Training Report

Internship Training Report
Submitted to
Lovely Professional University, Punjab
In partial fulfillment of the requirements
For the degree of
Master of Science in Clinical Microbiology

Submitted by:
Rubin Shil.
(Reg No. 11403765.)

SCHOOL OF PHYSIOTHERAPY AND PARAMEDICAL SCIENCES
LOVELY PROFESSIONAL UNIVERSITY, PUNJAB, INDIA
May, 2016

ISOLATION AND IDENTIFICATION OF BACTERIA FROM URINE SAMPLES AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN

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CERTIFICATE

This is to certify that the present thesis entitled "Isolation of bacteria from urine samples & their antibiotic susceptibility pattern" is the outcome of the original piece of work carried out by Mr. Rubin Shil (regd. No= 11403765) himself under my guidance & the contents of his thesis did not form any previous degree to him & to the best of my knowledge to anybody also. This thesis has not been submitted by the candidate for any research degree in any other university.

The project is fit for submission to the partial fulfillment of the conditions for the award of M.Sc. in Clinical Microbiology. Further certified that the candidate in habit & character is a fit & proper person for the award.

Dr. Tapan Majumder

MD. Microbiologist

I/C HOD & Associate Professor

Agartala Govt. Medical College

Miss Savita Devi

Lecturer

Department of Paramedical Sciences

Lovely Professional University

MLT-628 Practical training

LOVELY PROFESSIONAL UNIVERSITY

Transforming Education Transforming India

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21/4/16

Dr. Tapan Majumder

MD. Microbiologist

I/C HOD & Associate Professor

Agartala Govt. Medical College

(DR. TAPAN MAJUMDAR)
MD. (AIIMS)
Associate Professor,
Department of Microbiology
Agartala Govt. Medical College.

Miss Savita Devi

Lecturer

Department of Paramedical Sciences

Lovely Professional University

DECLARATION

I, Rubin Shil student of M. Sc Clinical Microbiology under Department of Paramedical Sciences in Lovely Professional University, Phagwara, Punjab hereby declare that the project work entitled " ISOLATION AND IDENTIFICATION OF BACTERIA FROM URINE SAMPLE AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN" submitted to AO office, Lovely school of Physiotherapy and Paramedical Sciences, LPU, Punjab is a record of an original work to the best of my knowledge done by me under guidance of Miss Savita Devi, Lecturer, Department of Paramedical sciences, LPU, Punjab and this project work is submitted in the fulfillment of the requirement for the award of the degree of Masters of Clinical Microbiology. This project report has not been submitted to any other University or Institute for the award of any degree or diploma.

Lovely Professional University — Rubin Shil

Phagwara, Punjab — Reg. No:-11403765

Date:-

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Rubin Shil

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ABSTRACT

Urinary tract infections (UTIs) are among the most common bacterial infections and account for a significant part of the workload in clinical microbiology laboratories. Enteric bacteria (in particular, *Escherichia coli*) remain the most frequent cause of UTIs, although the distribution of pathogens that cause UTIs is changing. More important is the increase in resistance to some antimicrobial agents, particularly the resistance to trimethoprim-sulfamethoxazole seen in *E. coli*. We can distinguish UTIs from other diseases that have similar clinical presentations with use of a small number of tests, none of which, if used individually, have adequate sensitivity and specificity. Among the diagnostic tests, urinalysis is useful mainly for excluding bacteriuria. Urine culture may not be necessary as part of the evaluation of outpatients with uncomplicated UTIs, but it is necessary for outpatients who have recurrent UTIs, experience treatment failures, or have complicated UTIs, as well as for inpatients who develop UTIs.

A secondary aim was to investigate whether the likelihood of treatment failure varied between different antibiotics and, in the case of trimethoprim (the antibiotic most frequently prescribed for UTIs) between prescriptions of different duration.

AIM & OBJECTIVE

Microorganisms such as bacteria cause urinary tract infections from the digestive tract. When these bacteria cling to the urethral opening, they multiply. The infection begins in the urethra. It can further go up in the bladder to cause cystitis. If it is left untreated, the infection may involve the ureters and eventually the kidneys, leading to pyelonephritis.

The objective of this work is to isolate and identify the bacteria from mid stream urine sample of suspended cases of Urinary Tract Infection patient and to assess antimicrobial sensitivity profile of bacteria causing Urinary Tract Infection.

INTRODUCTION

Urinary Tract Infection (UTI):

Urinary Tract Infections (UTIs) are one of the most prevalent extra-intestinal bacterial infections. Now a days, it represents one of the most common diseases encountered in medical practice affecting people of all ages from the neonate to geriatric age group, (Kumin, 1994). In UTI, it affects the lower urinary tract, which is known as a simple cystitis, which is called bladder infection and when it affects the upper urinary tract, it is known as pyelonephritis (a kidney infection**)**. Some studies carried out in the community have shown that uropathogens such as *Escherichia coli* (46.4 - 74.2%), *Klebsiella sp.* (6.0 - 13.45%), *Proteus* sp. (4.7 - 11.9%) and *Enterococcus* sp. (5.3 - 9.54%) represent the main causes of UTI,(Ines Linhares,2009).

Urinary tract infections occur more commonly in women than in men, with half of women having at least some infection in one point in their lives. Because the length of urinary tract of women is shorter than men. Recurrences are common. Risk factors include female anatomy, sexual intercourse and family history, (Salvatore and Cattoni, 2011).Pyelonephritis, if it occurs, usually follows a bladder infection but may also result from a blood borne infection. Diagnosis in young healthy women can be based on symptoms alone. In those with vague symptoms, diagnosis can be difficult because bacteria may be present without their belong an infection. In complicated cases or if treatment has failed, a urine culture may be useful. In those with frequent infections, low dose antibiotics may be taken as a preventive measure, (Lane and Tkhar, 2011). The treatment of UTI started empirically. Therapy based on the information determined from the antimicrobial resistance pattern of the urinary pathogens, (Safar Farajnia et.al., 2009).

Sign and symptoms:

The common symptoms of UTI are as follows

1. Burning during urination
2. Having to urination frequently
3. Significant pain etc.

These symptoms may vary from mild to severe and in healthy women last an average for six days. Some pain above the pubic bone or in the lower back may be present, (Nicolle, 2008).

Causes:

Different microorganisms can cause UTIs, including fungi and viruses, bacteria are the major causative organisms and are responsible for more than 95% of UTI cases, (Bonadio, 2001).*Escherichia coli* is the most prevalent causative organism of UTI and is solely responsible for more than 80% of these infections. An accurate and prompt diagnosis of UTI is important in shortening the disease course and for preventing the ascent of the infection to the upper urinary tract and renal failure.(National Committee for Clinical Laboratory Standards,2000).

Sex:

About 20% of women experience a single episode of UTI during their lifetime, and 3% of women have more than one episode of UTI per year, (Chandrasekhar et al., 2006).Women are more prone to UTIs than men because in females, the urethra is much shorter and closer to the anus. As a woman's estrogen levels decrease with menopause, her risk of urinary tract infections increases due to the loss of protective vaginal flora, (Sievert and Ricks, 2013).

Urinary Catheters:

Urinary catheterization is one of the main causes of urinary Tract Infection. If the insertion of Catheters is not proper or hygienic, than it can cause UTI. For that, the doctors should do all the work properly and hygienically, (Nicolle, 2011).Catheter-associated UTI is a trenchant problem, with about 5% of catheterized patients developing bacteriuria, (Chandrasekhar et al., 2006).

Pathogenesis:

The bacteria that cause urinary tract infections typically enter the bladder via the urethra. However, infection may also occur via the blood or lymph. It is believed that the bacteria are usually transmitted to the urethra from the bowel, with females at greater risk due to their anatomy. After gaining entry to the bladder, *Escherichia coli* is able to attach to the bladder wall and form a biofilm that resists the body's immune response, (Salvatore and Cattoni, 2011).

