Characterization of *Acinetobacter baumannii* from Hospital-Adjacent Waterbodies in Dhaka City

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of
Bachelor of Science in Microbiology & Bachelor of Science in Biotechnology

Department of Mathematics and Natural Sciences
Brac University
June 2023

Declaration

It is hereby declared that

1. The thesis submitted is our own original work while completing our undergraduate degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material that has been accepted or submitted, for any other degree or diploma at a university or other institution.
4. We have acknowledged all main sources of help.

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Abstract

Acinetobacter baumannii has become a significant pathogen over the last few decades. It is naturally found in food, soil, and surface water but in clinical settings, it has demonstrated increasing health problems and nosocomial infections with limited antibiotic treatment options. The presence of multidrug-resistant*A. baumannii* in hospital settings and from clinical samples have been well documented but their spread in the natural environment is still poorly understood. In this study, *A. baumannii* were isolated from four waterbodies adjacent (within a 100-200 meter range) to four different hospitals. The samples were collected consecutively for four months to observe the prevalence of the organism. Out of 16 samples 21 positive isolates were confirmed by PCR of the *blaOXA51* gene. Subsequently, susceptibility test was carried out for 13 antibiotics from seven different antibiotic classes. The test reported that all isolates (95%) except for one (LM2) were resistant to Ceftazidime meanwhile, 95% of the isolates were susceptible to Meropenem and Gentamicin except for MMY4 and LA7 respectively. The resistance pattern for other antibiotics was as follows: Ceftriaxone (48%), Piperacillin (33%), Trimethoprim (28%), and Cefepime (28%). Notedly, 33.33% of the isolates were in the intermediate category for Ciprofloxacin and Piperacillin. Moreover, the MAR index of 7 out of the 21 isolates was higher than 0.28. indicating the sources of these isolates are exposed to antibiotics at a higher level. Finally, among the 21 isolates, 8 isolates (38%) were found to be resistant to three or more categories of antibiotics making them multidrug-resistant.

Keywords: Acinetobacterbaumannii; surface water; antibiotic resistance; multidrug resistance

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List of Abbreviations

A.baumanni	*Acinetobacter baumannii*
spp.	Species
µl	Microliter
MDR	Multidrug Resistance
MAR	Multi-Antibiotic Resistance
MAC	MacConkey Agar Media
ICU	Intensive Care Unit
^{O}C	Degree Celsius
%	Percentage
mm	Millimeter
NA	Nutrient Agar
MHA	Muller Hinton Agar
hr	Hour

Chapter 1

1. Introduction

Acinetobacter baumannii is a Gram-negative, rod-shaped opportunistic bacterium responsible for various healthcare-associated infections, including bacteremia, meningitis, pneumonia, urinary tract infections, and wound infections (Lowe et al., 2022).

A. baumannii is considered a major source of nosocomial infections, especially in intensive care units worldwide. The mortality rate of *A. baumannii*-related infection confers to 26% in hospital settings and it goes up to 43% in Intensive care units (ICU). *A. baumannii* has also been found in bloodstream infections in 10-15% of cases (Asif et al., 2018).In fact, bloodstream infections and ventilator-associated pneumonia caused by multidrug-resistant *A. baumannii*fall between the ranges of 47% and 93% with mortality rates of 30% and 70% (Kovacic et al., 2017). Furthermore, it has become an emerging threat to neurosurgeon patients as *A. baumannii* is associated with 4% of all meningitis and shunt-related infections, with a mortality rate of 70%. Although it is regarded as a low-virulence pathogen, if isolated from neonates with low birth weights and elderly patients with malignancy, it can be deemed virulent. At the same time, *A. baumannii* is not a community pathogen but in children and immunocompromised individuals, it can cause community-acquired bronchiolitis and tracheobronchitis (Asif et al., 2018).

A. baumannii has become one of the most difficult pathogens to treat over the past ten years because an increasing number of multidrug-resistant isolates (i.e., not susceptible to three or more classes of drugs) are being reported worldwide(Neonakis et al., 2011). Major global health authorities such as the European Centre for Disease Prevention and Control (ECDC), Infectious Diseases Society of America (IDSA), World Health Organization (WHO), and

Center for Disease Control and Prevention of America (CDC) have declared multi-drug resistant *A. baumannii* a critical threat to global health. The latter has promoted Carbapenem-Resistant *Acinetobacter* from a “serious” threat level to "urgent" in 2019 (Mea et al., 2021).

Although the majority of *Acinetobacter* species have been isolated from various environmental matrices like soil and water, *A. baumannii* is rarely found outside of hospital settings (Fernando et al., 2016). In fact, it is the most frequently isolated species from human clinical specimens as well as hospital environments such as ventilators, moisturizing devices, catheters, and other medical equipment (Falah et al., 2019). The occurrence and susceptibility of these clinical isolates of *A. baumannii* have been extensively studied and well documented but their distribution and proportion of resistance in the aquatic environment is still to be explored (Kittinger et al., 2018).

Recently *A. baumannii* has been increasingly reported from environments associated with human wastes, especially various types of municipal wastewater such as domestic, industrial, and hospital. Among them hospital wastewater is regarded as the major source of clinically significant *A. baumannii*. since numerous researchers have found the presence of carbapenem-resistant*Acinetobacter* spp. (including *A. baumannii*) in hospital wastewater (Hubeny et al., 2022).However, hospital-adjacent municipal waterbodies have been rarely investigated for similar resistant isolates given the fact that these waterbodies are frequently exposed to hospital wastes.

Up until now *A. baumannii* related research in Bangladesh is observed to be limited to only hospital settings and clinical samples. Reports have been made on isolation, molecular characterization, and resistance profiling of *A. baumannii* exclusively from tertiary care hospitals. Most of the studies mainly focused on multidrug resistance *A. baumannii* while a few spread their focal point towards genetic characterization by investigating the distribution

of MDR genes such as *adeB* and *NDM-1* (Farzana et al., 2022; Jahirul et al., n.d.; Mn et al., 2015; Mohammad et al., 2020; Nahar et al., n.d.; Rahman et al., 2022).

Meanwhile, the majority of *A. baumannii* studies are also narrowed down to hospital settings, clinical samples, and hospital environments worldwide. However, the introduction of *A. baumannii*into the hospital environment is still poorly understood. Some studies reported acute community-acquired human infections which suggests a source of the pathogen outside of the hospital. Whereas other studies reported the isolation of multidrug-resistant (MDR) *A. baumannii* strains from hospital wastewater. Apart from these, only a few studies reported detection elsewhere in the environment (Hrenovic et al., 2016).

