

INVESTIGATION ON ANTIMICROBIAL RESISTANCE PATTERN OF
ACINETOBACTER SPP. ISOLATED FROM PATIENTS OF BANGLADESH

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for
the degree of Bachelor of Pharmacy (B. Pharm.)

School of Pharmacy
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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing 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 have acknowledged all main sources of help.

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The thesis titled “Investigation on Antimicrobial Resistance Pattern of *Acinetobacter Spp.* isolated from Patients of Bangladesh” submitted by Sadatul Islam (21346093), of Summer, 2021 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on 30 August, 2025.

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Ethics Statement

This study does not involve any animal and human trial. This project work has been funded by Research Seed Grant Initiative (RSGI), BRAC University and approved by the Biomedical Research Review Board (IRB), BRAC University (Ref# BRACUIRB125012).

Abstract

Antimicrobial resistance (AMR) in *Acinetobacter spp.* is becoming prevalent and poses a significant danger to worldwide public health. A total of 4247 culture sensitivity data were analysed from BIRDEM General Hospital Microbiology Laboratory, Dhaka, along with patient demographics for the last three years (2022 – 2024) from diverse biological specimens, including urine, pus, blood and other biological fluids. The most prevalent pathogen was *Escherichia coli* (n=1482), followed by *Klebsiella spp.* (n=1084), *Pseudomonas spp.* (n=550), *Acinetobacter spp.* (n=306) and *Staphylococcus aureus* (n=215). Highest level of antibiotic resistance was exhibited against ciprofloxacin (88.0%), amoxicillin (75.7%), cefixime (75.3%), and cefuroxime (74.2%). A subset of 18 *Acinetobacter spp.* clinical isolates was cultured for confirmatory testing by PCR. Among 18 *Acinetobacter spp.* clinical isolates, 16 were PCR-positive. There was 80% total MDR and 20% was non-MDR. Among the clinical isolates 88.9% (n=16) were MDR. Interestingly, there were a single PDR (n=1) isolate. Complete (100%) resistance was exhibited against ceftriaxone and ceftazidime, followed by amoxicillin (98%), meropenem (95%), and cefixime (94%). Overall findings highlight a deteriorating public health issue that necessitates urgent intervention to halt AMR.

Keywords: Antimicrobial resistance, Multi Drug Resistance, MAR index, *A. baumannii*, *E. coli*, *Klebsiella spp.*, *Pseudomonas spp.*

Dedication

I want to dedicate my thesis to my parents. Because of their enormous efforts, I am here today.

Acknowledgement

I want to thank Allah for helping me at every stage of my life with the submission of my project work. I'd like to thank my project supervisor, Dr. Muhammad Asaduzzaman, Professor at the School of Pharmacy, for all the time, direction, encouragement, and advice he gave me during my project and B. Pharm. career. I would like convey my special thanks to Dr. Fahim Kabir Monjurul Haque, Associate Professor, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University, for facilitating lab works to be conducted at the Microbiology Lab of MNS Department. I want to express my gratitude to Fazle Rabbi, Lecturer, School of Pharmacy, BRAC University for helping during my project work. Also, I want to give a thanks to Dr. Lovely Barai, Professor of Microbiology department at BIRDEM for helping me to collecting the clinical isolates from the hospital. I want to thankfully acknowledge Fahim Faisal Nakib, research assistant, for his daily lab supervision and for providing hands on training. Last but not the least, I would like to express my heartfelt gratitude to my friends Jarin, Jinia and Yeelma, for their continuous support and help while doing the lab works.

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List of Acronyms

MDR	Multi-Drug Resistance
MAR	Multiple Antibiotic Resistance
AST	Antimicrobial Susceptibility Test
ASM	American Society for Microbiology
WHO	World Health Organization
GLASS	Global Antimicrobial Resistance and Use Surveillance System
AMR	Anti-Microbial Resistance
XDR	Extensively Drug Resistance
PDR	Pan Drug Resistance
MHA	Mueller Hinton Agar
CLSI	Clinical Laboratory Standards Institute

Chapter 1: Introduction

1.1 Background

AMR, or antimicrobial resistance, is a growing threat to health around the world. *Acinetobacter Spp.* has become one of the most dangerous critical priority bacteria because it can become resistant to many antibiotics. AMR is having a bigger effect on public health worldwide, where *Acinetobacter Spp.* is the cause of many different illnesses. *Acinetobacter Spp.* infects patients with many different types of infections, but the clinical isolates' drug tolerance has not been fully checked. *Acinetobacter spp.* infections predominantly occur in critically ill patients within the intensive care unit (ICU) (Fournier and Richet, 2006) and constitute up to 20% of all infections globally in ICUs (Vincent et al., 2009). The objective of this systematic review and meta-analysis was to ascertain the antimicrobial resistance (AMR) of *Acinetobacter spp.* nationwide (Asmare Z et al., 2025).

1.2 Antibiotics

Antibiotics are compounds made by microorganisms like bacteria or fungus that can stop the growth of other microbes and kill their cells. The name "antibiotics" has been substituted by "antimicrobials" in modern times, referring to natural, semi-synthetic, and synthetic compounds that limit microbial multiplication, hence inducing apoptosis. Sir Alexander Fleming found Penicillin, the first antibiotic in 1929. It is also known as a "wonder drug" since it can treat bacterial infections very well, especially those caused by *Staphylococcus* and *Streptococcus* species. Antibiotic resistance is an emerging global issue. This means that medicines are no longer effective against the infectious infections they were meant to cure (Victor Chigozie et al., 2022). Antibiotics gradually emerged as therapeutic saviors. In subsequent decades, the excessive use or improper application of various antimicrobial agents exacerbated by the overprescription of antibiotics by healthcare professionals and their extensive utilization in industries such as animal husbandry and agriculture resulted in the rampant proliferation of antibiotic resistance among microbial populations. The most virulent, nosocomial, multidrug-resistant infections of clinical significance constitute a category known as "ESKAPE." This group comprises species including *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.*, with "ESKAPE" serving as an abbreviation. (Gajdács, M. et al., 2019)

1.3 Mechanism Action of Antibiotics

Antibacterial action is often classified into five mechanisms: interference with bacterial cell wall formation, inhibition of bacterial protein synthesis, inhibition of bacterial nucleic acid synthesis, disruption of metabolic pathways, and impairment of bacterial membrane integrity.

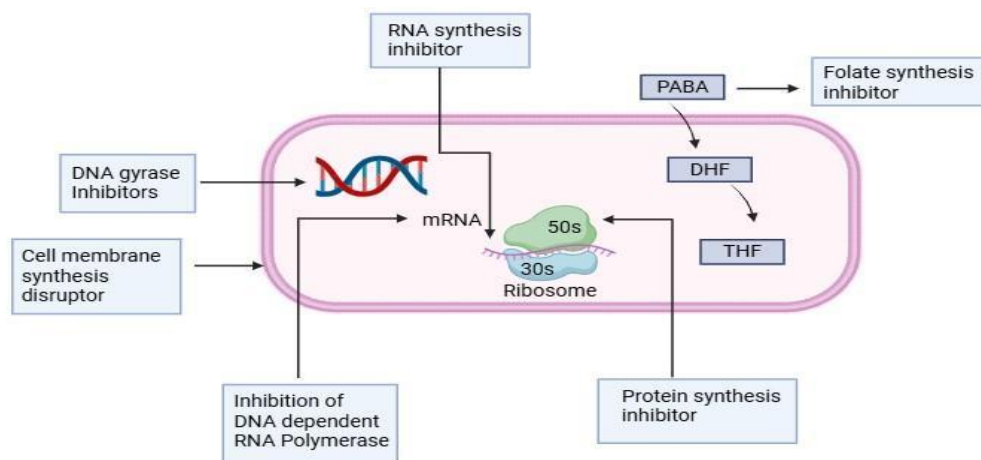


Figure 1: Mechanism Action of Antibiotics

1.4 Antibiotic Resistance Mechanisms

Antibiotic resistance occurs when bacteria, fungi, and other microbes undergo alterations that render antibiotics ineffective against them. This means that the infections are still there and may spread. Antibiotic resistance (AMR) is a worldwide health risk that makes antibiotics less efficient at fighting bacterial illnesses. AMR is also one of the most important features that bacteria have developed to survive. (Abbas A et al., 2024)

