Antibiotic-resistant *Acinetobacter baumannii* in Burn Wounds of Intensive Care Unit Admitted Patients: a Single Centered, Cross-Sectional Study

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of

Bachelor of Science in Microbiology

Department of Mathematics and Natural Sciences

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Declaration

It is hereby declared that

1. The thesis submitted is my/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. I/We have acknowledged all main sources of help.

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Ethics Statement

The ethical clearance to conduct this study was obtained from Sheikh Hasina National Institute of Burn and Plastic Surgery and the departmental review board of MNS Brac University.

Abstract

The purpose of the study was to identify *Acinetobacter baumannii* (*A. baumannii*) in burn wound patients admitted to the Intensive Care Unit (ICU) and to investigate the antibiotic resistance profile of these *A. baumannii* isolates. Wound samples were collected from patients at Sheikh Hasina National Institute of Burn and Plastic Surgery from September 2023 to March 2024, yielding 70 *A. baumannii* isolates. The samples were initially collected in saline-containing test tubes using Rayon swabs, then spread onto MacConkey agar. Pink round shaped colonies were picked and subcultured on Leed Acinetobacter Medium (LAM) overnight at 37 C. After the incubation plates were observed and those round shaped light pink color colonies with mauve color background colonies are considered as *A. bumannii* and these isolates were further confirmed by conventional PCR targeting the blaOXA-51 gene. Gel electrophoresis was performed to confirm the PCR results and antibiotic susceptibility testing was carried out using the Kirby-Bauer disc diffusion method. Among the 70 *A. baumannii* isolates, 100% were Multidrug Resistant (MDR) while 77.14% were Extensively Drug Resistant(XDR). There were no antibiotics that all the isolates were sensitive to. The isolates demonstrated the lowest resistance against the antibiotics from the tetracycline group, Tetracycline (58.57%) and Doxycycline (38.58%). However, all isolates exhibited complete 100% resistance to both Ceftazidime and Ampicillin-sulbactam. The high prevalence of MDR and XDR *A. baumannii* in hospital settings presents significant challenges in treatment management, complicating therapeutic options and highlighting the need for stringent infection control measures.

Keywords: *Acinetobacter baumannii*, Burn wound infections, Antibiotic resistance, Multidrug-resistant (MDR), Extensively drug-resistant (XDR).

Dedication

To my parents

- **Sayeed Khaled Sabbir**

To the Almighty Allah

- **Humayra Anjumee Elma**

To the Almighty Allah

- **Faria Islam Orpe**

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List of Acronyms

A. baumannii	*Acinetobacter Baumannii*
CLSI	Clinical and Laboratory Standards Institute
EtBr	Ethidium bromide
MDR	Multidrug resistance
XDR	Extensively drug-resistant
ICU	Intensive Care Unit
PCR	Polymerase chain reaction
MHA	Mueller–Hinton agar
MIU	Motility Urease test
MCT	Microcentrifuge tube
Bp	Base pair
DNA	Deoxyribonucleic acid
TE	Tris-EDTA
TBE	Tris-borate-EDTA
Min	Minute
Sec	Second

Chapter 01

Introduction

1.1 Background

Acinetobacter baumannii (A. baumannii) is an opportunistic bacterium that causes several types of infections acquired in healthcare settings. *A. baumannii* demonstrates a remarkable capacity for adapting to its surroundings and stands in different cleaning and disinfection techniques. As a result, it is extensively prevalent and capable of enduring for extended durations within hospital settings (Huang et al, 2023). *A. baumannii* is a significant pathogen in hospitals, *A. baumannii* is found throughout hospital settings as it is a nosocomial bacterium, but typically it is found mostly in the intensive care unit (ICU). It primarily affects patients who are debilitated and have weaker immune systems and/or imbalances in their natural flora. The most common clinical manifestations of this disease are pneumonia and bacteremia.(Almasaudi, 2018)

A. baumannii is becoming more antibiotic resistant in Bangladesh, which is causing considerable difficulties in the healthcare sector. Several studies reported recognizing different health care facilities in Bangladesh as breeding grounds of pathogenic *A. baumannii.* Several authors of Bangladesh cited the recovery of multidrug-resistant (MDR) *A. baumannii* strains from clinical samples from Bangladesh. (Dhar et al., 2017). Recent studies emphasize the concerning frequency of multidrug-resistant (MDR) strains of this disease in medical environments, specifically in intensive care units (ICUs). There is a significant lack of thorough data on the occurrence and resistance patterns of *A. baumannii* in Bangladesh. The majority of current research concentrates on particular types of infections or restricted geographical regions, so constraining the comprehension of the epidemiology of this bacteria. Conducting a comprehensive examination can yield crucial understanding of the dynamics of *A. baumannii* infections and resistance mechanisms, which are essential for devising precise therapies. Consequently, this study holds great significance.

The purpose of this study was to enhance the comprehension of *A.baumannii* by isolating and identifying strains from clinical samples obtained from a hospital in Bangladesh. This research will utilize molecular tools, such as Polymerase Chain Reaction (PCR) and biochemical testing, to both verify the existence of *A. baumannii* and evaluate its antibiotic resistance characteristics. The results will offer useful knowledge about the occurrence and mechanisms of resistance of *A. baumannii* in this area, thereby guiding future approaches for controlling infections and managing antibiotic use.

