

STUDY ON ANTIBIOTICS RESISTANCE FROM BACTERIA ISOLATED FROM UTI

Submitted in partial fulfilment of the requirements for the award of
Bachelor of Science degree in Biotechnology

by

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**SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
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This to certify that this project is the bonafide work of **P. Mahalakshmi (38770010)** who carried out the project entitled “**STUDY ON ANTIBIOTICS RESISTANCE FROM BACTERIA ISOLATED FROM UTI**” under my supervision from October 2020 to march 2021.

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DECLARATION

I P. Mahalakshmi (38770010) hereby declare that the project report entitled **“STUDY ON ANTIBIOTICS RESISTANCE FROM BACTERIA ISOLATED FROM UTI”** done by under guidance of Mr. G.Narendrakumar, Department of biotechnology is submitted in partial fulfilment of the requirements for the award of Bachelor of Science in Biotechnology.

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ABSTRACT

Urinary tract infection is one of the commonest infections to affect humans. Uncomplicated infections occur most commonly in otherwise healthy women when uropathogenic bacteria, usually *Escherichia coli*, ascend from the perineum into the bladder and overcome host innate immunity. Complicated infections occur in patients with an anatomical or functional abnormality of the urinary tract. The diagnosis is made on the basis of symptoms and diagnostic precision is improved by urinalysis. Urine culture is important with severe, recurrent or complicated infection and when the diagnosis is unclear, for example, in children and the elderly. Most women with symptoms that resolve quickly do not require further investigation but in children, men and patients with recurrent or severe infection, imaging of the renal tract, functional testing and cystoscopy should be considered to exclude an underlying abnormality. Empirical antibiotic treatment started on the basis of symptoms and directed by urinalysis is suitable for uncomplicated cystitis but should be altered based on culture results for more severe infections. Three days' antibiotic treatment is usually sufficient for uncomplicated cystitis in women. Long-term or post-coital antibiotics are effective treatments for patients with recurrent infection in whom non-antibiotic strategies have failed.

Keywords

Antibiotics; Cystitis; Host Defence; Pyelonephritis; Urinary Tract Infection

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CHAPTER-1

INTRODUCTION

Urinary tract infections (UTIs) are infections that begin in the urinary tract. UTIs that involve only your urethra or bladder are painful and inconvenient. However, if the infection spreads up your urinary tract and into your kidneys, it may have more serious consequences.

The kidneys, ureters, bladder, and urethra make up the urinary system. Both of these things contribute to the removal of waste from the body. The kidneys are a pair of bean-shaped organs located in the upper posterior abdomen that filter waste from the blood. Urine is transferred from your kidneys to your bladder through the ureters. Urine is stored in the bladder until it leaves the body via the urethra. Although the majority of urinary tract infections affect the lower tract, any or all of these components can become contaminated (the urethra and the bladder).

Antibiotics are often used to treat urinary tract infections. However, there are steps you can take to reduce the chances of having a UTI in the first place.

1.1 URINARY TRACT INFECTION

A urinary tract infection (UTI) is caused by microorganisms that invade the urethra and bladder and cause infection. The bladder and urethra are the most often infected parts of the urinary tract. Women are more likely than men to develop a urinary tract infection. UTIs are 50 times more common in women than in men among adults aged 20 to 50. Women are around 50 times more likely than men to have UTIs. The most common UTIs in this age group are cystitis or pyelonephritis. Most UTIs in men of the same age group are urethritis or prostatitis.

1.2 ORGANISM RESPONSIBLE FOR CAUSING UTI:

- *Escherichia coli*
- *Klebsiella species*
- *Pseudomonas*
- *Staphylococcus*

- *Enterococcus*
- *Proteus*

1.2.1 *ESCHERICHIA COLI*:

E. coli is a gram negative bacteria ,it has the ability to bind to the bladder wall .The bacteria *E. coli* will cause a urinary tract infection if they enter the urinary tract (UTI). According to a report published in Emerging Infectious Diseases, *E. coli* is responsible for over 85% of all UTIs. If *E. coli* is found in faeces that migrate to the opening of the urinary tract and into the bladder during intercourse, it is dangerous .*E.coli* bacteria is isolated in MacConkey agar.



Figure1.1 *Escherichia coli*

1.2.2 *KLEBSIELLA SPECIES*:

Klebsiella species are a diverse group of gram-negative, lactose-fermenting, non-motile bacilli that are encapsulated. They are significant urinary tract pathogens, especially in long-term hospital patients, and infection is frequently linked to urethral catheterization. The biochemical characterisation of these species in the laboratory is time-consuming and insufficient. According to antibiotic sensitivity tests, gentamicin is the best remedy for a *Klebsiella* urinary tract infection. It can also occur after a prolonged use of a urinary catheter. The *Klebsiella* species is isolated in MacConkey agar.



Figure 1.2 *Klebsiella* species

1.2.3 PSEUDOMONAS:

Pseudomonas infections are caused by bacteria belonging to the genus *Pseudomonas*. Bacteria can be found in a variety of places in the world, including soil, water, and plants. In healthy people, they rarely cause infections. If a healthy person develops an infection, it is normally minor. People who are already in the hospital with another illness or disease, or who have a poor immune system, are more likely to develop a more serious infection. Pseudomonas are a common pathogen found in hospital infections. A pathogen is a form of microbe that causes illness. Nosocomial infections are infections acquired in a hospital setting.

