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Isolation and characterization of *Acinetobacter baumannii* from environmental waters in Dhaka City, Bangladesh

Mohammed Aziz Hossain[1], Nayara Noor E. Fatima[2], Jahid H. Tushar[2], Hasib Mahmud[2] and Fahim Kabir Monjurul Haque[2*]

Abstract

Acinetobacter baumannii, a gram-negative bacterium commonly associated with nosocomial infections, has been relatively unexplored in the environmental context. The present study was conducted in Dhaka City, Bangladesh, with the primary objective of isolating and characterizing *A. baumannii* in environmental water sources. Surface water samples were collected from various water reservoirs to detect the presence of *A. baumannii*. Isolates were confirmed as *A. baumannii* using culture and PCR. Confirmed isolates were screened for antimicrobial susceptibility, antimicrobial resistance genes, and serum resistance. Results revealed that 32% of the water samples tested positive for *A. baumannii*. In total, 23 *A. baumannii* isolates were obtained. All isolates showed resistance to Cefepime. Varying degrees of resistance to other antibiotics were observed, and 56% showed resistance to the bactericidal effect of serum. This study underscored the remarkable adaptability of *A. baumannii* and its ability to flourish in diverse environmental conditions, highlighting public health concerns of increasing antibiotic resistant bacteria. The study concluded that, given the significance of effective infection control and sanitation and waste management measures, understanding the presence and behavior of *A. baumannii* in the environment is paramount. This study acts as the first report on environmental *A. baumannii* in Bangladesh and further research is warranted to elucidate the underlying mechanisms of antibiotic resistance and their implications for human health.

Highlights

- Molecular characterization confirmed 23 isolates as *A. baumannii* from 31 unique water sites in Dhaka City.
- Resistance to cefepime was observed in all isolates (100%), with a significant proportion also displaying resistance to ceftazidime (65%) and imipenem (56%).
- 30% of isolates were positive for NDM and OXA-48 genes, the latter being an atypical result.
- A majority of the isolates (56.5%) were resistant to human serum.

Keywords Environmental *Acinetobacter baumannii*, Antibiotic resistance, Serum resistant *Acinetobacter baumannii*, Antibiotic Resistant *Acinetobacter baumannii*

*Correspondence:
Fahim Kabir Monjurul Haque
fahim.haque@bracu.ac.bd
Full list of author information is available at the end of the article

Introduction

Acinetobacter baumannii (*A. baumannii*) is a gram-negative bacterium that causes nosocomial infections [1]. *A. baumannii* infections can range from mild, self-limiting infections such as urinary tract infections (UTIs) to severe infections such as pneumonia, meningitis, and sepsis [2]. This bacterium's potential severeness led the World Health Organization (WHO) to classify it as a priority pathogen for research and development of new antibiotics.

Globally, *A. baumannii* has been studied extensively in hospital settings. Infection with *A. baumannii* has increased Intensive Care Unit (ICU) admissions and hospital stays in the past decades [3, 4]. In India, a tertiary hospital found a high prevalence of A. *baumannii* infections common in the ICU with more extended stays and higher mortality [5]. In Wuhan, China, a retrospective study of hospitalized COVID-19 patients found that nearly half died from secondary bacterial infections, 36% of which were caused by *A. baumannii* [6].

The clinical significance of *A. baumannii* can be attributed to several factors, including antibiotic resistance. This bacterium tends to acquire resistance to multiple classes of antimicrobials [7]. Multi-drug-resistant (MDR) and extensively drug-resistant (XDR) is defined as resistance to three or more antimicrobial classes and resistance to all but 1 or 2 classes of antimicrobial agents [8]. The ability of *A. baumannii* to acquire resistance to antibiotics is due to a combination of factors, including its ability to acquire plasmids with resistance genes and produce efflux pumps that pump out antibiotics from the bacterial cell. All *A. baumannii* isolates possess an efflux system but resistance develops in case of hyperexpression [9]. In addition to the inherent resistance that *A. baumannii* possesses, the emergence of rapidly acquired resistance markers contributes to the emergence of a pan-resistant pathogen [10].

Resistance to human serum attributes *A. baumannii* to bacteremia with a high mortality rate [11]. Studies have shown polysaccharide capsules protect *Acinetobacter* species from unsettled environmental conditions and phagocytosis, complement-mediated lysis, or antimicrobial activity [12–15]. According to another research, *A baumannii* binds Factor H (FH) via the outer membrane porin OmpA, contributing to its serum resistance [16]. These contribute to a higher degree of pathogenicity.

In previous studies, the environmental reservoirs for *A. baumannii* outside hospitals were unclear [17], with a lack of reports on environmental *A. baumannii* in Bangladesh, and its presence outside the hospital was considered atypical. Studies have reported that *A. baumannii* was isolated from soil contaminated with hydrocarbons in countries with different climates and conditions [18] [19]. *A. baumannii* has also been isolated from vegetables, meat, unprocessed milk, and cheese obtained from supermarkets, greengrocers, and private gardens [20, 21]. As a result, this opens the possibility of studying *A. baumannii* in the environment. Some studies have found that *A. baumannii* can leach from hospital wastewater to hospital-adjacent community waters, and can survive for prolonged periods [22].

