ISOLATION AND IDENTIFICATION OF BACTERIAL PATHOGENS FROM URINARY TRACT INFECTION IN HUMAN

A THESIS

BY

SONDHYA MIKHALINA DAS

REGISTRATION NO. 1505253

SEMESTER: JULY-DECEMBER, 2017

MASTER OF SCIENCE (M.S.)

IN

MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY

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DECEMBER, 2017

Dedicated

To

My Beloved Parents and Teachers

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The author *December, 2017*

Abstract

The aim of the study was to diagnose the type of pathogens resposible for urinary tract infection (UTI) and test their sensitivity to different antibiotics that help the physician to choose the correct empirical treatment. Urine samples were

cultured on Nutrient agar, MacConkey agar, Mannitol Salt Agar, Blood agar, EMB Agar, Cetrimide Agar and *Staphylococcus* Agar No. 110 plates with overnight incubation. Identification of organisms was done by conventional methods. The sensitivity test was carried out on Nutrient agar, Mueller-Hinton agar plates unless otherwise stated. A total of 58 clinical samples, 16 (27.6 %) were male of which 6 (37.5%) were positive and that of 42 (72.4 %) were female of which 19 (45.2%) were positive for UTI. From total collected samples, 43.1 % were culture positive. The predominant organisms were *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp. and S*taphylococcus* spp. *E. coli* showed sensitivity to Ciprofloxacin 73.3%, Chloramphenicol 100%, Gentamycin 80% and Amikacin 80% respectively. *Klebsiella spp.* showed sensitivity to Ciprofloxacin 50%, Gentamicin 100%, Amikacin 50% and Chloramphenicol 50%. *Pseudumonas* spp. showed sensitivity to Ciprofloxacin 100%, Gentamicin 100% and Livofloxacin 100%. Where as *Staphylococcus* spp. showed sensitivity to Ciprofloxacin 100%, Gentamicin 85.7%, Chloramphenicol 100% and Amikacin 51.1%. It is concluded that females had more UTI than that of males specially, at the age of 31-45 years. More than 50% of the predominant isolates were sensitive to Chloramphenicol, Gentamicin, Ciprofloxacin and Amikacin.

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LIST OF ABBREVIATIONS AND SYMBOLS

-	:	Negative
%	:	Percentage
@	:	At the rate of
+	:	Positive
µg	:	Microgram
µl	:	Microlitre
^{0}C	:	Degree of celcius
BA	:	BloodAgar
BD	:	Bangladesh
EMB	:	Eosin Methylene Blue
ER	:	Erythromycin
et al.	:	Associated
etc	:	Etcetera
Gm	:	Gram
H_2O_2	:	Hydrogen peroxide
H_2S	:	Hydrogen sulphide
HSTU	:	Hajee Mohammad Danesh Science and Technology University
i.e.	:	That is
Ltd	:	Limited
M.S	:	Master of Science
MC	:	MacConkey Agar
MI	:	Milliliter
MIU	:	Motility Indole Urease
MR	:	Methyl Red
NA	:	Nutrient Agar
No.	:	Number
PBS	:	Phosphate Buffer Saline
Prof.	:	Professor
PSS	:	Physiological Saline Solution
SL	:	Serial number
Sp	:	Species

STVH	:	Saint Vincent Hospital
UTI	:	Urinary Tract Infection
v/v	:	Volume by volume
VP	:	Voges-Proskauer
w/v	:	Weight by volume

CHAPTER-I

INTRODUCTION

A urinary tract infection, or UTI, is a bacterial infection of any part of the urinary tract, which includes the bladder, kidneys, ureters (tubes that connect the kidneys to the bladder) and the urethra (the tube that allows the bladder to be emptied). Sometimes, the infection is carried through the bloodstream to other areas of the body.

General Information

Urinary tract infection is an inflammatory response of the urinary tract to bacterial invasion that is usually associated with bacteriuria. Most bacteria enter the urinary tract from the fecal reservoir via ascent through the urethra into the bladder. In approximately 50% of instances cystitis may ascend into the upper urinary tract. Urinary tract infections (UTI) affects any part of the urinary tract and include mainly cystitis (bladder infection), pyelonephritis (kidney infection) and ureteritis (urethra infection) showing tissue damage, burning, painful urination, urgency and urinary frequency, suprapubic pain, pain in renal angle, fever and other systemic manifestations but asymtomatic cases may also occur .The urinary system consists of the kidneys, ureters, bladder, and urethra. The key elements in the system are the kidneys. The kidneys remove excess liquid and wastes from the blood in the form of urine. Narrow tubes called ureters carry urine from the kidneys to the bladder, a sack-like organ in the lower abdomen. Urine is stored in the bladder and emptied through the urethra. The amount of urine varies, depending on the fluids and foods a person consumes. Although urine contains a variety of fluids, salts, and waste products, it usually does not have bacteria in it. **(Adult Health Advisor, 2007**). The most common characteristics of bacterial and fungal UTI are colonization of urinary tract with invasion and inflammation of one or more of the structures of the urinary tract. When bacteria get into the bladder or kidney and multiply in the urine, they cause a UTI. In many cases, bacteria first travel to the urethra causing an infection called urethritis . The pyelonephritis is much more serious than other types.

The Urinary System and Its Defenses

The Urinary System

The urinary system helps maintain proper water and salt balance throughout the body and also expels urine from the body. It is made up of the following organs and structures:

- The two kidneys, located on each side below the ribs and toward the middle-back, play the major role in this process. They filter waste products, water, and salts from the blood to form urine.
- Urine passes from each kidney to the bladder through thin tubes called ureters.
- Ureters empty into the bladder, which rests on top of the pelvic floor. This is a muscular structure similar to a sling running between the pubic bone in front to the base of the spine.
- The bladder stores the urine, which is then eliminated from the body via another tube called the urethra, which is the lowest part of the urinary tract. (In men it is enclosed in the penis. In women it leads directly out.)

Defense Systems against Bacteria

Infection does not always occur when bacteria are introduced into the bladder. A number of defense systems protect the urinary tract against infection-causing bacteria:

- Urine itself functions as an antiseptic, washing potentially harmful bacteria out of the body during normal urination. (Urine is normally sterile, that is, free of bacteria, viruses, and fungi).
- The ureters are structurally designed to prevent urine from backing up into the kidney.
- The prostate gland in men secretes infection-fighting substances.
- The immune system in both sexes continuously fights bacteria and other harmful micro-invaders. In addition, immune system defenses and antibacterial substances in the mucous lining of the bladder eliminate many organisms.

- In normal fertile women, the vagina is colonized by lactobacilli, beneficial microorganisms that maintain a highly acidic environment (low pH). Acid is hostile to other bacteria. Lactobacilli also produce hydrogen peroxide, which helps eliminate bacteria and reduces the ability of *E. coli* to adhere to vaginal cells. (*E. coli* is the major bacterial culprit in urinary tract infections).

- Some interesting research suggests that when bacteria infect the bladder, the cells that line the bladder make sacrifice themselves and self-destruct (a process called apoptosis). In so doing, they fall away from the lining, carrying the bacteria with them. This eliminates about 90% of the *E. coli*.

Some researchers have identified a possible natural antibiotic called human beta-defensin-1 (HBD-1), which fights E-coli within the female urinary and reproductive tracts.

Types of urinary tract infection

Each type of UTI may result in more-specific signs and symptoms, depending on which part of your urinary tract is infected.

Part of urinary tract affected	Signs and symptoms
Kidneys (acute pyelonephritis)	• Upper back and side (flank) pain • High fever • Shaking and chills • Nausea • Vomiting
Bladder (cystitis)	• Pelvic pressure • Lower abdomen discomfort • Frequent, painful urination • Blood in urine
Urethra (urethritis)	• Burning with urination • Discharge

Causes of UTI

Symptomatic urinary tract infections (UTI) are either uncomplicated or complicated. Uncomplicated infections occur in healthy women in the community and are usually caused by *Escherichia coli*. Complicated infections are associated with anatomical, functional, or metabolic abnormalities of the urinary tract that disable the natural innate host defences and lead to tissue injury. **(Jasmine B.L.LeeGuy H.Neild, 2007).** Common invading pathogens of UTI are with *Escherichia coli* followed by *Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus* and *Citrobacter freundii* (1%). (**Tambekar *et al.*, 2006).** Usually UTIs are caused by single type of polymicrobic infections are found in complicated cases with urinary stones and anatomical abnormality of urinary tract. Microorganisms called *Chlamydia* and *Mycoplasma* may also cause UTIs in both men and women, but these infections tend to remain limited to the urethra and reproductive system . The wall of urinary bladder is coated with various mannosylated proteins (Tamm-Horsfall proteins), that interfere the binding of bacteria to the uroepithelium. Allergy is also a hidden factor for UTIs i.e. food allergens cause bladder wall irritation increasing the susceptibility of the organ to UTI. UTIs after sexual intercourse is due to an allergy to latex condoms, spermicides, or oral contraceptives. Any other disorder that suppresses the immune system raises the risk of a urinary infection**.**

Uncomplicated UTI

The infections especially with non-resistant pathogens are termed as uncomplicated UTI.

Complicated UTI

Prostate infections (chronic bacterial prostatitis) are harder to cure because antibiotics are unable to penetrate infected prostate tissue effectively. For this reason, men with prostatitis often need long-term treatment with a carefully selected antibiotic. Complicated urinary tract infection is considered when simultaneous evidence of a metabolic disease, functional/anatomical abnormality of the urinary tract, or more severe infection of the urinary tract, especially with "resistant pathogens" are found. Urinary tract infection may also lead to an infection in the bloodstream (sepsis, septicemia) that can be life threatening.

Recurrent UTI

Usually, the latest infection stems from a strain or type of bacteria that is different from the infection before it, indicating a separate infection, known as Recurrent Infection. Even when several UTIs in a row are due to *E. coli*, slight differences in the bacteria indicate distinct infections. Research suggests that one factor behind recurrent UTIs may be the ability of bacteria to attach to cells lining the urinary tract.

UTIs in Pregnancy

A pregnant woman who develops a UTI should be treated promptly to avoid premature delivery of her baby and other risks such as high blood pressure. Some antibiotics are not safe to take during pregnancy. In selecting the best treatments, doctors consider various factors such as the drug's effectiveness, the stage of pregnancy, the mother's health, and potential effects on the f e t u s .

Symptoms of UTI

Urinary tract infections don't always cause signs and symptoms, but when they do they may include:

- A strong, persistent urge to urinate
- A burning sensation when urinating
- Passing frequent, small amounts of urine
- Urine that appears cloudy
- Urine that appears red, bright pink or cola-colored — a sign of blood in the urine
- Strong-smelling urine
- Pelvic pain, in women — especially in the center of the pelvis and around the area of the pubic bone

Distribution among Humans

UTIs are more common in women than in men though male over 60 years with prostatic hypertrophy are the exceptions (**Calvin 1994; Jawetz *et. al.*, 1995**). UTIs are most common in sexually active women and increase in diabetics and people with sickle-cell disease or anatomical malformations of the urinary tract.

Women are more prone to UTIs than men because in females, the urethra is much shorter and closer to the anus than in males **(Jawetz and Melnick *et. al.*, 1994)** and they lack the bacteriostatic properties of prostatic secretions. An increased sexual activity with vigorous intercourse with a new partner is the cause of so called “honeymoon cystitis”, the UTI. Most cases of lower urinary tract infections in females are benign and do not need exhaustive laboratory work-ups. People with diabetes have a higher risk of a UTI because of changes in the immune system. UTIs may occur in infants who are born with abnormalities of the urinary tract, which sometimes need to be corrected with surgery. UTIs are more rare in boys and young men. In adult women, though, the rate of UTIs gradually increases with age. A woman's urethral opening is near sources of bacteria from the anus and vagina.

Elderly individuals are more likely to harbor bacteria in their genitourinary system with UTI frequency of roughly equal proportions in women and men. One woman in five develops a UTI during her lifetime. Many women suffer from frequent UTIs. Nearly 20 percent of women who have a UTI will have another, and 30 percent of those will have yet another. Of the last group, 80 percent will have recurrences. Pregnant women seem no more prone to UTIs than other women. However, when a UTI does occur in a pregnant woman, it is more likely to travel to the kidneys. According to some reports, about 2 to 4 percent of pregnant women develop a urinary infection. Scientists think that hormonal changes and shifts in the position of the urinary tract during pregnancy make it easier for bacteria to travel up the ureters to the kidneys. For this reason, many doctors recommend periodic testing of urine during pregnancy.

UTIs in men are not as common as in women but can be very serious when they do occur. UTIs in men are often a result of an obstruction. i.e.- a urinary stone or enlarged prostate, or from a medical procedure involving a catheter. An enlarged prostate gland also can slow the flow of urine, thus raising the risk of infection.

Diagnosis

Patient with dysuria (painful voiding) and urinary frequency is usually subject to urinalysis with mid-stream urine sample for the presence of nitrites, leukocytes or leukocyte esterase. High bacterial load without the presence of leukocytes indicates the contamination. The diagnosis of UTI is confirmed by a urine culture.

Detection of UTI pathogen in case of negative urine culture

In case of negative urine culture, symptoms of urethritis may be due to *Chlamydia trachomatis* or *Neisseria gonorrheae* infection, or presence of unusual bacteria or viruses causing symptoms of UTI.

A urine culture is usually performed if the dipstick results are positive, but even if the results are negative, a culture may still be helpful under certain circumstances:

- If urinalysis or dipstick is negative but the patient has UTI symptoms, particularly if the patient has recurring infections or is in a high-risk group.
- If the doctor suspects complications.
- In girls less than 2 years of age with a high fever of unknown origin that lasts 2 days or more.

Even if bacteria are present in the culture, a diagnosis of UTI depends on symptoms and gender:

- The presence in a culture of at least 100,000 bacteria per milliliter of any single type of bacterium per milliliter of urine usually provides conclusive evidence of infection in women with symptoms.
- A count of 100,000 bacterial per milliliter in a woman without symptoms indicates asymptomatic bacteriuria. The decision to treat depends on the woman's risk factors for complications.
- In young women with symptoms of cystitis, a diagnosis of infection can reasonably be made with counts as low as 1000 bacteria per milliliter.
- Men are considered to have an infection with a count of only 1,000.

Other Diagnostic Tests for Kidney Infections and Severe UTIs

No noninvasive test will differentiate between upper and lower urinary tract infections. This is a particular problem because of the high percentage of women whose cystitis symptoms mask infections that also exist in the upper tract.

Antibiotic Trial- The best current test for pyelonephritis is the short-term antibiotic therapy given for cystitis. If the infection returns within 2 weeks after treatment, upper urinary tract infection is usually present.

Blood Cultures -If symptoms are severe, blood cultures will be taken to determine if the infection is in the blood stream and threatening other parts of the body.

Prevention

The following are measures may reduce the incidence of urinary tract infections. These may be appropriate for people, especially women, with recurrent infections **(Mayon-White *et. al.*, 1988; Fisher *et. al.*, 1995; Talukder *et. al.*, 1987)**.

(i) Avoiding the delay of urination.

(ii) Cleaning the urethral opening with an antiseptic after intercourse.

(iii) Fruit juice (cranberry, blueberries) containing tanin can decrease the incidence of UTI. Tanin prevents the adherence of certain pathogens (*E. coli*) to the epithelium of the urinary bladder.

(iv) Intravaginal application of topical estrogen cream and long courses of low-dose antibiotics taken at night can prevent recurrent cystitis

(V) Breastfeeding can reduce the risk of UTIs in infants

Early tests indicate that a vaccine helps patients build up their own natural infection-fighting powers.

Treatment

Treatment of UTI needs the use of antibiotics. Most uncomplicated UTIs can be treated with oral antibiotics such as trimethoprim, cephalosporins, nitrofurantoin, or a fluoroquinolone (*e.g.* ciprofloxacin or levofloxacin). Trimethoprim is one widely used antibiotic for UTIs and is usually taken for seven days. It is often recommended that trimethoprim be taken at night to ensure maximal urinary concentrations to increase its effectiveness. Trimethoprim/sulfamethoxazole was previously internationally used. The addition of the sulfonamide gave little additional benefit compared to the trimethoprim component alone but it may cause high incidence of mild allergic reactions, and other

rare but serious complications. A three-day treatment of trimethoprim/ sulfamethoxazole or ciprofloxacin is usually all that is be passed back and forth between partners. The single dosage is best complimented by the traditional 7-day treatment. Patient with pyelonephritis, intravenous antibiotics usually Aminoglycosides (Gentamicin) are used in combination with a beta-lactam, such as Ampicillin or Ceftriaxone with variable regimens for 48 hours after fever subsides. The patient may then be discharged home on oral antibiotics for a further 5 days. Children with simple UTIs, often respond well to a three-day course of antibiotics (**Monica , 1984**).

A pregnant woman who develops a UTI should be treated promptly to avoid premature delivery of her baby and other risks such as high blood pressure. Some antibiotics are not safe to take during pregnancy. Since indiscriminate use of antibiotics and many other factors are responsible for the evolution of many drug resistant microbial strains causing infections including UTI, the patterns of their antibiotic sensitivity are ever changing. Although most uncomplicated infections readily respond to anti-microbial agents to which they are susceptible, emergence of resistant strains and changing sensitivity pattern are also alarming concerns for the proper treatment of UTI. The purpose of the study was to focus on a scenario of UTI caused by bacteria and their sensitivity pattern to antimicrobial drugs in Bangladesh context.

Objectives

By justifing the research in their context of bangladesh and neaighbour country in the world the present study was conducted for their isolation and identification of bacterial pathogens from UTI infections by using morphological , culturals and biochemical techniques with antibiogram study of identify isolates there for-

1. To isolate and identify the bacterial pathogens from urinary tract infection (UTI) in human.
2. To evaluate the sensitivity and resistance pattern of commercially available antibiotics used against identified isolates

CHAPTER-II

REVIEW OF LITERATURE

Akhtar *et al.*, (2017) observed urinary tract infection (UTI) is one of the common bacterial infections in mankind. Out of 95 urine samples, 56 (58.9%) were found positive. The prevalence was significantly higher in females than in males (females: 58.9%; males: 41%). Age group of >48 years showed higher prevalence of UTI. The most common organisms isolated were *Escherichia coli, Klebsiella, Pseudomonas, Proteus* and *Staphylococcus aureus*. These represented 44.6%, 21.4%, 14.3%, 12.5%, and 7.14% of isolates respectively. Imipenem and Meropenem were found the most susceptible drug against isolated uropathogens. Most powerful antibiotics in our study were imipenem and meropenem. In conclusion, one can truly affirm that the choice of drugs in the treatment of UTI is becoming quite narrow today due to the wide scale resistance that the common UTI pathogens show to drugs which have been used previously.

