EMERGENCE AND ANTIBIOTIC SENSITIVITY PATTERN OF *ACINETOBACTER BAUMANNII* IN A HOSPITAL FACILITY

Internship Training Report

Submitted to

Lovely Professional University, Punjab

in partial fulfilment of the requirements

For the degree of

Master of Science in Clinical Microbiology

Submitted by:
Dinobandhu Nandi
(Reg. No. 11401162)

SCHOOL OF PHYSIOTHERAPY AND PARAMEDICAL SCIENCES
LOVELY PROFESSIONAL UNIVERSITY, PUNJAB, INDIA
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DECLARATION

I hereby declare that the work embodied in this internship report was carried by me under the supervision of Dr. Anania Arjuna (Internal supervisor), Lovely Professional University and Dr. Renuka Bajaj (External supervisor), SRL Ltd. Fortis Escort Hospital, Amritsar. This work has not been submitted in part or in full in any other university for any degree or diploma.

Name: Dinobandhu Nandi
Date: 03- 05- 2016
Place: Lovely Professional University

CERTIFICATE

This is to certify that Dinobandhu Nandi (registration no.- 11401162) student of M.Sc. Clinical Microbiology (course code- 240A) in Lovely Professional University, Phagwara, Punjab has actively worked in a practical training from 1st January, 2016 to 30th April, 2016 in SRL Ltd. Fortis Escort Hospital, Amritsar, Punjab under supervision and proper guidance He has worked in microbiology department with full responsibility. We wish him good luck and success in future endeavour.

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ABSTRACT

Hospital acquired infections (HAIs) are mostly caused by Gram-negative organisms and is one of the major issues in patient safety. These infections are often associated with the medical processes of hospitals such as invasive medical devices and various surgical procedures. Gram-negative organisms account for most infections in the hospital environment because of their ability to acquire resistant against multiple antibiotics. Through different mechanisms including the synthesis of β- lactamases, overexpression of transmembrane efflux pump, loss of porins, synthesis of antibiotic modifying enzymes, target mutations, ribosomal mutation or modifications, mutations in lipopolysaccharide structure etc. these organisms have developed drug-resistant property and the genes encoded in plasmids play a vital role in developing the resistant. Among all Gram-negative bacteria, *Acinetobacter baumannii* is an emerging pathogen that accounts for about 80% of all reported infections. Although other species of *Acinetobacter* are also often associated with HAIs. *Acinetobacter* is non-motile, obligate aerobic Gram-negative coccobacillus and are ubiquitous free-living saprophytes in soil and water. It is commonly transmitted through medical devices such as ventilators, urinary catheters and other invasive devices in hospitals but its ability to colonize on the skin of individuals often increases the rate of transmission through person to person contact. Patients admitted to Intensive Care Unit (ICU) are at the major risk of getting infected by *A. baumannii* and these includes pneumonia/ ventilator-associated pneumonia, bloodstream infections, wound abscesses, urinary tract infections etc. Lower respiratory tract infections were more prevalent (89.07%) in hospital facility followed by abscess (6.72%), septicaemia (2.52%), urinary tract infections (0.84%), and soft tissue infections (0.84%). Mechanical ventilation, intubations, and other medical devices increase the rate of infection. Ventilation is one of the major reasons for lower respiratory tract infection. Males have a high prevalence of infection when compare with females. *Acinetobacter baumannii* resist approximately all common antibiotics; biofilm production and their ability to survive for a long time on low nutritional requirement play an important role in their pathogenesis. Colistin is the promising drug for multidrug resistant *A. baumannii* infections. Tigecycline and Minocycline have shown a sensitive percentage of 52.94 and 22.69 respectively. Imipenem, Aztreonam and Ticarcillin- clavulanic acid have shown a resistant profile of 100%. Good housekeeping, sterilization of equipment, hand hygiene, water purification, isolation procedures and maintaining of the hospital environment, use of infection control practices should be implemented to control the rate *A. baumannii* infection.

Keywords: *A. baumannii*, biofilm, β- lactamases, HAI.

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CHAPTER – 1

INTRODUCTION

Hospital acquired infection (HAI) also called nosocomial infection is a kind of infection which is acquired in the hospital by a patient in whom the infection was not present at the time of admission but emerged after discharging and it also includes occupational infections among the staff of the organisation (1, 2). Nosocomial infections are mostly caused by Gram-negative organisms and are one of the major issues in patient safety. HAIs are one of the major causes of death (3) which have worldwide prevalence and affect developed, developing and resource-poor countries. The rate of morbidity and mortality is significantly increased and cause financial losses for health care system. The study has revealed that within hospital it is mostly prevalent is intensive care units (ICU) followed by acute surgical and orthopaedic wards (4), approximately 30% patients in ICU are affected by at least one nosocomial infection (5).

1.1 FACTORS RESPONSIBLE FOR HOSPITAL ACQUIRED INFECTIONS

A group of factors is influencing the development of HAI which mainly includes microbial agent and its drug-resistant ability, patient's health and immune status, recent advances in medical facilities and environmental factors.

1.1.1 THE MICROORGANISM AND THEIR ABILITY TO RESIST DRUGS

During the hospitalization, the patient is exposed to numerous microorganism and also depending on their health and immune status they itself getting infected by normal flora. Many different microorganism e.g. bacteria, viruses, fungi and parasites are causing infection in health- care system. The transmission plays a role in the development of infection, through cross- infection, environmental infection, endogenous infection and recent advanced surgical methods provide various routes for entry of pathogens. Multidrug- resistant (MDR) strains of different bacteria are new challenge to the health- care facility. Through genetic modification and because of using numerous antibiotics organisms develop resistant. Many strains of pneumococci, staphylococci, enterococci, Acinetobacter, and tuberculosis are currently resistant to most or all anti- microbial agents who were previously effective (4, 6).

1.1.2 PATIENT HEALTH AND IMMUNE STATUS

Many factors are contributing to influence the chance of nosocomial infection includes age, underlying diseases, immunity, and diagnostic, therapeutic, and surgical interventions. Patient suffering from chronic diseases like diabetes, malignant tumours, tuberculosis, leukaemia, acquired immune deficiency syndrome (AIDS) etc. increases the susceptibility to get infected in a hospital environment as all of these conditions are associated with weakening of immune system. Infants and older persons are more prone towards infection because of underdeveloped and degenerating immune response respectively. Socio- economic status of the patient is an important issue as malnutrition plays a crucial role in infection. Although modern techniques involved in medical facilities ease the diagnostic and therapeutic options but they also provide the route for an infection to develop such as catheterization, ventilation, endoscopic examination, and various surgical procedures. Major surgeries like

transplantation in which immunosuppressive drugs are used put the patient at a high risk of getting infected if preventive measures are not taken carefully.

1.1.3 HOSPITAL ENVIRONMENTAL FACTORS

A hospital is a place where patients with many different cases are admitted and also the visitors are important factors. Infected patients or the carriers of infection are the potent sources of infection to the patient and staff facility. Factors like overcrowding, shifting of the patients from one unit to another, the concentration of patients highly susceptible to infection in one area like burn patients, newborns etc. increases the risk of HAI (4). Inadequate environmental hygienic conditions, waste disposal policies, insufficient equipment, understaffing, poor knowledge and application of basic infection control measures, the absence of local and national guidelines and policies are the major issues towards patient safety. Some other factors like long- term hospitalization, home infusion therapy, long- term dialysis within 30 days, family member with multidrug- resistant pathogen; long- term antimicrobial therapy etc. increases the risk of HAIs (7).

1.2 ASSOCIATED ORGANISMS WITH HAIs

Many different organisms are associated with nosocomial infections and they vary according to patient health, health- care facility and epidemiologically. Mostly all kind of microorganisms are causing HAIs but bacteria are most common.