Diagnosis:

In most cases, the diagnosis is done by the study of patient history and by the study of the symptoms. In laboratory study, the diagnosis can be done by the culture of urine. In that case if the bacteria colony is greater or equal to 10^3, than we can say that it is the case of UTI. Diagnosis can also done by the presence of urinary nitrites, white blood cells (leukocytes), or leukocyte esterase. However, women with negative cultures may still improve with antibiotic treatment, (Nicolle, 2011). As symptoms can be vague and without reliable tests for urinary tract infections, diagnosis can be difficult in the elderly, (Woodford, 2011).

Preventions:

A number of measures have not been confirmed to affect UTI frequency including : urinating immediately after intercourse , the type of underwear used , personal hygiene methods used after urinating or defecating , or whether a person typically bathes or showers,(Nicolle, 2011) . There is similarly a lack of evidence surrounding the effect of holding ones urine, tampon used and douching, (Dielubanza, 2011). In those with frequent urinary tract infection, who use spermicidal or a diaphragm as a method of contraception, they are advised to use alternative methods, (Salvatore and Cattoni, 2011). Condom used without spermicidal or use of birth control pills does not increases the risk of uncomplicated urinary tract infections, (Gould and Agarwal, 2010).

Treatment:

For the treatment of Urinary Tract Infection, Co-trimoxazole, trimethoprim, ciprofloxacin, norfloxacin, nitrofurantoin, first and second-generation cephalosporin and semi synthetic penicillins with or without inhibitors and fosfomycin trometamol are the most commonly used antibacterial drugs in the treatment of UTI outside of the hospital, (Katarzyna Hryniewicz, 2001).

Women with recurrence simple UTIs may benefit from self-treatment upon occurrence of symptoms with medical follow up only if the initial treatment fails. A prescription for antibiotic can be delivered to a pharmacist by phone. AS complicated UTI is more difficult to treat and usually requires more aggressive evolution, treatment, and follow up, (Kang et.al., 2005).

Antibiotic Resistance:

Now-a-days, drug resistance is a huge growing problem in treating infectious disease like UTI.As suggested by Goldman and Huskin (1997), the improper and uncontrolled use of many antibiotics resulted in the occurrence of antimicrobial resistance, which becomes a major health problem worldwide. In the past days, many kinds of resistant strains have been discovered. For example, Methicillin Resistant *Staphylococcus aureus* (MRSA)**,** multidrug resistant *Pseudomonas aeruginosa*, Vancomycin resistant *Enteriococci* (VRE).

In last three decades, there have been many reports in the scientific literature on the in appropriate use of antimicrobial agents and spread of bacterial resistance among microorganisms causing UTI, (Manikandan Subramanian and Manoj Singh, 2011).

Genes for resistance to antibiotics, like the antibiotics themselves, are ancient. However, the increasing prevalence of antibiotic resistant bacterial infections seen in clinical practice stems from antibiotic use both within human medicine and within veterinary medicine. Any use of antibiotics can increase selective pressure in a population of bacteria to allow the resistant bacteria to thrive and the susceptible bacteria to die off. As resistance towards antibiotics becomes more common a greater need for alternative treatment arises, (Fagarasan et. al., 2012).

LITERATURE REVIEWS

Global Scenario

- Worldwide data show that there is increasing resistance among urinary tract pathogens to conventional drugs. A multicentre study of 141 pathogens from hospital-acquired infections and 460pathogens from community-acquired infections was carried out between July 1998 and May 1999 in Poland. The most prevalent aetiological agent was *Escherichia coli* (73.0%), followed by *Proteus* sp. (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* isolate from both community and hospital infections were highly susceptible to many antimicrobial agents with the exception of those isolates producing extended spectrum β-lactamases (ESBLs). Of all *Enterobacteriaceae* tested, 38 strains (6.9%) were capable of producing ESBLs, (Katarzyna Szczypa and Krzysztof Jankowsky, 2001).

- The empirical therapy of urinary tract infections (UTI) relies on the predictability of the agents causing UTI and knowledge of their antimicrobial susceptibility patterns. In a prospective study undertaken I Iran over a 14-month period, 5136 samples from patients suspected of having a UTI were analyzed, of which 676 were culture-positive. Isolated bacteria were identified by standard tests, and antibiotic susceptibility was determined by disk diffusion method, (Safar Farajnia, 2008).

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

- A total of 206 bacteria were isolated from 150 urine samples collected from the subjects. The prevalence of the *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* isolates from the urine samples is 53.4%, 43.3% and 40.7%, respectively. Commercial sex workers had the highest (30.6%) prevalence of bacteria in their urine samples while the students had the least. Multiple antibiotics resistance was highest for the bacteria isolates obtained from urine samples of the students and commercial sex workers, (Joseph Ehinmidu, 2003).

From 1986 to the time of analysis (September 1996), Microbe Base contained data on 802,301 urinary tract infections (UTIs)—the majority (62%) from general practitioner (GP) patients.

The majority of infections were due to *Enterobacteriaceae*, which caused, on average, 83% of infections in GP patients and 77% of hospital in-patient infections, (Limb and Hancock, 1997).

Indian scenario:

- Analyzing antibiotic susceptibility pattern of uropathogens helped to overcome the therapeutic difficulties created by the rising antimicrobial resistant bacteria and guides in choosing appropriate antibiotics. The aim of the study was to estimate the prevalence of various bacterial isolates and to understand the susceptibility patterns of the uropathogens. Mid-stream urine samples were collected, cultured, and subjected to microscopy and appropriate biochemical tests for proper identification. Antimicrobial susceptibility tests were carried out by this diffusion technique using Muller Hinton Agar as culture media. The most common isolated uropathogens were *Escherichia coli* (82.6%) *Klebseilla pneumonia* (14.6%) and other bacterial species, named *Morganella morganii*, *Enterobacter* spp., *Citrobacter freundii*, *Pseudomonas* species, *Yersinia Enterocoliticia*, were also found in patients with UTI, although they were least frequent. High level of sensitivity was found to imipenem, Nitrofurantion, Meropanem, Ceftazidime, Netilmicin, Zentamicin, Chloram phanicol and amikacin in most of the isolates, (Chandrasekhar et al., 2006).
- Dr. Chaudhuri (2011) states that in India, UTI affects as mainly as 50% women at least once during their lifetime. All individual susceptible to urinary tract infection; However, the prevalence of infection differs with age, sex and certain pre disposing factors. Women are more susceptible to UTI because a woman's urethra is short allowing quick accesses of bacteria to the bladder. In addition, a woman's urethral opening is near sources of bacteria from the anus and vagina. The incidence increases with age and sexual activity. Rates of infection are high in post-menopausal women, because of bladder or uterine prolapsed are causing incomplete bladder emptying; loss of estrogen with attendant changes in vaginal flora, loss of lacto bacilli, which allows pre urethral colonization with Gram negative aerobes, such *Escherichia coli* catheterization is also associated with a very high incidence of UTI.

- Gram-negative bacilli were responsible for UTI infections in our patients. The most common isolated bacteria from urinary tract infections were *Escherichia coli* and the most effective antimicrobial agents were amikacin, tobramicin and ciprofloxacin against Gram negative bacilli and also the most effective antibiotics against Gram positive cocci where kanamycin , tobramycin and ciprofloxacin. (Mansur Amin et al. , 2009).

  In an article Rama Sikka (2011) states that Gram negative organisms are the pre-dominant pathogens casing infections in surgical patients. The increasing trend of resistance to β-lactams is posing a great problem. So for proper management for critically oil patients and patients undergoing various operative procedures and other medical interventions, hospital antibiotic policies make frequent revisions.

