MDR of bacteria related to Nosocomial catheter associated

Urinary Tract Infection

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By

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CERTIFICATE

This is to certify that, the work entitled "MDR of bacteria related to nosocomial catheter associated urinary tract infection" was carried out by Ms. Karamjit kaur under my direct supervision. This is to further 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.Gurinder Singh*** *Assistant professor* 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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Karamjit kaur

M.Sc. Clinical Microbiology

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 E.coli, *Klebsiella, 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 45% E.coli and 22% *Klebsiella spp. and 19 Pseudomonas spp.* were isolated.

The pattern of antibiotic susceptibility suggested that 100% of the isolates were resistant to Norfloxacin and 27.2% Amikacin, 18.1% Ciprofloxacin, Imipenem, Meropenem and the least resistance was shown by Gentamicin, Tobramycin- Piperacillin 9.0%. Antibiotic resistance pattern shows that all of the 22 isolates were Multidrug resistance *Klebsiella* (MDRK).

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CHAPTER 1

INTRODUCTION

INTRODUCTION

Catheter related urinary tract infection is one of the common infection amongst the all[1].Urinary catheter is most commonly used in health care areas nursing homes.The symptomatic infection to individual refers to catheter associated urinary tract infection[2]. Many patients are at risk just because of the unnecessary use of indwelling catheter during hospitalization or with such devices[3].Urinary tract infection (UTI) is the single most common hospital-acquired infection, and the majority of cases of nosocomial UTI are associated with an indwelling urinary catheter[4].

1.1

The major determination of bacteriuria is depend on the time or duration of catheterization. The women and older persons are at higher risk[5].Bacteriuria is must once a catheter remains attached for several weeks. Patient will be bacteriuric with chronic indwelling catheter[6].

1.2 Nosocomial infection hospital has always acted as source of infection to patient admitted to them. The infection usually occur after the patient admitted to hospital. The incidence of hospital infection has been reported to be 2 to 12 percent in advanced countries but it is much higher in crowded hospitals. Such infection is evident during their stay in hospitals or only after discharge[7].

URINARY TRACT INFECTION

Urinary tract infections (UTI's) can be defined as bacteriuria (>105 CFU/mL in adults; >104 CFU/mL in children) of an uropathogen with associated clinical signs that include dysuria and urgency.Urinary tract infection (UTI) is also refers to invasion of the urinary tract by bacteria. Urinary tract infection is usually associated with catheterization or instrumentation or urethra, bladder or kidneys[8]. *Klebsiella* ranks second to *E. coli* for

urinary tract infections. It is also an opportunistic pathogen for patients with chronic pulmonary disease, enteric pathogenicity, nasal mucosa atrophy, and rhinoscleroma.

ETIOLOGY

Urinary tract infection (UTI) is the second most common infectious presentation in world. organisum involve in urinary tract infection is *E.coli(60.4%), Klebsiella(11.6%), Pseudomonas(8.3%) Proteus(2.9%),Serriatia,,* coagulase negative *Staphylococci(2.2%), Enterococci(2.3%)* and *Candida Albicans.*e. coli is the most common cause for the UTI ,and *klebsiella* is the second most organisms which cause the hospital acquired urinary tract infection[9,10].

PATHOGENESIS OF INFECTION:

On the catheter surface when there is biofilm formation that is most important cause of bacteriuria. Biofilm is basically a complex of micro-organisms growing in colonies within and extra-cellular muco polysaccharide substance which they produce. Tamm-Horsfall Protein (THP) is urine's most abundant protein and is known to bind to uropathogenic bacteria. The role of THP in the pathogenesis of catheter associated urinary tract infection (CAUTI) is not clear. Urine components, including Tamm-Horsfall protein and magnesium and calcium ions, are incorporated into this material.

When we insert catheter, then biofilm immediately forms, when organisms attached to conditioning film of host proteins which forms along the catheter surface. Both the interior and exterior catheter surfaces are involved. Bacteria usually originate from the periurethral area or ascend the drainage tubing following colonization of the drainage bag[11,12].Bacterial biofilms play an important role in medicine. According to the NIH, Bacteria which form biofilm involved up to 80% of all infections with urology being one of the main fields in which biofilm can become a serious problem[13].

pathogenesis of CAUTI is related to the susceptibility of inert catheter material to microbial colonization. On the surface of normal bladder mucosa, binding of bacteria triggers inflammatory response that results in an influx of neutrophils and sloughing of epithelial cells with bound bacteria[14].

FORMAL DEFINATION OF BIOFILM INCLUDES 3 COMPONENETS [15]:

(1) Adherence of the microorganisms, either to a surface or to each other.

(2) A change in gene expression resulting in a different phenotype from the planktonic state.

(3) An extracellular matrix composed of host.Components and secreted bacterials.

