Clonality of MDR/XDR *Acinetobacter baumannii*

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfilment of the requirements for the degree of Bachelor of Science in Microbiology

Department of Mathematics and Natural Sciences
BRAC University
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Declaration

It is hereby declared that

1. The thesis submitted is our own original work while completing a degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. We have acknowledged all main sources of help.

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Ethics Statement

The study was conducted following the Helsinki Declaration and subsequent revisions.

Abstract

The rapid expansion of multi drug-resistant (MDR) and extremely drug-resistant (XDR) *Acinetobacter baumannii* over the last few decades is a rising health concern. This study evaluated the efficacy of last-line antibiotics against *Acinetobacter baumannii* isolates collected from Birdem Hospital, Dhaka, Bangladesh. Current study analyzed data from 50 confirmed *A. baumannii* isolates collected between October-November 2024. *A. baumannii* were cultured on selective media to isolate pure colonies at 44°C and the antibiograms for carbapenem drugs including colistin and others were determined by Vitek®2 along with traditional Kirby-Bauer disk diffusion method. Among the 50 isolates, 60% (30) were from female patients and 40% (20) from males. A significant number of samples (40%) were collected from ICU patients. Among the patients, 90% (45) were diabetic and 5% (5) were non-diabetic. Ventilator-associated pneumonia (38%) was the most common infection, followed by chronic kidney disease (32%) and acute kidney infection (26%). Out of the 50 isolates tested, 88% were identified as MDR, while 14% were classified as XDR. The prevalence of XDR and MDR *A. baumannii* strains were influenced by hyperglycemia. Also, the majority (44%) of the age group in this study was 51-70, which is a very vulnerable age group in terms of immune response. However, the second highest (34%) age group was 31-50 years which was alarming because it denoteed the severity of AMR among different age groups. The findings emphasized the urgent need for antimicrobial stewardship programs and alternative treatment strategies to combat MDR/XDR *A. baumannii*. Ongoing surveillance and research on resistance mechanisms are crucial for guiding clinical and policy decisions.

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List of Acronyms

Acronyms	**Full Form**
MDR	Multi Drug Resistant
XDR	Extensively Drug Resistant
ICU	Intensive Care Unit
CRAB	Carbapenem Resistant *Acinetobacter Baumannii*
HAI	Healthcare Associated Infections
WHO	World Health Organization
TE	Tris EDTA
AKI	Acute Kidney Infection
CKD	Chronic Kidney Disease
PPN	Piperacillin
CEF	Ceftazidime
CES	Cefepime
CFP	Cefoperazone/Sulbactam
IMP	Imipenem
MRP	Meropenem
AMK	Amikacin
GEN	Gentamicin
CIP	Ciprofloxacin
COL	Colistin
TSL	Tigecycline
IC	International Clone
ST	Sequence Type

CHAPTER 1

1. Introduction

Acinetobacter baumannii is an important opportunistic pathogen that is rapidly evolving toward multidrug resistance and is involved in various nosocomial infections that are often severe. It is a type of bacteria that can cause severe infections in hospitals. These infections are called nosocomial infections. Pneumonia, bacteraemia, skin infections, urinary tract infections, meningitis and wound infections are the symptoms of nosocomial infection.

A. baumannii is a gram-negative bacterium that often causes infections in hospitals. It can live in tough conditions, survive without moisture, and quickly develop resistance to many antibiotics, which makes it very difficult to treat in healthcare settings. *A. baumannii* has emerged as a major concern in hospital environments, particularly in intensive care units where it often infects patients with compromised immune systems. Its ability to withstand treatment is alarming, as it shows resistance to multiple classes of antibiotics, including carbapenems, which has earned it the reputation of a dangerous "superbug." Clinically, it is linked to a variety of serious infections, ranging from pneumonia and septicaemia to urinary tract infections, wound complications, and meningitis. Another factor that makes this pathogen especially problematic is its remarkable capacity to survive for long periods on hospital surfaces, which greatly enhances its potential for spreading within healthcare facilities.

A. baumannii is an opportunistic pathogen affecting both humans and animals, representing a significant One Health concern by linking environmental, veterinary, and human health domains. It demonstrates a high capacity for rapid acquisition of antimicrobial resistance, including to carbapenems. Within healthcare environments, particularly intensive care settings, it is a leading cause of severe infections. Additionally, its presence in veterinary contexts highlights how antimicrobial usage in animals can contribute to resistance patterns with direct implications for human health. *A. baumannii* exhibits extensive resistance to multiple antimicrobial agents, notably β-lactams, quinolones, and carbapenems, posing major challenges in clinical therapy. The organism demonstrates resistance to commonly used β-lactams, including piperacillin and ceftazidime, as well as to broad-spectrum carbapenems such as imipenem, meropenem, and doripenem. Furthermore, resistance extends to additional drug classes, including aminoglycosides, tetracyclines, and colistin. (Howard *et al.*, 2012).

1.1 Impact of *A. baumannii* on Diabetes

A. baumannii is a significant concern for diabetic patients, as they are considered more susceptible to infections caused by this bacteria due to their compromised immune system, which can lead to more severe infections and higher mortality rates compared to non-diabetic individuals; research shows that diabetic patients are at a higher risk of acquiring *A. baumannii* infections, particularly the carbapenem-resistant strains, due to their impaired immune response and difficulty in managing the infection with limited antibiotic options. Patients with diabetes are at higher risk of *A. baumannii* infections due to weakened immune function, poor wound healing, and frequent hospital visits. This bacterium can cause severe and difficult-to-treat infections in diabetic patients, often leading to complications.

1.1.1 Why are diabetes patients more vulnerable?

1. **Compromised Immune System**: The functioning of white blood cells is weakened by elevated blood sugar so reducing the infection-fighting ability in the body.
2. **Impaired Wound Healing**: Because the healing rate is slowed down by diabetes, bacterial infection is usual for diabetic ulcers and for postoperative wounds.
3. **Hospitalization**: Diabetic patients shall most likely receive hospital treatment, thus exposing them to the risk of acquiring nosocomial infection.
4. **Peripheral neuropathy:** Nerve damage lowers pain perception, so you may not feel pain if you get an infection in the foot or some other location you cannot see.

1.1.2 Common Infections in Diabetic Patients

Diabetic Foot Infections: A. baumannii may cause osteomyelitis or cellulitis by colonizing diabetic foot ulcers.

Sepsis (Bloodstream Infections): Sepsis is a deadly infection caused by bacteria.

Pneumonia: Diabetics are frequent recipients of hospital stays for ventilator-associated pneumonia.

Urinary Tract Infections (UTIs): As high sugar in the urine is conducive to the growth of bacteria, urinary tract infections are more prevalent among diabetics.

Wound Infections: Infection by A. baumannii is more prevalent in diabetic patient surgical wounds.

1.1.3 Complications in Diabetic Patients

Increased Antibiotic Resistance: Multidrug-resistant (MDR) illness occurs among diabetics who are repeatedly receiving antibiotic treatment.

Gangrene & Amputation: Diabetic foot infection can result in death of tissues for which even amputation is performed.

Septic Shock: Untreated, bloodstream infection may result in multiorgan dysfunction

1.2 Sources of *Acinetobacter baumannii*

A.baumannii can be found in soil. It can also be found in water. It can be found in animals. It can be found in the skin and throat of humans. It is often found in hospitals, especially intensive care units (ICUs).

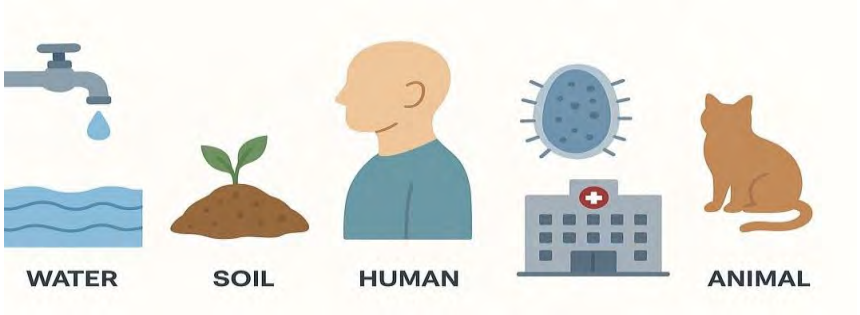

Figure 1.1: Sources of *A. baumannii*

1.3 Clonality of *Acinetobacter baumannii*

It implies the state of a cell or a substance being derived from one source or the other. Thus, there are terms like polyclonal that derived from many clones, oligoclonal that come from a few clones, and monoclonal that result from one clone. Such terms are generally applied when discussing antibiotics and immune cells.

Clonality plays an important role in studying and controlling *A. baumannii* infections, as it shows how certain genetically related strains circulate in hospitals. These strains are frequently linked to strong antibiotic resistance, making them capable of causing large outbreaks and serious complications through the fast spread of resistant variants.

This bacterium, *A. baumannii*, shows extensive drug resistance and tends to form highly similar clonal groups. It is a common cause of infections in hospitals, especially in intensive care units, with dominant global clones facilitating its persistence and resistance to treatment.