According to Bangladesh National Portal the capital Dhaka, houses around 193 hospitals as of 2022. Many of these hospitals are located near crucial urban water bodies, exclusively lakes. Depending on the area, some lakes are used for recreational purposes while others are used to commute. In this regard, hospital-adjacent lakes are regularly subjected to both poorly treated hospital waste as well as contact with patients due to a lack of proper management. Hence, these waterbodies may serve as a potential source of clinically significant pathogens.

Therefore, the aim of this study was to characterize *A. baumannii* from hospital adjacent lake surface water with the use of conventional and molecular microbiology methods. Samples were collected over a period of four months to analyze the prevalence of the organism. The confirmed isolates were studied for their antibiotic susceptibility to clinically relevant antibiotics.

1.2 Objective of the study

The specific aims and objectives of the study are as follows:

- To isolate and characterize *Acinetobacter baumannii.* from surface water samples collected from hospital-adjacent waterbodies in Dhaka city.
- To observe the prevalence of *Acinetobacter baumannii*positive samples over the period of four months.
- To study the antibiotic resistance and multi-drug resistance pattern of *Acinetobacter baumannii.* from surface water samples by comparing their MAR index.

CHAPTER 2

MATERIALS AND METHODS:

2.1. Sample site selection

Surface water samples were collected from four waterbodies within a range of 100-200 meters of four different hospitals. The collection was done consecutively for four months starting from February 2023 and ending in May 2023. A total of 16 samples were collected over the four-month study period. The collection procedure was based uponthe requirements of FDA's Bacteriological Analytical Manual and The Standard Methods for the Examination of Water and Wastewater(Andrews et al., n.d.,Standard Methods for the Examination of Water and Wastewater, 1999).

Serial No	Area (lake)	Hospitals Name	Distances
1	Dhanmondi Lake	Bangladesh Medical College Hospital	187.87m
2	Dhanmondi Lake	Japan Bangladesh Friendship Hospital	158.17m
3	Dhanmondi Lake	Medinova Medical Service	47.55m
4	Banani Lake	Life Care Medical Center Dhaka	60.11m

Table 1: Sample Collection: Places, Names, Maps, Distances

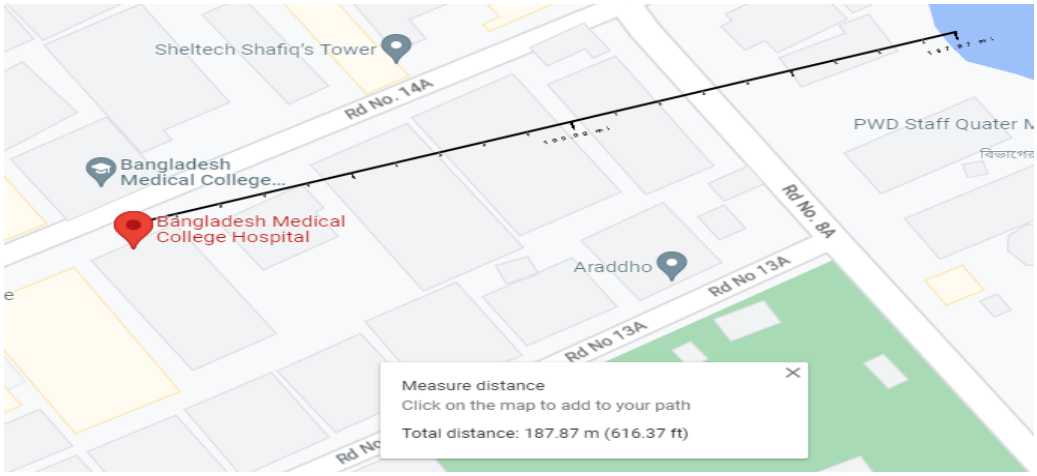


Figure 1: Measurement distance of Bangladesh medical college Hospital to Dhanmondi lake collection site

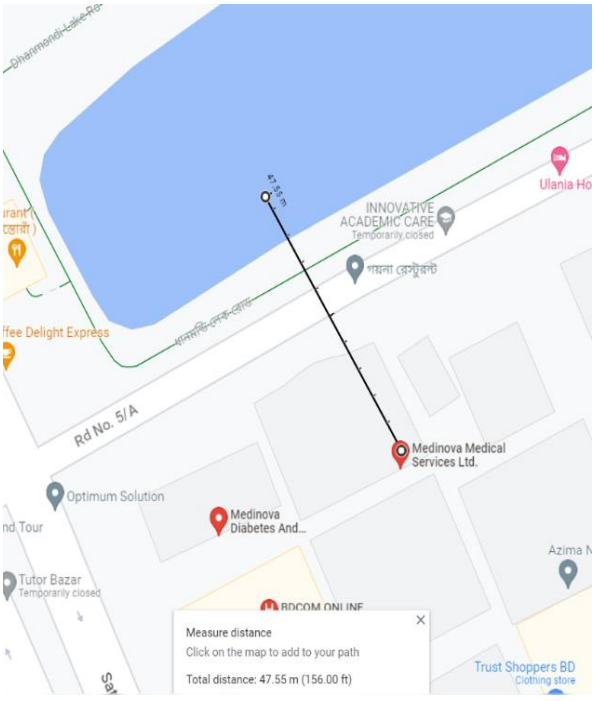


Figure2,3: Measurement distance of Japan Bangladesh Friendship Hospital and Medinova Medicals to Dhanmondi Lake collection site

