Antibiogram Profile of MDR *Klebsiella* in catheterized UTI patient in Punjab, India

Submitted in partial fulfillment of the requirements of the
Degree of

MASTER OF SCIENCE
(CLINICAL MICROBIOLOGY)

By

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May, 2015

CERTIFICATE

This is to certify that, the work entitled **"Antibiogram Profile MDR *Klebsiella* in catheterized UTI patient in Punjab, India"** was carried out by **Ms. Jaspreet 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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Lecturer ,
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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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ACKNOWLEDGEMENT

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.

It's my proud to express my deep gratification and sincere thanks of having being worked under the guidance of internal guide **Mr. Himal Sapkota.** I am grateful for his guidance, constant encouragement, keen interest, creative criticism and valuable suggestions during the observation and preparation of this manuscript.

Also I would like to thank all **Nursing Staff, Junior Doctors and Consultants** who inspired me to work hard at every step. My sincere thanks to all **Technical Staff** of Laboratory of **Oxford hospital** for their support.

Above all I thank my family who helped me in all the ways and motivated me from time to time to complete my degree successfully.

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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., *Klebsiella*. 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 22% *Klebsiella 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).

INDEX

LIST OF FIGURES

LIST OF TABLES

CHAPTER- I
INTRODUCTION

INTRODUCTION

1.1 URINARY TRACT INFECTION

Urinary tract infections (UTI's) can be defined as bacteriuria ($>10^5$ CFU/mL in adults; $>10^4$ CFU/mL in children) of an uropathogen with associated clinical signs that include dysuria and urgency.[1] Urinary tract infection (UTI) is also refers to invasion of the urinary tract by bacteria.[2] Urinary tract infection is usually associated with catheterization or instrumentation or urethra, bladder or kidneys.[3] *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. Feces are the most significant source of patient infection, after the contact with contaminated instruments.[17]

1.2 ETIOLOGY

Urinary tract infection (UTI) is the second most common infectious presentation in world. Organism 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.[3-5]

1.3 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 and lower urinary tract involve ureters, bladder and urethra.[6] Upper UTIs are infections of the kidneys and Symptoms of upper UTI include: pain and tenderness in the upper back and sides, chills, fever, nausea, vomiting. Lower UTIs are infections of the urethra and bladder and Their symptoms are :burning with urination, increased frequency of urination, bloody urine, cloudy urine, urine that looks like cola or tea, strong odor to urine, pelvic pain (women), rectal pain (men). If bacteria move from the infected kidney into the blood. This condition is called sepsis. Sepsis can cause low blood pressures, shock, and death.[7]

1.4 PATHOGENESIS OF URINAY TRACT INFECTION

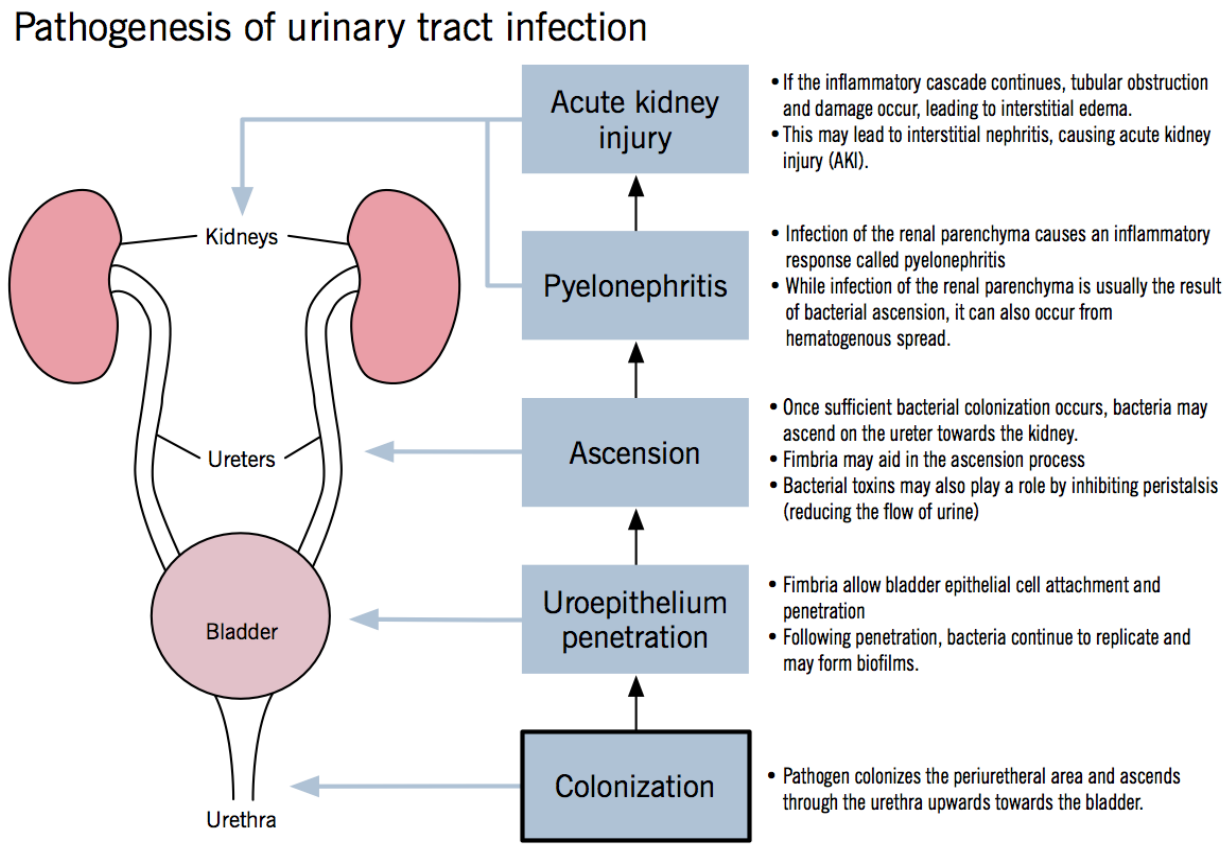
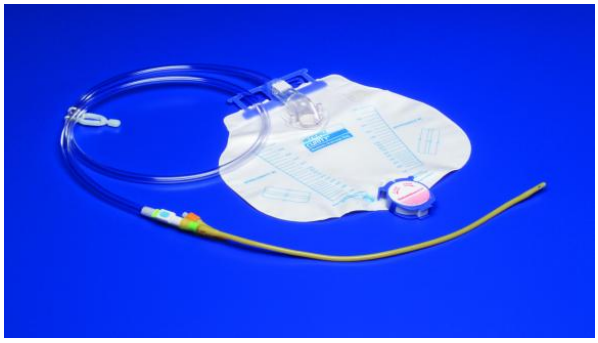


Fig.1: Pathogenesis of Urinary Tract Infection

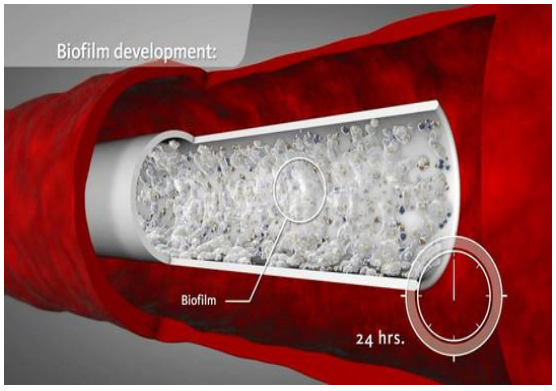
1.5 CATHETER

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, or dementia. 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.[8] if proper care is not taken during indwelling urinary catheterization this may lead for main cause of urinary tract infections.[9]