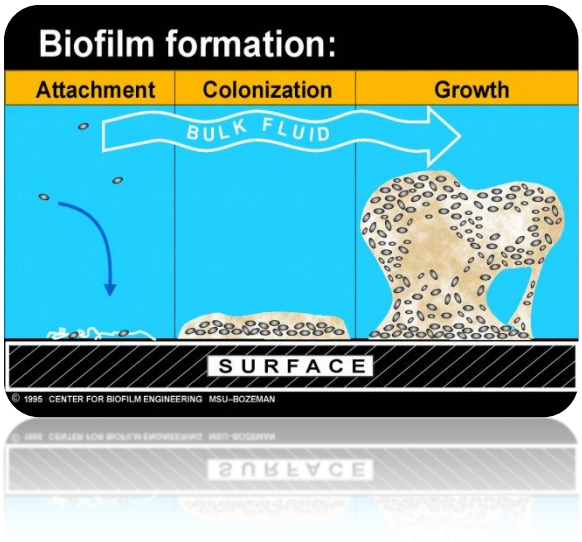

Fig: 1.1 Biofilm formation

The most common infecting organism is Escherichia coli. Other Enterobacteriaceae as well as *Enterococci spp, Staphylococcus, Pseudomonas aeruginosa*. Antimicrobial-resistant organisms are common. The urine of patients with indwelling catheters is the

major site of isolation of resistant gram negative organisms in both acute and long term care facilities [16].

SIGN AND SYMPTOMS OF URINARY TRACT INFECTION

The urinary tract infection is divided into two upper urinary tract infection and lower urinary tract infection.

In upper urinary tract involve **kidney**

Lower urinary tract involve **ureters, bladder and urethra**[17]

Lower UTIs are infections of the urethra and bladder. Their symptoms include : burning with urination, increased frequency of urination, bloody urine, cloudy urine, urine that looks like cola or tea, strong odour to urine, pelvic pain (women), rectal pain (men). Upper UTIs are infections of the kidneys. Symptoms of upper UTI include: pain and tenderness in the upper back and sides, chills, fever, nausea, vomiting. if bacteria move from the infected kidney into the blood. This condition is called sepsis. Sepsis can cause low blood pressures, shock, and death[18].

CATHETER

A urinary catheter is a tube placed in the body to drain and collect urine from the bladder. Urinary catheters are used to drain the bladder. It is mostly used in Urinary incontinence, Urinary retention, Surgery on the prostate or genitals and other medical conditions such as multiple sclerosis, spinal cord injury. An indwelling urinary catheter is one that is left in the bladder. Indwelling catheter can use for a short time or a long time. An indwelling catheter collects urine by attaching to a drainage bag if proper care is not taken during indwelling urinary catheterization this may lead for main cause of urinary tract infections[19].

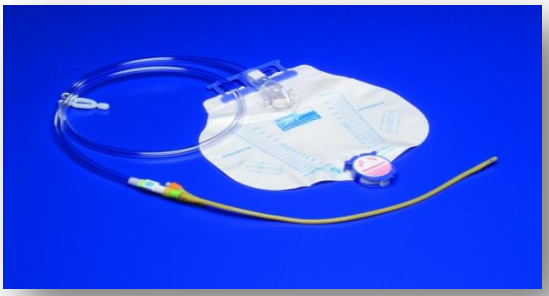

Fig: 1.2 Catheter

ROLE OF BIOFILM IN CATHETER IN ASSOCIATED URINARY TRACT INFECTION: "Biofilm" The primary form of life for the majority of microorganisms in any hydrated biologic system. A biofilm on an indwelling urinary catheter consists of adherent microorganisms, their extracellular products, and host components deposited on the catheter. The biofilm mode of life conveys a survival advantage to the microorganisms associated with it and, thus, biofilm on urinary catheters results in persistent infections that are resistant to antimicrobial therapy.

Chronic catheterization leads to routine treatment of bacteriuria and asymptomatic bacteriuria in persons who are catheterized is not recommended. In catheterized patient when symptoms of a urinary tract infection develop changing the catheter before collecting the urine. It will improves the accuracy of urine culture results. Changing the catheter may also improve the response to antibiotic therapy by removing the biofilm as biofilm probably contains the infecting organisms and that can serve as a nidus for reinfection[20].

CHAPTER: 2

REVIEW OF LITERATURE

MDR of bacteria related to nosocomial catheter associated urinary tract infection

Nosocomial infections problem is as old as the hospitalization. However this problem is an ongoing of modern medicine and depict a permanent danger even to hospitalized patient in the most modern hospitals. All organisms can be the causative agents of nosocomial infection but most often there is involvement of bacteria. Depending on the use of antibiotics and introduction of new diagnostic and therapeutic procedures, the types of bacteria change that cause hospital infection[21].

Escherichia coli in recurrent urinary tract infections, *Pseudomonas aeruginosa* in cystic fibrosis pneumonia, *Streptococcus pneumoniae* in chronic otitis media, *Staphylococcus aureus* in chronic rhinosinusitis, are linked to biofilm formation. Biofilms are usually defined as surface-associated microbial communities, surrounded by an extracellular polymeric substance (EPS) matrix. Mostly biofilms are found in chronic diseases. Biofilm basically resist host immune responses and antibiotic treatment[22].
Klebsiella pneumoniae is an important cause of nosocomial infection because of multiple antibiotic resistance. Patients with longer length of stay and greater total antibiotic exposure should be potential targets for stringent infection[23].

