Prevalence of Multidrug-resistant *Acinetobacter baumannii* in Intensive Care Unit Admitted Patients from a Hospital in Dhaka City, Bangladesh

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirement for the Degree of Bachelor of Science in Microbiology

Department of Mathematics and Natural Sciences

Brac University

December 2022

DECLARATION

It is hereby declared that

1. The thesis submitted is my/our own original work while completing the 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 that 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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ABSTRACT

Background: Multidrug-resistant *Acinetobacter baumannii* has become a concern in the world of healthcare. The most common source of infections acquired in hospitals is an opportunistic bacterial pathogen called *A. baumannii*. It has become a significant nosocomial pathogen that has claimed many lives throughout the world, including in Bangladesh. This study's goals were to determine the pathogen's prevalence and contribute to the development of a local antibiogram database so that future treatment approaches can be improved.

Materials and Method: From August 2022 to December 2022, a total of 72 pathogenic Gram-negative clinical isolates of *Acinetobacter spp.* were collected from ICU- admitted patients, and tested at the clinical microbiology laboratory of a private hospital in Dhaka, Bangladesh. Most of the isolates were recovered from tracheal aspirates, sputum, pus, and wounds. The collected isolates were further analyzed at BRACU MNS research laboratory presumptively by cultural methods for the presence of *A. baumannii* using highly selective Leeds Acinetobacter Medium (LAM) and *A. baumannii* was confirmed by conventional polymerase chain reaction (PCR) using the primers of $bla_{OXA\text{-}51}$.

***Findings*:** From all the 72 clinical isolates, a total of 24 *A. baumannii* isolates tested positive using conventional Polymerase Chain Reaction (PCR) and Agarose Gel Electrophoresis, determining the prevalence as 33%. All confirmed isolates were characterized by Antimicrobial Susceptibility Testing (AST), performed following the disk diffusion method as recommended by Clinical Laboratory and Standards Institute (CLSI). The *A. baumannii* isolates confirmed Multidrug-resistance (MDR) by showing antimicrobial resistance to more than three antimicrobial categories, such as 100% resistance to Ampicillin (AMP), 70.8% resistance to Cefepime (CPM), Ceftazidime (CAZ), and Levofloxacin (LE), 66.7% resistance to Imipenem (IMP), 62.5% to Gentamicin (GEN), Amikacin (AK) and Tetracycline (TE), and 58.3% resistance Piperacillin-tazobactam (PIT). However, the highest sensitivity result showed 62.5% for Doxycycline (DO) and 58.3% for Trimethoprim-sulfamethoxazole (COT).

***Conclusion*:** This research suggests that the supervision of antimicrobial resistance of *A. baumannii* is essential. The prevalence will help in the implementation of better infection control measures, and a local antibiogram update will increase our awareness of the patterns of antimicrobial resistance in healthcare facilities.

Keywords: *Acinetobacter baumannii;* antimicrobial resistance; bla_{OXA}-51

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Maliha Abdul Mannan Sharif, Nabila Tabassum & Md. Omar Faruque
December 2022

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ABBREVIATIONS

Abbreviation	Elaboration
bp	Base pairs
CLSI	Clinical & Laboratory Standards Institute
dH2O	Distilled water
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
CRE	Carbapenem resistant Enterobacteriaceae
et. al	and others
EtBr	Ethidium bromide
MDR	Multidrug-resistant
mm	Millimeter
OXA	Oxacillinase
PCR	Polymerase chain reaction
pH	Power of hydrogen
rpm	Revolutions per minute
rRNA	Ribosomal ribonucleic acid
TBE	Tris-borate-EDTA
TE	Tris-EDTA
MCT	Microcentrifuge tube

Chapter 1

Introduction

In many hospitals around the world, nosocomial infections have become a major health concern. Acinetobacter infections are a common cause of nosocomial infections in hospitals. The Gram-negative aerobic coccobacillus *Acinetobacter baumannii (A. baumannii*) is one of the most common causes of nosocomial bloodstream infections in Intensive Care Units (ICUs). Several clinical infections caused by *A. baumannii* have been identified, including nosocomial pneumonia, meningitis, endocarditis, skin and soft tissue infections, urinary tract infections, conjunctivitis, burn wound infections, and bacteremia. It is one of the most often isolated bacterium strains in intensive care unit (ICU) patients because its antibiotic resistance poses significant therapeutic challenges (Falagas & Rafailidis, 2007). The infections are difficult to control and treat because of their ability to survive in hospital environments for long durations and develop high resistance to broad-spectrum antibiotics. The transmission of this species in the ICU is facilitated by the special conditions of the ICU, artificial ventilation and other invasive procedures, exposure to antibiotics, colonization pressure, and underlying sickness (Zarrilli et al, 2009). Acinetobacter outbreaks are associated with contaminated bedding, intravascular access devices, breathing equipment, and bedding materials, as well as transmission through hospital staff members' hands (Wisplinghoff et al., 2000). Infections with *A. baumannii* have been linked to higher mortality rates (Lemos et al., 2014). Multiple antibiotic resistance can increase the pathogenicity of *A. baumannii*, making it difficult to determine the best treatment approaches (Grupper et al., 2007). According to the Chinese Meropenem Susceptibility Surveillance (CMSS) study from 2010, 180 strains of *A. baumannii* were found in 1259 samples of *Gram*-negative *bacilli* from 13 hospitals, making it the second most common infection after *Pseudomonas aeruginosa* (Wang et al., 2011).

