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Bacterial Profile of Urinary Tract Infection and Antimicrobial Susceptibility Pattern among the Patients attending a Tertiary Care Centre

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A B S T R A C T

Urinary Tract Infection (UTI) is a most cause of health issues in the world. It is the major cause of morbidity and mostly caused by bacterial infections from children to adults estimating the prevalence of UTI. To study the bacterial profile of urinary tract infection among the patients attending in a tertiary care center and also to assess the antimicrobial susceptibility patterns of the bacterial isolates. This study was conducted in the Pondicherry Institute of Medical Science Hospital, Pondicherry from 1st December 2020 to 30th January 2021. A total of 200 midstream urine samples were collected from suspected UTI patients attending in tertiary care settings. Urine samples were inoculated and the isolates were identified according to the standard microbiological techniques. The antimicrobial susceptibility tests were performed by Kirby-Bauer disk diffusion method on Mueller-Hinton agar according to the CLSI guidelines. Out of 200 samples, 107 positive urine cultures, UTI caused more in females (56%) when compared to males (44%). The most common bacteria frequently identified in this study were *E.coli* (57%) and followed by (12.15%) from gram negative isolates while Enterococcus species (7.48%) were common pathogen in gram positive organisms. The gram negative isolated were >70% sensitive to Amikacin, Nitrofurantoin and Piperacillintazobactam while >70% resistant to ampicillin, Nitrofloxacin and Cefazolin. The Gram positive isolates were 100% sensitive Vancomycin and Linezolid. The multi-drug resistance (MDR) was seen in most of the isolates. This study found a number of bacterial isolates with very high resistance to the commonly prescribed drugs from the patients with and without symptoms of UTI.

Introduction

A Urinary Tract Infection (UTI) is the most common infection inhuman experienced in highly specialized medical care usually over an extended period of time that involves advanced and complex procedures and treatments performed by medical specialists (Martha Medina and Edgardo Castillo-Pino, 2019; Michel Kengne *et al.,* 2017). UTI can identify in all aged groups and both genders. In global population, UTI reported as women affect more than the men. 40 to 60% of women recorded the UTI in a year. Urinary tract infection is most commonly caused by gram negative bacteria (Derese *et al.,* 2016); (Dave *et al.,* 2018); (Yonas Alem

Gessese *et al.,* 2017). The gram negative bacteria are *E.coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Proteus mirabilis, Citrobacter diversis, Acinetobacter baumanii and Enterobacter aerogens*. Some of the gram positive bacteria also cause UTI such as *Staphylococcus species, Streptococcus species and Enterococcus species* (Fenta *et al.,* 2020). Among the most common uropathogens identified in urinary tract infection is *E.coli*, which accounting for about 80% of cases (Bency *et al.,* 2017; Ashenafi Tula *et al.,* 2020). Usually urinary tract infection caused in lower urinary system – urinary bladder and urethra. Urinary tract infection which describes the presence of growth of bacteria in urine for greater than 10^5 CFU/ml (colony forming units) and also much lower than 10^3 CFU/ml in the urinary tract. Asymptomatic UTI is the presence of significant bacteria ($\geq 10^5$ cfu/ml) in two consecutive clean-catch mid-stream urine specimens in a patient without signs or symptoms.

Symptomatic UTI is defined as a condition whereby a patient has one or more of the following signs or symptoms with other recognized cause: fever (temperature, > 38 °C), urgency, frequency, dysuria, suprapubic pain or flank pain and a urine culture positive for 10^5 or more microorganisms per milliliter. Clean catch midstream urine, supra pubic aspirate, urine collected from patients on urinary catheter are the types of samples taken for testing urinary tract infection (Michel Kengne *et al.,* 2017; Gharavi *et al.,* 2021; Mohamed Abdel-Aziz Elzayat *et al.,* 2017). Although, various aspects of pathogens affecting urinary tract are genetic factors, age, gender, sexual activity, nocturnal enuresis and circumcision in boys, without making any symptoms. Most infections are caused by movement of bacteria through fecal from colon to urinary tract through urethra, peculiarly in females, due to presence of the genital tract and urethra. It is the most common cause of morbidity in the general population and hospital visit. We need to treat some peculiar patients before the lab results due to frequent misuse of antibiotics (Sharma *et al.,* 2011; Salwa H. Almukhtar, 2018). UTI spread by either ascending or descending bacterial movement into the urinary tract. The most common form of infection in the urinary tract is the ascending pathway of infection. Some of the specific urinary tract infection occurs by descending pathway also. Descending infection are the results of hematogenous spread of bacteria from a primary source of the body (Kalita *et al.,* 2016; Pegu *et al.,* 2014). Symptoms of a Urinary Tract Infection vary depending on the extent of the infection. Symptoms of UTI depend on what part of the urinary tract is infected. Although UTIs are commonly curable with antibiotics, the antibiotic susceptibility patterns of UTI vary according to regional and geographical locations and also change through time (Dnyaneshwari *et al.,* 2016; Timothy *et al.,* 2014).

Global burden of UTI

Globally, 150 lakh people are diagnosed with UTI yearly which causes an expense of greater than 6 crore dollars healthcare expenditure in treatment. UTI among university students is commonly to the general population and the majority of the females have recurrent infections within 1 year (Bency *et al.,* 2017; Yonas Alem Gessese *et al.,* 2017; Pharsuram Adhikari *et al.,* 2020). The prevalence of UTI in India and Saudi Arabia among College students was found to be 19.8 and 32.1% respectively. UTI among university students of Africa has been reported as 28%. It was estimated that in a year UTI was the cause of 1 million visits to the Emergency Departments, 7 million cases to Outpatient department and about 100,000 cases of hospitalizations all over the world. Leading cause of morbidity and health care expenditures in persons of all ages. An estimated 50 % of women report having a UTI at some point in their lives (Shashi Kant *et al.,* 2017; Timothy *et al.,* 2014; Mohamed Abdel-Aziz Elzayat *et al.,* 2017). UTIs are the most common outpatient infections in the United States. With the exception of a spike in young women aged 14−24 years old, the prevalence of UTIs increases with age. The prevalence in women over 65 years of age is approximately 20%, compared with approximately 11% in the overall population. Despite these clear increased risks of UTI, clinicians lack scientifically valid methods to identify and ultimately treat patients with UTI complains (Sharma *et al.,* 2011; Gharavi *et al.,* 2021). Therefore, UTI can cause serious complications such as frequent recurrences, bacteremia, and renal failure, preterm delivery. The diagnosis of UTI is very often missed in young children due to minimal and nonspecific symptoms (Nure Ishrat Nazme *et al.,* 2017; Dave *et al.,* 2018; Salwa H. Almukhtar, 2018). The developing renal cortex in young children is vulnerable to renal scarring resulting in hypertension and chronic renal failure. These morbidities in adults often have their origin in childhood (Debolina Chatterjee and Sarthak Kumar Sen, 2018; Gharavi *et al.,* 2021). A clinically suspected case of UTI should be defined and documented with urine culture report. After the diagnosis of UTI, its category should be defined. This helps in guiding a clinician about the

appropriate radio/nuclear imaging evaluation, choice of antimicrobial agent, duration of treatment and need of chemoprophylaxis (Derese *et al.,* 2016; Debolina Chatterjee and Sarthak Kumar Sen, 2018; Yonas Alem Gessese *et al.,* 2017).

Urinary tract infection

A Urinary tract infection is an illness caused by bacterial infection that affects any part of the urinary tract including your urethra, bladder, ureters, or kidneys. UTI is the second most common type of infection in the body. Our body has ways to defend against infection in the urinary tract. For example, urine normally flows from your kidneys, through the ureters to your bladder. Bacteria that enter your urinary tract are flushed out when you urinate. This one-way flow of urine helps to keep bacteria from infecting your urinary tract. Sometimes our body's defenses fail and the bacteria may cause a urinary tract infection (Chee Wei Tan and Maciej Piotr Chlebicki, 2016). Most UTIs are associated with a single bacterial pathogen, which usually arise from the gastrointestinal tract or the urinary tract. UTIs typically involve bacterial pathogens, but rarely fungi or viruses can cause infections of the urinary tract. Approximately 75% of bacterial UTIs are caused by a single species of pathogen, approximately 20% are caused by two species, and approximately 5% are caused by three species. *E. coli* is the most common uropathogen, and comprised greater than 40% of all laboratory isolates in the largest retrospective series on UTI. In addition to bacteria, viruses and fungi are other infectious agents that colonize the urinary tract (Pezzani and Antinori, 2018).

Urinary tract system

Urine typically doesn't contain bacteria. Urine is a byproduct of our filtration system—the kidneys. When waste products and excess water is removed from your blood by the kidneys, urine is created. Normally, urine moves through your urinary system without any contamination. However, bacteria can get into the urinary system from outside of the body, causing problems like infection and inflammation (Shamim and Ramalakshmi Sathiss, 2018). The urinary tract can be divided into the upper and lower tract. When it affects the lower urinary tract it is known as a bladder infection and when it affects the upper urinary tract it is known as a kidney infection. Most of the time, getting treatment right away for an infection in your urethra or bladder can prevent a kidney infection. A kidney infection can develop from a UTI that moves upstream to one or both of your kidneys. Kidney infections are often very painful and can cause serious health problems, so it's best to get early treatment for a UTI. When a bladder infection or other UTI is diagnosed and treated properly, most people won't have complications. Traditionally, UTIs are categorized as *uncomplicated* or *complicated*, or by site of infection. Infections may be symptomatic or asymptomatic. Lower UTIs include urethritis and cystitis and upper tract infections include pyelonephritis and renal abscesses (Ana L. Flores-Mireles *et al.,* 2015).

Uncomplicated UTI

In uncomplicated cases, UTIs are treated with a short course of antibiotics such as nitrofurantoin or trimethoprim/sulfamethoxazole. Resistance too many of the antibiotics used to treat this condition is increasing.

Complicated UTI

In complicated cases, a longer course or intravenous antibiotics may be needed. If symptoms do not improve in two or three days, further diagnostic testing may be needed. Phenazopyridine may help with symptoms (National Institute of Diabetes and Digestive and Kidney Diseases, National Kidney and Urologic Diseases Clearinghouse, 2011).