Urinary tract infection (UTI) accounts for up to 40% of nosocomial infections making it the most common hospital-acquired infection in the United States. Urinary catheter-related infection accounts for most nosocomial UTI. These are common in acute care hospitals (including ICUs); long-term care facilities; and in persons with injured spinal cords.

Urinary catheter-related infection leads to substantial morbidity and mortality.The incidence of bacteriuria in catheterized patients varies between 3% and 10% per day. Among patients with bacteriuria 10% to 25% develop symptoms of local infection whereas 1% to 4% develop bacteremia. Implementation of infection control measures to improve catheter function and remove catheter induce urinary tract infection and prevention of catheter associated urinary tract infection[24].

Majorly present bacteuria in symptomatic catheterized patients and most frequent isolate is *E.coli.* Diabetes is also a most common factor that may lead to UTIs in the catheterized patients the ratio is 44%.[25]

Specifically when the bladder is catheterized urinary tract is the commonest source of nosocomial infection. Patient's own normal flora is major cause of catheter-associated UTIs and other factor is catether predisposes. The most important risk factor for the development of catheter-associated bacteriuria is the Duration of catheterization play a pivotal role in catheter-associated bacteriuria this is the major risk because more duration more will be infection. So if the duration will be short-term the infection will be less and also caused by single organism mostly it will become asymptomatic. Further organisms tend to be acquired by patients catheterized for more than 30 days.

The clinician should be aware of two priorities: firstly the catheter system should remain closed and the duration of catheterization should be minimal or as less as possible. While asymptomatic catheter-associated bacteriuria does not require any antimicrobial treatment or impossible to recommended, except for some special cases.

In case of higher or symptomatic infection or chronic cases antibiotic treatment is recommended. Long-term antibiotic suppressive therapy is not effective. Avoiding the closed drainage bag, non return (flip) valve catheter use. Indwelling urethral catheters that are less causing symptomatic infection should be preferable. In appropriate patients,suprapubic catheters, condom drainage systems and intermittent catheterization are preferable to indwelling urethral catheterization[26].

E.coli was the first and usual common pathogen causing urinary tract infection. According to sex bacteria also varies that may lead to UTI. Majorly E.coli are the one who cause half of the infection of urinary tract. This is just because of their resistance to antibiotics was low when it is compared with any other pathogens like *Klebsiella* and *Pseudomonas spp*. which was implicated in UTI, along with this it also having the lowest percentage of multidrug resistant (MDR) isolates. Bacteria which were isolated from females were less resistant than those isolated from males and this difference increased with the patient age.[27].

MDR of bacteria related to nosocomial catheter associated urinary tract infection

Urinary tract infections are the most commonly acquired bacterial infections and they account for an estimated 25-40% of the nosocomial infections. The microbial biofilms pose a public health problem for the persons who require indwelling medical devices, as the microorganisms in the biofilms are difficult to treat with antimicrobial agents. Bacteuria in all the symptomatic catheterized patients and *E.coli* was the most frequent isolate. Diabetes (44%) was the most common factor which was associated with the UTIs in the catheterized patients[28].

Nosocomial infections affect about 30% of patients in intensive-care units and are associated with substantial morbidity and mortality. the persons who are more susceptible to nosocomial infection than others are the ones, in which such major several risk factors have been identified, including the use of catheters and other invasive equipment, and certain groups of patients – eg those with trauma or burns are recognised as being.

Awareness of these factors and simple preventive measures, such as adequate hygiene, can limit the occurrence of disease. Management of nosocomial infection relies on adequate and appropriate antibiotic therapy[29].

CHAPTER: 3

METHODOLOGY

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PATIENTS AND METHODS

PATIENTS

Patients participating in 2 randomized trials of 2 novel urinary catheters one nitrofurazone-impregnated silicone catheter and the other, a silver-polyurethane hydrogel catheter formed the study population. Neither medicated catheter was associated with any irritative urinary tract symptoms or with increased sterile pyuria, as compared with the control catheters used in each trial. Participants in both trials were hospitalized patients scheduled to receive an indwelling urethral (Foley) catheter who were expected to be catheterized for more than 24 hours; patient were excluded if they were younger than 18 years, pregnant, or had a known allergy to silicone, nitrofurazone, or silver.

Sample processing

The samples were selected on the basis of their growth on routine MacConkey medium which showed lactose Non-fermenting pale colonies which were oxidase test positive and on Nutrient agar pigmented and nonpigmented colonies with oxidase positive

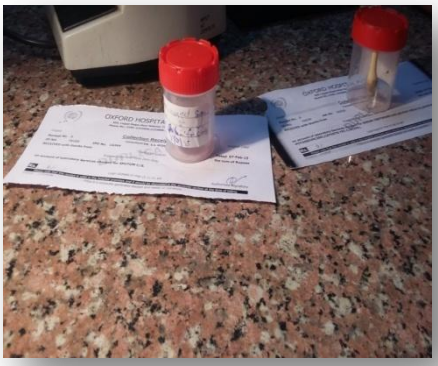

Fig:3 Cathtip specimen

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:

Urinary Catheter tip is collected from the catheterized patient .

Transport of specimen:

- Once collected specimen must be transported to the laboratory without delay.
- Sterile disposable container are used to transport specimen.