- This study was carried out from 8 Jan 2010 to 8 Feb 2011. 60 consecutive *Klebsiella pneumoniae* recovered during the study period in 100-urine sample of UTI patients. 22 isolates were ESBL producer and 38 isolates were non-ESBL producers. The prevalence of extended spectrum β-lactamase producing *Klebsiella pneumoniae* in urine sample of UTI patients was 22%. Detection of extended spectrum β-lactamase producing *Klebsiella pneumoniae* in urine sample of UTI patients was carried out by double disc diffusion method on Muller Hinton Agar. A susceptibility disk containing Piperacillin\ Tazobactum was placed as the inhibitor of β-lactamase in the center of the plate, Piperacillin were placed 30 mm from the Piperacillin\Tazobactum disk. Enhancement of zone of inhibition of disc of Piperacillin alone towards the disc containing Piperacillin\ Tazobactum, showing a figure of eight impression were considered as ESBL producer. All recovered isolates were resistant against ampicillin, amoxicillin, ceftazidime, ceftriaxone, tetracycline, chloramphenicol, gentamicin, and cefotaxime and sensitive against impenem, amikacin, and ciprofloxacin and meropenem. Antimicrobial activity of medicinal plants *Euphorbia heterophylla* and *Acalypha indica* were assessed for ESBL producing *Klebsiella pneumoniae*. (Singh, 2011).
- Urinary tract infection represents one of the most common diseases encountered in medical practice today and occurring from the neonate to the geriatric age group. Despite the widespread availability of antibiotics, it remains the most common bacterial infection in the human being. A total of 174 urine samples were analyzed for isolation and identification, 68 found to be significant bacteriuria with *Escherichia coli* (59%), followed by *Pseudomonas* a*eruginosa* (15%), *Klebsiella pneumoniae* (10%), *Proteus mirabilis* (9%), *Staphylococcus aureus* (6%) and *Citrobacter freundii* (1%). The urinary tract infections were found to most frequently in female (63%) than male (37%). The isolated uropathogens showed resistant to ampicillin (87%), co-trimoxazole (91%), nalidixic acid (88%) and sensitive to nitrofurantoin (52%), cephotaxime (54%) and norfloxacin (71%), (Tambekar, 2006).

METHODOLOGY

- Introduction – Clinical Microbiology is the study of microbes that infect humans, the diseases they cause, and their diagnosis, prevention and treatment. It also deals with the response of the human host to microbial and other antigens.

As microbes are invisible to the unaided eye, definitive knowledge about them had to await the development of microscope. The credit for having first observed and reported bacteria belongs to *ANTONY VAN LEEUWENHOEK*.

- Test done in the department:
  - ✓ Culture,
  - ✓ Gram stain
  - ✓ Acid fast stain.
- Instrument uses in microbiology department–
  - Micros can
  - Bactac- 9050
  - Laminar air flow
  - Microscope
  - Incubator
  - Autoclave
  - Sample culturing on Petri dish

PRINCIPLE OF CULTURING: To isolate & identify the organism after inoculation on the petri dish.

Procedure:

In bacteriology section I cultured different samples like urine, sputum, semen, blood, CSF, stool etc.

1. At first I arranged the plate as per sample,
   Like for Urine – CLED Agar.
   Sputum- Mac Conkey agar, chocolate agar, blood agar,
   Blood sample - Blood agar and Mac Conkey agar etc.
2. Then go for inoculation the particular sample on particular plate.
3. After inoculation keep the plate in incubator for 24 hour

4. After incubation check the growth on Petri plate.

5. If no growth on plate then it goes for negative result, if growth is there then go for MICRO SCANNING process. It is automated and within 24 hour it gives the result which organism is present there.

Fig: 1 & 2(Blood & Chocolate agar plate)

MICRO SCAN

Introduction:

The Micro Scan Walk Away 40 entails inoculation of a microbial suspension into prepared micro well plates for walk away system. These micro well panels contain a variety of conventional and proprietary biochemical substrates and antibiotics. Growth of bacteria within the micro wells results in biochemical substrate changes which can be interpreted by a specialized panel reader to produce a biochemical profile. This profile can be compared to the profiles of known micro-organisms to generate identification.

Micro-scan

Fig – 3

Operation of Micro-log system also involves inoculation of a microbial suspension into specialized micro-well plates. The wells of these plates contain buffered media with different carbon sources and indicator dye, tetrazolium violet. The dye is reduced when different carbon sources are utilized, resulting in a biochemical profile which can be compared to the profile of known micro-organism to generate an identification.

Principle: To establish the standard operating procedure to determine automatically the identification and sensitivity of organism isolated from clinical specimen to various drugs (Ab_s) by using Walk Away 40.

PROCEDURE OF MICROSCAN:

Panel Preparation Procedure

1. Remove the panel to be used from storage.
2. Allow panel to be equilibrate to room temperature prior to rehydration. All open panels should be used in the same day.

PROMPT SYSTEM;

1. Remove the required number of diluents bottle.
2. Label each bottle with the lab number/isolate number and the panel to be tasted (GP or GN).
3. Remove an inoculation wand.
4. Holding the wand straight up and down, touch 3-5 well isolated colonies.
5. Holding the wand by the handle with one hand, grasp the collar with the other hand and pull firmly to break the connection between the collar and the wand shaft.
6. Slide the collar down and off the collar wand shaft. Discard the collar.
7. While holding the prepared wand in one hand, remove the cap of the diluent bottle by bending the cap sideways.
8. Place the wand into the bottle and press down with a twisting motion to assure a tight seal.
9. Shake the bottle vigorously 8-10 times to release bacteria from the wand tip. The inoculums should sit for 15 minutes, but no longer than 4 hours, before inoculating the test panels.

PANEL RE-HYDRATION/ INOCULATION;

1. Remove the inoculation wand from the diluents bottle and use to inoculate a purity plate. Discard the wand in biohazards waste.
2. Pour the suspension into the seed tray. Squeeze the bottle gently as you pour.
3. Place the transfer lid over the seed tray.
4. Gently tap the transfer lid in all four corners to remove bubbles from underneath the prongs. Allow the transfer lid to equilibrate for a minimum of 20 second before proceeding.
5. Pick up the RENOK unit by depressing the pick-up levers with the thumb and finger. Place on the top of the transfer lid.
6. Release the pick-up levers.
7. Fully lift the center lever of the RENOK unit to draw inoculum into the transfer lid, then release hand pressure.
8. Pick up the RENOK unit/transfer lid assembly and position over the Micro Scan Panel.
9. Depress the center release button of the RENOK unit to release the inoculums into well.

10. Release the transfer lid into the seed tray and dispose into biohazard waste and repeat to each isolate to be tested. Processing panels in Micro Scan Walkaway 40 system.

BACTAC-9050

PRINCIPLE: The detection of microorganisms in a patient's blood has diagnostic and prognostic importance. Blood cultures are essential in the diagnosis and treatment of the etiologic agents of sepsis. Bacterial sepsis constitutes one of the most serious infectious diseases and, therefore, the expeditious detection and identification of blood borne bacterial pathogens is an important function of the diagnostic microbiology laboratory. The BACTEC 9000 series of blood culture instruments are designed for the rapid detection of microorganisms in clinical specimens. The sample to be tested is inoculated into the vial which is entered into the BACTEC instrument for incubation and periodic reading. Each vial contains a sensor which responds to the concentration of CO2 produced by the metabolism of microorganisms or the consumption of oxygen needed for the growth of microorganisms. The sensor is monitored by the instrument every ten minutes for an increase in its fluorescence, which is proportional to the increasing amount of CO2 or the decreasing amount of O2 present in the vial. A positive reading indicates the presumptive presence of viable microorganisms in the vial.

