Prevalence of Multi-drug Resistant *Acinetobacter baumannii* in Hospitalized Patients

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بسم الله الرحمن الرحيم

Dedication

To

MY GREAT AND BELOVED PARENTS

Whatever I am today could never become true without
their guidance, efforts and prayers.

MY DEAR BROTHERS

&

MY SWEET SISTER

Declaration

The material and information contained in this thesis is my original work. I have not previously presented any part of this work elsewhere for any other degree.

Shahzeera Begum

Certificate

This thesis submitted by ***Shahzeera Begum*** is accepted in its present form by the Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan; as satisfying the thesis requirements for the degree of Master of Philosophy in Microbiology.

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List of Abbreviations

MDR	Multi-drug resistant
HAIs	Health-care-associated infections
CDC	Center for disease control and prevention
ESBLs	Extended-spectrum-beta-lactamases
β	Beta
UTIs	Urinary tract inections
MRSA	Methicillin-resistant *Staphylococcus aureus*
CLSI	Clinical Laboratory Standards Institute
%	Percent
n	Sample size
N	Population size
NIH	National Institute of Health
PIMS	Pakistan Institute of Medical Sciences
ATCC	American Type Culture Collection
MIC	Minimum Inhibitory Concentration
spp	species
MHA	Muller Hinton Agar
PBP	Penicillin-binding protien
PCR	Polymerase chain reaction
≥	Greater than
≤	Less than
mL	milliliter
g	gram
°C	Degree Celsius
M	Molar
µg	micro gram
A. baumannii	*Acinetobacter baumannii*
AMC	Amoxacillin-C.acid
PRL	Piperacillin
CAZ	Ceftazidime
MEM	Meropenem
CN	Gentamicin

AK	Amikacin
TE	Tetracycline
CIP	Ciprofloxacin
TGC	Tigecyclice
MI	Minocycline
IPM	Imipenem
CTX	Cefotaxime
DO	Doxcycline
PB	Polymyxin B
TOB	Tobramycin
SAM	Ampicillin-sulbactam
TZP	Pipercillin-tazobactam
SXT	Tromrthoprim-sulfamethoxazole
PICU	Peads intensive care unit
PMW	Private medical ward
NICU	New natal intensive care unit
OPD	Out door patients
PWFF	Private ward first floor
PWSF	Private ward second floor
PWGF	Private ward ground floor
SW6	Surgical ward 6

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Shahzeera Begum

Abstract

ABSTRACT

The aim of study was to determine the prevalence of multi-drug resistant *Acinetobacter baumannii* and various resistant mechanisms in clinical samples of the patients, admitted in Pakistan Institute of Medical Scienes (PIMS). *A. baumannii* can result in a wide range of infections, including bacteremia, pneumonia, urinary tract infection, peritonitis, etc. This organism is becoming resistant to many and mostly all standard antibiotics. The reason for multi-drug resistance can be the production of extended spectrum β- lactamses(ESBLs), carbapenemases/ metallo β- lactamases or AmpC β-lactamases. A total of 91 isolates were obtained from PIMS during the period of February 2010 to December 2010. These isolates were identified to specie level by biochemical 20E kit, at Microbiology Laboratory, Pathology Department, Pakistan Institute of Medical Sciences (PIMS), Islamabad. The antibiotic susceptibility testing was performed by standard disc diffusion method as recommended by CLSI. The prevalence of MDRs was100% in *A. baumannii*. The antibiotic susceptibility profile showed that minocycline and tigecycline were the most effective drug against *A.baumannii*. Combination disc method, Modified Hodge test, EDTA disc synergy test and AmpC disc test were performed for detection of extended spectrum β-lactamases (ESBLs), Carbapenemases, Metallo β-lactamases, and AmpC β-lactamases respectvely. Carbapenemases/metallo β-lactamases production were 100% in *A. baumannii* isolates. AmpC prevalence was observed in 41.76%, while ESBL production was 0% in *A. baumannii* isolates. All β-lactamases were found to be more common in new born babies in new natal intensive care unit. All β-lactamases production represents clinical threat due to their resistance to the therapeutic inhibitors. Use of combined therapy may help in the treatment of infections due to resistant organisms. Predisposing factors that make patients susceptible to these infections include concurrent infections, prosthetic devices, surgery, administration of broad-spectrum antibiotics, and emergence of multidrug resistant pathogens most included *A. baumannii*. It is now recognized that *Acinetobacter spp.* plays a significant role in the colonization and infection of patients admitted to hospitals. Introduction of new antimicrobial agents is required to meet the challenge. Increased frequency of Multi drug resistance needs for continuous surveillance to determine prevalence and evolution of these enzymes in Pakistan.

Introduction

Hospital-acquired infections are a major challenge to patient safety. It is estimated that, a total of 1.7 million hospital-acquired infections occurred (4.5 per 100 admissions), and almost 99,000 deaths were associated with a hospital-acquired infection, making hospital-acquired infections the sixth leading cause of death in the United States Hospital-acquired infections are most commonly associated with invasive medical devices or surgical procedures (Klevens *et al.*, 2007). Lower respiratory tract and bloodstream infections are the most lethal; however, urinary tract infections are the most common (Kung *et al.*, 2008). Recent data from the U.S. National Healthcare Safety Network indicate that gram-negative bacteria are responsible for more than 30% of hospital-acquired infections, and these bacteria predominate in cases of ventilator-associated pneumonia (47%) and urinary tract infections (45%). In intensive care units (ICUs) in the United States, gram-negative bacteria account for about 70% of these types of infections, and similar data are reported from other parts of the world (Hidron *et al.*, 2008).

A range of gram-negative organisms are responsible for hospital-acquired infections, the Enterobacteriaceae family being the most commonly identified group overall. Unfortunately, multidrug-resistant organisms, including *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and extended-spectrum β-lactamase (ESBL)-producing or carbapenemase-producing Enterobacteriaceae, are increasingly being reported worldwide (Gaynes and Edwards, 2005).

Certain risk factors have been associated with nosocomial infections due to the identification of resistant organisms. In adult patients underlying disease and severity of illness, inter-institutional transfer of patients, prolonged hospitalization, gastrointestinal surgery and transplantation, as well as exposure to invasive devices of all types, and exposure to prior antimicrobials have been associated with nosocomial infection due to methicillin-resistant *Staphyloccocus aureus* (MRSA), vancomycin-resistant *enterococcus* (VRE), Gram-negative *bacilli*, *Clostridium difficile (C. difficile)* and *Candida* (Eric *et al.*, 2010). The bacteria that commonly cause nosocomial infections include *Staphylococcus aureus*, *Streptococcus* spp, *Bacillus cereus*, *Acinetobacter* spp, coagulase negative *staphylococci*, *enterococci*, *Pseudomonas aeruginosa*, *Legionella* and members of the *Enterobacteriaceae* family such as *Escherichia coli*, *Proteus mirabilis*, *Salmonella* spp, *Serratia marcescens* and

Klebsiella pneumonia. Predisposing factors that make patients susceptible to these infections include concurrent infections, prosthetic devices, surgery, immunosuppressive agents, and administration of broad-spectrum antibiotics and emergence of multidrug resistant pathogens. Other risk factors include age of patient, duration of hospitalization, underlying diseases like diabetes, tumors or overcrowding in the hospital wards (Chikere *et al.*, 2008).

Multiple mechanisms exist for ICU pathogens to acquire antibiotic resistance. These mechanisms include enzymatic inhibition of drugs, alteration of proteins targeted by antibiotics, changes in metabolic pathways, antibiotic efflux, and alterations in porin channels and changes of membrane permeability (Falagas *et al.*, 2007)

The control of hospital-acquired infection caused by multiply resistant gram-negative *bacilli* has proved to be a particular problem over the last 20 years in developed countries. An increasing incidence during the 1970s of resistant members of the family *Enterobacteriaceae* involved in nosocomial infections was followed by the therapeutic introduction of newer broad-spectrum antibiotics in hospitals and a subsequent increase in the importance of strictly aerobic gram-negative *bacilli*, including *Pseudomonas aeruginosa*, *Stenotrophomonas* (*Xanthomonas*) *maltophilia*, and *Acinetobacter* spp of these "newer" pathogens, it is now recognized that *Acinetobacter* spp plays a significant role in the colonization and infection of patients admitted to hospitals (Bergogn and Towner, 1996).

Global data reveal that antimicrobial resistance among *A. baumannii* species is increasing (Perez F *et al.*, 2007). Multidrug-resistant *A. baumannii* presently is emerging as a common hospital-and community- acquired infection that is difficult to treat. It is a very resistant and aggressive organism that infects patients with weakened defenses like ICU patients and those with invasive devices. It survives on wet and dry surfaces for long periods (Siegel, 2008). This organism has multiple mechanisms for resistance including an impermeable outer membrane, AmpC β-lactamases, class D OXA-type and class B metallo-β-lactamases which allow the organism to resist carbapenems, porin channels alterations as well as efflux pumps, and other genetic changes that may lead to resistance to fluoroquinolones (Maragakis and Perl, 2008). Species of *A. baumannii* that were resistant to all known commercially available antibiotics have been described (Doi Y *et al.*, 2009).

A. baumannii can result in a wide range of infections, including bacteremia, pneumonia, urinary tract infection, peritonitis, etc. Bacteremia is one of the most significant infections caused by *A. baumannii*, and is characteristically a nosocomial infection, particularly in intensive care units (ICUs) (Cisneros *et al.*, 2002).

The most prevalent mechanism of β-lactam resistance in *A. baumannii* is enzymatic degradation by β-lactamases. Inherent to all *A. baumannii* strains are chromosomally encoded AmpC cephalosporinases also known as *Acinetobacter*-derived cephalosporinases (ADCs). Unlike that of AmpC enzymes found in other gram-negative organisms, inducible AmpC expression does not occur in *A. baumannii*.

Extended-spectrum β-lactamases (ESBLs) from the Ambler class A group have also been described for *A. baumannii*, but assessment of their true prevalence is hindered by difficulties with laboratory detection, especially in the presence of an AmpC. More recent focus has been on VEB-1, which disseminated throughout hospitals in France (clonal dissemination) and was also recently reported from Belgium and Argentina (VEB-1a). Other ESBLs identified in *A. baumannii* include TEM-92 and -116 from Italy and The Netherlands, respectively, and SHV-12 from China and The Netherlands Also, CTX-M-2 and CTX-M-43 have been described from Japan and Bolivia, respectively. Narrow-spectrum β-lactamases, such as TEM-1 and TEM-2, are also prevalent in *A. baumannii*, but their current clinical significance is limited given the potency of other resistance determinant.

Of the β-lactamases, those with carbapenemase activity are most concerning and include the serine oxacillinases (Ambler class D OXA type) and the metallo-β-lactamases (MBLs) (Ambler class B). β-Lactam resistance, including carbapenem resistance, has also been ascribed to nonenzymatic mechanisms, including changes in outer membrane proteins (OMPs), multidrug efflux pumps and alterations in the affinity or expression of penicillin-binding proteins (Anton *et al.*, 2008).

This study gives us an insight to the current state of this causative pathogen and its sensitivity profile from the different clinical samples of PIMS. Since species distribution and their susceptibility to antibiotics vary temporally and spatially, as well as their resistance patterns at a local level is important for providing information for comparison with national trends. It also assists in choosing the most appropriate

empirical antimicrobial therapy for infections, in terms of its efficacy and safety. Secondly, the evaluation of the data regarding the testing for ESBLs, pAmpC beta lactamases, carbapanemases and metallo-β-lactamases (MBLs); and also aminoglycosides, quinolones, tetracyclines, sulfonamides and trimethoprime resistance provide information about the best therapeutic options for treating such infections.

AIMS AND OBJECTIVES

Aims

Considering these facts, the present study was designed to determine the magnitude, current trends and mechanisms of antibiotic resistance development among *A. baumannii* isolated from hospitalized patients against the antibiotics recommended by CLSI (2010).

Objectives

- Evaluation of the epidemiological data concerning the prevalence of *A. baumannii* isolated from hospitalized patients diagnosed at the pathology laboratory of Pakistan Institute of Medical Science (PIMS).
- Isolation and characterization of clinical isolates of *A. baumannii* based on their morphological and biochemical characteristics.
- Determination of resistance patterns of *A. baumannii* against antibiotics recommended by CLSI and isolation of multi-drug resistant isolates of *A. baumannii.*
- Determination of different beta-lactamase enzymes in resistant isolates.
- Determination of MICs of antibiotics by agar dilution method.

Literature Review

NOSOCOMIAL INFECTIONS

Nosocomial infections (also known as hospital associated/acquired infections) are those infections that develop in a patient during his/her stay in a hospital or other type of clinical facilities which were not present at the time of admission. Hence, pathogens that cause such infections are termed nosocomial pathogens (Prescott *et al.*, 2005). The hospital environment is a potential reservoir of infectious agents since it houses both patients with diverse pathogenic microorganisms and a large number of susceptible/immunocompromised individuals (Rhomberg *et al.*, 2006). The nosocomial pathogens that cause infections can come either from endogenous or exogenous sources. The most important means of transmission of nosocomial infections is by contact, usually directly but sometimes indirectly by means of secretions from the body (Bergogne-Berezin and Towner, 1996). Air can also be a route of transmission of air borne-nosocomial pathogens (e.g. in droplet nuclei and aerosols) that infect the respiratory tract. The faecal-oral route is a portal of entry for food-borne and water-borne infections (Pelczar *et al.*, 1993).

