CHAPTER I
INTRODUCTION

Pseudomonas aeruginosa is an opportunistic pathogen that is able to cause severe invasive diseases in critically ill and immunocompromised patients (Deplano *et al*., 2005). It continues to be a major cause of opportunistic nosocomial infections, causing around 9-10% of hospital infections (Hancock *et al*., 1996). In the US National Nosocomial Infections Surveillance (NNIS) system report, *P. aeruginosa* continues to represent the third most frequent organism associated with wound or pulmonary infections, the fourth most frequent organism causing urinary tract infection, and the fifth most frequent organism isolated from blood cultures in cases of septicemia (Richards *et al*.,2000).

P. aeruginosa is a gram negative rod measuring 0.5 to 0.8 um by 1.5 to 3.0 um. Almost all strains are motile by means of single pollar flagella. It is ubiquitous, with a predilection to most environments, primarily as waterborne and soil borne organism. *P. aeruginosa* is an extremely adaptive organism. It can grow on a wide range of subtrates and quickly respond to environmental alteration (Lambert, 2002). It has a large genome (6.26 Mbp encoding 5,567 genes) compared to other common human opportunistic pathogens such as *Escherichia coli* K12, *Haemophilus influenza* and *Staphylococcus aureus* N315 (Lambert, 2002).

Hospital acquired infections constitute a major fraction of the adverse events complicating hospital treatment (Gastmeier, 2004) The last two decades have witnessed significant changes in the spectrum of microorganisms causing nosocomial infections, gram negative enterobacteria gradually replaced by gram positive microorganisms such as *Staphylococci, enterococci* and *corynebacteria*. Inspite of these significant changes, *P. aeruginosa* has held a nearly unchanged position in the rank order of pathogens causing ICU- related infections for more than 4 decades (Spencer, 1996; Pfaller *et al*., 1999).

There have been a lot of recent advances in medicine, such as, the advent of more elaborate surgery and intensive care, the use of broad-spectrum antibiotics and immunosuppressive drugs, the availability of invasive procedures or instrumentations

and the increase in the number of immunocompromised patients (e.g. oncology patients on cytotoxic therapy / radiotherapy, patients with organ transplants and even patients with AIDS). A direct consequence of this is that there are rise in patients with impaired immune defenses (Hugbo *et al*., 1992) thereby leading to an increase in nosocomial infections especially by Gram-negative organisms such as *Pseudomonas.* Such organisms may be found in the patient's own flora, or in damp environmental sites or hospital equipments and medicaments. They exhibit natural resistance to many antibiotics and antiseptics in which they may survive for long periods, and may even multiply in the presence of minimal nutrients and have the ability to colonize traumatized skin (Richard, 1994).

According to the CDC (Centers for disease control), the overall incidence of *P. aeruginosa* infections in US hospitals averages about 0.4 percent(4 per 1000 discharges) and the bacterium is the fourth most commonly isolated nosocomial pathogen. It is the leading cause of nosocomial pneumonia and among the most virulent of the opportunistic pathogens (Salyers and Whitt, 1994). Upto 75% of all intensive care unit patients are colonized by *P. aeruginosa* and the mortality from Pneumonia is 50% even with antibiotic treatment (Malangoni *et al*., 1994). *P. aeruginosa* infection is also known to be a serious problem in patients hospitalized with cystic fibrosis, cancer, and burns. Half of these infections are fatal.

For an opportunistic pathogen such as *P. aeruginosa*, the disease process begins with some alteration or circumvention of normal host defenses. The bacterium never infects uncompromised tissues, yet there is hardly any tissue that it cannot infect if the tissue defenses are compromised in some manner. The ability of *P. aeruginosa* to invade tissues depends upon production of extracellular enzymes and toxins that break down physical barriers and damage host cells. The infections caused by the bacterium are endocarditis, respiratory infections, bacteremia and septicemia, central nervous system infection, ear infections including external otitis, eye infections, bone and joint infections, urinary tract infections, gastrointestinal infections, skin and soft tissue infections, including wound infections, pyoderma and dermatitis.

P. aeruginosa is notorious for its resistance to antibiotics and is, therefore, a particularly dangerous and dreaded pathogen. It has high intrinsic resistance to many antibiotics, antibiotics likely to most effective are the Aminoglycosides and

antipseudomonal Penicillins such as Ticarcillin, Azlocillin and Piperacillin, newer agents includes the Carbapenems, Imipenem, Meropenem and Monobactum. Of the Cephalosporins Ceftazidime has proved to be useful one and Quinolones in particular Ciprofloxacin, have provided a major advance as the first highly active antipseudomonal agents. The bacterium's resistance to antibiotics and disinfectants have been attributed to 1) intrinsic resistance to a wide variety of antimicrobial agents due to low membrane permeability, 2) genetic capacity to express a wide range of resistance mechanisms 3) acquisition of resistance to antibiotics through chromosomal mutation, and 4) acquisition of resistance genes from other organisms via plasmids, bacteriophages, and/or transposons (Lambert, 2002). The natural resistance to many antimicrobial agents is also due to active efflux systems that are present in all wild-type strain and chromosomal lactamase genes (Aires *et al*., 1999; Lambert, 2002; Livermore, 2002).

A major reason for its prominence as a pathogen is its high intrinsic resistance to antibiotics, such that even for the most recent antibiotics, a modest change in susceptibility can thwart their effectiveness (Hancock, 2000).

Excessive use of broad-spectrum antibiotics in hospitals has led to the emergence of highly resistant strains of *P. aeruginosa*. To reduce the selection pressure for resistance, it is important to determine the antibiotic susceptibility pattern of bacteria so that hospital patients can be treated with more narrow spectrum and target-specific antibiotics. Further the prevalence and sensitivity of *P. aeruginosa* often varies between communities, hospitals in the same community and among different patient population in the same hospital (Sexton, 2000). Faced with these variations, the physician in clinical practice should have asses to recent data in the prevalence and antimicrobial resistance pattern of the encountered pathogen (WHO, 2001).

National Institute of Neurological and Allied Sciences (NINAS) is established in 2006 to treat the traumatic and severely ill neurological patients. All the wards of NINAS have the facility needed for a severe patient and it follows all the standard international norms for the effective control of microorganisms (Karki, 2010). The purpose of this study was to determine the prevalence of *P. aeruginosa* in the hospitalized patients and to determine its antimicrobial sensitivity patterns from different clinical specimens of patients in ICU, General ward and Cabin of NINAS

over a period from May to December 2010. Secondary goals included comparing the incidence of the Pseudomonal infection with patients of different age groups, gender and their stay in the wards by statistical analysis.

The result of this study will help clinicians to make the most rational choices of empiric antibiotic regimes for this highly notorious pathogen. Additionally, a part of the present study will also help to bring awareness about cleaning and disinfection of the environment as well as improving the personal hygiene of HCWs and admitted patients in NINAS as well as in other hospitals. The present study is the first study at NINAS, assessing the prevalence of *P. aeruginosa* and its antibiotic sensitivity test profile.

CHAPTER II

OBJECTIVES

2.1 General objective

To determine the hospital based prevalence of *Pseudomonas aeruginosa* among the indoor patients of National Institute of Neurological and Allied Sciences and describe its antibiotic susceptibility profile.

2.2 Specific objectives

a) To describe *P. aeruginosa* from different clinical specimens collected from the indoor patients.

b) To assess the antibiotic susceptibility pattern of the isolates.

c) To determine proportion of the multidrug resistant *P. aeruginosa* and compare MDR pattern of *P. aeruginosa* between ICU and non ICU wards.

CHAPTER III

REVIEW OF LITERATURE

3.1 *Pseudomonas aeruginosa*

The Pseudomonads are a diverse bacterial group of established and emergent pathogens. Members of the genus are major agents of nosocomial and community acquired infections, being widely distributed in the hospital environment where they are particularly difficult to eradicate (Hugbo *et al*., 1992). *Pseudomonas aeruginosa* is member of the Gamma Proteobacteria class of bacteria. Like other members of the genus, *P. aeruginosa* is a free-living bacterium, commonly found in soil and water. *P. aeruginosa* has become increasingly recognized as an emerging opportunistic pathogen of clinical relevance. Several different epidemiological studies track its occurrence as a nosocomial pathogen and indicate that antibiotic resistance is increasing in clinical isolates (Todar, 2011).

P. aeruginosa is an opportunistic pathogen**,** meaning that it exploits some break in the host defenses to initiate an infection. In fact, it is the epitome of an opportunistic pathogen of humans. *P. aeruginosa* infection is a serious problem in patients hospitalized with cancer, cystic fibrosis, and burns. The case fatality rate in these patients is near 50 percent (Todar, 2011). Isolation of *P. aeruginosa* from healthy carriers or environmental sites is significant only when there is a risk of transfer to compromised patients, e.g. by nurses' hands or via respirators. Normally, human faecal carrier rate for *P. aeruginosa* is less than 10%; however, carrier rates increase with the length of stay of patients in hospital, reaching 30% after 3 weeks, and this represents a distinct risk of endogenous infection. Colonization is often iatrogenic and associated with prior instrumentation, e.g. Catheterization, tracheostomy, etc (Mackei and Mackatney, 2001).

3.2 Characteristics of *P. aeruginosa*

P. aeruginosa is a Gram-negative, aerobic rod which measures 0.5 to 0.8 µm by 1.5 to 3.0 µm. It belongs to the bacterial family *Pseudomonadaceae.* Almost all strains are motile by means of single polar flagella. The bacterium is ubiquitous in soil and water, and on surfaces in contact with soil or water. Its metabolism is respiratory and

never fermentative, but it will grow in the absence of O_2 if NO_3 is available as a respiratory electron acceptor. *P. aeruginosa* has very simple nutritional requirements. It is often observed "growing in distilled water", which is evidence of its minimal nutritional needs. In the laboratory, the simplest medium for growth of *P. aeruginosa* consists of acetate as a source of carbon and ammonium sulfate as a source of nitrogen (Todar, 2011). They are renowned for their metabolic versatility and flourishes as a saprophyte in warm moist situations in the human environment, including sinks, drains, respirators, humidifiers and disinfectant solutions. The temperature for *P. aeruginosa* to survive ranges between 37-42 degree celcius. But it is quite tolerant to temperature conditions and salt concentrations, which explains its environmental adaptability. It is resistant to high concentrations of salts and dyes, weak antiseptics, disinfectants and many commonly used antibiotics. *P. aeruginosa* has a predilection for growth in moist environments, which is probably a reflection of its natural existence in soil and water. The bacteria emits a fruity odor and contains two pigments- pyocyanin and pyoverdin, of which the former is responsible for the 'blue' pus formation and the later is low iron containing pigment that facilitates iron metabolism in the bacteria. These pigments also explain the florescence effect caused by it, when it is exposed to ultra violet radiation (Todar, 2011).

These natural properties of the bacterium undoubtedly contribute to its ecological success as an opportunistic pathogen. They also help explain the ubiquitous nature of the organism and its prominence as a nosocomial pathogen.

3.3 Virulence determinants of *Pseudomonas aeruginosa*

Adhesins: Adhesins are cell-surface components of appendages of bacteria that facilitate bacterial adhesion or adherence to other cells or to inanimate surfaces.

a) fimbriae (N-methyl-phenylalanine pili)
b) polysaccharide capsule (glycocalyx)
c) alginate slime (biofilm)

Invasins

a) Elastase: This is a kind of extracellular protease that breaks down proteins. It breaks down elastin, an elastic fiber that, together with collagen, determines the mechanical properties of connective tissue. Its biological significance for

the infected host also include cleavage of immunoglobin A and C, complement factors and alpha proteinase inhibitor (Peters *et al*., 1992; Galloway *et al*.,1991) and also potent proteolytic activation of human interferon-y and tumor necrosis factors alpha (Parmely *et al*.,1990).

b) Alkaline protease: It also appears to mediate potent, plasmin like anticoagulant activity based on the hydrolysis of fibrin and fibrinogen (Shibuya *et al*., 1991).

c) Hemolysins (phospholipase and lecithinase): Phospholipase is heat labile protein and exerts an important pathogenic effect in LRTI through enzymatic degradation of the phosphatidylcholine component of lung surfactant, leading to atelectasis and also comtibute on inflammation (Basil *et al*., 1991).

d) Cytotoxin (leukocidin): Cytotoxin is another toxin protein that is cytotoxic to most eukaryotic cells, acts on cell membrane, inhibit polymorphonuclear leukocyte function and may contribute to sepsis associated large injury and the development of acute respiratory distress syndrome (ARDS) (Grimminger *et al*., 1991).

e) Siderophores and siderophore uptake systems: Siderophores are small, high-affinity iron chelating compounds. It is released by the microbes to scavenge iron from the mineral phases by formation of soluble Fe^{3+} complexes that can be taken up by active transport mechanisms and thus rendering the host deprive of iron (Miethke *et al*., 2007).

f) Pyocynin: It is a blue phenazine pigment produced by the bacteria. It impairs the normal function of human nasal cilia(Kumar *et al*., 1993), disrupts respiratory epithelium, exerts proinflamatory effects in human phagocytes possibly neutrophil mediated tissue damage (Raj *et al*.,1990) and acts synergistically with the *Pseudomonas* siderophore ferripyochelin and generates hydroxyl radicals capable of producing endothelial damage (Britigan, 1992).

Motility/chemotaxis: Flagella, Retractile pili.

Toxins:

a) Exoenzyme S: It has the characteristic subunit structure of the A-component of a bacterial toxin, and it has ADP-ribosylating activity (for a variety of eucaryotic proteins) characteristic of many bacterial exotoxins. It contributes to bacterial virulence through impairment of local host defense mechanisms

(Nagaki *et al*., 1992). This extra cellular protein mediates bacterial attachment as well as has toxic and possibly immunosuppressive functions (Atkins *et al*., 1990). It has led to the suggestion that exoenzyme S may act to impair the ufunction of phagocytic cells in the bloodstream and internal organs as a preparation for invasion by *P. aeruginosa*.

b) Exotoxin A: It has exactly the same mechanism of action as the diphtheria toxin; it causes the ADP ribosylation of eucaryotic elongation factor 2 resulting in inhibition of protein synthesis in the affected cell. Although it is partially-identical to diphtheria toxin, it is antigenically-distinct. It utilizes a different receptor on host cells than diphtheria toxin, but otherwise it enters cells in the same manner and has the exact enzymatic mechanism. The production of Exotoxin A is regulated by exogenous iron, but the details of the regulatory process are distinctly different in *C. diphtheriae* and *P.aeruginosa.* Exotoxin A appears to mediate both local and systemic disease processes caused by *P. aeruginosa*. It has necrotizing activity at the site of bacterial colonization and is thereby thought to contribute to the colonization process. Toxinogenic strains cause a more virulent form of pneumonia than nontoxinogenic strains. In terms of its systemic role in virulence, purified Exotoxin A is highly lethal for animals including primates. Indirect evidence involving the role of exotoxin A in disease is seen in the increased chance of survival in patients with *Pseudomonas* septicemia that is correlated with the titer of anti-exotoxin A antibodies in the serum. Also, tox^- mutants show a reduced virulence in some models.

c) Lipopolysaccharide: LPS is the major component of the outer membrane of Gram negative bacteria, it is an endotoxin inducing a strong response from normal animal immune systems. It is also an exogenous pyrogen. *Pseudomonas* lipopolysachharides are biologically less potent than those of other gram negative bacteria.

Antiphagocytic surface properties: Capsules, Slime layers, LPS, Biofilm construction.

Defense against serum bactericidal reaction: Slime layers, capsules, biofilm, LPS, Protease enzymes.

Defense against immune responses: Capsules, slime layers, biofilm, protease enzyme

Genetic attributes

a) Genetic exchange by transduction and conjugation,

b) Inherent (natural) drug Resistance,

c) R factors and drug resistance plasmids.

Ecological criteria

a) Adaptability to minimal nutritional requirements,

b) Metabolic diversity

c) Widespread occurrence in a variety of habitats

3.4 Pathogenesis

For an opportunistic pathogen such as *P. aeruginosa*, the disease process begins with some alteration or circumvention of normal host defenses. The pathogenesis of *Pseudomonas* infections is multifactorial, as suggested by the number and wide array of virulence determinants possessed by the bacterium. Multiple and diverse determinants of virulence are expected in the wide range of diseases caused, which include septicemia, urinary tract infections, pneumonia, chronic lung infections, endocarditis, dermatitis, and osteochondritis (Todar, 2011).

Most *Pseudomonas* infections are both invasive and toxinogenic. The ultimate *Pseudomonas* infection may be seen as composed of three distinct stages: (1) bacterial attachment and colonization (2) local invasion (3) disseminated systemic disease. However, the disease process may stop at any stage. Particular bacterial determinants of virulence mediate each of these stages and are ultimately responsible for the characteristic syndromes that accompany the disease (Todar, 2011).

3.4.1Colonization

Although colonization usually precedes infections by *P. aeruginosa*, the exact source and mode of transmission of the pathogen are often unclear because of its ubiquitous presence in the environment. It is sometimes present as part of the normal flora of humans, although the prevalence of colonization of healthy individuals outside the hospital is relatively low (estimates range from 0 to 24 percent depending on the anatomical locale) (Todar, 2011).

The fimbriae of *Pseudomonas* will adhere to the epithelial cells of the upper respiratory tract and, by inference, to other epithelial cells as well. These adhesins appear to bind to specific galactose or mannose or sialic acid receptors on epithelial cells. Colonization of the respiratory tract by *Pseudomonas* requires fimbrial adherence and may be aided by production of a protease enzyme that degrades fibronectin in order to expose the underlying fimbrial receptors on the epithelial cell surface. Tissue injury may also play a role in colonization of the respiratory tract, since *P. aeruginosa* will adhere to tracheal epithelial cells of mice infected with influenza virus but not to normal tracheal epithelium. This has been called opportunistic adherence, and it may be an important step in *Pseudomonas* keratitis and urinary tract infections, as well as infections of the respiratory tract. The receptor on tracheal epithelial cells for *Pseudomonas* pili is probably sialic acid (N-acetylneuraminic acid). Mucoid strains, which produce an exopolysaccharide (alginate), have an additional or alternative adhesin which attaches to the tracheobronchial mucin (N-acetylglucosamine). Besides pili and the mucoid polysaccharide, there are possibly other cell surface adhesins utilized by *Pseudomonas* to colonize the respiratory epithelium or mucin. Also, it is possible that surface-bound exoenzyme S could serve as an adhesin for glycolipids on respiratory cells (Todar, 2011).

The mucoid exopolysaccharide produced by *P. aeruginosa* is a repeating polymer of mannuronic and glucuronic acid referred to as alginate. Alginate slime forms the matrix of the *Pseudomonas* biofilm which anchors the cells to their environment and in medical situations; it protects the bacteria from the host defenses such as lymphocytes, phagocytes, the ciliary action of the respiratory tract, antibodies and complement. Biofilm mucoid strains of *P. aeruginosa* are also less susceptible to antibiotics than their planktonic counterparts. Mucoid strains of *P. aeruginosa* are most often isolated from patients with cystic fibrosis and they are usually found in lung tissues from such individuals (Todar, 2011).

3.4.2Invasion

The ability of *P. aeruginosa* to invade tissues depends upon production of extracellular enzymes and toxins that break down physical barriers and damage host cells, as well as resistance to phagocytosis and the host immune defenses. As mentioned above, the bacterial capsule or slime layer effectively protects cells from

opsonization by antibodies, complement deposition, and phagocyte engulfment (Todar, 2011).

Two extracellular proteases have been associated with virulence that exerts their activity at the invasive stage: elastase and alkaline protease. Elastase has several activities that relate to virulence. The enzyme cleaves collagen, IgG, IgA, and complement. It also lyses fibronectin to expose receptors for bacterial attachment on the mucosa of the lung. Elastase disrupts the respiratory epithelium and interferes with ciliary function. Alkaline protease interferes with fibrin formation and will lyse fibrin. Together, elastase and alkaline protease destroy the ground substance of the cornea and other supporting structures composed of fibrin and elastin. Elastase and alkaline protease together are also reported to cause the inactivation of gamma interferon (IFN) and tumor necrosis factor (TNF) (Todar, 2011).

P. aeruginosa produces three other soluble proteins involved in invasion: a cytotoxin (mw 25 kDa) and two hemolysins. The cytotoxin is a pore-forming protein. It was originally named leukocidin because of its effect on neutrophils, but it appears to be cytotoxic for most eucaryotic cells. Of the two hemolysins, one is a phospholipase and the other is a lecithinase. They appear to act synergistically to break down lipids and lecithin. The cytotoxin and hemolysins contribute to invasion through their cytotoxic effects on neutrophils, lymphocytes and other eucaryotic cells (Todar, 2011).

One *Pseudomonas* pigment is probably a determinant of virulence for the pathogen. The blue pigment, pyocyanin, impairs the normal function of human nasal cilia, disrupts the respiratory epithelium, and exerts a proinflammatory effect on phagocytes. A derivative of pyocyanin, pyochelin, is a **s**iderophore that is produced under low-iron conditions to sequester iron from the environment for growth of the pathogen. It could play a role in invasion if it extracts iron from the host to permit bacterial growth in a relatively iron-limited environment. No role in virulence is known for the fluorescent pigments (Todar, 2011).

3.4.3Dissemination

Blood stream invasion and dissemination of *Pseudomonas* from local sites of infection is probably mediated by the same cell-associated and extracellular products responsible for the localized disease, although it is not entirely clear how the

bacterium produces systemic illness. *P. aeruginosa* is resistant to phagocytosis and the serum bactericidal response due to its mucoid capsule and possibly LPS. The proteases inactivate complement, cleave IgG antibodies, and inactivate IFN, TNF and probably other cytokines. The Lipid A moiety of *Pseudomonas* LPS (endotoxin) mediates the usual pathologic aspects of Gram-negative septicemia, e.g. fever, hypotension, intravascular coagulation, etc. It is also assumed that *Pseudomonas* Exotoxin A exerts some pathologic activity during the dissemination stage (Todar, 2011).

3.4.4Toxicogenesis

P.aeruginosa produces two extracellular protein toxins, Exoenzyme S and Exotoxin A. Exotoxin A appears to mediate both local and systemic disease processes caused by *P. aeruginosa*. It has necrotizing activity at the site of bacterial colonization and is thereby thought to contribute to the colonization process. Toxinogenic strains cause a more virulent form of pneumonia than nontoxinogenic strains. In terms of its systemic role in virulence, purified Exotoxin A is highly lethal for animals including primates. Indirect evidence involving the role of exotoxin A in disease is seen in the increased chance of survival in patients with *Pseudomonas* septicemia that is correlated with the titer of anti-exotoxin A antibodies in the serum (Todar, 2011).