Examples of a complicated UTI include:

- ✓ Infections occurring despite the presence of anatomical protective measures (UTI in males are by definition considered complicated UTI)
- ✓ Infections occurring due to anatomical abnormalities, for example, an obstruction, hydronephrosis, renal tract calculi, or colovesical fistula
- ✓ Infections occurring due to an immune compromised state, for example, steroid use, post chemotherapy, diabetes, elderly population, HIV)
- ✓ Atypical organisms causing UTI
- ✓ Recurrent infections despite adequate treatment (multi-drug resistant organisms)
- ✓ Infections are occurring in pregnancy (including asymptomatic bacteriuria)
- ✓ Infections are occurring after instrumentation, nephrostomy tubes, ureteric stents, suprapubic tubes or Foley catheters
- ✓ Infections in renal transplant patients
- ✓ Infections are occurring in patients with impaired

renal function
- ✓ Infections following prostatectomies or radiotherapy (Sabih and Leslie, 2021)

Types of infection

An infection can happen in different parts of your urinary tract. Each type has a different name, based on where it is. Infection is most often due to bacterial from patient's own bowel flora. Transfer to the urinary tract may be via the blood stream, the lymphatics or by direct extension, but the most often via the ascending transurethral rout. There are two different types of Urinary tract infection such as Lower urinary tract infection and Upper urinary tract infection (Chee Wei Tan and Maciej Piotr Chlebicki, 2016).

Lower UTI includes:

Cystitis
Urethritis

Upper UTI includes:

Pyelonephritis

Cystitis (bladder)

Cystitis usually occurs when the urethra and bladder, which are normally sterile, or microbe-free, become infected with bacteria. Bacteria fasten to the lining of the bladder and cause the area to become irritated and inflamed. Cystitis affects people of both sexes and all ages. It is more common among females than males because women have shorter urethras. Around 80 percent of all urinary tract infections are caused by bacteria from the bowel that reaches the urinary tract. Most of these bacteria form part of the healthy intestinal flora, but once they enter the sterile space in the urethra and bladder, they can cause a UTI (National Institute on Aging, 2011). UTIs are the most common hospital-acquired infections in the United States (U.S.), especially among patients using urinary catheters.

Pyelonephritis (kidneys)

Pyleonephritis is defined by inflammation of the kidney parenchyma and the renal pelvis, typically due to bacterial infection. Acute pyelonephritis can affect patients of all ages, however the highest incidence occurs in women aged 15-29. Pyelonephritis can be described as uncomplicated, when present in structurally or functionally normal urinary tract in a non-immunocompromised host, or complicated (Sabih and Leslie, 2021).

Urethritis (Urethra)

Urethritis is inflammation of the urethra and is a lower urinary tract infection. The urethra is a fibromuscular tube through which urine exits the body in both males and females, and semen in males. Urethritis has a strong association with sexually transmitted infections (STIs). Urethritis is characterized as gonococcal or nongonococcal infections. *Neisseria gonorrhea* and *Chlamydia trachomatis* are the most common causative organisms of STIs. The most common symptom of urethritis is urethral discharge (Shamim and Ramalakshmi Sathiss, 2018).

Recurrent UTI or Symptomatic bacteriuria

Recurrent UTIs are symptomatic UTIs that follow the resolution of an earlier episode, usually after appropriate treatment. They are common among young, healthy women even though these women generally have anatomically and physiologically normal urinary tracts. Recurrent UTIs can be diagnosed clinically without performing a urine culture, although urine cultures are essential in management. For women with recurrent UTIs, imaging of the upper urinary tract and cystoscopy are not routinely recommended for evaluation. However, they should be performed without delay in patients with atypical symptoms, such as obstructive symptoms or presence of haematuria after resolution of infection.

Asymptomatic bacteriuria

Asymptomatic bacteriuria (ABU) does not cause renal disease or damage. Several studies involving women and the paediatric population have demonstrated that treatment for ABU increases the risk of subsequent symptomatic UTIs; hence, it is not recommended except in diagnostic and therapeutic procedures involving entry to the urinary tract with a risk of mucosal damage, such as endoscopic urological surgery and transurethral resection of the prostate. Although screening and treatment of ABU in pregnant women are recommended in many guidelines to reduce the frequency of low-birth-weight infants and preterm delivery based on studies

done in the 1990s, recent studies have shown mixed results in improvement of outcomes following ABU treatment in pregnant women (Chee Wei Tan and Maciej Piotr Chlebicki, 2016).

Burden of UTI in Worldwide

UTIs are a severe public health problem and are caused by a range of pathogens. UTIs are a significant cause of morbidity in infant boys, older men and females of all ages. UTI are common, especially among women. Women are at higher risk of developing a UTI than are men. Around one in two women and one in 20 men will get a UTI in their lifetime (Pegu *et al.,* 2014). Each year, 8 million to 10 million visits to doctors are for urinary tract infections. It has been estimated that globally symptomatic UTIs result in as many as 7 million visits to outpatient clinics, 1 million visits to emergency departments, and 100,000 hospitalizations annually. 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 in urinary tract pathogens. The prevalence of antimicrobial resistance in urinary pathogens is increasing worldwide. Urinary tract infection is among the most prevailing infectious diseases with a considerable financial burden on society (Pezzani and Antinori, 2018; Sneka and Mangayarkarasi, 2019).

Symptoms

In those who have bacteria or white blood cells in their urine but have no symptoms, antibiotics are generally not needed, although during pregnancy is an exception. In those with frequent infections, a short course of antibiotics may be taken as soon as symptoms begin or long-term antibiotics may be used as a preventive measure (Ana L. Flores-Mireles *et al.,* 2015). Urinary tract infections don't always cause signs and symptoms, but when they do they may include:

Cystitis signs and symptoms often include:

- ✓ A strong, persistent urge to urinate
- ✓ A burning sensation when urinating
- ✓ Passing frequent, small amounts of urine
- ✓ Blood in the urine (hematuria)
- ✓ Passing cloudy or strong-smelling urine
- ✓ Pelvic discomfort
- ✓ A feeling of pressure in the lower abdomen
- ✓ Low-grade fever

Pyelonephritis

Symptoms usually appear within two days of infection. Common symptoms include:

- ✓ a fever greater than 102°F (38.9°C)
- ✓ pain in the abdomen, back, side, or groin
- ✓ painful or burning urination
- ✓ cloudy urine
- ✓ pus or blood in the urine
- ✓ urgent or frequent urination
- ✓ fishy-smelling urine

Other symptoms of Pyelonephritis can be included:

- ✓ shaking or chills
- ✓ nausea
- ✓ vomiting
- ✓ general aching or ill feeling
- ✓ fatigue
- ✓ moist skin

Symptoms of Urethritis

Symptoms in men

Males with urethritis may experience one or more of the following symptoms:

- ✓ burning sensation while urinating
- ✓ itching or burning near the opening of the penis
- ✓ presence of blood in the urine
- ✓ discharge from the penis

Symptoms in women

Some symptoms of urethritis in women include:

- ✓ more frequent urge to urinate
- ✓ discomfort during urination
- ✓ burning or irritation at the urethral opening
- ✓ abnormal discharge from the vagina may also be present along with the urinary symptoms

Causes of Urinary Tract Infecton

Uropathogenic *E. coli* from the gut is the cause of 80–85% of community-acquired urinary tract infections, with *Staphylococcus saprophyticus* being the cause in 5–

10%. Rarely this may be due to viral or fungal infections. Healthcare-associated urinary tract infections (mostly related to urinary catheterization) involve a much broader range of pathogens including: *E.coli* (27%), *Klebsiella* (11%), *Pseudomonas* (11%), the fungal pathogen *Candida albicans* (9%), and *Enterococcus* (7%) among others (Thass *et al.,* 2019). Urinary tract infections due to *Staphylococcus aureus* typically occur secondary to blood-borne infections. *Chlamydia trachomatis* and *Mycoplasma genitalium* can infect the urethra but not the bladder. These infections are usually classified as a urethritis rather than urinary tract infection (National Institute of Diabetes and Digestive and Kidney Diseases, National Kidney and Urologic Diseases Clearinghouse, 2011).

Causal organisms

The Gram-negative rods *Escherichia coli* is commonest cause of ascending UTIs about 60-90%; this is probably because they are often present in the colon and virulence factors which include: the possession of K antigens and specialized fimbriae. *Staphylococcus saprophyticus* is related to sexual active women. *Proteus mirabilis* and *Klebsiella* species are often multiply antibiotic-resistant. *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are cause infection especially after catheterization or instrumentation. Fastidious gram-positive bacteria (e.g. lactobacilli, streptococci, corynebacteria), which require incubation for 24–48 hour in the presence of CO2 for isolation, acute uncomplicated UTI is usually due to one type of organism and Chronic infection is often associated with more than one type of organism Obligate anaerobes are very rarely involved, other species may be found e.g.: *Salmonella typhi*, *Staphylococcus aureus*, and *Mycobacterium tuberculosis* (Sabih and Leslie, 2021).

Escherichia coli (*E. coli*)

E. coli is belong to the large group of gram negative rods referred to as enterobacteria, they are cause primary and opportunistic infections in humans belong mainly to lactose fermenting, often referred to as coliforms, they are aerobes and facultative anaerobes, non sporing and motile. *E. coli* is the cause of 60-90% of urinary tract infection. Certain serotypes of *E.coli* are particularly common in urinary infection. This is probably because they are often present in the colon, rather than because of inherently high pathogenicity for the urinary tract. Some strains are reputed to more invasive than other. Factor associated with virulence include: the possession of K (capsular) antigen, which inhibit phagocytosis and bactericidal effect of normal human serum, the ability to adhere to uro–epithelium due to specialize fimbriae.

Klebsiella species

Gram-negative and non- motile usually capsulated rods cause UTIs in hospital patients. Antigenic analyses for capsular polysaccharide reveals that more than 80 serotype are recognized. They grow well on ordinary media, with colonies which are often, but not always, large and mucoid.

Proteus species

Gram negative pleomorphic motile rods, they grow on selective enteric media *Proteus mirabilis* is main proteus species of medical importance. It causes urinary infection commonly in the elderly and young male often following catheterization or cytoscopy. It is often associated with urinary 8 stones, probably because this organisms produce ammonia rendering the urine alkaline.

Pseudomonas aeruginosa

Gram–negative motile aerobic bacilli some strain are capsulated have very simple growth requirement and limited fermentation activity *Ps. aeruginosa* being resistant to infections are often difficult to eradicate due to *Ps. aeruginosa* being resistant to many antimicrobials. Infection with *Ps. aeruginosa* usually following catheterization associated with chronic urinary disease.

Serratia marcescens

It has been reported to cause UTIs, and it is gram-negative rods, facultative anaerobe and it is resistant to cephalosporin.

Staphylococcus aureus

Gram-positive cocci are occurring in group. Non motile, non-capsulated and it is catalase, DNase and coagulase positive, and ferment mannitol, it is rarely cause UTI.

