Bacteriological profile and its antibiotic susceptibility in patients with Urinary Tract Infection in a diagnostic center in Dhaka

A DISSERTATION SUBMITTED TO BRAC UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE IN MICROBIOLOGY

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Declaration

I hereby declare that the thesis project titled **"Bacteriological profile and Antibiotic Susceptibility pattern of Bacteria from urine samples of Urinary Tract Infected patients from a diagnostic center in Dhaka"** has been written and submitted by me Ajrin Sultana Sraboni and has been carried out under the supervision of Namista Islam, Lecturer, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University, Dhaka.

It is further declared that this thesis has been composed solely by me and it has not been submitted, in whole or in part, in any previous institution for a degree or diploma. All explanations that have been adopted literally or analogously are marked as such.

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TO

MY

BELOVED MOTHER AND FATHER

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Abstract

Urinary tract infection (UTI) is a common bacterial infection known to affect the different parts of the urinary tract and the occurrence is found in both males and females. Despite the fact, that both the genders are susceptible to the infection, women are mostly vulnerable due to their anatomy and reproductive physiology. The infection is usually caused as a consequence of bacterial invasion of the urinary tract including the lower and the upper urinary tract. It is a frequent cause of morbidity and mortality, and a major driver of antibiotic resistance as antimicrobial drugs are often empirically prescribed. This study aimed isolation, identification of bacterial contamination and also determining the antibiotic susceptibility pattern of the isolates to some commonly used antibiotics and identifying the multidrug resistant bacteria isolated from urinary tract infected patients from a diagnostic centre. Samples were collected from both male and female Patients. Then the samples were processed and spreaded on nutrient agar and after incubation on the following day colonies were streaked on various selective media. Identification of bacteria was done through conventional biochemical tests according to Bergey's Manual of Systematic Bacteriology. Antibiotic susceptibility test was done by Kirby-Bauer method. Among the samples processed 100% of them showed bacterial growth. A total of about 91 bacterial isolates were found, among them most predominant bacteria were *Klebsiella* 19(20.88%) and *Staphylococcus* 19 (20.88%). *Enterobacter* 15(16.48%) found 2nd predominant and the rest were *Bacillus* 14(15.38%),*Shigella* 10(11%) *E.coli* 7(7.69%), *Micrococcus* 4(4.4%) and *Proteus* 3(3.29%) species were found. Antibiotic susceptibility pattern of the bacterial isolates showed that almost all of the isolates were resistant to at least one antibiotic. Among the isolates 37(40.65%) were gram positive and 54(59.35%) were gram negative bacteria. Highest resistance percentage of the isolates was observed to penicillin G (85.71%) followed by Cefepime (75.82%), Ampicillin (69.23%), Erythromycin (69.23%), Rifampicin (57.14%), Azithromycin (56%), Tetracycline (31.87%), chloramphenicol (11%) and Streptomycin (4.4%). Among the multi-drug resistant bacteria 91.31% were resistant to more than two antibiotics and 9.89% were resistant to at least two antibiotics.

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MSA	Mannitol Salt Aagar
MR	Methyl Red
VP	Voges-proskauer
TSI	Triple Sugar Iron
MDR	Multi-drug resistant
MHA	Muller Hinton Agar
MIU	Motility Indole Urease
µL	Microliter
Ml	Milliliter
spp.	Species

Chapter 1

Introduction

Introduction:

Urinary tract infection (UTI) is an infection from microbes. These are organisms that are too small to be seen without a microscope. Most UTIs are caused by bacteria, but some are caused by fungi and in rare cases by viruses. UTIs are among the most common infections in humans. A UTI can happen anywhere in your urinary tract. Our urinary tract is made up of your kidneys, ureters, bladder, and urethra. Most UTIs only involve the urethra and bladder, in the lower tract. However, UTIs can involve the ureters and kidneys, in the upper tract. Although upper tract UTIs are rarer than lower tract UTIs, they're also usually more severe.

Urinary infections are fairly common, especially lower urinary tract infections like cystitis. Often there is burning or stinging of the urine and urinary frequency. The urine may be quite offensive and sometimes contains blood. About 30% of women will be troubled by these distressing symptoms at some stage. By contrast, when less common infections like pyelonephritis occur in the upper part of the urinary tract, the person is usually quite sick...often with a high fever, back pain and shivers. This type of infection is far more likely to be associated with underlying abnormalities and follow-up investigations are always advised.

1.1 Background:

Urinary Tract Infections (UTI) are painful and uncomfortable, yet avoidable. Over 50% of women have had at least one UTI and over 20% have had multiple. UTI's are responsible for over 8 million doctor's visits per year Sexual activity is a high risk factor for developing UTI's in women Foxman *et al*, 1990.

UTI's are most typically caused by E. coli that has been transferred to the urinary tract from the bowel. When E. coli enters the urinary tract, the bacterium adheres to wall of the urinary mucosa using a type of fimbrial adhesin called P fimbriae. These P fimbriae are used by E. coli strands that colonize the urethra to specifically bind to glycoprotein receptors on urothelial cells. These glycoproteins have a mannose residue that is the binding site for the P fimbriae proteins. Current treatment for a UTI caused by the gram-negative E. coli is an antibiotic regiment. There are several common antibiotics used to treat UTI's, which are typically diagnosed on symptoms alone. Antibiotic resistance in gram-negative bacteria is of increasing concern, particularly for broad-

spectrum antibiotics, which are used more and more frequently for urinary infections. Recurrent and long-term antibiotic use risks increased bacterial resistance. As bacterial populations in the vagina are killed due to antibiotics taken to treat a UTI, vaginal yeast populations have the opportunity to proliferate. This leads to further infection, discomfort, and anti-fungal medication. This is a growing problem for treating UTI's specifically, which can lead to more serious infections. It is extremely important that antibiotics are used sparingly, and that alternative prevention options are made available.

1.2 Normal flora of human body:

Normal flora refers to the population of microorganisms that reside in the skin and mucus membranes of a healthy normal person without causing any disease (Jawetz *et al*, 2007). They protect us from disease by competing with invaders for space and nutrients, producing bateriocins which kill harmful bacteria and lowering the pH so that other bacteria can't grow.

Table 1.1: Classification of Normal flora of the human body (Eckburg *et al*, 2005)

Human body	Normal flora
Skin	*Sthaphylococci, micrococci*, diptheroids
Oral and upper respiratory tarct	*Neisseria*, *Bordetella*, *Corynebacterium*, and *Streptococcus* spp
Conjunctiva	*Haemophilus* and *Staphylococcus*
Gastrointestinal tract	*Enterococci*, non-haemolytic *streptococcus*, *E.coli,* lactobacillus
Genital tract	*Corynebacterium, Lactobacillus* spp, non-pathogenic *Neisseria* spp,

1.3 Causes of UTIs:

Urinary tract infections typically occur when bacteria enter the urinary tract through the urethra and begin to multiply in the bladder. Although the urinary system is designed to keep out such microscopic invaders, these defenses sometimes fail. When that happens, bacteria may take hold and grow into a full-blown infection in the urinary tract. The most common UTIs occur mainly in women and affect the bladder and urethra.

1.4 Classification of UTI:

It is understood that the infection targets the different parts of the urinary tract and as a consequence results in the contagion of the lower and the upper urinary tracts. The infection is named based on the site of infection. The infection of urethra and ureter are referred to as urethritis and ureteritis respectively whereas cystitis and phylonephritis corresponds to bladder and kidney infections. Cystitis is a common type of infection whereas the infection associated with the renal damage is an issue of serious concern. Therefore the infection of bladder and urethra are referred as the infection of the lower urinary tract whereas the kidney and ureter infection is an indication of upper tract infection. Generally UTIs are classified based on the factors that trigger the infection and the nature of occurrence. Taking these aspects in to consideration, UTIs can be classified as follows:

i. Uncomplicated or complicated (based on the factor that triggers the infection)
ii. Primary or recurrent (depending on the nature of occurrence)

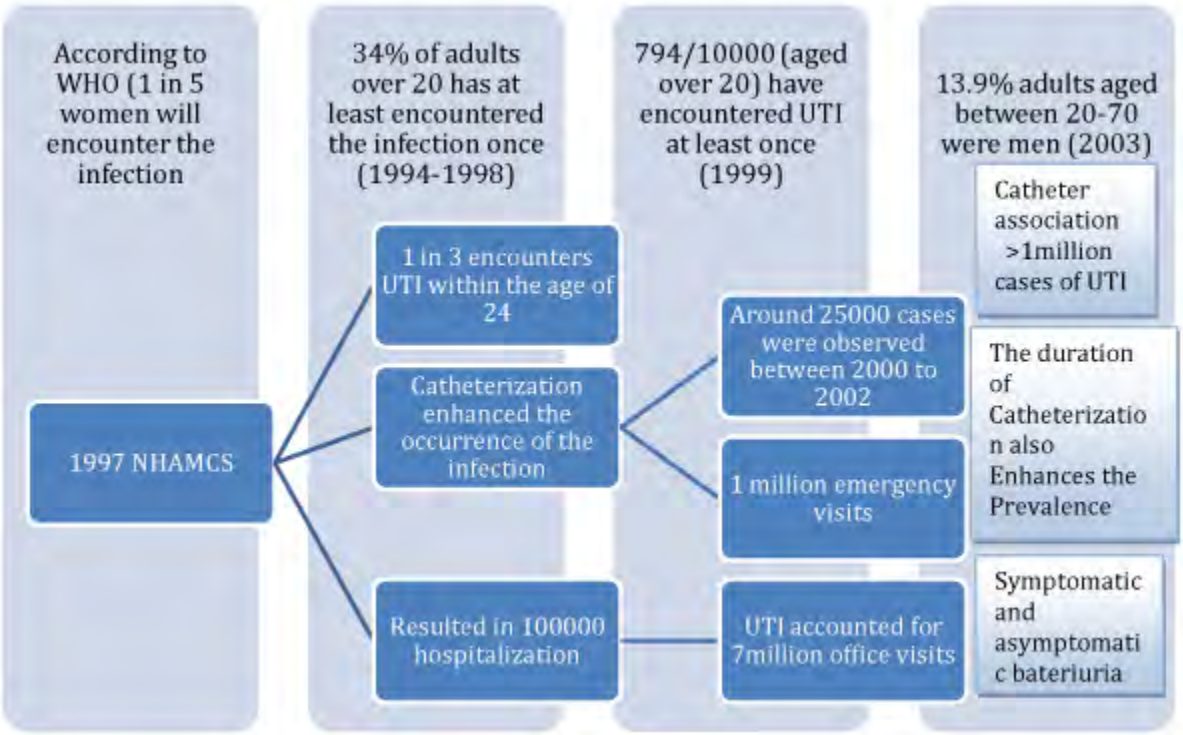
1.5 UTI symptoms:

Symptoms of a UTI depend on what part of the urinary tract is infected.

Lower tract UTIs affect the urethra and bladder. Symptoms of a lower tract UTI include:burning with urination, increased frequency of urination without passing much urine, increased urgency of urination, bloody urine, cloudy urine, urine that looks like cola or tea, urine that has a strong odor, pelvic pain in women, rectal pain in men

Upper tract UTIs affect the kidneys. These can be potentially life threatening if bacteria move from the infected kidney into the blood. This condition, called urosepsis, can cause dangerously low blood pressure, shock, and death.

1.6 Overview of UTI:



1.7 Risk factors of UTIs:

Urinary tract infections are common in women, and many women experience more than one infection during their lifetimes. Risk factors specific to women for UTIs include:

Female anatomy. A woman has a shorter urethra than a man does, which shortens the distance that bacteria must travel to reach the bladder.

Certain types of birth control. Women who use diaphragms for birth control may be at higher risk, as well as women who use spermicidal agents.

Menopause. After menopause, a decline in circulating estrogen causes changes in the urinary tract that make you more vulnerable to infection.

Urinary tract abnormalities. Babies born with urinary tract abnormalities that don't allow urine to leave the body normally or cause urine to back up in the urethra have an increased risk of UTIs.

Blockages in the urinary tract. Kidney stones or an enlarged prostate can trap urine in the bladder and increase the risk of UTIs.

A suppressed immune system. Diabetes and other diseases that impair the immune system — the body's defense against germs — can increase the risk of UTIs.

Catheter use. People who can't urinate on their own and use a tube (catheter) to urinate have an increased risk of UTIs. This may include people who are hospitalized, people with neurological problems that make it difficult to control their ability to urinate and people who are paralyzed.

A recent urinary procedure. Urinary surgery or an exam of your urinary tract that involves medical instruments can both increase your risk of developing a urinary tract infection.

1.8 Causative agent:

Urine is generally considered to be sterile and is believed to be germ free. Any source of possible infection occurs through urethra which initiates the incidence of the infection. The predominant pathogen responsible for UTI is *E. coli* which constitutes up to 80-85% and is followed by *Staphylococcus saprophyticus* which accounts to 5-10%(John *et a*l, 2017). The occurrence of the infection due to viral or fungal agents is a rare phenomenon. In addition to the above mentioned bacterial species, *Klebsiella*, *Proteus*, *Pseudomonas* and *Enterobacter* are associated with UTI. The bacteria enter the bladder through urethra and the infection can also occur through blood and lymph. The microbial etiology of UTIs is deemed to be well established and frequent Farajnia *et al*, 2009. Pathogens like *E. coli* and *S. saprophyticus* are associated with population acquired acute uncomplicated infection whereas *Klebsiella*, *Enterococcus*, *Proteus Species*, *Enterobacter, Bacillus, Shigella* are known to confer uncomplicated cystitis and phylonephritis that are sporadic Vasudevan *et al*, 2014.

1.9 Antibiotic resistance:

Antibiotics are type of antimicrobial drugs which are used in the treatment and prevention of bacterial infections caused by bacterial pathogens. Antibiotic resistance is one of the biggest threats to global health, food security, and development today. Multidrug resistance refers to

antimicrobial resistance shown by the organisms to multiple antimicrobial drugs usually at least two or more than two antibiotics.

1.9.1 Antibiotic resistance in bacteria:

The WHO list is divided into three categories according to the urgency of need for new antibiotics: critical, high and medium priority. (WHO, 2017)

Critical group:

Organisms	Resistant to antibiotics
Enterobacteriaceae,	carbapenem-resistant, cephalosporin-resistant
Pseudomonas aeruginosa,	carbapenem-resistant
Acinetobacter baumannii,	carbapenem-resistant, ESBL-producing

High group:

Organisms	Resistant to antibiotics
Enterococcus faecium,	vancomycin-resistant
Staphylococcus aureus	methicillin-resistant, vancomycin-intermediate and resistant
Salmonellae,	fluoroquinolone-resistant
Helicobacter pylori,	clarithromycin-resistant
Campylobacter spp	fluoroquinolone-resistant
Neisseria gonorrhoeae	cephalosporin-resistant, fluoroquinolone-resistant

Medium group:

Organisms	Resistant to antibiotics
Streptococcus pneumoniae,	penicillin-non-susceptible
Shigella spp.	fluoroquinolone-resistant
Haemophilus influenzae,	ampicillin-resistant

The high prevalence of multidrug resistant bacteria encoding various multidrug resistance genes has now become a major threat to public health. Without effective antimicrobials, medical procedures such as organ transplantation, cancer chemotherapy, diabetes management and major surgery (for example, caesarean sections or hip replacements) become very high risk. Globally, 480,000 people develop multi-drug resistance each year, and drug resistance is starting to complicate the fight against HIV and malaria, as well (Tanwar *et al.,* 2014). An influential report from the O'Neill Commission predicts that antibiotic resistance will lead to 10 million deaths per year by 2050, surpassing cancer as a source of human mortality.

1.9.2 Mechanisms of multi-drug resistance:

Antibiotic resistance genes might be transferred to pathogenic bacteria infecting humans, particularly under the selection pressure of antibiotics as well as via the "SOS" response (Beaber *et al.,* 2002; Ubeda *et al.,* 2005).Besides long term exposure of microorganisms to high concentration of antibiotics also giving rise to the multi-drug resistant organisms (Li *et al.,* 2002). Researchers have observed that there has been a "sigmoidal rise in resistance over time in the presence of a constant rate of antibiotic consumption" and a threshold level of antibiotic usage needed to "trigger the emergence of resistance to significant levels (Austin *et al.,* 1999)

1.10 Emergence of resistance among UTI pathogens:

Among UTI causing pathogens bacterial resistance have been going on for the last three decades and the available data and reports confirm that the increase in resistance to commonly employed antibiotics is a consequence of inappropriate use of the antimicrobial agent. Surfacing of resistance among the pathogens responsible for UTI is an issue of serious concern and requires an immediate attention in order to derive suitable remedy to overcome the problem Vasudevan *et al*, 2014. Bacterial urinary tract infections (UTIs) are frequent infections in the nosocomial setting. Nosocomial UTIs are almost exclusively complicated UTIs, although the complicating factors may be very heterogenous. The bacterial spectrum of nosocomial UTIs is broad and antibiotic resistance is common Naber *et al,* 2005.

1.11 Literature review:

Previous studies on bacteriological profile and antibiotic susceptibility pattern of bacterial isolates are given below.

Eliakim *et al,* (2013) investigated on Isolation, identification and characterization of urinary tract infections bacteria and the effect of different antibiotics of Masinde Muliro University of Science and Technology, Department of Medical Laboratory Science. This study focused on the frequency of uropathogens and their antibiotic susceptibility in different gender in Madurai District. 30 samples were collected from both male and female of different ages. They viewed the prevalence of both gram positive and gram negative bacteria among them *E.coli* was the predominant isolate along with *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Proteus mirabilis* and *Enterococcus faecalis*. Among the antibiotics tested, chloraphenicol and ciprofloxacin (100%) were found to be effective for empirical treatment of UTI and has covered the majority of urinary pathogens followed by tetracycline, gentamycin and kanamycin (83%), Ampicillin (67%). Streptomycin, Rifampicin and amoxicillin were less effective (50%).