1.2.1 BACTERIA

Bacteria are contributing about 90% infections and are the most common nosocomial pathogens, while viruses, fungi, and parasites are less contributing towards infection in health-care setting (8). Both commensals and pathogenic bacteria are causing serious infections. In an immune-compromised patient or patient with suppressed immune system normal flora can become a pathogen to cause infection such as cutaneous coagulase- negative staphylococci cause intravascular line infection and intestinal *Escherichia coli* (*E. coli*), cause urinary tract infection (UTI) (4). Pathogenic bacteria have greater virulence to breach host immune system and establish a well-developed infection. Gram- negative bacteria (GNB) are at major concern and include *Acinetobacter spp*., *Pseudomonas aeruginosa* (*P. aeruginosa*), and Enterobacteriaceae family members including *Enterobacter*, *Proteus mirabilis*, *Klebsiella pneumonia* (*K. pneumoniae*), *E. coli*, *Serratia marcescens*. Although the microbial agents are changing over time; for example, *Proteus* spp., *Klebsiella* spp. and *Escherichia* spp. were responsible for nosocomial infections in the 1960s, but from 1975 to 1980s, *Acinetobacter* spp. with *P. aeruginosa* created clinical difficulties (9). In 2011-2012, a study was conducted in japan to isolate bacteria from surgical infections and it ended up with the isolation of 523 strains from primary infections and 231 from surgical site infection. Anaerobic Gram- negative bacteria were most prevalent from primary infections, whereas aerobic *Enterococcus spp.* was most among Gram- positive bacteria (GPB) followed by *Streptococcus* and *Staphylococcus spp .E.coli* was the predominant organism among all aerobic Gram-negative followed by *K. pneumoniae*, *P. aeruginosa* and *Enterobacter cloacae* (10).

1.2.2 VIRUSES

Viruses are an important cause of nosocomial infection, near about 5% of all nosocomial infection is caused by viruses (11). The incidence of viral nosocomial infection is more common in pediatric wards (12-14) and wards with older patients (15, 16). In one study it is found that 32% of pediatric infections caused by viruses among which respiratory syncytial virus (RSV) was most common (11). Transmission occurs through respiratory route, fecal-oral route, perinatal route and person to person contact (17). The viral agents which are attributed to major infections caused by viruses include RSV, rotavirus, cytomegalovirus (CMV), herpes simplex virus (HSV), influenza viruses, varicella- zoster virus (VZV), hepatitis-B virus (HBV), hepatitis-C virus (HCV), human immunodeficiency virus (HIV) etc. HSV and CMV are the most frequent virus found in ICUs (18).

1.2.3 FUNGI

Fungi, the eukaryotic organism is also contributing to HAIs. *Candida spp.* is the most common fungi which have been reported to cause the majority of nosocomial infections (19-21). The other infection causing fungi include *Aspergillus spp.*; *Malasezzia spp*., non-*albicans Candida spp., Fusarium spp*., and *Trichosporon spp*. (22-28), including *Scedosporium spp* (29). These infections are difficult to diagnose and this attributed to high morbidity and mortality.

1.2.4 PARASITES

The protozoan and helminths are an important cause of nosocomial infection as they have been recognised to be the causative agent of diarrhea in12-17% nosocomial epidemics and 1% endemic outbreaks (30). *Cryptosporidium* spp., *Entamoeba histolytica, Giardia lamblia* and *Blastocystis spp*. is common in outbreaks. A study in Tunisia has reported 45% of children with a complex immune deficiency and has the symptom i.e. profuse diarrhoea was caused by *Cryptosporidium hominis* (31). In cases of blood transfusion *Toxoplasma gondii*, *Trypanosoma cruzi*, *Plasmodium spp.,* and *Leishmania* spp. are common.

1.3 POSSIBLE ROUTES OF TRANSMISSION IN A HEALTH CARE SETTING

In a health care setting the infections can be transmitted in several ways which are mainly divided into three following categories:

- Endogenous transmission- normal flora present in patient's body when move to unnatural places i.e. other than its habitat, cause infection. This may occur due to surgical procedures, inappropriate antibiotic therapy that allows overgrowth of organisms, invasive medical devices etc.
- Exogenous transmission (cross- transmission) - this occurs when organisms are transmitted between patients. It can be through different modes which include direct contact between patients through contaminated hands, body fluids etc., airborne transmission via infected droplets and dust, transmission through transient or permanent carriers e.g. staffs who have been contaminated during handling or nursing of patients.

- Environmental transmission- hospital environment is rich in different kind of organisms and a potent source of infection for patients as well as for the staff facility. Water, damp area, benches, disinfectants, food, linen, equipment, breast pumps, incubators, sinks, soap etc. are common sources of infection (4, 32). Bacteria like *Acinetobacter*, *Pseudomonas*, and *Mycobacteria* can remain in the air for several hours as fomites and droplets. Transmission of *Acinetobacter baumannii* is assumed to through contaminated fomites in the environment (33).

1.4 TYPES OF HOSPITAL ACQUIRED INFECTION (HAI)

HAI can be projected at any site in the body depending on the organism involved and clinical criteria. The major sites are- lower respiratory tract, gastrointestinal infections, urinary tract, surgical sites, skin and soft tissue, upper respiratory tract, and bloodstream infection (4, 34). *Acinetobacter*, an emerging pathogen is attributed to all of these infections and *A. baumannii* is more prevalent among all *Acinetobacter spp*. It is associated with hospital- acquired pneumonia (6) and ventilator-associated pneumonia (VAP), septicaemia, wound infection, urinary tract infection, endocarditis, skin and soft tissue infection and secondary meningitis (35, 36).

1.5 ACINETOBACTER- AN EMERGING NOSOCOMIAL PATHOGEN

Acinetobacter are Gram- negative coccobacilli (GNCB) who have become the most prevalent cause of hospital acquired infections. It has been identified to cause sporadic infections in health care settings and has a significant role in colonization in critically ill patients (37). The species *Acinetobacter* are associated with healthcare- related outbreaks (38-40). Among all species, *Acinetobacter baumannii* has been found to cause approximately 80% of reported Acinetobacter infections (41).

1.5.1 FACTORS THAT MAKE ACINETOBACTER A POTENT NOSOCOMIAL PATHOGEN

- They are commensal flora of human body and animals which are regular contaminants of healthcare environment (42).
- Ubiquitous in nature and have been found more commonly in soil, water and skin, throat and rectum of patients and also healthcare personals (43).
- They require minimal nutrition and can survive to extend period on various inanimate surfaces and aqueous environments (44-46).
- Most importantly their ability to resist nearly all kind of drug makes them a potent nosocomial pathogen.

1.5.2 MECHANISM OF DRUG RESISTANCE

Acinetobacter baumannii have developed resistance to a wide variety of antimicrobial drugs include β- lactams, aminoglycosides, quinolones, tetracyclines, and glycylcyclines.

1.5.2.1 β- LACTAMS

Acinetobacter baumannii uses two different modes to resist β- lactams include an enzymatic mechanism and non- enzymatic mechanism.

- **Enzymatic mechanism**- This is the most common mode used by *A. baumannii* to resist β- lactams. They produce β- lactamases and other phenotypes which degrade the drug and keep organism viable.
- **Non- enzymatic mechanism-** This includes changes in outer membrane proteins (OMPs), multidrug efflux pumps and alteration in the affinity or expression of penicillin- binding proteins (PBPs) (47). A 29- kDa protein named as CarO is shown to be associated with imipenem and meropenem resistance (48-50). *A. baumannii* has a wide variety of multidrug- efflux systems (51) among them AdeABC which is chromosomally encoded is best studied and it efflux substrates like β–lactams including carbapenems (52, 53), aminoglycosides, erythromycin, chloramphenicol, tetracyclines, fluoroquinolones, trimethoprim, and ethidium bromide (52- 54).

1.5.2.2 AMINOGLYCOSIDES

In multi-drug resistant *A. baumannii* aminoglycosides- modifying enzymes e.g. acetyltransferases, nucleotidyltransferases, and phosphotransferases encoded in class 1 integrons is prevalent (55-60). These all enzymes impair aminoglycoside (gentamicin, tobramycin, and amikacin) binding to its target and thus produce resistance.

1.5.2.3 QUINOLONES

Mutation in the *gyrA* and *parC* genes which results in modification of DNA gyrase have been found to cause resistance in *A. baumannii*. Quinolones are also substrates for efflux pump i.e. multi- drug efflux systems (AdeABC) and the MATE pump AdeM (53).

1.5.2.4 TETRACYCLINES AND GLYCYLCYCLINES

Resistance to these is mediated by efflux pumps or ribosomal protection (61). Tetracycline-specific efflux pump and multi-drug efflux systems e.g. AdeABC play a critical role to produce adequate resistance.

1.5.3 INFECTIONS ASSOCIATED WITH *ACINETOBACTER BAUMANNII*

A wide variety of infection is associated with *A. baumannii*. It is the major cause of hospital acquired pneumonia and particularly ventilator-associated pneumonia (VAP) is prevalent among hospitals. In a study from the United States has shown that 5-10% ICU- acquired pneumonia was caused by *A. baumannii* (62). Prolong stay in ICU increase the susceptibility of *A. baumannii* infections. It is also a successful pathogen to cause bloodstream infection, urinary tract infection (occasionally), wound infection, meningitis and in some cases it is also associated with endocarditis and keratitis. In a study from Unites States *A. baumannii* was found the 10^{th} most common etiological agent to cause mono-microbial nosocomial bloodstream infection (63).

