Assessment of the Antimicrobial Sensitivity of *Acinetobacter baumannii* Collected from the Environment of Neonatal Ward of a Rural Hospital of Bangladesh

Submitted By
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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the Bachelor's degree in Microbiology

Department of Mathematics and Natural Sciences
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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing a degree at Brac University.

2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.

3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.

4. I have acknowledged all main sources of help.

Student's Full Name and Signature:

_____

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Approval

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Abstract

Background

Healthcare associated infection caused by multi drug resistant *Acinetobacter baumannii* is not only becoming increasingly prevalent in hospital settings but also becoming a huge challenge for the healthcare system globally. It has already made space on the priority pathogen list by WHO for addressing growing global resistance to antimicrobial medicines (World Health Organization: WHO, 2017). Since it can survive under a wide range of environmental conditions for a prolonged period of time, it can cause outbreaks of nosocomial infection in healthcare settings.

Objective

The objective of this study is to investigate the prevalence and assess the antimicrobial sensitivity of *Acinetobacter baumannii* collected from the environment of the neonatal ward of a rural healthcare facility of Bangladesh.

Method

A total of 100 environmental samples were collected from different sites of the neonatal ward of the Zahid Memorial Children's Hospital of Faridpur district using proper protocol. After collection, these samples were transferred to the laboratory for proper identification and antimicrobial susceptibility testing which was done through Vitek-2 machine according to manufacturer's guideline.

Result

Among these 100 environmental samples, 31 *Acinetobacter baumannii* were isolated and 26% of these strains were multidrug resistant (MDR) and 10% were extremely drug resistant (XDR) bacteria. The antimicrobial susceptibility was determined based on minimum inhibitory concentration value according to the CLSI (Clinical and Laboratory Standards Institute) guideline and natural resistance across 14 different antimicrobial agents such as Piperacillin/Tazobactam, Ceftriaxone, Ceftazidime, Cefoperazone/Sulbactam, Cefepime, Imipenem, Meropenem, Amikacin, Gentamicin, Colistin, Ciprofloxacin, Levofloxacin, Minocycline, Trimethoprim/Sulfamethoxazole. Moreover, the presence of 13% carbapenem resistant *Acinetobacter baumannii* (CRAB) isolate is of great concern since according to CDC, the threat level of CRAB is urgent and requires immediate action (Carbapenem-resistant Acinetobacter | A.R. & Patient Safety Portal, n.d.).

Conclusion

The presence of multi-drug resistant and extremely drug resistant strains of *A. baumannii* in hospital environments is concerning and poses a grave danger to the residing patients. To gain critical information of these resistant strains, further study should be done including whole genome sequencing (WGS) and genetic analysis for the control of nosocomial *A. baumannii* infection.

Keywords:

Antimicrobial Resistance (AMR), Multidrug-Resistant (MDR), Extremely-drug Resistant (XDR) etc.

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CHAPTER 1

Introduction

Nosocomial or hospital acquired infections are becoming a leading health hazard in recent years, especially for developing countries like Bangladesh. Unregulated use of antibiotics by most of the population has led to antimicrobial resistance (AMR) where new strains of micro-organism are emerging that are resistant to most of the antimicrobial drugs. And the emergence of these resistant microorganisms has made nosocomial infections caused by opportunistic pathogens lethal for patients admitted to the healthcare facility (Dijkshoorn et al., 2007). To face this threat of AMR, the World health organization has published the global priority list of AMR Bacteria to guide research, discovery, and development of new effective antibiotics. The most critical group of this priority list include *Pseudomonas spp., Acinetobacter spp.* and various types of *Enterobacteriaceae,* like *Klebsiella spp., E. coli, Staphylococcus spp., Streptococcus spp.* etc. and multi drug resistant strains of these organisms present significant risks in hospitals and healthcare facilities. (World Health Organization: WHO, 2017).

CHAMPS or Child Health and Mortality Prevention Surveillance is a multi-country project with the aim of collecting and analyzing data to understand the causes of death of children who are under five years old. In Bangladesh, through minimally invasive tissue sampling (MITS) method, samples are collected from three different hospitals (BUHC- Baliakandi Upazila Health Complex, ZMCH -Zahid Memorial Children Hospital, FMCH- Faridpur Medical College Hospital) of Baliakandi sub-district of Faridpur and sent to the International Centre for Diarrheal

Disease Research, Bangladesh (icddr,b) for further investigation to identify the cause of child death (Sites: Bangladesh - CHAMPS Health, 2022). Since the start of the project during 2017, there were several specific cases where nosocomial pathogens (such as *Acinetobacter spp., Staphylococcus spp. etc.*) were a reason for the neonatal (infants under 28 days of age) deaths or stillbirths. According to CHAMPS Bangladesh Determination of Cause of Death (DeCoDe) data, neonatal infections are one of the leading causes of early neonatal death. Of all neonatal deaths determined by the CHAMPS Bangladesh Team, 9% showed infection as an underlying cause and 19% as an immediate cause of death. (Case Study: Addressing Neonatal Nosocomial Infections in a Bangladeshi Hospital Facility - CHAMPS Health, 2022). These infections generally result in sepsis, pneumonia, meningitidis etc. and most of these infections are caused by pathogenic bacteria. According to CHAMPS data of December 2016 to December 2021, the most common pathogen found among early neonatal death (24 hours to 7 days) and late neonatal death (7 to 27 days) were *Acinetobacter baumannii* and *Klebsiella pneumoniae* and most newborns who died from these pathogenic bacteria likely acquired their infections after birth from the healthcare setting (Bassat et al., 2023). Considering the severity of the presence of these pathogens in healthcare settings, the World Health Organization (WHO) developed a list of antibiotic resistant pathogens in 2017. And multi drug resistant *Acinetobacter baumannii* has made its place within the "Priority 1: Critical" pathogen group on the WHO's priority pathogens list. (Denissen et al., 2022) Moreover, CDC (Centers for disease control and prevention) has also acknowledged that *Acinetobacter* infection is a challenging threat to hospitalized patients because it frequently contaminates healthcare facility surfaces and shared medical equipment. (*Acinetobacter* in Healthcare Settings | HAI | CDC, n.d.) There are an estimated 45,000 (range, 41,400 to 83,000)

cases of *Acinetobacter* infections per year in the United States and 1 million (range, 600,000 to 1,400,000) cases globally per year (Wong et al., 2017).

Acinetobacter baumannii has emerged as a major problem pathogen for many institutions throughout the world. Because of its incredible potential to acquire or upregulate antibiotic drug resistance determinants, it has rightfully risen to the forefront of scientific research. (Peleg et al., 2008) *Acinetobacter baumannii* can survive in environments characterized by either dry or moist conditions and can remain viable under these conditions for several weeks, and even months. This bacterium is predominantly linked to nosocomial infections like pneumonia, bacteremia, meningitis, UTIs, stomach infections, and sepsis etc. It mostly affects people with weak immune systems who already have a number of other illnesses. It is important to note that *A. baumannii* can live for long periods of time in clinical settings, change and adapt its genome quickly, and acquire antibiotic resistant genes when it is under selective pressure from its environment. Because of these traits, this pathogen can contribute to higher morbidity and mortality rates and longer hospital stays. (Denissen et al., 2022) In this study, to understand the severity of bacterial presence of *Acinetobacter baumannii* in the hospital environment, its antimicrobial resistance pattern is analyzed.

CHAPTER 2

Literature review

2.1 Acinetobacter baumannii

Acinetobacter baumannii is a gram-negative, catalase-positive, oxidase-negative, nonmotile, nonfermenting coccobacilli bacteria known for its resilience and ability to cause a wide range of infections, particularly in healthcare settings. This bacterium is a significant opportunistic pathogen, often associated with hospital-acquired infections such as pneumonia, bloodstream infections, wound infections, and urinary tract infections, particularly in immunocompromised individuals or those with prolonged hospital stays. Understanding its biology, virulence factors, and mechanisms of antibiotic resistance is crucial in combating infections caused by this resilient pathogen. (Howard et al., 2012) *Acinetobacter* species of human origin grow well on solid media that are routinely used in clinical microbiology laboratories, such as MacConkey agar media, at a 37°C incubation temperature. The colonies are observed as non-lactose fermenting, opaque, elevated, and circular.