Annarita *et al,* (2017) studied on multi-drug-resistant Gram-negative bacteria causing urinary tract infection. Due to the high empiric use of antibiotics for the treatment of UTI, antibacterial resistance of *Enterobacteriaceae*, specifically the main uropathogens *Escherichia coli* and *Klebsiella pneumoniae*, has significantly increased worldwide. In this article the worldwide epidemiology of resistant Gram-negative bacteria causing UTIs, with a special focus on extended spectrum beta lactamase (ESBL) positive pathogens, as well as new threats such as multi-drug-resistant (MDR) clones (e.g. *E. coli* 131 (ST131) and *K. pneumoniae* ST258), are reviewed. The increased prevalence of MDR *Enterobacteriaceae*, limiting available treatment options for infections caused by these organisms, and the lack of new antibiotics provide good rationale for using older antibiotics, such as fosfomycin, that have been shown to retain some activity against MDR bacteria.

Salih, M.K., *et al* (2016) conducted an investigation on Isolation of Pathogenic Gram-Negative Bacteria from Urinary Tract Infected Patients. This study investigated the susceptibility pattern of different bacteria isolated from urinary tract infection to different antibiotics. 83 uropathogen bacteria were isolated from 300 urine samples taken from patients attended to Tikrit Teaching

Hospital. Bacteria obtained from urine samples were cultured and tested for antimicrobial susceptibility to 16 kinds of antibiotics. The results showed that the bacterial species of *Eschericia coli, Proteus mirabilis, Klebsiella pneumonia, Citrobacter diversus, Citrobacter freundii, Enterobacter aerogenes, Yersinia pestis, Pseudomonas aeruginosa, Klebsiella oxytoca* and *Hafnia alvei* were identified in 44 (53%), 18 (21.7%), 4 (4.8%), 4 (4.8%), 3 (3.6%), 3 (3.6%), 3 (3.6%), 2 (2.4%), 1 (1.2%) and 1 (1.2%), respectively, of the isolates. The results of antimicrobial susceptibility test showed that 83 (100%) isolates were resistant to Ampicillin, Rifampicin and Erythromycin. 75 (90.3%) isolates were resistant to Cefotaxime, 67 (80.7%) isolates were resistant to Tobramyci. 66 (79.5%), 65 (78.3%), 56 (67.4%) and 48 (57.8%) isolates showed susceptibility to Nalidixic acid, Tetracycline, Nitrofurantoin, Chloramphenicol, respectively. 45 (54.2%) isolates were resistant to Azithromycin, Norfloxacin and Ciprofloxacin. Meropenem, Gentamicin, Amikacin, and Imipenem show significant effect on 35 (42.1%), 32 (38.5%), 27 (32.5%) and 1 (1.2%) isolates, respectively.

Iqra jamil *et al,* (2014) investigated on Multi-drug resistant *Klebsiella pneumonia* causing urinary tract infections in children. One thousand and fifteen (1015) urine samples were collected aseptically from The Children Hospital Lahore, Pakistan. Multi-drug resistant (MDR)Klebsiella pneumonia has been associated with different types of infections and the most important aspect is the emergence of MDR strains particularly in hospitalized children. Antimicrobial susceptibility was determined using Kirby-Bauer disc diffusion method, Of the 1015 urinespe cimens, 230 (22.6%) were positive for bacterial growth Out of these positive cultures predominantly Gram-negative rods (90%) were isolated and major pathogens were K. pneumonia (40%) and Escherichia coli (33%). Antimi crobial susceptibility pattern of K. pneumoniae showed that m ore than 70% of these pathogens were resistant to cephalosporins, 69% to ciprofloxacin and amoxicillin-clavulanic acid and 63% to norfloxacin and nalidixic acid while most effective drugs were pipracillin-tazobactam and meropenem.

Kimberly .A *et al,* studied on Gram-Positive Uropathogens, Polymicrobial Urinary Tract Infection, and the Emerging Microbiota of the Urinary Tract. They found that Gram-positive bacteria are a common cause of urinary tract infection (UTI), particularly among individuals who are elderly, pregnant, or who have other risk factors for UTI. In this case the infection mostly caused by *Staphylococcus saprophyticus, Enterococcus faecalis*, and *Streptococcus agalactiae*. otherwise

underreported Gram-positive pathogens of the urinary tract including *Aerococcus, Corynebacterium, Actinobaculum,* and *Gardnerella*. UTI (>90% as defined by the culture of a uropathogen from urine with >100,000 colony forming units (CFU) per ml). They collected all the samples from elder patients and all the samples showed cfu more than 10^5 / ml. Here the studied showed that Gram-positive bacteria such as *Staphylococcus* and *Streptococcus* shows the higher prevalence in elderly patients.

Katarzyna *et al*,(2001) investigated on Antibiotic susceptibility of bacterial strains isolated from urinary tract infections. The aim of this study was to obtain data on susceptibility patterns of pathogens responsible for urinary tract infections (UTIs) in Poland to currently used antimicrobial agents. 141 pathogens from hospital-acquired infections and 460 pathogens from community-acquired infections was collected. The most prevalent aetiological agent was *Escherichia coli* (73.0%), followed by *Proteus* spp. (8.9%) and other species of Enterobacteriaceae (9.6%). Few community infections were caused by Gram-positive bacteria (2.2%). Gram-positive cocci were isolated more frequently from a hospital setting (14.1%) and the most common were *Enterococcus* spp. (8.5%). *Pseudomonas aeruginosa* was found only among hospital isolates and was responsible for 10.7% of infections. *E. coli* isolates from both community and hospital infections were highly susceptible to many antimicrobial agents. Of all Enterobacteriaceae tested, 38 strains (6.9%) were capable of producing ESBLs.

Raul *et a*l, 2011 investigated on UTI infection of women. In a multivariate analysis it was found that urinary incontinence, a history of UTI before menopause, and nonsecretor status were strongly associated with recurrent UTI in young postmenopausal women. Another study described the incidence and risk factors of acute cystitis among nondiabetic and diabetic postmenopausal women.The diabetic patients are also at high risk between the ages 40-65 years.

1.12 Aims and objectives:

The aims of this research work were to isolate, identify and evaluating the prevalence of bacterial contaminants from Urinary tract infected patients. Due to emerging incidence of multi-drug resistant organisms this study also aimed at determining the antibiotic resistance profile and detecting the multi-drug resistant organism from the isolated bacterial contaminants.

Chapter 2

Materials and Methods

2.1 Study area:

The study was conducted at the BRAC University in Dhaka, Bangladesh. The laboratory processing, analysis of data and the overall experimental work were done in Microbiology Research Laboratory of the Department of Mathematics and Natural Sciences of BRAC University.

2.2 Study duration:

The study was conducted during the period May-December, 2017.

2.3 Sample size:

A total of about 25 urine samples were collected from a Diagnostic Center.

2.4 Materials:

2.4.1 Culture media:

Culture media used for bacterial isolation and identification include**:**

2.4.1.1 Nutrient Agar (NA):

Nutrient agar is used for Total Viable Count (TVC) that means the number of colony forming units (cfu) per g (or per ml) of the sample.

2.4.1.2 MacConkey Agar:

MacConkey agar is used for the isolation and differentiation of non-fastidious gram-negative rods, particularly members of the family Enterobacteriaceae. It also can distinguish between lactose fermenting from non-fermenting bacteria.

2.4.1.3 Salmonella-Shigella Agar (SS):

Salmonella-Shigella (SS) agar is used for the isolation, cultivation and differentiation of gram-negative enteric microorganisms isolated from both clinical and non-clinical specimens.

2.4.1.4 Mannitol salt Agar (MSA):

Mannitol Salt agar is used as a selective media for the isolation of pathogens.It is is used as a selective and differential medium for the isolation and identification of *Staphylococcus aureus* and *S.epidermidis*.from clinical and non-clinical specimens.

2.4.1.5 Eosine Methylene Blue Agar (EMB):

This media can differentiate among lactose fermenters and lactose non fermenters bacteria. It is a selective media for gram-negative bacteria. Other coliform such as *Enterobacter aerogenes* and *Klebsiella spp* can also ferment lactose and grow on EMB media.

2.4.1.6 Bacillus cereus Agar (BC Agar):

Bacillus Cereus agar Base with added supplements is used as a selective medium for the isolation and identification of *Bacillus cereus*. It can also detect the other species of *Bacillus*.

2.4.1.7 Hi-Crome UTI agar:

This media is selective for urine infection causing microorganisms such as *Klebsiella pneumonia, Enterococcus fecalis, Staphylococcus aureus*, *Proteus mirabilis, E.coli*, *Pseudomonas aeruginosa* and they produce distinctive different colours on media.

2.4.1.8 Blood agar (BA):

Blood agar (BA) is an enriched medium used to culture those fastidious bacteria or microbes that do not grow easily. It is also a differential medium in allowing the detection of hemolysis (destroying the RBC) by cytolytic toxins secreted by some bacteria, such as certain strains of *Bacillus, Streptococcus, Enterococcus, Staphylococcus, Shigella, Proteus and Aerococcus*. It is used to differentiate bacteria based on their hemolytic properties (β-hemolysis, α-hemolysis and γ-hemolysis (or non-hemolytic). Hemolysis is determined by observing the clear zones around the bacterial growth.

2.4.2 Biochemical test media:

Table 2.1: Media used for biochemical tests

Media used for biochemical tests
Indole broth
Methyl Red (MR) broth
Voges-Proskauer (VP) broth
Simmons citrate agar
Triple Sugar Iron (TSI) agar
Motility Indole Urease (MIU) agar

2.4.3 Stock culture media:

Nutrient broth and glycerol are used as stock culture media. Isolates are inoculated into it and kept at -20^{o} for further use.

2.4.4 Antibiotics:

Antibiotic discs were used for identifying antibiotic sensitive and resistant bacteria, Antibiotics those were used in the study are given in table.

Table 2.2: List of antibiotics and their zone ranges

Serial no	Antibiotic	Disc potency (µg)	Inhibition zone diameter (in mm)		
			Resistant	Intermediate	Susceptible
1	**Azithromycin**	15	≤13	14-17	≥18 / ≥20
2	**Ciprofloxacin**	5	≤15 / ≤20	16-20/ 21-30	≥21 / ≤31
3	**Chloramphenicol**	30	≤12	13-17	≥18
4	**Erythromycin**	15	≤13	14-17	≥18/ ≥20
5	**Ampicillin**	10	≤13/≤28	10-13	≥14
6	**Penicillin-G**	10	≤23	24-28	≥29
7	**Rifampicin**	5	≤16	17-19	≥20
8	**Streptomycin**	10	≤11	12-14	≥15
9	**Cefepime**	30	≤14/≤21	15-17	≥18
10	**Tetracycline**	30	≤11	12-14	≥15

2.5Methods:

2.5.1 Sample collection:

The samples were collected from UTI suspected patients. Samples were collected by the method of the American Public Health Association (Skobe *et al*, 1999). The samples were mostly collected at early morning because it is more concentrated and abnormalities are easier to detect. Samples were mostly mid-stream urine that is not the first or last part of urine that comes out. This reduces the risk of the sample being contaminated with bacteria from hands, the skin around the urethra, the tube that carries urine out of the body. The samples must be transported by 24 hours and were carried in an ice box to suppress the growth of unwanted organisms. Then it was immediately transported to the Laboratory for further processing and analysis.

Table 2.3: Sample Collection: Patients Id, Date, Time, Number of the isolates found and their given name in the study

Sample No	Patient ID	Date	Time	Number of the isolates found	Isolates ID
1	R-17	7/5/2017	10:30 am	7	1a, 1b, 1c, 1d, 1e, 1f, 1g
2	R-16	7/5/2017	10:30 am	1	2
3	R-013586	7/5/2017	10:30 am	3	3a, 3b, 3c
4	R-013641	7/5/2017	10:30 am	1	4
5	D69542	11/16/2017	12:30 pm	3	a, b ,c
6	D69537	11/16/2017	12:30 pm	3	d, e, f
7	D69522	11/16/2017	12:30 pm	4	g, h, i, j
8	D69551	11/16/2017	12:30 pm	2	k l, m, n, o, p
9	D69499	11/16/2017	12:30 pm	2	q, r
10	D128346	9/7/2017	11:00 am	2	U_{15}, U_{18}
11	S7659	9/7/2017	11:00 am	6	U_{42}, U_{43}, U_{47}, U_{48}, U_{49}, U_{50}
12	D128496	9/7/2017	11:00 am	2	U_{11}, U_{14}
13	D128497	9/7/2017	11:00 am	7	U_4, U_5, U_6, U_7, U_8, U_9, U_{10}
14	D128358	9/7/2017	11:00 am	2	U_{20}, U_{24}
15	M26367	9/7/2017	11:00 am	2	U_{29}, U_{31}
16	S7710	9/7/2017	11:00 am	8	U_{33}, U_{34}, U_{35}, U_{36}, U_{38}, U_{39}, U_{40}, U_{41}
17	D128537	9/7/2017	11:00 am	1	U_2
18	B-1	10/9/2017	1:00 pm	4	B_3, B_6, B_{10}, B_{13}
19	B-2	10/9/2017	1:00 pm	2	B_{15}, B_{16}
20	E33220	15/10/2017	12:00 pm	7	I_1, I_3, I_4, I_5, I_6, I_8, I_9

21	M52249	15/10/2017	12:00 pm	6	$I_{12}, I_{17}, I_{18}, I_{19}, I_{21}, I_{22},$
22	M52923	15/10/2017	12:00 pm	1	I_{24}
23	E33170	15/10/2017	12:00 pm	5	$I_{25}, I_{26}, I_{28}, I_{31}, I_{32}$
24	E33199	15/10/2017	12:00 pm	4	$I_{33}, I_{35}, I_{36}, I_{37}$
25	M54970	15/10/2017	12:00 pm	2	I_{40}, I_{42}

2.6.1 Colony-forming unit:

In microbiology, a colony-forming unit (CFU) is a unit used to estimate the number of viable bacteria in a sample. Viable is defined as the ability to multiply via binary fission under the controlled conditions. Colony forming units are used as a measure of the number of microorganisms present in or on surface of a sample. Colony forming units may be reported as CFU per unit weight, CFU per unit area, or CFU per unit volume depending on the type of sample tested. To determine the number of colony forming units, sample was prepared and spreaded on a surface of an agar and then incubated at some suitable temperature for a number of days. The colonies that form are counted. CFU is not a measure for individual cells or spores as a colony may be formed from a single or a mass of cells or spores. Rene *et al*, 2013.

Table 2.4: CFU count of UTI patients from Urine samples.

Sample No	Patient ID	CFU/ml
1	R-17	$1.70x10^8$
2	R-16	$1.12x\ 10^7$
3	R-013586	$7.9x\ 10^7$
4	R-013641	TNTC
5	D69542	$2.9x\ 10^7$
6	D69537	$2.9x\ 10^7$
7	D69522	TNTC
8	D69551	$9.2x\ 10^6$
9	D69499	TNTC
10	D128346	$1.97x\ 10^8$
11	S7659	TNTC
12	D128496	TNTC
13	D128497	TNTC
14	D128358	$1.78x\ 10^8$
15	M26367	$9.7x10^7$
16	S7710	$2.62x\ 10^8$
17	D128537	$1.15x\ 10^8$

18	B-1	TFTC
19	B-2	TFTC
20	E33220	$1.75x\ 10^7$
21	M52249	$8.5x\ 10^6$
22	M52923	$1.10x\ 10^7$
23	E33170	TNTC
24	E33199	TNTC
25	M54970	$5.50x\ 10^6$

2.6.2 Sample Analysis:

The collected samples were processed to identify the bacteria through gram staining and biochemical test.

2.6.3 Biochemical tests:

2.6.3.1 Indole test:

Indole production test was done to determine the ability of microorganisms to convert tryptophan into indole.

For indole test each indole broth containing peptone, sodium chloride was taken. Using sterile technique, small amount of the experimental bacteria from fresh culture was inoculated into the tubes by means of loop inoculation method with an inoculating loop. After incubation on the following day by adding Kovacs reagent positive and negative test was detected. If it developed red colour layer then it indicated positive test. (Cappuccino & Sherman, 2005).

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2.6.3.2 Methyl red (MR) test:

Methyl red test was done to determine the ability of the bacteria to oxidize glucose with the production and stabilization of high concentration of acid end products.

For methyl red test each MR broth containing 5 ml of dipeptone, dextrose and potassium phosphate was taken. Using sterile technique, each tube was inoculated by fresh culture of experimental bacteria by means of loop inoculation method. After incubation on the following day after adding methyl red indicator If red colour develops then it indicates that the organism was capable of fermenting glucose with the production of high concentration of acid. If orange or yellow colour develops then it indicates methyl red negative result (Cappuccino & Sherman, 2005).

2.6.3.3 Voges-Proskauer (VP) test:

The Voges-Proskauer (VP) test was done to determine if an organism produces acetylmethyl carbinol from glucose fermentation.

For Voges-Proskauer test each VP broth containing dipeptone, dextrose and potassium phosphate was taken. Using sterile technique, each tube was inoculated by fresh culture of experimental bacteria by means of loop inoculation method. After incubation by adding of Barritt's reagent if red colour developed then it indicated that the organism was capable of fermenting glucose with ultimate production of acetyl methyl carbinol and it indicates positive result. If no colour developed then it indicated voges- proskauer negative result. (Cappuccino & Sherman, 2005)

2.6.3.4 Citrate utilization test:

Citrate utilization test was done to differentiate among enteric organisms on the basis of their ability to ferment citrate as a sole source of carbon by the enzyme citrase.

For citrate utilization test each vial containing 2.5 ml of simmons citrate agar was taken. Using sterile technique, small amount of the experimental bacteria from fresh culture was inoculated into the vials by means of a streak inoculation method with an inoculating loop. After incubation, if the Prussian blue colour developed then it indicated the citrate positive result which means the organism was capable of fermenting citrate as a sole source of carbon. If there was no colour change then it indicated citrate negative result. (Cappuccino & Sherman, 2005)

2.6.3.5 Triple sugar-iron (TSI) agar test:

Triple sugar iron agar test was done to differentiate between Gram negative enteric bacilli based on their ability to ferment carbohydrate and reduce hydrogen sulfide.