Staphylococcus saprophyticus

Gram-positive cocci of uniform size occurring in groups but also singly and pairs. They are non-motile and non-capsulated. *S. saprophyticus* cause UTIs in sexually

active women. It is coagulase and DNase negative and ferment mannitol. The organism causes are many as one quarter of symptomatic UTIs in women. The surface agglutinins of this pathogen appear to be a key determinant of the virulence promoting it colonizes urinary tract.

Enterococcus faecalis

It is gram-positive cocci, often found accompanying infection with coliforms.

Other bacteria

Bacteria species are not primarily in urinary tract but may found in urine e.g. salmonella species, Mycobacterium tuberculosis, *Neisseria gonorrhoeae*, *Leptospira interrogans*, Chlamydia and mycoplasma species

Parasites

Very few parasites can cause UTIs e.g. *Trichomonas vaginalis* which cause urethritis in both male and females, but most often considered as cause of vaginitis. *Onchocerca volvulus*, *Wuchereria bancrofti* and *Schistosoma haematobium* were also uncommon UTI agent.

Fungi

Candida albicans usually in diabetic patients and those with immunosuppression, cause bladder infection and source of infection is usually endogenous; however cross-infection may occur.

Viruses

Viral cause of UTIs appears to be rare although there are association with hemorrhagic cystitis and renal syndromes.

Intercourse

In young sexually active women, sexual activity is the cause of 75–90% of bladder infections, with the risk of infection related to the frequency of sex. The term "honeymoon cystitis" has been applied to this phenomenon of frequent UTIs during early marriage. In post-menopausal women, sexual activity does not affect the risk of developing a UTI. Spermicide use, independent of sexual frequency, increases the risk of UTIs. Diaphragm use is also associated. Condom use without spermicide or use of birth control pills does not increase the risk of uncomplicated urinary tract infection.

Urinary catheters

Urinary catheterization increases the risk for urinary tract infections. The risk of bacteriuria (bacteria in the urine) is between three and six percent per day and prophylactic antibiotics are not effective in decreasing symptomatic infections. The risk of an associated infection can be decreased by catheterizing only when necessary, using aseptic technique for insertion, and maintaining unobstructed closed drainage of the catheter.

Catheter-associated UTIs

CAUTIs are associated with increased morbidity and mortality, and are collectively the most common cause of secondary bloodstream infections. Risk factors for developing a CAUTI include prolonged catheterization, female gender, older age and diabetes (National Institute on Aging, 2011).

Cause of Pyelonephritis

Most cases of community-acquired pyelonephritis are due to bowel organisms that enter the urinary tract. Common organisms are *E. coli* (70–80%) and *Enterococcus faecalis*. Hospital-acquired infections may be due to coliform bacteria and enterococci, as well as other organisms uncommon in the community (e.g., *Pseudomonas aeruginosa* and various species of *Klebsiella*). Most cases of pyelonephritis start off as lower urinary tract infections, mainly cystitis and prostatitis. *E. coli* can invade the superficial umbrella cells of the bladder to form intracellular bacterial communities (IBCs), which can mature into biofilms. These biofilm-producing *E. coli* are resistant to antibiotic therapy and immune system responses, and present a possible explanation for recurrent urinary tract infections, including pyelonephritis. Risk is increased in the following situations.

Cause of Urethritis

Generally, most cases of urethritis are the result of an infection from either a bacteria or a virus. Bacteria are the most common causes. The same bacteria that can cause bladder and kidney infections can also infect the

lining of the urethra. Bacteria found naturally in the genital area may also cause urethritis if they enter the urinary tract. According to the Centers for Disease Control and Prevention (CDC) Trusted Source, bacteria associated with urethritis include:

- ✓ *Neisseria gonorrhoeae*
- ✓ *Chlamydia trachomatis*
- ✓ *Mycoplasma genitalium*

Pathogens are the biological agents that cause illness. The same pathogens that cause STIs can also cause urethritis. These include the bacteria that cause gonorrhea and chlamydia and the parasite that causes trichomoniasis. There are also viruses that can lead to the development of urethritis. These include the human papillomavirus (HPV), the herpes simplex virus (HSV), and the cytomegalovirus (CMV) (Chee Wei Tan and Maciej Piotr Chlebicki, 2016; National Institute on Aging, 2011).

Diagnosis of urinary tract infection

The diagnosis of acute uncomplicated cystitis can be made with high probability based on a focused history of lower urinary tract symptoms (dysuria, frequency and urgency) in a patient who has no risk factors for complicated UTI. Urine dipstick analysis, as opposed to urinary microscopy, is a reasonable alternative to urine culture to diagnose acute uncomplicated cystitis. Urine cultures are recommended for patients with risk factors for complicated UTIs and in the following situations: (a) suspected acute pyelonephritis; (b) symptoms that do not resolve or recur within 2–4 weeks after completion of treatment; (c) women who present with atypical symptoms; (d) pregnant women; and (e) male patients with suspected UTI (Sabih and Leslie, 2021).

Analyzing a urine sample

Your doctor may ask for a urine sample for lab analysis to look for white blood cells, red blood cells or bacteria. To avoid potential contamination of the sample, you may be instructed to first wipe your genital area with an antiseptic pad and to collect the urine midstream.

Growing urinary tract bacteria in a lab

Lab analysis of the urine is sometimes followed by a urine culture. This test tells your doctor what bacteria are causing your infection and which medications will be most effective.

Creating images of your urinary tract

If you are having frequent infections that your doctor thinks may be caused by an abnormality in your urinary tract, you may have an ultrasound, a computerized tomography (CT) scan or magnetic resonance imaging (MRI). Your doctor may also use a contrast dye to highlight structures in your urinary tract.

Using a scope to see inside your bladder

If you have recurrent UTIs, your doctor may perform a cystoscopy, using a long, thin tube with a lens (cystoscope) to see inside your urethra and bladder. The cystoscope is inserted in your urethra and passed through to your bladder.

Diagnosis of cystitis

Cystoscopy

During this test, your doctor inserts a cystoscope — a thin tube with a light and camera attached — through the urethra into your bladder to view your urinary tract for signs of disease. Using the cystoscope, your doctor can also remove a small sample of tissue (biopsy) for lab analysis. But this test most likely won't be needed if this is the first time you've had signs or symptoms of cystitis.

Diagnosis of Pyelonephritis

Laboratory examination

Analysis of the urine may show signs of urinary tract infection. Specifically, the presence of nitrite and white blood cells on a urine test strip in patients with typical symptoms are sufficient for the diagnosis of pyelonephritis, and are an indication for empirical treatment. Blood tests such as a complete blood count may show neutrophilia. Microbiological culture of the urine, with or without blood cultures and antibiotic sensitivity testing are useful for establishing a formal diagnosis, and are considered mandatory.

Imaging studies

If a kidney stone is suspected, a kidneys, ureters, and bladder x-ray may assist in identifying radioopaque stones. Where available, a noncontrast helical CT scan

with 5 millimeter sections is the diagnostic modality of choice in the radiographic evaluation of suspected nephrolithiasis (Shailaja and Mohankumar, 2017). All stones are detectable on CT scans except very rare stones composed of certain drug residues in the urine. In patients with recurrent ascending urinary tract infections, it may be necessary to exclude an anatomical abnormality, such as vesicoureteral reflux or polycystic kidney disease. Investigations used in this setting include kidney ultrasonography or voiding cystourethrography. CT scan or kidney ultrasonography is useful in the diagnosis of *Xanthogranulomatous pyelonephritis*; serial imaging may be useful for differentiating this condition from kidney cancer. Ultrasound findings that indicate pyelonephritis are enlargement of the kidney, edema in the renal sinus or parenchyma, bleeding, loss of corticomedullary differentiation, abscess formation, or an areas of poor blood flow on doppler ultrasound. However, ultrasound findings are seen in only 20% to 24% of people with pyelonephritis (Thass *et al.,* 2019; Fenta *et al.,* 2020).

A DMSA scan is a radionuclide scan that uses dimercaptosuccinic acid in assessing the kidney morphology. It is now the most reliable test for the diagnosis of acute pyelonephritis.

Diagnosis of Urethritis

Urethritis is usually diagnosed through collecting history on the individual and through a physical examination. In females, urethritis can be diagnosed with a number of tests including: urine test, blood test, vaginal culture, cytoscopy, or a nucleic acid test. Women will also have abdominal and pelvic exams to check for urethral discharge, and tenderness of the lower abdomen or urethra. In men, urethritis is diagnosed by at least one of the following: mucopurlent or purulent urethral discharge on examination, ≥ 2 white blood cells per oil immersion field from a Gram stain of a urethral swab, or positive leukocyte esterase and/or ≥10 white blood cells per high power field of the first-void urine. Men who meet the criteria for urethritis commonly get Nucleic Acid Amplification Testing (NAAT) for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* to determine the type of urethritis. Men will have an exam on the abdomen, bladder area, penis, and scrotom. Additionally, a digital rectal examination of the prostate may be used if rectal pain is reported or if the individual is of older age (Pezzani and Antinori, 2018; Manandhar *et al.,* 2020).

Differential Diagnosis

- ✓ Acute pyelonephritis
- ✓ Bladder cancer
- ✓ Chlamydial genitourinary infections
- ✓ Cystitis
- ✓ Herpes simplex
- ✓ Interstitial cystitis
- ✓ Pelvic inflammatory disease
- ✓ Urethritis
- ✓ Vaginitis

Treatment of Urinary Tract Infection

Antibiotics usually are the first line treatment for urinary tract infections. Which drugs are prescribed and for how long depend on your health condition and the type of bacteria found in your urine.

Simple infection

Drugs commonly recommended for simple UTIs include:

- ✓ Trimethoprim/sulfamethoxazole (Bactrim, Septra, others)
- ✓ Fosfomycin (Monurol)
- ✓ Nitrofurantoin (Macrodantin, Macrobid)
- ✓ Cephalexin (Keflex)
- ✓ Ceftriaxone

The group of antibiotic medicines known as fluoroquinolones — such as ciprofloxacin (Cipro), levofloxacin and others — isn't commonly recommended for simple UTIs, as the risks of these medicines generally outweigh the benefits for treating uncomplicated UTIs. In some cases, such as a complicated UTI or kidney infection, your doctor might prescribe a fluoroquinolone medicine if there are no other treatment options (Sneka and Mangayarkarasi, 2019; Shamim and Ramalakshmi Sathiss, 2018).

Frequent infections

If you have frequent UTIs, your doctor may make certain treatment recommendations,

- ✓ Low-dose antibiotics, initially for six months but sometimes longer
- ✓ Self-diagnosis and treatment, if you stay in touch with your doctor
- ✓ A single dose of antibiotic after sexual intercourse if

your infections are related to sexual activity
- ✓ Vaginal estrogen therapy if you're postmenopausal

Severe infection

For a severe UTI, you may need treatment with intravenous antibiotics in a hospital.