3.5 Hospital acquired infection

According to World Health Organisation (WHO, 1994), a hospital is a residential establishment that provides short term and long term medical care consisting of observational, diagnostics, therapeutics and rehabilitative services for person suffering from a disease or injury and for parturient. But the hospitalized patients are at unusually high risk of infections. The National Nosocomial Infection Surveillance System (NNIS) defines a NI as a localized or systemic condition that 1) results from adverse reaction to the presence of an infectious agent(s) or its toxin(s) and 2) was not present or incubating at the time of admission to the hospital (NNIS Manual, 1994). For most bacterial NIs, this means that the infection usually becomes evident in 48 hours (i.e., the typical incubation period) or more after admission. However, because the incubation period varies with the type of pathogen and to some extent with the patient's underlying condition, each infection must be assessed individually for evidence that links it to the hospitalization (Garner *et al*., 1988).

Nosocomial infections constitute an important worldwide health problem resulting in high morbidity and mortality as well as economic consequences. It is estimated that out of 100 admissions, approximately 5-6 admission develop nosocomial infections and that in 1995, nosocomial infections cost $4.5 billion and contributed to more than 88000 deaths –one death in every 6 minutes (Weinstein, 1998). WHO in 55 hospitals of 14 countries representing four WHO Regions (Europe, Eastern Mediterranean, South-East Asia and Western Pacific) showed an average of 8.7% of hospital patients had nosocomial infections. At any time, over 1.4 million people worldwide suffer from infectious complications acquired in hospital (Tikhomirov *et al*., 1987). The highest frequencies of nosocomial infections were reported from hospitals in the Eastern Mediterranean and South-East Asia Regions (11.8% and 10.0% respectively), with a prevalence of 7.7% and 9.0% respectively in the European and Western Pacific Regions (Mayon-white, 1988). Nosocomial infections typically affect patients who are immunocompromised because of age, underlying diseases, or medical or surgical treatments. Aging of our population and increasingly aggressive medical and therapeutic interventions, including implanted foreign bodies, organ transplantations, and xenotransplantations, have created a cohort of particularly vulnerable persons. As a result, the highest infection rates are in ICU patients (Weinstein, 1991).

3.6 Epidemiology and Role of *P. aeruginosa* in nosocomial infections

P. aeruginosa is the commonest cause of most of the NIs in the hospital. It accounts for about 11% of all nosocomial infections (Bennett, 1974). Since it causes disease primarily in persons whose health is compromised in some manner, it is considered an opportunistic pathogen. Mechanical ventilation, for instance, predisposes patients to pneumonia caused by *P aeruginosa*. Likewise, the presence of a urinary catheter is associated with an increased risk of urinary tract infections. Patients with cancer who have neutropenia resulting from chemotherapy or hematologic malignancies are prone to bacteremia, and burn patients often experience wound infections (Jarvis, 2003).This nonfermentative multidrug resistant bacterium can survive for long periods in equipments around the patients therefore is responsible for causation of many device associated infections in the hospitalized patients especially in the intensive care unit. It is the commonest pathogen causing VAP as well as bacteraemia, UTIs, etc in the ICU patients (Pollack, 2000). Rello *et al.* (1996)

reported that about 40-50% of the mortality of intubated patients in hospital is caused mainly due to infection by *P. aeruginosa*.

In hospitalized patients exposed to numerous antimicrobial agents, the intrinsic and acquired resistance of this organism undoubtedly confers on it a selective advantage and allows for colonization and subsequent infection. Once established, *P aeruginosa* infections often pose a therapeutic dilemma because of these same properties of resistance (Hauser and Sriram, 2005).

Infections due to *P. aeruginosa* are seldom encountered in healthy adults but in last two decades the organism has become increasingly recognized as the etiological agent in a variety of serious infection in hospitalized patients with impaired immune defense (Smith *et al.*, 2000). Despite advance in medical and surgical care and introduction of wide variety of antimicrobial agents against anti-pseudomonal activities, life threatening infection caused by *P. aeruginosa* continue to cause complications in hospital acquired infections (Mayhall, 1996).

3.6.1 Epidemiology of *P. aeruginosa* in ICUs

P. aeruginosa is one of the most important gram-negative pathogens causing infections in ICUs (Trautmann *et al.*, 2005). The last 2 decades have witnessed significant changes in the spectrum of microorganisms causing nosocomial infections. Gram-negative enterobacteria, which in the 1970s and 1980s accounted for 30% to 50% of all disease-associated isolates in ICUs (Allen *et al.*, 1981), have been to a large extent replaced by gram-positive microorganisms such as staphylococci, enterocci, and corynebacteria (Spencer, 1996). Techniques such as early enteral feeding, use of closed suctioning systems for aspiration of tracheal secretions during mechanical ventilation, and the concept of semiupright positioning of ventilated patients have contributed to create functional or physical barriers between gastrointestinal flora and the respiratory tract. In spite of these significant changes, *P. aeruginosa* has held a nearly unchanged position in the rank order of pathogens causing ICU-related infections for more than 4 decades. In the newest US National Nosocomial Infections Surveillance (NNIS) system report, *P. aeruginosa* continues to represent the third most frequent organism associated with wound or pulmonary infections, the fourth most frequent organism causing urinary tract infection, and the

fifth most frequent organism isolated from blood cultures in cases of septicemia (Richards *et al*., 2000). Studies performed in individual ICUs revealed a significant role of *P. aeruginosa* as a cause of ventilator-associated pneumonia (VAP) (Dupont *et al*., 2001).

A European survey showed that *P. aeruginosa* is one of the most frequent pathogens isolated from ICU-acquired infections (Vincent *et al*., 2000) and data derived from recent multicenter surveillance studies place this microrganism as the first Gram-negative species recovered in ICUs in Belgium (Glupczynski *et al*., 2001) and Italy (Jones *et al*., 2004). Several patient and pathogen-specific risk factors are associated with acquisition of this pathogen in ICUs, such as length of stay, severity of underlying disease and exposure to invasive procedures, on the one hand (Rello *et al*., 2002), and virulence, adherence, and antimicrobial drug resistance on the other (Aloush *et al*., 2006). The hospital environment particularly moist sites are known reservoirs of *P. aeruginosa* strains, often multidrug resistant (MDR) due to intrinsic and acquired determinants (Deplano *et al*., 2005).

ICUs are generally considered epicenters of multidrugresistant (MDR) organisms. Although one possible explanation of the spread of antibiotic-resistant strains in ICUs is the selection exerted by extensive use of antibiotics, increased spread of MDR *P. aeruginosa* may be due to transmissions of resistant clones between Patients (Meyer *et al*., 2003). Furthermore, it has been suggested that infection represents merely the tip of an iceberg, and that colonization reflects the submerged part. Colonization may be the first step of an endogenous infection, while the colonized patients represent a continuous exogenous source of microrganism for colonization/infection of other patients (Bertrand *et al*., 2001).

3.7 Different diseases caused by *P. aeruginosa*

3.7.1 Respiratory infections: Respiratory infections caused by *P. aeruginosa* occur almost exclusively in individuals with a compromised lower respiratory tract or a compromised systemic defense mechanism. It is a respiratory pathogen found most commonly in patients admitted to hospital, those undergoing prolonged mechanical ventilation, patients with neutropenia or on immunosuppressive drugs, and those with

cystic fibrosis (Doyle *et al*., 1995). Acute bronchitis or tracheitis of bronchopneumonia is regarded respiratory infection followed by tracheostomy (Gotsman and Whitby, 1964). Exposure to the hospital environment particularly in an ICU such as use of respiratory inhalation equipment and previous antibiotic therapy increases the likelihood of such infections (Brewer *et al*., 1996; Rello *et al*., 1996). Lower respiratory tract colonization of cystic fibrosis patients by mucoid strains of *P. aeruginosa* is common and difficult, if not impossible, to eradicate.

Pneumonia is the second most common NI affecting 27.0% of all critically ill patients in ICU, 86.0% percent of which are associated with mechanical ventilation (Richards *et al*., 1999) and is termed as Ventilator Associated Pneumonia (VAP). VAP is defined as pneumonia occurring in a patient within 48 hours or more after intubation with an endotracheal tube or tracheostomy tube and which was not present before (Wagh *et al.,* 2009). The incidence of nosocomial pneumonia in mechanically ventilated patients ranges from 9.0% to 68.0%, and mortality rates range from 33.0% to 71.0% (Bowton and Fagon *et al*., 1996). *P. aeruginosa* was one of the most frequently isolated agents in the study done by Camargo *et al.* (2004) in all the VAP episodes. Hospital acquired pneumonia (HAP) is the second most common nosocomial infection after urinary tract infections and has the highest fatality rate amongst nosocomial infection. HAP accounts for 15.0% of all nosocomial infections and affects 0.5-2.0% of hospitalized patients (Campbell *et al.*, 1996). The highest rates are seen in the intensive care units (ICU) where the rate is 15.0%-20.0%, particularly in intubated patients on mechanical ventilation (Vincent and Bihari, 1995). *P. aeruginosa* has been considered as an etiological agent in 21.0-30.0% of cases of HAP but it has a low incidence of CAP. Overall mortality rates for hospital acquired Pseudomonal pneumonia ranged from 42.0-75.0% (Arancibia *et al.*, 2002). The high mortality rate appears to be attributed to infection by *P. aeruginosa*, in addition to underlying illness. The estimated mortality rate of ventilator associated pneumonia caused by *P. aeruginosa* was 40.0-50.0% (Brewer *et al*., 1996). According to the National Nosocomial Infections Surveillance System in USA since the 1970s, the prevalence of *P. aeruginosa* remained constant over the period of 24 years, causing 17.0% HAP.

3.7.2 Urinary tract infections: UTI is an infection of the urinary tract that involves the kidney, ureter, urinary bladder, urethra; tissue surrounded the retro-peritoneal and peri-nephric spaces (Garner *et al*., 1996). Infections are usually defined by microbiological criteria: positive quantitative urine culture (≥105 microorganisms/ml, with a maximum of 2 isolated microbial species) (WHO, 2002). Catheterization and instrumentation of the urinary tract are considered to be the major predisposing factors in approximately 60.0-80.0% of the cases of nosocomial UTI (Arunodoya, 2001; Manley *et al*., 2000 and Ozinel *et al*., 2004). Study by Burke *et al*. (2004) showed that the vast majority of UTIs occur in patients with temporary indwelling bladder catheters and is frequently termed as catheter associated urinary tract infection (CA-UTI). Instillation of catheter may lead to damage of mucosal layer, which disrupts the natural barrier and allows bacterial colonization (Kalsi *et al*., 2003). Therefore, catheter serves as a direct conduit for pathogens which may be carried from the external meatus to the bladder when the catheter is introduced (Weise and Broek, 2005).

P. aeruginosa has been isolated as a pathogen responsible for UTIs representing 10.7% of isolates found exclusively in nosocomial UTIs, 3.5% in intensive care units, 35.6% in other hospital units and 27.7% in out patients and general practice (Henwood *et al*., 2001). *P. aeruginosa* is the third most common pathogen associated with hospital-acquired catheterassociated UTIs (Jarvis and Martone, 1992). Woods *et al*. (1986) showed high production of elastase and protease in Pseudomonal strains isolated from urinary tract infections in comparison to isolates from other infections like burn wounds infection; skin wound infection and acute pneumonia. In addition to other virulence factors, *P. aeruginosa* has a tendency to form biofilms on the surface of urinary catheters leading to higher incidence of UTIs in patients with long-term indwelling bladder catheterization. Growth of *P. aeruginosa* begins in the form of microcolonies, which later coalesce together to form biofilms (Hoiby *et al*., 2001). Despite advances in antimicrobial therapy the mortality and morbidity associated with *P. aeruginosa* induced UTIs still remains high. This unfavorable outcome is due to our incomplete understanding about the pathogenesis of the disease. Very limited studies are available in relation to the pathogenesis of *P. aeruginosa* induced UTI. Thus attention of the researchers need to be drawn to understand pathogenic

mechanisms of UTIs caused by *P. aeruginosa* in order to design effective treatment strategies (Mittal *et al*., 2009).

3.7.3 Bacteremia and septicemia: Bacterial bloodstream infections are serious infections associated with significant mortality and health-care costs (Weinstein *et al*., 1997). *P. aeruginosa* bloodstream infection is a serious infection with significant patient mortality and health-care costs. The hospital mortality associated with *P. aeruginosa* bloodstream infections is reported to be greater than 20.0% in most series and is highest among patients receiving inappropriate initial antimicrobial treatment (Bryan *et al*., 1983). *P. aeruginosa* causes bacteremia primarily in immunocompromised patients. Predisposing conditions include hematologic malignancies, immunodeficiency relating to AIDS, neutropenia, diabetes mellitus, and organ transplantations, ulceration of respiratory and gastrointestinal tracts, steroid administration, antibiotic therapy, surgery trauma, urinary tract instrumentation and severe burns. Most *Pseudomonas* bacteremia is acquired in hospitals and nursing homes.

3.7.4 Central nervous system infections: *P. aeruginosa* causes meningitis and brain abscesses. Pseudomonal meningitis is severe nosocomial meningitis associated with reported mortality of 21.0-40.0% that occurs predominantly secondary to invasive procedure (Huang *et al*., 2007). The organism invades the CNS from a contiguous structure such as the inner ear or paranasal sinus, or is inoculated directly by means of head trauma, surgery or invasive diagnostic procedures, or spreads from a distant site of infection such as the urinary tract. Primary central nervous system infections with *P. aeruginosa* are a relative rare. Involvement is almost always secondary to a surgical procedure or head trauma and occasionally bacteremia (Huang *et al.*, 2007).

3.7.5 Skin and soft tissue infections, including wound infections, pyoderma and dermatitis: *P. aeruginosa* can cause a variety of skin infections, both localized and diffuse. The common predisposing factors are breakdown of the integument which may result from burns, trauma or dermatitis; high moisture conditions such as those found in the ear of swimmers and the toe webs of athletes, hikers and combat troops, in the perineal region and under diapers of infants, and on the skin of whirlpool and hot tub users. Individuals with AIDS are easily infected. *Pseudomonas* has also been

implicated in folliculitis and unmanageable forms of acne vulgaris. Post operative wound infections seem prone to *P. aeruginosa* infections. There is an increasing incidence of *P. aeruginosa* in post-operative wound infections as observed by many scientists especially in recent years (Masaadeh and Jaran, 2009). Joshi *et al.* (2002) stated that *P. aeruginosa* has almost replaced *Staphylococcus aureus* in post-operative wound Infection.

3.7.6 Gastrointestinal infections: *P. aeruginosa* can produce disease in any part of the gastrointestinal tract from the oropharynx to the rectum. As in other forms of *Pseudomonas* disease, those involving the GI tract occur primarily in immunocompromised individuals. The organism has been implicated in perirectal infections, pediatric diarrhea, typical gastroenteritis, and necrotizing enterocolitis. The GI tract is also an important portal of entry in *Pseudomonas* septicemia and bacteremia (Todar, 2011).

3.7.7 Bone and joint infections: *Pseudomonas* infections of bones and joints result from direct inoculation of the bacteria or the hematogenous spread of the bacteria from other primary sites of infection. *P. aeruginosa* has a particular tropism for fibrocartilagenous joints of the axial skeleton. *P. aeruginosa* causes chronic contiguous osteomyelitis, usually resulting from direct inoculation of bone and is the most common pathogen implicated in osteochondritis after puncture wounds of the foot (Todar, 2011).

3.7.8 Ear infections including external otitis: *P. aeruginosa* is the predominant bacterial pathogen in some cases of external otitis, including "swimmer's ear". The bacterium is infrequently found in the normal ear, but often inhabits the external auditory canal in association with injury, maceration, inflammation, or simply wet and humid conditions (Todar, 2011).

3.7.9 Eye infections: *P. aeruginosa* can cause devastating infections in the human eye. It is one of the most common causes of bacterial keratitis, and has been isolated as the etiologic agent of neonatal ophthalmia blepharoconjunctivities, sceleral abscess and orbital cellulities (Atkins *et al*, 1990).

3.7.10 Endocarditis: *P. aeruginosa* infects heart valves of IV drug users and prosthetic heart valves. The organism establishes itself on the endocardium by direct invasion from the blood stream. *Pseudomonas* endocarditis may produce intractable congestive heart failure, large systemic arterial amboli, cardiac valve ring abscesses and high grade conduction disturbance. Brain abscess, cerebrities and mycotic ancurysm some times occur in *P. aeruginosa* endocarditis.

3.8 Therapeutic choices for *Pseudomonas aeruginosa*

3.8.1 Antibiotic options

Despite the fact that *P. aeruginosa* has high intrinsic resistance to antimicrobial agents, a number of drugs are available for treatment of infection.The major classes that have been used with success include aminoglycosides (such as gentamicin and tobramycin), semisynthetic penicillins (such as carbenicillin, ticarcillin and piperacillin) third generation cephalosporins (including ceftazidime and cefoperazone), quinolones (such as ciprofloxacin) and carbapenems (including meropenem and Imipenem).

In addition to the conventional antibiotics listed above, newer agents are being developed to counter the problem of antimicrobial resistance. Small cationic peptides, either natural or synthetic, are active against many strains of *P. aeruginosa*. These agents are present throughout nature (including within human neutrophils) and provide hope for treating infections caused by strains that are resistant to currently available drugs (Hancock and Lehrer, 1998). Human testing is underway, but these novel agents are not yet available for routine clinical use. Other agents to be considered are those which have not been thought to have useful antipseudomonal activity, e.g. the macrolides, but appear to improve the prognosis of patients with chronic *P. aeruginosa* pulmonary inections. Such agents may indeed have a slow bactericidal effect on this organism (Tateda *et al*., 1996).

3.8.2 Combination therapy

Therapy of serious infections caused by *P. aeruginosa*, usually consists of a combination of a semisynthetic penicillin, such as ticarcillin or piperacillin (with or without a β-lactamase inhibitor) and an aminoglycoside, such as tobramycin.These drugs have been shown to be synergistic in vitro against *P. aeruginosa* (Craig and

Ebert, 1994). Data on synergy is strain-specific, and there is debate regarding whether or not predictions about clinical efficacy can be made from in vitro observations. Nonetheless, most experts recommend that the two classes of drugs be used together for their possible synergistic effect. Other agents that have proven to be effective in treating pulmonary exacerbations in cystic fibrosis include quinolones, newer β-lactams such as cefoperazone or ceftazidime, and carbapenems (Imipenem or meropenem) (LeBel, 1991; De Boeck *et al*., 1989). These agents may be given singly or in combination with an aminoglycoside, but they should be chosen in the light of results from in vitro antimicrobial susceptibility testing. Combination therapy with agents other than penicillins and aminoglycosides has not been proven superior to use of single agents. Some of the newer agents, such as Imipenem, induce β-lactamase activity and should not be given in combination with other β-lactams. Often, a clinical improvement may be seen even when antimicrobial agents are administered to which the infecting strain of *P. aeruginosa* is resistant in vitro (Zabner and Quinn, 1992). Giamarellou (2002) stated that Combinations of β-lactams and aminoglycosides remain a sensible choice for therapy of invasive infection caused by *P. aeruginosa.* Similarly in the study done by Obritsch *et al.* (2004) the best combination was aminoglycoside or fluorquinolone in association with piperacillin/tazobactam.

3.9 Resistance to antibiotics

Given the number of compounds active against *P aeruginosa*, it might be expected that treatment of infections caused by this bacterium would be straightforward. The situation, however, is complicated by the predilection of *P aeruginosa* to develop resistance to nearly any antimicrobial agent. Resistance in *P. aeruginosa* can arise by various mechanisms including: i) reduced uptake of aminoglycosides across the outer and cytoplasmic membranes, ii) production of aminoglycoside-modifying enzymes, iii) loss of the outer membrane porin D2 (OprD) resulting in selective Imipenem resistance, iv) mutational derepression of AmpC chromosomal β-lactamases, v) acquisition of plasmid/transposon or class 1 integronborne mediated β-lactamases, (Weldhagen *et al*., 2003) vi) modification of DNA gyrase (in the case of quinolone resistance)

vii) class 1 integron-borne co-resistance factors (Recchia and Hall, 1995) and viii) multi-substrate efflux pumps (Bonfiglio *et al*., 1998). Resistance is problematic at

three levels: intrinsic resistance, acquired resistance, and emergence of resistance during therapy. Each of these must be considered when choosing an antibiotic regimen for patients infected with *P aeruginosa* (Hauser and Sriram, 2005)

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3.9.1 Intrinsic resistance

In general, *P aeruginosa* is naturally less susceptible than other gram-negative bacilli to many antibiotics, such as ampicillin (Principen), most cephalosporins, and the macrolides. This is because of its relatively impermeable outer membrane and its ability to actively transport some antibiotics out of the cell, preventing accumulation. *P aeruginosa* also harbors an inducible chromosomally encoded beta-lactamase, referred to as AmpC beta-lactamase that is capable of degrading many beta-lactams even though it is naturally expressed at very low levels. Biofilm formation by *P aeruginosa* may also contribute to antibiotic resistance in infections, although the importance of this mode of growth in hospital-acquired infections that do not involve foreign bodies is less well understood. When grown as biofilms, which are organized communities of bacteria that grow on surfaces, individual bacteria are much more resistant to antibiotics than when grown planktonically (dispersed in fluid) (Hauser; Sriram, 2005).