In a developing country like Bangladesh, MDR *A. baumannii* infection has been posing severe challenges among patients treated in hospitals in Bangladesh, over the past few years due to its rising prevalence. In Bangladeshi hospitals, infection control procedures and medical waste management are extremely inadequate or lacking (Hasan et al., 2015). *A. baumannii* strains have become more and more resistant over the past few years, requiring the use of broad-spectrum antibiotics like imipenem and ampicillin-sulbactam. These tigecycline- and colistin-resistant *A. baumannii* strains are resistant to all other antibiotics (Sengstock et al., 2010).

According to one of the recent studies in Bangladesh, *Acinetobacter baumannii* prevalence in DMCH, Bangladesh, was about 14%, which is slightly higher than prevalence rates in developing countries like India, which were 9.5%, 9.4%, and 11%, respectively. The prevalence rates in Kuwait (22.17 %), Saudi Arabia (31.71%), and Japan (18%16) are all greater than those found in the currently mentioned study (Uddin et al, 2021). An Indian study shows that ICU provided the greatest number of isolates; where tracheal samples were the most common location for *A. baumannii*, and the majority of the strains exhibited considerable antibiotic resistance (Islahi et al., 2014). The resistance profile of the *A. baumannii* isolates at one of the hospital base studies revealed that 96% were MDR, resistant to three classes of antibiotics, including cephalosporins, aminoglycosides, and fluoroquinolones; 85.0% were XDR. Colistin now has a sensitivity of 96.07% against *A. baumannii*, which is nearly identical to several previous studies (Cai, et al., 2012). Therefore, treating these infections has always been challenging and complex.

In our study, the prevalence of *A. baumannii* is almost 33.33% which is close to the other studies by Bhuiyan Mohammad Mahtab Uddin. The antibiotics that were used during AST were from seven different antimicrobial categories, showing MDR to more than three categories followed by Cefepime (CPM, 30mg), Piperacillin-tazobactam (PIT, 100/10mg), Ceftazidime (CAZ, 30mg), Imipenem (IMP, 10mg), Gentamicin (GEN, 10mg), Doxycycline (DO, 30mg), Levofloxacin (LE, 5mg)/ Ciprofloxacin (CIP, 5mg), Trimethoprim-sulfamethoxazole or Co-trimoxazole (COT, 1.25/23.75 mg), Amikacin (AK, 30mg), Tetracycline (TE, 30mg), and Ampicillin-sulbactam (AMP, 10/10mg). The antimicrobial susceptibility test findings were evaluated using the Clinical and Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute, 2018).

1.1 Objective

The variations in prevalence and resistance profiles among isolates emphasize the importance of local surveillance to choose the most effective antibiotic treatment for *A. baumannii* infections. In this study, *A. baumannii*, which was isolated from a variety of clinical specimens of ICU-admitted patients at a hospital (Popular Diagnostic Center Ltd.) in Dhaka, Bangladesh, was evaluated for prevalence and antibiotic resistance patterns including MDR.

Chapter 2

Materials and Methods

2.1 Sample Collection and Processing

This study was conducted in the Clinical Microbiology laboratory of a hospital and MNS Research Laboratory of BRAC University in Dhaka, Bangladesh, for a period of 4 months, from August 2022 to December 2022. From diverse clinical specimens, including sputum, wound swabs, blood, soft tissue, Central Venous Catheter Tip (CVC), etc., a total of 72 non-duplicate isolates of *Acinetobacter spp*. were recovered. The samples were collected and processed from hospital patients in the ICU department as part of standard clinical assessment. Initially, the specimens received in the hospital laboratory of Clinical Microbiology were inoculated on 5% Blood Agar and MacConkey Agar and incubated overnight at 37°C and were later identified by colonial morphology, Gram staining, a negative oxidase test, and oxidation of glucose.

2.2 Microbiological analysis of the isolates:

All the isolated strains from MacConkey were sub-cultured on a highly selective medium called "Leeds Acinetobacter Medium" (LAM, HiMedia, India), for growing pure species of *A. baumannii* from the clinical isolates. After sub-culturing on LAM at 37°C incubated for 24-48 hours, Acinetobacter species produce light pink colored colonies with mauve backgrounds, that have diffused into the medium (Jawad et al, 1994). The suspected pink colonies were observed. Confirmed *A. baumannii* isolates were tested for Antimicrobial Susceptibility afterward on a non-selective medium, NA (Nutrient Agar, HiMedia, India), and incubated at 37°C overnight for fresh culture which is a requirement for AST.

The following selective, differential, non-selective, and non-differential media were used during these processes-

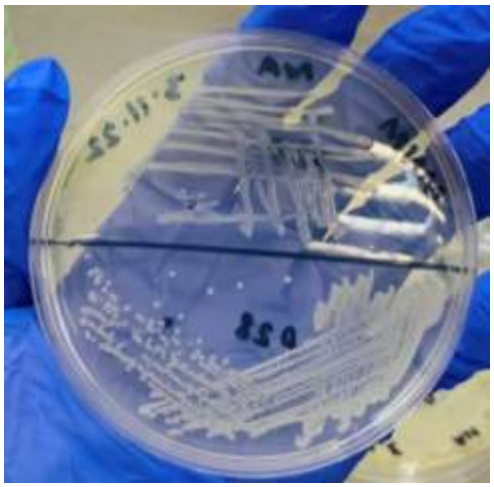
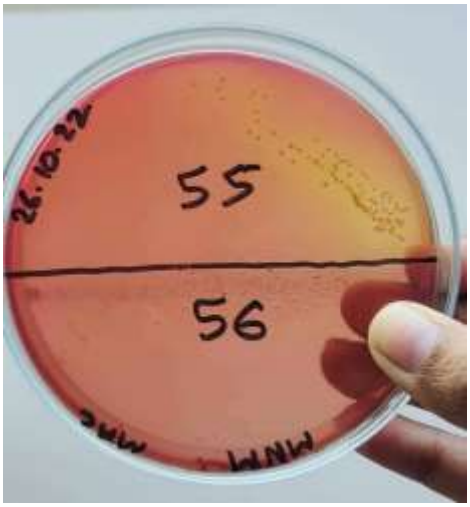
Selective and Differential Media	Non-Selective and Non-Differential Media
1. Leeds Acinetobacter Medium (Selective) 	**2. Nutrient Agar (Non-selective)** 
3. MacConkey Agar (Differential and selective) 	**4. Muller-Hinton-Agar (Non-selective and non-differential)** 

Figure 2.1: Name of the selective, differential, non-selective and non-differential media.

