T.R.N.C.
NEAR EAST UNIVERSITY
INSTITUTE OF HEALTH SCIENCES

ISOLATION IDENTIFICATION AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF *PSUEDOMONAS AERUGINOSA* STRAIN FROM VARIOUS CLINICAL SAMPLES OF NEAR EAST UNIVERSTY HOSPITAL NICOSIA CYPRUS

NADEEM ULLAH

MEDICAL MICROBIOLOGY
AND CLINICAL MICROBIOLOGY PROGRAMME

MASTER THESIS

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SUPERVISOR

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This study has been accepted by the Thesis Committee in Medical Microbiology and Clinical Microbiology Programme as Master Thesis.

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According to the relevant articles of the Near East University Postgraduate Study – Education and Examination Regulations, this thesis has been approved by the above mentioned members of the thesis committee and the decision of the Board of Directors of the institute.

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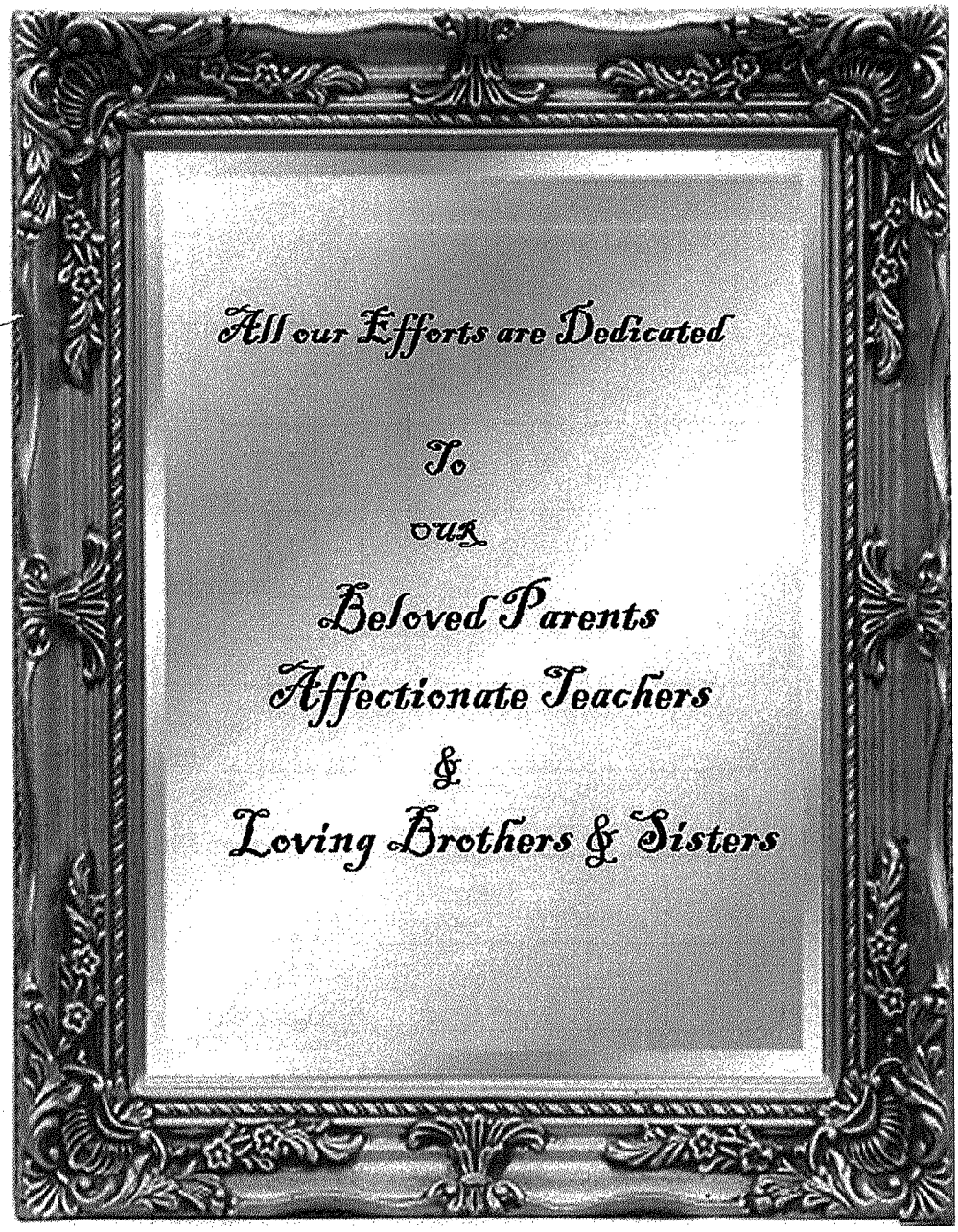
Director of the Institute of Health Sciences


All our Efforts are Dedicated
To
our
Beloved Parents
Affectionate Teachers
&
Loving Brothers & Sisters

CONTENTS

LIST OF ABBREVIATIONS

EMB	Eosin Methylene Blue
ESBL	Extended Spectrum Beta Lactamases
MIC	Minimum inhibitory concentration
GNR	Gram Negative Rods
ICU	Intensive Care Unit
NNIS	National Nosocomial Infections Surveillance System
TSI	Triple Sugar Iron
MIU	Motility Indole, Urease
TRNC	Turkish Republic of Northern Cyprus
SIR	Sensitive Intermediate Resistance
NCCLS	National Committee for Clinical Laboratory Standards
ENT	Ear Nose Throat
WHO	World Health Organization
EUCAST	European Committee on Antimicrobial Susceptibility Testing

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Abstract

Ullah, N. Isolation, Identification and Antibiotic Susceptibility Patterns of *Pseudomonas aeruginosa* Strain from Various Clinical Samples of Near East University Hospital Nicosia North Cyprus, Institute of Health Sciences, M.Sc. Thesis in Medical Microbiology and Clinical Microbiology Programme, Nicosia,2016.

Pseudomonas aeruginosa is an opportunistic pathogen causing serious nosocomial infections in patients. Today *P.aeruginosa* is feared as a dangerous opportunistic bacterium responsible for frequently lethal nosocomial infections. It is resistant to many disinfecting agents and highly resistant against most antibiotics. The main objective of this research was to isolate, screen and identify *P. aeruginosa* strain from various clinical samples of Near East University Hospital Nicosia North Cyprus. As a result of the analysis (n=152) samples were collected from various department of Near East University Hospital which include the samples of wound, blood, urine, ear nose and throat swab, Cerebro spinal fluid(CSF), sputum, aspiration fluids, and used nutrient agar for isolation of *pseudomonas aeruginosa* all the isolated samples was *P. aeruginosa* positive and were further screened for antibiotic sensitivity was tested by minimum inhibitory concentration(MIC) method The sensitivity pattern of Gram negative bacilli was determined against commonly used antibiotics using BD-Phoenix instrument *P. aeruginosa* were also Identified bases on their cultural, microscopic, morphological and biochemical characteristics. The antimicrobial susceptibility testing results shows that P. *aeruginosa* were highly sensitive to some antibiotics which are Amikacin(81.5%), Piperacillin Tazobactam(92.5%), Colistin (86.7%), Ticarcillin Clavuanate (86.6%), Imipenem (80.8%),Meropenem (87.2%),Cefepime (78.4%), Ceftazidime (76.0%),Ciprofloxacin(73.2%),Gentamicin(76.0%),Levofloxacin(73.5%), Norfloxcin(79.5%).The resistance rates to Ampicillin Sulbactam were found to be (98.7%), Cefoxitin(94.7%), Ceftriaxone (93.8%), Trimethoprim Sulfamethoxazole(94.7%), Amoxicillin (100.0%) Cefuroxime (97.7%) and Nitrofurantion (97.7%). The present thesis underline that the clinical isolates of *P. aeruginosa* are becoming resistant to commonly used antibiotics and also gaining more and more resistance to newer antibiotics it's a strong 'cross talk' between the drug, the bacteria and the environment has to be considered with multi disciplinary perspective for controlling of antimicrobial resistance development.

Key Words: *Pseudomonas aeruginosa*, Antibiotic patterns, MIC, North Cyprus.

1. INTRODUCTION

Pseudomonas aeruginosa is a gram negative bacillus, non- spore forming, straight or slightly curved rod shaped bacterium that occurs as a single form or in pairs and occasionally in short chains. It is widely divided in nature including soil, water and various types of vegetation throughout the world except that it has also revealed its presence in disinfectants, respiratory equipment, sinks, taps, and mops within the hospital as a biofilm. *P. aeruginosa* found its entry into the hospital environment either through outsider and patients or healthy person that enters in hospital. Vehicle transmission or contact transmission is common mode of transmission in hospital. Its infections is common in hospitalized patients, particularly those who are debilitated or immunocompromised for example in intensive care units, HIV-infected patients, particularly those in advanced stages are at risk groups *P. aeruginosa* infections can develop in many anatomic sites, including skin, subcutaneous tissue, bones, ears, eyes, urinary tract, and heart valves. *P. aeruginosa* is resistant to many disinfecting agents and highly resistant against most antibiotics (Ekrem et al., 2014).

In hospitals, patients are exposed to several types of exogenous pathogens such as bacteria, viruses, fungi, and protozoa from other sources like patients, health care personnel, or outsiders. Other sources of pathogens include the patient's endogenous flora i.e., bacteria residing on the patient's skin, mucous membranes, gastrointestinal tract, or respiratory tract that is difficult to exclude and remove from patient room touch surfaces, equipment, medication. The most common sources of infectious agents causing nosocomial infections are; the individual patient, medical surgical instruments, hospital environment, health care personnel, contaminated drugs, contaminated foods, and contaminated patient care equipments (Amy et al., 2006).

Development in antibiotic resistant against pathogenic microorganism is a serious threat to health care sector throughout the world. Beta lactam antimicrobial agents are the most commonly used antibiotics to treat bacterial infections. Clinical isolates of gram-negative rods have been shown to produce an enzyme called extended spectrum beta lactamases (ESBL) which cause these gram negative bacilli resistant to beta-lactam antibiotics. ESBLs (Plasmid mediated enzymes) mediate

resistance to extended-spectrum (third generation) cephalosporin's e.g. Ceftazidime, Cefotaxime, and Ceftriaxone and Monobactums e.g. Aztreonam ESBL were initially limited to P. *auerginosa*, *Escherichia coli* and *Klebsiella Spp.* then many microorganisms, particularly *Enterobacter sp.* and *Proteus* sp. acquired the genes responsible for ESBLs production. More than 150 species have been detected to produce ESBL (Sajjad et al., 2006).

