RESISTANCE PROFILE OF BACTERIAL PATHOGENS FROM URINARY TRACT INFECTIONS

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CERTIFICATE

This is to certify that the work titled **"Resistance profile of Bacterial pathogens from Urinary tract infections"** has been submitted by **Ms. Shivani Saxena** in partial fulfillment for the award of degree of **B.Tech Biotechnology** from Jaypee University of Information Technology, Solan has been carried out under our supervision. This work has not been submitted partially or wholly to any other university or Institute for the award of this or any other degree or diploma.

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ABSTRACT

UTIs (Urinary tract infection) are most common problem found in people most commonly in women. UTIs may cause chronic kidney infections, leading to permanent damage, including kidney scars, poor kidney function. UTIs are caused by microbes such as bacteria. UTIs have different names referring to the different parts of urinary tract.

- Bladder infection = Cystisis.
- Urethra infection = Urethritis.
- Kidney infection = Pyelonephritis

URINARY TRACT INFECTIONS are second most common infections after respiratory tract infections. UTI's can infect any part of the urinary tract. Infection is generally treated by short course of antibiotics. With increased use of antibiotics UTI has become difficult to treat because of appearance of pathogens with increasing resistance towards antibiotics. In present study urine samples were collected from various laboratories located in Himachal Pradesh region. The samples were further processed and the pathogens were isolated and further characterized. After characterization susceptibility tests were performed for various antibiotics. Results were obtained for various susceptible and resistant strains using Antibiotic Susceptibility Testing. In total 32 samples of UTI were collected and processed. The findings to this study showed that females were at greater risk to Urinary Tract Infections and also *E. coli* was the most common causative agent with a prevalence of causing around 59% infections.

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Chapter 1

INTRODUCTION

Urinary tract infections are most common and frequent bacterial infection .It affects people of all age groups. Urinary tract infection is a condition where a microbial pathogen is present in any part of the urinary tract. Infection can be symptomatic and asymptomatic. UTI'S that occur in normal genitourinary tract without prior instrumentation are called "uncomplicated" infections whereas "complicated" infections are those which are diagnosed in genitourinary tract having structural or functional abnormalities including instrumentation such as urethral catheters. Urinary tract infection is infection of kidneys, ureters, bladder, and urethra. It is seen that further up the urinary tract infection is located, more serious it is. The upper urinary tract infection includes infection of kidneys and ureters. Generally in upper urinary tract infection kidney is affected which is also called pyelonephritis. Symptoms of upper urinary tract infection are most common and include symptoms like fever, chills, nausea and vomiting. Lower urinary tract infection generally affects bladder and urethra. Symptoms of lower tract infection include dysuria, frequent urination, urinary urgency, abdominal pain, foul smelling urine. The infection generally starts at the opening of urethra and then moves upwards in urinary tract. People who are at increased risk of urinary tract infection generally include (i) People with kidney stones. (ii) People with suppressed immune system (iii) Women using diaphragm for birth control (iv) Men with enlarged prostate. Urinary tract infection (UTI) is one of the commonest infections encountered 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. Gram positive and Gram negative both can cause UTI'S but it is seen that Gram negative bacteria are most common. Bacteria most commonly causing UTI are *E. coli, Staphylococcus spp, Klebsiella spp, Enterococci ssp,* and *Proteus mirabilis*. *E. coli* is mostly responsible for bladder infection E. coli is generally harmless microorganism originating in intestine but if it spreads to the vaginal opening, it may invade and colonize the bladder causing infection. Staphylococcus spp is main cause of UTI'S in younger women. Other organisms like *Proteus mirabilis, Klebsiella spp, Enterococci spp* mostly infect older women. Females are found to be

more susceptible to UTI infection because of female anatomy (1). UTI'S are generally diagnosed on the basis of symptoms that appear. In complicated cases, diagnosis is confirmed by urinalysis or looking for presence of urinary nitrites, white blood cells. UTI'S are generally treated by course of antibiotics. Sometimes the condition may be asymptomatic, in such a case organism may present but no symptoms appear so no antibiotic should be prescribed in such cases. Urinary tract infections are treated by short course of antibiotics and resistance towards these antibiotics is increasing. Antibiotic resistance is most serious threat as resistant strains are increasing (10). Antimicrobial resistance can be a result of mutation or it can be due to transfer of genes causing resistance. Bacteria that are resistant to multiple drugs are termed as MDR (multiple drug resistant). Common type of multi drug resistant bacteria include methicillin-resistant *Staphylococcus aureus* (MRSA), Extented-spectrum producing β-lactamase (ESBL),Multidrug resistant gram negative rods, vancomycin- resistant *Enterococci spp.* Antibiotic resistance is a serious threat and causes of deaths worldwide (2). There are number of antibiotics used for treatment of UTI'S and choice depends in severity of infection and primary or recurrent infection. Development of resistant strains is a common problem and in case of uropathogens rate of resistance is high. Antibiotics generally used for treatment of UTI'S are amoxicillin, cephalosporins, tetracyclins, and fluoroquinolones. Knowledge of etiological agents of UTIs and their sensitivities to available drugs is of immense value to the rational selection and use of antimicrobial agents and to the development of appropriate prescribing policies. It is found that distribution of urinary pathogens and their antibiotic susceptibility varies regionally (3). This project is focused on study of the pathogens causing Urinary Tract Infection and also to study the susceptibility pattern of various antimicrobial agents used. In this study 42 urine samples were collected from various laboratories situated in Himachal region. Out of these 32 were positive for the infection. Further causative agent was isolated and characterized and were tested for their susceptibility patterns towards different antibiotics. In this study it was found that *E. coli* was the main causative agent (59%) followed by *Shigella spp (6%), Klebsiella spp (6%)* and *Proteus spp (3%).* Among antibiotics tested Amikacin was found to be most susceptible and gentamycin most resistant. Females were found to be at greater risk to Urinary Tract Infections.

CHAPTER 2

REVIEW OF LITERATURE

(2.1) Urinary Tract Infections

Most common bacterial infection, According to National Ambulatory Medical Care Survey and National Hospital Ambulatory Medical Care Survey, UTI accounted for 7 million office visits and 1 million emergency visits resulting in 100,000 hospitalizations. Women are at greater risk, nearly 1 in 3 women have had at least 1 episode of UTI (10). UTI's are generally classified by site of infection. Urinary tract infections can be complicated and uncomplicated. There are medical and financial implications associated with UTIs. In the non obstructed, non pregnant females, acute uncomplicated UTI is believed to be a benign illness with no long-term medical consequences. Studies concluded UTI elevates the risk of pyelonephritis, premature delivery, and fetal mortality among pregnant women, and is associated with impaired renal function and end-stage renal disease among pediatric patients. Financially, the estimated annual cost of community-acquired UTI is significant, at approximately $1.6 billion. Catheter-associated UTI is the most common nosocomial infection, accounting for >1 million cases in hospitals and nursing homes. Urinary tract is divided into upper urinary tract composed of kidneys, renal pelvis ureters and a lower urinary tract composed of urethra and bladder. Infection can occur in upper tract, lower tract or both. Bacteriuria is term used for presence of bacteria in urine. Pyuria is used when WBC'S are present in the urine (4).

(2.1.1) MANIFESTATIONS

Cystitis - It has been used to describe the syndrome involving dysuria, frequency, urgency. However these symptoms may be related to lower urinary tract inflammation caused by urethritis.

Acute pyelonephritis- It is a clinical syndrome characterized by abdominal pain, tenderness or both, and fever. It is often associated with dysuria, urgency and frequency. These symptoms can also occur in the absence of infection (renal infarction) (14).

Chronic pyelonephritis - This may rise from either infection or metabolic disorders. It refers to pathologic changes in the kidney caused by infection. In chronic pyelonephritis, one or both kidneys contain gross scars, but even when involvement is bilateral, the kidneys are not equally damaged. This uneven scarring is useful in differentiating chronic pyelonephritis from diseases that cause symmetrical contracted kidneys- for e.g.: chronic glomerulonephritis. In severe pyelonephritis, the kidney is somewhat enlarged and discrete, yellowish, raised abscesses are apparent on the surface (5).

Uncomplicated UTI – It refers to infection in a structurally and neurologically normal urinary tract (5).

Complicated UTI – It refers to infection in a urinary tract with functional or structural abnormalities, including indwelling catheters. Infection in men, pregnant women, children, and patients who are hospitalized or in health care associated settings may be considered complicated. In the patient with complicated infection, infecting microorganisms are more likely to be resistant to antimicrobial agents (5).

Recurrences of urinary tract infection - It is may be due to relapses or re-infections. Relapses of bacteriuria refer to a recurrence of bacteriuria with the same infecting microorganism that was present before therapy was started. It is caused by the persistence of the organism in the urinary tract. (14).

(2.2)PATHOGENICITY OF UTI'S

There are basically three possible routes whereby bacteria can invade and spread in the urinary tract**.** These are ascending route, haematogenous and lymphatic pathways (6).

(2.2).A Ascending Route

Some bacteria originate from bowel reservoir infects the perineal area and ascends from urethra towards the upper urinary tract. These infections are called infections by ascending route. The fact that women are at greater risk of UTI supports importance of ascending route of infection. Female urethra is short and in proximity to warm, moist vulvar and perineal areas making, It prone to infections.

(2.2).B HAEMATOGENOUS ROUTE

Infection is uncommon in normal individuals. Infection of the renal parenchyma by bloodborne organisms clearly occurs in humans. The kidney is frequently the site of abscesses in patients with *Staphylococcus aureus*, bacteremia or endocarditis or both.

(2.2).C LYMPHATIC ROUTE

On some rare occasions bacteria from adjacent organs may penetrate the urinary tract via the lymphatics. Conditions associated with the lymphatic route are retroperitoneal abscesses and severe bowel infections.

(2.3) EPIDEMIOLOGY (7)

Urinary tract infections are the most frequent bacterial infection in women. They occur most frequently between the ages of 16 and 35 years, with 10% of women getting an infection yearly and 60% having an infection at some point in their lives. Recurrences are common, with nearly half people getting a second infection within a year. Urinary tract infections occur four times more frequently in females than males. Pyelonephritis occurs between 20–30 times less frequently. They are the most common cause of Hospital Acquired Infections accounting for approximately 40%.Rates of asymptomatic bacteria in the urine increase with age from two to seven percent in women of child bearing age to as high as 50% in elderly women in care homes. Rates of asymptomatic bacteria in the urine among men over 75 are between 7-10%. Asymptomatic bacteria in the urine occur in 2% to 10% of pregnancies.

Urinary tract infections may affect 10% of people during childhood. Among children urinary tract infections are the most common in uncircumcised males less than three months of age, followed by females less than one year. Estimates of frequency among children however vary widely. In a group of children with a fever, ranging in age between birth and two years, two to 20% were diagnosed with a UTI (15).