1.3.1 Assessment of clonality

1. **Multilocus Sequence Typing (MLST)**

   MLST is widely applied to classify *A. baumannii* strains according to their sequence types. The most frequently observed types are ST1 (Sequigecycline) (IC1), ST2 (IC2), and ST3 (IC3). Around the world, additional sequence types, including ST25, ST79, and ST85, have started to appear.

2. **ICs (International Clones) & GCs (Global Clones)**

   Foreign & World Clones the majority of *A. baumannii* strains are classified into several main lineages, including IC1, IC2, and IC3. Of these, IC2 (also referred to as GC2) is the most widespread and is frequently connected to resistance against carbapenem-type drugs.

3. **PFGE (pulsed-field gel electrophoresis) and WGS (whole genome sequencing):**

   Clonal linkage among isolates upon the occurrence of localized outbreaks can be easily identified by PFGE.

   WGS is capable of providing high-resolution typing and is competent to differentiate resistance genes, thus potentially aiding large-scale epidemiological surveys.

4. **Antibiotic Resistance and Clonality**

   Since OXA-type carbapenems (OXA-23, OXA-24/40, OXA-58, OXA-235, and OXA-143) occur in various clones, especially IC2, they are associated with carbapenem-resistant *A. baumannii* (CRAB). They encompass PER, VIM, IMP, and NDM (New Delhi Metallo-β-lactamase) resistance genes for certain available clones.

5. **World-Wide Spread and Pandemics**

   Spread is attained by patient-to-patient transmission, by contaminated equipment, and hospital facilities. MDR and XDR *A. baumannii* outbreaks are generally related to extensive world-wide spread of clones

1.3.2 RAPD

The principle of Random Amplified Polymorphic DNA (RAPD) is a Polymerase Chain Reaction (PCR) based technique that uses single, short, arbitrary primers which amplify random DNA segments from a template DNA. In conventional PCR, primers are carefully designed according to a known DNA sequence which can only bind to its specific complementary sequence. On the other hand, RAPD uses a single arbitrary primer limiting from 8-12 nucleotides which can easily anneal at multiple locations of the genome where even partial complementarity is found. (Williams et al., 1990).

In RAPD, amplification occurs only when two primer sites are positioned on opposite DNA strands and are oriented towards one another having a distance which is short enough to be amplified under standard PCR conditions. This results in the production of a diverse set of DNA fragments which are of different sizes. When these amplified fragments are separated on an agarose gel, they form a distinct banding pattern which acts as a genetic fingerprint that is unique to each individual or strain. (Welsh & McClelland, 1990).

RAPD is particularly suitable to use because of its sensitivity to even minor genetic variations. A single base mutation, insertion or deletion at a primer binding site can prevent the primer from attaching which in turn eliminates a band from the fingerprint. Similarly, if a new site emerges, an additional band may appear which may have not appeared before. In this way, RAPD can find out the polymorphisms across individuals, populations or closely related species without the need for prior knowledge of the sequence. (Williams et al., 1990; Welsh & McClelland, 1990).

Fundamentally RAPD works like a broad genetic survey. Instead of focusing on one known gene it scans the whole genome in a random way which produces patterns that reflects the hidden genetic differences. Which means RAPD can identify variations even for species with insufficient DNA information. Its randomness, low cost and simple procedure have made it especially useful in studies of genetic diversity, plant and animal classification, and evolutionary research. (Nybom, 2004).

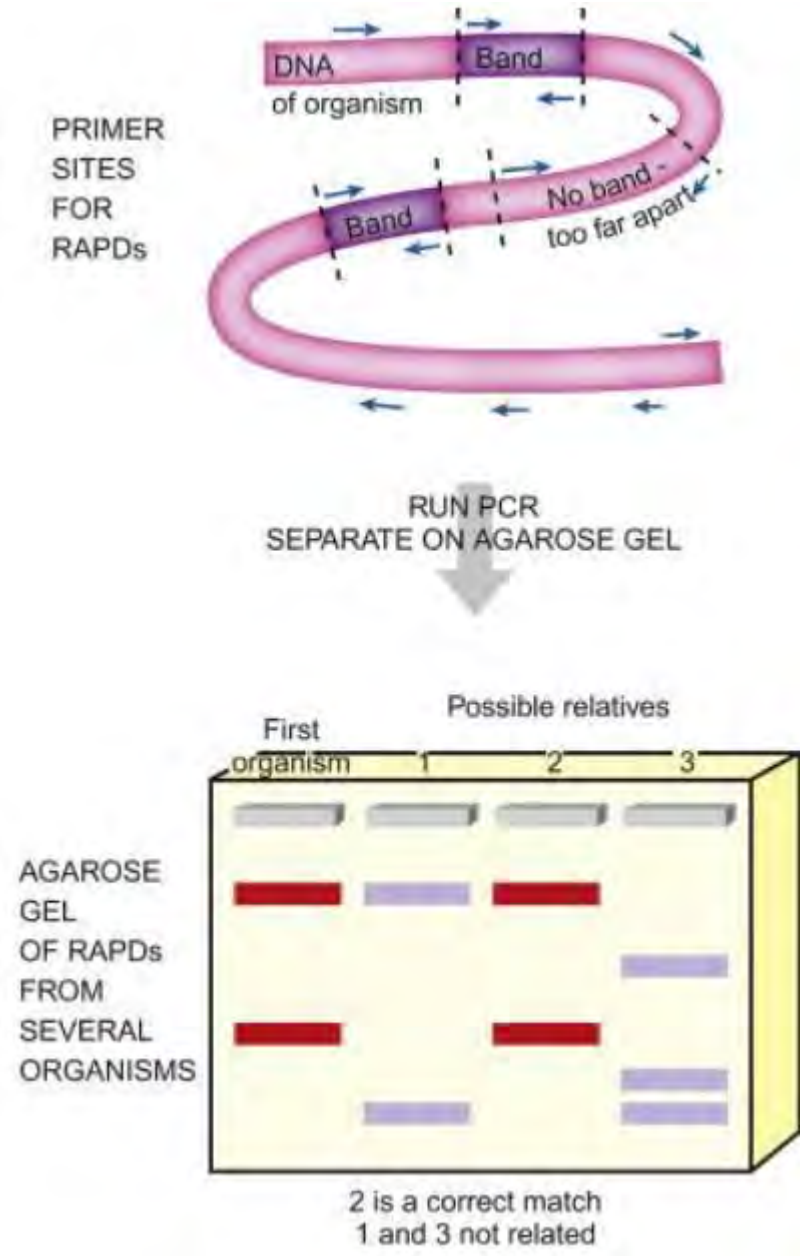

Figure 1.2: Visual representation of RAPD.

1.4 Transmission dynamics

Finding the infection's origin and possible channels of transmission within a medical facility are made easier with an understanding of the clonal structure. *A. baumannii* is a major cause of nosocomial (hospital-acquired) infections, particularly in critically ill patients. It spreads easily in healthcare settings due to its ability to survive on surfaces and resist antibiotics.

1. Common Routes of Transmission

- Hospital Surfaces & Equipment: It can persist on ventilators, catheters, IV lines, bedrails, and medical instruments.

- Healthcare Workers' Hands: Contaminated hands of doctors, nurses, or staff can transfer the bacteria to patients.
- Patient-to-Patient Transmission: Can spread through direct contact, often in intensive care units (ICUs). (Centers for Disease Control and Prevention, 2025)

2. Risk Factors for Infection
   - Prolonged Hospital Stay: The longer a patient stays, the higher the risk of exposure.
   - Ventilator Use: Leads to ventilator-associated pneumonia (VAP).
   - Urinary Catheters: Can cause urinary tract infections (UTIs).
   - Open Wounds or Surgery: Increases the risk of wound infections or surgical site infections.
   - Weakened Immune System: Critically ill, elderly, or immunocompromised patients are most vulnerable (Sikora, 2023)
3. Types of Infections Caused
   - Ventilator-Associated Pneumonia (VAP): A serious lung infection in intubated patients.
   - Sepsis (Bloodstream Infections): Life-threatening condition when bacteria enter the bloodstream.
   - Wound Infections: Common in burn patients or after surgery.
   - Meningitis: Rare but can occur after neurosurgery.

1.5 Epidemiology of *Acinetobacter Baumannii*

Among the many pathogens responsible for hospital-acquired infections *A. baumannii* has gained particular attention in recent years. It is a Gram-negative bacterium which is often described as an opportunistic pathogen and has been associated with conditions such as ventilator-associated pneumonia, wound and bloodstream infections, urinary tract infections (Antunes et al., 2014). What makes this organism especially concerning is not simply its ability to infect but the fact that it is increasingly detected in MDR and XDR forms which creates enormous challenges for clinicians particularly in intensive care units (ICUs) where patients are already in a fragile state (Howard et al., 2012).

Transmission most often occurs through contaminated hospital equipment, surfaces or even the hands of healthcare staff making strict hygiene and infection control protocols vital to prevention. Unlike many other bacteria, *A. baumannii* can survive for extended periods of time on dry surfaces and under harsh environmental conditions which explains its persistence and

recurring outbreaks in healthcare settings (Dijkshoorn et al., 2007). The situation is particularly severe in resource-limited countries where weak infection control systems and frequent misuse of antibiotics create an environment suitable for this pathogen to spread easily (Villar et al., 2020).

