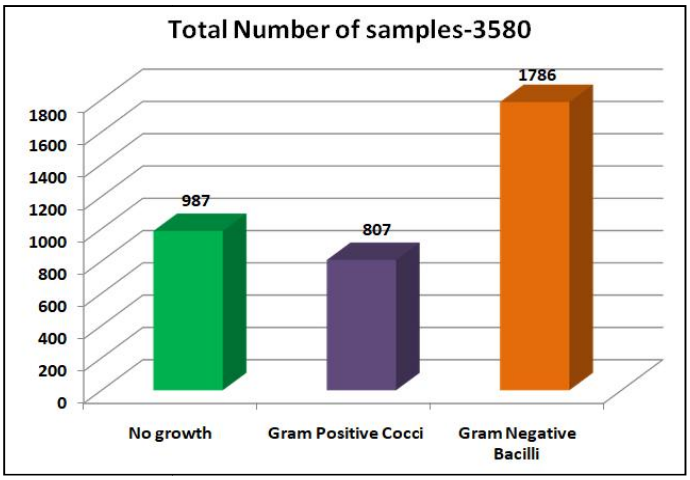

Fig. 2. Total number of samples-3580

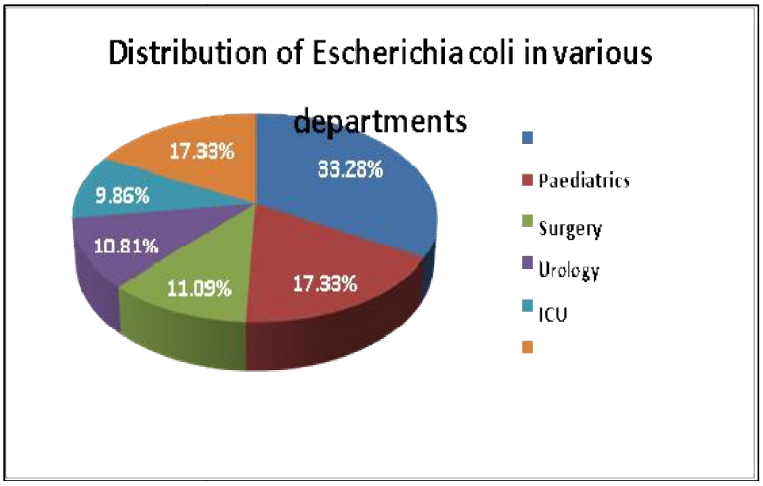

Fig. 3. Distribution of Escherichia coli in various

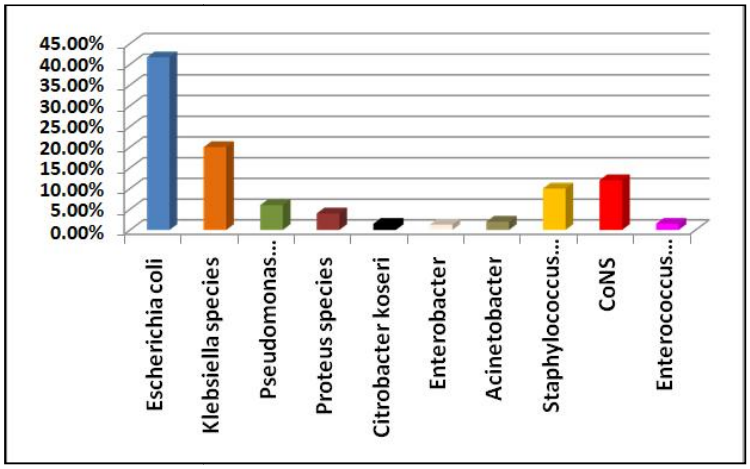

Fig. 4. Distribution of pathogens in UTI

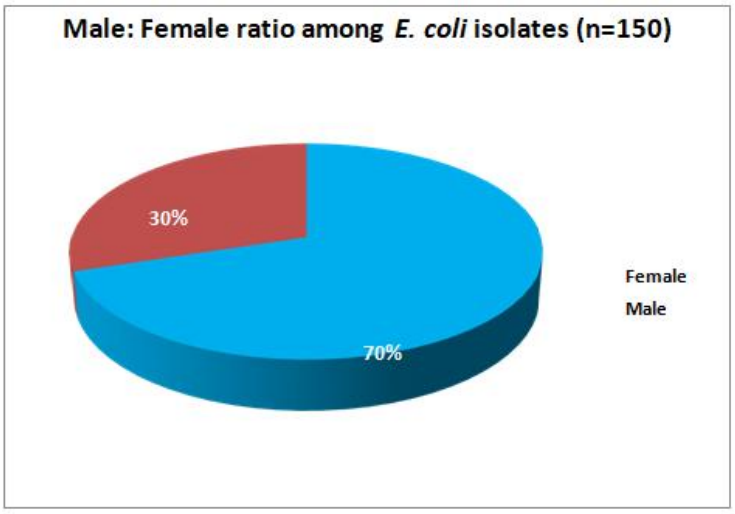