P. aeruginosa possesses a considerable rang of natural resistance to antibiotics. Antibiotics include aminoglycosides (Gentamicin, amikacin), Cephalosporins (cefotaxime, ceftazidime, cefoperazone), fluoroquinolones (ciprofloxacin, ofloxacin, perfloxacin), penicillins (piperacillin, ticarcillin, azlocillin) are non sensitive to *Pseudomonas* strains. The genes for drug resistance are present on both chromosome and plasmids of the bacteria for localized infections, topical colistin, polymyxin B or 1% acetic acid may be beneficial to control *Pseudomonas* strains (Elizabeth and Jean, 2002).

In the recent study characterize the multi drug resistant ability of *Pseudomonas aeruginosa* isolates from hospital and hospital free environment. Plasmid resistance genes often code for enzymes that damage or changed drugs for e.g. the hydrolysis of penicillin or the acetylation of chloramphenicol and aminoglycosides drugs. Plasmids associated genes have been implicated in resistance the aminoglycosides, Chloramphenicol, Penicillin and Cephalosporins, Erythromycin, Tetracycline, Sulfonamids and others (Mallea et al., 2000). Once a bacterial cell possesses an R-plasmid, the plasmid may be transferred to other cells quite rapidly through normal gene exchange processes such as transduction, transformation and conjugation (Hemalatha. N et al., 2010)

P. aeruginosa is widely found in natural environments and it is an Opportunistic pathogenic orginisms for human's leads to a wide spectrum of disease such as, respiratory infections, urinary, burn and septicemia. In recent years nosocomial infections caused by *P. aeruginosa* have been recognized as an acute problem in hospitals due to its intrinsic resistance to many antibiotic classes and its capacity to acquire practical resistance to all effective antibiotics. All these features in *P. aeruginosa* characterize it as a major microorganism to monitor antibiotic resistance in the clinical specimens. On the other side, the spread of these bacteria in hospital personnel, wet places, could be a reservoir of resistance genes. (Fazeli et al., 2012).

Pseudomonas aeruginosa is the second leading cause of gram-negative nosocomial infections is an important opportunistic pathogen, is the primary cause of hot tub folliculitis, otitis externa, as well as the principal cause of morbidity and mortality in cystic fibrosis patients it is highly ubiquitous in water systems, and has intrinsic antimicrobial resistance due to low outer membrane permeability, as well as an extensive efflux pump system. Indoor recreational water is an important reservoir for *P. aeruginosa* and is a meaningful exposure pathway for bacterial transmission, where wet skin and occlusion provide optimal conditions for *P. aeruginosa* to thrive *P. aeruginosa* has been implicated in numerous nosocomial and community outbreaks, with therapy tanks and whirlpools frequently acting as the environmental reservoir Complex or hard to clean piping has been noted as a factor in *P. aeruginosa* contamination; in nosocomial and household settings, contaminated sinks and shower heads have been a common reservoir for *P. aeruginosa*, where the inaccessible armature is nearly impossible to adequately decontaminate additionally, some *P. aeruginosa* strains exhibit mutations in fluoroquinolone binding sites, the loss of porin channels, and increased beta lactamase or cephalosporinase production *P. aeruginosa* frequently acquires additional resistance mechanisms (i.e., from plasmids) and routinely develops multidrug resistance throughout the course of a treatment regimen. The overall prevalence of antibiotic resistant *P. aeruginosa* is increasing, with up to 10% of global isolates found to be multidrug-resistant in addition, given the propensity of *P. aeruginosa* to proliferate rapidly when disinfectant levels fall below recommended levels, monitoring the prevalence of resistant strains may be important for the prevention of future outbreak (Jonathan et al., 2011)

P. aeruginosa has an intrinsic resistance against many antibiotics this resistance is mainly the result of a pressure selection due to abusive or bad use of antibiotics. The propagation of this resistance is elucidated in bacterial resistance by acquisition of *P. aeruginosa* of a transferable resistance to lactamines which presents a huge risk of dissemination to other bacteria (Gaouar et al., 2012).

In recent study, a notable increase in the prevalence and multi-drug resistant (MDR) of *P. aeruginosa* reported in critically ill hospitalized beta lactamases production and antimicrobial

resistance ratio of *P. aeruginosa* from hospitalized patients in Kahramanmaraş, Turkey (Torolugu et al., 2013).

The microbial pathogenic activity, as well as, their antibiotic sensitivity pattern, may change from time to time and place to place. Therefore knowledge of current drug resistance pattern of the common pathogenic bacteria in a particular region is beneficial in clinical practice. *P. aeruginosa* is one of the important bacterial pathogens isolated from various samples despite advances in medical and surgical care and introduction of broad variety of antimicrobial agents against having anti pseudomonal activities, life threatening infection caused by *P. aeruginosa* continues to cause complications in hospital acquired infections. *P. aeruginosa* is increasingly recognized as an emerging opportunistic pathogen of clinical relevance that causes infections in hospitalized patient particularly in burn patients, orthopedic related infections, respiratory diseases, immunosuppressed and catheterized patients. Several different epidemiological studies indicate that antibiotic resistance is increasing in clinical isolates. Being gram-negative bacteria, most *pseudomonas spp.* are naturally resistant to penicillin and majority of related betalactam antibiotics, but a number are sensitive to piperacillin, imipenem, tobramycin or ciprofloxacin. Nowadays more and more resistance P. *aeruginosa* are encountered in routine clinical practice, a serious problem, increase morbidity and mortality and also cost of treatment (Rajat et al., 2012).

Bacterial resistance to antibiotics is a burgeoning problem in the hospital setting, particularly in intensive care units. Infections caused by multidrug resistant bacterial strains are generally associated with increased morbidity and mortality as well as with the length of hospital stay and increased hospital cost (Vladimíra et al., 2011).

The number of isolates with acquired carbapenemases and metallo β lactamases emerged and spread during the early 1990s, and the detection of a considerable number of OXA, IMP and VIM-type carbapenemases have been reported in many countries. In addition, these genes are easily transferable because, in many times, they are inserted in motile structures, such as integrons due to this ability to spread; β lactamase production has become a serious concern. The most important clinically-significant carbapenemases in *P. aeruginosa* are class B metallo β lactamases such as VIM and IMP-type In fact, the presence of *P. aeruginosa* producing IMP enzymes was firstly described in Japan, and different IMP type enzymes have been described in

Japan, China, Canada, Italy, Brazil and USA. With regard to VIM enzymes, they were firstly identified in Italy, and different types of vim genes have been reported from other european countries and other regions like Asia and America There are few reports of carbapenem hydrolyzing OXA enzymes in *P. aeruginosa* isolates, being the O-XA-50 enzyme the only one identified in *P. aeruginosa* However, OXA-type enzymes are more frequently found in other non fermenting bacilli like Acinetobacter baumannii (Sevellan et al., 2006).

Pseudomonas aeruginosa exhibits intrinsic resistance to several antimicrobial agents. It posses some multi-drug efflux systems, including MexAB-OprM and MexXY-OprM. Furthermore, acquired antimicrobial resistance constitutes a major challenge for anti-pseudomonas therapy, especially when it is associated with resistance to other classes of drugs. Antimicrobial resistance among clinical isolates of *P. aeruginosa* may complicate the treatment of infections, and can adversely affect clinical outcomes and treatment costs for patients. New antimicrobial agents with activity against *P. aeruginosa* will not be available in the near future, making ongoing surveillance of the activities of currently available agents of critical importance (Rezvan et al., 2005).

P. aeruginosa is found almost everywhere that is in water, in soil and also on plants. It can also be present in tap water found in patient rooms. It can be isolated from various body fluids such as sputum, urine, wounds, and eye or ear swabs and from blood because it can infect almost any external part or organ of the body strains of *P. aeruginosa* which are Multidrug-resistant (MDR) are often isolated from the patients suffering from nosocomial infections, especially from those which are present in the intensive care unit. A narrow class of antibiotics is effective against *P. aeruginosa*, including the carboxypenicillins, quinolones (ciprofloxacin, levofloxacin), the antipseudomonal cephalosporin, and aminoglycosides. Beta-lactamase production by this organism present the major mechanism of resistance to β lactam antibiotics is and it is reported that more than 340 β-lactamase enzymes produced by *P. aeruginosa* have been detected Some enzymes like AmpC beta-lactamases, extended-spectrum beta-lactamases (ESBLs), and metallo-beta-lactamases, make *P. aeruginosa* as serious pathogens in hospitalized patients It is essential to determine the accurate bacterial susceptibility to antibiotics for the better management of bacterial infections (Jafar khan et al., 2014).

Otitis media is infection of middle ear caused by bacteria, fungi and virus resulting in inflammation of mucosal lining. Recurrent otitis media may cause damage of ossicles, facial nerve and cochlea, resulting in permanent hearing loss. It can be acute or chronic. The acute form usually associated with the infection in the upper respiratory tract whereas persistent form is known as chronic suppurative otitis media (CSOM). The chronic form is still a major problem in developing countries like Pakistan. It is more common in children belonging to lower socioeconomic group. Most common micro organisms found in CSOM are P. aeruginosa, Staphylococcus aureus, Proteus mirabilis, Klebsiella pneumonia, Escherichia coli, Aspergillus spp and Candida spp but these organisms vary in various geographical areas. (Tahira et al., 2009).

P. aeruginosa is one of the most frequent and dangerous pathogens involved in the etiology of severe nosocomial infections. It has been implicated in diverse nosocomial infections like nosocomial pneumonias, urinary tract infections (UTIs), skin and soft tissue infections, in severe burns and in infections in immunocompromised individuals. Infections caused by P. aeruginosa are often life threatening and difficult to treat because of its primary limited susceptibility to commonly used antimicrobial agents. Most strains of *P. aeruginosa* are multidrug resistant. The development of bacterial resistance is a major worldwide problem complicating the use of chemotherapeutic agents and the control of infectious diseases (AL-Salihi S et al., 2014).

P. aeruginosa is known for its ability to resist killing by a variety of antibiotics it is the second most common etiology of nosocomial pneumonia 3rd for urinary tract infections and 4th for surgical site infections. Likewise in a hospital wide surveillance of nosocomial infections conducted by the Infection Control Committee of the Philippine General Hospital in 1989. *P. aeruginosa* was the most common organism isolated from all sites of infection (37%) Resistance to antimicrobial agents is a development clinical problem and is a recognized public health threat *P. aeruginosa* has a particular propensity for the development of *resistance*. It is naturally resistant to many antibiotics because of its relatively impermeable outer membrane and it can also easily acquire resistance, creating challenging therapeutic scenarios. Outbreaks of multidrug resistant *P. aeruginosa* colonization or infection have been reported on urology wards, a burn unit, hematology/oncology units, and adult and neonatal critical care units. Various medical

devices and environmental reservoirs have been implicated in these outbreaks, including antiseptic solutions and lotions; endoscopy equipment; ventilator apparatus and mouth swab. These sources can easily be eliminated once identified. A greater challenge exists if the source of amount break involves permanent components of the hospital physical plant, such as plumbing fixtures (Paranjothi et al., 2010).