Infections can strike at any time and in any position on the body. The signs and symptoms differ depending on which part of the body is infected. The diseases are treated with antibiotics. Infection with *Pseudomonas* can be fatal in people who are already sick.



Figure 1.3 *pseudomonas*

1.3 ANTIBIOTIC RESISTANCE

Antibiotic resistance occurs when bacteria cease to react to antibiotics that were intended to kill them. This indicates that the germs are not destroyed and begin to multiply. It does not imply that our bodies are immune to antibiotics (McCaig et al., 2003).

On chromogenic agar, the Kirby-Bauer disc diffusion method defined in Clinical Laboratory Standards Institute (CLSI, 2015) guidelines was used to determine antibiotic susceptibility, intermediate tolerance, and resistance. Ampicillin 10 mg, augmentin (amoxicillin/clavulanic acid) 20 mg, Bactrim (trimethoprim/sulfamethoxazole) 1.25 mg, cefuroxime 30 mg, gentamicin 120 mg, norfloxacin 10 mg, ceftazidime 30 mg, ciprofloxacin 5 mg, and imipenem 10 mg were among the antimicrobial discs used (Karren et al., 1999).

According to CLSI guidelines, the inhibition zones were interpreted. The degree of improvement in microbe-specific antibiotic resistance over a three-year period was determined using a chi-square examination (Gilbert et al., 2004).

1.4 IMPORTANCE OF UTI:

- Women have a lifetime chance of contracting a urinary tract infection of more than 50%. (UTI).
- A heavy, persistent urge to urinate, as well as a painful and burning sensation when urinating, are common symptoms.
- A UTI is usually diagnosed based on symptoms and urine tests.
- UTIs may be healed in as little as two or three days.
- UTIs are not treated with cranberry extracts, although they may help to reduce the risk of recurrence.

CHAPTER-2

LITERATURE SURVEY

Urinary tract infections are the most common bacterial infections in women. Most urinary tract infections are acute uncomplicated cystitis. Identifiers of acute uncomplicated cystitis are frequency and dysuria in an immunocompetent woman of childbearing age who has no comorbidities or urologic abnormalities. Physical examination is typically normal or positive for suprapubic tenderness. A urinalysis, but not urine culture, is recommended in making the diagnosis. Guidelines recommend three options for first-line treatment of acute uncomplicated cystitis: fosfomycin, nitrofurantoin, and trimethoprim/sulfamethoxazole (in regions where the prevalence of *Escherichia coli* resistance does not exceed 20 percent). Beta-lactam antibiotics, amoxicillin/clavulanate, cefaclor, cefdinir, and cefpodoxime are not recommended for initial treatment because of concerns about resistance. Urine cultures are recommended in women with suspected pyelonephritis, women with symptoms that do not resolve or that recur within two to four weeks after completing treatment, and women who present with atypical symptoms (Colgan, and Williams, 2011).

The microbial etiology of urinary infections has been regarded as well established and reasonably consistent. *Escherichia coli* remains the predominant uropathogen (80%) isolated in acute community-acquired uncomplicated infections, followed by *Staphylococcus saprophyticus* (10% to 15%). *Klebsiella*, *Enterobacter*, and *Proteus* species, and enterococci infrequently cause uncomplicated cystitis and pyelonephritis (Gupta et al., 1999).

the pathogens traditionally associated with UTI are changing many of their features, particularly because of antimicrobial resistance. The etiology of UTI is also affected by underlying host factors that complicate UTI, such as age, diabetes, spinal cord injury, or catheterization. Consequently, complicated UTI has a more diverse etiology than uncomplicated UTI, and organisms that rarely cause disease in healthy patients can cause significant disease in hosts with anatomic, metabolic, or immunologic underlying disease. The majority of community-acquired symptomatic

UTIs in elderly women are caused by *E.coli*. However, gram-positive organisms are common, and polymicrobial infections account for up to 1 in 3 infections in the elderly. In comparison, the most common organisms isolated in children with uncomplicated UTI are Enterobacteriaceae. Etiologic pathogens associated with UTI among patients with diabetes include *Klebsiella* spp., Group B *Streptococci*, and *Enterococcus* spp., as well as *E.coli*. Patients with spinal cord injuries commonly have *E.coli* infections (Akram et al., 2007).

Other common uropathogens include *Pseudomonas* and *Proteus mirabilis*. Recent advances in molecular biology may facilitate the identification of new etiologic agents for UTI. The need for accurate and updated population surveillance data is apparent, particularly in light of concerns regarding antimicrobial resistance. This information will directly affect selection of empiric therapy for UTI (Ronald, 2002).

Up to 7 percent of girls and 2 percent of boys will have a symptomatic, culture-confirmed urinary tract infection by six years of age. Urinary tract infection may be suspected because of urinary symptoms in older children or because of fever, nonspecific symptoms, or failure to thrive in infants. Urine dipstick analysis is useful for ruling out urinary tract infections in cases with low clinical suspicion. However, urine culture is necessary for diagnosis of urinary tract infections in children if there is high clinical suspicion, cloudy urine, or if urine dipstick testing shows positive leukocyte esterase or nitrite activity. Despite current recommendations, routine imaging studies (e.g., renal ultrasonography, voiding cystourethrography, renal scans) do not appear to improve clinical outcomes in uncomplicated urinary tract infections. Oral antibiotics are as effective as parenteral therapy in randomized trials. The optimal duration of antibiotic therapy has not been established, but one-day therapies have been shown to be inferior to longer treatment courses (Alper and Curry 2005).