It is now well understood that in *P. aeruginosa*, as in other gram-negative bacteria, intrinsic resistance involves the collaboration of restricted uptake through the outer membrane, and secondary resistance mechanisms such as energy-dependent efflux and b-lactamase(s) (Hancock, 1997). The current explosion of information on efflux in *P. aeruginosa* might lead one to conclude that this is the only important element of intrinsic resistance. Two efflux systems have been described as having a role in intrinsic antibiotic resistance based on their apparent constitutive expression and the influence of knockout mutations and inhibitors. The first of these to be studied was the MexAB-OprM system (Zhao *et al*., 1998). This system is a prototype RND (resistance-nodulation-division) system with a cytoplasmic pump protein, MexB, a periplasmic linker protein, MexA, and an outer-membrane protein, OprM. Mutations that prevent expression of any or all of these genes results in hyper-susceptibility to quinolones, tetracyclines, chloramphenicol, sulfamethoxazole, trimethoprim, and some β-lactams, but not aminoglycosides, erythromycin, polymyxins or Imipenem and other β-lactams (Zhao *et al*., 1998). On the other hand, another efflux pump

operon, MexX-MexY, in apparent collaboration with OprM was recently discovered to have the capability to efflux many of the same substrates as MexAB-OprM but to have a primary role in intrinsic resistance to aminoglycoside antibiotics and erythromycin (Aires *et al*., 1999). Thus, mutation of MexXY led to increased susceptibility to these latter antibiotics, but affected a broader range of antibiotic classes only in a MexAB-deficient background (Wadman *et al*., 1999). Consistent with this observation, knockouts of OprM, which is weakly expressed from a secondary promoter and which collaborates with both MexXY and MexAB, have a far greater effect than knockouts of either MexAB or MexXY (Aires *et al*., 1999). Many studies have concluded that the susceptibility of *P. aeruginosa* to some β-lactams (e.g. ceftazidime, cefepime, piperacillin, aztreonam) is more strongly influenced by efflux, whereas susceptibility to others (Imipenem, panipenem) is more strongly affected by the presence of β-lactamase, while a third group (ceftriaxone, meropenem,moxalactam) is influenced only by knockout of both efflux and β-lactamase.

3.9.2 Acquired resistance

In addition to its intrinsic resistance, *P aeruginosa* has the ability, through mutational changes or acquisition of exogenous genetic material, to develop resistance to each of the antipseudomonal antibiotics. For example, the chromosomally encoded AmpC betalactamase is capable of degrading beta-lactams, such as piperacillin and ceftazidime, when mutations result in production of large amounts of this enzyme. Of note, the betalactamase inhibitors tazobactam, sulbactam sodium, and clavulanate are not active against AmpC beta-lactamase. Resistance to aminoglycosides may occur by mechanisms that differentially affect members of this class. Thus, resistance to gentamicin and tobramycin is often not accompanied by resistance to amikacin (Weinstein *et al*., 1980).

P aeruginosa can also express several efflux pump systems. Over-production of these pumps can prevent accumulation of antibiotics within the bacterium and result in simultaneous loss of susceptibility to multiple antibiotics. Not surprisingly, the many and varied ways by which *P aeruginosa* can resist the actions of antibiotics have resulted in documentation of relatively low susceptibility rates in several studies (Spencer, 1996). Although these studies were performed in different patient

populations and different geographic locations, two troubling trends are evident. First, susceptibility rates appear to be decreasing with time, especially with regard to piperacillin, ceftazidime, Imipenem, and ciprofloxacin. For example, in a study of *P aeruginosa* isolates from US intensive care units (Neuhauser *et al*., 2003), ciprofloxacin susceptibility rates decreased from 89.0% in 1990 through 1993 to 68.0% in 2000. Secondly, 10.0% or more of recovered isolates are not adequately treated with most empirically chosen single agents.

3.9.3 Emergence of resistance

Even when antimicrobial agents to which an isolate is susceptible are chosen, a successful therapeutic outcome is not ensured. One reason for this uncertainty is that *P aeruginosa* has shown a regrettable propensity to develop resistance to antibiotics during therapy. Resistance develops because of the natural occurrence of mutations essential for antibiotic penetration or activity. Within infected tissue, selection for individual bacteria that harbor these mutations occurs because the antibiotic is present, and eventually these organisms compose the majority of the bacterial population. As would be expected, antimicrobial agents for whom a single mutation is sufficient to compromise activity are most prone to the emergence of resistance during therapy. These include Imipenem and the fluoroquinolones. For example, resistance emerged in 25.0% to 50.0% of *P aeruginosa*-infected patients who received Imipenem monotherapy and 33.0% to 58.0% of such patients who received ciprofloxacin monotherapy (Lode *et al*., 1987).

3.10 Multidrug resistant *P. aeruginosa*

Organisms which confer resistance to two or more antibiotics are referred as MDRO (Stephen *et al*., 2005). Nowadays, prevalence of MDR strains of *P.aeruginosa* are seen mainly in hospital acquired infections due to the selective pressure exerted on the bacteria by over usage of broad-spectrum antibiotics. In a study carried out in Inan *et al*. isolated 60.0-83.0% multidrug-resistant *P. aeruginosa* strains from ICU patients. These strains were resistant to Ceftazidime (34.0%), Imipenem (26.0%), Gentamicin (67.0%), and Amikacin (26.0%).

P. aeruginosa is inherently resistant to many antibiotics and can mutate to even more resistant strains during therapy. Although numerous resistance mechanisms have been identified, the mutation of porin proteins constitutes the major mechanism of resistance. Penetration of antibiotics in to the Pseudomonad cell is primarily through pores in the outer membrane. If the proteins forming the walls of these pores are altered to restrict flow throw the channels, resistance to many classes of antibiotics can develop. *P. aeruginosa* also produces a number of different beta lactamases that can inactivate many beta lactam antibiotics (eg. penicillins, cepalosporins, and carbapenems). The extended spectrum of beta lactamases (ESBL) producing organisms are a breed of drug resistant pathogens that are rapidly becoming important globally in the area of hospital acquired infections. ESBL are transferable plasmid encoded, mutated beta lactamases enzymes, that have the capability to hydrolyze third generation cepalosporins. These enzymes are found in a variety of Enterobacteriaceae, most often in *Klebsiella pneumoniae* and *Escherichia coli*. ESBLs have been described in *P. aeruginosa* only recently (Jarlier *et al*., 1988; Nordmann & Naas, 1994). The ESBL enzymes described in *P.aeruginosa* belong to various families: the TEM and SHV types which are common among Enterobacteriaceae, the PER type which mostly originates from Turkey (Nordmann & Naas 1994); the VEB type from Southeast Asia (Poirel *et al*., 2000) or, more recently, the IBC type and the GES type which have been reported from various parts of the world, including France, Greece, South Africa and Brazil (Castanheir *et al*., 2004). Further multidrug resistance can be caused by regulatory mutations *nalB (mexR), nfxB* or *nfxC* (*mexT*) leading to overexpression of three separate RND efflux systems, MexAB-OprM, MexCD-OprJ and MexEF-OprN respectively (Germ *et al*., 1999).

3.10.1 Resistance to β-lactams

Multi drug efflux pumps in the inner and outer membrane of *P. aeruginosa* may protect the bacterium from to β-lactam agents (Srikumar *et al.*, 1997). All *P.aeruginosa* strains have a chromosomal AmpC β-lactamase that is normally inducible but may be derepressed by mutation, or can be induced by certain β-lactams. Such inducers include clavulanate, normally used as a β-lactamase inhibitor (but not against class C, AmpC-like β-lactamases) and the antibiotic Imipenem. Mutations resulting in β-lactamase derepression are the most common clinical cause of β-lactam resistance in *P. aeruginosa*.

The USA's national nosocomial infections surveillance system (NNIS) showed increases of 17.7%, 27.3% and 26.4% in resistance to Imipenem, quinolone and third-generation cephalosporin, respectively (Gaynes and Edwards, 2005). In various studies, it was reported that increased resistance rates have been detected against to carbapenems, and third-generation cephalosporins for *P. aeruginosa* worldwide (Hancock, 1998).

Carbapenem antibiotics are considered agents of choice to treat serious infections caused by *P. aeruginosa*. Progressive antimicrobial resistance to most classes of antibiotics, including carbapenems, has made treatment of infection caused by this bacteria particurlarly difficult, even with combination therapy. One growing factor leading to carbapenem resistance is the presence of carbapenemases.

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 has been reported in many countries. The most important clinically-significant carbapenemases in *P. aeruginosa* are class B metallo-β-lactamases such as VIM and IMP-type (Zhiyong *et al*., 2003). 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 (Walsh *et al*., 2005). In the study done by Lutfu *et al.* (2005), resistance rates against Imipenem and meropepem from carbanemem groups were determined as 15.0% and 20.0%, respectively. Imipenem is the first of a new class of beta-lactam antimicrobial agents with remarkable and extremely potent in vitro activity against most commonly isolated bacterial pathogens, including *P. aeruginosa* in that country. In the literature, it was reported that resistance to Imipenem was 14.0% in Spain (Bouza *et al*., 1999), 19.3% in Italy (Bonfiglio *et al*., 1998), and 68.0% in Saudi Arabia (Rotimi *et al*., 1998)). The National Nosocomial Infections Surveillance (NNIS) system reported the incidence of Imipenem resistance as 18.5% among isolates of *P. aeruginosa* from ICU patients (NNIS, 1999).

According to different reports, resistance to ceftazidime was 15.0%-22.0% in the world. Resistance to piperacillin was higher, similar to ceftazidime. While piperacillin resistance rate was 10.0% in Spain (Bouza, 1999), 12.0% in Italy (Bonfiglio *et al*., 1998), 14.0% in Latin America (Jones, 2001)), it was found as 28.7 in the study done by Lutfu *et al.* (2005).

3.10.2 Resitance to Aminoglycosides

One of the significant resistant groups detected against aminoglycosides was *P. aeruginosa.* Reports of the susceptibility of *P. aeruginosa* to gentamicin and tobramycin have ranged from as low as 49.8% and 77.7%, in Greece, to as high as 96.6% and 99.2%, respectively, in the United Kingdom (Van Landuyt *et al*., 1986). Previous studies reported that antipseudomonal effects of amikacin were greater than those of gentamicin (Akalõn *et al*., 1988). In the study done by Lutfu SAVAS *et al* (2005), the rate of aminoglycoside resistance was found to be relatively high (resistance to amikacin; 42.2%, netilmicin; 30.1%, tobramycin; 65.5% and gentamicin; 70.7%).

Most large studies have indicated that around 10.0% of *P. aeruginosa* isolates are aminoglycoside resistant, although higher levels of resistance occur in some studies for specific aminoglycosides (Hancock and Speert, 1996). Although enzyme-mediated aminoglycoside resistance is observed, at least 50.0% and up to 90.0% of isolates appear to carry the 'impermeability' type resistance. A mutation that appears to correlate with this phenotype involves up-regulation of the partial RND system MexXY (named AmrAB in the reported study) (Wadman *et al*., 1999). Interestingly, overexpression of MexXY alone from the cloned gene did not result in resistance. The lack of overexpression of OprM in aminoglycoside-resistant18 mutants was promoted as evidence that an outer membrane efflux protein other than OprM might participate with MexXY in determining impermeability type aminoglycoside resistance (Aires *et al*., 1999). Another relevant form of aminoglycoside resistance is adaptive resistance (Karlowsky *et al*., 1997) such resistance is reversible, after a post-antibiotic effect, upon removal of selective pressure. It was shown to occur in an artificial biofilm (Brooun *et al*., 2000).

3.10.3 Resistance to quinolones

Quinolone resistance is on the rise with reported frequencies of 12.0–20.0%. In most reported cases there was a missense mutation in the quinolone target (the *gyrA* subunit of DNA gyrase) at codon 83 (T83I), although other mutations are sometimes observed (Takenouchi *et al*., 1999; Mouneimné *et al*., 1999) Higher levels of resistance may involve additional mutations in *gyrB* (DNA gyrase B subunit) or *parC* (topoisomerase IV).These target site mutations affect susceptibility to all quinolones.

In a later study, it was reported that *P. aeruginosa* strains have developed relatively high resistance levels against to quinolone group. The resistance of *Pseudomonas* to the antibiotics in the quinolone group is variable in different centers (Snydman, 1991). In a prospective study, resistance to ciprofloxacin in ICU was reported as 8.0-31.0% (Tassios *et al*., 1988). In the study done by SAVAS *et al* (2005), resistance rates against ciprofloxacin and norfloxacin were found as 27.4%, 25.5%, respectively. Ciprofloxacin resistance rate was 23.0% in Spain (Bouza *et al*., 1999), 31.9% in Italy (Bonfiglio *et al*., 1988), and 26.8% in Latin America (Jones, 2001).

Thus, in ICUs, empirical antibiotic treatments should be avoided and treatment should be carried out using antibiotic susceptibility tests. ICUs should be regularly inspected for *Pseudomonas* colonization which shows a strong resistance pattern against the various antibiotics.

3.11 Strategies for Prevention of Emergence of Resistance

3.11.1 Combination therapy

Since cross-resistance between major classes of anti- Pseudomonal antibiotics is unlikely to develop; it is common practice to treat serious infections with a combination of a β-lactam and an aminoglycoside. Not only do the drugs appear to exert a synergistic antibacterial effect, but they may also delay or prevent the emergence of resistance during therapy. There are a few novel approaches under development in which the combination of agents would include an antibacterial agent and an inhibitor of a major resistance mechanism.The best example of this would be the use of a combination of β-lactamase inhibitor and β-lactam (Livermore and Chen, 1997) Another approach, being pursued by Microcide Pharmaceuticals (US) is the development of inhibitors of efflux pumps (e.g. MC-207, 110) as a method of

potentiating the activity of fluoroquinolones, and possible other drugs (Reneau *et al*., 1999). Similarly, the use of antimicrobial peptides that are able to break down the outer-membrane permeability barrier, but have little intrinsic antibiotic activity has been proposed as an approach to overcoming this type of intrinsic insusceptibility (Zhang *et al*., 1999).

3.11.2 Restriction of use

Good antibiotic stewardship plays an important role in limiting the emergence of antibiotic resistance. Some antimicrobial agents should be reserved for therapy of infections when all other agents have failed. For instance, it is inadvisable to use a drug such as meropenem for the initial infection with *P. aeruginosa* in a patient with cystic fibrosis; its use should be reserved for infections when the infecting strain is resistant to the first-line drugs: semisynthetic penicillin and an aminoglycoside. Some hospitals impose restrictions on certain antimicrobial agents to maximize the likelihood that they will be effective when a legitimate indication for their use arises (Hancock and Speert, 2000)

3.11.3 Prevention of infection

Infection control plays an exceedingly important role in preventing the spread of antimicrobial-resistant bacteria within hospitals. Since *P. aeruginosa* is a hydrophilic organism, it can thrive in moist environments in the hospital and be disseminated from a common source. Common source outbreaks linked to contaminated hydrotherapy water have been documented on burn and surgical wards. Additional outbreaks have been linked to contaminated endoscopes (Sagué *et al*., 1994). *P. aeruginosa* can be transiently carried on the hands of medical and nursing personnel resulting in the spread of infection among patients. Principles of good infection control such as careful hand washing and barrier precautions should be utilized when dealing with patients with antibiotic- resistant bacteria. There is conflicting data about the spread of *P. aeruginosa* among patients with cystic fibrosis, but epidemics have been documented (Cheng *et al*., 1996). Immunization against *P. aeruginosa* would be a strategy for preventing acquisition and spread of antibiotic-resistant strains. Whereas several different vaccine strategies have been considered and tested, none has yet entered clinical use (Hancock and Speert, 2000).

It appears likely that antibiotic resistance will continue to be a problem in dealing with *P. aeruginosa* infections, since the fundamental issues underlying this problem (i.e. the condition of the patients that are prone to such infections, and the high intrinsic resistance of this bacterium) have remained constant. It appears unlikely that there will be a large number of novel effective antibiotics to impact on this problem in the next decade. Instead we need a combination of good management of those agents we have and the application of innovative therapeutic approaches, such as the use of antiresistance strategies. In the longer term the recent sequencing of the genome of *P. aeruginosa* gives us hope to identify the basis for phenotypic resistance, and the discovery of novel targets for antimicrobial intervention (Hancock and Speert, 2000).

CHAPTER IV
MATERIALS AND METHODS

The present study was conducted from May 2010 to December 2010 at the microbiology laboratory of NINAS hospital, Bansbari, Nepal. The study was carried out on all the hospitalized patients at that duration. Altogether 1146 clinical specimens from 301 patients were analyzed microbiologically (Collee *et al*., 2001; Vandepitte *et al*., 2003).

4.1 Materials

List of materials used during study are as in appendix-II

4.2 Methodology

A Cross sectional study was done to determine the prevalence of *P. aeruginosa* and its antibiotic susceptibility profile from all the clinical specimens obtained from the hospitalized patients of National Institute of Neurological and Allied Sciences. Altogether 1146 clinical specimens from 301 patients were considered under study. The specimens included tracheal aspirate, urine, sputum, pus, catheter tip, cvp tip, blood, csf, and miscellaneous (RVD drain tip, Hemorrhagic fluid, Vertebral body aspiration, Chest tube drain aspiration, tip vp shunt, nasal swab, venous section tip, middle ear swab, sphenoid mucosa, ear discharge, etc).

4.3 Data collection

The clinical profiles and the personal details including age, sex, sign and symptoms, previous treatments if any, undergoing antibiotic therapy, etc were filled in a protocol prepared for this study(see appendix-I).

4.4 Laboratory methodology

All the clinical specimens from patients were collected by experienced physician on daily basis as per the guidelines of the department or when the attending physician suspected infection based on systemic signs (unexplained fever, chills, and hypotension), and/or local signs (purulent tracheal aspirates in mechanically ventilated patients or tracheostomised patients, purulent urinary drainage, or pus or pain at a vascular catheter insertion site).

4.4.1 Collection of sample

Tracheal aspirate

The tracheal aspirates were collected in Luken trap by applying negative pressure through automated machine, the sample was immediately transported in the same trap without adding any special transport media. 563 tracheal specimens from 78 patients were collected under this study.

Urine

Mid stream urine was collected into the sterile rubber capped bottles with all the careful precautions. In case of catheterized patients the catheter port was cleaned with 70% alcohol and 10 ml of urine was collected using a needle and syringe. The specimen was transported to the lab as soon as possible. A total of 147 urine specimens from 112 patients were collected under this study.

Sputum

A clean, dry and sterile wide mouth, leak proof container was provided to the patient and asked to cough deeply to get the sputum (not saliva) preferably in the morning (before any mouth wash was used). Then the specimen was collected into the container without smearing the outer surface of the container. The specimen was delivered to the laboratory as early as possible. Thirty three sputum samples from 21 patients were collected under this study.

Pus and swab specimens

All the pus and swab specimens from the wounds, either of accidental or surgical operation or any other type of wound infections, were collected aseptically. When the pus was being discharged 5 ml of pus was aspirated from the drainage tube and transferred to a leak-proof sterile container. When the pus was not being discharged the cotton sterile stick swabs within the test tubes with rubber stopper were used to collect the sample from the infected site. Usually two swabs were collected from each site, one for gram stain and other for culture. 80 pus specimens were collected form 51 patients.

Cerebrospinal fluid (CSF)

A sterile wide-bore needle was inserted between the fourth and and fifth lumbar vertebrae and CSF was allowed to drip into a dry sterile container. The CSF was collected by an experienced medical officer. A total of 139 CSF samples were collected from 87 patients.

Blood

After proper sterilization of skin desired 5-10 ml of blood was withdrawn by a sterile syringe. The blood was transferred immediately in sterile bottles fitted with rubber stoppers containing 50 ml to 100 ml BHI broth aseptically. The blood bottles were taken into the laboratory as soon as possible for proper culture. A total of 60 blood specimens were investigated within the time period.

Catheter tip, Cvp tip

The catheter and and Cvp was withdrawn aseptically and inserted directly into a sterile vial and the tip was cut and the vial was covered tightly with the cap. A total of 64 Cvp tip from 62 patients and 25 Catheter tip from 20 patients were collected during the study.

Miscellaneous

A total of 35 miscellaneous specimens were collected and studied. They include RVD drain tip, Hemorrhagic fluid, Vertebral body aspiration, Chest tube drain aspiration, tip vp shunt, nasal swab, venous section tip, middle ear swab, sphenoid mucosa, ear discharge, etc.

4.4.2 Processing of sample

Immediately after the specimens were received in the laboratory, they were provided with unique laboratory identification numbers and further proceeded.

4.4.2.1 Processing of Tracheal specimen and sputum

Macroscopic examination

All received specimens were visually observed for color, consistency, presence of blood and pus.

The collected sputum was classified as follows-

1. Purulent-green looking, mostly pus,

2. Mucopurulent-yellowish green looking with pus and mucus,

3. Mucoid-mostly mucus,

4. Mucosalivary-mucus with small amount of saliva.

Microscopic examination

Gram's stain of all received samples were prepared and observed under microscope for bacterial morphology to distinguish between gram positive and gram negative bacteria as well as presence of pus cell (Forbes *et al*, 2007). Sputum and tracheal aspirate containing more than 25 leukocytes or pus cells per low power field confirmed the reliability of the specimen indicating that it was not contaminated by saliva.

The specimens were inoculated into the Blood agar, Chocolate agar and MacConkey agar and. A 5µg Optochin disc was added in Blood agar and a 10 U Bacitracin disc in Chocolate agar was added to screen out *Streptococcous pneumonia* and *Haemophilus influenza* respectively. The chocolate agar and blood agar plates were incubated at 37ºC for overnight in 5-10% CO_2 environment whereas the MacConkey agar and cetrimide agar plate was incubated at 37ºC in an aerobic condition.

4.4.2.2 Processing of urine

Urine culture was done with the use of semi-quantitative method using standard loop technique with the help of 4.4 mm diameter loop. Approximately 0.001 ml urine was inoculated into Blood agar and Macconkey agar plate separately by mixing the specimen uniformly into the container. The both plates were incubated aerobically at 37ºC for overnight.

4.4.2.3 Processing of Pus

One pus swab was used for making gram stain and the other was inoculated on the Blood agar, MacConkey agar for the growth of aerobes and the cooked meat broth for the growth of anaerobes. The plates were incubated at 37ºC for 24 hours and cooked meat broth for 48 hours.

4.4.2.4 Processing of Blood

Blood culture bottles after receiving were incubated at 37ºC for overnight. Next day it was subcultured in Blood agar, MacConkey agar and Chocolate agar plates. The

plates were again incubated at the same temperature for another 24 hours and Chocolate agar in CO_2 rich environment. The negative bottles were incubated, checked for turbidity and again incubated till 7 days. On the 7^{th} day all turbid and non turbid culture bottles were subcultured and those showing negative growth were discarded.

4.4.2.5 Processing of CSF

Cerebrospinal fluid must be cultured as soon as possible after collection. When a delay is unavoidable, the fluid should be kept at 35-37ºC. Purulent cloudy fluids were examined immediately by a gram's stained smear. The specimen was centrifuged at 1500 rpm for few minutes and the sediment was used to inoculate in the culture media, Chocolate agar and Blood agar and incubated at 37ºC for upto 48 hours.

4.4.2.6 Processing of CVP tip, Cathetar tip and other miscellaneous

The CVP tips catheter tip and all other miscellaneous samples like body fluids and all surgical specimens were inoculated in Blood agar, MacConkey agar and Chocolate agar and incubated at 37ºC for 24-48 hours.

