Antibiotic susceptibility profile of MDR *Pseudomonas* in Nosocomial Urinary Tract Infection In Oxford Hospital, Jalandhar

Submitted in partial fulfillment of the requirements of the
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MASTER OF SCIENCE
(CLINICAL MICROBIOLOGY)

By

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CERTIFICATE

This is to certify that, the work entitled **"**Antibiotic susceptibility profile of MDR *Pseudomonas* By Nosocomial Urinary Tract Infection In Oxford Hospital, Jalandhar**"** was carried out by **Ms. Sumandeep Kaur ,** under my direct supervision. This is to futher certify that this report embodies the original work carried out by the candidate herself and has not been submitted elsewhere in any form or for any other degree.

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Date :

Place: Phagwara

DECLARATION

I hereby declare that work embodied in this Full Term Internship Training report was carried out by me under the direct supervision of **Mr.Himal Sapkota**,Lecturer, Department Of Paramedical Sciences, Lovely Professional University (Phagwara, Punjab). This work has not been submitted in part or in full in any other university for any degree.

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At the onset I would like to dedicate this work to the Endeavour of my family. I continue to add that the present report is result of efforts of many people who have support me at sundry level.

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ABSTRACT

One of the significant health problems in many areas of the world is Urinary Tract Infection and at high risk for nosocomial infection. The catheter represents a site susceptible to colonization of opportunistic pathogens, e.g *Pseudomonas.* The occurrence of infections in patients with UTIs have significantly decreased by present methods Catheter care, however, severely indwelling patient may still develop life-threatening infections and continue to be a general complication in Urinary tract infection morbidity and mortality worldwide.

100 samples were taken from the Catheterized patients for the study from which 19% *Pseudomonas spp.* were isolated.

The pattern of antibiotic susceptibility suggested that 100% of the isolates were resistant to Cefotaxime and 63.1% Norfloxacin, 31.5% Cefatazidime,Amikacin, Ciprofloxacin, Gentamicin, Meropenem, Cefapime and the least resistance was shown by Imipenem, Tobramycin, Piperacillin-tazobactam.

Antibiotic resistance pattern shows that all of the 19 isolates were Multidrug resistance *Pseudomonas* (MDRP).

INDEX

CHAPTER- I

INTRODUCTION

INTRODUCTION

Pseudomonas is Gram negative bacilli,non sporing, aerobic and also motile like polar flagella.There are found in soil,water or in most environment.Pseudomonas spp. is one of bacteria which is cause nosocomial infection[1].Urinary tract infection are bacterial infection which affecting humans throughout their life span.It also cause Urinary tract infection and affecting millions of strength of people each year[2].Mostly the infection is predispose the host by these infection.Catheterizaton of urinary tract is mostly affected.

Peudomonas spp. is the third most common pathogen associated with hospital-acquired catheter-associated UTIs [3]. Virulence of *Pseudomonas spp.*is multifactorial and has been attributed to cell-associated factors like alginate, lipopolysaccharide (LPS), flagellum, pilus and non-pilus adhesins as well as with exoenzymes or secretory virulence factors like protease, elastase, phopholipase, pyocyanin, exotoxin A, exoenzyme S, hemolysins (rhamnolipids) and siderophores[4] .

Urinary tract infection are bacterial infection and most commonly urological disease and second most disease of any organ[5].UTIs is most commonly in female than male.In female this incidence occur in the age of 20-40 years from 25-30% and in the older female above 60 years from 4-43% infection[6].The bacteria is commonly responsible for catheter-associated UTIs are *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Streptococcus faecalis*[7].

The predisposing factors in complicated UTIs are anatomic defects, obstruction, surgery, metabolic diseases like diabetes mellitus and generalized immunosuppression especially in patients of organ transplant[8]. Urinary tract infections (UTIs) are a serious health problem affecting millions of people each year. Infections of the urinary tract are the second most common type of infection in the body. Catheterization of the urinary tract is the most common factor, which predisposes the host to these infections. Catheter-associated UTI (CAUTI) is responsible for 40% of nosocomial infections,the commonly cause of nosocomial infection.CAUTI people for more than 1 million cases in hospitals and nursing homes annually involve uropathogens other than *Escherichia coli*. While the epidemiology and pathogenic

mechanisms of uropathogenic *Escherichia coli* have been studied, is known about the pathogenesis of UTIs caused by other organisms like *Pseudomonas spp*. [9].

Urinary tract infection patients are at high risk for nosocomial infections. And represents a site susceptible to colonization by opportunistic pathogens, e.g. *Pseudomonas spp.*[10]

Pseudomonas spp. also cause severe, acute and nosocomial infection in immunocompromised, catheterized or burn patients. Various types of virulent factors have been identified in *Pseudomonas spp*. [11].

By the data of the Centres for Disease Control and Prevention National Nosocomial Infection Surveillance System, in the USA, *Pseudomonas spp* was the second most common cause of nosocomial pneumonia, the third most common cause of nosocomial urinary tract infections, and the seventh most common cause of nosocomial bacteraemia. In Europe, *Pseudomonas spp.* was found to be the third most common isolate from nosocomial infections in intensive care units (ICUs) .[12]

Pseudomonas is serious problem that is cure by all effective antimicrobial drugs, is called a Multi drug Resistance (MDR).[13]Multidrug-resistance (MDR) are associated with nosocomial *Pseudomonas spp* strains.[14] Multi drug resistant bacteria have frequently been reported as the cause of nosocomial outbreaks of infection in UTI or as colonizers of the UTI patients.[15] UTI infections are largely hospital acquired and cross infection is very common.[16] Microbiological confirmation of a UTI takes 24–48 h. In the meantime, patients are usually given empirical antimicrobial therapy. [17]

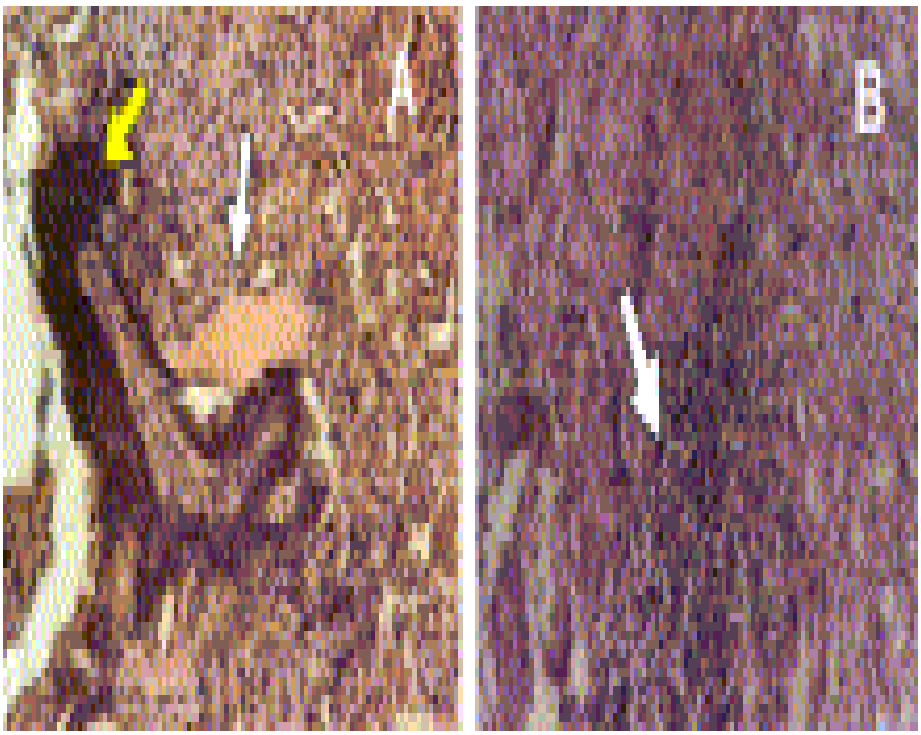
MORPHOLOGY OF *PSEUDOMONAS SPP*

Pseudomonas spp is a slender, gram-negative rod measuring 0.5 to 0.8 μm by 1.5 to 3.0 μm. All strains are motile and a single polar flagellum. The strains have two or three flagella. Clinical isolates are often piliated. It is non-capsulated but some strains have a mucoid slime layer. Mucoid strains, that is isolates from cystic fibrosis patients have an abundance of extracellular polysaccharides composed of alginate polymers. This forms a loose capsule (glycocalyx) in which micro colonies of the bacillus are enmeshed and protected from host defences.[18]