From a microbiological perspective, the bacterium is extremely adaptable. It can quickly acquire resistance genes through horizontal gene transfer. In addition, it uses mechanisms such as efflux pumps and enzymatic degradation. These traits have severely restricted available treatment options (Wong et al., 2017). As a result, developing new therapeutic approaches and optimizing existing strategies have become urgent priorities for researchers and clinicians.

1.6 Prevalence of *Acinetobacter baumannii*

Globally, *A. baumannii* has been recognized as one of the major causes of hospital-acquired infections. It is frequently reported in patients undergoing long-term hospitalization, especially those requiring invasive procedures or mechanical ventilation (Antunes et al., 2014). Data from multiple regions, including Southeast Asia, Europe, and North America, reveal that the prevalence of MDR and XDR strains continues to rise steadily (Perez et al., 2007).

A particularly alarming development is the growing presence of carbapenem-resistant *A. baumannii* (CRAB). In some countries, CRAB infections have been documented in as many as 50% of ICU patients, making it one of the most urgent antibiotic resistance threats in modern healthcare (Boucher et al., 2009).

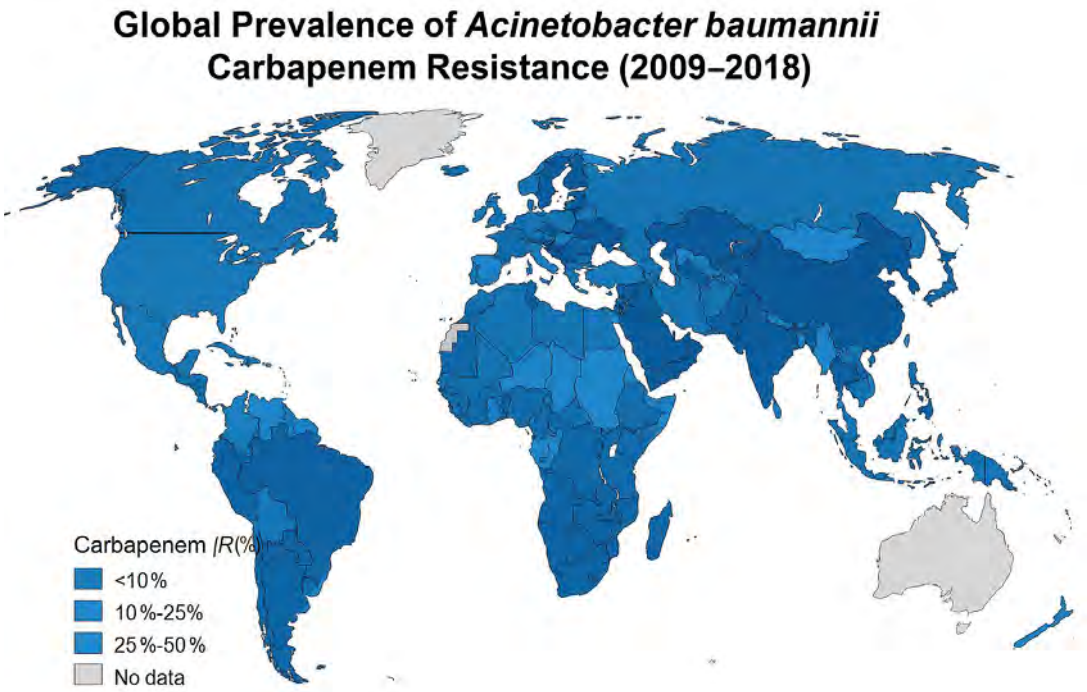

Figure 1.3: Prevalence map of CRAB in 2009-2018.

1.7 Prevalence of *A. baumannii* Nosocomial Infections

Nosocomial infections caused by *A. baumannii* account for a considerable proportion of hospital-associated illnesses worldwide. The impact is felt most strongly in ICUs and among immunocompromised patients who barely have the ability to withstand infections (Munoz-Price & Weinstein, 2008). The prevalence is even higher in under-resourced healthcare systems where sterilization practices and infection prevention measures are often insufficient (Villar et al., 2020).

Part of the difficulty lies in the organism's resilience. *A. baumannii* is not only resistant to multiple classes of antibiotics but also has the ability to persist on hospital surfaces despite the use of disinfectants. This capacity to remain viable for long periods explains the frequent recurrence of outbreaks in healthcare facilities (Davis et al., 2005).

1.8 Antimicrobial resistance of different microbes in comparison to *Acinetobacter baumannii:*

Table 1.1: Comparison table of antimicrobial resistance.

Antibiotics **Bacterial species**	**Beta lactams (penicillins)**	**Carbapenems**	**Aminoglycosides**	**Fluoroquinolones**	**Polymyxins(colistin)**	**References** **(In APA)**
Acinetobacter baumannii	High resistance	High	Variable	High	Emerging resistance	(Peleg et al., 2008; Doi et al., 2015; Tängdén & Giske, 2015)
Escherichia coli	Variable	Rare	Low	Increasing	Rare	(Pitout & Laupland, 2008; Logan & Weinstein, 2017)
Klebsiella Pneumoniae	High	Increasing	Variable	Increasing	Rare but reported	(Nordmann et al., 2009; Lee et al., 2016)
Pseudomonas aeruginosa	Variable	Increasing	Variable	High	Rare but emerging	(Oliver et al., 2015; Zilberberg et al., 2019)
Staphylococcus aureus	MRSA resistant to beta lactams	N/A	Variable	Variable	Intrinsically resistant	(Chambers & DeLeo, 2009)
Enterococcus Faecium	Intrinsically resistant	N/A	Variable	Variable	Intrinsically resistant	(Arias & Murray, 2012)
Streptococcus pneumoniae	Increasing resistance	N/A	Low	Increasing	Rare	(Lynch & Zhanel, 2009)
Neisseria gonorrhoeae	Increasing resistance	Rare	Low	High	Rare but emerging	(Unemo & Shafer, 2014)

1.9 Resistance of *A. baumannii* causing Nosocomial Infections in Bangladesh

The public health challenge posed by *A. baumannii* is especially evident in Bangladesh, where it has emerged as one of the most common hospital-associated pathogens. It is particularly widespread in ICUs where critically ill patients are most vulnerable. A genomic study of 82 isolates from Bangladeshi hospitals revealed that nearly all belonged to multidrug-resistant strains, underscoring the seriousness of the problem (Saha et al., 2023). Another investigation confirmed its status as a leading nosocomial pathogen, with extensively drug-resistant variants being frequently reported (Saha et al., 2021).

Alarmingly, clinical samples from the country demonstrate resistance to carbapenems, aminoglycosides and fluoroquinolones which significantly limits therapeutic choices (Rahman et al., 2020). Over-prescription and misuse of antibiotics have accelerated this resistance pattern (Islam et al., 2021). Supporting this concern, research from tertiary hospitals in Dhaka reported that more than 70% of the isolates were resistant to carbapenems (Haque et al., 2021). These findings not only highlighted the gravity of the crisis but also stressed the urgent need for robust antibiotic stewardship programs and stricter infection control practices across the healthcare system.

1.10 Mechanisms of Resistance

Acinetobacter baumannii is a nosocomial pathogen with extensively drug-resistant strains. Its resistance mechanism is diverse as it keeps altering time to time for adaptability. As a result it gets difficult to find a treatment in time for them. Their primary resistance mechanism includes:

Enzymatic Degradation: They produce enzymes like beta lactamases and metallo beta lactamases which provide them resistance to antibiotics like Carbapenems and other beta lactam antibiotics. (Nowak & Paluchowska, 2016). The widespread presence of OXA type carbapenemases, more specifically the presence of OXA-23, OXA-24/40, OXA-58 is the key reason for carbapenem resistance worldwide. (Evans & Amyes, 2014).

Efflux Pumps: The overexpression of the efflux pump system reduces the intracellular concentration of antibiotics making them more resistant to multiple drugs. (Bonomo & Szabo, 2006). These pumps contribute in building resistance against a broad range of antibiotics which includes fluoroquinolones and tetracyclines. (Coyne et al., 2011).

Porin Loss and Membrane Modification: Mutation in the proteins of outer membrane such as CarO decreases the permeability of the antibiotic preventing the effective drug intake. (Peleg et al., 2008). Moreover the modification or alteration of lipopolysaccharides makes colistin resistant. Which contributes to limiting the treatment options even more. (Moffat et al., 2010)

Biofilm Formation: Biofilms protect *A. baumannii* from host immune response and antibiotics which makes it difficult to eradicate the infection. (Gaddy & Actis, 2009). This resistance mechanism is a matter of worry for clinical settings as it can cause device-oriented infection from hospital ventilators and catheters. (Harding et al., 2018).