Procedure of BACTEC:

1. Take the sample and scan it by the scanner of the BACTEC.
2. Then put the sample on a particular position that is command the machine (red colour position).
3. Wait 24 hour

BACTEC 9050

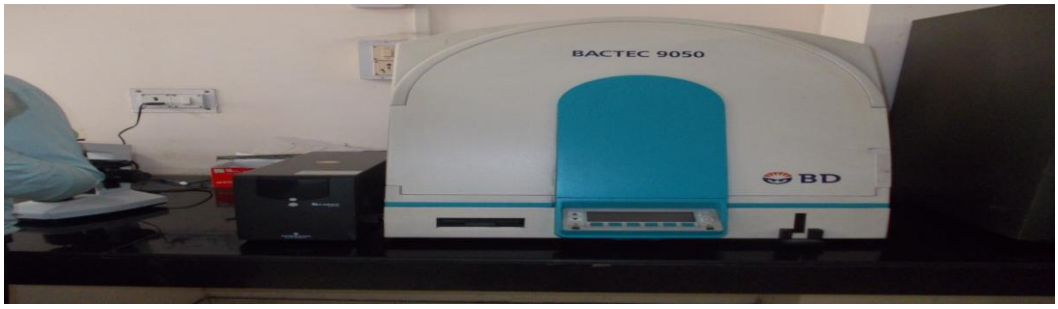

Fig-4

LAMINAR AIR FLOW (BIOSAFETY CABINET)

A laminar flow cabinet is a carefully enclosed bench designed to prevent contamination of semiconductor wafers, biological samples, or any particle sensitive device. Air is drawn through a HEPA filter & blown in a very smooth, laminar flow towards the user. The cabinet is usually made of stainless steel with no gaps or joints where spores might collect.

Such hoods exist in both horizontal & vertical configuration, & there are many different types of cabinets with a variety of air flow patterns & acceptable uses. NSF49 is the commonly accepted regulatory standard for these cabinets.

Laminar flow cabinets may have a UV-C germicidal lamp to sterilize the shell and contents when not in use. (It is important to switch this light off during use, as it will quickly give any exposed skin sunburn and may cause cataracts.)

A laminar flow cabinet or laminar flow closet or tissue culture hood is a carefully enclosed bench designed to prevent contamination of semiconductor wafers, biological samples, or any particle sensitive materials. Air is drawn through a HEPA filter and blown in a very smooth, laminar flow towards the user. The cabinet is usually made of stainless steel with no gaps or joints where spores might collect.

Such hoods exist in both horizontal and vertical configurations, and there are many different types of cabinets with a variety of airflow pattern and acceptable uses.

Laminar flow cabinets may have a UV-C germicidal lamp to sterilize the interior and contents before usage to prevent contamination of experiment. Germicidal lamps are usually kept on for 15 minutes to sterilize the interior and no contact is to be made with a laminar flow hood during this time. During this time, scientists normally prepare other materials to maximize efficiency. (It is important to switch this light off during use, to limit exposure to skin and eyes as stray ultraviolet light emissions can cause cancer and cataracts.

Fig- 5 & 6

REQUIRMENTS

Equipment required:

- Autoclave
- Hot air oven
- Laminar air flow
- BOD incubator
- Shaker incubator
- Micropipette tips (0.5 – 10 µl, 2-10 µl, 20 – 100 µl, 200 – 1000 µl)
- Micropipettes (0.5 – 10 µl, 2-10 µl, 20 – 100 µl, 200 – 1000 µl)

Antibiotic disks : Vancomycin(10µg), Penicilin(10µg), Novobiocin(10µg), Amikacin (30µg), Cepfotoxin (10µg), Ciprofloxacin (5µg), Ceftriaxone (30µg), Gentamicin (10µg), Ampicillin(10µg),Tetracycline(30µg), Doxycyclin(5µg), Cephalosporin(30µ), Amoxilin (10µg),Kanamycin(10µg), Chloramphenicol (5µg).

Media used:

- Urea Agar Media
- Starch Agar Media
- Triple Sugar Iron Agar (TSIA) Media
- Mannitol Agar Media
- MR-VP Broth Media
- Simmon's Citrate Agar Media
- Muller Hinton Agar (MHA) Media
- MacConkey Agar Media
- Blood Agar Media

Experimental methods:

Collection of clinical samples:

The study has been carried out on mid stream urine samples collected from patients admitted to the AGMC and GBP Hospital . A total number of 300 samples have been taken to carry out this experiment. Out of 300 samples, 180 samples are of female patients and 120 samples are of male patients.

Processing of clinical samples:

- Fresh mid-stream urine samples were aseptically collected in sterile containers.
- One loopful of the mid stream urine sample was inoculated on to CLED Agar ,Nutrient agar, MacConkey's agar and blood agar.
- Bacterial growth was seen in 126 samples from the total 300 samples collected. Five different types of bacterial colonies were present out of 126 samples where bacterial growth occurred and were subjected to Gram staining for identification.
- Staining result showed the presence of fifty six-Gram positive bacteria (cocci in cluster) and seventy -Gram negative bacteria, which were further, processed.

- Sub culturing was done on nutrient agar medium and pure culture was maintained for further use.

Gram's staining (Aneja, 2003):

This is the most common and important step in the identification of the bacteria. This is a type of differential staining in which we use two stains for staining of smear. It differentiates the bacteria mainly into two groups- Gram positive and Gram negative.

Principle:

The principle of Gram's staining is mainly based on the property of the bacteria to retain the primary stain because of the presence of cell wall. When mordent is added to the smear there is formation of a complex of primary stain and mordent. When Grams decolourizer is applied the primary stain does not come out of the cell wall of Gram positive bacteria and in case of Gram negative bacteria the stain comes out easily because of thinner cell wall and lesser peptidoglycan. When secondary stain is applied, the Gram-negative bacteria retain the secondary stain and appear pink.

Requirements:

- Primary stain (Crystal violet)
- Mordent (Grams Iodine)
- Decolourizer
- Secondary stain (Safranine)
- Glass slides, microscope

Procedure

1) The thin smear is prepared by transferring the loop full of culture aseptically on the clean glass slide and air dried.
2) Now the smear is heat fixed.
3) Then the primary stain (crystal violet) is applied to the smear for 1 min.
4) After this slide is washed with distilled water and mordent is applied for 60 seconds.
5) Grams decolourizer is applied till no more violet colour comes off the slide and then washed with distilled water.
6) Now the slide is flooded with secondary stain (safranin) for 30 seconds min and then washed with distilled water.

7) Slide is air dried and observed under oil immersion objective lens.

Biochemical analysis for identification of isolated bacteria: (Aneja, 2003):

Urease test:

Urea is a major organic waste product of protein digestion in most vertebrates and is excreted in the urine; some microorganisms have the ability to produce the enzyme urease.

Urease test was performed by growing the test organism on urea broth or agar medium containing the pH indicator phenol red (pH6.8). During incubation, microorganisms possessing urease will produce ammonia that raises the pH of the medium. As the pH becomes higher, the phenol red changes from a yellow colour to a red or deep pink colour. Failure of development of a deep pink colour due to no ammonia production is evidence of a lack of urease production by the microorganism.

Requirements:

- Urea agar medium
- Inoculation loop
- Bunsen burner
- Wax making pencil

Procedure:

1. Preparation of urea agar medium whose constituents (per liter) are as follows:
2. Peptone (1.0 gm)
3. Sodium Chloride (5.0 gm)
4. Potassium monohydrate phosphate (2.0gm)
5. Agar (20 gm)
6. Distilled water (1000 ml).
7. Dissolve the ingredients by heating, adjust the pH to 6.8 and autoclave 121°C for 15 minutes and cool to 50 ^{0}C.
8. Glucose (1 gm.).
9. Phenol red (0.2 % solution)
10. Add to the molten base and steam for one hour, cool to 50^{0}c
11. Urea, 20 % aqueous solution
12. Sterilize by filtration and add aseptically to the basal medium
13. Mix well, distribute into sterile containers, i.e., culture tubes, and allow the medium to solidify in slanting position to form slopes.
14. Keep a tube of urea agar medium as control and another tube with the name of the bacterial organism to be inoculated.
15. Inoculate the bacteria into the culture tube.
16. Incubate the two slants for 24 to 48 hours at 37^{0}c.