BIOFILM FORMATION BY *PSEUDOMONAS SPP*

Pseudomonas spp can form biofilms on the surface of urinary catheters. Growth of *Pseudomonas spp* begins in the form of microcolonies[19]. Alginate,that is an

acetylated polymer of beta-D mannouronic acid and alpha-Lguluronic acids, is important component of *Pseudomonas spp* biofilms. There are exopolysaccharides like psl and pel have been shown to play role in biofilm forming ability of non-alginate producing strains of *Pseudomonas spp* [20] Psl is a mannose-rich and galactose-rich polysaccharide, Psl structure has not been elucidated. Psl is a mannose-rich and galactose-rich polysaccharide, however the precise Psl structure has not been elucidated. With Psl, the Pel structure is unknown and further biochemical analyses of Pel polysaccharide is necessary[21]. Biofilms are resistant to antimicrobial agents as well as to host defense mechanisms and hence are difficult to eradicate. Biofilms contribute towards pathogenicity of *Pseudomonas spp* as these often lead to persistent and recurrent infections.[22]



CHAPTER- II

AIM OF STUDY

AIM OF THE STUDY

1. Isolation and identification of *Pseudomonas spp.*in catheterized patients.
2. To study antibiotic susceptibility patterning of *Pseudomonas spp.*

CHAPTER- III

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Studies have show how the effect of untreated bacteriuria during pregnancy can result in low birth weight and premature delivery [23]. Therefore, once need an accurate and routine screening for bacteriuria. This study was designed to check the sensitivity and specificity and to keep the balance between the costs for screening. This study has been designed to evaluate the diagnostic accuracy of the rapid urine dipstick test against urine culture. [24]

Studies have shown that pregnant women are at high risk between the week 6 and will increase mostly during the 22 to 24 week. It is usually happen due to the digestive ascending route through the urethra opening and bacteria get multiply there. Dipstick test is the easiest qualitative method to detect the UTI and easy to interpret the results that can be carried out in primary care giving facilities. This test was carried out at Felege Hiwot Referral Hospital to detect UTI between the pregnant women at the age between 17 to 40 years old. A total of 367 pregnant women (37 with complaints and 330 without complaints of UTI) were participated in the study and pregnant women who have taken antibiotics within seven days at the time of recruitment and who were not willing to participate were excluded from this study.Symptomatic UTI refers to patients whose urine is yielding positive culture ($\geq 10^5$ cfu/ml) and who have symptoms referable to the urinary tract infection, where as asymptomatic bacteriuria (ABU) refers to pregnant women whose two consecutive urine samples showed positive cultures ($\geq 10^5$ cfu/ml) of the same uropathogen and without showing sign and symptoms of UTI. However, this study has shown many false positive results as compared to the gold standard culture method. But this positive test might be able to help in the further evaluation of clinical manifestations for empirical treatment. On the other hand, a negative test is an indication for urine culture. The urine dipstick, if positive, will also be useful in follow-up of patient after treatment of urinary tract infection. This is useful in poor resource setting especially in the third world where

there is a dearth of trained personnel and equipment for urine culture. This study can only be useful for managing least positive cases empirically.[25]

This study has used two markers to determine the UTI. UTI are the most common of all bacterial infections. There can be many complications of urinary tract infection such as dehydration, sepsis, kidney failure, and death. So, there is a need of proper diagnostic method to diagnose the patients with UTI. Even though, culture method is the gold standard in diagnosis of UTI, but it is time and money consuming. Therefore, in this study, the quantitative method of XO and MPO levels had been used to determine urinary tract infection. Reactive oxygen species (ROS) could be generated by a variety of reasons at the cellular levels. An important source of ROS is known to be xanthine oxidase (XO) that could be formed from xanthine dehydrogenase either reversibly or irreversibly (via limited proteolysis) under pathological conditions. The elevated tissue or serum XO activities are thought to be responsible for mechanism of several pathological conditions including alcoholism and smoking. Myeloperoxidase (MPO) is an inflammatory marker. Studies indicated that MPO holds a central role in microbial killing in humans and animals. Upregulation of MPO gene expression in activated phagocytes would seem consistent with the presumed antimicrobial function of the enzyme in these cells. Recent investigations revealed a crucial role of MPO in chronic, nonmicrobial inflammatory processes such as neurodegenerative disease and atherosclerosis. This study has found that these markers can be useful to determine the sensitivity, specificity, positive predictive value and negative predictive value in diagnosing the urinary tract infection. In this study, the authors have shown the elevated levels of XO and MPO in patients with UTI. In view of the contribution of tissue-derived enzymes to the urinary enzymatic activities, the use of a urinary enzymatic marker for the detection UTI would require that urinary enzymatic activity be derived from the pathogenic bacteria only, with exclusion of tissues sources. For this reason they have chosen XO and MPO enzymes. In conclusion, this present study is the first to report significantly increased XO and MPO activities in urine derived from subjects with UTI, and they are tempting to speculate that urinary XO and MPO could be directly involved in the pathogenesis. Further studies should be directed towards evaluating their use in the diagnosis of UTI. [26]

Dt,Foutest et.al in 2012 concluded that UTI. UTI is a major infection and the most common urological disorder in the outpatient. There are patients with ABU and UTI in the health care. So, there is need to find out the bacteria that causes urinary tract

infection that requires antimicrobial treatment 16s rDNA sequence-processing method has been used to diagnose and predict the UTI genera. This study is composed of a selection of bioinformatics tools as an accurate and fast method. It is believe that 16S rDNA sequencing has the potential for translation to the clinic, offering significant clinical advancement over diagnostic urine culture because it provides a greater depth of understanding and sensitivity pertaining to the composition of commensal and potentially pathogenic microbes present in urine.Urinary metaproteomic profiles in parallel may contribute to the identification of a host inflammatory response utilizing urinary biomarkers with greater sensitivity and specificity for UTI than traditional measures of urinary leukocyte esterase production or white blood cell count detected by urinalysis. [27]

Pseudomonas aeruginosa has been an important uropathogen that causes complicated urinary tract infection. This study indicates that those patients with urinary tract catheterization had a higher incidence of fever than patients without catheterization. So we must improve not only the antimicrobial treatment of *Pseudomonas aeruginosa* but also care and hygine of catheters. [28]

Pseudomonas aeruginosa is a bacterium that is mostly in urinary tract infection (UTI) worldwide and has shown different antibiotic susceptibility patterns. In this study all the 100% isolates of *P. aeruginosa* were resistant to penicillin, cloxacillin, tetracycline, nitrofurantoin and nalidixic acid. While 67% were sensitive to augmentin, sensitivity to ofloxacin was 92%, ciprofloxacin 92% and cefuroxime (86%). The resistance pattern of *P. aeruginosa* from urine against antibiotics was extremely high. Antibiotic medication against UTI should be carefully weighed against this undesirable possible outcome (resistance).[29]

The indwelling urethral urinary catheter is used some 25% of patients admitted to hospitals in the United States.These catheters are mostly placed in surgical procedures and anesthesia for accurate measurements of urinary output, to relieve urinary retention , to improve nursing care, to facilitate urinary flow in neurologic patients that person are not for other forms of drainage, and for irrigation of the bladder or instillation of drugs. These catheters are usually left indwelling for a short period of time. The urethral catheter is the most cause of nosocomial infections and gram-negative bacteremia. The duration of catheterization is directly related to the

development of bacteriuria. Nosocomial infections associated with the urinary tract follow instrumentation, usually with the catheter.The clinician must be certain that the catheter inserted is absolutely necessary and should important to have the catheter removed as soon as possible. [30]

Nosocomial pathogens too much resistant to available antimicrobial agents is a cause in any hospital. Gram-negative organisms are being encountered as nosocomial pathogens with increasing factor. [31] The pattern for catheterization and care of indwelling urinary catheters should be reviewed with all personnel. [32]

CHAPTER- IV

METHODOLOGY

METHODOLOGY

METHODS FOR THE DIAGNOSIS OF URINARY TRACT INFECTION

A catheter tip is the most common type of specimen received by the clinical microbiological laboratories.

Specimen collection:

Catheter tip from the catheterized patients.

Transport of specimen:

- Once collected specimen must be transported to the laboratory without delay. When some time elapse before the cath tip swab is examined then the swab should be placed in transport medium such as Stuart's medium which prevents the viability of the pathogens. The medium is non-nutrient because the less delicate commensal bacteria present in the specimen would outgrow the pathogens in a nutrient medium.

- Transport medium which is made of semi-solid agar may be used in a screw capped universal or smaller bottle or a hermetically stoppered test tube. Immediately the specimen has been taken, the swab is plunged into the depth of the transport medium and the cap or stopper is firmly applied.

- Sterile disposable swab kits incorporating a transport medium are commercially available.

Microscopy:

Gram stained smear of cath tip is prepared to observe relative number of polymorphs and bacteria, different morphological forms of gram positive and gram negative bacteria.

GRAM'S STAINING

Introduction:

Gram staining is one of the most important and widely used differential staining techniques in diagnostic microbiology.

The gram staining procedure was developed by Christian Gram in 1883, a Danish physician who was working as a pathologist in Municipal Hospital in Berlin.

Principle:

Gram staining is a differential staining technique by which bacteria are classified as "Gram positive" or "Gram negative" depending upon whether they retain or loose the primary stain crystal violet when subjected to treatment with a decolourising agent such as alcohol.