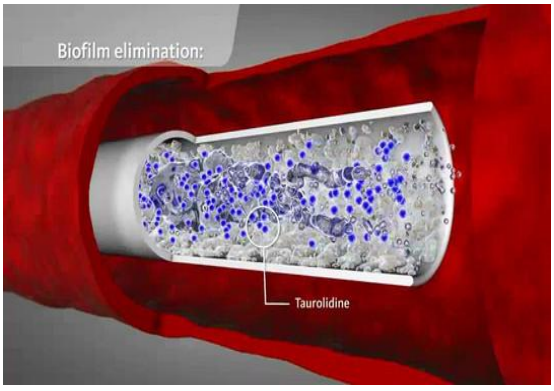


1.6 CATHETER ASSOCIATED URINARY TRACT INFECTION (CAUTIs) AND BIOFILM

According to the CDC, CAUTIs are defined as an UTI in a patient who had an indwelling urinary catheter in place at the time of or within 48 hours prior to infection onset[1]. After a month of catheterization, nearly all patients are bacteriuric. [3] the development of biofilm related to the use of indwelling urinary catheters. Biofilm development has important implications to catheter associated UTI.[10] Urinary catheters are tubular latex or silicone devices, which when inserted, may readily acquire Biofilms on the inner or outer surfaces.[11] Definition of biofilm includes 3 components: (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 bacterial products.[12]



Biofilm formation



Infected catheter

The bacteria which contaminate these devices and develop biofilms are *Staphylococcus epidermidis, Enterococcus faecalis, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae* and other gram-negative organisms.[11]

1.7 PATHOGENISIS OF BIOFILM

In the human body bacteria reside as biofilms on indwelling medical devices. To form a biofilm,Bacteria are attracted to the surface by environmental signals. On reaching the surface, thebacteria attach to it as single cells or as clusters. When single cells attach to a surface they form a monolayer biofilm. A monolayer biofilm can be defined as one in which the bacteria attach only to the surface. When bacteria attach to a surface as a cluster, they form a multilayerBiofilm. Multilayer biofilms can be defined as a microbial community, where the bacteria areattached both to the surface and the neighboring bacterial cells. The type of biofilm formed depends on the environmental conditions and surfaces that favor their development.[1]

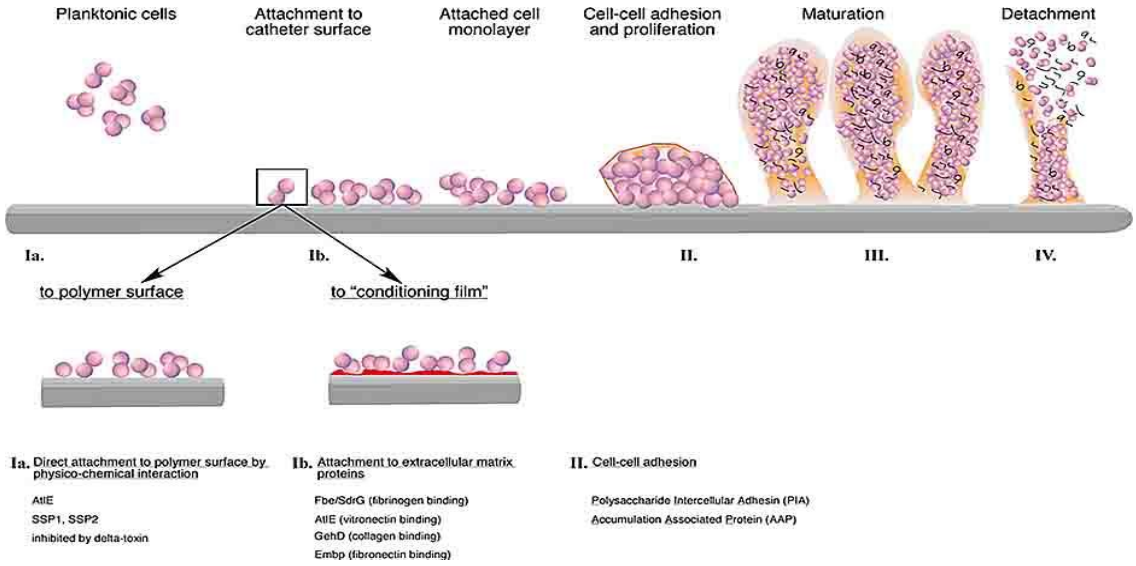


Fig 2: Pathogenesis of Biofilm

1.8 *KLEBSIELLA*

The genus *Klebsiella* is non-motile, non-sporing rod-shaped, gram-negative bacteria with a prominent polysaccharide capsule. This capsule provides resistance against many host defense mechanisms. Members of the *Klebsiella* genus typically express 2 types of antigens on their cell surface. The first is a lipopolysaccharide (O antigen), and capsular

polysaccharide (K antigen). The structural variability of these antigens forms the basis for classification into various serotypes. Three species in the genus *Klebsiella* are causing illness in human *Klebsiella pneumonia, Klebsiella oxytoca,* and *Klebsiella granulomatis.*[13]

K. pneumoniae are gram-negative, short and straight rods. They are non-motile and non-sporing. They are arranged singly or in pairs. Freshly isolated strains show a well-defined polysaccharide capsule. They are lactose- fermenting, urease-positive, and indole-negative organisms but some strain of these organisms are different they do not produce hydrogen sulphide, and they are both VP and MR tests positive. They produce large, dome-shaped, mucoid and lactose-fermenting red colonies on MacConkey agar. *K. rhinoscleromatis causing* Rhinoscleroma. It is a chronic inflammatory disease involving the nasopharynx. This is worldwide distributed organisum mostly in Europe, Central America, and India. Patient present with a purulent nasal discharge with formation of crusts and nodules that may lead to respiratory obstruction. *K. oxytoca* rarely isolated from clinical specimens. it is being increasingly isolated from patients with neonatal septicemia. The bacteria have also been associated with neonatal bacteremia, especially among premature infants and in neonatal intensive care units.[14]

1.9 NOSOCOMIAL INFECTION

The term nosocomial also called hospital acquired infection, hospital associated infection. Nosocomial meaning hospital and also defined as infection developing in patients after admission to the hospital, which was neither present nor in the incubation period at time of hospitalization. Such infections may become evident during their stay in hospital or sometime after their discharge. Hospital acquired infection is a great problem today. Many exogenous sources like another patient, hospital workers, and hospital environment, or some endogenous sources like patient own flora is responsible for nosocomial infection.urinary tract infection is most common nosocomial infection; 80% of infections are associated with the use of an indwelling bladder catheter. *E.coli, Klebsiella, Enterobacter, Proteus* and *serratia* are most important hospital pathogens. These are because of resistant factor conferring multiple drug resistance. [3, 15]

1.10 MULTI DRUG RESISTANT (MULTIPLE ANTIBIOTIC RESISTANT)

Multidrug resistance in bacteria occurs by the accumulation, on resistance plasmids or transposons, of genes, with each coding for resistance to a specific agent, and the action of multidrug efflux pumps, each of which can pump out more than one drug type. The ability of bacteria and other microorganisms to resist the effects of an antibiotic to which they were once sensitive are called antibiotic resistance. Antibiotic resistance is a major concern of overuse of antibiotics it also known as drug resistance.[16]Antibiotic resistance among nosocomial pathogens is a cause of major concern. Three aspects of this problem have been particularly challenging: the frequent emergence of resistance to the newest antibiotics, the presence of antibiotic resistance genes on bacterial plasmids, which may be transferred among different bacterial species; and the spread of resistant bacteria among patients not only in the hospital but also in the community.[9]