(2.4) URINARY PATHOGENS

Studies reveal that *E. coli* accounts for 85% of community acquired infections and 50% of Hospital Acquired Infections. In the *E. coli* species there are number of subgroups isolated from patients. Gram negative bacteria such as *Klebsiella spp* and *Proteus spp* and Gram positive *Staphylococcus spp* and *Enterococcus spp* are responsible for remaining community based infections. The remaining Hospital acquired infections are caused by *Klebsiella spp, Enterobacter spp, Citrobacter spp, Serratia spp, Pseudomonas aeruginosa, Providencia spp, E. faecalis, or S. epidermidis.* Patient's age is also a factor and influence type of infection causing organism. In case of uncomplicated UTI's, *E. coli* again is the major causative agent. Previously patients with uncomplicated UTI'S were sensitive to trimethoprim-sulfamethoxazole combination but recent studies have demonstrated increasing antibiotic resistance of uropathogens (10). One study investigated antimicrobial resistance among 4000 female patients with UTI isolates over a 5 year experimental time period. Results from this study demonstrated an increase in antimicrobial (*E. coli*) resistance from 9% to 18% in patients treated with trimethoprim-sulfamethoxazole. In addition, resistance to cephalothin (first generation cephalosporin) increased from 20% to 28% and resistance to ampicillin increased from 26% to 34%. Notably, resistance to nitrofrantoin and ciprofloxacin remained <1% after the 5 year period. This increase in bacterial resistance has been attributed to recent administration of trimethoprim-sulfamethoxazole, diabetes mellitus, recent hospitalization and recent administration of any other antibiotic. Based on these studies antimicrobial treatment with either a fluoroquinolone, nitrofurantoin or fosfomycin are currently recommended for uncomplicated UTIs. Importantly, clinicians should also be aware of the antimicrobial spectrum for these agents prior to administration as nitrofurantoin is not effective for treating uncomplicated pyelonephritis but highly effective for treating acute cystitis. Recurrent uncomplicated UTIs occur after 3 to 6 months in 25% to 35% of patients after their initial UTI (11). In complicated UTIs less virulent uropathogens (that rarely cause disease in a normal urinary tract) can cause significant damage. Studies have demonstrated associations between Group B *Streptococcal bacteraemia, Candida spp* and *Enterococci spp* with complicated UTIs in the elderly population to an abnormal urinary tract.. *Candida spp* and coagulase-negative *Staphylococci spp* are associated with complicated UTIs after instrumentation of the pediatric urinary tract. Common uropathogens causing complicated UTIs among patients with spinal cord injuries and indwelling catheters include *E. coli, Pseudomonas spp* and *Proteus mirabilis* (12).

(2.5)ANTIBIOTIC RESISTANCE

Increasing resistance against commonly used antibiotics has become a serious problem worldwide. Resistance can be intrinsic or acquired. Intrinsic resistance refers to bacterial ability to resist activity of a particular antimicrobial agent through its inherent structural or functional characteristics, which allow tolerance of a particular drug or antimicrobial class. This can also be called "insensitivity". Acquired resistance to antibiotics involves different mechanisms of drug resistance (17).

(2.5).A Mechanisms of resistance (8)

There are number of resistance mechanisms responsible for acquired bacterial resistance. Bacteria use these mechanisms for protection against antimicrobial agents.

i. Any enzyme that inactivates the antimicrobial agent.
ii. Presence of another enzyme for the enzyme that is inhibited by the antimicrobial agent.
iii. Any mutation in the target microorganism that reduces the binding of the antimicrobial agent
iv. Transcriptional modifications of the antimicrobial agent's target, which reduces binding of the antimicrobial agent.
v. Reduced uptake of the antimicrobial agent.
vi. Overproduction of the target of the antimicrobial agent
vii. Active efflux pump of the antimicrobial agent

(2.5).B TARGETED FUNCTIONS OF ANTIBIOTICS

Antibiotics target bacterial essential functions like protein synthesis, cell wall synthesis and genomic material of organism. Tetracycline inhibits protein synthesis by binding to the 30S ribosomal subunit, it prevents translation and the bacteria are not able to propagate.

Aminoglycosides bind to the ribosomal sites and thus interfere with protein synthesis. For instance, Chloramphenicol binds to the 50S ribosomal subunit and inhibits the peptidyltransferase step in protein synthesis (16).

Antibiotics prevent the cell wall synthesis by blocking cross-linking reactions of peptidoglycan necessary for the stabilization of the cell wall and thus causing the cells to rupture. The most common antibiotics that affect cell wall synthesis are the beta-lactams such as penicillin and cephalosporins. Vancomycin is a glycopeptide that also affects cell wall synthesis and is gaining increased attention. Some antibiotics interact with the genomic material of the organism, the DNA itself. Bacterial topoisomerases named DNA gyrase or bacterial topoisomerase II and topoisomerase IV are inhibited by quinolone antibiotics. These essential bacterial enzymes change the topology of double-stranded DNA (dsDNA) within the cell (17).

(2.6) MULTI DRUG RESISTANCE

This is an intense health problem occurring. Bacteria have developed the ability to share resistance to almost every antibiotic that has been developed by quite unexpected mechanisms. Certain efflux systems have been identified responsible for resistances to tetracyclines, chloramphenicol and/or florfenicol in Gram-positive and Gram-negative bacteria. Drug efflux is carried out by membrane transporters and is responsible for multidrug resistance in bacteria, even though many of these transporters are yet to be identified. Multidrug resistance can also be caused by reduced expression of porins and changes in the cell, causing reduced uptake or the expression of efflux pumps. Integrons are special case of MDR. The majority of genes encoding antibiotic resistance include resistance to cephalosporins, penicillin, trimethoprim-sulfhamethoxazole, tetracycline, chloramphenicol (18). An integron contains an integrase and an adjacent recombination site. Gene cassettes can be integrated by the integrase at the recombination site, and multiple gene cassettes can be present in one integron. Integrons can work as natural genetic engineering systems. Gene cassettes which are circularized open reading frames are converted into functional genes after incorporation into integrins. Transposons also have the capacity to carry multiple drug resistance genes. Among bacterial pathogens horizontal transfer of drug resistance genes is a successful mechanism. In the evolution of resistance, the resistance genes are transferred to the recipient cell in clusters. The associations of a highly

efficient gene capture and expression system, together with the capacity for vertical and horizontal transmission of resistance genes represents a powerful tool used by bacteria to combat the assault of antibiotics. Horizontal transfer of resistance genes contributed largely to the emergence of multidrug-resistant *Enterobacteriaceae spp* (20). Among clinical isolates of bacteria the rapid emergence of antibiotic resistances is due to horizontal transfer. The spread of resistant genes is more rapid when they form part of a mobile gene cassettes. These mechanisms drug resistance genes include (i) integrase encoded by integron facilitates the mobilization of individual gene cassettes (ii) by targeted transposition of integron containing gene cassettes (iii) dissemination of larger transposons such as Tn21 carrying integrons and (iv)movement of conjugative plasmids containing integrons among different bacterial species. It is therefore not surprising that many of the antibiotic resistance genes found in clinical isolates of gram negative microorganisms are part of a gene cassette inserted into an integron. Timely reporting of antibiotic resistance patterns and systemic surveillance among enteric pathogens from different regions of world is of paramount importance. Emergence of drug resistance will be an ongoing threat for developing and developed countries (19).

(2.6).A β LACTAMS

β Lactam class of antibiotics are most extensively used, these act on (PBPs) penicillin binding sites which are involved in cell wall synthesis. In case of gram-negative bacteria, β-lactamases are major cause of resistance to β-lactam antibiotics. Resistance can also be caused by mutations in PBP's resulting in reduced affinity for β Lactam antibiotics. Lactamase enzyme inactivate the antibiotics by hydrolysing the betalactam amide bond. Extended-spectrum β-lactamases (ESBLs) are highly prevalent; these enzymes hydrolyze and cause resistance to cephalosporins (9). These enzymes are derived from widespread broad spectrum β lactamases (TEM-1, SHV-1, CTX-M and OXA). Common plasmid mediated β-lactamase found in *Klebsiella pneumoniae* and *E. coli* is SHV-1. Resistance to cephalosporins can be reduced by employing the use of β-lactamase inhibitor, such as clavulanate, in combination with an oxyimino-cephalosporin such as ceftazidime or cefotaxime (20).

(2.6).B AMINOGLYCOSIDES

Aminoglycosides, which includes amikacin, gentamicin, streptomycin, and tobramycin are commonly used antibacterial agents in the treatment of infections caused by both gram positive and gram negative bacteria. After binding with the ribosomes these drugs interfere with the protein synthesis. Aminoglycoside resistance is widespread. More than 50 aminoglycoside modifying enzymes have been described (12). Majority of these drug resistance genes are present in gram-negative bacteria. These enzymes are classified depending on their type of modification as aminoglycoside adenylyltransferases (aad), aminoglycoside acetyltransferases (aac), aminoglycoside nucleotidyltransferases (ant) and aminoglycoside phosphotransferases (aph) (8).

(2.6).C Fluoroquinolone

Fluoroquinolone antibiotics play an important role by the inhibiting certain bacterial topoisomerase enzymes, namely, DNA gyrase or bacterial topoisomerase II and topoisomerase IV. Within the cell these important bacterial enzymes changes the topology of double-stranded DNA. Topoisomerase IV and DNA gyrase are heterotetrameric proteins composed of two subunits, A and B. The genes encoding the A and B subunits are referred to as gyrA and gyrB (DNA gyrase) or parC and parE (DNA topoisomerase IV) (13). Mechanisms of bacterial resistance to fluoroquinolones involve the changes in drug target enzymes that limits the permeation of drug to the target cell. The target enzymes are most commonly altered in domains near the enzyme active sites and in some cases reduced drug binding affinity has also been demonstrated. Both these enzymes are located in the cytoplasm of the bacterial cell. To reach their targets, fluoroquinolone antibiotics must traverse the cell envelope. Decreased uptake and increased resistance to fluoroquinolones in gram negative bacteria is associated with changes in the outer membrane (14). In gram-negative bacteria, DNA gyrase is the primary target for all quinolones. In expression of resistance to quinolones alterations of target enzymes appear to be the most dominant factor (8).