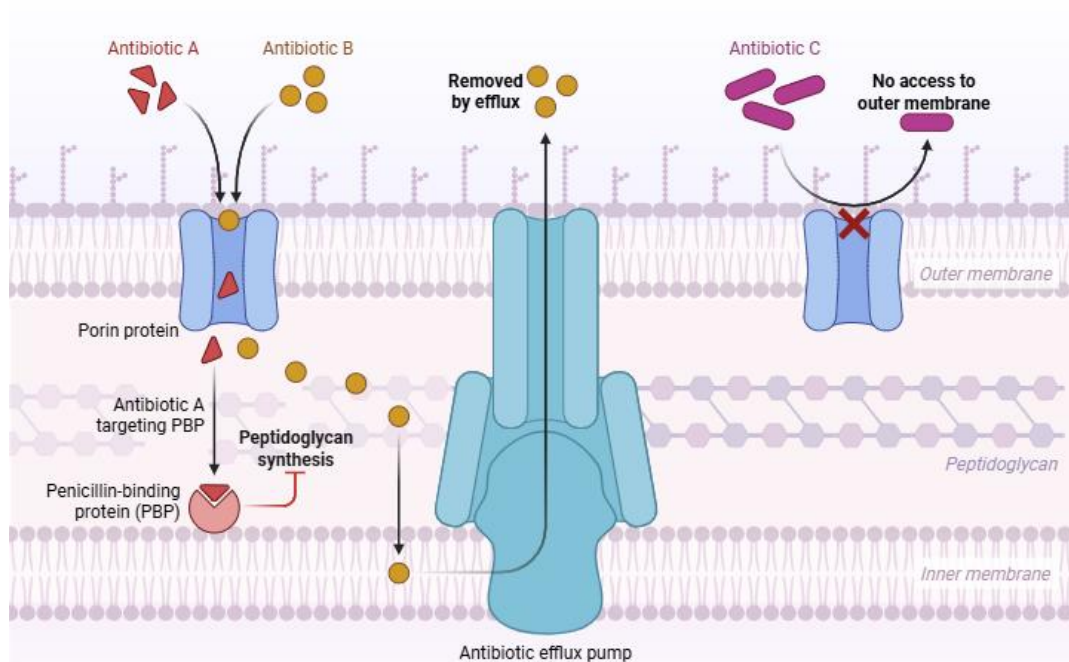


Figure 2: Mechanisms of antibiotic resistance

1.5 Major types of Antibiotics

The principal categories of antibiotics utilized in clinical practice are outlined in Table 1 as follows:

Antibiotic class	Examples	Bacteriostatic /Bactericidal	Gram (+/-)	MOA	Uses
Aminoglycosides	Gentamycin Streptomycin	Bactericidal	(-)	Inhibit Protein Synthesis (target 30s ribosomal subunit)	Bacteremia, Abdominal Infections
Cephalosporins	Ceftriaxone, Cefepime	Bactericidal	(+)/(-)	Inhibit Cell Wall Synthesis	Skin, Urinary & Respiratory Infections
Tetracyclines	Tetracycline, Doxycycline	Bacteriostatic	(+)/(-)	Inhibit Protein Synthesis (target 30s ribosomal subunit)	Lyme Diseases, PID, STIs
Penicillins	Ampicillin, Amoxicillin	Bactericidal	(+)/(-)	Inhibit Cell Wall Synthesis	ENT, Skin, Urinary Infections
Sulfonamides	Sulfasalazine, Sulfamethoxazole	Bacteriostatic	(+)/(-)	Inhibit Folate Synthesis	UTIs, Burns, Eye Infections
Fluoroquinolones	Ciprofloxacin, Levofloxacin	Bacteriostatic	(+)/(-)	Inhibit DNA Replication	Respiratory & Urinary Infections
Macrolides	Azithromycin, Erythromycin	Bactericidal	(+)	Inhibit Protein Synthesis (target 50s ribosomal subunit)	Pneumonia, Sinus, ENT, STIs
Carbapenems	Meropenem, Ertapenem	Bactericidal	(+)/(-)	Inhibit Cell Wall Synthesis	Urinary & Abdomen Infections
Lincosamides	Clindamycin	Bacteriostatic	(+)	Inhibits Protein Synthesis (50s)	Skin, Bone, Lung Infections
Glycopeptides	Vancomycin	Bactericidal	(+)	Inhibits Cell Wall Synthesis	MRSA, Skin, Endocarditis

Table 1: Major types of Antibiotics with Mechanism of Action and Uses

1.6 Antimicrobial resistance (AMR)

AMR has emerged as a significant global health issue in the 21st century. It makes antibiotics less effective, making it harder to treat common infections and making it more likely that diseases will spread, people will get very sick, and people will die. The World Health Organization (WHO) named antimicrobial resistance (AMR) one of the top ten global health challenges to humanity in 2019 (WHO, 2021). Bacterial infections that don't respond to medications mean longer hospital stays, higher medical costs, and higher death rates. This puts a lot of stress on health systems that are already stretched thin, especially in countries with low or middle incomes (LMICs), like Bangladesh. (WHO, 2021)

1.7 Multi Drug Resistance (MDR), non-MDR, XDR, and PDR

There are several ways to describe multidrug resistance patterns in Gram-positive and Gram-negative organisms. Gram-positive bacteria are classified as multidrug-resistant (MDR) when they exhibit resistance to several antimicrobial drugs, including multiple classes or subclasses. In contrast, Gram-negative bacteria are typically characterized as resistant to three or more classes of antimicrobial agents. XDR bacteria are important for public health since they are resistant to many antimicrobial drugs and probably all or almost all approved antimicrobials. The medical literature uses the letters XDR to mean "extreme drug resistance," "extremely drug resistant," and "extensively drug resistant." Pandrug resistant (PDR) means "resistant to all antimicrobial agents" from the Greek prefix "pan," meaning "all." In order to be classified as PDR, a species and its bacterial isolate must be evaluated and found to be resistant to all approved and beneficial treatments. However, PDR definitions vary in the literature. (Magiorakos et al., 2011)

1.8 GLASS Pathogen

The sixty-eighth session of the World Health Assembly approved this as the first global effort to standardize the monitoring of antibiotic resistance. GLASS was made to systematically include data from human AMR surveillance, such as monitoring resistance and how antibiotics are used. GLASS-AMR includes these pathogens: *Acinetobacter spp.*, *E. coli*, *Klebsiella pneumoniae*, *Neisseria gonorrhoeae*, *Salmonella spp.*, *Shigella spp.*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*.

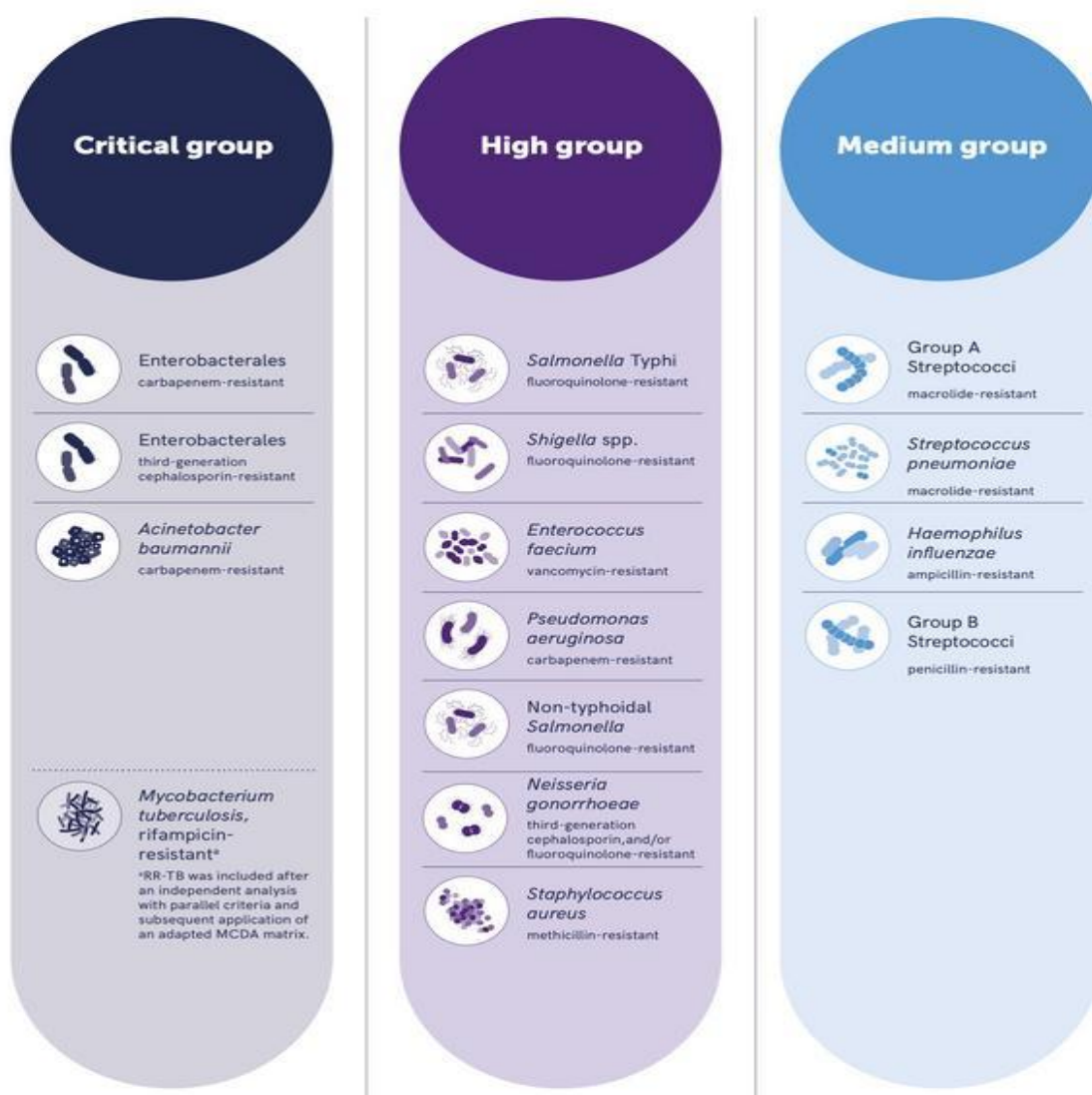


Figure 3: WHO Bacteria Priority List (WHO,2024)