Urinary tract infections (UTIs) are among the most common bacterial infections acquired in the community and in hospitals. In individuals without anatomical or functional abnormalities, UTIs are generally self limiting, but have a propensity to recur. Uropathogens have specialized characteristics, such as the production of adhesins, siderophores and toxins that enable them to colonize and

invade the urinary tract, and are transmitted between individuals both through person-to-person contact and possibly via food or water. Although generally self limiting, treatment of UTIs with antibiotics leads to a more rapid resolution of symptoms and is more likely to clear bacteriuria, but also selects for resistant uropathogens and commensal bacteria and adversely affects the gut and vaginal microbiota. As uropathogens are increasingly becoming resistant to currently available antibiotics, it may be time to explore alternative strategies for managing UTI (Foxman, 2010).

Urinary tract infection (UTI) remains very common. As many as 50% of women report having had at least one UTI in their lifetimes. Urinary tract infection is the most common cause of infection in nursing home residents and the most common source of bacteremia in the elderly population. Urinary tract infection occurs in patients with structurally or functionally abnormal urinary tracts (complicated UTI) and in patients with anatomically normal urinary tracts (uncomplicated UTI). *Escherichia coli* (*E.coli*) is the most common cause of uncomplicated UTI, whereas antibiotic-resistant Enterobacteriaceae enterococci, and *Candida* species often are the causes of complicated UTI. In this article we review current concepts of the epidemiology, microbiology, pathophysiology, clinical manifestations, diagnosis, and treatment of urinary tract infection (Barnett and Stephens, 1997)

Recurrent urinary tract infections (UTI) are common among young healthy women even though they generally have anatomically and physiologically normal urinary tracts. Women with recurrent UTI have an increased susceptibility to vaginal colonization with uropathogens, which is due to a greater propensity for uropathogenic coliforms to adhere to uroepithelial cells. Risk factors for recurrent UTI include sexual intercourse, use of spermicidal products, having a first UTI at an early age, and having a maternal history of UTIs. Inherited factors may be important in some women with recurrent UTI. Many factors thought to predispose to recurrent UTI in women, such as pre- and post-coital voiding patterns, frequency of urination, wiping patterns, and douching have not been proven to be risk factors for UTI. In contrast to the predominantly behavioral risk factors for young women, mechanical and/or physiological factors that affect bladder emptying are most strongly associated with recurrent UTI in healthy postmenopausal women. The management of recurrent

UTI is the same as that for sporadic UTI except that the likelihood of infection with an antibiotic resistant uropathogen is higher in women who have received recent antimicrobials. Strategies to prevent recurrent UTI in young women should include education about the association of recurrent UTI with frequency of sexual intercourse and the usage of spermicide-containing products. Continuous or post-coital prophylaxis with low-dose antimicrobials or intermittent self-treatment with antimicrobials have all been demonstrated to be effective in managing recurrent uncomplicated UTIs in women. Estrogen use is very effective in preventing recurrent UTI in post-menopausal women. Exciting new approaches to prevent recurrent UTI include the use of probiotics and vaccines. Further understanding of the pathogenesis of UTI will lead to more effective and safer methods to prevent these frequent infections (Hooton, 2001).

In a prospective study, 179 adult women (age range, 17–82 years) were followed up for 12 months after an index episode of community-acquired cystitis caused by *Escherichia coli*. Episodes of symptomatic urinary tract infection (UTI) were recorded, and urinary isolates were compared with the index episode isolate; 147 UTI episodes were detected during the follow-up. Of these episodes, 131 were classified as recurrences occurring at least 1 month after the index episode; 44% of the patients had recurrences. A history of UTI increased the risk of recurrence; only 11.8% of the 17 patients without previous episodes of UTI had at least one recurrence, while 47.5% of those with previous episodes had at least one recurrence (OR, 6.8; univariate logistic regression). *E. coli* caused 78% of the recurrent episodes. Phenotypic and genotypic analysis of *E. coli* strains showed that one-third of the recurrences were caused by the index episode strain, which could persist and cause recurrences throughout the 1-year follow-up period. The prevalence of adhesins or other identified virulence factors for UTI among the recurrence strains was identical to that among the index episode strains. The presence of these factors did not affect the risk of recurrence but did increase the likelihood that the index episode strain would persist and cause recurrent episodes of UTI (Ikähelmo et al., 1996)

Urinary tract infection is one of the most common bacterial infections seen in clinical practice particularly in developing countries. The causative agents for Urinary tract infection vary from place to place and they also vary in their susceptibility and

resistance patterns. Studies to know the most common causative agents and their susceptibility pattern will help the clinicians to choose the antibiotic for empirical treatment. This study was done to find out the common bacteria causing UTI and to determine the antibiotic susceptibility pattern of the urinary pathogens from a tertiary care hospital in South India. A total of 573 mid-stream urine samples from the suspected UTI patients were tested microbiologically and antimicrobial susceptibility test were performed for the isolated pathogens using Kirby-Bauer disk diffusion method. The rate of culture positivity in females was 87.82% and in males was 27.92%. *E.coli* was the most frequently isolated urinary pathogen (37.95%), followed by *Klebsiella* (21.41%) and *Acinetobacter* (10.94%). E-coli was highly sensitive to Nitrofurantoin(81.92%) and Amikacin(69.88%) and it was highly resistant to Ampicillin(1.0%). *Klebsiella* was highly sensitive to Impinem and it was highly resistant to Ampicillin. Higher prevalence of UTI was seen in females. Gram negative organisms were the most commonly isolated organisms in UTI. Urinary pathogens showed resistance to commonly used antibiotics like Ampicillin, Norfloxacin and Nalidixic acid. The susceptibility and resistance patterns of urinary pathogens should be considered before starting empirical treatment for UTI (Razak and Gurushantappa, 2012).