Predisposing factors that make patients susceptible to these infections include concurrent infections, prosthetic devices, surgery, immunosuppressive agents, administration of broad-spectrum antibiotics, and emergence of multidrug resistant pathogens (Courvaline and Weber, 2005). Other risk factors include age of patient, duration of hospitalization, underlying diseases like diabetes, tumors or overcrowding in the hospital wards (Prescott *et al.*, 2005) . The bacteria that commonly cause nosocomial infections include *Staphylococcus aureus*, *Streptococcus* spp, *Bacillus cereus, Acinetobacter* spp, coagulase negative *staphylococci, enterococci, Pseudomonas aeruginosa, Legionella* and members of the Enterobacteriaceae family such as *Escherichia coli, Proteus mirabilis, Salmonella* spp, *Serratia marcescens* and *Klebsiella pneumonia* (Esposito and Leone, 2007; Zhanel *et al.*, 2008).

The most frequently reported nosocomial pathogens have been *E. coli, S. aureus, enterococci and P. aeruginosa*. Pathogenic strains of *E. coli* can cause different forms of gastrointestinal tract infections. *P. aeruginosa* is a regular cause of nosocomial pneumonia, urinary tract infections, surgical site infections and infection of

severe burns. *S. aureus* is commonly associated with skin and soft tissue infections, surgical, lower respiratory tract infections and neonatal infections. (McCraig *et al.*, 2006; Pitout *et al.*, 2005 and 2007).

The occurrence of multi-drug resistance in hospital-associated pathogens has resulted in the emergence and reemergence of difficult-to-treat nosocomial infections in patients. Examples of bacteria possessing such drug resistance are methicillin-resistant *S. aureus,* penicillin-resistant *pneumococci,* vancomycin-resistant *enterococci, vancomycin* resistant *S. aureus and multi*-drug resistant *tuberculosis* (Moran *et al.*, 2005; Prescott *et al.*, 2005; McCraig *et al.*, 2006; Scheider-Linder *et al.*, 2007). In the developing countries, the incidence of nosocomial infections can be devastating resulting in major disease outbreaks in hospitals and other health care facilities. This may be attributed to poor infrastructure, over-crowding, inadequate personnel and management in most hospitals.

NOSOCOMIAL INFECTIONS DUE TO *A. BAUMANNII*

In most institutions, the majority of *A. baumannii* isolates are from the respiratory tracts of hospitalized patients. In many circumstances, it is very difficult to distinguish upper airway colonization from true pneumonia. There is no doubt, however, that true ventilator-associated pneumonia (VAP) due to *A. baumannii* occurs. In large surveillance studies from the United States, between 5 and 10% of cases of ICU-acquired pneumonia were due to *A. baumannii. A. baumannii* may occasionally cause skin/soft tissue infections outside of the military population. The organism caused 2.1% of ICU-acquired skin/soft tissue infections in one assessment. *A. baumannii* is an occasional cause of UTI, being responsible for just 1.6% of ICU-acquired UTIs in one study (Gaynes and Edwards, 2005). Community-acquired pneumonia due to *A. baumannii* is most typically occurs during the rainy season among people with a history of alcohol abuse and may sometimes require admission to an ICU. The source of infection may be throat carriage, which occurs in up to 10% of community residents with excessive alcohol consumption (Anstey *et al.*, 2002). In a large study of nosocomial bloodstream infection in the United States (1995–2002), *A. baumannii* was the 10th most common etiologic agent, being responsible for 1.3% of all monomicrobial nosocomial bloodstream infections (0.6 blood-stream infection per

10,000 admissions). *A. baumannii* was a more common cause of ICU-acquired bloodstream infection than of non-ICU-ward infection (1.6% versus 0.9% of bloodstream infections, respectively, in those locations). Crude mortality overall from *A. baumannii* bloodstream infection was 34.0% to 43.4% in the ICU and 16.3% outside the ICU. *A. baumannii* infections were the latest of all bloodstream infections to occur during hospitalization, occurring a mean of 26 days from the time of hospital admission (Wisplinghoff *et al.*, 2004).

MECHANISMS OF RESISTANCE TO SELECTED ANTIBIOTICS IN *A. BAUMANNII*

Resistance to Beta-Lactams

The mechanisms underlying resistance to beta-lactams in *A. baumannii* are:

1. Their hydrolysis by beta-lactamases.
2. Changes in penicillin-binding proteins (PBPs) that prevent their action.
3. Alterations in the structure and number of porin proteins that result in decreased permeability to antibiotics through the outer membrane of the bacterial cell.
4. The activity of efflux pumps that further decrease the concentration of antibiotic within the bacterial cell. (Federico *et al.*, 2007).

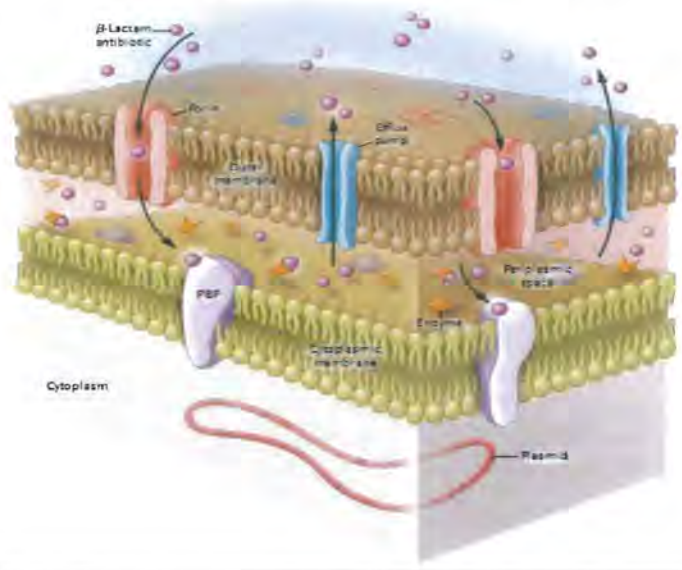

Figure 2: Various mechanisms of antibiotic resistance

1. Class A Beta-Lactamases

Although TEM-1 beta-lactamase is known to occur in *A. baumannii*, class A extended-spectrum beta-lactamases (ESBLs) have been found only more recently (Vila *et al.*, 1993). *A. baumannii* strains harboring PER-1, an ESBL, demonstrate high-level resistance to penicillins and extended-spectrum cephalosporin's but fortunately, PER-1 beta-lactamase does not confer resistance to carbapenems in *A. baumannii*. PER-1 is very prevalent among *A. baumannii* strains in Turkey and Korea (Kolayli *et al.*, 2005; Yong *et al.*, 2003). A recent molecular and epidemiological analysis described PER-1 for the first time in the United States (Hujer *et al.*, 2006).

In addition, a report of SHV-12 and TEM-116 in *A. baumannii* from The Netherlands was published (Naiemi *et al.*, 2005). CTX-M-2, an ESBL characterized by enhanced hydrolysis of cefotaxime and ceftriaxone, was found in epidemic strains of *A. baumannii* in a neurosurgical ward in Japan, as well as in strains of *A. baumannii* isolated in Bolivia (Celenza *et al.*, 2006; Nagano *et al.*, 2004). Interestingly, the dissemination of the blaCTX-M gene seems not to be as widespread in this organism as among Enterobacteriaceae. Since the clinical detection of ESBLs in *A. baumannii* is not standardized and is complicated by the presence of chromosomal cephalosporinases, it is uncertain to what extent class A ESBLs are distributed in *A. baumannii*. In our experience, many clinical isolates of *A. baumannii* test resistant to ceftazidime and cefepime. In *Enterobacter spp.* and *Klebsiella pneumoniae*, the class A ESBLs in an AmpC background is associated with clinical failure when cefepime is used for treatment despite in vitro susceptibility to that agent (Paterson *et al.*, 2001; Thomson *et al.*, 2001).

2. Class B Beta-Lactamases.

The increase in the number of metallo-beta-lactamases (MBLs) in *A. baumannii* is an ominous development in the global emergence of resistance to beta-lactams. MBLs are class B beta-lactamases that are able to hydrolyze carbapenems as well as every other beta-lactam antibiotic with the exception of aztreonam. They differ from class A and D carbapenemases by having a metal ion in the active site, usually zinc, which participates in catalysis. Mirroring the spread of other beta-lactamases, IMP MBLs

are now found around the world in different genera. In *A. baumannii* IMP MBLs are usually detected as part of a class 1 integron, as first discovered in the Far East. Although MBLs are not the predominant carbapenemases in *A. baumannii*, several have been described: IMP-1, IMP-2, IMP-4, IMP-5, IMP-6, and IMP-11. (Walsh, 2005; Walsh *et al.*, 2005) One of the early-described MBLs in *A. baumannii* in England was traced to an infected patient from Spain (Tysall *et al.*, 2002).

The diversity of MBLs in *A. baumannii* isolates from Korea is highlighted by the recent description of a Seoul imipenemase (SIM-1), a novel MBL (101). SIM-1 is a member of the B1 subclass. The broad-spectrum SIM-1 MBL possesses 69% identity with IMP-12 MBL and 64% identity with IMP-9 MBL. There is intriguing genetic evidence to suggest that the blaSIM-1 cassette may have originated from the *Pseudomonas alcaligenes* In55044 superintegron (Lee *et al.*, 2005).

3. Class C Beta-Lactamases.

Acinetobacter spp, like other gram-negative organisms, has a chromosomally encoded class C beta-lactamase. Recent phylogenetic analysis found that chromosomal AmpC genes in *Acinetobacter spp.* likely descend from a common beta-lactamase gene ancestor and are more closely related to each other than to AmpC genes present in other species of bacteria. It is proposed that these represent a distinct family of beta-lactamases, the Acinetobacter-derived cephalosporinases (ADCs). The bla genes code for class C cephalosporinases that hydrolyze penicillins and narrow-spectrum and extended-spectrum cephalosporins, but not cefepime or carbapenems. Thus, many clinical isolates of *A. baumannii* are resistant to ceftazidime. Given the genetic diversity of Acinetobacter spp., it is likely that more variants of the ADC will be found (Hujer *et al.*, 2005).

4. Class D Beta-Lactamases

Class D OXA beta-lactamases are usually robust penicillinases (oxacillinases). Some OXAs (i.e., OXA ESBLs) are also able to hydrolyze extended-spectrum cephalosporins (Aubert *et al.*, 2001; Walther and Hoiby, 2006). Most worrisome are OXA beta-lactamases that inactivate carbapenems. The first description of such an OXA carbapenemase in *A. baumannii* was OXA-23, which was obtained from a

clinical isolate found in Scotland in 1985 before the introduction of carbapenems. Since then, this plasmid-encoded enzyme, initially named ARI-1 (*acinetobacter* resistant to imipenem) has been discovered in England, Brazil, Polynesia, Singapore, Korea, and China (Brown Amyes 2006; Jeon *et al.*, 2005).

The outbreaks of *A. baumannii* harboring OXA-40 and OXA-58 in the United States reflect the dissemination and emergence of OXA enzymes in this organism in the Western hemisphere, raising their status as emerging carbapenemases (Hujer *et al.*, 2006; Lolans *et al.*, 2006).

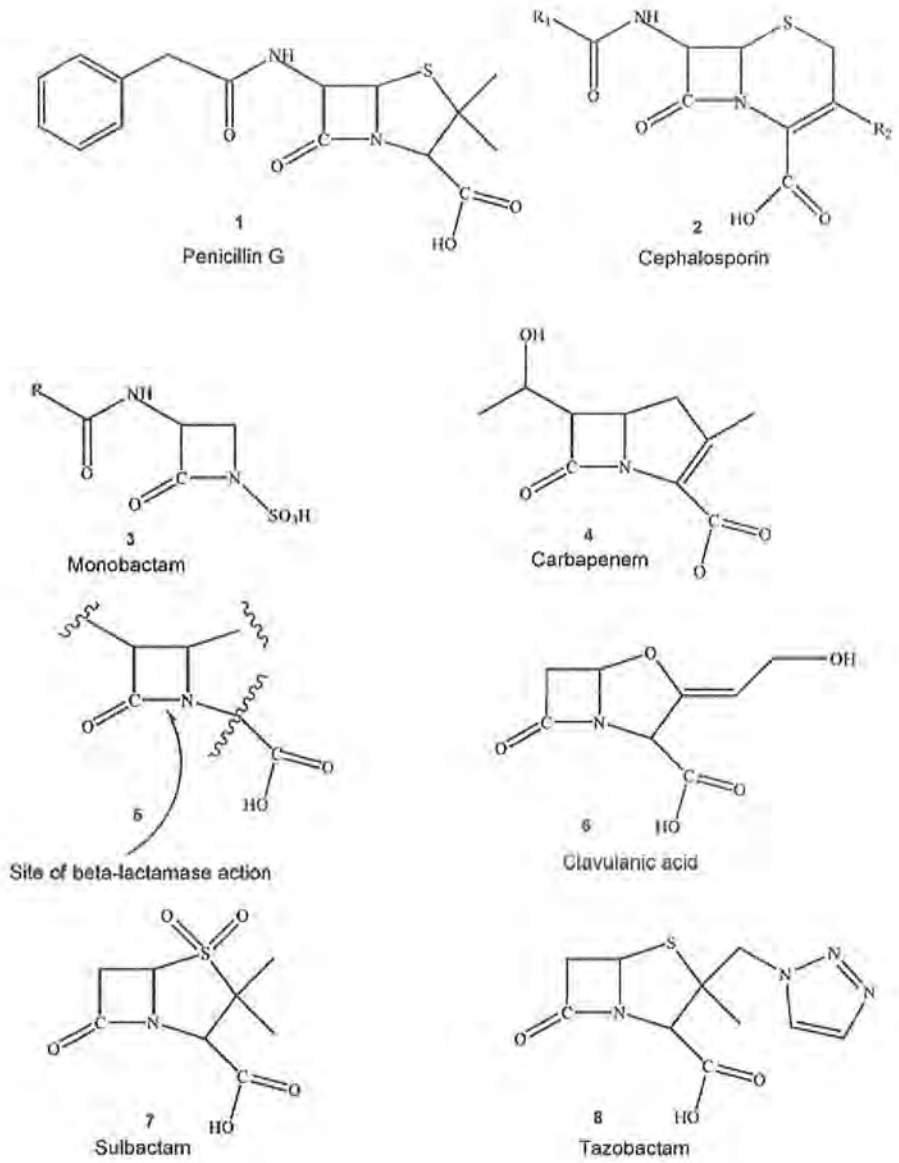

Figure 2: Chemical structures of beta-lactams (1–4), site of action of beta-lactamases (5), and chemical structures of beta-lactamase inhibitors used in clinical practice (6–8) (Maja *et al.*, 2006).