4.4.3 Identification of the isolates

After 24 hours of incubation, visual growth was observed for colony morphology and the isolated colonies were identified by conventional method. A positive culture was defined as identification of the organism on Gram stain followed by growth of the organism in the suitable culture medium (Hassanzadeh *et al*., 2009; Esen *et al*., 2009 and Eggimann *et al*., 2001).

4.4.4 Identification with staining reactions

Gram staining was performed for the presumptive identification of the bacteria according to standard technique using acid alcohol as decolorlizer. *P. aeruginosa* was identified as a gram negative rod. Staining procedure and reagents are given in appendix- IV.

4.4.5 Identification with biochemical test

Typical colonies of bacterial isolates were sub cultured on Nutrient broth and incubated at 37ºC for 4 hours. After incubation, fresh culture of test organism was inoculated into different biochemical media and also to Cetrimide agar for the confirmation of *P. aeruginosa*. Test organism was also cultured on Nutrient agar to

perform oxidase, catalase tests. *P. aeruginosa* isolates were characterised and identified using a combination of colonial morphology, Gram stain characteristics, motility test, oxidative- fermentation test, catalase, citrate and oxidase tests and pyocyanin production. Appropriate biochemical test were performed for identification of *P. aeruginosa* isolates as follows:

- Catalase test
- Oxidase test
- Indole test
- Methly Red test
- Voges Progeskaur test
- Citrate Utilization test
- Triple Sugar Iron Agar test
- Urease test

Result interpretation was done based on identifying characters of the isolates.

4.4.6 Antibiotic sensitivity testing

Antibiotic susceptibility test of all isolates was performed by Kirby Bauer disc diffusion method recommended by Clinical Laboratory Standard Institute (CLSI) guidelines using the antibiotic discs of HiMedia Company. In this technique a disc impregnated with antimicrobial agents are laid over the carpet culture of test organism, antimicrobial agent diffuses radially from the disc into the medium. Following overnight incubation, the culture was examined for areas of no growth around the disc. Bacterial strains sensitive to the antimicrobial were inhibited at a distance from the disc where as resistant strain grew up to the edge of the disc.

Procedure for AST:-

1. Preparation of medium
2. Preparation of inoculum
3. Performing sensitivity test
4. Observation and result interpretation

1. <u>Preparation of medium:</u>

Medium used for running AST was Muller Hinton agar. Medium was prepared as per directions provided by manufacturer. Care was taken to maintain the height of the medium while pouring (4mm in 90mm diameter plates i.e. 25ml per plate).

2. Preparation of inoculum:

   Inoculum was prepared by adding pure colonies of organisms to 5ml Nutrient broth and incubated at $37^{O}C$ for 4 hours. The prepared inoculum was compared with McFarland tube number 0.5 (Preparation of McFarland tube number 0.5 is given in appendix-III).

3. Performing sensitivity test:-

   After proper turbidity was achieved, a new sterile cotton swab was submerged in the suspension, lifted out of the broth, and the excess fluid was removed by pressing and rotating the swab against the wall of the tube. The swab was then used to inoculate the entire surface of the Mueller Hinton agar plate three times, rotating the plate 60 degrees between each inoculation. The inoculum was allowed to dry (usually taking only a few minutes but no longer than 15 minutes) before the discs are placed on the plates.

   The discs was then placed on the agar with sterile forceps and tapped gently to ensure the adherence to the agar. The plates containing the discs were incubated at 37ºC for 24 hours.

4. Observation and result interpretation

   After overnight incubation, the diameter of each zone of inhibition was measured with a ruler. The ruler was positioned across the center of the disc to make these measurements. The results were recorded in millimeters (mm) and interpretation of susceptibility was obtained by comparing the results to the standard zone interpretative chart provided by the company (appendix-V)

4.4.7 Purity plates

Purity plate culture of each batch biochemical test was performed to observe whether the tests were preceded in an aseptic condition or not. It was performed during each biochemical tests. Half nutrient agar plate was inoculated before the test and half after the biochemical test performed. This plate was incubated overnight at 37ºC. The

growth of same organism in pure form in both pre and post inoculation showed the maintenance of aseptic condition

4.4.8 Quality control (Forbes *et al.*, 2002)

Quality control was applied in various areas during the study period for the accurate interpretation of results.

a. Aseptic technique was followed during the collection and transportation of the specimens so as to avoid contamination.
b. During sample processing, all the tests were performed carefully in aseptic zone.
c. During the preparation, sterilization, storage and use of media, instructions provided by the manufacturer were strictly followed to avoid alteration of nutritional, selective, inhibitory and biochemical properties of the media.
d. The performance of newly prepared media were tested using the control species of bacteria (i.e., known organisms giving positive and negative reactions).
e. The QC of stains and reagents were maintained by preparing a control smear and staining it with the stains and reagents to be checked.
f. A Control strain of *P. aeruginosa* (ATCC 27853) was used for the standardization and correct interpretation of zone of inhibition of antibiotics during Antibiotic susceptibility testing.

4.4.8 Data analysis

All the data obtained were statistically analysed by using Statistical Package for Social Sciences (SPSS) version 16. The Chi-square test was used as per need to determine significant association between different factors for the causation of infection.

CHAPTER V

RESULTS

This study was conducted among the hospitalized patients of NINAS hospital, Bansbari. During the six months study period, a total of 1146 clinical specimens from 301 patients (ICU and other non ICU wards) were collected and microbiologically processed in the microbiology laboratory of the hospital. The clinical specimens included tracheal aspirate (563), urine (147), sputum (33), pus (80), catheter tip (25), CVP tip (64), blood (60), CSF (139), and other samples (35) which included RVD drain tip, hemorrhagic fluid, vertebral body aspiration, Chest tube drain aspiration, tip VP shunt, nasal swab, venous section tip, middle ear swab, etc. Out of the 1146 clinical specimens, 495 showed growth and 212 specimens showed positive growth with *Pseudomonas aeruginosa* which were subjected further to antimicrobial susceptibility test.

5.1 Microbiological investigation of clinical specimens

During this study period, a total of 301 patients admitted in the hospital were investigated. Of these 301 patients, 179 (57.7%) were male and 122 (42.3%) were female with age group ranging from 1-80 and more than that. The highest numbers of patients were from age group 21-30, followed by age group 31-40. The percentage of patients of age group 21-30 was found to be 20.9%

Table 1: Distribution of total clinical cases according to Gender and Age

Age groups	Male		Female		Total	
	n	%	n	%	N	%
<10	8	50.0	8	50.0	16	5.3
11-20	22	66.7	11	33.3	33	11.0
21-30	40	63.5	23	36.5	63	20.9
31-40	37	64.9	20	35.1	57	18.9
41-50	30	61.2	19	38.8	49	16.3
51-60	21	45.6	25	54.3	46	15.3
61-70	12	57.1	9	42.9	21	7.0
71-80	5	45.4	6	54.5	11	3.6
>80	4	80.0	1	20.0	5	1.7
Total	179	57.7	122	42.3	301	100

(n)=number of male or female clinical cases, (N) = total number of particular cases

Among the 301 patients, 101 (33.6 %) were admitted with Road traffic accident. Rest of the patients with their respective diagnosis in the hospitals were Craniotomy (16.6%), Infective discitis (13.0%), Fall Injury (10.3%), Cerebro-vascular diseases (8.6%), Post operative Complications (6.0%), Seizure disorder (4.0%), Subdural haematoma (3.3%), Aneurysm (2.0%), Ischaemic stroke (1.7%), and Meningitis (1.0%) (Table 2)

Table 2: Distribution of total clinical cases on the basis of diagnosis

Diagnosis	Male		Female		Total	
	n	%	n	%	N	%
RTA (Trauma)	61	60.4	40	39.6	101	33.6
Seizure disorder	5	41.7	7	58.3	12	4.0
Meningitis	3	100.0	0	0	3	1.0
Aneurysm	4	66.7	2	33.3	6	2.0
Subdural haematoma	6	60.0	4	40	10	3.3
Craniotomy	26	52.0	24	48	50	16.6
Infective discitis	19	48.7	20	51.3	39	13.0
Ischaemic stroke	3	60.0	2	40	5	1.7
Post operative complications	12	66.7	6	33.3	18	6.0
Cerebro-vascular diseases	20	76.9	6	23.1	26	8.6
Fall injury (Trauma)	20	64.5	11	35.5	31	10.3
Total	179	59.5	122	40.5	301	100

(n)= number of male or female cases, (N) = total number of particular cases

Among the 1146 clinical specimens received, 563 (49.1%) were tracheal specimens, 147 (12.8%) were urine, 33 (2.9%) were sputum, 80 (7.0%) were pus, 25 (2.1%) were catheter tip, 64 (5.6%) were CVP tip, 60 (5.2%) were blood, 138 (12.1%) were CSF, and 35 (3.0%) were miscellaneous (Table. 3). Most of the specimens of tracheal aspirate, sputum, catheter tip and blood were received from ICU patients while most of the specimens of urine, pus, CVP tip, and CSF were received from patients from other Non ICU wards (Table 3).

Table 3: Distribution of total clinical specimens under investigation

Types of Specimens	Total Specimens Received		ICU cases		Non ICU Cases	
	Number (n)	Percentage	Number (n)	Percentage	Number (n)	Percentage
Tracheal aspirate	563	49.1	328	58.3	235	41.7
Urine	147	12.8	57	38.8	90	61.2
Sputum	33	2.9	24	72.7	9	27.3
Pus	80	7.0	7	8.8	73	91.2
Catheter tip	25	2.2	15	60.0	10	40.0
CVP tip	64	5.6	15	23.4	49	76.6
Blood	60	5.2	35	60.0	24	40.0
CSF	139	12.1	39	28.1	100	71.9
Miscellaneous	35	3.0	11	31.4	24	68.6
Total	1146	100	531	46.4	614	53.6

Out of the total specimens investigated, significant growth of *P. aeruginosa* was obtained in 18.5% (n=212), 28.8 % (n=61) of whom had polymicrobial growth and 71.2% (n=151) had single growth. Microbiological analysis reflected that 29.7% (n=167) of the total tracheal aspirate, 6.1% (n=9) of the total urine, 36.4% (n=12) of the total sputum, 12.5% (n=10) of the total pus specimen, 7% (n=28) of the total catheter tip, 4.7% (n=3) of the total CVP tip, 1.4% (n=2) of the total CSF, and 5.7% (n=2) of the total miscellanous specimens showed the growth of Pseudomonas aeruginosa. However the blood specimen (60) showed no growth of any organism (Table 4).

Table 4: Distribution and analysis of *P. aeruginosa* in various specimens

Type of Specimen	Total	Growth obtained (*P. aeruginosa*)		Total (%)
		Single (%)	Polymicrobial (%)	
Tracheal aspirate	563	110 (65.9)	57 (34.1)	167 (29.7)
Urine	147	9 (100.0)	0	9 (6.1)
Sputum	33	12 (100.0)	0	12 (36.4)
Pus	80	8 (80.0)	2 (20.0)	10 (12.5)
Catheter tip	25	5 (71.4)	2 (28.6)	7 (28.0)
CVP tip	64	3 (100.0)	0	3 (4.7)
Blood	60	0	0	0 (0)
CSF	139	2 (100.0)	0	2 (1.4)
Miscellaneous	35	2 (100.0)	0	2 (5.7)
Total	1146	151 (71.2)	61 (28.8)	212(18.5)

Among the total positive growth of *P. aeruginosa*, 181 (85.4%) of the *P. aeruginosa* was found to be multidrug resistant (Table 5).

Table 5: Distribution and analysis of Multidrug Resistant *P. aeruginosa* in various specimens

Type of Specimen	Total *P. aeruginosa* isolated	Multidrug resistant	Percentage
Tracheal aspirate	167	140	83.8
Urine	9	9	100.0
Sputum	12	10	83.3
Pus	10	9	90.0
Catheter tip	7	7	100.0
CVP tip	3	2	66.7
Blood	0	0	0
CSF	2	2	100.0
Miscellaneous	2	2	100.0
Total	212	181	85.4

Among the 212 positive growths, the highest number of growth was obtained from age group 21-30 (27.8%), of whom 94.9% (n=56) were male and 5.1% (n=3) were female. In total 88.2% of the positive growth was from male patients and 11.8% of the positive growth was from female patients. The least percentage of positive growth was obtained from the age group >80 (0.5%). The highest number of positive growth in male patients (n=55) was obtained from age group 41-50 and that in female patients (n=7) was obtained from age group 51-60 (Table 6).

Table 6: Distribution pattern of culture positive specimens according to gender and age

Age category	Male		Female		Total	
	Number (n)	Percentage	Number (n)	Percentage	Number (N)	Percentage (%)
<10	2	66.7	1	33.3	3	1.4
11-20	45	93.8	3	6.2	48	22.6
21-30	56	94.9	3	5.1	59	27.8
31-40	23	85.2	4	14.8	27	12.7
41-50	55	96.5	2	3.5	57	26.9
51-60	1	12.5	7	87.5	8	3.8
61-70	2	40.0	3	60.0	5	2.3
71-80	2	50.0	2	50.0	4	1.9
>80	1	100.0	0	0	1	0.5
Total	187	88.2	25	11.8	212	100

n=number of male or female cases, (N) = total number of particular cases

Among the total 212 positive growth, 48.6% (n=103) of the specimens were from ICU and 51.4% (n=109) of the specimens were from other Non ICU units (General wards and cabins). The highest number of positive growth of *P. aeruginosa* in ICU (n=40) was from age group 21-30 and that from Non ICU units (n=45) were from age group 41-50 (Table 7).

Table 7: Distribution pattern of culture positive specimens according to wards

Age category	ICU		Non ICU		Total	
	Number (n)	Percentage	Number (n)	Percentage	Number (N)	Percentage
<10	0	0	3	100.0	3	1.4
11-20	21	43.8	27	56.2	48	22.6
21-30	40	67.8	19	32.2	59	27.8
31-40	15	55.6	12	44.4	27	12.7
41-50	12	21.1	45	78.9	57	26.9
51-60	8	100.0	0	0	8	3.8
61-70	5	100.0	0	0	5	2.4
71-80	2	50.0	2	50.0	4	1.9
>80	0	0	1	100.0	1	0.5
Total	103	48.6	109	51.4	212	100

(n)= number of ICU or non ICU positive cases, (N) = total number of positive cases

5.2 Antibiotic sensitivity pattern of *P. aeruginosa* isolated from different growth positive clinical specimens

The *P. aeruginosa* isolates exhibited maximum resistance to Cefepime (93.9%) followed by Cefotaxime (90.1%), Cefepime/tazobactum (84.4%), Ceftazidime (83.0%), Gentamicin (81.1%), Ofloxacin (75.9%), Amikacin (74.0%), Carbenicillin (58.5%), Ciprofloxacin (43.4%), Piperacillin/tazobactum (38.2%) and lastly Imipenem (9.4%). ICU isolates showed more resistance to Amikacin, Ceftazidime, Carbenicillin, Cefepime, Cefepime/Tazobactum, Imipenem, Piperacillin/Tazobactum and Meropenem in comparision to non ICU isolates. Similarly Non ICU isolates showed more resistance to drugs Cefotaxime, Ciprofloxacin, Gentamicin and Ofloxacin (Table-8).

Table 8: Resistance exhibited by *P. aeruginosa* isolates in total specimens.

Antibiotics	Percent resistance		
	Total	ICU	Non ICU
Amikacin(10mcg)	74.0	52.9	47.1
Cefotaxime(30 mcg)	90.1	49.7	50.3
Ciprofloxacin(5 mcg)	43.4	35.9	64.1
Ceftazidime(30 mcg)	83.0	51.7	48.3
Carbenicillin(100 mcg)	58.5	62.1	37.9
Cefepime(30 mcg)	93.9	50.2	49.8
Cefepime/Tazobactum(30/10 mcg)	84.4	51.4	48.6
Gentamicin(10 mcg)	81.1	49.4	50.6
Imipenem(10 mcg)	9.4	65.0	35.0
Ofloxacin(5 mcg)	75.9	47.8	52.2
Piperacillin/Tazobactum(100/10 mcg)	38.2	58.0	42.0
Meropenem(10 mcg)	71.2	50.3	49.7

Table 9: Antibiogram of the total *P. aeruginosa* isolates

Isolates	Antibiotics											
	AK	CE	CF	CA	CB	CPM	CPT	G	I	OF	PT	MR
Isolate-1	S	S	R	I	S	R	S	R	S	R	S	R
Isolate-2	S	I	R	R	I	R	R	I	S	R	S	R
Isolate-3	R	R	R	R	R	R	R	R	S	R	I	R
Isolate-4	R	R	R	R	R	R	R	R	S	R	R	R
Isolate-5	S	R	R	R	I	R	S	S	S	R	S	R
Isolate-6	R	R	R	R	R	R	R	R	S	R	R	R
Isolate-7	S	I	I	R	S	R	S	R	S	R	S	S
Isolate-8	S	R	S	R	S	R	R	R	S	R	R	S
Isolate-9	S	R	S	R	R	R	R	I	S	S	R	S
Isolate-10	R	R	I	R	R	R	R	R	S	R	I	R
Isolate-11	S	R	S	R	R	R	R	R	S	S	R	S
Isolate-12	R	R	R	R	R	R	R	R	S	R	R	R
Isolate-13	S	R	R	R	R	R	I	R	S	R	I	I
Isolate-14	S	R	S	I	S	S	S	S	S	S	R	S
Isolate-15	R	R	S	R	R	R	R	R	S	R	S	I
Isolate-16	S	R	S	R	I	R	S	R	S	R	S	S
Isolate-17	R	R	I	R	R	R	R	R	S	R	R	R
Isolate-18	R	R	I	R	R	R	R	R	S	R	I	R
Isolate-19	R	R	S	R	R	R	R	R	S	R	S	I
Isolate-20	R	R	R	R	R	R	R	R	R	R	I	R
Isolate-21	S	R	S	S	S	R	S	S	S	S	S	S
Isolate-22	R	R	R	R	R	R	R	R	S	R	R	R
Isolate-23	R	I	R	R	R	R	R	R	S	R	R	R
Isolate-24	R	R	I	R	R	R	R	R	S	R	R	R
Isolate-25	R	R	R	R	S	R	R	R	S	I	S	R
Isolate-26	R	R	I	R	R	R	R	R	I	R	I	R
Isolate-27	R	R	I	R	R	R	R	R	R	R	R	R
Isolate-28	R	R	R	R	S	R	R	R	S	R	S	R
Isolate-29	R	R	R	R	S	R	R	R	S	R	S	R
Isolate-30	R	R	R	R	R	R	R	R	S	R	S	R
Isolate-31	S	R	S	I	S	R	S	S	S	S	S	S
Isolate-32	R	R	I	R	R	R	R	R	S	R	R	R
Isolate-33	R	I	R	R	I	R	R	S	S	R	I	R
Isolate-34	S	R	R	I	I	R	S	S	S	R	R	I
Isolate-35	R	I	R	R	R	R	R	R	S	R	R	R
Isolate-36	S	R	I	R	S	R	R	S	S	R	R	S
Isolate-37	I	R	R	R	R	R	R	I	S	R	I	I
Isolate-38	S	R	R	R	R	R	R	R	S	R	S	I
Isolate-39	S	R	S	I	S	R	I	S	S	S	R	S
Isolate-40	S	R	S	R	S	R	I	S	I	S	R	I
Isolate-41	R	R	R	R	R	R	R	R	S	R	R	R

Isolates	Antibiotics											
	AK	CE	CF	CA	CB	CPM	CPT	G	I	OF	PT	MR
Isolate-42	R	R	R	R	R	R	R	R	S	R	R	R
Isolate-43	R	R	R	R	R	R	R	R	S	R	R	R
Isolate-44	R	R	R	R	R	R	R	R	S	R	R	R
Isolate-45	R	R	I	R	R	R	R	R	S	R	I	R
Isolate-46	R	R	S	R	R	R	R	R	S	R	S	R
Isolate-47	R	R	S	R	R	R	R	R	S	R	S	R
Isolate-48	R	R	S	R	R	R	R	R	S	R	S	R
Isolate-49	R	R	S	R	R	R	R	R	S	R	I	I
Isolate-50	I	R	S	R	I	R	R	R	S	R	I	I
Isolate-51	R	R	S	R	R	R	R	R	S	R	R	R
Isolate-52	R	R	I	R	R	R	R	R	S	R	I	R
Isolate-53	R	R	R	R	R	R	R	R	I	S	I	R
Isolate-54	R	R	I	R	R	R	R	R	S	R	I	R
Isolate-55	R	R	R	R	R	R	R	R	S	I	R	R
Isolate-56	R	R	I	R	R	R	R	I	S	I	R	R
Isolate-57	R	R	R	R	R	R	R	R	S	S	R	S
Isolate-58	R	R	I	R	I	R	R	R	S	S	R	R
Isolate-59	R	R	S	R	R	R	R	R	S	R	R	R
Isolate-60	R	R	I	R	I	R	R	R	S	S	R	R
Isolate-61	R	R	R	R	R	R	R	R	S	I	R	I
Isolate-62	R	R	R	R	R	R	R	R	S	R	I	R
Isolate-63	R	R	R	R	R	R	R	R	S	I	R	R
Isolate-64	R	R	R	R	R	R	R	R	S	R	R	R
Isolate-65	R	R	I	R	R	R	R	R	S	I	R	R
Isolate-66	R	R	R	R	R	R	R	R	S	R	R	R
Isolate-67	R	R	I	R	R	R	R	R	S	R	R	R
Isolate-68	R	R	R	R	R	R	R	R	R	R	S	R
Isolate-69	R	R	S	R	I	R	R	R	S	R	R	I
Isolate-70	R	R	I	R	R	R	R	R	I	R	R	R
Isolate-71	R	R	R	R	I	R	R	R	I	I	R	I
Isolate-72	R	R	I	R	R	R	R	R	R	R	R	R
Isolate-73	R	R	I	R	R	R	R	R	R	R	R	R
Isolate-74	R	R	I	R	R	R	R	R	S	R	R	R
Isolate-75	R	R	R	R	R	R	R	R	R	I	R	R
Isolate-76	R	R	R	R	R	R	R	R	I	I	R	R
Isolate-77	R	R	R	R	R	R	R	R	I	I	R	R
Isolate-78	R	R	I	R	R	R	R	R	S	R	R	R
Isolate-79	R	R	R	R	R	R	R	R	S	R	R	R
Isolate-80	R	R	R	R	R	R	R	I	R	R	R	R
Isolate-81	S	R	S	S	S	R	S	S	S	S	S	S
Isolate-82	R	R	I	R	R	R	R	R	S	R	I	R
Isolate-83	R	R	I	R	R	R	R	R	I	R	I	R
Isolate-84	R	R	R	R	R	R	R	R	I	R	I	R
Isolate-85	R	R	I	R	R	R	R	R	R	R	I	R