Urinary tract infections (UTIs) are one of the most common bacterial infections and are predominantly caused by uropathogenic *Escherichia coli* (UPEC). While UTIs are typically considered extracellular infections, it has been recently demonstrated that UPEC bind to, invade, and replicate within the murine bladder urothelium to form intracellular bacterial communities (IBCs). These IBCs dissociate and bacteria flux out of bladder facet cells, some with filamentous morphology, and ultimately establish quiescent intracellular reservoirs that can seed recurrent infection. This IBC pathogenic cycle has not yet been investigated in humans. In this study we sought to determine whether evidence of an IBC pathway could be found in urine specimens from women with acute UTI. We collected midstream, clean-catch urine specimens from 80 young healthy women with acute uncomplicated cystitis and 20 asymptomatic women with a history of UTI. Investigators were blinded to culture results and clinical history. Samples were analyzed by light microscopy, immunofluorescence, and electron microscopy for evidence of exfoliated IBCs and filamentous bacteria. Evidence of IBCs was found in 14 of 80 (18%) urines from

women with UTI. Filamentous bacteria were found in 33 of 80 (41%) urines from women with UTI. None of the 20 urines from the asymptomatic comparative group showed evidence of IBCs or filaments. Filamentous bacteria were present in all 14 of the urines with IBCs compared to 19 (29%) of 66 samples with no evidence of IBCs ($p < 0.001$). Of 65 urines from patients with *E. coli* infections, 14 (22%) had evidence of IBCs and 29 (45%) had filamentous bacteria, while none of the gram-positive infections had IBCs or filamentous bacteria. The presence of exfoliated IBCs and filamentous bacteria in the urines of women with acute cystitis suggests that the IBC pathogenic pathway characterized in the murine model may occur in humans. The findings support the occurrence of an intracellular bacterial niche in some women with cystitis that may have important implications for UTI recurrence and treatment. (Rosen et al., 2007)

Urinary tract infections (UTIs) are common in both inpatient and outpatient settings. This article provides an evidence-based, clinically relevant overview of management of UTIs, including screening, diagnosis, treatment, and prevention. Conditions covered include acute cystitis (both uncomplicated and complicated), catheter-associated UTI, and asymptomatic bacteriuria in both women and men.

CHAPTER-3

AIM AND SCOPE OF THE PRESENT INVESTIGATION

3.1 AIM OF THE STUDY

Isolation of bacteria causing urinary tract infection and their antibiotic resistance.

3.2 OBJECTIVE OF THE STUDY

- To determine the bacteriological profile of pathogens responsible for urinary tract infection.
- To evaluate the antibiotic sensitivity pattern of these pathogens.

3.3 SIGNIFICANCE

Severe bacteriuria in the presence of symptoms of cystitis or pyelonephritis is characterised as a urinary tract infection (UTI). UTIs are responsible for a large number of visits to the emergency room (ED), with 20% of women experiencing at least one infection. The majority of cases of uncomplicated cystitis are caused by *Escherichia coli*.

CHAPTER-4

MATERIALS AND METHODOLOGY

4.1 MATERIALS

4.1.1 Urine culture test:

- Urine sample
- Sterile container
- MacConkey agar
- Petri dish
- Incubator
- Laminar air flow
- Inoculation loop
- Bunsen burner
- Gloves
- Marker pen

4.1.2 Antibiotic sensitivity test:

- Muller Hinton agar
- Petri dish
- Antibiotic disc
- Incubator
- Isolated bacteria
- Sterile cotton swab
- Forceps

4.2 METHODOLOGY

4.2.1 COLLECTION OF SAMPLE

The urine sample is collected in a sterile container . To avoid contamination or deterioration of urine constituents, it is critical to collect urine specimens correctly. Ascertain that the urine collection container is clean and that the container manufacturer has guaranteed that no interfering substances will leach into the sample.

4.2.2 URINE CULTURE TEST

A urinary culture is a tool for detecting bacteria in the urine .The germs that cause a urinary tract infection can be found and identified with this test. The urethra is where bacteria that cause UTIs enter the urinary tract. Urine cultures may show the microorganisms that cause a UTI, most commonly bacteria (Butler et al.,2006).

The urine sample is collected in a sterile container. Container: should be clean , sterile ,screwed capped. Collection: should be midstream, avoid contamination, should be the genital area with soap .The proper labelling should be down in both the container and petri plates. Let sterilize our inoculation loop in Bunsen burner. After the sterilization, we should allow the inoculation loop for 30 seconds for cooling. After that, we have to mix the urine sample well. After 24 h, we can directly take out the readings by observing the plates. After observing the plates can we see the isolated organisms grown. we have to note the colony count for the grown isolated organisms (Karlowsky et al.,2001).

4.2.3 MACROSCOPIC OBSERVATION

The colony morphology of the bacteria were examined on the surface of the plate.

4.2.4 MICROSCOPIC OBSERVATION

4.3 Physical Characterization

4.3.1 GRAM'S STAINING

A loopful of overnight grown culture was taken and a uniform smear was made on a clear glass slide and it was heat fixed.

Crystal violet solution was added on to the slide and kept for a minute. The slide was then washed with water and flooded with a solution of Gram's iodine. After a minute, 95% ethanol was used on the slide for decolorization. Then, the washed slide was counter stained with saffranine and observed under a light microscope.