For TSI test each tube containing TSI agar was taken. Using sterile technique, small amount of the experimental bacteria from fresh culture was inoculated into the tubes by means of stab inoculation method with an inoculating needle. After incubation the results were recorded based on the following observation (Cappuccino & Sherman, 2005).

Table 2.5: Interpretation of Triple sugar iron (TSI) test result

TSI Test

Results (slant/butt)	Symbol	Interpretation
Red/yellow	K/A	Glucose fermentation only; Peptone catabolized
Yellow/yellow	A/A	Glucose and lactose and/or sucrose fermentation
Red/red	K/K	No fermentation; Peptone catabolized
Red/no color change	K/NC	No fermentation; Peptone used aerobically
Yellow/yellow with bubbles	A/A,G	Glucose and lactose and/or sucrose fermentation; Gas produced
Red/yellow with bubbles	K/A,G	Glucose fermentation only; Gas produced
Red/yellow with bubbles and black precipitate	K/A,G, H2S	Glucose fermentation only; Gas produced; H2S produced
Red/yellow with black precipitate	K/A, H2S	Glucose fermentation only; H2S produced
Yellow/yellow with black precipitate	A/A, H2S	Glucose and lactose and/or sucrose fermentation; H2S produced
No change/no change	NC/NC	No fermentation
A=acid production; K=alkaline reaction; G=gas production; H2S=sulfur reduction		

2.6.3.6 Catalase test:

Catalase test was done to determine the ability of the bacteria to degrade hydrogen peroxide by producing the enzyme catalase.

For catalase test a sterile microscopic slide was taken. A drop of the catalase reagent 3% Hydrogen peroxide was placed on the glass slide. Using a sterile inoculating loop, a small amount of bacteria from 24-hour pure culture was placed onto the reagent drops of the microscopic slide. An immediate bubble formation indicated a positive result and no bubble formation indicated catalase negative result (Reiner, 2010).

2.6.3.7 Oxidase test:

Oxidase test was done to determine the presence of the enzyme cytochrome oxidase in the bacteria.

Filter papers were taken, and two drops of oxidase reagent (p-Amino dimethyl aniline oxalate) were added onto the filter papers (Whatman, 1MM). Using an inoculating loop, a well isolated colony from pure 24-hour culture was picked and rubbed onto filter paper and observed for colour change. A positive reaction would turn the paper from violet to purple within 1 to 30 seconds. Delayed reactions should be ignored as that might give false positive result (Shields & Cathcart, 2010).

2.6.3.8 MIU (Motility-indole-urease) test:

MIU test was done for determining the motility of bacteria, indole production and urea degradation by means of the enzyme urease.

At first small amount of the experimental bacteria from fresh culture was inoculated into the tubes by means of stab inoculation method. The organism would spread throughout the test tube from downward to the upward of the test tube, if the organism is motile. The colour of the media will turn to deep pink if the organism is positive for urease test. If yellow colour develops then it indicates urease negative result. Motility was observes by the spreading of the organism from the stab line. (Cappuccino & Sherman, 2005).

2.7 Antibiotic susceptibility testing (AST):

Antibiotic susceptibility test is done to find the sensitivity or susceptibility and resistance pattern of bacteria to antibiotics.

2.7.1 Disk diffusion method:

Various methods can be used for antibiotic susceptibility testing but among them disk diffusion method is most common. Because of convenience, efficiency and cost, the disk diffusion method is probably the most widely used method for determining antimicrobial resistance. In this research work the antibiotic susceptibility testing of the organisms were performed by agar disc diffusion method by Kirby–Bauer antibiotic susceptibility testing, and interpreted according to CLSI standards and guidelines (Wayne, 2009).

2.7.1.1 Preparation of inoculum:

Pure culture plate of one of the organisms to be tested was selected. Using a sterile loop a colony from the plate was aseptically emulsified in the tube containing sterile saline solution and it was mixed thoroughly to ensure that no solid material from the colony is visible in the saline solution. The tube was vortexed properly so that the suspension becomes homogenous. (Labtronics; ISO 9001: 2008 Certified).

Then we compared it with the commercially available McFarland solution.

2.7.1.2 Inoculation of the Muller Hinton Agar (MHA) plates:

Muller Hinton agar plates were prepared. A sterile cotton swab was taken and was dipped into the broth culture of the organism. The swab was streaked at least four to six times onto the dried surface of the MHA plate to make a lawn culture and to ensure that the cotton swab is touched entirely on the agar surface. After the streaking is complete the plate is allowed to dry for 5 minutes.

2.7.1.3 Placing the antibiotic discs on MHA plates:

Sterilized forceps were used to place the antibiotic discs. After taking the discs, the discs were gently pressed onto the surface of the agar using flame sterilized forceps. Once all the discs were properly placed, the MHA plates were inverted and incubated.

2.7.1.4 Measuring zone:

After 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. A metric ruler is used to measure the diameter of the zone of inhibition for each antibiotic used. This zone is measured in millimeter and compared to a standard interpretation chart used to categorize the isolates as susceptible, intermediately susceptible or resistant.

Chapter 3

Results

3.1 Bacterial isolation and identification:

Samples were collected from UTI suspected patients. These samples were streaked on various selective and differential media for identifying the organisms present in urine sample. The results showed that all samples were UTI positive that means more than 10^5 bacteria/ml of urine. Both the Gram positive and Gram negative organisms were found from the samples. All the isolates were identified based on cultural, morphological and biochemical characteristics of the isolates. Physical and Biochemical characteristics of the isolates obtained from the study are shown in Table 3.1 and Table 3.2.

3.1.1 Cultural and morphological characteristics of bacterial isolates:

In Table 3.1 the colour, shape of the colonies on various selective, differential and enriched media and the morphology of the bacterial colonies on nutrient agar are explained.

Table 3.1: Cultural and Morphological Characteristics of the Bacterial Isolates from UTI suspected patients on various Selective, Differential, Enriched and Nutrient Media

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	**Mannitol Salt Agar (MSA)**	**Salmonella Shigella Agar (SS)**	**Eosine Methylene Blue Agar (EMB)**	**Bacillus Cereus Agar (BC Agar)**	**Hi-Chrome Agar**	**Blood Agar Hemo-Lysis**	**Size**	**Color**	**Form**	**Margin**	**Elevation**	
1. 1a					Blue colonies	Green colonies	Alpha Hemo-Lysis	Large	Off white	Regular	Undulate	Raised	***Bacillus spp.***
2. 1b	Pink colonies					Blue colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Undulate	Convex	***Enterobacter spp.***
3. 1c	Pink colonies					Blue colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Undulate	Convex	***Enterobacter spp.***
4. 1d	Pink colonies					Mucoid blue colonies	Alpha Hemo-Lysis	Small	Off white	Circular	Entire	Raised	***Klebsiella spp.***
5. 1e		White colonies				Green coloured colonies	Gamma Hemo-Lysis	Small	Yellow	Circular	Entire	Convex	***Micrococcus spp.***
6. 1f		Yellow colonies				Golden yellow colonies	Alpha Hemo-Lysis	Small	Yellow	Circular	Entire	Raised	***Staphylococcus spp.***

Table 3.1: Cultural and Morphological Characteristics of the Bacterial Isolates from UTI suspected patients on various Selective, Differential, Enriched and Nutrient Media

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	**Mannitol Salt Agar (MSA)**	**Salmonella Shigella Agar (SS)**	**Eosine Methylene Blue Agar (EMB)**	**Bacillus Cereus Agar (BC Agar)**	**Hi-Chrome Agar**	**Blood Agar Hemo-Lysis**	**Size**	**Color**	**Form**	**Margin**	**Elevation**	
7. 1g						Green colonies	Beta Hemo-Lysis	Large	Off white	Circular	Entire	Convex	***Micrococcus spp.***
8. 2		Yellow colonies				Golden yellow colonies	Alpha Hemo-Lysis	Small	Off white	Circular	Entire	Raised	***Staphylococcus spp.***
9. 3a					Blue colonies	Light green colonies	Beta Hemo-Lysis	Large	Off white	Irregular	Undulate	Raised	***Bacillus spp.***
10. 3b			Reddish orange colonies			White colonies	Beta Hemo-Lysis	Medium	Orange	Circular	Entire	Raised	***Shigella spp.***
11. 3c			Reddish orange colonies			White colonies	Beta Hemo-Lysis	Medium	Orange	Circular	Entire	Raised	***Shigella spp.***
12. 4			Reddish orange colonies			White colonies	Beta Hemo-Lysis	Medium	Orange	Circular	Entire	Raised	***Shigella spp.***

Table 3.1: Cultural and Morphological Characteristics of the Bacterial Isolates from UTI suspected patients on various Selective, Differential, Enriched and Nutrient Media

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Salmonella Shigella Agar (SS)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
13. a	Pink colonies					Blue colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Undulate	Convex	***Enterobacter spp.***
14. b		Yellow colonies				Golden yellow colonies	Gamma Hemo-Lysis	Medium	Orange	Irregular	Undulate	Convex	***Staphylococcus spp***
15. c	Pink colonies			Purple colonies		Mucoid blue colonies	Alpha Hemo-Lysis	Medium	Off white	Irregular	Undulate	Raised	***Klebsiella spp.***
16. d	Pink colonies					Blue colonies	Alpha Hemo-Lysis	Medium	Off white	Irregular	Undulate	Raised	***Enterobacter spp.***
17. e	Pink colonies					Blue colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Raised	***Enterobacter spp.***
18. f		Yellow colonies				Golden yellow colonies	Alpha Hemo-Lysis	Medium	Orange	Circular	Entire	Raised	***Staphylococcus spp.***

Table 3.1: Cultural and Morphological Characteristics of the Bacterial Isolates from UTI suspected patients on various Selective, Differential, Enriched and Nutrient Media

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Salmonella Shigella Agar (SS)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
19. g	Pink colonies			Purple colonies		Mucoid blue colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Raised	***Klebsiella spp.***
20. h	Pink colonies					Green colonies	Alpha Hemo-Lysis	Medium	Orange	Circular	Entire	Raised	***Enterobacter spp.***
21. i	Pink colonies					Blue colonies	Beta Hemo-Lysis	Medium	Off white	Irregular	Lobate	Convex	***Enterobacter spp.***
22. j	Pink colonies			Metallic green sheen		Purple colonies	Alpha Hemo-Lysis	Small	Off white	Circular	Entire	Raised	***E.coli***
23. k	Pink colonies			Metallic green sheen		Purple colonies	Alpha Hemo-Lysis	Medium	Off white	Irregular	Lobate	Convex	***E.coli***
24. l			Reddish orange colonies			White colonies	Alpha Hemo-Lysis	Medium	Yellow	Rhizoid	Filamentous	Raised	***Shigella spp.***

Table 3.1: Cultural and Morphological Characteristics of the Bacterial Isolates from UTI suspected patients on various Selective, Differential, Enriched and Nutrient Media

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	**Mannitol Salt Agar (MSA)**	**Salmonella Shigella Agar (SS)**	**Eosine Methylene Blue Agar (EMB)**	**Bacillus Cereus Agar (BC Agar)**	**Hi-Chrome Agar**	**Blood Agar Hemo-Lysis**	**Size**	**Color**	**Form**	**Margin**	**Elevation**	
25. m			Reddish orange colonies			Golden yellow colonies	Alpha Hemo-Lysis	Medium	Off white	Irregular	Undulate	Convex	***Shigella spp.***
26. n	Pink colonies			Metallic green sheen		Purple colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Raised	***E.coli***
27. o	Pink colonies					Blue colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Raised	***Enterobacter spp.***
28. p						Browh colonies	Gamma Hemo-Lysis	Small	Off white	Irregular	Undulate	Convex	***Proteus spp.***
29. q					Blue colonies	Light green colonies	Beta Hemo-Lysis	Large	Off white	Irregular	Undulate	Convex	***Bacillus spp.***
30. r			Reddish orange colonies			White colonies	Alpha Hemo-Lysis	Small	Transparent	Circular	Entire	Convex	***Shigella spp.***

Table 3.1: Cultural and Morphological Characteristics of the Bacterial Isolates from UTI suspected patients on various Selective, Differential, Enriched and Nutrient Media

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	**Mannitol Salt Agar (MSA)**	**Salmonella Shigella Agar (SS)**	**Eosine Methylene Blue Agar (EMB)**	**Bacillus Cereus Agar (BC Agar)**	**Hi-Chrome Agar**	**Blood Agar Hemo-Lysis**	**Size**	**Color**	**Form**	**Margin**	**Elevation**	
31. U_2	Pink colonies					Green colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Raised	***Klebsiella spp.***
32. U_4		Yellow colonies				Golden yellow colonies	Alpha Hemo-Lysis	Medium	Orange	Circular	Entire	Raised	***Staphylococcus spp.***
33. U_5				Metallic green sheen		Purple colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Raised	***E.coli.***
34. U_6				Metallic green sheen		Purple colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Raised	***E.coli***
35. U_7				Metallic green sheen		Purple colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Convex	***E.coli***
36. U_8				Metallic green sheen		Purple colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Convex	***E.coli***

Table 3.1: Cultural and Morphological Characteristics of the Bacterial Isolates from UTI suspected patients on various Selective, Differential, Enriched and Nutrient Media

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	**Mannitol Salt Agar (MSA)**	**Salmonella Shigella Agar (SS)**	**Eosine Methylene Blue Agar (EMB)**	**Bacillus Cereus Agar (BC Agar)**	**Hi-Chrome Agar**	**Blood Agar Hemo-Lysis**	**Size**	**Color**	**Form**	**Margin**	**Elevation**	
37. U_9		Yellow colonies				Golden yellow colonies	Gamma Hemo-Lysis	Medium	Off white	Circular	Undulate	Flat	***Staphylococcus spp.***
38. U_{10}		Yellow colonies				Green colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Convex	***Staphylococcus spp.***
39. U_{11}		Yellow colonies				Golden yellow colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Lobate	Raised	***Staphylococcus spp.***
40. U_{14}	Pink colonies			Purple colonies		Mucoid blue colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Lobate	Raised	***Klebsiella spp.***
41. U_{15}					Blue colonies	Green colonies	Alpha Hemo-Lysis	Large	Off white	Circular	Entire	Flat	***Bacillus spp.***
42. U_{18}	Pink colonies					Mucoid blue colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Raised	***Klebsiella spp.***

Table 3.1: Cultural and Morphological Characteristics of the Bacterial Isolates from UTI suspected patients on various Selective, Differential, Enriched and Nutrient Media

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Salmonella Shigella Agar (SS)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
43. U_{20}	Pink colonies					Blue colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Raised	***Enterobacter spp.***
44. U_{24}	Pink colonies					Mucoid blue colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Lobate	Raised	***Klebsiella spp.***
45. U_{29}	Pink colonies					Mucoid blue colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Lobate	Raised	***Klebsiella spp.***
46. U_{31}	Pink colonies					Mucoid blue colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Raised	***Klebsiella spp.***
47. U_{33}	Pink colonies			Purple colonies		Mucoid blue colonies	Alpha Hemo-Lysis	Medium	Off white	Irregular	Lobate	Convex	***Klebsiella spp.***
48. U_{34}	Pink colonies					Mucoid blue colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Lobate	Raised	***Klebsiella spp.***

Table 3.1: Cultural and Morphological Characteristics of the Bacterial Isolates from UTI suspected patients on various Selective, Differential, Enriched and Nutrient Media

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Salmonella Shigella Agar (SS)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
49. U_{35}	Pink colonies					Mucoid blue colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Lobate	Raised	***Klebsiella spp.***
50. U_{36}	Pink colonies					Mucoid blue colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Lobate	Raised	***Klebsiella spp.***
51. U_{38}						Brown colonies	Alpha Hemo-Lysis	Small	Off white	Irregular	Undulate	Convex	***Proteus spp.***
52. U_{39}		Yellow colonies				Golden yellow colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Convex	***Staphylococcus spp.***
53. U_{40}		Yellow colonies				Golden yellow colonies	Gamma Hemo-Lysis	Medium	Off white	Circular	Entire	Convex	***Staphylococcus spp.***
54. U_{41}						Brown colonies	Gamma Hemo-Lysis	Small	Off white	Irregular	Undulate	Convex	***Proteus spp.***

Table 3.1: Cultural and Morphological Characteristics of the Bacterial Isolates from UTI suspected patients on various Selective, Differential, Enriched and Nutrient Media

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Salmonella Shigella Agar (SS)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
55. U_{42}	Pink colonies					Mucoid blue colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Raised	***Klebsiella spp.***
56. U_{43}	Pink colonies			Purple colonies		Mucoid blue colonies	Alpha Hemo-Lysis	Medium	Off white	Irregular	Undulate	Convex	***Klebsiella spp.***
57. U_{47}			Reddish orange colonies			White colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Raised	***Shigella spp.***
58. U_{48}	Pink colonies					Mucoid blue colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Raised	***Klebsiella spp.***
59. U_{49}	Pink colonies					Blue colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Lobate	Raised	***Enterobacter spp.***
60. U_{50}	Pink colonies					Blue colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Raised	***Enterobacter spp.***

Table 3.1: Cultural and Morphological Characteristics of the Bacterial Isolates from UTI suspected patients on various Selective, Differential, Enriched and Nutrient Media

Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	**Mannitol Salt Agar (MSA)**	**Salmonella Shigella Agar (SS)**	**Eosine Methylene Blue Agar (EMB)**	**Bacillus Cereus Agar (BC Agar)**	**Hi-Chrome Agar**	**Blood Agar Hemo-Lysis**	**Size**	**Color**	**Form**	**Margin**	**Elevation**	
61. B_3					Blue colonies	Green colonies	Beta Hemo-Lysis	Large	Off white	Irregular	Undulate	Convex	***Bacillus spp.***
62. B_6					Blue colonies	Green colonies	Beta Hemo-Lysis	Large	Off white	Irregular	Undulate	Raised	***Bacillus spp.***
63. B_{10}					Blue colonies	Green colonies	Beta Hemo-Lysis	Large	Off white	Irregular	Lobate	Convex	***Bacillus spp.***
64. B_{13}		Yellow colonies				Golden yellow colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Convex	***Staphylococcus spp.***
65. B_{15}		Yellow colonies				Golden yellow colonies	Beta Hemo-Lysis	Medium	Off white	Circular	Entire	Convex	***Staphylococcus spp.***
66. B_{16}					Blue colonies	Green colonies	Beta Hemo-Lysis	Large	Off white	Irregular	Undulate	Convex	***Bacillus spp.***

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Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Salmonella Shigella Agar (SS)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
67. I_1	Pink colonies					Blue colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Raised	***Enterobacter spp.***
68. I_3	Pink colonies					Blue colonies	Beta Hemo-Lysis	Medium	Off white	Circular	Entire	Raised	***Enterobacter spp.***
69. I_4	Pink colonies					Blue colonies	Beta Hemo-Lysis	Medium	Off white	Circular	Entire	Raised	***Enterobacter spp.***
70. I_5	Pink colonies					Blue colonies	Beta Hemo-Lysis	Medium	Off white	Circular	Entire	Raised	***Enterobacter spp.***
71. I_6					Blue colonies	Green colonies	Beta Hemo-Lysis	Large	Off white	Irregular	Undulate	Convex	***Bacillus spp.***
72. I_8			Reddish orange colonies			White colonies	Gamma Hemo-Lysis	Medium	Off white	Circular	Entire	Raised	***Shigella spp.***

Table 3.1: Cultural and Morphological Characteristics of the Bacterial Isolates from UTI suspected patients on various Selective, Differential, Enriched and Nutrient Media

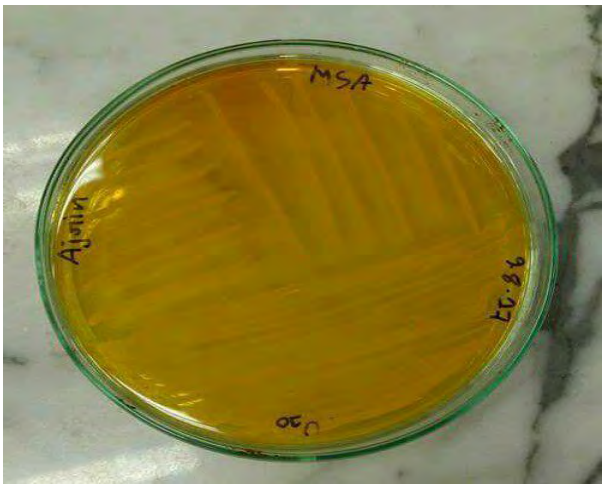
Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Salmonella Shigella Agar (SS)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
73. I_9		Yellow colonies				Golden yellow colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Convex	***Staphylococcus spp.***
74. I_{12}		Yellow colonies				Golden yellow colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Convex	***Staphylococcus spp.***
75. I_{17}	Pink colonies					Mucoid blue colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Raised	***Klebsiella spp.***
76. I_{18}			Reddish orange colonies			White colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Raised	***Shigella spp.***
77. I_{19}			Reddish orange colonies			White colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Raised	***Shigella spp.***
78. I_{21}		Yellow colonies				Golden yellow colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Convex	***Staphylococcus spp.***

Table 3.1: Cultural and Morphological Characteristics of the Bacterial Isolates from UTI suspected patients on various Selective, Differential, Enriched and Nutrient Media

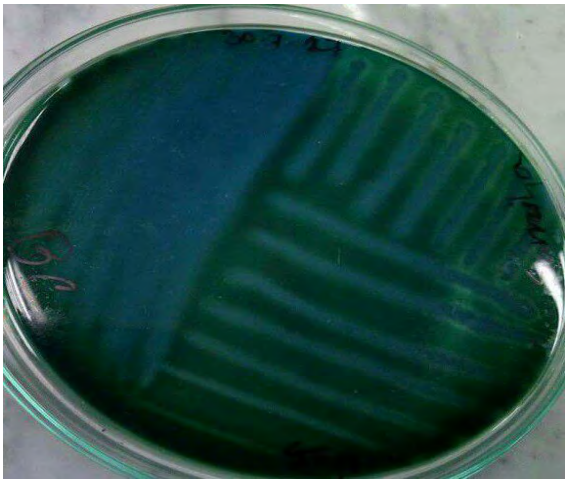
Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	Mannitol Salt Agar (MSA)	Salmonella Shigella Agar (SS)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Hi-Chrome Agar	Blood Agar Hemo-Lysis	Size	Color	Form	Margin	Elevation	
79. I_{22}		Yellow colonies				Golden yellow colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Convex	***Staphylococcus spp.***
80. I_{24}					Blue colonies	Light green colonies	Beta Hemo-Lysis	Large	Off white	Irregular	Undulate	Convex	***Bacillus spp.***
81. I_{25}		Yellow colonies				Golden yellow colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Convex	***Staphylococcus spp.***
82. I_{26}		Yellow colonies				Golden yellow colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Convex	***Staphylococcus spp.***
83. I_{28}		Yellow colonies				Golden yellow colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Convex	***Staphylococcus spp.***
84. I_{31}	Pink colonies			Purple colonies		Mucoid blue colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Raised	***Klebsiella spp.***

Table 3.1: Cultural and Morphological Characteristics of the Bacterial Isolates from UTI suspected patients on various Selective, Differential, Enriched and Nutrient Media

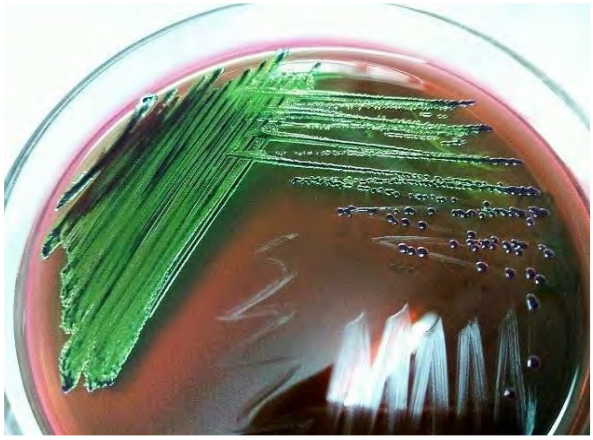
Isolates ID	Growth on Selective, Differential and Enriched Media							Colony morphology on Nutrient Agar					Suspected organism
	Mac-Conkey Agar	**Mannitol Salt Agar (MSA)**	**Salmonella Shigella Agar (SS)**	**Eosine Methylene Blue Agar (EMB)**	**Bacillus Cereus Agar (BC Agar)**	**Hi-Chrome Agar**	**Blood Agar Hemo-Lysis**	**Size**	**Color**	**Form**	**Margin**	**Elevation**	
85. I_{32}	Pink colonies			Purple colonies		Mucoid blue colonies	Alpha Hemo-Lysis	Medium	Off white	Circular	Entire	Raised	***Klebsiella spp.***
86. I_{33}						Yellow colonies	Gamma Hemo-Lysis	Large	Off white	Circular	Entire	Convex	***Micrococcus spp.***
87. I_{35}					Blue colonies	Green colonies	Beta Hemo-Lysis	Large	Off white	Irregular	Undulate	Convex	***Bacillus spp.***
88. I_{36}						Yellow colonies	Gamma Hemo-Lysis	Large	Off white	Circular	Entire	Convex	***Micrococcus spp.***
89. I_{37}					Blue colonies	Green colonies	Beta Hemo-Lysis	Large	Off white	Irregular	Undulate	Convex	***Bacillus spp.***
90. I_{40}					Blue colonies	Green colonies	Beta Hemo-Lysis	Large	Off white	Irregular	Undulate	Convex	***Bacillus spp.***
91. I_{42}					Blue colonies	Green colonies	Beta Hemo-Lysis	Large	Off white	Irregular	Undulate	Convex	***Bacillus spp.***



Growth of *Staphylococcus spp* on MSA



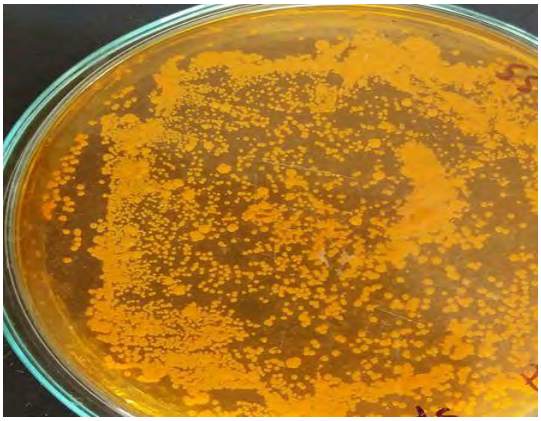
Growth of *Bacillus spp* on BC



Growth of *E. coli* on EMB

Growth of *Klebsiella spp* on MacConkey

Growth of *Enterobacter spp* on MacConky



Growth of *Shigella spp* on SS

Figure: **3.1: Bacterial growth on various selective media**

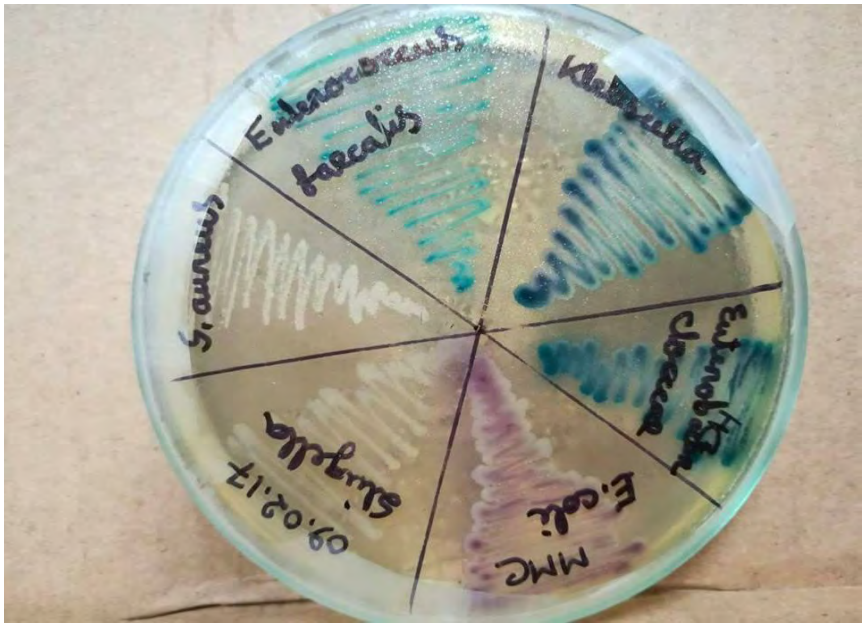
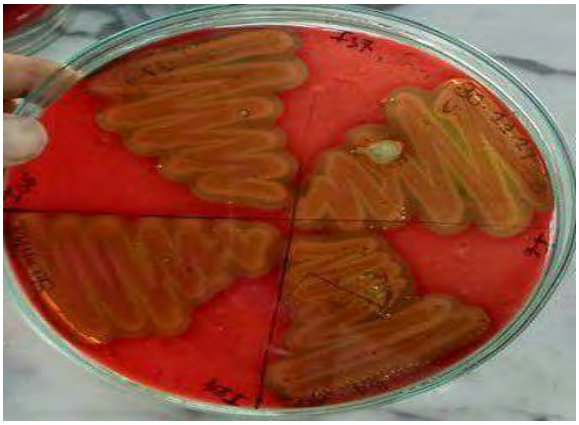
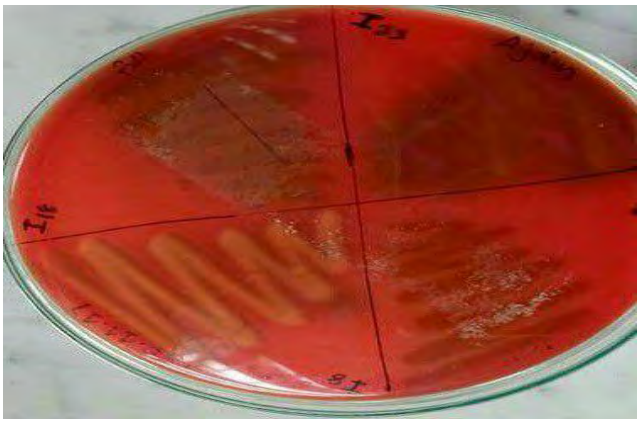


Figure 3.2: Growth of various organisms on Hi-Chrome agar



Beta hemolysis



Gamma hemolysis

Alpha Hemolysis

Figure: 3.3: Bacterial growth on Blood agar

3.1.2 Biochemical characteristics of the bacterial isolates:

Bacteria those were isolated from UTI suspected patients were tested by different types of biochemical tests. Organisms were analyzed and identified with the help of reference books including Bergey's manual of Systematic Bacteriology and Cappuccino and Sherman. The biochemical tests that were performed are described precisely in materials and method chapter 2 and the biochemical test results of the isolates are given below in Table 3.2.

Table 3.2: Biochemical characteristics of the bacteria isolated from UTI suspected patient

Isolates No	Isolates ID	Gram Staining		Indole test	Methyl Red (MR) Test	Voges- Proskauer (VP) Test	Citrate Test	Triple Sugar Iron test (TSI)						MIU Test			Catalase test	Oxidase Test	Suspected organism
		Gram Reaction	Shape					Slant/butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	Motility	Indole	Urease			
1	1a	+	Long rods	-	-	-	-	R/Y	+	-	-	-	-	+	-	-	+	-	***Bacillus spp.***
2	1b	-	Short rods	-	-	-	-	Y/Y	+	+	+	-	-	+	-	-	+	-	***Enterobacter spp***
2	1c	-	Short rods	-	-	-	-	Y/Y	+	+	+	-	-	-	-	-	+	-	***Enterobacter spp***
4	1d	-	Short rods	-	-	-	-	Y/Y	+	+	+	-	-	+	-	+	+	-	***Klebsiella spp***
5	1e	+	Cocci	-	-	-	-	Y/Y	+	+	+	-	-	-	-	-	-	-	***Micrococcus spp***
6	1f	+	Cocci in cluster	-	-	-	-	Y/Y	+	+	+	-	-	+	-	-	+	-	***Staphylococcus spp***
7	1g	+	Cocci	-	-	-	-	Y/Y	+	+	+	-	-	-	-	-	+	-	***Micrococcus spp***
8	2	+	Cocci in cluster	-	-	-	-	Y/Y	+	+	+	-	-	+	-	-	+	-	***Staphylococcus spp***
9	3a	+	Long rods	-	+	+	+	R/Y	+	-	-	-	-	+	-	-	+	-	***Bacillus spp***
10	3b	-	Rods	-	+	-	-	R/R	-	-	-	+	-	-	-	-	+	-	***Shigella spp***
11	3c	-	Rods	-	+	-	+	R/R	-	-	-	+	-	-	-	-	+	-	***Shigella spp***
12	4	-	Rods	-	-	-	-	R/R	-	-	-	+	-	-	-	-	+	-	***Shigella spp***
13	a	-	Short rods	-	-	+	+	Y/Y	+	+	+	-	+	+	-	+	-	-	***Enterobacter spp***
14	b	+	Cocci in cluster	-	+	-	-	Y/Y	+	+	+	-	-	+	-	-	+		***Staphylococcus spp***
15	c	-	Short rods	-	+	+	+	Y/Y	+	+	+	-	+	+	-	+	+	-	***Klebsiella spp***
16	d	-	Short rods	-	+	+	+	Y/Y	+	+	+	-	-	+	-	-	+	-	***Enterobacter spp***
'+'= Positive, '-'= Negative, Y= Yellow(Acidic), R=Red (Alkaline)																			

Table 3.2: Biochemical characteristics of the bacteria isolated from UTI suspected patient

Isolates No	Isolates ID	Gram Staining		Indole test	Methyl Red (MR) Test	Voges- Proskauer (VP) Test	Citrate Test	Triple Sugar Iron test (TSI)						MIU Test			Catalase test	Oxidase Test	Suspected organism
		Gram Reaction	Shape					Slant/butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	Motility	Indole	Urease			
17	e	-	Short rods	-	+	+(S)	-	Y/Y	+	+	+	-	-	+	-	+	+	-	***Enterobacter spp***
18	f	+	Cocci in cluster	-	+	-	-	Y/Y	+	+	+	-	-	-	-	-	+	-	***Staphylococcus spp***
19	g	-	Short rods	+	+	+	+	Y/Y	+	+	+	-	+	+	+	-	+	-	***Klebsiella spp***
20	h	-	Short rods	-	+	+	+	Y/Y	+	+	+	-	-	-	-	-	-	-	***Enterobacter spp***
21	i	-	Short rods	-	+	+	-	R/Y	+	-	-	-	-	-	-	+	+	-	***Enterobacter spp***
22	j	-	Short rods	+	+		-	Y/Y	+	+	+	-	-	+	+	+	+	-	***E.coli***
23	k	-	Short rods	+	+	-	-	R/Y	+	-	-	-	+	+	+	+	+	-	***E.coli***
24	l	-	Rods	-	+	-	-	R/Y	+	-	-	-	-	-	-	+	+	-	***Shigella spp***
25	m	-	Rods	-	+	-	-	Y/Y	+	+	+	+	-	-	-	+	+	-	***Shigella spp***
26	n	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	-	+	+	+	+	-	***E.coli***
27	o	-	Short rods	-	+	+	-	Y/Y	+	+	+	-	+	+	-	+	+	-	***Enterobacter spp***
28	p	-	Rods	+	+	-	-	Y/Y	+	+	+	-	-	+	+	-	+	-	***Proteus spp***
29	q	+	Long rods	+	+	+	-	R/Y	+	-	-	-	-	-	+	+	+	-	***Bacillus spp***
30	r	-	Rods	+	+	-	-	R/Y	+	-	-	-	-	-	+	+	+	-	***Shigella spp***
31	U_2	-	Short rods	-	-	+	+	R/Y	+	-	-	-	-	-	-	+	+	-	***Klebsiella spp***
32	U_4	+	Cocci in cluster	-	+	-	+	R/Y	+	-	-	-	-	+	-	-	+	-	***Staphylococcus spp***
'+'= Positive, '-'= Negative, Y= Yellow(Acidic), R=Red (Alkaline)																			