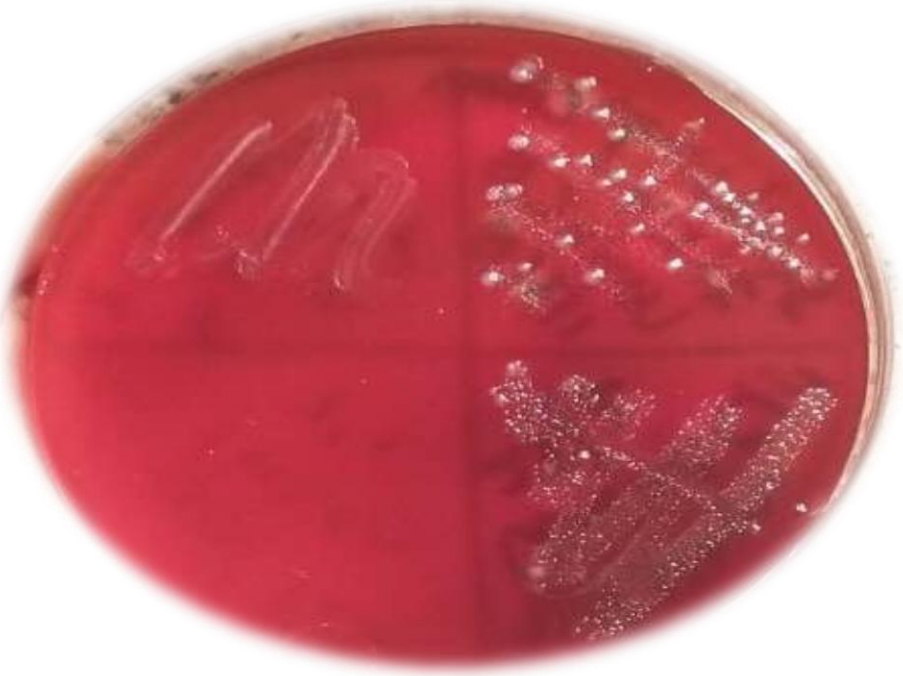


Fig 3: Isolation of *klebsiella spp* in MacConkey Agar

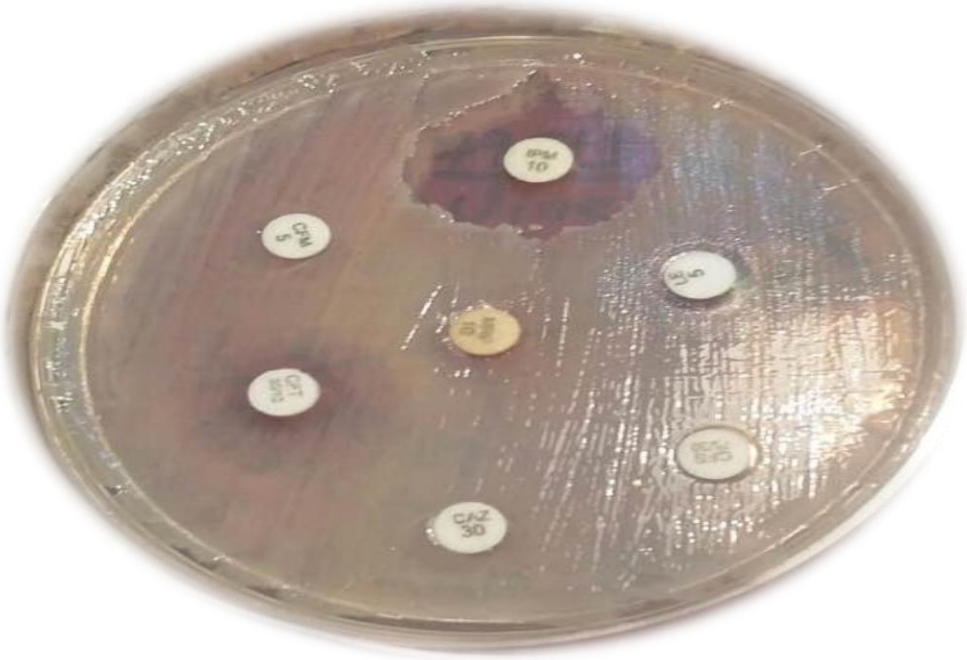


Fig no 4: Antibiotic sensitivity of klebsiella on MacConkey medium

CHAPTER- II
AIM OF STUDY

AIM OF THE STUDY

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

CHAPTER- III
REVIEW OF LITERATURE

REVIEW OF LITRATURE

Amer Custovic et al in 2014 stated that the problem of nosocomial infections is as old as the hospitalization. Yet it is an ongoing problem of modern medicine, and represents a permanent danger even to patients hospitalized in the most modern and well equipped hospitals. Medical, legal and economic consequences of nosocomial infections have begun to emphasize the importance of their control. The causative agents of nosocomial infections can be almost all organisms, but most often they involve bacteria. The types of bacteria that cause hospital infections can change over time depending on the use of antibiotics and introduction of new diagnostic and therapeutic procedures.[18]

In a study carried out by Stoodley L H et al in 2009 found out *Escherichia coli* in recurrent urinary tract infections 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 that resist host immune responses and antibiotic treatment. [19]

Filozov. A Visintainer et al in 2009, stated that *Klebsiella pneumoniae* is an important cause of nosocomial infection because of multiple antibiotic resistance. *K pneumoniae* easily acquire extended-spectrum b-lactamases (ESBLs). Tertiary care medical center experienced an outbreak of multiple antibiotic-resistant Klebsiella pneumoniae (MR-KP) that included extended-spectrum b-lactamase (ESBL)-producing and non-ESBL-producing strains. patients with MR-KP had a longer length of stay and greater antibiotic exposure. Patients with longer length of stay and greater total antibiotic exposure should be potential targets for stringent infection.[20]

Matthew P. Kronmanet al in 2014, stated that Multidrug-resistant (MDR) Enterobacteriaceae infections are associated with increased morbidity. A 20-year-old hematopoietic cell transplantation recipient with recurrent MDR *Klebsiella* pneumonia infection, prolonged intestinal colonization, and subsequent intestinal decontamination. Evaluate on the basis of stool surveillance, molecular typing, and fecal microbiota transplantation for patients with intestinal MDR Enterobacteriaceae carriage.[21]

In a study carried out by Rath.S et al in 2014 found out the Gram-negative pathogenic bacteria *Klebsiella oxytoca* and *Klebsiella pneumoniae* produce the extended spectrum-lactamase (ESBL) and cephalosporinase enzymes and are the major causes of hospital acquired (HA) infections and epidemics in non-hygienic communities in the majority of developing countries. The *K. oxytoca* strains were resistant to cefepime, gatifloxacin, ciprofloxacin, ceftazidime, levofloxacin and imipenem, whereas the *K. pneumoniae* strains were highly resistant to ampicillin, norfloxacin, ciprofloxacin, gatifloxacin, ofloxacin, amoxyclav, ceftazidime, cefepime, cefixime, piperacillin and imipenem. The ESBL-producing and fluoroquinolone-resistant *K. pneumoniae* strains were more prevalent than the *K. oxytoca* strains in the HA/CA samples. The minimum inhibitory concentration values of the third-generation cephalosporins: cefotaxime and ceftazidime and fluoroquinolones: ciprofloxacin and levofloxacin against both species of *Klebsiella* confirmed to resistance. Patients with other bacterial infections had a relatively higher probability of infection with ESBL-producing and fluoroquinolone-resistant *Klebsiella* strains.[22]

Sibhghatulla Shaikh et al in 2015, stated that the prevalence of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* at two North Indian hospitals and also focus to determine the risk factors associated with the acquisition of these organisms. ESBL producing isolates showed multiple drugs resistant. Length of hospital stay (more than 3 days) and previous exposure to antibiotics were found as significant risk factors associated with the acquisition of ESBL-producing *E. coli* and *K. pneumoniae* isolates.[23]

In a study carried out by Barbara W.Trautnera et al in 2005 found out the 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% develops 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. [10, 24]

Nivedita. S et al in 2012 stated that Bacteriuria is commonly present in 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.[25]

Bouamri M.C. et al in 2015 stated that the bacterial resistance rates of *K. pneumoniae* isolates not producing extended-spectrum-lactamase (ESBL) were: trimethoprim–sulfamethoxazole (61%), amoxicillin (51%), ciprofloxacin (32%), gentamicin (21%) and amikacin (11%). ESBL-producing *K. pneumonia* strains accounted for 25.5% of all the urinary *K. pneumoniae* isolates and showed resistance to (89%),gentamicin (89%), ciprofloxacin (84%) and amikacin (50%). It also carbapenem-resistant strains 7% of all the urinary ESBL-producing *K.pneumoniae* isolates.[26]

In a study carried out by Jan E.Patterson et al in 2000 found out that Antibiotic utilization measures and control of multi drug resistant (MDR) *Klebsiella pneumoniae* after emergence in two hospitals. The Antipseudomonal beta-lactam antibiotic before and after the interventions at both hospitals. For the strain identity Pulsed-field gel electrophoresis of whole cell DNA was used. Antibiotic-utilization interventions at both institutions included physician education regarding the association of ceftazidime use and MDR *K pneumoniae.* Continued decrease in piperacillin-tazobactam resistance despite increased use at both hospitals. Antibiotic-use measures may be particularly important for control of MDR *K pneumoniae,* whether emergence is clonal or polyclonal.[27]