CHAPTER- 2

LITERATURE REVIEW

2.1 HASSAN AHMED KHAN, AFTAB AHMAD, RIFFAT MEHBOOB (2015)

In the era of antibiotics, nosocomial infections are still uncontrollable. The causative agents associated with nosocomial infection should be controlled and managed as they cause both economic and production loss in the community. These infections are mostly caused by *Streptococcus spp.*, *Acinetobacter spp.*, enterococci, *Pseudomonas aeruginosa*, coagulase-negative staphylococci, *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Escherichia coli*, *and Serratia marcescens*. These organisms are associated with 13 types of nosocomial infection sites which commonly include urinary tract, surgical and soft tissues, respiratory tract, gastrointestinal tract and meninges. Transmission of nosocomial pathogens can occur through different routes e.g. person to person contact, environmental transmission (contaminated water and food), infected individuals and a contaminated population of hospital facilities and visitors. Improper and frequent uses of broad- spectrum antibiotics result in drug resistance which is the most common cause of all reported hospital- acquired infections. The drug-resistant ability of organisms makes them difficult to treat. The optimum practice is required to accomplish in hospitals and other health- care settings to control the rate of nosocomial infections.

2.2 ANTON Y. PELEG, M.B., B.S., M.P.H. AND DAVID C. HOOPER, M.D. (2010)

Hospital-associated infections are the sixth leading cause of death in the United States and Europe. In 2002, a total of 1.7 million HAI occurred and approximately 99,000 deaths resulted were because of HAI. Gram- negative bacteria are commonly associated with HAI. They account for approximately 30% of HAI and their ability to upregulate genes or acquiring genes responsible for drug resistance makes them a challenging duty to accomplish patient's safety in a health- care setting. Advances in surgical procedures and invasive medical devices open the gate for the pathogen to enter easily and develop a well-formed infection in critically ill patients. Among all nosocomial infections lower respiratory tract infections (particularly pneumonia and VAP) and bloodstream infections are fatal while urinary tract infection (UTI) accounts for most common infection.

2.3 WORLD HEALTH ORGANIZATION (WHO)- PREVENTION OF HOSPITAL- ACQUIRED INFECTIONS, A PRACTICAL GUIDE, 2ND EDITION

Nosocomial infections occur worldwide and affect developed, developing and resource- poor countries. The incidence is increasing at an alarming rate and it accounts for high morbidity rate among hospitalized patients. Economic costs have been considered in HAIs as the length of stay in hospital contributes to the cost. Many different factors are attributing to HAIs include the microbial agent and their ability to resist drugs, patient susceptibility, and environmental factors. The common sites of infection are a urinary tract, lower respiratory tract, surgical sites, skin and soft tissues, respiratory tract (other than lower respiratory tract),

and bloodstream. Patients in ICU are at major risk to get infected easily and VAP is common within ICUs. Viral bronchiolitis caused by Respiratory syncytial virus (RSV) is common in children unit and wards with elder patient may be infected with influenza and secondary bacterial pneumonia. Bacteria are the most common pathogen associated with HAI; more often commensals can cause serious infections in patients with weak immunity. Although viruses (HBV, HCV, RSV, rotavirus, and enterovirus), fungi (*Candida albicans*, *Aspergillus spp.*, *Cryptococcus neoformans*), and parasites (*Giardia lamblia*) are also attributed to HAIs. Viruses like Cytomegalovirus (CMV), HIV, Ebola, Influenza, Herpes simplex virus (HSV) and VZV may also be transmitted to hospital individual. Infected patients, staff members, hospital environment including air, water and food, medical devices are a potent source of infection.

2.4 CELIA AITKEN AND DONALD J. JEFFRIES (2001)

Nosocomial infections occur when microorganisms and viruses are acquired during a hospital stay. The infection can be transmitted both endogenously and exogenously. Community-acquired infection of patients or staff can be a source of HAI. Viruses are an important cause of nosocomial infection and they are associated with 5% of all reported infection. Particularly pediatric units and wards with elderly patients are affected. Patients infected with virus increase the risk of infection in the wards and strongly associated with nosocomial outbreaks. RSV, influenza, parainfluenza, coronaviruses, adenoviruses, rotavirus, HBV, HCV, Epstein - Barr virus (EBV) are the causative agent of nosocomial infection. These infections can be controlled by education, awareness of transmission routes and strict follow of infection control procedures.

2.5 SCOTT K. FRIDKIN AND WILLIAM R. JARVIS (1996)

New technologies in the medical field, bone marrow and solid organ transplantation, uses of chemotherapeutic drugs have helped to treat patients and increased the quality of life but simultaneously they have resulted in a number of severely ill, immunocompromised hospitalized patient populations. This makes them highly susceptible to the hospital- acquired infections caused by fungi which have low virulence or are generally non- pathogenic. Fungi are associated surgical wound infections, bloodstream infections, pneumonia, and UTI. The rate of nosocomial fungal infection has been increased. During 1980-1990, Centers for Disease Control and Prevention (CDC) and National Nosocomial Infections Surveillance (NNIS) system reported a constant increase of the rate of nosocomial fungal infection from 2.0 to 3.8 per 1000 discharges. The risk factors include uses of antimicrobial agents, chemotherapy, haematological or solid organ malignancy, prolong stay in ICUs, neutropenia, the indwelling of catheters, ventilation, haemodialysis, and malnutrition. *Candida albicans* is the most common fungus causing nosocomial infection worldwide. *Aspergillus spp*., *Trichosporon spp., Fusarium spp*., *Acremonium spp.* are associated with the hospital-acquired fungal infection.

2.6 KATARZYNA GORALSKA AND PIOTR KURNATOWSKI (2013)

Nosocomial infections continuously increasing the threat to patient's health and most of the time the associated risk factors are ongoing radio and chemotherapy, immunosuppression and infections in which the immune system gets depleted e.g. HIV infection. Parasites are contributing to 0.48% of overall nosocomial infections and in Western Europe, the rate is 0.25%. Diarrhoea has been recognised as an important sign of nosocomial infection. Viruses

are the most common cause of diarrhoea in hospitals followed by bacteria but parasites have been found to be causative agents of diarrhoea in 12-17% of nosocomial epidemics and 1% of endemic outbreaks. Surgical ward is mostly affected by parasitic outbreaks. The etiological agents are *Cryptosporidium spp.*, *Entamoeba histolytica*, *Giardia lamblia* and *Blastocystis spp.* The infection rate with parasites has been noticed to increase after chemotherapy and long-term antibiotic therapy. Oncological, ICUs, internal medicine, geriatric and pediatric wards are commonly affected. Transfusion is one of the major causes of the hospital-acquired parasitic infections e.g. post- transfusion malaria, post- transfusion and post-transplantation infection with *Toxoplasma gondii*, *Trypanosoma cruzi*, and *Leishmania spp*. may occur. Contaminated water and the food is also a common source of infections and transmitted by fecal- oral route.

2.7 PURTI C. TRIPATHI, SUNITA R. GAJBHIYE, GOPAL NANDLAL AGRAWAL (2014)

Acinetobacter has emerged as a prevalent nosocomial pathogen. It is the most common Gram- negative bacilli carried on the skin of hospital personnel. Factors make patient prone to Acinetobacter- associated infection include prosthesis, endotracheal intubation, intravenous catheters and prior antibiotic therapy. Pneumonia, septicemia, UTI, and peritonitis are common manifestations associated with Acinetobacter infection. *Acinetobacter spp*. are ranked 2nd after Pseudomonas aeruginosa to be found commonly in clinical specimens. They come in 4th position when to compare with *P. aeruginosa*, *S. aureus*, and *K. pneumoniae* to cause more frequent hospital- acquired infections. The ability to adhere to surfaces is one of the key factors helps in the pathogenesis of Acinetobacter spp. They are notorious for their ability to acquire antibiotic resistance. Resistant to all known antibiotics makes it a serious nosocomial pathogen. Patients with prolonged mechanical ventilation have a high risk of infection caused by multi- drug resistant *A. baumannii*. It is found that *Acinetobacter* strains were found in ICU most frequently than general wards. The maximum sensitivity of A. baumannii was seen to imipenem, amikacin, gatifloxacin, and tobramycin while maximum resistance was obtained to ceftazidime, cefotaxime, piperacillin, and piperacillin- tazobactam. *A. baumannii* was found to be more resistant to commonly used antimicrobials.