(2.7) Status of Research Proposed (National as well as International)

There are various studies conducted and it is found that prevalence of UTI is more in females than compared to males (2). It was also found that gram negative bacteria were found in much more prevalence than gram positive bacteria. *E. coli* was found to be one of major causative

agent of UTIs. Nitrofurantoin was found to be most resistant drug among all. In this study they found that 155 pathogens comprised of 140 gram negative and 15 gram positive bacteria. It was also found that UTIs infection were related to age as it was found to b more prevelant in elder people (>48 years)as compared to young age patients. They founded that drugs with inhibitors such as Augmentin may be tried for UTIs. It is also observed that microorganisms are developing resistance to other antibiotics like cephalosporin also. Studies conducted internationally also for instance in Southern Iran males were found to be more prone to UTIs and the causative bacteria which was most common in this case was again *E. coli*. They found Amikacin and Gentamicin most appropriate antibiotic for treatment. There are current researches going on as the resistance towards antibiotics is increasing as it is found that if antibiotics won't work UTIs can turn into a bloodstream infection (15). In other studies conducted nationally as well as internationally it has been found that some UTIs are even recurrent ones and reappear twice or thrice in year. In our case we will focus on Himachal Pradesh region and find out the results in context with the most common cause of UTIs in this region and their resistance profile. It was found that use of quinolones, made bacterial resistance to these drugs develop faster.

(2.8) Environmental impact assessment and risk analysis.

Bacteria with resistance genes can transfer these genes to pathogenic bacteria that enter same environment .The genetic elements that are transferred often carry factors that impart resistance to more than one kind of antibiotic. When such elements are transferred, they create "superbugs" resistant to distant kinds of antibiotics. Therefore it would become difficult to treat any of infectious disease by any of the antibiotic if bacteria gained resistance in same pace. It would become difficult to treat number of diseases by use of antibiotics (14).

CHAPTER 3

METHODS AND MATERIALS

SAMPLE COLLECTION

40 samples were collected from laboratories located in Solan District region. Out of these 32 samples (24 females and 8 males) were positive for the infection. Urine samples were collected in sterile container labeled properly.

BACTERIOLOGY

Urine samples were inoculated in nutrient broth and were left for growth overnight. After sufficient growth samples were streaked on MacConkey agar with help of a wire loop. Plates were incubated at 37°C and observed after 24 hours for colonies. After streaking there were different types of colonies observed. Morphology and characteristics of each isolated colony was noted.

MAcCONKEY AGAR

Composition: Peptone, Protease peptone, Lactose, Bile salts, Sodium chloride, Neutral red, Crystal violet, Agar, Water.

Enzymatic Digest of Gelatin, Casein and Animal tissue: provides nitrogen, vitamins, minerals and amino acids essential for growth.
Bile Salts: selective agents and inhibit Gram positive organisms.
Lactose: fermentable carbohydrate providing carbon and energy.
Crystal Violet: Use: Gram positive bacteria are generally inhibited by crystal violet.
Sodium Chloride: supplies essential electrolytes for transport and osmotic balance.
Neutral Red: pH indicator, which is red in color at pH's below 6.8.

When lactose is fermented, the pH of the medium decreases, changing the color of neutral red to pink

Agar: Solidifying agent

MacConkey is a selective and differential media which isolate gram negative bacteria and also differentiate them based on lactose and non lactose fermenting. Crystal violet and bile salts are incorporated in MacConkey Agar to prevent the growth of gram-positive bacteria and fastidious gram-negative bacteria.

BIOCHEMICAL TESTS (13)

IMVIC

IMVIC stands for four different tests

i) Indole
ii) Methyl red
iii) Voges-proskauer
iv) Citrate

Indole test determines the ability of an organism to split amino acid tryptophan to form the compound indole. Indole presence is detected by Kovac's reagent. Tryptophan is hydrolyzed by tryptophanase to produce 3 possible end products, one of them is indole. Kovac's reagent contains 4 (p)-dimethylamino benzaldehyde, this reacts with indole to produce a red colored compound.

Method

i) Inoculated the broth with culture.
ii) Incubated at 37℃ for 16 hours.
iii) Added 0.5ml of Kovac's reagent.

Positive: Pink colored ring appeared after addition of Kovac's reagent

Negative: No color change.

Methyl red determines if the microbe performs mixed acid fermentation or 2-3-butanediol fermentation. In mixed acid fermentation, three acids (acetic acid, lactic acid and succinic acid) are formed in significant amounts. The large amount of acid results in significant decrease in the pH of the medium below 4.4. This is visualized by using pH indicator, methyl red (p-dimethylaminoabenzene-O-carboxylic acid), which is yellow above pH 5.1 and red at pH 4.4.

MR Positive: When the culture medium turns red after addition of methyl red, because of a pH at or below 4.4 from the fermentation of glucose.
MR Negative: When the culture medium remains yellow, which occurs when less acid is produced (pH is higher) from the fermentation of glucose.

Method

i) Inoculate culture in 10 ml MR/VP broth.
ii) Incubated at 37℃ for 16 hours.
iii) From inoculated broth separated 5 ml media in another tube.
iv) Now in one tube added 5 drops of Kovac's reagent.
v) Check for color change red on addition of methyl red.

VOGES PROSKAUER

Pyruvic acid is a pivotal compound in the fermentative degradation of glucose, is further metabolized through various metabolic pathways. It depends on the enzyme systems possessed by different types of bacteria. One such pathways resulted in the production of acetoin (acetyl methyl carbinol), a neutral-reacting end product.

Media and Reagent

Media used again is MR/VP broth.

Reagents used are Alpha Naphthol, 5% intensifier and Potassium hydroxide, 40% oxidizing agent.

Method

i) Inoculated culture in MR/VP broth as defined earlier for methyl red test(10 ml)
ii) Incubated at 37℃ for 16 hours.
iii) Separated inoculated culture in another tube(5 ml for VP test)
iv) Added 0.6mL of 5% alpha naphthol, followed by 0.2 mL of 40% KOH.
v) Left undisturbed for 10-15 minutes

A positive test is confirmed by the development of a red color after 15 minutes or more on addition of the reagents indicating the presence of diacetyl, the oxidation product of acetoin Test should not be read after standing for over 1 hour because negative Voges-Proskauer cultures may produce a copper like color, potentially resulting in a false positive interpretation.

CITRATE TEST

This test is used to determine the ability of bacteria to use sodium citrate as sole carbon source. When an organic acid such as citrate is used as a carbon source, alkaline carbonates and bicarbonates are produced. Also, ammonium hydroxide is produced when the ammonium salts in the medium are used as the sole nitrogen source.

If exogenous citrate is to be used it needs presence of enzymes permeases .after uptake by the cell, lyase cleave it into oxaloacetate and acetate. Oxaloacetate is than metabolized to pyruvate and carbon dioxide. Further breakdown highly depend on pH of media

A. under alkaline conditions, Pyruvate metabolized to acetate and formate.

B. at pH 7.0 and below, lactate and acetoin are also produced.
The carbon dioxide that is released reacts with water and the sodium ion in the medium to produce sodium carbonate, an alkaline compound that will raise the pH. In addition, ammonium hydroxide is produced when the ammonium salts in the medium are used as the sole nitrogen source. Growth usually results in the bromothymol blue indicator, turning from green to

blue. The bromothymol blue pH indicator is a deep forest green at neutral pH. With an increase in medium pH to above 7.6, bromothymol blue changes to blue.

Method

i) Inoculated simmons citrate agar by streaking on it.
ii) Incubated for 16-18 hours at 37℃.
iii) Observed for change in color i.e. blue due to alkalization.

Citrate positive: Growth will be visible on the slant surface and the medium will be an intense blue. The alkaline carbonates and bicarbonates produced as by-products of citrate catabolism raise the pH of the medium to above 7.6, causing the bromothymol blue to change from the original green color to blue.

Citrate negative: Trace or no growth will be visible. No color change will occur; the medium will remain the deep forest green color of the uninoculated agar. Only bacteria that can utilize citrate as the sole carbon and energy source will be able to grow on the Simmons citrate medium.

TRIPLE SUGAR IRON

Triple Sugar Iron medium is a differential medium that can distinguish between a number of Gram-negative bacteria based on their ability or lack of:

a. metabolize lactose and/or sucrose
b. conduct fermentation to produce acid
c. produce gas during fermentation
d. generate H_2S.

The medium contains 1.0% each of sucrose and lactose and 0.1% glucose. If *only* glucose is fermented, acid produced in the butt will turn it yellow, but insufficient acid products are formed to affect the methyl red in the slant. However, if either sucrose or lactose is fermented, sufficient fermentation products will be formed to turn both the butt *and* the slant yellow. If gas is formed during the fermentation, it will show in the butt either as bubbles or as cracking of the agar. If no

fermentation occurs (as for an obligate aerobe) the slant and butt will remain red. The medium also contains ferrous sulfate. If the bacterium forms H_2S, this chemical will react with the iron to form ferrous sulfide, which is seen as a black precipitate in the butt (a black butt).

Method

i) Took sterilized straight wired loop.
ii) Inoculated TSI Agar by first stabbing through the center of the medium to the bottom of the tube and then streaking the surface of the agar slant.
iii) Incubated for 18 hours at 37℃

Interpretation of Triple Sugar Iron Agar Test

1. If lactose (or sucrose) is fermented, a large amount of acid is produced, which turns the phenol red indicator both in butt and in the slant. Some organisms generate gases, which produces bubbles/cracks on the medium.
2. If lactose is not fermented but the small amount of glucose is, the oxygen deficient butt will be yellow (remember that butt comparatively have more glucose compared to slant i.e. more media more glucose), but on the slant the acid (less acid as media in slant is very less) will be oxidized to carbon dioxide and water by the organism and the slant will be red (alkaline or Neutral pH).
3. If neither lactose/sucrose nor glucose is fermented, both the butt and the slant will be red. The slant can become a deeper red-purple (more alkaline) as a result of production of ammonia from the oxidative deamination of amino acids (remember peptone is a major constituents of TSI Agar).
4. H_2S is produced, the black color of ferrous sulfide observed.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

DISC DIFFUSION METHOD

Due to convenience, efficiency and cost, the disk diffusion method is probably the most widely used method for determining antimicrobial resistance. A growth medium, usually Mueller-

Hinton agar, is first evenly seeded throughout the plate with the isolate of interest that has been diluted at a standard concentration (approximately 1 to 2 x 10^8colony forming units per ml). Commercially prepared disks are pre-impregnated with a standard concentration of a particular antibiotic, are evenly dispensed and lightly pressed onto the agar surface. The test antibiotic immediately begins to diffuse outward from the disks, creating a gradient of antibiotic concentration in the agar such that the highest concentration is found close to the disk with decreasing concentrations further away from the disk. After an overnight incubation, the bacterial growth around each disc is observed. If the test isolate is susceptible to a particular antibiotic, a clear area of "no growth" will be observed around that particular disk. The zone around an antibiotic disk that has no growth is referred to as the zone of inhibition since this approximates the minimum antibiotic concentration sufficient to prevent growth of the test isolate. This zone is then measured in mm and compared to a standard interpretation chart used to categorize the isolate as susceptible, intermediately susceptible or resistant. MIC measurement cannot be determined from this qualitative test, which simply classifies the isolate as susceptible, intermediate or resistant.