P. aeruginosa, , is characterized as an aerobic, lactose negative, oxidase positive, and slightly curved gram-negative rod with varied morphology (e.g., non mucoid variants and less commonly mucoid variants associated with cystic fibrosis) The high mortality associated with *P. aeruginosa* infections, particularly with ineffective initial empiric therapy, emphasizes the need for reliable data on which to base the choice of empiric therapy. Significant declines in the susceptibility of *P. aeruginosa* to many antimicrobials were noted at our institution, primarily for cefepime, ciprofloxacin and tobramycin. Most alarming was the rapidly increasing resistance rates of *P. aeruginosa* to cefepime, which is considered to be the first-line antimicrobial agent for empiric nosocomial gram negative rod coverage at our institution. Optimal control and treatment of *P. aeruginosa* infections traditionally have been a focus of antimicrobial stewardship programs. Cefepime is currently approved for intensive care unit (ICU) empiric therapy when *P. aeruginosa* is suspected, while carbapenems require approval by the antimicrobial stewardship team (Brett H et al., 2010)

P. aeruginosa is an opportunistic pathogen characterized by an innate resistance to various groups of antimicrobials. Accurate *in vitro* susceptibility test methods are important to provide proper therapy and for the detection of newly emerging resistance. The BD Phoenix™ Automated Microbiology System (BD Diagnostic Systems, Sparks, MD) is an automated identification (ID) and antimicrobial susceptibility test (AST) system for both gram-positive and gram-negative organisms. The aims of this multi-center study were to evaluate the ability of the Phoenix System to detect resistant phenotypes of *P. aeruginosa* from demographically and geographically diverse strains. Seven antimicrobials with indications for *P. aeruginosa* (cefepime (FEP), cefoperazone (CFP), ceftazidime (CAZ), ceftizoxime (ZOX), ceftriaxone (CRO), piperacillin (PIP) and piperacillin tazobactam (TZP) were assessed for *in vitro* AST

performance in Phoenix gram-negative panels, as these agents are often problematic in automated AST systems (Denys G et al., 2005).

1.1 Aims and Objectives

(1) Isolation of *P. aeruginosa* strain from hospital environment.

(2) Identification of *P. aeruginosa* strain by using biochemical tests.

(3) To study the antimicrobial susceptibility pattern of *P. aeruginosa* strain from different clinical samples.

(4) To analyzed and provide data regarding hospital environment and related risks to the patient through which health care can be improved.

2. REVIEW OF LITERATURE

Kireçci et al., (2014) investigated those infections with *Pseudomonas aeruginosa* have high morbidity and mortality rates. Quick and efficient antibiotherapy can reduce these infections effectively. He study 420 samples from various clinical sites and was tested. Out of these 420 samples, 75 clinical isolates of P. aeruginosa were isolated. In this isolated isolates from various clinical samples were sensitive to gentamicin (54.7 %) followed by amikacin (62.7%), imipenem (96%), meropenem (98.7%), ceftazidime (82.7%), piperacillin (70.7%), tobramycin (69.3 %), ciprofloxacin (73.3 %), ceftriaxon (8%), and cefotaxime (0%). The results indicate that P. aeruginosa isolates have high susceptibility to meropenem, imipenem, and ceftazidime than other antibiotics.

Jafar et al., (2014) investigated that extended spectrum beta-lactamase enzymes are the increasing cause of resistance to penicillin's, cephalosporins, and aztreonam antibiotics in *P. aeruginosa.* A total of 200 specimens were received by the pathology laboratory of Pakistan Institute of Medical Sciences, Islamabad, Pakistan, which comprised of 50 tracheal 50 pus, 25 bloods, and 25 urine and 50 miscellaneous samples including sputum, swab, wounds, tissue and different body fluids. *P.aeruginosa* was tested against a panel of 14 antibiotics. The highest percentage of resistance to antibiotics amoxicillin + clavulanicacid, cefoperazone + sulbactam, ceftriaxone, ceftazidime, Piperacillin and tobramycin was measured. The most effective drug established were polymixine B, nalidixic acid, meropenem, amikacin, imipenem, aztreonam were found as more effective in the order respectively.

Firdous et al., (2013) determine *in vitro* synergistic effect of ciprofloxacin in combination with amikacin and gentamicin against MDR *P. aeruginosa* clinical isolates. Antibiotic resistance pattern of 100 identified clinical isolates of *P. aeruginosa* was determined against eight antibiotics by disc diffusion method at Microbiology Laboratory, Holy Family Hospital, and Rawalpindi. For 30 selected MDR isolates, minimum inhibitory concentrations (MICs) of amikacin in and gentamicin were determined separately by agar diffusion method followed by combined activity of ciprofloxacin with amikacin and gentamicin by checkerboard agar dilution technique. Antibiotic resistance pattern of *P. aeruginosa* isolates was; gentamicin and carbenicillin (94%), amikacin and piperacillin (92%), ceftazidime (90%), colistin (87%),

ciprofloxacin (79%) and imipenem (72%). MICs against 30 selected MDR isolates ranged from 32 to ≥128µg/ml for amikacin, and ≥128µg/ml for gentamicin. Synergistic effect was observed in 12/30(40%) isolates for AK+CIP and in 05/30 (16.7%) for CN+CIP. Ciprofloxacin in combination with amikacin and gentamicin showed synergistic effect and no antagonistic effect against MDR *P. aeruginosa*.

Vladimíra V et al., (2011), investigated that *P. aeruginosa* is one of the most frequent and dangerous pathogens involved in the etiology of severe nosocomial infections. A retrospective observational study was conducted at all intensive care units of the University Hospital in Olomouc, Czech Republic (155 ICU beds). Complete antibiotic utilization data of the ICUs in the period of 1999 to 2008 were processed according to ATC/DDD system and expressed in defined daily doses per 100 bed-days (DBD). Utilization of meropenem, imipenem, ciprofloxacin, ofloxacin, pefloxacin, gentamicin, amikacin, ceftazidime, cefoperazone, cefoperazone/sulbactam and piperacillin tazobactam was measured. And isolated from clinical material obtained from patients hospitalized in ICUs. During the ten year period, utilization of the entire group of antibiotics monitored grew. It increased from 23.52 DBD in 1999 to 27.48 DBD in 2008 with a peak of 33.04 DBD in 2007. *P. aeruginosa* accounted for as much as 42% of pneumonias and 23% of surgical wound infections.

Rakesh et al., (2012) investigated that *P. aeruginosa* is one of the important bacterial pathogens isolated from various samples. Despite advances in medical and surgical care and introduction of wide variety of antimicrobial agents against having anti-pseudomonal activities, life threatening infection caused by *P. aeruginosa* continues to cause complications in hospital acquired infections. During his study 630 samples were tested, in which 321 samples showed growth of bacteria. Out of 321 samples, 100 clinical isolates of *P. aeruginosa* were isolated. The samples were selected on the basis of their growth on routine MacConkey medium which showed lactose non-fermenting pale colonies which were oxidase test positive and on nutrient agar pigmented and non-pigmented colonies with oxidase positive. Antimicrobial susceptibility of all the isolates was performed by the disc-diffusion (Modified-Kirby Baur disc diffusion method) according to CLSIs guidelines *P. aeruginosa* isolated from various samples are resistant to tobramycin (68%) followed by gentamycin (63%), piperacillin (50%), ciprofloxacin (49%) and ceftazidime

(43%).

Tahira et al., (2009) investigated that chronic suppurative otitis media (CSOM) is a prevailing and notorious infection in developing countries causing serious local damage and threatening complications. *P. aeruginosa* is most common pathogen causing CSOM in Pakistan. A total of 275 bacterial isolates were studied. *P. aeruginosa* (40%) and *Staphylococcus aureus* (30.9%) were the most common bacterial agents found in CSOM. MIC was done for *P. aeruginosa* only as it was the commonest pathogen found in CSOM. Sensitivity pattern of *P. aeruginosa* showed that amikacin was active against 96% of isolates followed by ceftazidime 89%, ciprofloxacin 85%, gentamicin 81%, imipenem 76%, aztreonam 42% and ceftriaxone 21%. *Pseudomonas aeruginosa* was the most common bacteria isolated from chronic discharging ears followed by *Staphylococcus aureus*. Amikacin was found to be the most suitable drug followed by ceftazidime and ciprofloxacin for *Pseudomonas aeruginosa*.

Noel et al., (2013) investigated that *P. aeruginosa* is a germ of hospitalism responsible for nosocomial infections it is naturally resistant to many antibiotics and has a high susceptibility to the acquisition of acquiring new resistance. The observation of strains highly resistant to antibiotics, has led us to look for possible alternative therapeutics. Forty nine of 150 samples were positive to the cultivation of *P. aeruginosa* showing a prevalence of 32.66%. For the antibiotic susceptibility, we obtain amikacin 57.14%, ceftazidime 52.60%, imipenem 33%, colistin 97.95%, and ciprofloxacin 51%. Seven strains were resistant to all antibiotics tested other than colistin. One strain was resistant to colistin. Colistin retains high sensitivity to *P. aeruginosa*. However, there are some strains multi resistant to antibiotics.

Toroglu et al., (2013) investigated for a sixteen isolates of *P. aeruginosa* were collected from different hospitals in Kahramanmaraş among 2006-2007 and tested for the level of resistance to the widely used anti *pseudomonal* antibiotics and used in local medicinal and veterinary practice. These strains were mostly isolated from urine and few from tracheal aspirate, deep tracheal aspirate, sputum, mucus, bronchi alveolar lavage. The antibiotic resistance rates were as follows Penicillin (P) 100%, Amoxicillin (AM) 94%, Cefazolin (CEZ) 87.5%, Cefoxitin (CEF) 81%,

Nitrofrantoin (NIT) 75%, Chlorampenicol (C) 62.5 %, Tetracycline (TE) 56%, Oflaxain (OFX) Ceftriaxone (CEF) 44%, and Gentamycin (GE) 37.5%, Meropenem (MEM) and Streptomycine (SX) 31%. Among 16 isolates of P.aeruginosa from wounds showed 8 (50%) lactamase activities, where as 8 isolates of *P.aeruginosa* from urine showed no lactamase activity. All *P. aeruginosa* strains 16 (100%) isolates showed multiple antibiotic resistances towards three to eleven antibiotics.