2.3 Extraction of DNA from the Isolates

The genomic DNA of the selected isolates was extracted via the "Boiling method' due to its efficiency, simplicity, and cost-effectiveness (Dimitrakopoulou et al, 2020). The suspected *A. baumannii* isolates were grown in LB broth (Luria-Bertani Broth; HiMedia, India) overnight at 37°C using a Shaking Incubator. Later, the bacterial cultures went through a transfer of 700 microliters into MCTs, centrifuged at 13,000 rpm for 5 minutes. The supernatant was discarded from the top carefully, and the pellet was then suspended in 200 microliters of TE buffer [The major purposes of TE buffer are pH control, solubilization of DNA or RNA, and defense against enzymatic lysis of the nucleic acids. The components of TE (Tris-EDTA) buffer are the pH buffer Tris and the metal chelating ion EDTA. It is used to lyse, wash, and dissolve DNA during DNA extraction procedures] and boiled at 100°C for 12 minutes (Rice, 2021) The next step was followed by chilling for 10 minutes. The cell suspension went through the last round of centrifuge at 13,000 rpm for 5 minutes. The final DNA-rich supernatant was collected into new MCTs and stored at -20°C.

2.4 PCR Amplification

Presumptive *Acinetobacter baumannii* isolates were screened for confirmation by using *bla*$_{OXA-51}$ primers. PCR amplification was done with the following set of primers from Table 2.1-

Primer	Primer Sequence	PCR Condition	Number of Cycles	Amplicon Size	Reference
blaOXA-51F ***blaOXA-51R***	5'-TAATGCTTTGATCGGCCTTG-3' 5'-TGGATTGCACTTCATCTTGG-3'	94°C for 5 minutes 94°C for 30 seconds 55°C for 30 seconds 72°C for 30 seconds 72°C for 7 min at 4°C	30	253 bp	Falah et al.,2019

Table 2.1: Primers used for amplification of resistance genes by polymerase chain reaction (PCR).

2.4.1 PCR Preparation

The DNA extracts were used as a template for PCR amplification during this preparation. The final volume for all PCR reactions was 13 µl which contained 6.5 µl of MM (Master Mix), 0.26 µl of FP (Forward Primer), 0.26 µl of RP (Reverse Primer), 3.98 µl of NFW or dH2O (Nuclease-Free Water) and 2 µl of DNA template for each sample. The calculation given below is for 1 sample and for multiple samples, the amount will be multiplied with "n**".**

Name of the Reagent	**Total Volume= 13 µl**
Master Mix (2x)	6.5 µl
F Primer (10 µM)	0.26 µl
R Primer (10 µM)	0.26 µl
Nuclease-Free Water	3.98 µl
DNA Template	2 µl
Total	**13 µl**

Table 2.2: PCR Preparation calculation for 1 sample.

The following were the PCR cycling conditions:

Primer annealing at various temperatures (Table 2.1) 94°C for 5 min, 30 cycles (94°C for 30 s, 55°C for 30 s, 72°C for 30 s), and final temperature at 72°C for 7 min 4°C. Both positive (*A. baumannii,* ATCC 19606 or true positive) and negative controls were used in each PCR experiment. Aliquots of PCR products were analyzed by gel electrophoresis in 1.2% (w/v) agarose gel and the gel was stained with EtBr and run at a constant voltage of 110V for 60 min. after 60 min, the DNA bands were visualized under a UV transilluminator.

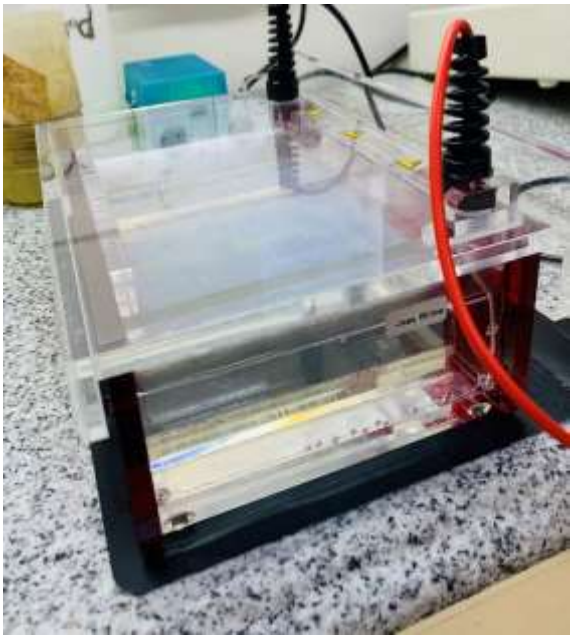
<table>
<tr><td>  </td><td>  </td></tr>
<tr><td>1. PCR tubes inside a Conventional PCR system.</td><td>2. Conditions setting parameters of PCR system.</td></tr>
<tr><td> 
  </td><td>  </td></tr>
<tr><td>3. 50/1000 bp DNA Ladder and PCR master mix.</td><td>4. Agarose Gel Electrophoresis system.</td></tr>
</table>

Figure 2.2: Some of the tools used during PCR amplification and Agarose Gel Electrophoresis.