Mueller-Hington agar

MH agar is considered the best medium to use for routine susceptibility testing of non fastidious bacteria for the following reasons:

- It shows acceptable batch-to-batch reproducibility for susceptibility testing.
- It is low in sulfonamide, trimethoprim and tetracycline inhibitors.
- It supports satisfactory growth of most non fastidious pathogens.

Susceptible

'The "susceptible" category implies that isolates are inhibited by the usually achievable concentrations of antimicrobial agent when the recommended dosage is used

Resistant

'The "resistant" category implies that isolates are not inhibited by the usually achieveable concentrations of the agent with normal dosage schedules.

Intermediate

'The "intermediate" category includes isolates with antimicrobial MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates. The intermediate category implies clinical efficacy in body sites where the drugs are physiologically concentrated (e.g. quinolones and beta-lactams in urine) or when a higher than normal dosage of a drug can be used (e.g. betalactams).

Method

i) Made Muller Hington Agar plates.
ii) With help of a spreader 100µl of inoculums is spread on plate.
iii) Plates left for 10 -15 minutes after spreading.
iv) Antibiotics discs placed.
v) Incubated for 16 hours.
vi) Noted zone of inhibition.
vii) Observed for resistant, sensitive and intermediate according to CLSI guidelines.

MIC (Minimum Inhibitory Concentration)

Minimum inhibitory concentration is defined as minimum concentration of antimicrobial drug that inhibit visible growth of microorganism after overnight incubation. To identify MIC via broth dilution method, identical doses of bacteria are cultured in wells with media containing progressively drug with lower concentration. Minimum inhibitory concentration is between the

concentration of last well in which no bacteria grew and next lower dose that allowed bacterial growth.

Because a lower MIC value indicates that less of the drug is required in order to inhibit growth of the organism, drugs with lower MIC scores are more effective antimicrobial agents. MIC scores are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. Clinicians use MIC scores to choose which antibiotics to administer to patients with specific infections and to identify an effective dose of antibiotic. This is important because populations of bacteria exposed to an insufficient concentration of a particular drug or to a broad-spectrum antibiotic (one designed to inhibit many strains of bacteria) can evolve resistance to these drugs. Therefore, MIC scores aid in improving outcomes for patients and preventing evolution of drug-resistant microorganisms.

MATERIALS

I) Liquid Cultures Of Bacteria At Suitable Growth Phase
II) Sterile Petri Dishes
III) Sterile 96-Well Microtitre Plates.
IV) Filter Sterilized Antibiotics
V) Sterile Diluents
VI) Test Tubes.

METHOD

1. Grow the test strains in the chosen medium to the right A_{600}. Have antibiotic solutions and plates ready before the cultures reach the desired growth phase.
2. Thaw and weigh the antibiotics. Take a note of the purity at this stage, e.g. gentamicin, 577ug/mg solid. Dis
3. Using the micropipette, dispense 100ul of medium into all wells of a microtitre plate. Label the plate and lid.
4. Pipette 100ul of appropriate 2x antibiotic solutions into the wells in column 1 (far left of plate).

5. Using the micropipette set at 100ul, mix the antibiotics into the wells in column 1 by sucking up and down 6-8 times. Do not splash.
6. Withdraw 100ul from column 1 and add this to column 2. This makes column 2 a twofold dilution of column 1.
7. Discard 100ul from column 10 rather than putting it in column 11.
8. Pour bacteria of the right A_{600} into a sterile petri dish. The bacteria may be diluted first depending on the desired inoculum. The appropriate inoculum size for standard MIC is 10^4 to 10^5 CFU/ml.
9. With the smaller micropipette set to 5ul, dispense bacteria into wells in columns 11 to 1 in that order. Do not add bacteria to column 12.
10. Incubate the plates at 37^oC or other desired temperature.
11. Streak the bacterial cultures on plates to check their purity.
12. When satisfactory growth is obtained (18-36 hours) scan the plates with an ELISA read. Use column 12 as the blank.
13. MIC can be taken as the lowest concentration of drug that reduces, by more than 50% or 90% for MIC_{50} or MIC_{90} respectively.

DNA ISOLATION

Many different methods and technologies are available for the isolation of genomic DNA. In general, all methods involve disruption and lysis of the starting material followed by the removal of proteins and other contaminants and finally recovery of the DNA. Removal of proteins is typically achieved by digestion with proteinase K, followed by salting-out, organic extraction, or binding of the DNA to a solid-phase support (either anion-exchange or silica technology). DNA is usually recovered by precipitation using ethanol or isopropanol. The choice of a method depends on many factors: the required quantity and molecular weight of the DNA, the purity required for downstream applications, and the time and expense. Several of the most commonly used methods are detailed below; although many different methods and variations on these methods exist (a comparison of methods is shown in Figure 7, page 13). Home-made methods often work well for researchers who have developed and regularly use them. However, they usually lack standardization and therefore yields and quality are not always reproducible.

Reproducibility is also affected when the method is used by different researchers, or with different sample types. The separation of DNA from cellular components can be divided into four stages:

1. Disruption

2. Lysis

3. Removal of proteins and contaminants

4. Recovery of DNA

METHOD

i) Inoculated 10µl Nutrient broth with bacterial culture.
ii) Incubate at 37℃ for 6 to 8 hours.
iii) Pellet down bacterial culture maximum at 7000rpm for 5 to 10 min.
iv) Discarded supernatant.
v) Added SDS lysis buffer (200µl) to pellet and re-suspend properly.
vi) Incubated at 60℃ for 1 hour.
vii) Added Phenol: Chloroform: Isoamylalcohol in equal volume (25:24:1).
viii) Centrifuge at 13000 rpm/10 min.
ix) Kept the supernatant.
x) Added equal volume of isopropanol and half volume of sodium acetate 0.5 M/0.5M NaCl.
xi) DNA threads visible.
xii) Kept at 4℃ for 1 hour.
xiii) Centrifuged at 13000 RPM for 10 min.
xiv) Washed with 70% ethanol/5minutes @ 13000rpm.
xv) Dried pellet and resuspended in nuclease free water.

GEL ELECTROPHORESIS

Theory: Agarose gel electrophoresis is the easiest and most popular way of separating and analyzing DNA. Here DNA molecules are separated on the basis of charge by applying an electric field to the electrophoretic apparatus. Shorter molecules migrate more easily and move faster than longer than molecules through the pores of the gel and this process is called sieving. The gel might be used to look at the DNA in order to quantify it or to isolate a particular band. The DNA can be visualized in the gel by the addition of ethidium bromide.

Agarose is a polysaccharide obtained from the red algae *Porphyra umbilicalis*. Its systematic name is (14)-3, 6-anhydro-a-L-galactopyranosyl-β-D-galactopyranan. Agarose makes an inter matrix. Most agarose gels are made between 0.7% and 2% of agarose. A 0.7%gel will show good separation for larger DNA fragments (5-10kb) and a 2% gel will show good resolution for small fragments with size range of (0.1-1kb). Low percentage gels are very weak but high percentage gels are usually brittle and do not set evenly.

Factors Affecting the Movement of DNA:

a) Voltage Applied: The migration rate of the liner DNA fragments through agarose gel is proportional to the voltage applied to the system. As voltage increase, the speed of DNA also increases but voltage should be limited because it heats and finally causes the gel to melt.

b) Ethidium Bromide (EtBr): It is an intercalating agent which intercalates between nucleic acid bases and allows the convenient detection of DNA fragments in gel. When exposed to UV light, it will fluoresce with an orange colour. When the gel is not cooled or not to hot in that condition EtBr should be added to the gel.

c) Buffers: several different buffers have been recommended for electrophoresis of DNA. The most commonly used buffers are Tris-acetate-EDTA (TAE) and Tris-borate-EDTA.

Method

A) Measured .4g of agarose.

B) Poured agarose powder into microwave flask along with 50ml of 1x TAE.

C) Microwave for 2minutes (until the agarose was completely dissolved and there was a nice rolling boil).
D) Let agarose solution cool down for 5minutes.
E) Added 0.35 µl ethidium bromide. EtBr binds to DNA and allows visualizing the DNA under UV light.
F) Poured the agarose into a gel tray with the well comb in place.
G) Placed newly poured gel at 4°C for 10-15minutes or let it solidify in room temperature for 20-30minutes.

Loading sample and Running an Agarose Gel:

1) Added loading buffer to each sample.
2) Once solidified, placed the agarose gel into the gel matrix (electrophoresis unit).
3) Filled gel matrix with 1x TAE until the gel fully marched.
4) Carefully load the molecular weight ladder into the first lane of the gel and samples.
5) Connected the plugs and ran the gel at 100V for faster movement of genomic DNA fragment.
6) When the dye moved to the middle of the gel carefully observe the gel image under gel doc system.
7) Save the pictures.

Polymerase Chain Reaction (PCR):

Kary Mulis invented PCR technique in 1985 while working as a chemist at the Cetus Corporation, a biotechnology firm in Emeryville, California. The procedure requires placing a small amount of the DNA containg the desire gene into tube. A large batch of loose nucleotides, which link into extract copies of the original gene, is also added to the tube. A pair of synthesized short DNA segments that match segments on each side of the desired gene is added. These “primers” find the right portion of DNA, and serve as starting points for DNA copying. When the enzyme *Thermus aquaticus* is added, the loose nucleotides look into a DNA sequence dictated by the sequence of that target gene located between two points.

When Mulis' completed PCR technique manually it was slow and labor-intensive. Therefore, Cetus scientists began looking for ways to automate the process. Before the discovery of the thermostable Taq enzyme, scientists needed to add fresh enzyme to each cycle. The first thermocycling machine, "Mr. Cycle" was developed by Cetus Engineers to address that need to add fresh enzyme to each test tube after the heating and cooling process and the purification of the Taq polymerase resulted in the need for machine to cycle more rapidly among different temperatures. In 1985 Cetus and Perkin-Elmer Corporation jointly introduced the DNA Themal cycler.

The polymerase chain reaction is a biochemical technology in molecular biology to amplify a single or a few nanogram concentration of DNA across several orders of magnitude, generating thousand to millions of copies of a particular DNA sequence.

The method relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Primers (short DNA fragment) containing sequence complementary to the target region along with a DNA polymerase (after which the method is named) is key components to enable selective and repeated amplification. As PCR progress, the DNA generated is itself used as a template for replication, setting in motion a chain reaction in which the DNA template is exponentially amplified. PCR can be extensively modified to perform a wide array of genetic manipulations.