1.9 *Acinetobacter baumannii*

This bacterium is a gram-negative, non-fermenting opportunistic pathogen primarily affecting critically ill patients in intensive care units (ICUs). This is associated with various hospital-acquired infections, including ventilator-associated pneumonia (VAP), bloodstream infections (BSIs), wound infections, and urinary tract infections (UTIs). A significant issue is the potential for resistance to multiple antibiotic classes, particularly carbapenems, regarded as the final line of defense. (World Health Organization, 2017) *Acinetobacter baumannii* represents the most critical species of *Acinetobacter* associated with hospital-acquired infections worldwide (Lin and Lan, 2014). This aerobic Gram-negative coccobacillus was once regarded as a low-grade pathogen; however, it is now recognized as an effective pathogen responsible for opportunistic infections in the skin, bloodstream, urinary tract, and other soft tissues (Peleg et al., 2008). Multidrug-resistant *A. baumannii* has spread to civilian hospitals, partly as a result of cross-infection among injured military personnel returning from conflict zones (Peleg et al., 2008). *A. baumannii* infections predominantly occur in critically ill patients within the intensive care unit (ICU) (Fournier and Richet, 2006) and account for up to 20% of all infections in ICUs globally (Vincent et al., 2009). The incidence of community-acquired *A. baumannii* infections has been increasing over time (Lin and Lan, 2014). Genomic and phenotypic analyses have identified multiple virulent factors, such as outer membrane porins, phospholipases, proteases, lipopolysaccharides (LPS), capsular polysaccharides, protein secretion systems, and iron-chelating systems (Antunes et al., 2011; McConnell et al., 2013; Lin and Lan, 2014).

Many investigations show that *A. baumannii* quickly becomes resistant to antimicrobials, and strains that are resistant to more than one antibiotic have been found (McConnell et al., 2013). According to the World Health Organization (WHO), *A. baumannii* is one of the most dangerous ESKAPE organisms (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, A. baumannii, Pseudomonas aeruginosa, and Enterobacter species) because it may be able to resist the effects of antibacterial drugs (Boucher et al., 2009). *A. baumannii* exhibits many resistance mechanisms, such as enzymatic drug degradation, target modifications, multidrug efflux pumps, and permeability defects (Gordon and Wareham, 2010; Kim et al., 2012).

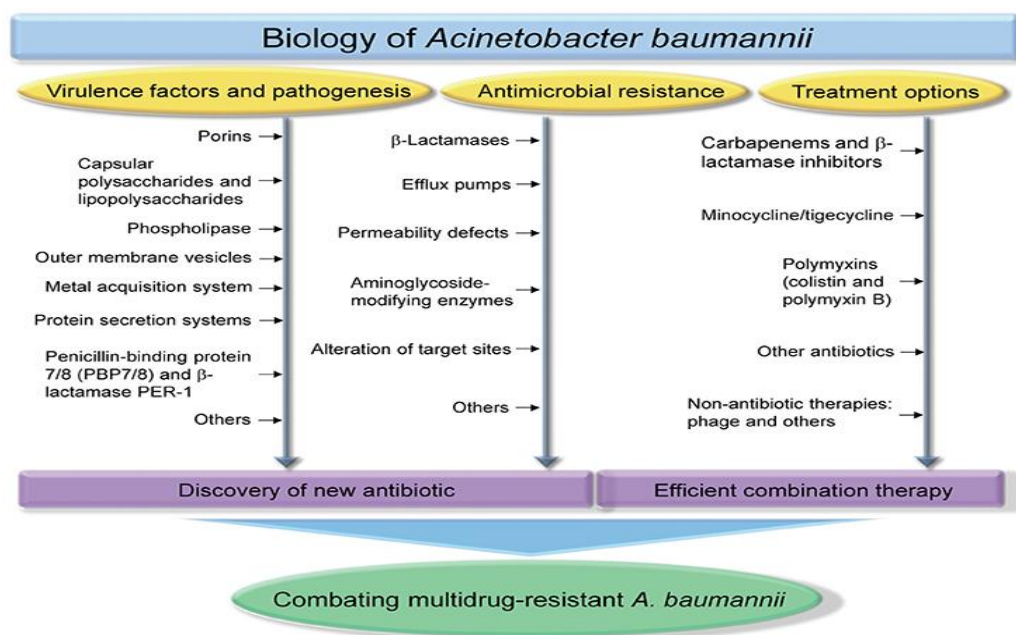


Figure 4 : Biology of *Acinetobacter baumannii* (McConnell et al., 2013)

1.10 Objective of the study

The aim of this investigation was to determine the prevalence of *Acinetobacter baumannii* spp. that are resistant to clinically available antibacterial agents among male and female patients at BIRDEM General Hospital in Dhaka, Bangladesh. Antibiotic resistance arises from the widespread use of antibiotics or may develop due to self-medication for infectious infections in underdeveloped nations or other factors. Antibiotic treatments frequently encounter resistant microorganisms. Thousands people are dying from resistant pathogens known as superbugs. Antibiotic-resistant bacteria are increasingly prevalent worldwide due to incorrect antibiotic usage by individuals. To mitigate antibiotic resistance, expenses, and patient distress, it is important to find out what bacteria are causing infections, how common they are, and how resistant they are in certain areas. This study will use epidemiological data to find out how bad and what kinds of resistance trends are present, find significant variables, and Propose strategies for infection control and monitoring antimicrobial activities.

Chapter 2: Methodology

2.1 Retrospective Study settings

A memorandum of understanding (MOU) was executed between the school of pharmacy at BRAC University and BIRDEM General Hospital in Dhaka prior to the collection of retrospective data. The retrospective analysis was conducted from the years 2022 to 2024 using 4247 clinical isolates from the BIRDEM General Hospital Microbiology Laboratory.

2.2 Type of samples

For both retrospective and prospective data, all isolates were derived from the following categories of biological specimens:

1. Urine
2. Blood
3. Pus
4. Sputum
5. Bronchoalveolar lavage
6. Wound swab
7. Tissue
8. Tracheal aspirate
9. Catheter tips

2.3 Data collection

Data collection was conducted via a pre-structured questionnaire (Figure 4) that encompassed information on the following:

1. Date
2. Sample code
3. Specimen Type
4. Organism name
5. Patient info
6. Antibigram result

An Excel file was made with the patient's age, the date the sample was taken, and whether the patient was resistant or susceptible to antibiotics. After that, the data was sorted by age, gender, and how well the antibiotics worked.

**Title: Investigation on Antimicrobial Resistance (AMR) pattern of
prevalent GLASS pathogens isolated from clinical samples of Bangladeshi
patients**

Data Collection Form

Date:

1. Sample Code:

2. Sample Type: Blood / Urine/ Stool/ Sputum/ Wound Swab/ Others
(.....)

3. Organism Name:

4. Patient Info:

a) Age:

b) Gender: M / F

5. Antibigram Result:

Name of the Antibiotics	R	S
Penicillin		
Amoxicillin		
Ampicillin		
Piperacillin		
Ceftriaxone		
Ceftazidime		
Cefotaxime		
Cefoxitin		
Cefuroxime		
Cefepime		

Figure 5: Data collection form

2.4 MAR index determination

To find the multiple antibiotic resistance (MAR) index (Ayandele et al., 2020), we used this formula:

The MAR Index is equal to a divided by b ,

where, a = is the number of antibiotics that an isolate is resistant to and

b = is the total number of antibiotics used in this research.

2.5 Analysis of statistics

All data comparing males and females were statistically analyzed using Microsoft Excel, 2024.

2.6 Prospective study Settings

A prospective research study was conducted utilizing 21 isolates of *Acinetobacter spp.* from the BIRDEM General Hospital Microbiology Laboratory between July and August 2025.

2.7 Collection of samples

The BIRDEM General Hospital in Dhaka, Bangladesh, gave us a total of 21 clinical samples of *Acinetobacter spp.* Following the standard procedures for the hospital's diagnostic laboratory, the isolates were collected from a range of clinical samples, such as blood, urine, wound swabs, sputum, and pus. All of the samples were taken in a clean environment to avoid contamination, and they were quickly taken to the microbiology lab to be processed.

2.8 Selective media for *Acinetobacter* spp.

Implementing appropriate culture-based procedures and techniques to allow the recovery and identification of *Acinetobacter* spp. from complicated soil environments is essential, particularly in research aimed at surveying and quantifying their contamination levels in the environment. These methods may also be used in clinical settings, enhancing the sensitivity of surveillance and diagnostics, hence mitigating the danger of *Acinetobacter* spp. infection epidemics in hospitals and communities. Leeds *Acinetobacter* agar was utilised for the selective isolation of *Acinetobacter* spp. All plates were incubated under optimal conditions according to the growth requirements of the particular species, providing dependable isolation for subsequent investigations.



Figure 6: Selective media subculture of Acinetobacter spp.

2.9 Subculture and identification confirmation of *Acinetobacter* spp. on Nutrient Agar

In order to further confirm the identity of *Acinetobacter* spp., the isolates were subculture on nutrient agar medium.



Figure 7: Subculture of *Acinetobacter* spp. on Nutrient Agar

2.10 Inoculation in Nutrient Broth

Nutrient broth is used to grow and cultivate a wide range of *Acinetobacter* spp. This makes it a useful and flexible tool for studying microorganisms because it gives *Acinetobacter* spp. the nutrients and growth factors they need to multiply.