Microscopy: Gram stained smear 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.
- Safranine 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

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

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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).

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

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.

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.

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.

. **ANTIBIOTIC SUSCEPTIBILITY TESTING**

The Kirby Bauer disc diffusion test was used to assess the antibiotic sensitivity pattern shown by the *klebsiella 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.

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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-	17	18-20	21
Cefepime	14	15-17	18
Ceftazidime	14	15-17	18
Norfloxacin	14	15-16	17

CHAPTER:4

RESULTS AND ANALYSIS

MDR of bacteria related to nosocomial catheter associated urinary tract infection

MDR of bacteria related to nosocomial catheter associated urinary tract infection

An observational study with 100 samples screened to estimate the prevalence of *E.coli* and Sensitivity and Resistance pattern for antibiotics. Out of which there is a lot percentage of *Klebsiella* and third one leads to the *Pseudomonas spp.* and many more candida albicans as well including *Streptococcus* also. But as result concluded that the *E.coli* leads more at the peak.

Total number of sample including male and female =100

Positive sample: 86

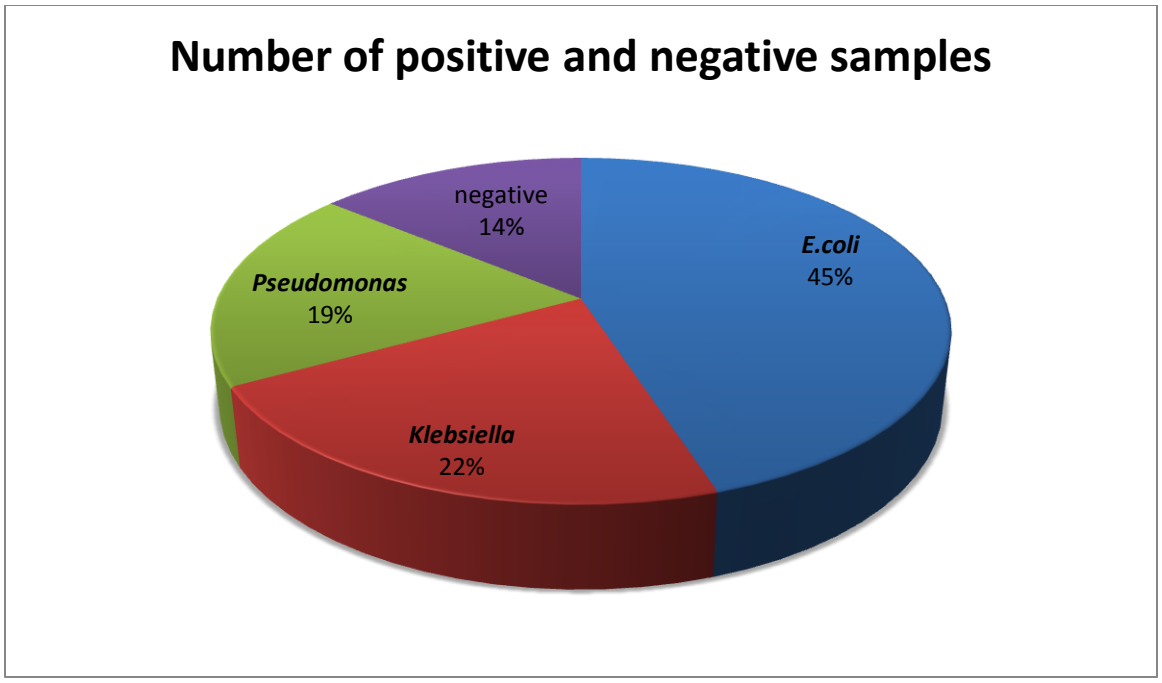
E.coli: 45

Klebsiella: 22

Pseudomonas : 19

Negative: 14

Total number of sample	100
Positive sample	86
Negative sample	14
Pseudomonas spp.	19
Klebsiella spp.	22
E.coli	45



E.coli is the cause of 80-85% of community acquired urinary tract infections. As urinary tract infections occur due to catheterization involve a much broader range of pathogens including ***E.coli, Pseudomonas, Klebsiella*** the fungal pathogen Candida albicans. As *E.coli* has a property to grow in round shape continuous margins. As *Pseudomonas* give odory colonies.

POSITIVE SAMPLE OF *E.COLI* SPP.