Changes in OMPs and PBPs

Understanding the contribution of porins or outer membrane proteins (OMPs) to antibiotic resistance in *A. baumannii* has been a particular challenge. Unfortunately, it is difficult to accurately compare the loss of OMPs. Laboratory studies reveal that there is variability in the number of observed OMPs (Cuenca *et al.*, 2003). The investigation of the epidemic of MDR *A. baumannii* in New York City demonstrated the presence of carbapenem-resistant isolates with reduced expression of 37-, 44-, and 47- kDa OMPs and increased expression of class C cephalosporinases (Quale *et al.*, 2003). Although in that report only a relatively small number of isolates were studied and MBL or OXA enzymes were not systematically investigated. Similarly, in isolates from Madrid, the loss of 22-kDa and 33-kDa OMPs combined with the production of OXA-24 resulted in resistance to carbapenems (Bou *et al.*, 2000).

The resistance of *A. baumannii* to carbapenems is also explained by reduced expression of PBP-2, as described for isolates from Seville, Spain. Of note, these strains had loss of OMPs and production of beta-lactamases, illustrating the interplay of several different mechanisms of resistance against one class of antibiotics. Establishing the relative contribution of the action of beta-lactamases, beta-lactam penetration through OMPs, and interaction with other mechanisms of resistance, and the control of their expression presents formidable challenges (Fernandez-Cuenca *et al.*, 2003).

Efflux pumps

Efflux pumps exemplify a unique phenomenon in drug resistance: a single mechanism causing resistance against several different classes of antibiotics. These multicomponent pumps mediate the efflux of compounds toxic to the bacterial cell, including antibiotics, in a coupled exchange with protons. Distinct families of efflux pumps widely found in various species of bacteria have been identified: the major facilitator super family, the small multidrug resistance super family the multidrug and toxic compound extrusion super family, and the resistance-nodulation-cell division family (Poole, 2005). In *A. baumannii*, the AdeABC efflux pump, a member of the resistance-nodulation-cell division family, has been well characterized. It pumps

aminoglycosides, cefotaxime, tetracyclines, erythromycin, chloramphenicol, trimethoprim, and fluoroquinolones (Magnet et al., 2001). The over expression of the AdeABC efflux pump may also confer high-level resistance to carbapenems (in conjunction with carbapenem-hydrolyzing oxacillinases) (Marque *et al.*, 2005). A mechanism that controls the expression of this pump was elucidated as a two-step regulator (adeR) and sensor (adeS) system; in the adeR or adeS gene, a single point mutation results in increased expression and hence in increased efflux. Recently, AbeM, another multidrug efflux pump from *A. baumannii* has been identified and characterized as a member of the multidrug and toxic compound extrusion family. Its spectrum of antibiotic substrates appears to be limited to fluoroquinolones, among other toxic compounds (Marchand *et al.*, 2004).

Aminoglycosides. Resistance

In addition to the AdeABC multidrug efflux pump, resistance to aminoglycosides in *A. baumannii* is mediated principally by aminoglycoside-modifying enzymes (AMEs). These include aminoglycoside phosphotransferases, aminoglycoside acetyltransferases, and aminoglycoside nucleotidyltransferases. In a study performed by Nemec and coworkers, aminoglycoside-resistant isolates from 13 countries were analyzed for the genes encoding AMEs (Nemec *et al.*, 2004). PCR mapping revealed that aphA1, aphA6, aacC1, aacC2, aacA4, aadA1, and aadB were present in these isolates. A United States-based study showed that aphA6, aadA1, aadB, aacC1, and aacC2 were present in the collection of isolates from Walter Reed Army Medical Center (Hujer *et al.*, 2006). Turton et al. examined isolates from military and civilian casualties from the Iraq conflict who were hospitalized in the United Kingdom and revealed aacC1, aadA1a, aadB, aacA4, and aadA1 genes encoding AMEs (Turton *et al.*, 2006).

Seward *et al.* further demonstrated that similar AMEs are found in unrelated isolates of *Acinetobacter spp.* and that particular genes are not restricted to specific areas of the world. Hence, similar integrons have been found in genotypically distinct isolates from different locations worldwide (Seward, 1999; Seward *et al.*, 1998).

Recently, a new type of AME, encoded by aac (6) -Iad, has been discovered and found to play a central role in amikacin resistance among *Acinetobacter spp.* in Japan

(Doi *et al.*, 2004). To date, bifunctional AMEs that modify more than one class of aminoglycosides have not been described in *A. baumannii*, as they have been in *Serratia marcescens, Enterococcus faecalis, Staphylococcus aureus, and P. aeruginosa* (Kim *et al.*, 2006).

Quinolones Resistance

Resistance of *A. baumannii* to quinolones is often caused by modifications in the structure of DNA gyrase secondary to mutations in the quinolone resistance-determining regions of the gyrA and parC genes (Seward and Towner 1998; Vila *et al.*, 1997; Vila *et al.*, 1995). These changes result in a lower affinity for the binding of the quinolone to the enzyme-DNA complex. As mentioned above, a second mechanism of resistance to the quinolones is mediated by efflux systems that decrease intracellular drug accumulation. It is unclear why some quinolones (clinafloxacin, gatifloxacin, levofloxacin, trovafloxacin, gemifloxacin, and moxifloxacin) display slightly increased activity against *A. baumannii* compared to ciprofloxacin (Heinemann *et al.*, 2000).

Tetracyclines Resistance

Two different mechanisms of resistance to tetracyclines have been widely described in *A. baumannii*. TetA and TetB are specific transposon-mediated efflux pumps; TetB determines the efflux of both tetracycline and minocycline, whereas TetA drives only the efflux of tetracycline (Guardabassi et al., 2000; Huys *et al.*, 2005). The second mechanism is the ribosomal protection protein, which shields the ribosome from the action of tetracycline. The Tet (M) gene encodes this protein, which serves to protect the ribosome from tetracycline, doxycycline, and minocycline. This ribosomal protection protein found in *A. baumannii* is 100% homologous to the Tet (M) protein of S. aureus (Ribera *et al.*, 2003). Neither efflux nor the ribosomal protector protein seems to interfere with the action of tigecycline, a representative of a new class of antibiotics, glycylcyclines, related to tetracyclines. However, tigecycline is a substrate for TetX (a plasmid-borne laving-dependent monooxygenase), although this enzyme has not been found in clinical isolates of *A. baumannii* (Moore *et al.*, 2005). Hence, it is concerning that bloodstream infections caused by non-tigecycline-susceptible *A. baumannii* are reported; such resistance appears to be at least partly attributable to an

efflux pump mechanism (Peleg *et al.*, 2007). A recent analysis by Ruzin et al. confirms the role of the AdeABC efflux pumps as a mechanism of resistance to tigecycline. The overexpression of the adeABC locus correlated with a three-fold increase in MIC in strains of the *Acinetobacter calcoaceticus-A. baumannii* complex. The efflux pump confers broad substrate specificity, which includes tigecycline, gentamicin, levofloxacin, and chloramphenicol. Furthermore, the AdeRS two-component system, which regulates the expression of AdeABC, was disrupted by an insertion sequence, ISAba1, in the tigecycline-resistant strains, whereas it remained intact in tigecycline-susceptible strains (Ruzin *et al.*, 2007).

ESBL detection

Detecting an ESBL producer is a major challenge for the clinical microbiology laboratory. The Clinical Laboratory Standards Institute (formerly, NCCLS) has published guidelines to assist in identifying the ESBL phenotype. Testing can be done using the broth micro dilution method, E-test or by disk diffusion. For MIC-based testing, a decrease of ≥3 doubling dilutions in an MIC for either cefotaxime or ceftazidime tested in combination with clavulanic acid confirms an ESBL-producing organism. Using disk diffusion testing, a ≥5mm increase in the inhibition zone diameter for either antimicrobial agent with clavulanate is also diagnostic. Despite clear guidelines, the proficiency of clinical microbiology laboratories to uniformly detect bacteria containing these resistance determinants has been poor (Maja *et al.*, 2006).

MBL Detection

Production of MβLs was screened by the disk approximation test Briefly, a 100mmMueller-Hinton-agar plate was inoculated using a 0.5McFarland suspension froma fresh culture. Imipenem, meropenem and ceftazidime disks were strategically aligned around disks contained either EDTA (750μg) or 2-mercaptopropionic acid (MPA, 360 μg). The appearance of a growth-inhibitory zone between the carbapenems and/or ceftazidime and either one of the disks containing the MβL inhibitor (EDTA or MPA) was considered a positive test . A. baumannii 54/97 was used as a Positive control. MβL Etest strips (AB BIODISK, Solna, Sweden) were used to quantitatively confirm the disk approximation test results. The strips contain

imipenem±EDTA. In addition, ceftazidime/ceftazidime-clavulanic acid and cefepime/cefepime-clavulanic acid ESBL Etest strips were used to evaluate the possible concurrent production of an extended-spectrum β-lactamase (ESBL) (Walsh *et al.*, 2002).

MULTI-DRUG RESISTANT *A. BAUMANNII*

Resistance mechanisms that are expressed frequently in nosocomial strains of acinetobacter include β-lactamases, alterations in cell-wall channels (porins), and efflux pumps. *A. baumannii* can become resistant to quinolones through mutations in the genes gyrA and parC and can become resistant to aminoglycosides by expressing aminoglycoside-modifying enzymes. Clinically most troubling have been *acinetobacter's* acquired β-lactamases, including serine and metallo-β-lactamases, which confer resistance to carbapenems (Bonomo *et al.*, 2006). AmpC β-lactamases are chromosomally encoded cephalosporinases intrinsic to all *A. baumannii*. Usually, such β-lactamases have a low level of expression that does not cause clinically appreciable resistance; however, the addition of a promoter insertion sequence, ISAba1, next to the AmpC gene increases β-lactamase production, causing treatment-limiting resistance to cephalosporins (Poirel *et al.*, 2006). Although porin channels in *A. baumannii* are poorly characterized, it is known that reduced expression or mutations of bacterial porin proteins can hinder passage of β-lactam antibiotics into the periplasmic space, leading to antibiotic resistance. Overexpression of bacterial efflux pumps can decrease the concentration of β-lactam antibiotics in the periplasmic space. To cause clinical resistance in *acinetobacter*, efflux pumps usually act in association with overexpression of AmpC β-lactamases or carbapenemases. In addition to removing β-lactam antibiotics, efflux pumps can actively expel quinolones, tetracyclines, chloramphenicol, disinfectants, and tigecycline (Peleg *et al.*, 2007). Acquired extended-spectrum β-lactamase carriage occurs in *acinetobacter* but is not as widespread as in *Klebsiella pneumoniae* or *Escherichia coli* (Jacoby *et al.*, 2005). Currently, the term "multidrug resistance" in reference to *acinetobacter* does not have a standard definition. It is sometimes used to denote resistance to three or more classes of drugs that would otherwise serve as treatments for *acinetobacter* infections (e.g., quinolones, cephalosporins, and carbapenems). The term "pan

resistance" has been used to describe strains of acinetobacter that are resistant to all standard antimicrobial agents tested (except colistin) (Paterson *et al.*, 2006).

Materials and Methods

The present study was carried out in the Microbiology Laboratory, Pathology Department, Pakistan Institute of Medical Sciences (PIMS), Islamabad. The study was conducted from February 2010 to December 2010. The aim of this study was to investigate the prevalence of multi- drug resistant gram negative, non lactose fermenter, coccobacilli *Acinetobacter baumannii* in the clinical samples collected from the patients admitted in ICU's, medical and surgical wards of PIMS. This hospital is the prime tertiary care facility in the public sector for Islamabad and its surrounding population. These pathological samples were proceeded for isolation and identification according to standard microbiological laboratory methods. 91 consecutive, non-duplicated Gram-negative *Acinetobacter baumannii* were finally selected for Antimicrobial sensitivity, extended spectrum β lactamases, Carbapanemases, Metallo β lactamases, AmpC β lactamases screening and MIC according to standard protocols.

BACTERIAL ISOLATES

Acinetobacter baumannii was found to be the most common bacteria isolated from clinical samples of different infectious sites; therefore this bacterium was selected in the current study.

SUBJECT POPULATION

The study population consisted of patients having infectious site cultures determined at the pathology laboratory of the hospital. These patients were identified from computerized laboratory reports. Demographic information (age, sex) and clinical data (medical diagnosis and antibiotics prescribed) were obtained from the patient's medical record. The resistance pattern of the selected *Acinetobacter baumannii* isolates was determined against 18 antibiotics by disc diffusion method.

SAMPLE PROCESSING AND IDENTIFICATION OF CULTURE ISOLATES:

Samples were initially cultured on MacConkey and blood agar medium. MacConkey agar medium helps in the differentiation of lactose fermenters and non-fermenters. The plates were incubated for 24 to 48 hours at 37 °C.

