DESIGN AND ANALYSIS OF COMPARATIVE SUSCEPTIBILITY OF UROPATHOGENIC ESCHERICHIA COLI AGAINST DIFFERENT ANTIMICROBIAL AGENTS

MUNENDRA PAL, ALOK KUMAR SRIVASTAV
Department of Microbiology, Dr. A.P.J. Abdul Kalam University, Indore, Madhya Pradesh, India.

[For Correspondence: aloksrivastav88@gmail.com]

ABSTRACT
Urinary tract infections (UTIs) are one of the most common pathological conditions in both community and hospital settings. It has been estimated that about 150 million people worldwide develop UTI each year, with high social costs in terms of hospitalizations and medical expenses. Among the common uropathogens associated to UTIs development, UroPathogenic Escherichia coli (UPEC) is the primary cause. UPEC strains possess a plethora of both structural (as fimbriae, pili, curli, flagella) and secreted (toxins, iron-acquisition systems) virulence factors that contribute to their capacity to cause disease, although the ability to adhere to host epithelial cells in the urinary tract represents the most important determinant of pathogenicity. On the opposite side, the bladder epithelium shows a multifaceted array of host defenses including the urine flow and the secretion of antimicrobial substances, which represent useful tools to counteract bacterial infections. The fascinating and intricate dynamics between these players determine a complex interaction system that needs to be revealed. This article will focus on the most relevant components of UPEC arsenal of pathogenicity together with the major host responses to infection, the current approved treatment and the emergence of resistant UPEC strains, the vaccine strategies, the natural antimicrobial compounds along with innovative anti-adhesive and prophylactic approaches to prevent UTIs.

Keywords: Antimicrobial Agents, Urinary Tract Infection, Susceptibility, Uropathogens, Escherichia coli community-acquired.

INTRODUCTION
Microorganisms play an important role in human body. They have mutual relationship with the host. They are either beneficial or pathogenic to the host. Among them, bacteria plays major role in causing a wide variety of infections in humans. Urinary tract infections (UTIs) are the most common bacterial infections affecting approximately 150 million people worldwide who need medical care, whereas in hospitals, they are the most common nosocomial infections accounting for about 30%-40%.
Figure 1: The Urinary Tract and Sites of Infection

About 10% of humans develop UTI in childhood. During the first year of life, the prevalence of UTI is around 2% in both females and males. After that, it is reduced in males and increased in females. UTI is predominantly a disease of females in reproductive age group. About 40-50% of women in the reproductive age group have had history of at least a single episode of UTI in their lifetime. Predisposing factors for UTI depends on age, gender, race, nutritional factors, hygiene and immune status of the patients. The high prevalence of UTI in females could be due to the anatomical structures like shorter urethra and its closeness to the anus which allows the entry of pathogen by fecal-perineal-urethral contamination. UTI during pregnancy is due to stasis of urine in the ureters, pressure effects and hormonal changes. Moreover, 25% of untreated women in pregnancy have asymptomatic bacteriuria (ASB) and pyelonephritis. unprotected sexual intercourse, poor hygiene and childbirth also contribute to recurrent UTI in females. Post-menopausal women have higher incidence for UTI due to uterine prolapse, less estrogen activity, altered vaginal biota and associated co-morbid condition like diabetes mellitus (DM).

In males, the UTI is common at extremes of life. After infancy, the incidence is low but it is complicated at older age due to prostate enlargement and comorbid conditions. Prolonged hospital stay due to other medical and surgical problems and urinary catheterization are the most important risk factors in older age of both sexes.

Based on the organs affected and clinical layout, they are grouped as upper UTI versus lower UTI and complicated versus uncomplicated UTI. Based on the presence or absence of symptoms, it has been classified into asymptomatic bacteriuria (ASB) and acute symptomatic UTI which further includes acute and chronic pyelonephritis, cystitis and urethritis in males and females, prostatitis in males. Depending on the number of episodes of UTI and treatment response, it is classified into recurrent infection/reinfection, relapse and treatment failure. Depending on the source of pathogen, it has been categorized into community acquired and hospital acquired UTI.

*E. coli* is the most common cause of uncomplicated UTI and causes 85% community acquired and 50% of hospital acquired infections (HAI). Other *Enterobacteriaceae* group, *Staphylococcus* spp, *Enterococcus* sp, *Pseudomonas aeruginosa* are the next most common causes. *Mycobacterium tuberculosis*, *Chlamydia trachomatis*, *Candida* species are other rare causes of UTI. Rarely UTI may be caused by viruses or fungi.