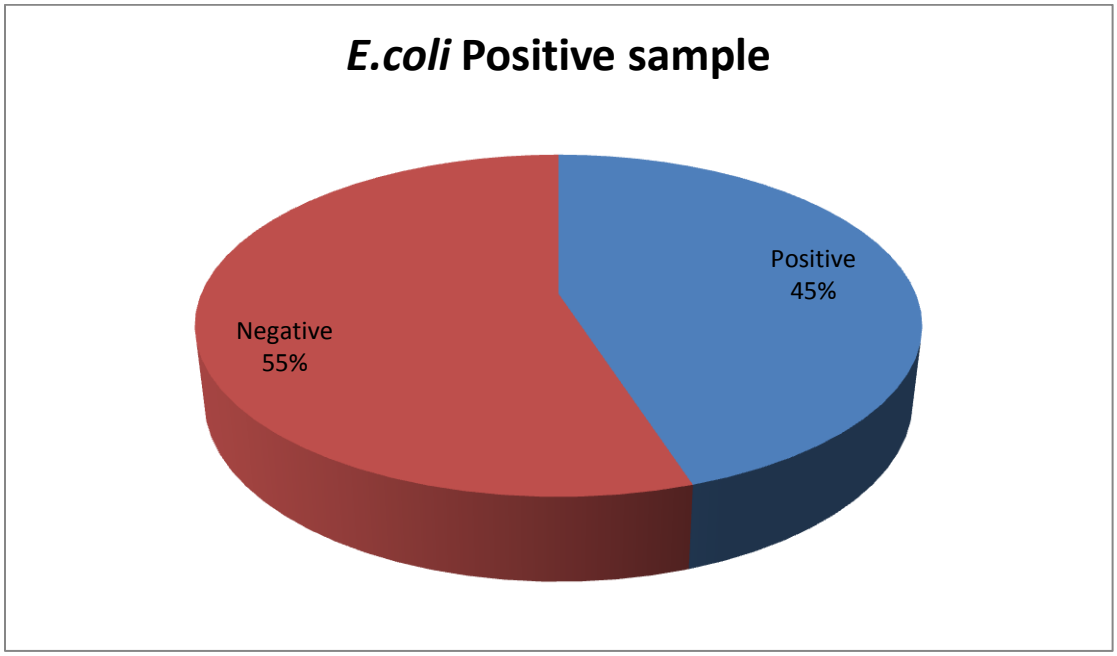
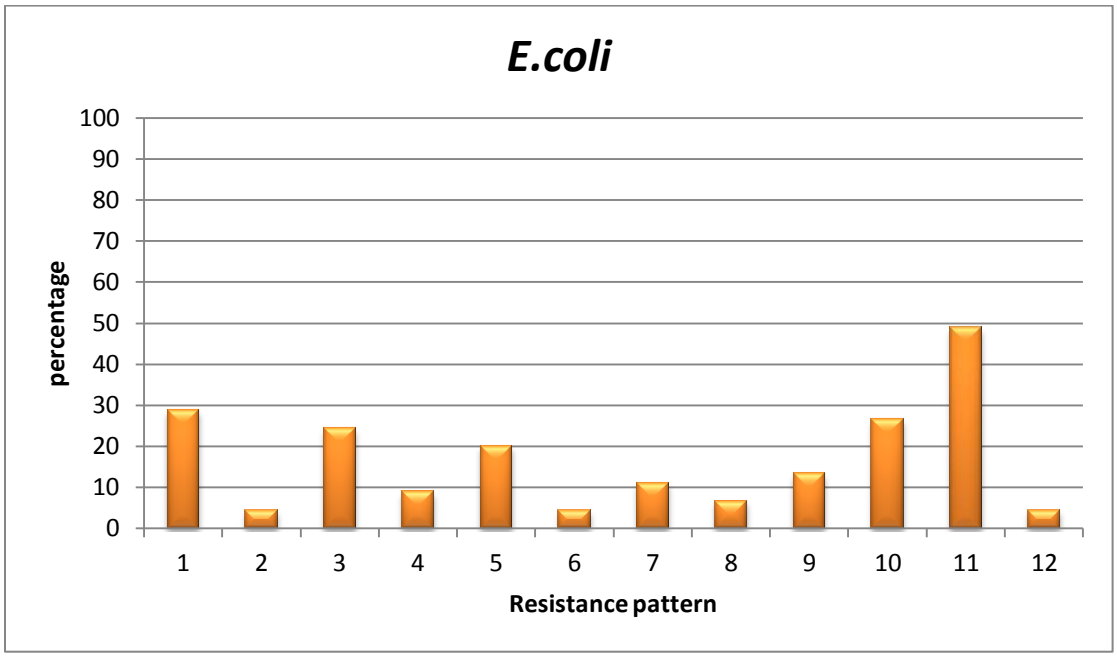


Table:1 Resistance pattern of *E.coli* positive samples studied

Resistance pattern	**Number of samples (n=45)**	**%**	**95%CI**
1.Amikacin	13	28.8	17.7-43.37
2.Ciprofloxacin	2	4.44	12.30-14.82
3.Gentamicin	11	24.44	14.23-38.67
4.Cefotaxime	4	8.88	35.10-20.73
5.Imipenem	9	20	10.90-33.82
6.Meropenem	2	4.44	12.30-14.82
7.Cefoperazone	5	11.11	48.40-23.50
8.Tobramycin	3	6.66	22.90-17.86
9.Piperacillin-tazobactam	6	13.33	62.50-26.17
10.Cefapime	12	26.66	15.97-41.04
11.Cefatazidime	22	48.8	34.96-63.00
12.Norfloxacin	2	4.44	12.30-14.82