Table 3.2: Biochemical characteristics of the bacteria isolated from UTI suspected patient

Isolates No	Isolates ID	Gram Staining		Indole test	Methyl Red (MR) Test	Voges- Proskauer (VP) Test	Citrate Test	Triple Sugar Iron test (TSI)						MIU Test			Catalase test	Oxidase Test	Suspected organism
		Gram Reaction	Shape					Slant/butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	Motility	Indole	Urease			
33	U_5	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	-	***E.coli.***
34	U_6	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	+	+	+	-	+	-	***E.coli***
35	U_7	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	-	+	+	-	+	-	***E.coli***
36	U_8	-	Short rods	+	+	-	-	Y/Y	+	+	+	-	-	+	+	-	+	-	***E.coli***
37	U_9	+	Cocci in cluster	+	+	+	-	R/Y	+	-	-	-	-	+	+	+	+	-	***Staphylococcus spp***
38	U_{10}	+	Cocci in cluster	+	+	-	-	Y/Y	+	+	+	-	-	-	+	+	+	-	***Staphylococcus spp***
39	U_{11}	+	Cocci in clustar	+	+	-	-	R/Y	+	-	-	+	-	+	+	+	+	-	***Staphylococcus spp***
40	U_{14}	-	Short rods	+	+	+	+	Y/Y	+	+	+	-	-	-	+	+	+	-	***Klebsiella spp***
41	U_{15}	+	Long rods;	+	+	-	-	R/R	-	-	-	-	-	-	+	-	+	-	***Bacillus spp.***
42	U_{18}	-	Short rods	-	+	+	+	R/Y	+	-	-	-	-	+	-	-	+	-	***Klebsiella spp***
43	U_{20}	-	Short rods	+	+	+	+	Y/Y	+	+	+	-	-	+	+	+	-	-	***Enterobacter spp***
44	U_{24}	-	Short rods	+	+	+	+	Y/Y	+	+	+	-	+	+	+	+	+	-	***Klebsiella spp***
45	U_{29}	-	Short rods	-	-	+	+	R/Y	+	-	-	+	-	+	-	+	+	-	***Klebsiella spp***
46	U_{31}	-	Short rods	-	-	+	+	R/Y	+	-	-	-	-	+	-	+	+	-	***Klebsiella spp***
47	U_{33}	-	Short rods	-	+	+	+	R/Y	+	-	-	-	-	-	-	+	+	-	***Klebsiella spp***
48	U_{34}	-	Short rods	+	+	+	-	R/Y	+	-	-	-	-	+	+	+	+	-	***Klebsiella spp***
'+'= Positive, '-'= Negative, Y= Yellow(Acidic), R=Red (Alkaline)																			

Table 3.2: Biochemical characteristics of the bacteria isolated from UTI suspected patient

Isolates No	Isolates ID	Gram Staining		Indole test	Methyl Red (MR) Test	Voges- Proskauer (VP) Test	Citrate Test	Triple Sugar Iron test (TSI)						MIU Test			Catalase test	Oxidase Test	Suspected organism
		Gram Reaction	Shape					Slant/butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	Motility	Indole	Urease			
49	U_{35}	-	Short rods	+	+	+	+	R/Y	+	-	-	-	+	+	+	+	-	-	***Klebsiella spp***
50	U_{36}	-	Short rods	-	+	+	+	R/Y	+	-	-	-	-	+	-	+	+	-	***Klebsiella spp***
51	U_{38}	-	Rods	+	+	+	-	Y/Y	+	+	+	-	-	+	+	+	+	-	***Proteus spp***
52	U_{39}	+	Cocci in cluster	+	+	-	-	Y/Y	+	+	+	-	-	-	+	+	+	-	***Staphylococcus spp***
53	U_{40}	+	Cocci in cluster	+	+	+	-	Y/Y	+	+	+	-	-	+	+	-	+	-	***Staphylococcus spp***
54	U_{41}	-	Rods	+	+	-	-	R/Y	+	-	-	-	-	+	+	+	+	-	***Proteus spp***
55	U_{42}	-	Short rods	+	+	-	+	Y/Y	+	+	+	-	-	+	+	+	+	-	***Klebsiella spp***
56	U_{43}	-	Short rods	+	+	-	+	R/Y	+	-	-	-	-	+	+	+	+	-	***Klebsiella spp***
57	U_{47}	-	Rods	+	+	-	-	R/Y	+	-	-	-	-	+	+	-	+	-	***Shigella spp***
58	U_{48}	-	Short rods	+	+	+	+	Y/Y	+	+	+	-	+	+	+	-	+	-	***Klebsiella spp***
59	U_{49}	-	Short rods	+	+	+	-	R/Y	+	-	-	-	-	+	+	+	-	-	***Enterobacter spp***
60	U_{50}	-	Short rods	+	+	-	-	R/Y	+	-	-	-	-	+	+	+	+	-	***Enterobacter spp***
61	B_3	+	Long rods	-	+	-	-	R/Y	+	-	-	-	-	-	-	+	+	-	***Bacillus spp***
62	B_6	+	Long rods	-	-	-	-	R/Y	+	-	-	+	-	-	-	+	+	-	***Bacillus spp***
63	B_{10}	+	Long rods	-	+	-	-	R/Y	+	-	-	-	-	-	-	+	+	-	***Bacillus spp***
64	B_{13}	+	Cocci in cluster	-	+	-	-	Y/Y	+	+	+	-	-	+	-	+	+	-	***Staphylococcus spp***
'+'= Positive, '-'= Negative, Y= Yellow(Acidic), R=Red (Alkaline)																			

Table 3.2: Biochemical characteristics of the bacteria isolated from UTI suspected patient

Isolates No	Isolates ID	Gram Staining		Indole test	Methyl Red (MR) Test	Voges- Proskauer (VP) Test	Citrate Test	Triple Sugar Iron test (TSI)						MIU Test			Catalase test	Oxidase Test	Suspected organism
		Gram Reaction	Shape					Slant/butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	Motility	Indole	Urease			
65	B_{15}	+	Cocci in cluster	-	+	-	-	Y/Y	+	+	+	-	-	+	-	-	+	-	***Staphylococcus spp***
66	B_{16}	+	Long rods	-	+	-	-	R/Y	+	-	-	-	-	-	-	+	+	-	***Bacillus spp***
67	I_1	-	Short rods	-	+	+	-	Y/Y	+	+	+	-	-	+	-	+	+	-	***Enterobacter spp***
68	I_3	-	Short rods	-	-	+	-	Y/Y	+	+	+	-	-	+	-	+	-	-	***Enterobacter spp***
69	I_4	-	Short rods	-	-	+	-	Y/Y	+	+	+	-	-	+	-	+	+	-	***Enterobacter spp***
70	I_5	-	Short rods	-	-	+	-	Y/Y	+	+	+	-	-	+	-	+	+	-	***Enterobacter spp***
71	I_6	+	Long rods	-	-	+	-	R/Y	+	-	-	-	-	-	-	-	+	-	***Bacillus spp***
72	I_8	-	Rods	-	+	-	-	Y/Y	+	+	+	-	-	+	-	-	+	-	***Shigella spp***
73	I_9	+	Cocci in cluster	-	+	-	-	Y/Y	+	+	+	-	-	+	-	-	+	-	***Staphylococcus spp***
74	I_{12}	+	Cocci in cluster	-	+	+	-	Y/Y	+	+	+	-	-	+	-	-	+	-	***Staphylococcus spp***
75	I_{17}	-	Short rods	-	-	+	+	Y/Y	+	+	+	-	+	+	-	+	+	-	***Klebsiella spp***
76	I_{18}	-	Rods	-	+	+	-	Y/Y	+	+	+	-	-	+	-	-	+	-	***Shigella spp***
77	I_{19}	-	Rods	-	+	-	-	Y/Y	+	+	+	-	-	+	-	-	+	-	***Shigella spp***
78	I_{21}	+	Cocci in cluster	-	+	-	-	R/Y	+	-	-	-	-	+	-	-	+	-	***Staphylococcus spp***
79	I_{22}	+	Cocci in cluster	-	+	-	-	Y/Y	+	+	+	-	-	+	-	-	+	-	***Staphylococcus spp***
80	I_{24}	+	Long rods	-	+	-	-	R/Y	+	-	-	-	-	-	-	-	+	-	***Bacillus spp***
'+'= Positive, '-'= Negative, Y= Yellow(Acidic), R=Red (Alkaline)																			

Table 3.2: Biochemical characteristics of the bacteria isolated from Urinary UTI suspected patient

Isolates No	Isolates ID	Gram Staining		Indole test	Methyl Red (MR) Test	Voges- Proskauer (VP) Test	Citrate Test	Triple Sugar Iron test (TSI)						MIU Test			Catalase test	Oxidase Test	Suspected organism
		Gram Reaction	Shape					Slant/butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	Motility	Indole	Urease			
81	I_{25}	+	Cocci in cluster	-	+	-	-	Y/Y	+	+	+	-	-	+	-	-	+	-	***Staphylococcus spp***
82	I_{26}	+	Cocci in cluster	-	+	-	-	Y/Y	+	+	+	-	-	+	-	+	+	-	***Staphylococcus spp***
83	I_{28}	+	Cocci in cluster	-	+	-	-	Y/Y	+	+	+	-	-	+	-	-	+	-	***Staphylococcus spp***
84	I_{31}	-	Short rods	-	+	+	+	Y/Y	+	+	+	-	+	+	-	+	+	-	***Klebsiella spp***
85	I_{32}	-	Short rods	-	+	+	+	Y/Y	+	+	+	-	+	+	-	+	+	-	***Klebsiella spp***
86	I_{33}	+	Cocci	-	-	-	-	R/R	-	-	-	+	-	-	-	+	+	-	***Micrococcus spp***
87	I_{35}	+	Long rods	-	+	-	-	R/R	-	-	-	-	-	-	-	-	+	-	***Bacillus spp***
88	I_{36}	+	Cocci	-	-	-	-	R/R	-	-	-	-	-	+	-	-	+	-	***Micrococcus spp***
89	I_{37}	+	Long rods	-	-	+	-	R/R	-	-	-	+	-	-	-	-	+	-	***Bacillus spp***
90	I_{40}	+	Long rods	-	-	+	-	R/R	-	-	-	+	-	-	-	-	+	-	***Bacillus spp***
91	I_{42}	+	Long rods	-	+	-	-	R/R	-	-	-	+	-	-	-	-	+	-	***Bacillus spp***
'+'= Positive, '-'= Negative, Y= Yellow(Acidic), R=Red (Alkaline)																			

After observing the cultural and morphological characteristics of bacterial isolates and performing the biochemical tests, 91 isolates had been identified from different samples collected from UTI patients. The isolates that have been confirmed include *Staphylococcus* species (found in 19 isolates), *Klebsiella* species (found 19 isolates), *Enterobacter* species (found 15 isolates), *Bacillus* species (found 14 isolates), *E.coli* (found 7 isolates), *Shigella* species (found 10 isolates), *Micrococcus* species (found 4 isolates) and proteus species (found 3 isolates). The total number and the percentage of the isolates obtained from the samples are shown in table 3.3 and figure 3.5

Table 3.3: Prevalence of bacteria isolated from urine samples.

Bacterial isolates	Number of the isolates	Total bacterial isolates	% Prevalence
***Staphylococcus* species**	19	**91**	**20.88**
***Klebsiella* species**	19		**20.88**
***Enterobacter* species**	15		**16.48**
***Bacillus* species**	14		**15.38**
***Shigella* species**	10		**11**
***E.coli* species**	7		**7.69**
***Micrococcus* species**	4		**4.4**
***Proteus* species**	3		**3.29**

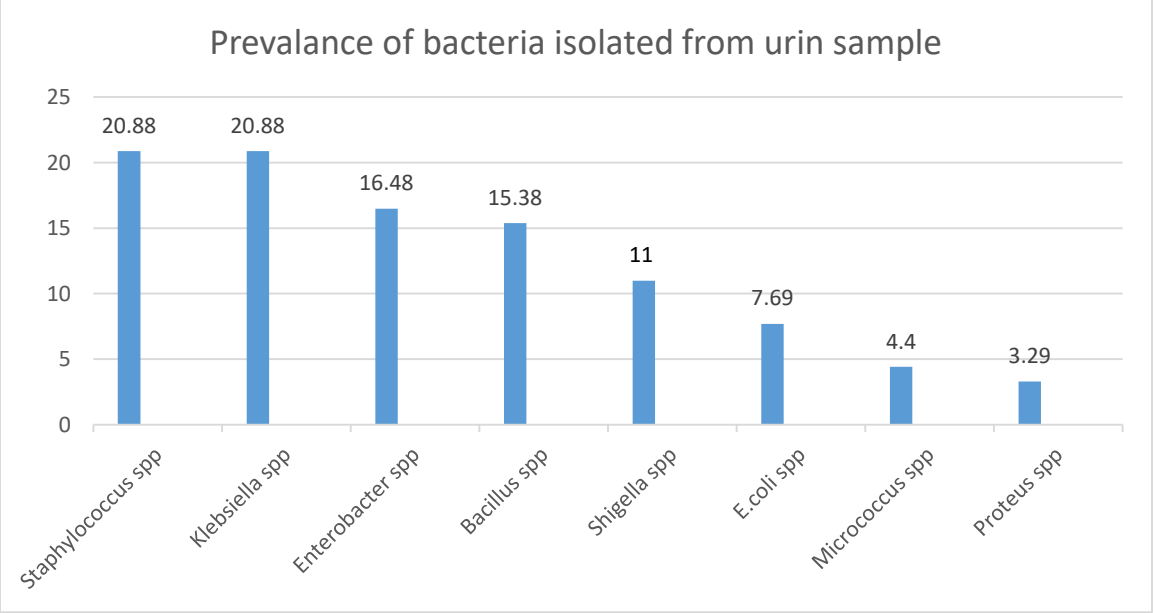


Figure 3.4: Percentage of prevalence of isolated bacteria from urine samples.

Among the identified isolates, both the Gram positive and Gram negative organisms were found. The Gram positive organisms that have been identified include *Staphylococcus* spp, *Bacillus* spp and *Micrococcus* spp. The Gram negative organisms that have been identified include *E.coli, Klebsiella spp, Enterobacter spp, Shigella spp and, Proteus spp* . The differentiation, number and the percentage of the identified bacterial isolates based on Gram reaction are shown in Table 3.4 and Figure 3.6.

Table 3.4: Distribution of the isolates according to Gram's Reaction

Gram's Reaction	**Number of isolates found**	**Percentage (%)**
Gram positive	37 (out of 91)	40.65%
Gram negative	54 (out of 91)	59.35%

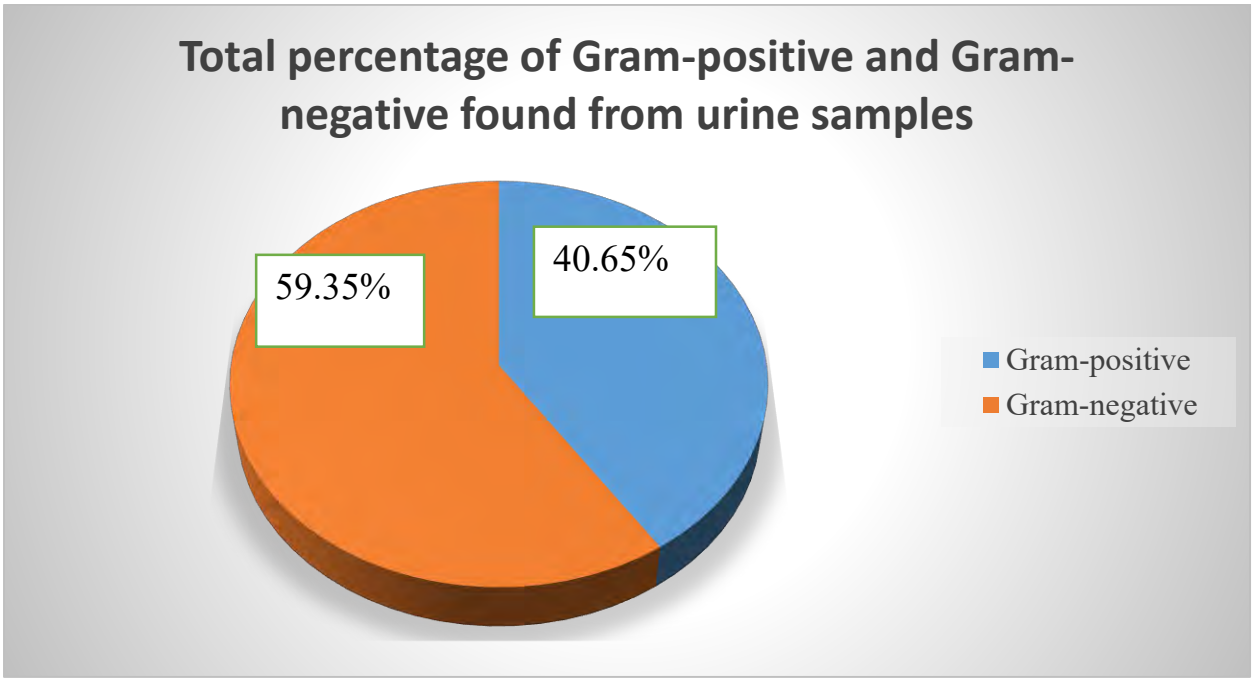


Figure 3.5: Total percentage of Gram positive and Gram negative bacteria identified from urine samples.