Isolation of *Acinetobacter baumannii*

The characteristic isolates were aseptically isolated and characterized using established microbiological methods, which included colony morphology, Gram stain reaction and biochemical characteristics

Identification of *Acinetobacter baumannii*

Colony Morphology

On the basis of presentation on the solid agar media, isolates were identified. Features, which were noticed on the respected media, include: Size (Pinpoint, small, moderate, large), Pigmentation (Color of colony) Form (Circular, irregular, rhizoid) Margin: (Entire, lobate, undulate, serrate, and filamentous) Elevation: (Flat, raised, convex)

Gram Staining

Isolated colonies, after purification, were initially Gram stained. Using sterile technique, smear of the isolates was prepared, dried and heat fixed on slides. The smear was flooded with crystal violet and allowed to stand for one minute. It was then washed with tap water and flooded with Gram's Iodine. The slide was then allowed to stand for one minute and then washed with tap water. The smear was then decolorized with 95 % ethyl alcohol and again washed with tap water. After that, it was counter stained with safranin for 30 seconds and washed with tap water. The slide was air dried and examined under the oil emulsion objective.

Preparation of McFarland Turbidity Standard

McFarland standard was prepared by adding specific volumes of 1 % sulphuric acid and 1.175 % barium chloride. McFarland 0.5 standard was used in this study. It contained 99.5 mL of 1 % sulphuric acid and 0.5 mL of 1.175 % barium chloride. Solution was dispensed into tubes comparable to those used for inoculum preparation, which were sealed tightly and stored in dark at room temperature.

Biochemical Characteristics

Isolates were identified by API 20 E kit (biomeriuex, USA). **Analytical profile index (API) is** a standardized identification system for Enterobacteriaceae. It has 20 miniaturized biochemical tests. A strip contains 20 micro tubes containing dehydrated substrates. These tests were inoculated with bacterial suspension. During incubation, metabolism produced color changes that were either spontaneous or revealed by the addition of the reagents. Adding distilled water in the incubation box provided humid environment. Bacterial suspension equal to 0.5 McFarland index was prepared by adding few colonies of isolates in 5ml of 0.85ml of NaCl solution. It is carefully emulsified to achieve homogeneous bacterial suspension. Wells in strips were charged with bacterial suspension. Tubes and cupules were filled according to the test requirement. Some tests were incubated anaerobically by overlaying mineral oil. After incubation for 18 to 24 hours AT 35±2°C, reagents were added in respected wells, positive results were noted, and seven digits numerical profile was determined which is looked up in Analytical Profile Index.

Table 1: Interpretation of biochemical tests on API 20E strip.

Test	Active	Reactions/ Enzymes	Result	
			Negative	Positive
ONPG	2-nitrophenyl- βD-galactopyranoside	Beta-galactosidase	Colorless	Yellow
ADH	L-arginine	Arginine Dihydrolase	Yellow	Red/orange
LDC	L-lysine	Lysine Decarboxylase	Yellow	Red/orange
ODC	L-ornithine	Ornithin Decarboxylase	yellow	Red/orange
CIT	Trisodium citrate	Citrate utilization	Palegreen/ yellow	Blue-green/blue
H2S	Sodium Thiosulfate	H2S production	Colorless	Blackdeposit
URE	Urea	Urease	Yellow	Red/orange
TDA	L-tryptophane	Tryptophane Deaminase		TDA/ immediate[1]
IND	L-tryptophane	Indole production	Yellow	Reddish brown /immediate[2]
VP	Sodium pyruvate	Acetoin production	Colorless	Pink (VP1 +VP2) 10min[3]
GEL	Gelatin	Gelatinase	No diffusion	Black pigment diffusion
GLU	D-glucose	F/O, glucose	Blue Green	Yellow
MAN	D-mannitol	F/O, mannitol	Blue Green	Yellow
INO	Inositol	F/O, inositol	Blue Green	Yellow
SOR	D-sorbitol	F/O, sorbitol	Blue Green	Yellow
RHA	L- rhamnose	F/O, rhamnose	Blue Green	Yellow
SAC	D-sucrose	F/O, saccharose	Blue Green	Yellow
MEL	D-melibiose	F/O, melibiose	Blue Green	Yellow
AMY	Amygdalin	F/O, amygdalin	Blue Green	Yellow
ARA	L-arabinose	F/O, arabinose	Blue Green	Yellow

1, 2, 3 -Reagents added after incubation & time to note result.

F/O=Fermentation/oxidation reactions

Motility test

The motility of test organism was determined by stabbing the semisolid motility media with sterile wire loop. Growth was observed along with the line (non-motile) or growth spreading away from the line in media (motile)

Oxidase test

Cytochrome oxidase enzymes activity was determined by using freshly prepared oxidase reagent and poured on filter paper. Colonies of isolates were spread on the paper and blue color within 10 second was observed.

DETERMINATION OF ANTIBIOTIC RESISTANCE PATTERNS OF *Acinetobacter baumannii:*

Antibiotic resistance patterns of the bacterial isolates conferring to be *A. baumannii* were studied. The pattern among different groups of antibiotics was determined by employing disc diffusion method of Bauer *et al.* (1996). Antibiotics tested with specified potencies are shown in Table 1. Bacteria were classified as susceptible, intermediate or resistant to antibiotics in accordance with current Clinical Laboratory Standard Institute (CLSI) recommendations (2010).

Control Strains

The control strains *Escherichia coli* (ATCC 25922) used for the study was obtained from National Institute of Health (NIH), Islamabad.

Table 2: Antimicrobial agents discs along with code and potencies used in the study.

Sr.No.	Antimicrobial agent	Antibiotic group	Code	Disc potency(µg)	Manufacturer
1.	Amoxacillin-C.acid	β-Lactam/βLactamase Inhibitor combination	AMC	20/10	OXOID
2.	Piperacillin	Penicillins	PRL	100	OXOID
3.	Ceftazidime	Cephalosporins	CAZ	30	OXOID
4.	Meropenem	Carbapenems	MEM	10	OXOID
5.	Gentamicin	Aminoglycosides	CN	10	OXOID
6.	Amikacin	Aminoglycosides	AK	30	OXOID
7.	Tetracycline	Tetracyclines	TE	30	OXOID
8.	Ciprofloxacin	Fluoroquinolones	CIP	5	OXOID
9.	Tigecyclice	Tetracyclines	TGC	15	OXOID
10.	Minocycline	Tetracyclines	MI	30	OXOID
11.	Imipenem	Carbapenems	IPM	10	OXOID
12.	Cefotaxime	Cephalosporins	CTX	30	OXOID
13.	Doxcycline	Tetracyclines	DO	30	OXOID
14.	Polymyxin B	Lipopeptides	PB	10	OXOID
.15.	Tobramycin	Aminoglycosides	TOB	10	OXOID
16.	Ampicillin-sulbactam	β-Lactam/βLactamase Inhibator combination	SAM	10/10	OXOID
17.	Pipercillin-tazobactam	β-Lactam/βLactamase Inhibator combination	TZP	100/10	OXOID
18.	Tromrthoprim-sulfamethoxazole	Folate pathway Inhibitor	SXT	1.25/23.75	OXOID

Kirby-Bauer Disc Diffusion Test Kirby-Bauer Disc Diffusion Test

Antimicrobial susceptibility testing was carried out by the standard Kirby-Bauer disk diffusion method following guidelines provided by the CLSI (2010). Muller-Hinton agar (MHA) was used as the growth medium and prepared according to the manufacturer's instructions and sterilized by autoclaving at 121°C for 15 minutes. First of all isolated colonies of *A. baumannii* in the nutrient agar were picked up with a sterile loop and cell suspension was made in sterile saline (0.85% NaCl) to obtain turbidity optically comparable to that of the 0.5 McFarland standards. A sterile cotton swab was dipped into standardized bacterial suspension and inoculum was spread evenly over the entire surface of the MHA plates by swabbing back and forth in three directions to give a uniform inoculum surface. The plates were allowed to dry, and within 15 minutes antibiotic impregnated disks (Oxoid) of given potency were applied on the inoculated plates at reasonable equidistance with the help of sterilized forceps. The plates were then incubated at 35°C for 18-24 hours in an inverted position. When applying antimicrobial impregnated paper disks on the surface of MHA plate, antimicrobials in these disks diffused into the medium and inhibited the development of growth around paper disks, forming zone of inhibition. After 18 hours of incubation, plates were examined and the diameters of zones of inhibition of growth were measured in mm. According to the diameter, isolates were classified into resistant, intermediate and susceptible.

DETERMINATION OF ESBL PRODUCTION

MHA was prepared and sterilized by autoclaving at 121° C for 15 minutes. About 25 mL of medium was poured into 90 mm diameter sterile petri dishes to a depth of 4 mm and left at 37°C overnight to check sterility. For inoculum, Nutrient broth was prepared and 5 mL broth dispensed in screw cap test tubes was sterilized by autoclaving at 121 °C for 15 minutes. The test tubes were cooled and kept in an incubator for 24 hours at 35 °C to check sterility. Each already identified cephalosporin resistant clinical isolate, was inoculated in sterilized test tubes containing the medium, and placed in an incubator overnight at 35 °C. The turbidity of broth cultures was adjusted according to 0.5 McFarland standards. A sterile cotton swab was saturated by dipping into standardized bacterial suspension. Inoculum was

spread evenly over the entire surface of the MHA plates by swabbing back and forth across the agar in three directions to give a uniform inoculum to the entire surface. The plates were allowed to dry before applying discs, and within 15 minutes discs of given potency were applied on the inoculated plates according to the double-disc diffusion test by placing a disk of amoxicillin/clavulanate (20 µg/10 µg) in the center and antibiotic discs of cefotaxime (30 µg), ceftazidime (30µg) and aztreonam (30 µg) placed, 25 mm (center to center) from the amoxicillin /clavulanate disk. The plates were then placed in an incubator at 35 °C for 18 hours in an inverted position. After 18 hours of incubation, plates were examined and a clear extension of the edge of the inhibition zone of any of the antibiotics towards the disk containing clavulanate was interpreted as synergy, indicating the presence of an ESBL.

DETERMINATION OF CARBAPENEMASE PRODUCTION

Modified Hodge Test

The lawn of the control strain *E. coli* ATCC 25922 was prepared on MHA plates, in the same way as in case of ESBL detection section. A sterile cotton swab was saturated by dipping into standardized bacterial suspension. Inoculum was spread evenly over the entire surface of the MHA plates by swabbing back and forth across the agar in three directions to give a uniform inoculum to the entire surface. The plates were allowed to dry for some time and then a 10-µg-carbapenem disc was applied. In a straight line, the carbapenem resistant test organisms were streaked from the edge of the disc to the edge of the plate.3 organisms were tested on a single plate. The plates were then placed in an incubator at 35 °C for 18 hours in an inverted position. After 18 hours of incubation, plates were examined and the indentation of the *E.coli* 25922 growing along the test organism growth streak within the disc diffusion zone was interpreted as a positive result indicating carbapenemase production (Lee et al., 2001).

DETERMINATION OF METALLO-β-LACTAMASES (MBL) PRODUCTION

EDTA test

The lawns of carbapenem resistant organisms were prepared on MHA plates. The plates were allowed to dry before applying discs, and within 15 minutes two imipenem discs (10μg) were applied on each inoculated plate. A 0.5 M EDTA solution was prepared by dissolving 186.1 g of disodium EDTA.2H_2O (Junsei Chemical, Tokyo, Japan) in 1000 ml of distilled water and adjusting it to pH 8.0 by using NaOH. The mixture was sterilized by autoclaving. 750μg of the EDTA solution was added to one of the imipenem discs, on the inoculated MHA plates, to obtain the desired concentration. The inhibition zones of imipenem and imipenem-EDTA discs were compared after 16-18 hours of incubation at 37°C. An increase of greater than 5mm in the size of imipenem-EDTA disc as compared to the imipenem alone disc was considered positive for MBL production (Lee et al., 2001).

DETERMINATION OF AmpC PRODUCTION

The principle of the test was to permeabilize a bacterial cell by using Tris-EDTA and release β-lactamase into the external environment. AmpC disk (i.e., filter paper disk containing Tris-EDTA) were prepared in the laboratory by applying 20ul of a 1:1 mixture of saline and 100×Tris-EDTA (Merck, Germany) to sterile filter paper disk, allowing the disks to dry, and storing them at 2 to 8 C. The surface of a Mueller-Hinton agar was inoculated with a lawn of cefoxitin- susceptible *E.Coli* ATCC 25922 according to the standard disk diffusion method. Immediately prior to use, AmpC disk were rehydrated with 20ul of saline and several colonies of each test organism were applied to a disk. A 30ug of cefoxitin disk was placed on the inoculated surface of the Mueller-Hinton agar. The inoculated AmpC disk was then placed almost touching the antibiotic disk with the inoculated disk face in contact with the agar surface. The plate was then inverted and incubated overnight at 35 C in ambient air. After incubation, plates were examined for either an indentation or a flattening of the zone of inhibition, indicating enzymatic inactivation of cefoxitin (positive result), or the absence of a

distortion, indicating no significant inactivation of cefxitin (negative result) (Black et al., 2005; Singhal et al., 2005).

Minimum Inhibitory Concentration (MIC):

Agar dilution method was used to determine the MICs of ciprofloxacin and tetracycline. Standard powder of antibiotics was used to make stock solution. Stock solution was prepared by using the formula

$$1000/P \times V \times C = W$$

Where P=potency given by the manufacture (ug/mg), V=volume required (ml), C= final concentration of the solution (multiples of 1000) (mg), and W=weight of antibiotic in mg to be dissolved in volume V (ml).

Stock solution of antibiotic was prepared by adding known quantity of antibiotic powder in respected sterile dilution. Ciprofloxacin and tetracycline powder were dissolved in water. These antibiotic stock solutions were used to make antibiotic dilution range according to antibiotic. According to the labeled potencies/concentration, stock solutions of different concentration of antibiotics were prepared. Stock solution was freshly used (Andrews, 2001).The volume of stick solutions that were added to flasks was calculated by formula.

$$C_1V_1=C_2V_2$$

C_1=*Concentration of stock solution*

V_1=*Volume of stock solution*

C_2=*Required concentration*

V_2=*Volume of media to be made*

Antibiotic dilution range of 1, 0, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024 ug/ml was prepared in flasks according to the antibiotic breakpoint for the particular species. No antibiotic was added to one flask which was antibiotic free growth control.