Fig. 5. Male: Female ratio among *E. coli* isolates (n=150)

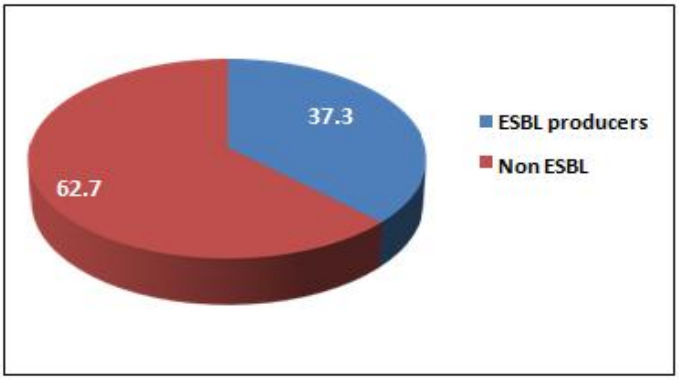

Fig. 6. ESBL Vs Non ESBL producers among theisolates

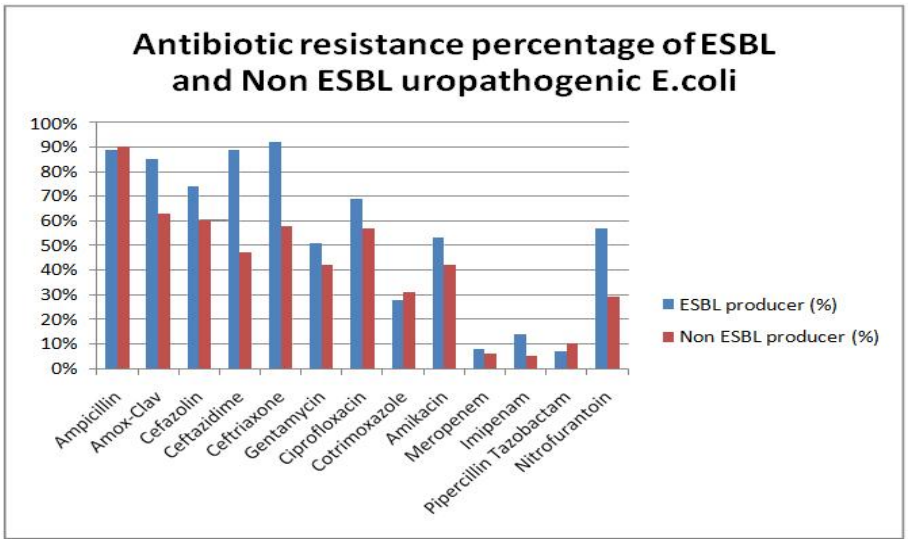

Fig. 7. Antibiotic resistance percentage of ESBL and Non ESBL uropathogenic *E. coli*

