Identification of *Acinetobacter baumannii* from clinical ACB complex isolates of nine sentinel surveillance sites of IEDCR: Focus on their distribution and antibiotic susceptibility pattern

by

Tarikul Islam -19126040

Md. Abir Hossen-19126032

A thesis submitted to the Department of Mathematics & Natural Science in partial fulfillment of the requirements for the degree of

Bachelor of Science in Microbiology

Department of Mathematics and Natural Sciences

Brac University

February 2024

Declaration

It is hereby declared that

1. The thesis submitted is our 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. We have acknowledged all main sources of help.

Student's Full Name & Signature:

Tarikul Islam 19126040	**Md. Abir Hossen** 19126032

Approval

The thesis titled "**Identification of *Acinetobacter baumannii* from clinical ACB complex isolates of nine sentinel surveillance sites of IEDCR: Focus on their distribution and antibiotic susceptibility pattern**" submitted by-

1. Tarikul Islam- 19126040
2. Md. Abir Hossen- 19126032

of Spring, 2023 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Science in Microbiology on 29th February of 2024.

Examining Committee:

IEDCR Supervisor:

Prof. Dr. Zakir Hossain Habib
Chief Scientific Officer (CSO)
Head, Department of Microbiology
IEDCR

University Supervisor:
(Member)

Mahbubul Hasan Siddiqee, PhD
Associate Professor, Microbiology Program
Department of Mathematics & Natural Science
BRAC University

Program Coordinator:
(Member)

Nadia Sultana Deen, PhD
Associate Professor, Microbiology Program
Department of Mathematics & Natural Science
BRAC University

Departmental Head:
(Chair)

A F M Yusuf Haider, PhD
Professor & Chairperson
Department of Mathematics & Natural Science
BRAC University

Ethics Statement

This research is conducted under appropriate supervision; thus, it is the original work of the author. During these tests, no living being were harmed in any way. The experiment was executed in accordance with all of the guidelines and restrictions outlined by the Microbiology laboratory inside the Department of Microbiology at Institute of Epidemiology, Disease Control and Research (IEDCR).

Abstract

ACB complex, a major concern in hospitals, particularly in intensive care unit (ICU), causes nosocomial infections & is multidrug-resistant (MDR), making infection management practices more challenging. (Acb) complex consists of four species. Infection with different (Acb) complex species may result in different risk factors and clinical consequences because these nosocomial pathogens species vary in terms of pathological and biological characteristics and for this accurate identification of species is necessary. It is quite challenging to accurately differentiate between various Acinetobacter species through biochemical tests and automated techniques like MALDI-TOF MS. *A. baumannii* is the most clinically significant species among the (Acb) complex, accounting for 80% of infections. Utilization of *blaOXA-51* gene through RT-PCR for molecular identification of *A. baumannii* proves to be significantly more accurate and reliable than conventional and automated methods. *A. baumannii* has emerged as a notorious pathogen in recent decades. It has been associated with a rise in health issues and healthcare-associated infections (HAIs) that are difficult to treat with antibiotics. In this study, nine different sentinel surveillance sites were selected to collect isolates. Isolates were taken in consideration from the year of 2017 to the month of May of 2023 to figure out the prevalence. In total, 133 clinical (Acb) complex isolates were taken as suspected *A. baumannii* in which 82 isolates were identified as *A. baumannii* by detecting the *blaOXA-51* gene through RT-PCR. Antibiotic susceptibility test (AST) was performed for the identified *A. baumannii* isolates through 12 antibiotics from the 7 antimicrobial agent groups of Acinetobacter spp. panel of CLSI 2022. Results showed that highest rate (79%) of resistant isolates were seen against Ceftazidime & highest rate (84%) of sensitive isolates were seen against Tigecycline. Fifty isolates (61%) and fifteen isolates (18%) were found to be MDR & suspected XDR.

Keywords: Clinical isolates, *(Acb) complex*, RT-PCR, *A. baumannii*, AST, MDR, suspected XDR.

Dedication

We would like to dedicate this research to our parents for their unwavering love, believing and supporting us during all the tough times.

Acknowledgement

First of all, we would like to express our sincere gratitude to the almighty for granting us the opportunity to embark on this amazing journey to conduct this research.

We are grateful to the Chairperson of the Department of Mathematics and Natural Sciences professor **Prof. A F M Yusuf Haider**.

We would like to express gratitude to our dear supervisor, **Dr. Mahbubul Hasan Siddiqee** sir, Associate Professor in the Microbiology Program in the Department of Mathematics and Natural Sciences (MNS) at BRAC University for providing us the opportunity to undertake our research at IEDCR, where his constant supervision, active support and suggestions helped us to improve & finish our undergraduate thesis.

We are thankful to the Director of IEDCR **Prof. Dr. Tahmina Shirin** mam and professor **Prof. Dr. Zakir Hossain Habib** sir, Chief Scientific Officer (CSO) & the Department head of Microbiology at IEDCR for allowing us to work in Microbiology lab of IEDCR while providing invaluable supportive instructions throughout the whole research journey that enlightened us to work on that topic.

We are thankful to **Dr. Nandita Banik** mam, National Consultant-Microbiologist, WHO at IEDCR & **Dr. Md. Abdullah Omar Nasif** sir, Medical Officer at Virology department at IEDCR for sharing their wealth of wisdom with us while accomplishing this research.

Our most profound gratitude to all the staff of Microbiology & Virology lab at IEDCR for helping us during our research tenure.

Table of Contents

List of Tables

List of Figures

List of Acronyms

ECDC	European Centre for Disease Prevention and Control
IDSA	Infectious Diseases Society of America
XDR	Extensively-Drug Resistant
MDR	Multi Drug-Resistant
ICU	Intensive Care Unit
HAIs	Hospital Acquired Infections
CDC	Centers for Disease Control and Prevention
A. baumannii	*Acinetobacter baumannii*
MALDI-TOF	Matrix Assisted Laser Desorption Ionization-Time of Flight
MS	Mass spectrometry
IPD	In-Patient Department
OPD	Out-Patient Department
AK	Amikacin
CPM	Cefepime
IMI	Imipenem
CIP	Ciprofloxacin
GM	Gentamycin
DO	Doxycycline
MEM	Meropenem
TS	Trimethoprim-Sulfamethoxazole
CAZ	Ceftazidime
TGC	Tigecycline
CRO	Ceftriaxone
PTZ	Piperacillin-Tazobactam
AMR	Anti-Microbial Resistance
RT-PCR	Real Time-Polymerase Chain Reaction
MARI	Multiple Antibiotic Resistance Index

Chapter 1

Introduction

1.1 Background of the study

(Acb) complex is a major concern in hospitals, particularly in intensive care units (ICU), making infection management practices more challenging. The (Acb) complex consists of four species: *Acinetobacter baumannii*, *Acinetobacter nosocomialis*, *Acinetobacter calcoaceticus* and *Acinetobacter pittii* while two new species, *Acinetobacter seifertii* and *Acinetobacter dijkshoorniae*, have been added to the (Acb) complex (Vijayakumar et al., 2019). Infection with different (Acb) complex species may result in different risk factors and clinical consequences because these nosocomial pathogens species vary in terms of pathological and biological characteristics along with colonizing the skin, antimicrobial resistance mechanisms and antimicrobial susceptibility and for this accurate identification of species is necessary (Fitzpatrick et al., 2015). At present, there are five Acinetobacter species (*A. baumannii*, *A. nosocomialis*, *A. seifertti*, *A. dijkshoorniae* and *A. pittii*) that are linked to human diseases, as well as one Acinetobacter species found in the environment (*A. calcoaceticus*). These species are highly similar and share common biochemical characteristics, making it challenging to differentiate them using traditional methods such as biochemical tests. Therefore, identifying Acinetobacter species at the individual level based on phenotypic methods has proven to be difficult. Furthermore, clear distinguishing between different Acinetobacter species using automated techniques such as MALDI-TOF MS also poses challenge because these species share very similar proteins among them. Only molecular techniques like PCR can accurately identify the clinically significant members of this group because of performing identification on species level. *A. baumannii* is the

most clinically significant species among the (Acb) complex, accounting for 80% of infections (Vijayakumar et al., 2019).

Acinetobacter baumannii, a gram-negative, rod-shaped opportunistic bacterium, that causes nosocomial infections such as endocarditis, urinary tract infections (UTI), pneumonia, skin, soft tissue and bone infections, bacteremia, wound infections and meningitis (Lowe et al., 2022).

A. baumannii is one of the "critical priority pathogen" of WHO along with IDSA, ECDC for its antimicrobial resistance mechanisms (Tiku, 2022). *A. baumannii* is one of the ESKAPE group pathogen which is a group of gram-negative and gram-positive pathogens where these pathogens can evade commonly used antibiotics due to their increasing multi-drug resistance (MDR) for which they are the leading cause of life-threatening hospital-acquired infections (HAIs) in critically ill and immunocompromised patients around the world (Santajit & Indrawattana, 2016). Carbapenem resistant *Acinetobacter baumannii* is one of the among top five antibiotic resistant (ABR) bacteria on the CDC's 2019 "urgent" threat list (Kadri, 2020). *A. baumannii* is commonly found in patients with unfavorable outcomes and higher resistance to antimicrobial agents compared to other pathogens in the (Acb) complex. In 2017, Liu et al. published a study on the mortality rate of *A. baumannii*, which was found to be quite high, ranging from 29.8% to 36.9%. In 2013, Lee et al. conducted a study that highlights significant differences in the clinical features and outcome of pneumonia caused by *A. nosocomialis* and *A. baumannii* where the study found that patients infected with *A. baumannii* had more intense illness, higher antimicrobial resistance and higher fatality rates compared to those infected with *A. nosocomialis*. So, these findings suggest that the pneumonia caused by *A. baumannii* and *A. nosocomialis* should be viewed as different clinical conditions. Hence, it is crucial to accurately identify the (Acb) complex at the

species level because this information is essential for understanding differences in clinical outcomes, antimicrobial resistance patterns and epidemiology (Vijayakumar et al., 2019).

By following this, the objective of this study was to identify *A. baumannii* from clinical (Acb) complex isolates with the use of traditional, automated and molecular microbiology techniques like biochemical tests, MALDI-TOF MS and RT-PCR and observe their distribution and antibiotic susceptibility pattern and for this, isolates were taken in consideration from the year of 2017 to the month of May of 2023 to assess the prevalence of the organism. *A. baumannii* isolates were analyzed to determine their susceptibility to clinically relevant antibiotics.

1.2 Objective of the study

The study outlines its specific aims and objectives as follows:

- To identify *Acinetobacter baumannii* from clinical (Acb) complex isolates.
- To find out the accuracy of biochemical tests, MALDI-TOF MS & RT-PCR in the detection of *A. baumannii*.
- To find out the antibiotic susceptibility pattern of *A. baumannii* isolates.
- To find out the antibiotic resistance of *A. baumannii* isolates among OPD, Ward and ICU patients.

Chapter 2

Materials & Methods

2.1 Isolate site selection

Nine different hospitals as sentinel surveillance sites under "AMR surveillance in Bangladesh" project was selected to collect isolates to conduct this study. Initially, in total 151 clinical (Acb) complex isolates were taken in consideration from the year of 2017 to the month of May of 2023 for this study. These sentinel surveillance sites identify clinical isolates from clinical specimens through following "The National Antimicrobial Resistance (AMR) Surveillance Strategy of Bangladesh" protocol based on WHO's Global Antimicrobial Resistance Surveillance System (GLASS) strategy (Sujan et al., 2023).