*E. coli* is the predominant commensal in the gastrointestinal tract which is the source for initiation of UTI. It has been proved that few consistent serotypes of *E. coli* causing UTI and hence was designated as uropathogenic *Escherichia coli* (UPEC). UPEC possesses its virulent property due to the presence of virulent genes carried by pathogenicity islands (PAIs), bacteriophage, transposons or plasmids.
History of UTI

Urinary tract infections (UTIs) had caused a large outbreak long back, even before the bacteria were identified and recognized as the causative agents. It was first mentioned around 1500BC28. They have been described since ancient times and Egyptians defined UTIs as "sending forth heat from the bladder.

<table>
<thead>
<tr>
<th>SL. No.</th>
<th>Contributors</th>
<th>Contributions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Egyptians (Ebers papyrus)</td>
<td>Documented UTI for the first time.</td>
</tr>
<tr>
<td>2.</td>
<td>Kahun papyrus</td>
<td>Suggested hematuria (due to worm in the belly)</td>
</tr>
<tr>
<td>3.</td>
<td>Arabs</td>
<td>Introduced uroscopy.</td>
</tr>
<tr>
<td>4.</td>
<td>Romans</td>
<td>Introduced surgery for renal calculi.</td>
</tr>
<tr>
<td>5.</td>
<td>Hippocrates (19th century)</td>
<td>Introduced urine analysis as diagnostic Procedure.</td>
</tr>
<tr>
<td>6.</td>
<td>Hippocrates (387 BC)</td>
<td>Found the association between UTI, calculi and groin abscess.</td>
</tr>
<tr>
<td>7.</td>
<td>Wilhelm Duschlan Lambl (1856)</td>
<td>Published for the first time on use of microscope in urine analysis.</td>
</tr>
</tbody>
</table>

Table 1: Historical Perspectives of UTI

UTI caused high morbidity in the preantibiotic period. Hippocrates said that cystitis appears and could last for a year before either resolving or worsening to affect the kidneys. Later on most of the UTIs were described and were thought to be the bacterial cause globally. They were gaining prevalence in many parts of the world. Many attempts were made to give the incidence rate of the infection. But accurate assessment was not possible since it was not a notifiable disease. Later it got many researchers’ attention to make their efforts in the study and to describe the causes, pathogenesis and treatment.

Many earlier investigators suspected renal involvement was very silent and coined the term for urinary infection as “pyelonephritis lenta”. They also stated them as persistent and insidious infection which could end up in End Stage Renal disease. In 1956, Kass developed his criteria that UTIs was based on significant and asymptomatic bacteriuria. Kass contribution in this field encouraged many researchers to develop the epidemiological investigations. Next step of investigations in UTI was to develop definite marker. This was carried out with the basis of Kass contribution. Later Kass observed that the growth was inhibited by pH and urine osmolality. Acute urethral syndrome which involved urethritis, vaginitis and cystitis was defined in 1980s.

Epidemiology of UTI

UTI causes enormous morbidity in the general population, and is the most common cause of community and hospital acquired infections.
The exact prevalence of UTIs is dependent on age, gender, socio economic status and other environmental factors. With advancing age, the incidence of UTI increases in males due to prostate enlargement and neurogenic bladder. About 20% of women experience a single episode of UTI during their lifetime, and 3% of women have more than one episode of UTI per year. The association of UTIs with sexual intercourse may also contribute to infection because sexual activity increases the chances of bacterial contamination of the female urethra. Pregnancy also makes them more susceptible to infection. Recurrent infections are not uncommon, and it leads to irreversible damage of the kidneys resulting in renal hypertension and renal failure in some. About 5% of catheterized patients develop bacteriuria, despite adequate aseptic precautions during instrumentation and in some it leads to septicemic death.

Figure 2: Global Prevalence of UTI (Wagenlehner F et al 2016)

Figure 3: Prevalence of UTI Among Females
Classification of UTI

- Community acquired UTI and Nosocomial UTI
- Upper UTI and Lower UTI
- Complicated UTI and Uncomplicated UTI

Community Acquired UTI

Episode of UTI may be detected at the time of admission or within the first 48 hours. It may occur without the above mentioned risk factors. Mostly they are caused by Enterobacteriaceae and recently the trends changed that MDR uropathogens are the common causes.