Sheikh Ajaz Rasool et al in 2003 stated that *Klebsiella* were isolated and identified on the basis of morphology, growth, and biochemical characteristics. 52% were identified as K. oxytoca, 42% as K. pneumoniae and 6% as K. ozaenae. All the isolates offered different resistance patterns against antibiotics including ampicillin, streptomycin, gentamicin, ofloxacin, tetracycline and chloramphenicol. Some of the representative isolates lost the antibiotic resistance after acridine orange mediated curing. Two methods were tried for in vivo gene transfer studies for determining the conjugative/ transferable nature of the drug resistance plasmid markers. In experiments, chloramphenicol and ampicillin resistance markers were transferred to the recipient *E. coli* MD40 cells. The rest of the plasmid

borne markers was non-conjugative/nontransferable. Conjugative plasmids carry a tremendous potential to disseminate resistance markers to distant recipient cells.[28]

Paul A.Tambyah in 2000 staed that the Catheter related urinary tract infection (CAUTI) is most common nosocomial infection, more than 1 million cases are accounting every year in United State hospital and nursing home .this study focus on catheter associated urinary tract infection in rarely symptomatic. Commonly symptom is fever and peripheral leukocytosis associated with community acquired urinary tract infection. 90% infected patients were asymptomatic, only 52% were detected by patient physicians by using hospital laboratory. During analysis there were no significant difference between patient with and without CAUTI in signs and symptoms commonly associated with urinary tract infection. Fever, dysuria, urgency or flack pain or in leukocytosis. CAUTI are major reservoir of antibiotic resistant organisum in the hospital. They are rarely symptomatic and infrequently cause blood stream infection. Symptoms of urinary tract, fever, or peripheral leukocytosis have small value for the diagnosis of CAUTI.[29]

Chaydhary B.L. et al in 2014 staed that to determine the Multidrug Resistance (MDR), Extended Spectrum –lactamases (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae*. ESBL production test was done by Double Disk Synergy Test and Confirmatory Method: National Committee for Clinical Laboratory Standard (NCCLS) Phenotypic confirmatory combination disc diffusion test. A total of 122 isolates *Klebsiella pneumoniae* 77 (63.11%) and *Escherichia coli* 45(36.88%) in which MDR 100(81.96%). *Klebsiella* spp 45.45% and *Escherichia coli* 50% was ESBL producer. This indicate the spread of Multidrug Resistance ESBL-producers nosocomial isolates.[30]

In a study carried out by Gales C. et al in 2002 found out the Urinary tract infections (UTI) are one of the most common infectious diseases diagnosed in outpatients as well as in hospitalized patients. Antimicrobial susceptibility testing was performed and results interpreted using reference broth micro dilution methods. *Esherichia coli* was the most frequent pathogen isolated followed by *Klebsiella* spp., *Pseudomonas aeruginosa*, and *Proteus mirabilis.* Among the *E. coli* isolates, piperacillin/tazobactam, aztreonam, extended-spectrum cephalosporins, carbapenems and amikacin constitute reasonable therapeutic options for treatment of serious UTI. High resistance rates to fluoroquinolones and trimethoprim/sulfamethoxazole were observed among the *E. coli.*

nitrofurantoin displayed susceptibility rate of 87.0%. Against *Klebsiella* spp. infections, the only effective therapeutic option would be the carbapenems due to the high number of isolates producing extended-spectrum -lactamases (ESBL). Even the new fluoroquinolones showed limited activity against *Klebsiella* spp. the uropathogens isolated high resistance to various classes of antimicrobial agents. Carbapenem-resistant *P. aeruginosa,* ciprofloxacin-resistant *E. coli,* ESBL-producing *K. pneumoniae* .[31]

David T.Bjork et al in 1984 staed that the Bacteria isolated from nursing home (NH) patients often displayed greater resistance to specific antibiotics than those isolated from hospitalized patients. urinary tract infections were associated with bacteremia. bacteremia were with highly-resistant organisms and also fatal. Use of urinary catheters and inappropriate use of antibiotics in NH patients results in urinary tract colonization and infection with resistant bacteria and is an important cause of morbidity and mortality.[32]

Richard platt et al in 2000 staed that the main factor for urinary tract infection duration of catheterization, lack of systemic antibiotic during short catheter courses, lack of urinemeter drainage, female sex, diabetes mellitus, microbial colonization of the drainage bag, serum creatinine >2 mg/dl at the time of catheterization, the reason for catheterization, and the use of catheters with sealed collection junctions when no antibiotic was administered. The persons with diabetes are more susceptible to urinary tract infection than are persons without diabetes. [33]

Marc Leone et al in 2008 stated that The frequency of catheter-associated bacteriuria was 9.6%. Five independent risk factors were determined: female sex, length of ICU stay, use of an antimicrobial therapy, severity score at admission, and duration of catheterization. This study states that the drainage system did not influence the occurrence of bacteriuria. To decrease the rate of catheter-associated bacteriuria in polyvalent ICU patients, removal of the bladder catheter must be performed as soon as possible.[34]

In a study carried out by Peter Tenke et al in 2008 found out the urinary tract is the commonest source of nosocomial infection, particularly when the bladder is catheterized. Most catheter-associated UTIs are arrived from the patient's own normal flora and the catheter predisposes. The most important risk factor for the development of catheter-associated bacteriuria is the duration of catheterization. Mostly short-term catheter-associated bacteriuria is asymptomatic and is caused by a single organism. Further

organisms tend to be acquired by patients catheterized for more than 30 days. The clinician should be aware of two priorities: the catheter system should remain closed and the duration of catheterization should be minimal. Antibiotic treatment is recommended only for symptomatic infection Avoiding the closed drainage bag, non return(flip) valve catheter use . Clinicians should always consider alternatives to indwelling urethral catheters that are less causing symptomatic infection. suprapubic catheters, condom drainage systems and intermittent catheterization are preferable to indwelling urethral catheterization.[35]

Ines Linhares et al in 2013 stated that *E. coli* was usual and the most common pathogen causing in UTI. The bacteria causes UTI varied with the sex of the patient. *E. coli* was responsible for more than an half of UTI; its resistance to antibiotics was low when compared with other pathogens implicated in UTI, also having the lowest percentage of multidrug resistant (MDR) isolates. Bacteria isolated from females were less resistant than those isolated from males and this difference increased with the patient age. the first line drugs (pivmecillinam and nitrofurantoin) and the alternative antibiotic amoxicillin-clavulanic acid (AMXCLA) are appropriate to treat community-acquired UTI, but the fluoroquinolones should not be suitable to treat male infections and the trimethoprim-sulfamethoxazole (SXT) shall not be used in the treatment of UTI.[36]

Sanjay Saint et al in 2009 stated that Catheter associated urinary tract infection, a common and potentially preventable complication of hospitalization. The hospital-acquired complications chosen by the Centers for Medicare & Medicaid Services (CMS). CMS examine the preventability of catheter-associated infection, and provide guidance for hospital-based administrators and clinicians. [37]

Richard A.Garibaldi et al in 1982 stated that the efficacy of bacteriologic monitoring for preventing symptomatic urinary tract infections (UTI) in hospitalized patients with temporary indwelling urethral catheters. 67%patients remained asymptomatic throughout their period of hospitalization. Remaining patients who developed symptomatic infections, only ten were potentially preventable. The routine daily bacteriologic monitoring of urine from all catheterized patients is not an efficient way to decrease the incidence of symptomatic, catheter-associated UTI.[38]

Hilmar Wisplinghoff et al in 2004 stated that intestinal decontamination can help to control an outbreak of intestinal colonization and infection with multi resistant gram-negative bacilli in the intensive care unit, but should not be recommended for routine prevention of endemic nosocomial infections. One of the largest multicenter studies performed, and found the nosocomial Blood stream infection (BSIs) due to antibiotic-resistant organisms in US hospitals. [37, 39]

A.M.Yinnon et al stated that after *E. coli, Klebsiella* was the leading cause of Gram-negative bacteraemia. There were 210 cases with *K. pneumoniae* and 31 with *K. oxytoca.* Community acquired *Klebsiella* bacteraemia (CAKB)58% and hospital acquired *Klebsiella* bacteraemia (HAKB)28%. In HAKB, as compared to CAKB, serious manifestations of illness were more common. Multiple drug resistance was very common: only 57% of all *Klebsiella* strains were susceptible to gentamicin, 66% to ceftriaxone, 70% to ciprofloxacin, and 83% to amikacin. The susceptibility rates of *Klebsiella* spp isolated from patients with HAKB were significantly lower. Sepsis due to multiple-drug-resistant *Klebsiella* has become frequent, carrying significant morbidity and mortality.[40]