Hospital Name	Hospital Codes
1) DHAKA MEDICAL COLLEGE & HOSPITAL	DMCH
2) UTTARA ADHUNIK MEDICAL COLLEGE & HOSPITAL	UAMCH
3) MYMENSINGH MEDICAL COLLEGE & HOSPITAL	MMCH
4) RAJSHAHI MEDICAL COLLEGE & HOSPITAL	RMCH
5) RANGPUR MEDICAL COLLEGE & HOSPITAL	RpMCH
6) SYLHET MAG OSMANI MEDICAL COLLEGE & HOSPITAL	SOMCH
7) KHULNA MEDICAL COLLEGE & HOSPITAL	KMCH
8) COX'S BAZAR MEDICAL COLLEGE & HOSPITAL	CoxMCH
9) BANGLADESH INSTITUTE OF TROPICAL AND INFECTIOUS DISEASES	BITID

Table 1: Hospital names & codes

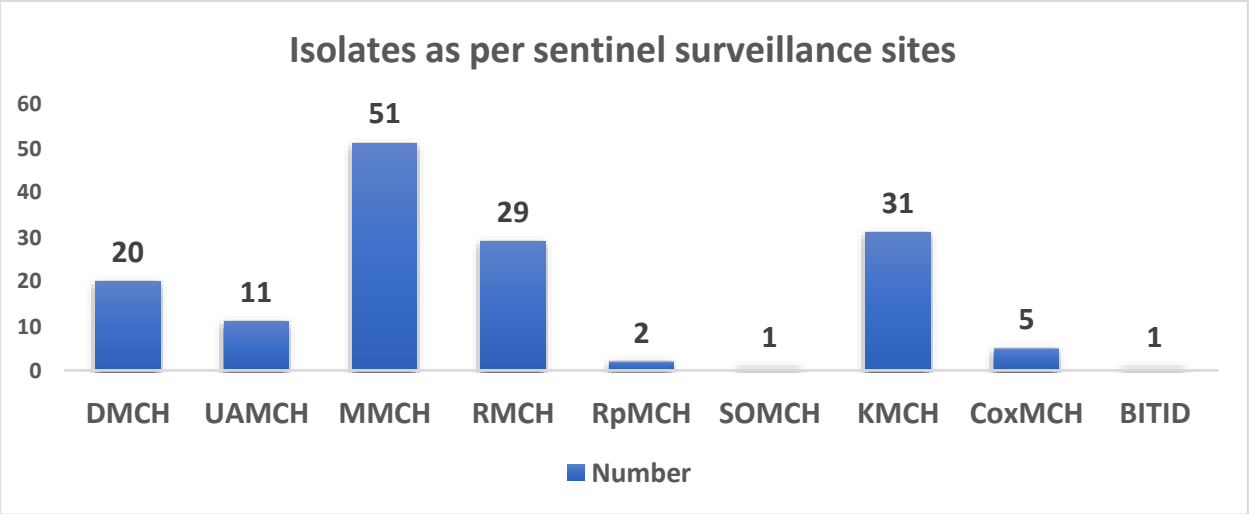
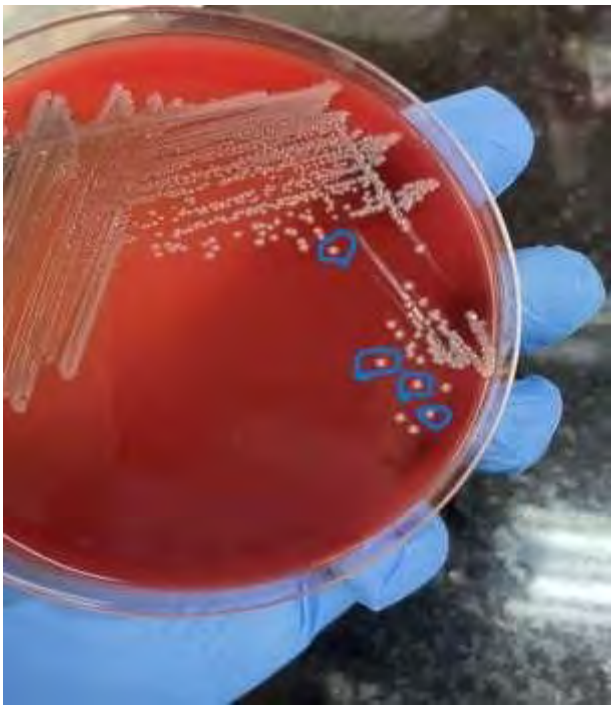


Figure 1: Isolates number as per sentinel surveillance sites

2.2 Isolate processing

Stocked isolates that were at -80 °C temperature refrigerator, was taken out & let in room temperature of the laboratory for a while. The screw capped tubes in which isolates were diluted & preserved with TSB + glycerol, from there through pipetting 10 µL diluted solution were taken for each isolate & streaked on blood agar and nutrient agar media for revival at 37°C for 24 hours. Whitish or grayish colonies were selected and streaked on MacConkey agar & incubated under aerobic conditions at 37°C for 24 hours (Kian et al., 2018).



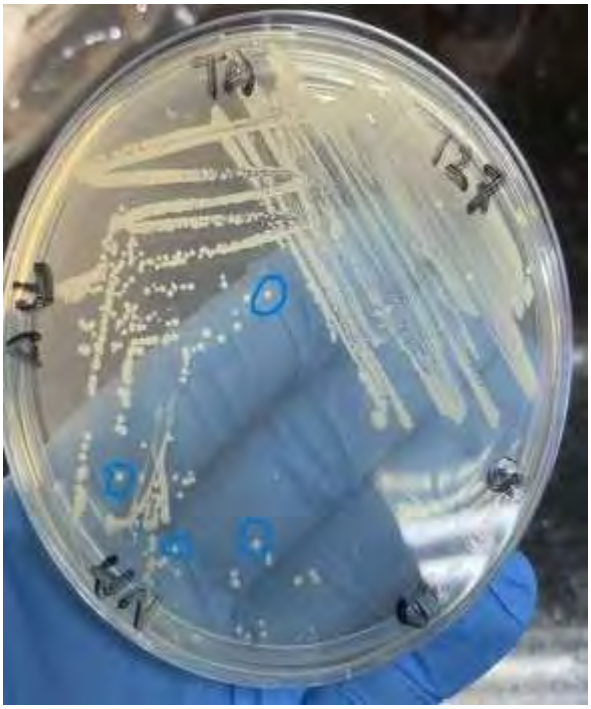


Figure 2,3,4: A. baumannii's growth on Blood, Nutrient & MacConkey agar media

2.3 Identification through biochemical tests

A series of biochemical tests were performed for the selected isolates to identify *Acinetobacter baumannii* that includes Gram staining, Catalase test, Oxidase test, Citrate utilization test, Triple Sugar Iron (TSI) test & Motility Indole Urease (MIU) test (D'Souza et al., 2019). After that, the results of those biochemical tests were compared with a reference chart of biochemical results.

<table>
<tr><td>Biochemical Tests</td><td colspan="4">Properties</td></tr>
<tr><td>Gram staining</td><td colspan="4">Gram-negative coccobacilli</td></tr>
<tr><td>Catalase</td><td colspan="4">Positive (+)</td></tr>
<tr><td>Oxidase</td><td colspan="4">Negative (-)</td></tr>
<tr><td>Citrate</td><td colspan="4">Positive (+)</td></tr>
<tr><td rowspan="2">TSI</td><td>Slant</td><td>Butt</td><td>H2S</td><td>Gas</td></tr>
<tr><td>K
Alkaline(red)</td><td>K
Alkaline(red)</td><td>Negative (-)</td><td>Negative (-)</td></tr>
<tr><td rowspan="2">MIU</td><td colspan="2">Motility</td><td>Indole</td><td>Urease</td></tr>
<tr><td colspan="2">Negative (-)</td><td>Negative (-)</td><td>Negative (-)</td></tr>
</table>

Table 2: Biochemical properties of Acinetobacter baumannii

2.4 Identification through MALDI-TOF MS

Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) method was applied to the selected isolates to identify *Acinetobacter baumannii*. For this, at first on sheep blood agar media plate each bacterial pure culture was grown overnight at 37°C for 24hrs to obtain isolated colony. In the next step, from bacterial pure culture small fraction of isolated single colony for each bacterial isolate were taken manually & spread carefully in parallel manner to spot on MALDI target plate through toothpick, which at room temperature were air dried. After that, saturated 1 µL of CHCA (alpha-cyano-4-hydroxy-cinnamic acid) matrix solution were added to the MALDI target plates by pipetting, that overlaid with spot platted isolated single colonies, and at room temperature were air dried (Marí-Almirall et al., 2017). VITEK® MS (bioMerieux) instrument was used to conduct MALDI-TOF MS for this study. Afterwards, MALDI target plates were inserted in VITEK® MS instrument.

VITEK® MS Results Reviewed

Patient ID	RMCH08
Patient Name	
Accession ID	SE04230018-1
Specimen Type	
Organism Name	Acinetobacter baumannii
Pathogenicity	
Organism Type	Protocol Bacteria
Confidence Value	99.9
Confidence Level	High
User Consolidated	No
Computation Engine	MS-CE 3.2.0
Review Status	Reviewed
Setup Operator	IEDCR
Setup Date	8/9/23 12:01 PM
Bench name	PREPSTATION1
Slide ID	DS223031516
Position	B1
Instrument	VITEKMSACQ01
Selection Operator	
Selection Date	
Review Operator	myla
Review Date	8/9/23 1:23 PM
Review Operator E-signature	Disabled
Comment	

Figure 5,6: Identification of Acinetobacter baumannii through VITEK® MS

2.5 DNA extraction

Boiling method as instructed by (Barbosa et al., 2016) was used for this study to extract DNA. Firstly, in 4 ml Luria Bertani (LB) broth's each screw capped tube, overnight culture of each selected isolate was mixed briefly through vortex machine. Then, bacterial culture containing 700 µL Luria Bertani (LB) broth was transferred into micro centrifuge tube (MCT) and the tubes were centrifuged for 10 minutes at 13,000 rpm. After centrifugation the supernatant was discarded.

Afterwards, at room temperature 300 µL (PBS) phosphate buffered saline was added in the broth containing bacterial culture, which was re-suspended through gently pipetting. Again, for 5 minutes at 14,000 rpm the tubes were centrifuged & the supernatant were discarded. After that, in each tube 200 µL TE buffer was added. Now, for 15 minutes at 100°C temperature through dry heater the tubes were heated. To provide cold shock to the tubes, immediately the tubes were taken off from dry heater & transferred to ice for 10 minutes. Again, for 5 minutes at 14,000 rpm the tubes were centrifuged and in a new labelled micro centrifuge tube the supernatant was transferred. Finally, at -20°C temperature, supernatant containing labelled micro centrifuge tubes were stored.

2.6 Identification through RT-PCR

Real time polymerase chain reaction (RT-PCR) was applied in this study for identification of *Acinetobacter baumannii* from the selected isolates.

A total volume of 15 µL reaction was made for each isolate that includes 8 µL Luna® Universal RT-PCR Master Mix ($BioLabs_{Inc}$, NEW ENGLAND), 0.5 µL forward & reverse primer each, 1 µL DNA template, 5 µL nuclease free water to amplify *blaOXA-51* gene.

In thermocycler (QuantStudio™ 3 and 5 Real-Time PCR Systems, USA) the RT-PCR protocol was set up as: 95 ºC for 5 min, 40 cycles at 95 ºC for 15 s, 58 ºC for 20 s and for final extension 72 ºC for 15 s. Afterwards, the melt curve protocol was added at 95 ºC for 5 s and at 60 ºC for 1 min (Depka et al., 2022).

RT-PCR products (10 µL) mixture for each reaction were transferred and placed on a 1% agarose gel in TBE buffer (40mM Tris, 20mM boric acid, 1mM EDTA, pH 8.3), which was examined by agarose gel electrophoresis at 80 V for 45 min. A gel image analysis system visualized gels under

ultraviolet light. The isolate was positive for *A. baumannii* because of the visualization of bands at the expected size for *blaOXA-51* (Falah et al., 2019).