Treatment of Urethritis

Antimicrobials are generally the drug of choice for gonococcal and non-gonococcal infections. The CDC in 2015 suggests using a dual therapy that consists of two antimicrobials that have different mechanisms of action would be an effective treatment strategy for urethritis and it could also potentially slow down antibiotic resistance.

Gonococcal urethritis

The CDC recommends administering an injection dose of ceftriaxone 250 mg intramuscularly and oral dose of azithromycin 1g simultaneously. Cefixime 400 mg oral single dose can be used as an alternative if ceftriaxone is not available.

Non-gonococcal urethritis

The CDC recommends administering an oral single dose of azithromycin 1g or a 7-day course of doxycycline 100 mg orally twice daily.

Alternative treatments can also be used when the above options are not available

- ✓ Erythromycin base 500 mg orally four times daily for 7 days
- ✓ Erythromycin ethylsuccinate 800 mg orally four times daily for 7 days
- ✓ Levofloxacin 500 mg orally once daily for 7 days
- ✓ Ofloxacin 300 mg orally twice daily for 7 days

If doxycycline was prescribed as initial therapy, give azithromycin 500 mg or 1 gram for the first day, then give azithromycin 250 mg once daily for 4 days plus metronidazole 400 – 500 mg twice daily for 5 days. If azithromycin was prescribed as initial therapy, then give doxycycline 100 mg twice daily for 7 days plus metronidazole 400 – 500 mg twice daily for 5 – 7 days. Moxifloxacin 400 mg orally once daily for 7 – 14 days can be given with use of caution, if macrolide-resistant *M. genitalium* infection is demonstrated (Manandhar *et al.,* 2020; Das *et al.,* 2018; National Institute on Aging, 2011).

Antibiotics used to treat UTI

Your body can actually get used to the antibiotics typically used to treat a urinary tract infection (UTI). This happens in people who have very frequent infections. With each UTI and use of antibiotics to treat it, the infection adapts and becomes harder to fight. This is called an antibiotic-resistant infection. Because of this, your healthcare provider may suggest alternative treatments if you have frequent UTIs. These could include:

Waiting: Your provider may suggest that you watch your symptoms and wait. During this time, you may be encouraged to drink plenty of fluids (especially water) in an effort to "flush out" your system.

Intravenous treatment: In some very complicated cases, where the UTI is resistant to antibiotics or the infection has moved to your kidneys, you may need to be treated in the hospital. The medicine will be given to you directly in your vein (intravenously). Once you're home, you will be prescribed antibiotics for a period of time to fully get rid of the infection (National Institute of Diabetes and Digestive and Kidney Diseases, National Kidney and Urologic Diseases Clearinghouse, 2011; Pezzani and Antinori, 2018).

Preventions of urinary tract infection

Drink plenty of liquids, especially water

Drinking water helps dilute your urine and ensures that you'll urinate more frequently — allowing bacteria to be flushed from your urinary tract before an infection can begin. Water is the best choice. Aim for 6 to 8 glasses per day. If it's hard for you to drink that much water, you can also increase your fluid intake by drinking sparkling water, decaffeinated herbal tea, milk, or smoothies made with fruits and vegetables. Try to limit or avoid alcohol and caffeinated drinks, which may irritate the bladder.

Drink cranberry juice

Although studies are not conclusive that cranberry juice prevents UTIs, it is likely not harmful. The berry has compounds called proanthocyanidins that may prevent

E.coli from adhering to tissues in the urinary tract. It's also thought that vitamin C in cranberries may increase the acidity of urine, which might reduce overgrowth of bad bacteria. Scientific research shows conflicting results. Some studies have found that cranberry extract reduces the frequency of UTIs, while others haven't found the same effect.

Though it's not clear if cranberries can prevent UTIs, it's a low-risk remedy. If you'd like to consume cranberries, opt for unsweetened, pure cranberry juice instead of sugary cranberry cocktails. You can also eat fresh or frozen cranberries (Sabih and Leslie, 2021).

Wipe from front to back

Doing so after urinating and after a bowel movement helps prevent bacteria in the anal region from spreading to the vagina and urethra. Since the rectum is a main source of *E.coli*, it's best to wipe your genitals from front to back after using the bathroom. This habit decreases the risk of bringing *E.coli* from the anus to the urethra.

It's even more important to do this if you have diarrhea. Having diarrhea can make it hard to control bowel movements, which may increase the chance of *E.coli* spreading to the urethra.

Empty your bladder soon after intercourse

Also, drink a full glass of water to help flush bacteria. Avoid holding in your urine, as this can encourage bacterial growth. Try not to wait more than 3 to 4 hours to urine, and completely empty your bladder each time. This is even more important if you're pregnant as pregnancy puts you at an increased risk for a UTI. Holding your urine can further increase the risk.

Avoid potentially irritating feminine products

Using deodorant sprays or other feminine products, such as douches and powders, in the genital area can irritate the urethra. Scented feminine products can disrupt this balance, allowing harmful bacteria to overgrow.

This can result in UTIs, bacterial vaginosis, and yeast infections. Scented bath oils, soaps, and bubble baths can also irritate the genital area and cause an imbalance in vaginal bacteria (Chee Wei Tan and Maciej Piotr Chlebicki, 2016).

Change your birth control method

Diaphragms, or unlubricated or spermicide-treated condoms, can all contribute to bacterial growth.

Take probiotics

Probiotics are live microorganisms that can increase good gut bacteria. They may also help promote the growth of good bacteria in the urinary tract. This could help protect you from getting a UTI. Generally, *Lactobacilli* strains have been associated with less frequent UTIs. There are several ways you can take probiotics to boost the health of your urinary tract, including:

- ✓ eating fermented foods, such as yogurt, kefir, sauerkraut, or tempeh
- ✓ taking probiotic supplements
- ✓ using probiotic suppositories

Get antibiotics

If you get UTIs that don't respond well to treatment or keep coming back, your doctor might recommend a small daily dose of oral antibiotics. This can help prevent a UTI by controlling harmful bacteria. You'll likely have to take the antibiotics after sex or when you first notice UTI symptoms. The drawback, however, is that prolonged antibiotic use can lead to antibiotic resistance. Your doctor can determine if this is the right prevention method for you (Ana L. Flores-Mireles *et al.,* 2015).

Materials and Methods

Study Area and Period

This study was conducted in the Bacteriology laboratory, Department of Microbiology, Pondicherry Institute of Medical Sciences (PIMS), Pondicherry, India over a period of two months from December 2020 to January 2021.

Sample Collection

The samples were collected from both inpatients and outpatients with complaints of fever, dysuria, burning micturition, lower abdominal or flank pain and post renal transplant. The Clean Catch Midstream urine samples (CCMSU) were collected in a sterile, screw capped

container from suspected patients of all age groups. In some of the cases where the patients are non-ambulatory urine sample has been collected from urinary catheter.

Study Population

The urine samples of two hundred patients were collected. Out of 200 patients 106 patients were female, 94 patients were male. The age of patients also reported in this study was 15% were 0-30 years old, 25% were 31-50 years old, 60% were above 50 years old. A total of 200 urine samples were collected 170 samples come from inpatients ward and 30 urine samples come from outpatients ward.

Requirements of Materials and Reagents

The materials used for urine culturing and sensitivity were Bunsen burner, Loop calibrated for 0.01 ml, straight wire, Incubator at 37^0C, Light microscope, Dispose jar, Applicator sticks, Immersion oil, Glass slides, Distilled water, Saline, Sterile glass tubes, tissue paper, staining rack, Antibiotic discs, Cover slips, Forceps and oxidase paper. The reagents used in culture such as gram stain reagents (gram's crystal violet, gram's iodine, acetone alcohol, dilute carbolfuschin), Hydrogen peroxide (H_2O_2), Kovac's reagent, plasma and Ferric chloride reagent. For biochemical tests, requirements of agar such as Peptone broth, nutrient broth, triple sugar iron agar, citrate, urease, mannitol motility medium, bile esculin agar, phenyl pyruvic acid medium, 2% sugars (dextrose, lactose, maltose, sucrose, glucose, galactose) and chromagar.

Macroscopic and Microscopic Appearance

When samples received for culture testing, first observe the sample and note down the macroscopic appearance of urine samples whether the samples were clear and turbid. Afterwards for microscopic observation, take a loop full of non-centrifuged urine sample was placed on a clean glass slide and the sample was covered with a coverslip and look down the number of pus cells under a High Power Fluorescence (pc/HPF) in light microscope.

Culture and Processing

Urine culture was done by a semi quantitative method in which sterile non-centrifuged urine samples was inoculated in a calibrated wire loop that held 0.01ml of urine. Before inoculating the petri plates, the surface of medium should be dried in incubator at 37°C. A loop full of urine sample was streak on a Cysteine-Lactose Electrolyte Deficiency agar (CLED agar) and Mac-Conkey agar plates. The inoculated agar plates were incubated for 24 to 48 hours at 37^0C. After incubation of plates; number of colonies grown has been counted and multiplies the number by 100 which give colony forming units per ml of urine. The number of bacterial colonies that grew $>10^5$ CFU/ml was considered as significant growth of bacteria. The count of bacterial colonies that grew between 10^3 CFU/ml-10^4CFU/ml was considered as probably significant growth in culture. If the plate has insignificant bacteriuria after 24 hours, it is considered as no growth in culture. The culture yields three or more than 3 types of colony have grown, it is considered as mixed growth and also patients has requested to repeat urine culture. The size, shape and color of these colonies help to identify which type of bacteria were present and the number of colonies represents the quantity of bacteria initially present in the urine sample. If a clean catch midstream urine sample was collected for the test, only bacteria causing a UTI were present. Generally, this will be a single type of bacteria that will be present in relatively large numbers. Sometimes, more than one type of bacteria will be present. This may be due to an infection that involves more than one pathogen. The lactose fermenting and non-lactose fermenting colonies were considered as Gram negative bacilli while the white opaque, yellow opaque, minute pink colonies and magenta pink colonies were considered as Gram positive cocci. The dry white colonies were considered as yeast.

Identification and Bacterial Isolation

The identification of bacterial colonies was done by gram staining and biochemical tests. After culturing, identify if the organisms has been gram positive or gram negative by the gram staining using microscope.

Preparation of smear: A drop of saline was mixed with single colony and spread evenly on a clean glass slide to make a thin layer of smear. The smear is allowed to air dry for some time and then fix the smear by passing the slide 2 to 3 times on a flame of spirit lamp of a Bunsen burner.

