PROSPECTIVE SUDY ON URINARY TRACT INFECTION ISOLATES FROM URINE SAMPLE AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN

Submitted in partial fulfilment of the requirements of Degree of

MASTER OF SCIENCE
(CLINICAL MICROBIOLOGY)

By

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RECOMMENDATION

This is to certify that Mr.Mahesh Kumar has completed this full-term training "**PROSPECTIVE STUDY ON URINARY TRACT INFECTION ISOLATE FROM URINE SAMPLE AND THEIR ANTIBIOTICSUSCEPTIBILITYPATTERN"** as a partial fulfillment of Master of Science in Clinical Microbiology under our supervision. To our knowledge, this work had been submitted to any other degree**.**

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PROSPECTIVE SUDY ON URINARY TRACT INFECTION ISOLATES FROM URINE SAMPLE AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN

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Date:-

CERTIFICATE OF APPROVAL

On the recommendation of Dr. Mamta Kumari (MD Microbiologist,SRL) and Mr Himal Sapkota (Lecturer, LFAMS) this full-term practical training by Mr. Mahesh Kumar entitled "PROSPECTIVE STUDY ON URINARY TRACT INFECTION ISOLATES FROM URINE SAMPLE AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN" has been approved for the examination and is submitted to the Lovely Professional University partial fulfillment of requirement for Master Degree of Science in Clinical Microbiology.

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PROSPECTIVE SUDY ON URINARY TRACT INFECTION ISOLATES FROM URINE SAMPLE AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN

Gurgaon Haryana

Date:-

DECLEARATION

I hereby decleared that full-term practical training entitled **"**PROSPECTIVE STUDY ON URINARY TRACT INFECTION ISOLATES FROM URINE SAMPLE AND OTHER ANTIBIOTICS SUSCEPTIBILITY PATTERN "submitted for MSc degree is entirely my work and all ideas and references have been duly acknowledged.

PROSPECTIVE SUDY ON URINARY TRACT INFECTION ISOLATES FROM URINE SAMPLE AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN

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PROSPECTIVE SUDY ON URINARY TRACT INFECTION ISOLATES FROM URINE SAMPLE AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN

MAHESH KUMAR

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LIST OF ABBREVATION

AST	Antimicrobial Susceptibility Testing
BA	Blood Agar
CFU	Colony Forming Units
CLED	Cysteine Lactose Electrolyte Deficient
E.Coli	*Escherichia coli*
C.freundii	*Citrobacter freundii*
E.Cloacae	*Enterobacter cloacae*
GNB	Gram Negative Bacilli
GPC	Gram Positive Cocci
K.Pneumoniae	*Klebsiella pneumoniae*
MDR	Multi Drug Resistant
Spp	species
UTI	Urinary Tract Infection
WHO	World Health Organization

ANTIBIOTICS

II

AK

Am

Aug

Cft

Cp

Cfz

Cpe

Cfx

Caz

Dap

E

Etp

Gm

Imp

Lvx

Mer

Mz

Nxn

Ox

P

Pi

Syn

Te

To

Va

Amikacin
Ampicillin
Amoxicillin
Cefotaxime
Ciprofloxacin
Cefazolin
Cefepime
Cefoxitin
Ceftazidime
Daptomycin
Erythromycin
Ertapenem
Gentamycin
Imipenem
Levofloxacin
Meropenem
Mezlocillin
Norfloxacin
Oxacillin
Penicillin
Piperacillin
Synercid
Tetracycline
Tobramycin
Vancomycin

PROSPECTIVE SUDY ON URINARY TRACT INFECTION ISOLATES FROM URINE SAMPLE AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN

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ABSTRACT

Urinary tract infection is one of the most common bacterial infections seen in clinical practice particularly in developing countries. The causative agents for urinary tract infection vary from place to place and they also vary in their susceptibility and resistance patterns. The objective was to assess the distribution of urinary tract bacterial pathogens with respect to age and gender and to determine the antibiotic susceptibility pattern of the isolates. Single positive cultures with a colony count of >105 CFU on CLED medium were identified and their antibiotic sensitivity and resistance pattern is represented in the form of an antibiogram.This study is done in SRL Diagnostic, Gurgaon, India. A total of 4362 mid-stream urine samples from the suspected UTI patients were tested microbiologically and antimicrobial susceptibility test were performed for the isolated pathogens using prompt system micro scan method.

The rate of culture positivity in females was 66.80% and in males was 33.20%. *E-coli* was the most frequently isolated urinary pathogen (58.68%), followed by *Klebsiella pneumoniae* (10.15%), *Enterococcus faecalis* (12.21%) and *Pseudomonas aeruginosa* (4.18%) *E-coli* was highly sensitive to Tigecycline (99%), Meropenem (98%), Ertapenem (97%), and Amikacin (93%) and it was highly resistant to Ampicillin (79%), *Klebsiella pneumoniae* is highly sensitive to Meropenem (78%) and it was highly resistant to Ampicillin (98%).

Higher prevalence of UTI was seen in females. Gram negative organisms were the most commonly isolated organisms in UTI. Urinary pathogens showed resistance to commonly used antibiotics like Ampicillin, Norfloxacin and Cefepime**.** The susceptibility and resistance patterns of urinary pathogens should be considered before starting empirical treatment for UTI.

Key Words: CFU=Colony Forming Units, CLED=Cysteine Lactose Electrolyte

Deficient agar, UTI= Urinary Tract Infection, uropathogens=urinary tract pathogens, IDSA=Infectious Diseases Society of America, spp=species.

CHAPTER-1

URINARY TRACT INFECTION

1.1 INTRODUCTION

A urinary tract infection is a condition in which one or more parts of the urinary system (the kidneys, ureters, bladder, and urethra) become infected. Urinary tract infection is the most common of all bacterial infections and can occur at any time in the life of an individual. Nearly 95% of cases of urinary tract infection are cause by bacteria that typically multiply at the opening of the urethra and travel up to the bladder. Much less frequently, bacteria spread to the kidney from the bloodstream. The male and female urinary tracts are similar except for the length of the urethra.

The urinary system helps maintain proper water and salt balance throughout body and also ejects urine from the body.

The ascending route is through fecal flora spreading to the perineum and from their ascend into the bladder. Urinary tract infection may be defined as the presence of bacteria undergoing multiplication in urine within the urinary drainage system. A count 105 organism/ml of urine denotes significant bacteriuria and indicate active urinary tract infection, Moreover, urinary tract infection is generallycaused by one species, while contaminants are generally of mixed species.

1.1.1 Types of Urinary tract infection

UTI are generally classified as:

- Uncomplicated or complicated, depending on the factors that cause the infections

- Primary or recurrent, depending on whether the infection is occurring for the first time or is a repeat occasion.

Uncomplicated Urinary Tract Infections (UTI)

Uncomplicated UTIs are due to a bacterial infection, most often *E. coli*. They affect women much more often than men.

Cystitis: - Cystitis, or bladder infection, is the most common urinary tract infection. It occurs in the lower urinary tract (the bladder and urethra) and almost always in women. In most cases, the infection is brief and acute and only the surface of the bladder is infected. Deeper layers of the bladder may be injured if the infection becomes chronic, or if the urinary tract is structurally abnormal.

Pyelonephritis (Kidney Infection):-Sometimes the infection spreads to the upper tract (the ureters and kidneys). This is called pyelonephritis, or more the commonly, a kidney infection.

Complicated Urinary Tract Infections

Complicated infections, which occur in men and women of any age, are also caused by bacteria but they tend to be more severe, more difficult to treat, and repeated. They are often the result of:

- Some anatomical or structural abnormality that harms the ability of the urinary tract to clear out urine and therefore bacterial infection takes place.
- Catheter use in the hospital setting or chronic indwelling catheter in the outpatient setting, also cause bacterial infection.
- Bladder and kidney dysfunction, or kidney transplant (especially in the first 3 months after transplant).

Reappearances occur in up to 50 - 60% of patients with complicated urinary tract

infection if the basic structural or anatomical abnormalities are not corrected.

1.1.2 Recurrent Urinary Tract Infections

Most women who have had an uncomplicated urinary tract infection have to occasional reappearances. About 25 - 50% of these women can expect another infection within a year of the previous one. Between3 - 5%of women have ongoing, recurrent urinary tract infections, which follow the resolution of a previous treated or untreated episode.

Recurrence is often categorized as either reinfection or relapse:

- **Reinfection**: - About 80% of recurring urinary tract infection is reinfections.Areinfection occurs several weeks after antibiotic treatment has cleared up the early period and can be caused by the same bacterial strain that caused the original episode or a different one. The infecting organism is usually introduced through fecal bacteria and moves up through the urinary tract.
- **Relapse:** - Relapse is the less common form of recurrent urinary tract infection. It is diagnosed when a urinary tract infection reoccurs with 2 weeks of treatment of the first period and is due to treatment failure. Relapse usually occurs in kidney infection (pyelonephritis) or is associated with obstructions such as kidney stones, structural abnormalities or, in men, Chronic prostatitis.

Asymptomatic Urinary Tract Infection (Asymptomatic Bacteriuria)

When a person has no symptoms of infection but significant numbers of bacteria have colonized the urinary tract, the condition is called asymptomatic urinary tract infection (also called asymptomatic bacteriuria). The condition is harmless in most people and rarely persists, although it does increase the risk for developing the

symptomatic urinary tract infection.

Screening for asymptomatic bacteriuria is not necessary during most routine medical examinations, with the following exceptions:

- Pregnant women: - Pregnant women with asymptomatic bacteriuria have an increased risk of acute pyelonephritis in their second or third trimester. Therefore, they need screening and treatment for this condition. Guidelines recommend that pregnant women be screened for asymptomatic bacteriuria at 12 - 16 weeks gestation or at the first pre-natal visit, if later.
- People undergoing urologic surgers (such as prostate surgery in men). The presence of an infection during surgery can lead to serious consequences.

1.2 GENERAL PRINCIPLE OF URINARY TRACT INFECTION

Infections in the urinary tract are relatively common. These infections are often referred to as “bladder infections”. They are also known as “UTI’s” or urinary tract infections. When an infection is restricted to the bladder, the correct term to use is cystitis.

The kidneys can also become infected. This is referred to as pyelonephritis. The urinary system (also called the excretory system) produces stores and eliminates urine. It includes two kidneys, two ureters, the bladder, and the urethra as well as two sphincter muscles.

- **Kidneys**: located in the upper abdomen close to the spine, at the level of or just below the ribcage. Kidneys filter water soluble waste products from the blood and form urine.
- **Ureters**: thin tubes through which urine travels from each kidney to the bladder. Small muscles that are present in the walls these tubes constantly

tighten and relax to force urine away from the kidney.

- **Bladder**: hollow organ shaped like a balloon. It is located in the pelvis and held in place by ligaments that attach to other organs and the pelvic bones. Its function is to store urine.It can swell to be quite large but generallyhold about two cups of urine comfortably for 2 to 5 hours.
- **Urethra**: tube that allows urine to pass from the bladder to outside the body.
- **Sphincters**: circular muscles that keep urine from leaking out of the bladder.

Nerves in the bladder signal the brain when it is time to urinate. The sensation the urinate becomes stronger as the bladder continues tofill and reaches its limit.When a person urinates, the brain signals the bladder muscles to tighten and squeeze the urine out of the bladder. It also signals the sphincter muscles to relax so that urine can exit through the urethra. Urine is normally clear, has no odor, and is the color of amber.

1.3 CAUSE OF URINARY TRACT INFECTION

Urinary tract infections usually occur when bacteria enter the urinary tract through the urethra. The bacteria then multiply in the bladder and can result in an infection. The bacteria that usually cause an infection come from the intestinal tract and live on the skin near the rectum or int hevagina in women.The most common organism

is *E. coli* but other bacteria can also cause infections. Sexually transmitted diseases such as *Chlamydia and Gonorrhea* can cause urethritis.

Generally the infection is limited to the bladder or urethra. However, mainly if left untreated, the bacteria can continue to multiply and travel up the ureters to one or both kidneys. This is a much more serious problem resulting in fever, chills, dehydration, and even sepsis.

1.4 RISK FACTOR OF URINARY TRACT INFECTION

1.4.1GENDER:-

- Females are more prone to urinary tract infections than are males. This is because the urethra is shorter which cuts down the distance that bacteria have to travel to reach the bladder. The urethral opening is also much closer to the anus and can come into contact with bacteria more readily. Females also lack the prostatic secretions which are present in males. Prostatic secretions are bacteriostatic which means that they keep the bacteria from growing and multiplying as readily.
- As males age, they often have enlargement of the prostate gland. This causes an obstruction to the flow of urine. When the bladder does not completely empty, bacteria are not fully flushed out and can multiply and cause an infection.
- In males who are not circumcised, there are more bacteria living closer to the opening of the urethra which increases their risk for developing an infection.
- After menopause, females are also more prone to infections due to lack of estrogen

1.4.2 SEXUAL ACTIVITY:-

Increased sexual activity leads to more frequent urinary tract infections women especially when a spermicide is used and there is more than one sexual partner.

1.4.3 URINARY TRACT ABNORMALITIES

- Neurologicals abnormalities such as a neurogenic bladder can lead the recurrent urinary tract infections the bladder does not empty correctly.
- Other anatomical variations that block the flow of urine and emptying the bladder cause an increase in infections.
- Having to use a catheter to empty the bladder leads infections mostly to introducing bacteria to the inside of the bladder when the catheter inserted. When a catheter is left in the bladder for more than a couple days, the bladder becomes colonized with bacteria and is prone more infections.