Isolates	Antibiotics											
	AK	CE	CF	CA	CB	CPM	CPT	G	I	OF	PT	MR
Isolate-86	R	R	S	R	S	R	R	R	S	R	S	R
Isolate-87	R	R	S	R	R	R	R	R	S	R	I	R
Isolate-88	R	R	I	R	S	R	R	R	S	R	I	R
Isolate-89	R	R	I	R	R	R	R	R	S	R	I	R
Isolate-90	R	R	I	R	R	R	R	R	S	R	I	R
Isolate-91	R	R	I	R	R	R	R	R	S	R	I	R
Isolate-92	R	R	I	R	R	R	R	R	R	R	S	R
Isolate-93	R	R	I	R	R	R	R	R	S	R	I	R
Isolate-94	R	R	I	R	R	R	R	R	R	R	I	R
Isolate-95	R	R	R	R	R	R	R	R	S	R	I	I
Isolate-96	R	R	I	R	R	R	R	R	R	R	I	R
Isolate-97	R	R	R	R	R	R	R	R	S	R	R	R
Isolate-98	R	R	I	R	R	R	R	R	S	R	I	R
Isolate-99	R	R	R	R	R	R	R	R	S	R	R	R
Isolate-100	R	R	I	R	R	R	R	R	S	R	I	R
Isolate-101	R	R	I	R	R	R	R	R	S	R	I	R
Isolate-102	S	R	R	R	R	R	R	R	R	R	I	I
Isolate-103	R	R	I	R	R	R	R	R	S	R	I	R
Isolate-104	R	R	I	R	R	R	R	R	S	R	I	R
Isolate-105	R	R	I	R	R	R	R	R	S	R	R	R
Isolate-106	R	R	R	R	S	R	R	R	S	R	S	R
Isolate-107	R	R	R	R	R	R	R	R	S	R	I	R
Isolate-108	R	R	I	R	R	R	R	R	S	R	R	R
Isolate-109	R	R	I	R	R	R	R	R	R	R	R	R
Isolate-110	R	R	I	R	R	R	R	R	S	R	I	R
Isolate-111	R	R	I	R	R	R	R	R	S	R	I	R
Isolate-112	R	R	I	R	R	R	R	R	S	R	I	R
Isolate-113	R	R	S	R	R	R	R	I	S	R	I	R
Isolate-114	R	R	I	R	R	R	R	R	S	R	R	R
Isolate-115	R	R	I	R	R	R	R	R	S	R	R	R
Isolate-116	R	R	I	R	R	R	R	R	S	R	R	R
Isolate-117	R	R	I	R	R	R	R	R	S	R	R	R
Isolate-118	R	R	I	R	R	R	R	R	R	R	R	R
Isolate-119	R	R	I	R	R	R	R	R	S	R	R	R
Isolate-120	R	R	I	R	R	R	R	R	S	R	R	R
Isolate-121	R	R	I	R	R	R	R	R	S	R	R	R
Isolate-122	R	R	I	R	R	R	R	R	S	R	R	R
Isolate-123	R	R	I	R	R	R	R	R	S	R	I	R
Isolate-124	R	R	I	R	R	R	R	R	S	R	R	R
Isolate-125	S	R	S	R	R	R	R	S	S	S	S	S
Isolate-126	R	R	I	R	R	R	R	R	S	R	R	R
Isolate-127	S	R	R	R	S	R	R	I	S	R	I	S
Isolate-128	R	R	I	R	R	R	R	R	S	R	R	R
Isolate-129	R	R	I	R	R	R	R	R	S	R	R	R
Isolate-130	S	R	S	R	R	R	R	S	S	S	S	S

Isolates	Antibiotics											
	AK	CE	CF	CA	CB	CPM	CPT	G	I	OF	PT	MR
Isolate-131	R	R	R	R	R	R	R	R	R	I	R	I
Isolate-132	R	R	I	R	R	R	R	S	S	R	R	R
Isolate-133	S	R	S	I	S	R	R	S	S	S	S	S
Isolate-134	R	R	I	R	R	R	R	S	S	R	R	R
Isolate-135	R	R	I	R	R	R	R	R	S	R	R	R
Isolate-136	R	R	I	R	R	R	R	R	S	R	R	R
Isolate-137	S	R	S	I	S	R	R	S	S	S	S	S
Isolate-138	R	R	I	R	R	R	R	R	S	R	R	R
Isolate-139	S	S	S	S	S	S	S	S	S	S	S	S
Isolate-140	S	S	S	S	S	S	S	S	S	S	S	S
Isolate-141	R	R	R	R	R	R	R	R	S	R	S	R
Isolate-142	R	R	R	R	R	R	R	R	I	R	R	R
Isolate-143	R	R	R	R	R	R	R	R	S	R	S	R
Isolate-144	R	R	R	R	R	R	R	R	I	R	I	R
Isolate-145	S	S	S	S	S	S	S	S	S	S	S	S
Isolate-146	R	R	R	R	R	R	R	R	S	R	S	R
Isolate-147	R	R	R	R	R	R	R	R	I	R	I	R
Isolate-148	S	S	S	S	S	S	S	S	S	S	S	S
Isolate-149	R	R	R	R	R	R	R	R	S	R	S	R
Isolate-150	S	S	S	S	S	S	S	S	S	S	S	S
Isolate-151	R	R	R	R	R	R	R	R	S	R	S	R
Isolate-152	R	R	R	R	R	R	R	R	S	R	S	R
Isolate-153	S	S	S	S	S	S	S	S	S	S	S	S
Isolate-154	S	S	S	S	S	S	S	S	S	S	S	S
Isolate-155	R	R	S	R	S	R	R	R	S	R	S	R
Isolate-156	S	S	S	S	S	S	S	S	S	S	S	S
Isolate-157	R	R	R	R	S	R	R	R	S	R	S	R
Isolate-158	S	R	S	I	S	S	S	R	S	R	S	R
Isolate-159	R	R	R	R	I	R	R	R	S	R	S	R
Isolate-160	R	R	R	R	I	R	R	R	S	R	S	R
Isolate-161	R	R	R	R	I	R	R	R	S	R	S	R
Isolate-162	R	R	R	R	I	R	R	R	S	R	S	R
Isolate-163	R	R	R	R	I	R	R	R	S	R	S	R
Isolate-164	R	R	R	R	I	R	R	R	S	R	S	R
Isolate-165	R	R	R	R	I	R	R	R	S	R	S	R
Isolate-166	R	R	R	R	I	R	R	R	S	R	S	R
Isolate-167	R	R	R	R	I	R	R	R	S	R	S	R
Isolate-168	R	R	R	R	R	R	R	R	S	R	S	R
Isolate-169	S	S	S	S	S	R	S	S	S	S	S	S
Isolate-170	R	R	R	R	I	R	R	R	S	R	S	R
Isolate-171	S	R	S	I	R	R	R	S	S	S	S	S
Isolate-172	R	R	R	R	R	R	R	R	S	R	R	R
Isolate173	R	R	R	R	I	R	R	R	S	R	R	R
Isolate174	R	R	R	R	R	R	R	R	S	R	R	R

Isolates	Antibiotics											
	AK	CE	CF	CA	CB	CPM	CPT	G	I	OF	PT	MR
Isolate-175	S	R	S	S	I	R	S	S	S	S	S	S
Isolate-176	R	R	R	R	I	R	R	R	S	R	R	R
Isolate-177	R	R	R	R	R	R	R	R	S	R	R	R
Isolate-178	R	R	R	R	S	R	R	R	S	R	R	R
Isolate-179	S	S	S	S	S	R	S	S	S	S	S	S
Isolate-180	R	R	R	R	S	R	R	R	S	R	R	R
Isolate-181	S	S	S	S	S	R	S	S	S	S	S	S
Isolate-182	R	R	R	R	S	R	R	R	S	R	R	I
Isolate-183	S	S	S	S	S	R	S	S	S	S	S	S
Isolate-184	R	R	R	R	S	R	R	R	S	R	I	R
Isolate-185	R	R	R	R	S	R	R	R	S	R	I	R
Isolate-186	S	R	S	S	S	R	S	R	S	R	S	S
Isolate-187	R	R	R	R	S	R	R	R	S	R	I	R
Isolate-188	S	I	S	S	S	R	R	R	S	S	S	S
Isolate-189	R	R	R	R	S	R	R	R	S	R	S	R
Isolate-190	S	R	S	S	S	S	S	S	S	S	S	S
Isolate-191	S	R	S	S	S	R	S	R	S	S	S	S
Isolate-192	S	R	S	S	S	R	S	R	S	S	S	S
Isolate-193	R	R	R	R	S	R	R	R	S	I	S	R
Isolate-194	R	R	R	R	S	R	R	R	S	I	I	R
Isolate-195	R	R	R	R	S	R	R	R	S	R	I	R
Isolate-196	S	R	S	R	S	R	R	R	S	R	S	S
Isolate-197	R	R	R	R	S	R	R	R	S	R	I	R
Isolate-198	S	R	S	R	S	R	R	S	S	S	S	S
Isolate-199	S	R	S	I	S	R	R	R	S	R	S	S
Isolate-200	S	R	S	I	S	R	R	R	S	R	S	S
Isolate-201	S	R	S	I	S	R	R	R	S	R	S	I
Isolate-202	S	R	S	I	S	R	R	S	S	S	I	S
Isolate-203	S	R	S	I	S	R	R	R	S	R	S	I
Isolate-204	S	S	S	S	S	S	S	S	S	S	S	S
Isolate-205	R	R	R	R	R	R	R	R	R	R	I	R
Isolate-206	R	R	R	R	S	R	R	R	R	R	S	R
Isolate-207	R	R	R	R	S	R	R	R	R	R	S	R
Isolate-208	R	R	R	R	S	R	R	R	S	R	S	R
Isolate-209	S	S	S	S	S	S	S	S	S	S	S	S
Isolate-210	R	R	R	R	S	R	R	R	S	R	S	R
Isolate-211	R	R	R	R	S	R	R	R	R	R	S	R
Isolate-212	R	R	R	R	S	R	R	R	R	R	I	R

(S)= sensitive, (I) = intermediate, (R)=resistant

AK-Amikacin, CE-Cephotaxime, CF=Ciprofloxacin, CA=Ceftazidime, CB=Carbenicillin, CPM=Cefepime, CPT= Cefepime/Tazobactum, G=Gentamicin, I=Imipenem, OF=Ofloxacin, PT= Piperacillin/Tazobactum, MR=Meropenem.

5.2.1 Antibiotic susceptibility pattern of *P. aeruginosa* from Tracheal specimens

P. aeruginosa isolated from tracheal specimens was found to be highly sensitive towards Imipenem (83.2%, n=139) followed by Piperacillin/Tazobactum (38.9%, n=65). The isolates showed maximum resistance to Cefepime (92.8%, n=155)

followed by Cefotaxime (91.0%, n=152). Intermediate resistance was shown maximum to Ciprofloxacin (31.7%, n=53) (Table 10).

Table 10: Antibiotic susceptibility pattern exhibited by *P. aeruginosa* in Tracheal specimen.

Antibiotics	Sensitive		Intermediate		Resistant	
	n	%	n	%	n	%
Amikacin(10mcg)	36	21.6	1	0.6	130	77.8
Cefotaxime(30 mcg)	14	8.4	1	0.6	152	91.0
Ciprofloxacin(5 mcg)	46	27.5	53	31.7	68	40.7
Ceftazidime(30 mcg)	21	12.6	9	5.4	137	82.0
Carbenicillin(100 mcg)	52	31.1	19	11.4	96	57.5
Cefepime(30 mcg)	12	7.1	0	0	155	92.8
Cefepime/Tazobactum(30/10 mcg)	21	12.6	1	0.6	145	86.8
Gentamicin(10 mcg)	24	14.4	4	2.4	139	83.2
Imipenem(10 mcg)	139	83.2	10	6.0	18	10.8
Ofloxacin(5 mcg)	31	18.6	12	7.2	123	73.6
Piperacillin/Tazobactum(100/10 mcg)	65	38.9	43	25.7	59	35.3
Meropenem(10 mcg)	33	19.8	11	6.6	123	73.6

(n)=number of sensitive, intermediate or resistant organism

5.2.2 Antibiotic susceptibility pattern exhibited by *P. aeruginosa* from Urine.

The isolates from urine were found to be highly sensitive to Imipenem (100.0%, n=9) followed by Piperacillin/Tazobactum (44.4%, n=4). They exhibited maximum resistance to Cefepime (100.0%, n=9) followed by Ceftazidime (88.9%, n=8) and Ofloxacin (88.9%, n=8). Intermediate resistance was shown to six antibiotics (Table 11).

Table 11: Antibiotic susceptibility pattern exhibited by *P. aeruginosa* from Urine.

Antibiotics	Sensitive		Intermediate		Resistant	
	n	%	n	%	n	%
Amikacin(10mcg)	6	66.7	0	0	3	33.3
Cefotaxime(30 mcg)	1	11.1	2	22.2	6	66.7
Ciprofloxacin(5 mcg)	2	22.2	1	11.1	6	66.7
Ceftazidime(30 mcg)	0	0	1	11.1	8	88.9
Carbenicillin(100 mcg)	3	33.3	2	22.2	4	44.4
Cefepime(30 mcg)	0	0	0	0	9	100.0
Cefepime/Tazobactum(30/10 mcg)	3	33.3	0	0	6	66.7
Gentamicin(10 mcg)	1	11.1	2	22.2	6	66.7
Imipenem(10 mcg)	9	100.0	0	0	0	0
Ofloxacin(5 mcg)	1	11.1	0	0	8	88.9
Piperacillin/Tazobactum(100/10 mcg)	4	44.4	1	11.1	4	44.4
Meropenem(10 mcg)	3	33.3	0	0	6	66.7

(n)=number of sensitive, intermediate or resistant organism

5.2.3 Antibiotic susceptibility pattern exhibited by *P. aeruginosa* from Sputum.

As shown in Table 11, the *P. aeruginosa* isolated from sputum showed maximum sensitivity to Imipenem (91.7%, n=11), while other drugs showed moderate sensitivity. The 100 percent resistance was shown to Cefotaxime (100.0%, n=12) followed by Cefepime (91.7%, n=11). It showed intermediate resistance to Ciprofloxacin (25.0%, n=3) and five other antibiotics (Table 12).

Table 12: Antibiotic susceptibility pattern exhibited by *P. aeruginosa* from Sputum.

Antibiotics	Sensitive		Intermediate		Resistant	
	n	%	n	%	n	%
Amikacin(10mcg)	5	41.7	0	0	7	58.3
Cefotaxime(30 mcg)	0	0	0	0	12	100.0
Ciprofloxacin(5 mcg)	6	50.0	3	25.0	3	25.0
Ceftazidime(30 mcg)	1	8.3	1	8.3	10	83.33
Carbenicillin(100 mcg)	2	16.7	1	8.3	9	75.0
Cefepime(30 mcg)	1	8.3	0	0	11	91.7
Cefepime/Tazobactum(30/10 mcg)	3	25.0	1	8.3	8	66.7
Gentamicin(10 mcg)	2	16.7	0	0	10	83.3
Imipenem(10 mcg)	11	91.7	0	0	1	8.3
Ofloxacin(5 mcg)	3	25.0	0	0	9	75.0
Piperacillin/Tazobactum(100/10 mcg)	4	33.3	4	33.3	4	33.3
Meropenem(10 mcg)	4	33.3	3	25.0	5	41.7

(n)=number of sensitive, intermediate or resistant organism

5.2.4 Antibiotic susceptibility pattern exhibited by *P. aeruginosa* from Pus.

P. aeruginosa isolated from pus exhibited maximum sensitivity to Imipenem (80.0%, n=8) followed by Piperacillin/Tazobactum (50.0%, n=5). 100 percent resistance was shown to Cefepime (100.0%, n=10) while maximum resistance was shown to other drugs also like Amikacin, Cefotaxime, Cefepime/Tazobactum, Ceftazidime, Gentamicin and Meropenem. Intermediate resistance was shown to six drugs (Table. 13)

Table 13: Antibiotic susceptibility pattern exhibited by *P. aeruginosa* from Pus.

Antibiotics	Sensitive		Intermediate		Resistant	
	n	%	n	%	n	%
Amikacin(10mcg)	1	10.0	0	0	9	90.0
Cefotaxime(30 mcg)	0	0	1	10.0	9	90.0
Ciprofloxacin(5 mcg)	1	10.0	3	30.0	6	60.0
Ceftazidime(30 mcg)	0	0	1	10.0	9	90.0
Carbenicillin(100 mcg)	4	40.0	0	0	6	60.0
Cefepime(30 mcg)	0	0	0	0	10	100.0
Cefepime/Tazobactum(30/10 mcg)	1	10.0	0	0	9	90.0
Gentamicin(10 mcg)	1	10.0	0	0	9	90.0
Imipenem(10 mcg)	8	80.0	1	10.0	1	10.0
Ofloxacin(5 mcg)	1	10.0	1	10.0	8	80.0
Piperacillin/Tazobactum(100/10 mcg)	5	50.0	1	10.0	4	40.0
Meropenem(10 mcg)	1	10.0	0	0	9	90.0

(n)=number of sensitive, intermediate or resistant organism

5.2.5 Antibiotic susceptibility pattern exhibited by *P. aeruginosa* from Catheter tip.

As shown in Table 14, the organism isolated from Catheter tip specimen exhibited 100 percent sensitivity to Imipenem (100.0%, n=7), followed by moderate sensitivity to Amikacin (42.8%, n=3) and to Gentamicin (42.8%, n=3). While the isolates showed 100 percent resistance to Cefepime and maximum resistance to other drugs like Ceftazidime (85.7%, n=6) and Cefipime/Tazobactum (85.7%, n=6). Maximum intermediate resistance was exhibited to Meropenem (42.8%, n=3) followed by seven other drugs (Table 14).

Table 14: Antibiotic susceptibility pattern exhibited by *P. aeruginosa* from Catheter tip.

Antibiotics	Sensitive		Intermediate		Resistant	
	n	%	n	%	n	%
Amikacin(10mcg)	3	42.8	1	14.3	3	42.8
Cefotaxime(30 mcg)	0	0	2	28.6	5	71.4
Ciprofloxacin(5 mcg)	0	0	2	28.6	5	71.4
Ceftazidime(30 mcg)	0	0	1	14.3	6	85.7
Carbenicillin(100 mcg)	1	14.3	2	28.6	4	57.1
Cefepime(30 mcg)	0	0	0	0	7	100.0
Cefepime/Tazobactum(30/10 mcg)	1	14.3	0	0	6	85.7
Gentamicin(10 mcg)	3	42.8	1	14.3	3	42.8
Imipenem(10 mcg)	7	100.0	0	0	0	0
Ofloxacin(5 mcg)	0	0	0	0	7	100.0
Piperacillin/Tazobactum(100/10 mcg)	1	14.3	2	28.6	4	57.1
Meropenem(10 mcg)	1	14.3	3	42.8	3	42.8

(n)=number of sensitive, intermediate or resistant organism

5.2.6 Antibiotic susceptibility pattern exhibited by *P. aeruginosa* from CVP tip

P. aeruginosa isolated from CVP tip did not showed 100 percent senstitivity to any of the antibiotics while it showed 100 percent resistance to Cefepime, Cefotaxime and Piperacillin/Tazobactum.

5.2.7 Antibiotic susceptibility pattern exhibited by *P. aeruginosa* from CSF

The total isolates from CSF were two which showed 100 percent sensitivity to Imipenem and 100 percent resistance to all other drugs except Imipenem. There was no intermediate resistance shown by the isolates obtained from CSF.

5.2.8 Antibiotic susceptibility pattern exhibited by *P. aeruginosa* from miscellanous

Of the two isolates obtained from the miscellaneous specimens, 100 percent sensitivity was shown to Imipenem, intermediate resistance was exhibited to Ciprofloxacin (50.0%, n=1) and Piperacilli/Tazobactum (50.0%, n=1). While 100 percent resistance was shown to Amikacin, Cefotaxime, Ceftazidime, Carbenicillin, Cefepime, Cefepime/Tazobactum, Gentamicin, Ofloxacin and Meropenem.

5.2.9 Antibiotic susceptibility pattern exhibited by *P. aeruginosa* isolated from ICU

Of the total 103 *P. aeruginosa* isolates obtained from ICU, 81.6 %(n=84) sensitivity was shown to Imipenem. It showed maximum resistance to Cefepime (97.1%, n=100), followed by Cefotaxime (92.2%, n=95) and also Cefepime/Tazobactum (89.3%, n=92), Ceftazidime (88.3%, n=91), Gentamicin (82.5%, n=85) and Amikacin (80.6%, n=83). Moderate intermediate resistance was exhibited to Ciprofloxacin and Piperacillin/Tazobactum (Table 15).

Table 15: Antibiotic susceptibility pattern exhibited by *P. aeruginosa* isolated from ICU.

Antibiotics	Sensitive		Intermediate		Resistant	
	n	%	n	%	n	%
Amikacin(10mcg)	19	18.4	1	1.0	83	80.6
Cefotaxime(30 mcg)	5	4.8	3	2.9	95	92.2
Ciprofloxacin(5 mcg)	25	24.3	45	43.7	33	32.0
Ceftazidime(30 mcg)	7	6.8	5	4.8	91	88.3
Carbenicillin(100 mcg)	16	15.5	10	9.7	77	74.8
Cefepime(30 mcg)	3	2.9	0	0	100	97.1
Cefepime/Tazobactum(30/10 mcg)	10	9.7	1	1.0	92	89.3
Gentamicin(10 mcg)	14	13.6	4	3.9	85	82.5
Imipenem(10 mcg)	84	81.6	6	5.8	13	12.6
Ofloxacin(5 mcg)	16	15.5	10	9.7	77	74.8
Piperacillin/Tazobactum(100/10 mcg)	23	22.3	33	32.0	47	45.6
Meropenem(10 mcg)	16	15.5	11	10.7	76	73.8

5.2.10 Antibiotic susceptibility pattern exhibited by *P. aeruginosa* isolated from non ICU.

Of the total 109 *P. aeruginosa* isolates obtained from Non ICU wards, 88.1% (n=96) was found to be sensitive to Imipenem which was greater than that exhibited by the organism isolated from ICU. It did not showed effective sensitivity to other drugs in comparison to Imipenem. In case of resistance shown by the isolates, 90.8 %(n=99) resistance was exhibited to Cefepime followed by Cefotaxime (88.1%, n=96), Cefepime/Tazobactum (79.8%, n=87) and Gentamicin (79.8%, n=87) (Table 16).