Gram stain: The smear fixed slide was placed on the staining rack. Then the smear was stained with crystal violet solution for 30 to 60 seconds. The slide was washed off with water. The slide was covered with gram's iodine for 30 to 60 seconds. The slide was again

washed off with water. Then decolorize with acetone alcohol for few seconds. The slide was rinse with water. Then the slide was stained using dilute carbolfuschin for 30 seconds. Again washed off with water and the slide was allowed to air dry. The organisms were observed under high power in the microscope through oil immersion objective. Gram positive organisms appear in dark purple color because gram positive bacteria which retain crystal violet stain even washed with acetone alcohol while the gram negative organisms would appear in pink color because gram negative bacteria which cannot retain crystal violet stain. Yeast cells also appear in dark purple color.

Biochemical tests: These biochemical tests were used for confirmation of organisms. For gram negative bacilli were identified by indole test, triple sugar iron agar, citrate agar, urease, Mannitol motility medium, phenyl pyruvic acid medium, and oxidase test. The gram positive cocci can be in two forms, one is GPC in clusters and second one is GPC in pairs and short chains. The GPC in clusters were identified by biochemical tests such as catalase, tube coagulase and Mannitol. For GPC in pairs and short chains were identified by catalase, bile esculin, mannitol motility medium and arabinose. Sometimes yeast is also present in culture. Yeasts were identified by chromagar, germ tube test and sugar fermentation test. A single colony was taken in straight wire and streak on the peptone broth, triple sugar iron agar, citrate, urease, mannitol motility medium, and phenyl pyruvic acid medium, bile esculin and arabinose. In tube coagulase test, add 0.5ml of plasma with nutrient broth and emulsify few colonies in the test tubes. The inoculated test tubes were incubated in incubator for overnight at 37^0C. The TSI agar changed into black color due to H_2S gas formation indicates identification of *Proteus mirabilis*.

Oxidase test was done by touching the colony using applicator sticks and smear it on oxidase paper. The bile esculin agar indicates identification of Enterococcus species. Then observe the development of purple color within few seconds. The positive results shown in oxidase test indicate confirmation of Pseudomonas Species. Catalase test was used to identify the Gram positive Cocci and a few drops of hydrogen peroxide solution placed on a slide and remove few colonies using applicator sticks on the solution. Observe the active bubbling in the solution on the slide. CAMP (Christie, Atkins, Munch and Peterson) test was used to identify the Group B Streptococcus. In a blood agar plate, streak a staphylococcal strain across center of the plate and the testing organism streak at right angles to it and also inoculate *Streptococcus agalactae* and *Streptococcus pyogenes*. This plate was incubated at 37^0C. For indole test, colony was inoculated in peptone broth. After incubation for overnight then add Kovac's reagent and observe the development of red/pink ring at the junction of the medium. The indole test indicates identification of *E.coli*. Germ tube test was done using 0.5 ml of human serum was taken in the test tube with the help of sterile calibrated loop inoculate the yeast colony in the serum. Then the tube was kept in incubated in incubator at 37ºC for 2 hours. After incubation, the drop of yeast culture was placed on the glass slide and covered using coverslip. The slide was observed under 10X and 40X to look for tube like outgrowth from the cells. The chromagar was used to identify the Candida species.

Antibiotic Susceptibility Testing (AST)

The antibiotic susceptibility testing of pathogenic isolates in urine culture was determined by the Kirby-Bauer Diffusion method on the Mueller-Hinton agar. Ampicillin-20µg, Gentamicin-10µg, Amikacin-30µg, Ciprofloxacin-5µg, Cefazolin-30µg, Imipenem-10µg, Meropenem-10µg, Cotrimoxazole-10µg, Piperacillintazobactam-10µg, Cefoperazon-sulbactam-10µg, Nitrofurantoin-300µg, Norfloxacin-10µg, Ceftriazone-30µg, Ceftazidime-30µg, Chloramphenicol-30µg, Cefotazime-30µg, Penicillin-15µg, Clindamycin-2µg, Cefoxitin-30µg.

Linezolid-30µg, Ofloxacin-5µg, Erythromycin-15µg, Vancomycin-30µg, Gentamicin (HLG)-120µg and Fluconazole-150µg were the following antibiotic discs mostly used in the susceptibility testing in urine. In broth, a single colony was inoculated and kept in incubator for 2 hours. Before streaking on agar antibiotic discs name was written in short form. For Gram Negative Bacteria, peptone broth was used while Gram Positive Bacteria and Yeast cells, nutrient broth were used. After 2 hours, a cotton swab was dipped in the broth and then streaking on the Mueller-Hinton agar. A cotton swab was properly discarded in dispose jar. Afterwards antibiotic discs were placed in the Mueller-Hinton agar using forceps. Then the agar was incubated in incubator for overnight at 37ºC. The next day, zone of inhibition was formed around the disks.

Then the diameter of zone was measured in the following laboratory standard operating procedures. For yeast cells,

fluconazole disc was placed in Mueller-Hinton agar with dextrose indicator. Results were interpreted as Sensitive, Resistant and Intermediate based on CLSI guidelines. Multi- drug resistance was considered as an isolates tested two or more than two antibiotic drugs shows resistance.

Results and Discussion

Statistical Analysis of the Urine Samples

In this observational study, 200 patients with the urinary sample, among these 106 patients (53%) were female and 94 patients (47%) were male. Out of 200 patients, 10 patients (4%) were belonging to the age of 1-19 years, 120 patients (60%) were belonging to the age of 20-60 years and 70 patients (36%) were belonging to the age of ≥60 years.

Microbial analysis of the urine samples

The processed urine specimens were analysed for various microbial growth. Majority (107,53.5%) of the samples were reported as significant growth, 70 (25%) were reported as no growth, 18 (9%) were reported as normal urethral flora, and 5 (2.5%) were reported as ≥ 3 types of organism grown in the culture. 57% of were caused by *E.coli*, 12% were caused by *Klebsiela pneumoniae*, 2.8% (3) were caused by *Klebsiela species*, and 1.8% (2) was Streptococcus, 0.9% (1) were affected *Acintobacter baumanii*, 0.9% (1) *Staphylococcus aureus,* 3.7% (4) were affected by *Proteus mirabilis*, 3.7% (4) were affected by *Pseudomonas aeruginosa*, 3.7% (4) were affected by *Enterococcus faecium*, 3.7% (4) were affected by *Enterococcus faecalis*, 1.8% (2) were affected by *Citrobacter diversus*, 0.9% (1) were affected by *Citrobacter freundii*, 2.8% (3) were affected by Candida species, 0.9% (1) were affected by *Candida krusei*, 2.8% (3) were affected by *Candida albicans.*

Analysis of Antibiotic Susceptibility Testing (AST)

The drug susceptibility testing for Gram Negative Bacteria shows most sensitive to Amikacin, Piperacillin-tazobactam, Nitrofurantoin and Cefoperazone-sulbactam while shows most resistant to Ampicillin, Cefazolin and Norfloxacin. The Enterococcus was most sensitive to Vancomycin and Linezolid. The Streptococcus was 100% sensitive to all drugs but resistant to Bacitracin. The Staphylococcus was 100% sensitive to all drugs but resistant to penicillin. The Pseudomonas species were 75% sensitive to Gentamicin, Norfloxacin, Imipenem, Meropenem, Piperacillintazobactam, Amikacin, Ciprofloxacin and Ceftazidime while 25% resistant to above mentioned drugs. The yeast was 86% sensitive to Flucanozole.

This study was demonstrates overall prevalence of UTI in examined urine specimens was 53.5%. Approximately 56% of women will suffer from UTI during their lifetime. According to patients' gender in this study, total uropathogens were significantly isolated from females 60/107 (56%) compared to males 47/107 (44%).

Females show higher percentage than the males because females were more susceptible to UTI because the urethra was shorter and closer to the anus i.e. bacteria can easily reach the urinary tract. The distribution of infection among patients' age groups was higher (62%) in adults (20-60 years) compared to other groups. This might be due to adults in this age group being more sexually active which might predispose them to UTI, followed by above 60 years (36%) then elderly under 20 years (2%). Regarding etiological agents, gram-negative bacteria were the most common isolates in this study, which is identical with other reports from different areas. *E.coli* was the most common bacterial isolates in both inpatients and outpatients.

The present study detected the dominance of *Escherichia coli* (57%) and *Klebsiella pneumoniae* (12%) which was with different rates when compared with other studies in Libya and other countries. In Northwest Libya, Abujnah *et al., 2015* has found predominance of *Escherichia coli* (55.8%) and *Klebsiella pneumoniae* (18.5%) (Das *et al.,* 2018). In Messalata, Libya, Mohammed *et al.,* has reported the predominance of *Escherichia coli* (55.6%) and *Klebsiella pneumoniae* (16.3%). In Southern Tunisia, The authors have found *Escherichia coli* (67.1%) and *Klebsiella pneumoniae* (12.8%) as predominance uropathogens among patients of UTIs. Studies in Poland have reported uropathogens with a predominance of *Escherichia coli* (73%) and *Proteus spp.* (8.9%). Similar prevalence rate have been reported by Urvasi Chongtham *et al.,* (2013) found *E.coli* (43.16%) was the predominant growth followed by *Klebsiella pneumoniaee* (17.89%) (Urvashi Chongtham *et al.,* 2013). Another study in North India, authors reported the predominant growth of in their study was *E.coli* (53.9%) followed by *Klebsiella* species (24.9%).