1.4.4 SUPPRESSED IMMUNE SYSTEM:-

- Diabetes and other diseases can impair the immune system and increase the risk of infection. The immune system is the body's defense against bacteria and developing infections.

1.5 SYMPTONS OF INFECTION:-

Most infections involve the lower urinary tract: the bladder and urethra. Infections in the urinary tract don't always cause symptoms. Frequent symptoms of bacterial infection include:

- Strong, persistent urge to urinate.
- Burning sensation when urinating.

- Passing urine frequently in small amounts.
- Cloudy, pink, or brown urine.

- Strong-smelling urine.
- Pelvic or rectal pain.

More specific symptoms may depend on which part of the urinary system is infected:

Kidneys (pyelonephritis):

- Upper back and side (flank) pain.
- High fever.
- Shaking and chills.
- Nausea and/or vomiting.

Bladder (cystitis):

- Pelvic pressure.
- Lower abdomen discomfort.
- Frequent, painful urination.
- Bloody or discolored urine

Urethra (urethritis):

- Burning with urination sometimes no symptoms are present. Elderly individuals may not have discomfort of any type.However,new onset confusion or worsening of confusion can frequently be seen when an infection is present in the elderly.

1.6 DIAGNOSIS OF INFECTION

Diagnosis of an infection iusually done by testing urine sample,The sample be tested for the presence of white blood cells, red blood cells, and bacteria.

are “test strips” that have reagents on the strip that react by changing color dipped into urine. These reagents react with many different things that

found in urine such as cells, bacteria, protein and glucose. Looking at the sample under a microscope is also done to see if cells and bacteria are present.

The urine may also be set up for a culture. A culture will grow the bacteria that are present in the urine on special media.The type of bacteria present can be identified and then tested to see which antibiotics wil lto be successful in fighting the bacteria. Since cultures take at least two days before results are available, physicians will often treat the infection empirically with one of several antibiotic that generally are effective.If the infection is not responsive to treatment, culture becomes necessary in order to determine which antibiotic will be effective.

1.7 TREATMENT

Antibiotics:

- **Cystitis**: Uncomplicated urinary tract infections are treated with the antibiotics. Generally oral antibiotics such as nitrofurantoin, and fluoroquinolones (Ciprofloxacin), cephalosporin, and trimethoprim sulfamethoxazole are used. Usually a three-day course of treatment adequate however nitrofurantoin requires a 5 to 7 day course.
- **Pyelonephritis** Pyelonephritis is treated more aggressively than simple bladder infection using either a longer course of oral antibiotics intravenous antibiotics.
- **Pyridium (phenazopyridine) and Uristat:** These medications numb the bladder and urethra.Pyridium is sometimes prescribed to reduce burning pain associated with infections.Uristat is an over-the-counter medication.These medications change the color of urine to either blue orange and interfere with laboratory testing of the urine.
- **Fluids:** It is often recommended to drink extra fluids when a bladder

infection is present to help flush bacteria from the bladder. However

also dilute the antibiotic in the bladder possibly making the medication less effective. No studies have been done to address this issue.

- **Bladder irritants:** Coffee, alcohol, and soft drinks containing citrus juices and caffeine can irritate the bladder and intensify symptoms.
- **Heating pads:** Warm heating pads applied to the abdomen may minimize bladder pressure and discomfort.

1.8 PREVENTION

Steps can be taken to reduce the risk of urinary tract infections.

- **Drink plenty of liquids, especially water**. Drinking water helps to dilute the urine and causes increased frequency of urination which flush out bacteria.
- **Avoid bladder irritants**. Carbonated beverages (soda), caffeine (coffee, tea, soda)and alcohol are the most common irritants.Tomatoes and tomato based products, sugar, chocolate, and highly spiced foods can also cause irritation in the bladder.Artificial sweeteners,especially aspartame should also be avoided.
- **Wipe from front to back.** After urinating or bowel movements, wiping from the front to the back helps prevent bacteria in the anal region from spreading to the vagina and urethra.
- **Empty bladder regularly**. Flushing the bacteria out of the bladder and urethra are important to prevent infections.
- **Cranberry products:** Cranberry juice or tablets are promoted as a way to help prevent infections.Reviews of literature have found evidence for this women but not in everyone.
- **Antibiotics:** A prolonged course (six months to a year) or low-dose

antibiotics may be prescribed and is effective in reducing the frequency recurrent urinary tract infections.

CHAPTER-2
AIM AND OBJECTIVES

OBJECTIVES

2.1 General Objectives

To determine the prevalence of UTI infection isolated from urine sample and antibiotic susceptibility pattern of isolates in patient visiting SRLdiagnostic center.

2.2 Specific Objectives

- To isolate and identify the pathogenic bacteria in urine sample collected from patient visiting SRL diagnostic center.
- To study the bacteriological episodes in different age group with relation to gender.
- To perform antibiotic susceptibility test of bacterial isolates.
- To estimate the prevalence of Multi Drug Resistant microorganism from the isolates.

CAPTER 3
3.1 LITERATURE REVIEW

Aghdam (2013) reported that urinary tract infections second to the respiratory infections founds the highest statistics of infections among patients and has ranked first in terms of adult patients visits with doctor. In infectious diseases such urinary tract infections, doctors often have to treat the infection before definitive identification of infectious agents and susceptibility testing to antibiotics; therefore, they have to have sufficient information in the context of infections and antibiotic susceptibility. The aim of this study was to determine the prevalence of bacterial agents of urinary tract infections and their antibiotic susceptibility pattern in Tabriz during the year 2011. In this study, 876 urine samples were cultured after being transferred in a completely sterile condition to the microbiology laboratory and finally the total of 135 samples were culture positive. Among the positive cases, the most frequency was related to *E. coli* with 63.7% of the whole positive samples and the highest sensitivity belonged to Ceftriaxone antibiotics with a sensitivity of 53.48% of all reported cases.

Bano.K et.al (2012) conduct a study of Urinary tract infections (UTI) are common and frequently encountered serious illness that affects its toll not only to all the segments of human population but also results in increasing antibiotic resistance due to persistence and mis-management of the ailment. Pathogenic organisms' isolation and determination of antibiotic resistance by bacterial uropathogens in a cross section of patients was investigated at National Institute of Health, Islamabad. A total of 115 samples were collected from June to the August 2009. Identification was conducted by conventional biochemical tests and API 20E system. Percentage identification of API-20E was 100% for *Enterobacter cloacae and Klebsiella*

pneumoniae while 98.9% for *E. coli.* Antibiotic sensitivity test was analyzed by disc diffusion method using different antibiotics and their zone othe inhibition

measured. The bacterial isolates were identified as *Escherichia coli* (46.98 %) and *E. cloacae,* methicillin resistant *Staphylococcus aureus* (MRSA), *Staphylococcus saprophyticus* (1.20 %). In this study it was found that *K. pneumoniae* showed highest sensitivity (80%) to cefapime and low susceptibility (13%) to ciprofloxacin, while the highest resistance (60%) to gentamicin and the lowest (6%) to meropenem, nitrofurantoin and ciprofloxacin was also observed. The susceptibility of *S. aureus* was highest (64%) to amikacin, augmentin and Oxacillin and lower sensitivity for ampicillin and moderate for erythromycin, methicillin, and cefotaxime with 45% outcome. The overall results obtained indicated varied patterns of antibiotic sensitivity and resistance, deserving therefore the judicious and rational use of antibiotic in the routine treatment of urinary tract infection to prevent the recurrence as well as resistant strains.

Beyen (2011) reported that 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.

Chaudhary and Murthy. (2013) reported that Urinary tract infection (UTI) is a serious health problem, affecting millions of people each year. This study had undertaken to study the profile of uro-pathogenic bacterial flora in adult, their antibiotic resistant pattern, adherence factors of uropathogens and in vitro adherence capabilities of Uropathogens.

The samples were tested microbiologically by standard procedure. Antibiotic susceptibility of the isolated pathogens was tested for commonly used antibiotics by Kirby-Bauer technique according to CLSI guidelines.
Significant bacteriuria was present in 40% of samples. The most common pathogens isolated were *Escherichia coli* (52%), followed by *Klebsiella pneumoniae* (16%), *Acinetobacter anitratus* (11%), *Proteus mirabilis* (6%),

Enterobacter species (5%), *Citrobacter* species (2%), *Pseudomonas aeruginosa* (2%), *Morganella morganii* (1%), *Enterococcus* species (3%) and *Staphylococcus aureus* (2%). The mean susceptibility of uropathogens was for amikacin (Ak-81%), nitrofurantoin (Nf-60%), cefotaxime (52%), ceftriaxone (Ci-47%), ciprofloxacin (Cf-45%), norfloxacin (Nx-33%), cotrimoxazole (Co-18%) and nalidixic acid (Na-17%). *Escherichia coli* are the commonest cause of UTI. Adherence is one of the essential pre-requisites to establish UTI. Majority of UTI in men are mono-microbial. Most of uropathogens are susceptible to amikacin (81%) and nitrofurantoin (60%).

Dash et al (2013): determined the antimicrobial resistance of urinary tract pathogens has increased worldwide. Empiric treatment of community acquire urinary tract infection is determined by antimicrobial resistance patterns of uropathogens in a population of specific geographical location. This study to conducted to determine the prevalence of community acquired urinary tract infection in rural Odisha, India, and the effect of gender and age on its prevalence as well as etiologic agents and the resistance profile of the bacterial isolate Consecutive clean mid-stream urine samples were collected from 1670 adult patients. The urine samples were processed and microbial isolates were identified by conventional methods.Antimicrobial susceptibility testing was performed on all

bacterial isolates by Kirby Bauer's disc diffusion method. The prevalence of UTI was significantly higher in females compared with males(females 45.2%, males

18.4%, Young females within the age group of 18to37 years and elderly males (≥68 years) showed high prevalence of UTI. *Escherichia coli* (68.8%) were the to prevalent isolate followed by *Enterococcus spp*. (9.7%). Amikacin and the nitrofurantoin were the most active antimicrobial agents which showed to low resistance rate of 5.8% and 9.8%, respectively.

Hasan (2007) conduct a study analyze the pathogenic organisms recovered from patients with urinary tract infection in a tertiary Indian hospital setting along with determination of the occurrence and antimicrobial sensitivity of uropathogens on retrospective basis during a period of one year.

A total of 5073 urine samples were processed. Urine culture was done using the Conventional microbiological techniques. Biochemical testing was used identify the organisms and antibiotic sensitivity was done by the Kirby Bauer method. A total of 2436 uropathogens were isolated. *E coli* were seen in50.7%samples followed by Klebsiella spp (27.6%).*Staphylococcus aureus* was the commonest Gram- positive isolate (1.5%). Urinary tract infection (UTI)was seen in70.5% females as compared to 29.5% males.A high recovery of isolates was noted from July to September. Multi drug resistance was commonest with Enterococcus (78.8%) followed Pseudomonas (65.1%). Drugs, which retained usefulness for Gram-negative isolates, were amikacin, norfloxacin and cefotaxime. For Gram-positive isolates,vancomycin, teicoplanin, lincomycin and Norfloxacin were very effective.

Khatri.B et al (2014) reported that Urinary tract infection (UTI) is the most common infection in both community and hospital patients. In majority the cases, empirical antimicrobial treatment practiced before the laboratory results of urine culture. Thus, antibiotic resistance may increase urinary bacterial pathogens due

to improper use of drugs. This study was designed find out the etiological agents of UTI and their prevalence, and determine the antimicrobial susceptibility

pattern of the bacterial pathogens isolated from urine culture. This study was conducted in Kathmandu Model Hospital, Kathmandu, Nepal from April to the October, 2009. Midstream Urine samples from 1323 patients suspected of UTI was analyzed by microscopy, and conventional semi-quantitative culture technique for the significant growth. Antimicrobial susceptibility test was performed for the isolates Modified Kirby-Bauer disk diffusion method. Data were analyzed using SPSS software window version 16.The overall prevalence of UTI was found to be 18.89%. The most frequent causative organisms isolated were *Escherichia coli* (82.30%), *Enterococcus faecalis* (5.60%), *Citrobacter freundii* (3.60%), *Enterobacter aerogenes* (2.40%), Coagulase Negative Staphylococci (2.40%), *Pseudomonas aeruginosa* (1.20%),*Proteus mirabilis*(0.8%)*Klebsiellapneumonia* (0.4%), and *Staphylococcus aureus* (0.4%). Nitrofurantoin and Amoxicillin found to the most effective antibiotic against gram negative and gram positive isolates respectively. *E. coli* was found to be the most common etiological agent UTI and Nitrofurantoin was the most effective drug among the isolates.