Previous research has also concluded that the presence of A. baumannii beyond hospital environments suggests multiple transmission routes, such as wastewater discharge and contamination, human and animal reservoirs, agricultural runoffs, and contact with contaminated food sources, all of which are likely sources of contamination in a densely populated city like Dhaka. [23]

Despite being a global health concern, in Bangladesh, *A. baumannii* studies are almost exclusively focused on nosocomial infections and hospital environments. Little to no effort has been given to study this organism in its natural habitat, which is concerning given the ease of transmission through frequently used waterbodies in the densely populated capital city of Bangladesh Thus.

The purpose of this study is to isolate *A. baumannii* from water reservoirs, particularly lakes and rivers in Dhaka City. The source of *A. baumannii* is not clinical; therefore, this work also aims to assess its antibiotic susceptibility to clinically relevant antibiotics and determine whether it possesses pathogenic determinants to provide insight regarding this organism's heterogeneity.

Method

Sample collection, processing, and isolation of bacteria

Water samples from 31 unique water sites which consist of the major waterbodies of Dhaka City, including lakes, rivers, and ponds were brought to the laboratory in sterile 50 mL falcon conical tubes following FDA guidelines for water samples [24]. All of the aforementioned sites are major hubs of public transport, irrigation, and water from the sites are regularly used for domestic purposes by the residents of Dhaka.1 mL of the water sample was mixed with sterile 9 mL saline to obtain a ten-fold serial dilution. This process was repeated 5 times, after which they were spread onto Leeds Acinetobacter Agar plates. The plates were then incubated at 44 °C for 24 h. Colonies suspected to be *A. baumannii* were selected according to the Leeds Acinetobacter Agar Base technical datasheet [25]. These colonies were multiplied in Nutrient Agar for further characterization.

Molecular characterization of *A. baumannii* isolates

DNA was extracted using a modified boiling method [26]. Briefly, 2–3 bacterial colonies suspected of being *Acinetobacter baumannii* were selected and suspended

into a microcentrifuge (MCT) tube containing 200 µL Tris–EDTA (TE) buffer. The tubes were boiled at 100 °C in a water bath for 10 min. Afterward, the tubes were centrifuged at 14,000 ×g for 5 min. Subsequently, the supernatant containing the DNA was collected. Polymerase Chain Reaction (PCR) was used to confirm the *A. baumannii* isolates by targeting two different *A. baumannii-specific* genes, $bla_{\text{OXA-51,}}$ and *16S-23SrDNA*.

PCR was performed based on the identification of the $bla_{\text{OXA-51}}$ gene for *A. baumannii* (primers: OXA-51-F: 5'TAATGCTTTGATCGGCCTTG-3' and OXA-51-R: 5'-TGGATTGCACTTCATCTTGG-3' [27]. PCR mixtures were made in a 15-µL volume comprising 4.9 µl Nuclease free water, 7.5 µL 2× PCR Master Mix (Takara-Bio) 0.3 µL of forward and reverse primers(10 µM), and 2 µL of DNA template. The PCR program was set up in an Applied Biosystem (Thermo Fischer) thermal cycler as follows: 94 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 10 min [27]. The expected size for the $bla_{\text{OXA-51}}$ gene is 353 bp. Additionally, PCR was performed by targeting the *16S-23SrDNA* gene (primers: P-Ab-ITSF: 5'-CATTATCACGGTAATTAGTG-3′ and PAb-ITSB: 5'-AGAGCACTGTGCACTTAAG-3′ [28]. PCR mixtures were prepared similarly to the process discussed above, and the PCR program was set up in an Applied Biosystem (Thermo Fischer) thermal cycler as follows: 94 °C for 6 min, followed by 34 cycles of 95 °C for 50 s, 58 °C for 70 s, 72 °C for 55 s, and a final extension at 72 °C for 10 min [28]. The expected size for the *16S-23SrDNA* gene is 930 bp.

The PCR products were subjected to electrophoresis (110 V, 45 min) in 2% agarose gel in TBE buffer (40 mM Tris, 20 mM boric acid, 1 mM EDTA, pH of 8.0) containing 0.5 µg/mL DNA ethidium bromide dye. The gels were examined with ultraviolet illumination and the resulting images were saved and stored. Images were modified using ImageJ [29]. In total, 23 isolates were confirmed to be *A. baumannii* via PCR.

Antimicrobial susceptibility testing

Pure cultures of the *A. baumannii* that were confirmed using PCR were used for antibiotic susceptibility tests. The test was carried out using the Kirby-Bauer disc diffusion method in Mueller–Hinton agar (MHA) according to the Clinical Laboratory Standards Institute guidelines [30]. The following antimicrobial discs were used: amikacin 30 µg(AK), cefepime 30 µg(CPM), ceftazidime 30 µg(CAZ), ciprofloxacin 5 µg(CIP), doxycycline 30 µg(DO), gentamicin 10 µg(CN), imipenem 10 µg(IMP), levofloxacin 5 µg(LE), meropenem 10 µg(MRP), piperacillin-tazobactam 100/10 µg(TZP), tetracycline 30 µg(TE), trimethoprim-sulfamethoxazole 1.25/23.75 µg (CoT).