2.5 Antibiotic Susceptibility Testing

The isolates' antimicrobial susceptibility was determined using the conventional Kirby-Bauer disc diffusion technique on Mueller-Hinton (MHA, HiMedia, India) agar in the presence of 11 different antibiotics from 7 antimicrobial categories. Initially, the bacterial suspension was adjusted to vequivalent to 0.5 McFarland standard based on the turbidity of the suspension, the suspension was inoculated onto MHA plates. Afterward, the 11 antibiotic disks were placed on MHA plates and incubated at 37◦C for 18–24 hours. The antibiotic disks that were used during this test-Cefepime (CPM, 30mg), Piperacillin-tazobactam (PIT, 100/10mg), Ceftazidime (CAZ, 30mg), Imipenem (IMP, 10mg), Gentamicin (GEN, 10mg), Doxycycline (DO, 30mg), Levofloxacin (LE, 5mg)/ Ciprofloxacin (CIP, 5mg), Trimethoprim-sulfamethoxazole or Co-trimoxazole (COT, 1.25/23.75 mg), Amikacin (AK, 30mg), Tetracycline (TE, 30mg), and Ampicillin-sulbactam (AMP, 10/10mg) were used for this test. The antimicrobial susceptibility test findings were evaluated using the Clinical and Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute, 2018).

Chapter 3

Results

3.1 Identification of *A. baumannii* through PCR

Targeted PCR analyses were carried out using the protocols and primers previously discussed. The results showed that $bla_{OXA\text{-}51}$ confirmatory primer resulted from the presence of *A. baumannii* in 24 out of 72 collected isolates via the conventional PCR method. **Figure 3.1** shows that the positive results were identified by giving a band size of 353 bp for each sample. As a positive control, ATCC 19606 was used and as a negative control, no DNA template was added to the PCR product (Figure 3.1.1). The results suggest that the *OXA-51* could be the main cause of the resistance of *A. baumannii* isolates from the ICU-admitted patients in the hospital. Other investigations also reported *OXA-51* group is one of the most prevalent carbapenemase-encoding genes (Leski, 2013).

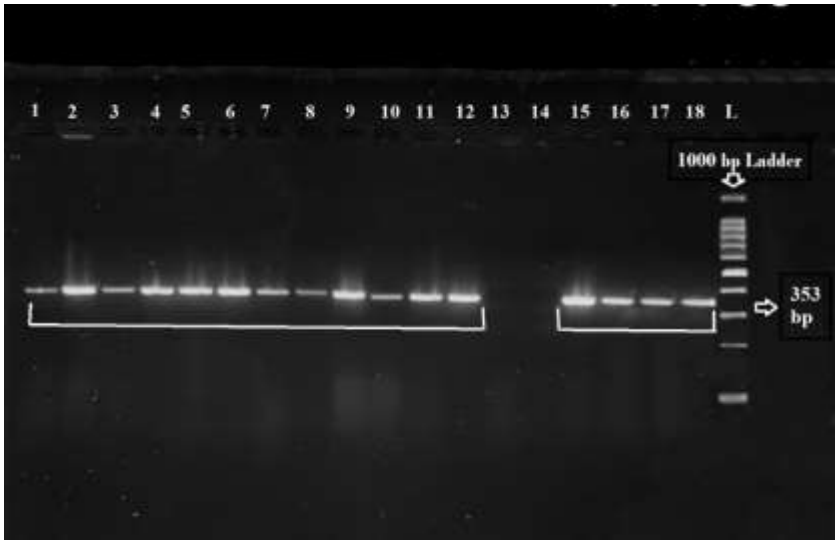


Figure 3.1: The gel electrophoresis image of PCR product of *bla*$_{OXA-51}$ (353 bp) DNA amplification in some isolates of *A. baumannii* under UV. 1–12: A. baumannii isolates; 13–14: negative control; 15-18: *A. baumannii* isolates; L: 1000 bp marker.

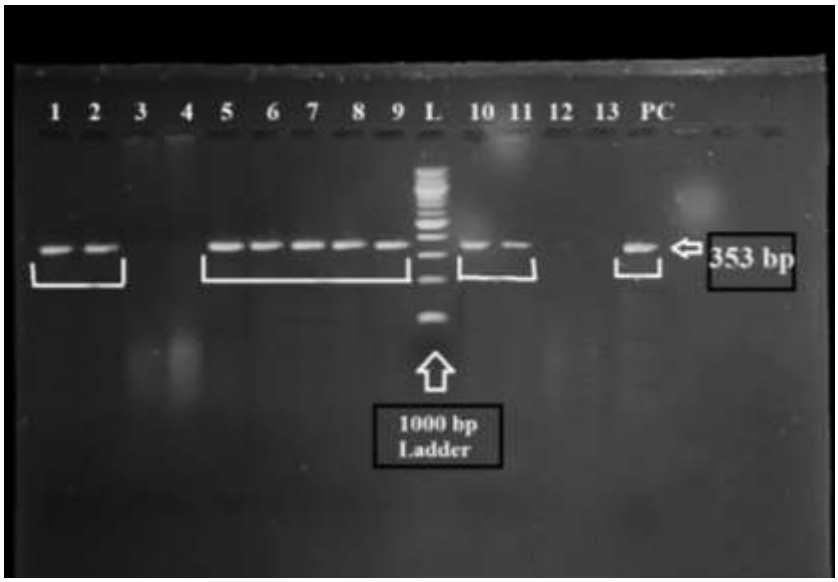


Figure 3.2: The gel electrophoresis image of PCR product of *bla*$_{OXA-51}$ (353 bp) DNA amplification in some isolates of *A. baumannii* under UV. 1–2: *A. baumannii* isolates; 3–4: negative result; 5-9 & 10-11*: A. baumannii* isolates, L: 1000 bp marker; PC: positive control (*A. baumannii*, ATCC19606).