ECDC et al., (2009) investigated that *P. aeruginosa* is an important cause of infection among patients with localized and systemic immune defects. Resistance to carbapenems in *P. aeruginosa* are high all over Europe, as almost three quarters of the countries reported more than 10% carbapenem resistance In Europe, multi-drug resistance is the dominant threat posed by invasive *P. aeruginosa.*

SAPG et al., (2008) investigated that a total of 196 cases of *P. aeruginosa* bacteraemia were recorded in 2008. *P. aeruginosa* is intrinsically resistant to a broad range of antimicrobials due to outer membrane and efflux systems that efficiently exclude antimicrobials from the bacterial cells. Resistance to all available anti-pseudomonal agents, including piperacillin-tazobactam, ceftazidime, carbapenems, fluoroquinolones and aminoglycosides was observed among the Scottish isolates Resistance to piperacillin-tazobactam (8.3%) in the Scottish isolates of *P. aeruginosa* was above what was reported for the UK (1.3-5.4% in 2005-2008) and Wales (3.9%) in 2007 Resistance to ceftazidime in *P. aeruginosa* was 8.3% among the Scottish isolates, while most recent figures reported from the UK and Wales were below 5%. Resistance to carbapenems (here meropenem) was 5.3% among the Scottish isolates of *P. aeruginosa*, which is within the same range as reported for the rest of the UK (6.4%) and Sweden (4.0%) in 2008. Resistance to fluoroquinolones (11.8%) among the Scottish isolates of *P. aeruginosa* was within the same range as reported by HPA (12%) and Wales (13.6%) in 2007, but above that of the UK resistance (7.6%) reported to EARSS in 2008. Resistance to amino glycosides among the Scottish isolates of *P. aeruginosa.*

Arshi Syed et al., (2006) determine the sensitivity and resistance patterns of *P. aeruginosa*

isolated from hospitalized patients and from hospital environment. A total of 120 patients of *P. aeruginosa* were isolated from selected group of patients during the period June 1999 to June 2001, by using conventional techniques. Out of 120 strains of *P. aeruginosa* 38 samples were from pus, 26 from urine, 24 from sputum, 20 from blood, 6 from CSF and 6 from catheter tips. All the strains of *P. aeruginosa* were subjected to in vitro sensitivity test by the standard Stokes disc diffusion method and in those isolates of *Pseudomonas aeruginosa* Cefoperazone (91.0%) was the most effective anti pseudomonal agent and Ciprofloxacin (33%) was the least sensitive antimicrobial agent after performing MIC, a high level of drug resistance was noted for Ciprofloxacin (66.5%), Gentamicin(54.4%), Amikacin(51.7%), Ceftazidime(50%) and Tobramycin(45.8%). The results of sensitivity of clinical isolates indicate that multiple antibiotic resistant *P. aeruginosa* is a major clinical problem.

Denys et al., (2005) investigated that increased resistance in *P. aeruginosa* (PSAE) continues to pose a significant threat to patient care because of limited therapeutic options. The ability to detect resistance in clinical PSAE isolates is critical for appropriate antimicrobial agent selection and eventual patient outcome. The BD Phoenix Automated Microbiology System (BD Diagnostics, Sparks, Maryland, USA), a rapid automated ID/AST system, was compared to the CLSI recommended standard broth micro dilution (SBM) method for performance with seven antimicrobials against PSAE. A total of 271 PSAE clinical and stock isolates, including nine challenge set strains, were tested for AST accuracy against four third generation cephalosporins (ceftazidime, CAZ; cefoperazone, CFP; ceftizoxime, ZOX; and ceftriaxone, CRO), a fourth-generation cephalosporin (cefepime, FEP), piperacillin (PIP) and piperacillin-tazobactam (TZP). Each isolate was simultaneously tested in Phoenix and the CLSI-recommended SBM reference method. Inocula densities were adjusted equivalent to a 0.5 McFarland standard, and then inoculated into both panel types. Phoenix panels were incubated and read every 20 minutes to completion in the BD Phoenix instrument, while SBM panels were incubated at 35°C for 18 – 20 hours in ambient air and read manually for MIC endpoint determination. Break points and QC (Quality Control) Strains were those recommended in the current CLSI standard (M100-S15) for each antimicrobial. Essential agreement (EA) between Phoenix and the SBM was between 92% and 98% for the seven antimicrobials. Exact categorical agreement (CA) was between 76 and 97%, while these rates improved to between 95 and 98% when agreement within +/- one dilution

was considered. The very major error (VME) rate ranged from 0% for CRO to 7.6% for TZP, though 4/5 TZP VMEs were within EA. Major Error rates were all less than 2.8%, except for CRO at 6% (2/33). The BD Phoenix System provides a satisfactory level of agreement to the reference method with PSAE and the seven antimicrobial agents tested The combined rate of resistance detection for these antimicrobial against PSAE was 97.9%.

Minhas et al., (2014) studied the drug resistance of different microbes from clinical isolates. 324 samples were collected from suspected patients visiting different hospitals at district Peshawar. For morphological identification, samples of clinical isolates were analyzed by blood agar, MacConkey agar and Eosine Methylene Blue, identified by gram staining and characterized by different biochemical tests. Antibiotic Sensitivity test by Modified Kirby-Bauer Disc diffusion method was used to test the *in-vitro* susceptibility of the identified isolates to different antibiotics such as Ceftazidime, Ceftazidime, Ceftriaxone, Cefepime and Imipenem. These resistant non-lactose fermenting gram negative bacteria were isolated from samples of pus/wound (33.30%, n = 108/324), blood (33.30%, n = 108/324), urine (23.30%, n = 75/324) and from ascetic/pleural fluids (10.20%, n = 33/324). The study revealed that the percentage of non-fermenting bacterial infection was higher in females (53%) as compared to males (47%) along with higher infection observed in the age group of 11 - 30 years. *P. aeroginosa* showed high resistance against Cefepime (88.80%), followed by Cefoperazone (55.50%), Ceftazidime (48.10%), Ceftriaxone (33.30%). Imipenem was active with low resistance (7.40).

Humera et al., (2014). Investigated that *P. aeruginosa* accounts for a significant proportion of nosocomial infection and are responsible for about 13 % of eye, ear, nose and throat infections. 237 samples of ear swabs were received at Dr. Ziauddin Hospital North Nazimabad (Campus) Karachi. Samples of pus from external auditory canal were taken with sterile cotton swab and are cultured on blood, chocolate and MacConkey agars media and were incubated for 24 to 48 hours. Antibiotic sensitivity was tested and interpreted by method according to CLISI criteria. *P. aeruginosa* was isolated from 37 samples and rests of 70 samples were positive for different microorganisms. Majority of organisms were sensitive to Meropenem (100%), Ceftazidime (100%), Polymyxin B (100%) and Colistin (100%).

Zulfiqar et al., (2005) studied that *P. aeruginosa* remains the leading pathogen causing burn wound infection. It is found as major colonizer of the burn wound because it thrives on moist burn wound surface and survives well in the hospital environment, once it is established, it can persist for months within a unit, and poses as multi drug resistant nosocomial infection threat for patients being treated there. The emergence of multi drug resistant *P. aeruginosa* in burn wound is becoming a challenging problem in infection control programmes. A total of 44 isolates of *P. aeruginosa* were recovered from burn patients. Most of them were resistant to multiple antibiotics. Their sensitivity against Imipenem was over all better than the other drugs i.e. 77.3%. Ciprofloxacin was the second most effective drug against this organism with a sensitivity of 54.5% while a 4th generation cephalosporin, Cefepime was effective against 22 (50%) isolates. About 30% *P. aeruginosa* were sensitive to Amikacin. Aztreonam showed inhibitory activity against (6.8%) strains. Piperacillin activity was 18.2%. The efficacy of Cefutaxime was 4.5%. Chloramphenicol and Septran were 100% inactive against *Pseudomonas* infection while > 95% strains of *P. aeruginosa* were resistant to Tobramycin.

Al-Marzoqi1 et al., (2013) determined that *P. aeruginosa* considered as most important bacteria which can isolated from various kinds of infection The isolates were obtained from different clinical specimens, including pus, urine, respiratory fluids, blood, tissue, and genitalia. All the clinically isolated samples were identified as *P. aeruginosa.* Out of 285, 74.04% are males and 25.96% are females. Most of patients were aged between 27-48 years. Approximately half the isolates tested were from community patients, mostly from infections of the wound/pus (22.46%), urinary tract (22.11%), swab (18.6%) and respiratory tract (15.09%). *P. aeruginosa* strains screened showed sensitivity to Amikacin, Erythromycin and Penicillin while showed resistance to penicillin, erythromycin, and norfloxacin, Amoxicillin, \Amoxicillin + Clavulanic acid, Azithromycin Antimicrobial susceptibility of all the isolates was performed using disc-diffusion (Modified-Kirby Baur method) according to CLSIs guideline

Mohanasoundaram et al., (2013) investigated that the isolation rate of *P.aeruginosa* was 5%, 6.8% and 5% in 2008, 2009 and 2010 respectively. Pus, tracheal aspirates and urine were important sources of *P.aeruginosa* isolation in ICU and non ICU inpatients. Resistance rates of

pseudomonas varied with the antibiotics and the high resistance observed was related to the increased use of broad spectrum antibiotics. Multidrug resistance *P.aeruginosa* is on the rise especially in nosocomial infections. Hence rigorous monitoring of MDR strains, restriction of inappropriate use of antimicrobial agents and adherence of infection control practices should be emphasized to delay the emergence of clinically significant MDR *P.aeruginosa* to conclude, although multidrug resistance has commonly been reported in nosocomial *P.aeruginosa*, community acquired data are less frequently reported. For this reason epidemiological studies on the prevalence and antimicrobial susceptibility pattern of resistant isolates in different geographical settings would provide useful information to guide clinicians in their choice of therapy and to contribute to the global picture of antimicrobial resistance.

Parmar H et al., (2013) investigated that *P. aeruginosa* has been emerged as an important pathogen and is one of the important causes of morbidity and mortality among hospital patients. Because of changing antibiotic sensitivity pattern, knowledge of current status of drug resistant is very important in clinical practice specifically in treatment of critically ill. Total 2811 samples were tested all of them were subjected to direct microscopy and culture for identification & isolation of *P. aeruginosa*. Isolated colonies of *P. aeruginosa* were further subjected to antibiotic sensitivity testing for 12 routine anti pseudomonal drugs. *Pseudomonas aeruginosa* were isolated from maximum resistant isolates (56.25%) were obtained from pus samples. It is evident that nowadays *P. aeruginosa* is becoming less sensitive to cephalosporins, imipenem, aminoglycosides and β-lactamase inhibitors.

