Genomic insights into multidrug-resistant *Acinetobacter baumannii* strains isolated from Dhaka, Bangladesh

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ABSTRACT

Acinetobacter baumannii is one of the most common nosocomial infections causing pathogens. Multidrug-resistant (MDR) *A. baumannii* is a growing concern to the healthcare system in Bangladesh, necessitating the elucidation of the genetic characteristics of this species. Five MDR, biofilm-forming *A. baumannii* strains isolated from clinical samples in Dhaka, Bangladesh, underwent a comprehensive whole-genome shotgun sequencing. *In silico* analyses determined strain types, antibiotic resistance, and virulence genes. The phylogenomic and pan-genome comparative analysis included the study samples along with an additional 15 Bangladeshi *A. baumannii* strains collected from the public database. The antibiotic-resistant profile showed that all the strains were resistant to meropenem. Two strains were resistant to polymyxin B up to 64 μg/ml. The average draft genome size of the strains was 3.93 Mbp, with about 39% GC content. Multilocus sequence typing characterized the strains as ST1, ST2, ST164, and ST623. All strains carried different classes of β-lactamase genes, including the carbapenem hydrolyzing enzyme coding genes bla_{oxa-23} and bla_{oxa-51}. One of the five strains studied harbored bla_{NDM-9} and bla_{NDM-23} genes. The phosphotransferase group of aminoglycosides modifying enzymes was the most common. All the strains had relatively similar virulence profiles. Pan-genome analysis revealed 1944 core and 1302 unique genes. Phylogenomic analysis revealed the distribution of these strains in different clusters with other MDR strains isolated worldwide. The study results suggest the implementation of antibiotic stewardship and genomic surveillance of MDR *A. baumannii* to control the spread of antibiotic resistance in Bangladesh.

INTRODUCTION

Antimicrobial resistance (AMR) presents a serious global health challenge, responsible for millions of deaths annually [1]. The widespread and often unregulated use of antibiotics in humans, farm animals, and agriculture increases AMR risks, affecting both human and animal health. Although considerable progress has been made in developing antimicrobial agents over recent decades, the rapid rise of multidrug-resistant (MDR) bacteria continues to complicate infectious disease management worldwide [2,3]. Given the rising prevalence of MDR pathogens, there is an urgent need to address this problem by resistant bacteria like *A. baumannii* across healthcare, agriculture, and environmental sectors [4].

A. baumannii is a Gram-negative opportunistic human pathogen responsible for nosocomial infection in hospital settings and intensive care units with a significant impact on mortality rates [5]. Several other studies have confirmed that *A. baumannii*, along with other clinically relevant Gram-negative bacteria, exhibited a marked decline

in sensitivity to most antibiotics over the past two decades [6,7]. Clinical isolates of *Acinetobacter* showed resistance to gentamicin (92.7%) and ceftriaxone (87.8%), imipenem (56%), and even colistin (19.5%) which is considered the last resort of treatment due to its high toxicity [4]. It was reported that more than half of these pathogens produced ß-lactamases and carbapenemases, resulting in resistance to the ß-lactam and carbapenem group of antibiotics [8].

A. baumannii is one of the most common pathogens causing nosocomial infections in Bangladesh and has recently emerged as an extensive drug-resistant in many clinical cases [4,8–10]. According to a study [9], the three most common pathogens isolated from patients with nosocomial infections in Bangladesh are *Klebsiella pneumoniae*, *A. baumannii*, and *Escherichia coli*. Although the antibiotic resistance pattern is established, little is known about the genomic makeup of this pathogen found in Bangladesh. The difficulty of treating resistant *A. baumannii* has made it urgent to understand and analyze the whole genomic sequence of this bacterium to treat and prevent the spread of hospital-acquired infections effectively. Hence, the primary objective of our research is to ascertain the genomic composition of *A. baumannii* strains obtained from healthcare facilities located in Dhaka, Bangladesh, to gain a more comprehensive understanding of the genetic variability and adaptability of *A. baumannii*.