William.R Jarvis et al in 1985 stated that *Klebsiella pneumoniae* causes serious epidemic and endemic nosocomial infections. 80% of the outbreaks involved infections of the bloodstream or urinary tract. Person-to-person spread was the most common mode of transmission, and 50% of the outbreaks occurred in neonatal intensive care units. The Centers for Disease Control (CDC) by hospitals participating in the National Nosocomial Infections Study (NNIS) to describe the epidemiology of endemic *K. pneumonia* infections. 184 deaths were caused by nosocomial *K. pneumonia* with higher ratios in pediatrics 5% where there was a 12% mortality in children infected with an aminoglycoside-resistant strain.[41]

In a study carried out by Niveditha S. et al in 2012 found out 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. bacteriuria 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.[42]

Jean Louis Vincent in 2003 stated that nosocomial infections affect about 30% of patients in intensive-care units and are associated with substantial morbidity and mortality. Several risk factors have been identified, including the use of catheters and other invasive equipment, and certain groups of patients—e.g. those with trauma or burns are recognized as being more susceptible to nosocomial infection than others. Awareness of these factors and simple preventive measures, such as adequate hand hygiene, can limit the occurrence of disease. Management of nosocomial infection relies on adequate and appropriate antibiotic therapy.[43]

Ronald N.Jones in 2001 stated that the important causes of Gram-negative resistance include extended-spectrum b-lactamases (ESBLs) in *Klebsiella pneumoniae, Escherichia coli*, and *Proteus mirabilis*, high-level third-generation cephalosporin (Amp C) b-lactamase resistance among Enterobacter species and *Citrobacter freundii*, and multidrug resistance genes observed in *Pseudomonas aeruginosa*, Acinetobacter, and *Stenotrophomonas maltophilia*. Antimicrobials used for the treatment of nosocomial infections should be effective against any likely resistant pathogens and should not further promote the development of resistance. The fluoroquinolones in treatment of nosocomial infections is also being limited by new resistance patterns and increasing resistance levels. Antimicrobials with good activity against many resistant pathogens include the carbapenems, piperacillin/tazobactam, and cefepime. Appropriate antimicrobial selection, surveillance systems, and effective infection-control procedures are key partners in limiting antimicrobial-resistant pathogen occurrence and spread.[44]

Scott K.Fridin et al in 1997 stated that nosocomial infections in ICU patients account for over 20% of all infections acquired in hospitals. The most common ICU-acquired infections include pneumonia, urinary tract infection, and bloodstream infection. [45]

In a study carried out by Emily P.et al in 2005 found out the increased resistance on carbapenem. The infection were associated with extended-spectrum b-lactamase–producing *Escherichia coli* and *Klebsiella* species (ESBL-EK) have been increasing. ESBL-EK infections are of clinical concern, because few antimicrobials are available as therapeutic options. Increased reliance on carbapenems has led to increasing carbapenem

resistance. Efforts to maintain current therapeutic options for ESBL-EK infections are essential. The emergence of MDR among ESBL-EK has important implications for the future ability to treat these infections. [46]

M.S.Ramirez et al in 2012 stated that *Klebsiella pneumoniae* clinical isolates, including an NDM-1 producer, and in silico-generated restriction maps of sequenced genomes revealed a highly heterogeneous region which is called 'high heterogeneity zone' (HHZ). The HHZ consists of several regions, including a 'hot spot' prone to insertions and other rearrangements. The HHZ is a characteristic genomic area that can be used in the identification of causing strains.[47]

Mohammed Akram et al in 2007 stated that Urinary tract infections (UTIs) remain the common infections diagnosed in outpatients as well as hospitalized patients. Knowledge on antimicrobial susceptibility pattern is essential for appropriate therapy. Extended-Spectrum beta-Lactamase (ESBL) producing bacteria may not be detected by routine disk diffusion susceptibility test, leading to inappropriate use of antibiotics and treatment failure. *E. coli* was the predominant bacterial pathogen of community acquired UTIs in Aligarh, India. It also an increasing resistance to Co-trimoxazole and production of extended spectrum β-lactamase among UTI pathogens in the community.[48]

John E.McGowanin 1983 stated that Organisms causing nosocomial infection are frequently resistant to antimicrobial agents. Major influences of resistant hospital bacteria include antimicrobial effects in treated individuals, mechanisms for transfer of resistance between bacteria, and routes of transmission with in the hospital for bacteria or their resistance factors. Barrier isolation techniques can help control resistant hospital bacteria. The careful, use of antimicrobial agents remains the key stone for minimizing the nosocomial infection. [49]

Noyal Mariya Joseph et al in 2015 stated that *Klebsiella pneumoniae*, has a significant association between the resistance rates and consumption of gentamycin, ceftazidime and meropenem. A linear relationship was noted between antimicrobial consumption and resistant isolates of *Escherichia coli* and *Klebsiella pneumonia*.[50]

In a study carried out by Matthew E Falagas et al in 2005 found out the increasing problem of infections due to multidrug-resistant Gram-negative bacteria has led to reuse of polymyxins in several countries. Gram-negative bacteria that is resistant to all

available antibiotics, including polymyxins. Colistin, in combination with beta lactam antibiotics, may be a useful agent for the management of pandrug-resistant Gram-negative bacterial infections. Tigecycline is active against almost all of the ESBL or MDR *E. coli* isolates and the great majority of ESBL or MDR *Klebsiella spp.* isolates. [51, 52]

Lindsay E Nicolle in 2014 stated that urinary tract infection related to the use of an indwelling urinary catheter is one of the most common infections acquired by patients in health care facilities. As biofilm ultimately develops on all of these devices, which development of bacteriuria. Catheter-acquired urinary infection is the source for about 20% of health-care acquired bacteremia in acute care facilities, and 50% in long term care facilities. To prevent bacteriuria and infection are to limit indwelling catheter use and, when catheter use is necessary, to discontinue the catheter as soon as clinically feasible.[53]

Barbara W.Traunter et al in 2004 stated that a biofilm on an indwelling urinary catheter consists of adherent microorganisms, their extracellular products, and host components deposited on the catheter. The biofilm on urinary catheters results in persistent infections that are resistant to antimicrobial therapy. Because chronic catheterization leads bacteriuria. When symptoms of a urinary tract infection develop in a person who is catheterized, changing the catheter before collecting urine improves the accuracy of urine culture results. Changing the catheter may also improve the response to antibiotic therapy by removing the biofilm that contains the infecting organisms.[54]

Hiroshi N in 2010 stated that large amounts of antibiotics used for human therapy resulted in the selection of pathogenic bacteria resistant to multiple drugs. Multidrug resistance in bacteria may be generated by one of two mechanisms. First, these bacteria may accumulate multiple genes, each coding for resistance to a single drug, within a single cell. This accumulation occurs typically on resistance plasmids. Second, multidrug resistance may also occur by the increased expression of genes that code for multidrug efflux pumps, extruding a wide range of drugs.[16]

In a study carried out by Podschun R et al in 1998 found out the *Klebsiella* spp. is ubiquitous in nature. Klebsiellae mostly have two common habitats, one is

environmental, which is found in surface of water, sewage, and soil and on plants and the other on mucosal surfaces of mammals such as humans, horses, or swine, which they colonize. In this respect, the genus *Klebsiella* is like *Enterobacter* and *Citrobacter* but unlike *Shigella* spp. or *E. coli*, which are common in humans but not in the environment. In humans, *K. pneumoniae* is present as a saprophyte in the nasopharynx and in the intestinal tract.[11]

William L S et al stated that the staff help in the transmission of *Klebsiella* in the hospital. preliminary survey the 17 % of hand washings from 28 staff taken on different days yielded *Klebsiella* spp. the *klebsiella* serotypes were identical with those colonizing or infecting patients in the ward on the day of sampling. In 47 observations *klebsiellae* were found to have been transmitted to nurses hands after varying nursing procedures. Other patients were found to have *klebsiellae* in the nasopharynx and on the skin (often hands and groin), as well as in their faeces. The serotypes (K47 and K10) colonising patient hands were transferred to the nurse's hands, but type 21, found in the patient's stool, was not. In all patients, except some patient, *klebsiellae* from the patient's hands could be transferred to the nurse undertaking simple, "clean" procedures.[2]

Adult ICU patients who received intravenous fosfomycin were prospectively examined to assess its safety and effectiveness and assess the antimicrobial therapy of life-threatening infections caused by carbapenem resistant *Klebsiella pneumoniae*. Fosfomycin was administered intravenously in patients for treatment of hospital-acquired infections caused by carbapenem-resistant *K. pneumoniae*. Fosfomycin was administered in combination with other antibiotics. All patients had good bacteriological and clinical outcome of infection. No patient experienced adverse events related to the administration of fosfomycin. Intravenous fosfomycin may be a beneficial and safe adjunctive treatment in the management of life-threatening ICU-acquired infections caused by carbapenem-resistant *K. pneumoniae.*[17]

In a study carrird out by Podschun r et al in 1998 found out *Klebsiella* accounts for 3 to 7% of all nosocomial bacterial infections, placing them among the eight most important infectious pathogens in hospitals. The urinary tract is the most common site of infection.