Reagents:

The gram stain has four different reagents:

1. Primary stain (Crystal violet): colours all cell a purple blue.
2. Mordant (Potassium iodine-Iodine solution): the bulkier iodine replaces chlorine in the crystal violet molecule; the complex formed becomes insoluble in water.
3. Decolouriser (Acetone or Alcohol): removes stain only from gram negative cells.
4. Counter stain (Saffaranine): stains the gram negative cells and makes them visible.

Procedure:

- Bacterial suspension is spread out in the form of thin film on the surface of the clean glass slide and allowed to dry. The air dried smear is fixed by passing over flames.
- Crystal violet is added for 1minute as primary stain and the slide is washed under running tap water.
- Gram's Iodine is added as mordant and left for 1 minute and washed under running tap water.
- Slide is decolourized by treating it with alcohol for 30 seconds and washed under running tap water.
- Saffaranine is added as counter stain for 1min and washed under running tap water.
- The slide is allowed to dry and finally focussed under oil immersion objective.

Observation:

Violet colour: Gram positive bacteria

Red/Pink colour: Gram negative bacteria

HANGING DROP PREPARATION

Purpose:

To study the motility and morphology of the bacteria in the given liquid culture (liquid medium)

Principle:

Live, unstained, motile cells will demonstrate their motility under 400 x magnifications by displaying individual movement amongst cells which means that movement is unique and directional. The hanging drop is used to distinguish this from browian movement which is just a random jiggling movement.

Procedure:

- Take a clean coverslip and apply vaseline to the four corners.
- Place a drop of liquid culture into the centre of coverslip using a sterile wire loop.
- Invert the clean depression (concavity) slide over the coverslip with its concavity facing one drop.
- Immediately turn slide so that the coverslip is uppermost in position and drop suspending from coverslip.
- Focus a drop under low power objective so that the edge of the drop is exactly in the centre of the microscopic field.
- Turn to high power and focus using fine knob and then observe for motility

MEDIA USED:

A. NUTRIENT AGAR

This is the simplest and routinely employed medium used in the laboratory for diagnostic purposes.

Ingredients:

Peptic digest of animal tissue : 5 gram/litre (g/l)

Sodium chloride	: 5 g/l
Beef extract	: 1.5 g/l
Yeast extract	: 1.5g/l
Agar	: 20g/l

Final pH (at 25^0C) – 7.4

Preparation:

Mix all the ingredients and autoclave at 121^0C for 15 minutes at 15 lbs pressure and pour into plates.

B. MACCONKEY AGAR

This is the differential media which is useful for the cultivation of enterobacteriaceae. It contains bile salt to inhibit non-intestinal bacteria and lactose with neutral red to distinguish the lactose fermenting coliforms from non-lactose fermenting groups like *Salmonella*, *Shigella*, and *Pseudomonas* etc. The concentration of sodium taurocholate may be reduced to suit less tolerant organisms. The omission of sodium chloride from the medium prevents the spreading of *Proteus* colonies.

Ingredients:

Peptone	: 20g
Sodium Taurocholate	: 5g
Agar	: 20g
Distilled water	: 1000ml (1lit)
Neutral red solution, 2% in 50% ethanol	: 3.5ml
Lactose, 10% aqueous solution	: 100ml

Preparation:

Dissolve the peptone and taurocholate (bile salt) in the water by heating. Add the agar and dissolve it in the steamer or autoclave. If necessary, clear by filtration. Adjust the pH to 7.5. Add the lactose and neutral red, which should be well shaken before use and mix. Heat the autoclave with 'free steam' (100^0C) for 1 hour, then at 115^0C for 15 minutes. Pour into Petri plates.

C.BLOOD AGAR

This is an enriched medium. This medium is prepared by adding sterile blood (horse blood or sheep blood) to sterile nutrient agar that has been melted and cooled to 50^0C.

The concentration of blood may vary from 5-10%, 10% is the more commonly used concentration.

Ingredients:

Peptone mixture	: 15g
Beef extract	: 5g
Tris base	: 1g
Monopotassium phosphate	: 1g
L-Tryptophan	: 1g
Chromogen mixture	: 0.96g
Enrichment	: 5g
Agar	: 16g
Distilled water	: 1000ml (1 lit). Final pH -7.2

D. CETRIMIDE AGAR

This is the selective medium for *P.aeruginosa.*

Ingredients:

Pancreatic digest of gelatin	: 20 g/l
Magnesium chloride	: 1.4 g/l
Dipotassium sulfate	: 10 g/l
Cetrimide	: 0.3 g/l
Agar	: 13.6g/l

Preparation:

Suspend 45.3 g in 1 l distilled water, heat until completely dissolved, and add 10ml/l Glycerin, Waterfree (TN1424). Autoclave at 121°C for 15 minutes.

Final pH at 25°C 7.2 ± 0.2

E. PEPTONE WATER

This is enrichment medium. It is used in routine culture, sugar fermentation tests

Ingredients:

Peptone	: 1g
Nacl	: 0.5g
Distilled water	: 100ml.

Final pH 7.4.

CONFIRMATION OF *PSEUDOMONAS SPP.*

After obtaining the pure strains, the strains were subjected to biochemical identification tests to identify Pseudomonas spp. For this purpose samples were inoculated in Triple Sugar Iron media (TSI), Citrate media, Peptone water, Urease media and kept in an incubator for 18 hrs at 37°C. Next day the results were noted on TSI, Citrate media and Urease media. Part of growth on Peptone water was subjected to Indole test with Kovac's Reagent and part for motility test by 'Hanging drop' method. A strain of Pseudomonas in the TSI medium showed alkaline slant, no reaction in butt. It showed negative reaction for indole test, negative urease test and positive citrate test. Glucose is utilised oxidatively, forming acid only Antimicrobial disc: susceptibility test Application of antibiotic discs to the inoculated agar plates: Antimicrobial susceptibility of all the isolates was performed by the disc-diffusion (Modified-Kirby Baur disc diffusion method) according to CLSIs guidelines. The following antibiotics were tested by disc diffusion method, Ceftazidime, Piperacillin, Piperacillintazobactam, Cefipime tazobactum, Imepenam, Aztreonam, Gentamicin**,** Ciprofloxacillin,Levofloxacillin,Cefoperazone, Tobramycin, PolymyxinB,

BIOCHEMICAL REACTION FOR IDENTIFICATION OF ISOLATES

A. OXIDASE TEST:

Purpose:

To determine the presence of an enzyme, cytochrome oxidase which catalyses the oxidation of reduced cytochrome by molecular oxygen

Principle:

When oxidase enzyme is present then substrate (1% Tetramethyl Paraphenylene Diamine Hydrochloride) is oxidized to give colour compound indophenol blue.

Method

1. **Plate method:** Freshly prepared oxidase reagent is directly poured on the surface of culture plate.

   Observation: Oxidase positive organism rapidly produce purple colour.

   NOTE: It should be performed only in Nutrient Agar plate.

2. **Dry filter paper method:** Strips of Whatman No.1 filter paper are soaked in freshly prepared oxidase reagent like strips are dried and stored in a dark bottle and for the use , the strip is taken on a petriplate and colonies on test organism is smeared on the surface with the help of glass rod/ capillary tube.

Observation: Oxidase positive organism gives deep purple colour within 5-10secs

3. **Wet filter paper method:** A sterile strip of filter paper is wet with oxidase reagent and test organism is smeared on surface of filter paper.
   Observation: Oxidase positive organism gives purple colour.

B. CATALASE TEST

Principle:

Certain bacteria have an enzyme catalase which acts on hydrogen peroxide to release hydrogen.

$$H_2O_2 \xrightarrow{\text{Catalase}} H_2O + O \text{ (Nascent oxygen)}.$$

Procedure (Method)

1. **Slide method:**

- Using a sterile glass rod/capillary tube transfer small amount of colony of test organism in a glass slide.
- Place one drop of 3% of H_2O_2 into the colony and observe for immediate effervescence.

2. **Tube method:**

- Take 2-3 ml of H_2O_2 in a clean slide using a sterile glass rod.
- Pick up a colony and inoculated into the solution.
- Observe the immediate effervescence.

Interpretation

Positive test **:** Immediate bubbling, easily observed (O_2 formed).

Negative test **:** No bubbling (no O_2 formed).