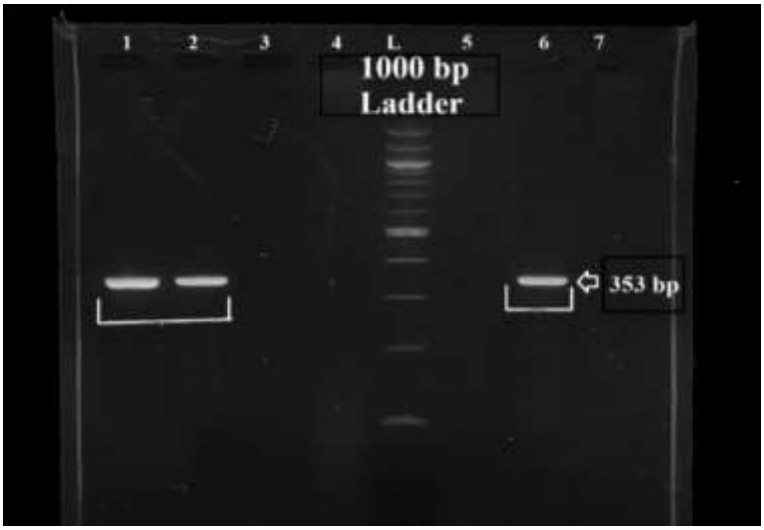


Figure 3.3: The gel electrophoresis image of PCR product of *bla*$_{OXA-51}$ (353 bp) DNA amplification in some isolates of *A. baumannii* under UV. 1–2: *A. baumannii* isolates, 3–5: negative result; 6: *A. baumannii isolates*, L: 1000 bp marker.

3.2 Antibiotic Susceptibility Profiling

For resistance profiling, antimicrobial susceptibility testing (AST) was done by following the Kirby-Bauer disk diffusion method as per the (CLSI) guidelines, using Muller-Hinton Agar (MHA) and 11 antibiotic-impregnated disks. Figure 3. shows the zone of inhibition of the antimicrobial susceptibility pattern from *A. baumannii* isolates. From picture A, the organism is showing resistance to Ampicillin (AMP) and sensitivity towards Piperacillin-tazobactam (PIT), Imipenem (IMP), Ceftazidime (CAZ), and Cefepime (CPM). From picture B, the organism showing high sensitivity to Trimethoprim-sulfamethoxazole or Co-trimoxazole (COT), Doxycycline (DO), Tetracycline (TE,) and Levofloxacin (LE) respectively, and shows less sensitivity to Amikacin (AK). For quality control, the standard strain of *A. baumannii* (ATCC 19606) was used.

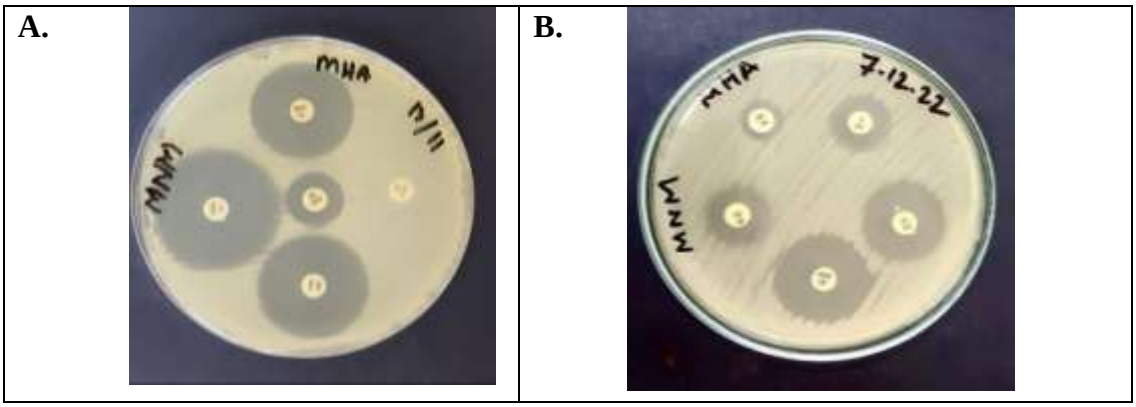


Figure 3.4: Disk Diffusion method to check antimicrobial susceptibility pattern.

The study showed that, out of 24 *Acinetobacter baumannii* samples, 17 samples showed 70.83% (17/24) resistance against the antibiotics, and 29.1% (7/24) showed sensitivity to most antibiotics. Here, 11 antibiotic discs were incorporated, and resistance and sensitivity profiles had been recorded. The total susceptibility of isolates for each antibiotic disc is shown in (**Table 3.1**) by following the CLSI chart. Total susceptibility percentages show that all 24 confirmed *A. baumannii* isolates showed 100% resistance to Ampicillin (AMP), 70.8% resistance to Cefepime (CPM), Ceftazidime (CAZ), and Levofloxacin (LE), 66.7% resistance to Imipenem (IMP), 62.5% to Gentamicin (GEN), Amikacin (AK) and Tetracycline (TE), and 58.3% resistance Piperacillin-tazobactam (PIT). However, the highest sensitivity result showed 62.5% for Doxycycline (DO) and 58.3% for Trimethoprim-sulfamethoxazole (COT). It is evident that antibiotics that were used during AST were from seven different antimicrobial categories, showing MDR to more than three categories making the isolates multidrug-resistant against the antimicrobial groups (**Figure 3.5).**