Klebsiella accounts for 6 to 17% of all nosocomial urinary tract infections (UTI) patients at risk, e.g., patients with neuropathic bladders or with diabetes mellitus. *Klebsiella* is second to *Escherichia coli*. Even hospital personnel have elevated rates of *Klebsiella* carriage. Reported carrier rates in hospitalized patients are 77% in the stool, 19% in the pharynx, and 42% on the hands of patients. The high rate of nosocomial *Klebsiella* colonization appears to be associated with the use of antibiotics. Widespread use of antimicrobial therapy has often been held responsible for the occurrence of multiply resistant *Klebsiella* strains in hospitals .[11]

Mary Anne Roshni Amalaradjou et al in 2013 stated that 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. The complications caused by biofilms can depend the patient's quality of life and threaten their health.[1, 12]

Mansour A et al in 2009 stated that 1 in 3 women will require antimicrobial treatment for a Urinary Tract Infection (UTI) before age 24. *Escherichia coli* are the most common cause of UTIs. Clean-Catch midstream urine of the patients was collected. Urine specimens were cultured for isolation of the microbial agents of UTI. The isolated bacteria were identified using biochemical tests. Disk diffusion susceptibility test was used to determine susceptibility of bacterial agents to antibiotics. The most isolated bacterium was *E. coli* with frequency rate of 59%. The other bacteria were Klebsiella spp. (11.6%), Enterobacter spp. (9.8%), Pseudomonas spp. (7.2 %), Proteus spp. (2.9%), Acinetobacter spp. (2.7%), Congolese positive Staphylococci (2.2%), Coagolase negative Staphylococci (2.3%), Citrobacter spp. (1.3%) and Streptococci α hemolytic (1.1%). All Gram-negative bacteria were more sensitive to amikacin .the Gram-negative bacilli were responsible for UTI infections in most of the patients. The most common isolated bacteria from urinary tract infections were *E. coli* and the most effective antimicrobial agents were amikacin, tobramicin and ciprofleoxacin against Gram-negative bacilli.[4]

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:

Urinary Catheter tip is collecting from the catheterized patient.

Transport of specimen:

- Once collected specimen must be transported to the laboratory without delay.
- Sterile disposable container is 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 lose the primary stain crystal violet when subjected to treatment with a decolorizing agent such as alcohol.

Reagents:

The gram stain has four different reagents:

1. Primary stain (Crystal violet): colors 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 cover slip and apply Vaseline to the four corners.
- Place a drop of liquid culture into the centre of cover slip using a sterile wire loop.
- Invert the clean depression (concavity) slide over the cover slip with its concavity facing one drop.
- Immediately turn slide so that the cover slip is uppermost in position and drop suspending from cover slip.
- 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 like *klebsiella* spp. 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. HICROME AGAR

This is the selective medium for *klebsiella spp.*

Ingredients:

Bile salt : 1.5 g/l

Cromogenic : 0.2 g/l

Peptone : 12.0 g/l

Sodium chloride : 5.0 g/l

Agar : 15.0g/l

Preparation:

Suspend 15g in l L distilled water, bile salt 1.5g/l, chromogenic0.2g/l, peptone12g/l, sodium chloride 5.0g/l. 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.

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).

B. OXIDATIVE- FERMENTATIVE (OF) 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.

B. 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**, **macro broth 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.

A.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.

A. 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 MDRK was established as isolates intermediate or resistant to at least 3 drugs in the following classes: β-lactams, carbapenems, aminoglycosides and fluoroquinolones.[60]

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^{0}C 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 and *klebsiella pneumonia* ATCC 700603 depending on the bacterium to be tested.

MATERIALS AND METHODOLOGY

One hundred (100) samples were collected from the catheterized patients consulting the Microbiology department of 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 patient.

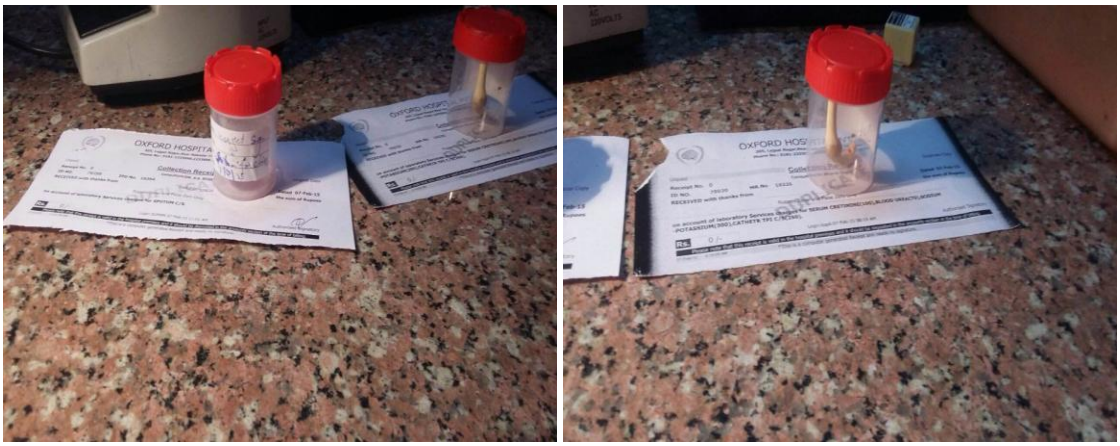


Fig.3: Collection of Sample

Culture:

- The samples were cultured on Nutrient agar, MacConkey agar
- 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, round, mucoid colonies.

MacConkey agar : - large, opaque, round, mucoid 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

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

C.HANGING DROP PREPARATION

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

Observation: - shows non-motile bacilli.

D. BIOCHEMICAL REACTIONS:

- Catalase Test : - Positive
- Oxidase Test : - Negative
- Oxidative Fermentative Test : - Oxidative
- Nitrate Test :- Reduced to nitrite
- Triple Sugar Iron Test(TSI) :- negative
- Sugar Fermentation Test:
    - ❖ Glucose: - Fermented with acid.
    - ❖ Lactose: - Fermented with acid and gas.
    - ❖ Mannitol: - Fermented with acid.
- Indole Test :- Negative
- Methyl Red Test :- Negative
- Voges Proskauer Test :- positive
- 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 *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.

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.

4. **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

5. 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.
6. The plates were inverted and incubated at 16 to 18 hrs at 35^0C.
7. The diameter of the inhibitory zone was measured using a ruler.
8. 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 millimeters:

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

Quality control

It is also necessary to check the potency of the discs periodically using as a control a standard bacterium of known sensitivity. In this study *klebsiella pneumoniae* ATCC 700603 was used to check for the quality control. The precision and accuracy of the test was counter checked by the parallel use of control strain with known susceptibility to the microbial agent. This quality control was performed using the same procedures as for the test organisms. Quality control was done for all the *klebsiella spp* isolated.

CHAPTER- V
RESULTS & ANALYSIS

RESULTS

100 clinical samples from the catheterized patient were processed out of which *klebsiella spp.* was isolated from 22 samples. These isolated *klebsiella* formed large, opaque, round, mucoid colonies on Nutrient Agar whereas large, opaque, round with non-lactose fermenting colonies on MacConkey agar.