Sedighi M et al., (2015) Studied the resistance rates of the isolates to various antibiotics were obtained. It was found that cefepime and cefotaxime had the highest resistance rates (100%). However, the resistance rates were also high for the drugs imipenem (58.5%), meropenem (58.5%), ceftazidime (89.6%), aztreonm (96.2%), ciprofloxacin (77.4%) and gentamicin (66%). Moreover, the lowest resistance rate was observed for amikacin (43.4%). The resistance rate of these bacterial strains to different antibiotics was then assessed by antibiogram (Kirby bauer method).

AL Salihi S et al., (2015) investigated that Wound and ear swab were important source for *P. aeruginosa* and isolated more frequently in inpatients than outpatients. The rate of isolation in

females 169/319 (52.97%) was higher than males 150/319 (47.01%). Antibiotic susceptibility test of these isolates was performed, and the results showed that all *Pseudomonas* isolates (100%) were resistant to ampicillin, cephradin and trimoxazole, followed by gentamycin (97.3%), amoxicillin (97.3), cephalexin (92.3%), neomycin (91.4%), nalidixic acid (89%), nitrofurantoin (87.5%), tobramycin (87.5%) and ciprofloxacin (84%), and the resistance to amikacin was (75%).

Juretschko S et al., (2007) investegated that contemporary clinical isolates and challenge strains of *P. aeruginosa* were tested by four automated susceptibility testing systems (BD Phoenix, MicroScan WalkAway, Vitek, and Vitek 2; two laboratories with each) against six broad spectrum lactams, and the results were compared to reference broth microdilution (BMD) and to consensus results from three validated methods (BMD, Etest [AB Biodisk, Solna, Sweden], and disk diffusion). Unacceptable levels of error (minor, major, and very major) were detected, some with systematic biases toward false susceptibility (piperacillin_ tazobactam and imipenem) and others toward false resistance (aztreonam, cefepime, and ceftazidime). They encourage corrective action by the system manufacturers to address test biases, and they suggest that clinical laboratories using automated systems should consider accurate alternative methods for routine use.

Iyad et al., (2006) investigated for hospital acquired infections in pediatric hospital settings at Karachi from July to December 2001. They isolated 124 isolates of *P. aeruginosa* and other *Pseudomonas sp.*, *Staphylococcus aureus* (MRSA/MSSA) and *Klebsiella* species, and stated that they are the commonest pathogen among the nosocomial infection causing organisms. The used kirby bar disc diffusion method, for antibiotic sensitivity and found Imipenem, Meropenem, Amikacin, Vancomycin (especially in MRSA), Fucidic acid (for burns and other infections) and some of the 3rd generation cephalosporin's are very effective.

3. MATERIALS AND METHODS

3.1. Samples Collection;

Samples used for the study were collected from the Near East University Hospital lefkosa Nicosia, North Cyprus in the duration of June 2014 to October 2015. A Total (n=152) samples were collected from the different wards such as (Neurosurgery, Ear Nose and Throat (ENT), Cardiology, Cardiovascular surgery, Plastic surgery, Gynecology, Pediatrics, Orthopedics, Medical Oncology, Geriatrics, Neurology, Urology, Chest Diseases, Internal Medicine, Infectious Diseases and Clinical Microbiology, Nephrology, Physiotherapy, Dermatology and Emergency services). These samples included the samples of urine, blood, nasal swab, sputum; aspiration Fluids, IV catheter and wound culture were investigated for *P. aeruginosa.*

The demographic information (age, sex) were obtained from the patient's medical record. The sensitivity pattern of Gram-negative bacilli was determined against commonly used antibiotics using BD-Phoenix instrument and disc diffusion method. The samples were labeled accordingly and were subjected for screening of *P. aeruginosa.*In Microbiology Laboratory at Near East University Hospital, Nicosia North Cyprus.

3.2. PURE CULTURE ISOLATION

All the samples were first processed to get pure culture by sub culturing using selective and differential media as EMB agar, MacConkey agar, MSA agar and Nutrient agar.

3.2.1. Nutrient Agar

Nutrient agar from OXIDE private limited was used. The media was prepared according to protocol provided by the company and autoclaved at 121°C for 20 minutes. After autoclave 25 ml of the media were poured into sterilized Petri plates (99 mm in diameter) under aseptic condition to leave it to get solidified the media after we recultured all *Pseudomonas aeruginosa* old culture through nutrient agar and incubated the plates at 37°C for 24 hours.

3.2.2. Eosin methylene blue (EMB) media

Eosin ethylene blue agar from OXIDE private limited was used. The media was prepared according to the protocol provided by the company and autoclaved at 121°C for 20 minutes. After autoclave 25 ml of the media were poured into sterilized Petri plates (99 mm in diameter) under aseptic condition. After media get solidified sample were inoculated under aseptic condition using sterile inoculating loop. After inoculation the plates were incubated at 37°C overnight.

3.2.3. Mannitol salt agar (MSA)

Mannitol salt agar from OXIDE private limited was used. The media was prepared according to company guideline and autoclaved at 115°C for 20 minutes. After autoclave, poured 25 ml of the media into sterilized Petri plates (99 mm in diameter) under aseptic condition, and kept under aseptic condition for 30 minutes so that the media get solidified. After solidification of media sample were inoculated under aseptic condition using sterile inoculating loop. The plates were incubated at 37 °C overnight.

3.2.4. MacConkey agar

MacConkey agar from OXIDE private limited was used. The media was prepared according to the protocol provided by the company and autoclaved at 121 °C for 20 minutes. After autoclave 25 ml of the media were poured into sterilized Petri plates (99 mm in diameter) under aseptic condition. After media get solidified samples were inoculated under aseptic conditions using sterile inoculating loop. After inoculation the plates were incubated at 37°C overnight.

3.2.5 Gram staining

Using sterile techniques, a smear of each isolate was prepared, dried and heat fixed on glass slides. The smear was flooded with crystal violet and allowed for one minute. It was then washed with distilled water and flooded with Gram's Iodine and allowed for one minute. Then it was washed with distilled water, decolorized with 95% ethyl alcohol and again washed with distilled water. After that, it was counter stained with safranin for 45 seconds and washed with

distilled water. The slide was dried and examined under compound microscope at 100 X using oil emulsion.

3.3. BIOCHEMICAL TESTS

3.3.1. Preparation of cell suspension

Cell suspension was prepared for running biochemical tests. Cell suspension was prepared in saline water (0.85% NaCl) and compared with McFarland turbidity standard solution (Gomes et al., 2001).

3.3.1. Catalase test

Catalase test was used for detection of catalase enzyme. This test was performed by taking 2-3 ml of hydrogen peroxide. Then take a colony of bacterial culture from nutrient agar plate by using glass or wood stick and put on hydrogen peroxide. Production of bubbles was considered as positive result (Saginur et al., 1982).

3.3.2. Oxidase test.

This test was performed by soaking a piece of filter paper using oxidase reagents. Pick some fresh growth from the culture plate with a disposable loop or stick and rub onto the filter paper. Examine for blue colour within 10 seconds for positive test (Tarrand et al., 1982)

3.3.3. Indole production test

Using sterile techniques experimental organism was inoculated into its appropriately labeled deep tube containing motility Indole urea (MIU) media with the help of wire loop. The tubes were incubated for 24 hours at 37°C. After that add Kovac's reagent and observe red colour within 10 min (Heizmann et al., 1988).

3.3.4. Citrate utilization test

Using sterile techniques, organisms were inoculated into Simmons citrate agar by mean of streak inoculation. Cultures were incubated for 24 hours at 37°C. Observed the change in colour of media from green to blue colour.

3.3.5. Motility test

Inoculated tubes contain semisolid nutrient agar with a pure culture by stabbing the center of the column of medium to greater than half the depth. Tubes were incubated for 24-48 hours at 35°C in an aerobic atmosphere.

3.3.6. Preparation of the inoculums

Bacterial suspension was prepared in 5 ml normal saline (0.85%NaCl solution). For this purpose fresh culture of 24 hours old was used. 2 to 3 well isolated colonies were taken with the help of platinum wire loop. After shacking the bacterial suspension was compared to 0.5% McFarland standard.

3.4. ANTIBIOTIC SENSITIVITY TEST

3.4.1. EUCAST Disk Diffusion Test

Since from 2011, more and more countries, mainly in Europe, have adopted the EUCAST clinical breakpoints and the EUCAST disk diffusion test. EUCAST encourages laboratories with expertise in susceptibility testing to participate in a network of collaborating laboratories interested in contributing to the development and maintenance of the disk diffusion test. With this network, the financial support of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and the support and interest of National Antimicrobial Susceptibility Testing Committees (NACs), the future of the EUCAST disk diffusion method is secured. Automated susceptibility testing may relieve laboratories of some AST work, but their lack of versatility, the unavailability of some agents and tests for some species, and their long development times, still favour the use of disk diffusion testing for many years to come. Matuschek E et al., (2013).

3.4.2. Inoculum Preparation

Inoculum was prepared by making a direct broth of isolated colonies selected from an 18-24 hours nutrient agar cultured plates. The suspension was adjusted to match the 0.5 McFarland turbidity standards, using saline and a vortex mixer.

3.4.3. The BD Phoenix System.

The BD Phoenix System consists of an instrument, software, disposable panels, broths for ID and AST, and an AST indicator. The ID method employs modified conventional, fluorogenic, and chromogenic substrates. The AST method is a broth based micro dilution test that utilizes a redox indicator to enhance the detection of organism growth. The NMIC/ID-26 panels were used in this study. A 0.5 McFarland suspension of the test organism was made in the ID broth. The Crystal Spec Nephelometer was used to verify the density of the suspension and 25µL of this suspension was added to the AST broth. One drop of AST indicator was previously added to the AST broth. The suspension in the ID broth was used to inoculate the ID wells of the panel and the suspension in the AST broth was used to inoculate the AST wells. After loading the panels into the instrument, the panels are read at 20-minute intervals by the instrument. IDs, minimal inhibitory concentrations (MICs), and category interpretations are generated. Organism identification is used in the interpretation of the MIC values of each antimicrobial agent producing Susceptible, Intermediate, or Resistant (SIR) result classifications. Final results are available in 2-12 hours for ID and 4-16 hours for AST, however, the majority of IDs were completed in 2-3 hours and MICs in 6-8 hours. The Phoenix system also includes the BD Xpert system software which analyzes ID and AST results against pre-defined rules and notifies the user of atypical results and patient conditions that may require further action. Nadarajah R et al., (2004).