The Gram-positive organisms appeared violet in colour and Gram-negative organisms, pink in colour.

4.3.2 MOTILITY TEST

A loopful of culture was stabbed onto semisolid Agar tubes. The tubes were incubated at room temperature for 24 hrs. Motile culture showed diffuse growth and non-motile culture showed growth only along the line of inoculation.

4.4 BIOCHEMICAL CHARACTERIZATION

4.4.1 CATALASE TEST

This test was performed to know whether aerobic bacteria possess Catalase enzyme or not. A small quantity of bacterial culture was scrapped out using a capillary tube and transferred to a test tube containing H₂O₂. Evolution of gas bubbles from H₂O₂ is positive for the presence of Catalase enzyme in the bacteria (Karlowsky et al.,2002).

4.4.2 OXIDASE TEST

This test was performed in order to know whether the bacteria possess Oxidase enzyme or not. A loopfull of bacterial culture was rubbed over the Oxidase disc (Hi-media) and development of purple blue colour within 30 seconds was taken as oxidase positive.

4.4.3 INDOLE PRODUCTION TEST

This test was performed to check the ability of bacteria to produce indole. The medium consisted of the following ingredients

Indole medium

Tryptone	-	10.0 g/l
Sodium chloride	-	0.5 g/l
Distilled water	-	1000 ml
pH	-	7.4 ± 0.2

Kovac's reagent

p-dimethyl amino benzaldehyde`	-	5.0 g/l
Amyl alcohol	-	750 ml
HCl - 250 ml		

Bacteria was inoculated on the indole medium and incubated for about 24-48 hrs at 37°C. To the bacterial culture, few drops of Kovac's reagent was added and shaken well. Development of pink or red coloured ring was recorded as positive for indole production.

4.4.4 PROSKAUER TEST-Methyl red and voges

This test was performed to know whether the organism produced has acid end products or neutral end products. The medium consisted of the following ingredients

Peptone	-	7.0 g/l
Dextrose	-	5.0 g/l
Dipotassium phosphate	-	5.0 g/l
Distilled water	-	1000 ml
pH	-	6.9 ± 0.2

5 ml of the above medium was poured into a test tube and autoclaved at 15 lbs for 15 minutes.

Methyl red reagent

Methyl red	-	0.1 g/l
Ethyl alcohol	-	300 ml
Distilled water	-	200 ml

Five drops of methyl red were added and development of red colour was recorded as positive for MR test.

Voges-Proskauer reagent

VP – Reagent A

Alpha-naphthol	-	5.0 g
Ethyl alcohol	-	100 ml

VP – Reagent B

Potassium hydroxide	-	40.0 g
Distilled water	-	100 ml

To another set of bacterial cultures, few drops of VP reagents A and B were added and mixed well. Development of pink colour was positive to VP test and indicated the production of acid by fermentation.

4.4.5 CITRATE UTILIZATION TEST

This test was performed to know whether the organism utilized citrate as the sole source of carbon. The medium consisted of the following ingredients

Sodium citrate	-	2.0 g/l
Magnesium sulphate	-	0.2 g/l
Sodium chloride	-	5.0 g/l
Ammonium dihydrogen phosphate	-	1.0 g/l
Dipotassium phosphate	-	1.0 g/l
Bromothymolblue	-	0.08 g/l
Agar	-	15.0 g/l
Distilled water	-	1000 ml
pH	-	6.8 ± 0.2

Slants of the above medium were prepared in test tube and bacteria were inoculated and incubated at 37°C for 24 hrs. Change of the colour of the medium from green to blue and it was taken as positive for utilization of citrate as a sole carbon source for growth (Schaeffer, 2002).

4.4.6 UREASE TEST

This test was performed to know whether the organism produced the enzyme urease or not. The medium consisted of the following ingredients

Peptone	-	1.0 g/l
Glucose	-	1.0 g/l
Sodium chloride	-	5.0 g/l
Mono potassium phosphate	-	0.2 g/l
Dipotassium phosphate	-	12 g/l
Phenol red (1.2%)	-	1 ml
Agar	-	15.0 g/l
Distilled water	-	1000 ml
pH	-	6.8 ± 0.2

The base was prepared and sterilized by autoclaving at 121°C for 15 lbs for 15 min. The media was cooled to 56°C, 40% Urea was added and allowed to solidify in a slant position, the slant was inoculated with a drop of 4-6 hours growth of bacterium in broth and incubated at 37°C for 24 hrs.

4.4.7 TRIPLE SUGAR IRON TEST

This test was performed to know whether the organism produced H₂S and gas formation. The medium consisted of the following ingredients

Beef extract	-	3.0 g/l
Yeast extract	-	3.0 g/l
Peptone	-	2.0 g/l
Sodium chloride	-	5.0 g/l-
Glucose	-	1.0 g/l
Lactose	-	10.0 g/l
Sucrose	-	10.0 g/l
Ferrous ammonium sulphate	-	2.0 g/l
Sodium thiosulphate	-	3.0 g/l
Phenol red	-	2.5 g/l
Agar	-	15.0 g/l
Distilled water	-	1000 ml
pH	-	7.4 ± 0.2

The ingredients were dissolved, autoclaved at 121°C for 15 lbs for min. Then pour the media in sterile test tubes in such a way that about 1 inch butt and slope is obtained. Colonies were picked up with a straight wire loop, and inoculate by stabbing down the centre of agar butt carefully. Incubate at 37°C for 24-48 hrs for 1 to 2 days.