Almost all PCR applications employ a heat-stable DNA polymerase, such as Taq polymerase, an enzyme originally isolated from the bacterium *Thermus aquaticus*. This DNA polymerase enzymatically assembles a new DNA strand from DNA building-blocks, the nucleotides, by using single-stranded DNA as a template and DNA oligonucleotides (also called DNA primers), which are required for initiation of DNA synthesis. The vast majority of PCR methods use thermal cycling, i.e. alternately heating and cooling the PCR sample in a DNA double helix at a high temperature steps. The first step is to physically separate the strands in a DNA double helix at a high temperature, the process called DNA melting. The second step is to lower the temperature. Then each strand is used as the template, in DNA synthesis by the DNA polymerase to selectively amplify the target DNA. The selectivity of PCR results from the use of primers that are complementary to the DNA region targeted for amplification under specific thermal cycling conditions.

CHAPTER 3

RESULTS AND DISCUSSION

S. No	Sample code	Sample Type	Gender
1)	68s	Urine	Female
2)	58s	Urine	Female
3)	48s	Urine	Female
4)	49sS	Urine	Female
5)	49sL	Urine	Female
6)	54S	Urine	Female
7)	L75	Urine	Female
8)	L75sUTI	Urine	Female
9)	SF 50s	Urine	Male
10)	SF50L	Urine	Male
11)	TMC U(10)	Urine	Female
12)	TMC U(27)	Urine	Female
13)	TMC U(24)	Urine	Female
14)	TMC U(3)	Urine	Female
15)	3F lab (U)	Urine	Female
16)	1 Lab (U)	Urine	Female
17)	4M	Urine	Male
18)	Anil (U)	Urine	Male
19)	6Fu	Urine	Female
20)	8Fu CRI	Urine	Female
21)	2F	Urine	Female
22)	8	Urine	Male

23)	Neelam	Urine	Female
24)	11	Urine	Female
25)	5M	Urine	Male
26)	F(v)	Urine	Female
27)	64	Urine	Female
28)	57	Urine	Male
29)	69	Urine	Female
30)	65	Urine	Female
31)	105	Urine	Male
32)	61	Urine	Female

Table 1: Table showing sample details

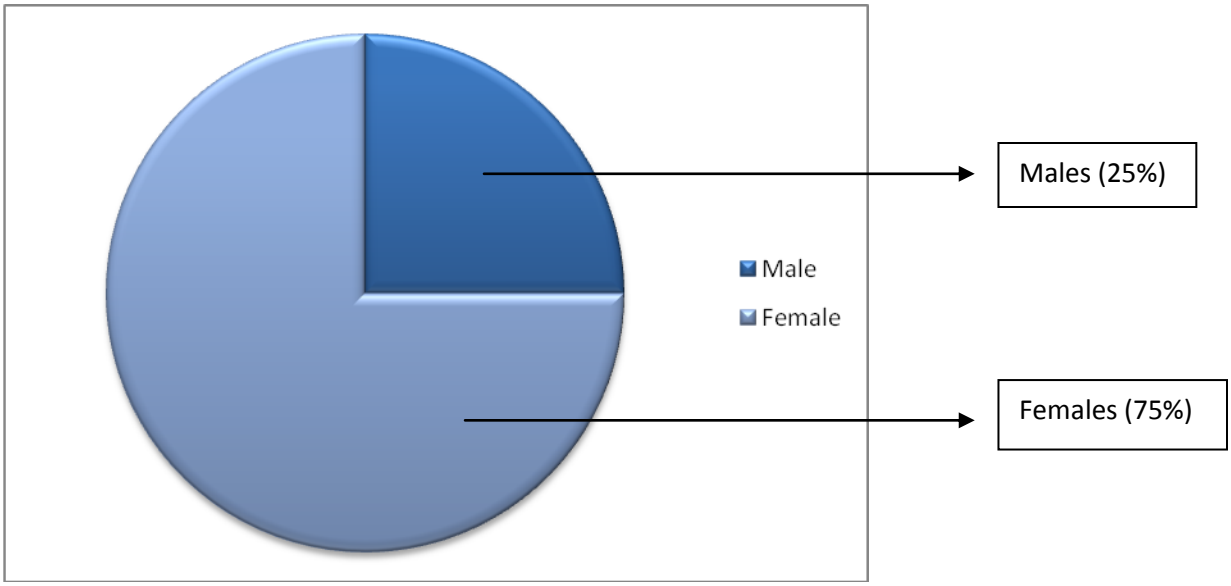

Figure 1: Pie chart showing sample distribution according to genders. It depicts that females suffering UTI'S are more in number.

2. Results after streaking of inoculated broth on MacConkey.

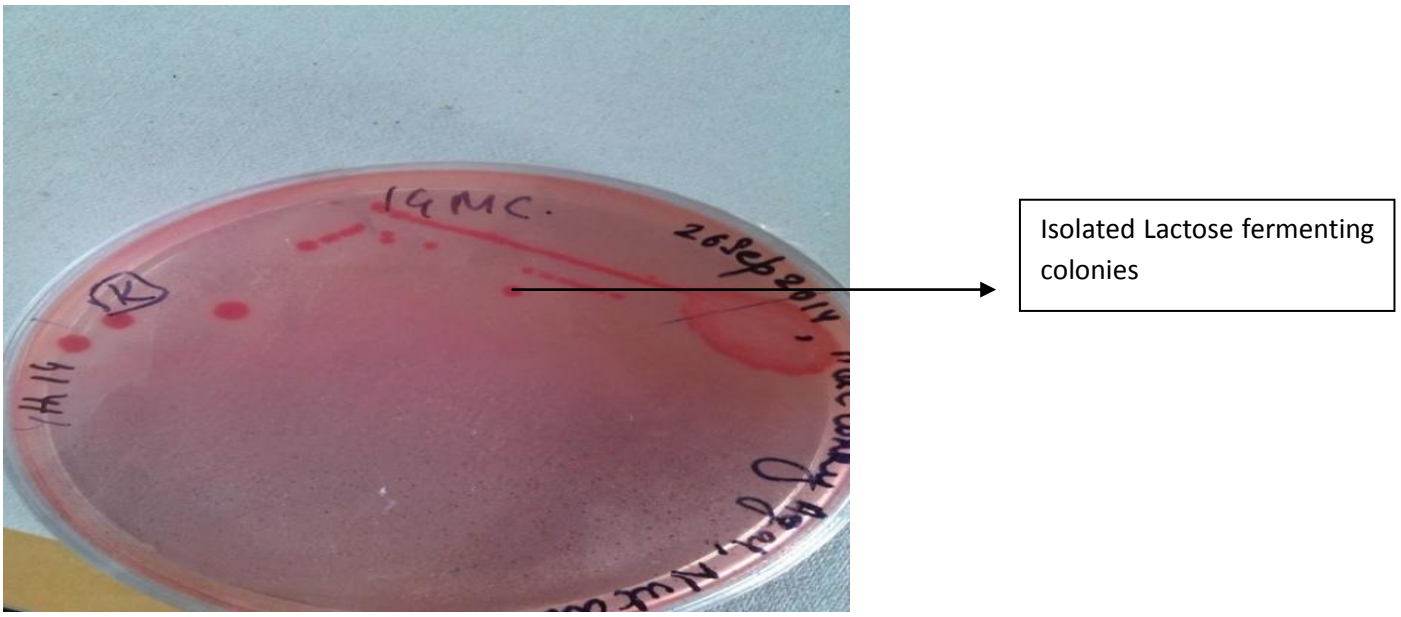

Figure 2: Picture showing streaking on MacConkey agar and isolated colonies

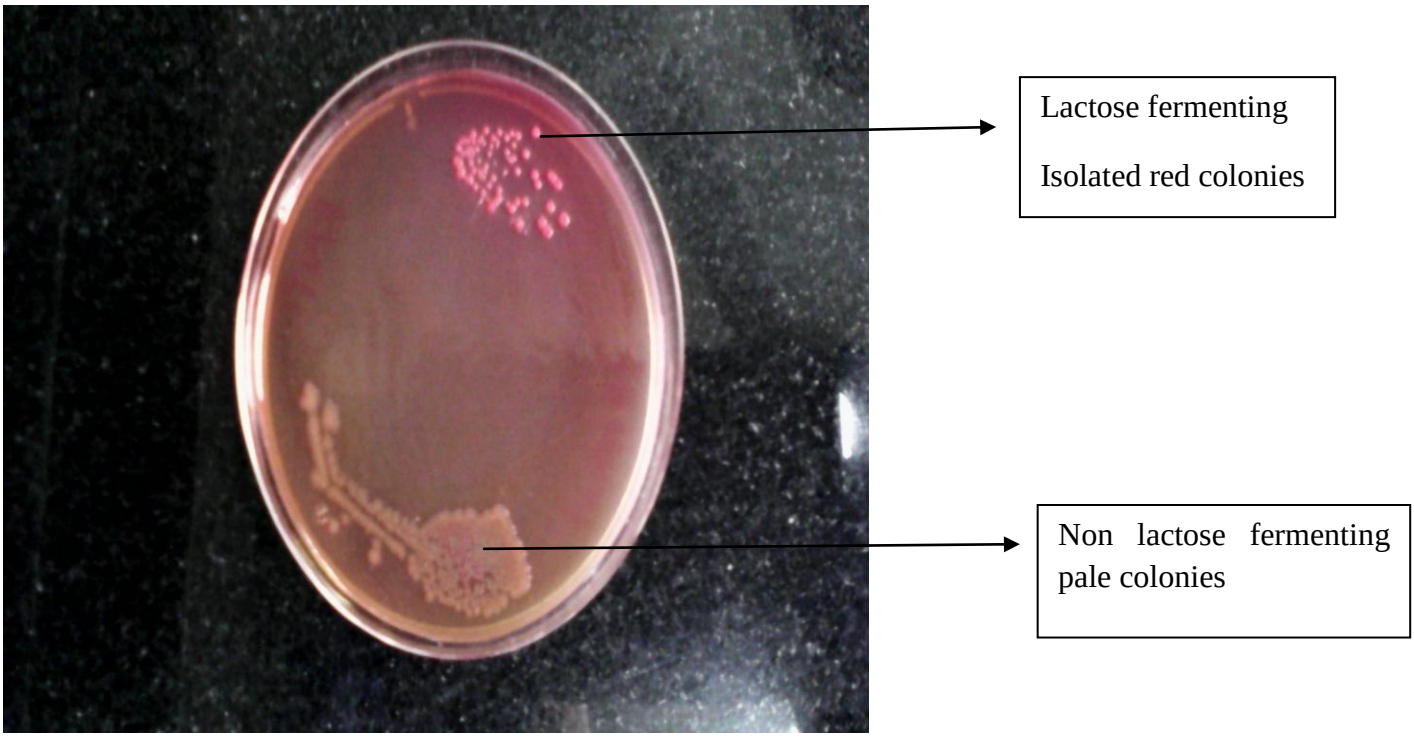

Figure 3: Picture showing streaking on MacConkey agar.