1.2 Overview of *Acinetobacter baumannii*

1.2.1 Characteristics

Acinetobacter baumannii are mainly rod shaped, aerobic coccobacillus bacteria which are 0.9 to 1.6 μm in diameter and 1.5 to 2.5 μm in length. In the stationary phase of the growth it becomes spherical. It can be observed in pairs and sometimes in chains. Generally *A. baumannii* is non-motile but may exist in twitching mortality controlled by type IV pili. *A. baumannii's* twitching pili are hair-like surface features that can stretch, adhere, and retract to facilitate twitching motility, a type of bacterial movement. This process facilitates the colonization of surfaces, the creation of biofilms, and may enhance the pathogen's pathogenicity and capacity to proliferate inside hospital settings (Jeong et al., 2023). *A. baumannii* has an encapsulated polysaccharide capsule that contributes to its virulence and immune response resistance. The capsule helps protect *A. baumannii* from being phagocytosed by immune cells like macrophages and neutrophils. Moreover, the capsule is also important for biofilm formation, which allows the bacteria to adhere to surfaces and medical devices, leading to persistent infections. Contains porins and efflux channels that contribute to its antibiotic resistance(Aryal, 2022).

1.2.2 Pathogenicity and Diseases

Because *Acinetobacter baumannii* can withstand most known antibiotics, it has become a very dangerous pathogen that can lead to deadly infections and severe epidemics, especially in hospital settings. It has a selective advantage in healthcare settings because of its exceptional metabolic flexibility, which allows it to live in difficult environments. The virulence mechanisms and host responses to *A. baumannii* infections are still poorly known, despite the infection's clinical relevance. Nonetheless, its pathogenicity is being investigated using a variety of model systems, including in vitro and in vivo techniques.

The vast majority of cases of Acinetobacter infections occur in hospitalized patients. In the U.S.-wide Surveillance and Control of Pathogens of Epidemiological Importance [SCOPE] study conducted between 1995 and 2002, *A. baumannii* was the tenth most common organism isolated and was responsible for 1.3% of all monomicrobial nosocomial bloodstream infections (0.6 bloodstream infections per 10,000 admissions). (Alsan & Klompas, 2010)

1.3 Source of *A. baumannii*

A. baumannii was first found and isolated from the environmental sources like soil, water and various surfaces. According to recent studies, *A. baumannii* can also be discovered in non-human environments, such as plants and animals. There are worries over these non-clinical isolates' ability to act as reservoirs because they may have virulence characteristics in common with clinical pathogens (Howard et al., 2012). It has probably been present in these natural niches for many years, but its consolidation as an important human pathogen only took place towards the end of the 20th century with hospital involvement. It’s genetic adaptations, such as antibiotic resistance, have contributed to its widespread presence in healthcare-associated infections. It was in the 1990s that *A. baumannii* first began to emerge as a significant pathogen in ICUs worldwide, including in Bangladesh, where multidrug-resistant strains have been implicated in outbreaks in both adult and pediatric units. These outbreaks highlight the pathogen's persistence on surfaces, medical equipment, and ability to form biofilms, which enhances its resistance to antibiotics and disinfectants (Sultana et al., 2022).

1.4 *A. baumannii* in Hospital and Intensive Care Unit (ICU)

A. baumannii bloodstream infections were more common in ICUs (1.6% of bloodstream infections) compared with non-ICUs (0.9% of bloodstream infections) (Alsan & Klompas, 2010) The majority of *A. baumannii* infections are opportunistic in character, affecting patients who require prolonged therapy with mechanical ventilation and antimicrobial drugs and who spend a lot of time in the intensive care unit (ICU) because of a serious underlying illness. The ICU setting's nosocomial spread of *A. baumannii* has frequently been linked to ventilatory equipment as well as hand transmission by infested nursing and respiratory professionals.

The most common etiological agents of healthcare-associated BSIs in 176 COVID-19 patients (193 episodes) in the COVID-19 intensive care unit at the Dubrava University Hospital during the second wave of COVID-19 pandemic are shown in Figure 1 ((Dobrović et al., 2023)

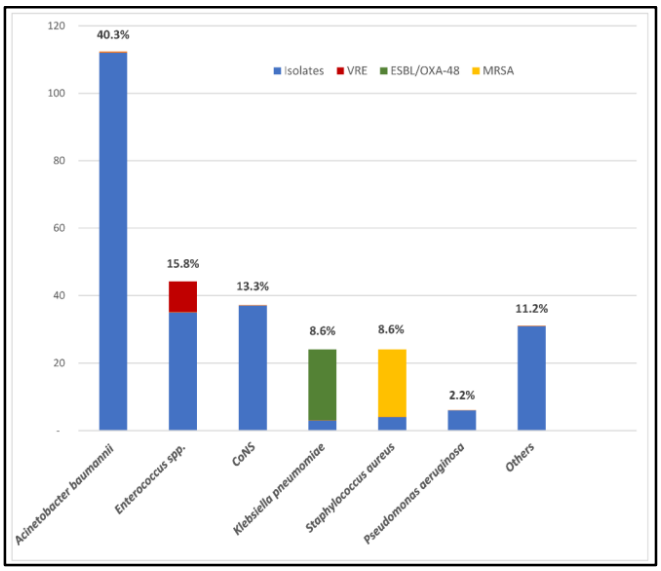

Figure 1 The most common etiological agents of healthcare-associated bloodstream infections in the COVID-19 intensive care unit at the Dubrava University Hospital during the second wave of the COVID-19 pandemic (1 October 2020–28 February 2021)
Source- (Dobrović et al., 2023)