Upper UTI vs Lower UTI

<table>
<thead>
<tr>
<th></th>
<th>Lower UTI</th>
<th>Upper UTI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sites involved</td>
<td>Urethra, Bladder</td>
<td>Kidney, Ureter</td>
</tr>
<tr>
<td>Route of spread</td>
<td>Ascending route</td>
<td>Both ascending and descending route</td>
</tr>
<tr>
<td>Occurrence</td>
<td>More common</td>
<td>Less common</td>
</tr>
</tbody>
</table>

Table 2: Comparison of Upper and Lower UTI

Uncomplicated UTIs

It occurs in patients with normal structural and functional urinary tract. Usually it is caused by antibiotic susceptible pathogens. It is seen in case of

- Immunocompetent patients.
- No co-morbid conditions.
- No congenital abnormalities.

Complicated UTIs

They are seen in individuals who have one or more structural and functional abnormalities. It may be seen in the following conditions.

- Immunosuppression.
- Obstruction due to tumor, Benign Prostatic Hypertrophy.
- Congenital abnormalities in the urinary tract.
- Renal calculi and renal failure.
- Renal transplantation.
- Foreign bodies (e.g., Indwelling catheters or other drainage tubes if kept)
- Infection in pregnant women and hospitalized patients.
Predisposing Factors

- Host Factors: Mutations in Toll-like receptors and the interleukin 8 receptor genes lead to recurrent UTI and pyelonephritis.
- Behavioural changes: Sexual activity can introduce the flora and can cause infection.
- Spermicidal agents (Nonoxynol-9) can alter the vaginal flora which interferes with the pathogen.
- Low level of CXCR2 expression on neutrophils is prone for recurrent UTI.

Demographic Factors

- Age: UTI may be experienced in neonatal age group, frequently seen in adults and reaches peak in old age group41.
- Gender: Females are more affected than men due to structural and anatomical changes.
- Genetic Factors: The susceptibility to colonization has been linked to an increased receptivity for the attachment of bacteria to the epithelium, and to an overrepresentation of the P1 blood group phenotype.

Routes of Infection

Three Possible Routes of Urinary Tract Infections are
- Ascending route.
- Haematogenous route.
- Lymphatic routes.

Ascending Route

It is the most common route where bacteria can ascend up and cause UTI. Mostly bacteria originating from bowel get colonized in urethra. Once it reaches the bladder, they can multiply and gain entry to the ureter and further invades renal parenchyma and pelvis.

Hematogenous Route

It is very uncommon in Immunocompetent individuals. Because the kidney receives 20% to 25% of the cardiac output, any microorganism that reaches the bloodstream can be delivered to the kidneys.

Lymphatic Route

It is less likely to be the route of infection. In retroperitoneal abscess, it may spread to the urinary tract through the lymphatic system.

Clinical Manifestations

Urinary tract infections have traditionally been viewed as acute and often self-limiting infections. However, this concept has been challenged by recent findings demonstrating that an acute bladder infection results from a complex series of host pathogen interactions that can lead to bacterial invasion and persistence and that ultimately can determine the course of the infectious disease. In general, UTIs can be classified as asymptomatic bacteriuria, cystitis, or acute pyelonephrities.
Cystitis predominantly involves colonization of the bladder. Patients with cystitis usually report dysuria, frequency, urgency, and supra-pubic pain. The urine often becomes grossly cloudy and malodorous, and it is bloody in about 30% of cases. White blood cells and bacteria can be detected by examination of un-spun urine in most cases. However, some women with cystitis have only 102 to 104 bacteria per milliliter of urine, and in these instances bacteria cannot be seen in a Gram stained preparation. Physical examination generally reveals only tenderness of the suprapubic area.

Figure 4: Anatomy of The Urinary Tract With Corresponding Terms and Diseases

Frequency of Uropathogens Causing UTI

The above figure depicts the commonly encountered uropathogens among complicated and uncomplicated UTI and also revealed that E.coli is the most common cause of both the complicated as well as uncomplicated UTI worldwide. Its prevalence in India varies from place to place.
Etiologic Agents of UTI