2.11 Glycerol Stock

Glycerol stock preparation starts with a pure culture. Put the bacteria in nutrient broth (NB) and let them grow. Once the visible growth is observed, prepare sterile Eppendorf tubes with glycerol. Mix 600 μ L culture solution with 400 μ L sterile glycerol in each tube. Prepare at least four stock tubes for long-term storage (usually at -20°C or -80°C).

2.12 DNA Extraction

To extract DNA, a boiling approach was employed. (Dashti AA. et al.,2009). In brief, cell lysis was achieved by adding 1X Tris-EDTA (TE) buffer as the basal solution for the DNA extraction procedure. Initially, 400 μ L of 1X TE buffer was introduced into an Eppendorf tube. A loopful of the bacterial culture was subsequently inoculated (using a sterile loop) into the tube containing the buffer. The sample was subsequently vortexed to ensure that the microbes were fully incorporated into the TE buffer. The bacterial organisms were pelleted by centrifuging the sample at 13,000 rpm for 10 minutes at 25°C after the initial mixing. The supernatant was removed following centrifugation, and 400 μ L of 1X TE buffer was added to the Eppendorf tube. Next, the bacterial particle was resuspended by vortexing the sample once more. The tubes were maintained at 100°C for a duration of 10 minutes in a heat block. This thermal treatment will guarantee that the bacterial cell wall and membrane are lysed properly, thereby releasing the DNA into the solution. The samples were then allowed to cool at ambient temperature for five minutes following this phase with the heat block. The remaining detritus or any cellular component was separated from the extracted DNA by centrifuging the cooled samples at 13,000 rpm for 10 minutes. Once these centrifugation phases were completed, 200 μ L of DNA supernatant was transferred to a new Eppendorf tube. The pellet located at the base of the tube was handled with the utmost care during the transfer. The supernatant is now containing the extracted DNA, which is being prepared for additional analysis. DNA concentration and purity was determined by using NanoDrop^R (Thermo Scientific™ NanoDrop™ 2000/2000c spectrophotometer.). This method ensured the efficient isolation of high-quality DNA from the bacterial cells for downstream applications, such as genome sequencing or PCR.

2.13 PCR (Polymerase Chain Reaction)

This is a way to quickly make millions or billions of copies of a section of section DNA in the lab. This process makes many copies quickly and easily, which is useful for tests in molecular biology, criminal analysis, and medical diagnosis (Britannica, T. Editors of Encyclopaedia 2023). In just a few hours, rounds of duplication were done on an Applied Biosystems 2720 Thermal Cycler PCR machine. For the procedure, two microlitres of forward primer, two microlitres of reverse primer, five microlitres of nuclease-free water, twelve microlitres of Emerald Amp PCR master mix, and four microlitres of template DNA were added to a 25 microlitre PCR mixture. The liquid was vortexed for three to five seconds to make sure it was mixed well. PCR was then done while the settings for each target gene were kept the same. After PCR was done, all the PCR products were sorted at -20°C so they could be resolved by agarose gel electrophoresis for gene analysis and detection.

Primer Used	Amount Per Reaction	Targeted Gene	Sequence	Product Size	Agarose gel Concentration	Reference
blaOXA-51	15-μL each	<i>bla_{OXA-51}</i>	5'- TAATGCTT TGATCGGC CTTG-3' 5'- TGGATTGC ACTTCATC TTGG-3'	353bp	1.65%	(Hossain, M.A et al., 2025)

Table 2: Primer and target gene information for Acinetobacter baumannii

2.14 Agarose Gel Electrophoresis

Agarose gel electrophoresis is thought to be one of the best ways for isolating DNA pieces that are between 100bp and 25kp long. The proteins are split up in the agarose by size. Charged molecules are pushed across a gel matrix by an electric field. There are many ways to find out the amount of DNA, and agarose gel electrophoresis is one of them. DNA moves through a highly cross-linked agarose substrate when an electric current flows through it. The molecule will move to the right because the DNA phosphates in the solution have a negative charge. (red) pole. The speed at which DNA moves through a gel is affected by three factors. This gives clues about the size of the DNA and confirms its appearance as a specific DNA ladder. For the tests, a gel is made by boiling the needed agarose 31 powder (mostly 1.5 to 2%) with 2000 μ L of Tris-acetate-EDTA Buffer and 98% distilled water. After that, 4 μ L of ethidium bromide, a chemical that sticks to cells, is used as a fluorescent tag. With the help of autoclaved tips, the PCR products that had already been made were then put into Agarose gel. Trace amounts of Tris-acetate-EDTA were used to run this test. DNA can move to the positive electrode with the help of a buffer. Agarose gel electrophoresis was used every time. Five microlitres of 50-base pair ladder (Biolab) and 100 volts of electricity were used to do it. Because MIDORI Green is used to colour the DNA, it is possible to follow the DNA through the gel. After the DNA goes through the gel, it is marked with something that sticks to it. When DNA molecules are exposed to visible light, they either reflect a certain colour or emit a certain colour when UV light hits them. So, the band sizes are found and written down with the help of the used DNA Ladder.

2.15 Antibiotic Susceptibility Testing

The Kirby-Bauer disc diffusion technique was employed to determine antimicrobial susceptibility profiles, offering a quicker and more practical alternative to the broth dilution method. This approach is widely employed to assess susceptibility and resistance in aerobes and facultative anaerobes to various classes of antibiotics. Standard antibiotic discs (HiMedia Laboratories Pvt. Ltd, India) were applied to Mueller-Hinton agar plates to evaluate the sensitivities of isolated strains of *Acinetobacter spp.*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas Aeruginosa*. Each isolate underwent testing with different antibiotics to assess their specific resistance or sensitivity profiles. As a result, the inhibitory zones surrounding each antibiotic disc were quantified (in mm) to assess the effectiveness of the antibiotics in suppressing bacterial growth. This method demonstrated significant utility in clarifying the susceptibility patterns of pathogens, thereby informing appropriate therapeutic choices. The information derived from these tests is crucial for healthcare professionals and public health authorities to address the challenges posed by antimicrobial resistance and to guarantee effective treatment options.

2.15.1 Inoculation and Isolation on Muller Hinton Agar Plates

This meant making a bacterial solution that met the McFarland Standard and then putting bacterial pathogens on Mueller-Hinton Agar (MHA) plates for isolation. First, 5 to 6 mL of normal saline was put into a test tube and put in an autoclave. In a clean way, an inoculating loop gathered up 3 to 5 bacteria colonies from a freshly streaked NA and put them in the saline. The test tube was then spun around quickly until the cloudiness of the suspended material was the same as what was called the 0.5 McFarland Standard, which is about 1.5×10^8 CFU/mL of bacteria. This criterion is necessary to make sure that the bacterial density is consistent and appropriate for testing for antimicrobial susceptibility. A new autoclaved cotton swab was put into the bacterial suspension after the right amount of turbidity had been reached. This helped the bacteria grow so that colony counts could be done. The swab was then rolled against the inside of the test tube to get rid of extra suspension. The swab made from the bacterial sample was streaked across the surface of a sterile Mueller Hinton Agar (MHA) plate to cover the whole petri plate. The cotton swab was turned several times, and at each angle, the plate was turned to make sure that the bacterial growth was even across the whole surface. After the grass was finished, sterile forceps were used to move the antibiotic discs to the top of the MHA plate. To keep the bacterial samples from getting dirty, the forceps used to place the antibiotic discs were cleaned with 70% ethanol and then burned before each antibiotic disc was picked up. The antibiotic discs were gently put on the agar so that they made good contact with the lawn of bacteria. After putting the plates in place, they were kept at 37 degrees Celsius, which is the temperature at which the bacteria proliferated and stopped the creation of the inhibitory zone. Because of all of these things, the exact and standardized way of inoculating the bacterial isolates gave a standard bacterial density and results that were fairly accurate for how susceptible the bacteria were to antimicrobial agents.

2.15.2 Zone of Inhibition

When the MHA plates are taken out after a day of incubation, there is bacterial growth around each disc. If the antibiotic works on the organism being tested, there will be no growth around it. Antibiotics may not be as effective in that situation as well; There will be a clear area of Inhibition there. In these situations, the zone of inhibitions is assessed with a scale, which serves to show what the permitted development restraint zones around the discs are. (Clinical and Laboratory Standards Institute, 1997, 1999). Using a standard table from Oxoid Limited (England), the results are then classified as sensitive, moderate, or resistant.

Chapter 3: Results and Discussion

3.1 Retrospective Study Results

3.1.1 Common Pathogens of positive culture and their distribution

Clinical specimens which fulfilled the standards established by the American Society for Microbiology (ASM) were prepared for culture and susceptibility testing. After getting samples from sample collection sites, they were quickly sent to the microbiology lab for more work. More than a thousand specimens processed, 4247 showed positive growth. The most common isolate was *Escherichia coli* (n=1482) followed by *Klebsiella spp.* (n = 1084), *Pseudomonas spp.* (n=550), *Acinetobacter spp.* (n=306), *Staphylococcus aureus* (n = 215), and others (n=610).

The percentage of female patients was much higher (58.5%) compared to the male patients (41.5%) in the hospitals. Among the GLASS pathogens *Acinetobacter spp.* was fourth highest in both female and male patients.