2.2 Virulence factors and pathogenesis of *Acinetobacter baumannii*

Acinetobacter baumannii employ a wide range of virulence factors to evade host defenses and cause infection. Among these factors, the polysaccharide capsule stands out for its ability to confer resistance to phagocytosis and desiccation, thus facilitating bacterial survival in the host environment. Moreover, *Acinetobacter baumannii* secretes various enzymes and toxins, such as

phospholipases, proteases, and hemolysins, which contribute to tissue damage and evasion of host defenses. Furthermore, outer membrane proteins (OMPs) play multiple roles in virulence, including mediating adhesion to host cells and modulating immune responses. Additionally, the bacterium employs sophisticated iron acquisition systems, such as siderophores and heme uptake systems, to scavenge iron from the host environment which is essential for bacterial growth and virulence. Understanding the process of these virulence factors is critical for developing effective therapeutic strategies against *Acinetobacter baumannii* infections (Moubareck & Halat, 2020). Survival and growth on host skin and mucosal surfaces requires that the organisms can resist antibiotics and inhibitory agents and the conditions that are exerted by these surfaces. Outgrowth on mucosal surfaces and medical devices, such as intravascular catheters and endotracheal tubes, can result in biofilm formation, which enhances the risk of infection of the bloodstream and airways. Quorum sensing might have a regulatory role in biofilm formation. (Dijkshoorn et al., 2007)

2.3 Antimicrobial agents against *Acinetobacter baumannii*

Antimicrobial agents play a crucial role in treating infections caused by *Acinetobacter baumannii*, especially considering its increasing resistance to multiple antibiotics. A joint initiative by the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC) has created a standardized international terminology that describes acquired resistance profiles of Acinetobacter spp. and other frequent bacteria often responsible for healthcare-associated infections and prone to multidrug resistance. According to

this initiative, there are a total of 9 antimicrobial categories and 15 antimicrobial agents to analyze the resistance of *Acinetobacter baumannii*. (Magiorakos et al., 2012)

SL no	Antimicrobial category	Antimicrobial agents
1	Antipseudomonal penicillin + b-lactamase inhibitors	Piperacillin/Tazobactam
2	Extended-spectrum cephalosporins	Ceftriaxone
		Ceftazidime
		Cefoperazone/Sulbactam
		Cefepime
3	Antipseudomonal carbapenems	Imipenem
		Meropenem
4	Aminoglycosides	Amikacin
		Gentamicin
5	Antipseudomonal fluoroquinolones	Ciprofloxacin
		Levofloxacin
6	Tetracycline	Minocycline
7	Folate pathway inhibitors	Trimethoprim/Sulfamethoxazole
8	Polymyxins	Colistin

Table 1. *Acinetobacter spp.* antimicrobial categories and agents used to define MDR, XDR and PDR

2.4 Definition of MDR, XDR and PDR

Many different definitions for multidrug-resistant (MDR), extensively drug-resistant (XDR) and pan drug-resistant (PDR) bacteria are being used in the medical literature to characterize the different patterns of resistance found in healthcare-associated, antimicrobial resistant bacteria. MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, XDR was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories) and PDR was defined as non-susceptibility to all agents in all antimicrobial categories. (Magiorakos et al., 2012)

2.5 Antimicrobial resistance mechanism of *Acinetobacter baumannii*

A. baumannii has several resistance mechanisms, including β-lactamases, aminoglycoside-modifying enzymes, efflux pumps, permeability defects, and modifications of target sites. The accumulation of several resistance mechanisms in *A. baumannii* has gradually decreased the number of antibiotic classes available to treat *A. baumannii* infections in clinical practice (Lee et al., 2017). In the table below a short summary of mechanisms of action of antimicrobial category and their resistance mechanisms are shown.

SL no	Antimicrobial category	Mechanism of Action	Resistance Mechanisms	References
1	Antipseudomonal penicillin + β-lactamase inhibitors	Inhibit bacterial cell wall synthesis by binding to penicillin-binding proteins (PBPs).	Resistance typically involves the production of β-lactamases, including class D oxacillinases (OXA) and class B metallo-β-lactamases (MBLs), which hydrolyze the β-lactam ring of penicillins and β-lactamase inhibitors.	(Kyriakidis et al., 2021)
2	Extended spectrum cephalosporins	Inhibit bacterial cell wall synthesis by binding to PBPs	Resistance involves the production of β-lactamases, alterations in PBPs, and upregulation of efflux pumps.	(Kyriakidis et al., 2021)
3	Antipseudomonal carbapenems	Inhibit bacterial cell wall synthesis by binding to PBPs.	Resistance is primarily mediated by the production of carbapenemases, including OXA carbapenemases, MBLs, and class D carbapenem-hydrolyzing oxacillinases.	(Kyriakidis et al., 2021)
4	Aminoglycosides	Inhibit bacterial protein synthesis by binding to the bacterial ribosome.	Resistance mechanisms include enzymatic modification of aminoglycosides, decreased permeability, and upregulation of efflux pumps.	(Kyriakidis et al., 2021)

5	Antipseudomonal fluoroquinolones	Inhibit bacterial DNA gyrase and topoisomerase IV, interfering with DNA replication.	Resistance arises due to mutations in DNA gyrase and topoisomerase IV genes, reducing drug binding, and upregulation of efflux pumps.	(Kyria kidis et al., 2021)
6	Tetracycline	Inhibit bacterial protein synthesis by binding to the bacterial ribosome.	Resistance involves the acquisition of tet genes encoding efflux pumps or ribosomal protection proteins.	(Kyria kidis et al., 2021)
7	Folate pathway inhibitors	Inhibit bacterial folate synthesis by targeting dihydropteroate synthase (DHPS) and dihydrofolate reductase (DHFR).	Resistance occurs through alterations in DHPS and DHFR, reducing drug affinity, and upregulation of efflux pumps.	(Lee et al., 2017)
8	Polymyxins	Disrupt bacterial cell membranes by binding to lipopolysaccharides (LPS).	Resistance mechanisms involve modifications of LPS structures, alterations in membrane charge, and thickness, limiting drug access to bacterial membranes.	(Kyria kidis et al., 2021)

Table 2. Summary of mechanism of action of antimicrobial category and their resistance mechanisms

CHAPTER 3

Methodologies

3.1 Environmental Sample collection

The Child Health and Mortality Prevention Surveillance (CHAMPS) Team conducted a small-scale laboratory investigation with the aim to understand the potential presence of pathogenic bacteria in the neonatal ward environment of Dr. Zahed Memorial Child Hospital (ZMCH) situated in the Faridpur district. A total of 100 Swab samples were collected from various designated sites within the Special Care and Neonatal Unit (SCANU) and neonatal ward of the hospital for the evaluation of environmental bacterial contamination. A detailed list containing all the collection sites are given below –

Sample ID	Sample Collection Site	Sample ID	Sample Collection Site
1	Operation Theater Table Mat (Operation Theater- 1)	51	Common Basin Neonatal Ward (6th Floor Neonatal Ward)
2	Oxygen Mask (Operation Theater- 1)	52	Photherapy Machine (6th Floor Neonatal Ward)
3	Baby sucker tube (Operation Theater- 1)	53	Common Bed (3rd Floor Neonatal Ward)
4	Operation Theater Baby Bed (Operation Theater- 1)	54	Common Oxygen Mask (3rd Floor Neonatal Ward)
5	Operation Theater Baby Tray (Operation Theater- 1)	55	Common Sucker (3rd Floor Neonatal Ward)
6	AC (Operation Theater- 1)	56	Bed (3rd Floor Neonatal Ward)
7	Sink Tap (Operation Theater)	57	Intravenous Pole (3rd Floor Neonatal Ward)
8	Mackintosh (Operation Theater)	58	Phototherapy Bed (3rd Floor Neonatal Ward)
9	Baby sucker tube (Operation Theater- 4)	59	Phototherapy Machine (3rd Floor Neonatal Ward)
10	Operation Theater Light (Operation Theater- 4)	60	Floor (3rd Floor Neonatal Ward)
11	Saline Stand (Operation Theater- 4)	61	Common Floor (3rd Floor Neonatal Ward)

12	Operation Theater Table Mat (Operation Theater- 4)	62	Head Box (3rd Floor Neonatal Ward)
13	AC (Operation Theater- 4)	63	Silicone Resuscitator (3rd Floor Neonatal Ward)
14	Door Handle (Operation Theater- 4)	64	Nebulizer Mask (3rd Floor Neonatal Ward)
15	Door Handle (Operation Theater- 1)	65	Nurse Desk (3rd Floor Neonatal Ward)
16	Operation Theater Table Mat (Operation Theater- 2)	66	Nurse Hand-1 (3rd Floor Neonatal Ward)
17	Weight Scale (Operation Theater- 2)	67	Nurse Hand-2 (3rd Floor Neonatal Ward)
18	AC (Operation Theater- 2)	68	Attendant's Hand-1 (3rd Floor Neonatal Ward)
19	Door Handle (Operation Theater- 2)	69	Wooden Desk (3rd Floor Neonatal Ward)
20	Floor (Operation Theater- 2)	70	Weight Machine (Incubator)
21	Floor (Operation Theater- 4)	71	Head Box with Tube (Incubator)
22	Floor (Operation Theater- 1)	72	Baby Sucker Tube (Incubator)
23	Common Floor (Operation Theater)	73	Oxygen Tube (Incubator)
24	Door Handle (Operation Theater)	74	Bed-1 (Incubator)
25	Operation Theater Table Mat (Operation Theater)	75	Bed-2 (Incubator)
26	AC (Operation Theater)	76	Floor (Incubator)
27	Common Sucker (Operation Theater)	77	AC (Incubator)
28	Baby Sucker (Operation Theater)	78	Tap and Basin Surface (Incubator)
29	Floor (Operation Theater)	79	Desk Nurse
30	Oxygen Tube (Operation Theater)	80	Common Door
31	Common Bed (6th Floor Neonatal Ward)	81	MITS Table
32	Weight Scale (6th Floor Neonatal Ward)	82	MITS Room Fridge
33	Nebulizer Mask (6th Floor Neonatal Ward)	83	MITS Room Floor
34	Oxygen Cylinder Pipe (6th Floor Neonatal Ward)	84	MITS Room Sink
35	Bed (R-605)(6th Floor Neonatal Ward)	85	MITS Room AC
36	Bed (R-606)(6th Floor Neonatal Ward)	86	MITS Room Table
37	Saline Stand (R-606)(6th Floor Neonatal Ward)	87	MITS Room Fridge