Khoshbakht.R et al; (2012) Urinary Tract Infection (UTI) is one of the most common bacterial diseases worldwide that can be presented asymptomatic symptomatic, characterized by a wide range symptoms from mild irritative voiding to bacteremia, sepsis, or death. In latter decades, widespread utilization of antibiotics resulted in increased incidence of antibiotic resistance among urinary tract pathogens all over the world.This descriptive study was conducted the city of Karaj from November 2009 to August 2010. Bacterial isolates identified by standard biochemical tests.Antibacterial susceptibility test was performed by Kirby-Bauer method against some common antibiotics Gram negative and Gram positive. In study, 230 (8.06%)out of 2852 patients showed positive urine cultures of which there 204 (88.69%) females and 26 (11.3%)

males. 180 (83.17%) cases of isolated bacteria Gram negative bacilli while 50 (21.73%) cases were Gram positive cocci. *E. coli* isolates, as the frequent

Gram negative bacteria, showed high resistance to cephalothin (88.16%) and high susceptibility to nitrofurantoin (90.78%). Also *Staphylococcus saprophyticus* isolates, as the most frequent Gram positive bacteria, exhibited high resistance to ampicillin, tetracycline, and erythromycin (92.31%) and high susceptibility to nitrofurantoin and vancomycin (92.3%).

Manikandan.S et al. (2011) studied that the current situation of antimicrobial resistance of Urinary Tract Infections (UTIs) caused by human pathogens.About 10 midstream urine samples were collected from adult patients were analyzed Multidrug Resistant (MDR) strain isolation and identified.The MDR strains were identified by the Kirby Bauer method following the definition of the National Committee 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 the antimicrobial agents were: 83.3% to SXT, 80.6% to Nalidixic acid, 67.3% Amoxicillin, 61% to Cotrimoxazole, 48.8% to Gentamycin,46%ciprofloxacin and 43% cephalexin. Isolated UTI strains were tested for susceptibility against antibiotics, few of the antibiotics were sensitive, but most antibiotics showed resistant to the MDRstrains. Among this *E. coli*,*K.pneumoniae* and *P.aeruginosa* were highly resistance to most the antibiotics, whereas *Staphylococcus* spp and *Serratia marcescens* exhibited sensitive to Cephalexin, Ciprofloxacin and Gentamycin.

Nivas (2014) studied that Urinary tract infection (UTI) is most common among all hospital acquired infections worldwide. In UTI, most common organism E.coli. Organism responsible for hospital acquired infection may have tendency

to develop multiple drug resistance. E.coli acquired from the Al-Quwayiyah General Hospital source may differ their resistant against antibiotics. This was

surveying study conducted in an Al-Quwayiyah General hospital to know the prevalence and antibiogram of E.coli. Samples received include mid-stream clean catch urine, suprapubic aspirate and from Foley's catheter. All the plates were inspected for growth and the isolates were identified by observing colony morphology, Gram-stain characteristics and relevant biochemical tests. The isolates were tested for their antimicrobial susceptibility and the results were interpreted by vitek 2 methods, according to the guidelines of Clinical and Laboratory Standards Institute. Out of 96 samples tested, 53 (55.2%) were positive for E.coli. E.coli was highly resistant to Ampicillin (85.1%) followed by Piperacillin (66.03%) and least resistance was seen with Nalidixic acid (5.6%) and Nitrofurantoin (5.6%) followed by and Ciprofloxacin (11.3%).

Patel et al (2012) reported that symptomatic urinary tract infections (UTI) occurs in as many as 7 million visits to emergency units and 100,000 hospitalization annually. UTI has become the most of common hospital-acquired infections, accounting for as many as 35% of nosocomial infections, and it is the second most common cause of bacteraemia in hospitalized patients. The prevalence of Urinary tract infections (UTI) was evaluated in 3046 patients attending G.G. Hospital Medical College, Jamnagar. Results showed 1416 (46.48%) patients were positive. The most common organisms were Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Proteus mirabilis. In-vitro antibiotic susceptibility tests revealed that the gram negatives bacteria were sensitive to quinolones (Gatifloxacin, levofloxacin) and meropenum, while the gram positive isolates were sensitive to linazolid, erythromycin & quinolones (Gatifloxacin, levofloxacin).

Rakesh et al (2013) Urinary tract Infection (UTI) is among the most common infections described in outpatient department and hospitals inpatients. *Klebsiella*

pneumoniae (*K. pneumoniae*) accounts for 2nd highest organism isolated from urine samples of UTI patients after *Escherichia coli*. The present study is undertaken to

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determine the antimicrobial susceptibility pattern of K.pneumoniae isolated from urine samples of UTI patients of Peoples College of Medical Science & Research Centre and hospital. Between January 2010 and December 2011, a total of 1450 urine specimens processed in the laboratory, of which 65 (15.4%) yielded *K. pneumoniae*. Organisms were identified by conventional methods. Antimicrobial susceptibility testing was done by the disk diffusion methods. K.pneumoniae is showing good antimicrobial susceptibility for imipenem, gatifloxacin, amikacin, gentamicin, ampicillin/Sulbactam, levofloxacin and amoxicillin/clavulinic acid. An attempt has been made in this study to recognize the K.pneumoniaein patients of UTI of tertiary care hospital, Bhopal M.P.; to record the antibiogram and probable drug of choice for K.pneumoniae considering the antibiotic susceptibility testing, cost, side effects and many other factors.

Razak (2012) reported that urinary tract infection is one the most common bacterial infections seen in clinical practice particularly in the developing countries.The causative agents for Urinary tract infection vary from place to place and they also vary in their susceptibility and resistance patterns. This study was done to find out the common bacteria causing UTI and to determine the antibiotic susceptibility pattern the urinary pathogens from a tertiary care hospital in South India. A total of 573 mid-stream urine samples from the suspected UTI patients were tested microbiologically and antimicrobial susceptibility test were performed for the isolated pathogens using Kirby-Bauer disk diffusion method.

The rate of culture positivity in females was 87.82% and in males was 27.92%. E-coli were the most frequently isolated urinary pathogen (37.95%), followed by Klebsiella (21.41%) and Acinetobacter (10.94%). E-coli were highly sensitive to Nitrofurantoin (81.92%) and Amikacin (69.88%) and it was highly resistant to Ampicillin (1.0%). Klebsiella was highly sensitive to imipenem and it was highly

resistant to Ampicillin. Higher prevalence of UTI was seen in females. Gram negative organisms were the most commonly isolated organisms in UTI. Urinary

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pathogens showed resistance to commonly used antibiotics like Ampicillin, Norfloxacin and Nalidixic acid. The susceptibility and resistance patterns of urinary pathogens should be considered before starting empirical treatment for UTI.

TulaM.Y (2014) conduct a study to determine the distribution and antimicrobial susceptibility of uropathogens among patients attending Mubi general hospital well as to determine the effect of gender on the etiology of bacterial uropathogens. Near about urine samples of 101 patients included of 46 males and 55 females were analyzed for bacterial growth, antibiogram and multiple antibiotic resistance index. Females showed higher prevalence of UTI than males. Gram negative bacteria (61.7%) were found in high prevalence than Gram positive (29.3%). *Staphylococcus aureus* (58.3%) has the highest prevalence rate among Gram positive organisms, while *Citrobacter freundii* (25.3%) was the most prevalent Gram negative isolates. *Citrobacter freundii* (17.9%) was the most prevalent uropathogens closely followed by *S. aureus* (17.1%). Antimicrobial susceptibility was performed on all isolated bacteria by the disc diffusion method employing multiple antibiotic discs differently for both Gram positive and Gram negative isolates. The results showed that *S. aureus* and Coagulase Negative Staphylococci (CONS) were more susceptible to Chloramphenicol (83%), followed by Streptomycin and Amoxicillin (78%). While their resistance profile showed that *S. aureus* and CONS are more resistant to Ampiclox, Gentamycin and Rifampicin (31%). Susceptibility to all the antibiotics by Gram positive organisms was significantly higher than their resistance to the same antibiotics ($p < 0.05$). Gram negative organisms are more susceptible to Streptomycin (62%), followed by Ciprofloxacin (47%) and Ofloxacin (44%), and while their resistance profile showed that they are more resistance to Nalixidic acid (79%) followed by Augmentin (76%), Ampicillin (75%) and Reflacine (74%). Resistance to all the antibiotics by Gram negative organisms is significantly higher

than their susceptibility to the same antibiotics ($p<0.05$). Also, there was significant correlation in resistance between *E. coli*, *Klebsiella* sp., *Citrobacter diversus* and *P.*

vulgaris ($p<0.05$). The multiple antibiotic resistances (MAR) index of each antibiotic was calculated. The MAR index for Gram positive antibiotics was significantly lower than that of Gram negative antibiotics ($p<0.05$).

Wasnik.D.D et al; (2013) study was conduct to detect common pathogens of urinary tract infection (UTI) and their susceptibility pattern the commonly used antimicrobial agents in local state. Urinary tract infection one of the common clinical conditions in the patient presenting the clinics and hospitals.Despite the widespread availability of antibiotics, it remains the most common bacterial infection in the human being. Detection of common pathogens and their antimicrobial susceptibility pattern is mandatory for effective treatment. In the present study,40 urine samples were collected from adult patient were analyzed for Multidrug Resistant (MDR) strain isolation and identified.The MDR strains were identified by Kirby Bauer method following the definition of the National Committee of Clinical Laboratory Standards.Out of total 40samples,32 (80%) samples grew potential pathogens causing UTI. *Escherichia coli* were the predominant (31.25%) isolates causing UTI,followed by*Staphylococcus aureus* -8 (25%), *Pseudomonas aeruginosa*- 5*(*15.62%),*Proteus mirabilis* -5(15.62%), *Klebsiella pneumoniae* -2(6.25%)and *Serratia marcescens* –2(6.25%)The mean sensitivity of the antibiotics was Tetracycline(76.66%),Penicillin(70.83%),and Ciprofloxacin (60%). *S.aureus* showed 75% resistance to Methicillin, Oxacillin Vancomycin. Uropathogens are sensitive to Norfloxacin, Co-trimoxazole and Ofloxacin. High prevalence drug- resistant urinary tract pathogens particularly Tetracycline, Penicillin and Ampicillin among local patients suggests cautious of antibiotic therapy for the treatment.

CHAPTER 4
MATERIAL & METHOD

4.1 MATERIALS

A list of materials used during the study is given appendix -I

4.2 METHODS

This study is carried out from 6th January to 22 April, 2015 in the microbiology laboratory of SRL diagnostic center in order to isolate and identify organism from urine sample along with their antibiotic susceptibility pattern there by covering a total period of 4 months. A total of 4362 urine sample were collected from the patient ranging in age from 3 month to 85 years.

4.3 COLLECTION OF SAMPLE

For this study, early morning mid-stream urine samples were collected using the Sterile,wide mouthed container with screw cap tops.On the urine sample bottles were indicated name, age,sex,and time of collection along with requisition forms.

4.4 PROCESSING OF SAMPLE

4.4.1Macroscopic Examination / Physical Examination

The physical characteristics of urine sample were noted.

4.4.2Microscopic Examination

The urine samples were mixed and centrifuged at5000rpm for5min. Discard the supernatant and place a drop of deposits on glass slide and put a cover slip over it and were examined by using both 10X and 40X objectives. Samples with ≥10 number of white blood cells/mm3were considered as pyuric.Avolume of the sample were applied to glass microscope slide,allowed air dry,stained with gram stain, and examined microscopically. Bacterial isolates were identified generally using biochemical reaction.

The composition and preparation of staining reagent are given I appendix-II

4.5 Culture of sample

The samples were culture for aerobic bacteria only and the study did not include anaerobic bacterial culture. The samples were inoculated in the CLED and the MacConkey agar or petriplates.

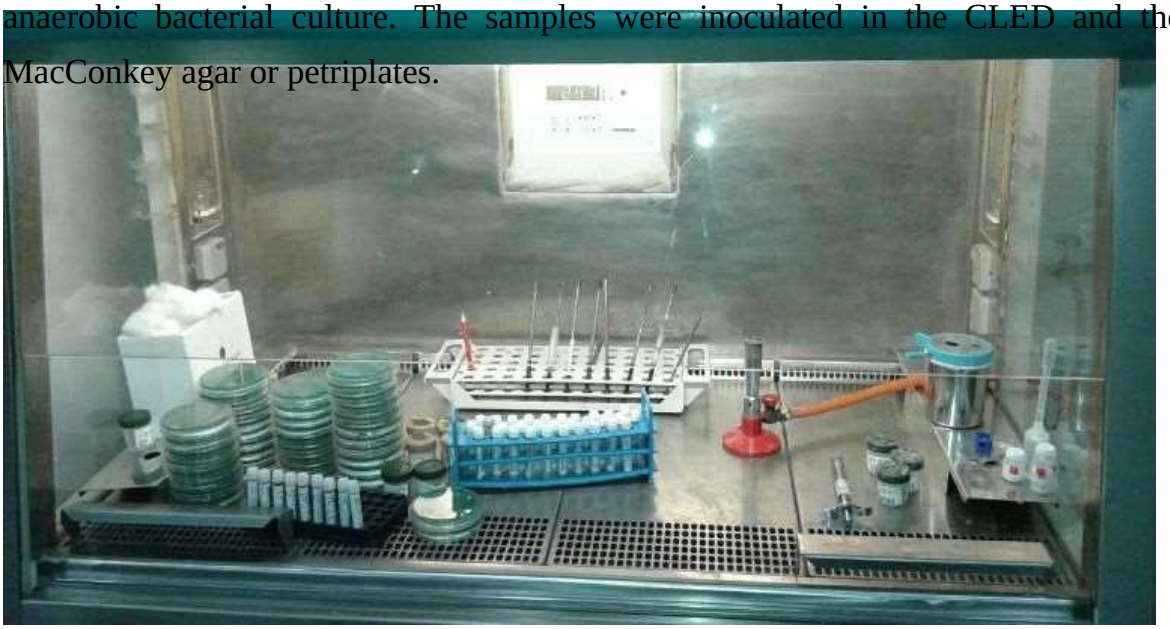

Fig 1: Sample processing in Bio septic cabinet.