Primer	Sequence	Amplicon size (bp)	Reference
blaOXA-51	F: 5'-TAATGCTTTGATCGGCCTTG-3' R: 5'-TGGATTGCACTTCATCTTGG-3'	353	(Niranjan et al., 2013)

Table 3: Primer sequence of blaOXA-51 gene

2.7 Antibiotic Susceptibility Test

Manually antibiotic susceptibility test (AST) through Kirby-Bauer disk diffusion method was performed for this study to see the sensitivity or resistance of desired bacterial isolates towards the given antibiotics (Sharma et al., 2023). To inoculate, colonies were selected using a sterile loop from an overnight pure culture on nutrient agar and suspended in sterile saline (0.85% NaCl w/v in water) until they reached the density of a McFarland 0.5 standard (Kittinger et al., 2017). Then, using sterile cotton swabs, the suspension was lawned on Muller Hinton agar (MHA). For 16–20 hours, the plates were incubated at 37ºC. Zones of inhibition were measured in millimeters following incubation. 12 different antimicrobial agents from *Acinetobacter* spp. panel of CLSI were taken & customized into 3 groups as Group-1, 2 & 3 where every group is composed of 4 antibiotics. Group-1 consists of amikacin (30 µg), cefepime (30 µg), imipenem (10 µg), ciprofloxacin (5 µg). Group-2 consists of gentamycin (10 µg), doxycycline (30 µg), meropenem (10 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg). Group-3 consists of ceftazidime (30 µg),

tigecycline (15 µg), ceftriaxone (30 µg), piperacillin-tazobactam (100/10 µg). All these antimicrobial agents represent 7 groups of *Acinetobacter* spp. panel of CLSI & these antimicrobial agents were tested at mentioned concentrations for each disk & by following CLSI 2022 guidelines the results were then interpreted (Syal et al., 2017).

Chapter 3

Results

3.1 *A. baumannii* identification on Blood, Nutrient & MacConkey agar

A total of 151 clinical (Acb) complex isolates were streaked on blood, nutrient & MacConkey agar plates in order to identify *A. baumannii* where 18 isolates didn't revive. After incubation period at 37ºC for 24 hours *Acinetobacter baumannii* isolates has shown grayish-white color colonies. On blood agar, gray color, opaque, circular, nonhemolytic colonies were observed whereas on nutrient agar, opaque, non-mucoid, circular colonies were observed & on MacConkey agar, circular, opaque, non-lactose fermenting colonies were observed (Aryal, 2022).

3.2 Biochemical tests result

Through biochemical tests 73 isolates as 55% were identified as suspected *Acinetobacter baumannii*.

Microorganism	**Number**	**Percentage**
Suspected *Acinetobacter baumannii*	73	55%

Table 4: Suspected Acinetobacter baumannii from biochemical tests

3.3 MALDI-TOF MS result

Through MALDI-TOF MS (VITEK® MS, bioMerieux) 71 isolates as 53% were identified as *Acinetobacter baumannii*.

Microorganism	**Number**	**Percentage**
Acinetobacter baumannii	71	53%

Table 5: Acinetobacter baumannii from MALDI-TOF MS

3.4 RT-PCR result

Through thermocycler (QuantStudio™ 3 and 5 Real-Time PCR Systems, USA) 82 isolates as 62% were identified as *Acinetobacter baumannii* through identifying their *blaOXA-51* gene.

Microorganism	**Number**	**Percentage**
Acinetobacter baumannii	82	62%

Table 6: Acinetobacter baumannii from RT-PCR

Amplification curves in red color above baseline are positive results for *blaOXA-51* gene indicating *A. baumannii* and amplification curves in green color equal to or below baseline are negative results for *blaOXA-51* gene indicating not *A. baumannii*.

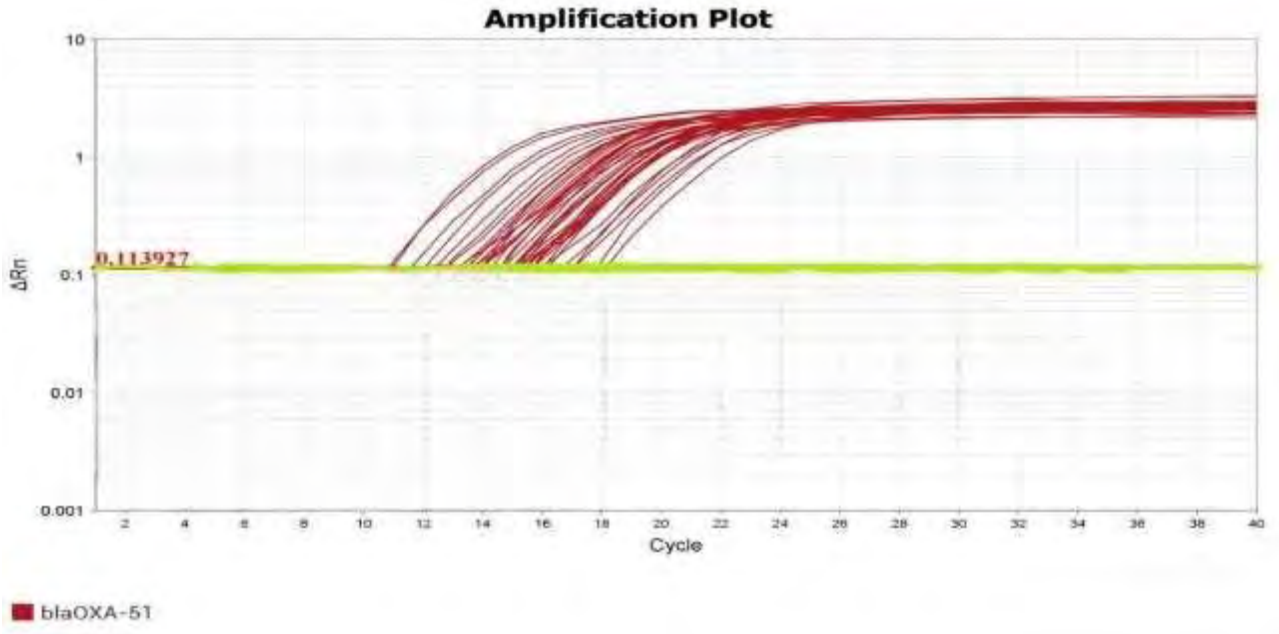


Figure 7: Logarithmic view of amplification plot of blaOXA-51 gene

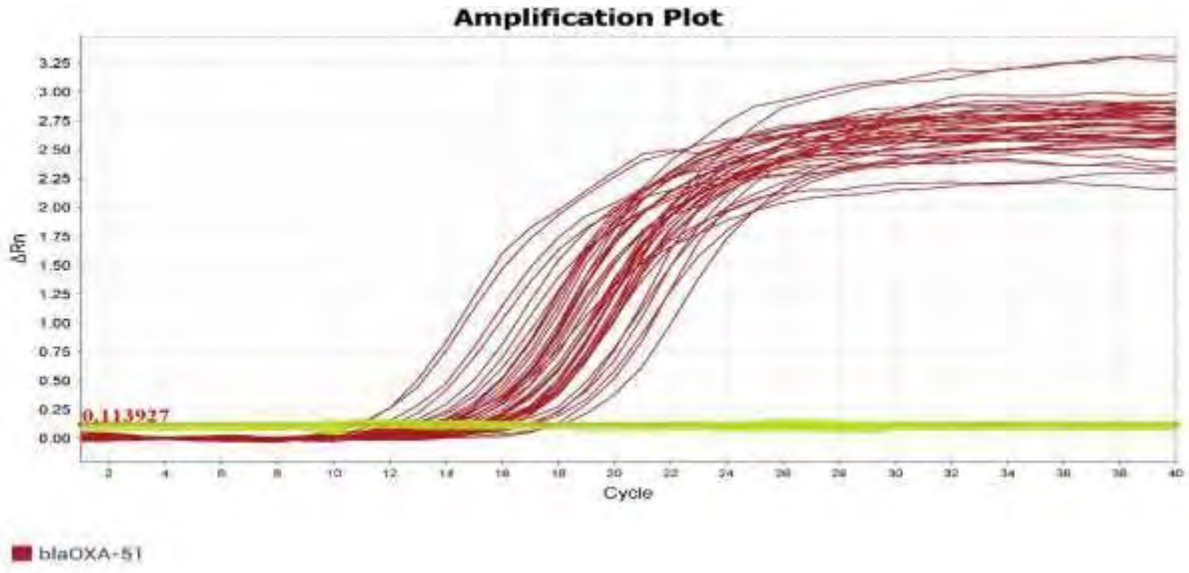


Figure 8: Linear view of amplification plot of blaOXA-51 gene

Melting curves at same position & almost same temperature are positive results for *blaOXA-51* gene indicating *A. baumannii* and melting curves at different position & different temperature are negative results for *blaOXA-51* gene indicating not *A. baumannii*.