Preparation of Agar dilution plates

20ml or 50ml of cooled molten agar (medium was cooled to 50 C before adding to the antibiotic) were added to each flask, including the antibiotic free control. Mixed well and poured into the 90mm or 150mm perti dish, allowed agar to set and then dry surface of plates and used immediately.

Preparation of Inoculum

Growth method was used to make bacterial suspension. Four colonies of isolates were transferred to nutrient broth. Broth was then placed in incubator shaker at 35-36 C until the visible turbidity was equal to or greater than the 0.5 Mc Farland standards. It was used within 30 minutes of preparation.

Inoculation

Multipoint inoculators were used to deliver 1-2ul of suspension on to the surface of the agar. The plates were then incubated at 35 C in ambient air.

MIC Determination

After incubation it was ensured that all of the organisms have grown on the antibiotic free control plates. Determined the MIC of each antibiotic, as the MIC is the lowest concentration of antibiotic at which there is no visible growth of organism.

Preservation of isolates

Isolates were preserved on the nutrient agar slants. Pure growth of respected pathogen from overnight-incubated blood agar plate was taken and inoculated on the slants. These slants were incubated overnight at 35°C and placed at 4°C.

Results

A total of 91 bacterial isolates were collected from the indoor and outdoor patients who visited or were admitted at PIMS and identified on the basis of colonial morphology and biochemical tests.

IDENTIFICATION OF ISOLATES

Colonial Morphology

Isolated colonies on Blood and MacConkey agar were used to study colony characteristics. On MacConkey colonies of *A. baumannii* was appeared as a non-lactose fermenter and on blood agar colonies were about 1 to 2 mm in diameter, non-pigmented, domed, and mucoid, with smooth to pitted surfaces (Figure 3, 4).

Gram staining

Under the microscope *A. baumannii* looked alike as pink short rods as these are the gram-negative bacteria.

Oxidase and Motility test

A baumannii were oxidase negative and non motile.

Biochemical characterization of *A. baumannii*

Identification by using API 20 E

Miniaturized biochemical test results for *A. baumannii* were as follows. These were looked in Analytical Profile Index (Figure 5) (Appendix Table 3).

Figure 3: Colonies of *A. baumannii* on blood agar

Figure 4: Colonies of *A.baumannii* on MacConkey agar

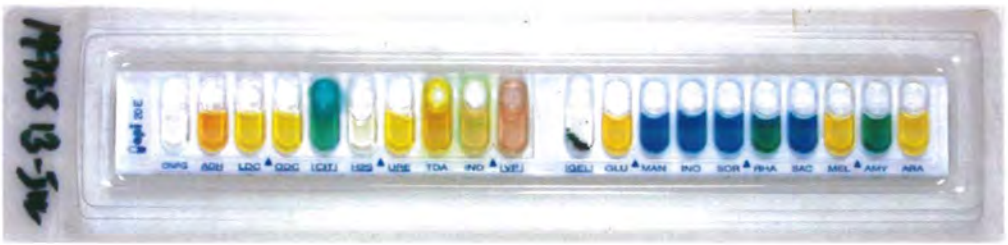

Figure 5: API 20E result of *A. baumannii*.

Table 3: Biochemical reactions of *A. baumannii*

Test Name	A.baumannii
Beta-galactosidase	-
Arginine dihydrolase	-
Lysine decarboxylase	-
Ornithine decarboxylase	-
Citrate utilization	+
H2S production	-
Urease	-
Tryptophane deaminase	-
Indole production	-
Acetoin production	-
Gelatinase	-
D-glucose fermentation	+
D-mannitol fermentation	-
Inositol fermentation	-
D-sorbitol fermentation	-
L-rhamnose fermentation	-
D-sucrose fermentation	-
D-melibiose fermentation	+
Amygdalin fermentation	-
L-arabinose fermentation	+

A.baumannii gave glucose, melibiose, and arabinose fermentations as well as positive citrate utilization.

PREVALENCE OF *A. BAUMANNII* AMONG PATIENTS

A Total 91 clinical isolates were identified as *A. baumannii.*

PREVALANCE OF MULTI-DRUG RESISTANT *A. BAUMANNII* AMONG PATIENTS

All 91 clinical isolates of *A. baumannii* were found to be multi-drug resistant.

Prevalence of multi-drug resistant *A. baumannii* in different specimens

Among the 91 samples of *A. baumannii*, the highest prevalence of *A. baumannii* (n=23, 25.27%) was observed in the endotracheal tubes secretion followed by tracheal secretion (n=18, 19.78%) and pus (n=15, 16.48%) (Figure 6) (Appendix Table 4).

Prevalence of multi drug resistant *A. baumannii* in different wards

Prevalence of *A. baumannii* at different hospital sites including medical, surgical, OPD, emergency, and ICUs (nicu, picu) was observed and prevalence was not similar in all the examined hospital sites. Highest prevalence of *A. baumannii* was found in NICU (n=37, 40.65%), followed by Medical (ICU) (n=18, 19.78%) and Emergency (n=9, 9.89%) (Figure 7) (Appendix Table 5).

Prevalence of muti-drug resistant *A. baumannii* in patients of different age groups

Prevalence of *A. baumannii* was found to be higher in new born babies as compared to the younger or older patients. Among 91 patients infected with *A. baumannii* , the highest percentage belonged to the age-group between 0-29 days babies (n=37,42.85%) followed by age groups between 40-60 years (17,18.68%) and age groups between 1-20 years (n=14,15.38%) (Figure 8) (Appendix Table 6).

Prevalence of multi- drug resistant *A. baumannii* in different genders

Among 91 clinical isolates of *A. baumannii* 37 (40.65%) were obtained from the new born babies, 35 (38.46%) were males while 19 (20.87%) were females (Figure 9, 10). (Appendix Table 7, 8).

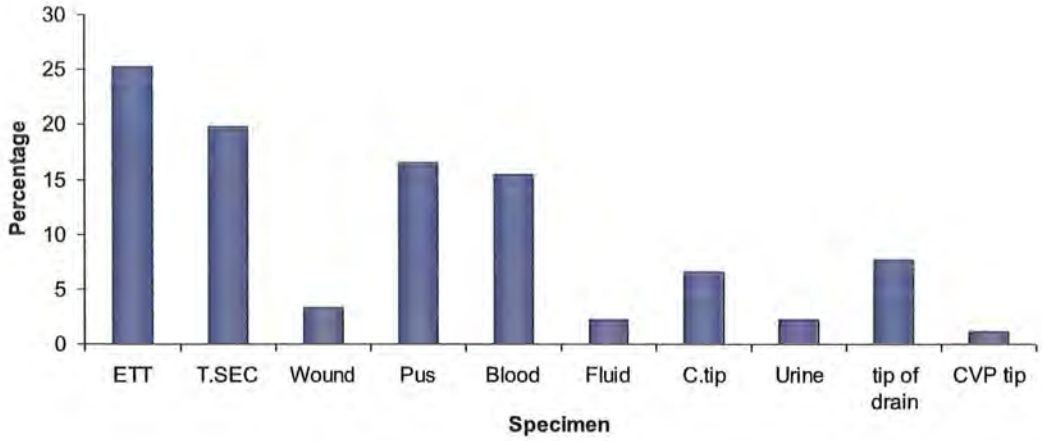

Figure 6: Prevalence of multi-drug resistant *A. baumannii* in different specimens.

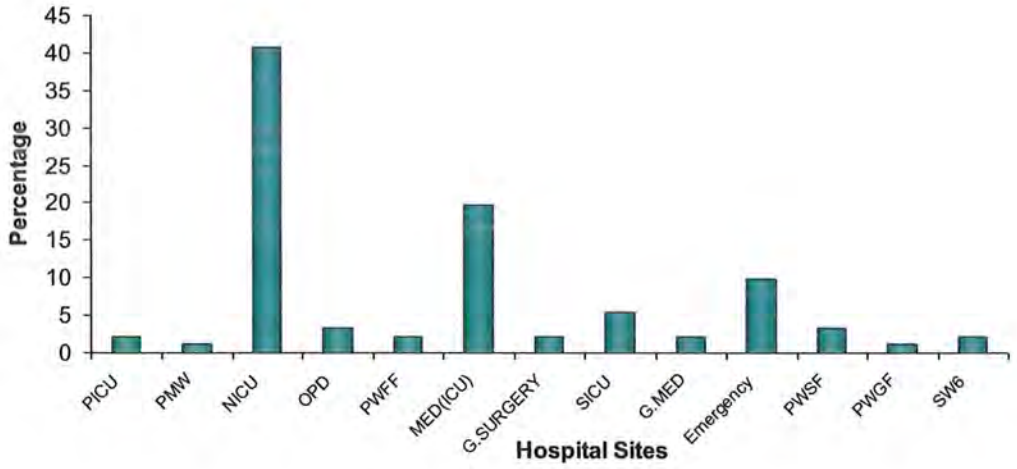

Figure 7: Prevalence of multi-drug resistant *A. baumannii* in different wards.

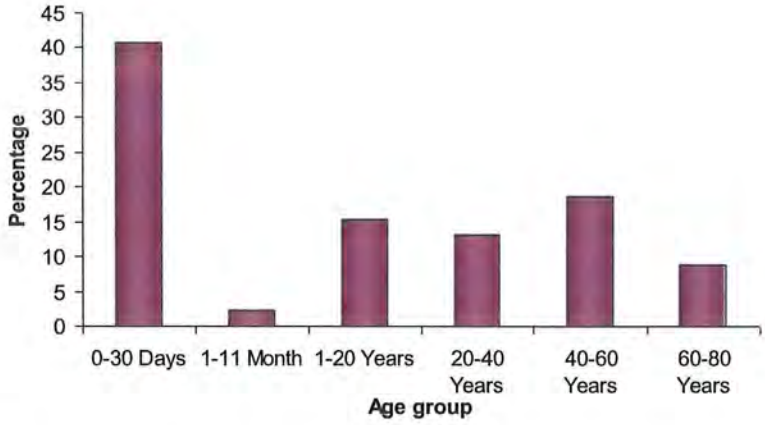

Figure 8: Prevalence of multi-drug resistant *A. baumannii* in patient of different age-groups.

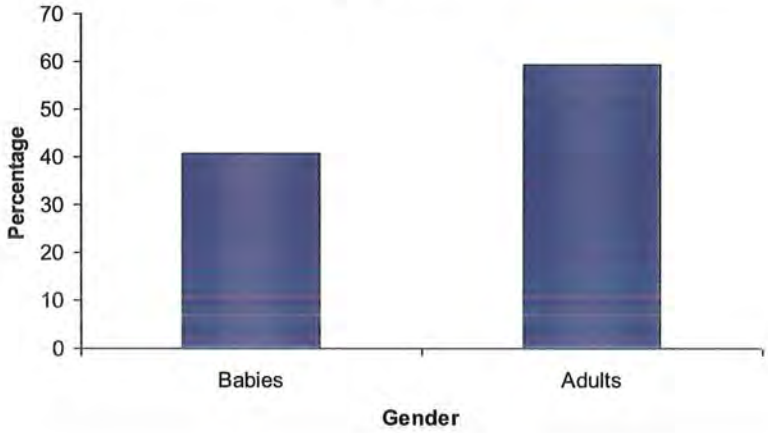

Figure 9: Prevalence of multi-drug resistant *A. baumannii* among babies and adults.

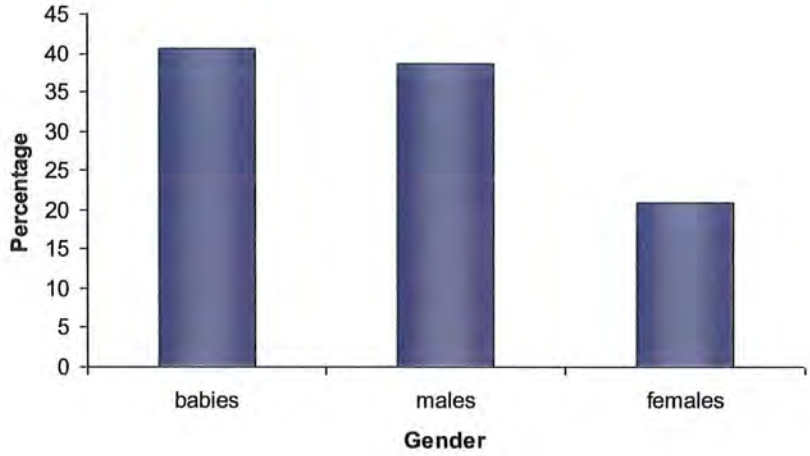

Figure 10: Prevalence of multi-drug resistant *A. baumannii* in different genders.

ANTIBIOTIC RESISTANCE PATTERNS OF *A. BAUMANNII*

The resistance patterns of *A. baumannii* against various antibiotics were determined by disc diffusion method. In this study *A. baumannii* exhibited the highest resistance (n=91,100%) against cephalosporin's, carbapenems, flouroquinolones and β-lactam drugs (Figure 11) (Appendix Table 9). In aminoglycosides, tobramycin showed better activity than amikacin. In tetracycline's, tetracycline also showed highest resistance (n=60, 65.93%) while tigecycline and minocycline showed a best antibacterial activity with a zero resistance (n=91, 0.00%).Among all antibiotics used in this study tigecycline and minocycline were found to be the most effective (Figure 12, 13) (Appendix Table 10).