2.8 SOFIA CONSTANTINIU, ANGELA ROMANIUC, LUMINITA SMARANDA IANCU, RALUCA FILIMON, IULIANA TARSI (2004)

Acinetobacter are strictly aerobic, non- motile, catalase positive, oxidase negative, Gram-negative coccobacilli. They play a significant role in colonization and infection of hospital personnel. They have been attributed to a variety of hospital- acquired infections include bacteremia, UTI, and secondary meningitis. But most commonly they are associated with VAP occur in ICU patients. It has been reported that *Acinetobacter spp.* are also associated with community- acquired infections. Their ability to survive in extreme condition e.g. drying and drug resistant makes them enable to cause severe infections in a health-care setup. They are difficult to treat because of their resistance property to all know and common antibiotics. *A. baumannii* has large metabolic activity. They produce acid from glucose, xylose, galactose, mannose, rhamnose and lactose. The study revealed that the susceptibility of Acinetobacter strains was ranged between 16.6% for ampicillin and 91.6% for imipenem. 16.6% of strains were sensitive to ampicillin, 20.8% to cephalotin, 25% to ceftriaxone, ceftazidime, gentamicin and kanamycin, and 41% to chloramphenicol and trimethoprim.

Most of the strains were sensitive to ciprofloxacin and imipenem i.e. 83.3% and 91.6% respectively.

2.9 RASHA .J .M.AL-WARID AND AZHAR A. L. AL-THAHAB (2014)

Acinetobacter spp. are the 2nd most common Gram- negative bacilli after *P. aeruginosa* encountered in human specimens. They are commensals on human and animal body, particularly on skin. A total of 458 samples were tested, among them, 11 isolates have been identified as *A. baumannii*. All isolates have been found to produce biofilm, gelatinase, and pellicle formation (100%). There was variation in production of lipase, protease, and lecithinase among isolates. All isolates have shown resistance to penicillin, piperacillin, ampicillin, amoxicillin, cefotaxime, tetracycline, ciprofloxacin, rifampicin, cefepime, ceftriaxone and ceftazidime (100%). It also has shown resistance to tobramycin, trimethoprim-sulfamethoxazole, amikacin, gentamicin, and imipenem. 9% has shown resistance to polymyxin- B. Its ability to grow on different medical devices makes it a successful pathogen to cause serious infections and also it has the capability to form a biofilm. Biofilm is a scavenging system that helps in trapping and concentrating all essential requirements from the environment. It also provides an ideal environment for horizontal gene transfer, including antibiotic resistant genes. Polymyxin B is one agent which is active against *A. baumannii*.

2.10 M. E. FALAGAS, E. A. KARVELI, I. I. SIEMPOS AND K. Z. VARDAKAS (2007)

Acinetobacter are non- fermentative, strictly aerobic, Gram- negative bacteria that have a minimal nutritional requirement. It recent years they have emerged as a successful nosocomial pathogen. Patients in ICU are mostly infected with this. Prolong stay in ICU is associated with increased rate of mortality and morbidity. It has been noticed that in last two decades clinicians from different countries have witnessed an increasing number of critically ill patients who suffer from an infection caused by *Acinetobacter spp.*; in most of the cases, the etiological agent associated with these infections is *A. baumannii*. It was found to be the common cause of ICU- acquired pneumonia and bacteremia. However, A. baumannii was also attributed to UTI and wound infections in ICU patients. Isolates of *Acinetobacter* are susceptible (*in vitro*) to piperacillin/ tazobactam, aminoglycosides, third generation cephalosporins, quinolones, and imipenem. Some studies have reported that sulbactam, meropenem, and polymyxin have shown sensitivity. A group of studies in Europe has come to the conclusion that 90% Acinetobacter isolates from critically ill patients were resistant to aminoglycosides. In Asia and Europe, the threat of Acinetobacter infections is increasing at a high rate.

CHAPTER- 3

AIM AND OBJECTIVES

3.1 AIM

To determine the emergence and antibiotic sensitivity pattern of *Acinetobacter baumannii* in a hospital facility.

3.2 OBJECTIVES

3.2.1 GENERAL OBJECTIVES

- To estimate the burden of *Acinetobacter baumannii* infection in patients admitted to hospital
- To evaluate the effect of antibiotics on *Acinetobacter baumannii* isolates.

3.2.2 SPECIFIC OBJECTIVES

- To isolate and identify *Acinetobacter baumannii* from clinical specimens.
- To study the distribution of infection caused by *Acinetobacter baumannii* in different age groups.
- To evaluate the effect of antibiotics on *Acinetobacter baumannii* isolates.

CHAPTER- 4

MATERIAL AND METHOD

4.1 MATERIAL

A list of material used during the study is mentioned in appendix- I

4.2 METHOD

A study was conducted from 1st January 2016 to 30th April 2016 in a tertiary care hospital of Punjab to find the burden of Acinetobacter baumannii in hospital acquired infections. The susceptibility of the organism to various drugs and the infections they are mostly associated with was recorded between that time of period.

4.2.1 SPECIMEN RECEIVED

Following specimens were received and they were collected in a sterile container by maintaining universal precautions

- Endo-tracheal secretions (ET secretions)
- Tracheal secretions (TT secretions)
- Bronchioalveolar lavage (BAL)
- Sputum
- Pus swab
- Endo-tracheal tip (ETT tip)
- Suction tip
- Oral secretions
- Bronchial aspirate
- Blood
- Urine
- Tissues
- Body fluids (CSF, pleural fluid, ascitic fluid)

4.2.2 SPECIMEN PROCESSING

The specimens are checked with the details mentioned on the container and in requisition form. Patient's name, age, sex, and type of specimens are checked before processing of the specimen.

- **Culture of specimen-** According to the specimens, they were inoculated on the culture plates. The specimens were inoculated on three solid (agar) medium and one liquid (broth) medium (except sputum).

Name of media	Media preparation
Solid (Agar) medium	
MacConkey agar (MA)	51.53 grams of MacConkey agar w/o CV with 0.15% bile salts provided by HiMideia Laboratories Pvt. Ltd. is dissolved in 1000 ml of distilled water. Set the pH at 7.1 +/- 0.2. Sterilize by autoclaving at 15 lbs pressure and 121˚C for 15 min. Cool to 45- 50˚C. Pour 20 ml of media to each Petri plate and allow to solidify.
Blood agar (BA)	Provided by bioMérieux
Chocolate agar (CA)	Provided by bioMérieux
Liquid (Broth) medium	
Fluid Thioglycollate medium (TG)	29.75 grams of Fluid Thioglycollate medium provided by HiMideia Laboratories Pvt. Ltd. is dissolved in 1000 ml of distilled water. Set the pH at 7.1 +/- 0.2. Sterilize by autoclaving at 15 lbs pressure and 121˚C for 15 min. Cool to 25˚C. 2-3 ml is poured into each sterile tube.

- **Inoculation of specimen-** Specimens were inoculated by Hi- FlexiLoop provided by HiMideia Laboratories Pvt. Ltd. having 4.4 mm diameter. 0.01 ml of the specimen was inoculated on the four media. Streak plate method was obtained to inoculate the specimen.

- **Incubation of plates-** Plates were incubated at 37˚C in an incubator for 24- 48 hours.

4.2.3 ISOLATION AND IDENTIFICATION OF ORGANISM

After incubation of 24-48 hours culture plates were examined for bacterial growth and identification and antibiotic sensitivity test was made by Vitek2 compact.

4.2.3.1 IDENTIFICATION WITH VITEK2 COMPACT

Vitek2 compact is an automated microbiology system which performs the identification and antibiotic sensitivity testing.

- **Principle**

  Vitek2 compact system identifies an organism by using an optical signal that has been generated by individual biochemical reactions contained within a set of reagent cards (micro identification cards). After inoculation the cards with the suspension of unknown organism they are incubated and read by the instruments internal optics. Through comparison of results with the database it completes the organism identification.

The method of antibiotic sensitivity testing performed by Vitek2 compact is based on the MIC (minimal inhibitory concentration) technique reported by MacLowry, Marsh, and Gerlach.

- ## Reagent cards

The reagent cards consist barcodes that have information about product type, lot number, date of expiry and a unique identifier. These cards have 64 wells; each contains an individual test substrate which measures metabolic activities such as acidification, alkalinization, enzyme hydrolysis and growth in the presence of inhibitory substances. On the both sides, an optically clear film is present that allows oxygen transmission and maintain a sealed vessel that prevents contact with the organism- substrate admixtures. A pre- inserted transfer tube is fixed to each card to inoculate the card with microbial suspension.