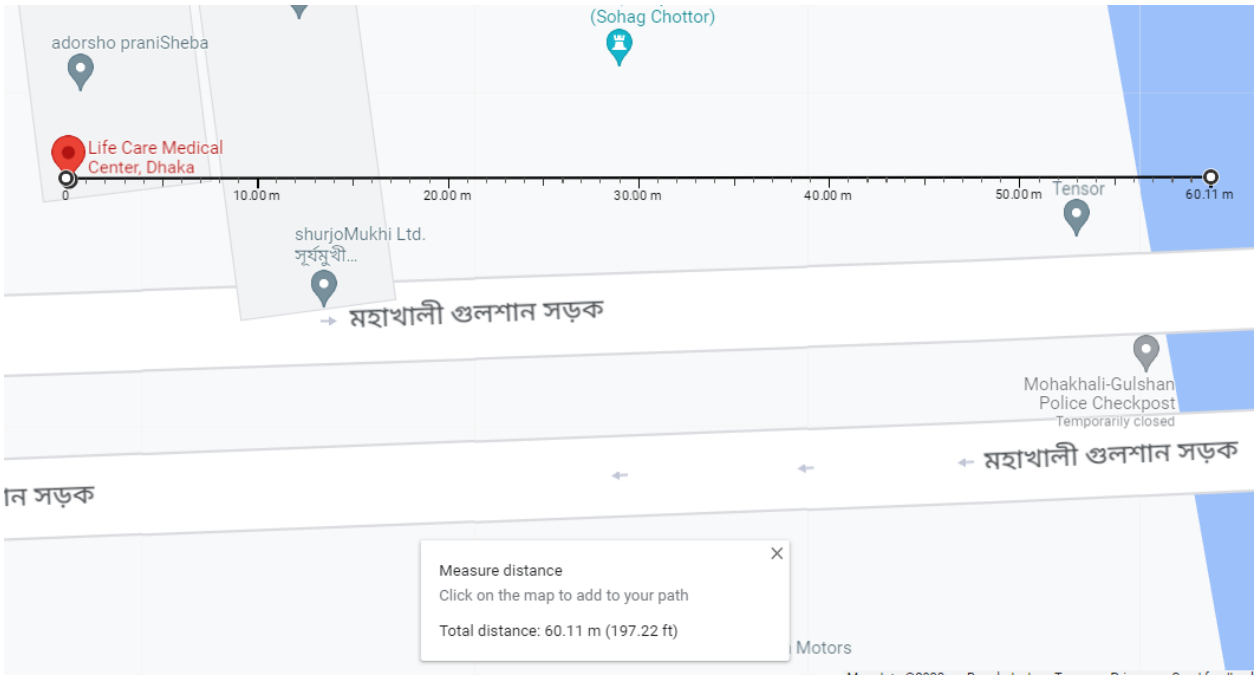


Figure4: Measurement distance of Life Care Medical Center Dhaka to Dhanmondi Lake collection site

2.2. Sample processing

The samples were collected via sterile screw-capped bottles aseptically and taken immediately to the BRAC University laboratory. All samples were diluted up to 10^{-5}-fold using 9ml normal saline and 1ml of water from each of the collected samples.(Jackie Reynolds, 2016) After that, all diluted samples (10^{-1}, 10^{-2},10^{-3},10^{-4} 10^{-5}) including the raw sample were spread on MacConkey media plates and incubated at 44°C for 24hrs. Grey or whitish colonies were selected as described by(Kian et al., 2018), streaked on MAC media again, and incubated at 37°C for 24hrs.

Figure 5,6: Samples in Falcon Tubes, Bacterial Growth on MAC media and Selected Colonies

2.3. DNA extraction

DNA extraction was carried out using boiling method as instructed by(Kian et al., 2018) Initially, suspected colonies were inoculated into NB (nutrient broth) and kept in a shaker incubator for 24 hours. When growth was noticed, 500µl solution was separated and centrifuged at12.5 X 100rpm for 5 minutes. Then the supernatant was discarded and 200µl of TE buffer was resuspended into the pallet. Next, the tubes were boiled at 100° C for 10

minutes. After boiling, the solutions were centrifuged again for 5 minutes. Lastly, the supernatants were transferred to new microcentrifuge tubes and stored at -20 degrees C.

2.4. PCR Assay

For PCR amplification of *blaOXA* gene, reaction was performed in a total volume of 13 µL which included 6.5 µL of Master Mix, 2µL of DNA, reverse and forward primer of 0.45 µL and 3.6 µL NW (nuclease-free water).

The PCR protocol was set up in the thermocycler (Senso-Quest Labcycler, Germany), as follows: 94 °C for 5 min, 30 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min, and then a final extension at 72 °C for 10 min.

Next, 10 µL of PCR products were transferred to a gel containing 2% agarose inTBE buffer (40mM Tris, 20mM boric acid, 1mM EDTA, pH of 8.3) and 0.5 µg/ml ethidium bromide dye. The gels were visualized under ultraviolet illumination using a gel image analysis system. Bands were visualized at the correct expected size for blaOXA-51 and the specimen was considered positive for *A. baumannii* (Falah et al., 2019).

Primer	Primer sequence	Amplicon size	Reference
blaOXA51	F: 5'-TAATGCTTTGATCGGCCTTG-3' R: 5'-TGGATTGCACTTCATCTTGG-3'	353	(Falah et al., 2019)

Table 2: Primer sequence of blaOXA51

2.5. Antibiotic Susceptibility Test

Disk diffusion method was used to check the resistance or sensitivity of bacterial strains towards the given antibiotics (Kirby-Bauer Disk Diffusion Susceptibility Test Protocol, 2009). For inoculation, colonies were picked from an overnight pure culture on Nutrient agar (non-selective medium) with a sterile loop and suspended in sterile saline (0.85% NaCl w/v in water)to the density of a McFarland 0.5 standard (Kittinger et al., 2018). The suspension was lawned on Muller Hinton agar using sterile cotton swabs. The plates were incubated at 37° C for 16–20 h. After incubation, zones of inhibitionweremeasured in millimeters. A total of 13 antibiotic agents (classified into 7 categories) were tested at specific concentrations per disk. Results were evaluated in accordance with CLSI standards(Weinstein & Clinical and Laboratory Standards Institute, n.d.).