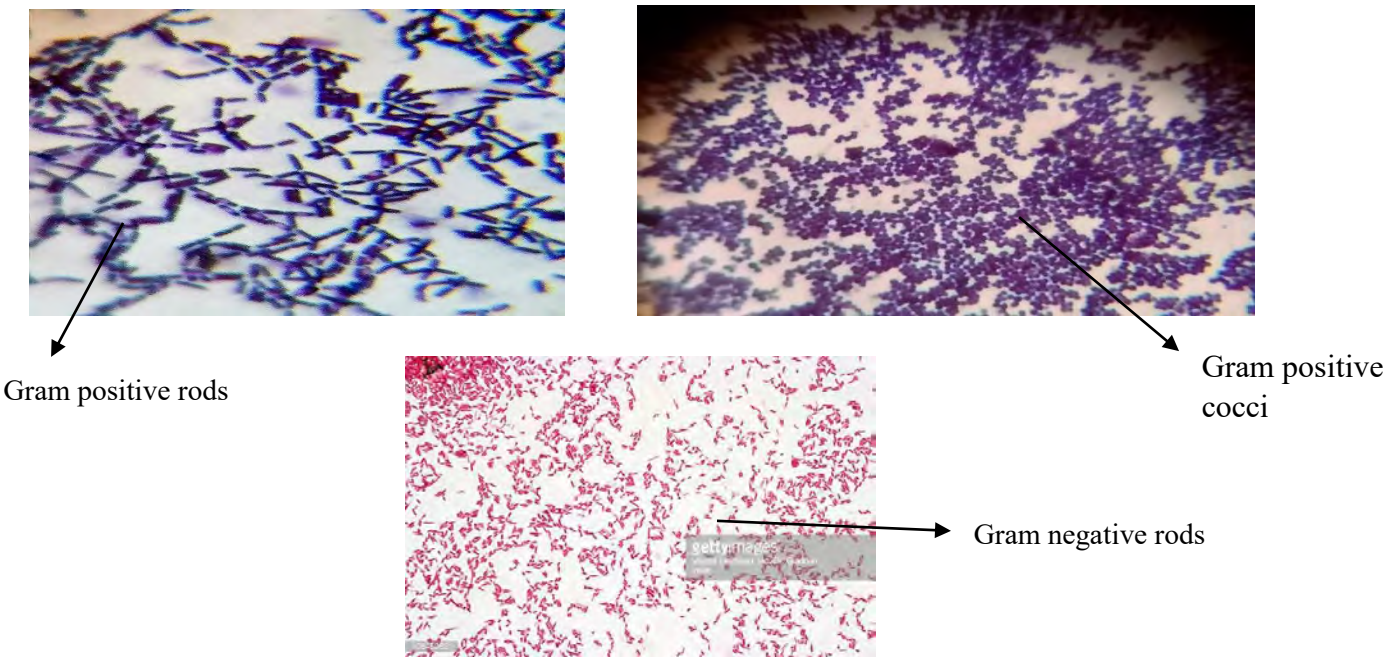
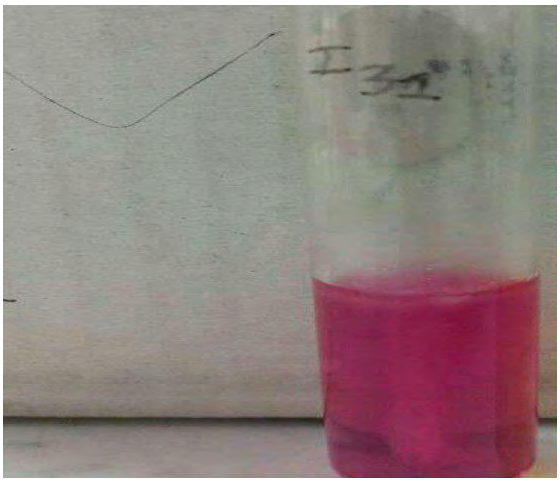
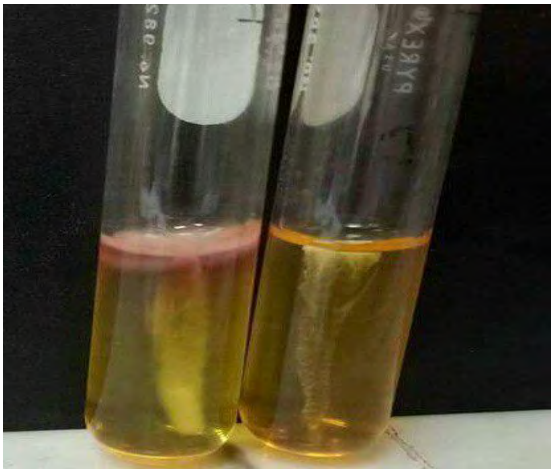


Figure3.6: Gram staining of bacterial isolates

Indole test



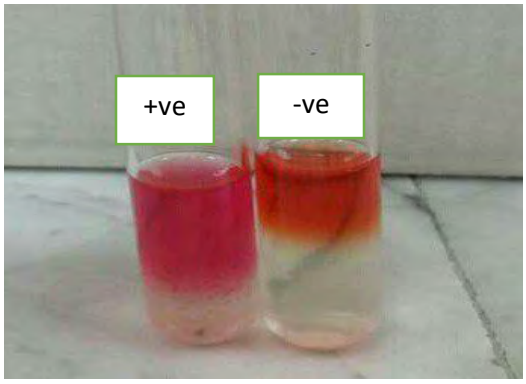
MIU test (Urease +ve, Non-motile)



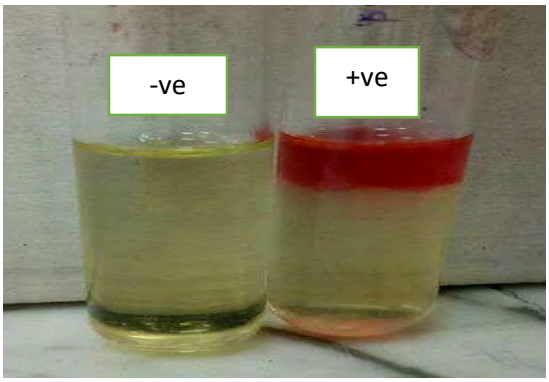
MIU test (Urease -ve, Motile)

Citrate test (positive

Citrate test (negative)



Methyl red test



Voges-Proskauer test

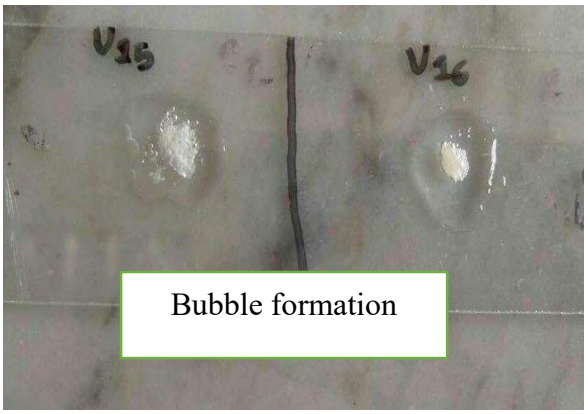
Yellow slant, Yellow butt

Red slant, yellow butt

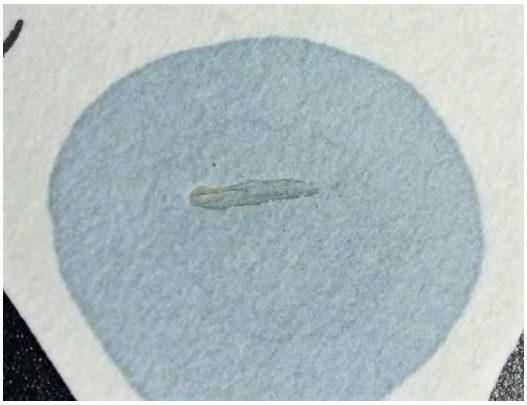
Yellow slant, yellow butt (gas produced)

Red slant, red butt, H_2S production

TSI test



Catalase test (positive)



Oxidase test (negative)

Figure 3.7: Biochemical test results of bacterial isolates

3.2 Antibiotic susceptibility test:

After identifying and confirming the organisms, the isolates were selected for antibiotic susceptibility test. Antibiotics were used for each of the 91 isolates isolated from Urinary tract infection patients. The sensitive and resistance pattern of the isolates to these antibiotics were determined.

In table the zone of inhibition of the isolates according to the zone range for resistance, intermediate and sensitive to different antibiotics are shown. The zone of inhibition is measured in millimeter. The interpretation of each bacterium either resistant or susceptible to antibiotic is shown in Table 3.5

Table 3.5: Antibiotic susceptibility pattern of various organisms isolated from urine sample of UTI suspected patient

Isolates Id	Suspected organisms	Penicillin G		Ciprofloxacin		Chloramphenicol		Ampicillin		Azithromycin		Rifampicin		Tetracycline		Erythromycin		Streptomycin		Cefepime	
		ZI	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**
1a	*Bacillus spp.*	Nil	R	34	S	27	S	15	I	20	S	17	I	29	S	24	S	20	S	Nil	R
1b	*Enterobacter spp.*	20	R	25	S	33	S	17	S	27	S	20	S	33	S	30	S	25	S	Nil	R
1c	*Enterobacter spp.*	22	R	24	S	30	S	16	I	26	S	20	S	30	S	30	S	24	S	Nil	R
1d	*Klebsiella spp.*	Nil	R	19	I	22	S	Nil	R	17	I	Nil	R	9	R	11	R	12	I	Nil	R
1e	*Micrococcus spp.*	36	S	25	S	26	S	28	S	35	S	19	I	32	S	24	S	25	S	Nil	R
1f	*Staphylococcus spp.*	16	R	25	S	36	S	16	R	13	R	37	S	20	S	Nil	R	24	S	Nil	R
1g	*Micrococcus spp.*	35	S	24	S	27	S	27	S	34	S	18	I	34	S	25	S	26	S	Nil	R
2	*Staphylococcus spp.*	17	R	27	S	32	S	14	R	15	I	35	S	21	S	Nil	R	22	S	Nil	R
3a	*Bacillus spp.*	Nil	R	34	S	27	S	16	I	20	S	17	I	29	S	24	S	20	S	Nil	R
3b	*Shigella spp.*	Nil	R	Nil	R	Nil	R	Nil	R	22	S	12	R	Nil	R	20	S	Nil	R	Nil	R
3c	*Shigella spp.*	Nil	R	Nil	R	Nil	R	Nil	R	Nil	R	15	R	Nil	R	Nil	R	Nil	R	Nil	R

ZI= Zone of Inhibition, INP= Interpretation, S= Sensitive, I= Intermediate, R=Resistant

Table 3.5: Antibiotic susceptibility pattern of various organisms isolated from urine sample of UTI suspected patient

Isolates Id	Suspected organisms	Penicillin G		Ciprofloxacin		Chloramphenicol		Ampicillin		Azithromycin		Rifampicin		Tetracycline		Erythromycin		Streptomycin		Cefepime	
		ZI	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**
4	*Shigella spp.*	Nil	R	Nil	R	Nil	R	Nil	R	Nil	R	19	I	41	S	Nil	R	21	S	Nil	R
a	*Enterobacter spp.*	Nil	R	23	I	25	S	Nil	R	16	I	Nil	R	24	S	9	R	20	S	34	S
b	*Staphylococcus spp.*	Nil	R	28	I	29	S	39	S	12	R	18	I	26	S	19	R	18	S	32	S
c	*Klebsiella spp.*	Nil	R	33	S	28	S	Nil	R	14	I	Nil	R	22	S	10	R	18	S	31	S
d	*Enterobacter spp.*	Nil	R	Nil	S	21	S	Nil	R	Nil	R	Nil	R	18	S	Nil	S	22	S	Nil	R
e	*Enterobacter spp.*	20	R	35	S	33	S	23	S	Nil	R	37	S	11	R	Nil	S	27	S	28	S
f	*Staphylococcus spp.*	Nil	R	30	I	30	S	Nil	R	Nil	R	28	S	32	S	11	R	19	S	33	S
g	*Klebsiella spp.*	Nil	R	Nil	R	22	S	Nil	R	Nil	R	Nil	R	17	S	Nil	R	18	S	Nil	R
h	*Enterobacter spp.*	26	R	Nil	R	11	R	12	R	Nil	R	22	S	Nil	R	12	R	Nil	R	14	R
i	*Enterobacter spp.*	36	S	36	S	24	S	35	S	24	S	22	S	31	S	26	S	24	S	Nil	R
j	*E. coli spp.*	Nil	R	30	I	23	S	8	R	11	R	7	R	24	S	Nil	R	22	S	31	S

ZI= Zone of Inhibition, INP= Interpretation, S= Sensitive, I= Intermediate, R=Resistant

Table 3.5: Antibiotic susceptibility pattern of various organisms isolated from urine sample of UTI suspected patient

Isolates Id	Suspected organisms	Penicillin G		Ciprofloxacin		Chloramphenicol		Ampicillin		Azithromycin		Rifampicin		Tetracycline		Erythromycin		Streptomycin		Cefepime	
		ZI	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**
k	*E. coli spp*.	Nil	R	32	S	25	S	Nil	R	13	R	9	R	24	S	8	R	20	S	Nil	R
l	*Shigella spp*.	34	S	23	I	27	S	31	S	Nil	R	28	S	23	S	9	R	28	S	10	R
m	*Shigella spp.*	31	S	26	I	30	S	30	S	Nil	R	27	S	25	S	11	R	27	S	11	R
n	*E. coli spp*.	Nil	R	32	S	27	S	19	S	11	R	10	R	22	S	10	R	19	S	32	S
o	*Enterobacter spp*.	Nil	R	24	I	30	S	Nil	R	15	I	8	R	11	R	10	R	20	S	14	R
p	*Proteus spp*.	17	R	37	S	37	S	20	S	Nil	R	38	S	13	I	Nil	R	24	S	23	S
q	*Bacillus spp.*	Nil	R	29	I	28	S	Nil	R	Nil	R	14	R	24	S	12	R	23	S	Nil	R
r	*Shigella spp*.	Nil	R	36	S	37	S	Nil	R	30	S	16	R	31	S	15	R	27	S	17	I
U_2	*Klebsiella spp.*	Nil	R	Nil	R	17	I	Nil	R	Nil	R	Nil	R	16	S	Nil	R	20	S	Nil	R
U_4	*Staphylococcus spp.*	31	S	27	S	25	S	32	S	22	S	25	S	26	S	28	S	20	S	14	R
U_5	*E. coli*	Nil	R	Nil	R	23	S	Nil	R	Nil	R	7	R	13	I	Nil	R	14	I	Nil	R

ZI= Zone of Inhibition, INP= Interpretation, S= Sensitive, I= Intermediate, R=Resistant

Table 3.5: Antibiotic susceptibility pattern of various organisms isolated from urine sample of UTI suspected patient

Isolates Id	Suspected organisms	Penicillin G		Ciprofloxacin		Chloramphenicol		Ampicillin		Azithromycin		Rifampicin		Tetracycline		Erythromycin		Streptomycin		Cefepime	
		ZI	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**
U_6	*E. coli*	Nil	R	Nil	R	26	S	Nil	R	11	R	9	R	23	S	Nil	R	20	S	Nil	R
U_7	*E. coli*	Nil	R	Nil	R	25	S	Nil	R	9	R	10	R	22	S	Nil	R	20	S	Nil	R
U_8	*E. coli*	Nil	R	Nil	R	26	S	Nil	R	Nil	R	8	R	23	S	Nil	R	18	S	Nil	R
U_9	*Staphylococcus spp.*	Nil	R	Nil	R	32	S	Nil	R	Nil	R	10	R	13	I	Nil	R	20	S	Nil	R
U_{10}	*Staphylococcus spp.*	Nil	R	Nil	R	19	S	Nil	R	11	R	10	R	24	S	Nil	R	21	S	Nil	R
U_{11}	*Staphylococcus spp.*	Nil	R	Nil	R	Nil	R	Nil	S	9	R	9	R	Nil	R	Nil	R	20	S	17	I
U_{14}	*Klebsiella spp.*	Nil	R	Nil	R	18	S	Nil	R	9	R	9	R	Nil	R	Nil	R	21	S	12	R
U_{15}	*Bacillus spp.*	Nil	R	36	S	20	S	18	R	17	I	22	S	34	S	21	I	27	S	15	I
U_{18}	*Klebsiella spp.*	Nil	R	Nil	R	16	I	Nil	R	Nil	R	Nil	R	17	S	Nil	R	17	S	Nil	R
U_{20}	*Enterobacter spp.*	Nil	R	Nil	R	16	I	Nil	R	7	R	Nil	R	18	S	Nil	R	17	S	Nil	R
U_{24}	*Klebsiella spp.*	Nil	R	Nil	R	Nil	R	Nil	R	Nil	R	Nil	R	Nil	R	Nil	R	18	S	15	I

ZI= Zone of Inhibition, INP= Interpretation, S= Sensitive, I= Intermediate, R=Resistant

Table 3.5: Antibiotic susceptibility pattern of various organisms isolated from urine sample of UTI suspected patient