Recently five types of identification card are available for different organism classes-

1. GN - Gram-negative fermenting and nonfermenting bacilli
2. GP - Gram-positive cocci and non-spore-forming bacilli
3. YST - Yeasts and yeast-like organisms

Five types of antibiotic sensitivity cards are available for different organism classes-

1. AST- N280- Lactose fermenting Gram- negative bacteria
2. AST- N281- Non- lactose fermenting Gram- negative bacteria
3. AST- P628- Gram- positive bacteria (except *Streptococcus spp*.)
4. AST- ST01- *Streptococcus spp*.
5. AST- YS 07- Yeasts and yeast-like organisms

- ## Substrates for GN card [Vitek2 compact]

Table- 1: Substrate details of GN card

Well	Test	Mnemonic	Amount/ well (mg)
2	Ala-Phe-Pro-ARYLAMIDASE	APPA	0.0384
3	ADONITOL	ADO	0.1875
4	L- Pyrrolydonyl- ARYLAMIDASE	PyrA	0.018
5	L- ARABITOL	IARL	0.3
7	D- CELLOBIOSE	dCEL	0.3
9	BETA- GALACTOSIDASE	BGAL	0.036
10	H_2S PRODUCTION	H_2S	0.0024
11	BETA-N-ACETYL- GLUCOSAMINIDASE	BNAG	0.0408
12	Glutamyl Arylamidase pNA	AGLTp	0.0324
13	D- GLUCOSE	dGLU	0.3
14	GAMMA- GLUTAMYL- TRANSFERASE	GGT	0.228
15	FERMENTATION/ GLUCOSE	OFF	0.45
17	BETA- GLUCOSIDASE	BGLU	0.036
18	D- MALTOSE	dMAL	0.3
19	D-MANNITOL	dMAN	0.1875
20	D- MANNOSE	dMNE	0.3
21	BETA- XYLOSIDASE	BXYL	0.0324
22	BETA- Alanine arylamidase pNA	BAlap	0.174

23	L- Proline ARYLAMIDASE	ProA	0.0234
26	LIPASE	LIP	0.0192
27	PALATINOSE	PLE	0.3
29	Tyrosine ARYLAMIDASE	TyrA	0.0276
31	UREASE	URE	0.15
32	D-SORBITOL	dSOR	0.1875
33	SACCHAROSE/ SUCROSE	SAC	0.3
34	D- TAGATOSE	dTAG	0.3
35	D- TREHALOSE	dTRE	0.3
36	CITRATE (SODIUM)	CIT	0.054
37	MALONATE	MNT	0.15
39	5- KETO- D- GLUCONATE	5KG	0.3
40	L- LACTATE alkalinization	ILATk	0.15
41	ALPHA- GLUCOSIDASE	AGLU	0.036
42	SUCCINATE alkalinisation	SUCT	0.15
43	Beta-N-ACETYL-GALACTOSAMINIDASE	NAGA	0.0306
44	ALPHA- GALACTOSIDASE	AGAL	0.036
45	PHOPHATASE	PHOS	0.0504
46	Glycine ARYLAMIDASE	GlyA	0.012
47	ORNITHINE DECARBOXYLASE	ODC	0.3
48	LYSINE DECARBOXYLASE	LDC	0.15
52	DECARBOXYLASE BASE	0DEC	NA
53	L- HISTIDINE assimilation	IHISa	0.087
56	COUMARATE	CMT	0.126
57	BETA- GLUCORONIDASE	BGUR	0.0378
58	O/ 129 RESISTANCE (comp. vibrio.)	O129R	0.0105
59	Glu-Gly- Arg- ARYLAMIDASE	GGAA	0.0576
61	L- MALATE assimilation	IMLTa	0.042
62	ELLMAN	ELLM	0.03
64	L- LACTATE assimilation	ILATa	0.186

- **Preparation of suspension**

  Through a sterile applicator stick required amount to pure growth was transferred to 3 ml sterile saline (0.45%- 0.50% aqueous NaCL, pH- 4.5-7.0) in a sterile test tube made of polystyrene (marked with ID). Colonies were emulsified well and mixed by vortex mixture. The turbidity was adjusted to 0.5- 0.63 McFarland turbidity range and was measured by using a turbidity meter called DensiChek™ plus. 145 µl of this suspension was transferred to another tube containing 3 ml of sterile saline (marked with AST).

- **Inoculation**

  The suspension tubes were placed in cassettes and GN card was inserted into the first tube that has marked with ID and the AST-281 card was inserted into the second tube that has marked with AST. These filled cassettes then load manually into the vacuum chamber station of the instrument. After the vacuum is applied and the air is re-introduced into the station, the organism suspension is forced through the transfer tube into micro-channels that fill all the test wells.

- **Incubation**

  After loading of suspension into the wells, the transfer tubes were cut and the cards were sealed by the machine itself. The cards then loaded into the carousel incubator where online incubation was given (35.5 +/- 1.0˚C). Each card was removed from the incubator once every 15 mins and transported to an optical system for reaction readings and then returned to the incubator until the next read time.

  When the identification and sensitivity test has completed a print out of the result was taken and examined.

CHAPTER- 5

RESULT

The study was conducted in SRL LTD. Fortis Escort Hospital, Amritsar from 1st January 2016 to 30th April 2016. Specimens were collected and processed by obtaining universal precautions and standard microbiological techniques.
In this study, a total of 2582 specimens were collected and processed for identification and sensitivity testing. Specimens of all age group (2 days- 93 years) and both sexes were processed for identification of *A. baumannii* and antibiotic sensitivity testing. The identification was done by Vitek2 compact [Table-2]

Table- 2: Biochemical details of *A. baumannii*

Well	Test	Mnemonic	Test result
2	Ala-Phe-Pro-ARYLAMIDASE	APPA	-ve
3	ADONITOL	ADO	-ve
4	L- Pyrrolydonyl- ARYLAMIDASE	PyrA	-ve
5	L- ARABITOL	IARL	-ve
7	D- CELLOBIOSE	dCEL	+ve
9	BETA- GALACTOSIDASE	BGAL	-ve
10	H_2S PRODUCTION	H_2S	-ve
11	BETA-N-ACETYL- GLUCOSAMINIDASE	BNAG	-ve
12	Glutamyl Arylamidase pNA	AGLTp	-ve
13	D- GLUCOSE	dGLU	+ve
14	GAMMA- GLUTAMYL- TRANSFERASE	GGT	-ve
15	FERMENTATION/ GLUCOSE	OFF	-ve
17	BETA- GLUCOSIDASE	BGLU	-ve
18	D- MALTOSE	dMAL	-ve
19	D-MANNITOL	dMAN	-ve
20	D- MANNOSE	dMNE	+ve
21	BETA- XYLOSIDASE	BXYL	-ve
22	BETA- Alanine arylamidase pNA	BAlap	-ve
23	L- Proline ARYLAMIDASE	ProA	-ve
26	LIPASE	LIP	-ve
27	PALATINOSE	PLE	-ve
29	Tyrosine ARYLAMIDASE	TyrA	+ve
31	UREASE	URE	-ve
32	D-SORBITOL	dSOR	-ve
33	SACCHAROSE/ SUCROSE	SAC	-ve
34	D- TAGATOSE	dTAG	-ve
35	D- TREHALOSE	dTRE	-ve
36	CITRATE (SODIUM)	CIT	+ve
37	MALONATE	MNT	+ve
39	5- KETO- D- GLUCONATE	5KG	-ve
40	L- LACTATE alkalinization	ILATk	+ve
41	ALPHA- GLUCOSIDASE	AGLU	-ve
42	SUCCINATE alkalinisation	SUCT	+ve

43	Beta-N-ACETYL-GALACTOSAMINIDASE	NAGA	-ve
44	ALPHA- GALACTOSIDASE	AGAL	-ve
45	PHOPHATASE	PHOS	-ve
46	Glycine ARYLAMIDASE	GlyA	-ve
47	ORNITHINE DECARBOXYLASE	ODC	-ve
48	LYSINE DECARBOXYLASE	LDC	-ve
53	L- HISTIDINE assimilation	IHISa	+ve
56	COUMARATE	CMT	+ve
57	BETA- GLUCORONIDASE	BGUR	-ve
58	O/ 129 RESISTANCE (comp. vibrio.)	O129R	+ve
59	Glu-Gly- Arg- ARYLAMIDASE	GGAA	-ve
61	L- MALATE assimilation	IMLTa	-ve
62	ELLMAN	ELLM	-ve
64	L- LACTATE assimilation	ILATa	+ve