3.4.4. Antimicrobial Susceptibility tests using BD phoenix

After the addition of Phoenix AST Indicator Solution to the AST broth tubes, mix by inversion. DO NOT VORTEX. Overtaxing may cause air bubbles to form in the AST broth, which can result in inappropriate filling of the Phoenix panel during inoculation. Because of the low

probability of occurrence or special growth requirements, some organisms included in the ID taxa are not included in the AST database. These organisms will display the message "Organism not included in the AST database, perform alternate method."

For some organism/antimicrobial combinations, the absence of resistant strains precludes defining any result categories other than "susceptible." For strains yielding results suggestive of a "no susceptible" category, organism identification and antimicrobial susceptibility test results should be confirmed. Subsequently, the isolates should be saved and submitted to a reference laboratory that will confirm the result using the CLSI reference dilution method.

3.5. PERFORMANCE CHARACTERISTICS

3.5.1. Gram Negative Identification

In two internal studies, the performance of the Phoenix Gram Negative identification was evaluated. The 0.5 inoculum density configuration and the 0.25 inoculum density configuration were tested with 165 strains (0.5) respectively. Enteric and non-enteric results were evaluated against commercial and non-commercial methods.

3.5.2. The Phoenix Gram Negative identification performance is outlined below:

	McFarland	Agreement	No Agreement	No ID
Species level	0.5	95.6%	3.6%	0.8%
	0.25	98.1%	1.4%	0.5%

An internal study was performed to simulate inter-site reproducibility. The identification results obtained using the Phoenix system was compared with expected results. This performance testing demonstrated intra-site and inter site reproducibility of at least 95% or greater.

3.5.3 Statistical Analysis:

After collecting the data were successfully analyzed through SPSS version 22 Statistical consults and the results will be compare to literature.

4. Results and Discussion

A Total (n=152) samples were collected from the different wards such as (Neurosurgery, Ear Nose and throat (ENT), Cardiology, cardiovascular surgeries, plastic surgery, Gynecology, Pediatrics, Orthopedics, Medical Oncology, Geriatrics, Neurology, Urology, Chest Diseases, Internal Medicine, Infectious Diseases and Clinical Microbiology, Nephrology, Physiotherapy, Dermatology and Emergency services).These samples included the samples of wound, Blood, Urine, Ear Nose and Throat swab, CSF, Sputum, Aspiration Fluids, all the isolated samples were *Pseudomonas* positive and were further screened for Antibiotic sensitivity was tested by Minimum inhibitory concentration(MIC) Method. The sensitivity pattern of Gram-negative bacilli was determined against commonly used antibiotics using BD-Phoenix instrument. The antibiotic susceptibility was determined according to CLSI standards *Pseudomonas spp* were also identified bases on their cultural, microscopic, morphological and biochemical characteristics. The distributions of *P. aeruginosa* isolates from different specimens were shown in figure (4.1).

In this study, with regards to gender, 152 (62.5%) subjects were male while 152 (37.5%) were female. The ages of the gender was ranged from less than 10> to more than 67< years old which shows in (Figure 4.3) and (Figure 4.4) in detail. The Antimicrobial susceptibility testing revealed that P. *aeruginosa* were highly sensitive to most of the antibiotics tested which are given in the table (4.1). The percentage of sensitivities were Amikacin (81.5%), Piperacillin Tazobactam (92.5%), Colistin (86.7%),Ticarcillin Clavuanate (86.6%), Imipenem (80.8%), Meropenem (87.2%),Cefepime (78.4%),Ceftazidime (76.0%), Ciprofloxacin(73.2%), Gentamicin (76.0%), Levofloxacin (73.5%), Norfloxcin (79.5%). The resistance rates to Ampicillin Sulbactum were found to be (98.7%), Cefoxitin (94.7%), Ceftriaxone (93.8%), Trimethoprim Sulfamethoxazole (94.7%), Amoxicillin (100.0%), Cefuroxime (97.7%), and Nitrofurantion (97.7%), in case of urine disorder we use nitrofurantion and norfloxcin antibiotics which are susceptible to microbial infection.

P. aeruginosa emerged as important pathogenic bacteria which is responsible for nosocomial infections. It is one of the important causes of morbidity and mortality among hospitalized

patients. *P. aeruginosa* in hospital infections is due to its resistance to common antibiotics and antiseptics, and its ability to establish itself widely in hospitals. Being an extremely adaptable organism, it can survive and multiply even with minimum nutrients, if moisture is available as P. *aeruginosa* causes serious diseases, and is one of the leading causes of nosocomial infections, various studies were carried out to detect antibiotic sensitivity pattern for the different drugs available such study helps clinicians for the better management of patients. In the present study sex wise prevalence of clinical isolates shows that infections caused by *P. aeruginosa* are more common in males (62.5%) compared to females (37.5%). This is comparable with study of Javia et al Jamshaid Ali Khan et al and Rashid *et al* (2007).

In our study, most of the patients age range from less than <20 to more than >60. This is comparable with study of Rajat et al. (2012) and Mohan et al. (2013). Our present study maximum resistant isolates of *P. aeruginosa* were isolated from urine samples all of the isolates of *P. aeruginosa* were resistant to Amoxicillin (100.0%) to Ampicillin Sulbactum (98.7%), to Cefoxitin (94.7%), to Ceftriaxone (93.8%) to, Trimethoprim Sulfamethoxazole to (94.7%), to Cefuroxime (97.7%), and Nitrofurantion (97.7%), in our study, highly sensitive antibiotics to *P. auerginosa* Amikacin (81.5%), Piperacillin Tazobactam (92.5%), Colistin (86.7%),Ticarcillin Clavuanate (86.6%), Imipenem (80.8%), Meropenem (87.2%), Cefepime (78.4%), Ceftazidime (76.0%),Ciprofloxacin(73.2%), Gentamicin (76.0%), Levofloxacin (73.5%), Norfloxcin (79.5%), similar study in Saudi Arabia by Ahmad *et al.,* (2014) also showed 85% of the *P. aeruginosa* isolates sensitive to ciprofloxacin. In our study we found similar results have been reported in a study from Saudi Arabia. Another study by Strateva et al. (2007) reported that clinical isolates of *P. aeruginosa*, resistance to clavulanic acid was 53% and to ticarcilin was 8.22%.the sensitivity pattern of *P. aeruginosa* antibiotics appeared as 100% sensitivity imipenem, meropenem, Ceftazidime, Polymyxin-B and colistin. One earlier study cited by Gales *et al* 2002 shows that the meropenem was the most effective antibiotic against *P. aeruginosa* Approximately half the isolates tested were from community patients mostly from infections of the wound/pus (22.46%), urinary tract (22.11%), swab (18.6%) and respiratory tract (15.09%). Tirodimos et al 2010 also found that, resistance rate to imipenem, meropenem, and aztreonam was 2.2%.

In a study conducted in 2001 by J. G. Pi'eboji at the Yaound'e Central Hospital, ceftazidime sensitivity was 71.6% and imipenem was 90%For Paramythiotou et al., (2004). The rate of

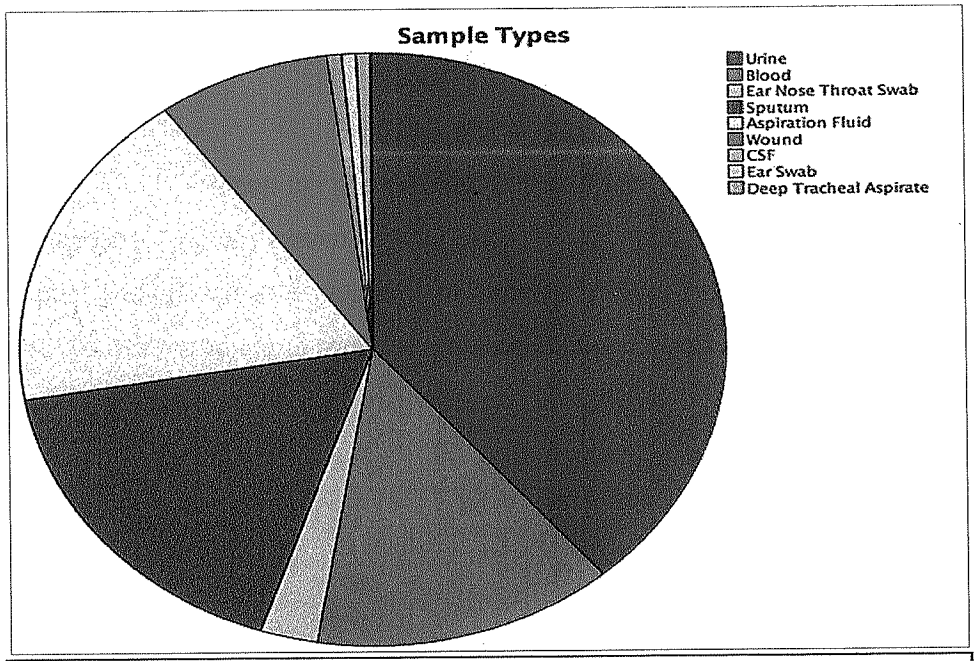
resistance to ceftazidime and imipenem was 10.5%.Contrary to reports, so far about Amikacin being 90% sensitive, our study has found it resistant in more than 50% of the isolates. Ceftazidime has been found to be more efficacious that other cephalosporins in urinary isolates which has been reported earlier also by Chopra GS et al in a similar type of study Sivaraj s et al 2012 among the seven antibiotics, maximum sensitivity was found with imipenem (82%) followed by amikacin (72%) while other drugs showed decrease in susceptibility pattern. Maximum sensitivity was demonstrated by these drugs in comparison to other antibiotics used in our study.

Similarly study by Farhat et al., (2009) reported ESBL producing *P. aeruginosa* that have 99% susceptibility to meropenem, 96% to imipenem, 70% to amikacin, 25% to gentamicin, 49% to ciprofloxacin, 47% to enoxacin, 21% to doxycycline and 16% to co-trimoxazole. This shows an increase in resistance of GNRs to meropenem and Trimethoprim / Sulphamethoxazole . Cephalothin was recommended as a drug of choice to treat both ESBL producing and non-producing GNRs.