Isolates Id	Suspected organisms	Penicillin G		Ciprofloxacin		Chloramphenicol		Ampicillin		Azithromycin		Rifampicin		Tetracycline		Erythromycin		Streptomycin		Cefepime	
		ZI	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**
U_{29}	*Klebsiella spp.*	Nil	R	30	S	26	S	8	R	15	I	8	R	23	S	10	R	18	S	27	S
U_{31}	*Klebsiella spp.*	Nil	R	30	S	26	S	9	R	14	I	9	R	21	R	9	R	18	S	28	S
U_{33}	*Klebsiella spp.*	Nil	R	Nil	R	18	S	Nil	R	Nil	R	Nil	R	16	S	Nil	R	20	S	12	R
U_{34}	*Klebsiella spp.*	Nil	R	Nil	R	24	S	Nil	R	Nil	R	8	R	Nil	R	Nil	R	14	I	20	S
U_{35}	*Klebsiella spp.*	Nil	R	10	R	27	S	Nil	R	Nil	R	7	R	Nil	R	Nil	R	14	I	16	I
U_{36}	*Klebsiella spp.*	Nil	R	Nil	R	15	I	Nil	R	Nil	R	Nil	R	15	S	Nil	R	18	S	Nil	R
U_{38}	*Proteus spp.*	Nil	R	Nil	R	Nil	R	Nil	R	Nil	R	10	R	Nil	R	Nil	R	22	S	17	I
U_{39}	*Staphylococcus spp.*	Nil	R	Nil	R	Nil	R	Nil	R	Nil	R	9	R	Nil	R	Nil	R	23	S	16	I
U_{40}	*Staphylococcus spp.*	Nil	R	Nil	R	30	S	32	S	Nil	R	24	S	13	I	Nil	R	Nil	R	Nil	R
U_{41}	*Proteus spp.*	Nil	R	Nil	R	Nil	R	Nil	R	Nil	R	11	R	Nil	R	Nil	R	23	S	17	I
U_{42}	*Klebsiella spp.*	Nil	R	Nil	R	24	S	Nil	R	16	I	9	R	22	S	Nil	R	22	S	Nil	R

ZI= Zone of Inhibition, INP= Interpretation, S= Sensitive, I= Intermediate, R=Resistant

Table 3.5: Antibiotic susceptibility pattern of various organisms isolated from urine sample of UTI suspected patient

Isolates Id	Suspected organisms	Penicillin G		Ciprofloxacin		Chloramphenicol		Ampicillin		Azithromycin		Rifampicin		Tetracycline		Erythromycin		Streptomycin		Cefepime	
		ZI	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**
U_{43}	*Klebsiella spp.*	Nil	R	Nil	R	28	S	Nil	R	Nil	R	Nil	R	22	S	Nil	R	22	S	12	R
U_{47}	*Shigella spp.*	Nil	R	Nil	R	19	S	Nil	R	Nil	R	9	R	16	S	Nil	R	14	I	Nil	R
U_{48}	*Klebsiella spp.*	13	R	39	S	20	S	50	S	35	S	33	S	23	S	37	S	28	S	12	R
U_{49}	*Enterobacter spp.*	Nil	R	Nil	R	22	S	Nil	R	Nil	R	8	R	18	S	Nil	R	14	I	Nil	R
U_{50}	*Enterobacter spp.*	Nil	R	Nil	R	22	S	Nil	R	Nil	R	Nil	R	16	S	Nil	R	13	I	Nil	R
B_3	*Bacillus spp*.	Nil	R	33	S	25	S	Nil	R	25	S	8	R	20	S	32	S	26	S	Nil	R
B_6	*Bacillus spp.*	15	R	34	S	24	S	12	I	20	S	15	R	27	S	22	I	24	S	Nil	R
B_{10}	*Bacillus spp*.	Nil	R	36	S	23	S	13	I	25	S	14	R	18	S	34	S	22	S	Nil	R
B_{13}	*Staphylococcus spp*.	41	S	32	S	31	S	40	S	Nil	R	27	S	38	S	Nil	R	26	S	22	S
B_{15}	*Staphylococcus spp*.	23	R	21	S	Nil	R	26	R	10	R	42	S	Nil	R	13	R	31	S	25	S
B_{16}	*Bacillus spp.*	Nil	R	27	S	21	S	Nil	R	22	S	14	R	23	S	30	S	20	S	Nil	S
ZI= Zone of Inhibition, INP= Interpretation, S= Sensitive, I= Intermediate, R=Resistant																					

Table 3.5: Antibiotic susceptibility pattern of various organisms isolated from urine sample of UTI suspected patient

Isolates Id	Suspected organisms	Penicillin G		Ciprofloxacin		Chloramphenicol		Ampicillin		Azithromycin		Rifampicin		Tetracycline		Erythromycin		Streptomycin		Cefepime	
		ZI	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**
I_1	*Enterobacter spp.*	16	R	24	S	35	S	15	S	16	I	36	S	14	I	11	R	27	S	Nil	R
I_3	*Enterobacter spp.*	42	S	37	S	30	S	29	S	33	S	19	I	33	S	32	S	26	S	Nil	R
I_4	*Enterobacter spp.*	32	S	40	S	25	S	30	S	32	S	20	S	33	S	30	S	25	S	Nil	R
I_5	*Enterobacter spp.*	38	S	40	S	22	S	32	S	28	S	23	S	34	S	32	S	26	S	Nil	R
I_6	*Bacillus spp.*	8	R	27	S	22	S	Nil	R	18	S	15	R	9	R	17	I	22	S	Nil	R
I_8	*Shigella spp.*	10	R	16	I	30	S	Nil	R	13	R	31	S	11	R	12	R	23	S	Nil	R
I_9	*Staphylococcus spp.*	12	R	19	I	32	S	11	R	Nil	R	33	S	8	R	Nil	R	25	S	Nil	R
I_{12}	*Staphylococcus spp.*	13	R	17	I	31	S	11	R	Nil	R	34	S	Nil	R	Nil	R	26	S	Nil	R
I_{17}	*Klebsiella spp.*	Nil	R	18	I	27	S	Nil	R	18	S	Nil	R	9	R	Nil	R	13	I	Nil	R
I_{18}	*Shigella spp.*	Nil	R	Nil	R	30	S	10	R	Nil	R	15	R	11	R	Nil	R	22	S	Nil	R
I_{19}	*Shigella spp.*	10	R	17	I	30	S	10	R	13	R	15	R	27	S	Nil	R	22	S	Nil	R

ZI= Zone of Inhibition, INP= Interpretation, S= Sensitive, I= Intermediate, R=Resistant

Table 3.5: Antibiotic susceptibility pattern of various organisms isolated from urine sample of UTI suspected patient

Isolates Id	Suspected organisms	Penicillin G		Ciprofloxacin		Chloramphenicol		Ampicillin		Azithromycin		Rifampicin		Tetracycline		Erythromycin		Streptomycin		Cefepime	
		ZI	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**
I_{21}	*Staphylococcus spp.*	9	R	20	I	30	S	Nil	R	10	R	37	S	10	R	Nil	R	22	S	Nil	R
I_{22}	*Staphylococcus spp.*	15	R	16	I	32	S	11	R	15	I	34	S	34	S	11	R	22	S	Nil	R
I_{24}	*Bacillus spp.*	35	S	25	S	32	S	30	S	36	S	14	R	15	S	30	S	25	S	Nil	R
I_{25}	*Staphylococcus spp.*	17	R	25	S	36	S	15	I	12	R	39	S	39	S	12	R	28	S	Nil	R
I_{26}	*Staphylococcus spp.*	13	R	10	R	32	S	9	R	7	R	32	S	32	S	Nil	R	24	S	Nil	R
I_{28}	*Staphylococcus spp.*	13	R	12	R	33	S	12	R	Nil	R	34	S	34	S	Nil	R	23	S	Nil	R
I_{31}	*Klebsiella spp.*	Nil	R	19	I	22	S	Nil	R	18	S	Nil	R	Nil	R	12	R	13	I	Nil	R
I_{32}	*Klebsiella spp.*	Nil	R	19	I	23	S	Nil	R	17	I	Nil	R	8	R	10	R	12	I	Nil	R
I_{33}	*Micrococcus spp.*	34	S	26	S	23	S	27	S	25	S	17	I	9	R	23	S	25	S	Nil	R
I_{35}	*Bacillus spp.*	Nil	R	26	S	22	S	Nil	R	17	I	15	R	Nil	R	15	I	23	S	Nil	R

ZI= Zone of Inhibition, INP= Interpretation, S= Sensitive, I= Intermediate, R=Resistant

Table 3.5: Antibiotic susceptibility pattern of various organisms isolated from urine sample of UTI suspected patient

Isolates Id	Suspected organisms	Penicillin G		Ciprofloxacin		Chloramphenicol		Ampicillin		Azithromycin		Rifampicin		Tetracycline		Erythromycin		Streptomycin		Cefepime	
		ZI	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**	**ZI**	**INP**
I_{36}	*Micrococcus spp.*	40	S	27	S	37	S	35	S	35	S	36	S	38	S	45	S	30	S	Nil	R
I_{37}	*Bacillus spp.*	Nil	R	17	I	24	S	Nil	R	16	I	18	I	Nil	R	17	I	23	S	Nil	R
I_{40}	*Bacillus spp.*	Nil	R	27	S	26	S	Nil	R	22	S	15	R	Nil	R	25	S	21	S	Nil	R
I_{42}	*Bacillus spp.*	Nil	R	28	S	28	S	Nil	R	18	S	14	R	25	S	22	I	20	S	Nil	R

ZI= Zone of Inhibition, INP= Interpretation, S= Sensitive, I= Intermediate, R=Resistant

3.2.1 Resistance pattern of the organisms to the tested antibiotics:

After determining the antibiotic resistant organisms, their percentage of the resistance to the antibiotics tested was also determined which are shown in Table 3.6 and in the following figure.

Table3.6: Antibiotic resistance pattern of total 91 bacterial isolates.

Antibiotics	Penicillin G	Ciprofloxacin	Chloramphenicol	Ampicillin	Azithromycin	Rifampicin	Tetracycline	Erythromycin	Streptomycin	Cefepime
Number of isolates resistant to antibiotics	78	33	10	63	51	52	29	63	4	69
Percentage of isolates resistant to antibiotics	85.71	36.26	11	69.23	56	57.14	31.87	69.23	4.4	75.82

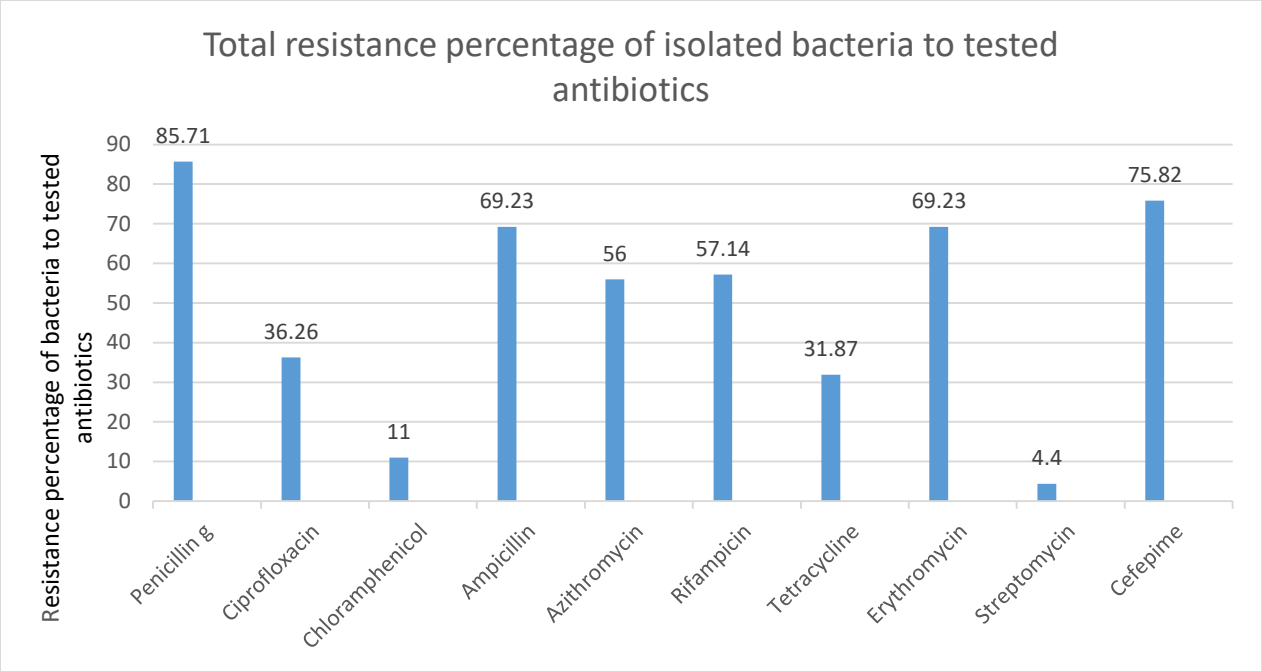


Figure 3.8: Resistance percentage of the isolated bacteria to tested antibiotics

After observing the antibiotic resistance pattern of the organisms, it was found that all organisms were resistant to at least one or two or more than two antibiotics. According to Magiorakos *et al* (2011), organisms that are susceptible to at least one agent in three or more antimicrobial categories are considered as Multi-drug resistant organisms. In this study all the bacterial isolates were found resistant to at least one agent in more than three antibiotic categories. Here most bacteria have the ability to resist the effects of drugs so they are antibiotic resistant.

Table 3.7: Total percentage of the isolates resistant to one antibiotic, two antibiotics and more than two antibiotics.

Total bacterial isolates	Number of Isolates Resistant To one antibiotic	Percentage of isolates Resistant to one antibiotic	Number of Isolates Resistant to two antibiotic	Percentage of isolates Resistant to two antibiotic	Number of Isolates Resistant to more than two antibiotics	Percentage of isolates Resistant to more than two antibiotics
91	8	8.8	9	9.9	74	81.30

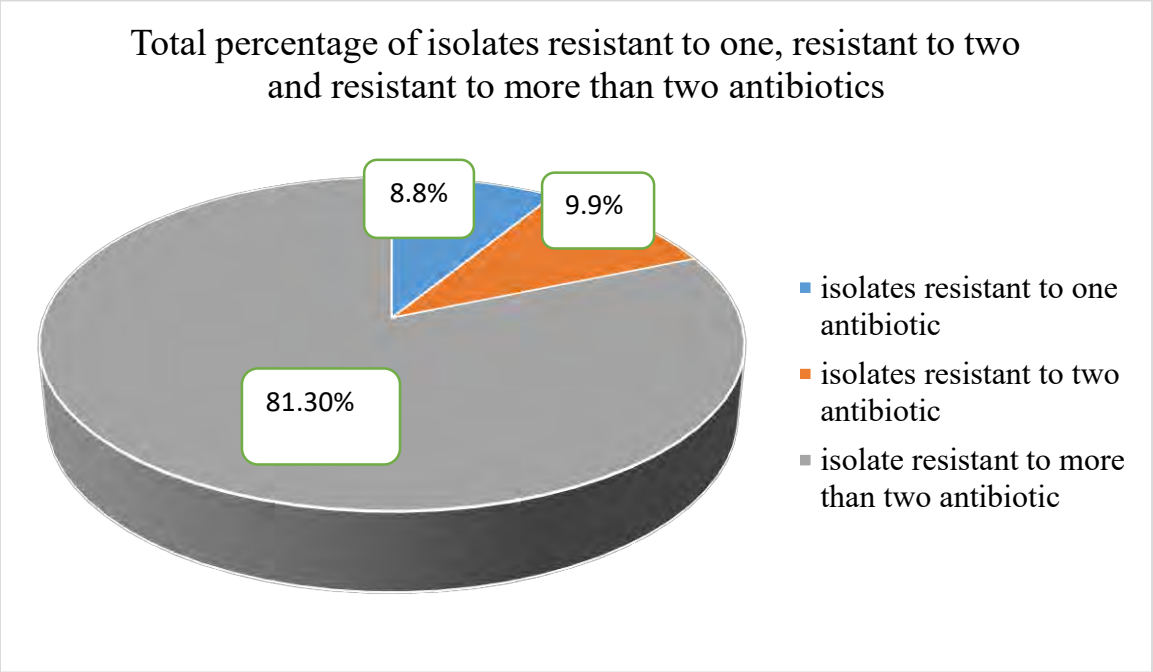
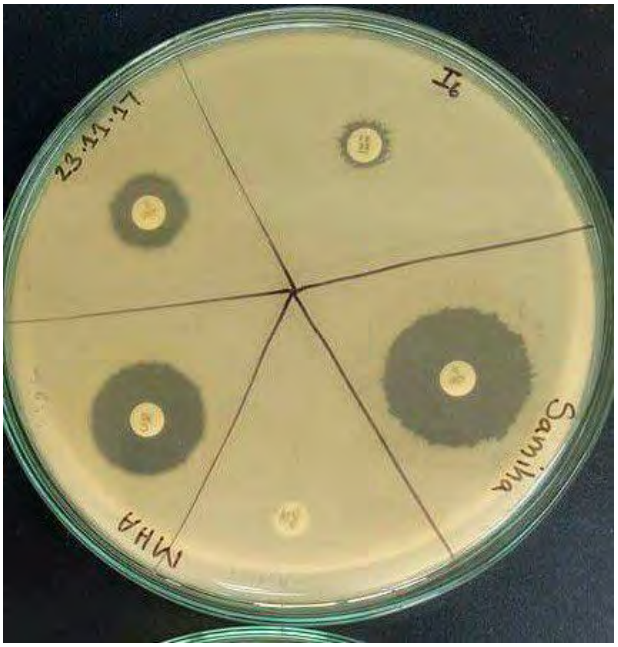
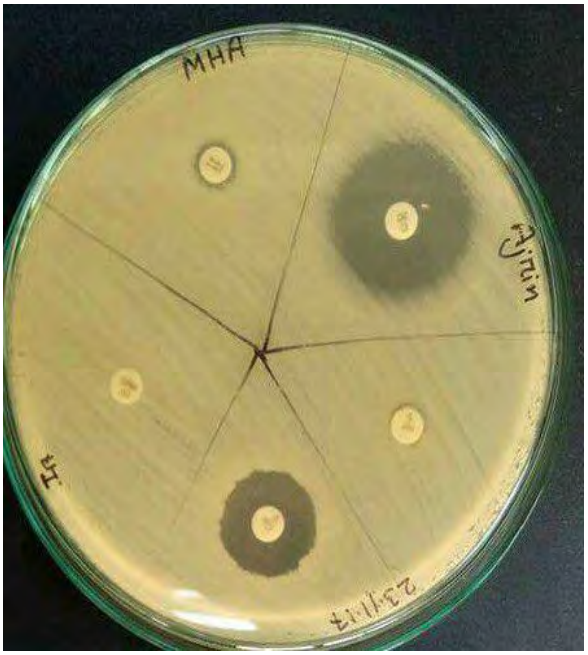


Figure 3.9: Total percentage of the isolates resistant to one, resistant to two and resistant to more than two antibiotics.