1.11 Global perspectives

Globally, *A. baumannii* is one of the leading causes of Healthcare Associated Infections (HAIs) particularly in the countries lacking infection control practices. The World Health Organization (WHO) has recognized *A. baumannii* as a priority pathogen which needs immediate attention for new antibiotics. (WHO, 2017). The prevalence of carbapenem-resistant *A. baumannii* (CRAB) is alarmingly high in regions such as the Middle East, Latin America, and parts of Europe. (CDC, 2020). In the United States approximately 2% of all hospital acquired infections are caused by *A. baumannii* having XDR or MDR strains. (Spellberg & Rex, 2013).

1.12 Treatment Strategies

A. baumannii is one of the most infectious pathogens which has the capability to cause a wide range of infectious diseases especially in immunity compromised individuals. Finding a suitable treatment that can be consistent for this particular pathogen is difficult because of its capacity of multi-drug resistance. Treatment choices can be influenced by knowledge about the most common clonal lineages, since some clonal groups may be more vulnerable to particular antibiotics. Treatment strategies that can be implied based on the susceptibility profile of the isolates are:

A. **Empirical Therapy**: Empirical therapy is the kind of treatment which is given before the exact diagnosis based on prior experience and guesses. Broad spectrum antibiotics like Carbapenems are given before receiving the antibiotic susceptibility test results. However, this line of treatment is not much effective anymore due to the emergence of Carbapenem resistant strains.
B. **Definitive Therapy:** Definitive Therapy is the kind of therapy that is implemented once the susceptibility test profile is in hands. This therapy can be designed as-

I. **Sulbactam based:** Sulbactam is a kind of medication which works as beta-lactamase inhibitors. Beta lactamase are enzymes that are produced by bacteria to be multi drug resistant against beta lactam antibiotics. Sulbactam is effective alone and in combination with other antibiotics like ampicillin.

II. **Tigecycline:** This antibiotic is utilized for *A. baumanni* with multiple drug resistance but its effectiveness depends on the infection site.

III. **Polymyxin**: Polymyxin and Colistin are a class of antibiotics that are used as the last resort of treatment for the Gram-Negative bacteria. Although they have a nephrotoxic effect, they are currently used as the backbone for multi-drug-resistant *A. baumannii.*

C. Combination of Therapies: Combination of antibiotics can increase the efficacy of treatment. Like sulbactam in combination with other antibiotics is more efficient.

D. Emerging Therapies: Much research is ongoing regarding the nontraditional approach of treatment like the Phage Therapy. Approaches like Phage Shearing have been effective in impairing bacterial revolutionary mechanisms to increase antimicrobial susceptibility.

1.13 Implications and future directions

The increasing prevalence of MDR and XDR strains of *A.baumannii* needs major attention. Improvement in antimicrobial surveillance, strict infection control measures and investment for new antibiotics has become crucial to address this global threat. (Doi et al., 2015). Moreover, development of other treatment strategies like immunotherapy, bacteriophage therapy, antimicrobial peptides can help in future clinical applications. (Asif et at., 2018). Recent advancement in genome sequencing and transcriptomic studies offers new insights into the resistance mechanism by potentially aiding the targeted therapeutic treatment strategy. (Wright et al., 2021).

1.14 Aims and objectives:

The main objective of this research is to study the clonality of the threatening *A. baumannii present* in Bangladesh locally. To specify our aim is:

1. To identify the strains of *A. baumannii* that are locally circulating in Bangladesh.

2. To look into the clonality and severity of infection in diabetic and non-diabetic patients.

3. To identify genetic differences or variation among the local strains using molecular typing method in Bangladesh.

CHAPTER 2

2. Methods and Materials

2.1 Study Design

This study was conducted with 50 samples from distinct patients from BIRDEM general hospital between October and November 2024. Entirety of the samples were confirmed diagnoses of *A.baumannii.* The study area is Dhaka, the urban capital of Bangladesh. It is located in the center of the country and is the largest city of the country. According to the World population review website, the total population of Dhaka is 24,652,900 as of 2025. Data were gathered from BIRDEM general hospital, the region's tertiary hospital; the hospital has 650-bed capacity in its hospital complex. The hospital also manages a large outpatient department (OPD) where approximately 3,000 patients are treated daily, making it a major center for diabetes care in Bangladesh (https://birdembd.org/)

2.2 Data Collection

All patients who were tested positive for *A. baumannii* were included in the study. The clinical diagnostic data and AST data were collected from the patients and the hospital database with consent from every sector. Those who did not meet the inclusion criteria, as well as those who had duplicate findings for the same patient were omitted. Data were obtained and analyzed retrospectively from patient file records and test findings. The primary data, which included age, gender, specimen type, and antibiotic susceptibility profile, were obtained from laboratory reports. Furthermore, the clinical data were collected from patient, patients' family members and confirmed by experienced medical staff.

2.3 Consent and Ethical Issues

The parents of the participants were fully informed about the study's purpose and procedures. Written consent was obtained from each patient's guardian by signing the consent form. Demographic and clinical data were collected directly from the participants' records in the presence of attending healthcare professionals. Additionally, maternal history was documented using a structured questionnaire. The study received approval from the International Review Board (IRB) of the Department of Mathematics and Natural Sciences, BRAC University.

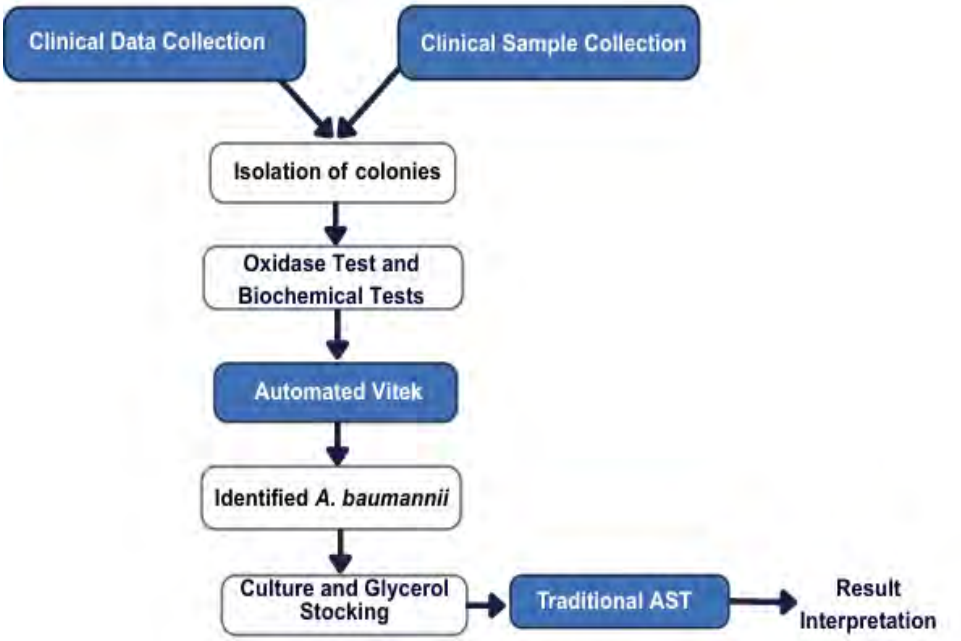

Figure 2.1: A Flowchart Depicting the methodology of this study.

2.4 Collection of Samples

Clinical samples were collected from 8 different sources: a) Wound swab, b) Tracheal aspiration, c) Sputum, d) Blood, e) Urine, f) Gastric lavage, g) Pus, h) Tissue sample.

The collected sample was immediately transferred into a sterile, labelled transport container and, if needed, placed in an anaerobic transport medium for suspected anaerobic pathogens. The specimen was promptly transported to the laboratory under appropriate conditions to ensure microbial viability. All procedures were conducted following biosafety guidelines to ensure accurate microbiological analysis and minimize the risk of infection transmission.

2.5 Isolation and Identification of *A. baumannii*

A. baumannii were isolated based on their morphological and biochemical characteristics. For biochemical characterization, Oxidase and Catalase tests were performed, upon finding oxidase-negative as *A. baumannii* lacks cytochrome c oxidase and catalase-positive as it produces catalase, breaking down hydrogen peroxide into water and oxygen, which met the criteria (Doughari et al., 2011) along with morphological observation, the selected specimens were sent without delay to VITEK. VITEK is an automated method to conduct AST as well as bacterial identification. It cross checks various biochemical test results with existing databases to determine the Bacteria. (Funke & Funke-Kissling, 2004).

2.5.1 Microscopic Identification

A. baumannii exhibits a small, rod-like morphology. However, our target organism shares similar characteristics with *Klebsiella neumoniae*. Therefore, an additional approach was employed to differentiate between the two species.

2.5.2 Life Technology (VITEK)

Vitek meaning life technology is used to determine the presence of certain organisms. The latin word 'viv' means life and ‘tek’ is the short form for technology. Finally, for definitive confirmation, we utilized the VITEK machine, which analyzes biochemical reactions, growth patterns, antibiotic susceptibility profiles, and database comparisons to accurately identify *A. baumannii*.