C. OXIDATIVE- FERMENTATIVE TEST

Purpose:

Oxidative-fermentation (OF) test medium supplemented with 1% glucose is used to differentiate different species of bacteria on their ability or mode of utilization of glucose i.e. either oxidation or fermentation. The medium is also used for testing acid production from other carbohydrates. This medium should not be used for testing *Staphylococci.*

Principle:

This test is done in Hugh and Leifson medium/OF media which contains a small amount of protein, Nacl, buffer, agar, carbohydrate and 1% glucose Bromothymol blue (indicator). The protein content of the medium is kept low to avoid neutralization of acid by alkaline products resulting from deamination of protein. The traditional method for determining the utilization of glucose is to inoculate two tubes of medium with the test organism. One tube is overlaid with sterile mineral oil. Bacteria that oxidize glucose require atmospheric oxygen for the utilization of the carbohydrate. Thus these bacteria are able to produce acid from glucose in the open tube of the medium but are unable to produce acid in the tube sealed with mineral oil. Bacteria that ferment glucose produce acid in both tubes. Other bacteria may be unable to ferment or oxidize glucose and thus do not produce acid in either tube; these bacteria are said to be asaccharolytic. Production of acid in either tube causes a lowering of the pH in the medium and a concomitant change in the colour of the indicator, phenol red. Phenol red is yellow at its acidic end point and red at its alkaline end point.

Ingredients:

Pancreatic digest of casein, USP	: 2g
Phenol red, 1.5% solutions	: 2ml
Agar	: 3g
Deionized water	: 1000ml
Final pH – 7.3 +/-0.1	

Procedure:

- Inoculate each tube of Oxidation-Fermentation carbohydrate medium with one drop of a broth culture or saline suspension of the test organism or alternatively with isolated colonies that are stabbed four times approximately 1cm below the surface of the agar.
- Also inoculate a control tube containing carbohydrate- free-basal medium.

- Incubate tubes at 35^0C and observe daily for 4 days and again after 7 days, for evidence of acid production.

Interpretation (Expected results):

- Fermentation : both tubes of the medium turn yellow.
- Oxidation : open tube of medium turns yellow; sealed tube stays red.
- Asaccharolytic : both tubes of the medium turn yellow.

Determination of the utilization of other carbohydrates by non- fermentative organisms may be performed using only one of the medium.

D. NITRATE REDUCTION TEST

Purpose:

Nitrate broth medium is used to determine an organism's ability to reduce nitrate to nitrite by producing enzyme nitrate reductase.

Principle:

This is a test for the presence of the enzyme nitrate reductase which causes the reduction of nitrate, in the presence of a suitable electron donor, to nitrite which can be tested for an appropriate colorimetric reagent. Almost all enterobacteriaceae reduce nitrate.

$$\text{Nitrate} \xrightarrow{\text{NITRATE REDUCTASE}} \text{Nitrite}$$

This is observed in two stages:

1. **Stage I:** Detects the presence of nitrite i.e. when sulphanilic acid and napthylamine are added to overnight broth culture of test organism in nitrate broth.

   Presence of nitrite is indicated by red colour.
2. **Stage II:** Absence of red colour in stage-I may be due to negative test, nitrate (NO_3) is not reduced to nitrite (NO_2) or may be due to further reduction of nitrate $\longrightarrow$ NH_3+N_2.

So, if nitrate was not reduced in stage-I by the test organism it will reduce after adding Zinc dust indicates the test and nitrite (NO_2) has been reduced to NH_3 and N_2.

Ingredients:

Potassium nitrate, KNO_3 (Nitrate-free)	: 0.2g
Peptone	: 5g
Distilled water	: 1000ml (1lit)

Preparation:

Tube in 5ml amounts and autoclave at 121^0C for 15 minutes.

Test Reagents:

Solution A: Dissolve 8.0g of sulphanilic acid in 1 lit of acetic acid 5mol/litre.

Solution B: Dissolve 5.0g of alpha-napthylamine in 1 litre of acetic acid 5mol/litre.

Procedure:

- Inoculate the medium and incubate for 96 hours.
- Add 0.1ml of the test reagent to the rest of the culture. A red colour developing within a few minutes indicates the presence of nitrite and hence the ability of the organism to reduce nitrate.

NOTE: alpha-napthylamine is potentially carcinogenic.

E. TRIPLE SUGAR IRON TEST (TSI)

Purpose:

Triple Sugar Iron (TSI) agar is a screening medium used to identify Gram negative bacilli based on their ability to ferment the carbohydrates glucose, sucrose and lactose to produce H_2S gas.

Principle:

TSI agar contains protein, Nacl, lactose, sucrose, dextrose, a sulphur source, H_2S indicator, a pH indicator and agar. Bacteria that ferment glucose produce a variety of acids, turning the colour of the medium from red to yellow. Larger amounts of acid are produced in the butt of the tube (fermentation) than in the slant of the tube (respiration). Organisms growing on TSI also form alkaline products from the oxidative decarboxylation of peptone. These alkaline products neutralize the small amounts of acids present in the slant but unable to neutralize the large amounts of acid present in the butt. Thus the appearance of alkaline (red) slant and an acid butt (yellow) after 24 hours incubation indicates that the organism is a glucose fermenter but is unable to ferment lactose and sucrose.

Bacteria that ferment lactose or sucrose (or both), in addition to glucose from such large amounts of acid that the oxidative deamination of protein that may occur in the slant does not yield enough alkaline products to cause a reversion of pH in the region. Thus these bacteria produce an acid slant and acid butt. It is impossible to determine

from TSI reaction whether both lactose and sucrose are being fermented; individual carbohydrate fermentation tests are required to make this assessment.

Gas production (CO_2 and hydrogen) is detected by the presence of cracks or bubbles in the medium. These are formed when the accumulated gas escapes.

H_2S gas is produced as a result of the reduction of thiosulphate. H_2S is a colourless gas and can be detected only in the presence of an indicator, in this case ferric ammonium sulphate. H_2S combines with the ferric ions of ferric ammonium sulphate to produce blackening. Although the black precipitate may frequently obscure the colour of the butt, it can be assumed that the organism is a glucose fermenter because of the requirement for an acid environment.

The reaction can be summarized as follows:

- Alkaline slant/Acid butt - only glucose is fermented.
- Acid slant/Acid butt - glucose and sucrose fermented or glucose and lactose fermented or glucose, lactose and sucrose fermented.
- Bubbles or cracks presents - gas produced.
- Black precipitate present - H_2S gas present.

Ingredients:

Pancreatic digest of casein, USP	: 10g
Peptic digest of animal tissue, USP	: 10g
Nacl	: 1g
Lactose	: 10g
Sucrose	: 10g
D-glucose	: 1g
Ferric ammonium sulphate	: 0.2g
Sodium thiosulfate	: 0.2g
Phenol red	: 25mg
Agar	: 13g
Distilled water	: 1000ml

Final pH- 7.3-7.4

Procedure:

- Inoculate test cultures to TSI agar by first touching a sterile bacteriologic needle to a colony and then stabbing the needle into the deep agar region of

the medium. When withdrawing the needles, move it from side to side over the surface of the medium.

- Incubate cultures at 35^0C for 18 to 24 hours.
- Examine the cultures for the colour of the slant, butt, gas cracks, and blackening caused by H_2S.

F. SUGAR FERMENTATION TEST

Purpose:

To differentiate aerobic gram negative bacteria based on their ability to ferment sugar with production of acid and gas.

Principle

The test is done in peptone water containing 1% of any carbohydrate (glucose, lactose, arabinose, mannitol, xylose and sucrose) to differentiate aerobic gram negative bacteria based on their ability to ferment sugar with production of acid and gas.

Andrade's is used as an indicator. The sugar media is a colourless liquid media and contains an inverted Durham's tube.

Ingredients

Peptic digest of casein, USP	: 10g
Test carbohydrate	: 10g
Nacl	: 5g
Andrade's indicator	: 10ml
Distilled water	: 1000ml (1 lit)

Final pH- 7.4

Preparation

- Mix the basal ingredients, heat to boiling, and sterilize at 121^0C for 15minutes.
- Cool to 50^0C and pre-sterilized solutions of carbohydrates to achieve a final concentration of 1%.
- Alternatively, as listed above the carbohydrate may be added directly to the medium. Carbohydrate-impregnated discs are commercially available.

Reagent:

Acid fuchsin	: 0.5g
NaOH, 1N	: 15-18ml

Distilled water : 100ml

Andrade's indicator, the reagent, is prepared as follows:

- Dissolve fuchsin in distilled water, and then add NaOH solution. If after several hours the fuchsin has not decolorized from a red colour to brown, add additional 1 or 2 ml of alkali, drop by drop, until a straw yellow colour is obtained. The reagents should be aged approximately 6 months before using.

Procedure

- Inoculate the test organism to the sugar media, and incubate at 35^0C for upto 5 days.

G. INDOLE TEST

Purpose:

To differentiate gram negative bacteria based on their ability to breakdown tryptophan into indole.

Principle:

The test is performed in peptone water which contains tryptophan. Some bacteria can breakdown tryptophan into indole in the presence of tryptophanase enzyme. The released indole is detected by a colorimetric reaction with Kovac's reagent (P- amino dimethyl amino benzaldehyde) to form red colour.

Ingredients

Peptone (containing sufficient tryptophan) : 20g

Sodium chloride, NaCl : 5g

Distilled water : 1000ml (1 lit)

Adjust pH to 7.4

Preparation

Dispense and sterilize by autoclaving at 121^0C for 15minutes.