Another similar study in Cyprus by European Antibimicrobial Resistance Surveillance study (EARSS) in (2009) that resistance to Piperacillin ± tazobactam was (19.6 %), Ceftazidime (23.5%), Fluoroquinolones (13.7%), Aminoglycosides (15.7 %), and Carbapenems (43.1 %) Similar result by Ekrem K et al 2014 in Iraq was determined that isolates from various clinical samples are sensitive to gentamicin (54.7 %) followed by amikacin (62.7%), imipenem (96%), meropenem (98.7%), ceftazidime (82.7%), piperacillin (70.7%), tobramycin (69.3 %), ciprofloxacin (73.3 %), ceftriaxon (8%), and cefotaxime (0%).

Another study report by (EARSS) in 2008 shows that Resistance to piperacillin-tazobactam (8.3%) in the Scottish isolates of *P. aeruginosa* was reported for the UK (1.3-5.4% in 2005-2008) and Wales (3.9% in 2007) Resistance to ceftazidime in *P. aeruginosa* was 8.3% among the Scottish isolates, while most recent figures reported from the UK and Wales were 5%.Resistance to carbapenems was 5.3% among the Scottish isolates of *P. aeruginosa*, which is within the same range as reported for the rest of the UK (6.4%) and Sweden (4.0%) in 2008.

Figure No4.1: the below pie diagram showing samples wise distribution of understudy specimens



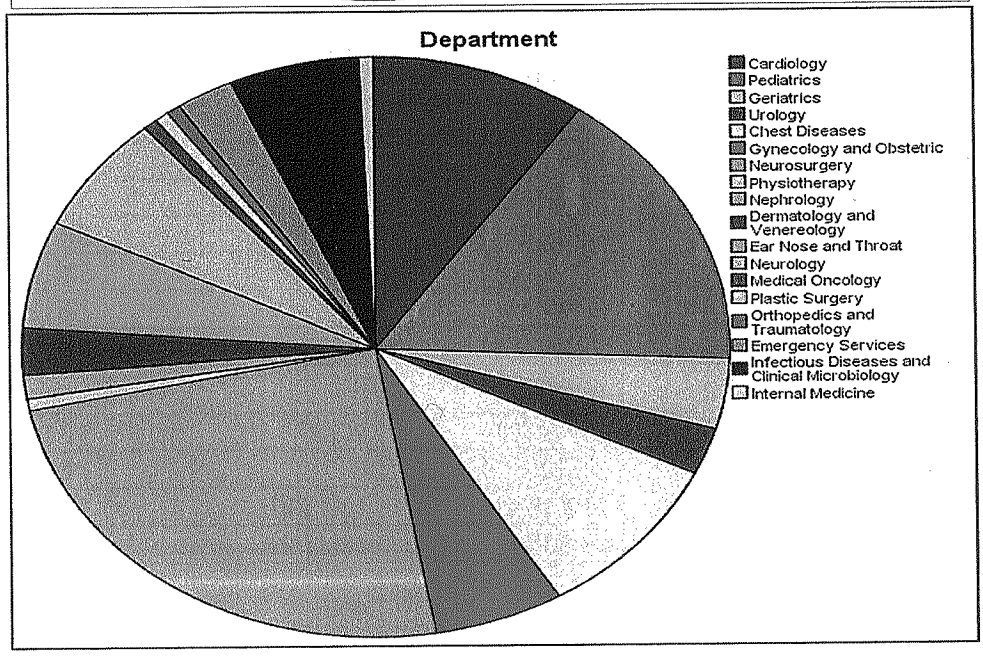


Figure No 4.2: The above pie diagram showing Department wise distribution of understudy sample

Table4.1: Antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* isolated from various clinical samples

DRUGS	TOTAL	SENSITIVE COUNT (%)	INTERMEDIATE COUNT (%)	RESISTANT COUNT (%)
Amikacin	151	123(81.5)	8(5.3)	20(13.2)
AmpicillinSulbactam	151	2(1.3)	00	149(98.7)
Aztreonam	152	76(50.0)	18(11.8)	58(38.2)
Cefepime	148	116(78.4)	19(12.8)	13(8.8)
Cefoxitin	151	8(5.3)	0	143(94.7)
Ceftazidime	150	114(76.0)	5(3.3)	31(20.7)
Ceftriaxone	113	7(6.2)	0	106(93.8)
Ciprofloxacin	149	109(73.2)	5(3.4)	35(23.5)
Colistin	113	98(86.7)	0	15(13.3)
Gentamicin	146	111(76.0)	8(5.5)	27(18.5)
Levofloxacin	113	83(73.5)	4(3.5)	26(23.0)
Imipenem	151	122(80.8)	7(4.6)	22(14.6)
Meropenem	149	130(87.2)	6(4.0)	13(8.7)
PiperacillinTazobactam	148	135(92.5)	0	17(11.5)
TicarcillinClavuanate	144	64(86.6)	0	46(42.6)
Trimethoprim Sulfamethoxazole	150	7(4.7)	1(.7)	142(94.7)
Amoxcicillin	152	0	0	47(100.0)
Cefuroxime	44	0	0	43(97.7)
Nitrofurantion	44	1(2.3)	0	43(97.7)
Norfloxcin	44	35(79.5)	3(6.8)	6(13.6)

The above table demonstrates Sensitivity, Intermediate and Resistance (SIR) percentage to various antibiotics which are used against understudy samples in the present study Amoxicillin is (100%) Ampicillin Sulbactum (98%) resistance to *pseudomonas aeruginosa*, and Piperacillin Tazobactam (92.5%) Meropenem (87.2%) Ticarcillin Clavuanate (86.6%) Colistin (86.7%) was sensitive to above antibiotics which are given in table no (4.1) in detail.

Table 4.2. MIC Breakpoint Values (µg/ml) determine Susceptibility to antibiotics against *Pseudomonas aeruginosa*

Drugs	Total	Breakpoint values(µg/ml)		
Amikacin	151	<=8	16	>=32
AmpicillinSulbactum	151	<=4	8	>=16
Aztreonam	91	<=4	8	>=16
Cefepime	148	<=1	8	>=16
Cefoxitin	151	<=4	8	>=16
Ceftazidime	152	<=1	4	>=16
Ceftriaxone	152	<=1	16	>=32
Ciprofloxacin	152	<0.5	1	>=2
Colistin	152	<=1	2	>=4
Gentamicin	146	<=2	4	>=8
Levofloxacin	152	<=1	2	>=4
Imipenem	152	<=1	2	>=8
Meropenem	152	<=0.5	1	>=8
PiperacillinTazobactam	152	<=4	32	>=64
TicarcillinClavuanate	152	<=8	64	>=128
Trimethoprim Sulfamethoxazole	150	<=19	38	>=76
Amoxcicillin	48	<=4	8	>=16
Cefuroxime	45	<=1	4	>=8
Nitrofurantion	152	<=8	32	>=64
Norfloxcin	152	<=2	4	>=8

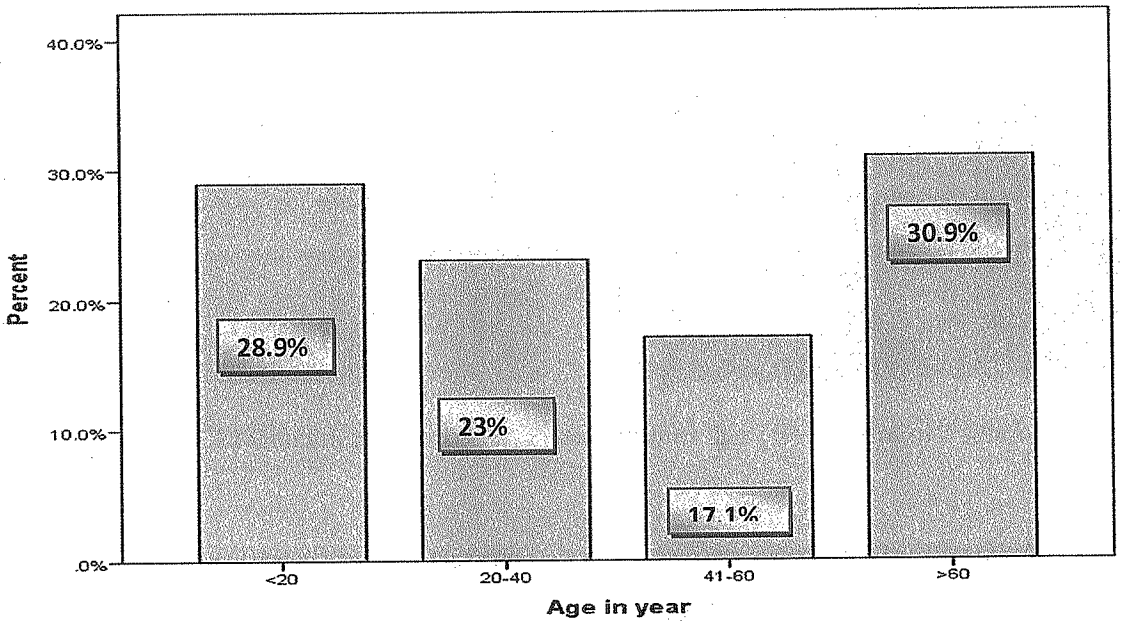
The above table demonstrates three different values of the given antibiotics which are the lowest intermediate and highest values showing susceptibility to *pseudomonas aeruginosa.*

Table 4.3. Minimum inhibitory concentrations (MICs %) of various Antibiotics against *Pseudomonas aeruginosa*

DRUGS	TOTAL	MIC Breakpoint Values (µg/ml) %		
Amikacin	151	108(71.5)	11(7.3)	32(21.2)
AmpicillinSulbactum	151	5(3.3)	6(4.0)	140(92.7)
Aztreonam	152	19(12.5)	42(27.6)	41(59.9)
Cefepime	148	14(9.5)	51(33.8)	34(23.0)
Cefoxitin	151	7(4.6)	6(4.0)	138(91.4)
Ceftazidime	150	26(17.3)	86(57.3)	38(25.3)
Ceftriaxone	113	16(14.2)	62(54.9)	35(31.0)
Ciprofloxacin	149	109(73.2)	12(8.1)	28(18.8)
Colistin	113	94(83.2)	7(6.2)	12(10.6)
Gentamicin	146	86(58.9)	26(17.8)	34(23.3)
Levofloxacin	113	65(57.5)	22(19.5)	26(23.0)
Imipenem	151	67(44.4)	53(35.1)	31(20.5)
Meropenem	149	85(57.0)	40(26.8)	24(16.1)
PiperacillinTazobactam	148	78(52.7)	47(31.8)	23(15.5)
TicarcillinClavuanate	152	51(46.4)	20(18.20)	39(35.5)
Trimethoprim Sulfamethoxazole	152	16(10.9)	36(24.5)	95(64.6)
Amoxcicillin	104	7(14.6)	3(6.3)	37(77.1)
Cefuroxime	45	3(6.7)	42(93.3)	40(30.3)
Nitrofurantion	152	44(100.0)	48(3.3)	36(4.2)
Norfloxcin	44	29(65.9)	8(18.2)	7(15.9)