Bar chart showing resistance of *E.coli*

POSITIVE SAMPLE OF *PSEUDOMONAS SPP.*

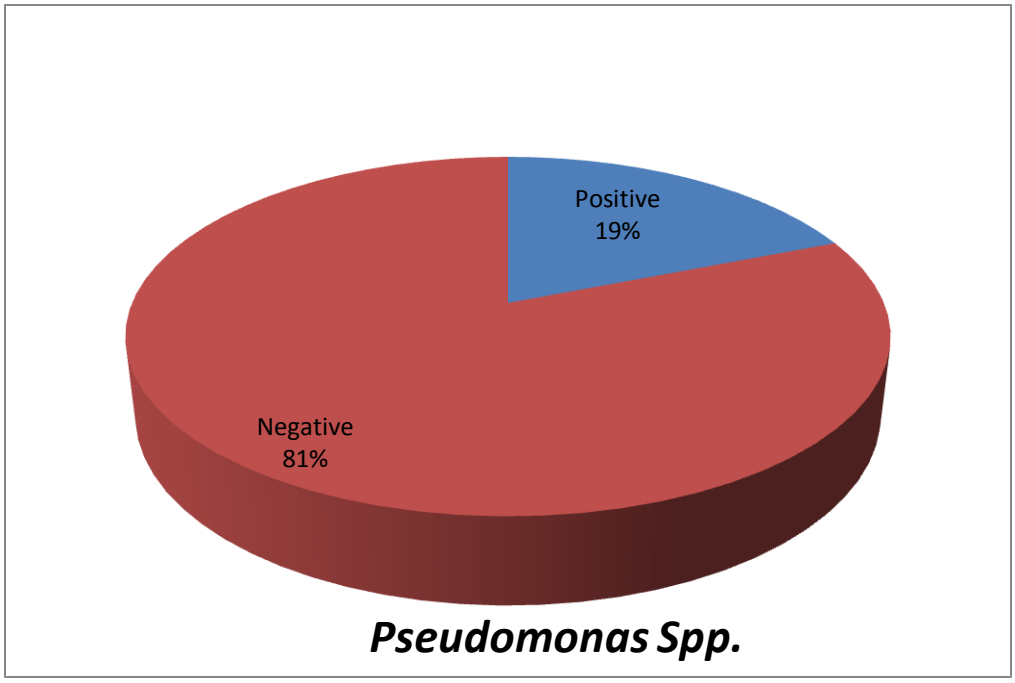
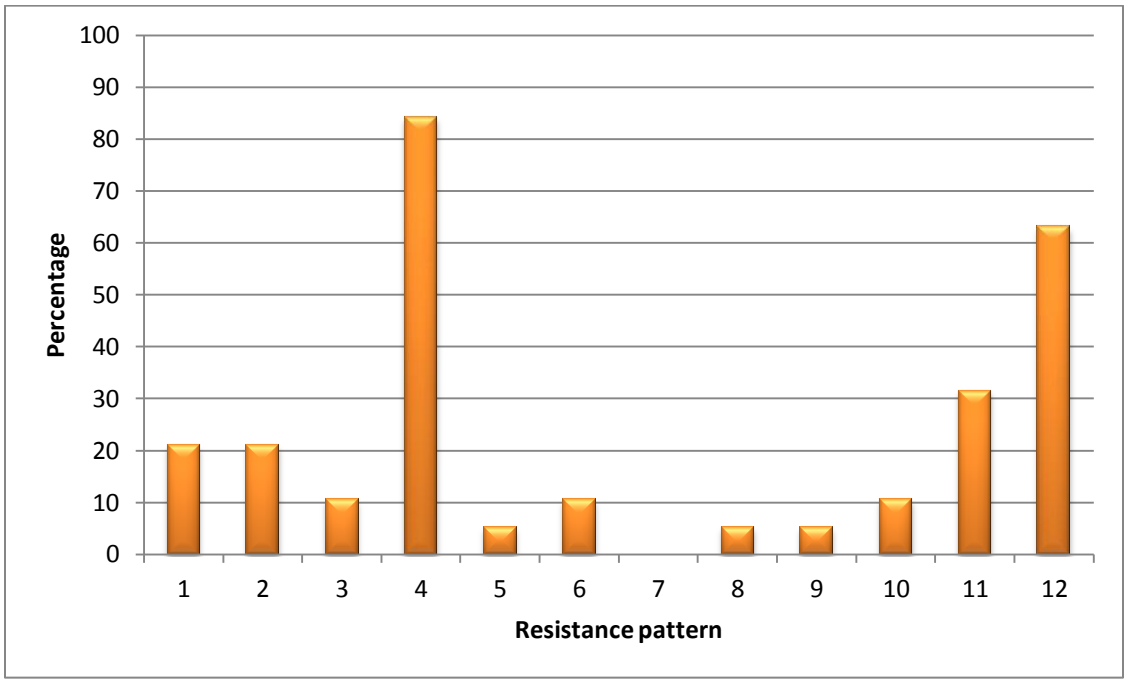


Table: 2 Resistance pattern of *Pseudomonas spp.* positive samples studied.

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



Bar chart showing resistance of *Pseudomonas spp.*

POSITIVE SAMPLE OF *KLEBSIELLA SPP*.