Observation:

Inoculated culture shows deep pink colorization of the medium thus showing positive reaction for the degradation of urea by means of the production of an enzyme urease.

Starch hydrolysis test:

Starch is a complex carbohydrate composed of two constituents – amylose and amylopeptin. Amylase is an exo-enzyme that hydrolyses starch a polysaccharide. Into Maltose a disaccharide and some monosaccharides such as glucose. Some bacteria can produce amylase enzyme.

The ability to degrade starch is used as a criterion for the determination of amylase production by a microbe. In the laboratory, it is tested by performing the starch test to determine the absence or presence of starch in the medium by using iodine solution as an indicator. Starch in the presence of Iodine produces a dark blue coloration of the medium, and a yellow zone around a colony in an otherwise blue medium indicates amylolitic activities.

Requirements:

- Starch agar medium
- Gram's Iodine solution
- Sterile Petri dishes (2)
- Dropper
- Inoculating loop
- Bunsen burner
- Wax marking pencil

Procedure:

1) Melt the starch agar medium, cool to 45^0c and pour into the sterile Petri dishes
2) Allow it to solidify
3) Level one plate as control and another plate with the name of the organism to be inoculated
4) Using sterile technique make a single streak inoculation of the organism into the center of its appropriately leveled plate.
5) Incubate the plates for 48 hours at 37^0c.
6) Flood the surface of the plates of the iodine solution with a dropper for 30 seconds.
7) Pour off the excess iodine solution

Observations:

A typical positive starch hydrolysis reaction (i.e., clear zone surrounding the microbial colonies) is observed.

Citrate test:

The citrate test utilizes Simmons's citrate media to determine if a bacterium can grow utilizing citrate as its carbon and energy sources. Simmons' citrate media contains bromthymol blue, a pH indicator with a range of 6.0 to 7.6. A bromthymol blue shows green colour at acidic pH and gradually changes the colour to blue at more alkaline pH . Uninoculated Simmons's citrate agar has a pH of 6.9. Therefore, it is an intermediate green colour. Growth of bacteria in the media leads to development of blue colour (positive citrate).

Observations:

Development of blue colour is observed in the media.

Triple sugar iron test:

Triple sugar iron agar (TSIA) medium is composed of three sugars: Lactose, Sucrose and very small amount of glucose, iron, and phenol red as an indicator. The indicator is employed for the direction for sugars indicated by the change in the colour of the medium due to the production of organic acids and hydrogen sulphide (H_2S). If an organism ferments any of the three sugars or any combination of them. The medium will become yellow due to the production of acid as end product of the fermentation. If the slanted portion of the tube remains red and the butt of the tube becomes yellow then it shows glucose fermentation only. If the slant and the butt both become yellow then the pathogen shows lactose and glucose fermentation. Hydrogen sulphide production by an organism is indicated by the reduction of ferrous sulphate of the medium to ferric sulphide, which is manifested as a black precipitate.

Observations:
Glucose and lactose fermentation is observed with no production of H_2S.

Catalase test:

During aerobic respiration in the presence of oxygen, Microorganisms produce Hydrogen peroxide (H_2O_2) which is lethal to the cell. The enzyme catalase present in some microorganisms breaks down hydrogen peroxide to water, hydrogen, and help-s them in their survival. Catalase test is performed by adding hydrogen peroxide (H_2O_2) . Release of free oxygen gas bubbles is a positive catalase test.

Observations:
Appearance of gas bubbles is observed.

Mannitol fermentation test:

The purpose of the test is to see if the microbes can ferment the carbohydrate in mannitol as a carbon source. If mannitol is fermented to produce acid end products, the pH of the medium will drop. A pH indicator in the medium changes colour to indicate the acid production.

Observations: The colour of the media turns pink to yellow.

Coagulase test:

Coagulase test is used to differentiate *Staphylococcus aureus* from coagulase negative *Staphylococci*. *Staphylococcus aureus* produces two forms of coagulase (i.e., bound coagulase and free coagulase) .Bound coagulase are otherwise known as 'clumping factor', can be detected by carrying out a slide coagulase test.

Observations:

If clumping occurred then the sample bacterium shows coagulase positive results.

MR-VP Test:

The Methyl Red (MR) & Voges Proskauer (VP) tests are read from a single inoculated tube of MR-VP broth. After 24-48 hours of incubation, the MR-VP broth is split into two tubes. One is used for MR test and the other is used for the VP test.

MR-VP media contains glucose and peptone. All enteric oxidize glucose for energy; however, the products vary depending on bacterial enzymes. Both the MR & VP tests are used to determine what end products result when the test organisms degrade glucose. When the pH indicator methyl red is added the acidic broth it will be cherry red (a positive MR test) .

The reagents used for the VP test are Barritt's (α napthol) & Barritt's B (potassium hydroxide). When these reagents are added to a broth in which acetyl methyl carbinol is present, they turned into a pink – burgundy colour (a positive VP test). It may take 20 – 30 minutes to develop.

RESULTS

RESULT OF BIOCHEMICAL TESTS FOR IDENTIFICATION:

ISOLATED BACTERIA 1 (S1):

EXPERIMENTAL TABLE OF BIOCHEMICAL TESTS OF ISOLATED BACTERIA (S1)	
Name of the tests	Results
Gram's staining	Gram positive
Catalase	Positive
Coagulase	Positive
MR test	Positive
VP Test	Negative
Simmon citrate Test	Positive
Urease Test	Positive
Starch Hydrolysis	Positive
Mannitol fermentation	Positive
TSI Test	Glucose fermentation Positive
H_2S Production	Negative

Isolated bacteria sample 1 (S1) shows in Gram's staining Gram positive cocci in clusters, which was positive for coagulase test, urease, Starch hydrolysis, Glucose fermentation, mannitol fermentation, catalase, Simmon's citrate.

Grams Staining:

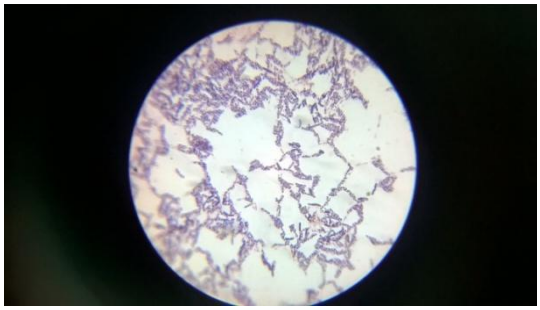

Fig: S1- Grams staining shows gram positive cocci in clusters

Coagulase Test**:**

Fig**:** S1- Shows Coagulase test positive

MR Test:

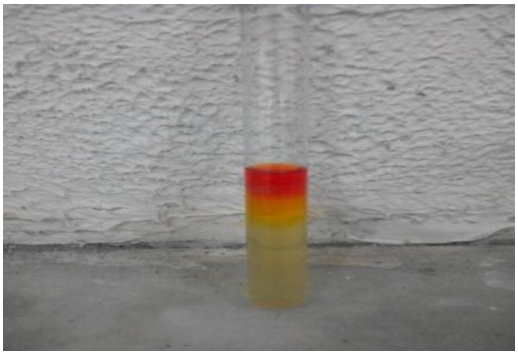

Fig: S1- Shows MR test Positive

Simmons Citrate:

Fig: S1- shows Simmons citrate test Positive

Urease Test:

Fig: S1- Shows Urease test Positive

TSI:

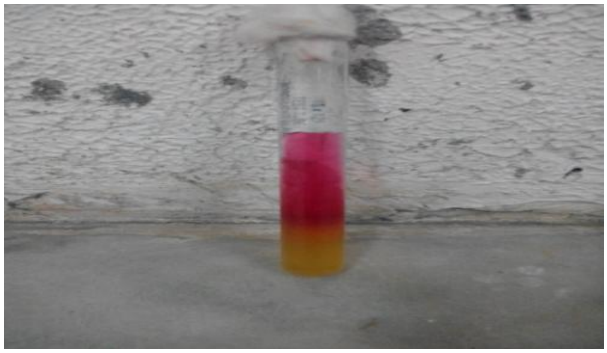

Fig: S1- Shows Glucose Fermentation test Positive

Starch Hydrolysis Test:

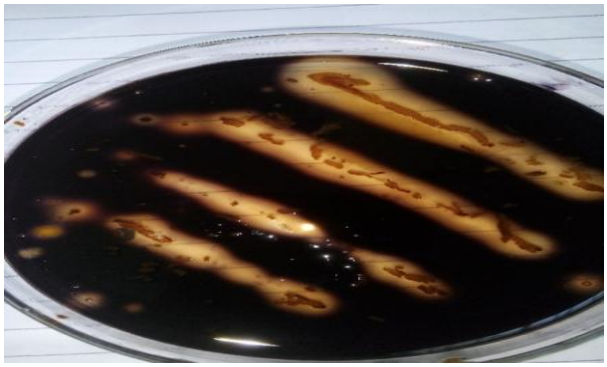

Fig: S1- Shows Starch Hydrolysis test Positive

ISOLATED BACTERIA 2 (S2):

EXPERIMENTAL TABLE OF BIOCHEMICAL TEST OF ISOLATED BACTERIA 2 (S2)	
Name of the tests	Results
Gram's staining	Gram Negative
Catalase	Positive
MR Test	Negative
VP Test	Positive
Simmons Citrate	Positive
Urease	Positive
Starch Hydrolysis	Positive
TSI Test	Glucose , lactose fermentation positive
H_2S	Positive
Oxidase Test	Negative

Isolated Gram negative sample 2 (S2) shows in Gram staining Gram negative rod, which was positive for VP test , Simon's citrate , Catalase, Urease , Starch hydrolysis , glucose and lactose fermentation and negative for MR Test & Oxidase test.

Grams staining:

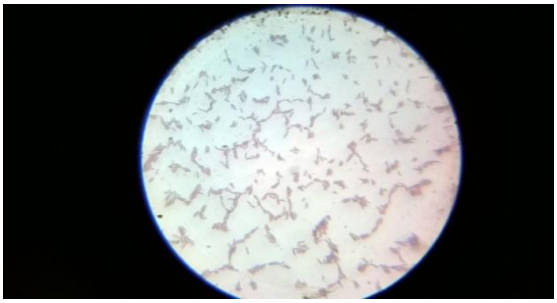

Fig: Gram Staining of S2 Bacteria

MR Test:

Fig: Isolated Bacteria S2 Shows MR Test Negative

VP Test:

Fig: Isolated Bacteria S2 Shows VP Test Positive

Simmons Citrate Test:

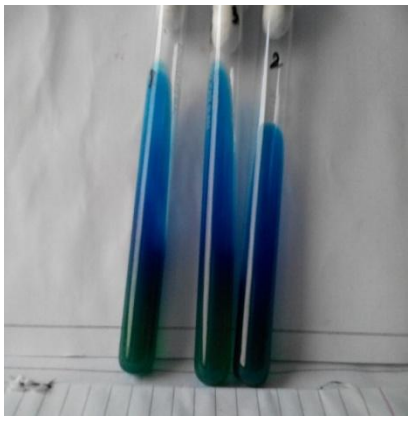

Fig: Isolated Bacteria S2 Shows Simmons Citrate Test Positive

Urease Test:

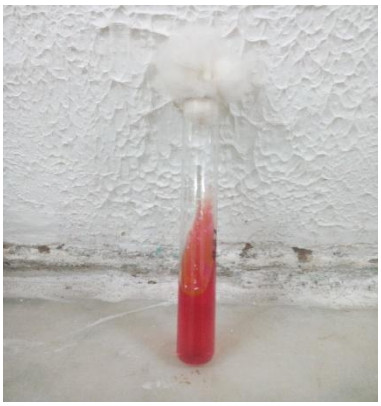

Fig: Isolated Bacteria S2 Shows Urease positive

TSI Test:

Fig: Isolated Bacteria S2 Ferments Glucose & Lactose

Starch Hydrolysis Test:

Fig: S2 Shows Starch Hydrolysis Test positive

Oxidase Test:

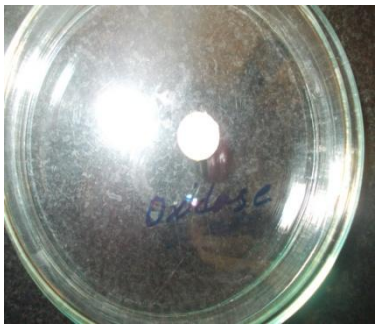

Fig: S2 Shows Oxidase Test Negative

ISOLATED BACTERIA 3 (S3):

EXPERIMENTAL TABLE OF BIOCHEMICAL TEST OF ISOLATED BACTERIA 3 (S3)	
Name of the tests	Results
Gram staining	Gram Negative
Catalase	Positive
MR Test	Positive
VP Test	Negative
Simmons Citrate	Negative
Urease	Negative
Starch Hydrolysis	Negative
TSI Test	Glucose positive , lactose negative
H_2S	Negative
Oxidase Test	Negative

Isolated Gram negative sample 3(S3) bacteria shows in Gram's staining Gram negative rod, which was positive for Catalase, MR test, glucose fermentation and negative for VP test, Simmon's Citrate test. Urease Test, Oxidase test and Starch hydrolysis

Grams Staining:

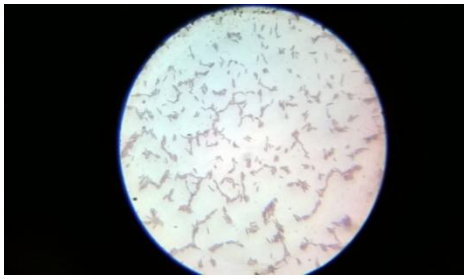

Fig: Grams Staining of S3 Bacteria

MR Test:

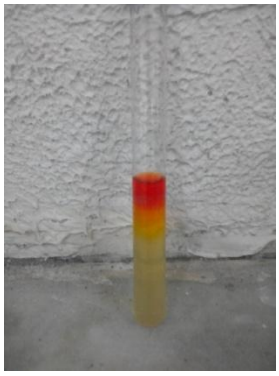

Fig: Isolated Bacteria Shows MR Test Positive

VP Test:

Fig: S3 Shows VP Negative

Simmons Citrate Test:

Fig: S3 Shows Citrate Negative

TSI Test:

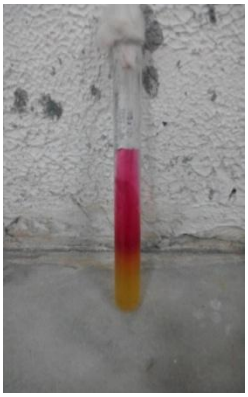

Fig: S3 Shows Glucose Fermentation

Oxidase Test:

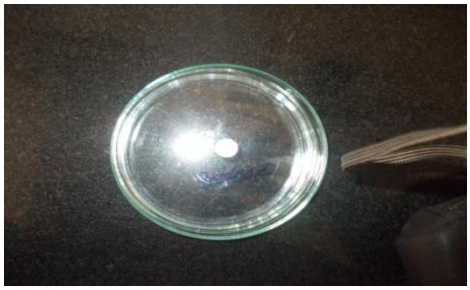

Fig: S3 Shows Oxidase Test Negative

ISOLATED BACTERIA 4 (S4):

EXPERIMENTAL TABLE OF BIOCHEMICAL TEST OF ISOLATED BACTERIA 4 (S4)	
Name of the tests	Results
Gram staining	Gram positive
Catalase Test	Positive
Coagulase	Negative
MR Test	Positive
VP Test	Negative
Simmon's Citrate Test	Positive
Urease	Positive
Mannitol Fermentation	Positive
TSI	Glucose fermentation positive
H_2S	Negative

Isolated bacteria 4 (S4) shows in Gram's staining Gram positive cocci in cluster, which shows positive result for Urease, Starch hydrolysis, Glucose fermentation, Mannitol fermentation, Catalase , Simmon's citrate and negative for Coagulase.