Overnight cultures of *A. baumannii* were used to make a 0.5 McFarland standard suspension before lawning the bacterial suspension onto MHA agar plates [31]. Selected antibiotic discs were placed onto the plates in a sterile manner. The plates were then incubated at 37 °C for 24 h before measuring the zone of inhibition for the antibiotics and the results were interpreted using the interpretive criteria provided by CLSI [30]. We defined multi-drug resistant (MDR) and extensively drug-resistant (XDR) as bacterial resistance to three or more antimicrobial classes and resistance to all but 1 class of antimicrobial agents [8].

Antibiotic-resistant genes

Presence of OXA-23, OXA-24, OXA-48, bla-GES, blaKPC, SPM and NDM were determined using PCR. PCR amplifications were performed in an Applied Biosystem (Thermo Fischer) thermal cycler, and all runs included negative DNA control consisting of Nuclease-free water.

Serum resistance assay

Serum resistance assay was performed using a slightly modified version of the described protocol [32]. A single colony grown overnight on Nutrient Agar plates of each isolate was added to 1 mL fresh LB broth and incubated in a shaker incubator at 37 °C for 2 h. The culture was centrifuged at 8,000 ×g for 7 min and the supernatant was removed. The pellet obtained was resuspended with 1 mL of sterile saline. In a sterile 96-well microtiter plate (Corning, USA), 20 µl aliquots of the culture-saline mix were treated with 180 µL of pooled normal human serum (NHS). A ten-fold serial dilution was performed up to 7 times before 10 µL of serum-treated cells was taken from each dilution and plated on Nutrient Agar plates. The initial plating was referred to as 0 h. Afterward, the microtiter plate was incubated at 37 °C for 3 h. This was followed by a ten-fold serial dilution performed up to 7 times before 10 µL of serum-bacteria mix was taken from each dilution and plated on Nutrient Agar plates again. Bacterial colonies were enumerated after the Nutrient agar plates had been incubated at 37 °C for 24 h. To assess the serum resistance, the colony-forming units (CFU) were compared from the plates at the beginning (0 h) and end (3 h) of the incubation time, and a paired T test was conducted between the 0-h and 3-h plates. If the CFU count remained the same or increased after 3 h of incubation, it was categorized as resistant, whereas a decrease in the count at the 3-h mark indicated sensitivity. The experiment for serum assay was performed in triplicates.

Data analysis

Data were transferred to Microsoft Excel spreadsheets (Microsoft Corp., Redmond, WA, USA) for analysis. GraphPad Prism 10 was used for generating graphs. Data obtained in this study are available in Supplementary Table S1.

Results

Sampling sites and bacterial identification

The purpose was to determine whether *A. baumannii* is present in environmental water sites. For this, water samples from 31 surface water sites (Fig. 1) were analyzed for the presence of *A. baumannii* which showed pink opaque colonies on Leeds Acinetobacter Agar Based on the PCR amplification of the $bla_{\text{OXA-51}}$ and *16S-23SrDNA* gene (Supplementary Figs. S1 A & B), *A. baumannii* was positive for 10 of the 31 samples, indicating a prevalence rate of 32% In addition, 23 isolates of *A. baumannii* were obtained from the positive samples. An overview of the sampling location is provided in Supplementary Table S2.

Antimicrobial susceptibility testing

All the isolates tested were found to be resistant to cefepime (Fig. 2). Varying levels of susceptibility to doxycycline, gentamicin, tetracycline, and amikacin were observed with sensitivities of 60%, 60%, 70%, and almost 90%, respectively. Sixty-five percent (65%) of isolates showed resistance to ceftazidime. When compared, imipenem had a significantly higher resistance of 56% than meropenem (4%). The resistance for ciprofloxacin, and levofloxacin were 52.5% and 47.8% respectively. The highest instance of intermediate resistance was seen with piperacillin-tazobactam, at around 45%, surpassing all other antibiotics tested (Fig. 2).

Resistance to antibiotic classes, MDR, and XDR distribution

The 13 antibiotics used fall under seven antibiotic classes and the goal was to observe how many of the isolates were resistant to different antibiotic classes. 14 isolates showed resistance to 3 or more antibiotic classes, with the highest levels of resistance being observed in 5 isolates for both 4 and 6 antibiotic classes respectively