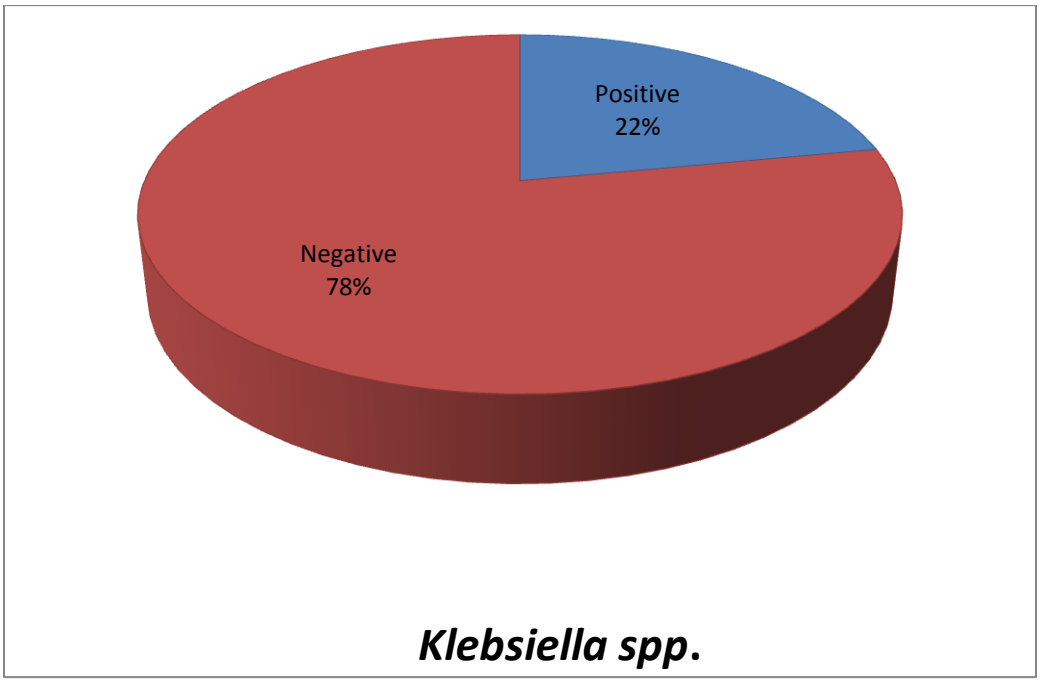
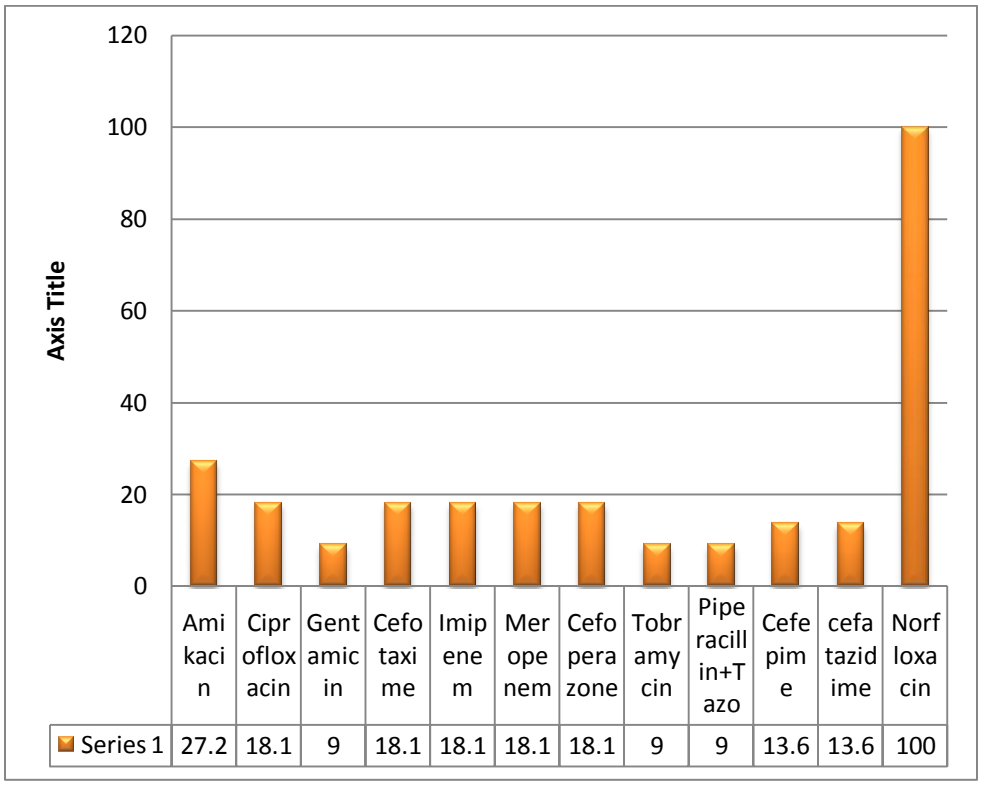


Table 3: Resistance pattern of *Klebsiella spp.* positive samples studied.