Kovac's Reagents

Amyl or isoamyl alcohol : 150ml

P- Dimethyl aminobenzaldehyde : 10g

Hydrochloric acid, concentrated, HCl : 50ml

Procedure

- Inoculate medium and incubate for 48 hours at 37^0C. Sometimes a period for 96 hours at 37^0C may be required for optimum accumulation of indole.
- Add 0.5 ml of Kovac's reagent and shake gently.

Observation

- After adding 5 drops of Kovac's reagent a red colour ring is formed in the upper portion of broth indicates positive test.

H. METHYL RED TEST

Purpose:

This test is used to differentiate gram negative bacilli based on their ability to ferment glucose and to produce strong acids (lactic acid/ acetic acid/ formic acid/succinic acid).

Principle:

Some members of enterobacteriaceae family produce large amount of acids when grown in medium containing glucose. These strong acids can be detected by methyl-red test.

Media used : Glucose Phosphate Peptone water (GPPW)

Reagent (Indicator) : Methyl Red.

Ingredients

Peptone	: 5g
Dipotassium hydrogen phosphate (K_2HPO_4)	: 5g
Distilled water	: 1000ml (1lit)
Glucose, 10% solution	: 50ml

Preparation:

Dissolve the peptone and phosphate, adjust the pH to 7.6, filter, dispense in 5ml amounts and sterilize at 121^0C for 15minutes. Sterilize the glucose solution by filtration and 0.25 ml to each tube (final concentration 0.5%).

Methyl Red Indicator solution

Methyl Red	: 0.1g
Ethanol	: 300ml
Distilled water	: 200ml

Procedure:

- Inoculate the organism into 1ml of GPPW and incubate at 37^0C for overnight.
- After incubation 2-3 drops of methyl red reagent is added and then observed.

Observation:

Pink/Red colour : Positive test (large amount of mixed acids are formed).

No change in colour : Negative test (no production of acid).

I. VOGES- PROSKAUER (VP) TEST

Purpose:

To differentiate gram negative bacteria based on their ability to ferment glucose and to produce pyruvic acid which is decarboxylated to produce acetonine.

Principle:

During fermentation of carbohydrate, some members of enterobacteriaceae produce acetonine which is one product of pyruvic acid and this is detected by VP test.

Media used : Glucose Phosphate Peptone Water (GPPW)

Reagents used : alpha (α) napthol and 90% KOH

Procedure:

- Test organism is inoculated into 0.05ml of GPPW and incubates at 37^0C for overnight.
- To broth culture add few drops of α- napthol and 90% KOH and shake well and observe the reaction.
- **Observation:**

Red colour : Positive test

No change in colour : Negative test

J. CITRATE TEST

Purpose:

To differentiate gram negative bacteria based on their ability to utilize citrate as a sole source of carbon and ammonia as a source of nitrogen.

Principle:

Organisms that utilizes as source of carbon by means of citratase enzyme to produce alkaline products. These alkaline products increase the pH of the medium and changes green colour to blue.

Media used: Koser's liquid citrate medium or Simmons' citrate agar may be used.

Ingredients (Koser's medium)

Sodium chloride, NaCl : 5g

Magnesium sulphate, $MgSO_4$: 0.2g

Ammonium Dihydrogen Phosphate, $NH_4H_2PO_4$: 1g

Potassium Dihydrogen Phosphate, KH_2PO_4 : 1g

Sodium citrate, $Na_3C_6H_5O_7.2H_2O$: 5g

Distilled water : 1000ml (1 lit)

Final pH- 6.8

Preparation

The medium is sterilized by autoclaving at 121^0C for 15 min.

Simmons' Citrate Medium

This is a modification of Koser's medium with agar and an indicator is added.

Koser's medium : 1 lit

Agar : 20g

Bromothymol Blue, 0.2% : 40ml

Preparation

Dispense and autoclave at 121^0C for 15 min and allow to set as slopes.

Procedure:

- Inoculate from a saline suspension of the organism to be tested.
- Incubate for 96 hour at 37^0C.

Observation:

1. **Koser's citrate medium:**
   - Turbidity : Positive Test
   - No Turbidity : Negative test
2. **Simmons' citrate medium**
   - Blue colour : Positive Test
   - No change in colour (green) : Negative Test

K.UREASE TEST

Purpose:

To demonstrate the ability of gram negative bacteria to hydrolyse urea into ammonia by urease enzyme.

Principle:

When urea is hydrolysed alkaline product is released which increases the pH of the media, this is brought about by urease enzyme.

Media used : Christensen media

Indicator : Phenol Red.

Ingredients:

Peptone	: 1g
Sodium chloride, NaCl	: 5g
Dipotassium hydrogen phosphate, K_2HPO_4	: 2g
Phenol red solution (1 in 500 aqueous solutions)	: 6ml
Agar	: 20g
Distilled water	: 1000ml (1 lit)
Glucose, 10% solution, sterile	: 10ml
Urea, 20% solution, sterile	: 100ml

Preparation:

Sterilize the glucose and urea solutions by filtration. Prepare the basal medium without glucose or urea, adjust to pH 6.8-6.9 and sterilize by autoclaving in a flask at 121^0C for 30 minutes. Cool to about 50^0C, and add the glucose and urea and tube the medium as deep slopes.

Procedure:

- The medium is inoculated by striking the surface of the slope by the test organism.
- Incubate at 37^0C for overnight.

Observation:

Pink colour : Positive test

No change in colour : Negative test.

L. PHENYLALANINE DEAMINASE TEST (PPA)

Purpose:

PPA is used to differentiate *Proteus, Morgenella* and *Provendencia* species from other enterobacteriaceae based on their ability to determine phenylalanine. It may be used to differentiate selected non-fermentative organisms.

Principle:

PPA contains yeast extract, NaCl, buffer agar and DL-Phenylalanine. Bacteria that are capable of oxidatively deaminating phenylalanine grow on this medium and produce keto acid phenyl pyruvic acid as a deaminating by product. The α- keto reacts with Ferric chloride ($FeCl_3$) reagent to form a green reaction product. Immediate appearance of this intense green colour is a positive result for deamination of phenylalanine.

Ingredients:

Yeast extract : 3g

DL-Phenylalanine : 2g

Disodium hydrogen phosphate, Na_2HPO_4 : 1g

Sodium Chloride, NaCl : 5g

Agar : 12g

Distilled water : 1000ml (1 lit)

Final pH-7.4

Preparation:

Distribute and sterilize by autoclaving at 121^0C for 15 min. Allow to solidify in tubes as slopes.

Procedure:

- Inoculate with a fairly heavy inoculum.
- Incubate for 4 hours or if required for upto 24 hours at 37^0C.

- Allow a few drops of a 10% solution of ferric chloride to run down over the growth on the slope.
- **Observation:**

Green colour : Positive test

No change in colour : Negative test.

M. LYSINE IRON AGAR (LIA) TEST

Purpose:

LIA is prepared on a started agar medium which is used to differentiate number of enterobacteriaceae family on the ability to decarboxylate or deaminate lysine and produce H_2S.

Principle:

LIA contains a small amount of protein, glucose, lysine, a sulphur source, and H_2S indicator, agar and pH indicator. The pH indicator, bromocresol purple is yellow in the presence of acid and purple in basic conditions. All organisms used in LIA must be glucose fermenters. As these organisms ferment glucose, they produce turning bromocresol indicator yellow. This reaction is observed only in the butt of the tube because fermentation is anaerobic and not enough acid is produced to extend throughout the medium.

Organisms may either decarboxylate or deaminate lysine, they do not do both. Decarboxylation of lysine yields the alkaline products cadaverine which neutralizes the acids from glucose fermentation and leads to reversion of the butt from yellow to purple. Organisms that deaminate lysine do so in the presence of oxygen, i.e. in the slant area of the medium, and cause the slant to turn red. The reason for the red colour is incompletely understood. However, because the omission of the indicator from the medium results in the production of an orange slant by lysine-deaminase positive organisms, the red slant may result from an interaction between the purple colour of the slant and the orange pigment.

Some bacteria are able to produce H_2S gas from sodium thiosulphate. H_2S reacts with the ferric ions of ferric ammonium citrate to yields ferrous sulphide. This insoluble compound is detected as a blackening throughout the medium.

Ingredients:

Pancreatic hydrolysate of gelatin : 5g
Yeast extract : 3g
D- Glucose : 1g
L-lysine : 10g
Ferric ammonium citrate : 0.5g
Sodium thiosulphate : 0.04g
Bromocresol purple : 0.02g
Agar : 13.5g
Distilled water : 1000ml (1lit)
Final pH 6.7

Preparation:

Mix the following ingredients in 1 lit of distilled water, heat to boiling and dispense into sterile tubes. Sterilize the tubes of medium at 121^0C for 15min. Cool in a slanted position.