Samiah (2017) conducted urinary tract infection (UTI) is one of the commonest infections encountered by clinicians and despite the widespread availability of antimicrobial agents.. This study describes the relationships between sex, isolated bacterial agents and antibiotic resistance of UTIs. Out of 116 samples of out patients, urinary tract infection (UTI), of these 70 (60.35%) belonged to female and 46 (39.66%) samples belonged to male patients, while adult patients included (22.41%) of female as well as male patients with the same percentage. It was found that old adult women have a higher prevalence of UTI than men. *Escherichia coli* was the most common isolate (78.45%) followed by *Klebsiella pneumoniae* (21.56 %) amongst the gram-negative bacilli. Also, the results showed that, *E. coli* occurred more frequently in women (50%) than in men (28.45%). All isolates of *E. coli* and *K. pneumoniae* were high susceptible to Meropenem, Imipenem, Colistin, Ertapenem and Amikacin. This study showed that *E. coli* isolates were the predominant pathogens and showed increasing resistance pattern to the commonly prescribed drugs in private practice that in turn leaves the clinicians with very few alternative options of drugs for the treatment of UTIs

Akter *et al.*, (2016) studyed urinary tract infection (UTI) is the most common community-acquired bacterial infection affecting people of all age groups and both

sexes. This study was performed to isolate bacterial pathogens usually cause community-acquired uncomplicated UTI and to evaluate their sensitivity against 9 different antibiotics. Pathogenic bacteria were isolated and identified using conventional cultural and biochemical methods. Kirby-Bauer disc diffusion method on Mueller Hinton agar and Nutrient Agar media was used for the determination of sensitivity of the positive isolates to commonly prescribed antibiotics. Statistical Package for Social Sciences (SPSS) software, version 20 was used for statistical analysis. Our research showed that *Escherichia coli* was the most common causative agent of UTI (50.68%), followed by *Pseudomonas* species (17.81%), *Streptococcus* species (13.70%), *Staphylococcus aureus* (10.96%), *Klebsiella* species (4.11%) and *Proteus* species (2.74%). The number of Gram-negative bacteria (75.34%) was higher than the Gram-positive bacteria (24.66%). In this study, UTI was more prevalent in females (84.93%) in comparison to males (15.07%). Antimicrobial susceptibility results for *E. coli* are as follows: Cefixime (94.59%), Cephalexin (91.89%), Azithromycin (89.19%), Ciprofloxacin (83.78%), Co-trimoxazole (81.08%), Gentamycin (75.68%), Amikacin (51.35%), Amoxicillin (21.62%) and Nalidixic acid (8.10%). More than 90% of the isolated uropathogens were susceptible to Cefixime (94.52%), Cephalexin (94.52%) and Azithromycin (93.15%) and less than 20% were susceptible to Nalidixic acid (13.69 %). Among the uropathogens, *E. coli* (50.68%) was the most predominant bacteria in both gender and different age groups. Cefixime (94.52%), Cephalexin (94.52%) and Azithromycin (93.15%) were the most effective drugs and Nalidixic acid (13.69%) was the least effective drug for the treatment of UTI.

Mahmood *et al.*, (2016) investigated the susceptibility pattern of different bacteria isolated from urinary tract infection to different antibiotics. The isolated strains of bacteria were tested for their susceptibility to some antibiotics using disk diffusion method. The results showed that the bacterial species of *Eschericia coli , Proteus mirabilis, Klebsiella pneumonia, Citrobacter di-versus , Citrobacter freundii , Enterobacter aerogenes , Yersinia pestis , Pseudomonas aeruginosa, Klebsiella oxytoca* and *Hafnia alvei* were identified in 44 (53%), 18 (21.7%), 4 (4.8%), 4 (4.8%), 3 (3.6%), 3 (3.6%), 3 (3.6%), 2 (2.4%), 1 (1.2%) and 1 (1.2%), respectively, of the isolates. The results of antimicrobial susceptibility test showed that 83 (100%) isolates were resistant to Ampicillin, Rifampicin and Erythromycin. 75(90.3%) isolates were resistant to Cefotaxime, 67 (80.7%) isolates were resistant to Tobramyci. 66 (79.5%), 65 (78.3%),

56 (67.4%) and 48 (57.8%) isolates showed susceptibility to Nalidixic acid, Tetracycline, Nitrofurantoin, Chloramphenicol, respectively. 45 (54.2%) isolates were resistant to Azithromycin, Norfloxacin and Ciprofloxacin. Meropenem, Gentamicin, Amikacin, and Imipenem show significant effect on 35 (42.1%), 32 (38.5%), 27 (32.5%) and 1 (1.2%) isolates, respectively. In conclusion, significant bacteria count isolated from urine samples is pathogenic. The most effective antibiotic in inhibiting the bacterial growth.

Amany Sh. Jabber *et al.*, (2016) studied of urinary tract infection has been planned to isolation and identification of bacterial pathogens. The urine were collected from 60 patients for a Suq-Alshukh hospitals they were including 23 male and 37 female. Patients aged between 15 to 70 years. Urine culture had been done for all the 60 patients who were included in this study. Only 45 patients had positive urine culture they were including 15 male and 30 females. The most common organism was *Esherichia coli* which was isolated from 14 patients with percentage of 31.1%. *E.coli* was the most prevalent followed by *Proteus mirabilis* 22.2% (10), *Pseudomonas aeruginosa* 15.5% (7), *Staphylococcus aureus* 11.1% (5), *Klebsiella pneumonia* 13.3 % (4), *Staph. saprophyticus* 4.4% (2) and *Serratia marcescenes* 2.2% (1). The antibiotics susceptibility test was done for all isolates to 8 antibiotics, Amikacin was more antibiotic that effect on all kinds of isolate , the sensitivity of isolates to this antibiotic was registered (95.7%) followed by Ciprofloxacin (80.7%), while the highest resistant of all isolates was to Amoxicillin, it was registered (9.2%).

Yasmeen *et al.*, (2015) observed urinary tract infection (UTI) is one of the most important causes of morbidity and mortality in the developing countries like Bangladesh. Antimicrobial agents are the frequently used drug for its treatment. In this study, 182 (20.73%) out of 878 urine sample were positive for pathogenic organisms. Of the various pathogenic organisms isolated, *Escherichia coli* constituted for 85.16% followed by *Pseudomonas* spp, *Acinatobacter* spp., Group D *Streptococcus, Staphylococcus aureus, Klebsiella* spp., *Enterobacter* spp. and others. *E.coli* was found to be most sensitive to Imipenem, Amikacin and Meropenem and resistant to most commonly used oral drugs like Azithromycin, Cefexime, cotrimoxazole and Ciprofloxacin and Levofloxacin. Mainly Gram negative bacilli is responsible for UTI and most frequent isolated bacteria was *E-coli* . The most effective antibiotics were Imipenem, Amikacin, Meropenem,all of them are parentral. Majority *E-coli* were resistant to commonly used oral drugs like

Azithromycin, Cefexime, cotrimoxazole and Ciprofloxacin. Therefore the choice of antibiotic therapy in UTI should be depends on the local sensitivity pattern of the infecting organisms.

Goli *et al.*, (2015) determined *Escherichia coli* (*E. coli*) is the most frequent infecting organism in acute infection. 5093 (62.47%) of them were female and 3060 (37.53%) of them male. Urine specimens were cultured for isolation of the microbial agents of UTI. The isolated bacteria were identified using biochemical tests. Disk diffusion susceptibility test was used to determine antimicrobial susceptibility. *E. coli* (55.38%) was the most common isolated pathogen, followed by *Enterobacter* spp. (29.61%), *Pseudomonas* spp. (4.9%), S. aureus(3.21%), *Enterococcus* spp. (2.3%), fungi (1.5%) and *Klebsiella* (0.48%). The sensitivity rates of isolated gram negative bacteria were for Amikacin (95.7%), Nitrofurantoin (91.5%), Gentamicin (64.1%), Ceftizoxim (56.8%), Ciprofloxacin (37.6%), Cotrimoxazole (31.4%) and Nalidixic acid (23.5%). This study showed that the frequency of *E. coli* and *Enterobacter* spp . increases the probability of urinary tract infection. Also this survey indicates the emergence of antibiotic resistant infections in the studied hospital. So, there is a need to improve the effectiveness of integrated infection control programs to control and manage nosocomial infections caused by highly resistant organisms.

Khan *et al.*, (2014) determined the antimicrobial susceptibility pattern of bacterial pathogens in the patients of urinary tract infection reporting at a tertiary care hospital. Out of the 440 culture positive urine samples, 152 (34.6%) were from indoor patients whereas 288 (65.4%) from outdoor patients. Gram negative bacteria accounted for 414 (94%) of the total isolates while rest of the 26 (6%) were Gram positive bacteria. The most prevalent bacterial isolate was Escherichia (E.) coli 270 (61.3%) followed by *Pseudomonas* (P.) aeruginosa 52 (12%) and *Klebsiella* (K.) pneumoniae 42 (9.5%). The susceptibility pattern of *E. coli* showed that 96.2% of the bacterial isolates were sensitive to imipenem, 85.1% to amikacin, 80.7% to piperacillin/tazobactam and 72.6% to nitrofurantoin. In case of P. aeruginsosa, 73% bacterial isolates were sensitive to tazobactam/piperacillin, 69.2% to sulbactam/ cefoperazone and 65.38% to imipenem. The antibiogram of K. pneumoniae has revealed that 76.1% of the bacterial isolates were sensitive to imipenem and 52.3% to piperacillin/tazobactam. Nitrofurantoin and imipenem were the most effective antimicrobials amongst the Enterococcus spp. as

92.3% showed susceptibility to this bacterial isolate.Majority of the bacterial isolates were sensitive to imipenem and piperacillin/tazobactam while susceptibility to most of the commonly used oral antibiotics was very low. Among the oral antimicrobials, nitrofurantoin showed good susceptibility against Enterobacteriaceae family and Gram positive organisms.

Rahman *et al.*, (2014) studied urinary tract infection (UTI) is commonly experienced by women of various age groups especially elderly ones. We planned to find out the prevalent microbial strains causing UTI in slum inhabitant adolescent and adult women in Dhaka City, Bangladesh. Urine sample was collected from 462 UTI suspected female subjects. Pathogenic bacteria were identified using standard microbiological tests, and antimicrobial sensitivity profiles of the pathogens were determined. Bacteriuria was present in 9% of the subjects. A higher incidence (16.8%) of UTI was noted among adult women aged above 19 years. *Escherichia coli* (69%), *Streptococcus* spp. (15%) and *Pseudomonas aeruginosa* (7%) were more frequently isolated from the urine samples compared to *Enterococcus faecalis* (3%), *Staphylococcus aureus* (2%), *Klebsiella pneumoniae* (2%) and *Hafnia alvei* (2%). The *E. coli* isolates showed complete resistance to commonly used drugs, and 58% of these isolates were multidrug resistant (MDR). Minimum Inhibitory Concentration (MIC) values for ciprofloxacin ranged between 64µg/ml and 512µg/ml, and the Minimum Bactericidal Concentration (MBC) values against the isolates were 128µg/ml or above. Isolated strains of *E. coli* exhibited equal extent of ciprofloxacin resistance irrespective of the presence or absence of plasmid in them.

Mulugeta and Abera, (2014) to determine the prevalence and antimicrobial susceptibility of bacteria from suspected urinary tract infections. A retrospective analysis of bacterial pathogens and their antimicrobial susceptibility was done on urine samples at Dessie Regional Laboratory in the period 2003 to 2010. Antimicrobial susceptibility tests were done using disc diffusion technique as per the standard of Kirby-Bauer method. The male to female ratio of the patients was 1:1.96. Of the total 1404 samples, 319 (22.7%) were culture positive. *Escherichia coli* was the dominant isolate (63.6%) followed by *Klebsiella* spp. (8.5%) and *Proteus* spp. (8.2%). The overall resistance rates to erythromycin, amoxycillin, and tetracycline were 85.6%, 88.9% and 76.7%, respectively. The three most frequently isolated bacteria had resistance rates of 80.1%-

90.0% to, amoxycillin, and tetracycline and sensitivity rates of 0 to 25% to nitrofurantoin, ciprofloxacin and gentamicin. Antibiogram of isolate showed that 152 (47.85%) isolates were resistance to two and more antimicrobials. In the study area resistance rates to erythromycin, amoxycillin and tetracycline were high. Since most isolates were sensitive to nitrofurantoin and gentamicin, they are considered as appropriate antimicrobials for empirical treatment urinary tract infections.

Anbarasu (2014) found of his study 250 known diabetics patients among them 124 were males and 126 were females. Those individuals whose fasting blood sugar >140 mg/dl, postprandial blood sugar >200mg/dl and HbA1C levels >7 were grouped as uncontrolled diabetes; while those with fasting blood sugar <140 mg/dl, postprandial blood sugar <200 mg/dl and HbA1C levels <7 were grouped as controlled diabetes. He found that 176 (70.4%) persons had uncontrolled diabetes and 74 (29.6%) had controlled diabetes. Asymptomatic bacteriuria was present in 26.7% of uncontrolled diabetes and 12% (9) among controlled diabetes. *Escherichia coli* (44.6%) were the predominant pathogen isolated followed by *coagulase negative Staphylococci.*

Abdul-Hameed *et al.*, (2014) determined the prevalence of uropathogens in diabetic patient and their antibiotic susceptibility. He showed that females are more vulnerable to pathogenic attack than males throughout a wide age distribution. *Escherichia coli* were the most common pathogen had been isolated followed by *Staphylococcus aureus*, *Enterobacter* species, *Klebsiella pneumoniae* and a few others. The isolates showed moderate to high level of sensitivity to various antibiotics tested.

Hossain *et al.*, (2014) investigated Catheter-associated urinary tract infection (CAUTI) is the most common device-associated nosocomial infection worldwide. Bacteria, which exist as a biofilm inside catheters, show higher anti-microbial resistance when compared to non-CAUTI pathogens. The present study was conducted to determine the antibiotic susceptibility patterns of CAUTI and non-CAUTI bacteria. The antibiotic susceptibility patterns of 102 uropathogens from noncatheterized patients and 100 uropathogens from catheterized patients were compared using the disc diffusion method. A higher incidence of uropathogens was correlated with catheter use in male patients. *Escherichia coli* was the predominant isolate obtained from catheterized (81%) and noncatheterized (67%) patients. This was followed by *Pseudomonas* aeruginosa, with rates of 28% and 15% in non-CAUTI and CAUTI patients, respectively. Overall, the *E. coli* isolates from CAUTI

patients showed significantly higher resistance ($p<0.05$) than those from non-CAUTI patients against all antibiotics tested, except for trimethoprim/sulfamethoxazole and gentamicin. Catheter-associated P. aeruginosa isolates showed significantly higher resistance ($p<0.05$) against most antibiotics tested compared to non-catheter-associated isolates. Uropathogens from CAUTI patients exhibit significantly higher resistance to most antibiotics than non-CAUTI isolates. This is an important factor to take into consideration when choosing correct treatment options for patients with urinary tract infection.

Ouno *et al.*, (2013) conduced urinary Tract Infection (UTI) defined a condition in which the urinary tract is infected with a pathogen causing inflammation which is a common, distressing and occasionally life threatening condition. Cultural and biochemical characterization of uropathogens revealed the prevalence of both gram-positive and gram-negative organisms. *E. coli* was the predominant isolate isolated from the urine specimen followed by *Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Proteus mirabilis* and *Enterococcus faecalis.* Among the antibiotics tested, chloraphenicol and ciprofloxacin (100%) were found to be effective for empirical treatment of UTI and has covered the majority of urinary pathogens followed by tetracycline, gentamycin and kanamycin (83%), Ampicillin (67). Streptomycin, Rifampicin and amoxicillin were less effective (50%). Some of the isolates were resistant to penicillin-G, Streptomycin, rifampicin and amoxicillin which are more frequently prescribed and indicates that increased consumption of a particular antibiotic leads to acquisition of resistance by the uropathogens.

Akter *et al.*, (2013) Diagnosed of urinary tract infection (UTI) causing pathogens with their sensitivity to different antibiotics was performed with a total of 96 samples from both male (n=31; 32.3%) and female (n=65; 67.7%) of different age groups. Out of 96 urine samples, 55 (57.3%) were found positive after culturing in MacConkey agar plates. The percent distribution of positive cases against collected samples was higher for female (67%) than male (32%). However, female and male at the reproductive age of 16-30 years were more susceptible to UTI. A total of 55 bacterial isolates were identified by conventional methods and their antibiotic sensitivity was tested using Mueller-Hinton agar plates. The predominant isolates were *Escherichia coli* (34.5%), *Klebsiella* sp. (18.2%) and *Staphylococci* (20.0%). The sensitivity pattern for most of the isolated

organisms showed 50% and/or higher sensitivity to imipenem, azithromycin and cephalexin, except *Staphylococci* (only 9.09% to azithromycin).

Sharif (2013) observed "Prevalence and Antibiotic Susceptibility Pattern of Microbial Agents That Cause Urinary Tract Infection". The sensitivity against various antibiotics was determined by using antibiotic sensitivity discs; namely imepenem, gentamicin, ciprofloxacin and co-trimoxazole. The most common pathogen was *Escherichia coli* (45.2%) and *Klebsiella* sp (18%). Our study showed 8.5%, 29.6% and 40.9% resistance rate of *E coli* to Imipenem, ciprofloxacin and co-trimaxazole. Overall bacterial resistance rate to imipenem, gentamycin, ciprofloxacin and co-trimoxazole was high especially in mixed specious cultures. Resistance of co-trimoxazole is needs more attention because it is more than other studies and shows unwise use of this antibiotic. He utilized 221 cultures, 157 had showed significant bacterial growth and entered our study. 110 (70%) with mean age of 40.1 (SD=11.3) and 47 were male with the mean age of 31 (SD=4.4). the most common pathogen was *Escherichia coli* (70 isolates, 45.2%) and *Klebsiella* sp (20 isolates, 18%).

Nerurkar *et al.*, (2012) investigated urinary tract infection (UTI) is one of the commonest infections encountered by clinicians and despite the widespread availability of antimicrobial agents UTI has become difficult to treat because of appearance of pathogens with increasing resistance to antimicrobial agents. The aim and objectives of this study were to determine the etiological Bacterial pathogens of the UTI and to determine the antibiotic sensitivity pattern of pathogens isolated. The present study was a cross sectional study carried out in a private pathology laboratory situated in western Mumbai from January 2 008 to December 2010. Total 280 urine samples were tested bacteriologically and for antibiotic susceptibility using standard procedures. Out of 280 urine samples 168 (60%) patients tested positive for culture. E.coli was the most common isolate (44.96%) followed by Enterobacter spp (17.83 %) and *Klebsiella* spp (14.72%) amongst the gram negative bacilli. Amongst the gram positive bacteria *Staphylococcus aureus* (92.3%) was commonest. *E. coli* which was the main isolate identified was found to be highly susceptible to Amikacin (82.2%) followed by Ciprofloxacin (78.2 %), Gentamicin (80.4 %), Ampicillin (59 %) and Nitrofurantoin (57 %). This study finding showed that *E. coli* isolates were the predominant pathogens and showed increasing resistance pattern to the commonly prescribed drugs in private

practise that in turn leaves the clinicians with very few alternative options of drugs for the treatment of UTIs.