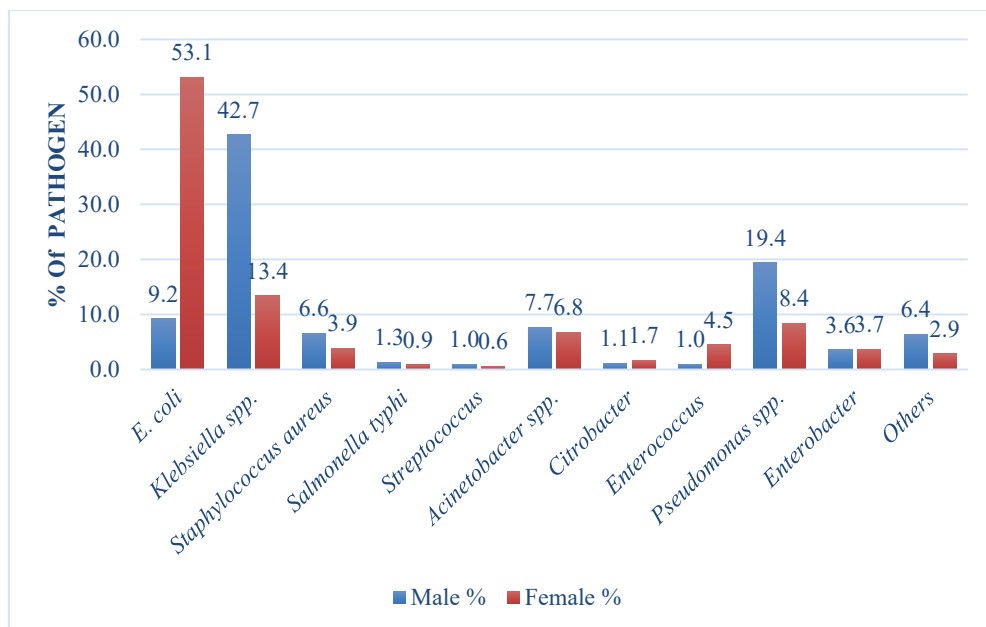


Figure 8: Percentage of Pathogens among Male and Female

For both genders the maximum number of patients infected with the age group of 51-60 years. Furthermore, the minimum age for the male and female patients among the culture

positive test results obtained to be only 7 months respectively. Contrarily, the maximum ages were 99 and 93 years.

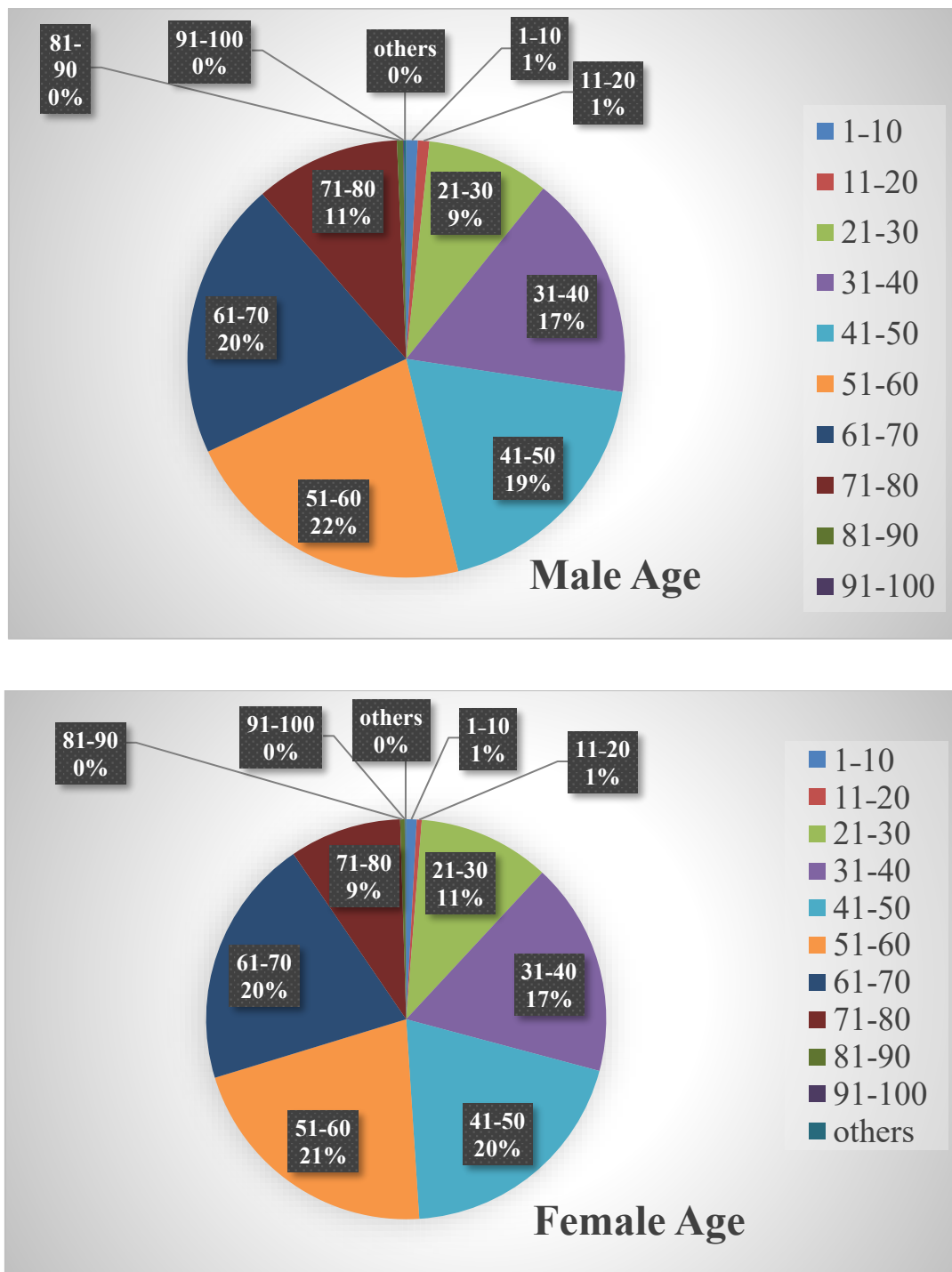


Figure 9: Pathogens distribution in different age groups of males and females

Several different clinical specimens were tested in the hospital from which showed most positive cultures from Urine, Blood, Pus, Sputum, Bronchoalveolar lavage, Wound swab, Tissue, Tracheal aspirate, and Catheter tips.

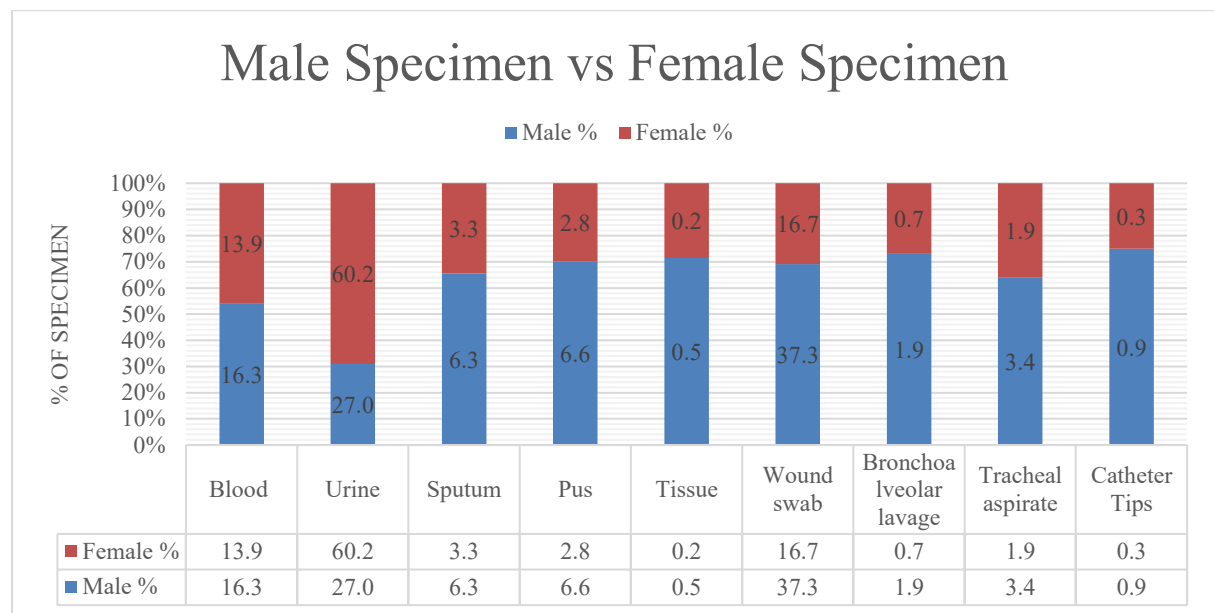


Figure 10: Pathogen Distribution among culture positive specimen

3.1.2 Distribution of *Acinetobacter spp.*

According to figure 7 *Acinetobacter spp.* is seen in 14.5% of the population in Dhaka. Also, female patients are at higher risk compared to the male patients.