Procedure:

- Inoculate cultures of bacteria into LIA by touching a sterile bacteriological needle to a colony and then stabbing the needle into the deep agar of the medium.
- During withdrawal the needle is streaked in a back and forth motion over the surface of the agar.
- Cultures are incubated at 35^0C for 18-24 hrs.

Observation:

Purple (slant)/yellow (butt) : Lysine decarboxylase : Negative
Purple/purple : Lysine decarboxylase : Positive
Red/Yellow : Lysine deaminase : Positive
Blackening : H_2S produced

N. MALONATE UTILIZATION TEST

Principle:

Malonate broth tests for utilization of sodium malonate as a sole of carbon. Medium contains buffer pH indicator, sodium malonate, requires salts and a small amount of yeast extract and glucose. Bacteria that are capable of using malonate as a source of

energy and carbon produced alkaline by-products that change the colour of the medium blue.

Ingredients:

Yeast extract : 1 g
Ammonium sulphate $(NH_4)_2SO_4$: 2g
Dipotassium hydrogen phosphate KH_2PO_4 : 0.6g
Potassium dihydrgen phosphate KH_2PO_4 : 0.4g
Sodium chloride, NaCl : 2g
Sodium malonate : 3g
Bromothymol blue : 0.025g
Distilled water : 1000ml (1 lit)

Adjust pH to 7.4 if necessary. Sterilize by autoclaving at 121^0C for 15 min.

Procedure:

Inoculate from a young agar slope culture and incubate at 37^0C for 48 hrs.

Observation:

Positive result is indicated by change in colour of the indicator from green to blue due to rise in pH consequent upon the utilization of sodium malonate.

ANTIBIOTIC SUSCEPTIBILITY TESTING

Antimicrobial susceptibility test for the bacteria can be either quantitative or qualitative. In quantitative test the minimum amount of antimicrobial agents that inhibits the visible growth of bacterial isolate or minimal inhibitory concentration (MIC) is determined. Qualitative tests such as the disc diffusion test such as the disc diffusion test categorize a bacterial isolate as susceptible, intermediate or resistant to particular antimicrobial agents.

Muller-Hinton agar is widely used for antibiotic susceptibility testing.

Ingredients:

Beef infusion : 300ml
Casein hydrolysate : 17.5g
Starch : 1.5g

Agar : 10g

Distilled water : 1000ml

Final pH -7.4

Preparation:

Emulsify the starch in a small amount of cold water, pour into the beef infusion then add the casein hydrolysate and the agar. Make up the volume to 1lit with distilled water. Dissolve the constituent by heating gently at 100^0C with agitation. Dispense in screw capped bottle and sterilise by autoclaving 121^0 C for 20 min and pour on the plate.

The methods most commonly used in clinical laboratories are **disc diffusion**, **agar diffusion**, **macrobroth dilution**. Additionally, automated methods are becoming widely recognized.

A. DISC DIFFUSION

In the disc diffusion test, bacteria are spread over the surface of an agar plate, and then paper discs to which antimicrobial agents have been added are placed on the agar surface. The plates are incubated at 35^0C and the zones of inhibition are examined the following day. The disc diffusion method currently recommended by the National Committee for Clinical Laboratory Standards (NCCLS) is based on the original Kirby-Bauer test.

Kirby-Bauer disc diffusion test:

1. 3-5 isolated colonies are inoculated into 4-5 ml nutrient broth, Muller-Hinton broth or Trpticase soy broth and incubated for 2-8 hours until the turbidity of the suspension reaches or exceeds that of 0.5Mcfarland turbidity standard (approximately 1.5x 108 colony forming unit [CFU] /ml). The turbidity is adjusted to match the McFarland standard, if necessary, by diluting with additional broth.

2. Muller-Hinton plates (150mm diameter, 3-5mm thick, pH 7.2-7.4) are inoculated by dipping a sterile cotton swab into suspension, expressing excess

broth by rotating the swab firmly against the inside of the tube and evenly streaking the entire surface of the plate.

3. The antimicrobial-containing discs are placed on the agar plates within 15 min of inoculation. This is accomplished by using either a sterile forceps to apply individual disc or multiple disc dispensers. The discs are pressed firmly against the agar surface to ensure contact and subsequent antimicrobial diffusion.
4. The plates are inverted and incubated at 16 to 18 hrs at 35^0C.
5. The diameter of the inhibitory zone is measured using either a ruler or callipers.
6. The zone size around each antimicrobial disc is interpreted as susceptible, intermediate or resistant based on the criteria indicated in tables provided by NCCLS.

B. STOKES DISC DIFFUSION METHOD

1. Prepare the inoculum from material picked up with a loop from 5-10 colonies to be tested.
2. This material should be suspended in saline or broth, grown as an overnight culture in broth.
3. The suspension and culture should then be diluted to yield the correct weight of the inoculum. The density of the suspension to be inoculated should be measured by comparing with 0.5 McFarland standards.
4. The control inoculum should be spread into two bands on either side of the plate, leaving a central band uninoculated. This is best achieved with swabs impregnated with the control organisms.
5. Alternatively, a loopful of inoculum may be placed on both sides of the plate and spread with a dry sterile swab. The test organism is inoculated onto the central area of the e plate in a similar manner. An uninoculated gap, 2-3mm wide, should separate the test and control areas. Antibiotics discs are placed.

C. DILUTION TEST

- Serial dilutions of drugs are prepared and inoculated with the test bacterium. Dilution may be done by tube dilution or agar dilution methods.

Tube dilution

1. In the tube diffusion method, serial dilutions of the drug broth are taken in tubes and a standard standardized suspension of the test bacterium inoculated.
2. After overnight incubation, the 'minimum inhibitory concentration' (MIC) is read by noting the lowest concentration of drug that inhibits the growth. The 'minimum bactericidal concentration' (MBC) is the lowest concentration of the drug that kills the bacterium. It can be estimated by subculturing from the broth tubes that show no growth onto suitable solid media.

Agar dilution:

The agar dilution method is more convenient when several strains are to be tested at the same time. Here, serial dilutions of the drug are prepared in agar and poured in agar and poured into plates. The advantage is that many strains can be inoculated on each plate containing an antibiotic dilution.

The definition of MDRPA was established as isolates intermediate or resistant to at least 3 drugs in the following classes: β-lactams, carbapenems, aminoglycosides and fluoroquinolones.

QUALITY CONTROL

High standards can be maintained only when quality control procedures are in operation.

- Internal quality control requires that control organisms be stored freeze-dried or frozen at -20^0C or below. Fresh working cultures of controls should be prepared every few months or earlier if contamination is suspected. Working cultures should be stored on agar slopes and subcultured every 2 weeks. Maximum and minimum zone sizes should be predetermined. The mean of a series of control zone sizes should be close to a mid-point of the acceptable range. Batches of antibiotic discs and new batches of media must be tested before routine use. The depth and pH of each medium should be monitored.

- It is also necessary to check the potency of the discs periodically using as control a standard bacterium of known sensitivity such as *Staphylococcus*

aureus ATCC 25923, *Escherichia coli* ATCC 25922 or *Pseudomonas aeruginosa* ATCC 27853 depending on the bacterium to be tested.

MATERIALS AND METHODOLOGY

One hundred (100) samples were collected from the cathetrization patients consulting the Microbiology department Oxford Hospital Jalandhar

Inclusion criteria:

- Age- All groups
- Gender- both sexes

Exclusion criteria:

- Patient already on antibiotic therapy.

Collection of specimen:

Catheter tip from the catheterized patients.

Culture:

- The samples were cultured on Nutrient agar, MacConkey agar (composition, method of preparation has been mentioned in page number 23 and 24).
- Inoculation was done with the help a 0.001 ml calibrated loop.
- All the samples were incubated for 24hrs at 37^0C.

Microscopy:

- Suspected colonies were identified by Gram's stain.
- Bacterial identification was done by standard biochemical tests.

IDENTIFICATION OF THE ISOLATE:

A. COLONY MORPHOLOGY:

Nutrient agar : - large, opaque, irregular colonies with a pigmentation.

MacConkey agar : - large, opaque, irregular with non-lactose fermenting colonies.

B. GRAM'S STAINING:

- Bacterial suspension was spread out in the form of the thin film on the surface of clean glass slide and allowed to dry.
- The heat fixed smears were then stained by Gram's staining and observed under oil immersion objective (principle, reagents and procedure has been described in previous pages.

  Observation: - pink coloured gram negative straight rods arranged singly.

B. HANGING DROP PREPARATION

Hanging drop preparation was done to study the motility and morphology of the bacteria.

Observation: - shows motile bacilli.

D. BIOCHEMICAL REACTIONS:

- Catalase Test : - Positive
- Oxidase Test : - Positive
- Oxidative Fermentative Test : - Oxidative
- Nitrate Test :- Reduced to nitrite
- Triple Sugar Iron Test(TSI) :- Alkaline slant/Acidic butt (K/A)
- Sugar Fermentation Test:
  - ❖ Glucose: - Fermented with acid.
  - ❖ Lactose: - Not fermented.
  - ❖ Mannitol: - Fermented with acid.
- Indole Test :- Negative
- Methyl Red Test :- Negative
- Voges Proskauer Test :- Negative
- Citrate Test : - Citrate is utilised.
- LIA : - Decarboxylated.
- PPA : - Not deaminated.