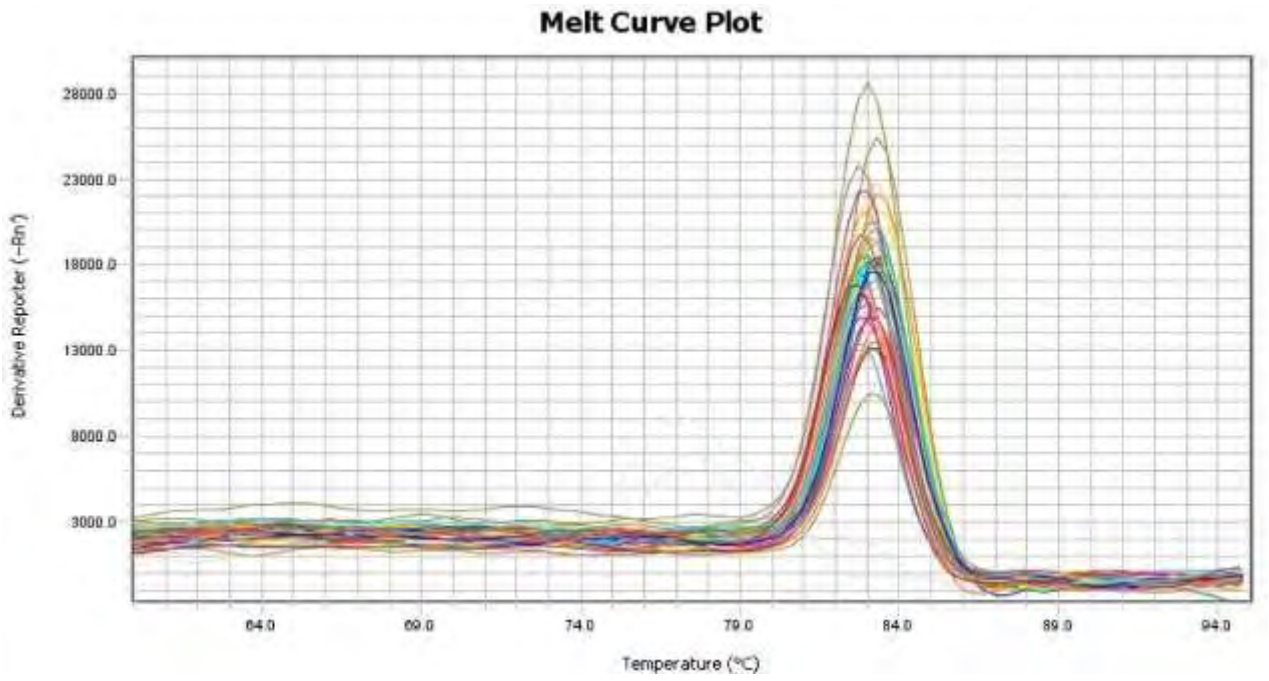


Figure 9: Melt curve plot of blaOXA-51 gene

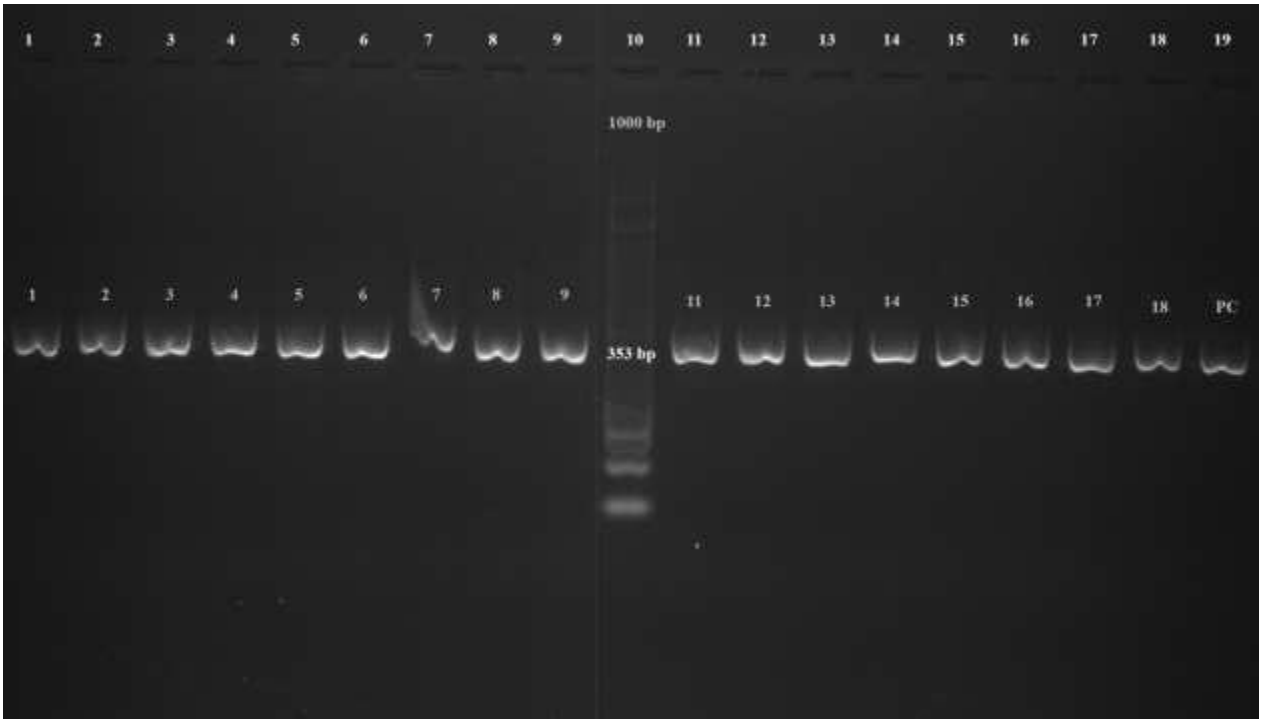


Figure 10: RT-PCR amplification of *blaOXA-51* gene of *A. baumannii*. Lanes 1-9, 11-18: clinical isolates (1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18) show amplified DNA bands at 353bp; Lane 10: 1000 bp size marker; Lane 19: Positive control *A. baumannii* NCTC12156.

3.5 Comparison of identification methods

Three methods named as biochemical tests, MALDI-TOF MS and RT-PCR were applied on the selected isolates for accurate identification of *Acinetobacter baumannii*. Among them biochemical tests identify bacteria on genus or group level through assessing the presence or absence of specific molecules and enzymes that is involved in metabolism of bacteria and for this reason species level accurate identification of bacteria is challenging by this method. MALDI-TOF MS (Matrix Assisted Laser Desorption Ionization-Time Of Flight Mass Spectrometry) identify bacteria on species level through analyzing and comparing the bacterial protein profile with the known database but some bacterial species share very similar proteins among them which makes this method difficult for the accurate identification of the species of the bacteria. RT-PCR (Real Time-Polymerase Chain Reaction) identify bacteria on species level through amplifying specific DNA sequence of the bacteria that is highly specific to the species of the bacteria and gives result in real time where RT-PCR provides a higher level of specificity when compared to MALDI-TOF MS particularly in distinguishing closely related (Acb) complex species and for this reason among these three methods this is the only reliable method that can accurately identify the species of the bacteria and for this study identified *Acinetobacter baumannii* isolates from RT-PCR has been taken in consideration for subsequent test.

3.6 Distribution of *Acinetobacter baumannii*

In total 82 *Acinetobacter baumannii* isolates were identified from 133 isolates from nine different hospitals as sentinel surveillance sites. In between these sentinel surveillance sites; highest rate of isolates (22%) with 29 isolates was from Mymensingh medical college & hospital whereas lowest rate of isolates (1%) with 1 isolate was from both Sylhet Mag Osmani medical college & hospital and Cox's bazar medical college & hospital.

Hospitals as sentinel surveillance sites	**Number of *A. baumannii* isolates**
DHAKA MEDICAL COLLEGE & HOSPITAL	13
KHULNA MEDICAL COLLEGE & HOSPITAL	18
MYMENSINGH MEDICAL COLLEGE & HOSPITAL	29
RAJSHAHI MEDICAL COLLEGE & HOSPITAL	12
SYLHET MAG OSMANI MEDICAL COLLEGE & HOSPITAL	1
UTTARA ADHUNIK MEDICAL COLLEGE & HOSPITAL	8
COX'S BAZAR MEDICAL COLLEGE & HOSPITAL	1

Table 7: Acinetobacter baumannii isolates as per sentinel surveillance sites

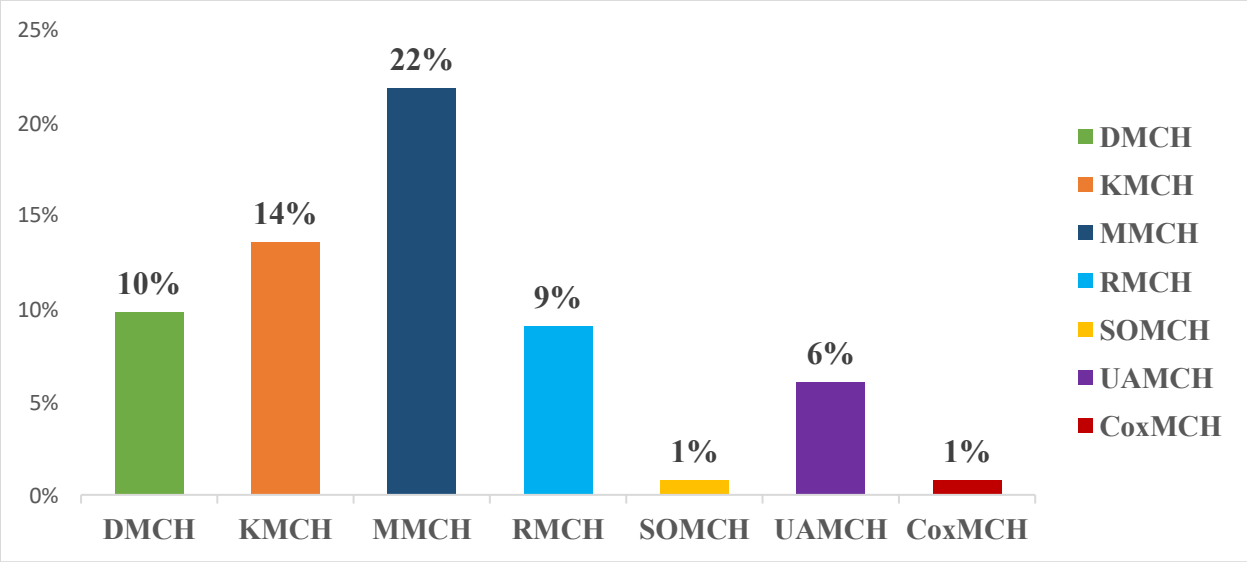


Figure 11: Percentage of Acinetobacter baumannii isolates as per sentinel surveillance sites

Among these *A. baumannii* isolates highest rate of specimen type (50%) was endotracheal aspirate & lowest rate of specimen type (5%) was sputum.

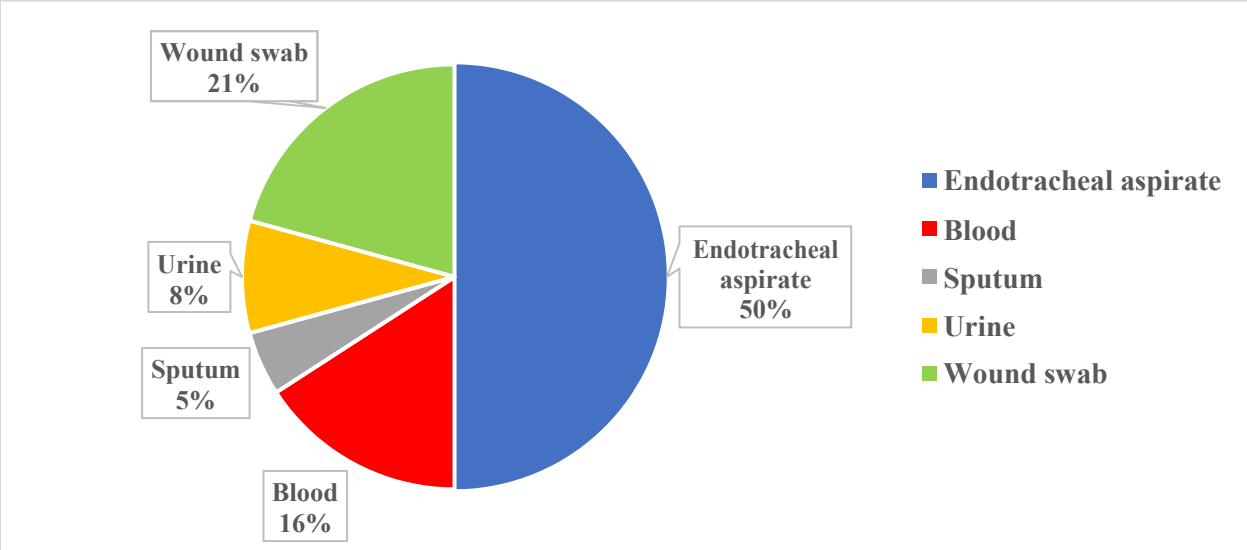


Figure 12: Specimen type of A. baumannii isolates (n=82)

Number of *Acinetobacter baumannii* isolates were counted in consideration from the year of 2017 to the month of May of 2023 to observe the prevalence of *A. baumannii* isolates according to year trend. Highest number of *A. baumannii* with 25 isolates were from the year of 2023 whereas second highest with 19 isolates were from the year of 2022 & the lowest number of *A. baumannii* with 1 isolate were from the year of 2017.