38	Bed Handle (R-606) (6th Floor Neonatal Ward)	88	MITS Room Floor
39	Floor (R-605) (6th Floor Neonatal Ward)	89	MITS Weight Scale
40	Floor (R-606) (6th Floor Neonatal Ward)	90	MITS Room Sink
41	Nurse's Hand-1 (6th Floor Neonatal Ward)	91	MITS Room AC
42	Nurse's Hand-2 (6th Floor Neonatal Ward)	92	Water (Neonatal Ward 6th Floor Sister Basin)
43	Nurse's Hand-3 (6th Floor Neonatal Ward)	93	Water (Neonatal Ward 6th Floor Common Basin)
44	Nurse's Apron-1 (6th Floor Neonatal Ward)	94	Hexisol (Neonatal Ward 6th Floor)
45	Tiles (6th Floor Neonatal Ward)	95	Water (Neonatal Ward 3rd Floor Sister Basin)
46	Attendant's Hand-1 (6th Floor Neonatal Ward)	96	Hexisol (Neonatal Ward 3rd Floor)
47	Attendant's Hand-2 (6th Floor Neonatal Ward)	97	Water (Incubator)
48	Attendant's Cloth (6th Floor Neonatal Ward)	98	Savlon (Incubator)
49	Common Gate Handle (6th Floor Neonatal Ward)	99	Hexisol (4th Floor Operation Theater)
50	Nurse Basin (6th Floor Neonatal Ward)	100	Water (4th floor Operation Theater)

Table 3: List of the sample collection sites

3.2 Sample collection process

The environmental samples were collected by swabbing the given 100 different locations of the hospital with cotton swab sticks. The transport cotton swab sticks were first dampened in PBS or phosphate buffer saline and then held at the sample collection site. The swab tip was rotated slowly throughout the sampling area and samples were collected. Then these swabs were collected and preserved in commercially available Amies Transport Medium and the specimens were transported at 2-8 degree Celsius to the virology laboratory of Infectious Disease Division of icddr,b within a 24-hour time frame.

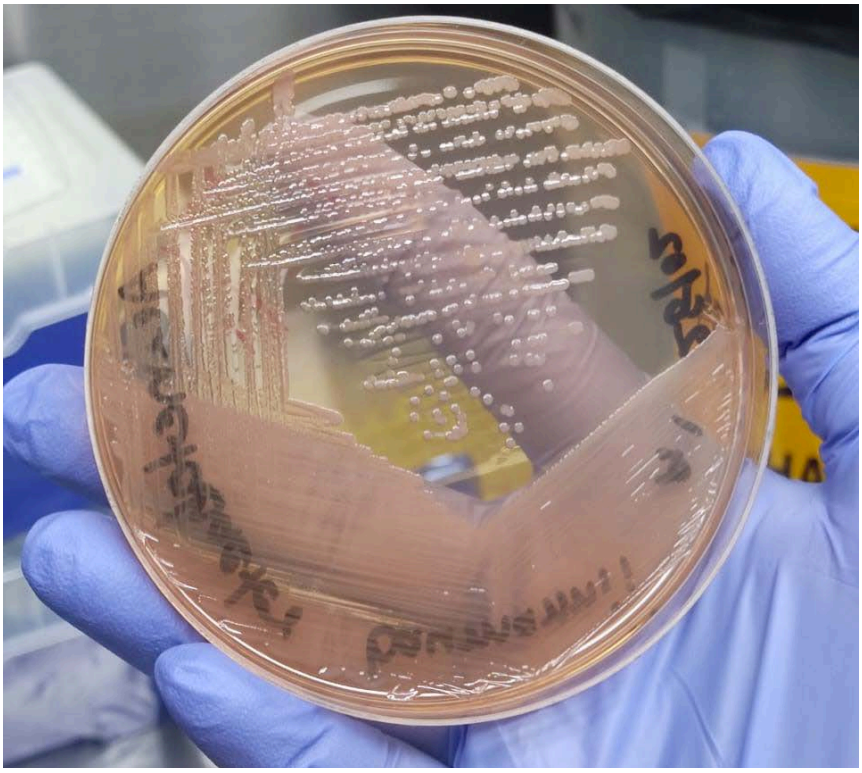

Figure 1. *Acinetobacter baumannii* on MacConkey agar media

3.3 Isolation of organism and preservation

After the arrival, the swab sticks were inoculated on MacConkey agar and blood agar which was then incubated at 37°C for 24 hours. After 24 hours, the plates were observed for microbial growth. Microbiological observation of these samples revealed colonies like *Acinetobacter sp.* (Non-lactose fermenting, small, circular, elevated colonies), *Pseudomonas sp.*(Small, circular, colorless, dry and alligator scale like colonies), *Klebsiella sp.*(large, circular, mucoid colonies) etc. To obtain isolated colonies of these organisms, quadrant streaking technique was done using a sterile microbiological loop again on MacConkey agar media. The plates were subsequently placed in an incubator at 37°C temperature, for 18 to 24 hours. These colonies were subcultured

on Mueller-Hinton agar (MHA) media and isolates were preserved on STGG (skim milk, tryptone, glucose, glycerin) and Skim milk media for future investigation.

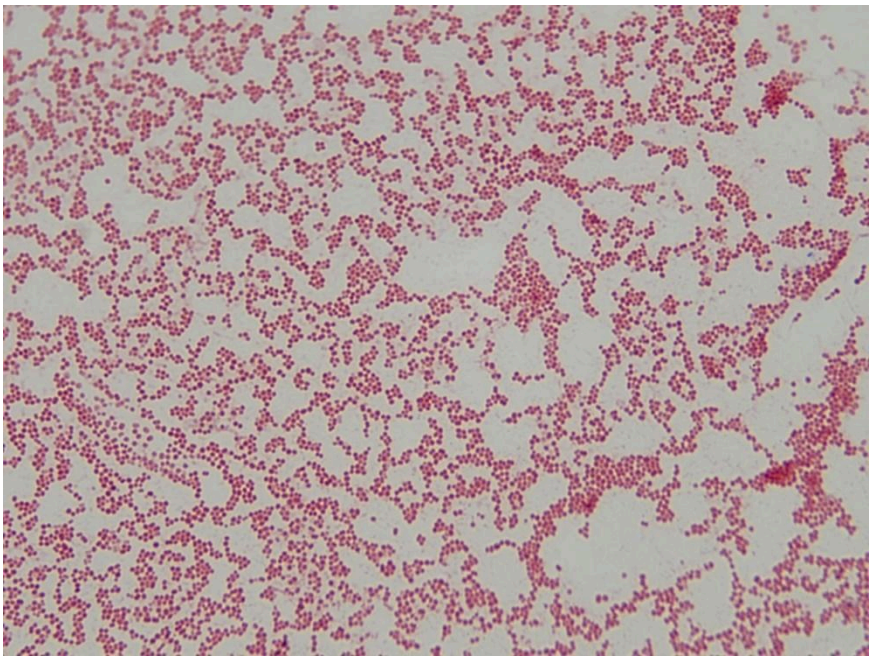

Figure 2. Microscopic observation of *Acinetobacter baumannii*

3.4 Identification and Antimicrobial susceptibility testing

Isolates that gave *Acinetobacter-like* colonies were subcultured on MacConkey agar media. For primary identification, colony morphology and characteristics of the bacterial isolates on MacConkey agar media were observed and gram stain was done. For confirmatory identification, automated bacterial identification system (Vitek-2 system) was used according to the manufacturer guidelines. Vitek-2 system is an automated microbiology system utilizing growth base technology to accurately identify microorganisms through biochemical testing, fluorescence, and turbidity measurement. Along with identification, to assess the microbial susceptibility against antibiotics, it uses the broth microdilution method for generating minimum inhibitory concentration (MIC) against specific antibiotics and compares the results against the

extensive database of Clinical and Laboratory Standard Institute (CLSI). (Pincus, n.d.) According to the results of the Vitek 2 system, antibiotic resistance patterns of these organisms were analyzed based on their antimicrobial categories.