The Gram-negative rods *E. coli*, *Proteus*, *Klebsiella*, *Pseudomonas aeruginosa* and other *Enterobacteriaceae* are mostly found in hospital. They are common cause of UTI in hospital because of their resistance to antibiotics. *Klebsiella pneumoniae* strains cause lesions such as urinary infections, nosocomial infection, respiratory tract infection and wound infection. The family of *Enterobacteriaceae* especially in *Klebsiella* spp. and *E. coli* cause most nosocomial infections, including urinary tract infection and are known to be antimicrobial resistance. Acquisition of plasmid that encode for the production of extended spectrum β-lactamase (ESβC) from cephalosporin and penicillin, by *K. pneumoniae* causes resistance to antibiotics mainly cephalosporins and penicillins. *Proteus mirabilis* isolates cause severe UTIs leading to acute pyelonephritis, chronic inflammation and bacteremia. There is frequent infection by *P. mirabilis* in inpatients and outpatients due to contaminated hospital equipments which increase the risks of nosocomial infection in hospital staff. Due to increased antibiotic resistance, it has become necessary to control the spread of *P. mirabilis* strain isolated from community infections and in hospital environment. *Proteus mirabilis* strains are usually resistance to β-lactams and with prolonged use of these drugs will increase their resistance which can be reduced by culturing and setting susceptibility testing and use of correct prescription of the right antibiotics.

Uropathogenic *Escherichia coli* (UPEC)

Although UTIs are caused by many species of microorganisms, most are caused by *E. coli*. Non-pathogenic and pathogenic *E. coli* which migrate from the colon colonizes the urinary tract and persists for a long time. Hence, UTI starts with colonization of periurethral region which are derived from host fecal flora. Genes coding for various urovirulence factors of *E. coli* are often duplicated in uropathogens and grouped as pathogenicity islands which are not present in coliforms. These genetic changes enable the pathogenic *E. coli* to adopt and persist in the urine. Single or multiple genes encoding a single virulence factor is not sufficient to make the bacteria producing infections. All together enhances the survival of bacteria and their multiplication within the urinary tract. Significant virulence factors expressed by UPEC like adhesins, haemolysins, siderophore production, capsular polysaccharide and outer membrane proteins which help to maintain the extra intestinal survival and enable it to colonize the urinary tract and cause UTIs.

![Figure 6: Escherichia coli Adhesins and Harboring/Motile Structures](image-url)
Virulence Factors (VF) of UPEC

Virulence factors of UPEC that have been potentially implicated as important in establishing UTIs can be divided into two groups:

- Virulence factors associated with the surface of bacterial cell and
- Virulence factors, which are secreted and exported to the site of action.

<table>
<thead>
<tr>
<th>Surface VF</th>
<th>Exported VF</th>
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<tbody>
<tr>
<td><strong>Adhesins</strong> – Fimbrial and afimbrial antigens</td>
<td><strong>Toxin Genes</strong></td>
</tr>
<tr>
<td><strong>Flagellum Flagella</strong> – H Antigen</td>
<td>• Hemolysin</td>
</tr>
<tr>
<td><strong>Capsular Polysaccharide</strong>- K Antigen</td>
<td>• Cytotoxic necrotizing factor-1(CNF)</td>
</tr>
<tr>
<td><strong>Somatic</strong> – O Antigen</td>
<td>• Uropathogenic-specific protein(USP)</td>
</tr>
<tr>
<td>Outer membrane proteins</td>
<td>• Secreted autotransporter toxin (SAT)</td>
</tr>
<tr>
<td></td>
<td><strong>Siderophore</strong></td>
</tr>
<tr>
<td></td>
<td>• Enterobactin</td>
</tr>
<tr>
<td></td>
<td>• Aerobactin</td>
</tr>
<tr>
<td></td>
<td>• Yersiniabactin</td>
</tr>
<tr>
<td></td>
<td>• Salmochelin</td>
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</tbody>
</table>

Table 3: List of Virulence Factors Produced By UPEC

Summary of Pathogenesis of UTI by UPEC

The term UTI covers a variety of conditions with different causes and makes the survival of bacteria in the urinary tract. The severity of a UTI depends on the protective mechanisms of the host, the virulent property of the agent and the supporting environmental factors.

**Step1:** Colonization of coliforms.

**Step2:** Invasion of *E.coli*.

**Step3:** Attaining virulent property.

**Step4:** Adhesion to the uroepithelial cells.

**Step5:** Lysis of the uroepithelial cells.

**Step6:** Evades immune mechanism.

**Step7:** If not properly treated, dissemination from lower urinary tract to upper urinary tract.
Colonization of the upper urinary parcel requires a firmly regulated interplay between virulence factors, supposed phase variety. Flagellae are activated when the bacteria ascend towards the kidneys. To join to the renal epithelial cells, P fimbriae are turned on and type 1 fimbria turned off. Complex interactions between P and type 1 fimbriae might be seen when P fimbriae join to kidney epithelial cells at the same time as type 1 fimbriae are responsible for inter-bacterial restricting in the renal tubule, causing rounded check.
MANAGEMENT OF UTI

➢ Before Discovery of Antibiotics
The origin of UTI was not recognized and no specific antimicrobial therapies were available so the treatment of UTI was only palliative in the pre-antibiotic era.