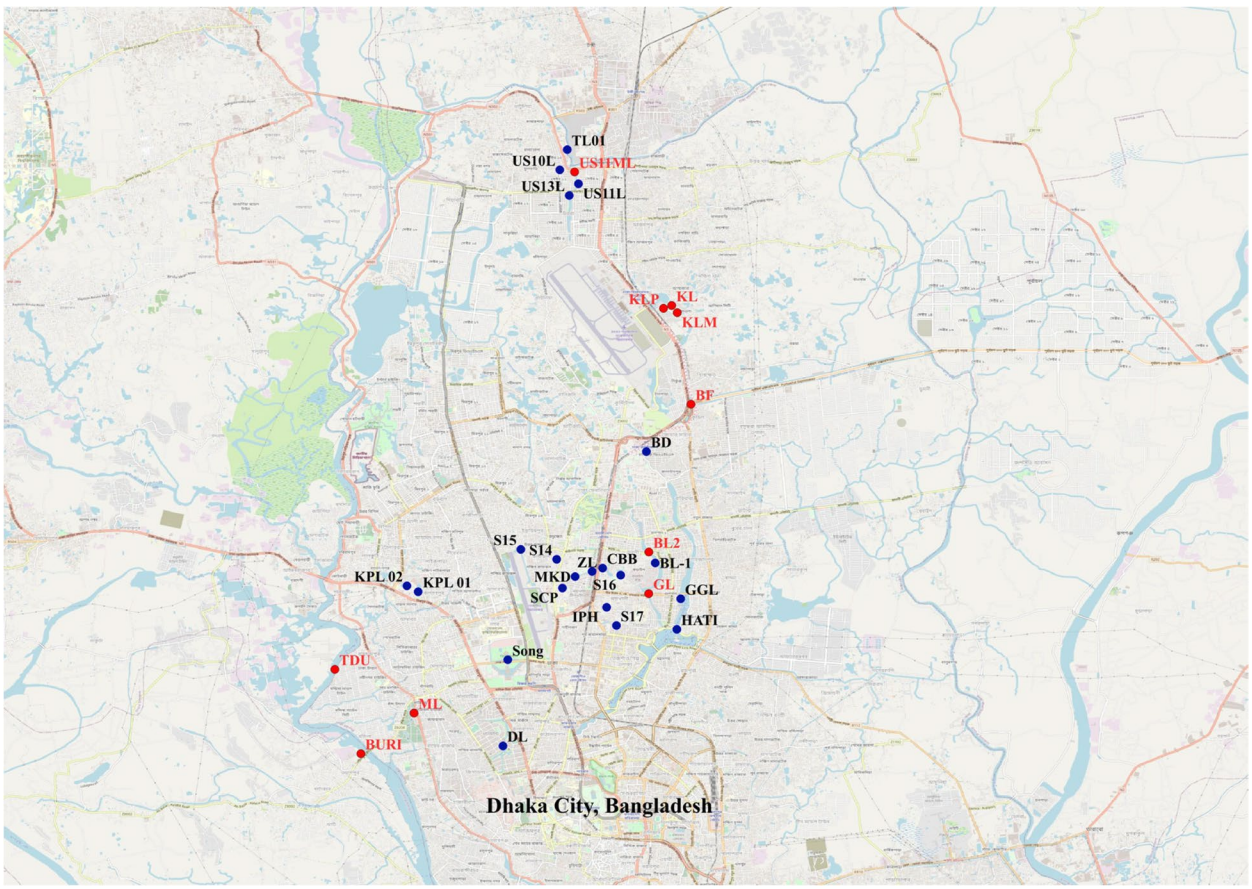

Fig. 1 The sample collection sites pointed on QGIS using GPS coordinates. Blue dots represent sample points that yielded negative results, while the red dots indicate sample points from which *A. baumannii* was isolated