1.4.1 Prevalence and Antibiotic Resistance Patterns of *Acinetobacter baumannii* in Bangladeshi Hospitals

A. baumannii has emerged as a significant nosocomial pathogen in Bangladesh, causing serious healthcare-associated infections, especially in critical care environments. Recent investigations indicate that *A. baumannii* constitutes around 14.66% of all pathogenic Gram-negative isolates in hospitals nationwide. Its prevalence is particularly significant in intensive care units (ICUs), where it accounts for 75% of infections. *A. baumannii* accounts for 18% of infections among inpatients and 7% of infections in outpatient settings (Uddin et al., 2022). The pathogen is predominantly isolated from respiratory specimens, with 75% of isolates obtained from tracheal aspirates, highlighting its significant correlation with ventilator-associated pneumonia and other respiratory illnesses. The issue is further exacerbated by the high levels of antibiotic resistance exhibited by strains of *A. baumannii* in Bangladesh. Several critical classes of antibiotics show alarmingly high resistance rates. Resistance to cephalosporins is found in 96–100% of the isolates, while aminoglycosides display resistance rates ranging from 88% to 92%. Fluoroquinolones have reached a staggering 100% resistance rate, and for carbapenems, resistance rates range from 88% to 92% (Farzana et al., 2022b). In another study, the resistance profile of *Acinetobacter baumannii* isolates at Dhaka Medical College Hospital (DMCH) revealed that 96% of the isolates were multidrug-resistant (MDR), exhibiting resistance to three major classes of antibiotics: cephalosporins, aminoglycosides, and fluoroquinolones (Uddin et al., 2022). This widespread resistance severely limits treatment options, complicating the management of infections caused by *A. baumannii*. The restricted treatment options for *A. baumannii* infections are reflected in these resistance patterns. This is particularly relevant in intensive care unit settings, which are characterized by the highest prevalence of the pathogen.

The antibiotic colistin continues to be the most effective treatment for *A. baumannii* in Bangladesh, despite the fact that the disease is resistant to a wide variety of other medication classes. Due to the fact that it is able to target bacterial cell membranes, it is considered a treatment of last resort. On the other hand, a greater reliance on Colistin presents a risk of resistance development, which has already been demonstrated in other regions of the world. The development of Colistin-resistant strains in Bangladesh is something that must be prevented by careful management and antibiotic stewardship (Uddin et al., 2022). This is necessary in order to maintain the effectiveness of the antibiotic.

1.5 Knowledge gap

- **Lack of Comprehensive Epidemiological Data in Bangladesh:** Although various studies have indicated the presence of *A. baumannii* in hospital environments, especially in ICUs, there is a notable gap in comprehensive epidemiological data throughout several areas of Bangladesh.
- **Environmental Sources and Reservoirs:** There is not much research on *A. baumannii* in Bangladesh's non-clinical settings—that is, soil, water, plants. Particularly in view of research implying non-clinical isolates may operate as reservoirs for virulent strains, it is imperative to understand how environmental sources could contribute to the persistence and spread of this virus in hospital settings.
- **Host-Pathogen Interactions and Virulence Factors:** Despite the well-documented resistance of *A. baumannii* to antibiotics, there is limited research on the specific virulence factors that contribute to its pathogenicity in hospitalized patients in Bangladesh.

Objective

The primary goal of this investigation was to examine the prevalence and antibiotic resistance patterns of *A. baumannii* in clinical samples from a hospital in Bangladesh, with a particular emphasis on critical care settings. The following are the specific objectives:

- To identify and isolate *A. baumannii* from wound samples obtained from intensive care units (ICU).
- To evaluate the antibiotic resistance profiles of *A. baumannii* isolates, with a particular emphasis on resistance to commonly used antibiotics, including carbapenems, aminoglycosides, fluoroquinolones, and cephalosporins.
- Analyze the multidrug-resistant (MDR) and extensively drug-resistant (XDR) profiles of *A. baumannii* isolates, with a focus on identifying resistance to various antibiotic classes commonly used in clinical settings.

These objectives will contribute to the development of critical insights into the occurrence and resistance mechanisms of *A. baumannii* in Bangladesh, which will inform future treatment guidelines and infection control measures.

Chapter 2

Materials and methods

2.1 Study Design

This single centered cross-sectional study was conducted in Sheikh Hasina National Institute of Burn and Plastic Surgery in Bangladesh. The patient sample was collected between September 2023 - March 2024 inclusive. Total 87 wound samples were collected from the patients from this hospital. The population of this study are burn patients in the Intensive care unit (ICU).

2.2 Workflow

Sample Collection

- Wound swabs from burn patients

⬇

Culture of *A. baumannii*

- *A. baumanni*i was cultured onto selective media

⬇

DNA Extraction

- Extraction of bacterial DNA

⬇

Polymerase Chain Reaction (PCR)

- Amplification using blaOXA-51 primers

⬇

Agarose Gel Electrophoresis

- Detection of PCR products

⬇

Antibiotic Susceptibility Testing

- Kirby-Bauer disk diffusion method following CLSI guidelines

2.3 Sample collection

The research was conducted in a hospital's clinical microbiology laboratory and the MNS Research Laboratory at BRAC University in Dhaka, Bangladesh. Over a period of approximately Six months, a total of 70 samples were collected and isolated from clinical specimens obtained from patients, particularly from wounds.