MATERIALS AND METHODS

Sample collection and identification

A total of five clinical isolates (SDL03, SDL07, SDL32, SDL35, and SDL40) of *A. baumannii* were collected from Dhaka, Bangladesh (Table 1). The samples were collected in T1N1 soft agar (Oxoid, UK) from the hospital facilities and transferred to the Department of Biochemistry & Microbiology, North South University, where they were grown in MacConkey agar (Oxoid, UK). The molecular confirmation of the pure colony of strains was done by a conventional 16s rRNA gene sequencing using universal primer set 27F/1492R [11]. The amplified 16s rRNA gene sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) to confirm the identification of the obtained samples as *A. baumannii*. The sample acquisition and subsequent use were approved by the Institutional Review Board, with approval number 2019/OR-NSU/IRB-No.0504.

Antibiotic susceptibility test

The antibiotic resistance patterns of the confirmed *A. baumannii* strains were determined by the Kirby-Bauer disc diffusion method, as stated in the Clinical and Laboratory Standards Institute (CLSI) guidelines [12]. In this study, antibiotics from various classes were chosen based on the current national guidelines for treating *A. baumannii* infections [13]. This selection aimed to assess bacterial resistance profiles and establish whether the isolates met the criteria for MDR or extensive drug resistance (XDR) [14]. The antibiotic disks used in this study were amikacin (AMK 30 μg), gentamicin (GEN 10 μg), imipenem (IPM 10 μg), meropenem (MEM 10 μg), ceftriaxone (CRO 30 μg), cefepime (FEP 30 μg), ciprofloxacin (CIP 5 μg), levofloxacin (LVX 5 μg), trimethoprim-sulfamethoxazole (SXT 25 μg), ampicillin (AMP 10 μg), piperacillin-tazobactam (TZP 110 μg), and tigecycline (TGC 15 μg) (Oxoid, UK). Additionally, the minimum inhibitory concentration (MIC) against colistin and polymyxin-B (Sigma-Aldrich, USA) of all *A. baumannii* strains were determined by broth-microdilution (BMD) quantitatively, following the CLSI guidelines [12]. All experiments were

performed in triplicate on three separate occasions. Here, *E. coli* DH5α, a strain sensitive to colistin, was used as a control strain [15] to ensure test accuracy. Cation-adjusted Mueller-Hinton broth (Oxoid, UK) acted as a negative control to ascertain suitable conditions for reliable MIC determination.

Biofilm formation assay

The biofilm production ability of the studied strains was determined by the qualitative and quantitative methods described with slight modifications [16]. The biofilm formation was determined by using the following methods: $OD_{cut} = OD_{avg}$ of negative control + 3 × standard deviation (SD) of OD of negative control; $OD \leq OD_{cut}$ = non-biofilm former; $OD_{cut} < OD \leq 2 \times OD_{cut}$ = weak-biofilm former; $2 \times OD_{cut} < OD \leq 4 \times OD_{cut}$ = moderate-biofilm former; $OD > 4 \times OD_{cut}$ = strong-biofilm former [17]. Briefly, a single colony from each culture plate of the samples was inoculated and incubated overnight at 37°C in a shaking incubator at 220 rpm. A 1:1000 dilution of the inoculum in the nutrient broth was incubated again at 37°C for 48 hours in a static place. The presence of biofilm was determined by 0.1% crystal violet staining. The biomass was solubilized using 30% acetic acid to quantify the biofilm. The absorbance of the solubilized biomass was measured at 590 nm using a microplate reader (Multiskan EX, Thermo Scientific, Finland) (Supplementary Table 1). All the processes were carried out at room temperature and were repeated four times under the same conditions. Furthermore, a bivariate analysis was conducted to examine the correlation between the degree of biofilm formation and antibiotic resistance patterns across all five studied strains (Supplementary Table 2), using SPSS Version 26.0 (IBM Corp., NY, USA) and following the protocol outlined by Ullah *et al.* [18].

DNA extraction and preparation for whole genome sequencing

Bacterial genomic DNA was extracted using Wizard® Genomic DNA Purification Kit according to the manufacturer's instructions (Promega, USA). The quantity and quality of extracted DNA was determined using a NanoDrop™ 2000 (Thermo Scientific, USA).