Table 16: Antibiotic susceptibility pattern exhibited by *P. aeruginosa* isolated from non ICU.

Antibiotics	Sensitive		Intermediate		Resistant	
	n	%	n	%	n	%
Amikacin(10mcg)	34	31.2	1	0.9	74	67.9
Cefotaxime(30 mcg)	10	9.2	3	2.8	96	88.1
Ciprofloxacin(5 mcg)	32	29.4	18	16.5	59	54.1
Ceftazidime(30 mcg)	15	13.8	9	8.2	85	78.0
Carbenicillin(100 mcg)	48	44.0	14	12.8	47	43.1
Cefepime(30 mcg)	10	9.2	0	0	99	90.8
Cefepime/Tazobactum(30/10 mcg)	19	17.4	3	2.8	87	79.8
Gentamicin(10 mcg)	19	17.4	3	2.8	87	79.8
Imipenem(10 mcg)	96	88.1	6	5.5	7	6.4
Ofloxacin(5 mcg)	23	20.2	3	2.8	83	76.1
Piperacillin/Tazobactum(100/10 mcg)	56	51.4	19	17.4	34	31.2
Meropenem(10 mcg)	42	38.5	17	15.6	50	45.9

(n)=number of sensitive, intermediate or resistant organism

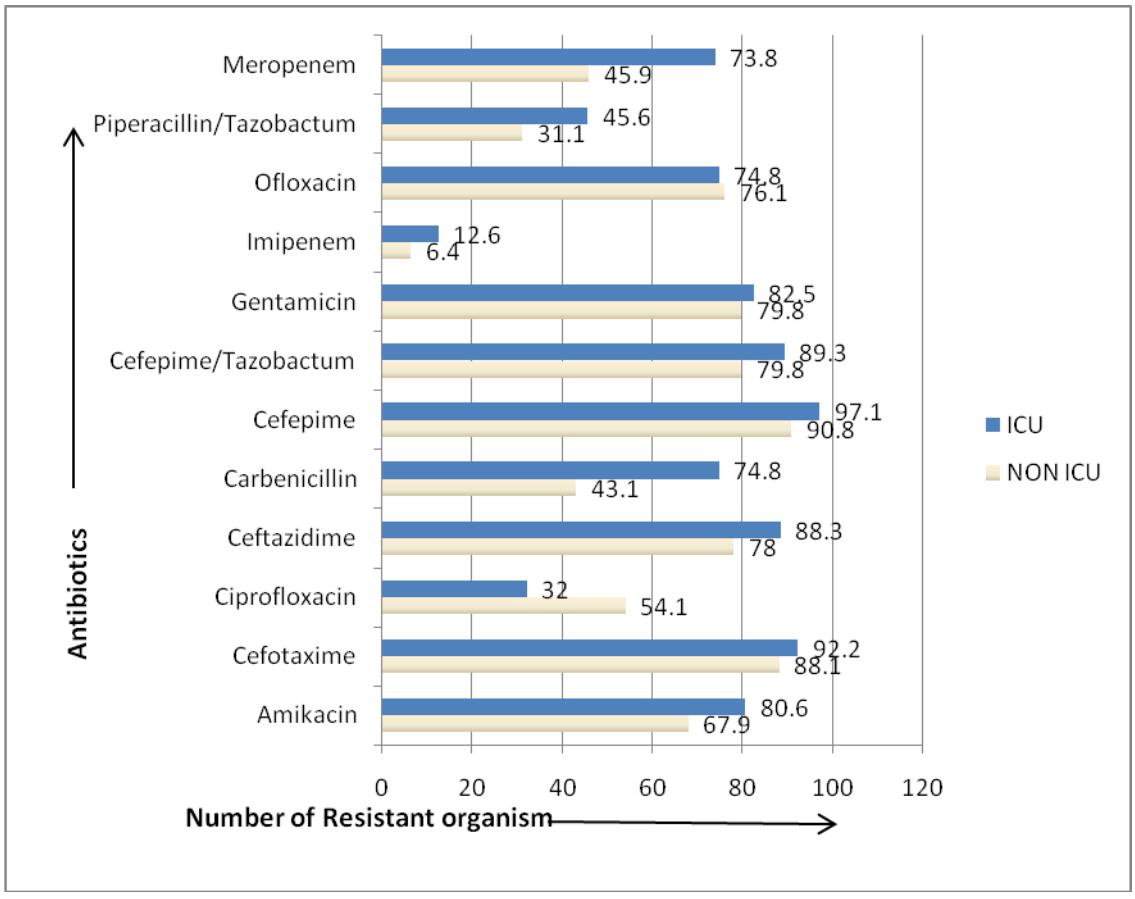

Fig 3. Comparision of Antibiotic resistance pattern exhibited by isolates from ICU and non ICU wards.

5.3.1 Association between sex of patients and infection status

A statistically significant association ($p<0.05$) was found between the sex of the patients and infection status (Table 17).

Table 17: Chi- square test of sex of patients and infection status.

Infection status	Sex of patients		p-value
	Male	Female	
Non infected	638(77%)	296(92%)	P<0.05
Infected	187(23%)	25(8%)	

5.3.2 Association between ward and infection status of patients:

There was no significant association ($p>0.05$) found between the ward and the infection status of patients (Table 18).

Table 18: Chi-square test of ward and infection status of patients

Infection status	Wards		p-value
	ICU	Non ICU	
Non infected	429(81%)	505(82%)	p= 0.484
Infected	103(19%)	109(18%)	

5.3.3 Association between MDR and Wards of patient.

There was no significant association ($p>0.05$) seen between the MDR isolates and the wards from where the organisms were isolated (Table 19).

Table 19: Chi-square test of Ward of patients and MDR

MDR	Wards		p-value
	ICU	Non ICU	
Yes	90(88%)	93(85%)	p= 0.484
No	12(12%)	17(15%)	

CHAPTER-VI
DISCUSSION AND CONCLUSION

6.1 Discussion

Nosocomial infections constitute an important worldwide health problem resulting in high morbidity and mortality as well as economic consequences. It is estimated that out of 100 admissions, approximately 5-6 admission develop nosocomial infections. Nosocomal infection rates range from as low as 1% in a few countries in Europe and the Americas to more than 40% in parts of Asia, Latin America and sub-saharan Africa (Lynch *et al.*, 1997). In 1987, a prevalence survey involving four WHO Regions (Europe, Eastern Mediterranean, South-East Asia and Western Pacific) found an average of 8.7% of all hospital patients had nosocomial infections. At any time, over 1.4 million people worldwide suffer from infectious complications acquired in hospital (Tikhomirov *et al*., 1987). The WHO study and others also found that the highest prevalence of nosocomial infections occurs in intensive care units and acute care surgical and orthopedic wards.

P. aeruginosa accounts for about 11% of all nosocomial infections (Bennett, 1974). *P. aeruginosa* isolation accounted for 13.9% of the 25,266 consecutive aerobic bacteria isolates and for 20.9% of the 16,863 Gram negative bacteria isolates examined in an Italian survey of clinically significant isolates from in and out patients (Bonfiglio, 1998). It has proved to be an important nosocomial pathogen, causing high mortality in susceptible patients. Although it causes disease in healthy individuals, it is a major threat to hospitalised and immunocompromised patients, particularly those with diseases such as cancer and burns. The high mortality associated with these infections is due to a combination of weak host defense system and bacterial resistance to antibiotics. The high incidence of this notorious, opportunistic pathogen in causing nosocomial infections are due to multiple reasons including disruption of barrier to infection by invasive instrumental procedures such as endotracheal intubation and tracheostomy, urinary bladder catheterization, central venous catheterization etc. (Shannon, 2005; Shaikh *et al*., 2008). Every hospital follows various standard international guidelines to control the growth of microorganisms and so does the well equipped wards of NINAS hospital. But despite following all the norms, persistence

of organisms is possible; hence it is essential to check the efficacy of such guidelines followed.

P. aeruginosa is known to cause a wide spectrum of diseases. It can infect almost any external site or organ, and therefore can be isolated from various body fluids such as sputum, urine, wounds, and eye or ear swabs and from blood (Hugbo and Olurinola, 1992). Constant bacteriological monitoring of the pathogens isolated from clinical specimens from patients in special units is necessary to draw attention of clinicians and infection control specialists to their current antibiotic susceptibility pattern and how often specific pathogens are isolated (Shanson, 1989; Vandepitte *et al*., 1991). In the previous two studies carried out in the hospital the most prevalent microorganism was found to be *P. aeruginosa*. In the study carried out by Koirala (2008) in tracheal aspirates, *P. aeruginosa* was found in majority of the cases (40.3%), similarly Karki (2009) also reported *P. aeruginosa* to be the most frequent organism (61.4%) isolated from different clinical and environmental specimens of ICU. Thus this cross-sectional study was carried out to determine the prevalence of *P. aeruginosa* in different clinical specimens of the hospitalized patients admitted to different wards, along with the antibiotic susceptibility profile of isolates and to estimate its Multi-drug resistant pattern. The study included all the clinical specimens from ICU as well as Non ICU wards so that any difference in the prevalence and drug resistant pattern of the organism between the two wards, if any, could be studied.

This study was conducted in the patients of 6-91 years who were admitted to National Institute of Neurological and Allied Sciences, Bansbari Kathmandu. A total of 301 cases were enrolled in the period during May 2010 to December 2010. Majority of the cases were from the age group 21-30 (20.9%) and were male (57.7%). The diagnosis showed that most of the cases were of Road Traffic accident (33.6%).

A total of 1146 clinical specimens were collected from 301 hospitalized patients and were sent for microbiological investigation by the clinicians during the study period. Tracheal aspirates occupied the majority of the specimens (49.1%) obtained for investigation while Catheter tip was the least frequent specimens (2.1%) obtained during the study period. 53.6% of the total clinical specimens were obtained from Non ICU wards and the remaining 46.3% were received from the ICU. Out of 1146 clinical specimens investigated, significant growth of *P. aeruginosa* was obtained in

only 18.5% (n=212). The percentage of the organism isolation is different in various studies. Study by Anupurba *et al.* (2006) showed 32.0% of the Pseudomonal isolation while in a similar study done by Khan *et al*. (2004) the rate of isolation of *P. aeruginosa* was found to be 6.7%. In a study conducted by Savas *et al.* (2005) the isolation rate of the organism was 16.40%. Deep *et al*. (2002) showed 19.0% of the *P. aeruginosa* isolation from different clinical specimens under study. These results were somewhat similar to our study.

Out of the 563 tracheal aspirate received 29.7% (n=167) showed positive growth with *P. aeruginosa*. Thus the majority of the isolates were from tracheal aspirates which are in accordance to several other studies (Zhanel *et al*., 2008; Reshedko *et al*., 2007). Tracheostomy has been found to play an important role in the airway management of patients with severe head trauma (Chintamani *et al*., 2005). Tracheostomy is required in these patients because of patient's inability to protect the airway and persistence of excessive secretions (Siddiqui *et al*., 2011). Despite high level of hygiene, exogenous colonization with or without subsequent infection is common. In the study done in lower respiratory tract infections in trachestomozed patients by Siddiqui *et al.* (2011), 30.0% of the isolates were *P. aeruginosa.* Similar results was shown in research of Nseir *et al.* (2008) giving 32.0% isolation, these studies can be correlated to our results. Among these isolates 83.8% (n=140) were multidrug resistant. The high prevalence of the MDR isolates in tracheostomized patients could be due to their long term hospital stay and a long term antibiotic therapy. Study by Niederman *et al*. (1984) and study by Patrizia *et al*. (2000) showed *Pseudomonas* as the most predominant pathogen of the tracheal aspirates. Morar *et al*. (2000) also suggested the *Pseudomonas* sps as the most important pathogen to be colonized in tracheobronchial trees. Nseir *et al*. (2002) had suggested *P. aeruginosa* as the most common aetiological agent of tracheobronchitis. As *P. aeruginosa* is not the normal flora of the body, the source of the species most be the hospital environment. Whenever the tracheostomized patients were infected with *P. aeruginosa,* the infection had persisted and was really problematic for both the patients and the physician. The persistance of *P. aeruginosa* in these patients for a long time may be attributed to its capacity to form biofilm in the tracheostomy tube and the calcium alginate capsule around it worked for reservoir for the organism and interfered the action of antibiotics (Inglis *et al.,* 2002). Further there might be several reasons behind the high rate of positive

yield. As the patients were critically ill they had a weak immune system due to various factors, like bypassing of upper airway resistance and defected mucociliary escalator. The respiration in natural air after tracheostomy might also have increased the infection rate among the tracheostomized patients. Other route of infection might be leakage of contaminated secretion around the endotracheal tube, and passing of inocula by the health professional during the respiratory therapy.

The growth of multiple organisms from tracheal specimen had been mentioned by Niederman *et al*. (1984). In our study 28.8% (n=61) of the isolates showed polymicrobial growth. Of these most portions was occupied by Enteric gram negative bacteria along with the *P. aeruginosa*, however, *P. aeruginosa* had persisted more than other Gram negative enteric bacteria. In tracheostomized patients from whom multiple samples were obtained, first few samples had not shown any growth or non significant growth. This evidence also suggests trachestomy and infection association. Super-infection (Infection one after another) was the important finding of the work in tracheostomized patients. Out of the 167 positive samples almost more than 50.0% were the result of super infection. A study conducted by Gotsman *et al*. (1964) also showed super infection in patients who were selected under discussion.

Out of the 147 urine specimens received from patients with indwelling urethral catheter 6.1% (n=9) showed positive growth with *P. aeruginosa*. Among these isolates 100% (n=9) showed multidrug resistant pattern. Urethral catheter is a major predisposing factor in the development of nosocomial UTI. About 80.0% of UTI are associated with the use of urethral catheters (Mandell *et al*., 1990). *P. aeruginosa* is the third most common pathogen associated with hospital acquired catheter-associated UTIs (Jarvis *et al*., 1992). *P. aeruginosa* has an innate propensity to stick to the surfaces of catheters and form biofilms leading to higher incidence of UTIs in patients with long term indwelling bladder catheterization (Hoiby *et al*., 2001). In many studies it has been found that *P. aeruginosa* plays an important role in bladder infection and is considered as primary pathogens in compromised host (Dolan *et al*., 1989; Jones *et al*., 1999) and also in uncomplicated urinary tract infection (Kosakai *et al*., 1990). A research done by Alavaren *et al*. (1993) has reported *P. aeruginosa* as the most predominant bacteria (20.5%) causing UTI in his study of patients with indwelling catheter. Similar report of high prevalence of *P. aeruginosa* was obtained

from the research of Olayinka *et al.* (2004) showing 51.1% prevalence. On the other hand Okonko *et al.* (2010) reported 5.3% of *P. aeruginosa* isolation in their study of UTI which is somewhat similar to our findings. The variation in the percentage may have occurred due to difference in the prevalence of prevailing local flora of the hospital.

In our study 36.4% (n=12) of the sputum specimens showed positive growth with *P.aeruginosa* of which 83.3% (n=10) were MDR isolates. This finding corelates to the results of Suwangool *et al*. (1999) showing 37.5% isolation and of Tripathi *et al*. (2011) showing 34.2% isolation of *P. aeruginosa* from the sputum. However there are other researches which vary significantly from our result. *P. aeruginosa* infections of the lower respiratory tract can range in severity from colonization (without an immunological response) to a severe necrotizing bronchopneumonia (Banerjee *et al*., 2000). Suwangool *et al*. (1999) have stated *P. aeruginosa* as the principal causative agent of nosocomial pneumonia. The significance of the mucoid form of *Pseudomonas* as a cause of suppuration, and not merely as a passenger in the respiratory tract, needs to be emphasized. Such strains are actually capsulated and more virulent (Sonnenschein, 1927).

In the pus specimens investigated under our study, 12.5% (n=10) showed growth positive with *P.aeruginosa*. Joshi *et al*. stated that *P. aeruginosa* has almost replaced *Staphylococcus aureus* in post-operative wound infection. Also it is revealed that the increasing incidence of *P. aeruginosa* in post-operative wound infections is observed by other scientists especially in recent years. The incidence of *P. aeruginosa* in postoperative wound infection is becoming more serious in developing countries because of relaxation in general hygienic measures, mass production of low quality antiseptic and medicinal solutions for treatment, difficulties in proper definition of the responsibility among the hospital staff (Bertrand *et al*., 2002). Maximum clinical isolates of *P. aeruginosa* were isolated from the pus samples (57.6%) in a research done by Khan *et al*. (2008). Similarly high pecentage isolation of the organism (41.0%) has been reported in other researches done by Farida Anjum and Asif Mir (2010). The result of our study is lesser than that reported by most of the researchers. This could be attributed to differences in geographical location and hygienic measures.

Out of the 89 specimens of different tip cultures of clinical devices being inserted into the patients (Catheter tip-25, CVP tip-64), 11.2% of them showed positive growth with *P. aeruginosa*. Anjum and Mir (2010) and Javiya *et al*. (2008) showed mimimum isolation of the pathogen from the catheter tip in their studies, 3.0% and 1.8% respectively. While Taiwo and Aderunmu reported 20.6% of the *P. aeruginosa* isolates in their research which was only slightly distant from our findings which has resulted 28.0% (n=7) of the *Pseudomonas* isolation from the Catheter tips. On the other hand 4.7% (n=3) of the CVP tip showed positive growth with the organism which was in sharp contrast to the 50.0% isolation of the *P. aeruginosa* from CVP tips in a study done by Deep *et al*. (2004). Studies report that urinary catheterization is associated with nosocomial UTI and central venous catheterization is associated with the development of nosocomial BSI (Habibi *et al*., 2008; Tennant *et al*., 2005 and Caglayan *et al*., 2005). The presence of the isolates in the tip cultures of medical devices indicate the possible source of nosocomial pathogen in hospitalized patients which can cause infections in the patients at the respective site of insertion. These tip cultures may have become colonized with the pathogen present within the patients or may have become contaminated via the HCWs hands or by means of water through which it was rinsed.

Bacterial bloodstream infections are serious infections associated with significant mortality and health-care costs (Weinstein, 1997). The hospital mortality associated with *P. aeruginosa* bloodstream infections is reported to be greater than 20.0% in most series and is highest among patients receiving inappropriate initial antimicrobial treatment (Bryan *et al*., 1983; Chatzinikolaou *et al*., 2000; Osmon *et al*., 2004 and Vidal *et al*., 1996). Out of the 60 blood samples received during this study period not a single growth of any pathogen was seen. Research done by Olayinka *et al*. (2004) showed 1.1% and similar study done by Ergin and Mutlu (1999) showed 0.6% growth in blood.

Out of the 139 CSF specimens studied, 1.4% (n=2) showed positive growth with *P. aeruginosa* as a single isolate. Nosocomial *Pseudomonas* meningitis is a serious complication after neurosurgery and is associated with high mortality (Huttova *et al*., 2007). Studies showed that *P. aeruginosa* was responsible for 8.3%-10.7% of meningitis in post-surgical patients (Erdem *et al*., 2008; Huang *et al*., 2007). In a

study done by Juhi *et al*. (2009) *P. aeruginosa* was the most common Gram negative isolates recovered from the CSF, representing 40.0% of gram negative isolates and 9.9% of all CSF isolates. Our result showed somewhat lesser isolates than these researches. May be the proper hygienic practices and guidelines followed during surgery contibuted to this less prevalence of *P. aeruginosa* in CSF samples.

In our study 48.6% (n=103) of the total Pseudomonal isolates were obtained from ICU. Intensive care units (ICUs) are generally considered epicenters of antibiotic resistance and the principal sources of outbreaks of multi-resistant bacteria. The most important risk factors are excessive consumption of broad spectrum antibiotics exerting selective pressure on bacteria, the frequent use of invasive devices and relative density of a susceptible patient population with severe underlying diseases (Kumar, 2006; Hassazadeh *et al*., 2009). Balikaran *et al. (*2010) reported that the prevalence of *P. aeruginosa* was found to be highest in ICU giving 28.4% (Balikaran *et al*., 2010). A European survey on the prevalence of nosocomial infection in ICU patients showed that *P. aeruginosa* was one of the most frequent pathogens isolated from 29.0% of ICU-acquired infections (Vincent, 2000). The high percentage isolation from ICU in our study may be due to the origination of the organism from exogenous sources of the ICU such as tap water, fomites and/or patient-to-patient transmission, or as an endogenous phenomenon related to antibiotic use.

On the other hand isolation of *P. aeruginosa* from other Non ICU units (General ward I and II, and Cabin) were slightly higher than from the ICU units (51.4%, n=109). More than 70.0% of the patients in these units had been transferred from the ICU, thus these patients had been undergoing longer antibiotic therapy since their time of admisson to ICU initially. *P. aeruginosa* is not generally considered to be part of the normal human flora (Kerr and Snelling, 2009), and in most patients admitted to hospital for the first time, *P. aeruginosa* is not usually isolated from bacteriological specimens until the patient has been in the hospital for several days (Johnson *et al*., 2009; Fourrier *et al*., 2000 and Ewig *et al*., 1999). Within this period *P. aeruginosa* could be acquired from the environment through repetitive daily healthcare procedures or from the invasive devices. Thus the longer stay in the hospital could be correlated to the higher prevalence of the organism in the Non ICU units. However

the relation of wards and infection status was found to be statistically insignificant ($p>0.05$) in our study.

In the present study, the rate of growth positivity with *P. aeruginosa* was found to be 88.2% in males which is much greater than the rate in females, 11.8%. This higher growth positivity seen in males was found to be statistically significant ($p<0.05$) in this study. These high infected males belong to the age group 41-50. In the research of Khan *et al.* (2008), the gender-wise prevalence of clinical isolates showed that infections caused by *P. aeruginosa* are very common in male (61.8%) compared with female (38.2%). On the other hand the Anjum and Mir (2010) reported 43.0% isolation of the organism from the males and 47.0% isolation from the females. Also, the age group of 21-30 showed highest percentage of growth positivity (27.8%) in our study. This finding corelates to the results of Rashid *et al.* (2007). The second highest percentage of the *Pseudomonas* was exhibited by the age group 41-50 (26.9%). In disease like cystic fibrosis (CF) lung disease, the prevalence of *P.aeruginosa* in respiratory cultures increases with age, reaching approximately 80.0% by adulthood (Rosenfeld *et al*., 2003).