Antibiotics	**Isolates Resistant (%)**	**Isolates Sensitive (%)**	**Isolates Intermediate (%)**
Ampicillin-sulbactam (AMP)	24 (100 %)	0 (0 %)	0 (0 %)
Cefepime (CPM)	17 (70.8 %)	6 (25 %)	1 (4.2 %)
Ceftazidime (CAZ)	17 (70.8 %)	6 (25 %)	1 (4.2 %)
Levofloxacin (LE)/ Ciprofloxacin (CIP)	17 (70.8 %)	7 (29.2 %)	0 (0 %)
Imipenem (IMP)	16 (66.7 %)	7 (29.2 %)	1 (4.2 %)
Amikacin (AK)	15 (62.5 %)	9 (37.5 %)	0 (0 %)
Tetracycline (TE)	15 (62.5 %)	9 (37.5 %)	0 (0 %)
Gentamicin (GEN)	15 (62.5 %)	8 (33.3 %)	1 (4.2 %)
Piperacillin-tazobactam (PIT)	14 (58.3 5)	8 (33.3 %)	2 (8.3 %)
Trimethoprim-sulfamethoxazole (COT)	9 (37.5 %)	14 (58.3 %)	1 (4.2 %)
Doxycycline (DO)	4 (16.7 %)	15 (62.5 %)	5 (20.8 %)

Table 3.1: Antimicrobial Susceptibility Pattern of isolated *A. baumannii.*

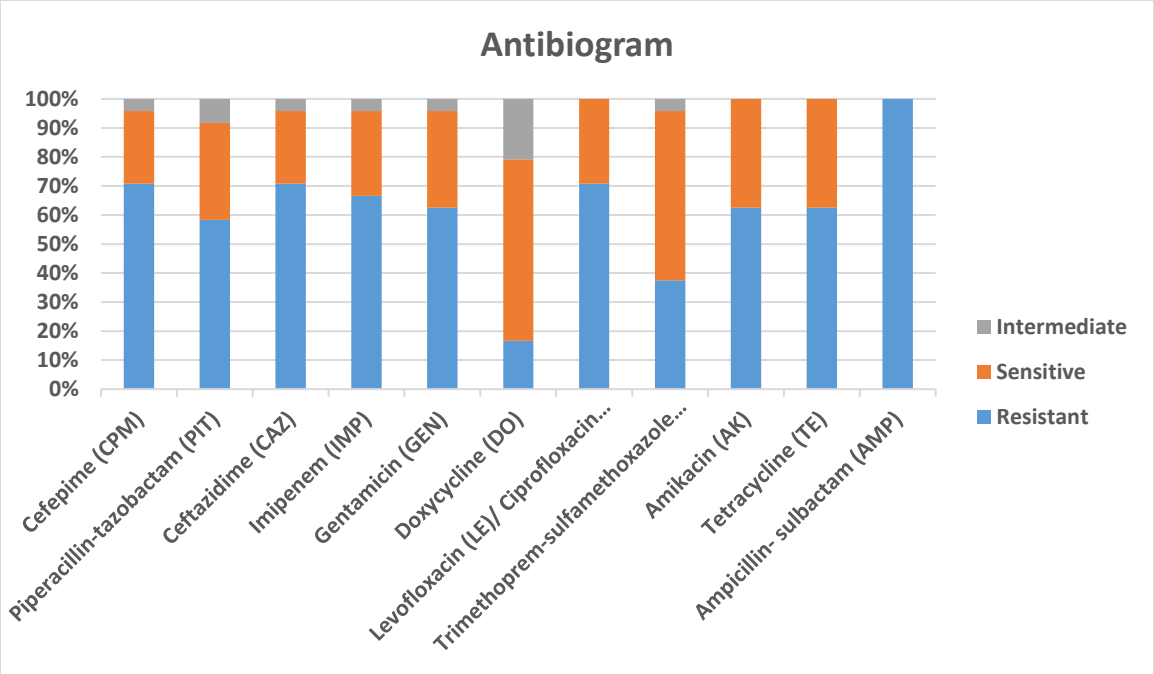


Figure 3.5: **Resistance and sensitivity patterns of all confirmed *A. baumannii* isolates.**

Chapter 4

Discussion:

Antibiotic susceptibility testing is used to identify the potential efficiency of certain antibiotics on bacteria and whether the bacteria have evolved any resistance to specific antibiotics. The findings of this test can be used to determine which medications are most likely to be beneficial in treating an infection (Altun et al, 2014). Based upon these considerations, the current study was conducted to identify the prevalence of *A. baumannii* from ICU-admitted patients, which was 33.33% (24/72) over a period of 4 months (**Figure 3.6**). The study showed that, out of 24 *A. baumannii* samples, 17 samples showed resistance of 70.83% (17/24) against the antibiotics and five out of seven antimicrobial categories showed multidrug resistance as well. The remaining isolates showed 29.1% (7/24) sensitivity to most antibiotics. The confirmation of *A. baumannii* isolates was done via traditional PCR amplification, and how frequently isolated resistant bacteria include one of the genes $bla_{OXA\text{-}51}$ that codes for carbapenemase of the OXA type.