All the clinical samples were collected from the patient's wound secretion admitted to the intensive care unit (ICU). Rayon swabs were used directly to the wounds for taking those samples. Then these swabs are further dipped into the normal saline in the sterile test tube and all the samples are then vortexed to create a suspension. This suspension then spread further with a sterile glass rod onto the plate of MacConkey agar media in the hospital lab and transported immediately to the BRAC University Microbiology laboratory and incubated overnight at 37°C. for further detection.

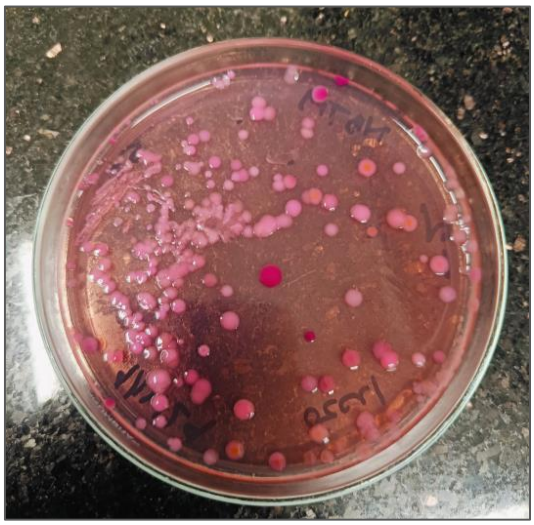

Figure 2 - MacConkey Agar (Differential and selective)

2.4 Culture of *A. baumannii*

Following the incubation all the plates are observed for growth and those suspected colonies with pink round shape colonies are considered and further sub-cultured in Leeds Acinetobacter Medium (LAM) media. The plates were again incubated at 37°C overnight. In LAM media *A. baumannii* shows light pink color colonies with mauve color background and the colonies are round, smooth, and slightly raised. These pink colored colonies indicated the presence of *A. baumannii.*

Figure 3- Leeds Acinetobacter Media (LAM)

Isolates confirmed by the plate observation morphology are further performed biochemical tests to identify. Following incubation, the morphology of the bacterial colonies was examined using gram staining, along with conducting tests such as the oxidase test and catalase test for identification purposes.

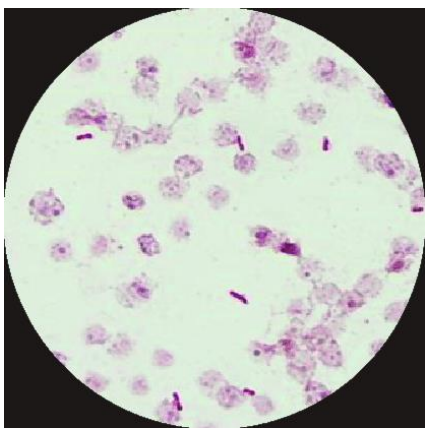

Figure 4- Gram staining

In Gram staining, the organism is stained pink, indicating it is Gram-negative. The oxidase test was negative, meaning the organism does not produce cytochrome oxidase. The catalase test was positive, showing the presence of the catalase enzyme, which breaks down hydrogen peroxide into water and oxygen.

2.5 DNA Extraction from Isolates

To begin the DNA extraction process, all samples were sub-cultured in Nutrient agar medium and incubated for 24 hours at 37°C. The following day, the samples were prepared for DNA extraction using the boiling method. A loopful of colonies from each sample was transferred into a microcentrifuge tube (MCT) containing 400 µl of Tris-EDTA (TE) buffer. The tubes were vortexed for 15 seconds to thoroughly mix the contents and then centrifuged at 13,000 RPM and 25°C for 10 minutes. After centrifugation, the supernatant was carefully discarded. Another 400 µl of TE buffer was added to the pellet, and the mixture was vortexed again for 15 seconds. The tubes were then placed in a 100°C water bath for 10 minutes. Once the water bath step was completed, the samples were allowed to cool to room temperature for 5 to 10 minutes. Subsequently, the tubes were centrifuged again at 13,000 RPM and 25°C for 10 minutes. After this centrifugation, the supernatant was collected into a new sterile 1.5 ml MCT, while the pellet was discarded.

2.6 Molecular Detection

PCR (Polymerase Chain Reaction)

All the DNA extraction products are performed with polymerase chain reaction (PCR).

2.6.1 Master Mix Preparation

In a PCR tube containing 6 µl master mix 1 µl of forward primer and 1 µl reverse primer including 3 µl of nuclease free water has been taken then added 2µl DNA extraction as a template in each PCR tube. All the tubes have a short spine for 10 to 15 seconds. The total volume for a PCR product is 13 µl. After that the PCR products are ready to be amplified in thermal cycler PCR machines. The calculation given below is for 1 sample and for multiple samples, the amount will be multiplied with "n".