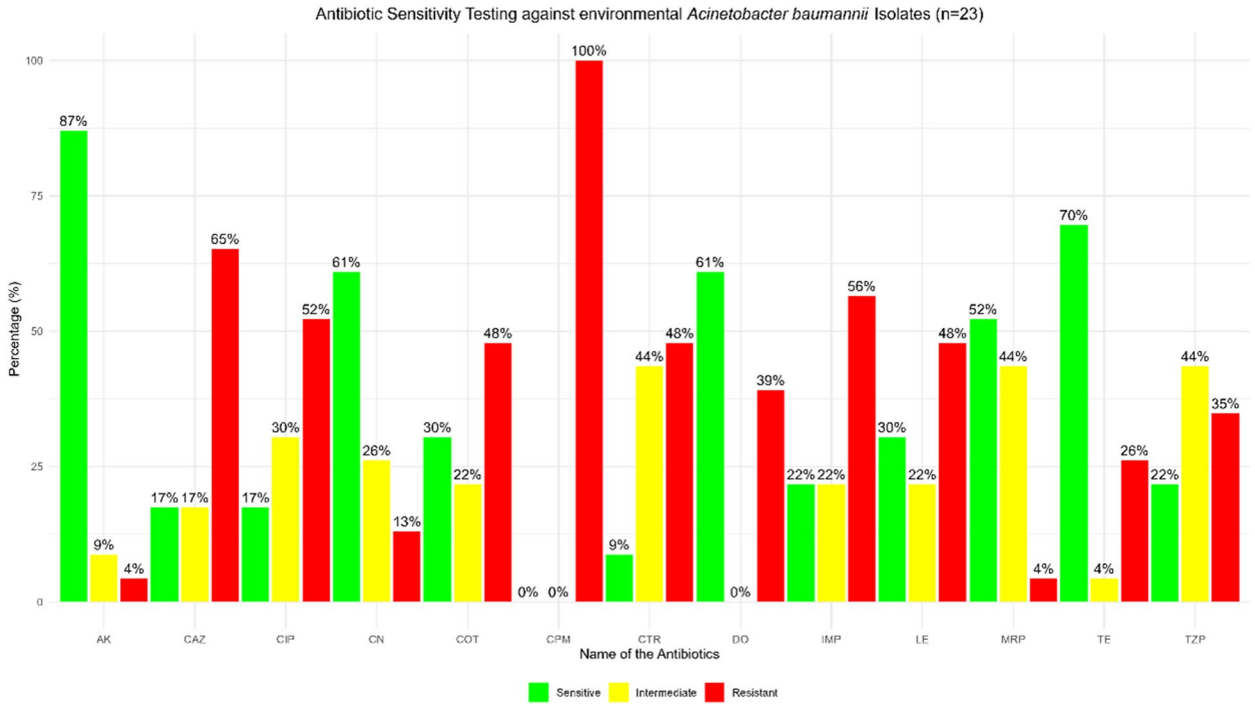

Fig. 2 Antimicrobial susceptibility (AST) pattern against environmental *A. baumannii* isolates. COT = Trimethoprim-sulfamethoxazole, DO = Doxycycline, TE = Tetracycline, AK = Amikacin, N = Gentamicin, CAZ = Ceftazidime, CPM = Cefepime, CTR = Ceftriaxone, CIP = Ciprofloxacin, LE = Levofloxacin IMP = Imipenem, MRP = Meropenem, TZP = piperacillin-Tazobactam

(Fig. 3a). No isolate was resistant to all 7 classes of antibiotics. Based on the findings in this study, the isolates can be categorized and distributed into multi-drug resistant (MDR) and Extensively Drug-resistant (XDR) based on resistance to antibiotic classes (Fig. 3b, c).

MAR index

The multiple antibiotic resistance (MAR) index was calculated as a ratio of resistance to the number of antibiotics by the isolates ('a') to that of the number of antibiotics used('b) [33]. Data show that there is a wide range of

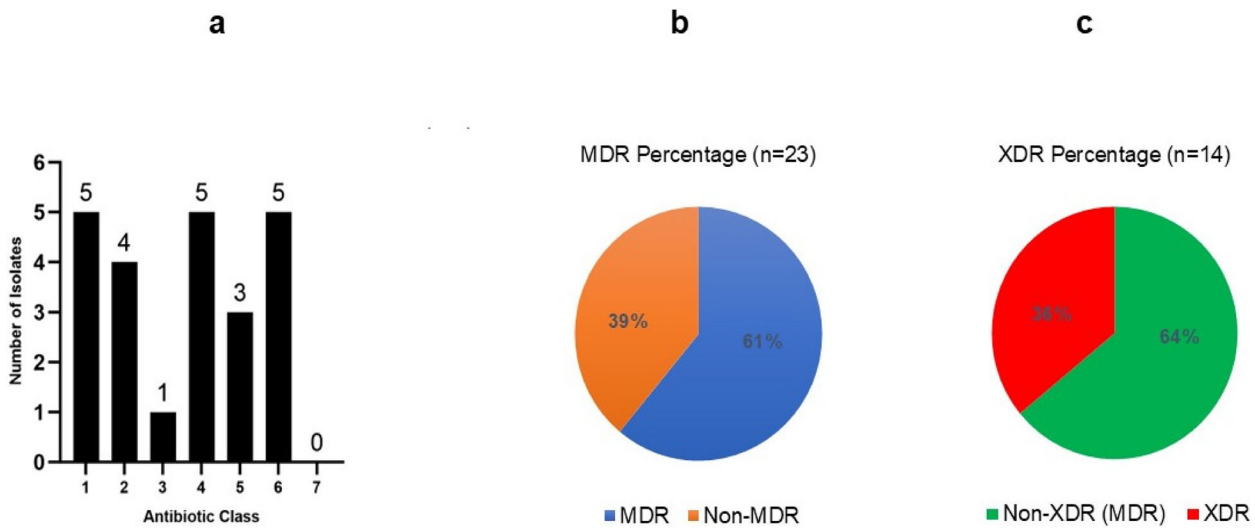

Fig. 3 **a** Resistance to antibiotic classes by environmental *A. baumanni* isolates. **b, c** Distribution of MDR and XDR of environmental *A. baumanni* isolates

MAR indices among the isolates (Table 1). Notably, none of the isolates had a MAR index of 1.0, which indicates resistance to all 13 antibiotics tested, or 0.9, which means resistance to 12 antibiotics. Similarly, there were no cases of MAR indices at 0.8, which would indicate resistance to 11 or 10 antibiotics. However, there is a trend of MAR indices ranging from 0.1 to 0.8, indicating different levels of resistance. Approximately 13% of the isolates had MAR indices of 0.8 and 0.7, which implies resistance to 10 and 9 antibiotics, respectively. A prevalence of 9% was observed for MAR indices of 0.6, 0.5, and 0.2, corresponding to resistances against 8, 7, and 3 antibiotics, respectively. In addition, 4% of the isolates had a MAR index of 0.4, indicating resistance to 5 antibiotics, while 9% showed MAR indices of 0.3 (4 antibiotics) and 0.2 (2 antibiotics).