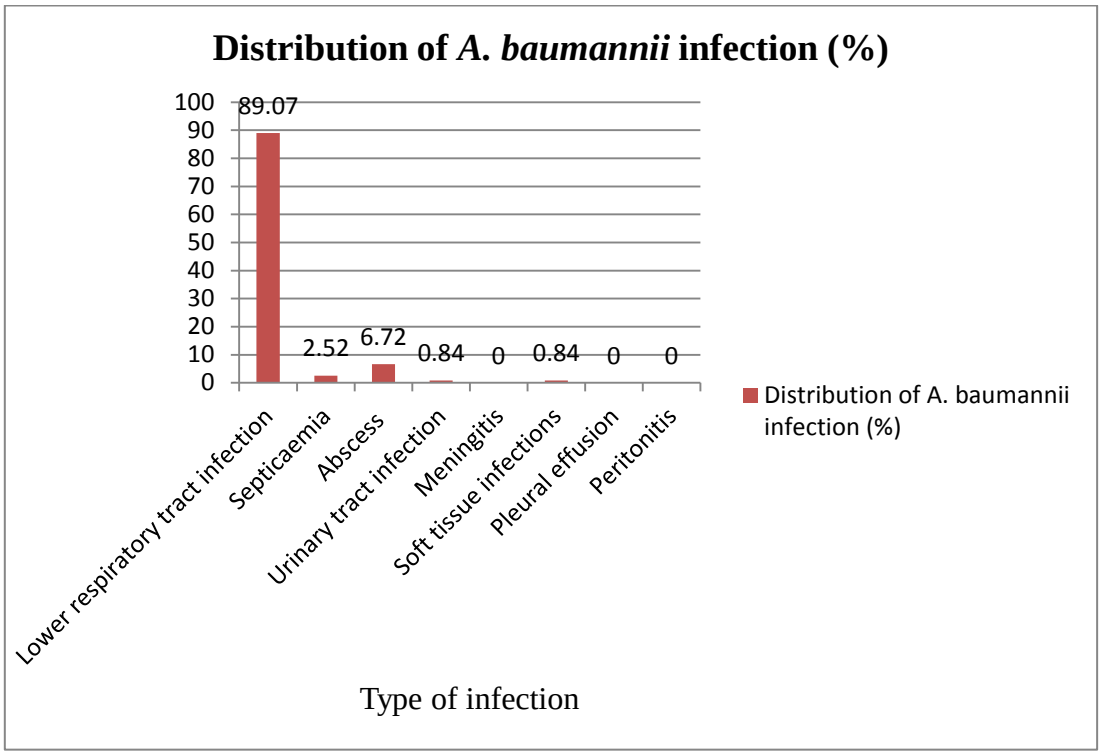
5.1 DISTRIBUTION OF *A. BAUMANNII* INFECTIONS

A total of 119 isolates (4.60%) of *A. baumannii* were obtained from 2582 clinical specimens including BAL, blood, body fluids, bronchial wash, E.T. secretion, E.T.T tip, oral secretions, pus, pus- swab, sputum, suction tip, tracheal secretion, and urine. The most common infection *A. baumannii* was found as lower respiratory tract infection (89.07%) followed by abscess (6.72%), septicaemia (2.52%), urinary tract infections (0.84%), and soft tissue infections (0.84%) [Table- 3, Chart- 1].

Table- 3: Distribution of *A. baumannii* infections

Type of infection	**Type of sample**	**Total no. of sample**	**Positive cases**	
			Total number	**Percentage (%)**
Respiratory tract infection	E.T. secretion, tracheal secretion, BAL, E.T.T. tip, sputum, bronchial aspirate, suction tip	445	106	89.07
Septicaemia	Blood	968	3	2.52
Abscess	Pus	279	8	6.72
Urinary tract infection	Urine	820	1	0.84
Meningitis	CSF	6	0	0.00
Soft tissue infections	tissue	24	1	0.84
Pleural effusion	Pleural fluid	19	0	0.00
Peritonitis	Ascitic fluid	21	0	0.00
TOTAL		2582	119	4.60

Chart-1: Distribution of *A. baumannii* infections



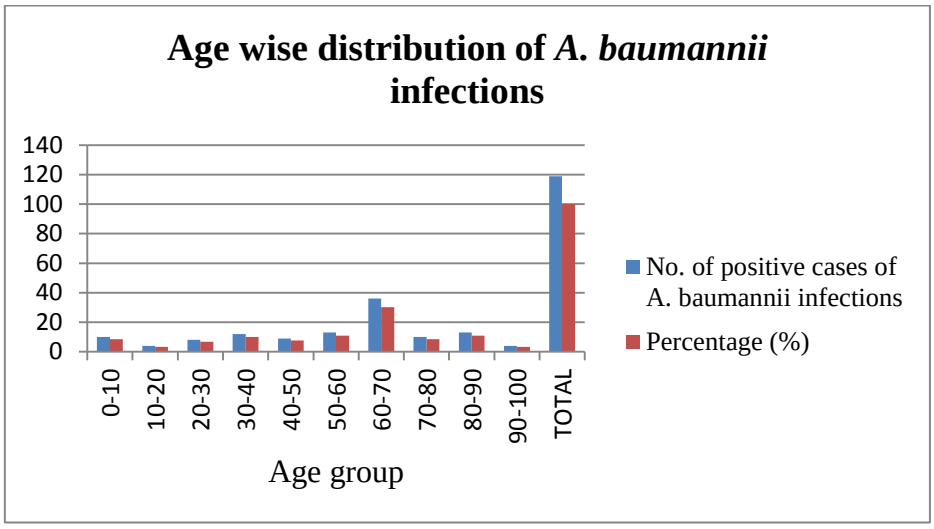
5.2 DISTRIBUTION OF *A. BAUMANNII* ACCORDING TO AGE AND GENDER

The age group of 60-70 year was most affected with *A. baumannii* infections (36, 30.25%) followed by 50-60 year (13, 10.92%), 80-90 year (13, 10.92%) 30-40 year(12, 10.08%), 0-10 year (10, 8.40%), 70-80 year (10, 8.40%), 40-50 year (9, 7.56%), 20-30 year (8, 6.72), 10-20 year (4, 3.36%), and 90-100 year (4, 3.36%) [Table-4, Chart-2].

Tabel-4: Age wise distribution of *A. baumannii* infections

Age group (year)	No. of positive cases of *A. baumannii* infections	Percentage (%)
0-10	10	8.40
10-20	4	3.36
20-30	8	6.72
30-40	12	10.08
40-50	9	7.56
50-60	13	10.92
60-70	36	30.25
70-80	10	8.40
80-90	13	10.92
90-100	4	3.36
TOTAL	119	100

Chart- 2: Age wise distribution of *A. baumannii* infections

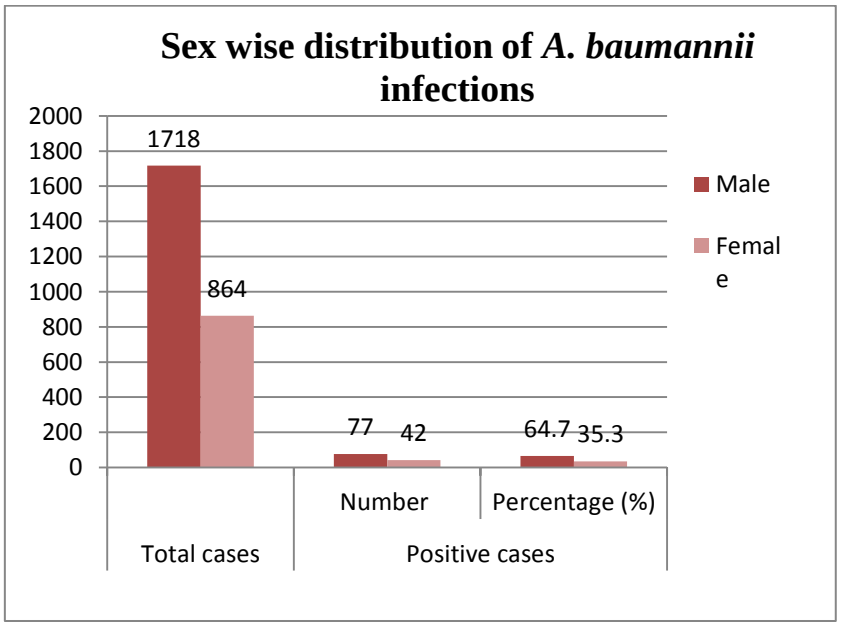


Out of 2582 specimens processed 1718 (66.54%) were from male with positive A. baumannii infections 77 (64.70%) and 864 (33.46%) specimens were from female with 42 (35.30%) positive *A. baumannii* infections. Males (64.70%) were more commonly infected with *A. baumannii* than females (35.30%) [Table- 5, Chart- 3].