Reagents	Total Volume
Master mix	6 µl
Reverse Primer (RP)	1 µl
Forward Primer (FP)	1 µl
Nuclease Free Water	3 µl
DNA sample	2 µl
Total	**13 µl**

Table- 1: PCR product preparation for 1 sample

2.6.2 PCR product amplification

Using blaOXA-51 primers, suspected *Acinetobacter baumannii* isolates were examined for confirmation. The primer set from Table 2.1 was used for PCR amplification-

Primer	Primer Sequence	PCR Condition	Number of Cycles	Amplicon Size	Reference
blaOXA-51F	5'-TAATGCTTTGATCGGCCTTG-3'	94°C for 5 minutes, 94°C for 1 minutes, 55°C for 1 minutes, 72°C for 1 minutes and a final step at 72°C for 10 minutes	30	353 bp	(Falah et al., 2019)
blaOXA-51R	5'-TGGATTGCACTTCATCTTGG-3'				

Table 2: Primers used for amplification of resistance genes by polymerase chain reaction (PCR)

- **The cycles for PCR conditions are** 94°C for 5 minutes, 94°C for 1 minutes, 55°C for 1 minutes, 72°C for 1 minutes and a final step at 72°C for 10 minutes

After the PCR all the products are further analyzed in Gel electrophoresis.

2.6.3 Agarose Gel Electrophoresis

The findings of a PCR reaction are typically visualized (made visible) using gel electrophoresis. The agarose gel is prepared by 1.2g agarose powder, 2000 µL Tris-acetate-EDTA (TAE) buffer along with 98% distilled water. TAE is used as a running buffer to migrate the DNA in the positive electrode. Next there is added 4µL Ethidium Bromide (EDTA). Ethidium Bromide is a cargeogenic fluorescence dye which helps the bands to show under the UV light. All the PCR products of 4µL are then loaded into the gel with the help of autoclaved tips. 5µL of 100 bp ladder has been used to identify the size of unknown DNA molecules.

2.7 Antibiotic Susceptibility Test (AST)

For antimicrobial susceptibility testing all the isolates are subcultured and streaked in the non-selective media Nutrient agar and incubated in 37°C for 24 hours. All the isolates are performed using the Kirby-Bauer disc diffusion technique on Mueller-Hinton agar in the presence of 10 different antibiotics from 7 antimicrobial categories for antimicrobial susceptibility testing. Antibiotics and groups and interpretive criteria are shown below-

Groups	**Antibiotics**	**Disk Code**	**Disk Potency**	**Interpretive Criteria**		
				Sensitive (mm)	**Intermediate (mm)**	**Resistance (mm)**
Aminoglycosides	Amikacin	AK	30	≥ 17	15-16	≤ 14
	Gentamicin	GEN	10	≥15	13-14	≤ 12
Penicillins with Beta-Lactamase Inhibitors	Piperacillin-tazobactam	PIT	100	≥ 21	15–20	≤ 14
	Ampicillin-sulbactam	AMP	10	≥17	12-16	≤ 13
Tetracyclines	Tetracycline	TE	30	≥15	12-14	≤ 11
	Doxycycline	DO	30	≥14	11-13	≤ 10
Beta-lactam	Cefepime	CPM	30	≥18	15-17	≤ 14
Cephalosporins	Ceftazidime	CAZ	30	≥18	15-17	≤ 14
Fluoroquinolone/ Ciprofloxacin	Levofloxacin	LEV	05	≥17	14-16	≤ 13
Carbapenems	Imipenem	IMP	10	≥16	14-15	≤ 13

Table 3- Concentrations and diffusion zones of the antibiotics.

All the sample organisms are first streaked into nutritional agar (NA) culture plates following a 24-hour incubation period using autoclave cotton swabs. The swabs are dipped into distilled water and then mixed with the liquids. The suspension was adjusted using equivalent to 0.5 McFarland standard based on the turbidity of the suspension, the suspension was inoculated onto MHA plates.Then the suspension was swiped with the cotton swabs from different angles to guarantee that the bacteria are evenly spread across the plates. Finally, the plates are held for a brief period of time to allow the media to absorb the suspension. Using a sterile tweezer, the necessary antibiotic disks are then placed on the plates. Following that, the discs are placed on it and gently pressed on it to guarantee direct contact with the medium. After that, the tweezer is immersed to sanitize it for future usage. This is how all the disks are placed into the MHA plate, and all the plates are then incubated for 24 hours in 37 degree celsius.

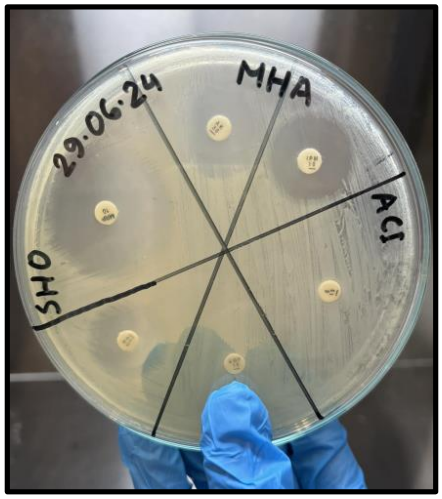

Figure 5- Antibiotic susceptibility Test

After 24 hours of incubation the MHA plates are observed and try to find out the zone of inhibition. If the particular antibiotic is effective on the tested organism, then there will be ‘no growth’ visible around it. A clear zone of inhibition surrounding the antibiotic disk on the plate will be apparent if the bacteria are antibiotic-susceptible. Greater zones indicate that the bacteria are more sensitive to the antibiotic. The size of this zone represents the degree of susceptibility.There won't be a visible zone of inhibition surrounding the disk if the bacteria are resistant to the antibiotic.
The zone of inhibition is measured by millimeters and whether they are sensitive, resistant or intermediate by using a standard table by Clinical and Laboratory Standards Institute (CLSI) guidelines. After the tested sample's data has been collected, it is all arranged in a Microsoft Excel spreadsheet for recording purposes and the creation of factual graphs and charts.