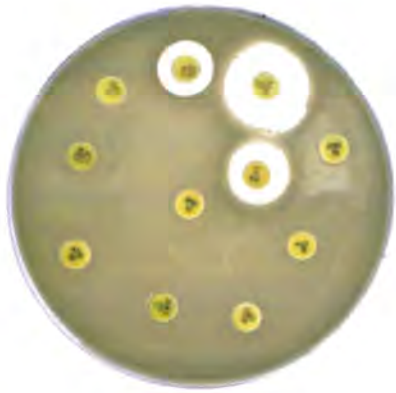

Figure 11: Kirby-Baur Disc Diffusion Test.

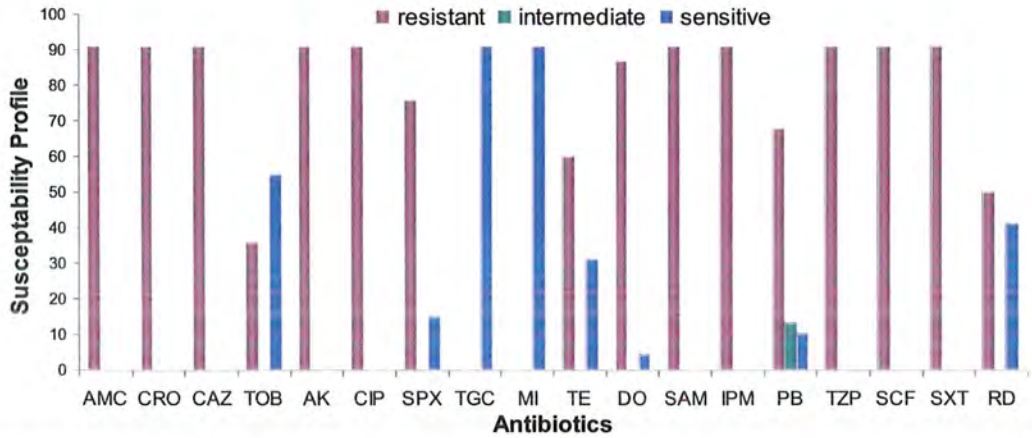

Figure 12: Antibiotics susceptibility profile of multi drug resistant *A. baumannii.*

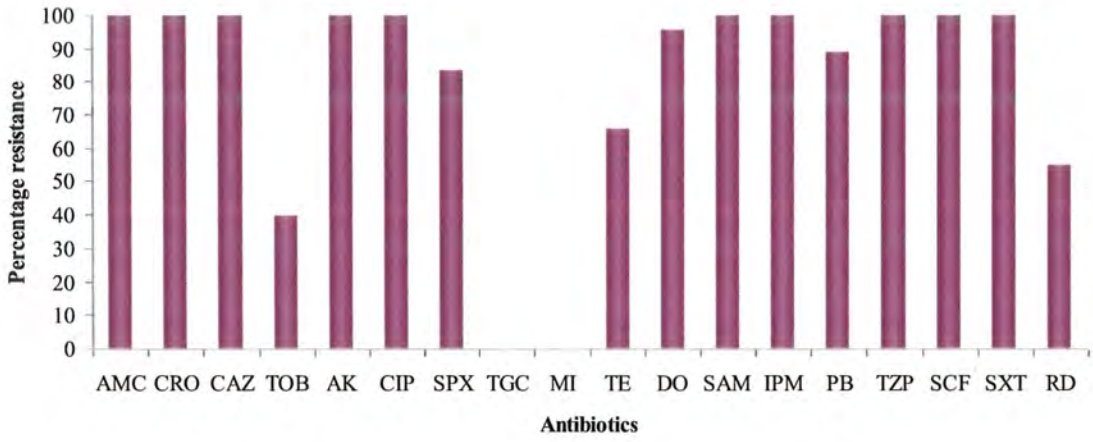

Figure 13: Antibiotic resistance pattern of *A. baumannii.*

DETERMINATION OF ESBL PRODUCTION

PREVALENCE OF ESBL PRODUCING *A. BAUMANNII* AMONG PATIENTS

Among the 91 cephalosporin resistant isolates of *A. baumannii*, no one was found to ESBL positive. All were ESBL negative (Figure 14) (Appendix Table 11).

DETERMINATION OF CARBAPENEMASE PRODUCTION

PREVALENCE OF CARBAPENEMASE PRODUCING *A. BAUMANNII* AMONG PATIENTS

Among the 91 carbapenem resistant *A. baumannii* isolates, 91 (100%) were found to be carbapenemase producers (Figure 15, 16) (Appendix Table 12).

PREVALENCE OF METALLO-BETA-LACTAMASE (MBL) PRODUCING *A. BAUMANNII* AMONG PATIENTS

All the 91 (100%) carbapenemase positive isolates were tested for MBL production test. All isolates belonged to the metallo-beta-lactamase group of carbapenemases (Figure 17, 18) (Appendix Table 13).

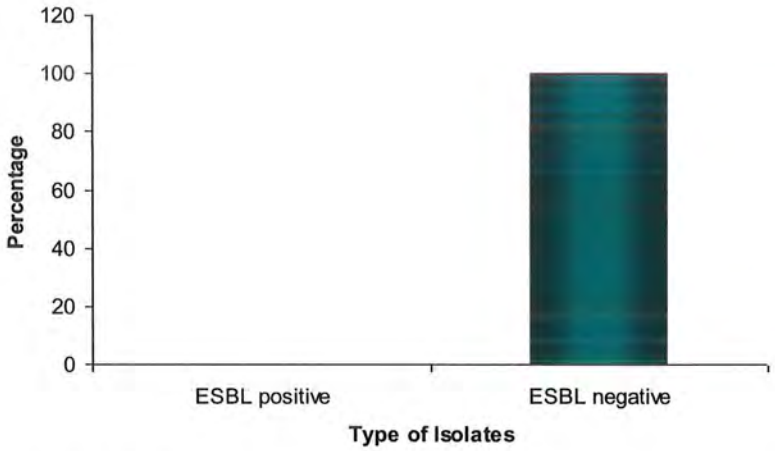

Figure 14 : Prevalence of ESBL producing *A. baumannii* among patients.

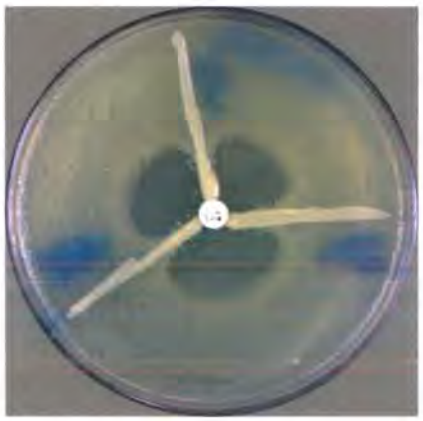

Figure 15 : Positive result of carbnapenemase detection test.

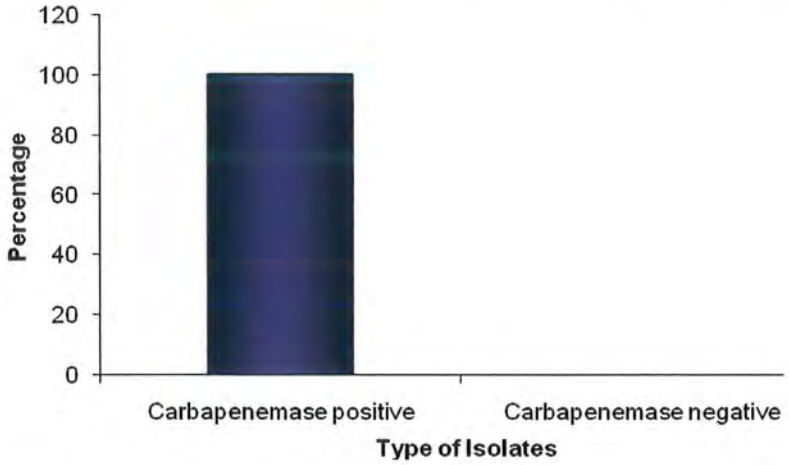

Figure 16: Prevalence of carbapenemase producing *A. baumannii* among patients.

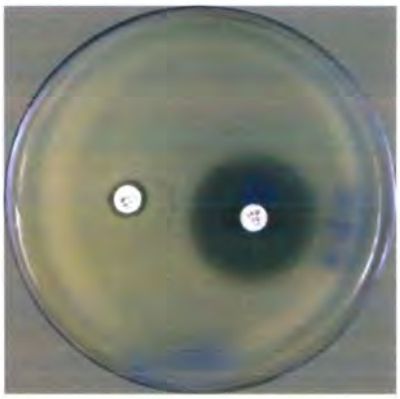

Figure 17 : positive result of MBL detection test.

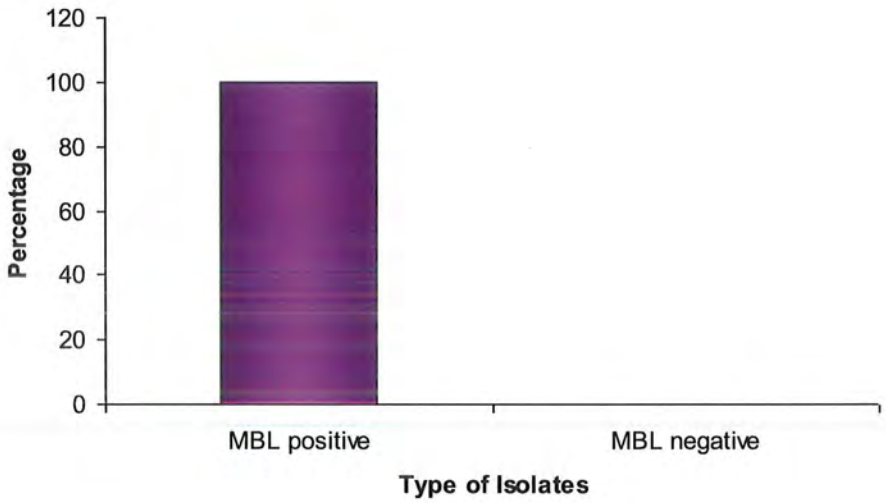

Figure 18: Prevalence of MBL producing *A. baumannii* among patients.

DETERMINATION OF Amp C β- LACTAMASE PRODUCTION

PREVALENCE OF Amp C β- LACTAMASE PRODUCING *A. BAUMANNII* AMONG PATIENTS

Cefoxitin resistance screening results showed that all 91 *A. baumannii* isolates were resistant to it. AmpC β-lactamase detection showed that 21 (23.07%) isolates were strongly positive, 17 (18.68%) isolates were weakly positive and 53 (58.24%) isolates were negative (Figure 19, 20) (Appendix Table 14). Different isolates have shown prevalence of different β-lactamases production. (Figure 21) (Appendix Table 15).

Figure 19: Positive result of AmpC detection test.

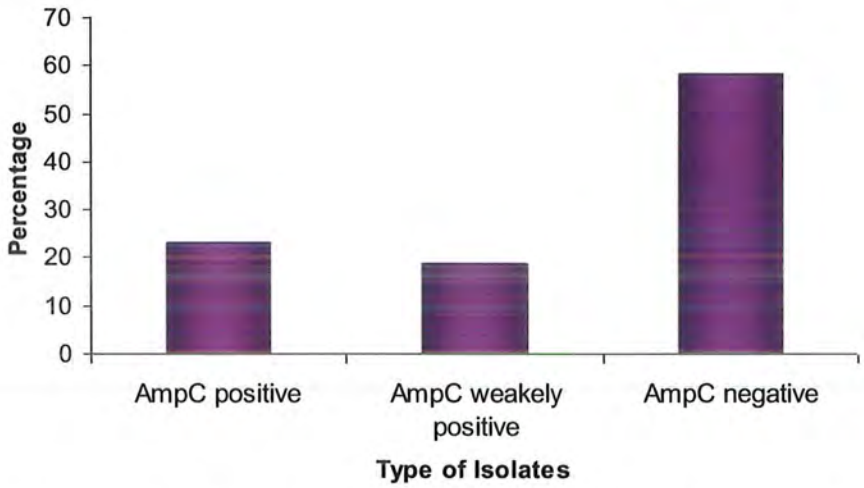

Figure 20: Prevalence of AmpC lactamase producing *A. baumannii* among patients.

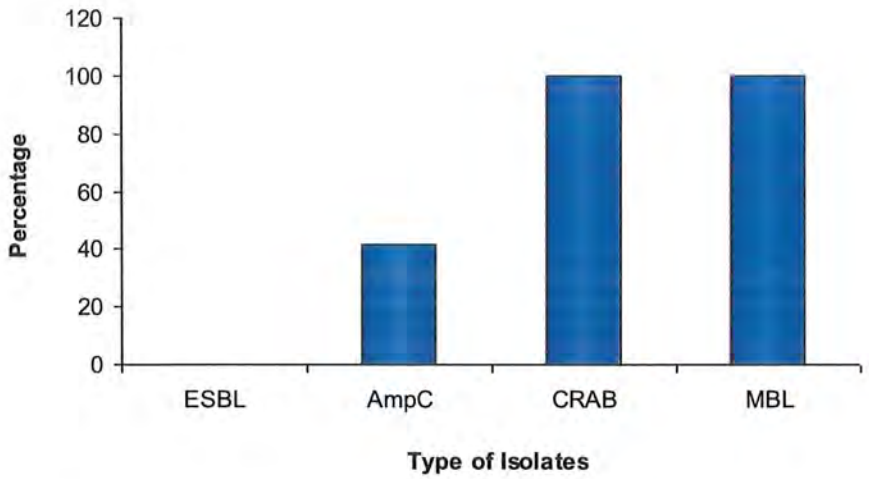

Figure 21: Prevalence of different β-lactamases producing *A. baumannii* among patients.

MIC DETERMINATION

MICs of ciprofloxacin and tetracycline were determined. MIC of ciprofloxacin was 64μg/ml to ≥512μg/ml. MICs for tetracycline range from 8 to 128μg/ml. Control plates were without any antibiotic dilutions, showed growth of all inoculated isolates. Different antibiotic dilution ranges inhibit the growth of inoculated isolates at different dilutions (Figure 22) (Appendix Table 16, 17).