Table 1: Shows the prevalence of *klebsiella spp* in catheterized patients which indicates that 22% of the cases were positive i.e. out of 100 samples *klebsiella* was isolated only from 22 samples.

Table 2: Shows the resistance pattern of positive samples with Norfloxacin being 100% resistant, Amikacin 27.2%, Cefoperazone,Imipenem,Ciproflaxacin and Meropenem being 18.1% resistant and the least resistant was shown by Gentamicin, Tobramycin-Piperacillin 9.0%.

Table 3: Shows the multidrug resistance pattern of *Klebsiella spp* from the positive samples i.e. all the 22 isolates were resistant to more than at least 3 drugs in the following classes: β-lactams (Cefotaxime, Imipenem, Meropenem, Cefoperazone Piperacillin-Tazobactum, Cefepime, Ceftazidime), carbapenems, aminoglycosides (Amikacin, Gentamicin Tobramycin), and fluoroquinolones (ciprofloxacin, Norfloxacin) [58] so all the isolates were Multidrug Resistance *Klebsiella spp*.

Table 4: Shows the incidence of Multi drug resistance of the positive samples. Out of 100 samples 22 samples were isolated as *Klebsiella spp.* and 100% were Multi Drug Resistant (i.e. MDRK).

ANALYZED DATA

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

Table 1: Prevalence of *Klebsiella spp.* In samples studied

Klebsiella spp	**Number of samples**	**%**
Positive	22	22.0
Negative	78	78.0
Total	100	100.0

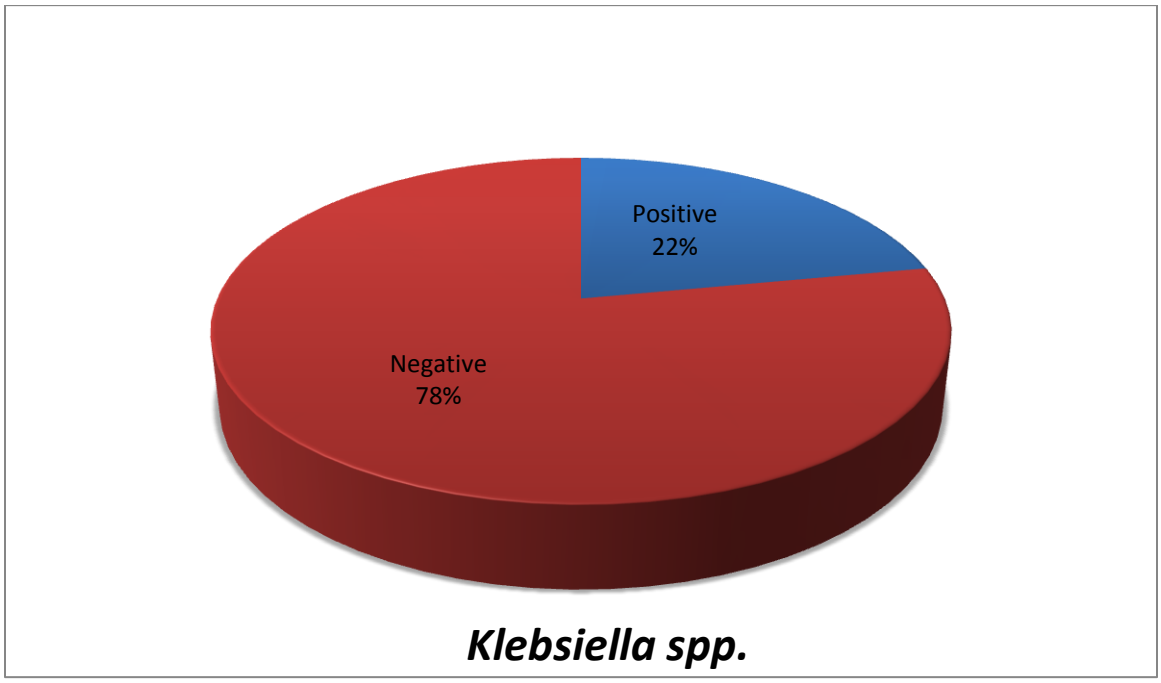


Table 2: Resistance pattern of positive samples studied

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

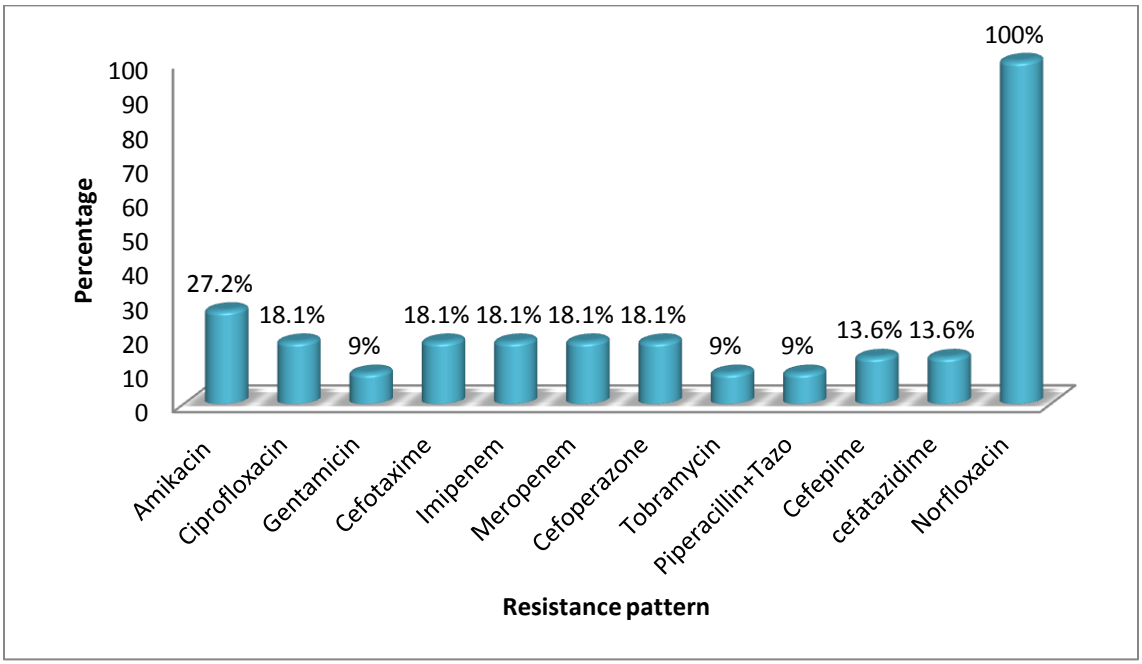
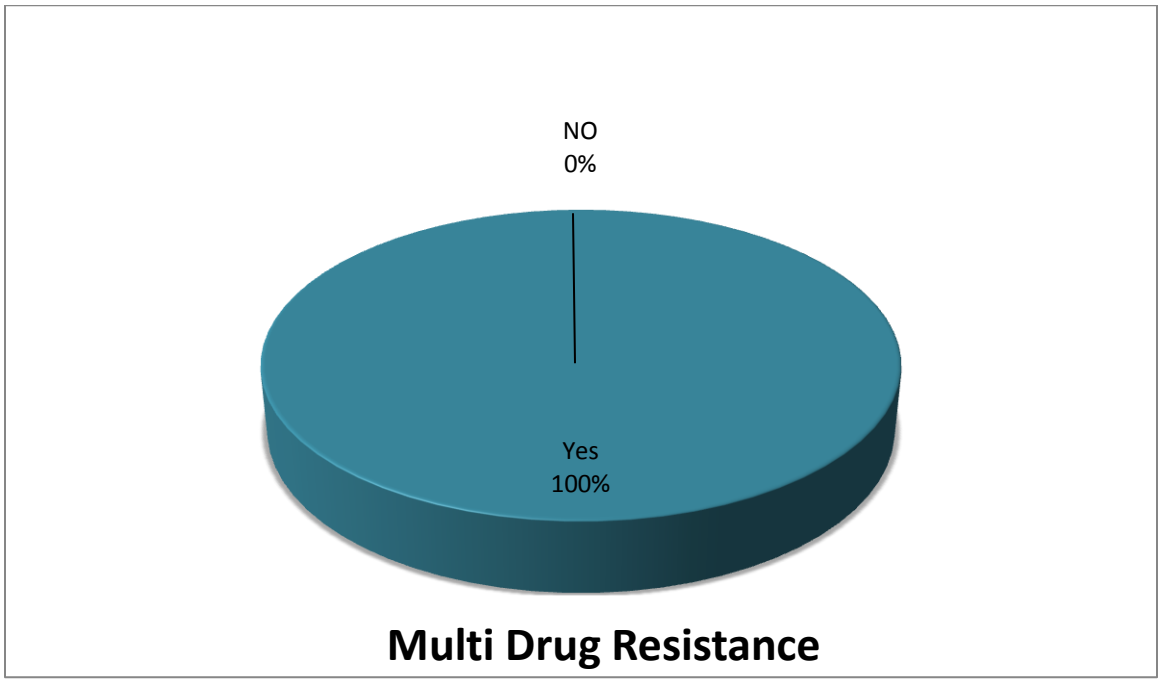