E. ANTIBIOTIC SUSCEPTIBILITY TESTING

The Kirby Bauer disc diffusion test was used to assess the antibiotic sensitivity pattern shown by the Pseudomonas spp. isolated from the clinical specimen.

Procedure:

1. 3-5 isolated colonies were inoculated into 4-5 ml nutrient broth, Muller-Hinton broth or Trpticase soy broth and incubated for 2-8 hours until the turbidity of the suspension reaches or exceeds that of 0.5Mcfarland turbidity standard (approximately 1.5x 108 colony forming unit [CFU] /ml). The turbidity is adjusted to match the McFarland standard, if necessary, by diluting with additional broth.

2. Muller-Hinton plates (150mm diameter, 3-5mm thick, pH 7.2-7.4) were inoculated by dipping a sterile cotton swab into suspension, expressing excess broth by rotating the swab firmly against the inside of the tube and evenly streaking the entire surface of the plate.

3. Whatman No.2 filter paper circular discs (6mm) impregnated with known concentration of antibiotics was prepared.

Antibiotics used:

Name	Strength (mcg)
Amikacin	30
Ciprofloxacin	5
Gentamicin	10
Cefotaxime	30
Imipenem	10
Meropenem	10
Cefoperazone	75
Tobramycin	10
Piperacillin- Tazobactam	100/10
Cefepime	30
Ceftazidime	30
Norfloxacin	10

4. The antimicrobial-containing discs were placed on the agar plates within 15 min of inoculation by using a sterile forceps. The discs were pressed firmly against the agar surface to ensure contact and subsequent antimicrobial diffusion.
5. The plates were inverted and incubated at 16 to 18 hrs at 35^0C.

6. The diameter of the inhibitory zone was measured using a ruler.
7. The zone size around each antimicrobial disc is interpreted as susceptible, intermediate or resistant based on the criteria indicated in tables provided by NCCLS

Antibiotics and their zone size in millimetres:

Antibiotics	**Resistant**	**Intermediate**	**Sensitive**
Amikacin	14	15-16	17
Ciprofloxacin	15	16-20	21
Gentamicin	12	13-14	15
Cefotaxime	14	15-17	23
Imipenem	13	14-15	16
Meropenem	13	14-15	16
Cefoperazone	15	16-20	21
Tobramycin	12	13-14	15
Piperacillin-Tazobactam	17	18-20	21
Cefepime	14	15-17	18
Ceftazidime	14	15-17	18
Norfloxacin	14	15-16	17

CHAPTER- V

RESULTS & ANALYSIS

RESULTS

100 clinical samples from the Catherised UTI patient were processed out of which *Pseudomonas spp* was isolated from 19 samples. These isolated *Pseudomonas spp* formed large, opaque, irregular colonies with a pigmentation on Nutrient Agar that is large, opaque, irregular with non-lactose fermenting colonies on MacConkey agar.

Table 1: Shows the prevalence of *Pseudomonas spp* in UTI patients which indicates that 19% of the cases were positive i.e. out of 100 samples *Pseudomonas spp* was isolated only from 19 samples.

Table 2: Shows the resistance pattern of positive samples with Cefotaxime(84.2%) , Norfloxacin (63.1%) resistant and the least resistant was shown by Cefoperazone (0.0%)

Table 3: Shows the multidrug resistance pattern of *Pseudomonas spp* from the positive samples i.e. all the 19 isolates were resistant (Amikacin, Ciprofloxacin, Gentamicin, Cefotaxime, Imipenem, Meropenem, Cefoperazone, Tobramycin, Piperacillin-tazobactam, Cefapime, Cefatazidime, Norfloxacin) these all the isolates were Multidrug Resistance *Pseudomonas spp*.

Table 4: Shows the incidence of Multi drug resistance of the positive samples. Out of 100 samples 19 samples were isolated as *Pseudomonas spp* and 100% were Multi Drug Resistant (i.e. MDRPA).

Fig-a

Fig-b

ANALYZED DATA

Study design: An observational study with 100 samples screened to estimate the prevalence of *Pseudomonas spp* and Sensitivity and Resistance pattern for antibiotics.

Table 1: Prevalence of *Pseudomonas spp* in samples studied

Pseudomonas spp	Number of samples	%
Positive	19	19.0
Negative	81	81.0
Total	100	100.0

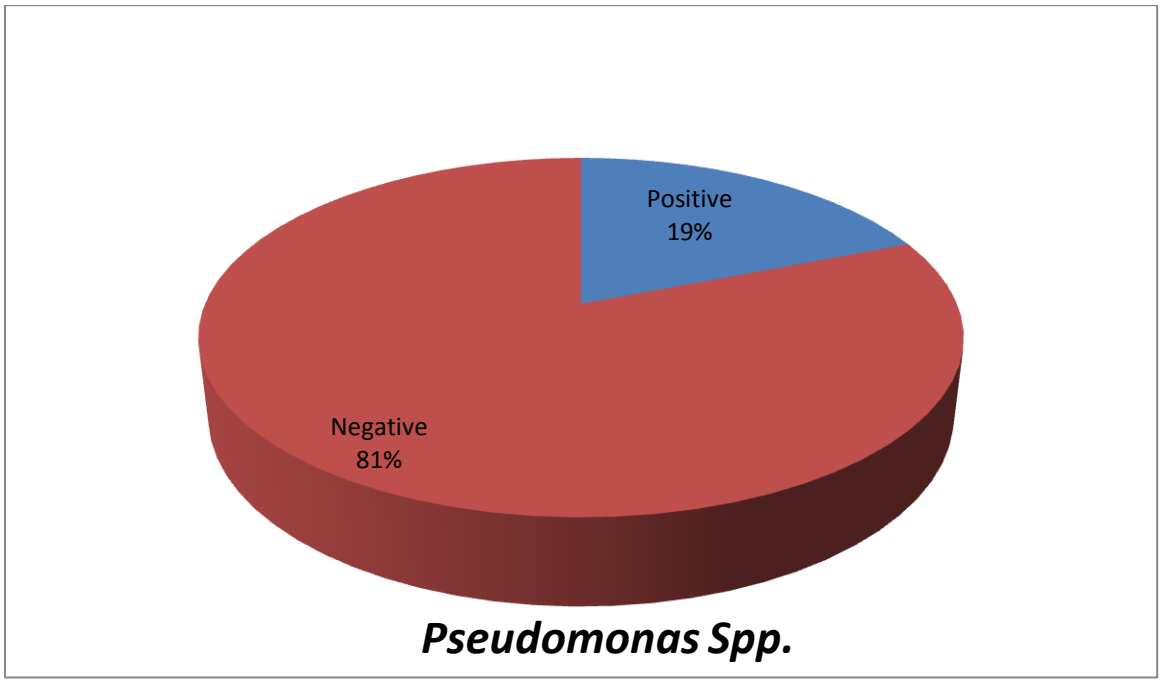


Table 2: Resistance pattern of positive samples studied

Resistance pattern	**Number of samples (n=19)**	**%**	**95%CI**
1.Amikacin	4	21.0	85.10-43.33
2.Ciprofloxacin	4	21.0	85.10-43.33
3.Gentamicin	2	10.5	29.40-31.40
4.Cefotaxime	16	84.2	62.43-94.48
5.Imipenem	1	5.2	09.30-24.63
6.Meropenem	2	10.5	29.40-31.40
7.Cefoperazone	0	0.0	0-16.82
8.Tobramycin	1	5.2	09.30-24.63
9.Piperacillin-tazobactam	1	5.2	09.30-24.63
10.Cefapime	2	10.5	29.40-31.40
11.Cefatazidime	6	31.5	15.37-53.99
12.Norfloxacin	12	63.1	41.04-80.85