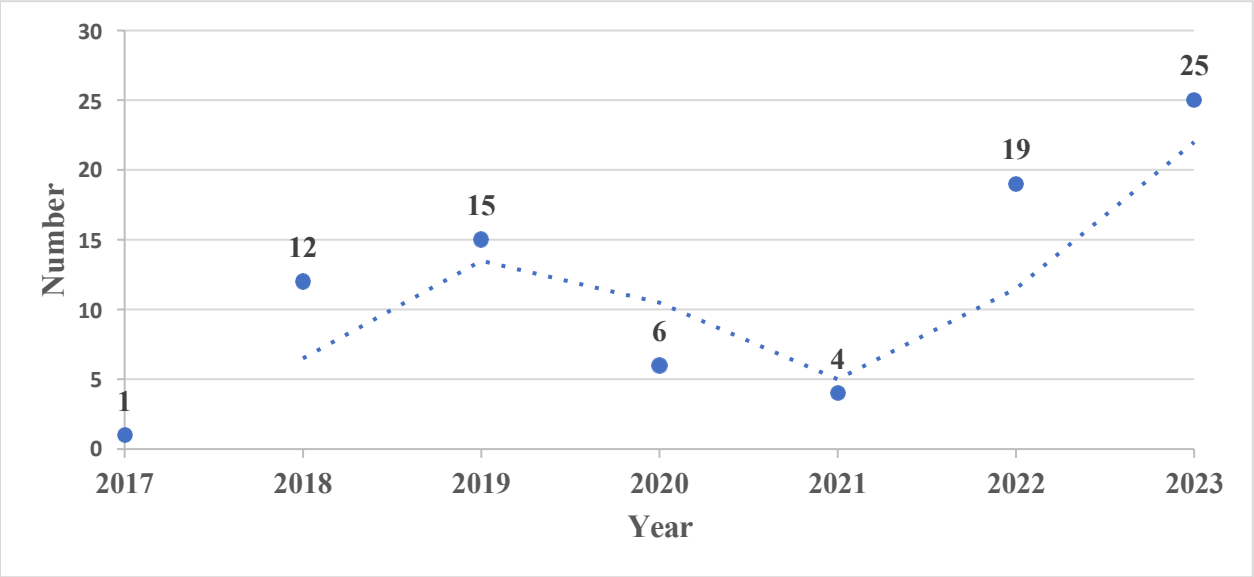
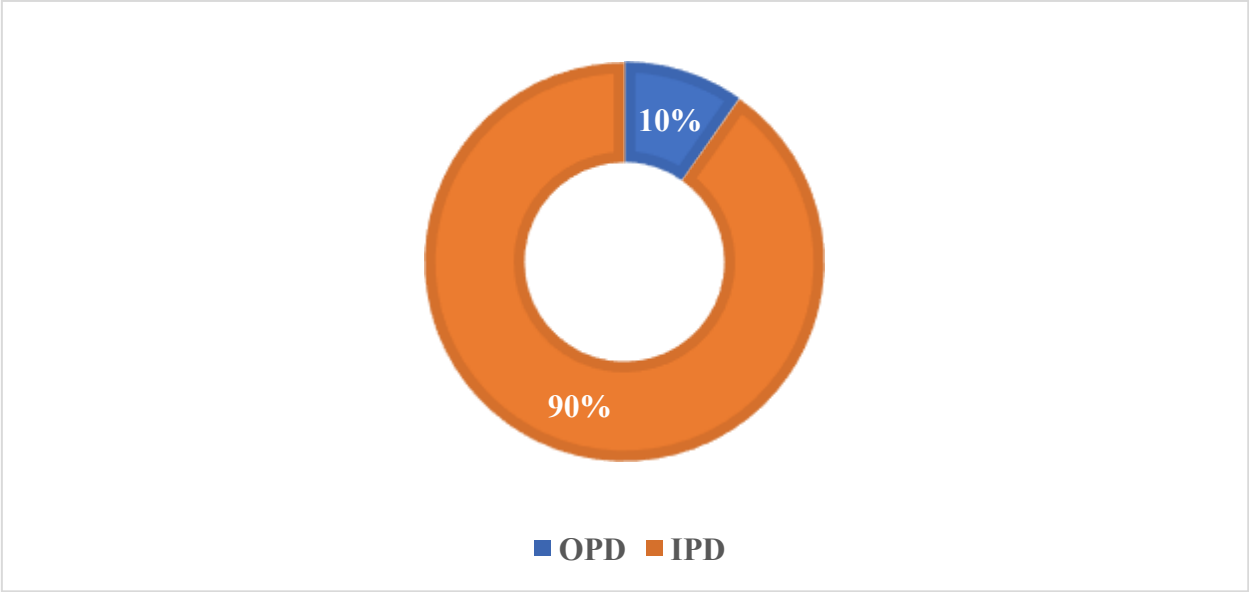


Figure 13: Acinetobacter baumannii isolates as per year (n=82)

Among 82 *A. baumannii* isolates 74 isolates as 90% were from indoor (IPD) patients and 8 isolates as 10% were from outdoor (OPD) patients.



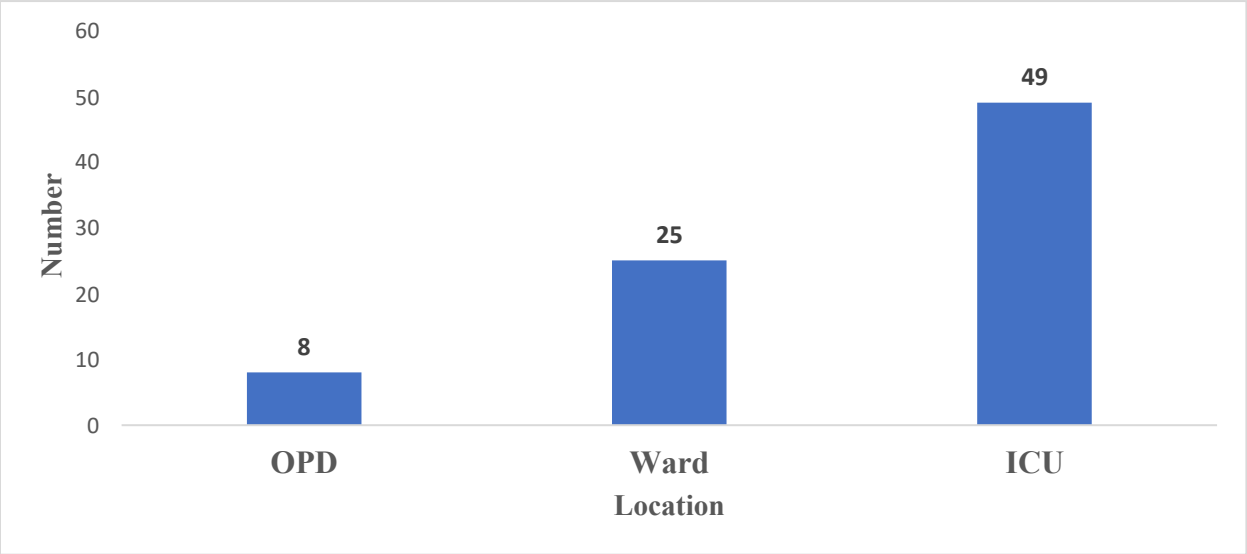


Figure 14, 15: Patients distribution (n=82)

Highest number of indoor patients with 49 *A. baumannii* isolates were from ICU ward patients, contributing highest proportion as 66% whereas surgery unit as second highest with 15 isolates contributed 20% and lowest number of indoor patients with 1 isolate contributing 1% were from burn unit.

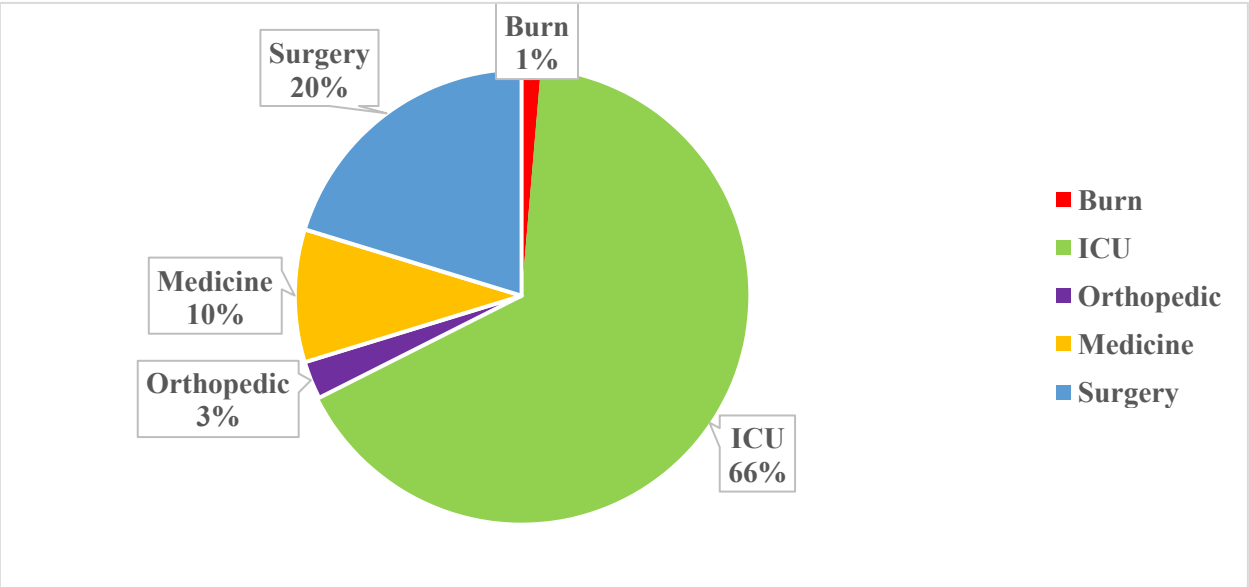


Figure 16: Patients distribution within the ward (n=74)

Among 82 *A. baumannii* isolates 49 isolates were from male patients and 33 isolates were from female patients. Highest number with 14 patients were from 21-30 and 61-70 age group.

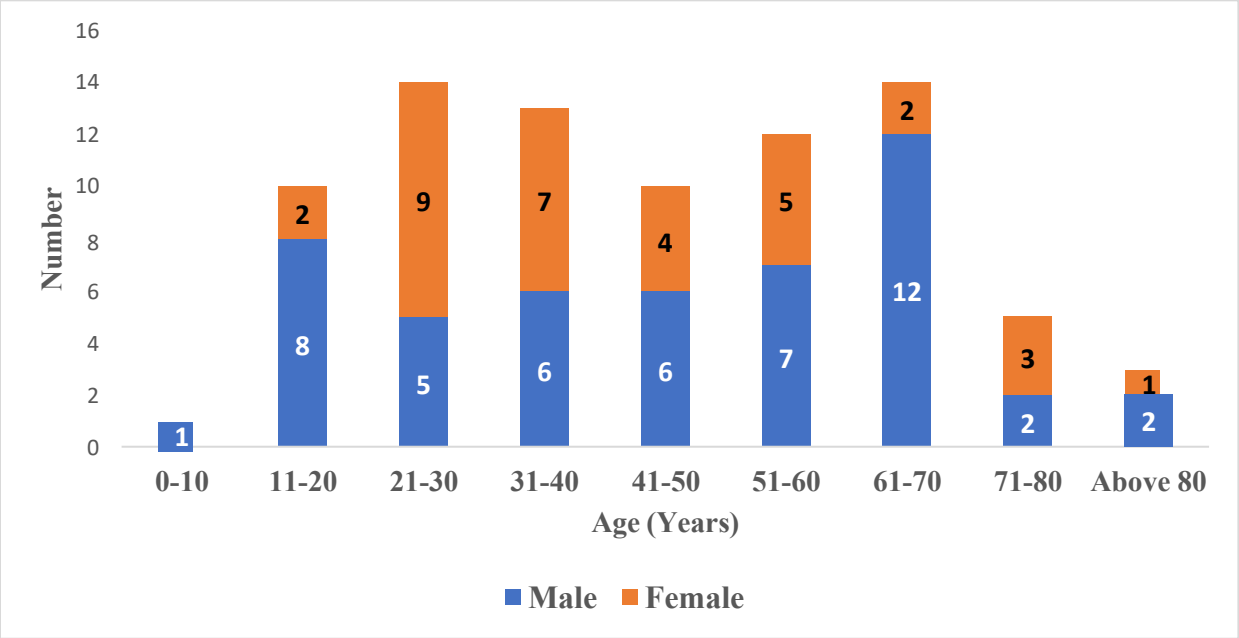


Figure 17: Patients distribution based on gender and age (n=82)

3.7 Antibiotic susceptibility profile of *Acinetobacter baumannii* isolates

Antibiotic susceptibility test was performed for all the 82 *A. baumannii* isolates through 12 antibiotics from the 7 antimicrobial agent groups of *Acinetobacter spp.* panel as: aminoglycosides, cephems, carbapenems, fluoroquinolones, tetracyclines, folate pathway antagonists and β-lactam combination agents. In this study the antibiotics were customized into 3 groups as Group-1, 2 & 3. The highest rate (79%) of resistant isolates were seen for Ceftazidime & the lowest rate (0%) of resistant isolates were seen for Tigecycline. Resistance from group-1 antibiotics were observed as Amikacin (72%), Cefepime (76%), Imipenem (77%), Ciprofloxacin (76%). Resistance from group-2 antibiotics were observed as Gentamycin (63%), Doxycycline (38%), Meropenem (77%), Trimethoprim-Sulfamethoxazole (56%). Resistance from group-3 antibiotics were observed as Ceftazidime (79%), Tigecycline (0%), Ceftriaxone (77%), Piperacillin-Tazobactam (73%). Also, the highest rate (84%) of sensitive isolates were seen for Tigecycline whereas the lowest rate of sensitive isolates (79%) was seen for Ceftazidime. Meanwhile, 77% isolates were assumed to be suspected carbapenemase enzyme producer because of their high resistance to Imipenem & Meropenem. Additionally, the highest rate (17%) of intermediate isolates were seen for Trimethoprim-Sulfamethoxazole where the lowest rate of intermediate isolates (0%) was seen for Imipenem, Ciprofloxacin, Meropenem & Ceftazidime. All isolates zone of inhibition (ZOI) was measured in millimeter (mm) as: Sensitive (S), Intermediate (I), Resistant (R) according to the guidelines of CLSI 2022 (Weinstein & Lewis, 2020).