Tabel-5: Sex wise distribution of *A. baumannii* infections

Gender	Total cases	Positive cases	
		Number	Percentage (%)
Male	1718	77	64.70
Female	864	42	35.30
Total	2582	119	100

Chart- 3: Sex wise distribution of *A. baumannii* infections



5.3 EFFECT OF ANTIBIOTICS ON *A. BAUMANNII* ISOLATES

In this study, the following drugs were used against *A. baumannii*

- Amikacin (AK)
- Cotrimoxazole (COT)
- Tigecycline (TGC)
- Levofloxacin (LEV)
- Ciprofloxacin (CIP)
- Colistin (CL)
- Meropenem (MER)
- Imipenem (IMI)
- Aztreonam (AZT)
- Cefoperazone- Sulbactam (CFS)
- Piperacillin- tazobactam (P/T)
- Cefepime (CPM)
- Ceftazidime (CAZ)
- Ticarcillin- clavulanic acid (TIC)
- Doripenem (DOR)
- Minocycline (MIN)
- Gentamicin (GEN)

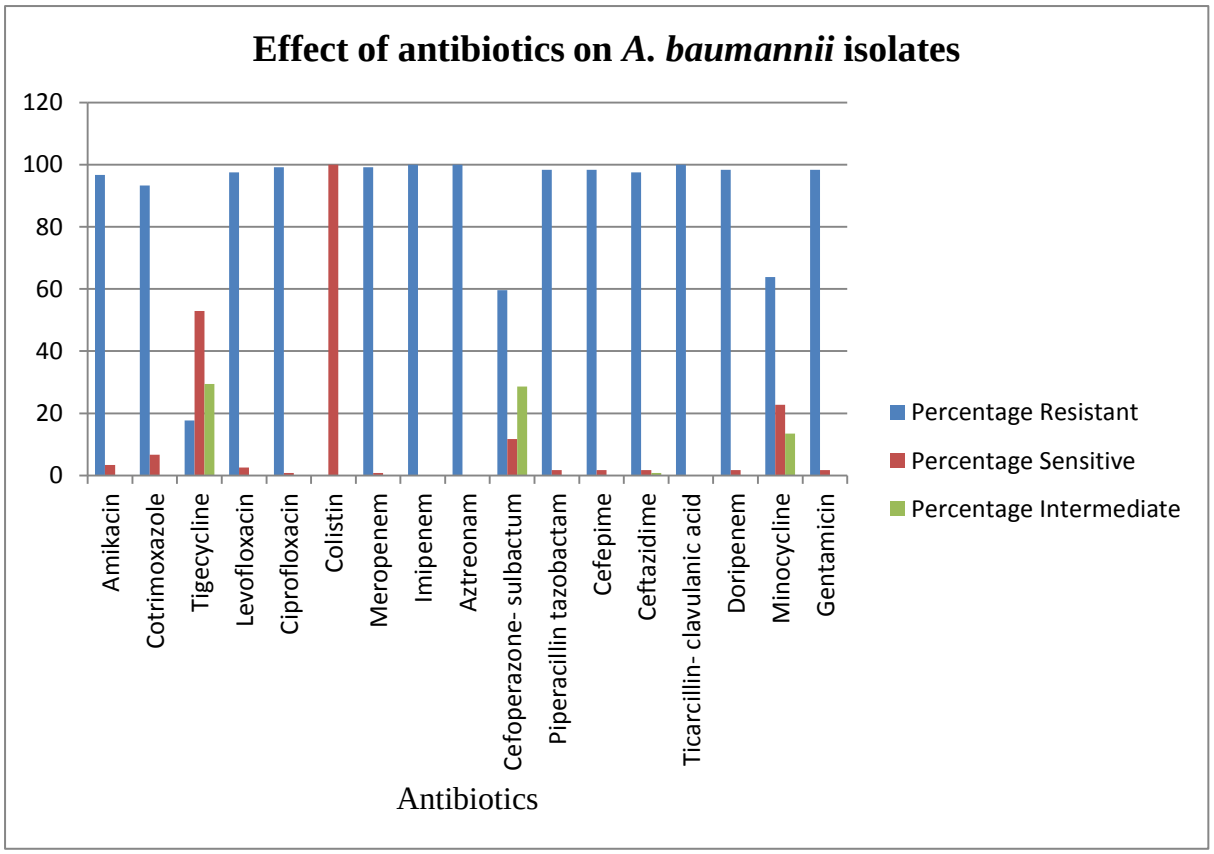
The maximum sensitivity of *A. baumannii* isolates were seen to CL (119, 100%), followed by TGC (63, 52.94%) and MIN (27, 22.69%) whereas the sensitivity to CFS was 11.8%. Isolates were appeared to be intermediately sensitive to TGC (35, 29.41%), CFS (34, 28.6%), MIN (16, 13.45%), and CAZ (1, 0.84%). The maximum resistant was observed for IMI, AZT and TIC (119, 100%), followed by CIP (118, 99.16%), MER (118, 99.16%), PT (117, 98.32%), CPM (117, 98.32%), DOR (117, 98.32%), GEN (117, 98.32%), and LEV (117, 97.48%) [Table-6, Chart-4].

Table-6: Effect of antibiotics on *A. baumannii* isolates

Name of drug	Resistant		Sensitive		Intermediate	
	Number	%	Number	%	Number	%
Amikacin	115	96.64	4	3.36	0	0
Cotrimoxazole	111	93.3	8	6.7	0	0
Tigecycline	21	17.65	63	52.94	35	29.41
Levofloxacin	116	97.48	3	2.52	0	0
Ciprofloxacin	118	99.16	1	0.84	0	0
Colistin	0	0	119	100	0	0
Meropenem	118	99.16	1	0.84	0	0
Imipenem	119	100	0	0	0	0
Aztreonam	119	100	0	0	0	0
Cefoperazone-Sulbactum	71	59.6	14	11.8	34	28.6
Piperacillin-tazobactam	117	98.32	2	1.68	0	0
Cefepime	117	98.32	2	1.68	0	0
Ceftazidime	116	97.48	2	1.68	1	0.84

Ticarcillin-clavulanic acid	119	100	0	0	0	0
Doripenem	117	98.32	2	1.68	0	0
Minocycline	76	63.86	27	22.69	16	13.45
Gentamicin	117	98.32	2	1.68	0	0

Chart- 4: Effect of antibiotics on *A. baumannii* isolates



CHAPTER- 6

DISCUSSION

Acinetobacter baumannii is Gram- negative coccobacillus that has emerged as a successful nosocomial pathogen in past two decades. Its ability to resist drug, minimal nutrition requirements and ubiquitous distribution in nature made it a superbug. They are ubiquitous inhabitants of soil, water, and sewage environments. It is a commensal flora of human and animal skin that eases its transmission to critically ill patients in a hospital facility. Advance facilities in treatment and use of broad- spectrum antibiotic therapy increases the rate of morbidity and mortality of *Acinetobacter* infections. *Acinetobacter* adheres to surfaces and survives for a long time on surfaces of inanimate substances which play a vital role in their pathogenesis. They are a strong producer of biofilm which helps them in trapping and concentrating all essential requirements from the environment and also provide a gate for horizontal gene transfer. Their ability of rapid acquiring genes that encoded drug resistant property has made them a potent pathogen in a hospital environment. Biofilm prevents bacteria from host immune defence and also diminish the action of antibiotics. The ratio of multi- drug resistant *A. baumannii* is increased, particularly in ICU patients (64). In last two decades, the incidence of *Acinetobacter* infections has increased at a high rate that made them be ranked as 2nd most common nosocomial pathogen found in all clinical specimens after *P. aeruginosa* and 4th according to the frequency of infections after *P. aeruginosa*, *S. aureus*, and *K. pneumoniae* (65).