Chapter 3

Results

3.1Confirmation of*A. baumannii*

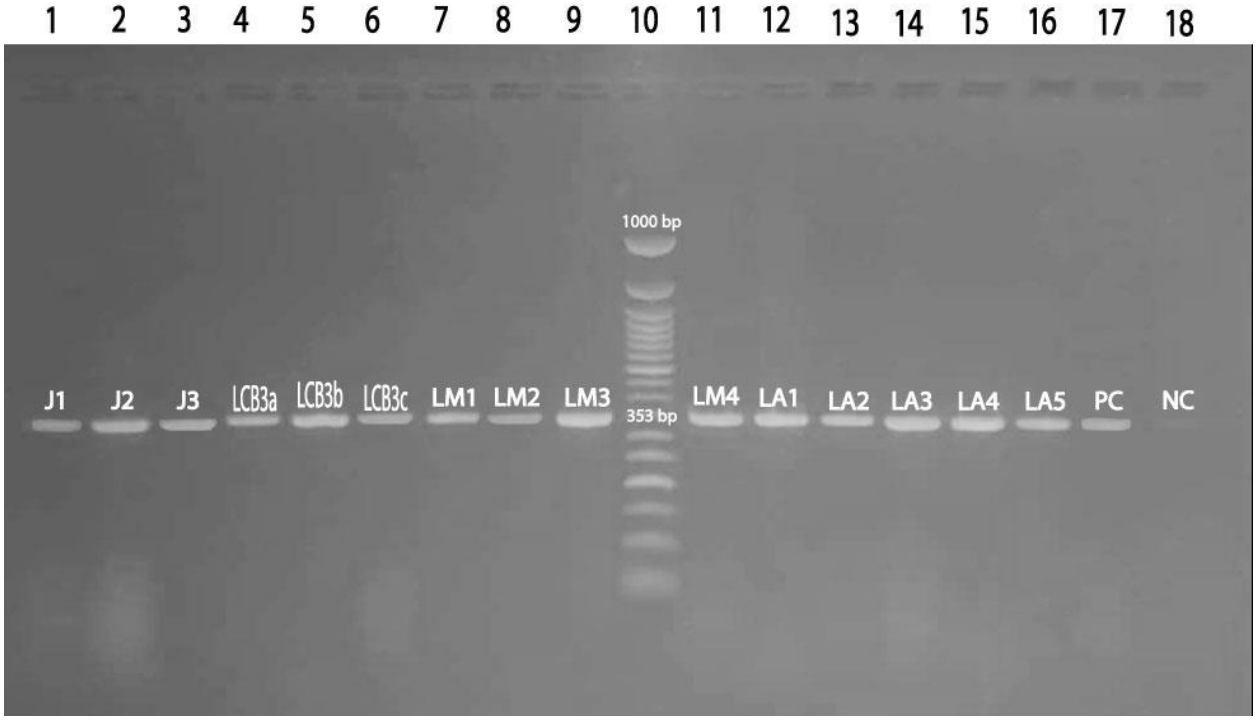


Figure 7: PCR amplification of blaOXA51 gene of A. baumannii. Lanes 1-9, 11-16: environmental isolates (J1, J2, J3, LCB3a, LCB3b, LCB3c, LM1, LM2, LM3, LM4, LA1, LA2, LA3, LA4, LA5) show amplified DNA bands at 353bp; Lane 10: 1000 bp Size marker; Lane 17: Positive control A. baumannii NCTC12156 and Lane 18: Negative control.

3.2 Distribution of *A. baumannii*

A total of 21 positive *A. baumannii* were isolated from 16 samples from four different hospital-adjacent waterbody sites over the period of four months (February to May). Among the four hospital sites, the highest (72%) rate of isolates was from Life Care Hospital –

Banani Lake while the lowest (0%) was from Bangladesh Medical College Hospital – Dhanmondi Lake. Meanwhile, 14% of the total isolates were recovered from both Japan Bangladesh Friendship Hospital – Dhanmondi Lake and Medinova -Dhanmondi Lake.

Hospital/ Lake	**Number of Samples**	**Number of *A. baumannii* positive isolates**
Bangladesh Medical College Hospital – Dhanmondi Lake	4	0
Japan Bangladesh Friendship Hospital – Dhanmondi Lake	4	3
Medinova -Dhanmondi lake	4	3
Life Care Hospital – Banani Lake	4	15
Total	**16**	**21**

Table 3: Acinetobacter baumannii isolates from hospital adjacent lake surface water.

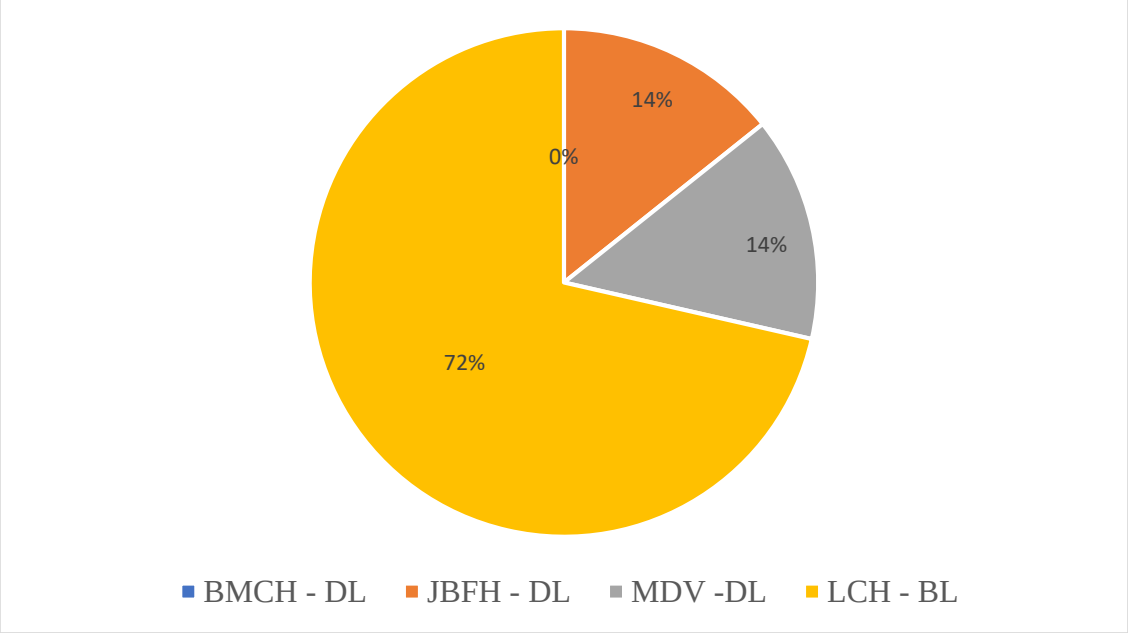


Figure 8: Percentage of positive A. baumannii per hospital

In terms of waterbodies, 28% of isolates were recovered from Dhanmondi Lake whereas 72% of the isolates were recovered from Banani Lake.

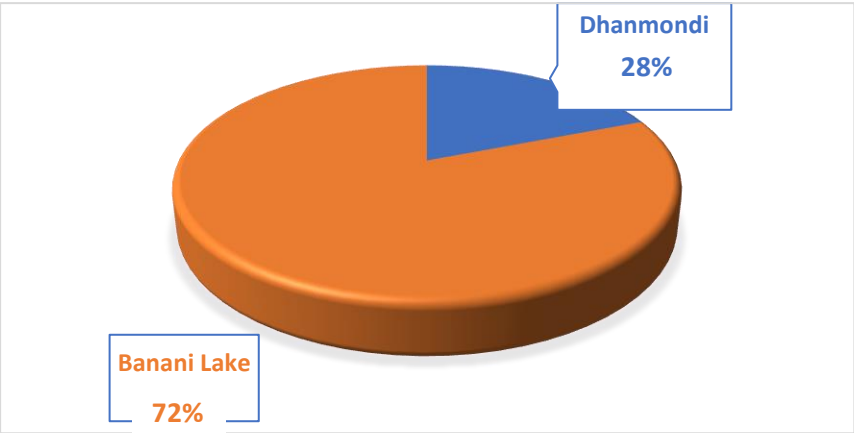


Figure 9: Percentage of positive A. baumannii per lake

To evaluate the prevalence of *A. baumannii* over the four-month study period, the number of positive isolates was counted in each month. The highest number of isolates (7) were recovered in April followed by the second highest (6) in February. Similar prevalence of the organism was seen in March and May with a number of 4 isolates each month.