Fig 2: sample processing on CLED agar.

The inoculated plates were incubated at 370C for 24 hours in incubator.

The composition and preparation of culture media are given in appendix-III.

4.6 ISOLATION AND IDENTIFICATION OF ORGANISM

After overnight incubation, the culture plate incubated aerobically were examined for bacterial growth and identified by using standard microbial technique which involves colony characteristics, staining reaction and biochemical properties and also the antibiotic susceptibility pattern were noted.

4.6.1 Identification with micro scan walkaway 40si

To perform biochemical test, the isolated organism should be pure culture. From the pure culture plate, grams staining, catalase test and oxidase test were perform Organisms were identified and their antibiotic susceptibility test was done by Mi-Cro Scan Walk Away 40 system, which is fully automated.

4.6.2 Procedre for Micro scan Walkaway 40si

Panel preparation

- Panels are going to be removed from the storage area.
- Then allowed the panel to be equilibrated at room temperature prior to rehydration. All open panels should be used in the same day.

Prompt System

- The required number of diluent bottle is going to be removed.
- Label each bottle with the lab number/isolate number and the panel to be tasted (GP or GN).
- An inoculation wand is going to be removed.
- Holding the wand straight up and down, touch 3-5 well isolated colonies.
- Holding the wand by the handle with one hand, grasp the collar with the other hand and pull firmly to break the connection between the collar and the wand shaft.

- Slide the collar down and off the collar wand shaft. Discard the collar.

- While holding the prepared wand in one hand, remove the cap of the diluent bottle by bending the cap sideways.
- Place the wand into the bottle and press down with a twisting motion to assure a tight seal.
- Shake the bottle vigorously 8-10 times to release bacteria from the wand tip. The inoculum should sit for 15 minutes, but no longer than 4 hours, before inoculating the test panels.

Panel re-hydration/ inoculation

- Removed the inoculation wand from the diluent bottle and use to inoculate purity plate. Discard the wand in biohazards waste.
- Poured the suspension into the seed tray. Squeeze the bottle gently as pour.
- Place the transfer lid over the seed tray.

- Gently tap the transfer lid in all four corners to remove bubbles from underneath the prongs. Allow the transfer lid to equilibrate for a minimum of 20 second before proceeding.
- Pick up the RENOK unit by depressing the pick-up levers with the thumb and finger. Place on the top of the transfer lid.
- Release the pick-up levers.
- Fully lift to the center lever of the RENOK unit to draw inoculum into the transfer lid, then release hand pressure.
- Pick up the RENOK unit/transfer lid assembly and position over the Micro Scan Panel.
- Depress the center release button of the RENOK unit to release the inoculums into well.
- Release the transfers into the seed tray and dispose into biohazard waste.

- Repeat to each isolate to be tested.
- Processing panels in Micro Scan Walkaway 40si system.

4.6.3 Quality control for test

Quality control is considered is one of the important factors for the correct result interpretation. In this study, strict aseptic condition was maintained during collection and processing of the sample to maintain quality control. The quality control of Micro Scan Walkaway 40 was done by inoculating known strains of bacteria in both Gram Positive Cocci and Gram Negative Bacilli (GPC & GNB) panel respectively as quality control. These quality control strains are tested exactly the same procedure as for the test organisms.

Fig 3: (Micro Scan) Walk Away 40si System is attached with a Labpro System through which we can access the Micro Scan system.

Fig 4: Renok

Fig 5: Autoclaved sterile water

Fig 6: Grams positive Cocci Panel

Fig 7: Grams negative Cocci Panel

Fig 6: **Positive B Combo Panel 23 panel:** MicroScan panels are designed for use in determining antimicrobial agent susceptibility and identification to the species level of rapidly growing aerobic gram positive Cocci.

Fig 7: **Negative B Combo Panel 34 Panel:** These panels are designed for use in

determining antimicrobial agent suspecibility or identification to the species level of aerobic and facultative anaerobic gram-negative bacilli.

Fig 8: CLED and MacConkey Agar.

PHOTOGRAPH OF BACTERIAL ISOLATES

Fig 9: Culture of E.Coli in CLED agar.

Fig 10: E.Coli in MacConkey agar.

Fig 11: Klebsiella in MacConkey agar

Fig 12: Klebsiella in blood agar.

Fig 13: Proteus in MacConkey agar

Fig 14: Proteus in blood agar

Fig 15: S.aureus grown in blood agar.

Fig 16: Pseudomonas in MacCankey agar

CHAPTER 5
RESULT

The study was carried out in Microbiology Laboratory of Super Religare Laboratory from 6th January to 22nd April 2015 thereby covering a total period of 4 months. Urine samples collected were processed and analyzed by the standard microbial techniques.

In this study a total of 4362 urine sample were collected and processed of the culture And sensitivity testing. Urine samples of patients of all age groups (1day-85years) and both sexes were processed. A total 1244 different organisms were isolated from 4362 urine samples thus the culture positive was 28.51 %(i.e.,1244) and negative is 71.41% (i.e. 3118) as shown in Table.

Table 1: Organisms wise distribution of Positive cases (n=1244)

Types of Organisms	Positive Cases	
	No.	%
E.Coli	730	58.68
Klebsiella pneumoniae	126	10.15
Pseudomonas aeruginosa	52	04.18
Proteus mirabilis	36	02.89
Proteus vulgaris	10	00.80
Enterococcus faecalis	152	12.21
Enterococcus faecium	26	02.10
Enterobacter	34	02.73
Acinetobacter	28	02.25
Staphylococcus aureus	11	00.88
Staphylococcus seprophyticus	16	01.28
Staphylococcus haemolyticus	23	01.85
TOTAL	1244	100

More than half (58.68%) cases reported as *E.coli* followed by *Klebsiella* (10.15%),

Pseudomonas aeruginosa (04.18%), *Proteus vulgaris* (00.80%).

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Proteus merabilis (02.89%), *Enterococcus faecalis* (12.21%), *Enterococcus faecium* (02.10%), *Enterobacter spp* (02.73%), and *Acinetobacter* (02.25%), *Staphylococcus haemolyticus* (01.85%), *Staphylococcus seprophyticus*(01.28%), *and Staphylococcus aureus* (8.10%) respectively.

Table 2: Sex wise Distribution of the Cases according to their test results (n =1244)

Gender	Total cases	Positive cases	
		No	%
Male	1745	413	33.20
Female	2617	831	66.80
Total	4362	1244	100

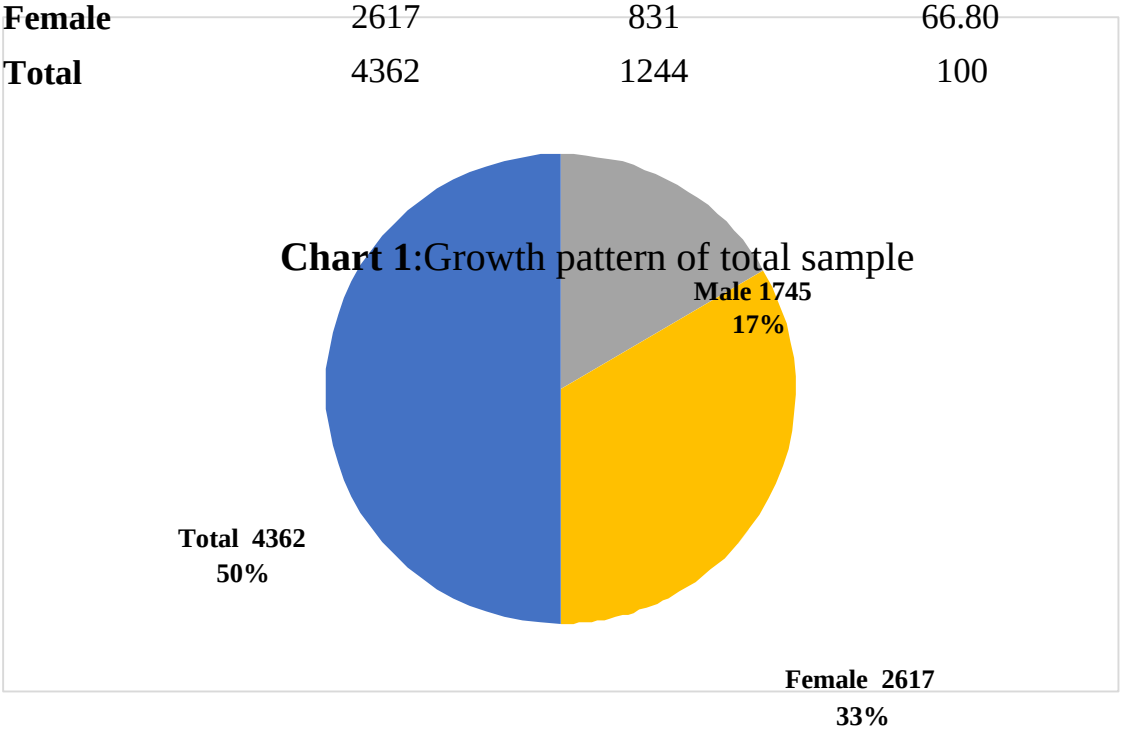

Chart 1:Growth pattern of total sample

[NOTE: Percentage in the chart is against total sample]

5.1 AGE AND GENDER WISE DISTRIBUTION OF PATENT WITH POSITIVE CASES.

Table 3: Age and gender wise distribution of patent with positive Cases.

AGE	GENDER				M+F	M+F (+ VE CASE)	M+F %	M+F (+ VE CASE) %
	Male (M)		FEMALE (F)					
	No.	+Ve Case	No.	+Ve Case				
Below 11	280	52	356	76	636	128	14.58	10.28
11-20	297	73	467	126	764	199	17.51	15.99
21-40	570	155	928	298	1498	453	34.34	36.41
41-60	308	60	468	182	776	242	17.80	19.47
>60	290	73	398	149	688	222	15.77	17.85
Total	1745	413	2617	831	4362	1244	100	100

[Percentage in the table is against total 4362 cases of male and female percentage and against 1244 positive cases for male and female positive cases]

Out of 4362 urine sample collected 1745 (40%) were from male patient with 413 (9.47%) positive cases and (2617, 59.99%) were from female patients with (831, 19.05%) positive cases. The samples were collected from age group ranging from 3month to 8 years. Age group21-40 year was found to be most affected age group (453, 34.34%) followed by41-60 years and age group bellow11years being the list affected age group.

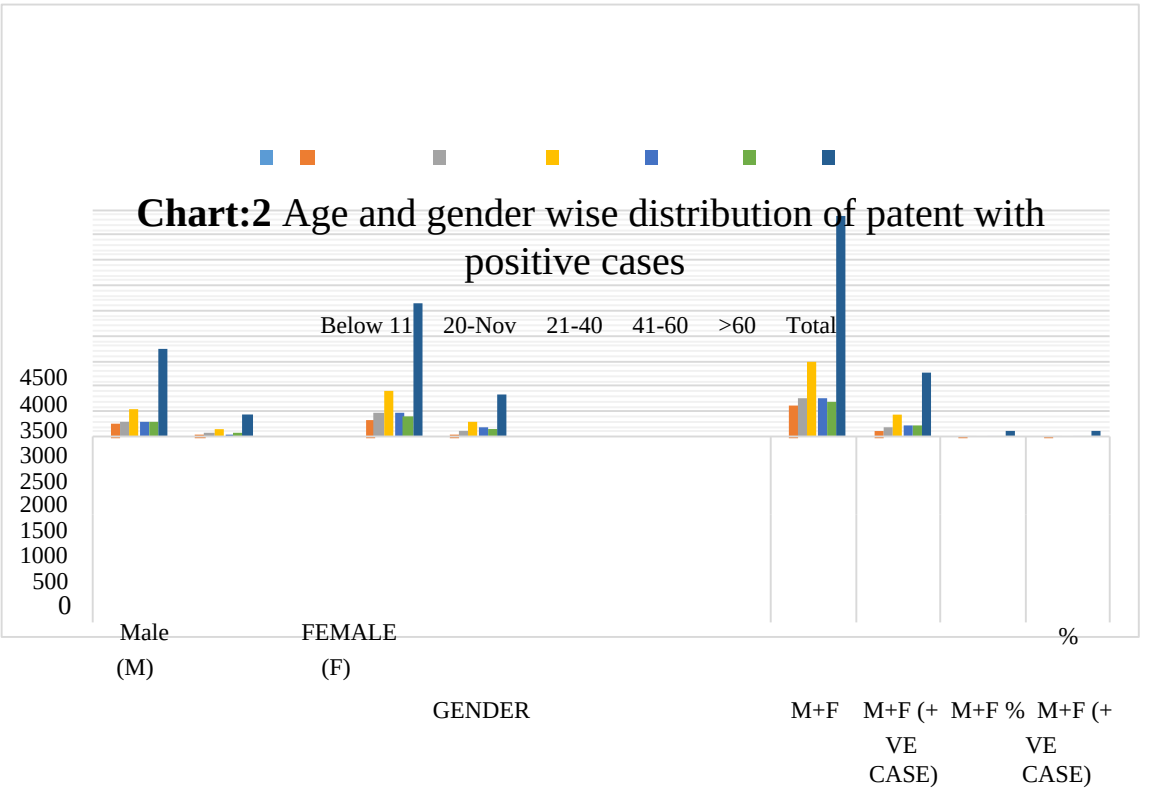

Chart:2 Age and gender wise distribution of patent with positive cases

5.2 ANTIBIOTIC SUSCEPTIBILITY PATTERN OF THE BACTERIAL ISOLATES

5.2.1 Antibiotic susceptibility patterns of *E.coli* (N-730) amongst the bacterial isolates.

Table 4: Antibiotic susceptibility patterns of *E.coli* (N-730) amongst the bacterial isolates.