4.4.8 NITRITE TEST

This test was performed to know whether the organism reduced nitrate. The medium consisted of the following ingredients

Beef extract	-	0.3 g/l
Peptone	-	0.5 g/l

Potassium nitrate	-	0.1 g/l
Distilled water	-	1000 ml

Before adding potassium nitrate and glucose the other ingredients of the medium were boiled to dissolve them, and the pH was adjusted to 7.4. Then the medium was poured in test tubes and autoclaved at 15 lbs for 30 min. The bacteria were inoculated and incubated at 37°C for 24 hrs.

To 5 ml of the bacterial culture, 0.5 ml of 1% sulphanilic acid prepared in 5M acetic acid, followed by 0.5 ml of 0.6% dimethyl-naphthalamine in 5M acetic acid was added.

Development of red colour indicated the reduction of nitrate into nitrite. If no colour was developed, 5 mg of zinc dust was added to each test tube. Development of red colour indicated that the nitrate has not been broken down by the bacterial isolates.

4.4.9 CARBOHYDRATE FERMENTATION TEST

Carbohydrate fermentation test is used to identify the fermentation of sugars. The medium consisted of the following ingredients

Carbohydrate broth

A. Base

Peptone	-	1.0 g/l
Beef extract	-	1.0 g/l
NaCl	-	0.5 g/l
Distilled water	-	100 ml.

B. Carbohydrate Solution

Carbohydrate	-	10 g/l
Distilled water	-	100 ml

C. Indicator

Bromocresol purple	-	1.6 g/l
Ethanol	-	100 ml

Solution A	-	900 ml
Solution B	-	100 ml
Solution C	-	1ml

One ml of mixed solution was taken in a test and autoclaved for 10 min at 121°C. Filter sterilized (5-10%) carbohydrates were added aseptically to the sterile base to make 1% Solution. Lactose, Sucrose, Glucose, Mannitol and Maltose were used for sugar fermentation.

4.5 SENSITIVITY TEST

A sensitivity analysis is a test that measures a bacteria's "sensitivity" to an antibiotic. It also determines the drug's ability to destroy bacteria. Your doctor will use the results of the test to assess which medications are most likely to be successful in treating the infection (Garau et al.,1999).

Take a sterile Muller Hinton agar plate . The proper labelling should be done in the plate . Let sterilize our cotton swab for swabbing. After sterilization , using cotton swab take some colonies. After that open the plate wipe the swab inside gently .After completely swabbing the plate in one direction slightly rotate the plate and continue swabbing. Close the plate and then dispose the swab in a biohazard container. The next step is to place antibiotic disc on the plate for that first select the antibiotics .After that sterilize the tip of the forceps .Pick up the disc carefully from one antibiotic disc and place the disc onto the swabbed plate. Each disc should be slightly pressed down to ensure the contact with agar. The disc should not keep closer and keep atleast 25 millimeter distance from this to disk maximum 6 discolour should be placed on a 90 mm plate. Close the plate and keep the plate in an incubator at 37 C in inverted position for 12 hours. After 12 hours we can observe the result.

4.5.1 Drugs used for antibiotic sensitivity test

- Ciprofloxacin
- Amikacin
- Cefixime
- Ceftriaxone/ sulbactam
- Imipenem
- Levofloxacin

- Co-trimoxazole
- Nitrofurantoin
- Ofloxacin
- Piperacillin/tazobactam
- Norfloxacin
- Amoxycylav
- Cephalexin