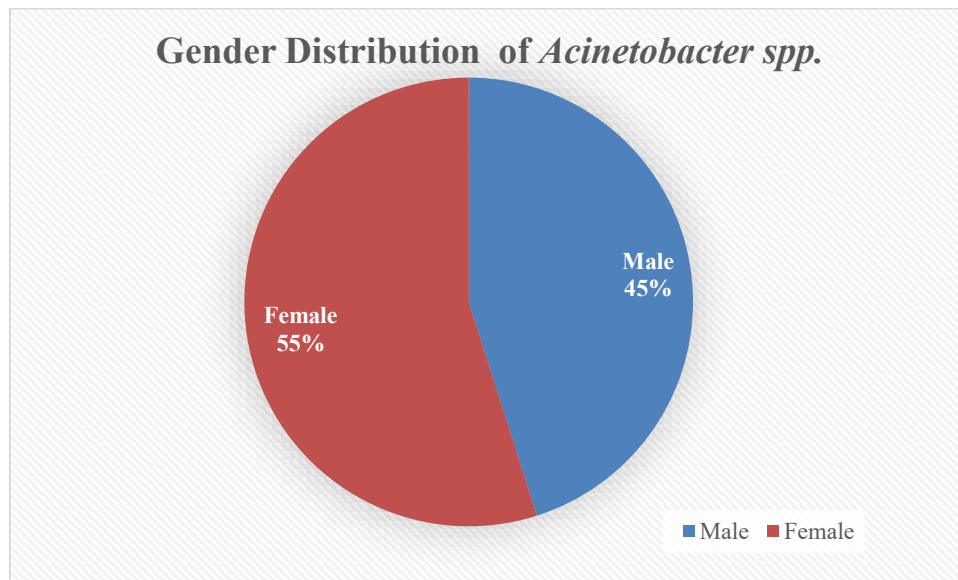
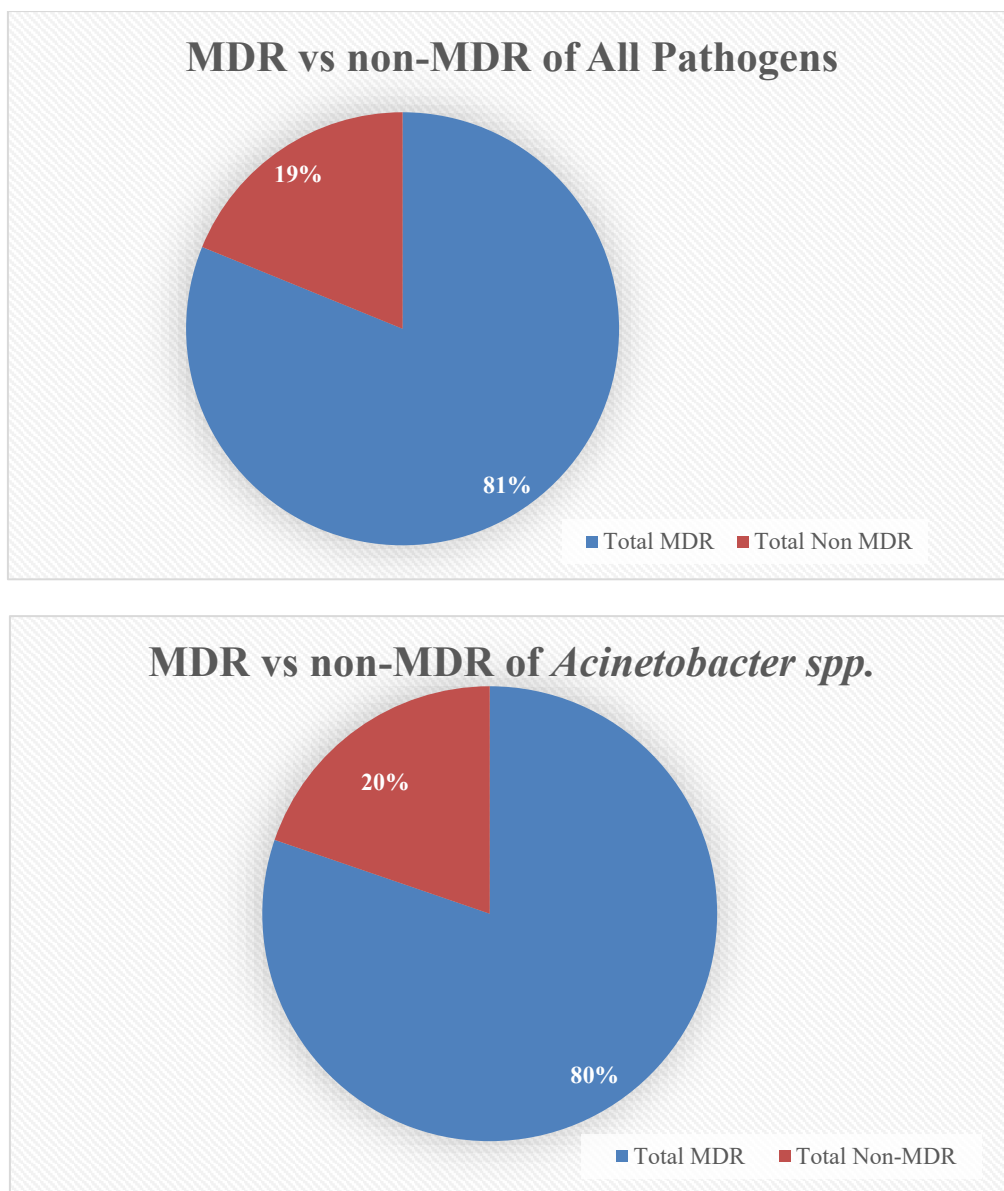


Figure 11: Distribution of *Acinetobacter spp.* according to gender

3.1.3 Multi-Drug Resistance and Non- Multi-Drug Resistance (MDR and Non-MDR)

Multi-drug resistance cases were found to be highly common among the positive cultures.

For the *Acinetobacter spp.* there were 80% Total MDR and the rest of the 20% was total non-MDR.



*Figure 12: Comparison between the percentage of MDR vs non-MDR of *Acinetobacter* spp. and MDR vs non-MDR of all pathogens*

3.1.4 Antibiotic Susceptibility Patterns

3.1.4.1 Antibiotic sensitivity and resistance patterns between males and females

A total of 21 antibiotics of various classes were used in the antimicrobial susceptibility tests (AST) of diverse specimens of patients in the BIRDEM General Hospital Dhaka, Bangladesh. Antibiotics such as amoxicillin (male=75.7%, female=65.9%), ceftriaxone (male=73.1%, female =61.9%), cefixime (male=75.3%, female=62.7%), cefuroxime (male =74.2%, female =62.4%) etc showed higher resistance patterns in both genders. Simultaneously antibiotics that showed greater sensitivity patterns were vancomycin (male=97.0%, female=95.1%), meropenem (male=67.9%, female=83.7%), ampicillin (male=79.5%, female=82.1%), amikacin (male=61.2%, female=78.6%), etc. However, many antibiotics showed moderate sensitivity patterns, such as cotrimoxazole, imipenem etc. termed as intermediate resistant (designated “I”).

Resistant bacteria to carbapenems rose from 4.5% in 2005 to 100% in 2016 in Iran. (Shakib P et al., 2020). In the AST for *Acinetobacter spp.* isolates from various specimens, a total of 21 antibiotics were used based on different cases that overall showed higher resistance patterns to Cephalosporins beta-lactams, and other antibiotics such as colistin, and gentamicin. Some antibiotics were used very rarely. but all of them showed resistance (100%). e.g. cloxacillin, vancomycin and erythromycin. The highest percentage of resistance among the commonly used antibiotics were ciprofloxacin (81.5%). meropenem (80.75%), etc. However, a high percentage of sensitivity was seen against amikacin (92.56%). Followed by piperacillin-tazobactam (80.57%).

3.2 Prospective study results

3.2.1 Culture positive isolates from diverse clinical specimens

From BIRDEM General Hospital, Dhaka, Bangladesh, a total of 73 clinical isolates under GLASS pathogens were obtained. which were subsequently tested in the lab. Besides, multi-centred sampling was conducted from which a significant number of *Acinetobacter spp.* were collected (n=21). It was obtained from Wound swab (n=8), Pus (n=2), Sputum (n=2), Blood (n=3), Tracheal aspirate (n=5), Gastric lavage (n=1).

3.2.2 Distribution *Acinetobacter spp.* and AMR categorization

Most of the clinical isolates showed Multi Drug Resistance (MDR) (n=16). Additionally, there were cases of Extended Drug Resistance or XDR (n=4) and Pan Drug Resistance or PDR (n=1).