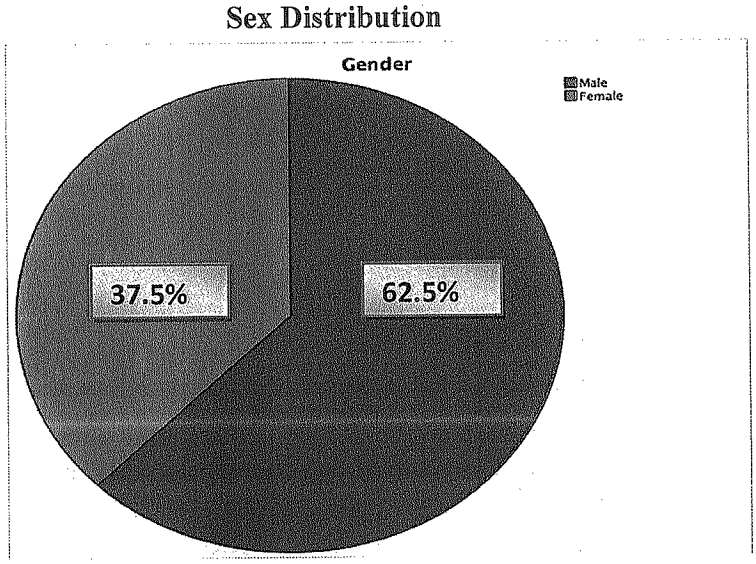
In the above table shows Minimum inhibitory concentrations (MICs) to 24 clinically-relevant antimicrobial agents for *pseudomonas aeruginosa* (MICs) values are different like Susceptibility lower bound susceptibility upper bound and intermediate susceptibility breakpoint zone respectively.

Figure No 4.3: *Pseudomonas aeruginosa* isolates among different patient age groups



The above figures demonstrate percentage of different age patients which are susceptible to variouse groups of antibiotics.

Figure No 4.4: Sex wise distribution of the isolation score among the study population



The above figure demonstrates percentages of sex wise distribution which shows male are more susceptible then female.

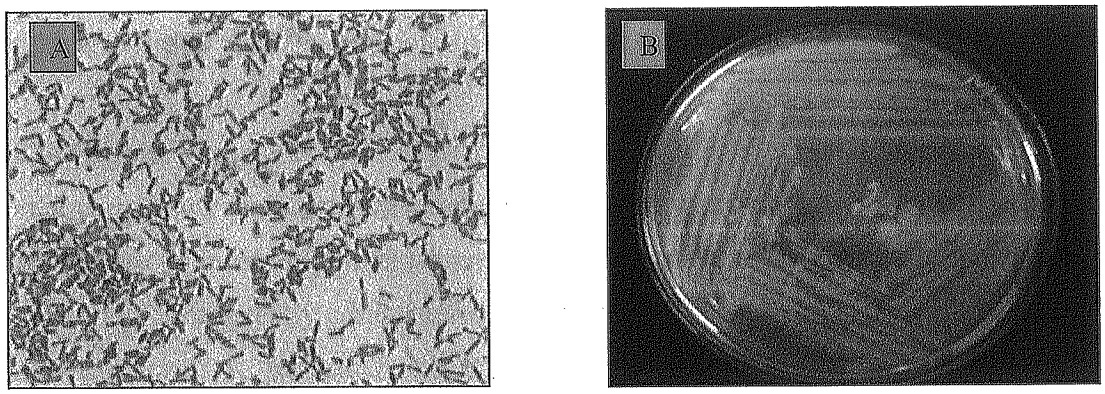


Figure 4.5 (A) Gram negative rods of *Pseudomonas* spp **(B)** Culture of *Pseudomonas* spp

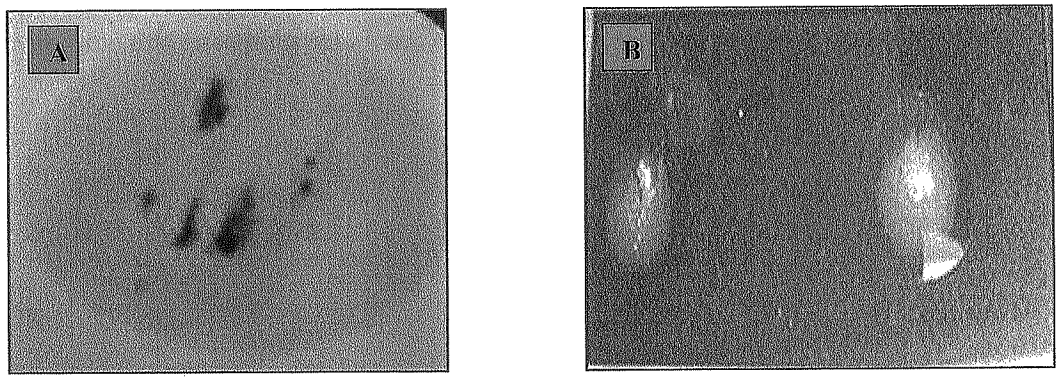


Figure 4.6. (A) Oxidase test **(B)** Catalase test

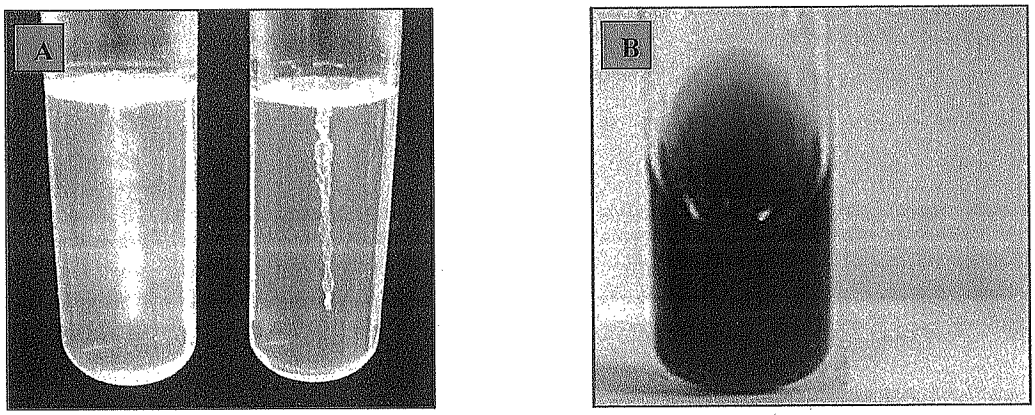


Figure 4.7(A): Motility test **(B)** Citrate utilization test

5. CONCLUSION

The present study shows that the clinical isolates of *Pseudomonas aeruginosa* are becoming resistant to commonly used antibiotics and also achieving more and more resistance to newer antibiotics. In Cyprus Piperacillin, Tazobactam, Ceftazidime, Colistin was less resistance against *Pseudomonas aeruginosa* for practicing physicians, medical microbiologists and public health officials, knowledge of local antimicrobial resistance patterns is necessary to guide empirical therapy. More antibiotics recently administered in our hospitals should be included in the study to determine the level of resistance to microorganisms. Regarding treatment imipenem, meropenem, ciprofloxacin, ceftazidime, and amikacin may be beneficial to control the difficult to treat *P.aeruginosa* infections in local area to combat the seriousness of *pseudomonal* infection. It is the need of time that antibiotic policies should be formulated and implemented intensely to resist and overcome this emerging problem.

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APPENDIX

1. List of Chemicals and Equipments

S.N0	Chemicals	Manufactured
1	Eosin Methylene Blue Agar	OXIDE
2	Nutrient Agar (NA)	OXIDE
3	Macconkey Agar	OXIDE

1	BD Phonex TM	USA
2	Laminar flow-hood	K&K scientific supplier Korea
3	Incubater	Pansonic UK
4	Electronic balance	Kern Germany
5	Autoclave	Wisd Korea
6	Hot plate stirrer	Jenway England
7	Microscope	Motic BA210 USA-CANADA

2. Nutrient Agar Media (OXIDE)

This media is best prepared from ready to use dehydrated powder.

Nutrient agar is usually used at concentration of 28g per liter of distilled water.

Ingredients	Gm/ Litre
Peptic digest of animal tissue	5.000
Sodium chloride	5.000
Beef extract	1.500
Yeast extract	1.500
Agar	15.000
Final pH (at 25°C)	7.4±0.2

1. Suspend 28 grams in 1000 ml distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Dispense as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
4. Mix well before pouring.

3. Eosin Methylene Blue Agar (OXIDE)

Ingredients	Gm/ Litre
Pancreatic Digest of Gelatin	10.0 g/L
Lactose	5.0 g/L
Sucrose	0 g/L
Dipotassium Phosphate	2.0 g/L
Eosin Y	0.4 g/L
Methylene Blue	65.0 Mg
Agar	13.5 g/L

4. Macconkey Agar(OXIDE)

Ingredients	Gm/ Litre
Pancreatic Digest of Gelatin	17.00g/L
Bile Salts	1.50 g/L
Lactose Monohydrate	10.00 g/L
Neutral Red	0.03 g/L

Sodium Chloride	5.00 g/L
Crystal Violet	0.001g g/L
Peptones (Meat & Casein)	3.00 g/L
Bacteriological Agar	13.50g/L

5. **Gram's staining reagents and preparation:**

Reagents:

Crystal violet, the primary stain, iodine, the mordant, a decolorizer made of acetone

And alcohol, safranin, the counter stain.

Ingredients	**Amount**
a. Crystal Violet, Stain	
Crystal Violet	20 gm
Ammonium Oxalate	8 gm
Ethanol, Denatured	200 ml
Water, deionized	800 ml
b. Iodine, Mordant	
Iodine	3.3 gm
Potassium Iodine	6.6 gm
Water, deionized	1000 ml

c. **Alcohol-Acetone, Decolorizer**	
Ethanol, denatured	500 ml
Acetone	500 ml
d. . **Safranin, Counter stain**	
Safranin O	2.5 gm
Ethanol, denatured	100 ml
Water, deionized	900Ml

6. <u>Peptone Water</u> (Per Liter)

Peptone	10 g
Sodium Chloride	5 g