VITEK 2 system is a computerized system that provides quick and accurate identification of microorganisms, along with antimicrobial susceptibility. The process begins with the preparation of a standardized microbial suspension, which is inoculated in a closed plastic reagent card. The card contains a number of wells containing biochemical substrates that serve as small test chambers. Once loaded onto the instrument, the system incubates the card and monitors well-by-well changes constantly using Advanced Colorimetry™. This color change occurs as the microbe is metabolizing certain substrates and deposits a unique biochemical fingerprint for each microbe. The system is available in different configurations, such as the Compact, typical VITEK® 2, and XL versions, with the latter differing mainly by throughput but otherwise running on the same fundamental technology (Pincus,2006).

When the biochemical reactions are read, information is interpreted by the Advanced Expert System (AES) based on a comparison with a large internal database. The database contains over 2,000 microbial phenotypes, over 20,000 MIC (minimum inhibitory concentration) distributions, and more than 100 known mechanisms of resistance. With such reaction patterns and growth curves examined, the AES not only determines an identification result but also flags up unusual or borderline results, hence being more accurate. The final identifications are normally achieved in less than 10 hours, and in most instances, sooner, making the process much quicker than with many traditional manual techniques. Notably, the system is also capable of antimicrobial susceptibility testing, giving laboratories and clinicians the capacity to identify both the identity of an organism and its resistance pattern with rapidity, an important determinant of successful therapeutic choice.

Extensive studies of validation have proven the VITEK® 2 system highly accurate, with identification greater than 95% for gram-negative and gram-positive organisms in most studies. While Gram-negative bacteria are identified more reliably than gram-positive organisms, overall error and misidentification are very low. Beyond its accuracy, VITEK® 2 prevents manual handling, reduces potential for contamination, and maximizes laboratory workflow. Because reagent cards are pre-sealed, biohazard risk is decreased, and system automation reduces technician workload and training requirements. Together, these features make the VITEK® 2 a worthwhile tool for today's microbiology laboratories, combining speed, precision, and safety to deliver on-time diagnostic data that supports clinical decision-making and antimicrobial stewardship programs.

2.1 Storage of the *A. baumannii* isolates

Nutrient Agar (NA), MacConkey agar, T1N1 agar media and Glycerol ($C_3H_8O_3$) for stocking were used. Furthermore, for performing Antibiotics Susceptibility Test (AST), Muller Hinton Agar (MHA) was incorporated.

2.2 Antibiotic Susceptibility Test (AST)

The antibiotic susceptibility test (AST) is a standard in vitro method of assessing the effectiveness of antibiotics for treating a specific bacterial pathogen. The test is used to guide effective antibiotic therapy by ascertaining whether a microorganism is susceptible, intermediate, or resistant to an antibiotic. There are several methods for AST, but one of the most widely used qualitative methods is the Kirby-Bauer disc diffusion test. In this technique,

discs impregnated with antibiotics are placed on a lawn of bacteria cultured on Mueller-Hinton Agar (MHA), a standardized agar for antibiotic sensitivity testing.

Mueller-Hinton Agar is the choice for AST because it possesses several distinct characteristics. First, Mueller-Hinton Agar includes starch, which can bind to any toxins that are secreted by bacteria and render them ineffective against inhibiting antibiotic action. Second, Mueller-Hinton Agar has the ideal diffusion rate, making antibiotics diffuse evenly throughout the agar so that results are reproducible and uniform. Finally, MHA is a non-selective medium, supporting the growth of fastidious and non-fastidious bacteria alike, and thus applicable to a broad spectrum of microorganisms.

Following incubation under the proper conditions, plates are observed for the formation of zones of inhibition, appearing as clear bands around the antibiotic discs. Zone diameter will determine if the bacterial strain is sensitive, intermediate, or resistant to the test antibiotic. Larger the zone diameter, more susceptible is the organism. Smaller or no zones indicate resistance. Results are interpreted in accordance with standard guidelines, e.g., from the Clinical and Laboratory Standards Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Twelve different antibiotics were used in the current study to test the susceptibility pattern of the bacterial isolates. These kinds of data are pertinent when choosing therapeutic options and monitoring antibiotic resistance trends, which are imperative in the battle against drug-resistant infections.

2.3 Extraction of DNA

DNA extraction is a basic molecular technique. It is the method where genomic DNA is isolated from the whole cell. This process includes breakage of the cell membrane and the nuclear envelope to release the DNA in pure form without any protein or cellular contamination for using it in the downstream processes (Wilson, 2010). After extraction we get the genomic DNA in the form of supernatant whereas the other parts of the cell form pellets at the bottom of the tube. This supernatant is separated very carefully without disturbing the pellet for pure extraction. For following the protocol that is to be used for extraction of genomic DNA we need various equipment which are mostly sterile. We need fresh bacterial culture, TE buffer

with a pH 8.0, autoclaved micro centrifuge tubes, centrifuge machine for centrifugation, crushed ice for cooling and heat block for boiling.

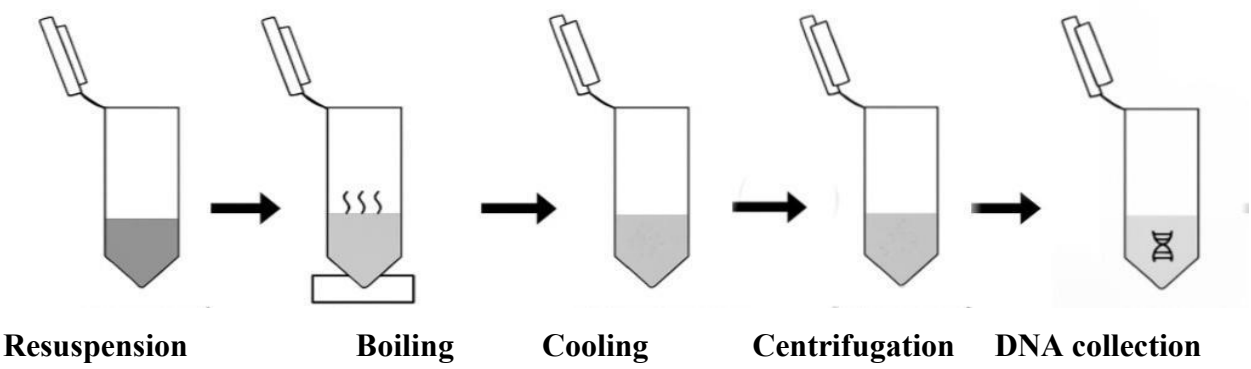

Figure 2.2: DNA extraction

For extraction of genomic DNA from the isolates of *A. baumannii* we need to culture the isolates in fresh Luria Bertani agar. Then select and pick 3-5 colonies and resuspend them in 100 uL of TE buffer. After that we have to place the microcentrifuge tube in the heat block having a temperature of 95-100 degree Celsius for boiling to cause lysis of the cell. This will cause the DNA fragment to come out of the cell in the process. Right after boiling we have to transfer the tubes in crushed ice for 5 minutes to make sure that the DNA does not degrade. Then centrifuge it for 5 minutes at 13,500 rpm to pellet the cell debris. Finally, after that we have to separate the supernatant containing the genomic DNA carefully without disturbing the pellet which contains cell debris. We can store the extracted DNA in -20 degrees Celsius for its future application if not used immediately after extraction. It is important to maintain one certain temperature to make sure that the DNA does not degrade.

2.4 Quantification Of DNA

After DNA extraction, quantification is the process which is used in order to assess the concentration and purity of DNA. The most widely used approach for the quantification process is spectrophotometry where absorbance at 260nm is often used to measure the concentration of DNA and the 260/280 ratio helps in identifying the protein contamination (Wilfinger et al., 1997). DNA is quantified based on its ability to absorb the ultraviolet. After the extraction of DNA, DNA quantification is the process we went through where the purity and the concentration of the extracted genomic DNA is measured by taking the absorbance value at

260 nanometres (A260) and 280 nm(A280). And the pure DNA ratio of absorbance is A260/A280 which is 1.8-2. In our study we took the absorbance value thrice to finally average them and have the proper result to be sure of their purity and concentration.

2.5 Random Amplified Polymorphic DNA (RAPD) PCR

The RAPD-PCR method is used for this study. RAPD-PCR stands for Random Amplified Polymorphic DNA Polymerase Chain Reaction. It is denoted as random because primers bind at multiple non-specific sites, producing unique patterns of amplified fragments for different genomes. This molecular technique is used to compare between organisms depending on genetic polymorphisms without prior knowledge of their DNA sequences.(Williams et al., 1990) Short and arbitrary primers are used to amplify random segments across the genome and later PCR products are visualised by agarose gel electrophoresis This method has been proven to be very effective to determine microbial typing, genetic diversity analysis, and taxonomic studies due to production of unique banding patterns which act as genetic fingerprints. (Naqvi et al., 2014). Though it is a very rapid and accurate method, it has its limitations. RAPD-PCR suffers from low reproducibility due to its high sensitivity to PCR conditions, and it produces dominant markers that cannot distinguish between homozygous and heterozygous states (Hadrys et al., 1992). Yet RAPD-PCR is more suitable for clonal typing of *A. baumannii* because of its ability to differentiate strains rapidly without needing any prior genetic sequence information (Turton et al., 2007). In contrast with other methods, such as PFGE, RAPD is faster, simpler, and cost-effective, making it practical for routine epidemiological investigations despite its lower reproducibility (Higgins et al., 2000). The specific protocol which was used in this particular study in explained below:

RAPD-PCR Protocol for Clonal Typing: The purpose was to preliminarily determine the clonal types of bacterial isolates using RAPD-PCR with a single random 10-bp primer.