CHAPTER 4

Result and Observation

4.1 Microbiological observation of samples of 100 collection site

During the microbiological observation of blood agar plate and MacConkey agar plate of these 100 samples after collection, a total of 62% (62 of100) *Acinetobacter sp.* Like colonies were found among other bacterial colonies such as *Klebsiella* like colonies (5%), *Pseudomonas* like colonies (1%) and *non-Acinetobacter* like colonies (6%) which reflects that these bacteria were present at these sample collection sites. No growth was seen on 23% of the plates of both media indicating the absence of bacteria at the collection site of these samples.

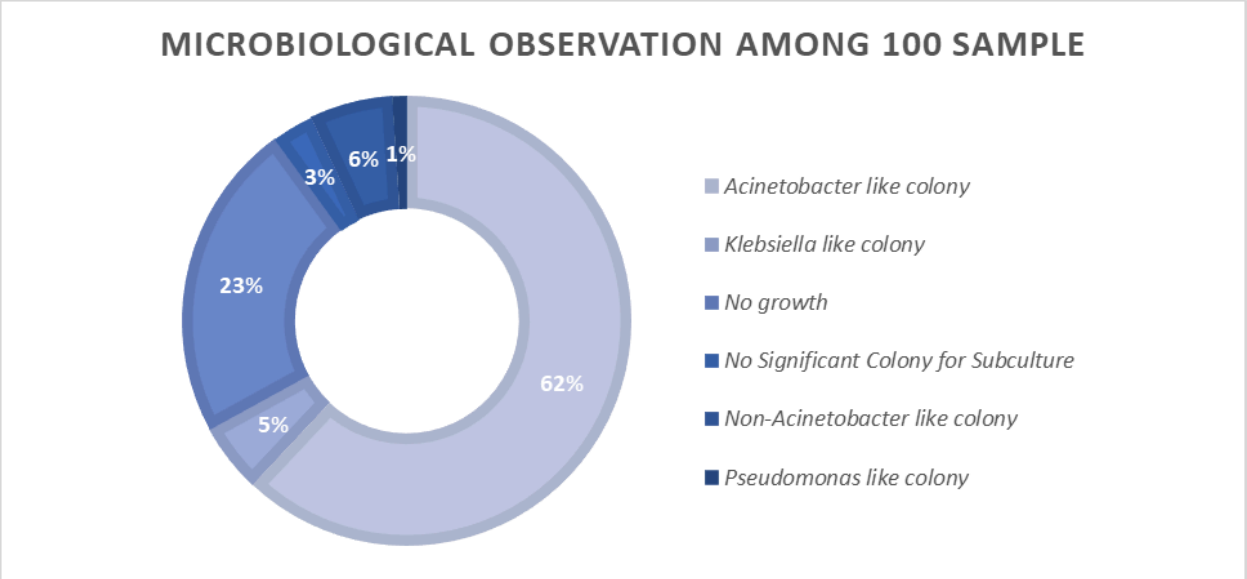

Figure 3. Microbiological Observation of 100 samples

4.2 Confirmatory identification of the isolates

The isolates that gave *Acinetobacter sp.* microbiological properties such as medium, circular, elevated and light pink colonies in MacConkey agar media and gram-negative coccobacillus during the gram staining process were selected for confirmatory identification through the Vitek-2 system. According to the Vitek-2 result, a total of 39 isolates were determined as *Acinetobacter spp.* and the rest of them were *non-Acinetobacter* (NA). Among these 39 isolates 31 of them were identified as *Acinetobacter baumannii* and the rest were *Acinetobacter lwoffii, Acinetobacter junii, Acinetobacter ursingii* etc.

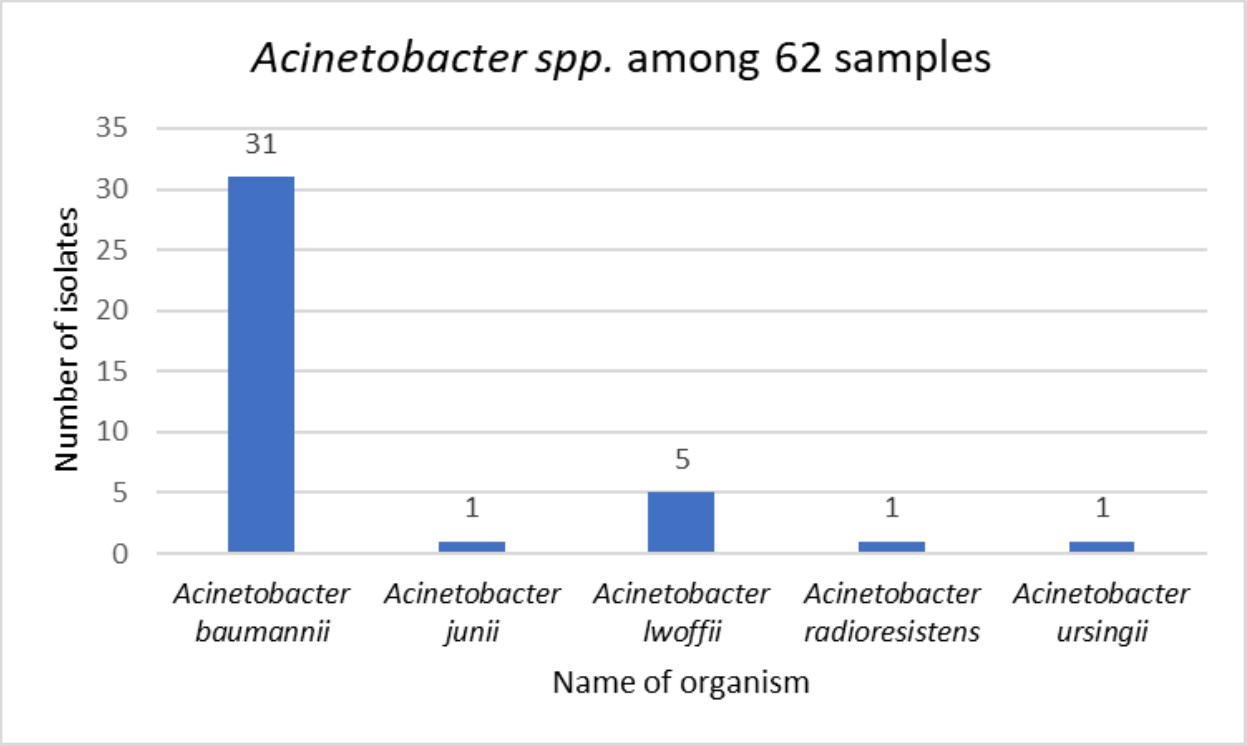

Figure 4. *Acinetobacter spp*. among 62 samples

4.3 Result of Antimicrobial Susceptibility Testing

Acinetobacter sp. is commonly found in the environment which also increases the chances of these bacteria being highly resistant because of their ability to adapt in adverse conditions. Among these 39 isolates *Acinetobacter baumannii* was found from the samples of 31 different collection sites. To determine the resistance pattern of these 31 *Acinetobacter baumannii* against known antibiotics, antimicrobial susceptibility test (AST) profile was done by vitek-2 system according to manufacturer guidelines. The antimicrobial agents include Piperacillin/Tazobactam, Cefepime, Amikacin, Gentamicin, Trimethoprim/Sulfamethoxazole and many more antibiotics of various antimicrobial category which were used to determine the AST pattern of rest of the total 31 *Acinetobacter baumannii* isolates.

4.3.1 Resistance against Antipseudomonal penicillin and beta-lactamase inhibitors

Out of these 31 *Acinetobacter baumannii* isolates 42% (13 of 31) are resistant and the rest of 58% (18 of 31) isolates are sensitive against this combined antibiotic called Piperacillin/Tazobactam which belongs to the antimicrobial category of antipseudomonal penicillin (Piperacillin) and β-lactamase inhibitors (Tazobactam). The collection sites of these isolates are mentioned in table 4.