2.8 Definition of Multidrug-resistance (MDR) and Extensively drug resistance (XDR)

Multidrug-resistance (MDR) is defined as nonsusceptibility to at least one agent in three or more antimicrobial categories.Extensively drug resistant (XDR) is defined as nonsusceptibility to at least one agent in all but two or fewer antimicrobial categories.

Chapter 3

Results

3.1 Isolation of *A. baumannii*

Out of 87 clinical samples that were collected and put through conventional PCR analysis using the blaOXA-51 primers, 70 samples showed the presence of a 353 bp band confirming the presence of *A.baumannii* in 80.46% of samples. No amplifications were given by the remaining 12 samples indicating the absence of *A.baumannii*.

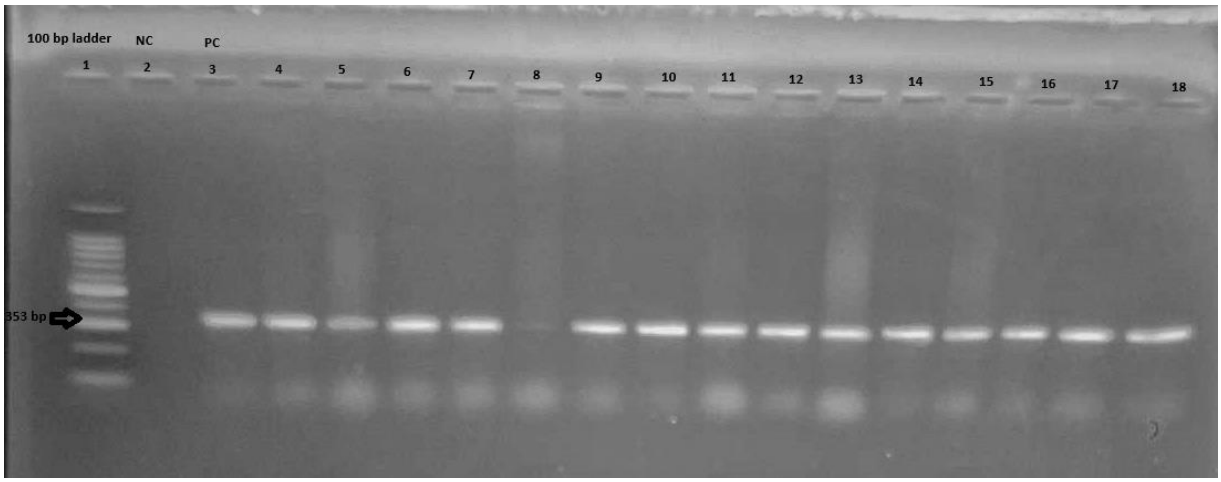

Figure 6: The gel electrophoresis image of PCR product of blaOXA-51 (353 bp) DNA amplification in some isolates of *A. baumannii* under UV. Lane 1: 100bp marker, Lane 2: negative control, Lane 3: positive control, Lane 4-7;9-18: confirmed *A.baumannii* isolates showing amplified bands at 353 bp.

3.2 Antibiotic susceptibility patterns of the isolates

Resistance to Amikacin was detected in 82.86% of the isolates, while Gentamicin exhibited resistance in 80% of the isolates. Imipenem resistance was observed in 88.57% of the isolates. Remarkably, 100% of the isolates were resistant to both Ceftazidime and Ampicillin-sulbactam. Additionally, 94.28% of the isolates showed resistance to Cefepime, and 97.14% were resistant to Levofloxacin. Resistance to Piperacillin-tazobactam was found in 90% of the isolates. In contrast, lower resistance rates were noted for Tetracycline (58.57%) and Doxycycline (38.58%).
The table below presents the antibiotic susceptibility patterns of the isolates.