Fig. 8. *E. coli* colonies on Blood agar plate

Fig. 9. *E. coli* colonies on MacConkey agar plate

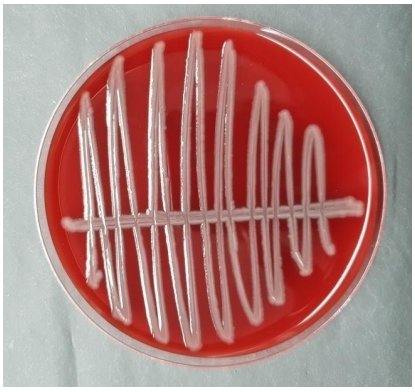

Fig. 10. Semi Quantitative urine culture on BAP and MacConkey agar

Fig. 11. Biochemical Reactions

Indole-Positive	**Citrate - Not Utilised**
Urease-Not Hydrolysed	**TSI- Acid/Acid**
Manitol Motility-Motile, Fermented	

Fig. 12. Sugar fermentation test

Fig. 13. Decarboxylation test

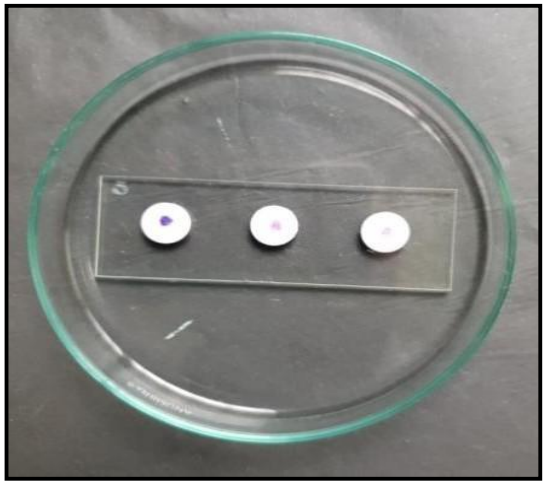

Fig. 14. Oxidase test negative by *E. coli*

A- Positive Control, Pseudomonas aeruginosa, ATCC 27853
B- Test Negative, *E. coli*
C-Negative control, *E. coli* , ATCC 25922

Fig 15. Antibiotic Sensitivity Pattern

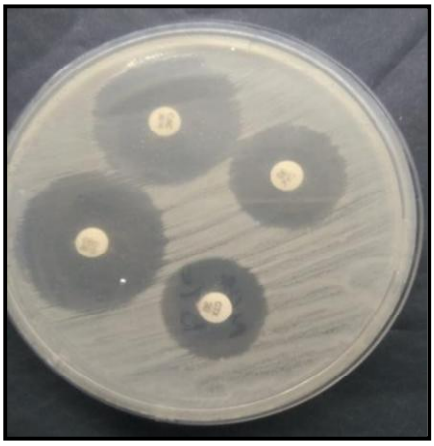

Fig. 16. ESBL detection by phenotypic confirmatory disc diffusion method

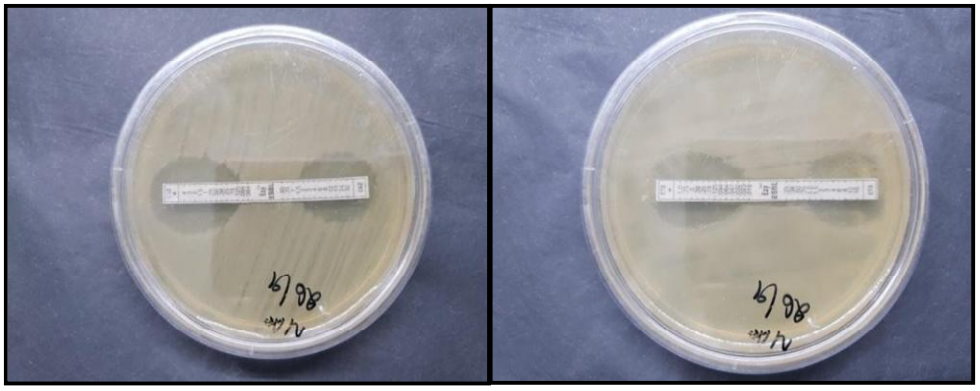

Fig. 17. ESBL detection by E strip method

According to Foxman et al 26, USA the ratio was 1:4.2. In every age group there was a higher incidence of UTI in females than male. In the present study, more number of UTI was found in reproductive age group between 21 years to 40 years (35%) followed by middle age group of 41 -60 years (29%) and then the elders accounts for around 24%. This result is comparable to a study by Kiffer et al, they found that the higher percentage of *E. coli* isolates in people of age group 13-60 years and lower percentage in people younger 13 years or older than 60 years 55. Another study found that the lowest percentage of *E. coli* was among age group 10