In this study, a total of 119 (4.60%) *A. baumannii* isolates were isolated by using Vitek2 compact from a total of 2582 processed clinical specimens including lower respiratory samples, blood, urine, body fluids, and tissues. Respiratory tract infections (89.07%) were most common infection observed followed by abscess (6.72%), septicaemia (2.52%), urinary tract infections (0.84%), and soft tissue infections (0.84%) [Table- 3]. The reason for the respiratory infections to be more frequent is may be because of the use of mechanical ventilators and intubations. Tripathi et al reported a total number of 107 (1.02%) *Acinetobacter* isolates from all processed clinical specimens and Rasha .J .Mal- Warid and Azhar A. L. - Thahab reported 11 (2.40%) isolates of *A. baumannii* from a total of 458 clinical samples which is well comparable with our study.

In the present study, *A. baumannii* infections were common in males (64.70%) as compared with females (35.30%). A more frequent visit to the hospitals might be the possible cause of infections for the males (36).

The notorious nature of *A. baumannii* to acquire resistant genes are accounting for drug resistance to most common antibiotics. This has created a major challenge in patient safety. *Acinetobacter spp.* is universally resistant to penicillin, ampicillin, and cephalothin. (36). *Acinetobacter* resists mostly all the classes of drug including β- lactams, aminoglycosides, quinolones, and tetracyclines. Through the production of enzymes (β- lactamases), changes in their OMPs, using of multidrug efflux pumps and alteration in the affinity or expression of PBPs *Acinetobacter spp.* resist β- lactams and carbapenems. They produce enzymes that modify aminoglycosides and through mutation in the genes (*gyrA* and *parC*) they modify DNA gyrase which in turn cause resistant. They implicate many efflux systems e.g. chromosomally encoded multidrug efflux systems (AdeABC), MATE pump AdeM, Tet (A), and Tet (B) to eliminate the drug.

In this study IMI, AZT and TIC has shown maximum resistant (100%) followed by CIP (99.16%), MER (99.16%), PT (98.32%), CPM (98.32%), DOR (98.32%), GEN (98.32%), and LEV (97.48%). Colistin was the most sensitive drug (100%) obtained. Sensitivity to TGC and MIN was 52.94% and 22.69% respectively. Isolates were appeared to be intermediately sensitive to TGC (29.41%), CFS (28.6%), MIN (13.45%), and CAZ (0.84%). Development of new therapies, well- managed clinical trials of existing antibiotics for combination therapy, prevention of transmission of the hospital- associated infections, hospital hygiene are essential to control *Acinetobacter* infections.

CHAPTER- 7

CONCLUSION

The Gram- negative coccobacillus, Acinetobacter baumannii poses a formidable threat to patients. It has emerged as a superbug in hospital environment particularly in ICU units. They account for most of the hospital- associated infections e.g. lower respiratory tract infections, hospital- associated pneumonia, bloodstream infections, abscess, soft tissue infections, urinary tract infections etc. The chances of *A. baumannii* infections increase in the presence of iatrogenic factors like inadequate long- term antibiotic therapy and new interventions in a medical facility. The notorious nature of acquiring genes made them multi-drug resistant organism which has limited treatment options. Colistin and Tigecycline are the choice of drug for *A. baumannii* according to our study. Early identification of organism is necessary for proper treatment and it also prevents the iatrogenic effects. To control the burden of *Acinetobacter* infections new therapies such as combine therapy must be obtained and followed with proper dose as recommend by physicians; along with awareness of the importance of this infection should be implicated. Proper sanitation, good housekeeping, sterilization of equipment, hand hygiene, water purification, isolation procedures and maintaining of the hospital environment, use of infection control practices are some of the measures to control the transmission of *Acinetobacter spp.* among hospital personnel.

CHAPTER- 8

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APPENDIX

Appendix- I

LIST OF ABBREVIATIONS

%	Percentage
˚C	Degree Centigrade
+ve	Positive
-ve	Negative
5KG	5- KETO- D- GLUCONATE
A. baumannii	*Acinetobacter baumannii*
ADO	ADONITOL
AGAL	ALPHA- GALACTOSIDASE
AGLTp	Glutamyl Arylamidase pNA
AGLU	ALPHA- GLUCOSIDASE
AIDS	Acquired immune- deficiency syndrome
AK	Amikacin
APPA	Ala-Phe-Pro-ARYLAMIDASE
AST	Antibiotic sensitivity testing
AZT	Aztreonam
BA	Blood agar
BAL	Bronchioalveolar lavage
BAlap	BETA- Alanine arylamidase pNA
BGAL	BETA- GALACTOSIDASE
BGLU	BETA- GLUCOSIDASE
BGUR	BETA- GLUCORONIDASE
BNAG	BETA-N-ACETYL- GLUCOSAMINIDASE
BXYL	BETA- XYLOSIDASE
CA	Chocolate agar
CAZ	Ceftazidime
CDC	Centers for Disease Control and Prevention
CFS	Cefoperazone- Sulbactam
CIP	Ciprofloxacin
CIT	CITRATE (SODIUM)
CL	Colistin
CMT	COUMARATE
CMV	Cytomegalovirus
COT	Cotrimoxazole
CPM	Cefepime
CSF	Cerebrospinal fluid
CV	Crystal violet
dCEL	D- CELLOBIOSE
dGLU	D- GLUCOSE
dMAL	D- MALTOSE
dMAN	D-MANNITOL
dMNE	D- MANNOSE
DNA	Deoxyribonucleic acid
DOR	Doripenem
dSOR	D-SORBITOL

dTAG	D- TAGATOSE
dTRE	D- TREHALOSE
E. coli	*Escherichia coli*
EBV	Epstein- Barr virus
ELLM	ELLMAN
ET	Endo- tracheal
ETT	Endo- tracheal tract
GEN	Gentamicin
GGAA	Glu-Gly- Arg- ARYLAMIDASE
GGT	GAMMA- GLUTAMYL- TRANSFERASE
GlyA	Glycine ARYLAMIDASE
GN	Gram negative
GNB	Gram- negative bacteria
GNCB	Gram- negative coccobacilli
GP	Gram positive
GPB	Gram- positive bacteria
HAI	Hospital- acquired infection
HBV	Hepatitis- B virus
HCV	Hepatitis- C virus
HIV	Human immunodeficiency virus
HSV	Herpes simplex virus
H_2S	H_2S PRODUCTION
IARL	L- ARABITOL
ICU	Intensive Care Unit
ID	Identification
IHISa	L- HISTIDINE assimilation
ILATa	L- LACTATE assimilation
ILATk	L- LACTATE alkalinization
IMLTa	L- MALATE assimilation
IMI	Imipenem
K. pneumoniae	*Klebsiella pneumoniae*
Lbs	
LDC	LYSINE DECARBOXYLASE
LEV	Levofloxacin
LIP	LIPASE
MA	MacConeky agar
MDR	Multi-drug resistant
MER	Meropenem
min	Minute
MIN	Minocyclin
MNT	MALONATE
mg	Milligram
ml	Millilitre
NaCL	Sodium chloride
NAGA	Beta-N-ACETYL- GALACTOSAMINIDASE
NNIS	National Nosocomial Infections Surveillance
O129R	O/ 129 RESISTANCE (comp. vibrio.)
ODC	ORNITHINE DECARBOXYLASE
OFF	FERMENTATION/ GLUCOSE
OMP	Outer membrane protein

P. aeruginosa	*Pseudomonas aeruginosa*
PBP	Penicillin- binding protein
PHOS	PHOPHATASE
PLE	PALATINOSE
ProA	L- Proline ARYLAMIDASE
PyrA	L- Pyrrolydonyl- ARYLAMIDASE
P/T	Piperacillin- tazobactam
RSV	Respiratory syncytial virus
S. aureus	*Staphylococcus aureus*
SAC	SACCHAROSE/ SUCROSE
spp.	Species
SUCT	SUCCINATE alkalinisation
TIC	Ticarcillin- clavulanic acid
TG	Thioglycollate
TGC	Tigecycline
TT	Tracheal tract
TyrA	Tyrosine ARYLAMIDASE
URE	UREASE
UTI	Urinary tract infection
VAP	Ventilator associated pneumonia
VZV	Varicella- zoster virus
WHO	World Health Organisation
YST	Yeast

Appendix- II

LIST OF MATERIALS

1. ECUIPMENT

- Autoclave
- Hot air oven
- Bio- safety cabinet- II
- Incubator
- Microscope
- Refrigerator
- Vitek2 compact
- DensiChek™ plus
- Dispensette®
- GN card
- AST- N281 card
- Electronic weighing balance

2. GLASS WARES AND PLASTIC WARES

- Petri plates
- Tubes
- Glass slides
- Glass rods
- Conical flask

3. OTHERS

- Cotton
- Scissors
- Hi- FlexiLoop
- Cassettes
- Racks
- Face mask
- Gloves
- Spatula
- Forceps