Table 1 Multiple Antibiotic Resistance (MAR) Index of each *A. baumannii* isolate obtained from the environment

No of Antibiotics Resistant to (a)	No. Antibiotics tested (b)	MAR index (a/b)	Frequency of MAR isolates ($n = 23$)	Percentage of MAR
13	13	1.0	0	0%
12	13	0.9	0	0%
11	13	0.8	0	0%
10	13	0.8	3	13%
9	13	0.7	3	13%
8	13	0.6	1	4%
7	13	0.5	3	13%
6	13	0.5	2	9%
5	13	0.4	1	4%
4	13	0.3	2	9%
3	13	0.2	2	9%
2	13	0.2	1	4%
1	13	0.1	5	22%

AMR Genes

Based on the AST findings, our goal was to determine if the isolates were carrying any known AMR genes for *A. baumannii*. The distribution of OXA-23, OXA-24, blaGES, blaKPC, SPM, and NDM genes is shown (Fig. 4). PCR was positive for NDM (Supplementary Fig. S3) and OXA-48 (Supplementary Fig. S4). Notably, 7 out of 23 isolates (30%) were positive for NDM and OXA-48.

Serum resistance assay

The final objective was to observe if the isolates were capable of surviving against serum treatment. The 23 isolates were tested against human pooled serum (shown in Fig. 5). Findings reveal that most of the isolates were resistant to human serum at a rate of 56.5% (13 out of 23), which was evident by the increase in CFU/ml in the 3 h plates compared to the 0 h plates, with GL-2 showing the highest increase in CFU/ml. The rest of the isolates