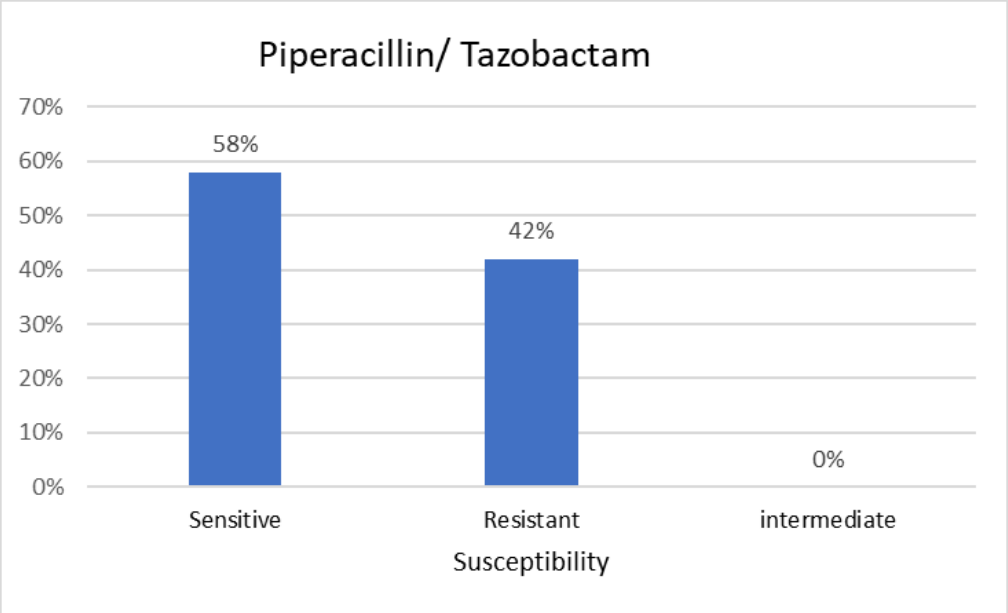

Figure 5. Resistance against Piperacillin/Tazobactam

No. of Sites	Isolate Collection Sites
1	Operation Theater Table Mat (Operation Theater- 1)
2	AC (Operation Theater- 1)
3	Operation Theater Table Mat (Operation Theater)
4	Floor (Operation Theater)
5	Nebulizer Mask (6th Floor Neonatal Ward)
6	Bed (R-605)(6th Floor Neonatal Ward)
7	Bed (R-606)(6th Floor Neonatal Ward)
8	Nurse's Apron-1 (6th Floor Neonatal Ward)
9	Common Gate Handle (6th Floor Neonatal Ward)
10	Weight Machine (Incubator)

11	Baby Sucker Tube (Incubator)
12	Bed-1 (Incubator)
13	Operation Theater Table Mat (Operation Theater- 4)

Table 4: List of collection sites of isolates resistant against antipseudomonal penicillin (Piperacillin) and β-lactamase inhibitors (Tazobactam)

4.3.2 Resistance against extended spectrum cephalosporins

In this study, resistance against third (ceftriaxone, ceftazidime, cefoperazone/sulbactam) and fourth generation (cefepime) of cephalosporins are observed. Out of these 31 *Acinetobacter baumannii* isolates 13%, 23% and 10% isolates are resistant against third generation extended spectrum cephalosporin Ceftriaxone, Ceftazidime and cefoperazone/sulbactam respectively whereas 13% isolates are resistant against fourth generation extended spectrum cephalosporin Cefepime. The collection sites of these resistant isolates are also mentioned in table 5.

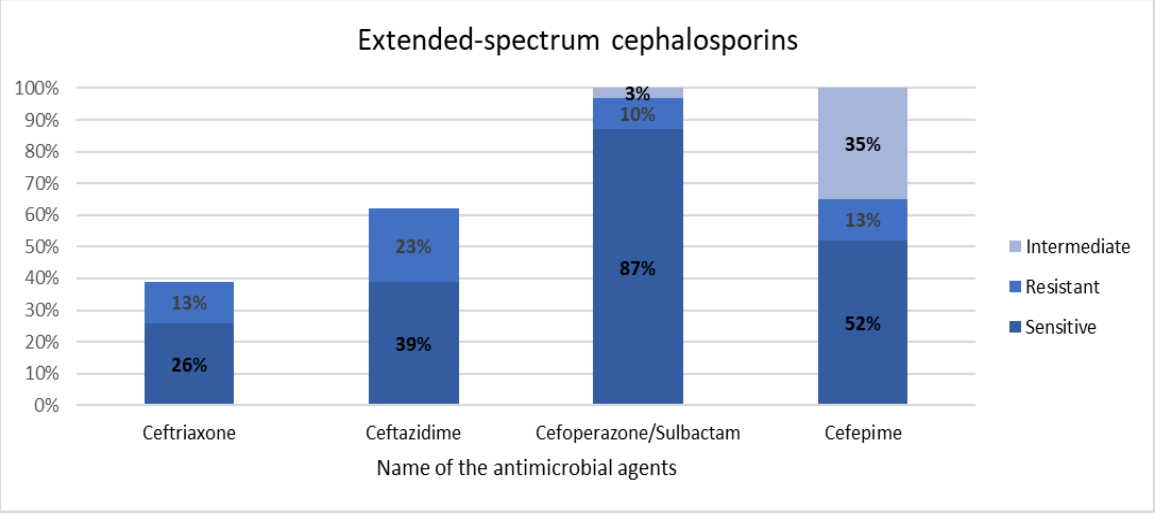

Figure 5. Resistance against extended spectrum cephalosporins

No. of Sites	Isolate Collection Site	Resistant against Antimicrobial agents of Extended-spectrum cephalosporins
1	Operation Theater Table Mat (Operation Theater- 1)	Ceftriaxone
2	AC (Operation Theater- 1)	Ceftriaxone
3	Operation Theater Table Mat (Operation Theater)	Ceftazidime
4	Floor (Operation Theater)	Ceftazidime
5	Nebulizer Mask (6th Floor Neonatal Ward)	Ceftazidime
6	Bed (R-606)(6th Floor Neonatal Ward)	Ceftazidime, Cefoperazone/Sulbactam, Cefepime
7	Nurse's Apron-1 (6th Floor Neonatal Ward)	Ceftazidime
8	Common Gate Handle (6th Floor Neonatal Ward)	Ceftazidime, Cefoperazone/Sulbactam, Cefepime
9	Baby Sucker Tube (Incubator)	Ceftriaxone, Cefepime
10	Bed-1 (Incubator)	Ceftriaxone, Cefoperazone/Sulbactam, Cefepime
11	Operation Theater Table Mat (Operation Theater- 4)	Ceftazidime

Table 5: List of collection sites of isolates resistant against Extended spectrum cephalosporins

4.3.3 Resistance against antipseudomonal carbapenem

Imipenem and Meropenem are potent antibiotic agents of the Antipseudomonal category. But in this study, among these 31 *Acinetobacter baumannii* isolates 13% (4 of 31) isolates are resistant to both of these agents and the rest 87% (27 of 31) are sensitive. The collection sites of these resistant isolates are mentioned in table 6.

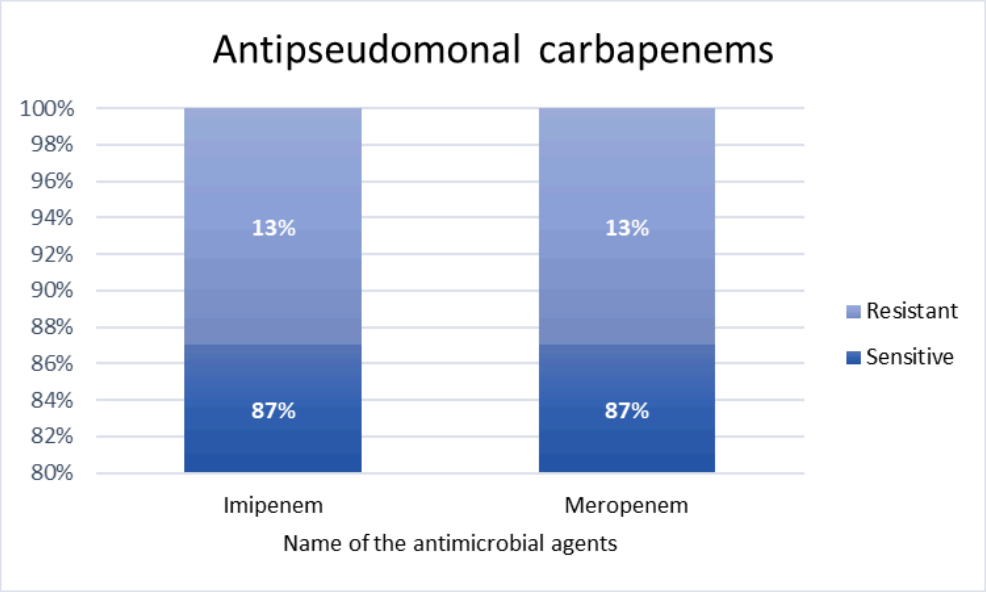

Figure 7. Resistance against antipseudomonal carbapenems

No. of Sites	Isolate Collection Sites
1	Bed (R-606)(6th Floor Neonatal Ward)
2	Common Gate Handle (6th Floor Neonatal Ward)
3	Baby Sucker Tube (Incubator)
4	Bed-1 (Incubator)

Table 6: List of collection sites of isolates resistant against Carbapenems

4.3.4 Resistance against Aminoglycosides

Resistance against two effective antibiotic Amikacin and Gentamicin of the aminoglycoside category is present among these isolates of *Acinetobacter baumannii.* 10% (3 of 31) of the isolates are resistant against Amikacin and 35% (11 of 31) of the isolates are resistant against

gentamicin. Gentamicin resistant isolates are much higher than Amikacin resistant isolates. The collection sites of these isolates are also mentioned in table 7.