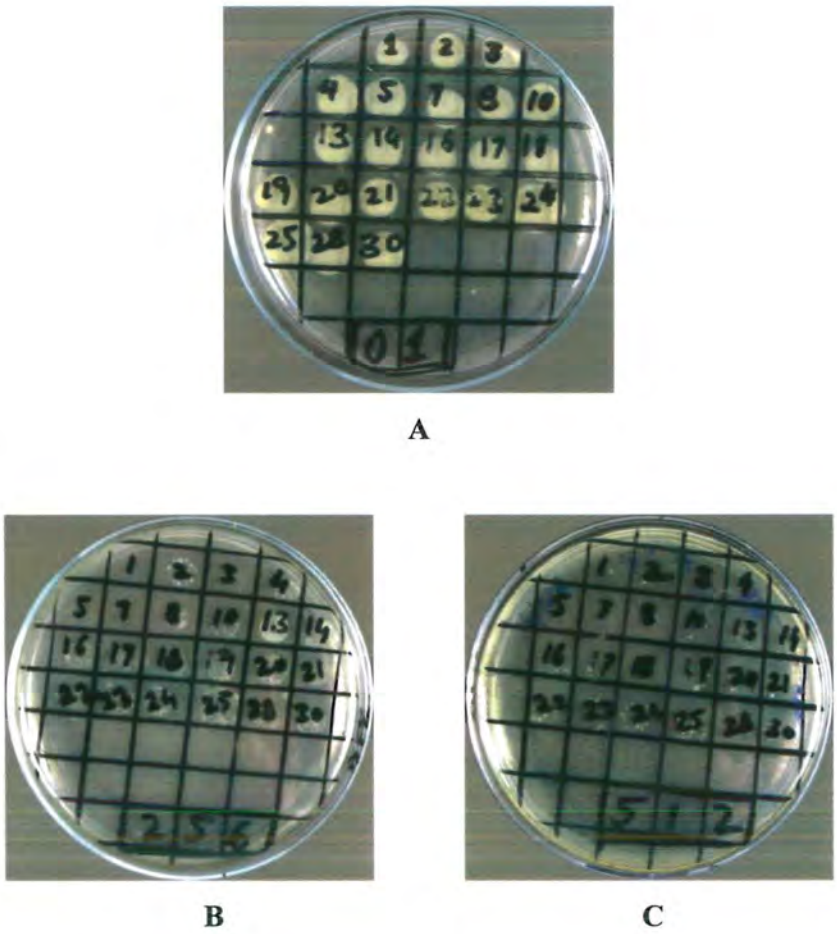

Figure 22: Agar dilution methods, MICs of ciprofloxacin and tetracycline antibiotic, A: Antibiotic free control plate having growth of all inoculated isolates. B: plate having antibiotic concentration 256µg/ml, C: plate having antibiotic concentration 512µg/ml.

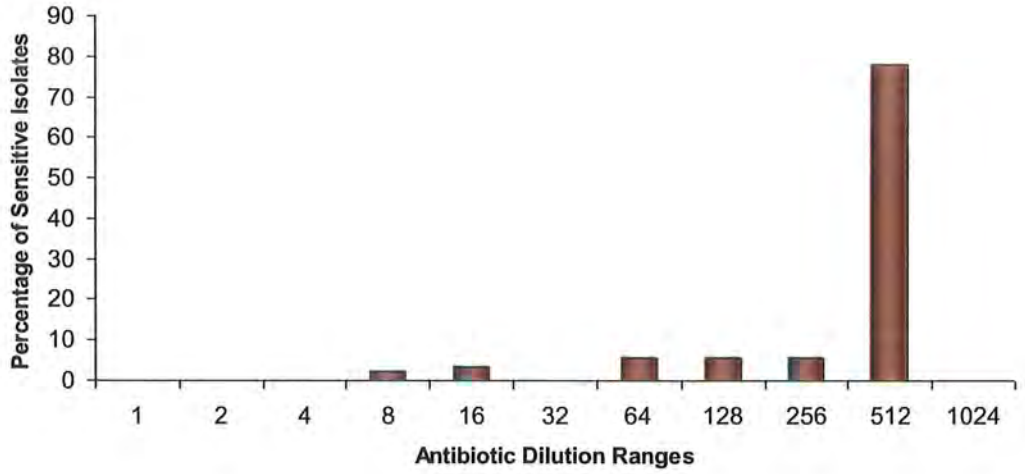

Figure 23: MICs of Ciprofoxacin.

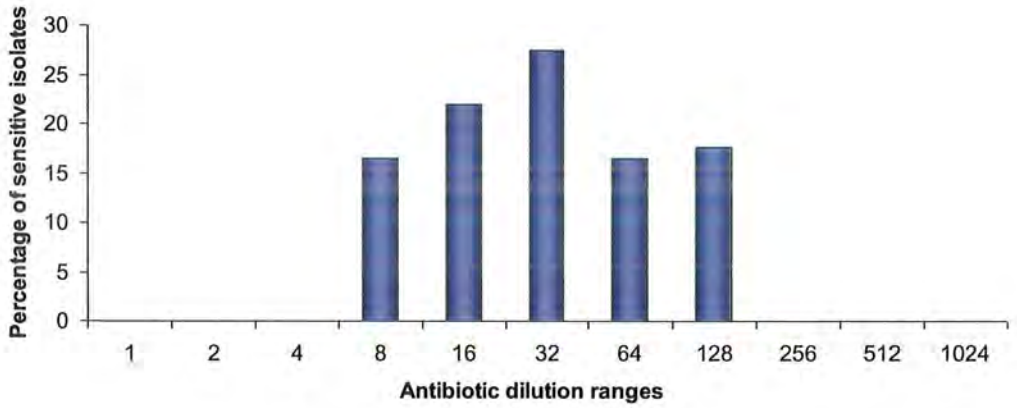

Figure 24: MICs of Tetracycline.

Discussion

The hospital environment is a potential reservoir of infectious agents since it houses both patients with diverse pathogenic microorganisms and a large number of susceptible/immunocompromised individuals (Rhomberg *et al.*, 2006; Zhanel *et al.*, 2008). The nosocomial pathogens that cause infections can come either from endogenous or exogenous sources. Endogenous sources are those that are from the patient's own microbial flora while the latter are from the surrounding hospital environment. A patient may be infected by his/her body flora following surgical manipulation, chemotherapy and diagnostic or therapeutic procedures which in most cases suppress the natural body defensive mechanisms (Pelczar *et al.*, 1993). Animate and inanimate sources of exogenous infections include hospital staff, other patients, visitors, food, water, formites, urinary catheter, intravenous devices, respiratory equipment and other prostheses (Prescott *et al.*, 2005). The most important means of transmission of nosocomial infections is by contact, usually directly but sometimes indirectly by means of secretions from the body (Bergogne-Berezin and Towner, 1996).

The present study was conducted to determine the prevalence of infections caused by multi drug resistant *A. baumannii* and to ascertain the various mechanisms of its resistances. These clinical isolates were collected from the patients admitted at different wards of Pakistan Institute of Medical Sciences (PIMS) from February 2010 to December 2010. In the present study *A. baumannii* was found to be most frequent cause of nosocomial infections. These results are in line with the findings of Bergogne-Berezin, (2005) and Henwood *et al.*, (2002).

In the present study infections due to *A. baumannii* were found to be more prevalent among new borns than adults that are in accordance with the finding of Regev *et al* (1993). *Acinetobacter* spp have emerged as particularly important organisms in intensive care units (ICUs), and this is probably related, at least in part, to the increasingly invasive diagnostic and therapeutic procedures used in hospital ICUs in recent years (Bergogne-Berezin and Towner, 1996). The proportion of newborns to adults' patients varies from study to study and area to area. The prevalence of infections due to *A. baumannii* was higher in the new born as compared to young and very old patients. Risk factors for acquisition of *Acinetobacter* spp include hospitalization, poor general medical status of patients, mechanical ventilation,

cardiovascular or respiratory failure, previous infection or antimicrobial therapy, and the presence of central venous or urinary catheters (Husni *et al.*, 1999). In our study, the highest prevalence of infections due to *A. baumannii* was observed in the ETT (endo tracheal tube) specimens (25.27%), followed by tracheal secretions (19.78%), and pus (16.48%). Shanthi and Sekar (2009) reported that most of the isolates of *A. baumannii* were obtained from the respiratory tract (41.8%) followed by urinary tract (25.5%), wound (20%) and blood (12.7%). The highest prevalence of infections was observed in the NICU (42.85%), followed by medical ICUs (19.78%) and emergency (9.89%). Using a national point prevalence study in US hospitals, Banerjee *et al* (2006) calculated the nosocomial infection prevalence to be 3.7 to 25.0 cases per 100 patients for PICUs and 2.7 to 23.8 cases per 100 patients for NICUs, with average prevalence rate of 14 cases per 100 patients in both settings.

The resistance patterns of *A. baumannii* towards various antimicrobial agents were determined by disc diffusion method. In the present study, *A. baumannii* exhibited the highest resistance 100% against cephalosporins, carbapanems, β-lactam inhibitors (Fig 11.12). These finding are in line with the study of Cisneros and Rodriguez, (2002) and Cisneros *et al.*, (2005). The specific issue of in vitro testing of β-lactam-β-lactamase inhibitor combinations has been assessed by Higgins *et al*. Higgins *et al* (2004) showed that in vitro results for β-lactam-β-lactamase inhibitor combinations against *A. baumannii* are determined mainly by the activity of the inhibitors alone and therefore influenced by whether a fixed ratio of β-lactam to inhibitor or a fixed concentration of inhibitor is used Among aminoglycosides amikacin showed 100% resistance, while tobramycin was found to be effective against *A. baumannii* with (39.6%) resistance. Among fluoroquinolones ciprofloxacin showed more resistance as compared to sparfloxacin. Resistance mechanisms that are expressed frequently in nosocomial strains of *acinetobacter* include β-lactamases, alterations in cell-wall channels (porins), and efflux pumps. *A. baumannii* can become resistant to quinolones through mutations in the genes gyrA and parC and can become resistant to aminoglycosides by expressing aminoglycoside-modifying enzymes (Bonomo and Szabo, 2006).

Polymyxins b showed (89%) resistance in this study. Appleman et al., (2000) reported that Polymyxins b and colistin remains the mainstay of treatment for multi-drug

resistant *Acinetobacter baumannii*. The alternative therapeutic strategies for MDR Acinetobacter baumannii include the use of rifampicin, travofloxcin, doxycycline, minocycline or tigecycline with sulbactam. Tetracyclines were found to be the most effective drugs with the lowest resistance, while tigecycline and minocycline were found to be most effective among all tetracyclines with 100% susceptibility against *A. baumannii* which is in accordance with study Jamal et al., (2009). Unlike the experience of Enoc et al., who reported persistence of *A. baumanni* till death or discharge, although tigecycline was used in only one of their patients.

CLSI (2007) recommends that MICs for antibiotics versus *Acinetobacter* spp. be determined in broth, using cation-adjusted Mueller-Hinton broth, or on agar, using Mueller-Hinton agar. Disk diffusion should also be performed using Mueller-Hinton agar. Swenson *et al.*, (2004) assessed these CLSI-recommended methods and identified several problems in testing β-lactam antibiotics. First, very small colonies or a star-like growth was frequently observed in wells containing high concentrations of β-lactam antibiotics. This apparent growth beyond a more obvious end point makes determining an MIC by broth micro dilution methods quite difficult. Second, there were many discrepancies between results obtained by broth micro dilution and those obtained by disk diffusion. In contrast to the findings with these β-lactams, there was little MIC and zone diameter discrepancy for carbapenems, aminoglycosides, fluoroquinolones, and trimethoprim-sulfamethoxazole.

Talbot *et al.*, (2006) reported the wide array of intrinsic and acquired resistance determinants that have emerged in *A. baumannii* have justifiably brought it great scientific attention. As determined by the Infectious Diseases Society of America, *A. baumannii* is one of the "red alert" pathogens that greatly threaten the utility of our current antibacterial armamentarium.

According to Bergogne and Towner, (1996) prior to the 1970s, it was possible to treat *Acinetobacter* infections with a range of antibiotics, including aminoglycosides, β-lactams, and tetracyclines. However, Landman *et al.*, (2002) and Falagas *et al.*, (2007) reported resistance to all known antibiotics has now emerged in *A. baumannii*, thus leaving the majority of today's clinicians in unfamiliar territory. Unfortunately, at this stage, very little is in the therapeutic pipeline, and the new agents with activity against gram-negative organisms are all modifications of existing classes. Novel

antibiotic targets and mechanisms of action are urgently required. Garcia-Garmendia *et al.*, (2001) reported, MDR *A. baumannii* infections tend to occur in immunosuppressed patients, in patients with serious underlying diseases, and in those subjected to invasive procedures and treated with broad-spectrum antibiotics. The frequency of *A. baumannii* isolates that were MDR in our study was 100% which is high as compared to the the study conducted by Lahiri *et al.*, (2004) who reported 69.7% isolates to be multi-drug-resistant.

Acinetobacter infections are often difficult to treat, because of the widespread antibiotic resistance. Further, these bacteria survive for a long time in the hospital environment, with enhanced opportunities for transmission between patients (Bergogne-Berezin and Towner, 1999). The transferable nature of ESBL in *Acinetobacter* may lead to increase in the basal level of multidrug resistance among other nosocomial pathogens by dissemination and integration of the R-plasmids (Joshi *et al.*, 2003). Vila *et al.*, (1993) reported frequency of ESBL production in *A. baumannii* recently. Aubert *et al.*, (2001) and Walther-Rasmussen and Hoiby (2006) reported Class D OXA beta-lactamases are usually robust penicillinases (oxacillinases). Some OXAs (i.e., OXA ESBLs) are also able to hydrolyze extended-spectrum cephalosporin. In our study no single isolates was found to be ESBL producer.