ANTIBIOTIC	SENSITIVE %
Amikacin	93
Amoxicillin	52
Ampicillin	21
Cepfotaxime	43
Cefoxitin	82
Ceftazidime	43
Ciprofloxacin	28
Colistin	96
Ertapenem	97
Gentamycin	65
Imipenem	98

Le cillin
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m
Me
zlo

29		
98	**INTERMEDIAT E**	**RESISTANT %**
22	**%**	04
	04	16
	32	79
	<1	56
	>1	18
	00	57
	>1	71
	>1	04
	00	03
	00	34
	1	02
	00	68
	03	02
	00	77
	01	
34		

Nitrofurantoin	50	20	30
Norfloxacin	29	>1	71
Pip/Tazo	75	14	11
Piperacillin	23	01	76
Tetracycline	37	>1	63
Tigecycline	99	>1	>1
Tobramycin	54	02	44
Trimeth/Sulfa	43	00	57
Trim Trimethoprim	38	00	62

In the invitro antibiotic susceptibility pattern of *E.Coli* isolates Tigecycline (99%) was found to be most sensitive followed by Imipenem (98%) , Ertapenem (97%), Colistin (96%), Amikacin (93%) while Gentamycin(65%),and ampicillin (21%) being the least sensitive antibiotic respectively.

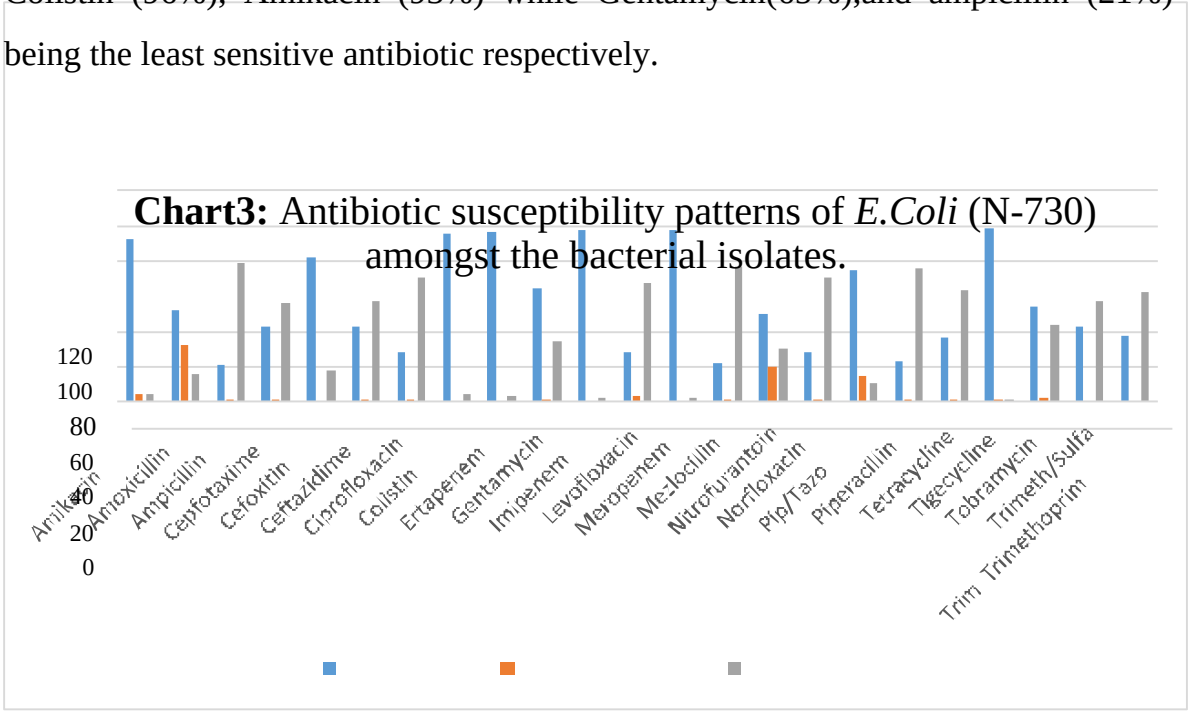

Chart3: Antibiotic susceptibility patterns of *E.Coli* (N-730) amongst the bacterial isolates.

5.2.2 Antibiotic susceptibility patterns of *Klebsiella pneumonia* (N-126) amongst the bacterial isolates.

Table 5: Antibiotic susceptibility patterns of *Klebsiella pneumonia* (N-126) amongst the bacterial isolates.

ANTIBIOTIC	SENSITIVE %	INTERMEDIATE %	RESISTANT %
Amikacin	67		03
Amoxicillin	52		12
Ampicillin	>1		>1
Cefepime	00		20
Cepfotaxime	45		>1
Cefoxitin	58		00
Ceftazidime	44		02
Ciprofloxacin	49		06
Colistin	80		00
Ertapenem	73		00
Gentamycin	58		00
Imipenem	79		00
Levofloxacin	60		03
Meropenem	78		00
Mezlocillin	08		09
Norfloxacin	53		05
Pip/Tazo	68		06
Piperacillin	10		10
Tetracycline	62		11
Tigecycline	79		15
Tobramycin	52		02
Trimeth/Sulfa	42		00
TrimeThoprim	40		00

PROSPECTIVE SUDY ON URINARY TRACT INFECTION ISOLATES FROM URINE SAMPLE AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN

30
35
98
80
54
42
55
44
20
27
42
21
37
22
83
42
27
80
27
06
47
58
60

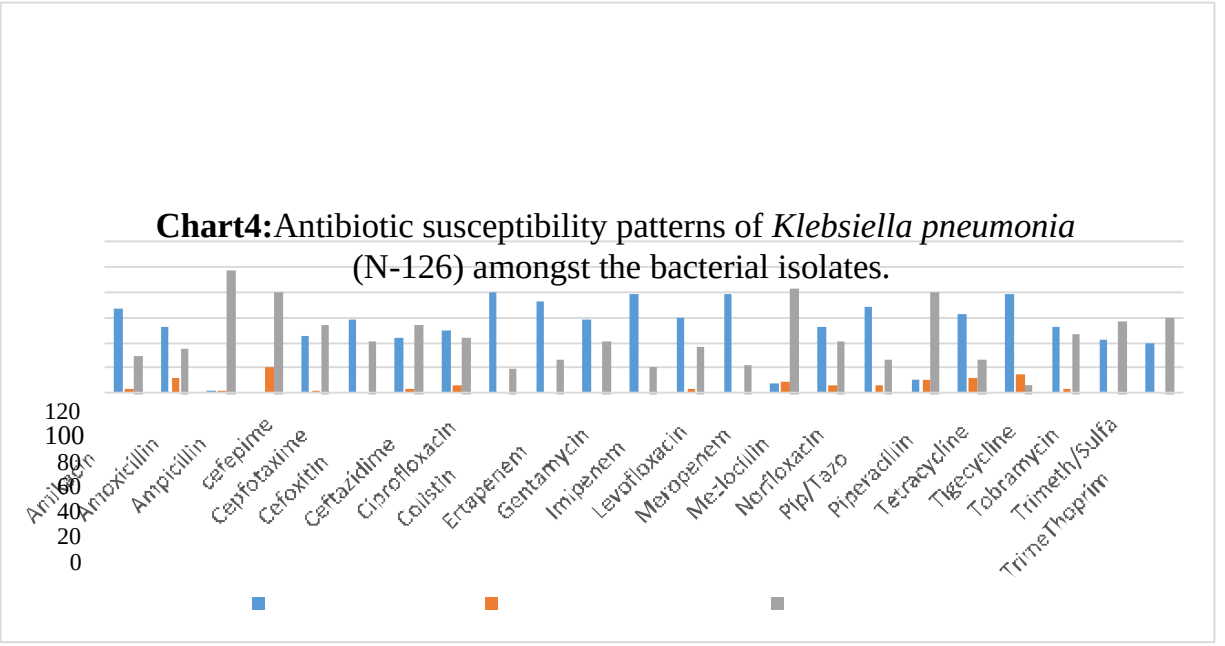

Chart4: Antibiotic susceptibility patterns of *Klebsiella pneumonia* (N-126) amongst the bacterial isolates.

SENSITIVE % INTERMEDIAT %E RESISTANT %

5.2.3 Antibiotic susceptibility patterns of *Pseudomonas aeruginosa* (N-52) amongst the bacterial isolates.

Table 6: Antibiotic susceptibility patterns of *Pseudomonas aeruginosa* (N-52) amongst the bacterial isolates.

	M	**c cin**	
	e	**i**	
	r	**l**	
ANTIBIOTIC	**o**	**i**	**SENSITIVE**
	p	**n**	**%**
Amikacin	**e**	**N**	76
Cefepime	**n**	**o**	20
Cepfotaxime	**e**	**r**	10
Ceftazidime	**m**	**f**	74
Ciprofloxacin		**l**	63
Colistin	**M**	**o**	61
Gentamycin	**e**	**x**	67
Imipenem	**z**	**a**	82
Levofloxacin	**l**		59
	o		

78
71
63

IN RESISTANT

TE **%**
R 22
M 60
ED 47
IA 18
TE 33
39
27
%
14
37
0
22
29
35
0
4
37 3
0
8
0
4
0
0
0
6
0
4
0
4
0
0
0
0
0
2

Pip/Tazo	86	00	14
Piperacillin	80	00	20
Tobramycin	73	02	49

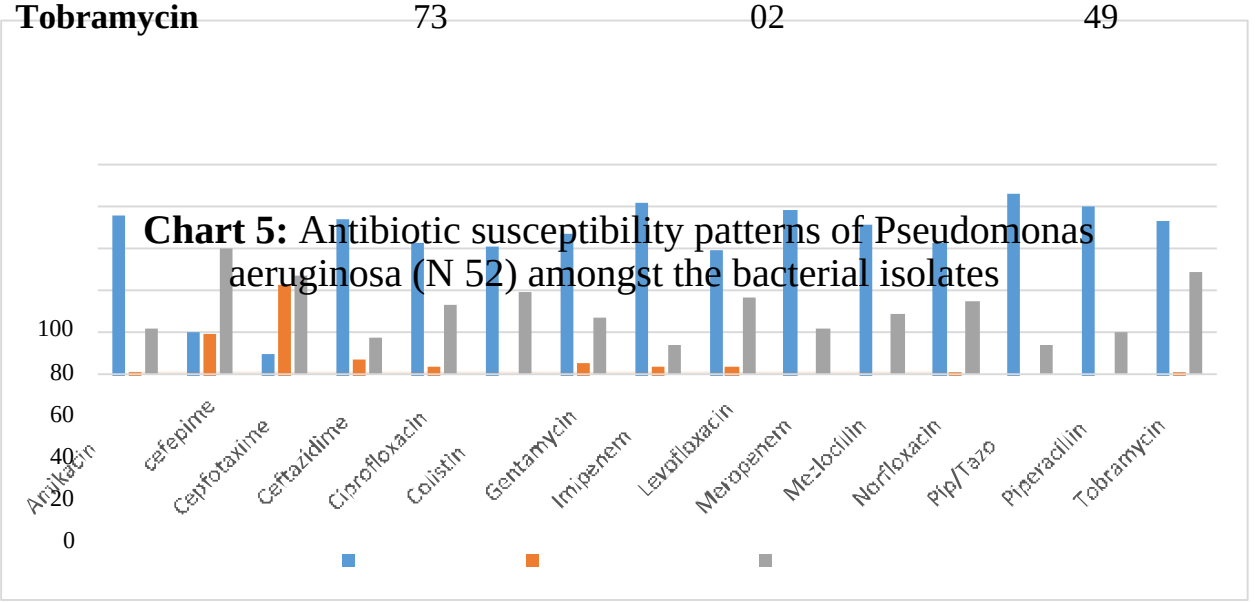

Chart 5: Antibiotic susceptibility patterns of Pseudomonas aeruginosa (N 52) amongst the bacterial isolates

5.2.4 Antibiotic susceptibility patterns of *Proteus mirabilis* (N-36) amongst the bacterial isolates.

Table 7: Antibiotic susceptibility patterns of *Proteus mirabilis* (N-36) amongst the bacterial isolates.