Alemu *et al.*, (2012) indicated the prevalence of UTI in pregnant women was 11.6% and Gram negative bacteria was the predominant isolates and showed multi drug resistance. This study aimed to assess bacterial profile that causes urinary tract infection and their antimicrobial susceptibility pattern among pregnant women visiting antenatal clinic at University of Gondar Teaching Hospital, Northwest Ethiopia.A cross-sectional study was conducted at University of Gondar Teaching Hospital from March 22 to April 30, 2011. Mid stream urine samples were collected and inoculated into Cystine Lactose Electrolyte Deficient medium (CLED). Colony counts yielding bacterial growth of 105/ml of urine or more of pure isolates were regarded as significant bacteriuria for infection. Colony from CLED was sub cultured onto MacConkey agar and blood agar plates. Identification was done using cultural characteristics and a series of biochemical tests. A standard method of agar disc diffusion susceptibility testing method was used to determine susceptibility patterns of the isolates.The overall prevalence of UTI in pregnant women was 10.4%. The predominant bacterial pathogens were *Escherichia coli* 47.5% followed by coagulase-negative *Staphylococci* 22.5%, *Staphylococcus aureus* 10%, and *Klebsiella* pneumoniae 10%. Gram negative isolates were resulted low susceptibility to co-trimoxazole (51.9%) and tetracycline (40.7%) whereas Gram positive showed susceptibility to ceftriaxon (84.6%) and amoxicillin-clavulanic acid (92.3%). Multiple drug resistance (resistance to two or more drugs) was observed in 95% of the isolates. Significant bacteriuria was observed in asymptomatic pregnant women. Periodic studies are recommended to check the outcome of asymptomatic bacteriuria and also monitor any changes in the susceptibility patterns of urinary tract pathogens in pregnant women

Getenet and Wondewosen (2011) observed urinary tract infection (UTI) is one of the most common bacterial infections encountered by clinicians in developing countries. Area-specific monitoring studies aimed to gain knowledge about the type of pathogens responsible for urinary tract infections and their resistance patterns may help the clinician to choose the correct empirical treatment. Therefore, the aim of this study was to determine the type and antibiotic resistance pattern of the urinary pathogens isolated from patients attending Jimma University Specialized Hospital from April to June 2010.

A hospital based cross sectional stud was conducted and urine samples were collected using the mid-stream "clean catch" method from 228 clinically-suspected cases of urinary tract infections and tested bacteriologically using standard procedures. Antimicrobial susceptibility test was performed for the isolated pathogens using Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute guidelines. Significant bacteria were detected from 9.2% of the total patients. The most common pathogens isolated were *Escherichia coli* (33.3%), *Klebsiella pneumoniae* (19%) and S. saprophyticus (14.3%). *E. coli* and *Klebsiella* pneumoniae showed the highest percentage of resistance to ampicillin and amoxacillin (100%) however, all isolates of *E. coli* and *K. pneumoniae* were susceptible to ciprofloxacin. *S. saprophyticus* and *S. aureus* were resistant to ampicillin (100%) and amoxicillin (66.7%). For all UTI isolates, least resistance was observed against drugs such as ceftriaxone, gentamycin and chloramphenicol. This study finding showed that *E. coli* isolates were the predominant pathogens and the presence of bacterial isolates with very high resistance to the commonly prescribed drugs that in turn leaves the clinicians with very few alternative options of drugs for the treatment of UTIs. As drug resistance among bacterial pathogens is an evolving process, routine surveillance and monitoring studies should be conducted to provide physicians knowledge on the updated and most effective empirical treatment of UTIs.

Nyambane and Ontita (2011) conducted urinary Tract infection (UTI) is a serious infection causing illness in infants and children. It represents one of the most common diseases encountered in medical practice today. A total of 186 urine samples were collected from in and out -patients attending Kisii level 5 Hospital, Kisii County, Kenya between December 2012 and March 2013. Urine samples accompanied by microbiology request forms were delivered directly to the laboratory. All sample processing and patient's biodata were carried out centrally in Kisii level 5 hospital microbiology laboratory. The samples were cultured on Cystein lactose electrolyte deficient (CLED) media and incubated for 18 hours at 37^0C. Criteria for defining significant bacteria was the presence of 105 colony forming units per millimetre of urine. The bacterial isolates recovered were tested against Ampicilin, Tetracycline, Nitrofurantion, Nalidixic acid, Streptomycin, Co-Trimoxazole and Gentamicin using Kirby Bauer disc diffusion technique.Data was presented as frequencies. Chi square analysis (χ^2) was used in comparing of positive UTI cases according to individual characteristics. Evaluations

were carried out at 95 % confidence level and P< 0.05 was considered statistically significant. Among the 186 samples examined 63.4 % of them were from female patients and 36.6 % from male patients; 26 (14 %) samples had positive bacteriuria with *Escherichia coli* isolates being the highest with 13 (50 %), *Klebsiella* 8 (30.8 %), *Staphylococcus aureus* 4 (15.4 %) and *Pseudomonas aureginosa* 1 (3.8 %). The isolates were sensitive to Nitrofurantion, Nalidixic acid, and Streptomycin while resistant to Ampicilin, Tetracycline, Gentamicin and Cotrimoxazole.

Neil (2011) observed 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.

Saber and Barai (2010) found Department of Microbiology, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorder (BIRDEM). "The Pattern of Organism Causing Urinary Tract Infection in Diabetic and Non Diabetic Patients in Bangladesh". Out of 351 urine samples, 288 patients (196 female and 92 males) were type 2 diabetic and 63 (43 females and 20 males) were non-diabetic patients. The mean ages of diabetic and non diabetic patients were 49.5 ± 8.3 years and 43.4 ± 17.4 years respectively. Majority of patients (263/351) were in between 20 to 60 years

age and the culture positivity rate was 45.6%. Out of 351, 76 patients were above 60 years, culture positivity rate 42.1% and 12 were below 20 years whose culture positivity rate was only 8.3%. A total of 153 (43.6%) sample showed significant growth.

Manikandan *et al.*, (2010) observed the present study aimed to ascertain the current situation of antimicrobial resistance of Urinary Tract Infections (UTIs) caused by human pathogens. 10 midstream urine samples were collected from adult patients were analyzed for Multi drug Resistant (MDR) strain isolation and identified. The MDR strains were identified by the Kerby Bauer method following the definition of the National Committee of Clinical Laboratory Standards. This result was clear that *E. coli* was the predominant pathogen (31.5%) causing UTI, followed by *Staphylococcus aureus* (20.5%), *Klebsiella pneumonia* (15.8%), *Proteus mirabilis* (7.4%) and *Pseudomonas aeruginosa* (7.5%). The percentages of resistance of all isolates to the antimicrobial agents were: 83.3% to SXT, 80.6% to Nalidixic acid, 67.3% to Amoxycillin, 61% to Cotrimoxazole, 48.8% to Gentamycin, 46% to ciprofloxacin and 43% to cephalexin. Isolated UTI strains were tested for susceptibility against antibiotics, few of the antibiotics were sensitive, but most of antibiotics showed resistant to the MDR strains. Among this *E. coli* , *K. pneumoniae* and *P. aeruginosa* were highly resistance to most of the antibiotics, whereas *Staphylococcus* spp, and *Serratia marcescens* exhibited sensitive to Cephalexin, Ciprofloxacin and Gentamycin. The present study was evaluated for the prevalence of micrograms implicated in UTI to ascertain their antimicrobial resistance patterns and indicates emerging multi drug 6+resistance among UTI bacterial pathogens.

Farjana *et al., (* 2009) found the empirical therapy of urinary tract infections (UTI) relies on the predictability of the agents causing UTI and knowledge of their antimicrobial susceptibility patterns.In a prospective study undertaken over a 14-month period, 5136 samples from patients suspected of having a UTI were analyzed, of which 676 were culture-positive. Isolated bacteria were identified by standard tests, and antibiotic susceptibility was determined by disk diffusion method. According to our results, *Escherichia coli* was the most common etiological agent of UTI (74.6%), followed by *Klebsiella spp* (11.7%), *Staphylococcus saprophyticus* (6.4%), and *Pseudomonas aeruginosa* (2.2%). Analysis of the frequency of isolated bacteria according to the age of the patients revealed that *Klebsiella* infections are more prevalent

in the older age groups (>10 years) and *Pseudomonas* infections are more prevalent in children and the elderly (<9 years and >60 years). Results of antimicrobial susceptibility analysis for *E. coli*, as the most prevalent cause of UTI, to commonly used antibiotics are as follows: amikacin (97.8%), gentamicin (97%), ciprofloxacin (94%), nitrofurantoin (87.1%), nalidixic acid (93.7%), trimethoprim–sulfamethoxazole (48.2%), cephalexin (76%), and ampicillin (6.9%). The results show that the antimicrobial resistance patterns of the causes of UTI are highly variable and continuous surveillance of trends in resistance patterns of uropathogens is important.

Imirzaligolu *et al.*, (2008) observed urinary tract infections (UTIs) are the most common kidney and urologic diseases in industrial nations and are usually caused through faecal contamination of the urinary tract. In this study, we have examined 1449 urine specimens both by culture and by PCR. The majority of UTIs examined were caused by *Escherichia coli* (35.15%), followed by miscellaneous bacteria (23.03%), and by *Enterococcus faecalis* (19.39%). A large fraction of fastidious and anaerobic bacteria (22.43%) was not detected under culture conditions but only by using PCR. This group of bacteria evade the standard culture conditions used in routine diagnostic laboratories examining urine specimens. The molecular approach used broad-range 16S rDNA PCR, denaturing high-performance liquid chromatography analysis, sequencing, and bioinformatic analysis to uncover these ‘hidden’ pathogens and is recommended in particular when examining leukocyte esterase-positive and culture-negative urinary tract specimens.

Rosen *et al.*, (2007) conducted 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.

Jasmine and Guy (2007) identified symptomatic urinary tract infections (UTI) are either uncomplicated or complicated. Uncomplicated infections occur in healthy women in the community and are usually caused by *Escherichia coli*. Complicated infections are associated with anatomical, functional, or metabolic abnormalities of the urinary tract that disable the natural innate host defences and lead to tissue injury. Patients with symptomatic infections will have $>10^5$ bacteria/ml and inflammatory cells in freshly voided urine. A third group is commonly seen whose symptoms may suggest UTI, but in whom there is no objective evidence for infection. Careful history, examination and investigation are important to avoid repeated and unnecessary courses of antibiotics. Infection is determined by bacterial virulence offset by a complex of innate host defences and some acquired immunity. Urine flow and regular and complete bladder emptying are the first priority; any cause of urine stagnation will promote infection. Investigation is, therefore, primarily aimed at ensuring there is no obstruction and that the bladder voids to completion. This is achieved with plain X-ray, ultrasound of kidneys and the bladder after voiding, and urine flow rate. Acute uncomplicated infection does not require more than 3 days antibiotics. Asymptomatic bacteriuria requires treatment only in infants, pregnancy and before urological intervention. For recurrent and complicated infections, it is mandatory to identify the organism and its sensitivity.

Tambekar *et al.*, (2006) found urinary tract infection represents one of the most common diseases encountered in medical practice today and occurring from the neonate to the geriatric age group. Despite the widespread availability of antibiotics, it remains the most common bacterial infection in the human being. A total of 174 urine samples were analyzed for isolation and identification, 68 found to be significant acteriuria with *Escherichia coli* (59%), followed by *Pseudomonas aeruginosa* (15%), *Klebsiella* pneumoniae (10%), *Proteus mirabilis* (9%), *Staphylococcus aureus* (6%) and *Citrobacter freundii* (1%).The urinary tract infections were found to most frequently in female (63%) than male (37%). The isolated uropathogens showed resistant to ampicillin (87%), co-trimoxazole (91%), nalidixic acid (88%) and sensitive to nitrofurantoin (52%), cephotaxime (54%) and norfloxacin (71%).

Allan Ronald (2003) done 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.The pathogens traditionally associated with UTI are changing many of their features, particularly because of antimicrobial resistance. 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. 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.

Orenstein and Wong (1999) studied urinary tract infections remain a significant cause of morbidity in all age groups. Recent studies have helped to better define the population groups at risk for these infections, as well as the most cost-effective management strategies. Initially, a urinary tract infection should be categorized as complicated or uncomplicated. Further categorization of the infection by clinical syndrome and by host (i.e., acute cystitis in young women, acute pyelonephritis, catheter-related infection, infection in men, asymptomatic bacteriuria in the elderly) helps the physician determine the appropriate diagnostic and management strategies. Uncomplicated urinary tract infections are caused by a predictable group of susceptible organisms. These infections can be empirically treated without the need for urine cultures. The most effective therapy for an uncomplicated infection is a three-day course of trimethoprim-sulfamethoxazole. Complicated infections are diagnosed by quantitative urine cultures and require a more prolonged course of therapy. Asymptomatic bacteriuria rarely requires treatment and is not associated with increased morbidity in elderly patients.

Giammanco and Sarina (1994) conducted Two commercially available media recommended for the isolation and rapid identification of *Escherichia coli* from urinary tract infections were supplemented with L- phenylalanine and L-tryptophan. The non-selective medium proved suitable for the direct detection of lactose fermentation, P-glucuronidase and phenylalanine deaminase activities, indole production and the oxidase

test. It was highly efficient in making a presumptive identification at species level of the most common gram-negative urinary pathogens, E. coli, *Proteus mirabilis* and *Pseudomonas* aeruginosa, that account for 85 YO of all urinary isolates. Among the gram-positive isolates, most colonies were non-fluorescent and could be separated into *Staphylococci* and *enterococci* on the basis of the catalase test. Fluorescent colonies were found to be *Staphylococcus* haemolyticus isolates, 61 YO of which were fluorescent. The selective medium proved suitable for the same biochemical tests, with the exception of indole, which was not visible against the red colour of the medium. Therefore, the differentiation of P. mirabilis from other Proteus-Providencia species was impossible on this medium.

Johnson and Stamm (1989) observed that acute urinary tract infection is a major health problem among women, accounting for considerable morbidity and health care costs. We review recent developments in the diagnosis and treatment of these infections. In acute lower urinary tract infection, empiric short-course therapy (single-dose or 3-day therapy) with one of several antibiotics is recommended in the absence of complicating factors. When complicating factors are present, the antibiotic susceptibility profile of the infecting organism should be determined and therapy with an appropriate agent should be provided for 7 days. Ampicillin and related drugs are probably inferior to trimethoprim-sulfamethoxazole in the treatment of occult renal infection. In acute pyelonephritis, most patients require hospitalization and treatment with intravenous antibiotics until they can take oral medications. In uncomplicated cases, a single broad-spectrum intravenous agent can be used initially, followed by an oral agent selected on the basis of antibiotic-susceptibility testing results. Patients with uncomplicated acute pyelonephritis who are less ill can be managed with oral therapy as outpatients, again with reference to the results of antibiotic-susceptibility testing. Complicated acute pyelonephritis requires more aggressive diagnostic and therapeutic measures. Therapy for uncomplicated acute pyelonephritis should be given for 14 days. The role of post-therapy cultures in the management of urinary tract infection is not well defined, but cultures probably can be safely omitted in most cases of uncomplicated acute cystitis.

CHAPTER-III

MATERIALS AND METHODS

The present study was conducted in the Saint Vincent Hospital, Mission Road, Kosba, Dinajpur-5200 and Department of Microbiology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science & Technology University, Dinajpur during the period of July 2016 to June 2017. The detailed outline of the Materials and Methods are given bellow:

Materials and Methods

3.1 Materials used

3.1.1 Selection of study area

This study was conducted during the period of July 2016 to June 2017 at ST. Vincent Hospital Dinajpur. The samples were collected from the suspected patients with the sterile urine collection pot and brought to the Department of Pathology for routine examination (physical, chemical and microscopic) at ST. Vincent Hospital, Dinajpur.

3.1.2 Collection of data and samples

A total of 58 samples were collected from suspected be infected patients at ST. Vincent Hospital, Dinajpur during the period of July 2016 to June 2017. The sample were collected based on age, sex for the isolation and identification of bacterial pathogens by morphology, staining, cultural and biochemical properties.

3.1.3 Laboratory preparations

All items of required glassware including test tubes, pipettes plate, slides, cylinder, flasks, conical flasks, glass and vials soaked in a household discwashing detergent solution (‘Trix’ Recket and Colman Bangladesh Ltd.) overnight. Contaminated glassware was disinfected with 2% sodium hypochloride solution prior to cleaning. The glassware were then cleaned by brushing, washed thoroughly in running tape water, rinsed within distilled water and finally sterilized either by dry heat at 160˚C for 2 hours or by autoclaving for 15 minutes at 121˚C under 15 lbs pressure per sq inch. Autoclaved items were dried in a hot air oven over at 50˚C. Disposable plastic (items e.g.

micropipette tips) was sterilized by autoclaving. All the glassware was kept in oven at 50˚C for future use.

3.1.4 Media for culture

The media and reagents that have been used for the isolation and identification of the bacteria are mentioned below.

3.1.4.1 Solid media

- Nutrient Agar Medium, (HI-MEDIA, India)
- Eosin Methylene Blue, (EMB) (HI-MEDIA, India)
- Blood Agar Medium, (HI-MEDIA, India)
- Mac Conkey Agar medium, (HI-MEDIA, India)
- S-110 Agar Medium, (HI-MEDIA, India).
- Mannitol Salt Agar Medium, (HI-MEDIA, India).
- Cetrimide Agar Medium, (HI-MEDIA, India).

3.1.4.2 Liquid media

- Methyl Red-Voges Proskauer(MR-VP) broth, (HI-MEDIA, India)
- 1% Pepton water (HI-MEDIA, India)

3.1.5 Chemicals and reagents

3.1.5.1 Reagents

The chemicals and reagents used during the study were-

- Gram's staining reagents (Crystal violet, Gram's iodine, Acetone alcohol, Safranin)
- Potassium- di-hydrogen phosphate (0.2M, KH_2PO_4 $2H_2O$)
- Dehydrated sodium citrate
- Phosphate Buffered Saline (PBS)
- Physiological Saline Solution (PSS)
- Methylene Blue stain
- Di-sodium hydrogen phosphate (0.2M, $Na_2HPO_4 12H_2O$)
- Voges-Proskauer (VP) Solution

- Sugar media (Dextrose, Maltose, Lactose, Sucrose, and Mannitol) and other chemicals and reagents as when required during the experiment.
- Indol Solution
- Methyl Red Solution

3.1.6 Glasswares and appliances

The different kinds of glassware's and appliances used during the course of the experiment were as follows:

Test tubes (with or without Durham's fermentation tube and stopper)

- Pipette
- Cover slips
- Hanging drop slide
- Glass rod spreader
- Test tube stand Water bath
- Autoclave
- Refrigerator
- Hot air oven
- Compound microscope
- Micropipette
- Centrifuged tube
- Spirit lamp
- Slide
- Conical flask
- Inoculating loop
- Petridishes Conical flask
- Stopper of test tube
- Immersion oil
- Electric Balance
- Bacteriological loop
- Microscope

3.1.7 Antimicrobial Sensitivity Discs

To determine the drug sensitivity pattern of different bacterial isolate with different types of antimicrobial. Commercially available antimicrobial discs (Oxoid Ltd., UK) were used. The method allowed for the rapid determination of the efficacy of the drug by measuring the diameter of the zone of inhibition that result from different diffusion of the agent into the medium surrounding the disc. The followings are the antibiotics that were tested against, the selected organism with their disc concentration.

Table 1. Antimicrobial agents with their disc concentration.

Sl. No.	Name of the Antibiotic	Letter Code	Disc Concentration (µg/disc)	Source
1	Amoxycillin	AMC	10	Becton Dickinson, USA
2	Ciprofloxacillin	CIP	5	Oxoid Ltd., UK
3	Vancomycin	VA	30	Oxoid Ltd., UK
4	Gentamicin	GEN	10	Oxoid Ltd., UK
5	Amikacin	AK	30	Oxoid Ltd, UK
6	Streptomycin	S	10	Oxoid Ltd, UK
7	Erythromycin	E	15	Oxoid Ltd., UK
8	Tetracycline	TE	30	Oxoid Ltd., UK
9	Chloramphenicol	C	30	Oxoid Ltd., UK
10	Levofloxacin	LF	5	Oxoid Ltd., UK
11	Norfloxacin	NX	10	Oxoid Ltd., UK

Legend: µg = Microgram

3.2 Methods

The following methods were used for the isolation and identification of bacteria.