years and high within the age group 26 -36 years. In this study, the prevalence of UTIs in young less than 20 years of age was 17% which correlates with different studies conducted worldwide. In the present Study, only 1.3% of Enterobacter was isolated, but the study conducted 56 showed prevalence of 14% and Tamil Nadu showed 35%.

Worldwide, *E. coli* was the predominant pathogen isolated from patients with community acquired UTI. The current study shows a prevalence of 41.7 % of *E. coli* is olated from urine samples of patients suffering from urinary tract infections. This result is in agreement with different studies conducted worldwide. Mohanthy et al 2005 58 documented a prevalence rate of 46% *E. coli* in New Delhi among UTI patients while a study conducted by Baby Padmini and Appalaraju in Chennai in 2004 showed a prevalence similar to our present study (49.3%) [9].

3.2 Antibiotic Susceptibility Pattern

Antimicrobial resistance pattern in uropathogenic *E. coli* is of major concern worldwide due to its ever increasing resistance to several commonly prescribed antibiotics [13]. In our study, *E. coli* isolates were various in their susceptibility to different antibiotics belonging to different groups. 90% isolates were resistance to Ampicillin and 80% resistant to Amoxicillin clavulanic acid derivative.It is also revealed that 70 % of ESBL isolates were resistant to Ciprofloxacin, one of the most commonly used fluoroquinolone drug which might be indicative of fluoroquinolones being prescribed at a higher frequency. Although fluoroquinolones should not be prescribed routinely for UTIs such a high degree of resistance clearly shows the misuse of antibiotics by healthcare professionals.

An individual is at a significantly higher risk of being infected by ESBL-producing uropathogens if he/she is exposed to antibiotics for a long period of time, suffers from any severe illness, undergoes instrumentation or catheterization procedures.Under such circumstances, fluoroquinolones become the drug of choice. However, the study shows a higher resistance pattern among the quinolones and it should be used when bacterial load is extremely high [14].

In the outpatient setting, oral antibiotics are preferred for administration but we are left with very limited options of oral drugs for treatment of UTI except to some extent Cotrimoxazole and Nitrofurantoin, which shows good sensitivity pattern .Surprisingly, Cotrimoxazole showed good sensitivity in ESBL positive cases with 72 % sensitive cases. Whereas, 92% isolates were susceptible /sensitive to Meropenam, 90% isolates were sensitive to both Imipenam and Piperacillin Tazobatam antibiotics.
This result was comparable to different studies around the world. This high resistance may be due to spontaneous and uncontrollable use of these antibiotics. The carbapenems (Meropenem and Imipenem) are known to be stable ag ainst ESBL enzymes and effective in the treatment of infections caused by ESBL-producing bacteria 86.90% of ESBL producing *E. coli* strains were resistant to all four generations of Cepahlosporins. *E. coli* and other genera of gram negative bacteria possess a naturally occurring, chromosomally mediated β-lactamase and plasmid mediated β-lactamase [15,16-26]. The plasmid mediated resistance has spread all over the world and into many different members of the Enterobacteriaceae family. In our study, *E. coli* isolates susceptibility pattern against the β-lactamase inhibitor was found to be in agreement with the results obtained by Kumar et al 88.The efficacy of β- lactam-β-lactamase inhibitor combination may be reduced for organisms producing multiple ESBLs.

4. CONCLUSION

The incidence and causative uropathogenic *E. coli* are comparable with reports and studies from around the world. Females are more susceptible to UTI than males. The reproductive age group of 21 to 40 years showed higher percentage of *E. Coli*. Additionally educational programs and improving the hygienic measures are necessary to prevent contamination and cross infection with *E. coli* and minimize the use of β-lactam and Cephalosporin antibiotics in order to minimize the emergence of ESBLs producing bacteria.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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