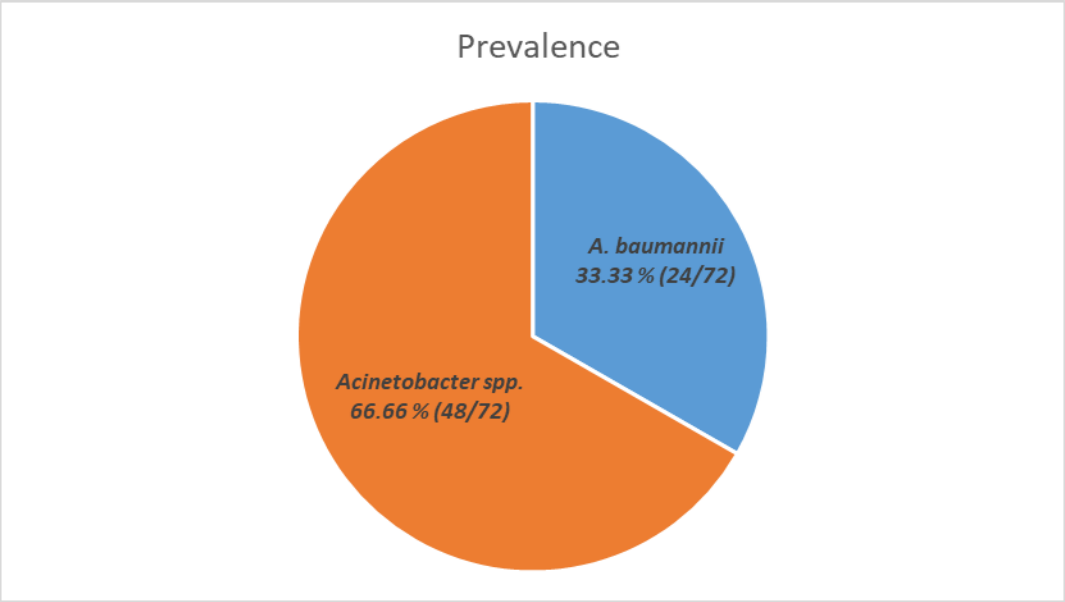


Figure 3.6: Prevalence of *A. baumannii* in ICU-admitted patients.

Most typically, β-lactamase enzymes, also known as carbapenemases are able to hydrolyze carbapenem compounds differ in the range of host microorganisms they can digest, the types of substrates they prefer, and how sensitive they are to β-lactamase inhibitors (Patel & Bonomo, 2011). Additionally, carbapenemases are typically present in pathogenic strains that have other genetic elements that give resistance to aminoglycosides, tetracyclines, β-lactams, and fluoroquinolones, which can cause intractable infections with significant fatality rates (Poirel et al., 2011). In our study, it was found that most resistance showed antibiotic groups were β-lactam combination agents (Ampicillin 100%, Piperacillin-tazobactam 58.3%), Cephems (Ceftazidime 70.8%, Cefepime 70.8%), Carbapenems (Imipenem 66.7%), Aminoglycosides (Gentamicin 62.5%, Amikacin 62.5%), and Fluoroquinolones (Levofloxacin 70.8%). In case resistance showing antibiotic groups, Folate pathway antagonists (Co-trimoxazole 58.30%) and Tetracyclines (Doxycycline 62.30%) showed the most sensitivity. Therefore, our study evidenced that *bla*$_{OXA-51}$ was confirmed in 24 isolates using the primers and the isolates showed resistance to aminoglycosides, β-lactam combination agents, cephems, carbapenems, and fluoroquinolones and these cause infections in compromised patients at ICU, which is similar to the study by Laurent Poirel. Undoubtedly, antimicrobial resistance is the biggest problem right now. Drugs that reduce the morbidity and mortality caused by major and life-threatening infections become less effective, endangering human health. Multidrug resistance (MDR) is a widespread issue that presents

difficulties for infections. It is essential to determine an isolate's multidrug resistance pattern in order to resolve this problem. In this study, an effort was made to separate 24 *A. baumannii* strains from clinical samples to analyze their MDR patterns as well. Additionally, our research was successful in allowing a PCR-based technique that would make it simple and effective to identify the significant $bla_{OXA\text{-}51}$ present in the clinical isolates from the ICU admitted patients.

In conclusion, based on our study's findings, multidrug-resistant *A. baumannii* can be found in the hospital environment. Therefore, it is essential to identify *A. baumannii* infections early and implement appropriate control measures to stop their spread throughout hospital settings, particularly in intensive care units.

References

1) AbdAllah S. Nosocomial infections and their risk factors at Mubarak Al-Kabeer hospital. Kuwait. Medical Journal of Cairo University. 2009;78:123-31.
2) Altun, H. U., Yagci, S., Bulut, C., Sahin, H., Kinikli, S., Adiloglu, A. K., & Demiroz, A. P. (2014). Antimicrobial Susceptibilities of Clinical Acinetobacter baumannii Isolates With Different Genotypes. Jundishapur journal of microbiology, 7(12), e13347. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4335573/
3) Cai Y, Chai D, Wang R, Liang B, Bai N. Colistin resistance of Acinetobacter baumannii: Clinical reports, mechanisms and antimicrobial strategies. J Antimicrob Chemotherapy 2012;67:1607-1615
4) Dimitrakopoulou M E, Stavrou V, Kotsalou, and Vantarakis A (2020). Boiling Extraction Method VS Commercial Kits for Bacterial DNA Isolation from Food Samples. Journal of Food Science and Nutrition Research. 03. 10.26502/jfsnr.2642-11000057.
5) Endo S, Yano H, Hirakata Y, Arai K, Kanamori H, Ogawa M, et al. Molecular epidemiology of carbapenem-non-susceptible *Acinetobacter baumannii* in Japan. J Antimicrob Chemother 2012;67(7):1623-1626 Villers D, Espaze E
6) Falagas M E and Rafailidis (2007). PI: Attributable mortality of Acinetobacter baumannii: No longer a controversial issue. Retrieved from https://doi.org/10.1186/cc5911
7) Falah F, Shokoohizadeh, L, and Adabi, M (2019). Molecular identification and genotyping of Acinetobacter baumannii isolated from burn patients by PCR and ERIC-PCR. Scars, Burns & Healing. 5. 205951311983136. 10.1177/2059513119831369.