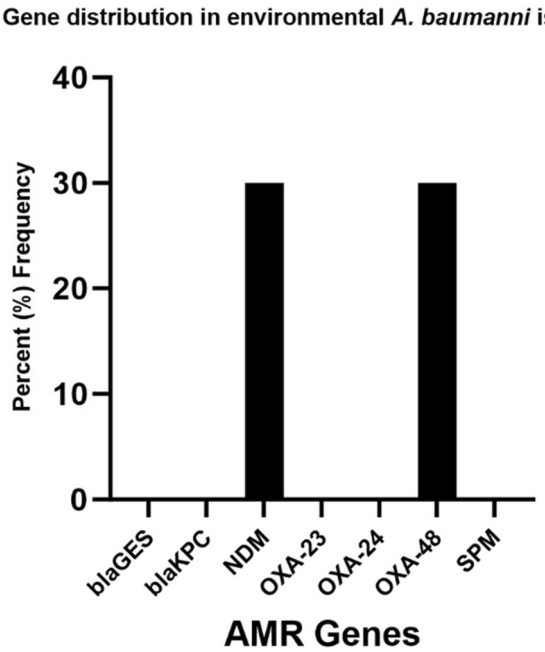

Fig. 4 Percentage frequency distribution of AMR

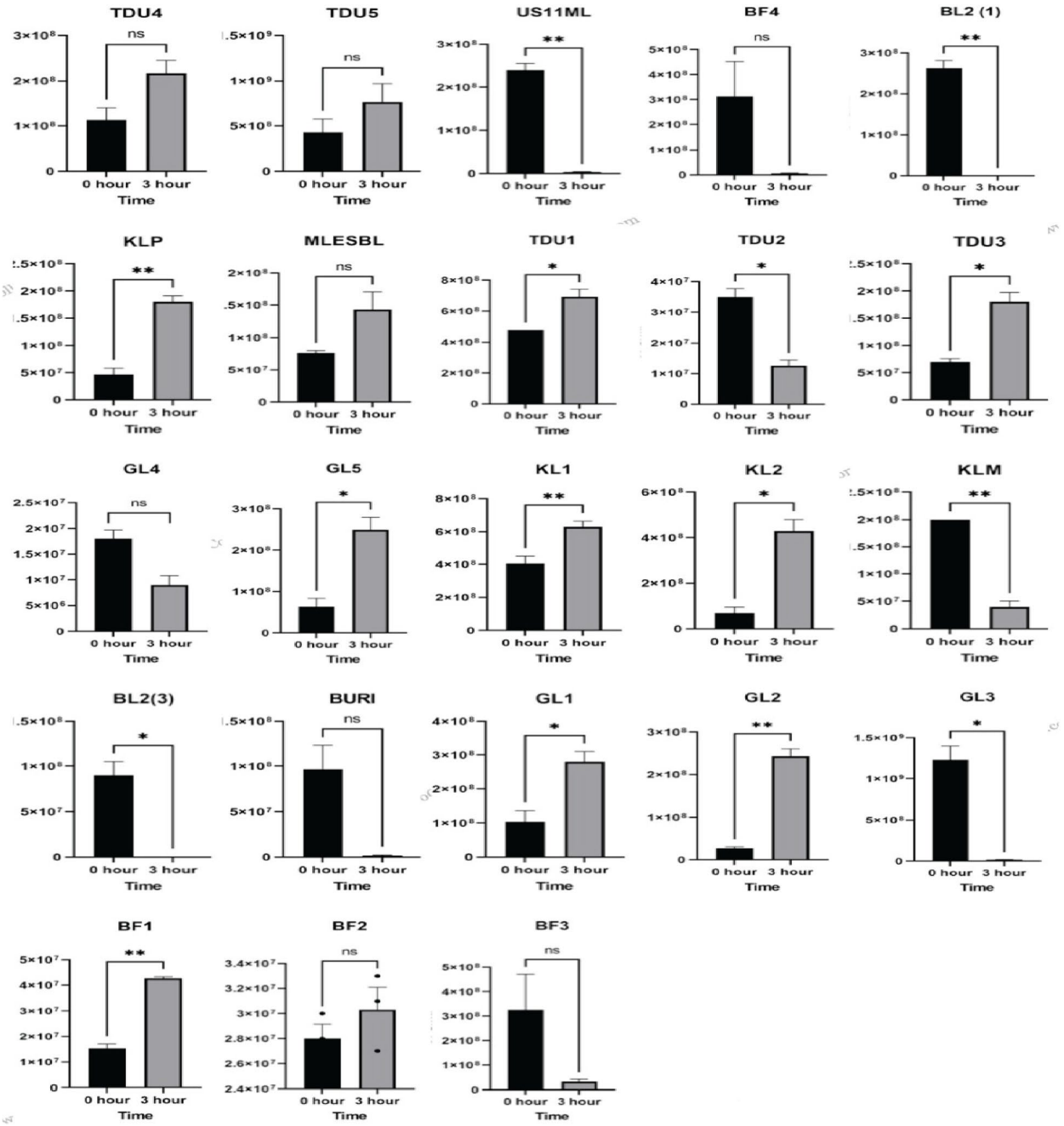

Fig. 5 Serum Resistance Assay against environmental *A. baumannii* isolates with *p* values. The bar chart is plotted against mean values with Standard Deviation of the triplicates

(10 out of 23) were serum-sensitive with 6 isolates showing almost no growth after 3 h.

Discussion

Acinetobacter baumannii has historically been widely associated with clinical settings. To that end, *A. baumannii* has been investigated in hospitals and among patients in Bangladesh [34, 35]. Researchers have previously isolated SPM and OXA-23 carrying *A. baumannii* in urbanized rivers in Southeastern Brazil and Paris, fortifying the capacity of rivers as a reservoir for multi-drug resistant bacteria [36–38]. In Bangladesh however, *A. baumannii's* existence and diversity in environmental water sources remain largely unexplored. To address this knowledge

gap, surface water samples from various water reservoirs were collected between December 2021 and October 2022 in Dhaka City, Bangladesh. Sampling sites were selected randomly. To the best of our knowledge, this report represents the first identification of *A. baumannii* in environmental water sources exclusive to Bangladesh. 32% of the water samples tested positive for *A. baumannii*, highlighting the potential role of environmental water sources as a vehicle for the spread of *A. baumannii*.

The presence of multi-drug-resistant *A. baumannii* through environmental water sources should be a concern for public health because antibiotic resistance makes it difficult to treat infections. This study found varying degrees of antibiotic resistance by *A. baumannii* isolates. Previous studies conducted in Bangladesh and Romania on clinical *A. baumannii* isolates reported resistance rates to Cefepime at 95% and 93%, respectively [34, 35]. In this study, we noted a 100% resistance to cefepime, although this higher percentage is likely influenced by a smaller sample size ($n = 23$). Previous studies conducted in Bangladesh with a similar sample size of 20 clinical ESBL-producing *A. baumannii* isolates have reported high levels of resistance to Imipenem and Ceftriaxone (81.81% for both) [39], with one study having 22 isolates reporting 100% resistance to Ciprofloxacin [40]. Although the isolates in this study did not show such high levels of resistance to the aforementioned antibiotics, more than 50% of the isolates were resistant to Imipenem, and Ceftriaxone had the second-largest resistance rate. None of the studies had isolates with 100% resistance to Cefepime, and there have been no reports of XDR *A. baumannii* in clinical settings, but the resistance could be attributed to ESBL production, and needs to be investigated further.The presence of MDR and XDR *A. baumannii* in surface water suggests increased antimicrobial resistance compared to previous studies, and will likely pose a significant challenge to infection control in the future. Recent research underscores the rapid spread of antimicrobial resistance (AMR) genes, such as *blaNDM, blaKPC*, and *mcr*, among pathogens like *A. baumannii*, *Klebsiella pneumoniae*, and *Escherichia coli*, facilitated by plasmids and transposons. If more comprehensive research necessitates future studies, the limited sample number may be addressed by expanding the sampling sites to Dhaka-adjacent waterbodies, and conducting more advanced molecular screening of retrieved isolates.

The MAR index provides a quantitative measure of resistance, and is a simple and effective tool in communicating resistance, to help clinicians take appropriate drug therapy measures. Incorporating MAR indices in antibiotic stewardship and monitoring results in detailed surveillance, allowing doctors to provide better treatment options [41], which, in Bangladesh, could be used to designed effective public health measures. The proportion of MAR indices greater than 0.2 was 65% (15), while those with MAR indices of 0.2 or less were 35% (8). These results suggest that a significant portion of our isolates likely originated from an environment where multiple antibiotics were frequently employed before eventually reaching the sampling sites, suggesting that the AMR genes are mobile. This finding is alarming as antibiotic-resistant bacteria can potentially be transferred from the environment to humans who come into contact with these water bodies, posing significant health threats. Due to the limited number of isolates obtained from sampling points, we did not calculate a MAR index for these sites. In the future, it will be taken into consideration should a comprehensive study become necessary.