In the study done by karki (2010), out of 404 culture positive clinical specimens, *P. aeruginosa* occurred in more than half (61.4%, 248 out of 404) of them. Simultaneously, *P. aeruginosa* was also isolated as the frequent isolate from 562 culture positive environmental samples accounting for 80.0% (n=24) of air samples, 100.0% (n=10) of water samples each before maintenance and after maintenance, 71.7% (n=33)and 67.5% (n=27) of Hand imprint samples each respectively for before wash and after wash, similarly, 32.1% (n=61) of fabrics/clothing samples and 29.8% (n=54) of inanimate surface samples. However, it did not show growth in Nasal sample of HCWs. Statistical analysis showed that there existed significant association between *P. aeruginosa* isolated from various clinical specimens and environmental samples ($p<0.05$) except the hand imprints of HCWs ($p>0.05$). So these inanimate sources could be the major source of the organism in the hospital.

Today, the antibiotics remain the front line therapy for conquering the bacterial infections. However, their indiscrimination use is no longer viewed as benign. As antimicrobial agents have been misused and overused, bacteria have fought back with

a selection process by which certain strains are now no longer susceptible to one or more agents. As with the wide spread use of antibiotics and the increase in number of immunosuppresed host, *P. aeruginosa* has become a leading cause of gram negative bacterial infections especially in immunocompromised patients who need prolonged hospitalization (Schimpff *et al*., 1970; Korvick *et al*., 1991 and Griffith, 1989).

In our study the isolates were subjected to three classes of antibiotics namely β-lactams (extended spectrum Penicillins: Carboxypenicillin-Carbenicillin, Ureidopenicillin-Piperacillin; Carbapenems-Imipenem and Meropenem; Cephalosporins: Third generation cephalosporin- Ceftazidime and Cefotaxime, fourth generation cephalosporin- Cefepime and Cefepime/Tazobactum), Aminogycosides (Amikacin and Gentamicin) and Quinolones (Ciprofloxacin and Ofloxacin). The susceptibility of *P. aeruginosa* to some β-lactams (e.g. ceftazidime, cefepime, piperacillin, aztreonam) is more strongly influenced by efflux, whereas susceptibility to others (Imipenem, panipenem) is more strongly affected by the presence of β-lactamase, while a third group (ceftriaxone, meropenem, moxalactam) is influenced only by knockout of both efflux and β-lactamase (Masuda *et al*., 1999; Nakae *et al*., 1999). In our study the isolates showed more than 50.0% resistance to nine antibiotics. These decreased sensitivity values may also be explained by antimicrobial use. More than 95.0% of the patients under investigation were receiving antibiotics when the sample was collected for analysis, and the majority of them were broad-spectrum antibiotics.

The Antibiotic sensitivity test of *P. aeruginosa* isolated in this study, in overall showed higher sensitivity towards Imipenem followed by Piperacillin/Tazobactum whereas highest resistance was observed towards Cefepime (93.9), followed by Cefotaxime (90.1%) and Cefepime/Tazobactum (84.4%) all three belonging to subclass Cephalosporin of β-lactam class. Resistance by Gentamicin (81.1%), Ofloxacin (75.9%), Amikacin (74.0%), Meropenem (71.2%), Carbenicillin (58.5%) and Ciprofloxacin (43.4%) were shown by the isolates. In an overall analysis, *Pseudomonas* exhibited high resistance to Cephalosporin groups in comparision to other group of β-lactam antibiotics. Two main categories of β-lactamases mediate resistance to third-generation cephalosporins among the common gram-negative nosocomial pathogens: chromosomal β-lactamases and plasmid-associated extended-

spectrum β-lactamases (ESBLs) (Livermore, 1995). Enzymes that can confer resistance to most penicillins, cephalosporins, and monobactams, ESBLs belong to Bush-Jacoby-Medeiros functional group 2, whose enzymes are generally inhibited in vitro by β-lactamase inhibitors. By contrast, the chromosomal β-lactamases present in *Pseudomonas* belong to group 1, whose enzymes are not inhibited by β-lactamase inhibitors (Bush, 2001). Thus the resistance exhibited by Cefepime and Cefepime/Tazobactum did not showed marked difference though cephalosporin with β lactamase inhibitor showed slight less resistance than the Cefepime alone. Though Cephotaxime has a good activity against the gram negative bacteria, *P. aeruginosa* isolated were 90.1% resistant to it so that question of its use in therapy against *Pseudomonas* is always thinkable.

The isolates exhibited 58.5% resistance to Carbenicillin and 38.2% resistance to Piperacillin/Tazobactum. Both of them are extended spectrum Antipseudomonal drugs. Carbenicillin is recently developed semisynthetic penicillin which has moderate activity against *P. aeruginosa* (Acred *et al*., 1967). However increase in the proportion of carbenicillin-resistant strains of *P. aeruginosa* isolated from patients has also been recently reported from England (Lowbury *et al*., 1969). Piperacillin/Tazobactum is frequently used to treat *P. aeruginosa* infections in critically ill patients. In the report by Laure and Guillou *et al.* (2010), piperacillin/tazobactam was the most potent antibiotic against *P.aeruginosa.* Our finding too showed that it was the second most effective drug against the isolates.

In our study the isolates exhibited high resistance to Aminoglycosides as well, showing 81.1% resistance to Gentamicin and 74.0% resistance to Amikacin. Hancock *et al.* (1998) has stated that Amikacin seems to be a promising therapy for *Pseudomonas* infection. However, our data suggests that resistance in amikacin is increasing progressively in our country. The production of aminoglycoside-modifying enzyme N'acetyl transferase (ACC6'-[I]) hydrolyses amikacin, tobramycin, and netlimicin, but not gentamicin (Miller *et al*., 1999). However, our study suggests Amikacin more sensitive to Gentamicin. This may be due to selective pressure associated with high rate of consumption of Gentamicin than Amikacin by the hospitalized patients. Similar result was observed in study of Smitha *et al.* and Poole *et al*. Koirala (2008) also reported similar result in his research in this hospital.

Resistance to Quinolones showed marked difference between the two antibiotics. The isolates showed 75.9% resistance to Ofloxacin while 43.4% resistance to ciprofloxacin. Now increase in the levels of resistance to the fluoroquinolones among nosocomial isolates, like *P. aeruginosa* has been reported worldwide (Acar and Goldstein, 1997). Principal mechanisms of bacterial resistance to quinolones are modification of target enzymes, DNA gyrase (gyr A) and topoisomerase IV (parC), or reduction of intracellular concentration due to mutations in the regulatory genes for efflux systems, such as mexR and nfxB (Shah *et al*., 1998). The study done by Kemmerich *et al.* (1985) and Shalit *et al.* (1992) also showed ciprofloxacin as an effective agent than ofloxacin against *P. aeruginosa*.

The isolates showed 71.2% resistance towards Meropenem while only 9.4% resistance was shown towards Imipenem. Carbapenem antibiotics are considered agents of choice to treat serious infections caused by *P. aeruginosa*. Progressive antimicrobial resistance to most classes of antibiotics, including carbapenems, has made treatment of infection caused by this bacteria particurlarly difficult, even with combination therapy (Sevillano *et al*., 2006). Although the antibiotic resistance is caused by multiple mechanisms that this bacteria presents, one growing factor leading to carbapenem resistance is the presence of carbapenemases (Livermore, 2002 and Tenover, 2006). The most important clinically-significant carbapenemases in *P. aeruginosa* are class B metallo-β-lactamases such as VIM and IMP-type (Zhiyong *et al*., 2003). Study done by Sevillano *et al.* (2006) showed that resistance to Meropenem by *P. aeruginosa* was 11.5% which is much less than our results. While in another research by Agodi *et al.* (2007) 67.6% reistance was seen towards Meropenem which was somewhat similar to our results. Imipenem is the broadest-spectrum parenteral antimicrobial agent that is commercially available and has remained a useful drug for gram-negative bacilli. It had been anticipated that the greater stability of Imipenem to hydrolysis by commonly encountered chromosomal and plasma-mediated beta-lactamases would make development of resistant organisms during Imipenem therapy less of a clinical problem (Neu and Labthavikul *et al*., 1982). In our study as well, Imipenem was consistently the most active against most ot the Pseudomonal isolates. Despite its effectiveness, resistant pattern of Imipenem during the study period was observed to be 9.4%. Karki (2010) reported

8.3% resistance to Imipenem by the Pseudomonal isolates in her study in this hospital. The slight increase in the resistance in one year may be due to the wide usage of this antibiotic among the hospitalized patients giving a selective pressure to the bacteria.

Further in comparision between the antibiotic resistance pattern exhibited by ICU and Non ICU isolates, *P. aeruginosa* isolated from ICU showed more resistance to eight antibiotics (Amikacin, Ceftazidime, Carbenicillin, Cefepime, Cefepime/Tazobactum, Imipenem, Piperacillin/Tazobactum and Meropenem) than by the isolates from Non ICU isolates.

In tracheal specimens, the antibiotic susceptibility profile showed highest susceptibility to Imipenem (83.2%) followed by Piperacillin/Tazobactum (38.9%) and then Carbenicillin (31.1%). While highest resistance was shown towards Cefepime (92.8%) followed by Cephotaxime (91.0%). Highest intermediate resistance was shown by Ciprofloxacin (31.7%). Researches done by various scientists has shown Imipenem as the most effective antibiotic against *P. aeruginosa* infections, Arora *et al.* (2011) showed 96.3% sensitivity, and Anjum and Mir (2010) showed 97% sensitivity of the isolates to Imipenem.

Among the urine isolates the most effective antibiotic was found to be Imipenem giving 100.0% susceptibility and the greatest resistance was shown towards Cefepime (100.0%). This result was similar for almost all the isolates under this study. In resistance pattern Cefepime was followed by Ofloxacin (88.9%), Ceftazidime (88.9%). Ciprofloxacin also showed 66.7% resistance. Repeated use of quinolone therapy for complicated UTI, particularly *P. aeruginosa* infection results in emergence of quinolone resistant organisms (Ena *et al.*, 1995). In sputum specimens, again Imipenem was the most sensitive (91.7%). Amikacin was the second most sensitive drug giving 41.7% sensitivity. In this case Cephotaxime was the most resistant agent (100.0%) for the isolates followed by Cefepime (91.7%). Tripathi *et al.* (2011), in his study of lower respitaroty tract infections, reported the *Pseudomonas* isolates were sensitive to 87.3% of Imipenem, 54.9% of Ciprofloxacin, 21.6% of Gentamicin which was similar to our studies while sensitivity pattern to other antibiotics did not correlated to our results.

The ten isolates from Pus specimens showed maximum susceptibitlity to Imipenem (80.0%) followed by Piperacillin/Tazobactum (50.0%) and Carbenicillin (40.0%). Amikacin, Ciprofloxacin, Cefepime/Tazobactum, Gentamicin, Ofloxacin and Meropenem showed only 10.0% sensitivity which was different from the other researches done. Masaadeh and Jaran (2009) showed that the isolates were 78.0% sensitive to Amikacin, 72.0% to Gentamicin, 66.0% to Ciprofloxacin, 16.0% to Meropenem and 9.0% to Cefepime. Among the Pseudomonal isolates obtained from the tip cultures, isolates of Catheter tip showed 100.0% sensitivity to Imipenem and 100.0% resistance to Ofloxacin and Cefepime. Cephotaxime, Ciprofloxacin and Ceftazidime did not showed sensitivity to any of the isolates but intermediate resistance was observed in these drugs. The three isolates obtained from CVP tips showed 66.7% sensitivity to Amikacin, Ciprofloxacin, Carbenicillin, Gentamicin, Imipenem and Ofloxacin while 100.0% resistance was shown to Piperacillin/Tazobactum, Cefepime and Cefotaxime.

P. aeruginosa isolated from cerebrospinal fluid was susceptible to only one drug Imipenem; all the other antibiotics were resistant to the bacteria. In a study by Juhi *et al.* (2009) the most active agents against *P. aeruginosa* were Imipenem (90.0% susceptible), which correlates to our results. But the isolates were susceptible to other antibiotics also like Meropenem (70.0% susceptible) and Amikacin (70.0% susceptible). In the study done by Huang *et al.* (2007) the susceptibility rates of the tested *P. aeruginosa* strains to Ceftazidime, Cefepime, Imipenem, Meropenem and ciprofloxacin were 91.7%, 83.3%, 83.3%, 83.3%and 66.7% respectively. Like CSF, the isolates from miscellaneous samples also showed sensitivity to only one drug Imipenem. From the results we had known that Imipenem was the most potent drug for all *P. aeruginosa* isolates obtained from all the clinical specimens. Most of the researches till date have shown similar results.

Comparing the Antibiotic resistance pattern of the *P. aeruginosa* isolates from the ICU and Non ICU wards, it was observed that the isolates from ICU wards exhibited more resistance to all the twelve antibiotics in comparision from the isolates of Non ICU wards. Studies have demonstrated that rates of antimicrobial resistance are greater in bacteria isolated from ICUs compared with other hospital wards and outpatient clinics (Archibald *et al*., 1997). Our finding correlates to the findings of

Ergin and Mutlu (1999) where the rate of *Pseudomonas* infections in IW-ICU was higher than in IW-nonICU in the hospital. Similarly ICU *P. aeruginosa* isolates were highly multidrug resistant compared to ORL ward isolates in the study done by Slama *et al.* (2011) and in a study carried out in Turkey. Inan *et al*. isolated 60-83% multidrug-resistant *P. aeruginosa* strains from ICU patients. However in our study, statistically there was no corelation between the wards and the MDR pattern of the isolates.

Nowadays, prevalence of MDR strains of *P.aeruginosa* are seen mainly in hospital acquired infections due to the selective pressure exerted on the bacteria by overusage of broad-spectrum antibiotics. Multidrug resistance can be caused by regulatory mutations *nalB (mexR), nfxB* or *nfxC* (*mexT*) leading to overexpression of three separate RND efflux systems, MexAB-OprM, MexCD-OprJ and MexEF-OprN respectively (Germ *et al*., 1999). Isolates were considered multi-drug resistant if they showed resistance to two or more of the tested antibiotics. In our study 85.4 % (181/212) of the *P. aeruginosa* were multi-drug resistant. In the study done by R Moniri *et al.* (2006) Multi-drug resistant (MDR) isolates accounted for 73.9% of isolates.

MDR isolates were found from all the specimens and from both the ICU and non ICU wards. Tracheal specimens (140/167), Urine (9/9), Sputum (10/12), Pus (9/10), Catheter tip (7/7), CVP tip (2/3), CSF (2/2) and Miscellaneous (2/2) showed MDR isolates. Outcome of prevalence of MDR depends on various factors, MDR criterion being the chief one followed by the types of antibiotics used in antibiogram and study population. The emergence of MDR is clearly related to the quantity of antibiotics and how they are being used (Levy, 1997).

In the present study, all the MDR isolates (100.0%) showed resistance to at least 1 third generation cephalosporins. Subha and Ananthan (2002) and Amutha *et al.* (2009) reported 95.0% resistance to at least 1 third generation cephalosporin's amongst the MDR isolates. In Europe, significant decline in susceptibility rates to β-lactams, aminoglycosides, and quinolones was recently observed in this pathogen, and nosocomial outbreaks of MDR *P. aeruginosa* have been described in various European hospitals (Spencer, 1996; Sidorenko *et al*., 1999).

High prevalence of MDR *P. aeruginosa* isolates in this study may be due the long term hospital stay of the patients and a long term broad spectrum antibiotic therapy. Another important aspect was the persistent of these MDR infections so we should think for amendable therapy. A consensus statement by American thoracic society suggested that in case of Gram negative enteric bacteria, the monoantibiotics therapy is adequate but when there is infection with highly resistant Gram negative bacteria such as *P. aeruginosa* a combined drug therapy is required. During the study we encountered a high frequency of MDR isolates as well as persisting Pseudomonal infection, so it's important to evaluate drug therapy. Most experts recommend that the two classes of drugs be used together for their possible synergistic effect against Pseudomonal infections. Therapy of serious infections caused by *P. aeruginosa* usually consists of a combination of semisynthetic penicillin, such as ticarcillin or piperacillin (with or without a β-lactamase inhibitor) and an aminoglycoside, such as Tobramycin. These drugs have been shown to be synergistic in vitro against *P. aeruginosa*. (Craig, 1994). The use of acombination of β-lactamase inhibitor and β-lactam is also a novel approach under development. For example in our study Piperacillin/Tazobactum has proved to be the second most potent antibiotic against the bacteria.

Before proceeding therapy, consideration of Pharmacological features like penetration of antibiotics to the site of infection is always important. Some antibiotics penetrate respiratory secretion than others. Aminoglycosides have relatively poor penetration, while fluroquinolones can achieve concentration in brochial secretion that is equal to or exceeds serum level (LaForce, 1989; Honeybourne, 1994). Thus a combination of Aminoglycosides and fluroquinolones is one option. These agents are bactericidal in concentration dependant fashion, killing more rapidly in high concentration. In addition these have a prolonged post antibiotic effect (PAE) allowing them to suppress bacterial growth even after antibiotic concentration is below the minimum inhibitory concentration of target organisms (Craig, 1993).

Since this study showed Imipenem and Piperacillin/Tazobactum as most effective antibiotics in vitro these agents may be given singly or in combination with the aminoglycosides for their effectiveness. The prevalence and antibiotic sensitivity

pattern of the isolates keep on varying with time and environment, thus the physician in clinical pratice should have access to up-to-date data on these things in order to augment the successful therapy and also to prevent from emergence of the multidrug resistant bacteria.

6.2 Conclusion

From the study of microbiological analysis of all the clinical specimens received from the hospitalized patients, the prevalence level of *P. aeruginosa* was found to be 18.5%. The isolates were found predominantly in lower respiratory secretions (tracheal aspirates and sputum) followed by pus. Imipenem was the drug showing high sensitivity to most of the isolates while 9.4% isolates exhibited resistance to this drug. Similarly isolates exhibited maximum resistance to Cefepime showing 93.9%. A high prevalence of MDR isolates, 85.4%, was found in the specimens. The organism obtained from clinical specimens of ICU patients showed maximum resistance to most of the antibiotics in comparison to the organism from non ICU units. However no significant difference ((P>0.05) was found between the relation of wards and infection with the organism and the MDR status. Infection with *P. aeruginosa* was found to be more in males than females and mostly in age group 21-30. A significant association (P<0.05) between the gender and infections status was found in our study.

CHAPTER VII

SUMMARY AND RECOMMENDATIONS

7.1 Summary

1. *P. aeruginosa* is the commonest cause of most of the NIs in the hospital. It is a major threat to hospitalised and immunocompromised patients. Despite advance in medical and surgical care and introduction of wide variety of antimicrobial agents against anti-pseudomonal activities, life threatening infection caused by *P. aeruginosa* continue to cause complications in hospital acquired infections. Hence this study was conducted to determine the prevalence and the antibiotic sensentivity test profile of *P. aeruginosa*.

2. Out of the 1146 clinical specimens obtained from 301 hospitalized patients, tracheal aspirate was the most regularly sent specimens (n=563) followed by urine (n=147) and CSF (n=139). The prevalence of *P. aeruginosa* was found to be 18.5% (n=212), of which 28.8% (n=61) showed polymicrobial growth.

3. The prevalence of *P. aeruginosa* was found to be 29.7% in tracheal specimens, 6.1% in urine, 36.4% in sputum, 12.5% in pus, 28.0% in catheter tip, 4.7% in CVP tip, 1.4% in CSF and 5.7% in miscellaneous specimens.

4. The prevalence of MDR *P. aeruginosa* was 85.4%. Most of the MDR isolates were obtained from tracheal aspirates.

5. Among the 212 positive growths, the highest number of growth was obtained from age group 21-30 (27.8%). 88.2% of the Pseudomonal isolates were from male and 11.8% isolates were from the female patients.

6. Of the total growth, 48.6% (n=103) of the Psedomonal isolates were from the ICU and 51.4% (n=109) were from the Non ICU wards.

7. Regarding antibiotics, Imipenem was most effective against most of the clinical isolates followed by Piperacillin/Tazobactum. Isolates exhibited maximum resistance to Cefepime (93.9%) followed by Cephotaxime (90.1%). However, 9.4% resistance to Imipenem was found in this study. In contrast to Imipenem a high resistance to

meropenem (71.2%) was found. Thus Carbapenem resistance was a significant finding in our study.

8. *P. aeruginosa* isolated from all the clinical specimens showed maximum sensitivity to Imipenem and maximum resistance to Cefepime. Thus in our study Imipenem was found to be the most effective drug against *P. aeruginosa* obtained from all the specimens and from all the wards of the hospital.

9. *P. aeruginosa* isolated from specimens obtained from non ICU wards showed more sensitivity to Imipenem (88.1%) in comparision to the isolates from ICU (81.6%) while the isolates from non ICU showed less resistance to Cefepime (90.8%) in comparision to the the ICU isolates (97.1%). Thus the variance in the antibiotic sensitivity pattern among the isolates from the different wards was seen.

10. In our study, a statistically significant association ($p<0.05$) was found between the sex of the patients and infection status but no association was found ($p>0.05$) between the MDR isolates and the wards from where the organisms were isolated and among the infection status of patients and wards of their stay.

7.2 Recommendations

1. Most of the Pseudomonal isolates were obtained from the tracheal specimens, thus the source of the organism could be the hands of the physicians while performing tracheostomy and of the HCWs while suctioning the aspirates. Therefore strict hand washing guidelines should be followed by regular monitoring and supervision is highly recommended so as to reduce contact and cross transmission by HCWs.

2. The high prevalence of MDR *P. aeruginosa* indicates that MDR isolates are emerging. Antibiotic therapy should be given carefully to the patients so as to control the emergence of MDR strains.

3. This dissertation work is representative of the *P. aeruginosa* infections in patients by analyzing the clinical specimens alone. It is recommended to study the association of the isolate with the hospital environment as well, in order to get a complete picture of nosocomial infection caused by *P. aeruginosa* in the hospital.