- **The Egypt (Ebers papyrus) Physicians** - recommended mostly the herbal products.
- **Roman Medicine** - explained the conservative approach (bed rest, diet, narcotics and herbs)
- **Greek Physicians** - introduced some invasive techniques like lithotomy for stones and catheterization for retention.
- **The Arabian Physicians** - introduced the method uroscopy.
- In 19th century many physicians managed UTI by hospitalization, bed rest, diet changes, narcotics, herbal enema products and douches and with surgical procedures for stones, abscess and retention.
- Later various antibacterial agents like hexamine, mercurochrome were introduced. But their outcomes in clinical practices were not satisfactory.

➢ Post Antibiotic Era
In the 1950s, Nitrofurantoin was first used. It was the first tolerable and effective drug available for the treatment of UTIs. During 1970s, Amoxicillin and other β-lactams were introduced. Slowly the developed resistance and shifted to Cotrimoxazole as the drug of choice in UTI.

➢ Resistance to antibiotic agents is not a modern concept. Penicillinase producing *E.coli* was first isolated in 1940 even before penicillin entered to the clinical use. Since then, patients developed resistance to third generation cephalosporins. Later on, extended spectrum cephalosporins developed resistance rapidly due to the emergence of ESBLs. ESBLs were first reported from Europe. Several sporadic cases and outbreaks have been reported from France in early 1980s.

Molecular Identification of Uropathogens
Urine samples are collected from UTI patients with clean catch midstream technique. The samples are centrifuged and cotton swabs used for inoculation of brain heart infusion broth. The media then incubated for 3h at 37°C and the culture saved in refrigerator for deoxyribonucleic acid (DNA) extraction purpose. DNA is extracted from brain heath infusion broth by participation of bacteria by 7000 rpm/min. and extraction using genomic DNA kit (Geneaid, China). Polymerase chain reaction (PCR) assay is performed to detect **Lac Z** gene which is specific for the identification of *E. coli*. PCR reaction is conducted in 50μl of reaction mixture containing 25μl of green master mix, 2μl of each primer, 10μl DNA template and 11μl of deionised water. Amplification is conducted using thermo cycler Epen-droff programmed cycler for initial denaturation at 94°C for 3 min., 35 cycle of denaturation at 94°C for 30sec., annealing 59°C 30sec., extension 72°C 30sec, and 7min. of final extension at 72°C. PCR products were resolved by electrophoresis on 2% w/v analytical grade agarose gels (Promega, USA) stained by ethidium bromide, with the use of 100 bp DNA ladder from (Intron, Korea) visualized using UV transeliminator and documented using digital camera (Sony, Japan) and run in TBE (1X) buffer, Gels were stained with ethidium
bromide (0.5 μg/ml) and analyzed using UV eliminator. The molecular weight identification of resolved band was based on their correspondence to the ladder bands. *E.coli* represent the highest UTI’s causal organisms among other causal organisms in both classical culture method (66%) and in PCR method (60%). Most of the bacteria which often seen in UTI are faecal bacteria, these bacteria were mostly found in feces, while anaerobic bacteria rarely cause UTI. Most of UTI (90%) in patient with normal anatomic structure are caused by *E.coli*, 10-20% of UTI infection is caused by *Staphylococcus saprophyticus* (young sexually active females) and 5% is caused by *Enterobacter*.

**Prevention of UTI and Control**

Creating awareness to women on the effects of frequently using low dose antibiotics to treat symptomatic UTIs and prevent recurrent infections will be of great importance. Women have high risk of contracting recurrent UTI than men and they are advised use a single dose of trimethprime-sulfamethoxazole (160/800mg) before and soon after intercourse. Other antibiotics used for prophylaxis for recurrent UTIs are Norfloxacin and Fluoroquinolone. They can only be used after bacteriuria has been eradicated with a full dose treatment regimen.