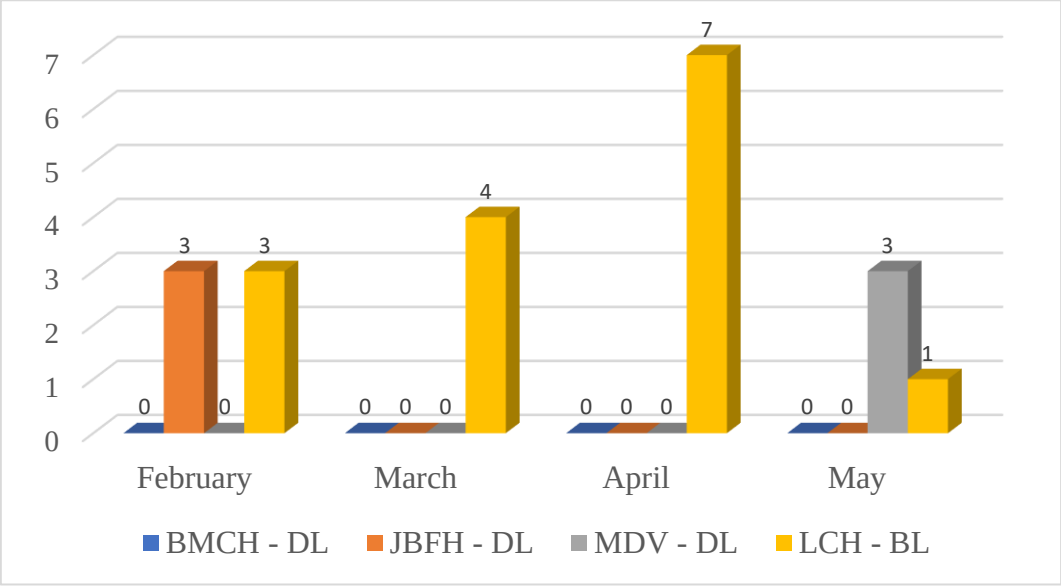


Figure 10: Percentage of positive A. baumannii per month

3.3 Antibiotic resistance analysis

Antibiotic susceptibility test was performed for all 21 isolates using 13 antibiotics belonging to the groups – Beta-Lactam combination agents, Cephalosporins, Carbapenems, Aminoglycosides, Tetracyclines, fluoroquinolones, and Folate pathway antagonists. It was observed from the antibiotic susceptibility test that all isolates (95%) except for one (LM2) were resistant to Ceftazidime. Resistance to other antibiotics was as follows - Ceftriaxone (48%), Piperacillin (33%), Trimethoprim (28%), and Cefepime (28%). Meanwhile, 95% of the isolates were susceptible to Meropenem and Gentamicin except for MMY4 which was resistant to Meropenem, and LA7 which was intermediately resistant to Gentamicin. Additionally, 33.33% of the isolates were in the intermediate category for Ciprofloxacin and Piperacillin. The most antibiotic-resistant isolate was reported to be LCB3c from Life Care Hospital -Banani Lake and MMY3 from Medinova – Dhanmondi Lake. Both the isolates were resistant to 6 of the 13 antibiotics.

All the isolates were divided into three groups after measuring the zone of inhibition (mm): Resistant (R), Intermediate Resistant (I), and Sensitive (S) according to CLSI (2021) guidelines(Weinstein & Clinical and Laboratory Standards Institute, n.d.).