3.2.1 Experimental design

The experimental work was divided into two steps: The first step was performed for the isolation and identification of the organisms of the collected sample using cultural, staining and biochemical characteristics. The second step was conducted for the determination of antibiotic sensitivity and resistant pattern of isolated organisms of various samples by using different antibiotic discs available in the market. The layout of the diagrammatic illustration of the present study is shown in figure-1.

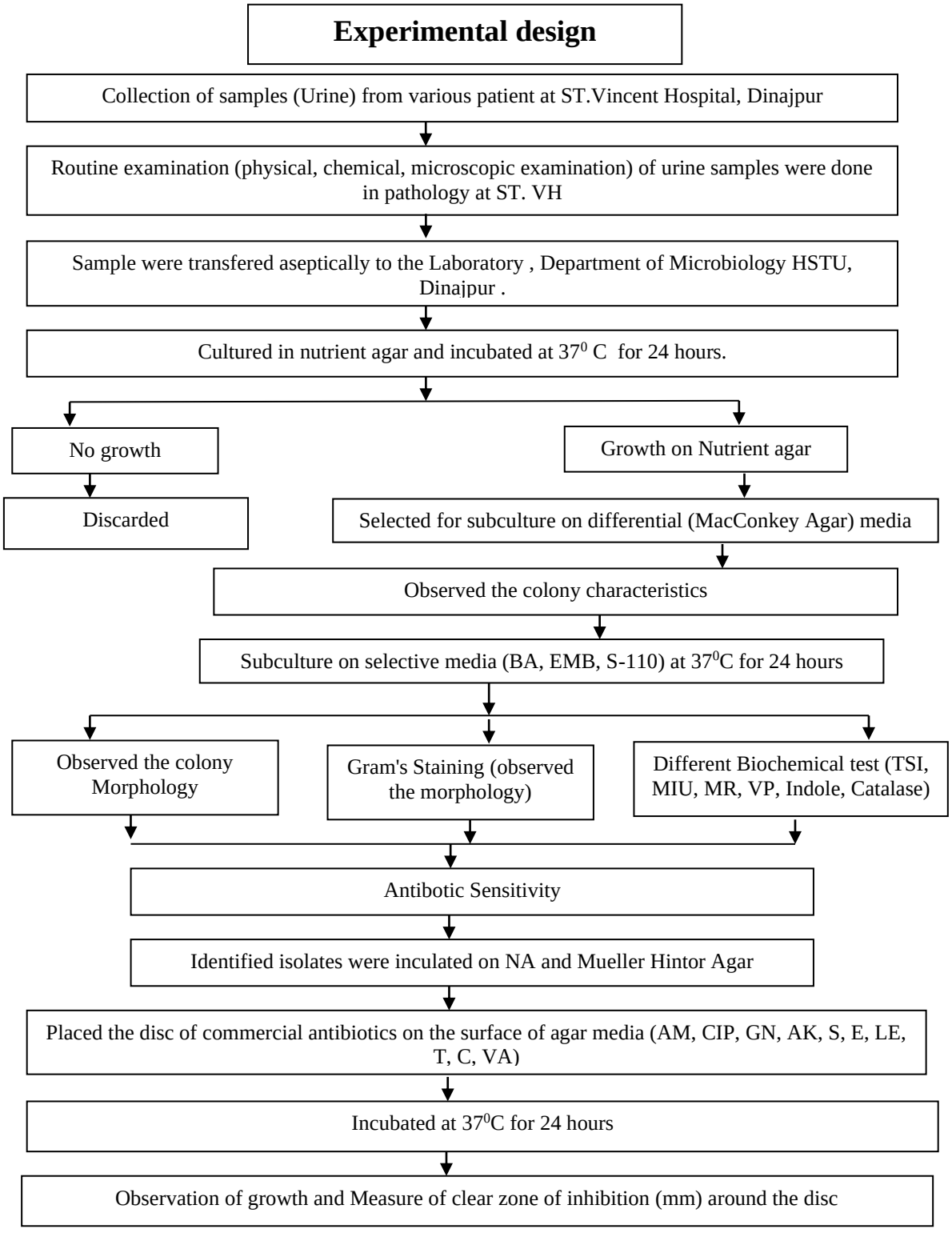


Figure 1. The schematic illustration of the experimental layout.

(Legends: BA-Blood agar, EMB-Eosin methylene blue agar,s-110-Staphylococcus agar no.-110, TSI-Triple sugar iron, MIU-Motility Indole Urease, MR-Methyl Red, VP-Voges-Proskauer).

3.2.2 Collection and transportation of samples

3.2.2.1 General concepts for specimen collection and handling

3.2.2.1.1 Appropriate collection techniques

Specimens should be collected during the acute phase and illness (of within 2 to 3 days for bacterial infections), and before antibiotics and administered. Specimens collected by the patient are handled properly .Most urine collection kits contain instructions in several languages, but nothing substituting for a concise set of verbal instructions.

Plate 1: Specimen collection from patient

After collection and recording of data all of the sample transferred to the Microbiological laboratory, HSTU, Dinajpur.

3.2.2.1.2 Specimen transport

Specimens were transported to the laboratory within 2 hours of collection. All specimens containers should be leak-proof, and the specimens should be transported within sealable, leak-proof, plastic bags with a separate section for paperwork; resalable bags or bags with a permanent seal were common for this purpose. Bags should be market with a biohazard label.

3.2.2.1.3 Specimen storage

Specimens cannot be processed as soon as they were received, they must be stored. Several storage methods are used (i.e., refrigerator temperature 4°C) ambient room temperature are [22°C], body temperature [37°C], and freezer temperature [either- 20 or- 70°C], depending on the type of transport media (if applicably) and the etiologic (infectious) agents sought specimens suspected of containing anaerobic bacteria, for

example, should never be stored in the refrigerator urine stool, viral specimens sputum sweeps, and foreign devices such as catheters should be stored at 4°C.

3.2.3 Preparation of reagents

3.2.3.1 Methyl- Red solution

The indicator MR solution was prepared by dissolving 0.1 gm of Bacto methyl- red in 300 ml of 95% alcohol and diluted to 500 ml with the addition of distilled water.

3.2.3.2 Methyl Red - Voges Proskauer broth

A quantity of 17 gms of MR-VP medium (HI-MEDIA) was dissolved in 1000 ml of distilled water, dispensed in 2 ml amount in each tube and the tubes were autoclaved. After autoclaving, the tubes containing medium were incubated at 37^{o}C for overnight check their sterility and then in refrigerator for future use.

3.2.3.3 Voges – Proskauer solution

Alpha- naphthol solution was prepared by dissolving 5 gm of Alpha- naphthol in 100 ml of 95% ethyl alcohol.

3.2.3.4 Potassium hydroxide solution

Potassium hydroxide (KOH) solution was prepared by adding 40 grams of Potassium hydroxide crystals in100 ml of cooled water.

3.2.3.5 Phosphate buffered saline solution

Eight grams of sodium chloride (NaCl), 2.89 grams of di-sodium hydrogen phosphate Na_2HPO_4, $12H_2O$), 0.2 gram of potassium chloride (KC1) and 0.2 gram of potassium hydrogen phosphate (KH_2PO_4) were suspended in 1000 ml of distilled for the preparation of phosphate buffered saline solution. The solution was heated to dissolve completely. Then the solution was sterilized by autoclaving at 1.2 kg / cm^2 pressure and 121°C for 15 minutes and stored for future use.

3.2.3.6 Preparation of physiological saline solution

For the preparation of this solution procedures suggested by Cowan (1985) were followed. A 0.85% PSS was prepared by dissolving 8.5 gms of chemically pure sodium chloride (NaCl) in 1000 ml of distilled water in a conical flask. The physiological saline solution was then sterilized by autoclaving at $l2l^0$ C under 15 lbs, for 15 minutes.

Following sterilization, the saline was cooled and then kept at 4^0 C -8^0 C in the refrigerator until used.

3.2.4 Preparation of culture media and broth

All the media, broth and reagents used in this experiment were prepared according to instruction of the manufacturer.

3.2.4.1 Nutrient agar medium

Twenty eight grams of nutrient agar powder (HI-MEDIA) was suspended in 1000 ml of cold distilled water in a flask and heated to boiling for dissolving the medium completely. The medium was then sterilized by autoclaving. After autoclaving, the medium was poured into each sterile Petridish and allowed to solidify. After solidification of the medium in the petridishes, these were incubated at 37°C for overnight to check their sterility and used for cultural characterization or stored at 4°C refrigerator for future use.

3.2.4.2 Mannitol salt agar media

111 grams manitol salt agar powder (HI-MEDIA) was suspend in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. If desired, add 5% v/v Egg Yolk Emulsion (FD045). Mix well and pour into sterile Petri plates or dipense as desired. After solidification of the medium in the petridishes, these were incubated at 37°C for overnight to check their sterility and used for cultural characterization or stored at 2-8°C refrigerator for future use.

3.2.4.3 MacConkey agar medium

51.5 grams MacConkey agar base (HI- MEDIA, India) powder was added to 1000 ml of distilled water in a flask and heated until boiling to dissolve the medium completely. The medium was then sterilized by autoclaving at 1.2 kg/cm^2 pressure and 121° C for 15 minutes. After autoclaving the medium was put into water bath at 45^0- 50^0C to decrease the temperature. Then medium was poured in 10 ml quantities in sterile glass petridishes (medium sized) and in 15 ml quantities in sterile glass Petridishes (large sized) to form thick layer there in. To accomplish the surface be quite dry, the medium was allowed to solidify for about 2 hours with thennn/bn/n / covers of the Petridishes partially removed.

The sterility of the medium was checked by incubating at 37°C for overnight. The sterile medium was used for cultural characterization or stored at 4°C in refrigerator for future use petridishes, these were incubated at 37°C for overnight to check their sterility and used for cultural characterization or stored at 4°C in refrigerator for future use.

3.2.4.4 Blood agar medium

Forty grams of Blood agar base (HI-MEDIA, India) powder was suspended in 1000 ml of distilled water and boil to dissolve the medium completely. The medium was sterilized by autoclaving at 1.2 kg/cm^2 pressure and 121°C for 15 minutes and 45°C. Then 5-10 % sterile defibrinated blood was added to the medium and distributed to sterile petridishes and allowed to solidify.

3.2.4.5 Staphylococcus agar No.110

Suspend 149.5 grams Staphylococcus Agar No.110 in 1000 ml of distilled water. Mix thoroughly. Heat, to boiling, to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Resuspend the precipitate by gentle agitation to avoid bubbles and pour the plates while the medium is hot. Alternatively, cool the medium to 45 - 50°C. This medium may also be used without sterilization; it should be boiled for 5 minutes and used at once . After solidification, the Plates were incubated at 37^{0}C in the incubator for 24 hours to check sterility of the media and were kept at 2^{0}C-8^{0}C in the refrigerator until used.

3.2.4.6 Eosin methylene blue agar (EMB)

Thirty six grams of EMB agar base (HI-MEDIA, India) was added to 1000 ml of water in a flask and boil to dissolve the medium completely. The medium was sterilized by autoclaving at 1.2 kg/cm^2 pressure and 121° C for 15 minutes and I to 50°C and shake the medium in order to oxidize the methylene blue (i.e. to restore its blue colour). Then 10 ml of medium was poured into each sterile Petridish sized and allowed to solidify. After solidification of the medium in the petridishs, these were incubated at 37°C for overnight to check their sterility and petridishes without contamination were used for cultural characterization or stored at 4°C in refrigerator for future use.

3.2.4.7 Cetrimide agar media

The CA agar plates were prepared and stored following the procedure of Crowan, (1985). An amount suspend 46.7 grams in 1000 ml distilled water containing 10 ml

glycerol. Heat, to boiling, to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. If desired, rehydrated contents of 1 vial of Nalidixic Selective Supplement (FD130) may be added aseptically to 1000 ml medium. Mix well and pour into sterile Petri plate. The media was then poured into sterile petridishes (75 mm diameter) in 20 ml quantities to form thick layer. The sterile of the media was checked by incubating at 37^0C over-night and stored at 2^0C -8^0C.

3.2.4.8 Mueller Hinton agar

Mueller Hinton Agar is used in antimicrobial susceptibility testing by the disk diffusion method. 38 grams of Mueller Hinton agar powder was suspended in 1000 ml of distilled water. It was mixed well. It was heated agitating frequently and boiled for about one minute. It was dispensed and sterilized in autoclave at 116 - 121°C (15 lbs. sp) for 15 minutes. It was cooled to 45° or 50° C (Carter, 1979).

3.2.5 Isolation and identification of bacteria

3.2.5.1 Culture of urine samples

Nutient agar, MacConkey agar, Mannitol Salt agar, Blood agar , Staphylococcus Agar No.110 (S-110 agar), Eosin Methylene Blue agar (EMB), Cetrimide Agar media(CA) were used.

3.2.5.2 Culture in ordinary media

Samples were inoculated separately into ordinary media like Nutrient agar and were incubated at 37^OC for overnight.

3.2.5.3 Isolation of bacteria in pure culture

For isolation of bacteria in pure culture, The colonies on primary cultures were repeatedly sub-cultured on Nutrient Agar by streak plate method (Cheesbrough, 1985) until the pure culture with homogenous colonies were obtained.

Step-1: An inoculum was picked up with a sterile loop and spread on an area of the medium in the petridish.

Step-2: The loop was sterilized by being heated as red hot in a flame.

Step-3: The inoculum was spread over the reminder of the plate by drawing the cooled parallel line.

This method was repeated as many times as necessary to obtain a culture containing only one type of colony and usually at least two more times to ensure purity.

3.2.5.4 Morphological characterization of organisms by Gram's staining method

The grams staining was followed to study the morphological and staining characteristics of bacteria and to provide information about the presumptive bacterial identification as per recommendation of Cowan and Steel (1979).

Technique:

A drop of sterile normal saline was taken on the middle of the clear slide. Then a loopful bacterial suspension (young culture) was transferred to the sterile drop of normal saline and a very thin film was prepared on the slide by spreading uniformly. The film was fixed by passing it gently over flame for two or three times.

- The slide was flooded with crystal violet solution for up to one minute. Wash off briefly with tap water (not over 5 seconds). Drained.
- The slide was flooded with Gram’s Iodine solution, and allow to act (as a mordant) for about one minute. Wash off with tap water. Drained.
- Excess water was removed from slide and blotted, so that alcohol used for decolorization was not diluted. Slide was flooded with 95% alcohol for 10 seconds and washed off with tap water. (Smears that are excessively thick may require longer decolorization. This is the most sensitive and variable step of the procedure, and requires experience to know just how much to decolorize). Drained.
- The slide was flooded with safranin solution and allowed to counter stain for 30 seconds. Washed off with tap water. Drained and blotted with bibulous paper.

All sides of bacteria were examined under the oil immersion lens.

3.2.5.5 Culture into differential media

3.2.5.5.1 Mac-Conkey agar

Gram negative cultures were sub-culture seperately on Mac-conkey agar media and incubated at 37°C for overnight. After that both lactose fermenter bacteria (rose pink color colony) and lactose non fermenter bacteria (pale color colony) were selected.

3.2.5.5.2 Mannitol salt agar media

Gram positive culture were sub-cultured on mannitol Salt Agar. Both mannitol fermenter bacteria (yellow color colony) and mannitol non- fermenter bacteria (pink color colony) were selected.

3.2.5.5.3 Blood Agar

Then colony from Mannitol Salt Agar were subcultured on Blood agar media and incubated at 37^0C for overnight.

3.2.5.6 Culture on selective media

3.2.5.6.1 Staphylococcus agar No.110

Colonies from Mannitol Salt Agar were taken and sub-culture on S-110 agar media and incubated at 37^OC for overnight. Some S-110 agar plate characteristics by good growth and yellowish colonies.

3.2.5.6.2 Cetrimide agar media (CA)

Lactose non fermental organism from MacConkey Agar were sub-culture on Citrimide Agar.

3.2.5.6.3 Eosin methylene blue (EMB) agar

Samples of positive lactose fermenter were taken and sub-culture on EMB agar media and incubated at 37°C for overnight.

Some EMB agar plate showed slightly circular colonies with dark center metallic sheen. Also in some EMB agar, the growth was indicated by smooth, characteristics mucoid and

pink colored colonies which are a consequence of the organism's abundant polysaccharide capsule.

3.2.5.7 Microscopic study for identification of *E. coli.*, *Klebsiella* spp., *Pseudomonas* spp., *Staphylococcus* spp., and suspected colonies by Gram's staining

Gram's staining was performed by taking colony from selected media to determine the size, shape, and arrangement of bacteria according to the methods described by Merchant and Packer (1967). Stained slides were examined under light microscope at 100 x magnification.

3.2.5.8 Identification of isolated bacteria by different biochemical Tests

Isolated organisms with supported growth characteristics of *E. coli, Klebsiella spp., Pseudomonas spp.,Staphylococcus spp.,* were maintained in pure culture and subjected to biochemical tests.

3.2.5.8.1 Procedure of Catalase test

This test was performed by taking 2-3 drops of 3 per cent H_2O_2 on clean grease-free glass slide and single colony was mixed with the help of a wire loop. Immediate formation of gas bubbles was considered as positive test.

3.2.5.8.2 Procedure of Indole test

2 ml of peptone water was inoculated separately with 5 ml of culture of each of the isolated bacteria and incubated for 48 hours. 0.5 ml Kovac's reagent was added, shakes well and examined after 1 minute. A red color ring at the top of the reagent indicated production of the indole by the organisms (Cowan, 1985).

3.2.5.8.3 Procedure of MR test

The test was performed by inoculating separately a colony of the each of the isolated test organisms in 0.5 ml sterile glucose phosphate broth. After overnight incubation at 37^0C, a drop of methyl red solution was added. A positive methyl red test was shown by the appearance of a bright red color. A yellow or orange color was a negative test (Cowan, 1985).

3.2.5.8.4 Procedure of VP test

2 ml of sterile glucose phosphate peptone water were inoculated separately with 5ml of each of the isolated organisms and incubated at 37°C for 48 hours. A very small amount (knife point) of creatine was added and mixed. 3 ml of 40% potassium hydroxide were added and shacked well. The bottle cap was removed and left for an hour at room temperature. It was observed closely for the slow development of a pink color for positive cases. In negative cases there was no development of pink color (Cowan, 1985).

3.2.5.8.5. Procedure of Motility Indole Urease Test (MIU)

MIU media were prepared in test tubes. Then the isolated organisms were inoculated separately into the media by stabbing method with the help of sterile straight wire. Then the test tubes were incubated 37°C overnight. Single stick that is no turbidity throughout the medium indicate gram negative organism (non motile) and turbidity throughout the medium indicate gram positive case (Cowan, 1985).

3.2.5.8.6 Procedure of Triple Sugar Iron Test (TSI)

Triple sugar iron contains three sugars (Glucose, Sucrose and Lactose). At first TSI agar slant were prepared in a test tube. Then the isolated organisms were inoculated separately into the butt with a sterilized wire and on the slant with a wire loop producing zigzag streaking. The tubes were incubated for 24 hours at 37°C. Yellow color of butt and slant of the test tube indicate fermentation of Glucose, Sucrose and Lactose fermentation and butt shows blacking indicate H_2S production (Cowan, 1985).