Table 2.1: Materials for RAPD and gel electrophoresis

Materials Needed
• DNA template (100 ng per reaction)
• 10-bp RAPD primer (0.2 µM final concentration)
• Hotstart Taq polymerase enzyme (1 U/20 µL) – Lot No. 1817560
• dNTPs (provided in Hotstart kit or added separately)
• PCR buffer (provided with the enzyme)
• Nuclease-free water
• PCR tubes (0.2 mL)
• Thermocycler
• Pipettes and sterile tips
• Ice box for reagent preparation

Table 2.2: PCR Reaction Setup

PCR Reaction Setup (per 20 µL reaction)
• Primer: 0.2 µM
• Template DNA: 100 ng
• Hotstart Taq enzyme: 1 U

dNTPs, Buffer, Water: As per kit instructions or adjusted to 20 µL final volume

Table 2.3: Thermocycling Conditions

1. Initial Denaturation: 94°C for 10 minutes
2. Denaturation: 94°C for 10 seconds
3. Annealing: 36°C for 30 seconds
4. Extension: 72°C for 1 minute
5. Final Extension: 72°C for 2 minutes

40 Cycles of denaturation, annealing, and extension, Total PCR run time: ~2 hours and 30 min.

Table 2.4: Materials for Post PCR analysis

Materials Needed
● Agarose powder (1.5–2% recommended)
● 1X TAE or TBE buffer
● Gel casting tray and comb
● DNA ladder (100 bp or similar)
● Gel loading dye
● Ethidium bromide or safe DNA stain (e.g., GelRed, SYBR Safe)
● Gel documentation system

To initiate the process, 2% agarose gel is cast in 1X TBE buffer, and a suitable DNA stain is incorporated into the gel if staining within the gel is preferred. The molten agarose is poured into a casting tray containing a comb, which creates wells for sample loading, and left to set completely. Once the gel is ready, the PCR products are ready for loading by combining them with loading dye, in case the dye was not included during amplification. Approximately 5–10 µL of each sample is carefully loaded into individual wells of the gel, with a DNA ladder loaded into an additional lane to serve as a molecular size standard. The gel is then placed in an electrophoresis chamber filled with the same buffer in which it was prepared and subjected to an electric current of about 80–100 volts for 45–60 minutes, or longer if the gel is large and/or high resolution is needed. As this is done, the DNA fragments move through the agarose matrix at speeds directly proportional to the size of the fragments. Following electrophoresis, the gel is put in a UV or blue-light transilluminator to visualize the DNA bands and take photographs to record the results and for future analysis.

Interpretation of data

When we compare gel electrophoresis data from RAPD analysis, we compare cautiously banding patterns generated by different isolates. Each band is an amplified DNA fragment resulting from the amplification by a short arbitrary primer, and the fragments together give a unique fingerprint to each genome. If two isolates show highly similar or even identical banding patterns, we consider this as an indicator of genetic relatedness or clonality, but distinct differences in the number or size of the bands reflect greater genetic diversity among the samples. For the purpose of reliable interpretation, we always do a DNA ladder for comparison of molecular size and repeat amplifications to ascertain that the bands obtained are reproducible due to the fact that RAPD has been shown to be sensitive to reaction conditions (Williams et al., 1990; Welsh & McClelland, 1990). The reproducible bands serve as markers to make similarity matrices from which we can group isolates and present their relationships. Therefore, RAPD allows us to distinguish between closely related strains and to estimate the greater genetic diversity within our sample set, providing valuable information on microbial population structure and epidemiology.

CHAPTER 3

3. Results

From our study we see out of 50 isolates, 60% (30) were from female patients and 40% (20) were from male patients. Forty percent of the samples were collected from ICU patients. Among the patients, 90% (45) were diabetic and 5% (5) were non-diabetic. Female patients are most likely to have diabetic as 28 of them were diabetic and 2 were non-diabetic. On the other hand, out of 20 male patients, 18 were diabetic. This indicates that women are slightly more vulnerable to diabetic.

Samples of these patients were collected from several sources where samples from blood, G/L, Pus, Tracheal Aspiration and wound swab being the hardest to handle showed the maximum resistance to the common antibiotics whereas samples from urine showed lesser resistance. Ventilator-associated pneumonia is the most common infection with a rate of 38%, followed by chronic kidney disease 32% and acute Kidney Infection with a rate of 26%. Meropenem Resistance was seen in 88% of MDR isolates and 12% of XDR isolates, indicating its limited efficacy. Colistin is shown to be 100% Intermediate. Raising concerns about emerging colistin resistance. Out of the 50 isolates tested, 88% were identified as MDR, while 14% were classified as XDR.

The prevalence of XDR and MDR *A. baumannii* strains are influenced by hyperglycaemia as the study denotes 90% patients were diabetic. Hyperglycaemia state impairs an individual's immunity which plays a pivotal role for nosocomial infections to occur. Moreover 22 patients, the majority (44%), are of the age group 51-70 where all of them are diabetic and commonly diagnosed with diseases like pneumonia and heart diseases in this study. Which is a very vulnerable age group in terms of immune response. However, the second highest (34%) age group is 31-50 years where mostly the patients are diabetic with one case of non-diabetic who are commonly diagnosed with diseases like AKI, CKD and hypertension which is alarming because it denotes the severity of AMR among different age groups. We see fewer patients, only 6 for the age group of 71-90 and 5 patients, where 3 are diabetic and 2 are non-diabetic having been diagnosed mostly with pneumonia and fewer cases of CKD and Hernia in the range of age 10-30 years. That means that older patients are more prevalent to diabetic.

It is observed that diabetic patients show 92% resistance to antibiotics like piperacillin or Tazobactam and ciprofloxacin whereas non-diabetic patients are 80% resistant to them. For

these conditions bacteria are more resistant and it makes it more difficult to treat them effectively with these drugs. Our findings indicate that diabetic patients diagnosed commonly with diseases like pneumonia, AKI, CKD and heart diseases are mostly vulnerable to antibiotic resistance.

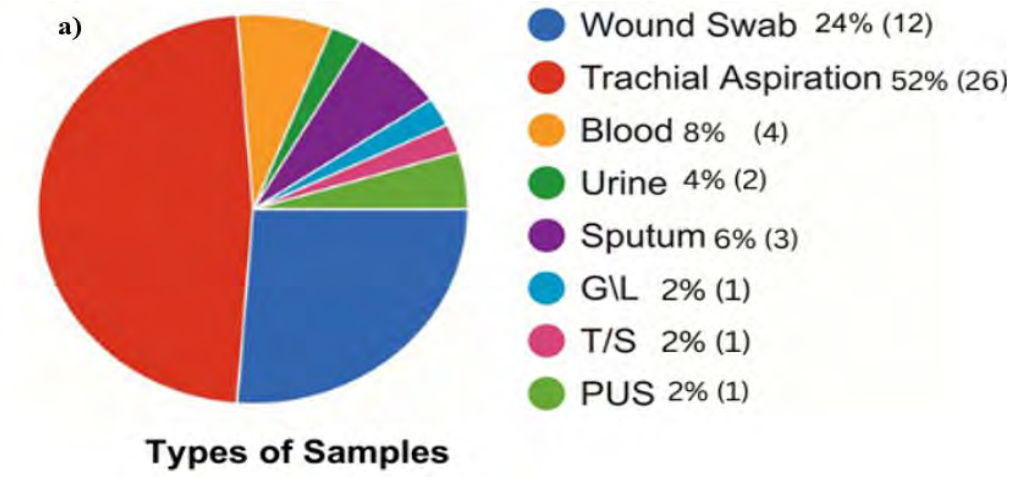

Figure 3.1: Pie chart showing the 8 clinical sample sources for this study.

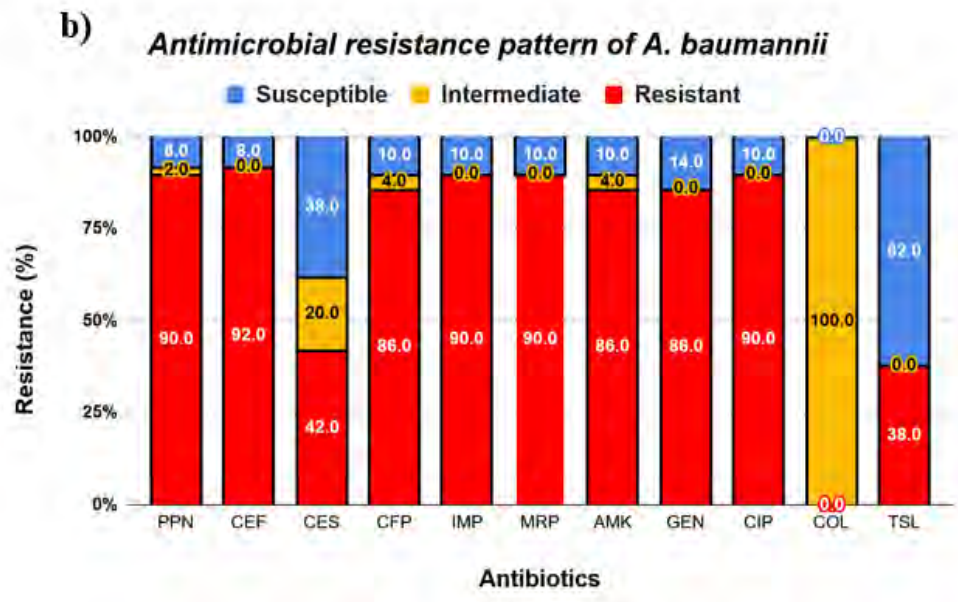

Figure 3.1 (b): Bar diagram showing the antimicrobial resistance pattern of *A. Baumannii* isolates from our study.