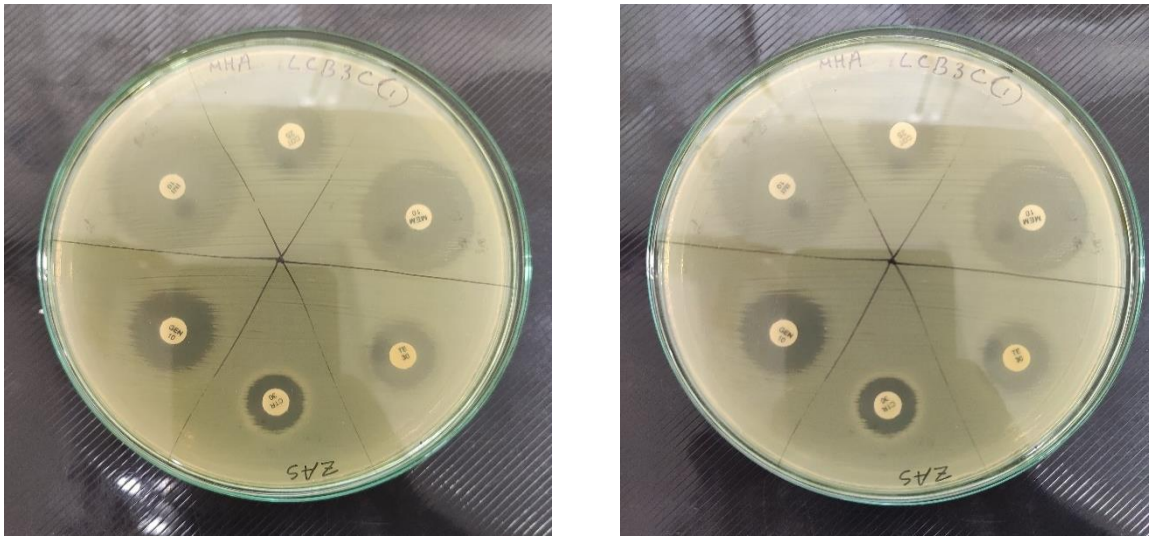
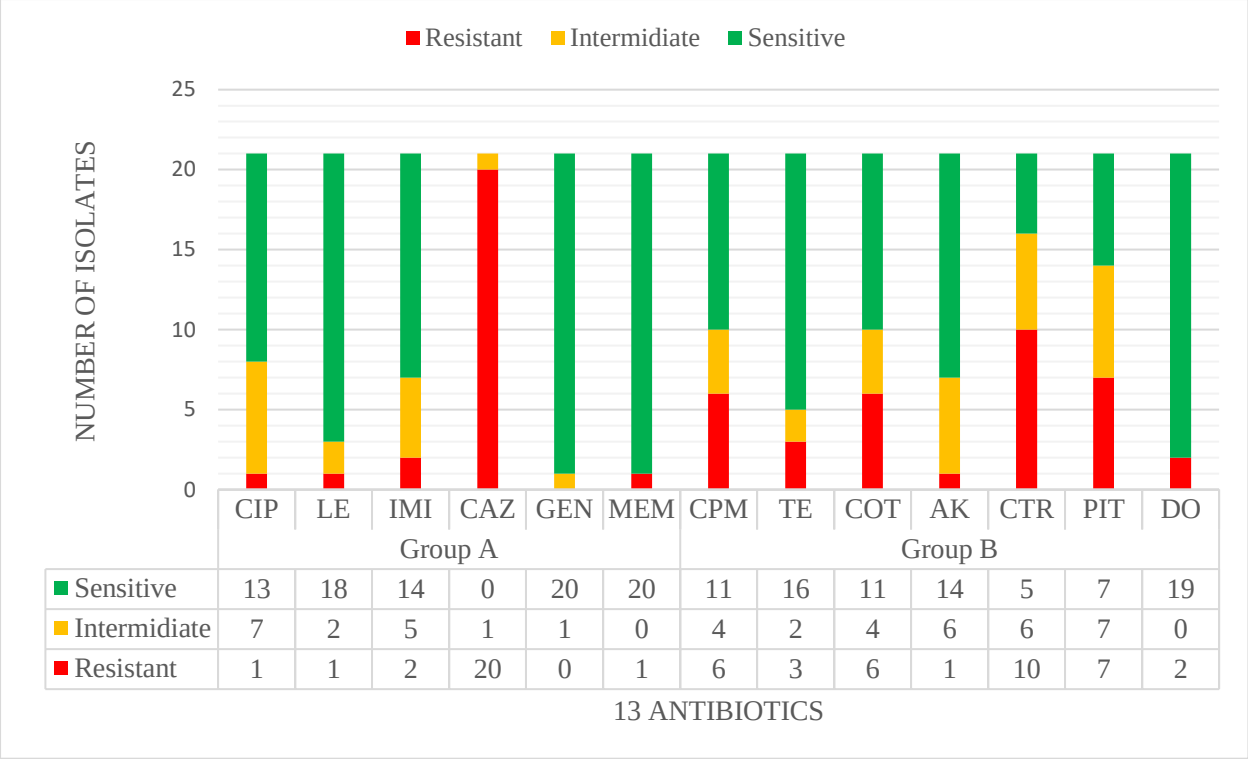


Figure 11: Most Resistant Isolate LCB3c

	Combination Agents	Cephalosporin			Carbapenems		Aminoglycosides		Tetracyclines		Fluoroquinolones		Antagonists
	PIT	CAZ	CPM	CTR	IMI	MEM	GEN	AK	TE	DO	CIP	LE	COT
J1	24	0	20	15	30	30	24	21	17	17	26	26	16
J2	24	0	20	16	30	27	24	20	16	16	27	26	17
J3	25	0	20	16	35	34	25	20	16	16	23	25	18
LCB 3a	16	0	16	13	25	20	22	17	14	19	22	22	14
LCB 3b	15	0	14	5	22	20	20	16	5	15	20	20	5
LCB 3c	15	0	13	5	25	21	22	16	16	19	25	24	6
LM 1	16	0	14	5	26	20	22	17	7	18	25	24	0
LM 2	17	15	20	21	26	31	24	15	14	7	35	26	23
LM 3	20	0	16	7	24	24	20	19	15	20	24	23	0
LM 4	19	9	16	11	25	27	20	16	7	19	25	25	7
LA 1	22	10	25	19	24	23	16	18	19	17	21	20	22
LA 2	20	9	23	22	23	22	16	17	21	16	20	19	21
LA 3	22	9	23	17	20	21	15	16	20	16	20	19	21
LA 4	21	9	21	22	20	21	15	17	18	14	19	18	20
LA 5	20	10	22	21	18	21	15	16	19	14	19	17	17
LA 6	21	9	20	15	20	21	15	17	19	14	19	16	19
LA 7	20	9	22	21	17	20	14	14	19	14	18	15	19
LMY10	20	0	0	0	21	20	19	16	16	9	26	26	0
MMY2	18	0	15	12	21	20	20	17	15	22	27	25	15
MMY3	17	0	14	12	23	26	18	17	15	21	0	0	15
MMY4	15	0	12	10	22	12	19	18	15	20	25	24	11

***Table 4:** Resistant, Intermediate, and Sensitive isolates for all 13 antibiotics*



	CIP	LE	IMI	CAZ	GEN	MEM	CPM	TE	COT	AK	CTR	PIT	DO
Sensitive	13	18	14	0	20	20	11	16	11	14	5	7	19
Intermidiate	7	2	5	1	1	0	4	2	4	6	6	7	0
Resistant	1	1	2	20	0	1	6	3	6	1	10	7	2

Figure 12: The number of Resistant, Intermediate, and Sensitive isolates for each of the 13 antibiotics