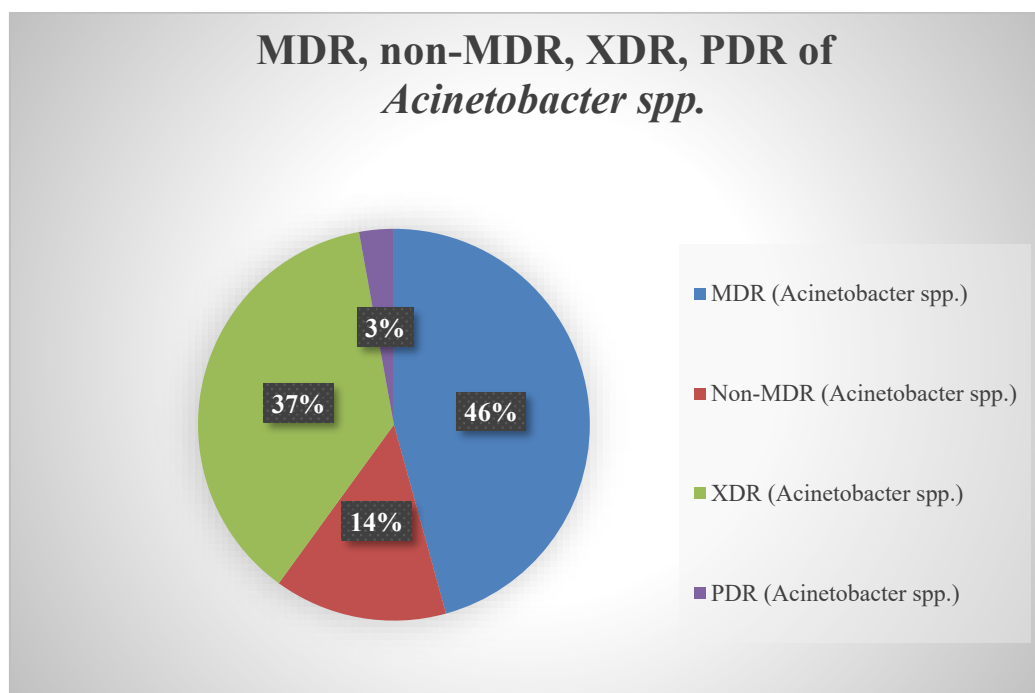


Figure 13: MDR, non-MDR, XDR and PDR Pathogens among *Acinetobacter spp.* Isolates

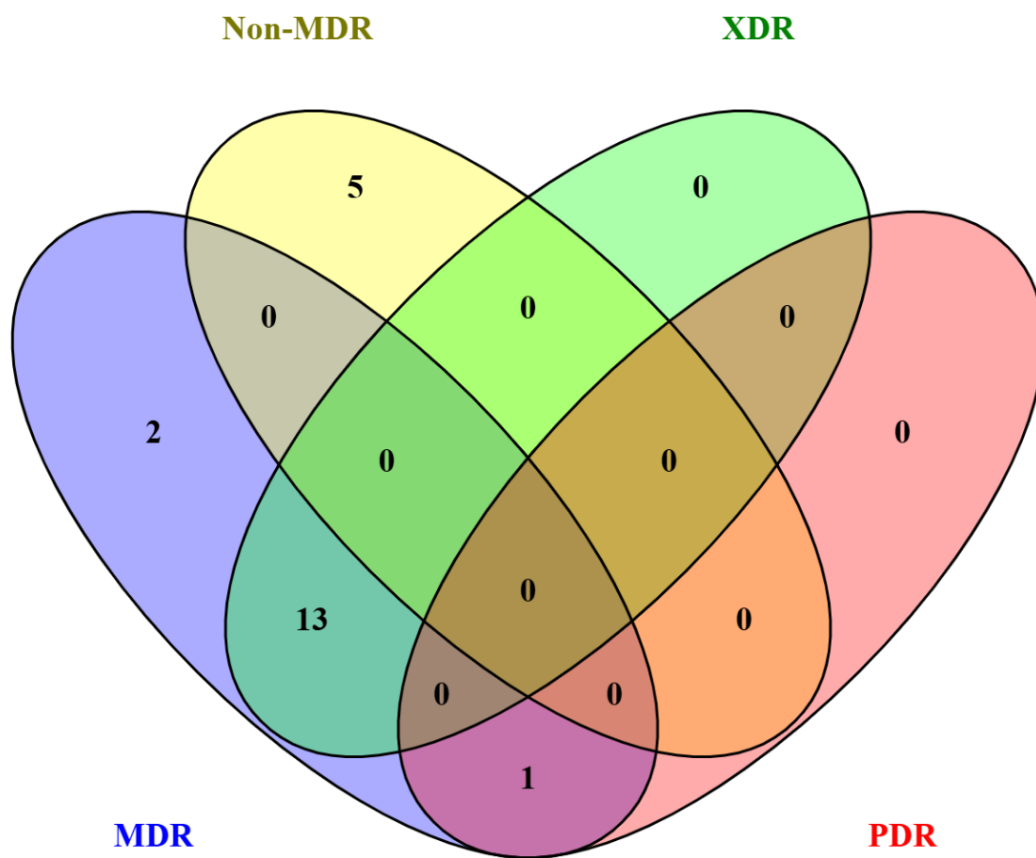


Figure 14: Venn Diagram of MDR, non-MDR, XDR and PDR Pathogens among *Acinetobacter* spp. Isolates

3.2.3 Multi Antibiotic Resistance Index (MAR Index) of *Acinetobacter* spp.

The prospective study shows that *Acinetobacter* spp. isolates show a very high MAR index. The highest showing was 1 (which corresponds to the PDR case) followed by 0.9.

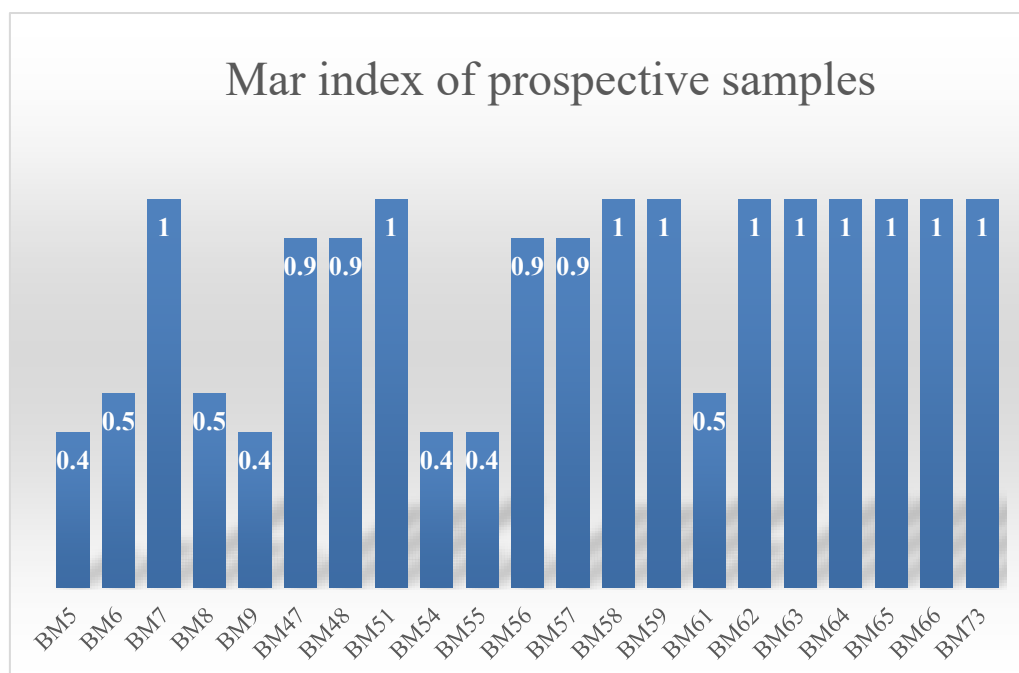


Figure 15: MAR Indexes of the test isolates of *Acinetobacter* spp.

3.2.4 Nucleic Acid (DNA) Purity Assessment

The purity of genomic DNA extracted from *A. baumannii* isolates was evaluated through spectrophotometric absorbance ratios, specifically A260/A280 and A260/A230. DNA concentrations showed variability, ranging from 31.53 mg/L to 121.74 mg/L, which might be because of variability in bacterial load or, the efficiency of the extraction process. The A260/A280 ratios across all isolates were within the range of 2.01 to 2.41, slightly exceeding the standard benchmark of 1.8-2.0 typically associated with pure DNA. These elevated values

suggest minimal levels of protein contamination. On the other hand, A260/A230 ratios were Ranged between 0.85 to 1.11, indicating the possible presence of residual polysaccharides.

Sample ID	Conc. (mg/L)	A260/A280	A260/A230
BM51	31.53	2.41	1.06
BM7	61.31	2.27	0.89
BM48	45.34	2.45	1.01
BM64	60.16	2.28	0.90
BM57	49.26	2.11	0.91
BM8	121.74	2.01	1.00
BM54	80.58	2.11	0.95
BM56	67.92	2.10	0.86
BM9	79.08	2.26	1.07
BM59	37.64	2.16	0.87
BM62	50.75	2.22	0.90
BM66	64.40	2.29	0.95
BM58	52.18	2.06	0.85
BM65	81.86	2.12	0.91
BM63	72.25	2.17	0.94
BM61	53.63	2.13	0.88
BM55	77.53	2.09	0.89
BM47	54.70	2.34	1.11

Table 3: *Purity Assessment of Acinetobacter baumannii spp. DNA Using NanoDrop Spectrophotometer*

3.3 Discussion

3.3.1 AST Results Discussion

Out of all the positive *Acinetobacter spp.* isolates, most strains showed comparatively high susceptibility to antibiotics, including cephalosporins, monobactam, carbapenems, and Some beta-lactams like Piperacillin-Tazobactam out of the isolates were tested with 13 broad-spectrum antibiotics to correlate with the hospital antibiogram data. Most of the Isolates showed similar susceptibility data.