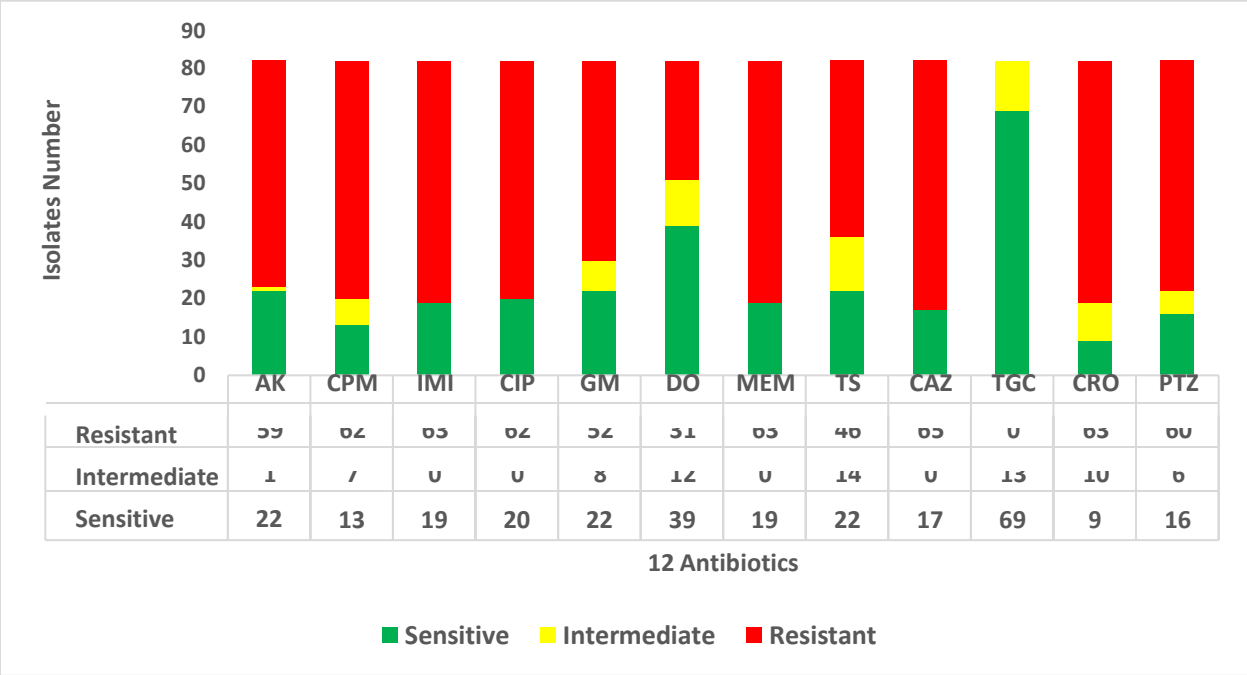


Figure 18: Number of Sensitive, Intermediate and Resistant isolates for 12 antibiotics (n=82)

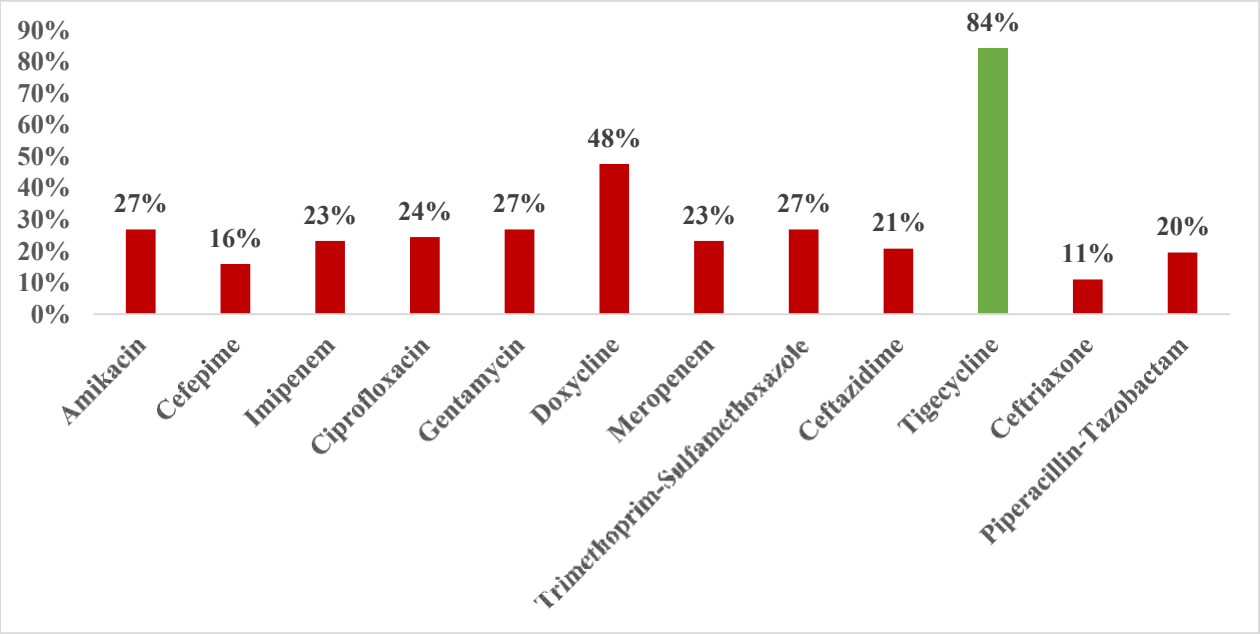


Figure 19: Susceptibility pattern of Acinetobacter baumannii isolates (n=82)

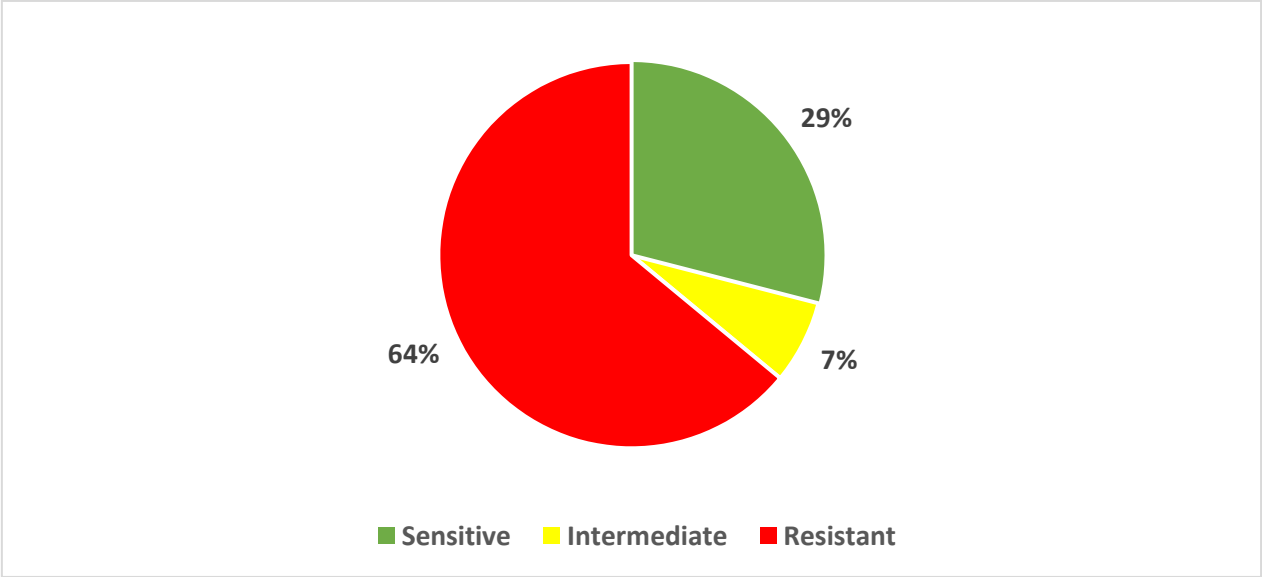


Figure 20: Percentage of Sensitive, Intermediate, Resistant isolates for 12 antibiotics (n=82)

The majority among 12 antibiotics showed notable difference in resistance among OPD, ward and ICU patients isolates, regardless of samples. Antibiotic resistance was highest in intensive care unit (ICU) patients isolates, followed by the ward and the outpatient department (OPD) patients isolates.

Antibiotic Resistance			
Antibiotics name	**OPD**	**Ward**	**ICU**
	%	**%**	**%**
Amikacin	46	57	70
Cefepime	52	43	73
Imipenem	39	62	75
Ciprofloxacin	27	65	72
Gentamycin	35	49	60
Doxycycline	38	45	48
Meropenem	32	56	72
Trimethoprim-Sulfamethoxazole	49	33	58
Ceftazidime	61	65	76
Tigecycline	0	0	0
Ceftriaxone	50	34	71
Piperacillin-Tazobactam	22	36	69

Table 8: Antibiotic resistance among OPD, Ward and ICU patients (n=82)

3.8 Multiple Antibiotic Resistance (MAR) Index

The multiple antibiotic resistance (MAR) index was calculated for each isolate using the formula MAR = a/b, where a represents the number of antibiotics to which the test isolate has demonstrated resistance and b represents the total number of antibiotics to which the test isolate was exposed (Sandhu et al., 2016). MAR index value (>0.2) implies that the test isolate came from a high-risk source of contamination where multiple antibiotics uses are practiced. A MAR index value of (≤0.2) indicates that the test isolate came from sources where antibiotics are never or rarely used (Anane A et al., 2019). Except for 9 isolates, the MAR index of 73 isolates among 82 *Acinetobacter baumannii* isolates was significantly higher than 0.2.

***A. baumannii* isolates number**	**Resistant antibiotics=a**	**Total antibiotics tested=b**	**MAR index**
1	10	12	0.83
2	9	12	0.75
3	0	12	0
4	8	12	0.67
5	11	12	0.92
6	10	12	0.83
7	10	12	0.83
8	5	12	0.42
9	9	12	0.75
10	10	12	0.83
11	10	12	0.83
12	11	12	0.92
13	9	12	0.75
14	11	12	0.92
15	11	12	0.92
16	4	12	0.33
17	8	12	0.67

18	0	12	0
19	11	12	0.92
20	0	12	0
21	9	12	0.75
22	11	12	0.92
23	10	12	0.83
24	10	12	0.83
25	10	12	0.83
26	11	12	0.92
27	11	12	0.92
28	9	12	0.75
29	10	12	0.83
30	11	12	0.92
31	10	12	0.83
32	11	12	0.92
33	8	12	0.67
34	9	12	0.75
35	8	12	0.67
36	11	12	0.92
37	9	12	0.75
38	9	12	0.75
39	9	12	0.75
40	11	12	0.92
41	8	12	0.67
42	0	12	0
43	9	12	0.75
44	10	12	0.83
45	0	12	0
46	10	12	0.83
47	10	12	0.83
48	10	12	0.83
49	0	12	0
50	9	12	0.75
51	11	12	0.92
52	10	12	0.83
53	10	12	0.83
54	10	12	0.83
55	10	12	0.83
56	0	12	0
57	7	12	0.58

58	9	12	0.75
59	0	12	0
60	9	12	0.75
61	9	12	0.75
62	9	12	0.75
63	0	12	0
64	10	12	0.83
65	10	12	0.83
66	9	12	0.75
67	10	12	0.83
68	7	12	0.58
69	10	12	0.83
70	6	12	0.5
71	8	12	0.67
72	10	12	0.83
73	9	12	0.75
74	9	12	0.75
75	10	12	0.83
76	7	12	0.58
77	11	12	0.92
78	9	12	0.75
79	8	12	0.67
80	8	12	0.67
81	7	12	0.58
82	11	12	0.92

Table 9: All 82 Acinetobacter baumannii isolates MAR index

3.9 MDR *Acinetobacter baumannii*

The term "multi drug resistant"(MDR) refers to a species of microorganism that exhibits resistance to at least one antimicrobial drug in three or more categories of antimicrobial drugs (Magiorakos et al., 2012). In between 82 isolates 50 isolates (61%) have shown resistance to at least one antimicrobial drug in three or more antibiotic categories indicating them as MDR isolates.