Grams Staining:

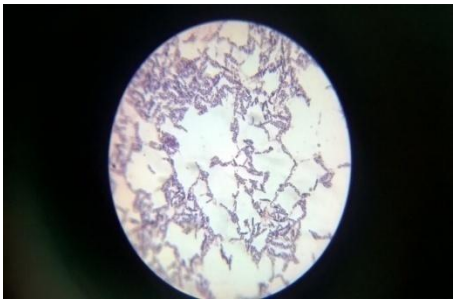

Fig: Grams Staining Shows Gram Positive Cocci in Clusters

Coagulase Test:

Fig: S4 Bacteria Shows Coagulase Test negative

MR Test:

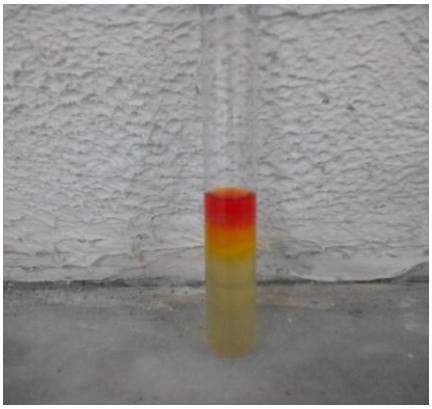

Fig: S4 Shows MR Test Positive

Simmons Citrate Test:

Fig: S4 Shows Simmons Citrate Positive

Urease Test:

Fig: S4 Shows Urease Test Positive

TSI Test:

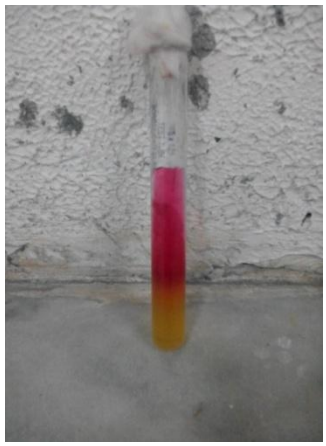

Fig: S4 Ferments Glucose

Starch Hydrolysis Test:

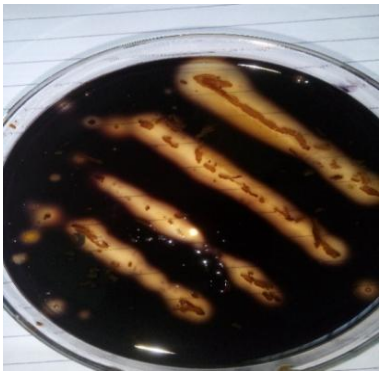

Fig: S4 Bacteria Starch Hydrolysis Test Positive

ISOLATED BACTERIA 5 (S5):

EXPERIMENTAL TABLE OF BIOCHEMICAL TEST OF ISOLATED BACTERIA 5 (S5)	
Name of the tests	Results
Gram's staining	Gram Negative
Catalase Test	Positive
MR Test	Positive
VP Test	Negative
Simmons citrate	Positive
Urease Test	Positive
Starch Hydrolysis	Negative
Mannitol Fermentation	Negative
TSI Test	Glucose Positive
H_2S Test	Negative
Oxidase Test	Negative

Isolated bacteria 5 (S5) shows in Gram's staining Gram negative rod, which shows positive result for Urease, Glucose Fermentation, Catalase, Simmon's citrate, MR test and negative for VP test, Starch hydrolysis, Oxidase test and Mannitol fermentation.

Grams Staining:

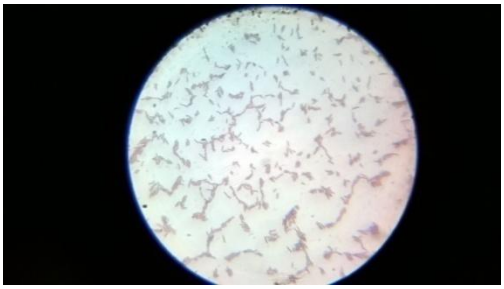

Fig: Grams Staining Of S5 Bacteria

MR Test:

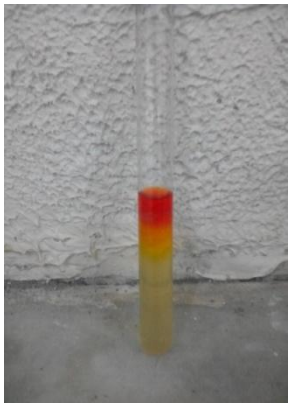

Fig: Isolated Bacteria S5 Shows MR Positive

VP Test:

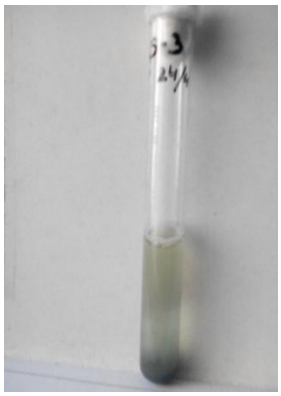

Fig: S5 Shows VP Negative

Simmons Citrate Test:

Fig: Isolated Bacteria S5- Shows Simmons Citrate Positive

Urease Test:

Fig: Isolated Bacteria S5 Shows Urease Test Positive

TSI Test:

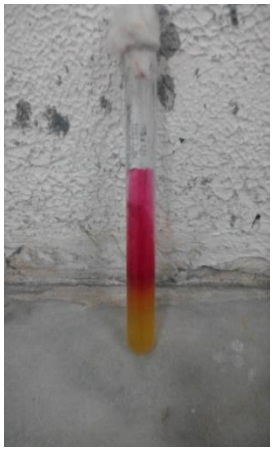

Fig: Isolated Bacteria S5 Ferments Glucose

Oxidase Test:

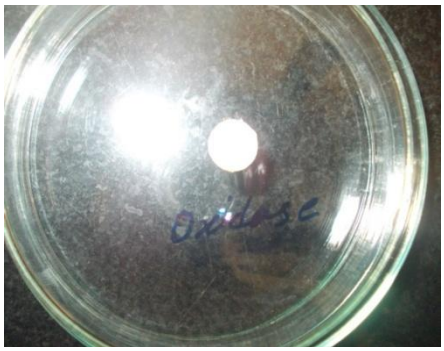

Fig: Isolated Bacteria S5 Shows Oxidase Test Negative

- Isolated Bacteria S1:

The characteristics of isolated bacteria S1 mostly matched with *Staphylococcus aureus.* So, S1 Bacteria is suggestive of *Staphylococcus aureus.*

- Isolated Bacteria S2**:**

The characteristics of isolated bacteria S2 mostly matched with *Klebsiella sp.* So, the isolated S2 Bacteria is suggestive of *Klebsiella pneumoniae.*

- Isolated Bacteria S3:

The characteristics of isolated bacteria S2 mostly matched with *Escherichia coli.* So, the S3 Bacteria is suggestive of *Escherichia coli.*

- Isolated Bacteria S4:

The characteristics of isolated bacteria S4 mostly matched with *Staphylococcus sp.* But this bacteria shows coagulase test negative. So, the isolated bacterium is suggestive of *Staphylococcus saprophyticus.*

- Isolated Bacteria S5:

The characteristics of isolated bacteria S5 mostly matched with *Protius sp.* Therefore, the bacteria is suggestive of *Protius vulgaris*

ANTIBIOTIC SUSEPTIBILITY TEST:

1) In 5 tubes, (3 ml peptone water each) 5µl of stock culture for 5 samples were inoculated and kept for overnight incubation at 37^0c.
2) The overnight culture were spread in five Muller Hilton Agar Petri plates and then kept for drying for 5 minutes.
3) Antibiotics were placed at equal distance within the Petri plates containing culture.
4) Then the plates were incubated at 37^0C for overnight.
5) After overnight incubation the zone of inhibition for each antibiotic discs in culture plates were measured and tabulated for interpretation.