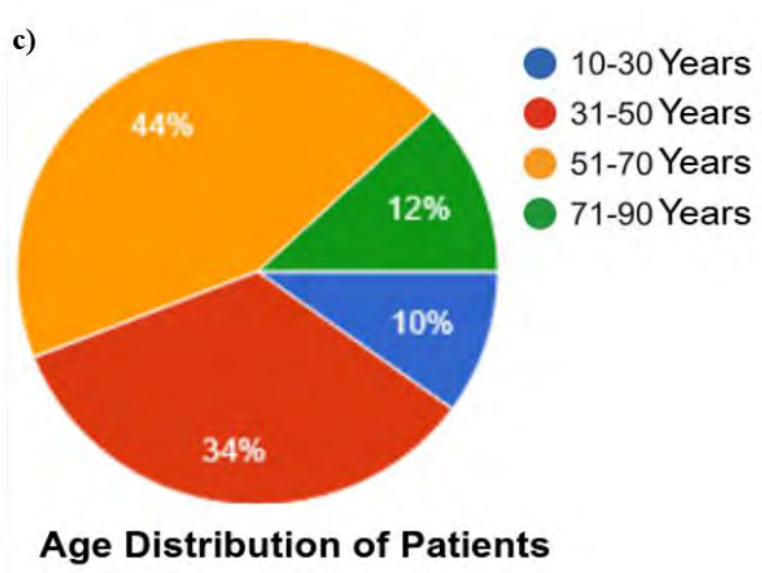

Figure 3.1 (c): Pie chart showing the age distribution of patients of this study.

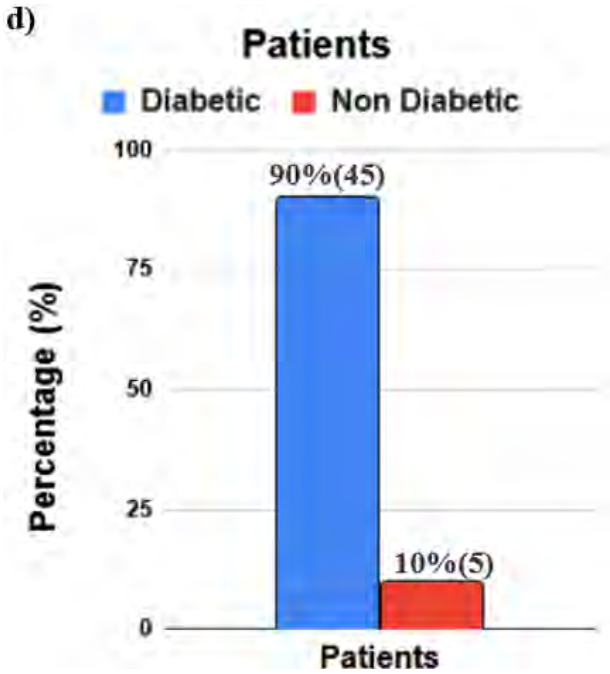

Figure 3.1 (d): Bar chart showing the percentage of diabetic and non-diabetic samples.

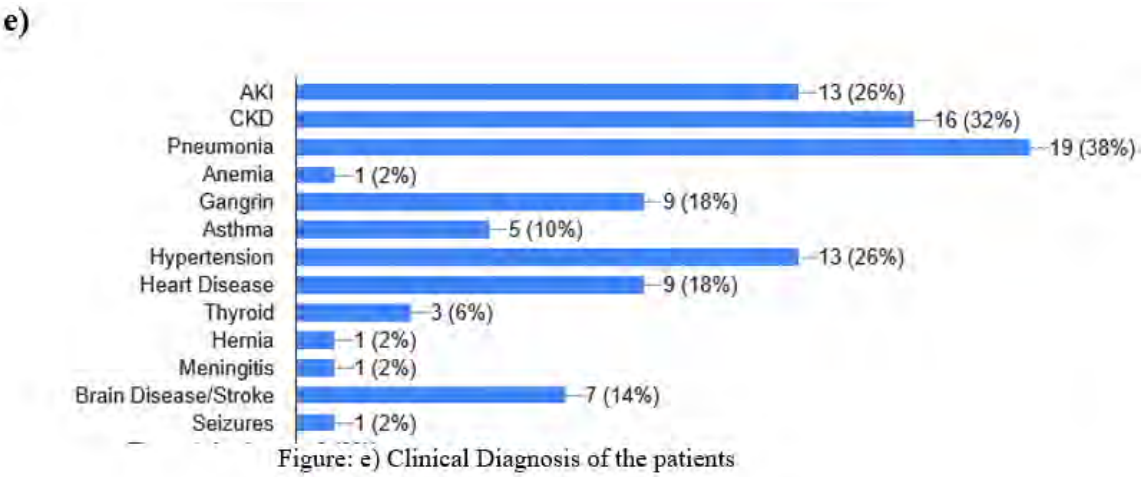

Figure 3.1(e): Clinical diagnosis of the patients of this study.

Figure 3.1 (e) showed that the most common case is pneumonia. Followed by AKI and CKD. In several Asian studies, Acinetobacter spp. comprised 33.5 % of VAP (Ventilator associated pneumonia) pathogens (Jabbar et al., 2022). Furthermore, treatment for MDR/XDR A. baumannii often involves nephrotoxic drugs. These can cause drug-induced AKI, and repeated or prolonged injury increases CKD risk. (Mirjalili et al., 2022; Wikipedia, 2025).

The dendrogram groups isolates based on their genetic similarity. Branch lengths represent the degree of similarity. Clusters indicate groups of isolates with closely related band patterns (potential clonal relationships). The XDR/MDR isolates cluster together, it suggests clonal spread. If they are spread across many branches, it suggests diverse sources or multiple clones circulating simultaneously.

Firstly, we can see the shocking prevalence of resistance among various banding patterns. Moreover, we can observe that the resistance pattern is quite identical among RAPD type 4 and 5, denoting a clonal relationship between them.

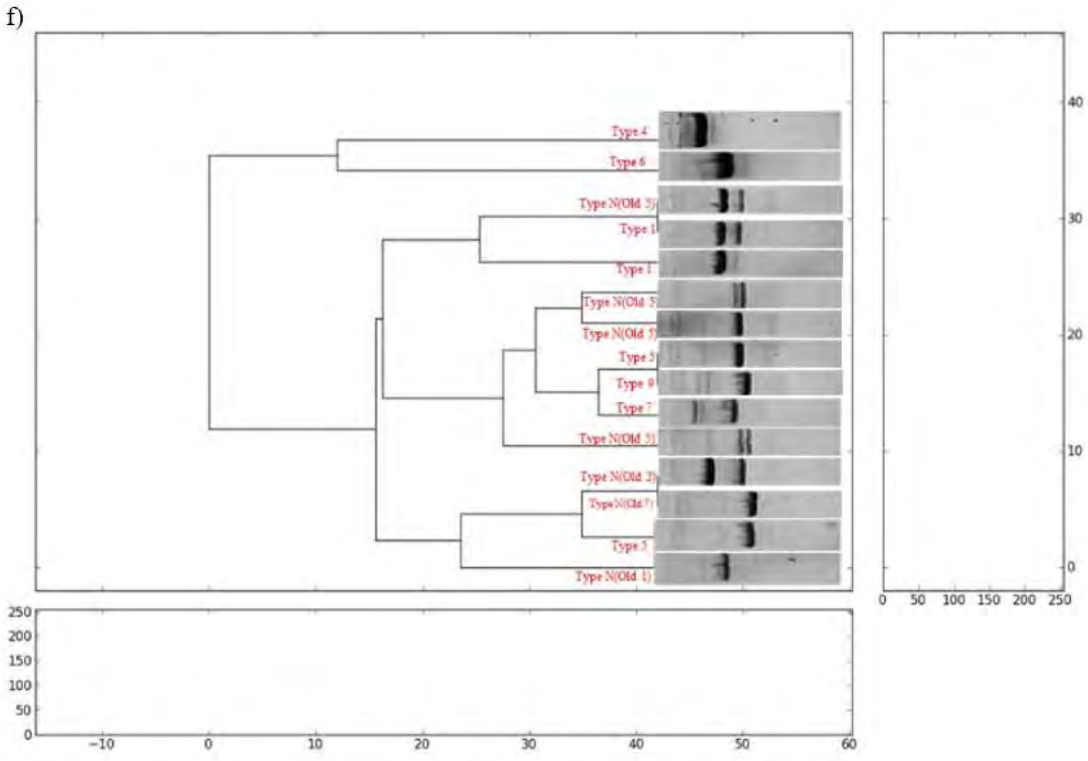

Figure 3.1 (f): Figure showing a dendrogram representation of banding patterns.