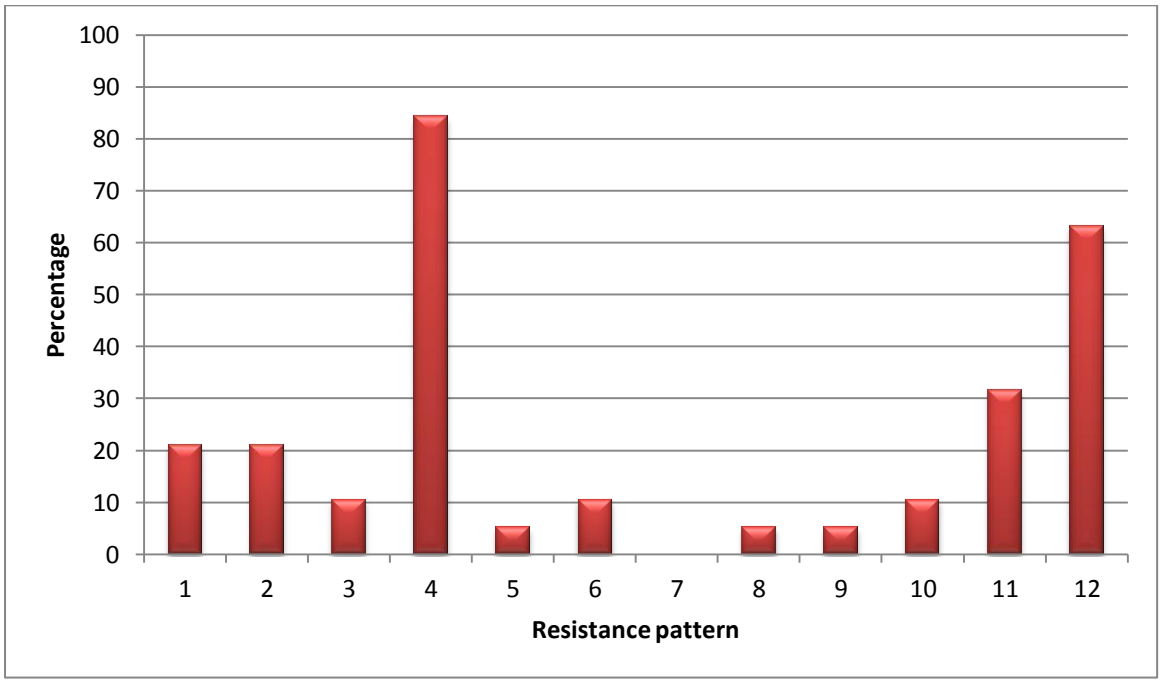
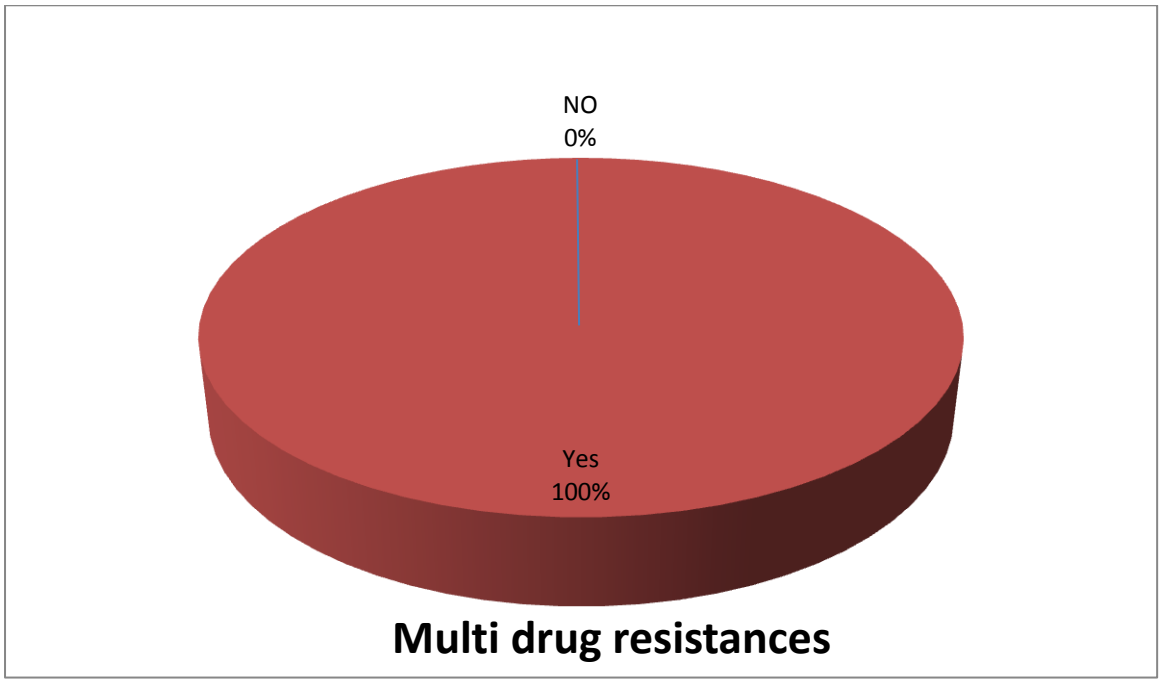


Table 3: Incidence of Multidrug resistance of sample studied

Isolation	**Number of antibiotics –resistance (n=12)**
Isolate 1	4
Isolate 2	5
Isolate 3	12
Isolate 4	10
Isolate 5	7
Isolate 6	3
Isolate 7	7
Isolate 8	7
Isolate 9	12
Isolate 10	8
Isolate 11	15
Isolate 12	6
Isolate 13	9
Isolate 14	11
Isolate 15	10
Isolate 16	9
Isolate 17	5
Isolate 18	11
Isolate 19	12

Table 4: Incidence of Multidrug resistance of sample studied

Multi drug resistance	**Number of isolation**	**%**
Yes	19	100.0
No	0	0.0
Total isolates	19	100.0



ANNEXURES-I

MASTER CHART

	AK	CF	G	CE	I	MR	CS	TB	PT	CPM	CA	NX
Isolate 1	S	S	S	S	S	S	S	S	R	S	R	R
Isolate 2	R	S	R	R	S	R	S	S	S	S	R	S
Isolate 3	S	S	S	R	S	S	S	S	S	S	R	S
Isolate 4	S	R	S	R	S	S	S	S	S	S	R	S
Isolate 5	S	S	S	R	S	S	S	R	S	R	R	S
Isolate 6	R	R	S	R	S	S	S	S	S	S	R	R
Isolate 7	S	S	S	S	S	S	S	S	S	R	S	R
Isolate 8	S	S	S	R	S	S	S	S	S	S	S	R
Isolate 9	R	R	S	R	S	S	S	S	S	S	S	R
Isolate 10	S	S	S	R	S	S	S	S	S	S	S	R
Isolate 11	R	R	S	R	S	S	S	S	S	S	S	R
Isolate 12	S	S	S	R	S	R	S	S	S	S	S	R
Isolate 13	S	S	S	R	R	S	S	S	S	S	S	R
Isolate 14	S	S	S	R	S	S	S	S	S	S	S	R
Isolate 15	S	S	S	R	S	S	S	S	S	S	S	R
Isolate 16	S	S	R	R	S	S	S	S	S	S	S	R
Isolate 17	S	S	S	R	S	S	S	S	S	S	S	S
Isolate 18	S	S	S	S	S	S	S	S	S	S	S	S
Isolate 19	S	S	S	R	S	S	S	S	S	S	S	S

R-Resistance

S-Sensitive

CHAPTER- VI

DISCUSSION & CONCLUSION

DISCUSSION

Pseudomonas spp. is an important pathogen and it may responsible for the nosocomial urinary tract infections.This is cases of morbidity among hospital patients. The pre-eminent of *pseudomonas spp* in hospital infections is due to its resistance to common antibiotics and antiseptics, and its ability to establish itself widely in hospitals. It can survive and multiply even with minimum nutrients, or can survive anywhere where ever less conditions, if moisture is available.*Pseudomonas spp.* causes serious infections, and is one of the leading causes of hospital acquired infections, several studies were carried out to detect antibiotic sensitivity pattern for the various drugs available.The present study was conducted to determine the antibiotic sensitivity pattern of *Pseudomonas spp* isolated from various clinical samples.The sex and age wise distribution of patients diagnosed with infections followed the natural epidemiological pattern. In present study, the maximum clinical isolates of *Pseudomonas spp.* were isolated from followed by urine (16%).[32]

In present study the highest percentage (48%) of *Pseudomonas spp* infections were observed in the surgical ward, and medical ward (17%).This study shows that the clinical isolates of *Pseudomonas spp* are becoming resistant to commonly used antibiotics and gaining more and more resistance to newer antibiotics.[33]

Due to indiscriminate use of antibiotics, patient non compliance and unhygienic condition. It is the need of the time that antibiotic policies should be formulated and implemented to resist and overcome this emerging problem. Every effort should be made to prevent spread of resistant organisms.[34]

CONCLUSION

The present study was to describe the isolation and identification of *Pseudomonas spp* in urinary tract infection patients and to study antibiotic susceptibility patterning of *Pseudomonas spp.*

Out of 100 samples, 19 samples were isolated as *Pseudomonas spp*. The percentage of Multi drug resistance *Pseudomonas spp* from the positive samples was 100% since all these positive samples were resistant .

The percentage of resistance by antibiotics: Amikacin (21.05%), Ciprofloxacin (21.05%), Gentamicin (10.52%), Cefotaxime (84.21%), Imipenem (5.2%), Meropenem (10.52%), Cefoperazone (0%), Tobramycin (5.26%), Piperacillin-tazobactam (5.26%), Cefepime (10.52%), Ceftazidime (31.57%), Norfloxacin (63.15%).

CHAPTER- VII

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