RESULT OF ANTIBIOTIC SENSITIVITY:

Staphylococcus aureus (S1):

Antibiotic Used: Vancomycin(10µg), Penicilin(10µg), Novobiocin(10µg)

Fig: Antibiotic Susceptibility Test for *Staphylococcus aureus*

Klebsiella pneumonae (S2):

Antibiotic Disc Used: Amikacin(30µg), Cepfotoxin(10µg),Ciprofloxacin(5µg), Ceftriaxone(30µg), Gentamicin(10µg), Ampicillin(10µg) And Tetracycline(30µg)

Fig: Antibiotic Susceptibility Test for *Klebsiella pneumonia*

Escherichia coli (S3):

Antibiotic Used: Tetracyclin(30µg), Doxycyclin(5µg), Cephalosporin(30µg), Ampicilin(10µg)

Fig: Antibiotic Susceptibility Test for *E.coli*

Staphylococcus saprophyticus (S4):

Antibiotic Used: Vancomycin(10µg), Penicilin(10µg), Tetracyclin(30µg), Ampicilin(10µg), Amikacin(30µg), Novobiocin(10µg).

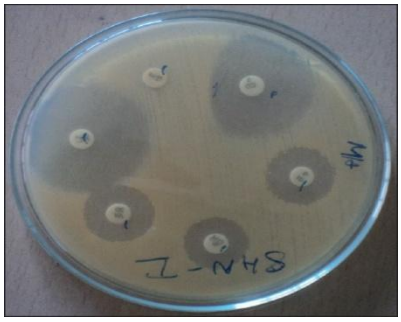

Fig: Antibiotic Susceptibility Test for *Staphylococcus saprophyticus*

Protius vulgaris (S5):

Antibiotic Used: Tetracyclin(30µg), Ciprofloxacin(5µg), Chloramphenicol(5µg), Kanamicin(10µg), Vancomycin(10µg), Amoxilin(10µg).

Fig: Antibiotic Susceptibility Test for *Protius vulgaris*

TABLE FOR ANTIBIOTIC SENSITIVITY:

Staphylococcus aureus (S1):

VAN	PEN	NOV
16mm	22mm	12mm

Klebsiella pneumoniae (S2):

AMK	CEF	CIP	CFT	GNT	AMP	TET
14 mm	21mm	23 mm	3mm	1 mm	R	R

Escherichia .coli (S3):

DOX	CEP	AMP	TET
19mm	14mm	17mm	22mm

Staphylococcus saprophyticus (S4):

VAN	PEN	TET	AMP	AMK	NOV
25mm	27mm	12mm	10mm	7mm	R

Proteus vulgaris (S5):

TET	VAN	CLP	AMX	KNM	CIP
R	27mm	8mm	13mm	1mm	9mm

DISCUSSION

Urinary tract infection (UTI) is one of the most important causes of morbidity in the general population, and is the second most common cause of hospital visits, (Chandrasekhar, 2006).

Bacterial infection of urinary tract is one of the common causes for seeking medical attention in the community**,** (Getenet Beyene and Wondewosen Tesaye, 2010).

In this study, the isolation rate of bacteria from urine was 70% (7 out of 10). *Escherichia coli* and *Klebsiella pneumoniae* are the major causing agent for UTI. *Escherichia coli* was found in 85.71% cases. *Klebsiella pneumoniae* is found in 71.42% cases, where *Staphylococcus aureus* (14.28%), *Staphylococcus saprophyticus* (28.57%) and *Proteus* sp. (28.57%) were less common.

But the study of Joseph O Ehinmidu,(2003) states that result of bacterial isolation from urine samples from the samples investigated indicated that the order of prevalence of these bacteria is *Pseudomonas aeruginosa* (53.3%) >*Staphylococcus aureus* (43.3%) > *Escherichia coli* (40.7%).

Another study of Getenet Beyene and Wondewosen Tsegate(2010) from Southwest Ethiopia reported that *Escherichia coli* is the main aetiological agent in causing UTI, which accounts for up to 90% cases, which is in agreement with the findings of this study.

The study of antibiotic sensitivity shows that *Klebsiella pneumoniae* and *Proteus vulgaris* shows 100% resistant to tetracyclin antibiotic. On the other hand, Vancomycin and penicillin shows effective result against *Staphylococcus aureus, Staphylococcus saprophyticus,* and *Protius vulgaris*. But the study of Joseph O Ehinmidu(2003) from Nigeria States that Penicillin is 0% sensitive to *Staphylococcus aureus*.

Mansur Amin et al.,(2009) state that Gram negative bacilli were responsible for UTI infections. The most common isolated bacteria from urinary tract infections were *Escherichia coli* and the most effective antimicrobial agents were amikacin, tobramicin and ciprofleoxacin against Gram negative bacilli. But, in my study I have observed that Tetracyclin and Doxycyclin is most effective against *Escherichia coli.* and Cepfotoxin and Ciprofloxacin against *Klebsiella pneumoniae.*

Chandrasekhar and Hari Shanker Joshi (2006) conducted a study and the result has revealed that *Escherichia coli*, which was the principal pathogen isolated, showed high susceptibility to common antibiotics ampicillin.But the present study *Escherichia coli* showed high susceptibility to Tetracyclin.

In this study, Gram-positive isolates show most susceptibility to Penicillin antibiotic. But the another study of(Ehinmidu , 2003) showed that Gram positive bacteria showed also higher resistance to Penicillin than to the other tested antibiotics and The isolates of *Staphylococcus aureus* showed high resistance to penicillin (average of 55.1%).

In this study, I have found that *Klebsiella pneumoniae* was found to be highly susceptible to Amikacin, Cepfotoxin and Ciprofloxacin and resistant to Ampicilin and Tetracyclin. 1But the

study of Singh(2011) states that the antibiotics which are found to be resistant against *Klebsiella pneumoniae* were Carbapenem, Cephalosporin, Ampicillin,,Penicillin Amoxicilin, Gentamicin, Kanamycin, Cofoxitin,Naldixic acid and the antibiotics which are found to be sensitive were Cefotaxime, Ceftazidime, Ceftriaxone,Tetracyclin and Amikacin.

CONCLUSION

In the present study, a high rate of isolates from mid stream urine sample of suspected cases of UTI is observed to be Gram-negative bacilli, Viz., *Escherichia coli* and *Klebsiella pneumoniae* are the major causing agent for UTI. Data presented in this study indicate that antibiotics commonly used for the treatment of UTIs are effective, but the common antibiotic tetracyclin is less effective against *Klebsiella pneumoniae* and *Proteus vulgaris.* So, further regular monitoring is required to establish reliable information about resistance pattern of urinary pathogens for optimal empirical therapy of patients with UTI.

FUTURE PROSPECTS

- Use of chemically synthesized antibiotic has bad effect to human health. That is why we need to find some alternative treatment module by using medicinal plants, which is effective against UTI causing bacteria.
- We need to find the age and sex specific trends of antibiotic sensitivity against UTI causing bacteria.
- In future, we can study on ESBL producing bacteria.
- We can study on multi drug resistant UTI causing bacteria and design new medicine against the bacteria.
- We need to know how the bacteria acquire resistance against a particular antibiotic.
- The compilation of the data would be helpful in studying the epidemiological patterns of the infection with reference to the emergence of the drug resistant strains and could be used as base level information to control indiscriminate usage of antibiotics.

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