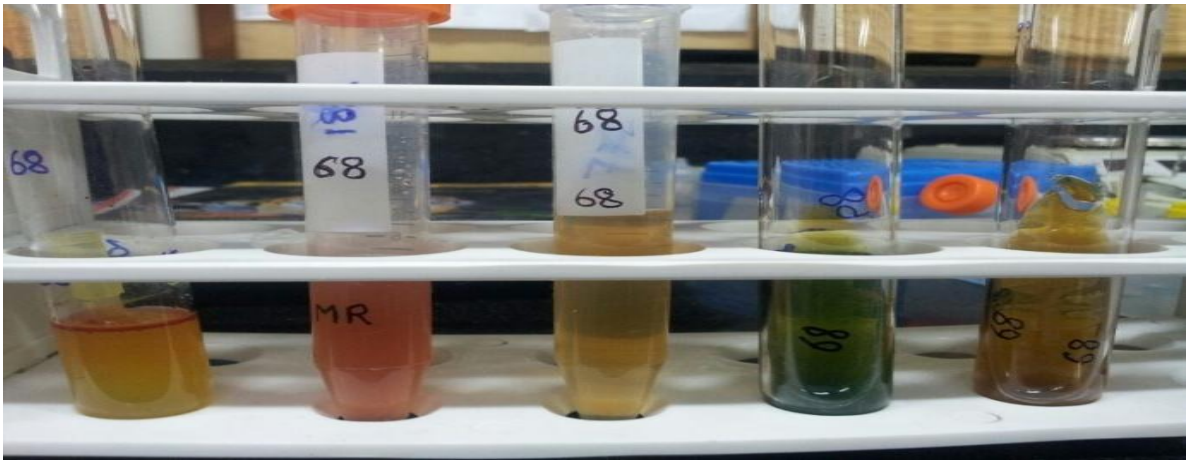

Figure 4: results of biochemical test IMVIC.

Table2: Results of biochemical test

S.NO	SAMPLE CODE	SAMPLE TYPE	INDOLE TEST	METHYL RED	VOGES PROSKAEUR	CITRATE TEST	TRIPLE SUGAR IRON	INTERPRETATION
1)	68s	Urine	+	+	−	−	A/A	*E. coli*
2)	58s	Urine	+	+	−	−	A/A	*E. coli*
3)	48s	Urine	+	+	−	−	A/A	*E. coli*
4)	49sS	Urine	+	+	+	−	K/A	*
5)	49sL	Urine	+	+	+	−	K/A	*
6)	54S	Urine	+	+	−	−	A/A	*E. coli*
7)	L75	Urine	+	+	−	−	A/A	*E. coli*
8)	L75sUTI	Urine	+	+	−	−	A/A	*E. coli*
9)	SF 50s	Urine	−	−	+	+	A/A	*Klebsiella pneumonia*
10)	SF50L	Urine	−	+	+	+	K/A	*
11)	TMC U(10)	Urine	+	+	−	−	A/A	*E. coli*
12)	TMC U(27)	Urine	+	+	−	−	A/A	*E. coli*
13)	TMC U(24)	Urine	+	+	−	−	A/A	*E. coli*
14)	TMC U(3)	Urine	+	+	−	−	A/A	*E. coli*
15)	3F lab (U)	Urine	+	+	−	−	A/A	*E. coli*
16)	1 Lab (U)	Urine	−	+	−	−	A/A	*Shigella boydii*
17)	4M	Urine	+	+	−	−	A/A	*E. coli*
18)	Anil (U)	Urine	+	+	−	−	A/A	*E. coli*
19)	6Fu	Urine	−	+	+	+	K/A	*
20)	8Fu CRI	Urine	−	+	+	+	K/A	*

21)	2F	Urine	−	+	−	−	A/A	*Shigella boydii*
22)	8	Urine	−	+	−	+	A/A	*Proteus*
23)	Neelam	Urine	+	+	−	−	A/A	*E. coli*
24)	11	Urine	+	+	−	−	A/A	*E. coli*
25)	5M	Urine	+	+	−	−	A/A	*E. coli*
26)	F(v)	Urine	+	+	−	−	A/A	*E. coli*
27)	64	Urine	−	−	+	+	A/A	*Klebsiella pneumonia*
28)	57	Urine	+	+	+	−	K/A	*
29)	69	Urine	+	+	+	−	K/A	*
30)	65	Urine	+	+	−	−	A/A	*E. coli*
31)	105	Urine	+	+	+	−	K/A	*
32)	61	Urine	+	+	−	−	A/A	*E. coli*

* Further confirmation required

TSI RESULT SLANT/BUTT	SYMBOL	INTERPRETATION/SUGAR FERMENTED
Red/yellow	K/A	Glucose only
Yellow/yellow	A/A	Glucose, lactose, sucrose

Table 3: Triple Sugar Iron agar Interpretation

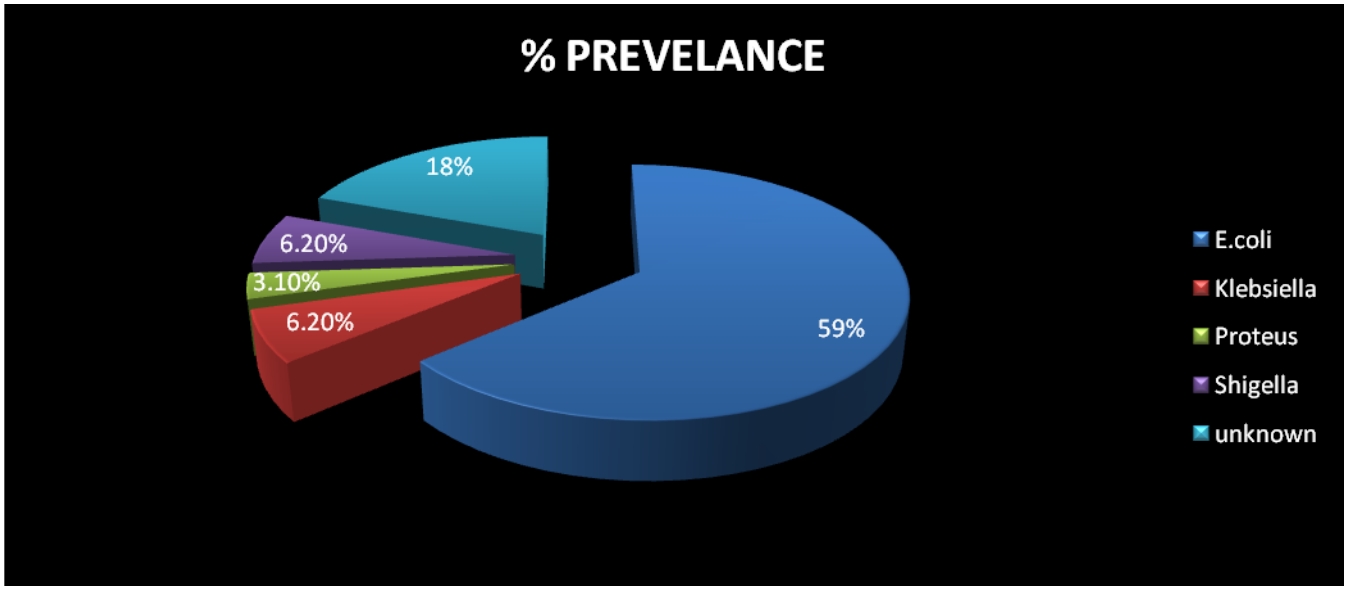

Figure 5: Pie chart depicting % prevalence of various organisms.

It is clear from above data and table that prevalence of *E. coli* is maximum in infected samples that is 59%.Occurence of Proteus is the least that is 3% in one male infected sample.

Table 4: RESULTS OF AST

S.NO	REGION	SAMPLES	Amikacin (30µg/ml)	Streptomycin 30µg/ml	Gentamicin 30µg/ml	Kanamycin 30µg/ml
1	Shimla	49sL	17mm/S	12mm/R	10mm/R	0mm/R
2	Shimla	68s	18mm/S	10mm/R	8mm/R	15mm/I
3	Shimla	48s	20mm/S	8mm/R	8mm/R	0mm/R

4	Shimla	58s	15mm/I	11mm/R	8mm/R	0mm/R
5	Shimla	54	23mm/S	0mm/R	15mm/S	0mm/R
6	Shimla	49sS	24mm/S	8mm/R	10mm/R	0mm/R
7	Shimla	75LeL	24mm/S	12mm/R	13mm/I	0mm/R
8	Shimla	75LeS	22mm/S	22mm/S	13mm/I	0mm/R
9	Shimla	50sSF	24mm/S	22mm/S	11mm/R	8mm/R
10	Kasauli	CRI KIII	26mm/S	8mm/R	10mm/R	0mm/R
11	Shimla	50SFl	24mm/S	26mm/S	12mm/R	0mm/R
12	Shimla	U(3)	21mm/S	0mm/R	12mm/R	0mm/R
13	Shimla	U(10)	23mm/S	0mm/R	8mm/R	0mm/R
14	Shimla	U(24)	25mm/S	0mm/R	16mm/S	0mm/R
15	Shimla	U(27)	27mm/S	0mm/R	16mm/S	0mm/R

Table 5: Results susceptibility testing of antibiotics

S.No	**Region**	**Samples**	**Amikacin**	**Norfloxacin**	**Ciprofloxacin**	**Gentamicin**
1	Shimla	3F lab (U)	14mm/R	10mm/R	12mm/R	14mm/I
2	Shimla	1 Lab (U)	24mm/S	9mm/R	8mm/R	8mm/R
3	Shimla	4M	22mm/S	10mm/R	6mm/R	10mm/R
4	Kasaulli	Anil CRI (U)	21mm/S	8mm/R	10mm/R	14mm/I
5	Shimla	6 Fu	16mm/I	14mm/I	20mm/I	8mm/R
6	Kasaulli	8 Fu CRI	14mm/R	10mm/R	12mm/R	10mm/R

7	Shimla	2F	26mm/S	16mm/I	16mm/I	9mm/R
8	Shimla	8	22mm/S	8mm/R	14mm/R	10mm/R
9	Shimla	Neelam U(9)	12mm/R	10mm/R	12mm/R	10mm/R
10	Shimla	11	16mm/I	20mm/S	26mm/S	14mm/I
11	Shimla	5M	24mm/S	10mm/R	14mm/R	12mm/R
12	Kasaulli	F(v) CRI	14mm/R	20mm/S	24mm/S	12mm/R
13	Shimla	64	15mm/I	22mm/S	22mm/S	0mm/R
14	Shimla	57	20mm/S	0mm/R	26mm/S	11mm/R
15	Shimla	69	25mm/S	31mm/S	29mm/S	19mm/S
16	Shimla	65	25mm/S	28mm/S	26mm/S	18mm/S
17	Shimla	105	22mm/S	33mm/S	14mm/R	12mm/R
18	Shimla	61	0mm/R	0mm/R	0mm/R	0mm/R