Generally most of the patients experience UTI in their life time; especially in females. Hence, prophylactic measures are often needed only for the women who are suffering from recurrent infections. A prophylactic dose of antibiotic may be needed in case of acute UTI. Antibiotic treatment should be used when culture results become available to avoid drug resistance and therefore antimicrobial sensitivity test should be used to direct therapy. Management of uncomplicated UTIs should be done on two important principle organisms especially *E.coli* which accounts for more than half of all urinary isolates and *Staphylococcus saprophyticus* which accounts for less than a quarter of the urinary isolates. Nosocomial and uncomplicated community acquired UTIs rate the highest in antibiotic resistance.

**Antimicrobial Resistance**

Factors favouring antimicrobial resistance are mutations, acquiring new genetic material, exposure to cells with new genetic material and use of antimicrobial agents as growth promoters in animal feeds destined for human consumption give rise to multidrug resistance. However, misuse of antimicrobial agents has led to a post antibiotic era which is a current situation worldwide.

**Susceptibility to Antimicrobial Agents**

In spite of the availability and use of the antimicrobial drugs, UTIs caused by bacteria have been showing increasing trends in recent years. Much of the increase has been related to emerging antibiotic resistance among urinary tract pathogens. Increasing multidrug resistance in bacterial uropathogens is an important and evolving public health challenge. Accurate bacteriologic records of culture results provide guidance on empirical therapy before sensitivity patterns are available. Since most UTIs are treated empirically, the criteria for the selection of antimicrobial agents should be determined on the basis of the most likely pathogen and its expected resistance pattern determined.
MATERIALS AND METHOD

Collection of Urine Specimen

Midstream urine samples were collected. Every patient was given a sterile wide mouth container and explained the proper method of collection of urine to maintain a strategic distance from tainting. Male patients were instructed to clean their genital area before voiding. Female patients were instructed to clean the vulva and perineum with cleanser and water, dry the area. They were advised to provide 10 ml of urine sample. Collected urine samples were processed immediately. The specimens were processed by standard bacteriological methods and identified by standard conventional methods.

Specimens Included In The Study

Samples recovered from in patient and out patients of the clinics were received from different specialties like Medicine, Surgery, Obstetrics and Gynecology, Pediatrics, Orthopedics, Dermatology, Nephrology, and Intensive Care Units. Patient’s history and temporary conclusion of the infection were obtained from emergency clinic records.

1. Macroscopic Examination of Urine

- Urine was observed with naked eye for altered tone, turbidity, scent.
- Urine was tested for pH.
- Presence of protein, sugar and nitrite was seen.

2. Plating of Urine Sample by Standard Loop Procedure

The sample was inoculated by a standard circle (with an internal diameter of 4 mm) on well dried plates of blood agar and MacConkey agar. Plates were incubated overnight at $37\,^\circ C$. Next day number of colonies and their morphology were noted and recorded. The colonies of same type were counted on Blood agar. Presence of more than 100 colonies of comparative morphology was considered huge. Just those samples that produced a single type of province resembling to that of \textit{E.coli} were selected. Samples containing multiple types of creatures were not included in this examination.

Isolation, Identification and Confirmation of \textit{E.coli}.

\textbf{Indole Test:} Peptone water was inoculated with test life form and incubated at $36\,^\circ C$ for 24 hours. 10 ml of KOVAC”s reagent was added along the side of the test tube to frame a layer on the top. A positive reaction was indicated by the arrangement of pink ring at the intersection.

\textbf{Carbohydrate Fermentation:} Pure cultures were inoculated from the agar plates to sugar media and inoculated at $36^\circ C$ for 1-2 days. Positive test was appeared by corrosive and gas creation by change in shade of the media (pink with pointer) and gas inside the Durham”s tube.

\textbf{Citrate Utilization Test:} Test organic entity was inoculated in Simmons citrate agar and incubated at $36\,^\circ C$ for 3 days. Blue medium with a streak of development was indicated in citrate using bacteria (positive reaction).

\textbf{Urease Test:} Christensen Urea Agar: The test organic entity was inoculated heavily over the entire slope surface and incubated at $36\,^\circ C$. A positive reaction was indicated by a pink shade of the medium. The alkaline pH produced changes the shade of the
medium to pink or red.

**Triple Sugar Iron Agar Test:** TSI agar was stabbed in the center of the butt and streaked on the slope with a needle charged with a single province of the test creature. The tube incubated at 36 for 24 hours. A yellow butt and red inclination showed glucose fermentation, yellow butt and yellow inclination showed glucose, lactose and sucrose fermentation. Gas produced was trapped inside the medium.