ANTIBIOTIC	**SENSITIVE %**	**INTERMEDIATE %**	**RESISTANT %**
Amikacin		**Cefiditaz**	**Ciprofloxacin** 64
Amoxicillin			83
Ampicillin			31
Cepfotaxime			**Colistin** 61
Cefoxitin			92

61	03	33
31	00	17
00	00	69
	03	36
	00	08
	00	39
	08	61
	00	100

Ertapenem	94	00	06
Gentamycin	56	6	44
Imipenem	92	00	08
Levofloxacin	47	06	47
Meropenem	97	00	03
Mezlocillin	39	06	56
Nitrofurantoin	00	15	85
Norfloxacin	33	36	31
Pip/Tazo	94	06	00
Piperacillin	39	11	50
Tetracycline	03	00	97
Tobramycin	53	11	36
Trimeth/Sulfa	28	00	72
72TriTrimethoprim	19	00	81

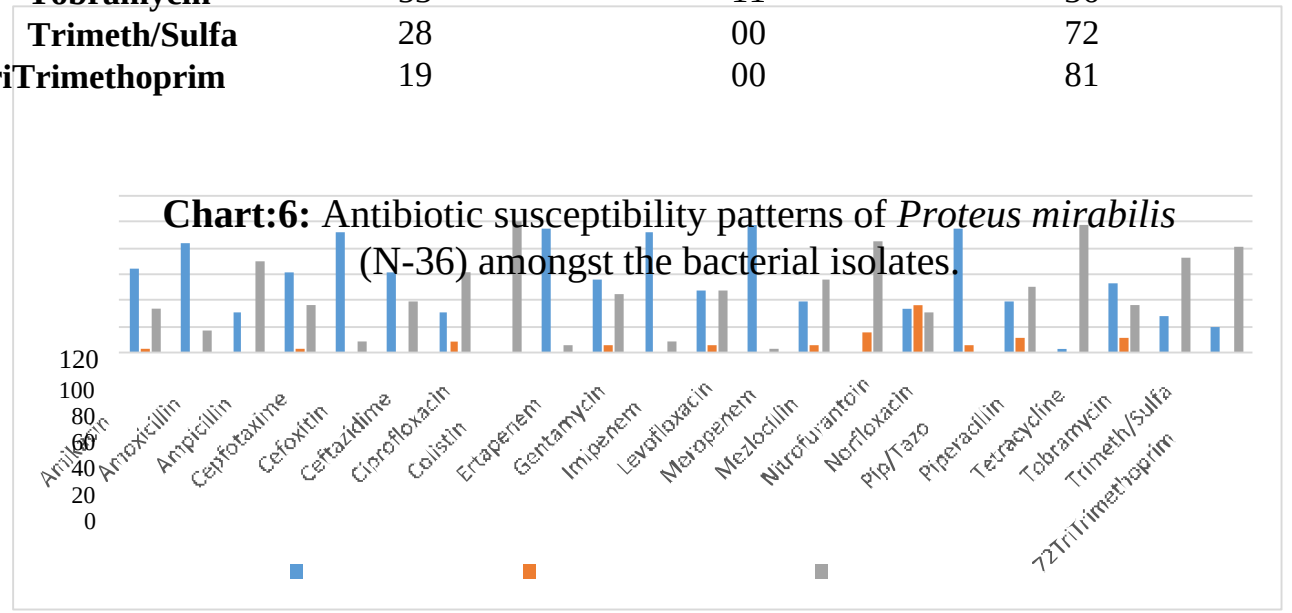

Chart:6: Antibiotic susceptibility patterns of *Proteus mirabilis* (N-36) amongst the bacterial isolates.

SENSITIVE % INTERMEDIATE % 39

PROSPECTIVE SUDY ON URINARY TRACT INFECTION ISOLATES FROM URINE SAMPLE AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN

RESISTANT %

5.2.5 Antibiotic susceptibility patterns of *Proteus vulgaris* (N-10) amongst the bacterial isolates.

Table8: Antibiotic susceptibility patterns of *Proteus vulgaris* (N-10) amongst the bacterial isolates.

ANTIBIOTIC	SENSITIVE	INTERMEDIATE	RESISTANT
	%	%	%
Amikacin	100		00
Amoxicillin	75		00
Ampicillin	00		00
Cepfotaxime	25		00
Cefoxitin	100	40	00
Ceftazidime	25		00
Cefazolin	00		00
Ciprofloxacin	75		00
Colistin	00		00
Ertapenem	100		00
Gentamycin	100		00
Imipenem	100		00
Livofloxacin	100		00
Meropenem	100		00
Mezlocillin	25		00
Nitrofurantoin	15		00
Norfloxacin	75		25
Pip/Tazo	100		00
Piperacillin	25		00
Tetracycline	75		00
Tigecycline	75		25
Tobramycin	100		00
Trimeth/Sulfa	75		00
Trim Trimethoprim	75		00

PROSPECTIVE SUDY ON URINARY TRACT INFECTION ISOLATES FROM URINE SAMPLE AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN

00
25
100
75
00
75
100
25
100
00
00
00
00
00
75
85
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00
75
25
00
00
25
25

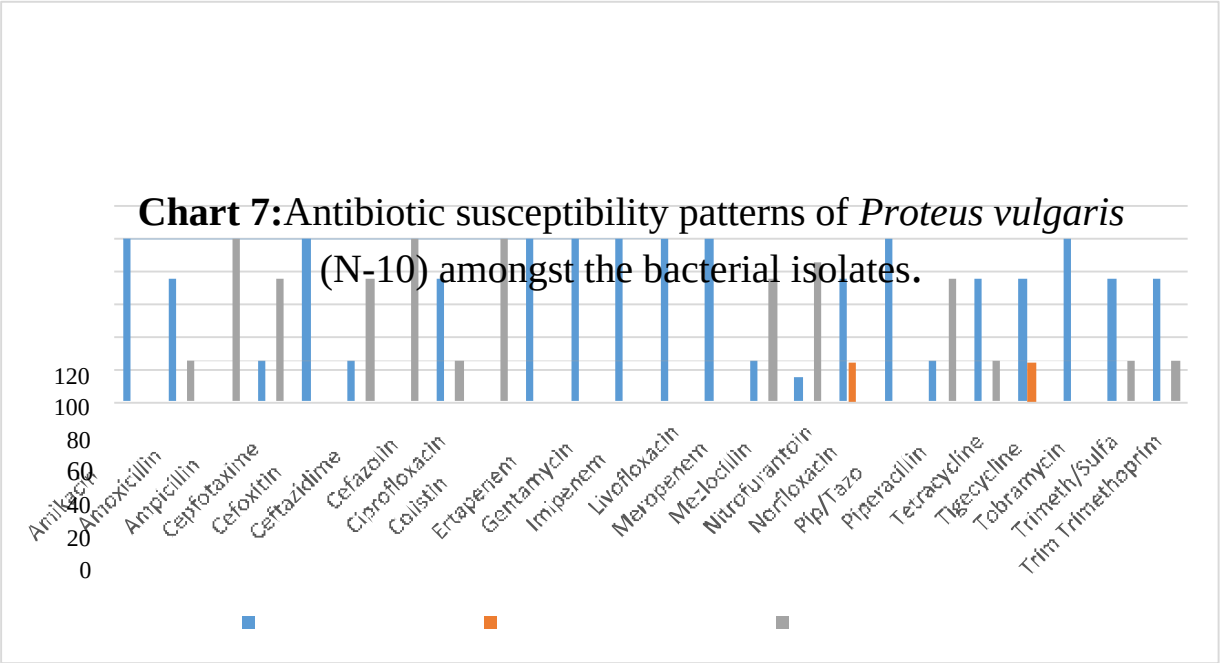

Chart 7: Antibiotic susceptibility patterns of *Proteus vulgaris* (N-10) amongst the bacterial isolates.

5.2.6 Antibiotic susceptibility patterns of *Enterococcus faecalis* (N-152) amongst the bacterial isolates.

Table 9: Antibiotic susceptibility patterns of *Enterococcus faecalis* (N-152) amongst the bacterial isolates.

ANTIBIOTIC	SENSITIVE %
Ampicillin	93
Ciprofloxacin	24
Daptomycin	98
Levofloxacin	32
Linezolid	98
Nitrofurantoin	60
Norfloxacin	25
Penicillin	93
Rifampin	53
Tetracycline	n
Synercid	
Vancomycin	

14
<1
97

INTERMEDIA RESISTANT	
TE	%
%	07
00	68
09	02
00	66
01	01
>1	30
10	68
07	07
00	22
25	85
01	91
09	03
<1	

41

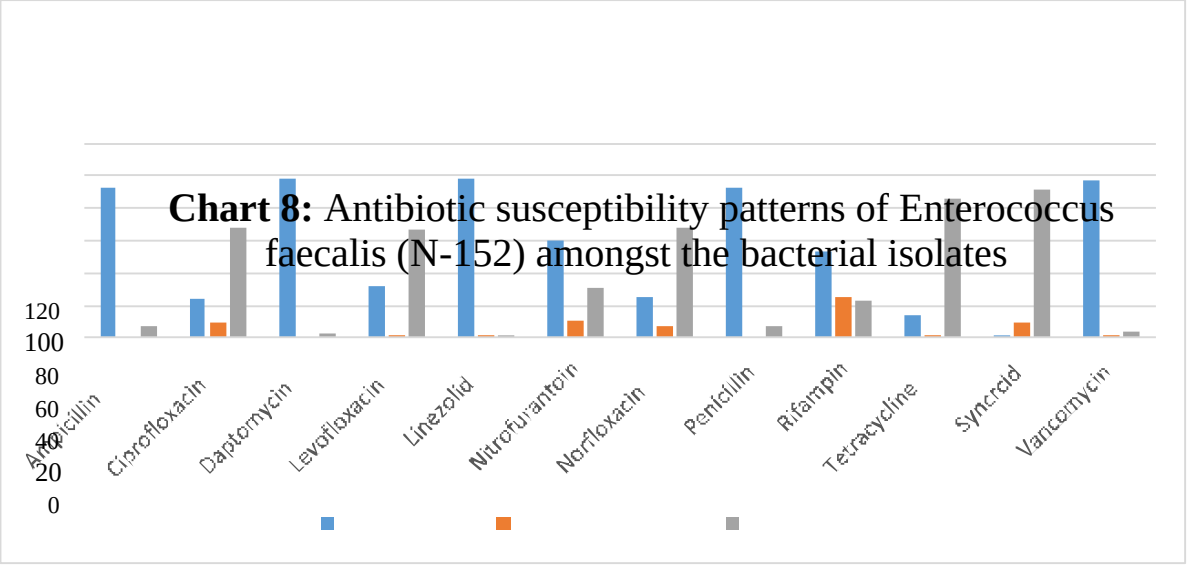

Chart 8: Antibiotic susceptibility patterns of Enterococcus faecalis (N-152) amongst the bacterial isolates

5.2.7: Antibiotic susceptibility patterns of *Enterococcus faecium* (N-26) amongst the bacterial isolates.

Table 10: Antibiotic susceptibility patterns of *Enterococcus faecium* (N-26) amongst the bacterial isolates

ANTIBIOTIC	SENSITIVE %	INTERMEDIATE %	RESISTANT %
Amikacin	15		00
Ampicillin	65		10
Ciprofloxacin	08		08
Daptomycin	92		00
Levofloxacin	12	42	08
Linezolid	100		00
Nitrofurantoin	60		20
Norfloxacin	04		08
Penicillin	15		00
Rifampin	15		08
Tetracycline	50		00
Vancomycin	92		00

PROSPECTIVE SUDY ON URINARY TRACT INFECTION ISOLATES FROM URINE SAMPLE AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN

85
25
85
08
81
00
20
88
85
77
50
08

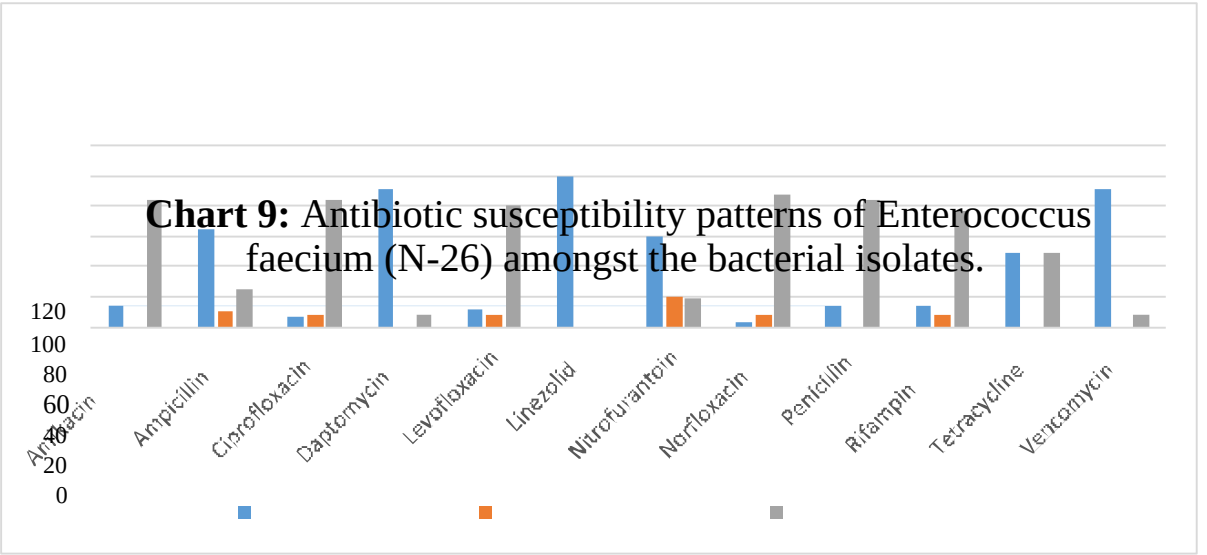

Chart 9: Antibiotic susceptibility patterns of Enterococcus faecium (N-26) amongst the bacterial isolates.