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APPENDICES

Appendix-I

Clinical and Microbiological profile of patient

Name of patient: Date:

Age: sex:

Specimen

Lab no.:

Clinical history of patient:

Antibiotics taken

- Yes/No
- Name the antibiotic if taken

Microbiological investigation:

Day-1

Appearance of specimen

- color
- clear
- cloudy

Microscopic examination of specimen

- Bacteria
- White blood cell
- Yeast cell
- epithelial cell

Gram's staining

- Gram positive cocci / bacilli
- Gram negative cocci / bacilli

Inoculation of sample into BA, MA and CA

Day-2

Cultural characteristics

MA	Colony characteristics	Catalase	Oxidase	Coagulase	Gram reaction	Morphology	Inference
1.							
2.							
3.							
4.							
BA	**Colony characteristics**	**Haemolysis**	**Gram reaction**			**Morphology**	**Inference**
1.							
2.							
3.							
4.							
CA	**Colony characteristics**	**Haemolysis**	**Gram reaction**			**Morphology**	**Inference**
1.							
2.							
3.							
4.							

Culture on NB for 4 hours (from MA if gram negative, from BA if gram positive)

Day-3

Biochemical characteristics

S.No.	MR/VP	Citrate utilization	SIM	TSI	Urea hydrolysis	Inference
1.						
2.						
3.						
4.						
5.						

Organism identified as:

Antibiotic Sensitivity Testing: Kirby-Bauer method

S.No.	Antibiotics used	Zone of inhibition (mm)	Interpretation

Performed by.....

Appendix-II

List of equipments and materials used during the study

Autoclave	
Hot air oven	Chitransh (India)
Incubator	Associated Scientific Technologist (India)
Refrigerator	LG (Korea)
Microscope	Olympus (Japan)
Weighing Machine	Tanita (Japan)
Centrifuge	Gemmy international corporation (Taiwan)
Glasswares	
Antibiotics	HiMedia Company
Media	HiMedia Company
Chemicals and Reagents	

Appendix - III

A. Composition and Preparation of Different Culture Media

The culture media used were from HiMEDIA company.

1. Nutrient Agar (NA)

Composition	gram/litre
Peptic digest of animal tissue	5.00
Beef Extract	1.50
Yeast Extracts	1.50
Sodium Chloride	5.00
Agar	15.00
Final pH (at 25°C)	7.4 ± 0.2

28 gm of the medium was suspended in 1000ml of the distilled water and boiled to dissolve completely. Then medium was autoclaved at 121°C (15lbs pressure) for 15min. The sterilized medium was then poured into the sterilized petridishes and then was allowed to cool.

2. Blood Agar Base (Infusion Agar)

Composition	gm/litre
Beef heart, infusion from	500
Tryptose	10.0
Sodium Chloride	5.00
Agar	15.0
Final pH (at 25°C)	7.3 ± 0.2

40gms of the medium was suspended in 1000ml of the distilled water, dissolved by boiling and sterilized by autoclaved at 121°C (15lbs pressure) for 15minutes. After cooling to 50^{o}C, 5%v/v sterile defibrinated blood was added aseptically, then mixed with gentle rotation and poured into the sterilized petridishes and was allowed to cool.

3. MacConkey Agar (MA)

Composition	gm/litre
Peptic digest of animal tissue	17.00
Proteose Peptone	3.00
Lactose	10.00
Bile Salt	1.50
Sodium Chloride	5.00
Neutral Red	0.03
Agar	15.00
Final pH (at 25°)	7.1 ± 0.2

51.53gm of the medium was dissolved in 1000ml of the distilled water and then boiled to dissolve completely. The media was autoclaved at 121°C (151bs pressure) for 15min. The sterilized medium was then poured in sterilized petridishes and was allowed to cool.

4. Nutrient Broth (NB)

Composition	gm/litre
Peptic digest of animal tissue	5.00
Sodium Chloride	5.00
Beef Extract	1.50
Yeast Extracts	1.50
Final pH (at 25°)	7.4 ± 0.2

13gm of the medium was dissolved in 1000ml of the distilled water and then boiled to dissolve completely. The medium was then dispensed in test tube in amount of 3ml in each and autoclaved at 121°C (151bs pressure) for 15 minutes. The sterilized medium was then cooled to room temperature.

5. Muller Hinton Agar (MHA)

Composition	gm/litre
Beef infusion form	300.0
Casein Acid Hydrolysate	17.50
Starch	1.50
Agar	17.00
Final pH (at 25°)	7.3 ± 0.2

38gm of the medium was dissolved in 1000ml of the distilled water and then boiled to dissolve completely. The medium was autoclaved at 121°C (15lbs pressure) for 15minutes. The sterilized medium was then poured in sterilized petridishes and was allowed to cool.

6. Cetrimide Agar (CA)

Composition	gm/litre
Pancreatic digest of gelatin	20
Magnesium chloride	1.4
Dipotassium sulfate	10
Cetrimide	0.3
Agar	13.6

Suspend 45.3 g in 1 l distilled water, heat until completely dissolved, and add 10 ml/l Glycerin, Waterfree (TN1424). Autoclave at 121°C for 15 minutes. The sterilized medium was then poured in sterilized petridishes and was allowed to cool.

B. Composition and preparation of different biochemical media

1. Simmon Citrate Agar

Composition	gm/litre
Magnesium Sulfate	0.20
Monoammonium Dihydrogen Phosphate	1.00
Dipotassium Phosphate	1.00
Sodium Citrate	2.00
SodiumChloride	5.00
Agar	15.0
Bromothymol Blue	0.08
Final pH (at 25°C)	6.8 ± 0.5

24.2 grams of the medium was dissolved in 1000ml of the distilled water and boiled to dissolve completely. 3 ml of the medium was dispensed in each test tube and autoclaved at121°C (15lbs pressure) for 15minutes. The sterilized medium in the test tube was then allowed to set in slopes or slant.

2. Urea Agar base (Christensen urea agar)

Composition	gm/litre
Peptic digest of animal tissues	1.000
Dextrose	1.000
Monopotassium Phosphate	0.800
Dipotassium Phosphate	1.200
Sodium Chloride	5.000
Agar	15.00
Phenol Red	0.012
Final pH (at 25°C)	6.8 ± 0.2

24 grams of the medium was suspended in 950 ml of distilled water, dissolved by boiling and autoclaved at 121^0C (15 lbs pressure) for 15 minutes. After cooling to 50^0C, 50 ml of sterile 40% urea solution was added aseptically, mixed with gentle rotation. Then 5ml of the medium was dispensed in test tube and set at slant position.

3. Sulphide Indole Motility (SIM) Agar

Composition	gm/litre

Peptic digest of animal	30.00
Beef Extract	3.00
Peptonized Iron	0.20
Sodium Thiosulfate	0.025
Agar	3.00
Final pH (at 25°C)	7.3 ± 0.2

36.23grams of the medium was dissolved in 1000ml of the distilled water, boiled to dissolve completely and then dispensed in test tubes to a depth of about 3 inches. Then the medium in tubes was autoclaved at121°C (15lbs pressure) for 15 minutes.

3. MR-VP Medium

Composition	gm/litre
Buffered peptone	7.00
Dextrose	5.00
Di-potassium phosphate	5.00
Final pH (at 25°C)	6.9 ± 0.2

17 grams of the medium was dissolved in 1000ml of the distilled water, boiled to dissolve completely and then 3ml of the medium was dispensed in each test tubes and then autoclaved at121°C (15lbs pressure) for 15 minutes.

4. Triple Sugar Iron (TSI) Agar

Composition	gm/litre
Peptic digest of animal tissue	10.00
Casein Enzymatic Hydrolysate	10.00
Yeast Extracts	3.00
Beef Extract	3.00
Lactose	10.00
Sucrose	10.00
Dextrose	1.00
Sodium Chloride	5.00
Ferrous Sulphate	0.20
Sodium Thiosulphate	0.30
Agar	12.00
Phenol red	0.024
Final pH (at 25°C)	7.4 ± 0.2

65grams of the medium was dissolved in 1000ml of the distilled water. The medium was then dispensed in test tubes and autoclaved at 121°C (15lbs pressure) for 15minutes. The sterilized medium in the test tube was then allowed to set in slant with a butt of about 1 inch of thickness.

C. Composition and preparation of different staining reagent

1. Gram Stain

(a) Crystal Violet Solution

Crystal Violet	20.0 g
Ammonium Oxalate	9.0 g
Ethanol or Methanol	95.0 ml
Distilled Water (D/W) to make	1 litre

Preparation: 20 grams of Crystal Violet was weighed in a clean piece of paper and transferred to a clean brown bottle. Then 95 ml of ethanol was added and mixed until the dye is completely dissolved. To the mixture, 9 grams of Ammonium Oxalate dissolved in 200ml of D/W was added. Finally the volume was made 1 litre by adding D/W.

(b) Lugol's Iodine

Potassium Iodide	20.00g
Iodine	10.00g
Distilled Water	1000.0 ml

Preparation: To 250 ml D/W, 20 grams of Potassium Iodide was dissolved. Then 10 grams of iodine was mixed to it until it was dissolved completely. Finally the volume was made 1 litre by adding D/W.

(c) Acetone-Alcohol Decolorizer

Acetone	500ml
Ethanol (absolute)	475ml
Distilled Water	25.0 ml

Preparation: 475 ml of ethanol (absolute) was added to 25ml of D/W, mixed and transferred into a clean bottle. Then immediately, 500ml of acetone was added to the bottle and mixed well.

(d) Safranin (Counter Stain)

Safranin (2.5% solution in 95% ethyl alcohol)	10.00ml
Distilled Water	100.0 ml

Preparation: 2.5 % of Safranin solution was prepared in 95% ethanol. 10 ml of this solution was then suspended in 100 ml of D/W.

2. Normal saline

Sodium Chloride	0.85g
Distilled Water	100ml

Preparation: 0.85 grams of Sodium Chloride was weighed and added to a bottle containing 100ml of D/W and mixed well to dissolve the salt completely. The bottle was well labeled and stored at room temperature.

3. Biochemical Test Reagents

a. For catalase test

Catalase reagent (3%H2O2)

Hydrogen Peroxide	1ml
Distilled Water	9ml

Preparation: To 9ml of D/W, 1ml of Hydrogen Peroxide was added and mixed well so as to make a 3% solution of Hydrogen Peroxide.

b. For oxidase test

Oxidase reagent (impregnated in Whatman's No. 1 filter paper)

Tetramethyl *p*-phenylene diamine dihydrochloride(TPD)	1.00g
Distilled Water	100ml

Preparation: 1 gram of TPD was dissolved in 100 ml of D/W. To this solution, strips of Whatman's No.1 filter paper were soaked and drained for about 30 seconds. Then these strips were freeze dried and stored in a dark bottle tightly sealed with a screw cap.

c. For indole test

Kovac's Indole Reagent

p-dimethyl aminobenzyldehyde	2.00gm
Isoamyl alcohol	30.0ml

Concentrated Hydrochloric Acid	10.0ml

Preparation: In 30 ml of isoamyl alcohol, 2 grams of *p*-dimethyl aminobenzaldehyde was dissolved and transferred to a clean brown bottle. Then to this solution, 10ml of concentrated Hydrochloric Acid was added and mixed well.

d. For methyl red test

Methyl red solution

Methyl red	0.05gm
Ethylalcohol(absolute)	28.0ml
Distilled Water	22.0ml

Preparation: 0.05 gram of methyl red was dissolved in 28 ml ethanol and transferred to a clean brown bottle. To this, 22 ml of D/W was added and mixed well.

e. For Voges Proskauer test

Barrit's reagent

Solution A

α-Napthol	5.0gm
Ethyl alcohol (absolute)	100ml

Preparation: 5 gram of α napthol was dissolved in 25 ml ethanol and transferred into a clean brown bottle.Then the finel volume was made 100ml by adding ethanol.

Solution B

Potassium hydroxide (KOH)	40.0gm
Distilled Water	100ml

Preparation: 40 gram of KOH was dissolved in 25 ml D/W and transferred into a clean brown bottle. Then the final volume was made 100 ml by adding D/W.

4. Turbidity standard equivalent to McFarland 0.5

1% v/v solution of sulphuric acid was prepared by adding 1 ml of concentrated sulphuric acid to 99ml of water. Then to 99.4 ml of this solution, 0.6 ml of 1% w/v solution of barium chloride prepared by dissolving 0.5 gram dihydrate barium chloride (BaCl2.2H2O) in 50 ml of D/W, was added and mixed well. Then the standard was transferred into screw capped tubes of the same size and volume as those used for preparing the test and control inocula. The tubes were then sealed tightly to prevent loss by evaporation and stored protected from light at room temperature. The turbidity standard was then vigorously agitated before use. This

standard when stored in well sealed container in the dark at room temperature (20-28°C) may be kept for up to 6 months.

Appendix - IV

A. Procedure for Gram Staining (Forbes *et al*., 2007)

Gram staining is a differential staining that differentiates all bacterial species into two large groups: gram positive and gram negative. The following steps were involved in gram staining:

1. A thin film of the material to be examined was prepared and dried.
2. The material on the slide was heat fixed and allowed to cool before staining.
3. The slide was flooded with Crystal Violet stain and allowed to remain without drying for 10-30 seconds.
4. The slide was rinsed with tap water, shaking off excess.
5. The slide was flooded with iodine solution and allowed to remain on the surface without drying for twice as long as the crystal violet was in contact with the slide surface.
6. The slide was rinsed with tap water, shaking off excess.
7. The slide was flooded with alcohol acetone decolorizer for 10 seconds and rinsed immediately with tap water until no further colour flows from the slide with the decolorizer. Thicker smear requires more aggressive decolorization.
8. The slide was flooded with counter stain (safranin) for 30 seconds and washed off with tap water.
9. The slide was blotted between two clean sheets of bibulous paper and examined microscopically under oil immersion at 100X.

B. Procedure for Antibiotic Sensitivity Testing (AST) by Disc Diffusion Method

In the treatment and control of infectious disease, antimicrobial susceptibility test is done to select effective antimicrobial drugs against suspected organisms. Disc diffusion method is a standard method for antimicrobial susceptibility test.

The following steps are involved in AST by Disc diffusion method:

1. An isolated colony of organism was suspended in the nutrient broth and incubated at 37^{o}C for 4 hours. The turbidity was matched with 0.5 McFarland standard.
2. A sterile cotton swab was introduced into the standardized tube and swabbed onto the MHA plate.
3. 3. The pate was allowed to dry and the antibiotics discs were placed over the media in the plate and incubated at 37^{o}C. The result was noted after 18 hours.

Appendix-V

Zone size interpretative chart of antibiotics for *P. aeruginosa*

Antibiotics used	Symbol	Disc potency (mcg)	Diameter of zone of inhibition in mm		
			Resistant	Intermediate	Sensitive
Amikacin	Ak	10	14	15-16	17
Carbenicillin	CB	100	13	14-16	17
Cefepime	CPM	30	14	15-17	18
Cefepime / Tazobactam	CPT	30/10			
Cefotaxime	CE	30	14	15-22	23
Ceftazidime	CA	30	14	15-17	18
Ciprofloxacin	CF	5	15	16-20	21
Gentamicin	G	10	12	13-14	15
Imipenem	I	10	13	14-15	16
Meropenem	MR	10	13	14-15	16
Ofloxacin	OF	5	14	15-17	18
Piperacillin / Tazobactam	PT	100/10	17	18-20	18

Appendix-VI

Methods of biochemical test used for the identification of pathogens

a. Catalase test:

The enzyme Catalase is present in most cytochrome containing aerobic and facultative anaerobic bacteria; the main exception is Streptococcus species (catalase negative). Usually organisms which lack the cytochrome system also lack the Catalase enzyme and therefore are unable to break down hydrogen peroxide. Catalase is a heme protein. The prosthetic group is made up of four atoms of trivalent iron (ferric) per molecule, which retains its oxidized state during enzyme activity. Hydrogen peroxide is formed as an oxidative end product of the aerobic breakdown of sugars. Reduced flavoprotein reacts directly with gaseous oxygen by way of electron reduction to form hydrogen peroxide and not by direct action between hydrogen and molecular oxygen.

With the help of a sterile glass rod, a small amount of culture from the Nutrient Agar was transferred to a clean glass slide and a drop of 3% Hydrogen peroxide solution was dropped on the surface of the slide. The positive test is indicated by the formation of active bubbling of the oxygen gas. The lack of Catalase was evident by lack of or weak bubble production.

b. Oxidase test :

The Oxidase test is based on the bacterial production of an Oxidase enzyme. The oxides reaction is due to the presence of a cytochrome oxidase system which activates the oxidation of reduced cytochrome by molecular oxygen, which in turn acts as an electron acceptor in the terminal stage of the electron transport system.The cytochrome system is usually present only in aerobic organisms which make them capable of utilizing oxygen as a final hydrogen acceptor to reduce molecular oxygen to hydrogen peroxide, the last link in the chain of aerobic respiration.

A piece of filter paper soaked in Oxidase reagent and dried was moistened with distilled water and a colony from the fresh culture was picked up with a sterile glass rod and smeared on the paper. The positive test is indicated by the appearance of blue-

purple color within 10 seconds. The Oxidase reagent (Tetra methyl *p*- phenylene diamine dihydrochloride) is a dye that is primary aromatic amines and diamine derivatives of benzene. Cytochrome oxidase in the presence of atmospheric oxygen oxidizes the Oxidase reagent to form a colored compound called indophenol. The Oxidase test is based on the bacterial production of an Oxidase enzyme.

c. Indole production test :

Tryptophan is an amino acid that can be oxidized by certain bacteria to form three major indolic metabolites: indole, skatol (methyl indole), and indole acetic acid. Intracellular enzymes involved in this oxidation process are collectively called as Tryptophanase. The enzyme tryptophanase catalyses the deamination reaction attacking the tryptophan molecule in its side chains and leaving the aromatic ring intact in the form of indole.

For this test, organism was stabbed in SIM (Sulfide Indole Motility) medium from the nutrient broth and incubated at 37^{o}C for24 hours . After incubation, 2-3 drops of Kovac's reagent (*p*- dimethyl aminobenzaldehyde in acid ethanol) was added and resulting color was noted .Indole, if present combines with the aldehyde present in Kovac's reagent to give a red color in the alcohol layer. The color reaction is based on the presence of pyrrole structure present in the indole.

d. Methyl red test

This test is used to determine the ability of an organisms to produce and maintain the stable acid end product from glucose fermentation, and to overcome the buffering capacity of the system.The methyl red test uses a pH indicator in the form of methyl red, to determine the hydrogen ion concentration (pH) arising out of fermentation of glucose by an organism. The hydrogen ion concentration depends on gas ratio (CO2 and H2), which in turn is an index to the different pathways of glucose metabolism exhibited by various organism. The different fermentation patterns are due to variation in enzymes concerned with pyruvic acid metabolism present in the organism. Methyl red positive organisms produce stable acids, maintaining a high concentration of hydrogen ions until a sudden concentration is reached. The validity of methyl red test depends upon a sufficient incubation period in order to permit the differences in glucose metabolism to occur. The organisms to be tested should be

incubated at least 35ºC -37ºC. Methyl red is an indicator which is already acidic and well denotes changes in degree of acidity by color reactions over a pH range of 4.4-6.0.A pure colony of the test organism was inoculated into 2 ml of MRVP medium and was incubated at 37ºC for 24 hours. After incubation, about 5 drops of methyl red reagent was added and mixed well. The positive test was indicated by the development of bright red color, indicating acidity and negative with yellow color.

e. Voges Proskauer test

The principle of this test is to determine the ability of some organisms to produce a neutral end product, acetyl methyl carbinol (acetoin), from glucose fermentation.Glucose is metabolized to pyruvic acid which is a key intermediate in glycolysis. From pyruvic acid, there are many pathways that a bacterium may follow. The production of acetoin is one pathway for glucose degradation occurring in bacteria. The VP test for acetoin is used primarily to separate *Escherichia coli* from *Klebsiella* and *Enterobacter* spp.A pure colony of the test organism was inoculated into 2 ml of MRVP medium and was incubated at 37ºC for 24 hours. After incubation, about 5 drops of Barrit's reagent was added and mixed well and kept for 15 minutes, positive test shows development of pink red color.

f. Citrate utilisation test :

This test is done to determine if an organism is capable of utilizing citrate as a sole source of carbon for metabolism with resulting alkalinity .The organism whose ability was to be tested were inoculated in the slant of Simmon's Citrate agar media and incubated at 37ºC for 24 hours. Result was interpreted as positive if there was a growth or change in color of slant from green to intense blue and negative if there is no growth and no change in color.

g. Triple sugar Iron (TSI) Agar:

Triple sugar Iron (TSI) agar is a medium used in the identification of Gram negative enteric rods. The medium measures the ability of a bacteria to utilize sugars: glucose, sucrose and lactose, the concentration of which are in 0.1%, 1.0% and 1.0% respectively. A pH indicator (Phenol Red) included in the medium can detect acid

production from fermentation of test carbohydrates. The test organism was streaked and stabbed on the surface of TSI and incubated at 37°C. for 24 hours. The results are interpreted as:

a. Yellow (Acid) / Yellow (Acid), Gas, H2S → Glucose, Lactose/ Sucrose fermenter, H2S producer.

b. Red (Alkali) / Yellow (Acid), No Gas, No H2S → Glucose fermenter, Lactose/Sucrose nonfermenter, Anaerogenic, H2S nonproducer.

c. Red (Alkali) /No Change → Glucose, Lactose and Sucrose nonfermenter,

d. Yellow (Acid)/ No Change → Glucose oxidizer.

e. No Change / No Change → Nonfermenter.

h. Motility test:

Bacteria are motile by means of flagella. Flagella occur primarily among the bacilli; however a few cocci forms are motile. Motile bacteria may contain single flagella. The motility media used for motility are semi solid, making motility interpretation macroscopic. Motile organisms migrate from the stab- line and diffuse into the medium causing turbidity. They may exhibit fussy streaks of growth. Whereas nonmotile bacteria show the growth along the stab-line, and the surrounding media remains colorless and clear.

j. Urea hydrolysis test

This test demonstrates the urease activity present in certain bacteria which decomposes urea, releasing ammonia. The test organism was inoculated in a medium containing urea and the indicator Phenol red. The inoculated medium was incubated at 37°C overnight. Positive organism shows pink red due to the break down of urea to ammonia. With the release of ammonia the medium becomes alkaline as shown by a change in color of the indicator to red pink.

Appendix-vii

Identification of Pseudomonas aeruginosa

Growth at 42°C, Growth at 37°C	**Positive**
Gelatin hydrolysis	**Positive**
Indole	**Negative**
MethylRed	**Negative**
Citrate	**Positive**
Triple Sugar Iron Agar	**Red/Red**
Catalase	**Positive**
Oxidase	**Positive**
Urease	**Negative**
Motility	**Positive**
Morphology	**Bacillus**
Gram stain	**Gram negative**
Pigment	**Positive**
Odour	**Positive**