**Methyl Red Test:** Test organic entity was inoculated on glucose phosphate stock and incubated at 36 for 42 hrs. 5 to 6 drops of methyl red reagent was added to the culture. A red shading indicated positive reaction. negative tests were yellow in shading. Positive reaction indicated the capacity of the organic entity to produce and keep a corrosive Ph.

**Voges-Proskauer Test:** The test creature was inoculated in glucose phosphate stock and incubated at 36 for 42 hours. Then VP reagent (1ml of 40 % potassium hydroxide 4 ml of 6% alpha napthol in absolute ethanol) was added. The tube was shaken overwhelmingly to ensure greatest aeration. A positive result was indicated by the development of pink tone in 2-5 minutes becoming dark red in 25 minute.

**RESULTS AND DISCUSSION**

**Data Collection**

**Table 4: Components of Uropathogenic**

<table>
<thead>
<tr>
<th>COMPONENTS</th>
<th>SAMPLES</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>2100</td>
<td>29.1 %</td>
</tr>
<tr>
<td>Pus</td>
<td>1620</td>
<td>22.4 %</td>
</tr>
<tr>
<td>Blood</td>
<td>996</td>
<td>13.8 %</td>
</tr>
<tr>
<td>Body Fluid</td>
<td>890</td>
<td>12.3 %</td>
</tr>
<tr>
<td>Sputum</td>
<td>1220</td>
<td>16.9 %</td>
</tr>
<tr>
<td>Stool</td>
<td>380</td>
<td>5.27 %</td>
</tr>
</tbody>
</table>
Figure 9: Components of Uropathogenic
Table 5: Distribution of Uropathogens (n=280)

<table>
<thead>
<tr>
<th>COMPONENTS</th>
<th>NO. OF SAMPLES</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.coli</td>
<td>280</td>
<td>40.6 %</td>
</tr>
<tr>
<td>Klebsiella spp</td>
<td>98</td>
<td>14.24 %</td>
</tr>
<tr>
<td>Proteus spp</td>
<td>68</td>
<td>9.88 %</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>91</td>
<td>13.22 %</td>
</tr>
<tr>
<td>Enterobacter spp</td>
<td>25</td>
<td>3.63 %</td>
</tr>
<tr>
<td>Citrobacter spp</td>
<td>21</td>
<td>3.05 %</td>
</tr>
<tr>
<td>Other NFGNB</td>
<td>12</td>
<td>1.74 %</td>
</tr>
<tr>
<td>S.aureus</td>
<td>19</td>
<td>2.76 %</td>
</tr>
<tr>
<td>CONS</td>
<td>22</td>
<td>3.19 %</td>
</tr>
<tr>
<td>Enterococci spp</td>
<td>27</td>
<td>3.92 %</td>
</tr>
<tr>
<td>Candida spp</td>
<td>25</td>
<td>3.63 %</td>
</tr>
</tbody>
</table>

UPEC was the predominant uropathogen isolated in our examination and it is compared with other studies and is furnished in the accompanying. The second most normal uropathogen was *Klebsiella spp* followed by *Pseudomonas aeruginosa, Proteus spp* and other *Enterobactericeae*. It is firmly supported by the examination done in Pattukkottai area in Tamilnadu in which the second most basic uropathogen was *Klebsiella pneumonia* followed by *Pseudomonas aeruginosa* and *Proteus spp*. It is interestingly with the results of study done where UPEC was followed by *Citrobacter spp.*, and *Pseudomonas aeruginosa* showed that UPEC was followed by *Citrobacter spp.*, and *Pseudomonas aeruginosa*. 
Figure 10: Graphical representation of Quantification of Uropathogens.

Table 6: Gender Wise Distribution of UPEC Isolates

<table>
<thead>
<tr>
<th>GENDER</th>
<th>NO. OF SAMPLES</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEMALE</td>
<td>166</td>
<td>59.28</td>
</tr>
<tr>
<td>MALE</td>
<td>114</td>
<td>40.71</td>
</tr>
</tbody>
</table>
Figure 11: Gender Wise Distribution of UPEC Isolates

Gender Wise Distribution

Our examination showed that the prevalence of UTI in females (59.28%) was higher than males (40.71%). It unequivocally correlates with other discoveries which revealed that the frequency of UTI is greater in females as compared to males observed a prevalence in females when compared to in males respectively. The reason behind this high prevalence of UTI in females is shorter urethra, due to its close nearness to butt, sexual intercourse, incontinence and other comorbid condition.