5.2.8 Antibiotic susceptibility patterns of *Enterobacter* (N-34) amongst the bacterial isolates.

Table 11: Antibiotic susceptibility patterns of *Enterobacter* (N-34) amongst the bacterial isolates.

ANTIBIOTIC			SENSITIVE %
Amikacin	**Ceftazidime**	**Ciprofloxacin**	100
Amoxicillin			60
Ampicillin			20
Cefepime	**Cefazolin**		70
Cepfotaxime			60
Cefoxitin			80

60
60
60

INTERMEDIATE	RESISTANT
%	%
00	00
20	20
40	40
10	20
00	40
00	20
00	40
00	40
00	40
00	

Colistin	100	00	00
Ertapenem	73	00	27
Gentamycin	67	00	33
Imipenem	87	00	13
Levofloxacin	80	00	20
Meropenem	100	00	00
Mezlocillin	60	00	40
Nitrofurantoin	70	10	20
Norfloxacin	60	20	20
Pip/Tazo	80	20	00
Piperacillin	60	20	20
Tetracycline	80	00	20
Tigecycline	100	00	00
Tobramycin	60	20	20
Trimeth/Sulf	60	00	40

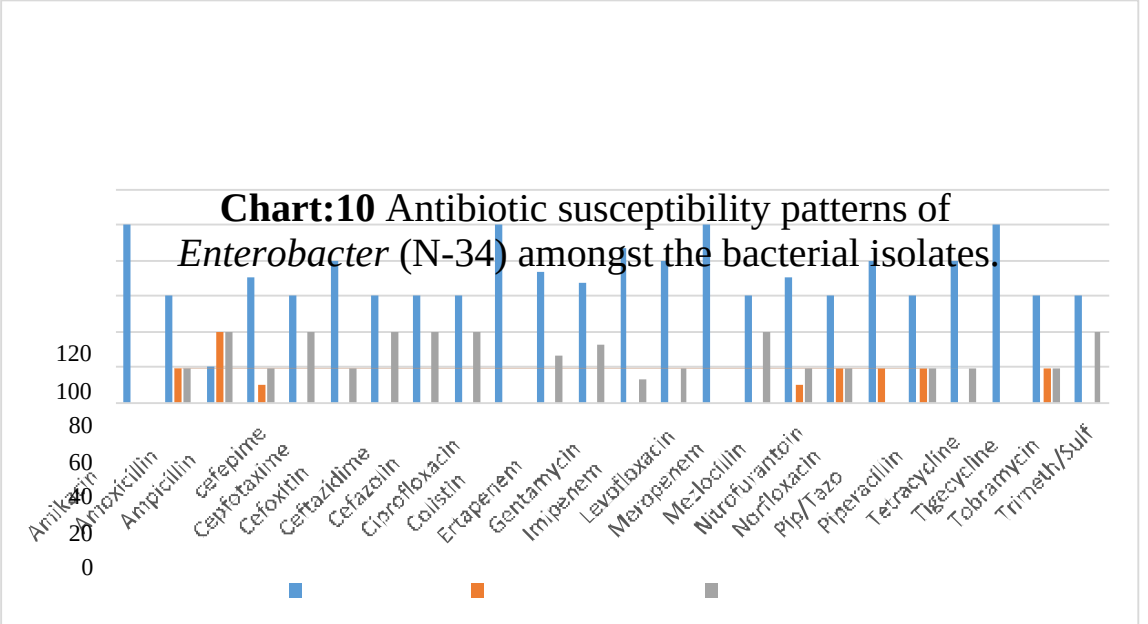

Chart:10 Antibiotic susceptibility patterns of *Enterobacter* (N-34) amongst the bacterial isolates.

SENSITIVE % INTERMEDIATE % RESISTANT %

PROSPECTIVE SUDY ON URINARY TRACT INFECTION ISOLATES FROM URINE SAMPLE AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN

5.2.9 Antibiotic susceptibility patterns of Acinetobacter (N-28) amongst the bacterial isolates.

Table 12: Antibiotic susceptibility patterns of *Acinetobacter* (N-28) amongst the bacterial isolates.

ANTIBIOTIC	SENSITIVE %	INTERMEDIATE %	RESISTANT %
Amikacin	93	00	03
Cefepime	98	00	02
Cepfotaxime	90	00	10
Ceftazidime	100	00	00
Ciprofloxacin	75	25	00
Colistin	73	00	27
Gentamycin	93	00	07
Levofloxacin	100	00	00
Mezlocillin	100	00	00
Piperacillin	95	00	05
Tetracycline	87	13	00
Tobramycin	75	15	10
Trimeth/Sulfa	80	00	20

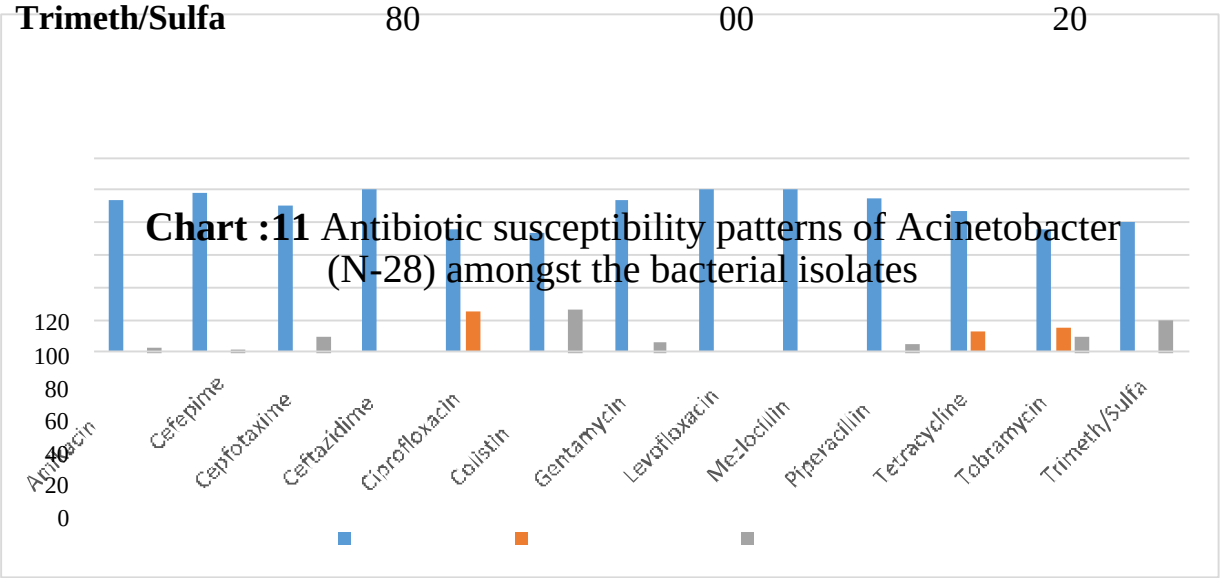

Chart :11 Antibiotic susceptibility patterns of Acinetobacter (N-28) amongst the bacterial isolates

5.2.10 Antibiotic susceptibility patterns of *Staphylococcus aureus* (N-11) amongst the bacterial isolates

Table 13: Antibiotic susceptibility patterns of *Staphylococcus aureus* (N-11) amongst the bacterial isolates.

ANTIBIOTIC	SENSITIVE %	INTERMEDIATE %	RESISTANT %
Amoxicillin	73	00	27
Ampicillin	9	00	91
Cefuroxime	73	00	27
Ciprofloxacin	09	09	82
Daptomycin	100	00	00
Gentamycin	64	00	36
Levofloxacin	09	08	73
Linezolid	100	00	00
Nitrofurantoin	100	00	00
Norfloxacin	09	00	91
Oxacillin	73	00	27
Penicillin	09	00	91
Rifampin	100	00	00
Synercid	100	00	00
Tetracycline	82	00	18
Teicoplanin	100	00	00
Trimeth/Sulfa	91	00	09
Vancomycin	91	09	00

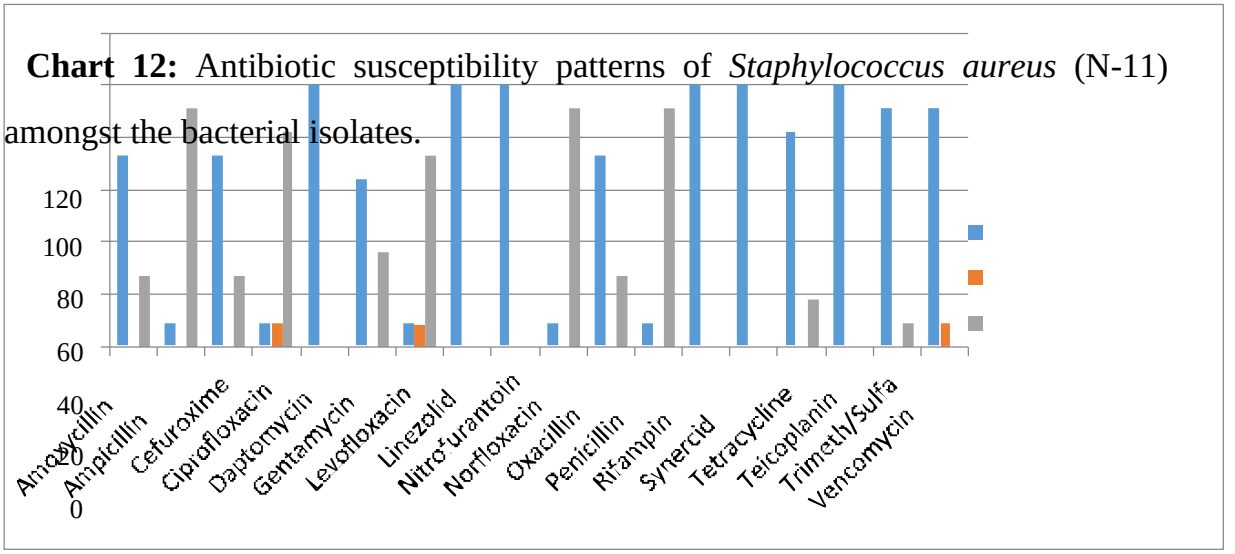

Chart 12: Antibiotic susceptibility patterns of *Staphylococcus aureus* (N-11) amongst the bacterial isolates.

PROSPECTIVE SUDY ON URINARY TRACT INFECTION ISOLATES FROM URINE SAMPLE AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN

SENSITIVE %

INTERMEDIATE %

RESISTANT %

5.2.11 Antibiotic susceptibility patterns *Streptococcus saprophyticus* (n-16) amongst the bacterial isolates

Table14: Antibiotic susceptibility patterns *Streptococcus saprophyticus* (n-16) amongst the bacterial isolates.

ANTIBIOTIC	**SENSITIVE %**	**INTERMEDIATE%**	
Amoxicillin	31	00	
Ampicillin	19	00	
Cefuroxime	31	00	
Cefoxitin	55	15	
Ciprofloxacin	100	00	
Daptomycin	100	00	
Gentamycin	100	00	
Levofloxacin	100	00	
Linezolid	100	00	
Netlimicin	100	00	
Nitrofurantoin	100	00	
Norfloxacin	100	00	
Oxacillin	31	00	
Penicillin	81	00	
Rifampin	100	00	
Synercid	94	06	
Tetracycline	94	00	
Teicoplanin	100	00	
Trimeth/Sulfa	80	00	
Vancomycin	100	00	

RESISTANT

%

69
81
69
30
00
00
00
00
00
00
00
00
69
19
00
00
06
00
20
00

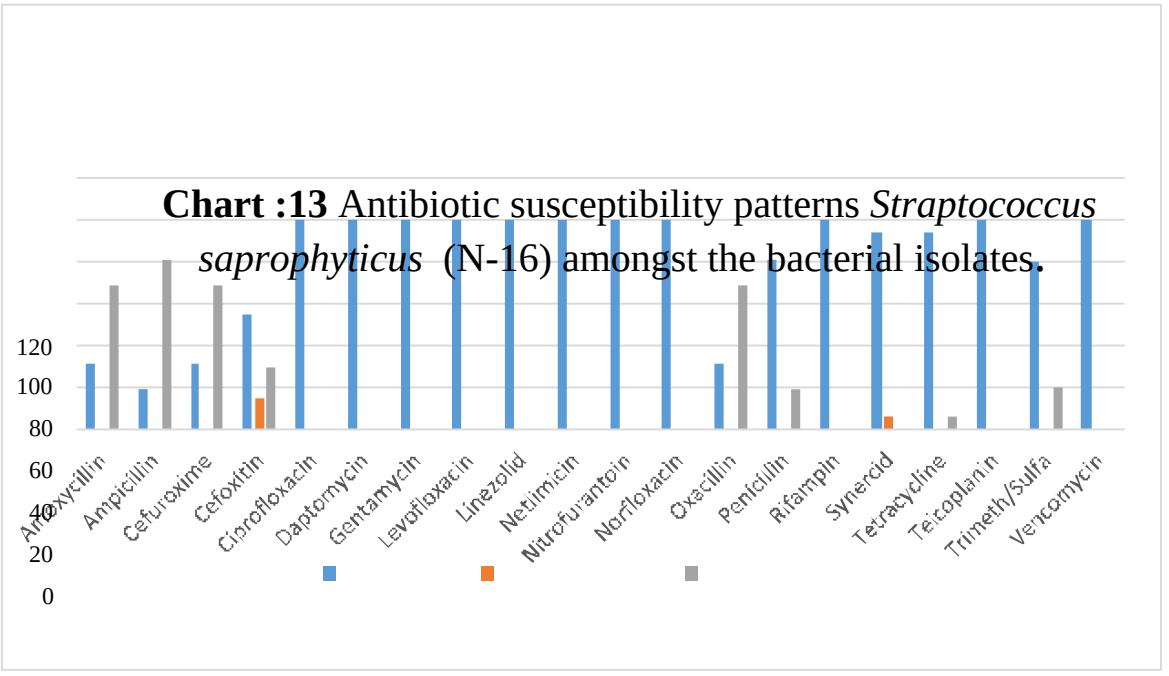

Chart :13 Antibiotic susceptibility patterns *Straptococcus saprophyticus* (N-16) amongst the bacterial isolates.