Resistance pattern	**Number of samples (n=22)**	**%**	**95%CI**
1.Amikacin	6	27.2	13.15-48.15
2.Ciprofloxacin	4	18.1	73.10-38.51
3.Gentamicin	2	9.0	25.30-27.81
4.Cefotaxime	4	18.1	73.10-38.51
5.Imipenem	4	18.1	73.10-38.51
6.Meropenem	4	18.1	73.10-38.51
7.Cefoperazone	4	18.1	73.10-38.51
8.Tobramycin	2	9.0	25.30-27.81

9.Piperacillin-tazobactam	2	9.0	25.30-27.81
10.Cefapime	3	13.6	47.50-33.34
11.Cefatazidime	3	13.6	47.50-33.34
12.Norfloxacin	22	100.0	85.13-100.0



Bar chart showing resistance of *Klebsiella spp.*

CHAPTER: 5

DISCUSSION AND CONCLUSION

MDR of bacteria related to nosocomial catheter associated urinary tract infection

DISCUSSION

Indwelling catheter are basically use in hospitals widely and also across the united states as well. It is basically a necessary intervention, but along with this this urinary catheters unfortunately are the leading cause of UTIs in hospitalized patients.This may lead to major incidence of bacteriuria. In addition, economic burdens associated with catheter-related infection.

Person who is already catheterized from last 2 to 10 days, in that patient catheter-related bacteriuria is common. Symptoms of local infection develop in approximately 1 in 5 whereas most patients with catheter associated bacteriuria remain free of symptoms. In addition, bacteremia (presence of bacteria in the blood) from the same urinary tract organism will develop in 1 of 27 patients with bacteriuria.

The use of aseptic technique in catheter insertion and maintenance Infection control professionals and hospital epidemiologists play an important role in reducing the incidence of this important complication and by advocating for methods with demonstrated effectiveness in preventing this complication. In the future, it is hoped that new technologies will be developed that will reduce the significant health care burden of urinary catheter-related infection[30].

MDR of bacteria related to nosocomial catheter associated urinary tract infection

CONCLUSION

The present study was to describe the isolation and identification of *Pseudomonas spp*, *Klebsiella spp* and *E.coli* in urinary tract infection patients and to study antibiotic susceptibility patterning of these as well. As *E.coli* is first major pathogen that may lead to UTI after its *Klebsiella* and *Pseudomonas* play role.

Out of 100 samples, 19 samples were isolated as *Pseudomonas spp* and 22 *Klebsiella* and *E.coli* 47. 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%) and *Klebsiella* and *E.coli* have also.

CHAPTER: 6

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MDR of bacteria related to nosocomial catheter associated urinary tract infection

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APPENDIX

PREPARATION OF MEDIA:

Blood Agar

Blood agar is an **enriched**, bacterial growth **medium**. **Fastidious** organisms, such as streptococci, do not grow well on ordinary growth media. Blood agar is a type of growth medium that encourages the growth of bacteria, such as streptococci, that otherwise wouldn't grow well at all on other types of media.

Composition:-

Meat extract	10g/L
NaCl	8.5g/L
Peptone	10gL
Agar	20gL
Distilled Water	1000ml
Defibrinated blood	50-100ml

C.L.E.D media:

Composition:

Peptic digest of animal tissue	4g/L
Casein enzyme hydrolysate	4g/L
Beef extract	3g/L
Lactose	10g.L
L –Cystine	0.128g/L
Bromothymol blue	0.02g/L
Andrade indicater	0.10g/L
Agar	15.00g/L
PH	7.5+0.2

Mix all the ingredients in distilled water and heat to dissolve properly .Autoclave at 121°C at 15lb pressure for 20min.

Mueller Hinton Agar

Composition

Beef infusion from	300g/L
Casein acid hydrolysate	17.5g/L
Starch	1.7g/L
Agar	17.00g/L
Final PH at 25C	7.3+0.1

Mix all the ingredients in distilled water and heat to dissolve properly .Autoclave at 121°C at 15lb pressure for 20 minutes.

Nutrient Agar

Composition:

Meat extract	10g/L
NaCl	8.5g/L
Peptone	10gL
Agar	20gL
Distilled Water	1000ml
pH	7.4-7.6

Mix all the ingredients in distilled water and heat to dissolve properly .Autoclave at 121°C at 15lb pressure for 20min.pour the media in Petri dishes when temperature reaches to 40-45°C.

XLD (Xylulose Lysine Deoxycholate Agar)

Composition:-

Yeast extract	03g/L
Lysine hydrochloride	05g/L
Xylose	3.7g/L
Lactose	7.5g/L
NaCl	5.0g/L
Sodium deoxycholate	2.5g/L
Sodium thiosulphate	6.0g/L
Ferric ammonium citrate	0.8g/L
Phenol red	0.08g/L
Agar	15g/L
Distilled Water	1000ml

Mix all the ingredients in distilled water adjust the pH to 7.4. Heat the media at 100°C for 10min.When cooled to 40-45°C pour the media in Petri dishes.

GRAM STAINING:

Introduction :

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

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

Reagents:

(A) 1% CRYSTAL VIOLET SOLUTION

Crystal violet - 1.0 g

Distilled water - 100 mL

(B) GRAM'S IODINE SOLUTION

Iodine - 1.0 g

Potassium iodide - 2.0 g

Distilled water - 300 mL

(C) ACETONE

(D) 0.5% Safranin Solution