Table 3: Incidence of Multidrug resistance of sample studied

Isolation	**Number of antibiotics –resistance (n=12)**
Isolate 1	4
Isolate 2	6
Isolate 3	6
Isolate 4	8
Isolate 5	5
Isolate 6	7
Isolate 7	5
Isolate 8	6
Isolate 9	9
Isolate 10	6
Isolate 11	10
Isolate 12	4
Isolate 13	10
Isolate 14	9
Isolate 15	8
Isolate 16	7
Isolate 17	6
Isolate 18	8
Isolate 19	10
Isolate 20	7
Isolate 21	6
Isolate 22	6

Table 4: Incidence of Multidrug resistance of sample studied

Multi drug resistance	**Number of isolation**	**%**
Yes	22	100.0
No	0	0.0
Total isolates	22	100.0



CHAPTER- VI
DISCUSSION & CONCLUSION

DISCUSSION

Urinary tract infection is the most common nosocomial infection; 80% of infections are associated with the use of an indwelling bladder catheter. Urinary infections are associated with less morbidity than other nosocomial infections, but can occasionally lead to bacteraemia and death. Infections are usually defined by microbiological criteria: positive quantitative urine culture ($\geq 10^5$ microorganisms/ml, with a maximum of 2 isolated microbial species). The bacteria responsible arise from the gut flora, either normal (*Escherichia coli*) or acquired in hospital (multiresistant *Klebsiella*).

Urinary tract infection (UTI) is the most common nosocomial infection among hospitalised patients. The most common pathogens isolated were *Escherichia coli* (59.4 %), *Klebsiella* spp (15.7 %) and *Enterococcus faecalis* (8.1 %). The mean susceptibility was high for amikacin (87.2 %), ciprofloxacin (74.8 %), ceftazidime (71.5 %) and gentamicin (70.4 %) but low for nitrofurantoin (35 %), cephalexin (49.7 %) and ampicillin (50.5 %) [56]

The Gram-negative pathogenic bacteria *Klebsiella oxytoca* and *Klebsiella pneumoniae* produce the extended spectrum-lactamase (ESBL) and cephalosporinase enzymes and are the major causes of hospital acquired (HA) infections and epidemics in non-hygienic communities in the majority of developing countries. The *K. oxytoca* strains were resistant to cefepime, gatifloxacin, ciprofloxacin, ceftazidime, levofloxacin and imipenem, whereas the *K. pneumoniae* strains were highly resistant to ampicillin, norfloxacin, ciprofloxacin, gatifloxacin, ofloxacin, amoxyclav,ceftazidime, cefepime, cefixime, piperacillin and imipenem. The ESBL-producing and fluoroquinolone-resistant *K. pneumoniae* strains were more prevalent than the *K. oxytoca* strains in the HA/CA samples. The minimum inhibitory concentration values of the third-generation cephalosporins: cefotaxime and ceftazidime and fluoroquinolones: ciprofloxacin and levofloxacin against both species of *Klebsiella* confirmed to resistance. Patients with other bacterial infections had a relatively higher probability of infection with ESBL-producing and fluoroquinolone-resistant *Klebsiella* strains.[22]

Bacteriuria is common in chronically catheterized patients. Maximum catheterized patients, 98%contained bacteria at high concentrations and 77% were poly microbial. The episodes of bacteriuria caused by nonenterococcal gram-positive cocci, more than 75% lasted less than one week. Mean durations of episodes of bacteriuria due to *Escherichia coli, Proteus mirabilis,* and *Pseudomonas aeruginosa* were four to six weeks, whereas those due to *Providencia stuartii* averaged 10 weeks and ranged up to 36 weeks. Thus, the very high prevalence of bacteriuria virtually 100% was a result of a high incidence caused by many different species combined with the prolonged residence of some gram-negative bacilli in the catheter and urinary tract.[49]

Klebsiella is one of a number of gram-negative bacilli that has caused an increase in the number of infections in hospital. In the hospital, colonization of humans is the major reservoir of *Klebsiella.* The *Klebsiella* is acquired by patients after admission to the hospital. The bowel is the body site most frequently colonized; *Klebsiella* has been cultured from the bowel of up to 35% of normal adults. The prevalence and numbers of *Klebsiella* have been shown to increase in the bowel flora of patients receiving antibiotics. Infections in patients on urological wards, infected urine has been considered a significant reservoir of *Klebsiella* and a source of infection. The relation of urinary tract infections to the presence of *Klebsiella* in the bowel flora is normally seen. It is likely that widespread use of antibiotics in urological patients increases the colonization of the bowel with the organism. Infection of the urinary tract of patients who have indwelling urethral catheters is likely if *Klebsiella* is predominant in the bowel flora.[46]

Multidrug resistance and production of extended spectrum b-lactamases (ESBLs) by enteric gram negative rods in hospitals and community continue to be a matter of scientific concern. Assess the prevalence of ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* at two North Indian hospitals and to determine the risk factors associated with the acquisition of these organisms. 48.27% isolates were confirmed to be ESBL producers while 51.73% were non ESBL producers. Among the ESBL producers, 55.69% were *E. coli* and 44.31% were *K. pneumoniae.* ESBL producing isolates showed co-resistance to multitude of antibiotics tested. Length of hospital stay more than three days risk factors for ESBL-producing *E. coli* and *K. pneumoniae* isolates.[24]

Infections with ESBL *K. pneumoniae* are increasing, particularly among patients in ICUs. This pathogen is usually multidrug-resistant and limited treatment available. ESBL-producing pathogens in high-risk populations which are using antimicrobial techniques. Disease progression has occurred while on treatment with antibiotics. The carbapenems, that is, imipenem and meropenem, are safe and effective antibiotics for the treatment of severe ESBL-producing *K. pneumoniae* infection.[59]

In this study 100 random samples were taken. The relative frequency of *klebsella spp* was 22%. Out of 100 samples from 22 samples *klebsiella* were isolated and all these isolated *klebsiella* were resistant to more than at least 3 drugs in the following classes: β-lactams, carbapenems, aminoglycosides and fluoroquinolones, so all these isolates were Multi-drug resistance (i.e. MDRK).The highest resistance was shown by Norfloxacin (100%) and the least resistance was shown by Gentamicin, Tobramycin- Piperacillin 9.0%.

CONCLUSION

This study was to describe the isolation and identification of *klebsiella* by nosocomial urinary tract infection in catheterized patients and to study antibiotic susceptibility patterning of *klebsiella spp.*

Out of 100 samples, 22 samples were isolated as *Klebsiella spp*. The percentage of Multi drug resistance *Klebsiella spp* from the positive samples was 100% since all these positive samples were resistant to at least 3 drugs in the following classes: β-lactams, carbapenems, aminoglycosides and fluoroquinolones

The percentage of resistance by antibiotics: Amikacin (27.2%), Ciprofloxacin (18.1%), Gentamicin (9.0%), Cefotaxime (18.1%), Imipenem (18.1%), Meropenem (18.1%) Cefoperazone (18.1%), Tobramycin (9.0%), Piperacillin-tazobactam (9.0%), Cefepime (13.6%), Ceftazidime (13.6%), Norfloxacin (100%).

CHAPTER- VII
REFERENCE & BIBLOGRAPHY

REFERENCES (& BIBLOGRAPHY)

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