Table.1

Age	No of patients	Percentage
1-19 years	10	4
20-60 years	120	60
≥60 years	70	36

Figure.1

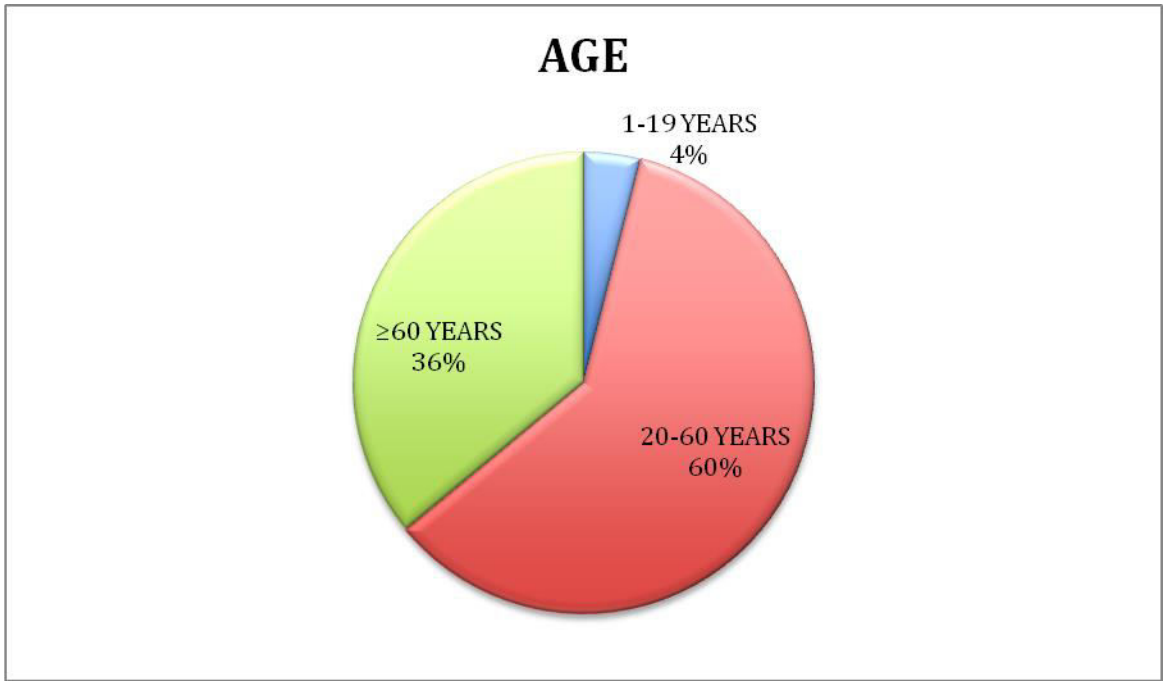


Table.2

Sex	No of patients	Patients with significant bacteria	Percentage
Male	106	60	57%
Female	94	47	43%

Figure.2

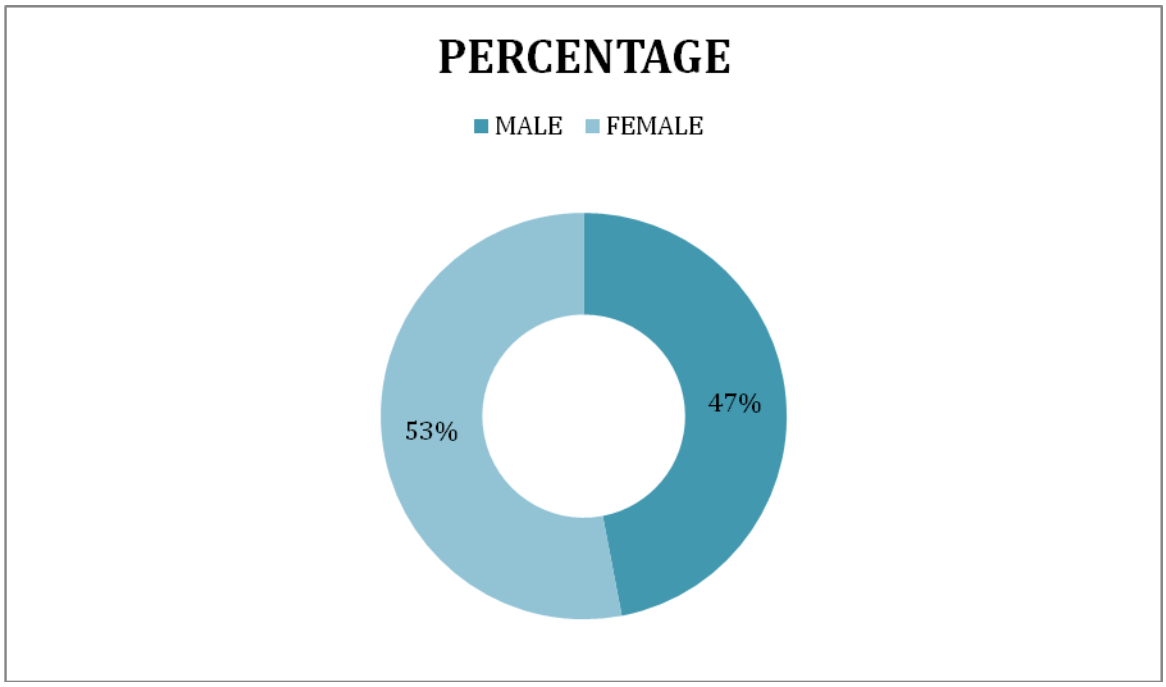


Table.3 Morphological characteristic on CLED agar and gram reaction

Organisms	CLED agar	Gram Stain Reaction
E.coli	Moist, Yellow LF colonies	Gram – negative bacilli (rod-shaped bacteria)
K.pneumoniae	Large, Mucoid, LF colonies	Gram – negative bacilli (rod-shaped bacteria)
Pseudomonas aeruginosa	Moderate, moist, NLF colonies	Gram – negative bacilli (rod-shaped bacteria)
Proteus mirabilis	Moderate, moist, blue-green NLF colonies	Gram – negative bacilli (rod-shaped bacteria)
Staphylococcus aureus	Small, moist, White opaque colonies	Gram – positive cocci (round-shaped bacteria)
Streptococcus agalactae	Minute pink colonies	Gram – positive cocci (round-shaped bacteria)
Enterococcus species	Pink colonies	Gram – positive cocci (round-shaped bacteria)
Candida species	Small, Dry, white colonies	Gram positive

Table.4 Biochemical tests of isolated Gram- negative bacteria

Species	Biochemical tests								
	Indole test	Citrate test	Urease test	MMM		Triple sugar iron agar (TSI)			
				Motility	Ferment	Slant	Butt	Gas	H_2S
E.coli	+	-	-	M	+	A	A	+	-
K.pneumoniae	-	+	+	M	-	A	A	+	-
Ps.aeruginosa	-	+	-	NM	+	K	K	-	-
P. mirabilis	-	-	+	M	+	K	A	+	+
C. diversis	+	+	-	M	+	A	A	+	-
C.frondii	-	+	V	M	+	A	A	+	+
A.baumanii	-	+	-	NM	-	K	K	-	-
Enterobacteraerogens	-	+	-	M	+	A	A	+	-

Species	Biochemical tests	
	Oxidase test	PPA medium
E.coli	Negative	Negative
Pseudomonas aeruginosa	Positive	Not applicable
Klebsiella pneumoniaee	Negative	Negative
Proteus mirabilis	Negative	Positive
Citrobacter diversis	Negative	Negative
Citrobacter freundii	Negative	Negative
Acinetobacter baumanii	Negative	Negative

Table.5 Biochemical tests of isolated Gram-positive bacteria

Species	Catalase	Coagulase	Arabinose	Mannitol	CAMP test	Bile esculin
E.faecium	NA	NA	Positive	Positive	NA	Positive
E. faecalis	NA	NA	Negative	Positive	NA	Positive
Staphlococcus aureus	Positive	Negative	Negative	Positive	NA	Negative
Streptococcus agalactae	Negative	Negative	Negative	Negative	Positive	Negative

Table.6 Biochemical tests of isolated fungi or yeasts

Species	Glu	Lac	Suc	Mal	Gal	Tre	GTT	Chromagar
Candida albigans	F	NF	F	F	NF	NF	(+)	Green colonies
Candida krusei	F	NF	F	NF	NF	NF	(-)	Pink Colonies

Table.7

Category	No. of Patients	Percentage	Female	Male
Significant Bacteriuria	107	53.5%	60	47
Probably significant Bacteriuria	70	35%	33	37
Normal Urethral Flora	18	9%	10	8
More than 3 types of organisms	5	2.5%	3	2

Table.8

Gram Negative Organisms				
Organisms		**Percentage**		**Total**
Escherichia coli		57%		61
Klebseilla pneumoniae		12%		13
***Klebseilla* species**		2.8%		3
Pseudomonas aeruginosa		3.7%		4
Acinetobacter baumanii		0.9%		1
Citrobacter diversis		1.8%		2
Citrobacter freundii		0.9%		1
Proteus mirabilis		3.7%		4

Table.9

Gram Positive Organisms			
Organisms		**Percentage**	**Total**
Enterococcus faecium		3.7%	4
Enterococcus feacalis		3.7%	4
Staphylococcus aureus		0.9%	1
Streptococcus		1.8%	2

Table.10

Yeast				
Organisms		**Percentage**		**Total**
***Candida* species**		2.8%		3
Candida albicans		2.8%		3
Candida krusei		0.9%		1

Table.11 Antibiotic susceptibility testing for Gram Negative Organisms

***E.coli, Proteus mirabilis, Klebsiella pneumoniaee, Klebsiella* species, *Acinetobacter baumanii*, *Citrobacter diversus* and *Citrobacter freundii* (n=85)**			
Antibiotics	**Sensitive**	**Resistant**	**Intermediate**
Ampicillin	8	77	-
Gentamicin	59	24	2
Cefazolin	21	64	-
Nitrofloxacin	31	54	-
Nitrofurantoin	64	18	3
Piperacillintazobactam	68	13	4
Ceftriaxone	32	53	-
Amikacin	77	8	-
Ciprofloxacin	29	52	4
Cefoperazone-sulbactam	60	19	6