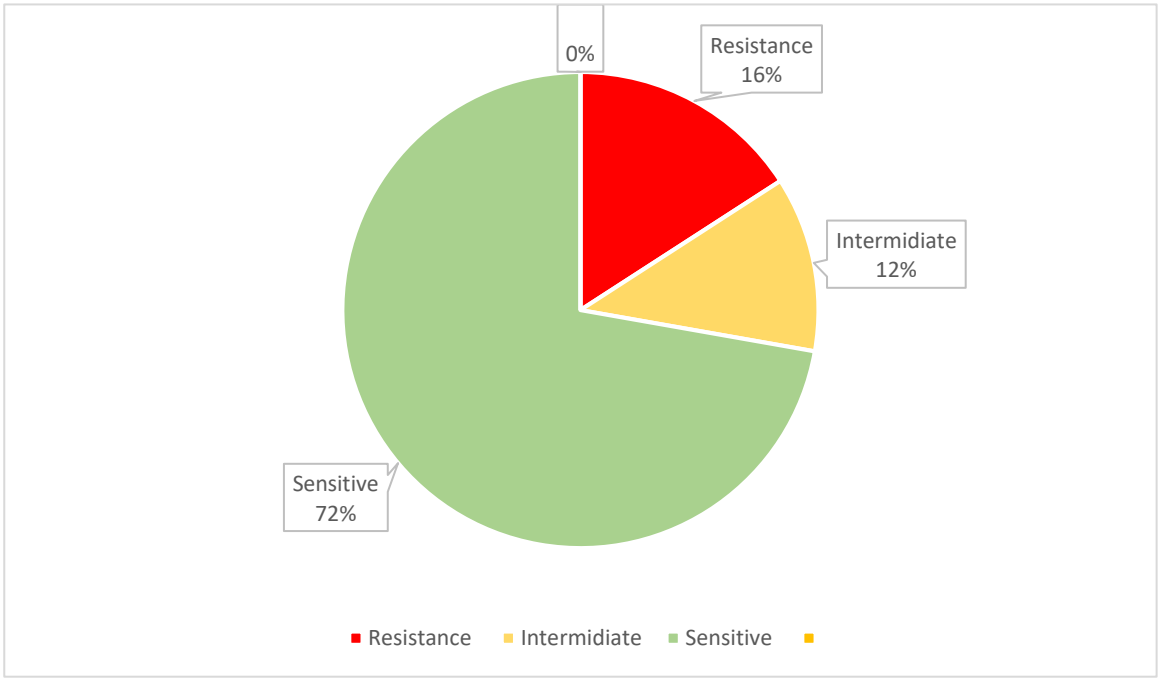


Figure 13: The Percentage of Resistant, Intermediate, and Sensitive isolates for all 13 antibiotics

3.4 MAR Index

The MAR index is determined by dividing the number of antibiotic isolates that are resistant by the total number of antibiotics the organism has been tested for. A MAR larger than 0.2 indicates that areas, where antibiotics are commonly used, are the sources of contamination with the most risk(Ayandele et al., 2020). In this study, the MAR index of 7 of the 21 isolates was higher than 0.28.

Samples of *A. baumannii*	**Resistant Antibiotics (a)**	**Total Tested Antibiotics (b)**	**MAR**
J1	1	13	0.076923
J2	1	13	0.076923
J3	1	13	0.076923
LCB 3a	3	13	0.230769
LCB 3b	6	13	0.461538
LCB 3c	5	13	0.384615
LM 1	6	13	0.461538
LM 2	2	13	0.153846
LM 3	3	13	0.230769
LM 4	4	13	0.307692
LA 1	1	13	0.076923
LA 2	1	13	0.076923
LA 3	1	13	0.076923
LA 4	1	13	0.076923
LA 5	2	13	0.153846
LA 6	1	13	0.076923
LA 7	3	13	0.230769
LMY10	5	13	0.384615
MMY2	2	13	0.153846
MMY3	6	13	0.461538
MMY4	5	13	0.384615

Table 5: MAR index of all 21 A. baumannii Isolates

3.5 Multidrug Resistant *A. baumannii*

A species of microorganism demonstrating resistance to at least one antimicrobial drug in three or more antimicrobial categories is considered a multidrug resistance microorganism (Magiorakos et al., 2012). Among the 21 isolates, 8 isolates (38%) were found to be resistant to three or more categories of antibiotics making them multidrug-resistant isolates. The isolates are LCB3b, LCB3c, LM1, LM4, LA7, LMY10, MMY3 and MMY4. The graph below shows the number of antibiotics each of the MDR isolates is resistant to. The highest pattern of resistance was shown by LCB3c, LM1, and MMY3 with 46% resistance; followed by LCB3b and MMY4 (38%), MMY4 and LMY10 (31%), and LA7 (23%).