3.2.6 Antibiotic sensitivity test

Materials:

- Test tube rack
- Bunsen burner
- Inoculating loop or needle
- Forceps
- Sterile swabs
- Mueller-Hinton or Nutrient agar plates
- Antibiotic disks

- Stock broth cultures of experimental bacteria
- 35°C to 37°C non-CO_2 incubator

Antimicrobial drug susceptibility against eleven commonly used antibiotics was performed by disc diffusion or Kirby–Bauer method (Bauer *et. al.*,1966). The procedure of disc diffusion method is presented below:

i. One well isolated colony was selected from the agar plate.

ii. Colony was touched with a sterile loop and streaked onto nutrient agar and incubated overnight at 37^0C

iii. 4 or 5 well isolated colonies were transferred into a tube of sterile physiological saline and vortex thoroughly.

iv. A sterile cotton swab was dipped into the bacterial suspension. The excess fluid of swab was removed by pressing firmly against the inside of the tube just above the fluid level.

v. The swab was streaked over the entire surface of Mueller-Hinton agar (Himedia, India) / Nutrient agar(Himedia, India) medium three times, rotating the plate approximately 60 degrees after each application to ensure an even distribution of the inoculums.

vi. The antimicrobial discs were placed individually using sterile forceps and then gently press down onto the agar.

vii. The plates were inverted and incubated at 37^0C temperature for overnight. After incubation the diameter of the zone of complete inhibition (including diameter of the discs) was measured in millimeters with a ruler. The measurements were made with a ruler on the undersurface of the plate without opening the lid.

viii. The value was compared with the zone-size table. The zones of growth inhibition were provided by Clinical and Laboratory Standards Institute (CLSI, 2013).

3.2.7 Recording and interpretating results

The zones of growth inhibition was compared with the zone-size interpretative table standard for *E. coli*, *Klebsilla* spp., *pseudomonas* spp., and *Staphylacoccu*s spp., (Table 2, 3, 4 and 5) provided by Clinical and Laboratory Standards Institute (CLSI, 2013). Isolates were classified as susceptible, intermediate and resistant categories based on the standard interpretation tables updated according to the Clinical and Laboratory Standards Institution (CLSI, 2013). Antimicrobial testing results were recorded as resistant, intermediate and sensitive according to zone diameter interpretive standards provided by CLSI, (2013).

Table 2.: Zone diameter imperative standards for *E. coli*

Antimicrobial agents	**Zone Diameter**		
	Resistant (mm)	**Intermediate (mm)**	**Sensitive (mm)**
Amoxicillin (AMC)	≤13	14-17	≥18
Chloramphenicol (C)	≤12	13-17	≥18
Streptomycin (S)	≤15	12-14	≥11
Erythromycin (E)	≤13	14-19	≥20
Cefixime (CFM)	≤15	16-18	≥19
Livofloxacin (LE)	≤13	14-16	≥17
Ciprofloxacin (CIP)	≤15	16-20	≥21
Vancomycin (VA)	≤09	10-11	≥12
Gentamycin (GEN)	≤12	13-14	≥15
Amikacin (AK)	≤14	15-16	≥17

Notes: mm= Millimeter

Table 3.: Zone diameter imperative standards *Klebsiella spp.*

Antimicrobial agents	Zone Diameter		
	Resistant (mm)	**Intermediate (mm)**	**Sensitive (mm)**
Amoxicillin (AMC)	≤13	14-17	≥18
Ciprofloxacin (CIP)	≤15	16-20	≥21
Chloramphenicol (C)	≤12	13-17	≥18
Gentamycin (GEN)	≤12	13-14	≥15
Amikacin (AK)	≤14	15-16	≥17
Livofloxacin (LE)	≤13	14-16	≥17
Erythromycin (E)	≤13	14-19	≥20

Notes: mm= Millimeter

Table 4. : Zone diameter imperative standards for *Pseudomonas spp.*

Antimicrobial agents	Zone Diameter		
	Resistant (mm)	**Intermediate (mm)**	**Sensitive (mm)**
Amikacin (AK)	≤14	15-16	≥17
Ciprofloxacin (CIP)	≤15	16-20	≥21
Ciprofloxacin (CIP) U-Cast	≤22	23-24	≥25
Livofloxacin (LE)	≤13	14-16	≥17
Gentamycin (GEN)	≤12	13-14	≥15
Erythromycin (E)	≤13	14-19	≥20
Vancomycin (VA)	≤09	10-11	≥12
Norfloxacin (NX)	≤12	13-16	≥17

Notes: mm= Millimeter

Table 5.: Zone diameter imperative standards for *Staphylococcus spp.*

Antimicrobial agents	Zone Diameter		
	Resistant (mm)	**Intermediate (mm)**	**Sensitive (mm)**
Amoxicillin (AMC)	≤19	-	≥20
Chloramphenicol (C)	≤12	13-17	≥18
Erythromycin (E)	≤13	14-22	≥23
Livofloxacin (LE)	≤15	16-18	≥19
Ciprofloxacin (CIP)	≤15	16-20	≥21
Gentamycin (GEN)	≤12	13-14	≥15
Amakacin (AK)	≤14	15-16	≥17

Notes: mm= Millimeter

CHAPTER-IV

RESULT

The current study was performed as per experimental layout mention in page no. (33). As per experimental layout the bacterial pathogens were isolated and identified from the 58 clinical samples suspected to be infected with UTI. The pathogens were confirmed by using morphology (staining), cultural and biochemical techniques and evaluate the sensitivity and resistance pattern of commercially available antibiotics used against identified isolates. The results of above mentioned all experiments were presented bellow:

Out of 58 clinical samples, 16 (27.6 %) samples were collected form male and 42 (72.4 %) were from female. In this 58 samples 6 (37.5 %) were positive for male and 19 (45.2 %) were positive for female (Table-6 & 7).

Table-6 Samples collected from different sex groups

Sex	Number of Samples	Percentage (%)
Male	16	27.6
Female	42	72.4
Total	**58**	**100.0**

Table-7 Positive samples found in different sex groups

Sex	Number of samples	Positive cases	Percentage (%)	X^2 Value	Level of Significance
Male	16	06	37.5	0.283	0.595
Female	42	19	45.2		
Total	**58**	**25**	**82.7**		

In this study it was observed that their was a relationship in UTIs age and sex (Table-7). The UTIs were very high in female (45.2%) in comparison with male (37.5%).

4.1 Identification of UTI infection by using the physical, chemical and microscopic examination based on their age and sex

Table- 8: Physical, chemical and microscopic examination of collected sample at STVH based on their age and sex

Characteristics/ Sample No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Physical Examination	Color	Straw	Straw	Straw	Deep Straw	Straw	Straw	Deep Straw	Deep Straw	Straw	Deep Straw	Deep Straw	Deep Straw	Deep Straw	Deep Straw	Deep Straw	Deep Straw	Deep Straw	Deep Straw	Deep Straw	Deep Straw	Deep Straw	Deep Straw	Deep Straw	Deep Straw	Deep Straw
	Odour	ar	pun	pun	pun	pun	put	put	put	pun	pun	put	put	put	put	put	put	put	put	put	pun	put	put	put	put	put
	Sp.Gra.	1014	1014	1014	1014	1014	1014	1014	1014	1014	1014	1014	1014	1014	1014	1014	1014	1014	1014	1014	1014	1014	1014	1014	1014	1014
	Sediment	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Present	Nil	Present	Nil	Nil	Present	Nil	Present	Nil	Nil	Present	Nil
Chemical Examination	Reaction	Aci.	Aci.	Aci.	Aci.	Aci.	Aci.	Aci.	Aci.	Aci.	Aci.	Aci.	Aci.	Aci.	Aci.	Aci.	Aci.	Aci.	Aci.	Aci.	Aci.	Aci.	Aci.	Aci.	Aci.	Aci.
	Albumin	+	+	+	+	+	++	++	+	+	+	++	++	++	++	++	++	++	++	++	+	++	++	++	++	++
	Sugar	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	++	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	+	Nil	++	Nil
Micros-Copical Examination	Eip. Cell	6-7	7-8	7-8	Few	7-8	6-7	Few	Few	7-8	Few	Few	Few	Few	Few	Few	Plenty	Few	Plenty	Few	Plenty	Few	Few	Plenty	Few	Few
	Pus Cell	Few	Few	Few	Few	Few	Few	Few	Few	Few	Few	Few	Few	Few	Few	Few	Plenty	Few	Plenty	Plenty	Few	Few	Few	Few	Few	Few
	Cast	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	Crystals (Calcium-oxalate)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	3-4	Nil	Nil	3-4	Nil	Few	Nil
Age Group Male	A=16-30			M-A																						
	B=31-45				M-B			M-B																		
	C=46-60					M-C			M-C																	
	D=>60										M-D															
Age Group Female	A=16-30	F-A	F-A				F-A			F-A																
	B=31-45											F-B	F-B		F-B	F-B		F-B	F-B	F-B						F-B
	C=46-60													F-C			F-C				F-C	F-C				
	D=>60																						F-D	F-D	F-D	

Legends: Sp.Grivity= Specific Gravity,F-A= Female Age Group(16-30), F-B Female Age Group(31-45), F-C=Female Age Group(46-60), F-D=Female Age Group(>60), M-A= Male Age Group(16-30), M-B Male Age Group(31-45), M-C=Male Age Group(46-60), M-D=Male Age Group(>60); Aci- Acidic, Microscopic examination found cell by every High Power Field.,odour-ar-aromatic,put-putrid,pun-pungent.

In this study, the urine samples of UTI were physically examined by observing the color (straw, deep straw), odour (aromatic, pungent, putrid), specific gravity (1010, 1012, 1014), sediment (nil, present) respectively. In this table showed, the urine samples of UTI were chemical examined by heat and acetic acid test [albumin- nil, (+), (++), benedict test (sugar-nil, green color, orange color)] respectively. In this study, the urine samples of UTI were microscopical examined by observing under microscope (epithelial cell, pus cell, cast, crystals) respectively.

Table-9 Distribution of positives cases for UTI among different age groups

Age Group	Positive for UTI	
	Male	**Female**
16-30	01 (16.7 %)	04 (21.1 %)
31-45	02 (33.3 %)	08 (42.1 %)
46-60	02 (33.3 %)	04 (21.1 %)
>60	01 (16.7 %)	03 (15.7 %)
Total	**06 (100%)**	**19 (100%)**
X^2 Value	0.889	4.14
Level of Significance	0.828	0.247

4.2 Isolation and Identification of bacteria from urine sample

The following of the bacteria such as *Escherichia coli, Klebsiella spp., Pseudomonas spp.,Staphylococcus spp* were isolated from UTI samples. Identification of bacteria was performed by determining cultural characteristics, staining and different biochemical properties.

4.2.1 Identification of isolates by cultural and morphological characteristics

Table-10. Colony characters of the isolated organisms on different media

Name of media	Colony characteristics	Remerks
Nutrient Agar	White, moist, glistening growth	*E. coli*
MacConkey Agar	Rose pink colonies	
EMB agar	Metallic sheen (greenish black) colony	
Nutrient Agar	White, translucent, raised growth	*Klebsiella* spp.
MacConkey Agar	Pink moist colonies	
EMB agar	Pink colonies	
Nutrient Agar	Smooth, raised, irregular and semi-translucent colony	*Pseudomonas* spp.
Cetrimide Agar	Yellow-green to blue color (Pyocyanin color)	
Nutrient Agar	Grey-white to yellowish colonies	*Staphylococcus.* spp.
MS agar	Good growth yellowish colonies	
Blood agar	Beta haemolytic colonies	
S-110 agar	Showing yellowish colonies	

(Legends: Sl. No= Serial Number, EMB= Eosin Methylene Blue; MS =Mannitol Salt)

In this table, it was observed that white, moist, glistening colonies on nutrient agar, rose pink colonies on MacConkey agar, green metallic sheen colonies on EMB agar which indicate the organism might be *E. coli* (Plate-10, Plate-11, Plate-12). White, translucent, raised growth colonies on nutrient agar, pink moist colonies on MacConkeys agar, pink colonies on EMB agar which indicate the organism might be *klebsiella* spp. (Plate-13, Plate-14, Plate-15). Smooth, Raised, irregular and semi-translucent colonies on nutrient agar, yellow-green to blue color (Pyocanin color) colonies on cetrimide agar which indicate the organism might be *Pseudomonas* spp. (Plate-16, Plate-17). Grey-white to yellowish colonies on nutrient agar, good growth yellowish colonies on mannitol salt agar, beta haemolytic colonies on blood agar and yellowish colonies on S-110 agar which indicate the organism might be *Staphylococcus* spp. (Plate-18, Plate-19, Plate-20, Plate-21).

4.2.2 Gram's staining

Most of the isolates were observed as small rod shaped gram negative and cocci shaped with grapes like cluster gram positive by Gram's staining techniques (Plate-22, Plate-23, Plate-24, Plate-25).

Table-11. Morphological and staining properties of the bacterial isolates of UTI infections by Gram's staining

Characteristics				**Bacterial Isolates**
Smple No.	**Shape**	**Arrangement**	**Gram's Staining**	
1, 4, 7, 8, 11, 13,14, 15, 16, 17, 19, 20, 22, 23, 25	Short plump rods	Single, paired or in short chain	Gram negative (-) ve	*E. coli*
6,10	Small rod	Single, pairs or short chain	Gram negative (-) ve	*Klebsiella* spp.
2	Rod in shape	Single	Gram negative (-) ve	*Pseudomonas* spp.
3, 5, 9, 12,18, 21, 24	Cocci in shape	Arranged in grapes like cluster	Gram positive (+) ve	*Staphylococcus* spp.

4.2.3 Biochemical characteristics of all of the isolates

Biochemical characteristics of 25 cultural positive urine samples out of 58 samples. The growth and morphology characteristics indicated that the isolated organism might be *E. coli* (Table-12), which was later confirm by different biochemical tests.

Table: 12. Identification of *E. coli* by different biochemical tests

Biochemical test	Changes in reaction	Results
Catalase test	Gas bubble	Positive
Indole test	Pink color ring at the top of the media	Positive
MR test	Bright red color	Positive
MIU test	Diffuse, hazy growth, slightly opaque media	Positive
Triple sugar iron (TSI) test	S-yellow, B-yellow; S-A, B-A, gas (+), H_2S (-)	Positive
VP test	No color change	Negative

(Legends: S=Slant, B=Butt, A = Acid, MR = Methyl-Red test, MIU= Motility Indole Urease**,** VP = Voges-Proskauer test, + = Positive reaction, - = Negative reaction)

In this table it was observed that, all of the biochemical tests were positive for *E. coli*. (Table No: 12, Plate: 26, Plate-27, Plate-28, Plate-29, Plate-30, Plate-31).

The organism might be *Klebsiella* spp that were differentiated by observing the growth and morphological characteristics, which was later confirmed by different biochemical tests (Table-13).

Table: 13. Identification of *Klebsiella* spp. by different biochemical tests

Biochemical test	Changes of the reaction	Results
Catalase test	Bubble formation	Positive
Indole test	No color change	Negative
MR test	No color change	Negative
MIU test	No color change	Negative
Triple sugar iron (TSI) test	S-yellow, B-yellow; S-A, B-A, gas (+), H_2S (-)	Positive
VP test	Red color	Positive

(Legends: S=Slant, B=Butt, A = Acid, MR = Methyl-Red test, MIU= Motility Indole Urease**,** VP = Voges-Proskauer test, + = Positive reaction, - = Negative reaction).

In this table it was observed that, all of the biochemical tests were positive for *Klebsiella* spp. (Table No:13 and Plate: 32, Plate-33, Plate-34, Plate: 35, Plate-36, Plate-37).

The growth and morphology characteristics indicated that the isolated organism might be *Pseudomonas* spp (Table-14), which was later confirmed by different biochemical tests.

Table: 14. Identification of *Pseudomonas* spp. by different biochemical tests

Biochemical test	Changes of the reaction	Results
Catalase test	Gas bubble	Positive
Indole test	No color change	Negative
Triple sugar iron (TSI) test	S-Al, B-Al	Positive
MR test	No color change	Negative
MIU test	No color change	Negative
VP test	No color change	Negative

(Legends: Al= Alkaline, MR = Methyl-Red test, MIU= Motility Indole Urease**,** VP = Voges-Proskauer test).

In this table it was observed that, all of the biochemical tests were positive for *Pseudomonas* spp. (Table No: 14 and Plate: 38, Plate-39, Plate-40, Plate: 41, Plate-42, Plate-43).

The organism might be *Staphylococcus* spp that were differentiate by observed the growth and morphological characteristics, which was later confirmed by different Biochemical tests (Table-15).

Table: 15. Identification of *Staphalococcus* spp. by different biochemical tests

Biochemical test	Changes of the reaction	Results
Catalase test	Gas bubble	Positive
Triple sugar iron (TSI) test	S-yellow, B-yellow; S-A, B-A, gas (+), H_2S (-)	Positive
Indole test	No color change	Negative
MR test	Red color	Positive
MIU test	No color change	Positive
VP test	Red color	Positive

(Legends: S=Slant, B=Butt , A= Acid, MR = Methyl-Red test, MIU= Motility Indole Urease**,** VP = Voges-Proskauer test, + = Positive reaction, - = Negative reaction)

In this table it was observed that, all of the biochemical tests were positive for *Staphylococcus* spp. (Table No: 15 and Plate: 44, Plate- 45, Plate-46, Plate-47, Plate-49, Plate-49).

4.3 Prevalence of pathogens isolated from patients with UTIs infection

The predominant organisms isolated from the positive samples were *E. coli, Klebsiella* spp. , *Pseudomonas* spp. and *Staphylococcus* spp. with 60.0 %, 8.0 %, 4.0 % and 28 .0 % respectively (**Table-16**).

Table-16: Different organisms found in urine samples

Organisms	**Number of isolates**	**Percentage (n=25)**	**X^2 Value**	**Level of Significance**
E. coli	15	60.0 %	26.187	0.00
Klebsiella sp.	02	08.0 %		
Pseudomonas spp.	01	04.0 %		
Staphylococcus spp.	07	28.0 %		
Total	**25**	**100%**		

4.4 Antibiotic resistance pattern of *E .coli.*

Table-17: Antibiotic resistance pattern of *E .coli.*

Name of the Antibiotics/ Samples No.	Amoxicillin (AMC)			Ciprofloxacin (CIP)			Vancomycin (VA)			Gentamicin (GEN)			Amikacin (AK)			Streptomycin (S)			Erythro-mycin (E)			Chloram-phenicool			Levo-floxacin			Cefixime		
	Zone size of Inhibiton			Zone size of Inhibiton			Zone size of Inhibiton			Zone size of Inhibiton			Zone size of Inhibiton			Zone size of Inhibiton			Zone size of Inhibiton			Zone size of Inhibiton			Zone size of Inhibiton			Zone size of Inhibiton		
	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I
Sample 1		12		21				09		15			18				11		20			18				12		19		
Sample 2		11			13			08			13			11			11			13		19				13			14	
Sample 3		11		22				09			12			12			11				15	18			19			15	
Sample 4		09		24				09		16			17				10				14	19			18		19			
Sample 5		13			12			08		15			17				11				15	18					16		15	
Sample 6		14		22				09		17			19				10				14	19					15		14	
Sample 7		09		23				08		16			18			15					14	18					14		14	
Sample 8		11			12			09		18			17				11				15	19					14		14	
Sample 9		12		21				08		16				11		15					17	18					15		13	
Sample 10		14		22				09		17			18				11				18	18					14		14	
Sample 11		13		21				08		18			17			16					17	19					15		14	
Sample 12		11		21				09		16			18			16					16	18					14	19		
Sample 13		12			13			08		17			18			15					14	19					15	19		
Sample 14		11		21				08			12		18			16				13		18				12		19		
Sample 15		12		21				09		17			18				11			12		19			19			19		

(Legends: S= Sentive, R= Resistance, I= Intermediate)

Table 18: Antibiotic resistance pattern (%) of *E. coli.*

Antimicrobial agents	Total isolate (15)			Percentage of R, S and I		
	Resistant (No.)	Sensitive (No.)	Intermediate (No.)	R %	S %	I %
Amoxycillin	15	00	00	100	00	00
Ciprofloxacillin	04	11	00	26.7	73.3	00
Vancomycin	15	00	00	100	00	00
Gentamicin	03	12	00	20	80	00
Amikacin	03	12	00	20	80	00
Streptomycin	09	06	00	60	40	00
Erythromycin	03	01	11	20	6.7	73.3
Cefixime	09	06	00	60	40	00
Chloramphenicol	00	15	00	00	100	00
Levofloxacin	03	03	09	20	20	60

(Legends: S= Sentive, R= Resistance, I= Intermediate and %= Percentage)

This results of antimicrobial susceptibility of the isolates were summarized in the Table- 18. Out of 25 samples 15 were *E.coli* positive. Among them Ciprofloxacin sensitive to 73.3%, Chloramphenicol 100%, Gentamicin 80% and Amikacin 80% and 100% resistance to Amoxycilin and Vancomycin respectively.