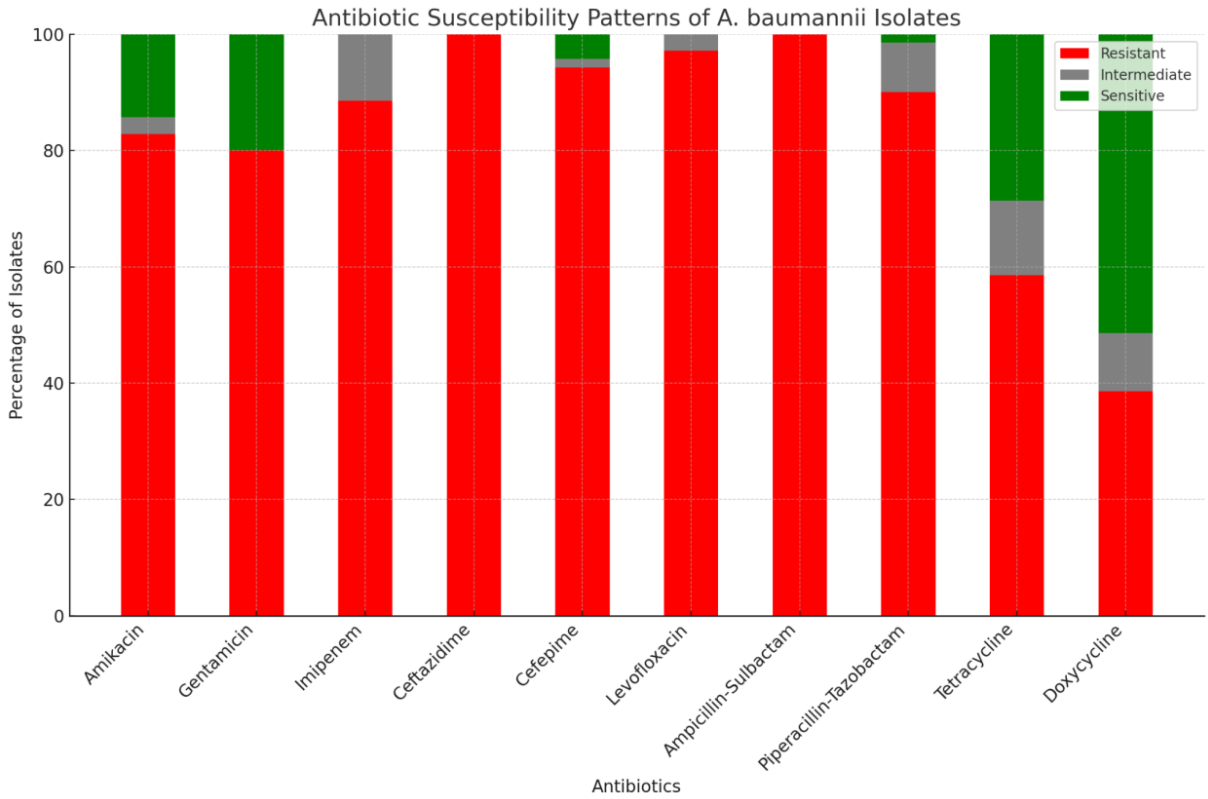

Figure 7: Antibiotic susceptibility patterns of all confirmed *A. baumannii* isolates

3.3 Multidrug resistance and Extensively drug resistance patterns

Multidrug-resistance (MDR) is defined as nonsusceptibility to at least one agent in three or more antimicrobial categories. Among the 70 isolates all 70 (100%) were found to be resistant to 3 or more categories of antibiotics making them multidrug-resistant isolates. Extensively drug resistant (XDR) is defined as nonsusceptibility to at least one agent in all but two or fewer antimicrobial categories. Out of the 70 isolates 54 (77.143%) were found to be resistant to at least one antibiotic in all but two or fewer antimicrobial categories making them extensively drug resistant isolates.

Chapter 4

4.1 Discussion

The study was conducted to identify Multidrug-resistance and Extensively drug resistance properties of samples collected from burn wounds of ICU patients admitted in Sheikh Hasina National Institute of Burn and Plastic Surgery. The isolates were confirmed to be *A. baumannii* via traditional PCR amplification and blaOXA-51 was used as a primer. This gene is a beta-lactamase that codes for carbapenemase of the OXA type. Which gives the *A. baumannii* strains the ability to hydrolyse carbapenems (Patel & Bonomo,2011). blaOXA-51 is a class D beta-lactamase which are effective oxacillinases (Tooke et al., 2019). This shows that these samples were present with this enzyme which made them able to break down carbapenems.The presence of blaOXA-51 makes these samples both beta-lactamase and carbapenemase. This translates in results as none of the isolates were sensitive to Imipenem of Carbapenem group of antibiotics and Ampicillin-sulbactam of Penicillins and beta-lactamase inhibitors group of antibiotics. On the other hand, the isolates showed the least resistance to the antibiotics of Tetracycline group Tetracycline (38.58%) and Doxycycline (58.57%). When we compare our results to those of Zhao et al., which was conducted on *Acinetobacter baumannii* isolates collected from clinical specimens in a comprehensive hospital from Jiangsu province in China, we see some important differences in antibiotic resistance. Our study shows higher resistance rates for some key antibiotics. For instance, Ceftazidime and Ampicillin-sulbactam had 100% resistance in our isolates, while Zhao et al. reported 92.3% and 93.8%, respectively. This suggests that resistance is increasing in our area. Additionally, Levofloxacin resistance in our study was 97.14%, much higher than the 80.0% reported by Zhao et al., indicating a growing problem. Although the study of Zhao et al. showed 53% MDR among the isolates compared to our very concerning 100% MDR rate. These differences may reflect variations in regional antibiotic use practices, hospital infection control policies, or genetic differences in local strains. On the other hand, resistance to Gentamicin and Piperacillin-tazobactam was similar in both studies, with our rates being 80.00% and 90.00%, respectively, compared to 87.7% and 89.2% in Zhao et al.'s study. This suggests that resistance to these antibiotics is fairly consistent. Imipenem resistance was slightly lower in our study (88.57%) compared to 92.3% in the study of Zhao et al., which might show some regional differences or improvements.