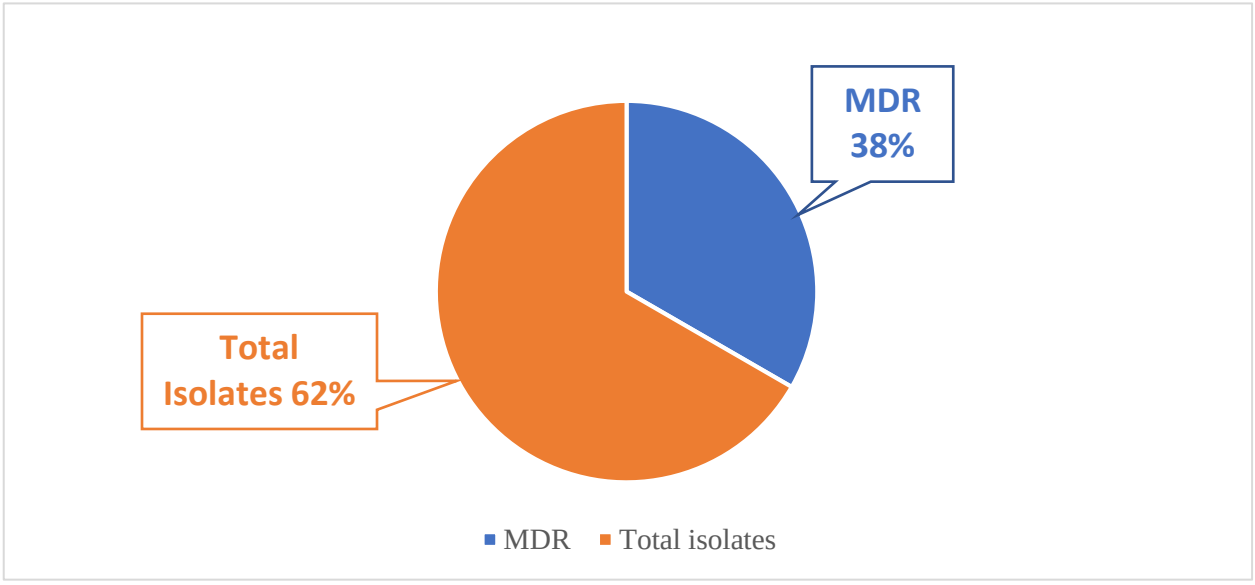


Figure 14: Percentage of Multi-Drug Resistant in totalA. baumanniiIsolates

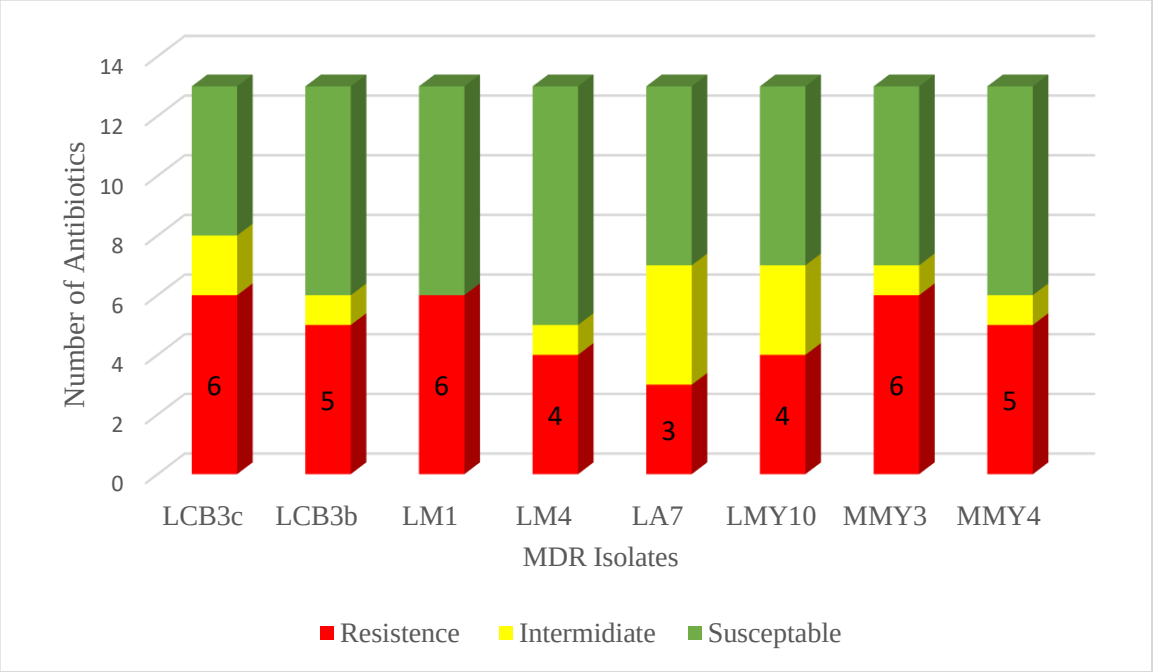


Figure 15: Multidrug-Resistant Isolates and the number of antibiotics they are resistant to

CHAPTER 4

Discussion

The aim of this study was to isolate and characterize *Acinetobacter baumannii* from hospital-adjacent lake surface water. In Bangladesh, there have been multiple studies associated with clinical *A. baumannii* but there is relatively little to no data regarding environmental isolates of the organism which limits the potential risk assessment and future safety protocols. In this research, fourwaterbodies that were within a 100-200meters range of hospitals were selected. Samples were collected consecutively for four months starting in February and ending in May to assess the prevalence of *A. baumannii*. A total of 16 samples were taken from which 21 positive isolates were confirmed.

Following the confirmation of the isolates antibiotic susceptibility tests were conducted against 13 antibiotics belonging to 7 distinct groups: Beta-Lactam combination agents, Cephalosporins, Carbapenems, Aminoglycosides, Tetracyclines, fluoroquinolones, and Folate pathway antagonists. The tests revealed that the isolates were 72% sensitive, 12% intermediate, and 16% resistant against the 13 antibiotics. It was also observed that 8 isolates (38%) were found to be resistant to three or more categories of antibiotics making them multidrug-resistant isolates. During a two-month-long study in Croatia, a total of 19 isolates were recovered from patients, hospital wastewater, urban sewage, and river water, and all the isolates were classified as extensively drug-resistant (XDR)(Seruga Music et al., 2017). Compared to that, this study reported a lower percentage of resistant isolates and only multidrug-resistant patterns. However, the most promising antibiotic for the environmental isolates found in this study could be Meropenem belonging to the carbapenem class of antibiotics as all the isolates (95%) were sensitive to it. In contrast, the resistance of 95% of

isolates to Ceftazidime may reveal the ineffectiveness of the antibiotics against environmental *A. baumannii*.

Moreover, this study successfully isolated *A. baumannii* from hospital-adjacent waterbodies and found several multidrug-resistant isolates. Similarly, multidrug-resistant *A. baumannii*has also been reported in hospital wastewater from Brazil, China, and Croatia(Ferreira et al., 2011; Hrenovic et al., 2016; Zhang et al., 2013). In fact, in Croatia, carbapenem-resistant isolates of *A. baumannii* were reported from municipal wastewater treatment plants(Kovacic et al., 2017).Meanwhile, multiple studies conducted in Bangladesh have already reported MDR, and XDR isolates of *A. baumannii* from hospital settings(Farzana et al., 2022; Jahirul et al., n.d.; Mn et al., 2015; Mohammad et al., 2020; Nahar et al., n.d.; Rahman et al., 2022).Hence, it can be hypothesized that transmission of multidrug-resistant *A. baumannii* may occur through hospital settings to the natural environment. One plausible cause could be that municipal waterbodies adjacent to hospitals like the ones selected in this study are being constantly polluted.MDR *A. baumannii* has been found in animal feces and urine, as well as in the digestive tracts of hospitalized patients. As a result, the colonized patients' and animals' digestive systems could be important epidemiological reservoirs for the organism. Moreover, hospital wastes, and industrial waste all are associated with the use of high levels of antibiotics for treatment, production, and preservation purposes. Misuse and overuse of such drugs result in residual antibiotics leaching out and accumulating in the environment. Due to the adaptation property of microbes to their environment, horizontal gene transfer occurs resulting in gene shifts among different or similar species. As a result, resistant microbes emerge in the environment. According to Seruga Music et al., 2017 they found close relatedness between the clinical and environmental isolates and suggested that untreated hospital wastes may be the reason for XDR *A. baumannii* in the natural environment. This may also be the case in Bangladesh for which further investigation and research are needed focusing on environmental samples. Environmental isolates of *A. baumannii* should be

compared with hospital and community-acquired strains by using molecular assays like whole genome sequencing. Finally, epidemiological studies and larger screenings must be performed in order to investigate the spread of MDR *A.baumnnii* as it may help design any response plan for controlling the pathogen in hospital settings, communities, and environments.

Conclusion

In conclusion, the data from this study suggests that antibiotic-resistant*A. baumannii* may be slowly emerging into the environment. Although the exact reason still requires further studies, it can be implied that the discharge of hospital wastes and unsupervised human contact with municipal waterbodies may inevitably contribute to the issue. On the contrary, *A. baumannii* can survive up to 50 days in natural water (Seruga Music et al., 2017).Thus conferring that the presence of *A. baumannii* in the lakes could be associated with community-acquired infections. Nevertheless, proper management of hospital wastes, disinfecting of hospital wastewater, minimal human pollution, and ensuring proper use of antibiotics in every sector can be crucial steps for preventing the spread of MDR *A. baumannii* into the environment.Last but not least, the effectiveness of new innovative methodsshould be continuously improved upon to mitigate the antibiotic resistance problem globally.

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