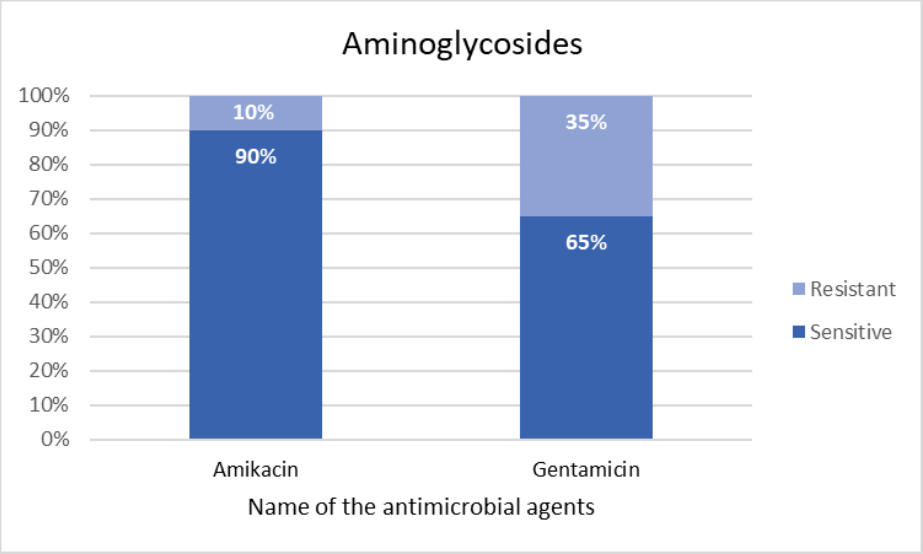

Figure 8. Resistance against aminoglycosides

No. of Sites	**Isolate Collection Site**	**Resistant against Antimicrobial agents of Aminoglycosides**
1	Operation Theater Table Mat (Operation Theater- 1)	Gentamicin
2	AC (Operation Theater- 1)	Gentamicin
3	Operation Theater Table Mat (Operation Theater)	Gentamicin
4	Floor (Operation Theater)	Gentamicin
5	Nebulizer Mask (6th Floor Neonatal Ward)	Gentamicin
6	Bed (R-606)(6th Floor Neonatal Ward)	Gentamicin, Amikacin
7	Nurse's Apron-1 (6th Floor Neonatal Ward)	Gentamicin

8	Common Gate Handle (6th Floor Neonatal Ward)	Gentamicin, Amikacin
9	Baby Sucker Tube (Incubator)	Gentamicin
10	Bed-1 (Incubator)	Gentamicin, Amikacin
11	Operation Theater Table Mat (Operation Theater- 4)	Gentamicin

Table 7: List of collection sites of isolates resistant against Aminoglycosides

4.3.5 Resistance against Antipseudomonal fluoroquinolones

In this study, out of 31 *Acinetobacter baumannii* isolates 32% (10 of 31) are ciprofloxacin resistant whereas 13% (4 of 31) are levofloxacin resistant. Moreover, 10% (3 of 31) of the isolates are levofloxacin intermediate. Ciprofloxacin and Levofloxacin are two broad spectrum antibiotic agents belonging to the antipseudomonal fluoroquinolone category. The collection sites of these isolates are mentioned in table 8.

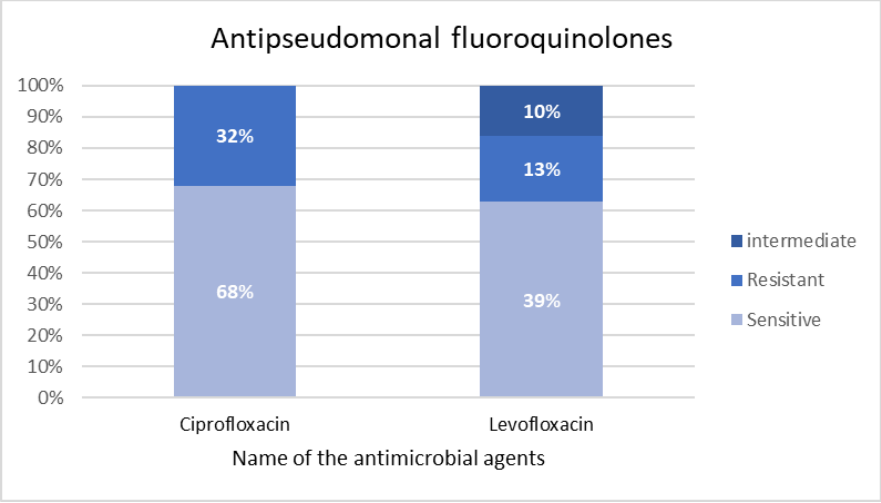

Figure 9. Resistance against antipseudomonal fluoroquinolones

No. of Sites	Isolate Collection Site	Resistant against Antimicrobial agents of Antipseudomonal fluoroquinolones
1	Operation Theater Table Mat (Operation Theater-1)	Ciprofloxacin
2	AC (Operation Theater- 1)	Ciprofloxacin
3	Operation Theater Table Mat (Operation Theater)	Ciprofloxacin, Levofloxacin
4	Floor (Operation Theater)	Ciprofloxacin, Levofloxacin
5	Nebulizer Mask (6th Floor Neonatal Ward)	Ciprofloxacin, Levofloxacin
6	Bed (R-606)(6th Floor Neonatal Ward)	Ciprofloxacin, Levofloxacin
7	Nurse's Apron-1 (6th Floor Neonatal Ward)	Ciprofloxacin
8	Common Gate Handle (6th Floor Neonatal Ward)	Ciprofloxacin
9	Bed-1 (Incubator)	Ciprofloxacin
10	Operation Theater Table Mat (Operation Theater- 4)	Ciprofloxacin

Table 8: List of collection sites of isolates resistant against antipseudomonal fluoroquinolones

4.3.6 Resistance against Tetracycline

Minocycline antimicrobial agent belongs to the broad-spectrum tetracycline antibiotic class where the antibiotic binds to the 16S rRNA of the 30S ribosome to inhibit protein synthesis of the bacteria (Kanel, 2024). Among these 31 isolates only 3% (1 of 31) *Acinetobacter baumannii* isolate is resistant against minocycline. This single isolate was collected from the common gate handle of the sixth floor neonatal ward.

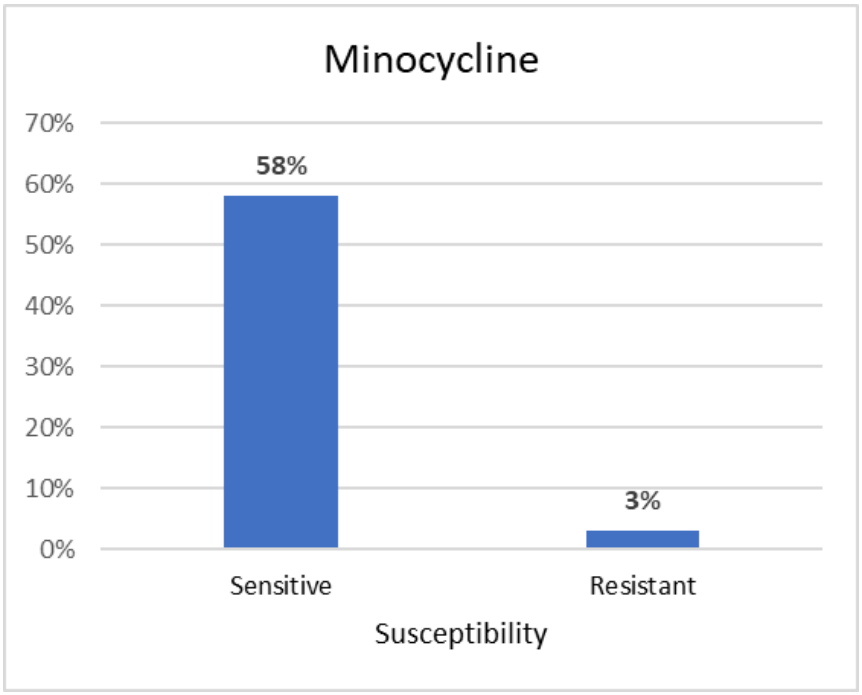

Figure 6. Resistance against tetracycline

4.3.7 Resistance against Folate pathway inhibitors

Trimethoprim/Sulfamethoxazole blocks the synthesis of folate which results in bactericidal activity. From the 31 isolates, 35% (11 of 31) *Acinetobacter baumannii* is resistant to Trimethoprim/Sulfamethoxazole. The collection sites of these isolates are mentioned in table 9.