4.5 Antibiotic resistance pattern of *klebsiella* spp.

Table- 19: Antibiotic resistance pattern of *klebsiella* spp.

Name of the Antibiotics / Samples No.	Amoxicillin (AMC)			Ciprofloxacin (CIP)			Chloram-phenicol (C)			Gentamicin (GEN)			Amikacin (AK)			Erythro mycin (E)			Levo-floxacin		
	Zone size of Inhibiton			Zone size of Inhibiton			Zone size of Inhibiton			Zone size of Inhibiton			Zone size of Inhibiton			Zone size of Inhibiton			Zone size of Inhibiton		
	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I
Sample 1		12		21			18			16				05			12		17		
Sample 2		11			15			09		15				18			13		17		

(Legends: S= Sentive, R= Resistance, I= Intermediate)

Table 20:Antibiotic resistant pattern (%) of *Klebsiella* spp.

Antimicrobial agents	Total isolate (02)			Percentage of R, S and I		
	Resistant (No.)	Sensitive (No.)	Intermediate (No.)	R %	S %	I %
Amoxicillin	02	00	00	100	00	00
Ciprofloxacillin	01	01	00	50	50	00
Chloramphenicol	01	01	00	50	50	00
Gentamicin	00	02	00	00	100	00
Amikacin	01	01	00	50	50	00
Livofloxacin	02	00	00	100	00	00
Erythromycin	02	00	00	100	00	00

(Legends: S= Sentive, R= Resistance, I= Intermediate and %= Percentage)

In the present study , *Klebsiella* spp. showed sensitivity to Ciprofloxacin, Gentamicin, Amikacin and Chloramphenicol at 50 %, 100 %, 50 % and 50 % and 100% resistance to Amoxicillin, Livofloxacillin and Erythromycin respectively (Table: 20).

4.6 Antibiotic resistance pattern of *Pseudomonas* spp.

Table-21: Antibiotic resistance pattern of *Pseudomonas* spp

Name of the Antibiotics/ Samples No.	Amikacin (AK)			Ciprofloxacin (CIP)			Vancomycin (VA)			Gentamicin (GEN)			Norfloxacin (NX)			Erythro-mycin (E)			Levo-floxacin		
	Zone size of Inhibiton			Zone size of Inhibiton			Zone size of Inhibiton			Zone size of Inhibiton			Zone size of Inhibiton			Zone size of Inhibiton			Zone size of Inhibiton		
	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I
Sample 1		12		21				09		16				11				14	17		

(Legends: S= Sentive, R= Resistance, I= Intermediate)

Table-22: Antibiotic resistance pattern (%) of *Pseudomonas* spp.

Antimicrobial agents	Total isolate (01)			Percentage of R, S and I		
	Resistant (No.)	Sensitive (No.)	Intermediate (No.)	R %	S %	I %
Norfloxacin	01	00	00	100	00	00
Ciprofloxacillin	00	01	00	00	100	00
Vancomycin	01	00	00	100	00	00
Gentamicin	00	01	00	00	100	00
Amikacin	01	00	00	100	00	00
Erythromycin	00	00	01	00	00	100
Livofloxacin	00	01	00	00	100	00

(Legends: S= Sentive, R= Resistance, I= Intermediate and %= Percentage)

From the study , the *Pseudumonas* spp. isolated from the UTI samples showed sensitivity to Ciprofloxacin 100%, Gentamicin 100% and Livofloxacin 100%. On the other hand , 100% isolares were resistant to Norfloxacin ,Vancomycin and Amikacin respectively.

4.7 Antibiotic resistance pattern of *Staphylococcus* spp.

Table-23: Antibiotic resistance pattern of *Staphylococcus* spp.

Name of the Antibiotics/ Samples No.	Amoxicillin (AMC)			Ciprofloxacin (CIP)			Gentamicin (GEN)			Amikacin (AK)			Erythro-mycin (E)			Chloram-phenicool (C)		
	Zone size of Inhibiton			Zone size of Inhibiton			Zone size of Inhibiton			Zone size of Inhibiton			Zone size of Inhibiton			Zone size of Inhibiton		
	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I
Sample 1		12		21			15				11			12		18		
Sample 2		11		22			16				18			13		19		
Sample 3		13		22			15				10			12		18		
Sample 4		11		22					14		11			13		19		
Sample 5		12		21			15				17			12		18		
Sample 6		11		21			17				19			12		19		
Sample 7		09		22			16				18			11		18		

(Legends: S= Sentive, R= Resistance, I= Intermediate)

Table 24: Antibiotic resistance pattern (%) of *Staphylococcus* spp.

Antimicrobial agents	Total isolate (07)			Percentage of R , S and I		
	Resistant (No.)	Sensitive (No.)	Intermediate (No.)	R %	S %	I %
Amoxycillin	07	00	00	100	00	00
Ciprofloxacillin	00	07	00	00	100	00
Gentamicin	00	06	01	00	85.7	14.3
Amikacin	03	04	00	42.9	51.1	00
Erythromycin	07	00	00	100	00	00
Chloramphenicol	00	07	00	00	100	00

(Legends: S= Sentive, R= Resistance, I= Intermediate and %= Percentage)

This results of antimicrobial susceptibility of the isolates *Staphylococcus* spp. were summarized in the Table: 24. Out of 07 representative sample of field isolates, sensitivity to Ciprofloxacin, Gentamicin, Chloramphenicol and Amikacin were 100 %, 85.7 %, 100 % and 51.1% respectively. On the other hand, 100% isolates were resistance to Amoxycilin and Erythromycin .

Physical , Chemical and Microscopic Examination of Collected sample at STVH.

Plate 2: Collected samples from patients.

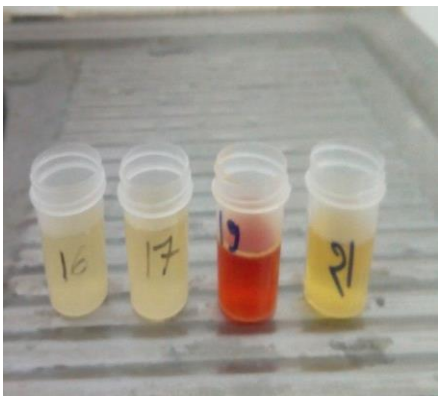


Plate 3 : Collected samples from patients.

Plate 4: Chemical examination of urine (Protein/Albumin test) positive (right) and control (left)

Plate 5: Chemical examination of urine (Sugar test) green color (right) and control (left)

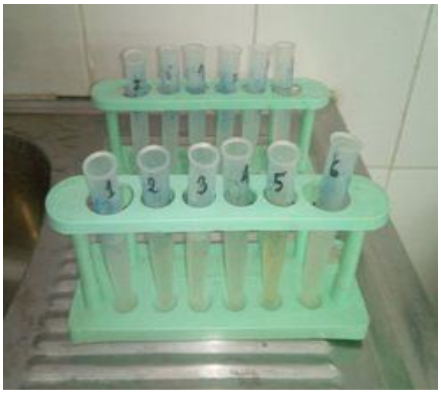


Plate 6 : After centrifugation the sample were ready to microscopic.

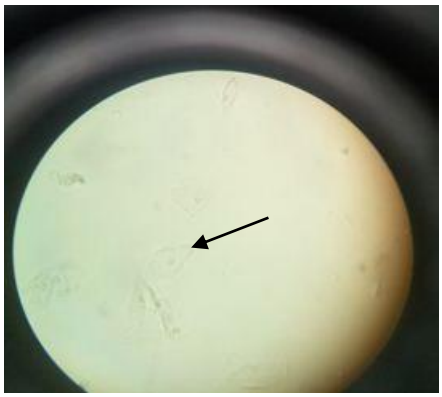


Plate 7 : Epithelial cells showing at 40X magnification.

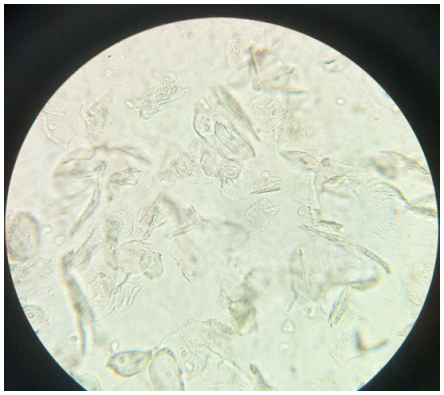


Plate 8 : Plenty of Epithelial cells showing at 40X magnification.

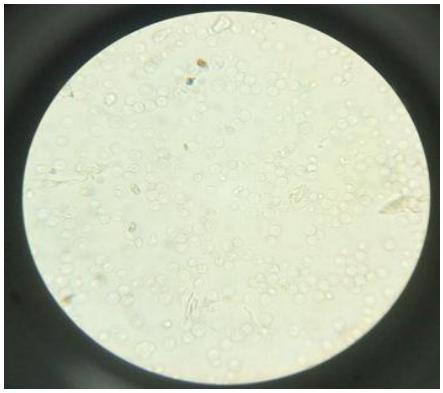
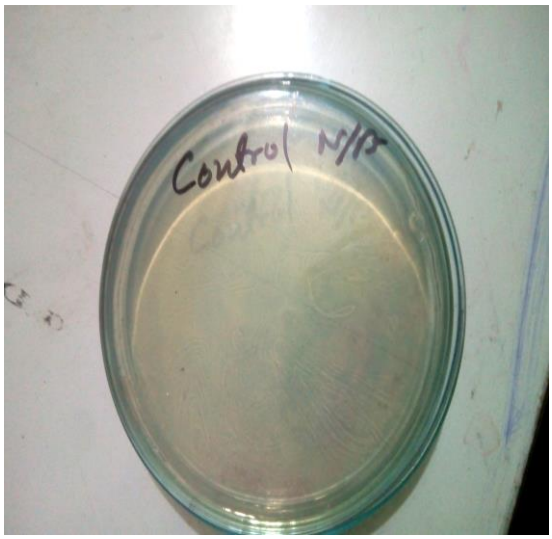


Plate 9 : Plenty of Pus cells showing at 40X magnification.



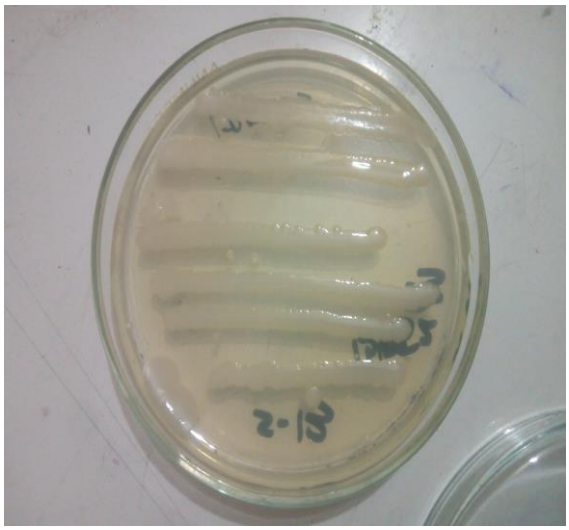


Plate 10 : Thick grayish white colony of *E. coli* on Nutrient agar (right) and uninoculated control (left).

Plate 11 : Growth of *Escherichia coli* on MacConkey agar plate (right) and uninoculated control (left).

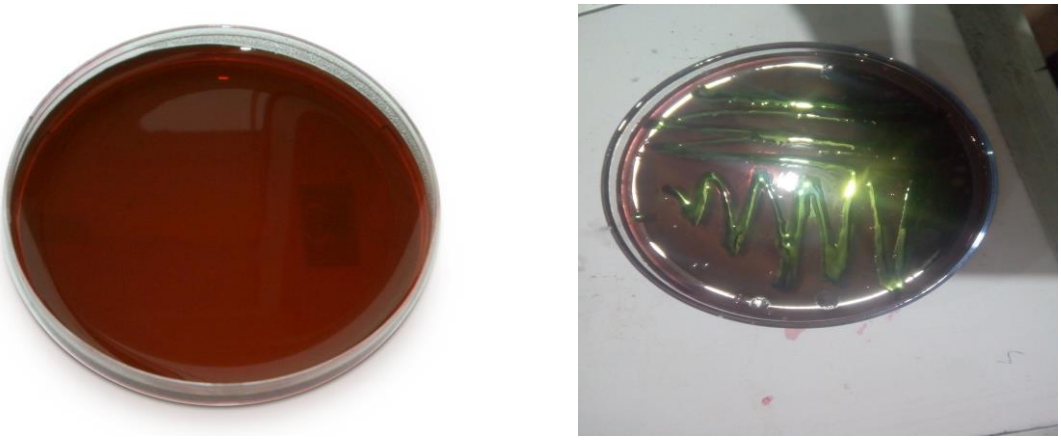


Plate 12 : Metallic sheen (greenish black) colonies of *E.coli* on EMB media(right) and uninoculated control (left).

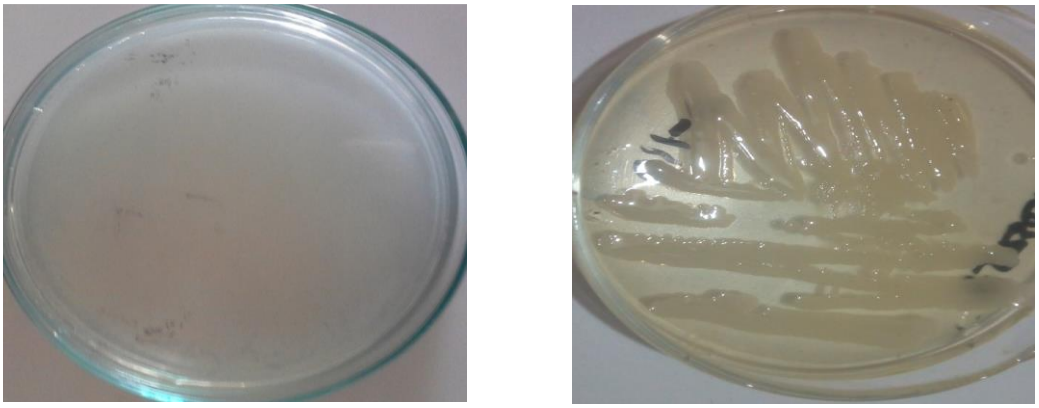


Plate 13 : White, translucent, raised growth of *Klebsiella* spp. on Nutrient agar (right) and uninoculated control (left).

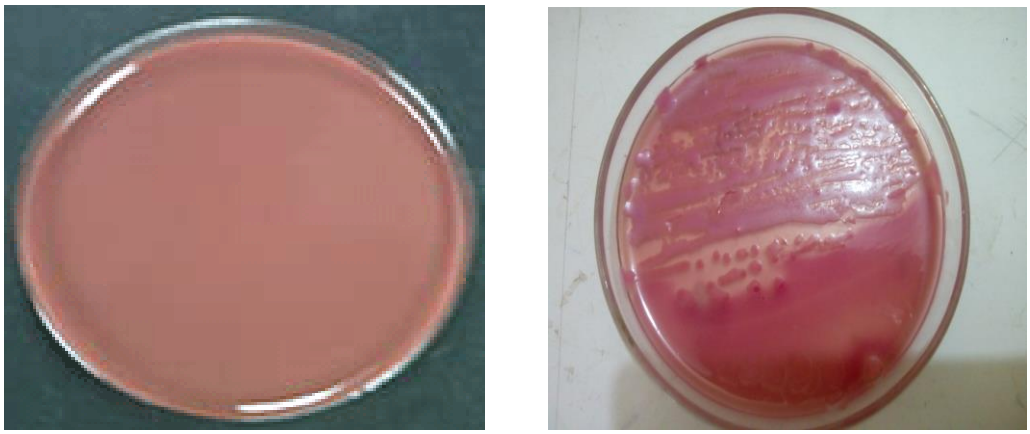


Plate 14: Pink moist colonies of *Klebsiella* spp.on MacConkey agar (right)and uninoculated control (left)

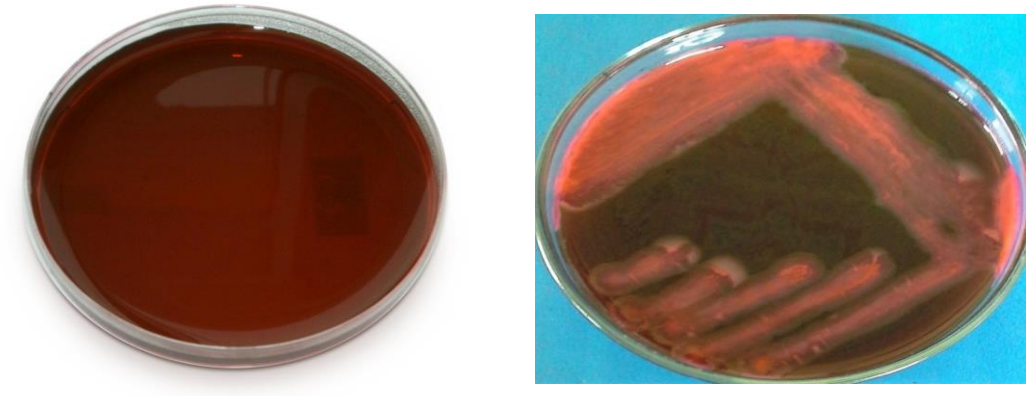


Plate 15: Pink colonies produced by *Klebsiella* spp. on EMB agar (right) and uninoculated control (left).

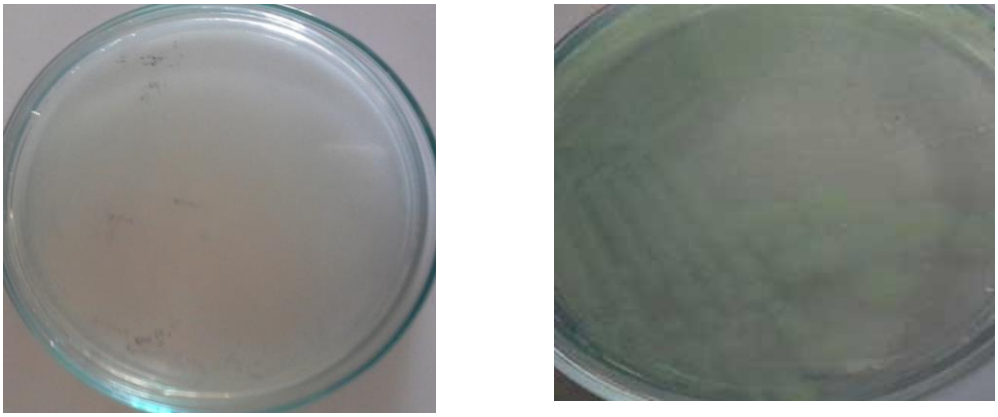


Plate 16: Smooth, raised irregular and semi translucent colonies by *Pseudomonas* spp. on Nutrient agar (right) and uninoculated control (left).