8) Grupper M, Sprecher H, Mashiach T, and Finkelstein R (2007). Attributable Mortality of Nosocomial Acinetobacter Bacteremia. Infection Control & Hospital Epidemiology, 28(3), 293-298. doi:10.1086/512629

9) Hasan B, Alam A, Akter L. Melhus A (2015). Dissemination of multidrug resistant ESBL-producing E. coli O25b-ST131 Clone and role of House Crow (Corvus splendens) foraging on hospital waste in Bangladesh. Clin Microbiol Infect. http://www.clinicalmicrobiologyandinfection.com/article/S1198-743X(15)00661-8/abstract.

10) Hudzicki, J. (2009,08 December). Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. American Society for Microbiology. https://asm.org/getattachment/2594ce26-bd44-47f6-8287-0657aa9185ad/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Protocol-pdf.pdf

11) Islahi S, Ahmad F, Khare V, Mishra N, Yaqoob S, Shukla P, and Singh Y I (2014). Prevalence and resistance pattern of Acinetobacter species in hospitalized patients in a tertiary care centre. Journal of Evolution of Medical and Dental Sciences. 3(17):4629-36

12) Jawad A., Hawkey P M., Heritage J., and Snelling A M. (1994). Description of Leeds Acinetobacter Medium. *Journal of clinical microbiology*. 32. 2353-8. 10.1128/JCM.32.10.2353-2358.1994.

13) Lemos, E V., Hoz, F P, Einarson T R, McGhan W F, Quevedo E, Castañeda C, and Kawai K (2014). Carbapenem resistance and mortality in patients with Acinetobacter baumannii infection: systematic review and meta-analysis. Clinical Microbiology and Infection, 20(5), 416-423.

14) Leski T A, Bangura U, Jimmy D H, Ansumana R, Lizewski S E, Li R W, Stenger D A, Taitt C R, Vora G J (2013). Identification of blaOXA-$_{51}$-like, blaOXA-$_{58}$, blaDIM-$_{1}$, and blaVIM carbapenemase genes in hospital Enterobacteriaceae isolates from Sierra Leone. J Clin Microbiol. doi: 10.1128/JCM.00832-13. Epub 2013 May 8. PMID: 23658259; PMCID: PMC3697688.

15) Maragakis L L, Cosgrove S E, Song X, Kim D, Rosenbaum P, Ciesla N, Srinivasan A, Ross T, Carroll K and Perl T M (2004). An outbreak of multidrug-resistant Acinetobacter baumannii associated with pulsatile lavage wound treatment. JAMA. 292:3006–3011

16) Patel G, Bonomo RA. 2011. Status report on carbapenemases: challenges and prospects. Expert Rev. Anti Infect. Ther. 9:555–570

17) Poirel L, Dortet L, Bernabeu S, Nordmann P. 2011. Genetic features of blaNDM-1-positive Enterobacteriaceae. Antimicrob. Agents Chemother. 55:5403–5407

18) Rice G (2021). DNA Extraction. *Microbial Life*. Retrieved from https://serc.carleton.edu/15925

19) Sengstock D M, Thyagarajan R, Apalara J, Mira A, Chopra T, Kaye K S (2010). Multidrug resistant Acinetobacter baumannii: An emerging pathogen among older adults in community hospitals and nursing homes. Clinical Infectious Diseases. 50:1611-1616 13. AbdAllah S. Nosocomial infections and their risk factors at Mubarak Al-Kabeer hospital. Kuwait. Medical Journal of Cairo University. 2009;78:123-31.

20) Shamsizadeh Z, Nikaeen M, Esfahani B M et al. (2017). Detection of antibiotic resistant Acinetobacter baumannii in various hospital environments: potential sources for transmission of Acinetobacter infections. *BioMed Central*. Retrieved from https://doi.org/10.1186/s12199-017-0653-4

21) Siau H, Yuen KY, Wong SSY. The epidemiology of Acinetobacter infections in Hong Kong, J Med Microbiol 1996; 44:340-347

22) Uddin B M M, Akhand F, Bari S, Suchi S E, Saha R, and Ahmed M U (2022). Prevalence and Resistance Profile of Acinetobacter baumannii Isolated from Clinical Specimens at a Tertiary

Care Hospital in Bangladesh. Journal of National Institute of Neurosciences Bangladesh, 7(2), 177–180. https://doi.org/10.3329/jninb.v7i2.58117

23) Wang H, Zhao C J, Wang Z W, Ni Y X, Chen M J, Xu Y C, Yu Y S, Zhang LY, Mei Y N, Chu Y Z, et al. (2011). Report from Chinese Meropenem Susceptibility Surveillance in 2010 Antimicrobial resistance among nosocomial Gram-negative bacilli. Zhong Hua Jian Yan Yi Xue Za Zhi. 34:897–904

24) Wisplinghoff, H, Edmond M B, Pfaller M A., Jones R N, Wenzel R P, and Selfert H (2000) Nosocomial blood stream infections caused by Acinetobacter species. Clin Infect Dis 2000; 3: 690-7

25) Xiao D, Wang L, Zhang D, Xiang D, Liu D, and Xing X (2017). Prognosis of patients with Acinetobacter baumannii infection in the intensive care unit: A retrospective analysis. Experimental and Therapeutic Medicine, 13, 1630-1633. Retrieved from https://doi.org/10.3892/etm.2017.4137

26) Zarrilli R, Giannouli M, Tomasone F, Triassi M, and Tsakris (2009). A. Carbapenem resistance in Acinetobacter baumannii: the molecular epidemic features of an emerging problem in health care facilities.J Infect Dev Ctries;3(5):335e41.