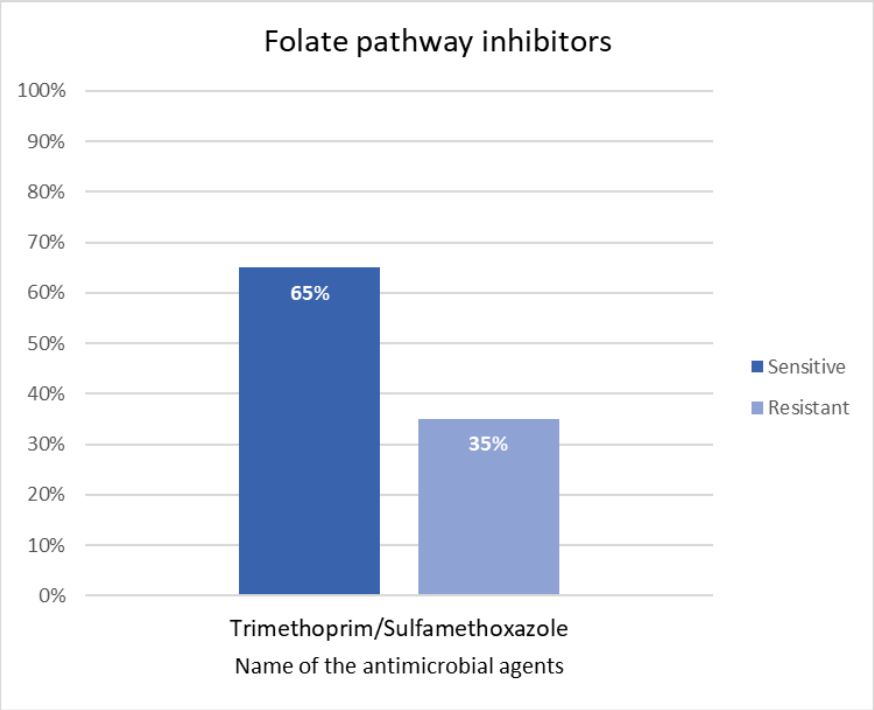

Figure 11. Resistance against folate pathway inhibitors

No. of Sites	Isolate Collection Site
1	Operation Theater Table Mat (Operation Theater- 1)
2	AC (Operation Theater- 1)
3	Operation Theater Table Mat (Operation Theater)
4	Floor (Operation Theater)
5	Nebulizer Mask (6th Floor Neonatal Ward)
6	Bed (R-606)(6th Floor Neonatal Ward)
7	Nurse's Apron-1 (6th Floor Neonatal Ward)
8	Tiles (6th Floor Neonatal Ward)
9	Common Gate Handle (6th Floor Neonatal Ward)

10	Bed-1 (Incubator)
11	Operation Theater Table Mat (Operation Theater- 4)

Table 9: List of collection sites of isolates resistant against folate pathway inhibitors

4.4 Percentage of Multidrug resistant *Acinetobacter baumannii*

In this study, a total of 13 microbial agents or antibiotics were used across seven antimicrobial categories to determine the antimicrobial susceptibility pattern of these 31 isolates of *Acinetobacter baumannii.* Summary of the AST pattern of these isolates are given below-

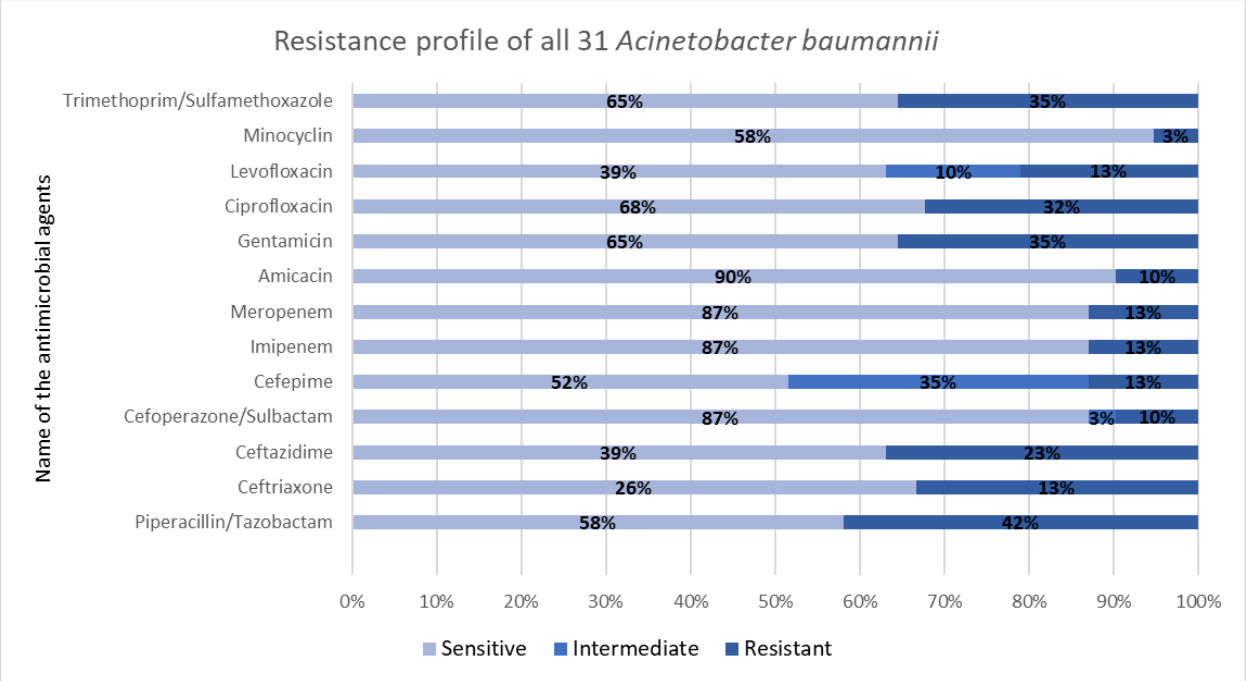

Figure 12. Summary of Number of *A. baumannii* resistant against antimicrobial agents

Figure 7.

The above chart summarizes the susceptibility profile of all 31 *Acinetobacter baumannii* strains, whereas the chart below summarizes the susceptibility profile of the identified MDR and XDR strains.

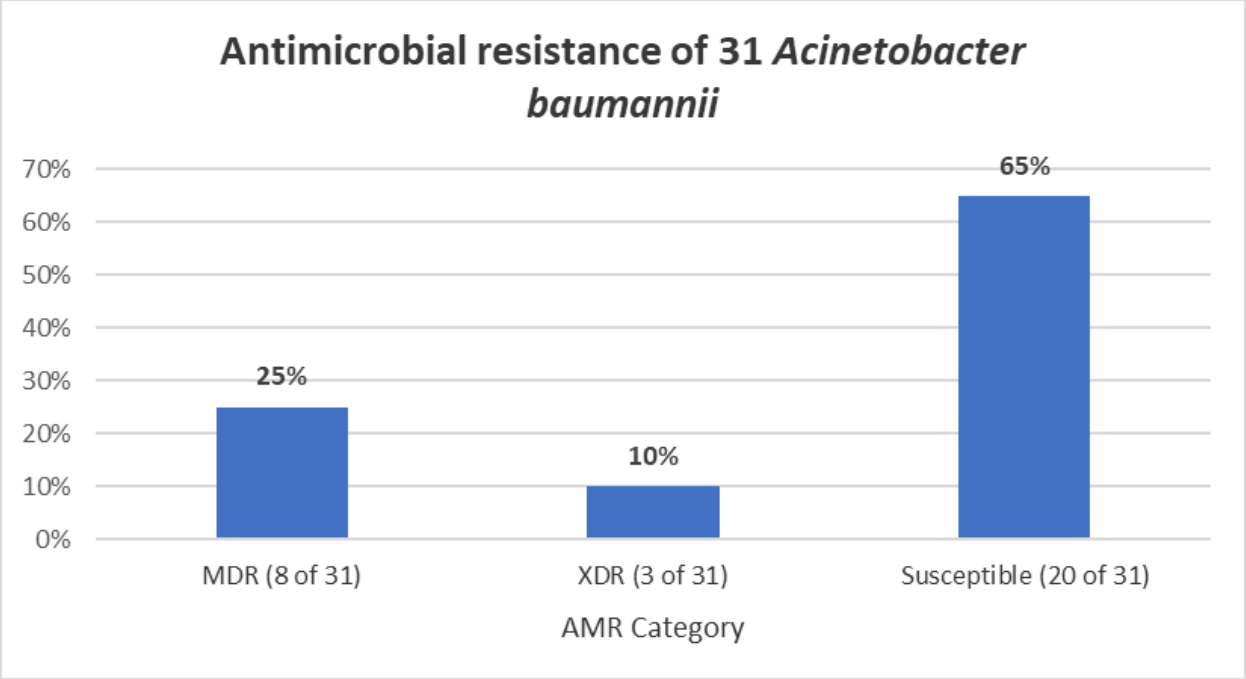

Figure 8. Antimicrobial Susceptibility Profile of 31 isolates

After analyzing the AST pattern of these 31 isolates, 26% (8 of 31) of the total *Acinetobacter baumannii* were found multidrug resistant or MDR, 10% (3 of 31) were extremely drug resistant or XDR and rest of the 65% (20 of 31) isolates were susceptible to most of the antibiotics according to the available data. The antimicrobial categories were determined by the standard of European Society of Clinical Microbiology and Infectious Diseases and CSLI (Clinical and Laboratory Standards Institute) guidelines (Magiorakos et al., 2012).