Antibiotic	Disc Content (µg)	ZOI (mm) for <i>A. baumannii spp.</i>				Hospital AST Results		Laboratory AST Results	
		≥ S	I	≤ R	Source	BM47	BM48	BM47	BM48
Azithromycin	AZM 15	-	-	-	-	-	-	R (0)	R (0)
Chloramphenicol	C 30	-	-	-	-	-	-	R (0)	R (0)
Piperacillin–Tazobactam	TPZ 110	21	18-20	17	CLSI	R	R	R (0)	R (0)
Cefepime	FEP 30	18	15-17	14	CLSI	R	R	R (0)	R (0)
Levofloxacin	LEV 5	17	14-16	13	CLSI	-	-	R (8)	R (9)
Imipenem	IMP 10	22	19-21	18	CLSI	-	-	R (0)	R (0)
Meropenem	MEM 10	23	20-22	19	CLSI	R	R	R (0)	R (0)
Ciprofloxacin	CIP 5	21	16-20	15	CLSI	R	R	R (0)	R (0)
Gentamicin	GEN 10	15	13-14	12	CLSI	S	S	S (23)	S (22)
Amikacin	AK 30	17	15-16	14	CLSI	R	R	R (10)	R (10)
Ceftazidime	CAZ 30	18	15-17	14	CLSI	R	R	R (0)	R (0)
Clindamycin	CL 30	-	-	-	-	-	-	R (14)	R (13)
Co-trimoxazole	COT 25	16	11-15	10	CLSI	R	R	R (0)	R (0)

Table 4: Comparison of AST Results of Hospital and Laboratory of A. baumannii spp.

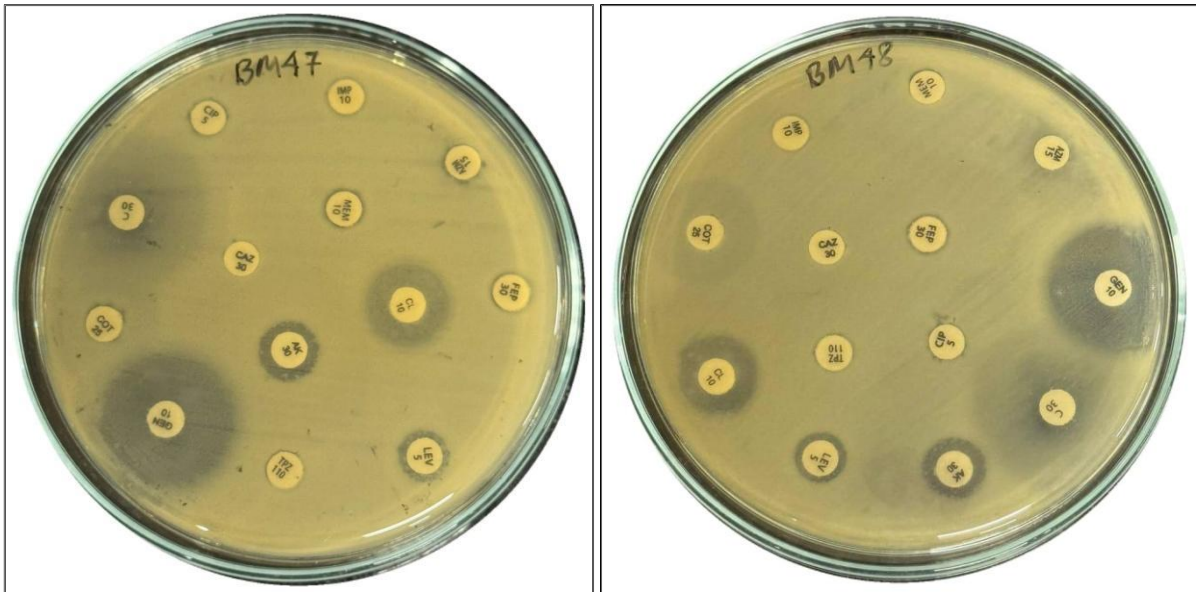


Figure 16: AST results highlighting multidrug resistance patterns in the tested Acinetobacter spp. isolates.

From the figure we can see that, among the 13 broad spectrum antibiotics disk we have found that gentamicin is more susceptible compared to the other antibiotics. We have also found that the hospital antibiogram test data and our laboratory antibiogram test data matches the same which indicates that our laboratory antibiogram test data is genuine and accurate.

Sample ID (<i>Acinetobacter</i> spp.)																					
Antibiotics	BM05	BM06	BM07	BM08	BM09	BM47	BM48	BM51	BM54	BM55	BM56	BM57	BM58	BM59	BM61	BM62	BM63	BM64	BM65	BM66	BM73
AMC	R	R	R	R	R	R	R	R		R	R	R	R	R	R	R			R		R
TZP	S	S	R	S	S	R	R	R	S	S	R	R	R	R	S	R	R	R	R	R	R
CRO	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
CTX									R					R	R	R	R	R	R	R	R
CAZ	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
FEP	R	R	R	R	S	R	R	R	R	R	R	R	R	R							R
CFM	R	R	R	R	R	R	R	R		R	R	R	R	R	R				R	R	R
CIP	S	R	R		S	R	R		S	S	R	R	R	R	S	R	R	R	R	R	R
ATM	R	R	R	R	R																
MEM	S	S	R	S	S	R	R	R	S	S	R	R	R	R	S	R	R	R	R	R	R
NET	S	S	R																		
AK	S	S	R	S	S	R	R	R	S	S	R	R	R	R	S	R	R	R	R	R	R
GEN	S	S	R	S	S	S	S	R	S	S	S	R	R	R	S	R	R	R	R	R	R
AZM														R		R		R			R
SXT	S	S	R	S	S	R	R	R	S	S	R	S	R	R	R	R	R	R	R	R	R
CL			R	R	R																

Here, R= Resistant, S= Sensitive

Table 5: Interpretation of Antimicrobial Susceptibility Testing of the *Acinetobacter* spp.

3.3.2 PCR and Agarose Gel Electrophoresis Results Discussion

Out of 18 samples, 16 showed positive results as Isolates of *Acinetobacter baumannii*, whereas 2 were confirmed to be species of the *Acinetobacter* genus. Therefore, the PCR followed by gel electrophoresis detected the presence of *Acinetobacter* isolates and identified their species by targeting a specific gene (BlaOXA-51). It Is noteworthy that the PCR products were stored at- 20°C for further examination.

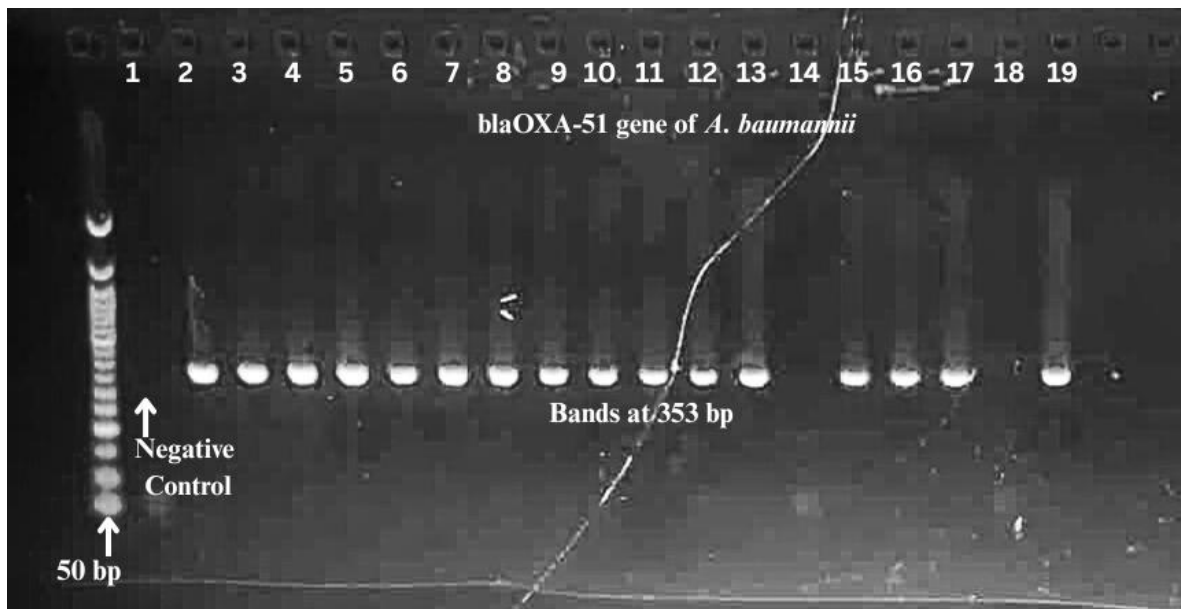


Figure 17: PCR amplification of *blaOXA-51* gene of *Acinetobacter baumannii*

Chapter 4: Limitations and Conclusion

4.1 Limitations

This study utilized data from a single hospital. This study investigated just retrospective data of 4247 samples, indicating a small sample size. Also, part of the data in the hospital's database were missing. Without this, data won't be available, and access will be limited. Furthermore, for the prospective study, the hospitals as well as our microbiology lab had both resources and time limitations.

4.2 Concluding Remarks

The retrospective and prospective study findings indicate an important link between the frequency of *Acinetobacter spp.* in the Division of Dhaka, Bangladesh, and the global urgency surrounding this WHO Priority group 1 pathogen. The extensive proliferation of this species surpasses the complex nature of antimicrobial resistance (AMR) and will ultimately result in a significant healthcare issue in the future. The resistance patterns and increasing cases of MDR isolates among *Acinetobacter baumannii* indicate the urgent necessity for future investigation to develop measures to counteract the resistant variants and modify their processes. As a treatment guideline for MDR isolates, ampicillin and amikacin can be recommended while meropenem and vancomycin can be reserved as last resort-treatment as revealed from their high sensitivity against *Acinetobacter baumannii*. To protect public health and hospitals, we need to take a holistic approach to this global catastrophe and focus on the organisms that are most likely to cause problems.

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