Carbapenem resistance in *Acinetobacter* species is caused by the production of OXA-type carbapenemases and Metallo-beta lactamase (MBL) [42]. While Carbapenamase such as $bla_{\text{OXA-51}}$ is intrinsic to *Acinetobacter baumannii*, the other carbapenemase and MBL genes such as OXA-48 and NDM are of plasmid origin [43, 44]. All 23 *A. baumannii* isolates were positive for the $bla_{\text{OXA-51}}$ gene which was the basis for determining *A. baumnnii*. 7 (30%) of the isolates were positive for NDM and OXA 48, which is an unusual finding, as OXA 48 genes are typical in Enterobacterales, not *A. baumannii*. Previously, NDM positive *A. baumannii* had been isolated from clinical samples in Bangladesh [39], but there have been no reports of NDM carrying *A. baumannii* in the environment, alluding to possible transfer of antibiotic-resistant genes through horizontal gene transfer. Of 13 isolates that showed resistance against imipenem, only 5 of them tested positive for the NDM gene, which suggests unscreened genes could be attributed to imipenem resistance. Only 3 (13%) isolates harbored both NDM and OXA 48 gene where 2 of them were categorized as XDR (one showed meropenem resistance, the other showed intermediate zone), and the remaining one was denoted as MDR. Conclusive results for other AMR genes were not obtained, as the established protocols for PCR resulted in non-specific bands for those genes, which acts as a limitation of this study and may be investigated further should more comprehensive reporting be deemed necessary.

Resistance to bactericidal effects of serum is an important virulence determinant by a bacterium [16]. Although numerous studies have explored serum resistance in clinical *A. baumannii,* we found no pertinent data concerning environmental isolates. The majority of *A. baumannii* isolates (56.5%) in this study were resistant to human serum, with a few being susceptible to the efficacy of serum. While serum resistance is expected in clinical strains due to their origin, it is difficult to ascertain

whether the serum-resistant isolates found in this study are of clinical origin or naturally developed.

Serum resistance accounts for multiple health-related complications in humans, resulting in bacteremia with a high mortality rate. Serum-resistant *A. baumannii* is thought to survive better in vivo and can cause systematic infections, as a result of evading the bacteriocidal effects of human serum [11, 16]. As a result, *A. baumannii* has been designated as a "red alert" human pathogen, which may cause severe infections, given that serum-resistant *A. baumannii* isolated from clinical settings have been found to be ESBL-producing [2]. The water bodies that surround Dhaka City are readily accessible to the general populace, facilitating transport, tourism, and even providing water for domestic purposes for Dhaka citizens. Serum resistance in environmental isolates is highly unlikely and needs to be investigated further.

Conclusion

This study establishes the MDR and serum-resistant patterns of *A. baumannii* isolated from the major waterbodies of Dhaka City, acting as the first report of antibiotic-resistant *A. baumannii* in Bangladesh. Based on the results presented in this study, A. *baumannii* isolates display varying levels of antibiotic resistance. Resistance to cefepime was observed in all isolates (100%), with a significant proportion also displaying resistance to ceftazidime (65%) and imipenem (56%). Additionally, 30% of isolates were positive for NDM and OXA-48 genes, the latter being an atypical result. The environmental origin of the samples indicates that there is the possibility that mobile AMR genes may have been passed to the environmental isolates. The prevalence of multidrug-resistant strains was also observed, with some isolates showing resistance to three or more antibiotic classes. The MAR indices also indicate a moderate level of resistance to multiple antibiotics. This study also found that serum resistance varied among isolates, with 56.6% being resistant to serum. These findings suggest that *A. baumannii* is a highly adaptive pathogen with a wide range of responses to various conditions. Given the limited number of isolates this research worked with, further investigations using whole genome sequencing (WGS) are warranted to conclude whether the origin of these isolates is clinical or not, and investigate the transmission routes of antibiotic and serum-resistant *A. baumannii* to design comprehensive response measures. Future studies should take these considerations in mind and include a larger sample size, analyze additional antibiotic resistance genes, and conduct more comprehensive MAR indexing for sampling sites.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-04029-w.

Supplementary Material 1.

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Authors' contributions
MAH and FKMH conceptualized and designed the manuscript. MAH, JHT and HM conducted the material preparation, laboratory experiments, and data collection and analysis. MAH, NNEF and FKMH wrote the main manuscript. All authors reviewed the manuscript.

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Data availability
The datasets generated in this study have been included in the study. Raw data are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Biotechnology program, Department of Mathematics and Natural Sciences, BRAC University, Kha-224 Pragati Sarani, Merul Badda, Dhaka 1212, Bangladesh. [2]Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University, Kha-224 Pragati Sarani, Merul Badda, Dhaka 1212, Bangladesh.

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