Table 7: Age and Gender Distribution in UPEC Infection

<table>
<thead>
<tr>
<th>YEARS</th>
<th>MALE</th>
<th>FEMALE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO. OF SAMPLES</td>
<td>PERCENTAGE</td>
</tr>
<tr>
<td>&lt;20 Years</td>
<td>5</td>
<td>1.78</td>
</tr>
<tr>
<td>21-39 Years</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>41-59 Years</td>
<td>40</td>
<td>14.28</td>
</tr>
<tr>
<td>&gt;60 Years</td>
<td>57</td>
<td>20.35</td>
</tr>
</tbody>
</table>
Age and Gender Wise Analysis

In the present investigation, UPEC was the most frequent pathogen seen in both the genders and furthermore on the whole age gatherings. Incidence is high in females in the age gathering of 41 to 60 years (26.42%) due to comorbid conditions like Diabetes mellitus, catheterization and incontinence and furthermore due to alteration of ordinary vaginal vegetation in older age observed of females were affected in the age gathering of 15-59 years. The investigation observations are like our examination and proved that UTI is more typical in older age gathering (41-50 years). Further stated that asymptomatic UTI is extremely regular in older age. UTI is normal in older age due to associated hazard factors, for example, urinary incontinence etc. Males above 61 years (20.35%) were discovered to be affected in the present examination. it is emphatically supported by where males were affected after 48 years and furthermore justified the reasons for higher incidence of UTI in the elderly males that could be due to BPH, incontinence and neurogenic bladder.

![Age and Gender Distribution in UPEC Infection](image)

Figure 12: Age and Gender Distribution in UPEC Infection

Patients Profile UPEC Infection:
Generally inpatients were affected in the present investigation which is as opposed to the examination who observed more of outpatients were infected.

Distribution of UPEC Among Complicated and Uncomplicated UTI:
In the investigation it was discovered that 55.78% and 56.5% of patients had complicated and uncomplicated UTI respectively whereas in the examination carried was 20% had complicated and 80% had uncomplicated UTI.

Association of UPEC with Various Risk Factors in Complicated UTI:
In the examination catheterization was the most well-known danger factor of complicated UTI in UPEC infection followed by DM and associated illness whereas in 48.36% were catheterized among the complicated UTIs.
Figure 13: Macroscopy Turbid

Figure 14: CLED Plate-Lactose Fermenting Colonies
Figure 15: Biochemical Test Showing Reactions of *E.coli* Indole-Produced, Methyl Red-Positive, Voges Prousker-Negative, Simmons Citrate (SC)-Utilised, Christensens Urease (CU)-not produced, Triple Sugar Iron Agar Slant-Acid Slant/Acid Butt with no H₂S Gas, Mannitol Motility Medium-Fermented and Motile.

Figure 16: Muller Hinton Agar Plate Showing Zone of Inhibition of *E.coli*
FUTURE PROSPECTS

It was experimented with whole killed vaccine or vaccines based on single or multiple VF that have been used in many animal models to show that they were protected against the strains expressing the respective virulence factors.

CONCLUSION

During the study period, a total of 7106 clinical samples were received, out of which 1989 (27.9%) were urine samples. Of these, significant growth was observed in 676 isolates (34%) which determines the prevalence of UTI. The most common organism causing UTI was found to be UPEC (41.1%). In our study it was observed that both extremes of age were affected with the mean age 51.75 years. Males were more affected with UTI after 60 years of age. Females were more affected in the age group of 41-60 years. In this study, females (59.35%) were more commonly affected than males (40.64%). UPEC were more commonly isolated from inpatients (77.33%) than outpatients (22.66%). UPEC were more commonly isolated in patients with uncomplicated UTI (156) than complicated UTI (122). Among the complicated UTI, patients with indwelling catheter were found to be at high risk (32.78%) followed by Diabetes mellitus (17.21%). Acute cystitis (33.8%) were frequently encountered in uncomplicated UTI than acute pyelonephritis (22.3%). Of these HA positive isolates, Mannose Sensitive Hemeagglutination (63.85%) were more common than Mannose resistant Hemeagglutination (36.14%). Multiple VF (more than 4) were seen in 106 isolates.

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I pay tribute to My Parents for lifting me up till this phase of life. I thank them for their love, trust, patience, support and bearing all kind of stress to make me what I am.

REFERENCE