4.5.2 positive drugs

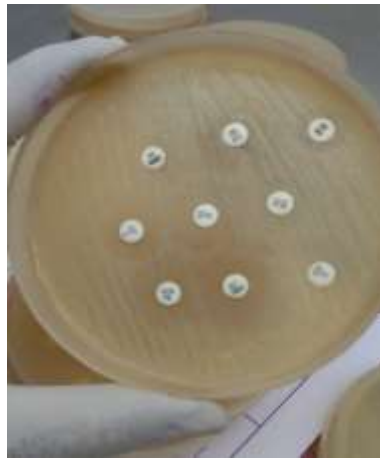


Figure 4.1 positive drugs

4.5.3 Negative drugs

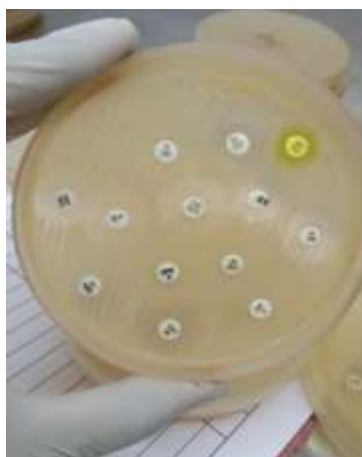


Figure 4.2 Negative drug

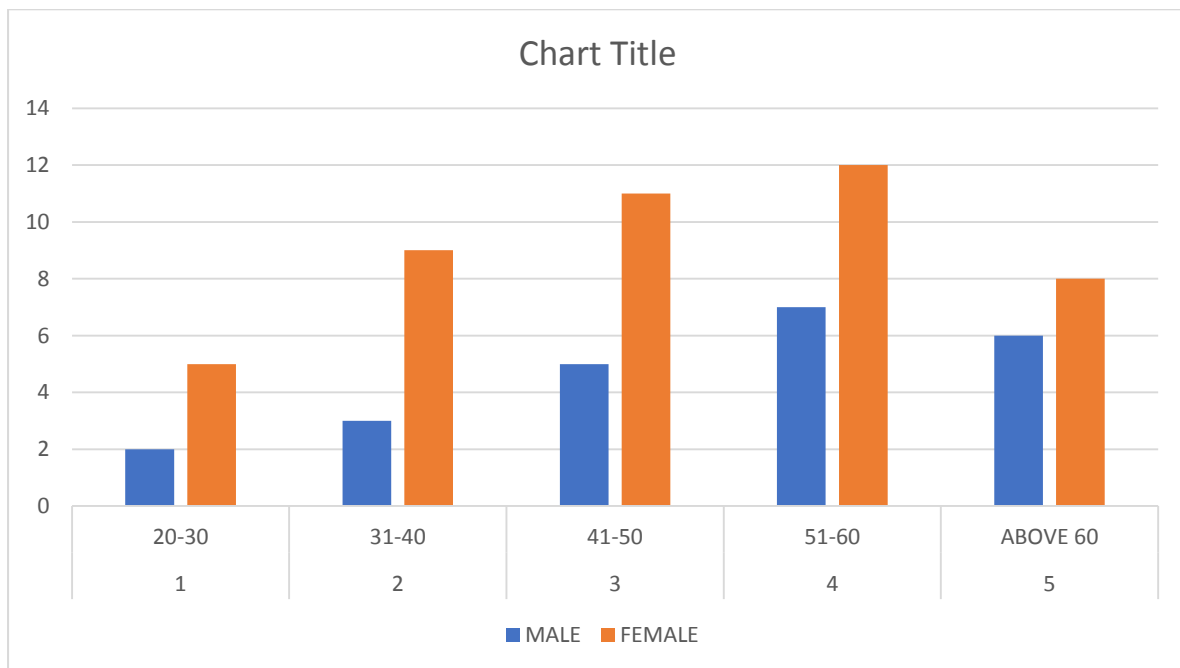
CHAPTER-5

RESULTS AND DISCUSSION

- Total number of samples= 68
- Number of positive samples = 54
- Number of negative samples= 14

S.NO	AGE GROUP	MALE	FEMALE
1	20-30	2	5
2	31-40	3	9
3	41-50	5	11
4	51-60	7	12
5	ABOVE 61	6	8

Table 5.1 list of reports of patient



Flow chart 5.1 list of reports of patient

5.2 List of reports of patient

Since the prevalence of uropathogen-caused pathogenesis and the production of drug resistance caused by uropathogens is growing at an alarming pace, UTI management is crucial. *Klebsiella* species was found to be the most prevalent uropathogen isolated from patients with UTI and the development of multi drug resistance among uropathogens that cause a complicated UTI, according to lab reports (Mazzulli et al., 2001).

When the urine specimens were subjected to physical inspection, they showed more variation in colour, odour, shape, and pH. When the urine specimens were examined physically, they displayed more variance in colour, odour, shape, and pH. Different selective and differential media were used on urine samples. Color morphology was used to identify the uropathogens. Biochemical experiments were used to confirm the existence of the uropathogen species from the various groups. The results show that *Klebsiella* was the most common isolate isolated from the urine sample (Bahadin et 2009).

Antibiotics were found to be effective against the uropathogens that were isolated. Among the isolated pathogens all of them shows 100% sensitivity towards ceftazidime , cephalexin and co-trimoxazole (Tang et al., 2019).

Urinary tract infections (UTIs) are severe health issues that impact millions of people each year. UTIs are classified as either complicated or uncomplicated. Patients with structurally or clinically irregular urinary tracts (complicated UTI) and patients with anatomically normal urinary tracts both get urinary tract infections (uncomplicated UTI) (Gupta et al., 2001).

Urinary tract infection pathogens are the product of a complex relationship between species, the environment, and the potential host. Infections of the urinary tract are caused by Gram negative aerobic species. These species are normally present in the normal microbial flora, and *Klebsiella* species is usually isolated in 80-90 percent of cases.

Table showing antibiotic resistance and biochemical results

S.NO	INDOLE	MOTILITY	CATALZE	OXIDASE	MR	VP	NITRATE	UREASE	ORGANISM	ANTIBIOTICS
1	-	-	-	-	-	+	-	+	<i>Klebsiella</i>	Ceftazidime
2	+	+	-	-	+	-	+	-	<i>E.coli</i>	Co-trimoxazole
3	-	-	-	-	-	+	-	+	<i>Klebsiella</i>	Ofloxacin
4	-	-	-	-	-	+	-	+	<i>Klebsiella</i>	Ceftazidime
5	-	-	-	+	-	-	-	-	<i>Pseudomonas</i>	Piperacillin
6	+	+	-	-	+	-	+	-	<i>E.coli</i>	Ceftazidime
7	+	+	-	-	+	-	+	-	<i>E.coli</i>	Co-trimoxazole
8	-	-	-	-	-	+	-	+	<i>Klebsiella</i>	Ceftazidime
9	-	-	+	+	-	-	-	-	<i>Pseudomonas</i>	Cephalexin
10	-	-	-	-	-	+	-	+	<i>Klebsiella</i>	Ofloxacin
11	-	-	-	-	-	+	-	+	<i>Klebsiella</i>	Norfloxacin
12	+	+	-	-	+	-	+	-	<i>E.coli</i>	Ceftazidime
13	+	+	-	-	+	-	+	-	<i>E.coli</i>	Co-trimoxazole
14	-	-	-	-	-	+	-	+	<i>Klebsiella</i>	Norfloxacin
15	-	-	-	+	-	-	-	-	<i>Pseudomonas</i>	Piperacillin
16	+	+	-	-	+	-	+	-	<i>E.coli</i>	Ceftazidime
17	-	-	-	+	-	-	-	-	<i>Pseudomonas</i>	Cephalexin
18	-	-	-	-	-	+	-	+	<i>Klebsiella</i>	Ofloxacin