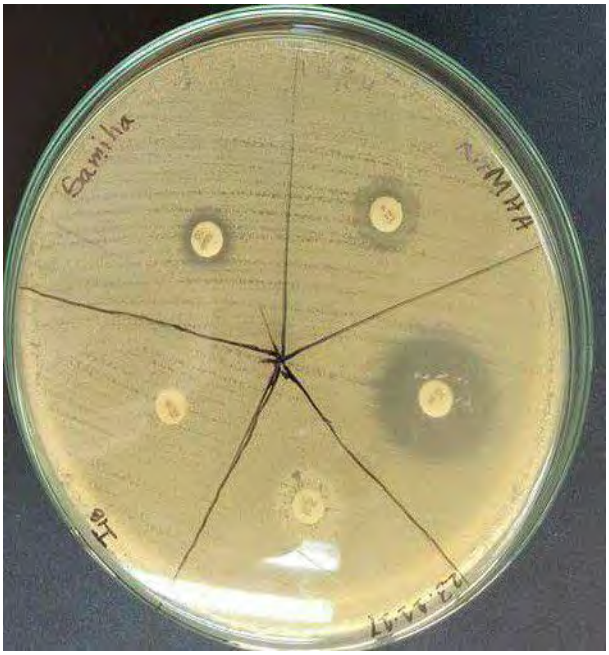
***Staphylococcus* spp**



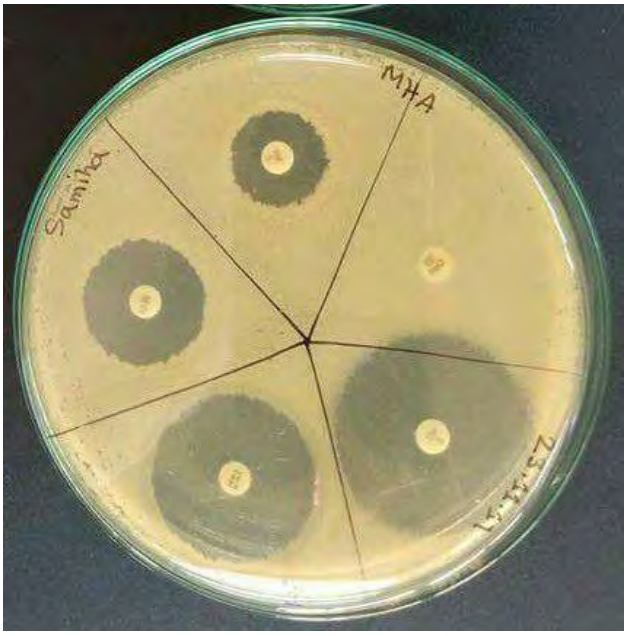
***Bacillus* spp**



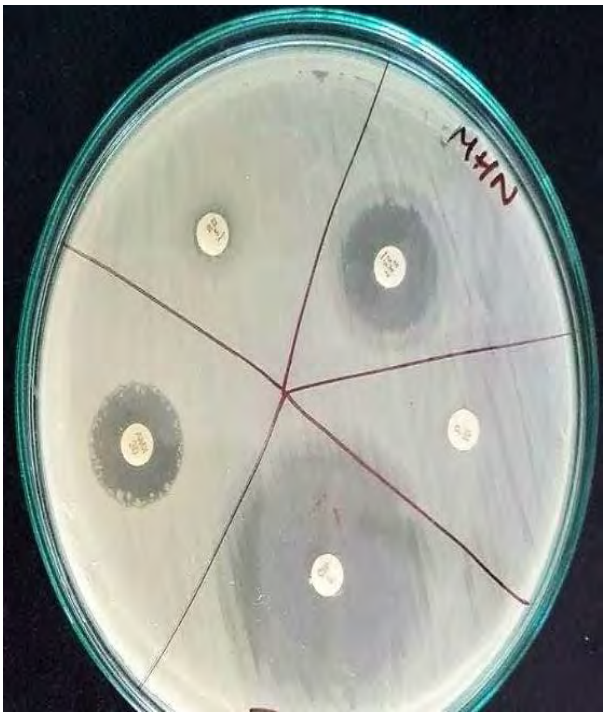
***Klebsiella* spp**



***Shigella* spp**



***Enterobacter* spp** ***E. coli* spp**



***Proteus* spp** ***Micrococcus* spp**

Figure3.10: Antibiotic susceptibility test of bacterial isolates

Chapter 4

Discussion and Conclusion

Discussion:

Urinary tract infections (UTIs) are a leading cause of morbidity and a severe public health problem in persons of all ages. Urinary Tract Infections are a serious health problem affecting millions of people every year. UTI represents a significant burden for the National Health Service. Extensive research has been directed towards rapid detection of UTI in the last thirty years. The prevalence of UTI varies by age, race, sex and temperature (Guernion et al 2001). According to Foxman, (2010) about 40% of women and 12% of men experience at least one symptomatic UTI during their lifetime, and approximately 25% of affected women show recurrent UTI (RUTI). The purpose of this study was to isolating, identifying the bacterial contaminants and identifying the resistance pattern of bacteria isolated from UTI suspected patients. Considerable number of Gram positive bacteria and Gram negative bacteria were found. However, Gram negative bacteria overtaken Gram positive bacteria. The study showed a statistically significant difference in this regard.

Among the samples processed all showed bacterial contamination which is in accordance with the reports of some researchers (Bates 2013) who observed 90% positive culture from urinary tract

Infected patients of Lima memorial Hospital, Lima ohio.

After 24 hours incubation of various selective and differential medium, some different morphological characteristic showing colonies from nutrient agar, pink colonies from MacConkey agar (considered as coliforms), yellow colonies from Mannitol Salt Agar (considered as *Staphylococcus spp*.), Blue colonies from Bacillus cereus Agar (considered as *Bacillius spp*.), Reddish orange colonies from SS agar (considered as *Shigella spp*) were initially isolated. Isolates from MacConkey and EMB agar were observed as Gram negative, single, short rods, compared to the characteristic of coliforms whereas isolates from Mannitol salt agar (MSA) were Gram positive in a cluster arrangement which were typical for Staphylococcus spp. After performing the biochemical characteristics, the isolates were finally confirmed as *E. coli, Klebsiella spp, Enterobacter spp, Staphylococcus spp., Micrococcus spp, Bacillus spp, Shigella spp and Proteus spp.*

In this study among the isolates found *Klebsiella spp* 20.88% and *Staphylococcus spp* 20.88% were by far the most predominant organisms. The frequency of infection caused by Klebsiella, and

Enterobacter species; and by enterococci and staphylococci is higher in recurrent UTIs, especially in the presence of structural abnormalities of the urinary tract. 77.3 percent of the urinary tract infections (UTIs) were caused by a single bacteria species. (Ejobi et al 2001). Now this two are the most predominant of bacteria responsible for urinary tract infection. *Staphylococcus aureus* is frequently isolated from urine samples obtained from long-term care patients. The significance of staphylococcal bacteriuria is uncertain. A hypothesis showed that that S. aureus is a urinary pathogen and that colonized urine could be a source of future staphylococcal infection. (Brennen et al 2006). Enterobacter was found 16.48%.

A high percentage of *Bacillus spp* (15.38%) was also investigated in this study and its high prevalence could be demonstrated by the fact that Bacillus species are ubiquitous in nature and also they are spore forming bacteria. Other organisms found are *Shigella spp* 10.98%, *E. coli* 7.69%, *Micrococcus spp* 4.4% and *Proteus spp* 3.29%. In previous study Salih *et al* 2016 found 53% *E. coli*. In another study Joan et al 2013 found 70% *E. coli* but now *Staphylococcus spp* and *Klebsiella spp* are mostly found among UTI suspected patients but previously *E. coli* was predominant.

Gram positive organisms compared to Gram negative organisms correspond with previous studies (Kiprono et al., 2013) and in favor with the statement that Gram-negative bacteria have overtaken the Gram-positive this is probably because Gram-negative bacteria are predominant for urinary tract infection. In this study Gram-negative bacteria was found 59.35% and Gram-positive bacteria was found 40.65%.

Determination of antibiotic susceptibility pattern revealed that all bacterial isolates tested were resistant to at least one antibiotic. Among the 91 bacterial isolates, 74(81.30%) of them were found to be resistant to more than two antibiotics and 9(9.9%) of them were found to be resistant to two antibiotics and 8(8.8%) isolates were found resistant to one antibiotic. Among the antibiotics tested penicillin was found to be least effective because 85.71% bacterial isolates showed resistance to penicillin. Cefepime can also be considered less effective because 75.82% bacterial isolates showed resistance to this antibiotic. The resistance percentage of the isolates to other antibiotics included Ampicillin 69.23%, Erythromycin 69.23%, Rifampicin 57.14%, Azithromycin 56%, Ciprofloxacin 36.26%, Tetracycline 31.87% Chloramphenicol 11% and Streptomycin 4.4%. In this study among the tested antibiotics streptomycin seems to be most effective for treating UTI

patients. Then Chloramphenicol could also be effective. In another study Erythromycin was highly effective against streptococci (9%). Ciprofloxacin was also most active drug against the different organisms for UTI (Ejobi et al, 2006). Ciprofloxacin can work effectively against *E. coli, Staphylococcus spp, Enterobacter spp, Klebsiella spp and Proteus spp.* (Ejobi 2016). Salih et al 2016 found Ampicillin 100% and also Chloramphenicol 57.8% effective from hospital sample. The improper use of antibiotics in human and livestock, wrong and substandard prescriptions by unqualified medical personnel along with poor diagnosis or lack of it all have been reported to be among the main factors contributing to the development of resistant microbes (Kimang'a, 2012).

Conclusion:

The findings of this research work indicate that Urinary tract infection is a common among both genders with higher prevalence among women due to their physiology and can be caused by various types of organisms both Gram-positive and Gram-negative. In addition, age is an important factor where elderly people are more affected. In addition, diabetes enhances the incidence due to elevated blood sugar levels and other factors like parity, gravidity, hormonal imbalance, immunosuppressant and geographical location also has a significant role in the incidence of the infection. Though antibiotic usage has proven to be beneficial in counteracting the infection but consuming more antibiotics are harmful for our body. By consuming more antibiotics bacteria turns into resistant then antibiotics will be no more effective for treating disease. Rather we should drink plenty of water and maintain hygiene to stay healthy. Patients undergoing long term treatment are also vulnerable to the infection due to moist hospitalized conditions, plant source like cranberry juice is equally effective in fighting the infection and can be used as an alternative to counteract the pathogen causing UTI.

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Appendices

Appendix- I

Media compositions

The composition of all media used in the study is given below:

Nutrient Agar

Component	Amount (g/L)
Peptone	5.0
Sodium chloride	5.0
Beef extract	3.0
Agar	15.0
Final pH	7.0

Saline

Component	Amount (g/L)
Sodium Chloride	9.0

Nutrient broth

Component	Amount (g/L)
Peptic digest of animal tissue	5.0
Sodium chloride	5.0
Beef extract	1.5
Yeast extract	1.5
Final pH	7.4±0.2 at 25ºC

Mannitol Salt Agar

Component	Amount (g/L)
Proteose peptone	10.0
Beef extract	1.0
Sodium chloride	75.0
D-mannitol	10.0
Phenol red	0.025
Agar	15.0
Final pH	7.4 ± 0.2 at 25°C

MacConkey Agar

Component	Amount (g/L)
Peptic digest of animal tissue	1.5
Casein enzymatic hydrolysate	1.5
Pancreatic digest of gelatin	17.0
Lactose	10.0
Bile salt	1.50
Crystal violet	0.001
Neutral red	0.03
Agar	15.0
Final pH	7.1 ± 0.2 at 25°C

Blood Agar Base

Component	Amount (g/L)
Beef heart infusion from (beef extract)	500.0
Tryptose	10.0
Sodium chloride	5.0
Agar	15.0
Final pH	6.8 ± 0.2 at 25°C

Eosine Methylene Blue Agar (EMB):

Component	Amount (g/L)
Peptone	10.0
Dipotassium Phosphate	2.0
Lactose	5.0
Sucrose	5.0
Eosin yellow	0.14
Methylene Blue	0.065
Agar	13.50
Final pH	7.1 ± 0.2 at 25°C

Bacillus cereus Agar (BC Agar):

Component	Amount (g/L)
Peptic digest of animal tissue	1.0
Mannitol	10.0
Sodium chloride	2.0
Magnesium sulphate	0.1
Disodium phosphate	2.5
Monopotassium phosphate	0.25
Sodium pyruvate	10.0
Bromo thymol blue	0.12
Agar	15.0
Final pH	7.12± 0.2 at 25°C

Muller Hinton Agar

Component	Amount (g/L)
Beef, dehydrated infusion form	300
Casein hydrolysate	17.5
Starch	1.5
Agar	17.0
Final pH	7.3± 0.1 at 25°C

HiCrome UTI Agar:

Component	Amount (g/L)
Peptic digest of animal tissue	15.0
Chromogenic mixture	26.80
Agar	15.0
Final pH	7.1 ± 0.2 at 25°C

Simmon's Citrate Agar

Component	Amount (g/L)
Magnesium sulphate	0.2
Ammoniun dihydrogen phosphate	1.0
Dipotassium phosphate	1.0
Sodium citrate	2.0
Sodium chloride	5.0
Bacto agar	15.0
Bacto bromo thymol blue	0.08

Methyl Red -Voges Proskauer (MR-VP) Media

Component	Amount (g/L)
Peptone	7.0
Dextrose	5.0
Dipotassium hydrogen phosphate	5.0
Final pH	7.0

Triple Sugar Iron Agar (TSI)

Component	Amount (g/L)
Bio-polytone	20.0
Sodium chloride	5.0
Lactose	10.0
Sucrose	10.0
Dextrose	1.0
Ferrous ammonium sulphate	0.2
Sodium thiosulphate	0.2
Phenol red	0.0125
Agar	13.0
Final pH	7.3

Motility Indole Urease (MIU) Agar

Component	Amount (g/L)
Tryptone	10
Phenol red	0.1
Agar	2.0
Sodium chloride	5.0
pH (at 25°C)	6.8 ± at 25°C

Indole broth

Component	Amount (g/L)
Peptone	10.0
Sodium chloride	5.0

Appendix – II

Reagents and buffers

Gram's iodine (300 ml)

To 300 ml distilled water, 1 g iodine and 2 g potassium iodide was added. The solution was mixed on a magnetic stirrer overnight and transferred to a reagent bottle and stored at room temperature.

Crystal Violet (100 ml)

To 29 ml 95% ethyl alcohol, 2 g crystal violet was dissolved. To 80 ml distilled water, 0.8 g ammonium oxalate was dissolved. The two solutions were mixed to make the stain and stored in a reagent bottle at room temperature.

Safranin (100ml)

To 10 ml 95% ethanol, 2.5 g safranin was dissolved. Distilled water was added to the solution to make a final volume of 100 ml. The final solution was stored in a reagent bottle at room temperature.

Malachite green (100 ml)

To 20 ml distilled water, 5 g malachite green was dissolved in a beaker. The solution was transferred to a reagent bottle. The beaker was washed two times with 10 ml distilled water separately and a third time with 50 ml distilled water and the solution was transferred to the reagent

bottle. The remaining malachite green in the beaker was washed a final time with 10 ml distilled water and added to the reagent bottle. The stain was stored at room temperature.

Kovac's Reagent (150 ml)

To a reagent bottle, 150 ml of reagent grade isoamyl alcohol, 10 g of p-dimethylaminobenzaldehyde (DMAB) and 50 ml of HCl (concentrated) were added and mixed. The reagent bottle was then covered with an aluminum foil to prevent exposure of reagent to light and stored at 4°C.

Methyl Red (200 ml)

In a reagent bottle, 1 g of methyl red powder was completely dissolved in 300 ml of ethanol (95%). 200 ml of destilled water was added to make 500 ml of a 0.05% (wt/vol) solution in 60% (vol/vol) ethanol and stored at 4°C.

Barrit's Reagent A (100 ml)

5% (wt/vol) a-naphthol was added to 100 ml absolute ethanol and stored in a reagent bottle at 4°C.

Barrit's Reagent B (100 ml)

40% (wt/vol) KOH was added to 100 ml distilled water and stored in a reagent bottle at 4°C.

Oxidase Reagent (100 ml)

To 100 ml distilled water, 1% tetra-methyl-*p*-phenylenediamine dihydrochloride was added and stored in a reagent bottle covered with aluminum foil at 4°C to prevent exposure to light.

Catalase Reagent (20 ml 3% hydrogen peroxide)

From a stock solution of 35 % hydrogen peroxide, 583 µl solution was added to 19.417 ml distilled water and stored at 4°C in a reagent bottle.

Urease Reagent (50 ml 40% urea solution)

To 50 ml distilled water, 20 g pure urea powder was added. The solution was filtered through a HEPA filter and collected into a reagent bottle. The solution was stored at room temperature.

APPENDIX – 3

Instruments:

The important equipment used through this study are listed below

Autoclave	Model: WIS 20R Daihan Scientific Co. ltd, Korea
Sterilizer	Model no: NDS-600D, Japan
Balance machine: Adam	UK
Freezer (-20^{o} C)	Siemens Germany
Incubator	Model-0SI-500D, Digi system Laboratory Instruments Inc. Taiwan
Laminar Airflow Cabinet	Model-SLF-V, vertical, SAARC group Bangladesh
Micropipettes	Eppendorf, Germany
Oven (Universal drying oven)	Model: LDO-060E , Labtech, Singapore
Refrigerator	Samsung
Vortex mixture	Digi system Taiwan, VM-2000