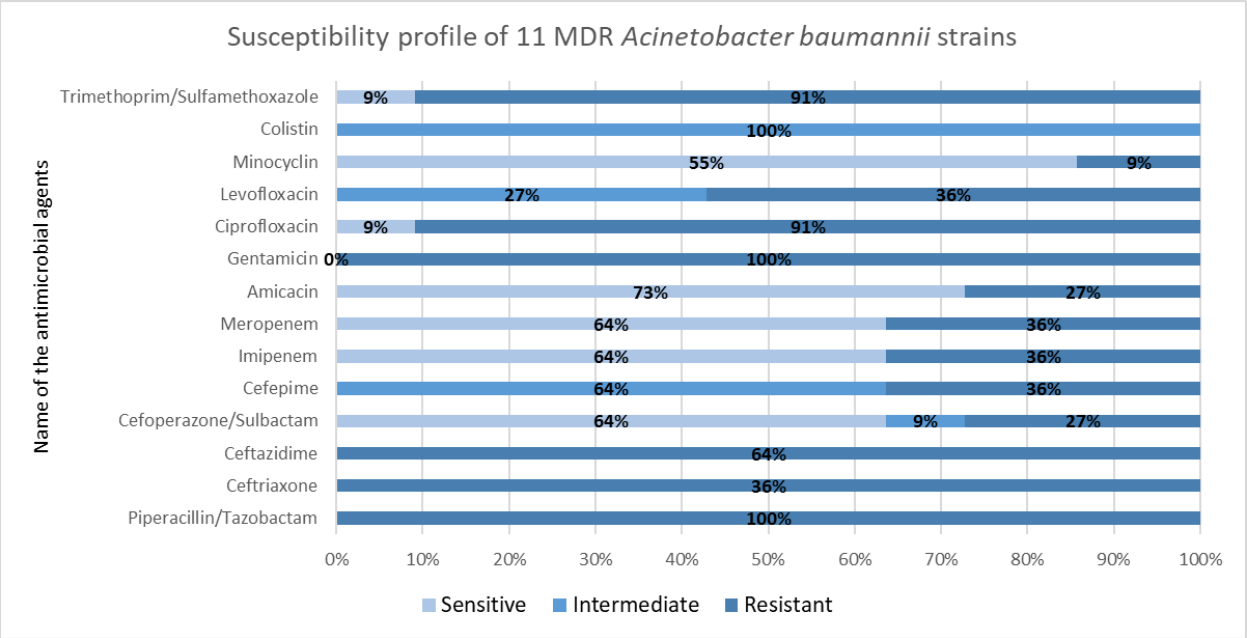

Figure 9. Antimicrobial Susceptibility Profile of MDR and XDR isolates

The above chart summarizes the susceptibility profile of identified MDR and XDR strains. These strains of *Acinetobacter baumannii* exhibited resistance to multiple antibiotics across various antimicrobial classes. All of these strains were resistant to Piperacillin/Tazobactam and Gentamicin, while 91% (10 out of 11) showed resistance to Ciprofloxacin and Trimethoprim/Sulfamethoxazole. Additionally, 36% (4 out of 11) were resistant to Ceftriaxone, Cefepime, Imipenem, Meropenem and Levofloxacin whereas 27% (3 out of 11) were resistant to Amikacin and Cefoperazone/Sulbactam.Moreover, 64% (7 of 11) were resistant against Ceftazidime and only 9% (1 of 11) were resistant against Minocycline. All of these strains were sensitive against Colistin.

The collection sites from which the MDR and XDR strain of *Acinetobacter baumannii* are shown in the table below-

SL no.	**Swab Collection Site**	**Antimicrobial category**
1	Operation Theater Table Mat (Operation Theater- 1)	MDR
2	AC (Operation Theater- 1)	MDR
3	Operation Theater Table Mat (Operation Theater)	MDR
4	Floor (Operation Theater)	MDR
5	Nebulizer Mask (6th Floor Neonatal Ward)	MDR
6	Bed (R-606) (6th Floor Neonatal Ward)	XDR
7	Nurse's Apron-1 (6th Floor Neonatal Ward)	MDR
8	Common Gate Handle (6th Floor Neonatal Ward)	XDR
9	Baby Sucker Tube (Incubator)	MDR
10	Bed-1 (Incubator)	XDR
11	Operation Theater Table Mat (Operation Theater- 4)	MDR

Table 10: List of the collection site of MDR and XDR *Acinetobacter baumannii*

CHAPTER 5

Discussion

In this study, out of 100 different collection sites of a neonatal ward environment of a rural hospital, 31 isolates of *Acinetobacter baumannii* were identified and their antimicrobial susceptibility pattern shows that 26% (8 of 31) of these strains are multi-drug resistant or MDR bacteria and 10% (3 of 31) were extremely drug resistant or XDR bacteria. In another study at the clinical microbiology laboratory of Dhaka Medical College and Hospital found various sequence types of MDR *A. baumannii* strains from clinical and environmental samples, posing a serious threat to patient health but no XDR strain was identified (Farzana et al., 2022).

Moreover, in a separate study by, Shamsizadeh reported that, among the 42 samples taken from hospital environment surfaces, 17% (7/42) of them were identified as *A. baumannii* isolates in the ICU. This study also emphasized the significance of *A. baumannii* isolates originating from mechanical ventilators, as they have the potential to serve as airborne pollutants that can lead to ventilator related pneumonia. (Shamsizadeh et al., 2017). But in our study, MDR isolates were identified from the operation theater table mat, floor and AC of the operation theater, common gate handle of the neonatal ward and various other places which allows the smooth transmission of these MDR strains and increases the risk of healthcare- associated infections and patient morbidity. According to Zahornacký et al. (2022), commonly used medical equipment and frequently touched surfaces in healthcare settings play a critical role in the cross-transmission of antimicrobial-resistant (AMR) bacteria and healthcare- associated infections (HAIs).

In our study, the antimicrobial susceptibility pattern also shows the presence of 13% (4 of 31) carbapenem resistant *Acinetobacter baumannii* (CRAB) isolate, which is of great concern since according to CDC, the threat level of CRAB is urgent and requires immediate action (Carbapenem-resistant Acinetobacter | A.R. & Patient Safety Portal, 2021). And due to its high level of concern, especially their development against the last resort antibiotics like carbapenem and colistin, *Acinetobacter baumannii* has also been classified as a red alert pathogen (Custovic et al., 2014).

Furthermore, our study also revealed that all these MDR and XDR strains of *Acinetobacter baumannii* (n=11) from the environment of the neonatal ward were resistant against multiple antibiotics of different antimicrobial categories such as Piperacillin/Tazobactam, Gentamicin, Ciprofloxacin and many more. All of them were resistant against Piperacillin/Tazobactam and Gentamicin whereas 91% (10 of 11) of these strains were resistant against Ciprofloxacin and Trimethoprim/Sulfamethoxazole. 36% (4 of 11) of these strains were resistant against Imipenem, Meropenem and 27%(3 of 11) against Amikacin. But in previously mentioned study on MDR *Acinetobacter baumannii* conducted at Dhaka Medical College revealed all environmental MDR *A. baumannii* (n=10) were resistant to imipenem, meropenem, gentamicin, amikacin, and ciprofloxacin. These MDR *A. baumannii* (n=10) were collected from various hospital surfaces like bed rails, switchboards, toilets, sewage drains etc. whereas the environmental samples from our study were isolated only from the neonatal ward (Farzana et al., 2022).

These isolated XDR and MDR *Acinetobacter baumannii* strains should be further investigated through whole genome sequencing (WGS) and genotypic analysis. With proper genotypic analysis, antibiotic resistance genes could be found and further studied to understand the

resistance mechanism of these strains. Moreover, these genotypic analyses could also reveal the presence of biofilm associated genes. Furthermore, WGS will reveal a high-resolution view of epidemiological relationships between isolates, which will help in controlling transmission of healthcare-acquired infections (Mao et al., 2021).

CHAPTER 6

Conclusion

In the perspective of Bangladesh, Infection prevention and control (IPC) in hospitals are inadequately managed or entirely neglected because of overcrowding, understaffing, inadequate environmental cleaning, insufficient hand washing stations, poor ventilation and various reasons (Harun et al., 2022). Primarily due to limited economic and technical resources, this lack of proper IPC results in a high prevalence of ESBL-producing bacteria in patients and hospital environments (Hasan et al., 2015). As such *Acinetobacter baumannii* has become a significant concern due to its spread in hospital environments, posing a serious threat to immunocompromised individuals, particularly neonates, due to their underdeveloped immune systems. The dissemination of these multidrug-resistant (MDR) *A. baumannii* strains could spread antimicrobial resistance, potentially rendering all antibiotics ineffective against infections. Consequently, the hospital faces a substantial amount of risk of an outbreak and an increased likelihood of antimicrobial resistance in nosocomial pathogens. Thus, it is imperative that hospital authorities should implement safety measures to prevent such fatality. Thorough cleaning of hospital surfaces and more frequent hygiene inspections are necessary. Additionally, hospital staff must adhere to strict hygiene and cleanliness protocols to prevent acting as vectors for pathogen transmission. With these precautionary measures, it is hoped that the spread of *A. baumannii* and other dangerous pathogens can be effectively controlled.

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