S.NO	INDOLE	MOTILITY	CATALZE	OXIDASE	MR	VP	NITRATE	UREASE	ORGANISM	ANTIBIOTICS
19	-	-	-	+	-	-	-	-	<i>Pseudomonas</i>	Piperacillin
20	-	-	-	-	-	+	-	+	<i>Klebsiella</i>	Ceftazidime
21	-	-	-	+	-	-	-	-	<i>Pseudomonas</i>	Cephalexin
22	+	+	-	-	+	-	+	-	<i>E.coli</i>	Ceftazidime
23	+	+	-	-	+	-	+	-	<i>E.coli</i>	Co-trimoxazole
24	-	-	-	-	-	+	-	+	<i>Klebsiella</i>	Ofloxacin
25	-	-	-	-	-	+	-	+	<i>Klebsiella</i>	Norfloxacin
26	-	-	-	+	-	-	-	-	<i>Pseudomonas</i>	Cephalexin
27	+	+	-	-	+	-	+	-	<i>E.coli</i>	Co-trimoxazole
28	+	+	-	-	+	-	+	-	<i>E.coli</i>	Co-trimoxazole
29	-	-	-	-	-	+	-	+	<i>Klebsiella</i>	Ceftazidime
30	-	-	-	+	-	-	-	-	<i>Pseudomonas</i>	Cephalexin
31	-	-	-	+	-	-	-	-	<i>Pseudomonas</i>	Cephalexin
32	-	-	-	-	-	+	-	+	<i>Klebsiella</i>	Ceftazidime
33	-	-	-	-	-	+	-	+	<i>Klebsiella</i>	Norfloxacin
34	-	-	-	-	-	+	-	+	<i>Klebsiella</i>	Ofloxacin
35	+	+	-	-	+	-	+	-	<i>E.coli</i>	Co-trimoxazole
36	-	-	-	+	-	-	-	-	<i>Pseudomonas</i>	Cephalexin
37	+	+	-	-	+	-	+	-	<i>E.coli</i>	Co-trimoxazole

S.NO	INDOLE	MOTILITY	CATALZE	OXIDASE	MR	VP	NITRATE	UREASE	ORGANISM	ANTIBIOTICS
38	-	-	-	-	-	+	-	+	<i>Klebsiella</i>	Ceftazidime
39	-	-	-	-	-	+	-	+	<i>Klebsiella</i>	Ceftazidime
40	+	+	-	-	+	-	+	-	<i>E.coli</i>	Co-trimoxazole
41	-	-	-	-	-	+	-	+	<i>Klebsiella</i>	Ceftazidime
42	-	-	-	-	-	+	-	+	<i>Klebsiella</i>	Ceftazidime
43	+	+	-	-	+	-	+	-	<i>E.coli</i>	Co-trimoxazole
44	-	-	-	-	-	+	-	+	<i>Klebsiella</i>	Norfloxacin
45	+	+	-	-	+	-	+	-	<i>E.coli</i>	Co-trimoxazole
46	-	-	-	+	-	-	-	-	<i>Pseudomonas</i>	Cephalexin
47	-	-	-	+	-	-	-	-	<i>Pseudomonas</i>	Cephalexin
48	-	-	-	-	-	+	-	+	<i>Klebsiella</i>	Ofloxacin
49	+	+	-	-	+	-	+	-	<i>E.coli</i>	Co-trimoxazole
50	+	+	-	-	+	-	+	-	<i>E.coli</i>	Co-trimoxazole
51	-	-	+	+	-	-	-	-	<i>Pseudomonas</i>	Cephalexin
52	-	-	-	-	-	+	-	+	<i>Klebsiella</i>	Ceftazidime
53	+	+	-	-	+	-	+	-	<i>E.coli</i>	Co-trimoxazole
54	-	-	-	-	-	+	-	+	<i>Klebsiella</i>	Ceftazidime

CHAPTER - 6

SUMMARY AND CONCLUSION

MICROSCOPIC OBSERVATION:

A sample of your urine is examined under a microscope in this examination. It can see urinary tract cells, blood cells, crystals, bacteria, parasites, and tumour cells. This test is often used to validate the results of other tests or to complement a diagnosis.

MACROSCOPIC OBSERVATION:

Macroscopic urinalysis is the direct visual observation of the urine, noting its quantity, colour, clarity or cloudiness, etc.

URINE CULTURE TEST:

A urine culture is a test that looks for germs (such as bacteria) that can cause an infection in the urine. Bacteria can enter the urinary tract via the urethra and cause infection (UTI). A sample of urine is mixed with a substance that encourages germ formation.

SENSITIVITY TEST:

An antibiotic sensitivity test is done to help choose the antibiotic that will be most effective against the specific types of bacteria or fungus infecting an individual person.

CONCLUSION

Make sure you drink plenty of water. The most direct way to stop a UTI is to flush bacteria out of the bladder and urinary tract before they can develop. It would be difficult to go too long without urinating if you are well hydrated. Antibiotics for 1-2 days; patients infected with bacteria, which is treated with antibiotics, kidney infections will require antibiotic therapy for many weeks.

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