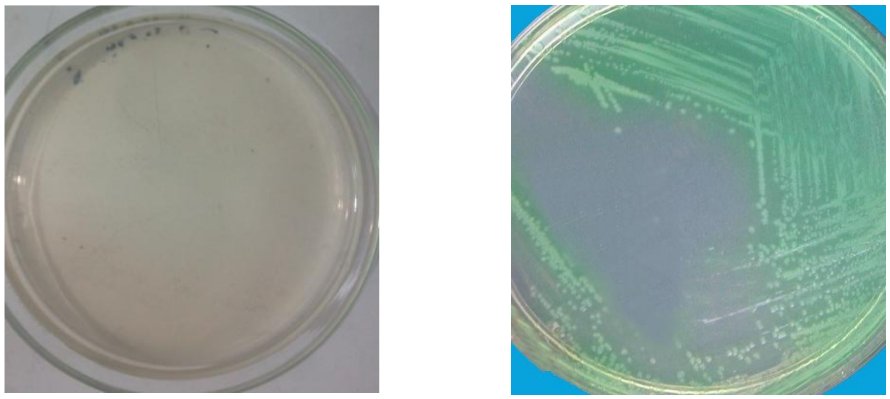


Plate 17: Yellow-green to blue color (pyocyanin color) colonies by *Pseudomonas* spp. on cetrimide agar (right) and uninoculated control (left).

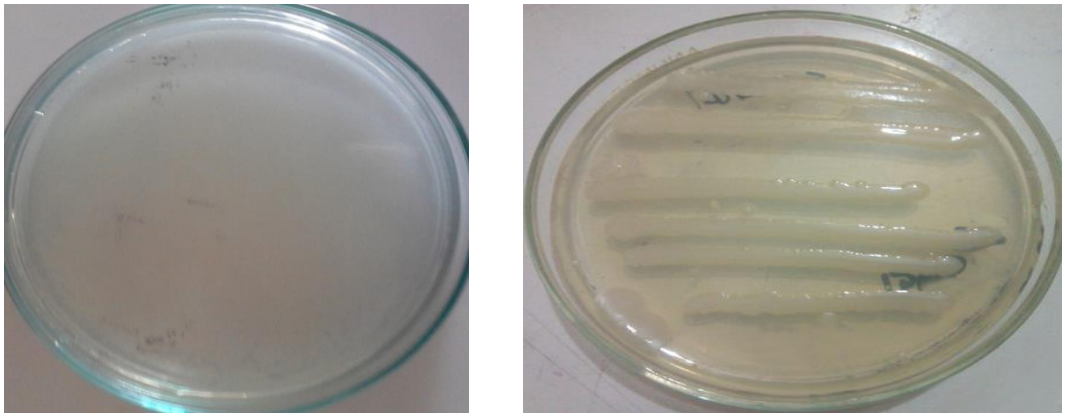


Plate 18: Culture of Small whitish *Staphylococcus* spp. on Nutrient agar (right) and uninoculated control (left).

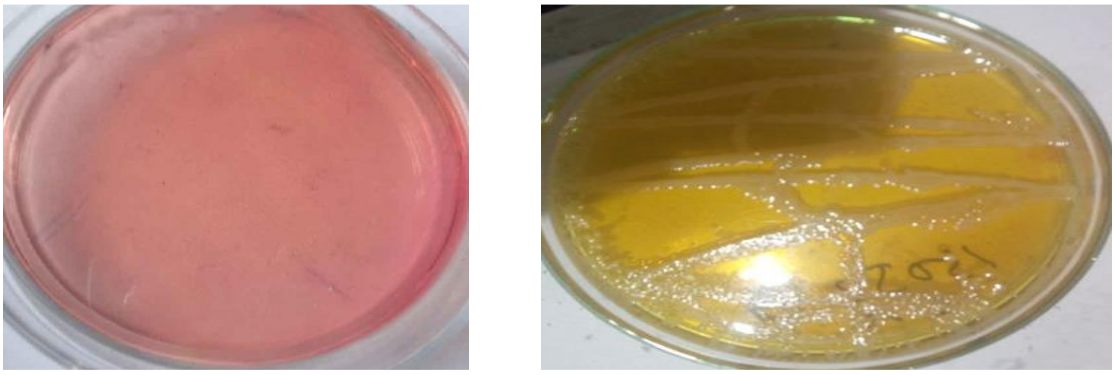


Plate 19 : Yellowish colonies by *Staphylococcus spp.* on Manitol salt agar (MSA) (right) and uninoculated control (left).

Plate 20 : Beta haemolytic colonies by *Staphylococcus* spp. on Blood agar (right) and uninoculated control (left).

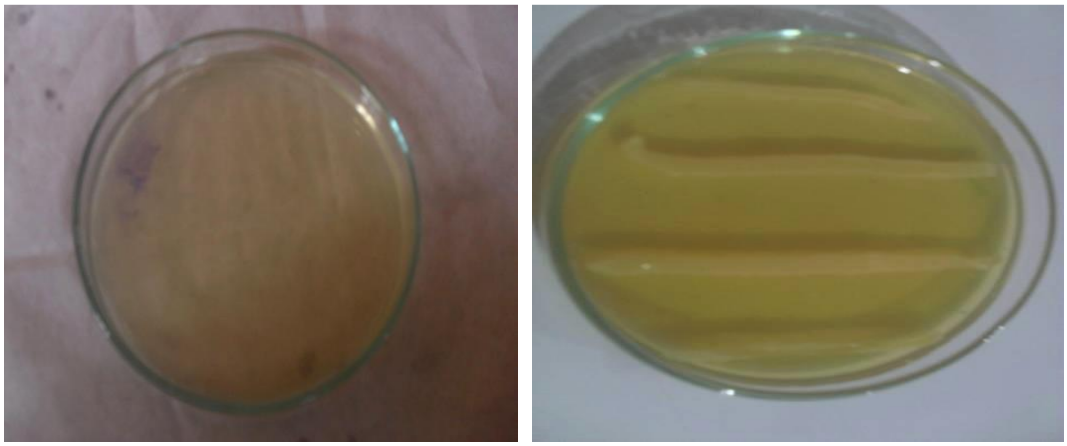


Plate 21 : Yellowish colonies by *Staphylococcus* spp. on *Staphylococcus* agar no.-110 (right) and uninoculated control (left).

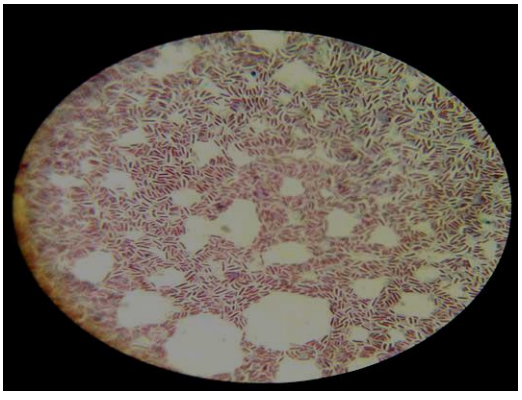


Plate 22: Light Microscopic image of ***E.coli*** showing at 100x magnification (Gram's staining)

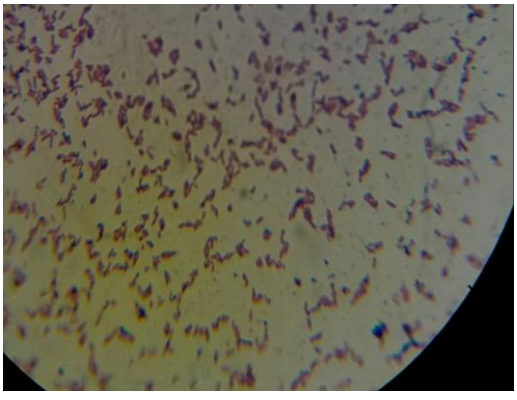


Plate 23: Light Microscopic image of *Klebsiella* spp. showing at 100x magnification (Gram's staining)

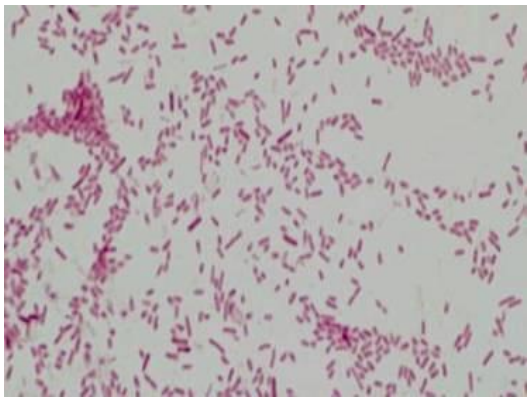


Plate 24: Gram negative single rods of *Pseudomonas* spp.(Gram's staining)

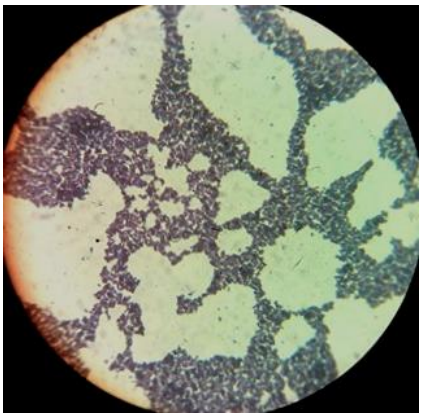


Plate 25 : *Staphylococcus* spp. showing at 100x magnification (Gram's staining)

Plate 26: Catalase (positive) for *E. coli* with gas bubble formation.

Plate 27: Indole test showing positive results with a red color ring on the top of the reagent indicating indole production with reaction of *E. coli* (right) and uninoculated control (left).

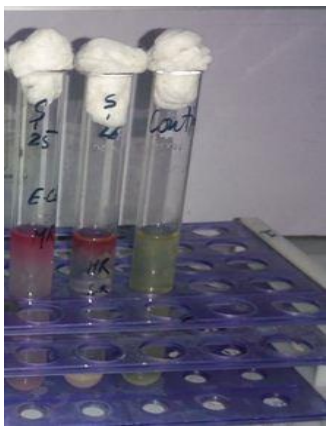


Plate: 28. Methyl-Red test for *E. Coli* showing bright red color (left) and uninoculated control (right)

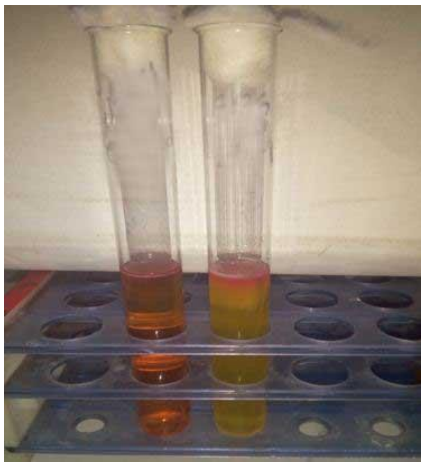


Plate 29: Motility Indole Urease test causing turbidity urease production with indole positive by *E. Coli* (right) and uninoculated control (left).

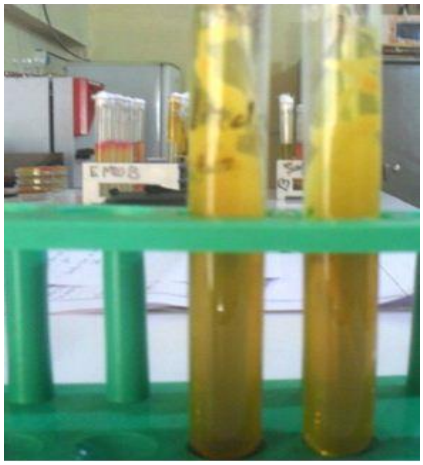


Plate 30. Culture in Triple Sugar Iron (TSI) agar slant reaction showing yellow slant and yellow butt and production of gas by *E. coli*.

Plate 31 : VP test causing no color change by *E. Coli* (right) and uninoculated control (left).

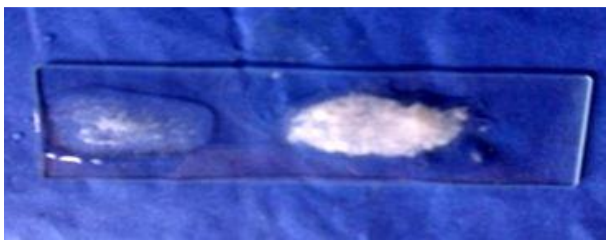


Plate 32 : Catalase test of *klebsiella* spp.showing bubble formation indicating positive reaction (right) and no bubble formation indicating negative reaction (left).

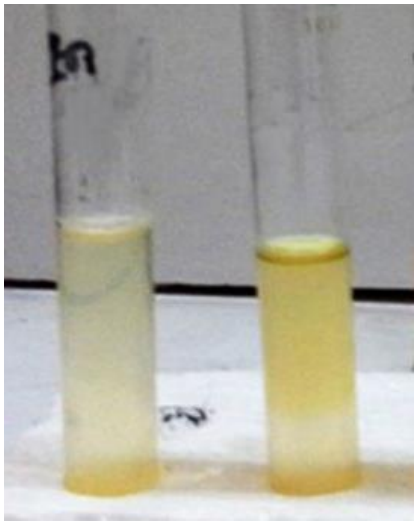


Plate 33 : Indole test *Klebsiella* spp. results negative (right) and uninoculated control (left).

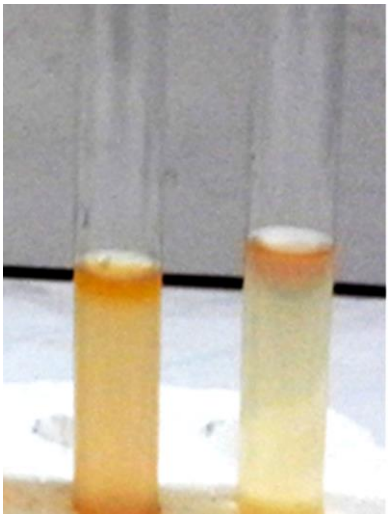


Plate 34 : MR test results *Klebsiella* spp. negative (left) and uninoculated control (right).

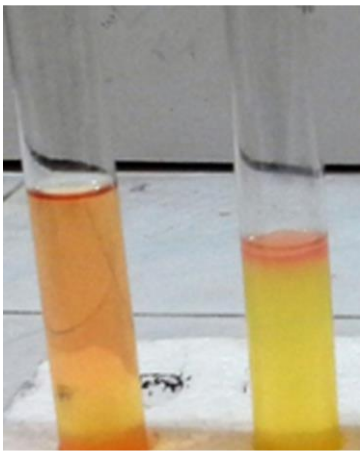


Plate 35: MIU test *Klebsiella* spp. results negative (right) and uninoculated control (left).

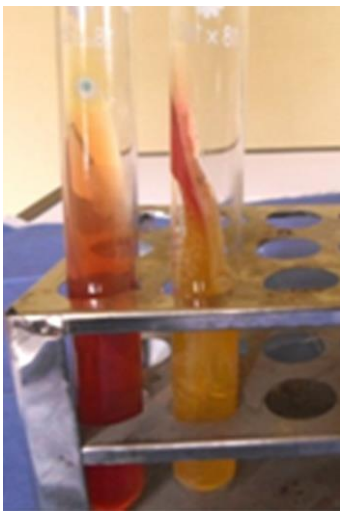


Plate 36: TSI test *Klebsiella* spp. (+) Ve (right) and uninoculated control (left) TSI test showing [Slant-Acid, Butt-Acid, gas (+), H_2S (-)]

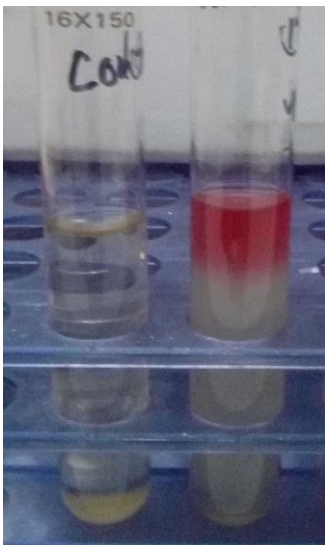


Plate 37: *Klebsiella* spp. VP test showing positive result (right) with controle(left)

Plate 38: Catalase test of *Pseuomonas* spp. showing bubble formation (left), and no bubble formation (right).

Plate 39: *Pseuomonas* spp. in indole test showing negative result (right) with controle (left)

Plate 40 : *Pseuomonas* spp. in Triple Sugar Iron (TSI) TSI agar showing slant and butt alkaline left with controle (right)

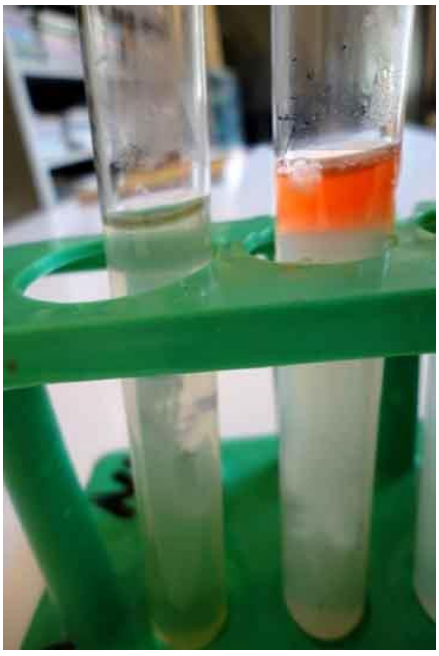


Plate 41 : *Pseudomonas* spp. showing MR Negative result (right) with Control (left).

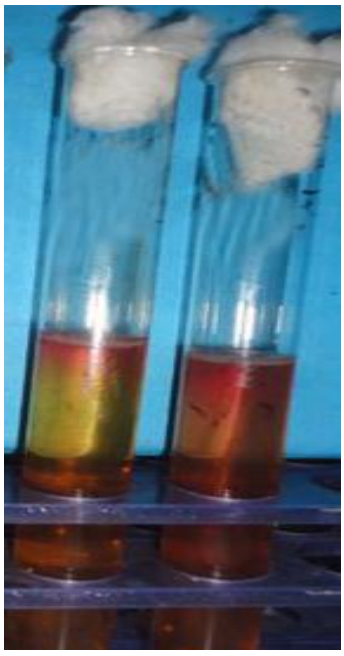


Plate 42 : *Pseudomonas* spp. showing MIU Negative result (right) with Control (left).

Plate 43 : *Pseudomonas* spp. in VP test showing negative result with controle

Plate 44 : Catalase test of *Staphylococcus* spp. showing bubble formation indicating positive reaction (left) and catalase test of *E. coli* showing no bubble formation indicating negative reaction (right).

Plate 45 : Culture in Triple Sugar Iron (TSI) agar slant reaction showing yellow slant and yellow butt (right) and no gas production by *Staphylocccus spp.* and uninoculated control (left).