ANTIBIOTICS	**CONCENTRATION**
Amikacin	30µg/ml
Norfloxacin	10µg/ml
Ciprofloxacin	5µg/ml
Gentamycin	30µg/ml
Kanamycin	30µg/ml
Streptomycin	30µg/ml

Table 6: Results of concentration of antibiotics used

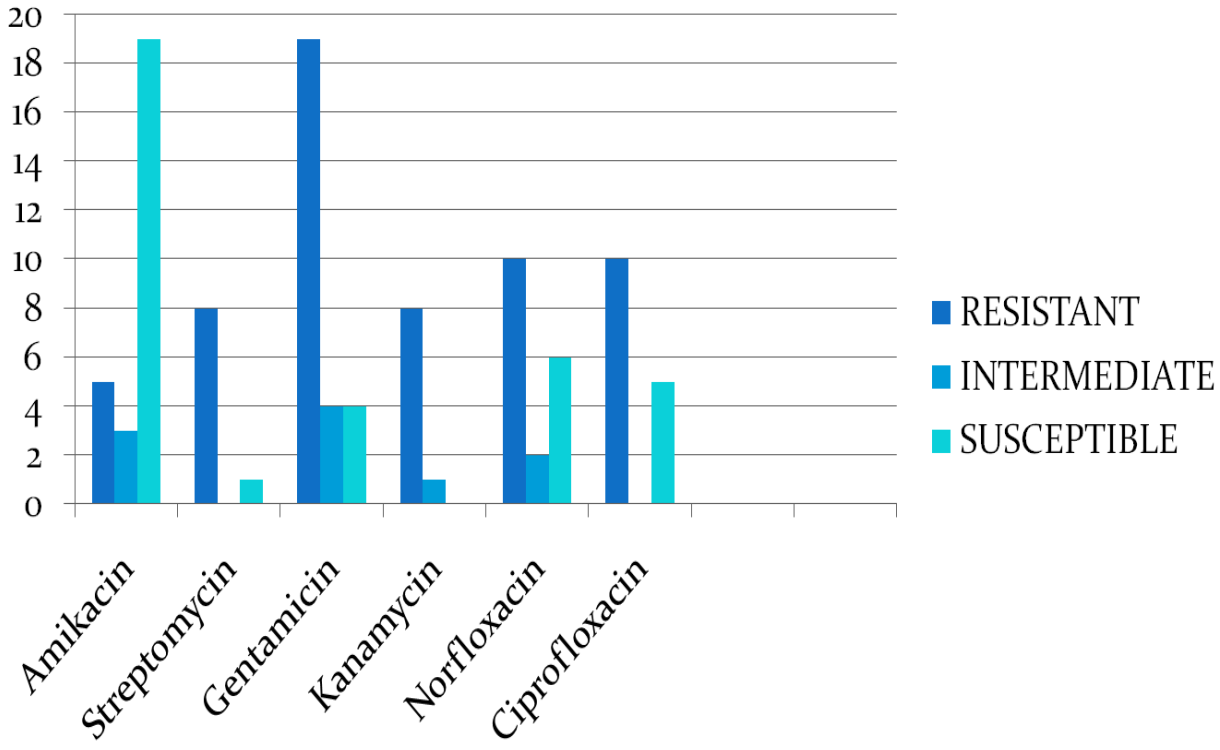

Figure 6: Graph depicting susceptibility pattern of various antibiotics

PICTURES DEPICTING AST RESULTS:

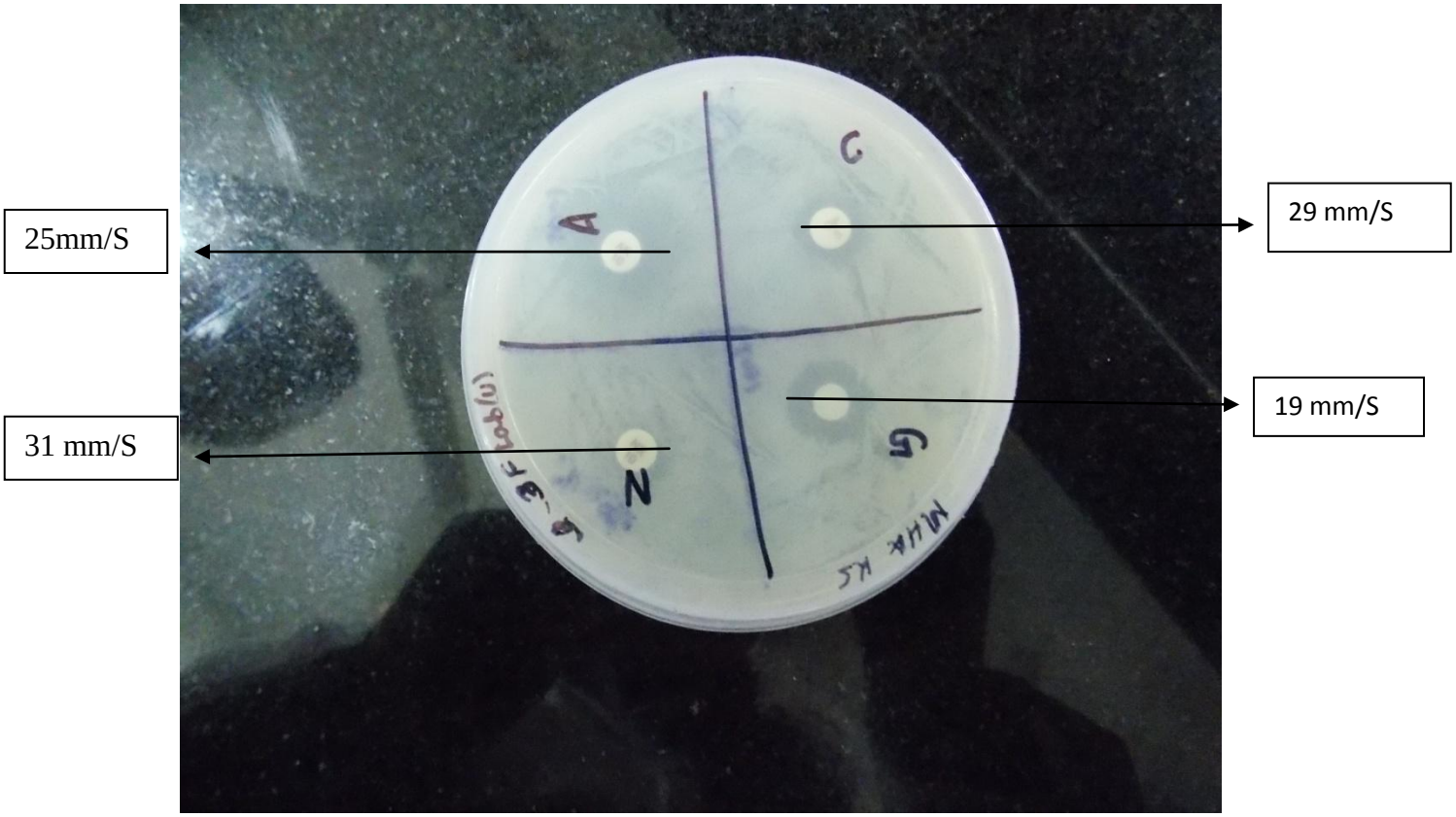

Figure 7: Picture showing sample number: 15

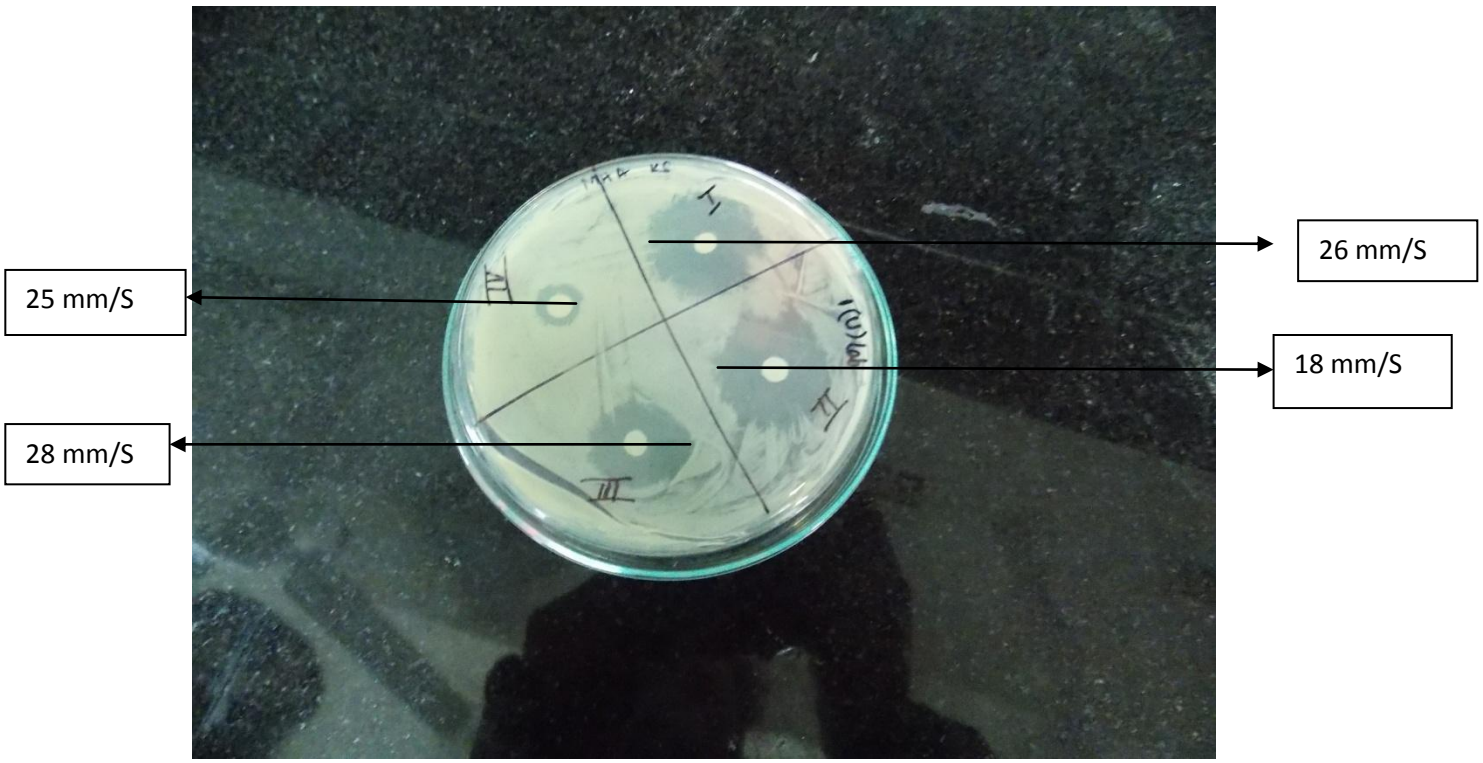

Figure 8: Picture of sample number: 16

Sample code	DNA conc. (ng/µl)	OD 260/280 ratio	OD 260/230 ratio
49sL	**737**	**2.16**	**2.05**
68s	**963**	**1.07**	**1.68**
48s	**386**	**2.17**	**2.15**
58s	**642**	**1.65**	**1.12**
54	**878**	**2.03**	**1.36**
49sS	**348**	**1.71**	**1.76**
CRI KII	**765**	**1.06**	**1.82**
$75Le_L$	**662**	**2.08**	**1.04**
$75Le_S$	**598**	**1.69**	**1.68**