Figure.3

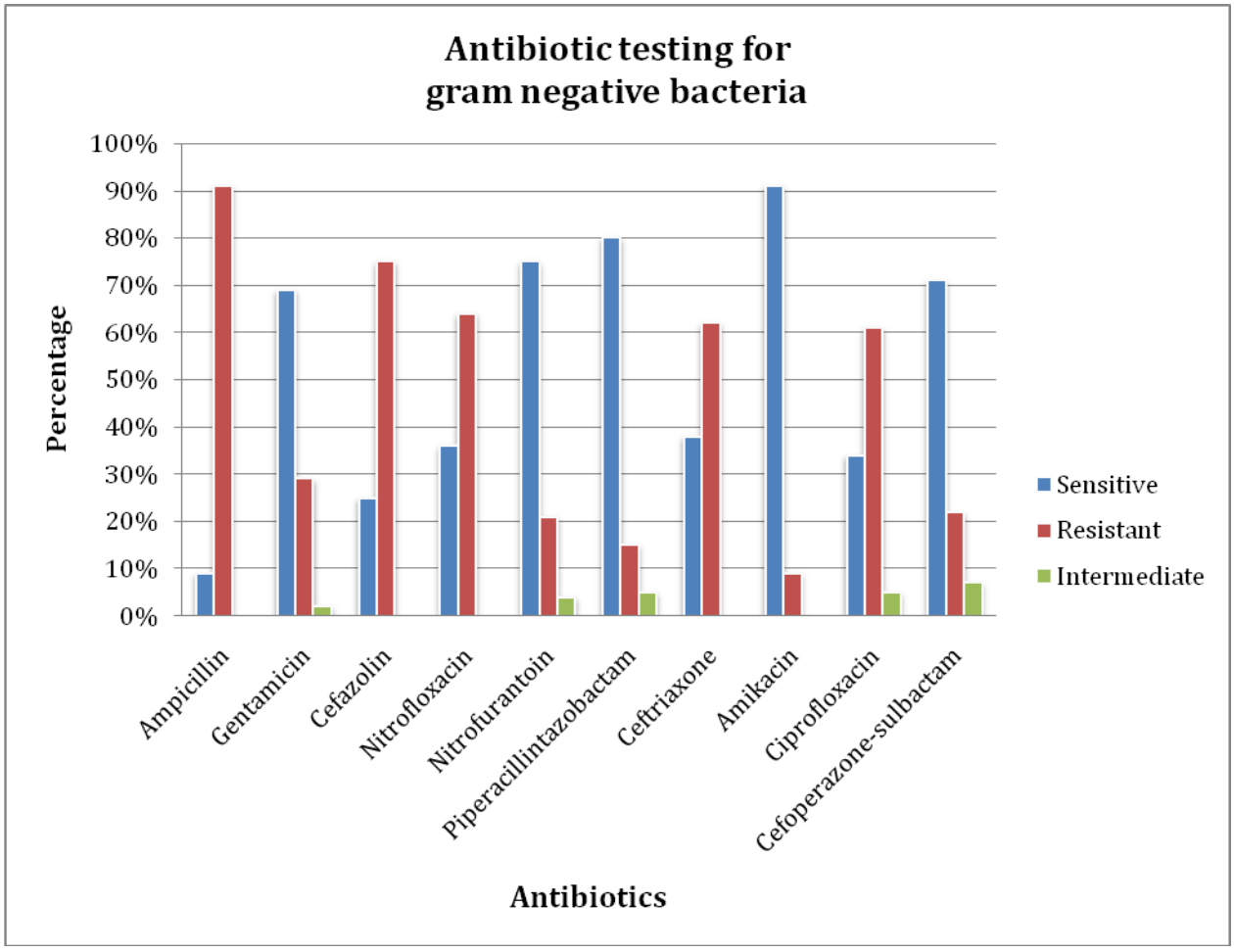


Table.12

Pseudomonas aeruginosa (n=4)		
Antobiotics	**Sensitive**	**Resistance**
Gentamicin	3	1
Nitrofloxacin	3	1
Imipenem	3	1
Meropenem	3	1
Piperacillintazobactam	3	1
Amikacin	3	1
Ciprofloxacin	3	1
Ceftazidime	3	1

Figure.4

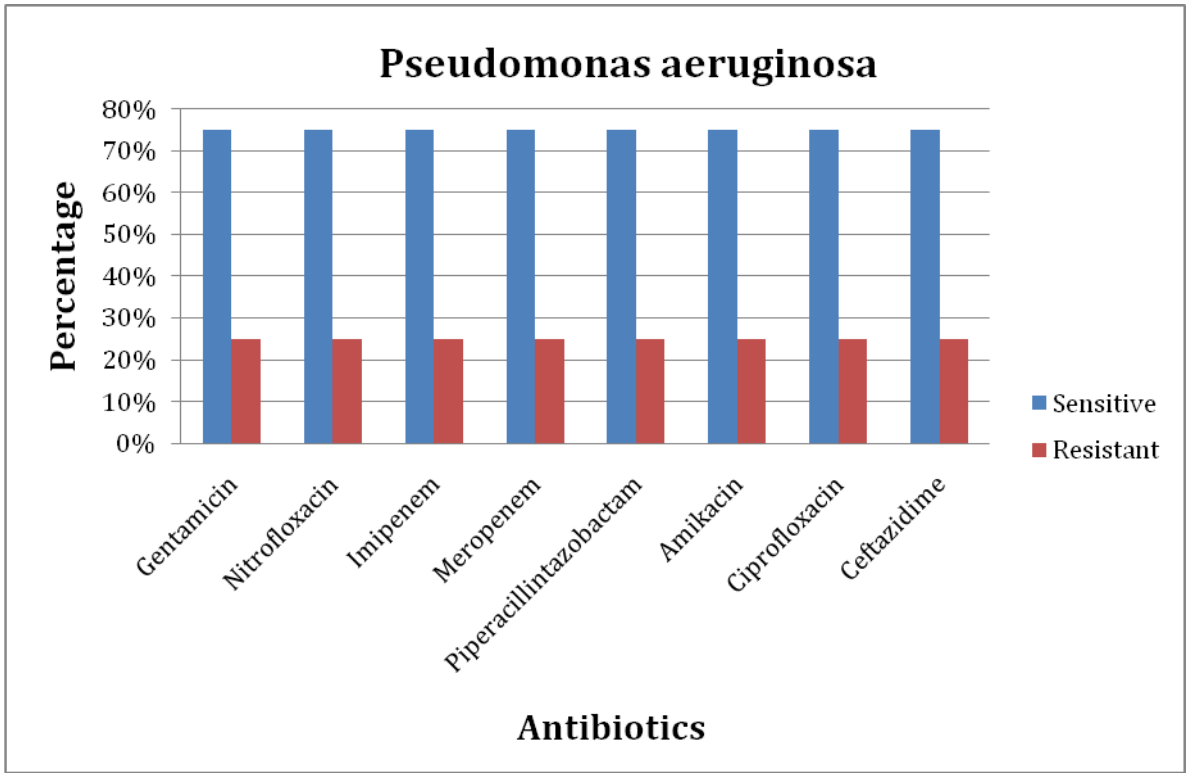


Table.13 Antibiotic sensitive and resistance pattern for Gram Positive Organisms

Enteroccoccusfaecium and Enterococcus faecalis (n=8)		
Antibiotics	**Sensitive**	**Resistance**
Ampicillin	6	2
Nitrofloxacin	5	3
Nitrofurantoin	6	2
Ciprofloxacin	5	3
Vancomycin	7	1
Linezolid	7	1
Gentamicin (HLG)	6	2

Figure.5

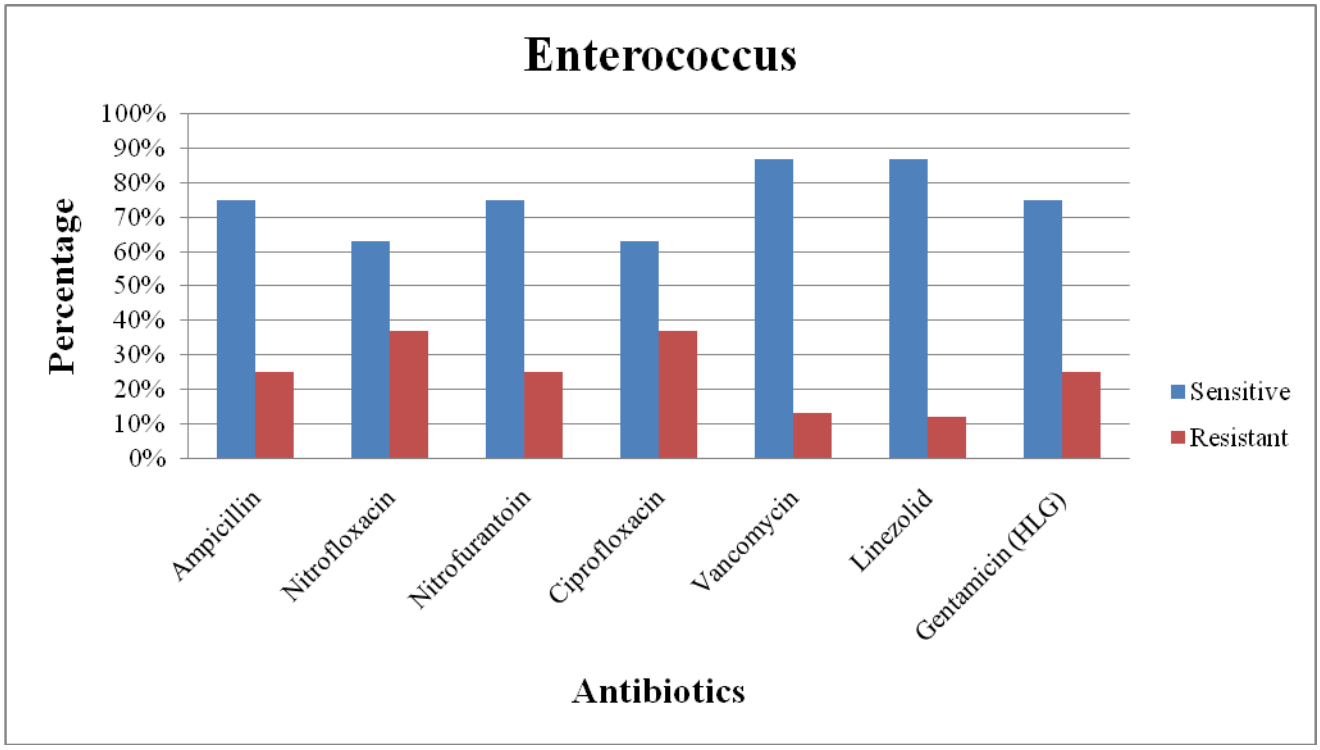


Table.14

Staphylococcus (n=1)		
Antibiotics	**Sensitive**	**Resistant**
Gentamicin	1	-
Cefazolin	1	-
Nitrofloxacin	1	-
Ciprofloxacin	1	-
Co-trimoxazole	1	-
Linezolid	1	-
Penicillin	-	1
Cefoxitin	1	-
Clindamycin	1	-
Novobiocin	1	-