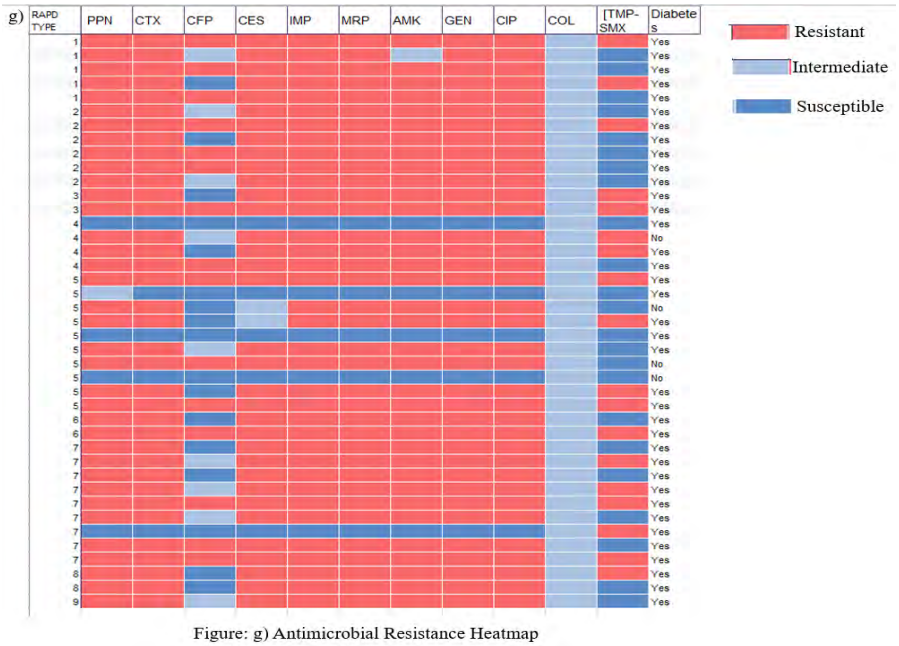

Figure 3.1 (g): This heatmap shows the resistance pattern against the different banding types.

CHAPTER 4

4. Discussion

The age distribution of affected patients showed that most cases involved individuals aged 51 to 70 years, which takes 44.9% of the total. This was followed by those aged 31 to 50 years at 36.7%. This confirms that older adults bear the highest burden of the disease. Patients aged 71 to 90 years accounted for 12.2% of cases, while the 10 to 30 years age group had the smallest share at 10.2%. These findings suggest a significant age-related vulnerability. The middle-aged and older people are being affected more. This trend aligns with other studies that have found older age groups to be more vulnerable. This increased risk may be due to a decline in immune function or existing health issues that worsen the severity of the disease. Diabetic patients tend to face more severe health issues than non-diabetics. Among diabetics, the most frequent conditions include pneumonia (18 cases), chronic kidney disease (16), hypertension (14), acute kidney injury (13), gangrene (9), and heart disease (9). This highlights a strong link between diabetes and serious infections or organ complications, especially involving the lungs and kidneys. In contrast, non-diabetic patients show a much lower disease burden, with only a few cases like pneumonia (2) and hernia (1) reported. The difference clearly shows that diabetics are more vulnerable to complex, multiple health issues, while non-diabetics generally present with fewer and less severe conditions.

The most common co-existing conditions found in patients infected with *A. baumannii* are pneumonia, chronic kidney disease (CKD), and acute kidney injury (AKI). We noticed that more patients had pneumonia (40%) and CKD (32%) compared to those with hypertension (28%) and AKI (26%). The high number of pneumonia cases shows a strong connection between this organism and hospital-acquired respiratory infections, the notable presence of CKD and AKI highlights increased risk among patients with kidney issues who often face long hospitalizations, frequent invasive procedures, and weakened immune systems. Cardiovascular and soft tissue problems were also relevant, with gangrene and heart disease reported in 18% of cases. Neurological and lung conditions were seen at moderate rates, with brain disease or stroke affecting 14% of patients and asthma reported in 10%. Asthma, though less common, can still make respiratory issues worse due to ongoing inflammation and possible use of corticosteroids. Less frequently reported conditions included thyroid disorders (6%). Meningitis, seizures, anemia, and hernia each affected 2% of the patient group. While these were not as widespread, their presence may still point to a background of complex medical

histories and overall physiological weakness, which could indirectly increase the risk of infections.

This study shows a significant level of resistance to antibiotics, especially to Piperacillin/Tazobactam and Ciprofloxacin. Notably, diabetic patients exhibit considerably higher resistance to these antibiotics compared to non-diabetic individuals. This means not only that diabetes increases the risk for infection but also that it may contribute to antibiotic resistance. This makes treatment more complicated, and infection control in diabetic patients is harder. These findings explain the need for targeted antibiotic stewardship and improved infection control measures in diabetic patients to reduce the risk of resistant infection and maximize clinical outcomes. AKI, CKD, pneumonia, and heart disease patients often have multiple comorbidities. They are also more at risk for MDR and XDR bacterial infections. For instance, AKI or CKD patients often require broad-spectrum antibiotics, which have selective pressure and enhance the emergence of resistant strains. Similarly, pneumonia and cardiac disease patients are repeatedly exposed to antibiotics at hospital admission, augmenting risk of resistance further. The findings underscore the urgent need for active antimicrobial stewardship and infection control practices in such vulnerable populations to prevent transmission of MDR and XDR microorganisms and improve clinical outcomes. We have several strengths for this study. It is the first RAPD-based study in the country to our knowledge. Secondly, the study also has a variety of clinical samples from different sources. They were from blood, sputum, wound swabs, and tracheal aspirates. That gives us a very good impression of the clinical impact of the pathogen. Apart from that, there are some limitations to this research. Firstly, the sample size was small, only 50 clinical isolates. That may not be enough statistical information to reflect on the larger epidemiological patterns and *A. baumannii* resistance patterns from Bangladesh. Second, the study was done in just a single hospital that is BIRDEM General Hospital in Dhaka. This limits the spatial coverage of samples and confines the applicability of how well the findings can be applied to other clinical settings, especially in rural hospitals where other strains might exist. Third, the study did not have a control group of healthy subjects. This made it hard to understand results seen and determine the impact of *A. baumannii.* Fourth, follow-up data on patient results like recovery status, treatment success, or mortality was unavailable. Because of this, the clinical significance of MDR and XDR infections were not well examined, particularly the antibiotic medications given.

CHAPTER 5

5. Conclusion

To conclude, *A. Baumannii* is a Gram-negative, opportunistic pathogen commonly associated with nosocomial (hospital-acquired) infections, particularly in intensive care units. It can survive on surfaces for extended periods and is frequently isolated from ventilators, catheters, and wounds. Its ability to acquire resistance to multiple antibiotics makes it a significant threat in healthcare settings, especially among immunocompromised patients. The term "clonality of MDR/XDR *A. baumannii"* refers to the study of genetic relatedness or similarity among strains of *A. baumannii* that exhibit MDR or XDR. Understanding clonality helps identify whether resistant strains come from a common ancestor (are genetically identical or very similar) or if they are diverse, which has implications for tracking the spread of resistant infections and understanding outbreak dynamics. To track such trends, we think our study provides vital information. Upon future research and determine a conclusive correlation between circulating strains and their clonality, we hope we would be able to help the most vulnerable spectrum of patients immunologically.

5.1 Strength of study

This cohort study puts emphasis on the growing antimicrobial resistance of *A. baumannii*. Which is a global phenomenon of recent times. Our study can add new insights on this very novel field of research. For instance, the correlation between ventilator associated pneumonia and infection of MDR/XDR *A. baumannii* can be established. Which can help in prevention of such infection. Correlation between Certain diseases such as Diabetes and *A. baumannii* can be determined upon further extensive research. Which can incorporate global strain data in order to create a reinforced combined strategy to combat this resistance phenomenon. Counter treatment options can be tailored based on such research data.

5.2 Limitations of study

The number of samples is insufficient to determine a comprehensive relation between diabetic patients and *A. baumannii* as there is no proper number of non-diabetic samples. Identifying *A. baumannii* is quite challenging since the morphological attributes and also some biochemical attributes are identical to other organisms such as *Klebsiella*. Lab conditions can sometimes be somewhat problematic. For example, the media preparation can be tricky due to availability of different types of media powder as well as Agarose Gel production can be quite challenging.

5.3 Future Prospects

Whole Genome Sequencing will be conducted upon receiving a satisfactory number of samples. Non-diabetic samples numbers should be raised. Genome sequences will be evaluated with other strains around the world to establish a correlation.

CHAPTER 6

6. References

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