Similar studies from Europe and the Middle East have also shown worrying resistance patterns. The study of Nowak et al. (2017) on high incidence of pandrug-resistant A. baumannii isolates collected from patients with ventilator-associated pneumonia in Greece, Italy and Spain show resistance rate of 98.5% and 96.9% to key antibiotics like Levofloxacin and Imipenem. Parallely, resistance rate of 94% and 92.6% was observed in the study of Gharaibeh et al. (2024) which was based on samples from Jordan. These results truly represent the global threat that antibiotic resistance A. baumannii carries.

On the other hand, more similarities between the results are found when compared to the findings of a recent study in Bangladesh by Farzana et al. (2022). The study reported a similar tendency in clinical *A. baumannii* isolates in Bangladesh. The findings emphasize the extreme resistance patterns of the pathogen. In their study the isolates were 100% resistant to Ciprofloxacin, 91.8% resistant to both Imipenem and Meropenem, and 91.8% resistant to Gentamicin. Furthermore, 87.7% of their isolates were resistant to Amikacin, and 61.2% to trimethoprim-sulfamethoxazole, with 59% classified as MDR. Once more, the rate of MDR found in our study is significantly higher even when compared to studies done in Bangladesh. Both studies show critical resistance to Imipenem and significant rates of MDR. The rapidly increasing threat of *A. baumannii* in healthcare settings in Bangladesh are highlighted by these patterns.This further signifies the need for improved antibiotic administration and infection control actions to battle this public health challenge.

Overall, our study points to higher resistance levels for several important antibiotics, which highlights the need for continuous monitoring and updated treatment strategies.

In this study, the prevalence of *A. baumannii* found in burn wounds of ICU admitted patients and the rate of MDR and XDR among them is highly alarming. Antimicrobial resistance is a major issue at the moment. Drugs that lower the morbidity and death from serious and potentially fatal illnesses start to lose their effectiveness, posing a risk to public health. To solve this issue, figuring out an isolated multidrug resistance pattern is crucial.

This study was conducted on 70 *A. baumannii* strains to determine their MDR patterns. Furthermore, this study successfully used a PCR based technique to identify blaOXA-51 present in the clinical isolates from burn wounds of the ICU admitted patients.

Conclusion

Our study found that the hospital environment hosts multidrug-resistant *A. baumannii*. Hence, it is crucial to rapidly identify *A. baumannii* infections and enforce suitable precautions to prevent them from spreading within hospital environments, especially in ICUs. Moreover, numerous published journals and reports have given evidence that *A. baumannii* infections are responsible for nosocomial infections such as nosocomial pneumonia, meningitis, and endocarditis. Therefore, it is essential to quickly detect these infections and implement appropriate prevention strategies to prevent their transmission within hospital settings, especially in intensive care units. The prevalence of our study will assist in the implementation of more effective infection control measures, while an immediate update on antibiotics will improve our understanding of the patterns of antimicrobial resistance in healthcare settings. In conclusion, it is apparent that *Acinetobacter baumannii* strains collected from Sheikh Hasina National Institute of Burn and Plastic Surgery were resistant to multiple drugs and were present in patients admitted to the intensive care unit (ICU).

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Glossary

MacConkey Agar: Since Acinetobacter baumannii is a gram-negative bacteria that can not ferment lactose, it cannot create acid on MacConkey agar. This enables it to be distinguished from bacteria that ferment lactose, which causes colonies of bacteria on MacConkey agar to turn pink or red. To differentiate itself from other non-lactose fermenters, Acinetobacter baumannii grows colorless to light pink colonies on MacConkey agar (Ajao et al., 2011).

Leeds Acinetobacter Media (LAM): It is a selective and differential media, it contains fructose, and sucrose that cannot be fermented by Acinobacter species resulting in pink or radish colonies. Moreover, some chromogenic substances in this medium allow to grow and differentiate *Acinetobacter baumannii* from other acinetobacter species. Acinatobacter Baumani gives reddish pink or bright pink color colonies in this media (McConnell et al., 2011).

PCR: Polymerase chain reaction, or PCR, is a technique for producing multiple copies of a given DNA sequence in vitro (in a test tube rather than an organism). PCR uses a thermostable DNA polymerase called Taq polymerase and DNA primers specific especially for the DNA region of interest. In PCR, the reaction is repeatedly cycled through a series of temperature changes, resulting in numerous copies of the target region. PCR has numerous research and practical uses. It is widely utilized in DNA cloning, medical diagnostics, and forensic analysis of DNA (PCR, n.d.).

Gel electrophoresis: Gel electrophoresis is a technique that uses an electric current to draw DNA fragments through a gel matrix and separate them based on their size. A standard, also known as a DNA ladder, is generally included to determine the size of the fragments in PCR samples. Agarose gel electrophoresis is mostly used to separate fragments according to their size (Khan Academy, n.d.).
When current is applied, negatively charged DNA/RNA migrates through the pores from the well of an agarose gel to the positive charged end.

Mular Hinton Agar (MHA): MHA agar is a non selective, non differential media. It contains starch and loose agar. Starch absorbs all the bacterial toxins so that it does not interfere with the antibiotics. To get the better zone of inhibition Muller Hinton agar contains loose agar which gives a better diffusion(Aryal, 2022).