Figure.6

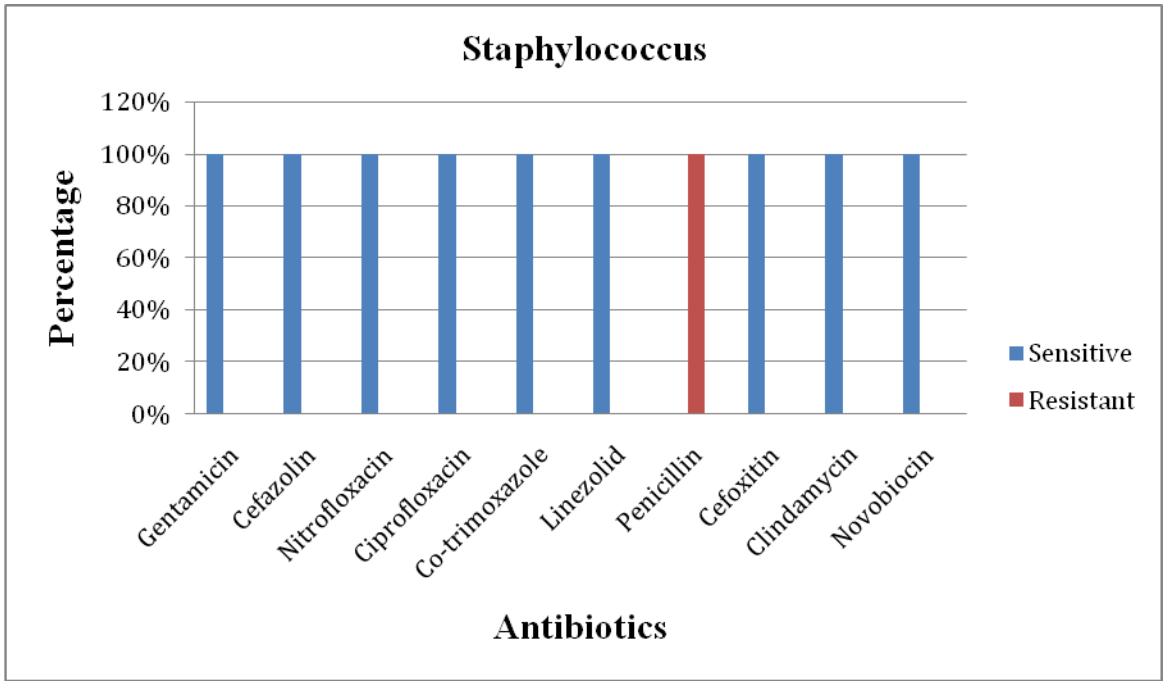


Table.15

Streptococci (n=2)		
Antibiotics	**Sensitive**	**Resistant**
Ampicillin	2	**-**
Co-trimoxazole	2	-
Cefotaxime	2	-
Clindamycin	2	-
Erythromycin	2	-
Bacitracin	2	2

Figure.7

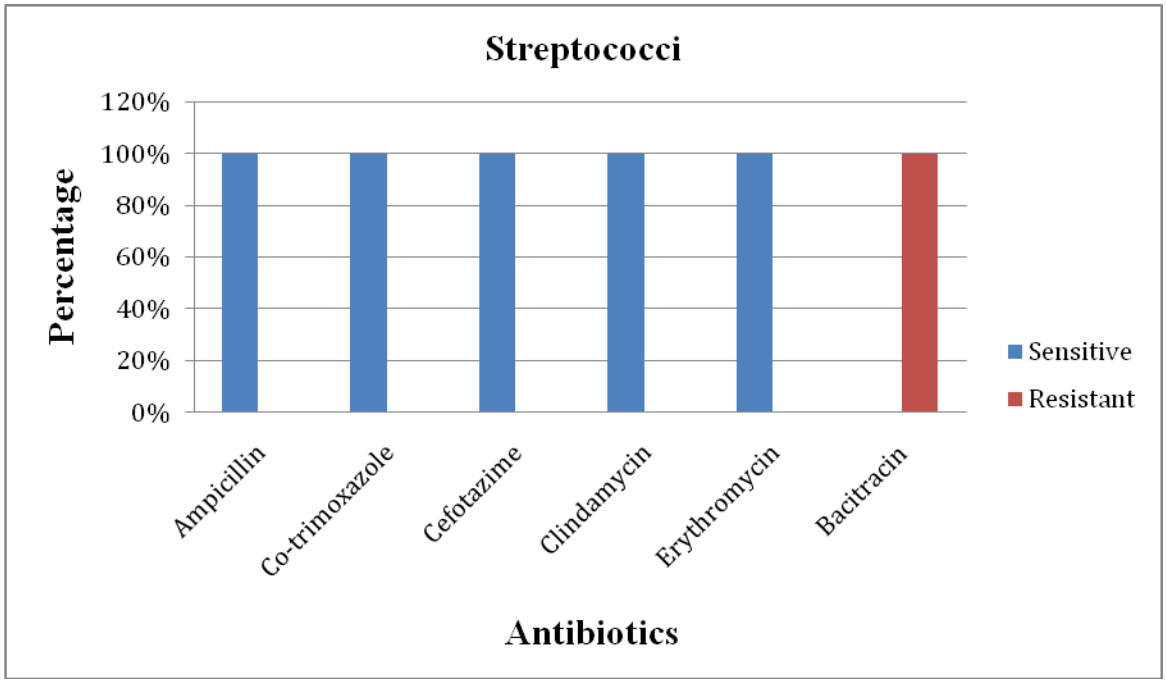
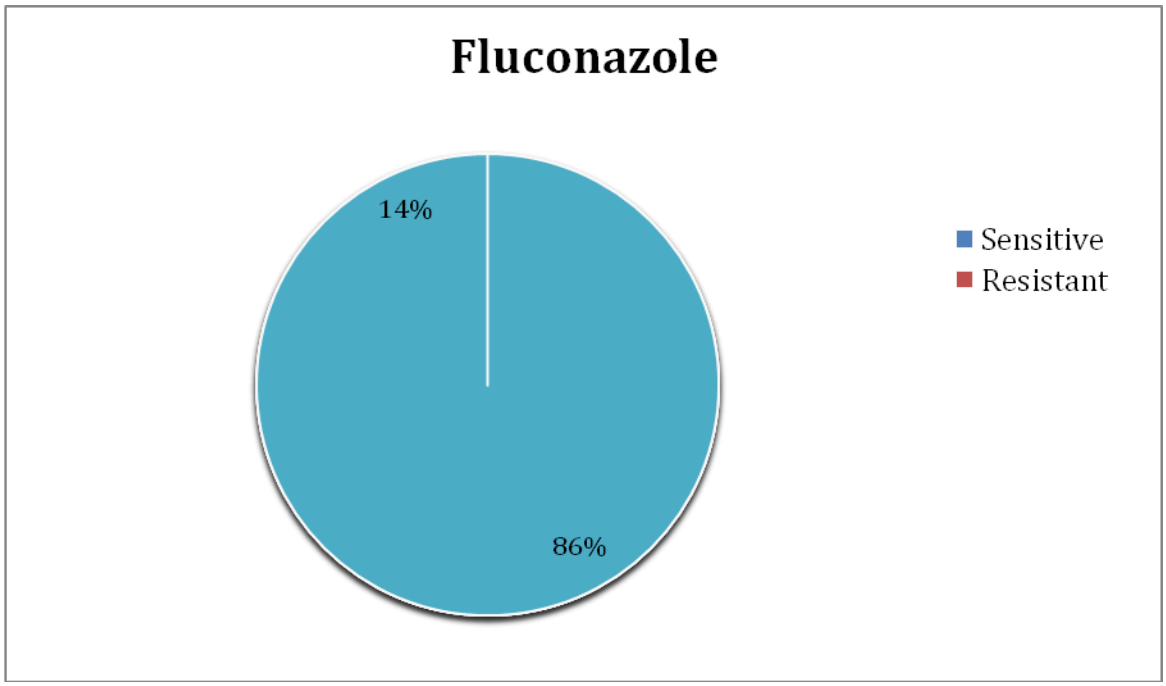


Table.16 Antibiotic Sensitive and Resistant pattern in yeast

Candida species, Candida albigans and Candida krusei (n=7)		
Antibiotics	**Sensitive**	**Resistant**
Flucanozole	6	1

Figure.8



In this study, isolation of some other organisms to cause UTI such as Citrobacter species and other non-lactose fermenter gram negative bacilli including *Pseudomonas aeruginosa*, *Acinetobacter baumanii* and *Proteus mirabilis*. In this current study, gram positive isolates detected the dominance of Enterococcus species (7.48%) (Thass *et al.,* 2019). Pseudomonas isolates in the current study showed 75% sensitivity to Amikacin, Piperacillintazobactam, Gentamicin and Norfloxacin, a compatible result with that in a study that has been conducted in Messalata, Libya (Pharsuram Adhikari *et al.,* 2020; Mohamed Abdel-Aziz Elzayat *et al.,* 2017).

On the other hand, other gram negative uropathogens exhibited high sensitivity to Cefoperazone-sulbactam, followed by Ceftazidime, Imipenem and Meropenem, then Trimethoprim/sulfamethoxazole, which was inconsistent with the result in other studies in Libya, however, gram positive isolates (*S. aureus*) were extremely resistant to Penicillin (Dave *et al.,* 2018; Fenta *et al.,* 2020). In this current study, gram positive isolates fully sensitive to Vancomycin, Linezolid, Clindamycin and Cotrimoxazole. UK. Das *et al.,* (2018) reported Gram positive isolates was sensitive to Vancomycin (95.33%) and Linezolid (93.62%). Acquiring of UTI starts with periurethral contamination by uropathogens inhabiting in the gut, followed by colonization of the urethra and successive migration of the pathogen to the bladder (Timothy *et al.,* 2014; Pegu *et al.,* 2014). The emergences of resistant strains among uropathogens are increasing with different resistant pattern. Acquisition of resistance might be either mutational or acquisition of new genetic material from other bacteria (Urvashi Chongtham *et al.,* 2013). Multiple drug resistance (MDR) was observed in most of the isolated bacteria. Some of the bacilli like *Klebsiella pneumoniaee*, *Escherichia coli* and *Acinetobacter baumanii* were resonance to MDR.

In *Klebsiella* species, uropathogens show high rate of 100% resistant to Ampicillin, Cefazolin, Nitrofurantoin and 75% resistant to Nitrofloxacin, Ciprofloxacin, Cefoperazone-sulbactam, etc.,. Some rare case, *Pseudomonas aeruginosa* shows Multi-drug resistant in this present study. Most strains of *Klebsiella pneumoniae* and *E.coli* in this present study found very high resistance to more than two drugs (Shamim and Ramalakshmi Sathiss, 2018). reported multiple drug resistance in uropathogenic *E.coli*, which could be due to its widespread prevalence in the community. Lack of guidelines for appropriate use of Antimicrobial agents is another factor responsible for spread of resistance (Lautika Sonkar *et al.,* 2020). The presence of complicated UTI improved the predictable symptoms to diagnose UTI such as diabetes and fever, dysuria and diabetes, dysuria and fever (Sneka and Mangayarkarasi,

2019). Diabetic mellitus has a number of long term effects on the genitourinary system. Diabetic nephropathy is one of the many factors that make these patients more prone to UTI than non-diabetes (National Institute of Diabetes and Digestive and Kidney Diseases, National Kidney and Urologic Diseases Clearinghouse, 2011).

Author Contribution

S. Kowsalya: Investigation, formal analysis, writing—original draft. C. Meghna: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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