Thus far, carbapenems have been thought of as the agents of choice for serious *A. baumannii* infections. However, although these drugs are still active against the vast majority of *A. baumannii* strains worldwide, the clinical utility of this class of antimicrobial is increasingly being jeopardized by the emergence of both enzymatic and membrane-based mechanisms of resistance (Bou *et al.*, 2000; Quale *et al.*, 2003).

The increase in the number of metallo-beta-lactamases (MBLs) in *A. baumannii* is an ominous development in the global emergence of resistance to beta-lactams (Walsh, 2005). Rasmussen and Bush, (1997) reported, Carbapenem resistance can be due to acquired carbapenemase production. Carbapenemases include molecular class B metallo-β-lactamases (MBL), and A and D serine enzymes in our study, all isolates were carbapenem resistant. All were carbapenemase as well as MBLs positive suggesting that all were class B carbapenemases.. Class A and D carbapenemase-producing Gram-negative bacilli are less prevalent, but a significant proportion of

carbapenem resistance in *P. aeruginosa and Acinetobacter spp.* is due to the production of acquired MBL (Livermore and Woodford, 2000). Carbapenem resistance is mainly due to either reduced levels of drug accumulation or increased expression levels of the pump efflux (Livermore, 1992; Hancock, 1998). Other risk factors responsible for colonization and infection with MBL producers include age of patient, duration of hospitalization, underlying diseases like diabetes, tumours or overcrowding in the hospital wards (Chikere *et al.*, 2008).

Heritier et al.,(2005) and Quale et al., (2003) reported *acinetobacters* may develop resistance to carbapenems through various combined mechanisms, including AmpC stable depression, decreased permeability, altered penicillin-binding proteins (PBPs) and, rarely, efflux pump over expression. Resistance to extended-spectrum cephalosporin's owing to production of these enzymes appears to be rarer than up regulation of AmpC, although difficulties in detection in the presence of other modes of resistance mean that their true prevalence may be underestimated Peleg et al., (2008). The frequency of AmpC β-lactamase producing *A. baumannii* isolates in our study was 41.75%. The prevalence of AmpC β-lactamase producer was higher in new born in new natal intensive care unit. Poire and Nordmann, (2006) reported, AmpC β-lactamases are chromosomally encoded cephalosporinases intrinsic to all *A. baumannii.* Usually, such β-lactamases have a low level of expression that does not cause clinically appreciable resistance; however, the addition of a promoter insertion sequence, ISAba1, next to the AmpC gene increases β-lactamase production, causing treatment-limiting resistance to cephalosporins. Over expression of bacterial efflux pumps can decrease the concentration of β-lactam antibiotics in the periplasmic space. To cause clinical resistance in acinetobacter, efflux pumps usually act in association with over expression of AmpC β-lactamases or carbapenemases. In addition to removing β-lactam antibiotics, efflux pumps can actively expel quinolones, tetracyclines, chloramphenicol, disinfectants, and tigecycline (Peleg et al., 2007).

Bacteria possessing AmpC beta-lactamases are resistant to penicillins, beta-lactamase inhibitors, cefoxitin, cefotetan, ceftazidime, ceftriaxone, and cefotaxime. Aztreonam and cefepime are usually more active against bacteria possessing class C beta-lactamases. Imipenem is an inducer, it remains stable in the presence of increased AmpC production (resists hydrolysis) (Livermore *et al.*, 2004). The presence of

AmpC beta-lactamases in pathogens known not to have chromosomal AmpC genes (like *Klebsiella, Proteus* and *Salmonella*) eventually led to the discovery of plasmid-borne AmpC enzymes (Philippon *et al.*,1997).

The primary goals for the control of multidrug resistant *acinetobacter* infection are recognizing its presence in a hospital or long-term care facility at an early stage, controlling spread aggressively, and preventing the establishment of endemic strains. Control measures are based almost entirely on experiences from outbreaks of *acinetobacter* infection and generally address the organism's major epidemic modes of transmission and the excessive use of broad-spectrum antibiotics (Villegas and Hartstein, 2003).

Conclusions

CONCLUSIONS

- *A. baumannii* was isolated as a nosocomial pathogen from the different sites of hospital environment.
- According to susceptibility profile 100% *A. baumannii* isolates were multi-drug resistant.
- Resistance to beta lactams was due to the production of Carbapenemases/MBLs and AmpC beta-lactamases. No single isolate gave ESBL production.
- Co-production of Carbapenemases and MBLs was observed in 100% *A. baumannii* isolates.
- Different factors gender, age, hospital site, and infection area relates with the patients stay in hospital making himor her more prone to infection by *A. baumannii*
- All β-lactamases were found to be more common in new born babies in new natal intensive care unit.
- All β-lactamases production represents clinical threat due to their resistance to the therapeutic inhibitors.
- Minocycline and tigecycline was found to be the most effective drugs against *A. baumannii* infections.

Recommendations

Following recommendations are suggested;

- Preventive measures rather than curative measures i.e. good hygienic conditions, better hospital and post operative care should be ensured and regular clinical meetings and discussions should be organized to educate health care personnel and the patients.
- Important goals must include eradicating the source of the organism, preventing their spread and treatment of infections due to these resistant strains.
- Development of methods to prevent the formation of biofilm on indwelling medical devices may help in reducing the spread and resistance of particular bacteria in hospital settings.
- Changes in the antibiotic utilization policies have to be made only after careful consideration and their effects should be monitored under close microbiological monitoring.
- Appropriate use of antibiotics must do, that will delay and in many cases prevent the emergence of resistance.
- Over use of antibiotics should be avoided.
- Use of combined therapy may help in the treatment of infections due to resistant organisms.
- Introduction of new antimicrobial agents is required to meet the challenge.
- Increased frequency of Multi drug resistance needs for continuous surveillance to determine prevalence and evolution of these enzymes in Pakistan.

Future Prospects

- Determination of beta lactamase type genes through PCR to confirm the phenotypic beta-lactamases.
- Important goals include eradicating the source of the organism and preventing their spread and treatment of infections due to these resistant strains.
- Evaluation of different phenotypic methods as there is no standardized assay for detection of ESBLs in organisms other than *E. coli* and *K. pneumoniae*, MBLs and AmpC β-lactamases, recommended by CLSI. The discovery of second generation β-lactamase inhibitors is eagerly awaited.
- By molecular analysis of the enzymes, we can establish the standards for antibiotic regime and sensitivity patterns of the β-lactamase producing strains, which can help in better diagnosis and treatment.

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Appendices

The Composition of MacConkey agar medium

Agar	12.0 g
Lactose	10.0 g
Peptone	20.0 g
Bile salts	5.0 g
NaCl	5.0 g
Neutral red	0.075 g
Distilled water	1000 ml
pH	7.4

The Composition of *MHA medium*

Beef dehydrated infusion	30.0 g
Casein hydrolysate	17.5 g
Starch	1.5 g
Agar	17.0 g
Distilled water	1000 ml
pH	7.3

The Composition of Blood Agar Medium

Agar	15.0 g
Heart Muscle Infusion	2.0 g
Pancreatic digest of casein	13.0 g
Yeast Extract	5.0 g
NaCl	5.0 g
Blood	100ml
Distilled water	1000 ml
pH	7.3±0.2

Table 4: Prevalence of multidrug resistant *A. baumannii* in different specimens (N=91)

Sample	Number of Isolates	Percentage %
ETT*	23	25.27
Tracheal Secretion	18	19.78
Wound	3	3.30
Pus	15	16.48
Blood	14	15.38
Fluid	2	2.20
Catheter tip	6	6.59
Urine	2	2.20
tip of drain	7	7.69
CVP tip**	1	1.10

*Endotracheal tube tip **Central venous pressure tip

Table 5: Prevalence of multidrug resistant *A. baumannii* in different wards (N=91)

Type of Wards	Number	Percentage%
Peads intensive care unit (PICU)	2	2.20
Private medical ward (PMW)	1	1.10
New natal intensive care unit (NICU)	37	40.66
Out door patients (OPD)	3	3.30
Private ward first floor (PWFF)	2	2.20
Medical (ICU)	18	19.78
General Surgery	2	2.20
Surgical (ICU)	5	5.49
General Medicine	2	2.20
Emergency	9	9.89
Private ward second floor (PWSF)	3	3.30
Private ward ground floor (PWGF)	1	1.10
Surgical ward 6 (SW6)	2	2.20

Table 6: Prevalence of multidrug resistant *A. baumannii* in patients of different age groups (N=91)

Age Groups	Number	Percentage%
0-30 Days	37	40.66
1-11 Month	2	2.20
1-20 Years	14	15.38
20-40 Years	12	13.19
40-60 Years	17	18.68
60-80 Years	8	8.79

Table 7: Prevalence of multidrug resistant *A. baumannii* in patients of different Genders (N=91)

Gender	Number	Percentage%
Babies	37	40.66
Males	35	38.46
Females	19	20.88

Table 8:Prevalence of multidrug resistant *A. baumannii* in babies and adults(N=91)

Gender	Number	Percentage%
Babies	37	40.66
Adults	54	59.34

Table 9: Antibiotic susceptibility profile of *A. baumannii*

Antibiotics	Total	Resistant	Intermediate	Sensitive
AMC	91	91	0	0
CRO	91	91	0	0
CAZ	91	91	0	0
TOB	91	36	0	55
AK	91	91	0	0
CIP	91	91	0	0
SPX	91	76	0	15
TGC	91	0	0	91
MI	91	0	0	91
TE	91	60	0	31
DO	91	87	0	4
SAM	91	91	0	0
IPM	91	91	0	0
PB	91	68	13	10
TZP	91	91	0	0
SCF	91	91	0	0
SXT	91	91	0	0
RD	91	50	0	41

Table 10: Antibiotic resistance pattern of *A. baumannii*

Antibiotic	No. of Isolates	Percentage Resistance%
AMC	91	100
CRO	91	100
CAZ	91	100
TOB	36	39.6
AK	91	100
CIP	91	100
SPX	76	83.5
TGC	0	0
MI	0	0
TE	60	65.9
DO	87	95.6
SAM	91	100
IPM	91	100
PB	81	89
TZP	91	100
SCF	91	100
SXT	91	100
RD	50	54.9

Table 11: Prevalence of ESBL producing *A. baumannii* (N=91)

A. baumannii (N=91)	ESBL positive	ESBL negative
Number	0	91
Percentage	0	100

Table 12: Prevalence of Carbapenemase producing *A. baumannii* (N=91)

A. baumannii (N=91)	Carbapenemase positive	Carbapenemase negative
Number	91	0
Percentage	100	0

Table 13: Prevalence of MBL producing *A. baumannii* (N=91)

A. baumannii (N=91)	MBL positive	MBL negative
Number	91	0
Percentage	100	0

Table 14: Prevalence of AmpC β-lactamase producing *A. baumannii* (N=91)

A. baumannii (N=91)	AmpC positive	AmpC weakely positive	AmpC negative
Number	21	17	53
Percentage	23.08	18.68	58.24

Table 15: Prevalence of different β-lactamases production in *A. baumannii*

Test	ESBL	AmpC	CRAB	MBL
Results	0	38	91	91
%age	0.00	41.76	100.00	100.00

Table 16: MICs Breakpoints (CLSI.2010)

(µg/ml)

Sr. #	Antimicrobial agent	R	S
1	Ciprofloxacin	≥4	≤1
2	Tetracycline	≥16	≤4

Table 17: MICs of β-lactamase producing *A. baumannii*

S.#	ID#	Ciprofloxacin	Tetracycline
1	1A	512	8
2	2A	16	16
3	3A	8	8
4	4A	128	16
5	5A	64	16
6	7A	512	8
7	8A	128	64
8	10A	16	16
9	13A	8	64
10	14A	128	8
11	16A	512	16
12	17A	64	64
13	18A	512	8
14	19A	128	16
15	20A	16	32
16	21A	512	8
17	22A	128	32
18	23A	64	16
19	24A	512	8
20	25A	512	32
21	28A	256	16
22	30A	512	8
23	1M	512	64
24	2M	512	16
25	4M	512	64
26	5M	512	32
27	6M	512	64
28	7M	512	16
29	8M	512	8
30	10M	512	32
31	11M	512	64
32	15M	512	16
33	17M	512	32
34	20M	64	8
35	21M	256	64
36	22M	512	128
37	23M	512	64
38	24M	512	64
39	25M	512	32
40	26M	512	8
41	27M	256	128
42	28M	512	16
43	31M	512	128
44	32M	512	32
45	33M	512	128
46	34M	512	8

47	35M	256	16
48	36M	64	32
49	1J	512	128
50	2J	512	16
51	3J	512	128
52	4J	512	32
53	5J	512	128
54	6J	512	8
55	7J	256	16
56	8J	512	128
57	9J	512	128
58	10J	512	128
59	11J	512	16
60	12J	512	128
61	13J	512	8
62	14J	512	128
63	15J	512	16
64	16J	512	128
65	17J	512	128
66	18J	512	16
67	19J	512	32
68	20J	512	128
69	22J	512	16
70	23J	512	32
71	24J	512	128
72	25J	512	8
73	26J	512	32
74	27J	512	16
75	28J	512	64
76	29J	512	32
77	30J	512	64
78	1JU	512	64
79	2JU	512	64
80	3JU	512	64
81	4JU	512	32
82	5JU	512	32
83	6JU	512	32
84	7JU	512	32
85	8JU	512	32
86	9JU	512	32
87	10JU	512	32
88	11JU	512	32
89	12JU	512	32
90	13JU	512	32
91	14JU	512	32