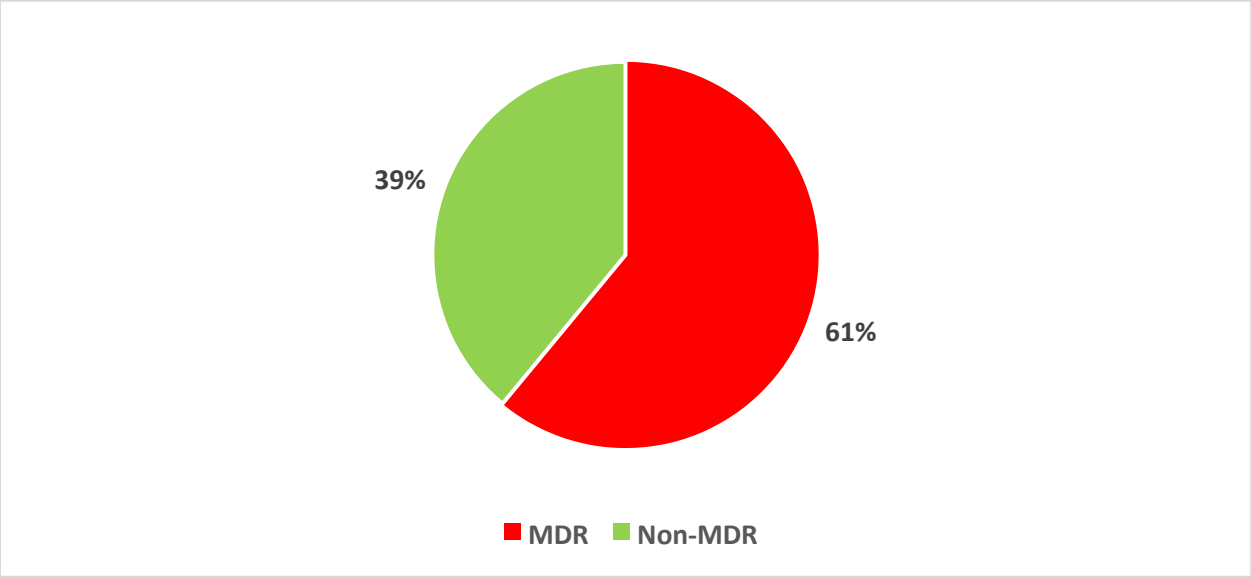


Figure 21: Percentage of MDR Acinetobacter baumannii isolates (n=50)

3.10 Suspected XDR *Acinetobacter baumannii*

The term "extensively drug resistant"(XDR) refers to a species of microorganism that exhibits resistance to at least one agent in all but two or fewer antimicrobial categories of antimicrobial agents. XDR microorganisms remain effective against only one or two categories of antimicrobial agents (Magiorakos et al., 2012). Among 82 isolates 15 isolates (18%) have shown resistance to at least one agent in all but two or fewer antimicrobial categories indicating them as suspected XDR isolates.

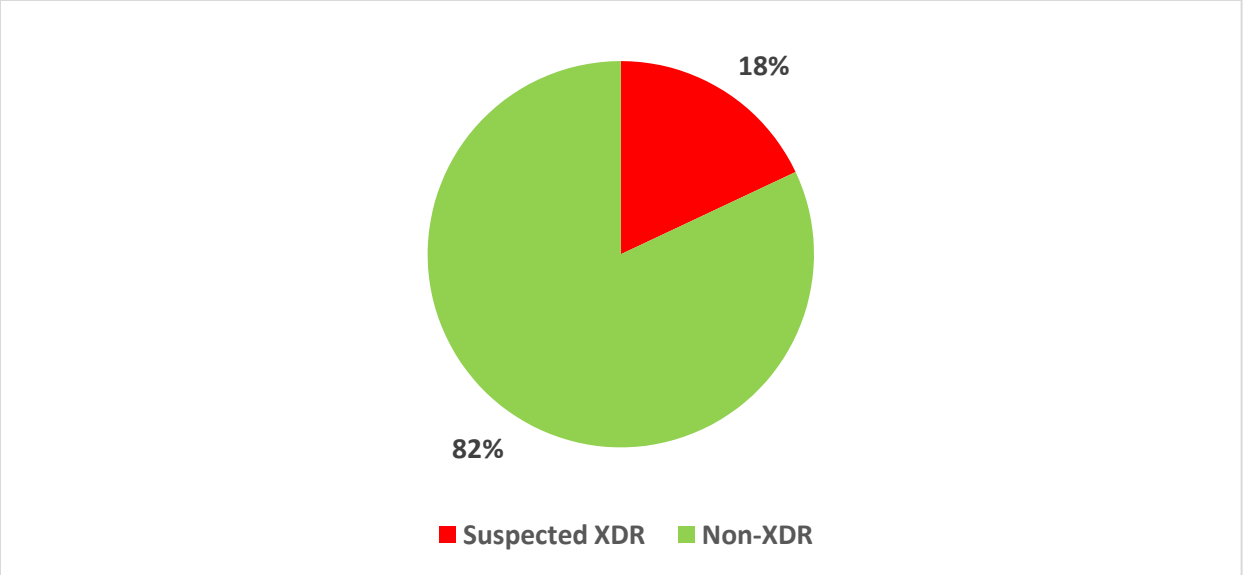


Figure 22: Percentage of suspected XDR Acinetobacter baumannii isolates (n=15)

Chapter 4

Discussion

The objective of this study was to identify *Acinetobacter baumannii* from clinical (Acb) complex isolates and observe their distribution and antibiotic susceptibility pattern. To conduct this study, nine different hospitals as sentinel surveillance sites under "AMR surveillance in Bangladesh" project was selected to collect isolates. Isolates were taken in consideration from the year of 2017 to the month of May of 2023 to figure out the prevalence of *Acinetobacter baumannii*. In total, 133 isolates were taken as suspected *Acinetobacter baumannii* in which 82 isolates were identified as *Acinetobacter baumannii*. In a study it is reported that biochemical tests have the sensitivity and specificity range from 70% to 90% and 75% to 95% in identification of *A. baumannii* (Visca et al., 2016). In comparison to that study, this study reported 55% specificity which is somewhat almost close compared to that study in identification of *A. baumannii* by biochemical tests but biochemical tests identify bacteria on genus or group level through assessing the presence or absence of specific molecules and enzymes that is involved in metabolism of bacteria and for this reason species level accurate identification of bacteria is challenging by this method. Studies have shown that MALDI-TOF MS is highly accurate in identification of *A. baumannii* with sensitivities and specificities ranging from 93% to 100% and 94% to 99% (Bou et al., 2010). In comparison to those studies, this study reported 53% specificity which is way below compared to that study in identification of *A. baumannii* by MALDI-TOF MS and behind this one possible reason could be that as some bacterial species share very similar proteins among them so it makes this method difficult for the accurate identification of the species of the bacteria. RT-PCR studies show sensitivity ranging from 75% to 95% and high specificity, often over 99% which minimizes the risk of misidentifying

other (Acb) complex species as *A. baumannii* (Lai et al., 2016). Additionally, RT-PCR provides a higher level of specificity when compared to MALDI-TOF MS particularly in distinguishing closely related species of (Acb) complex. In comparison to those studies, this study reported 62% specificity in identification of *A. baumannii* by RT-PCR which is the highest compared to other two methods of the study because RT-PCR identify bacteria on species level through amplifying specific DNA sequence of the bacteria that is highly specific to the species of the bacteria and gives result in real time and for these reason among these three methods this is the only reliable method that can accurately identify the species of the bacteria and for this study identified *Acinetobacter baumannii* isolates from RT-PCR has been taken in consideration for subsequent test.

Among 82 *A. baumannii* isolates 74 isolates as 90% were from indoor (IPD) patients and 8 isolates as 10% were from outdoor (OPD) patients. During a study conducted in Tehran, a total of 100 samples were collected from the ICU of Rasoul Akram hospital and out of these samples highest rate with 21% were found to be *A. baumannii* (Moghim et al., 2015). In comparison to that study, this study reported that highest number of indoor patients with 49 *A. baumannii* isolates were from ICU ward patients, contributing highest proportion as 66%. However, surgery unit as second highest with 15 isolates contributed 20% and lowest number of indoor patients with 1 isolate contributing 1% were from burn unit. A study conducted by Tsakris, et al., (2008) revealed that the age range of the patients varied from 22 to 83 years, with a higher number of males than females (Moghim et al., 2015). In comparison to that study, this study reported that among 82 *A. baumannii* isolates 49 isolates were from male patients and 33 isolates were from female patients which implies that males outnumbered females where highest number with 14 patients were from 21-30 and 61-70 age group. In a study conducted by Amudhan, et al. (2011), it was discovered that the

respiratory secretions had the highest isolation percentage with 53.49% (Moghim et al., 2015). In comparison to that study, this study reported that highest rate of specimen type (50%) was endotracheal aspirate.

Antibiotic Susceptibility Test (AST) was performed on the *A. baumannii* isolates through 12 antibiotics from the 7 antimicrobial agent groups of *Acinetobacter spp.* panel as: aminoglycosides, cephems, carbapenems, fluoroquinolones, tetracyclines, folate pathway antagonists and β-lactam combination agents where the antibiotics were customized into 3 groups as Group-1, 2 & 3 for this study. According to antibiotic susceptibility test result among the *A. baumannii* isolates 29% were sensitive, 7% were intermediate & 64% were resistant to the selected 12 antibiotics. Besides, the clinical isolates of this study showed promising results to Tigecycline, a reserved member of the Tetracyclines class of antibiotics, where it was found that 84% of the isolates were sensitive to this antibiotic. On the other hand, it is worth noting that 79% of isolates showed resistance to Ceftazidime, which suggests that the effectiveness of this antibiotic against clinical *A. baumannii* isolates may be limited. The majority among 12 antibiotics showed notable difference in resistance among OPD, ward and ICU patients isolates, regardless of samples. Antibiotic resistance was highest in intensive care unit (ICU) patients isolates, followed by the ward and the outpatient department (OPD) patients isolates. Throughout a comprehensive study conducted in Croatia a total of 19 isolates were identified from patients where it is noteworthy that all of these isolates were found to be extensively drug-resistant (XDR) (Musić et al., 2017). In comparison to that study, this study reported that 50 isolates (61%) among 82 *Acinetobacter baumannii* isolates have shown resistance to at least one antimicrobial drug in three or more antibiotic categories which indicates that they are MDR isolates and 15 isolates (18%) have shown resistance to at least one agent in all but two or fewer antimicrobial categories which indicates that they are suspected XDR

isolates which denotes that a higher percentage of resistant isolates are showing multi drug resistant (MDR) pattern and these resistant isolates are emerging to evolve as extensively drug resistant (XDR).

Identifying Acinetobacter species within the (Acb) complex remains a difficult task. Currently, the existing phenotypic methods are inadequate. Despite the widespread use of commercially available automated identification systems in clinical laboratories, these systems still lack effectiveness in accurate identification. Expanding the range of mass spectra from various species in the (Acb) complex will enhance the MALDI-TOF MS database, leading to more precise identification of the (Acb) complex at the species level. When it comes to molecular characterization, relying on just one gene target as a biomarker is not enough to ensure accurate identification of the species of (Acb) complex. Besides, genetic identification of acquired antibiotic-resistant genes is crucial for understanding resistance mechanisms and developing targeted strategies to combat the growing threat of antibiotic resistance. However, whole-genome sequencing shows potential as a valuable tool, but its application is restricted in settings with limited resources. In addition, conducting comparative genomic studies will enable us to pinpoint species-specific targets that can serve as reliable markers for molecular identification.