Plate 46 : *Staphylococcus* spp. *sh*owing Indole negative result (right) with control (left)

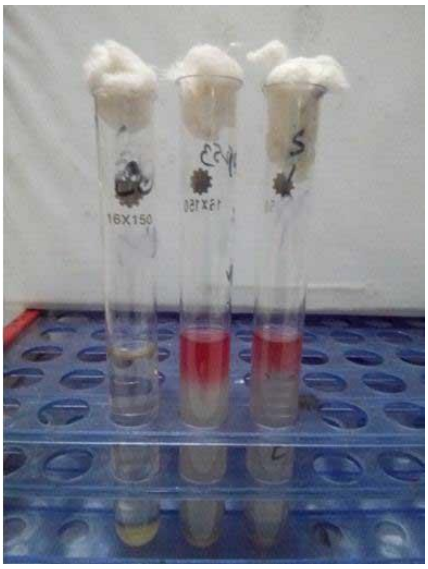


Plate 47 : Methyl-Red test for *Staphylocccus* spp. showing the medium was changed to bright red colour (right) and uninoculated control (left).

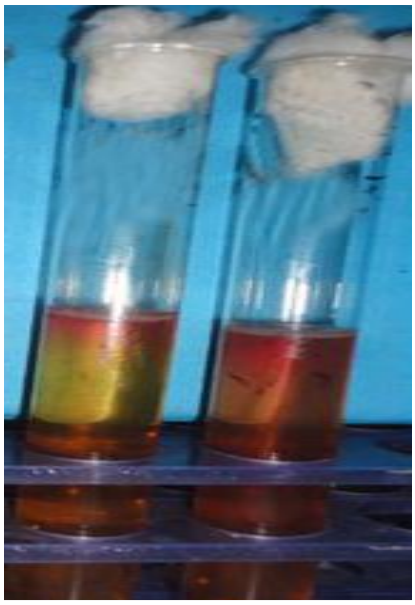


Plate 48 : Methyl Indole Urease test for *Staphylocccus* spp. showing the medium was no color changed (right) and uninoculated control (left).

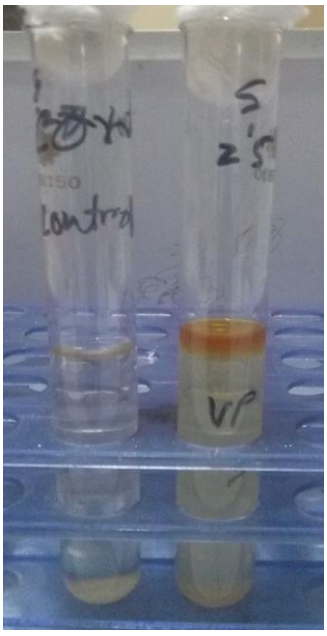


Plate 49 : VP test negative (no color change after adding reagent) by *staphylococcus spp*. (right) and unionculated control (left)

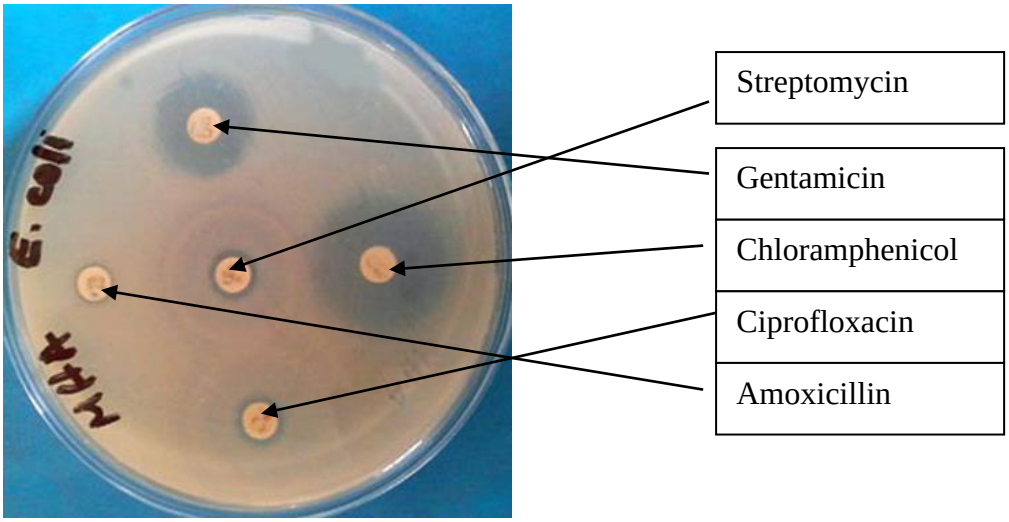
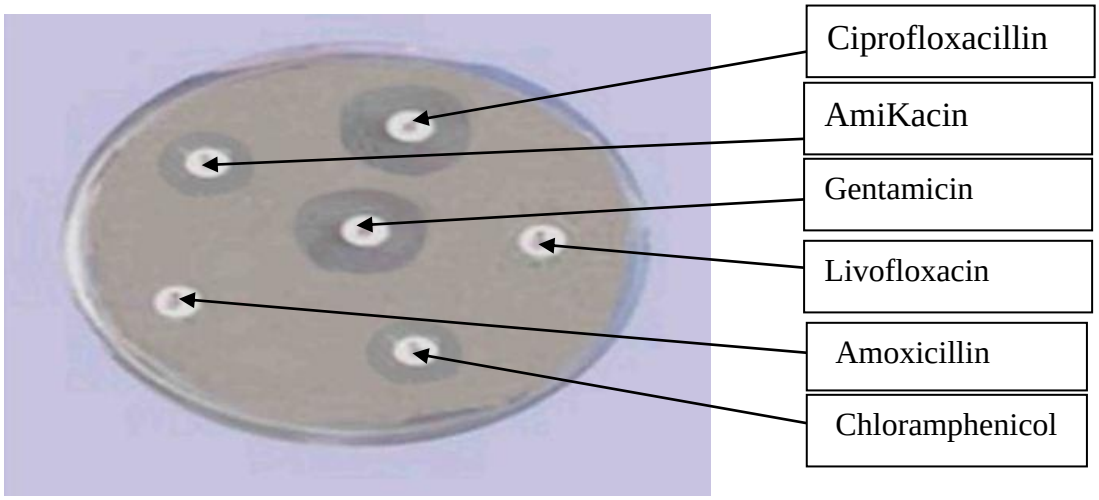
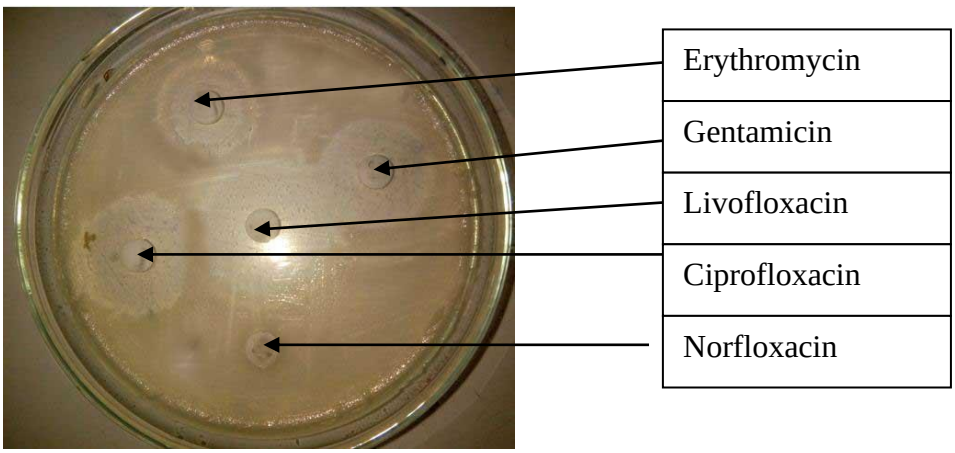


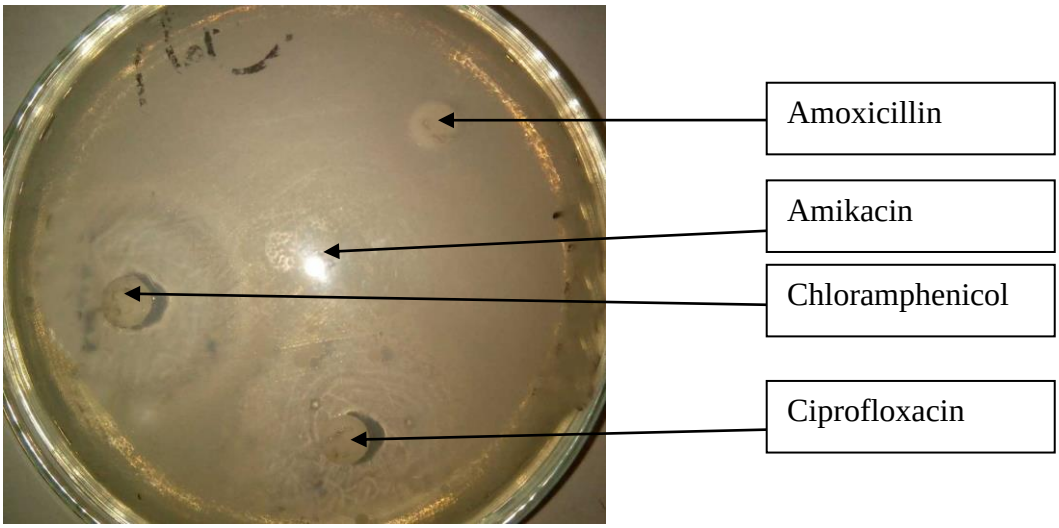
Plate 50: Antibiotic sensitivity test for *E.coli* on nutrient agar



Plat 51 : Antibiotic sensitivity test for *Kledsiella* spp. on nutrient agar



Plat 52 : Antibiotic sensitivity test for *Pseudomonas* spp. on nutrient agar



Plat 53 : Antibiotic sensitivity test for *Staphylococcus* spp. on nutrient agar

CHAPTER- V

DISCUSSION

The research topic were entiled as “Isolation and identification of bacterial pathogens from urinary tract infection in human” with determination of sensitivity and resistance pattern of commercially available antibiotics used against identified isolates.

In this current study a total of 58 clinical samples were collected from those patient suffering with UTIs. Out of 58 samples, 16 were from male of which 6 (37.5%) were positive, and 42 were from female of which 19 (45.2%) were positive with UTIs . In this present study it was observed that, 43.1 % samples were culturally positive for UTIs which is similar to the findings of **Akhtar *et al.*, 2017** and **Samiah 2017.** In our present study the ration of infection in male and female was 1:3.1 which was supported by **Mulugeta and Abera, 2014.**

In this study, the urine samples of UTI were physically examined by observing the color (straw, deep straw), odur (aromatic,pungent,putrid), specific gravity (1010,1012,1014), sediment (nil, present) respectivly. The urine samples of UTI were chemically examined by heat and acetic acid test[albumin-nil, (+), (++)], benedict test (sugar-nil, green color) respectivly. In this current study, the urine samples of UTI were microscopically examined by observing under microscope(epithelial cell-3-4/ 5-6/ Few/ +/plenty per high power field, pus cell- Few/ +/plenty per high power field, cast, crystals,) respectively. Which was supported by several authors **P B Godkar and D P Godkar, August 2004, K L Mukherjee, Volume-II, 2000.**

In our present study, pathogenic bacteria *E.coli, Klebsiella* spp., *Pseudomonas* spp. and *Staphylococcus* spp. were isoltaed and identified by observing conventional cultural, morphological and biochemical techniques this findings were supported by several authors (**Alemu *et al.*, 2012, Mahmood *et al.*, 2016), (Freeman, 1985 and I.A Marchant and Packer 1967) and (Goli *et al.*, 2015, Khan *et al.*, 2014** and **Ouno *et al.*, 2013).**

The most prevalent bacterial isolates were *E. coli* 270 (61.3%) followed by *P.aeruginosa* 52 (12%) and *Klebsiella pneumoniae* 42 (9.5%) **Khan *et al.*, 2014.** In our present study, the observed predominant organism`s isolated from the positive samples were *E. coli, Klebsiella*

spp., *Pseudomonas* spp. and *staphylococcus* spp. which was supported by several authors **Goli *et al.*, 2015, FICMS *et al.*, 2014 and Akhtar *et al.*, 2017.**

In this present study , the antibiotic sensitivity test was performed by disk diffusion method according to the procedure discribed by Kirby-Bauer (2011) and Clinical and Laboratory Standards Institution (CLSI, 2013)

In our study, it was observed *E. coli* were sensitivite to Ciprofloxacin 73.3%, Chloramphenicol 100%, Gentamycin 80% and Amikacin 80% respectively. It was also observed that 100% isolates were resistance to Amoxycilin and Vancomycin. This findings have similarities with several authors (**Akter *et al.*, 2016, Amany *et al.*, 2016 and Nerurkar *et al.*, 2012)** and dissimilarities with other authors **(Samiah 2017**and A**kter *et al.*, 2013).**

In the present study, *Klebsiella* spp. showed sensitivity to Ciprofloxacin, Gentamicin, Amikacin and Chloramphenicol at 50 %, 100 %, 50 % and 50 % which is supported by **Getenet and Wondewosen (2011).**

Similarly , the *Pseudumonas* spp. showed 100% sensitivity to Ciprofloxacin, Gentamicin, and Livofloxacin and 100% isolates were also found resistant to Norfloxacin, Vancomycin and Amikacin. This findings were supported by the several authors **(Goli *et al.*, 2015, Ouno *et al.*, 2013)** and disagreed by **Khan *et al.*, 2014.**

On the other hand *Staphylococcus* spp were sensitive to Ciprofloxacin, Gentamycin and Chloramphenicol and Amikacin at 100%, 85.7%, 100% and 51.1% respectively and 100% isolates were resistant to Amoxycilin and Erythromycin. This findings were similar to the several authors (**Amany *et al.*, 2016**, **Ouno *et al.*, 2013)** and different from **Alemu *et al.*, 2012.**

CHAPTER- VI

SUMMARY AND CONCLUSION

Urinary Tract Infections (UTI) is the second most common type of infection in the human population. Infections in the urinary tract are a serious health problem affecting millions of people every year. Treatment of UTI is becoming as a challenge due to the development of resistance in bacteria against many of the drugs that are available in market.Midstream urine samples were collected from 58 patients, among them urinary pathogens were isolated from 25 samples. The isolated uropathogens cultured and identified with four genera of *Escherichia, Klebsiella, Pseudomonas* and *Staphylococcus.* In this study, prevalence of UTI was found 43.1 % from 58 patients' samples. Female suffers more than male from UTI. The rate of infection in female is more at age of 31-45 years. Most effective antibiotics against *E. Coli* , *Klebsiella* spp. and *Staphylococcus* spp. were Chloramphenicol , Gentamicin, Ciprofloxacin and Amikacin. *Pesudomonas* spp. were sensitive to Ciprofloxacin, Gentamycin and Livofloxacin. Overall, most of the isolated microorganisms were sensitive to Ciprofloxacin, Gentamicin, Chloramphenicol and Amikacin except *Pseudomonas* spp. The results of this study focus on better management and treatment of patients with UTI.

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APPENDIX-I

Composition of the media used:

Nutrient Agar	**Grams/Liter**
Peptone	5.0
Bacto beef extract	3.0
NaCl	5.0
Agar	15.0
Distilled water	1000 ml
P^H	7.2

Sterilized at 121^0C under 151b/in^2 pressure for 15 minutes.

MacConkey Agar	**Grams/Liter**
Bacto Peptone	17.0
Proteas Peptone	3.0
Lactose	10.0
Bile Salt	1.54
Agar	15.0
Neutral red	0.03
Crystal violet	0.001
Distilled water	1000 ml
P^H	7.1

Sterilized at 121^0C under 151b/in^2 pressure for 15 minutes.

Eosine methylene blue(EMB) agar	**Grams/Liter**
Peptone	10.0
Lactose	10.0
K_2HpO_4	2.0
Eosin	0.4
Methylene blue	0.065
Agar	20.0
Distilled water	1000ml
P^H	6.8

Sterilized at 121^0C under 151b/in^2 pressure for 15 minutes.

Eosine methylene blue(EMB) agar	**Grams/Liter**
Proteas peptone	10.0
Beef extract	1.0
D-Mannitol	10.0
NaCl	75.0
Phenol red	0.025
Agar	20
Distilled water	1000ml

Sterilized at 121^0C under 151b/in^2 pressure for 15 minutes.

Blood agar

Ingredients	**Grams/Liter**
Agar	15.0
Beef extract	10.0
Peptone	10.5
Sodium chloride	5.0
Final pH	7.3±0.2

Normal Saline	**Grams/Liter**
NaCl	0.85
Distilled water	1000 ml

Autoclaved at 121^0C for 15 minutes.

APPENDIX-II

Composition of the media used in biochemical test

MR-VP broth	**Grams/Liter**
Peptone	7.0
Dextrose	5.0
Dipotassium phosphate	5.0
Distilled water	1000ml
P^H	6.9

Sterilized at 121^0C under 151b/in^2 pressure for 15 minutes.

Triple Sugar Iron (TSI) Agar	**Grams/Liter**
Peptone	10.0
Tryptone	10.0
Yeast Extract	3.0
Lactose	10.0
Saccharose	10.0
Dextrose	1.0
Ferrous Sulphate	0.2
Sodium Chloride	5.0
Sodium Thiosulphate	0.3
Phenol Red	0.024
Agar	12.0
P^H	7.4

Sterilized at 121^0C under 151b/in^2 pressure for 15 minutes.

Urea broth medium	**Grams/Liter**
Urea	20.0
Yeast extract	0.1
KH_2PO_4	9.0
K_2HPO_4	9.5
Phenol red	0.01
Distilled water	1000ml
P^H	6.8

Sterilized at 121^0C under 151b/in^2 pressure for 15 minutes.

Urea broth medium	**Grams/Liter**
Tryptone	10.0
Distilled water	1000ml

Sterilized at 121^0C under 151b/in^2 pressure for 15 minutes.

APPENDIX-III

Composition of chemicals and reagents

Crystal violet

Solution-A

Crystal violet (90% dye content)	2.0 g
Ethyl alcohol (95%)	20.0 ml

Solution-B

Ammonium oxalate	0.8
Distilled water	80.0 ml

Note-Mix the solution A and B

Gram's iodine

Iodine	1.0g
Potassium iodide	2.0g
Distilled water	300.0ml
Ethyl alcohol (95%)	
Ethyl alcohol (100%)	95.0 ml
Distilled water	5.0 ml

Safranin

Safranin O	0.25ml
Ethyl alcohol (95%)	10.0ml
Distilled water	100.0ml

Kovac's reagent (for detection of indole)

P-Dimethylaminobenzaldehyde	5.0g
Amyl alcohol	75.0 ml
Hydrochloric acid (concentrated)	25.0 ml

Concentrated P-Dimethylaminobenzaldehyde was dissolved in the amyl alcohol and HCl was added slowly.

Methyl red solution

Methyl red	0.04 g
Ethanol	40.0 g
Distilled water	100 .0 ml

Methyl red dissolved in ethanol and diluted water.

Barrit's reagent

Solution-A

α- naptho	l5.0 g
Ethanol (Absolut)	95.0 g

α- naptho was dissolved in ethanol with constant stirring.

Solution-B

KOH	40.0 g
Creatine	0.3 g
Distilled water	100.0 ml

Hydrogen peroxide

3% aqueous solution of H_2O_2 was prepared from the H_2O_2 absolute solution.