5.2.12 Antibiotic susceptibility patterns of *Staphylococcus haemolyticus* (n-23) amongst the bacterial isolates.

Table 15: Antibiotic susceptibility patterns of *Staphylococcus haemolyticus* (n-23) amongst the bacterial isolates.

		O	**a**	
		x	**m**	
ANTIBIOTIC		**a**	**p**	**SENSITIVE**
		c	**i**	**%**
Amoxicillin		**i**	**n**	13
Cefuroxime		**l**	**S**	13
Ciprofloxacin		**l**	**y**	17
Daptomycin		**i**	**n**	96
Gentamycin		**n**	**e**	26
Levofloxacin			**r**	22
Linezolid		**R**	**c**	96
Netlimicin		**i**	**i**	87
Norfloxacin		**f**	**d**	17

13
83
91

INRESISTANT

T	**%**
E	87
R	87
M	78
E	04
DI	70
A	78
T	04
E	09
	82
	%
	87
	0
	09
	0
	04
	0
	0
	0
	4
	0
	0
	0
	4
	0
	0
	0
	0
	0
	4
	0
	0
	0
	0
	0
	9
	0
	4

48

Tetracycline	43	00	57
Teicoplanin	94	04	00
Trimeth/Sulfa	52	00	48
Vencomycin	100	00	00

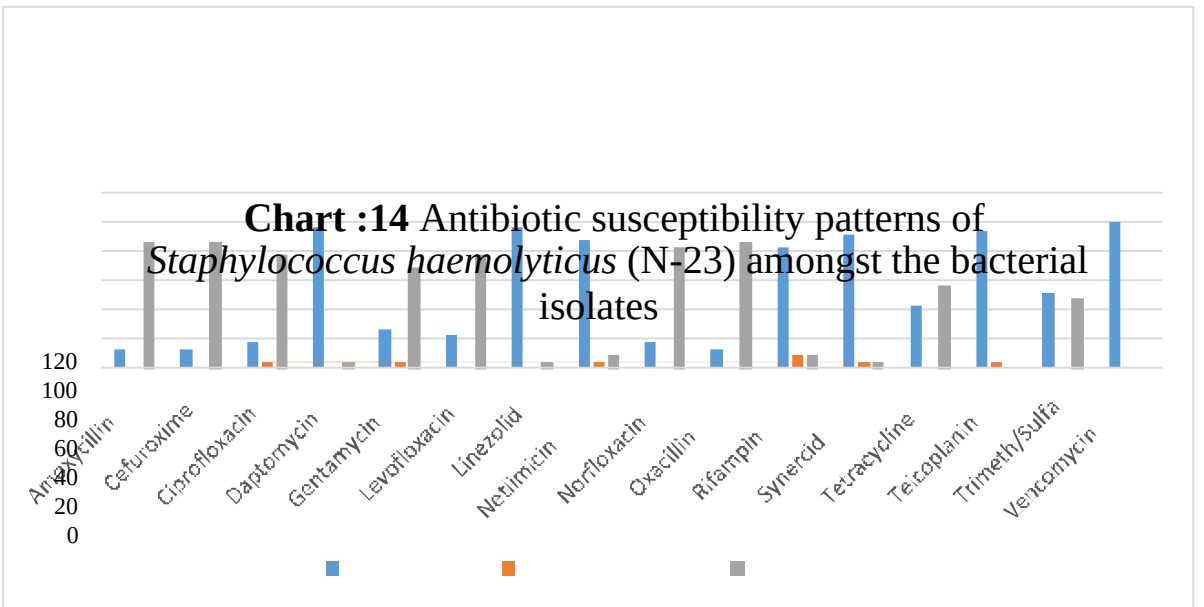

Chart :14 Antibiotic susceptibility patterns of *Staphylococcus haemolyticus* (N-23) amongst the bacterial isolates

SENSITIVE % INTERMEDIATE % RESISTANT %

PROSPECTIVE SUDY ON URINARY TRACT INFECTION ISOLATES FROM URINE SAMPLE AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN

CHAPTER 6

DISCUSSION

Urinary tract infection is one of the commonest bacterial infections. The enterobacteriaceae are the most frequent pathogen detected causing 80%of urinary tract infection .The present study was conduct to achieve resistance summary of clinical isolates from SRL Diagnostic Ltd against commonly prescribed antibiotic.

A total of 4362 urine specimens were received in SRL Diagnostic during January 2015 toApril 2015 and these were processed in the laboratory.Significant bacteria (cultures with >105 colony forming units (cfu) of bacteria/ml of urine) was found in 1244/4362(28.51%)of the urine specimens. Gram-negative bacteria were more prevalent (68.46%) than Gram-positive bacteria (31.54%).

Identification was done the basis of morphological,biochemical,and phenotypic characteristics.

Of the 1244 isolates,the most commonly isolated bacteria were *Escherichia coli* 730(58.68%), *Klebsiella pneumoniae* 126 (10.15%), *Enterococcus faecalis is* 152(12.21%), *Pseudomonas aeruginosa* 52(4.18%), *Proteus mirabilis*36(2.89%), *Proteus vulgaris* 10(0.80%), *Enterococcus faecium* 26(2.10%), *Enterobacter* 34(2.73%), and *Acinetobacter* is 28(2,25%) coagulase negative staphylococcus

39(3.13%) and *Staphylococcus aureus* 11 (0.88%) respectively.

This study showsthe distribution of microbial species isolated from patients with urinary tract infection at the SRL diagnostic laboratory and their susceptibility pattern to antimicrobial agents. Also, we described relationship between sex and isolated bacterial agent of urinary tract infection. Microbial infection the urinary tract infection is one of the most common infectious diseases worldwide. Approximately 1 in 3 women will require antimicrobial treatment a urinary tract infection before age 24, and 40% to 50% of women will suffer from urinary tract

infection during their lifetime.The sex distribution of patients in our study is consistent with those in other studies showing a statistically majority of females

(66.80% of the positive cultures). It is expected that the short urethra in girls predisposes them to ascending infection. Moreover, management of micturition in women is important. Management mistakes made by women include cleaning perineum forward from the anus to the vulva that can cause urinary tract infection. Sexual activity has been reported to influence higher prevalence of urinary tract infection in females. Considering the fact that most of infecting organisms are commensals of the perianal and vaginal regions, emphasis on personal hygiene especially in females may be important in reducing the incidence of urinary tract infection. Males are less prone to urinary tract infection possibly because of their longer urethra and the presence of antimicrobial substances in prostatic fluid. Our study indicate that *E. coli* is still the most common cause of urinary tract infection in India.

In addition ,coagulase negative *Staphylococcus spp* .was the most common cause urinary tract infection among Gram positive bacteria.Recent studies have revealed the importance of *coagulase negative Staphylococcus spp.* in urinary tract infections.

Our results confirmed that these pathogens play an important role in urinary tract infection as 10.15% cases in our study were affected by these bacteria *Klebsiella pneumoniae* which is the second species that caused urinary tract infection. Our study, *E. coli* demonstrated a very high microbial resistance to antibiotics. The analyzed results of antibiotic susceptibility test showed that *E. coli* was highly resistant to Ampicillin (79%), Mezlocillin (77%), Ciprofloxacin (71%), Levofloxacin (68%), Tetracycline (63%) and Norfloxacin (71%).

On the other hand, very low resistance was detected to antibiotics such as Cepfotaxime (56%),Tobramycin(44%)Gentamycin (34%),Nitrofurantoin (30%), and Amoxicillin (16%).

In the present study,*Coagulase negative Staphylococcus* showed 89%resistance

to Amoxicillin, Cefuroxime, and Oxacillin, respectively.

Results showed that the best activity against these bacteria was achieved by Tigecycline, Meropenem, Ertapenem, and Amikacin**.** *Klebsiella pneumoniae* exhibited varying antibiotic resistance and showed higher resistance to Ampicillin (98%) and Cefepime (80%). Also *Klebsiella pneumoniae* showed is the lowest resistance to Ertapenem, Tetracycline, and Amikacin (27.28%). Unpredictably, in the present study the susceptibility is *Enterobacteriaceae* to gentamicin is low and India reported high sensitivity to this antibiotic which can be due to uncontrolled administration of the drug.

Moreover,considering susceptibility pattern of antibiotic agents for urinary tract infection therapy, particularly caused by the *E.coli* as the most significant uropathogens, it seems that the drugs like ampicillin and should be prescribed cautiously especially against to those, show suitable respond to other antibiotics such as ciprofloxacin and nitrofurantoin.

CHAPTER 7

CONCLUSION

It studied that higher prevalence of urinary tract infection was seen in females.The females urinary tract infection is seen in patients between 21- 40 years age group and in males it was seen in older age group between 60-80 years.Gram negative organisms were the most commonly isolated organisms in urinary tract infection among which *E. coli* was the most frequent causative agent. Urinary pathogens showed resistance to commonly used antibiotics like Ampicillin,Norfloxacin and Ciprofloxacin. The susceptibility and resistance patterns of urinary pathogens should be considred before starting empirical treatment for urinary tract infection. Development of resistance to commonly used antibiotic fort reating urinary tract infection alert us against indiscriminate usages of antibiotics to prevent development of resistance against an antibiotic.

CHAPTER 8
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APPENDIX

Appendix I

A. LIST OF MATERIALS

1. Equipment

- Autoclave.
- BacTec 9060 & 9120.
- Micros scan Walkaway.
- Bunsen burner.
- Biosafety cabinet II.
- Incubator.
- Microscope.
- Refrigerator.
- Weighing machine.
- Face mask.
- Gloves

2. Glass wares

- Petri plates.
- Tubes.
- Slides.
- Glass rods.
- Conical flask.

3. Others

- Cottons.
- Scissors.
- Forceps.
- Inoculating loops.
- Racks.

APPENDIX-II

B. COMPOSITION AND PREPARATION OF GRAM'S STAIN REAGENT.

1. Crystal violet reagent

Composition	**gram/lit**
Solution A	
Crystal violet	2.00 gm.
Ethanol	20.00 ml.
Solution B	
Ammonium oxalate	0.80 gm.
Distilled water	8.00 ml.

Preparation

a) Mix solution A to solution B and store 24 hours to dissolve the stain completely.

b) Filter the solution through filter paper after 24 hours and then use.

c) Level the reagent bottle with preparation data and reagent name.

2. Gram's Iodine Solution

Composition	**gram/lit**
Potassium iodide	2.00 gm.
Iodine	1.00 gm.
Distilled water	100.00 ml.

Preparation

1. Dissolve the potassium iodide in distilled water and then add iodine.

2. Store in a tightly stopper bottle with preparation date and reagent name.

3. Acetone-alcohol decolorizer

Composition	Volume (ml)
Acetone	250 ml
Ethanol (absolute)	250 ml

Preparation

a) Mix the 250 ml of acetone into a 250 ethanol.

b) Store in a tightly stopper bottle with preparation date and reagent name.

4. Counterstain solution

Composition	gram/lit
Safranine	0.34 gm.
Absolute alcohol	10.0 ml.
Distilled water	90.0 ml.

Preparation

a) Dissolve the safranine in 0.34 gm. into 10 ml absolute alcohol and then add 90 ml distilled water.

b) Filter the solution through filter paper and then use.

c) Label the reagent bottle with preparation date and reagent name.

Appendix-III

C. COMPOSITION AND PREPARATION OF DIFFERENT TYPES OF CULTURE MEDIA

1. CLED Agar (Cysteine-Lactose-Electrolyte-Deficient Agar) (Hi-Media)

Composition	gram/lit
Pancreatic digest of Gelatin	4.0 gm.
Pancreatic digest of casein	4.0 gm.
Beef extract	3.0 gm.
Lactose	10.0 gm.
L-cystine	0.128 gm.
Bromthymol blue	0.02 gm.
Agar	15.0 gm.

Preparation

As directed by manufacturing company 40 gram of medium is dissolved in 1000 ml of distilled water and autoclave it at 15 lbs. pressure (121 c) for 15 minutes.

2. MacConkey agar (Hi-Media)

Composition		
Pancreatic digest of animal	N	red
Peptone	e	Final Ph
Lactose	u	
Sodium chloride	t	
Bile salt	r	
Agar	a	
	l	

	gram/lit
	17.0 gm.
	3.0 gm.
	10.0 gm.
	5.0 gm.
	1.0 gm.
60	
	15.0 gm.
	0.03 gm.
	6.9-7.3 gm.

Preparation

As directed by manufacturing company 51.53gram of medium is dissolved in 1000ml of distilled water and autoclave it at15 lbs.pressure(1210C)for15min.