Conclusion

In conclusion, it is challenging to differentiate between species within the (Acb) complex. Species-level identification is necessary due to the differences in clinical relevance of the (Acb) complex and their antimicrobial susceptibility profile. Traditional phenotypic methods, such as culture and biochemical tests, have limitations in terms of speed, reliability and efficiency when it comes to distinguishing the most clinically significant *A. baumannii* within the (Acb) complex. RT-PCR method focuses on specific targets, such as the *blaOXA-51* gene, which is widely recognized as a reliable marker for identifying *Acinetobacter baumannii* species. MALDI-TOF MS can prove to be quite valuable in identification at a species level if equipped with a precise database. Utilization of *blaOXA-51* gene through RT-PCR for molecular identification proves to be significantly more accurate than conventional and automated methods in identifying *Acinetobacter baumannii* species within the (Acb) complex. However, the findings of this study indicates that *A. baumannii*, which is highly resistant to antibiotics, is potentially evolving into its more dangerous forms known as MDR, XDR, and PDR. So, it is crucial to consistently enhance the effectiveness of new and innovative methods in order to address the global issue of antimicrobial resistance.

References

- Abbo, A., Navon-Venezia, S., Hammer-Muntz, O., Krichali, T., Siegman-Igra, Y., & Carmeli, Y. (2005, January). Multidrug-resistant Acinetobacter baumannii. Emerging infectious diseases. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3294361/

- Anane A, Y., Apalata, T., Vasaikar, S., Okuthe, G. E., & Songca, S. (2019, November). Prevalence and molecular analysis of multidrug-resistant Acinetobacter baumannii in the extra-hospital environment in Mthatha, South Africa. The Brazilian Journal of Infectious Diseases, 23(6), 371–380. https://doi.org/10.1016/j.bjid.2019.09.004

- Aryal, S. (2022, May 3). Acinetobacter baumannii- An Overview. Microbe Notes. https://microbenotes.com/acinetobacter-baumannii/#:~:text=On%20nutrient%20agar%2C%20non%2Dmucoid,circular%20colonies%20will%20be%20observed.

- Asif, M., Alvi, I. A., & Rehman, S. U. (2018, August). Insight into Acinetobacter baumannii: pathogenesis, global resistance, mechanisms of resistance, treatment options, and alternative modalities. Infection and Drug Resistance, Volume 11, 1249–1260. https://doi.org/10.2147/idr.s166750

- Barbosa, C., Nogueira, S., Gadanho, M., & Chaves, S. S. (2016, January 1). DNA extraction: finding the most suitable method. Elsevier eBooks. https://doi.org/10.1016/b978-0-12-416999-9.00007-1

- Castillo-Ramírez, S. (2022, December). Zoonotic Acinetobacter baumannii: the need for genomic epidemiology in a One Health context. The Lancet Microbe, 3(12), e895–e896. https://doi.org/10.1016/s2666-5247(22)00255-5

- Chapartegui-González, I., Lázaro-Díez, M., Bravo, Z., Navas, J., Icardo, J. M., & Ramos-Vivas, J. (2018, August 22). Acinetobacter baumannii maintains its virulence after long-time starvation. PLOS ONE, 13(8), e0201961. https://doi.org/10.1371/journal.pone.0201961

- D'Souza, R., Pinto, N. A., Phuong, N. L., Higgins, P. G., Vu, T. N., Byun, J.-H., Cho, Y. L., Choi, J. R., & Yong, D. (2019, March 5). Phenotypic and genotypic characterization of Acinetobacter spp.. panel strains: A Cornerstone to facilitate antimicrobial development. Frontiers. https://www.frontiersin.org/articles/10.3389/fmicb.2019.00559/full

- Depka, D., Mikucka, A., Bogiel, T., Rzepka, M., Zawadka, P., & Gospodarek-Komkowska, E. (2022). Conventional and real-time PCR targeting BLAOXA genes as reliable methods for a rapid detection of carbapenem-resistant Acinetobacter baumannii clinical strains. Antibiotics, 11(4), 455. https://doi.org/10.3390/antibiotics11040455

- Falah, F., Shokoohizadeh, L., & Adabi, M. (2019, January). Molecular identification and genotyping of Acinetobacter baumannii isolated from burn patients by PCR and ERIC-PCR. Scars, Burns & Healing, 5, 205951311983136. https://doi.org/10.1177/2059513119831369

- Farzana, R., Swedberg, G., Giske, C. G., & Hasan, B. (2022, June 1). Molecular and genetic characterization of emerging carbapenemase-producing Acinetobacter baumannii strains from patients and hospital environments in Bangladesh. Infection Prevention in Practice. https://doi.org/10.1016/j.infpip.2022.100215

- Fernando, D. M., Khan, I. U. H., Patidar, R., Lapen, D. R., Talbot, G., Topp, E., & Kumar, A. (2016, November 18). Isolation and Characterization of Acinetobacter baumannii Recovered from Campylobacter Selective Medium. Frontiers in Microbiology. https://doi.org/10.3389/fmicb.2016.01871

- Fitzpatrick, M. A., Ozer, E. A., Bolon, M. K., & Hauser, A. R. (2015, February 1). Influence of ACB complex genospecies on clinical outcomes in a U.S. hospital with high rates of multidrug resistance. Journal of Infection. https://doi.org/10.1016/j.jinf.2014.09.004

- Hrenović, J., Goić-Barišić, I., Kazazić, S., Kovačić, A., Ganjto, M., & Tonkić, M. (2016, April 14). Carbapenem-resistant isolates of Acinetobacter baumannii in a municipal wastewater treatment plant, Croatia, 2014. Eurosurveillance. https://doi.org/10.2807/1560-7917.es.2016.21.15.30195

- Kadri, S. S. (2020, July). Key Takeaways From the U.S. CDC's 2019 Antibiotic Resistance Threats Report for Frontline Providers. Critical Care Medicine, 48(7), 939–945. https://doi.org/10.1097/ccm.0000000000004371

- Kian, B., Mirnejad, R., Moradli, G., Mirkalantari, S., & Golmohammadi, R. (2018). Molecular genotyping of acinetobacter baumannii species isolated from patients in Tehran,

Iran, by repetitive element PCR fingerprinting. Iranian journal of pathology. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6339497/

- Kittinger, C., Kirschner, A. K. T., Lipp, M., Baumert, R., Mascher, F., Farnleitner, A. H., & Zarfel, G. (2017, December 30). Antibiotic Resistance of Acinetobacter spp. Isolates from the River Danube: Susceptibility Stays High. International Journal of Environmental Research and Public Health. https://doi.org/10.3390/ijerph15010052

- Kovačić, A., Musić, M. E., Dekić, S., Tonkić, M., Novak, A., Rubić, A., Hrenović, J., & Goić-Barišić, I. (2017, December 1). Transmission and survival of carbapenem-resistant Acinetobacter baumannii outside hospital setting. PubMed. https://doi.org/10.2436/20.1501.01.299

- López-Durán, P. A., Fonseca-Coronado, S., Lozano-Trenado, L. M., Araujo-Betanzos, S., Rugerio-Trujillo, D. A., Vaughan, G., & Saldaña-Rivera, E. (2020, April 17). Nosocomial transmission of extensively drug resistant Acinetobacter baumannii strains in a tertiary level hospital. PLOS ONE. https://doi.org/10.1371/journal.pone.0231829

- Lowe, M., Singh-Moodley, A., Ismail, H., Thomas, T., Chibabhai, V., Nana, T., Lowman, W., Ismail, A., Chan, W., & Perovic, O. (2022, August 5). Molecular characterisation of Acinetobacter baumannii isolates from bloodstream infections in a tertiary-level hospital in South Africa. Frontiers in Microbiology. https://doi.org/10.3389/fmicb.2022.863129

- Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S. J., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., Rice, L. B., Stelling, J., Struelens, M., Vatopoulos, A., Weber, J. T., & Monnet, D. (2012, March 1). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection. https://doi.org/10.1111/j.1469-0691.2011.03570.x

- Marí-Almirall, M., Cosgaya, C., Higgins, P. G., Van Assche, A., Telli, M., Huys, G., Lievens, B., Seifert, H., Dijkshoorn, L., Roca, I., & Vila, J. (2017, March 1). MALDI-TOF/MS identification of species from the Acinetobacter baumannii (Ab) group revisited: inclusion of the novel A. seifertii and A. dijkshoorniae species. Clinical Microbiology and Infection. https://doi.org/10.1016/j.cmi.2016.11.020

- Michael, C. A., Dominey-Howes, D., & Labbate, M. (2014, September 16). The Antimicrobial Resistance Crisis: Causes, Consequences, and Management. Frontiers in Public Health. https://doi.org/10.3389/fpubh.2014.00145

- Moghim, S., Ghajavand, H., Esfahani, B., Havaei, S., & Fazeli, H. (2015). Molecular identification of Acinetobacter baumannii isolated from intensive care units and their antimicrobial resistance patterns. Advanced Biomedical Research, 4(1), 110. https://doi.org/10.4103/2277-9175.157826

- Musić, M. E., Hrenović, J., Goić-Barišić, I., Hunjak, B., Škorić, D., & Ivanković, T. (2017, August 1). Emission of extensively-drug-resistant Acinetobacter baumannii from hospital settings to the natural environment. Journal of Hospital Infection. https://doi.org/10.1016/j.jhin.2017.04.005

- Neonakis, I. K., Spandidos, D., & Petinaki, E. (2011, February 1). Confronting multidrug-resistant Acinetobacter baumannii: a review. International Journal of Antimicrobial Agents. https://doi.org/10.1016/j.ijantimicag.2010.10.014

- Niranjan, D., Singh, N. P., Manchanda, V., Rai, S., & Kaur, I. (2013, July 1). Multiple carbapenem hydrolyzing genes in clinical isolates of Acinetobacter baumannii. Indian Journal of Medical Microbiology. https://doi.org/10.4103/0255-0857.115626

- Sandhu, R., Dahiya, S., & Sayal, P. (2016). Evaluation of multiple antibiotic resistance (MAR) index and Doxycycline susceptibility of Acinetobacter species among inpatients. Indian Journal of Microbiology Research, 3(3), 299. https://doi.org/10.5958/2394-5478.2016.00064.9

- Santajit, S., & Indrawattana, N. (2016). Mechanisms of Antimicrobial Resistance in ESKAPE Pathogens. BioMed Research International, 2016, 1–8. https://doi.org/10.1155/2016/2475067

- Sharma, R., Kibii, T., & otieno, S. (2023, May 17). Kirby Bauer disc diffusion method for antibiotic susceptibility testing. Microbe Notes. https://microbenotes.com/kirby-bauer-disc-diffusion/

- Sujan, M. J., Habib, Z. H., Rahman, A., Rizvi, S. M., Barua, H. T., Aboushady, A. T., Hasnat, M. A., Rasul, S. B., Joh, H. S., Prifti, K., Chi, K. K., Kwon, S. Y., Clark, A., Gautam, S., Holm, M., Marks, F., Stelling, J., Shaw, A., & Poudyal, N. (2023). Antimicrobial resistance

surveillance methods in Bangladesh: Present and way forward. Clinical Infectious Diseases, 77(Supplement_7). https://doi.org/10.1093/cid/ciad561

- Syal, K., Mo, M., Yu, H., Iriya, R., Jing, W., Guodong, S., Wang, S., Grys, T. E., Haydel, S. E., & Tao, N. (2017). Current and emerging techniques for antibiotic susceptibility tests. Theranostics, 7(7), 1795–1805. https://doi.org/10.7150/thno.19217

- Tiku, V. (2022, January 1). Acinetobacter baumannii: Virulence Strategies and Host Defense Mechanisms. DNA And Cell Biology, 41(1), 43–48. https://doi.org/10.1089/dna.2021.0588

- Vijayakumar, S., Biswas, I., & Veeraraghavan, B. (2019, July 1). Accurate identification of clinically important Acinetobacter spp.: an update. Future Science OA. https://doi.org/10.2144/fsoa-2018-0127

- Visca, P., Seifert, H., & Towner, K. J. (2011, October 18). Acinetobacter infection – an emerging threat to human health. IUBMB Life. https://doi.org/10.1002/iub.534

- Weinstein, M. P., & Lewis, J. S. (2020, February 24). The Clinical and Laboratory Standards Institute Subcommittee on Antimicrobial Susceptibility Testing: Background, Organization, Functions, and Processes. Journal of Clinical Microbiology, 58(3). https://doi.org/10.1128/jcm.01864-19