$50_{S}SF$	**959**	**1.54**	**1.12**
CRI KIII	**288**	**1.69**	**1.39**
Pus ND_2S	**692**	**1.93**	**1.78**
Pus ND_1L	**788**	**2.13**	**1.86**
$50SF_{L}$	**396**	**1.97**	**1.47**
U(3)	**248**	**2.24**	**2.39**
U(10)	**195**	**1.53**	**2.97**
U(24)	**457**	**1.41**	**2.55**
U(27)	**168**	**1.0**	**0.626**
3Flab(U)	**783**	**1.03**	**1.02**
1Lab(U)	**289**	**0.982**	**0.8**
4M	**322**	**0.893**	**0.522**
ACRI (U)	**416**	**0.25**	**0.364**
6 Fu	**697**	**0.848**	**0.667**
8Fu CRI	**180**	**1.03**	**0.63**
2F	**63**	**1.06**	**0.502**
8	**563**	**0.937**	**0.542**
NU(9)	**263**	**1.13**	**0.521**
11	**542**	**1.17**	**0.704**
5M	**42**	**2.23**	**0.947**
F(v) CRI	**192**	**1.87**	**0.749**
64	**367**	**1.44**	**1.04**
57	**544**	**0.947**	**1.26**
69	**278**	**0.893**	**0.825**
65	**424**	**1.87**	**1.45**
105	**259**	**1.16**	**1.20**

Table 7: Nanodrop Reading

DISCUSSION

This project was undertaken to evaluate the prevalence and susceptibility patterns of bacterial strains isolated from patients diagnosed with UTIs in Laboratories located in District solan region. A total of 40 urine specimens were collected from patients suspected of having UTI, out of which a total number of 32 showed significant bacterial growth. The bacteria isolated were *E. coli, Proteus mirabilis, Klebsiella spp, Shigella spp*. Therefore *E. coli* was found to be the major cause of UTI'S as concluded by other studies too. This was confirmed that there was resistance to antibiotics tested. Gentamycin was found to be most resistant among isolated organisms followed by Norfloxacin and ciprofloxin. Amikacin was found to be most susceptible. Increase in resistance towards above antibiotics is due to increased use and misuse of these antibiotics. It must be borne in mind that the variations in antimicrobial susceptibility in parts of countries may depend on awareness among local population. This study is helpful in evaluating the drugs that are becoming resistant and also to come up with other drugs that can be mixtures and are more susceptible. The significance of this study was providing information about microorganisms which are causing Urinary tract infection and their resistance towards various antibiotics in Himachal region, Introduction of new antibiotics and increasing treatment options, Understanding of emergence and spread of resistance pattern.

CHAPTER 5

Conclusion

UTI's are very common in women, associated with morbidity and recurrent infections. The pathogenesis of UTI is complex and influenced by many biological and behavioral factors and characteristics of infecting uropathogens. However there remains much to learn about this common disorder. There is a better need of understanding the pathogenesis and risk factors associated with UTI's.

As drug resistance among bacterial pathogens is an evolving process, regular surveillance and monitoring is necessary to provide physician's knowledge on the updated and most effective empirical treatment of UTIs. Periodic reassessment of *in vitro* susceptibility pattern of urinary pathogens to serve as a guide for antibiotic therapy since these organisms exhibit resistance to first-line drugs used for UTI infection. In order to prevent or decrease resistance to antibiotics, the use of antibiotics should be kept under supervision, should be given in appropriate doses for an appropriate period of time.

REFERENCES

1) StammWE,HootonTM,management of urinary tract infections N Enld J Med 1993;329:1328-1334.

2) Mathai D, Jones RN, Pfaller MA. Epidemiology and frequency of resistance among pathogens causing uri-nary tract infection in 1,510 hospitalized patients: a re-port from the SENTY Antimicrobial Surveillance Pro-gram (North America) Diag Microbiol Infect Dis. 2001; 40: 129-136.

3) Kahlmeter G. ECO.SENS. An international survey of the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections: the ECO.SENS Project. J Antimicrobial Chemother. 2003; 51:69-76.

4) Ryan KJ. Urinary tract infections In: Ryan KJ, Ray CG, ed.Sherris Medical Microbiology. New York: McGraw-Hill: 2004: 867–715)

5) Bonadio M, Meini M, Spetaleri P, Gilgi C. Current microbiological and clinical aspects of urinary tract infections. *Eur J* Urol. 2001; 40: 439-45.

6) Ronald AR, Nicolle LE, Stamm E, *et al*. Urinary tract infection in adults: research priorities and strategies. *Int J Antimicrob Agents*. 2001; 17:343

7) Nicolle LE. Epidemiology of urinary tract infection. Infect Med 2001; 18: 153–62.

8) Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's Diagnostic microbiology. Mosby Elsevier. 12th ed. 2007; 842-55.

9)Supriya S. Tankhiwale, Suresh V. Jalgaonkar, Sarfraz Ahamad & Umesh Hassani Evaluation of extended spectrum beta lactamase in urinary isolates Indian J Med Res 120, December 2004, pp 553-556.

10) Manikandan S., Ganesapandian S., Manoj Singh and Kumaraguru A.K. Antimicrobial Susceptibility Pattern of Urinary Tract Infection Causing Human Pathogenic BacteriaAsian Journal of Medical Sciences 3(2): 56-60, 2011.

11)Clinical and Laboratory Standard Institute ;(2005). Performance standards for antimicrobial disc susceptibility tests. NCCLS documents M 100 S 15. Wayne, PA, USA: Clinical and Laboratory Standard Institute.

12) Tambekar D.H., Dhanorkar D.V, Gulhane S.R, Khandelwal V.K., Dudhane M.N. Antibacterial susceptibility of some urinary tract pathogens to commonly used antibiotics African Journal of Biotechnology Vol. 5 (17), pp. 1562-1565, 4 September 2006.
13) MacFaddin JF. Biochemical tests for identification of medical bacteria. Philadelphia: Lippincott Williams and Wilkins. 3rd ed., 2000.

14) Gorbach SL, Bartlett JG, Blacklow NR. Infectious Diseases. Philadelphia: Lippincott Williams & Wilkins; (2004.15) Gastmeir P, Kampf G, Wischnewski N, Hauer T, Schulgen G, et al. Prevalence of nosocomial infections in representative German hospitals. *J. Hosp. Infect* 1998; 38:37-49.

15) Barisˇic´ Z, Babic´-Erceg A, Borzic´ El, *et al*. Urinary tract infections in South Croatia: aetiology and antimicrobial. *Intl J Antimicrob Agents*. 2003; 22: S61-S4.

16)Wilson ML, Gaido L. Laboratory Diagnosis of Urinary Tract Infections in Adult Patients. *Clin Infect Dis* 2004; 38:1150–8.

17) El-Mahmood Muhammad Abubakar. Antimicrobial susceptibility pattern of pathogenic bacteria causing urinary tract infections at the Specialist Hospital, Yola, Adamawa state, Nigeria Journal of Clinical Medicine and Research Vol. 1(1) pp. 001-008, October, 2009.

18) Orret FA, Davis GK. A comparison of antimicrobial susceptibility profile of urinary pathogens for two years, 1999 and 2003. West Indian Med J 2006; 55: 95–9.

19) Uwaezuoke JC, Ogbulie JN. Antibiotic Sensitivity Pattern of Urinary Tract Pathogens in Port – Harcourt, Nigeria. J. ApplSciEnvironm Manage 2006; 10: 103–7.

20) Tankhiwale SS, Jalgaonkar VS, Atimad S, Hassani U. Evaluation of extended spectrum of beta lactamase in urinary isolates. Indian J Med Res 2004; 120: 553–6.

APPENDIX

- **Nutrient Broth Medium (HiMedia Laboratories)**

  Composition :

Ingredients	**gms/Litre**
Peptone	10.000
Beef extract	10.000
Sodium chloride	5.000
pH after sterilization	7.3±0.1

- **Nutrient Agar (HiMedia Laboratories)**

  Composition :

Ingredients	**gms/Litre**
Peptic digest of animal tissue	5.000
Sodium chloride	5.000
Beef extract	1.500
Yeast extract	1.500
Agar	15.000
Final pH (at 25°C)	7.4±0.2

- **MacConkey Agar(HiMedia Laboratories)**

  Composition :

Ingredients	**gms/Litre**
Peptones (meat and casein)	3.000
Pancreatic digest of gelatin	17.000
Lactose monohydrate	10.000
Bile salts	1.500
Sodium chloride	5.000
Crystal violet	0.001
Neutral red	0.030

Agar	13.500
pH after sterilization (at 25°C)	7.1±0.2

- **Xylose–Lysine–Deoxycholate Agar (HiMedia Laboratories)**

  Composition :

Ingredients	**gms/Litre**
Xylose	3.500
L-Lysine	5.000
Lactose monohydrate	7.500
Sucrose	7.500
Sodium chloride	5.000
Yeast extract	3.000
Phenol red	0.080
Sodium deoxycholate	2.500
Sodium thiosulphate	6.800
Ferric ammonium citrate	0.800
Agar	13.500
pHafter heating (at 25°C)	7.4±0.2

- **TCBS Agar (HiMedia Laboratories)**

  Composition :

Ingredients	**gms/Litre**
Proteose peptone	10.000
Yeast extract	5.000
Sodium thiosulphate	10.000
Sodium citrate	10.000
Oxgall	8.000
Sucrose	20.000
Sodium chloride	10.000
Ferric citrate	1.000
Bromothymol blue	0.040

Thymol blue	0.040
Agar	15.000
Final pH (at 25°C)	8.6±0.2