Sequence assembly and annotation

The *A. baumannii* clinical strains were subjected to Whole Genome Shotgun (WGS) sequencing in an Ion GeneStudio™ S5 System (Thermo Fisher Scientific, USA) by DNA Solution Ltd., Dhaka, Bangladesh. Quality control and adapter trimming were performed for the multiple reads generated, as described [19]. Multiple reads were generated for each sample, with quality control and adapter trimming carried out using Torrent Suite™ Software version 5.10.0. The raw reads were uploaded to the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) [20] server and assembled using Unicylcer v0.4.8 [21], Spades v3.13.0 [22], Bandage v0.8.1 [23], Quast v5.0.2 [24], and Samtools v1.3_31 [25]. The assembled genome sequences were uploaded to the National Center for Biotechnology Information (NCBI) genome sequence database under the BioProject number PRJNA823880 and further annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) [26]. Proksee, a comprehensive system utilized for assembly, annotation, analysis, and visualization purposes, was employed to create a circular map representing the local alignment of the strains [27] (Supplementary Figure 1).

Predictions of antibiotic resistance genes

Antibiotic resistance genes (ARGs) were predicted from each annotated nucleotide sequence based on homology and single nucleotide polymorphism (SNP) models by using the Resistance Gene Identifier (RGI) tool of the Comprehensive Antibiotic Resistance Database (CARD) with the default parameters [28], except for including nudge ≥95% identity loose hits to strict. A heatmap was generated from http://www.bioinformatics.com.cn/srplot, an online platform for data analysis and visualization, using the predicted ARGs of the five *A. baumannii* strains used in this study and publicly available WGS data of 15 other *A. baumannii* strains isolated from Bangladesh (Supplementary Table 3) [29,30].

Genomic characterization

Multilocus Sequence Typing (MLST) 2.0.9 tool in the Center for Genomic Epidemiology Server [31,32] was employed with the Pasteur MLST scheme for *A. baumannii* to determine the sequence type (ST) of each sequence. The insertion sequences (IS) and prophages in bacterial genomes offer critical insights into the mechanisms behind bacterial evolution, adaptation, and AMR - key factors in controlling bacterial infections and developing effective antimicrobial strategies. The presence of any IS elements and prophage regions were predicted using ISFinder [33] and PHASTER [34], respectively. Both the tools predicted the presence and position of IS elements or prophages in the corresponding contigs of the assembled sequences. The pathogenicity was determined using PathogenFinder 1.1, also from the Center for Genomic Epidemiology [35]. The presence of pathogenicity islands was analyzed using IslandViewer 4 [36]. Pan-core genome analysis was performed to understand the genetic variation and evolutionary insights of the five strains included in this study plus 15 publicly available *A. baumannii* genome sequences in the NCBI genome database from Bangladesh using the Bacterial Pan-Genome Analysis tool (BPGA), version 1.3 [37].

Phylogenomic tree construction

The Average Nucleotide Identity (ANI) of the sequences of this study was calculated using FastANI v1.3 [38] (Supplementary Figure 2). From the ANI match, complete sequences were selected with a cutoff score of >97%. The genome BLAST Distance Phylogeny (GBDP) strategy was used by the Type strain genome server (TYGS) to contract the phylogenomic tree using 50 strains of *A. baumannii* (Supplementary Table 3) [39] utilizing default parameters followed by annotating the tree in Interactive Tree of Life (iTOL) [40].

RESULTS

MDR phenotype and biofilm production rate among *A. baumannii* strains

The antibiotic susceptibility test revealed high resistance against all the major antibiotic groups except polymyxins and tetracycline. Tigecycline and polymyxin B appeared to be the most effective antibiotics against studied *A. baumannii* isolates (Figure 1). However, two of these isolates (SDL32 and SDL40) exhibited resistance to polymyxin B. The disk diffusion test results for five studied *A. baumannii* strains against various antibiotics are shown in Supplementary Figure 3. As all the isolates in this study were resistant to at least three classes of antibiotics, they are characterized as MDR [14].

Besides, susceptibility towards the polymyxin (polymyxin B, colistin) group of antibiotics was determined by the BMD method (Figure 1). The MIC values for colistin against strains SDL03, SDL07, and SDL32 were 0.5 mg/L, 1 mg/L, and 0.5 mg/L, respectively, all were below 2 mg/L. In contrast, strains SDL35 and SDL40 had MIC values of 2 mg/L. According to earlier CLSI guidelines, MIC values <2 mg/L were categorized as colistin-susceptible. However, CLSI (2020) revised this classification, removing the 'susceptible' category for *Acinetobacter spp*. and defining MIC ≤2 mg/L as intermediate and MIC ≥4 mg/L as resistant. For Polymyxin B, strains SDL03, SDL07, and SDL35 were classified as sensitive, with an MIC of 2 mg/L. In contrast, strain SDL32 (MIC = 32 mg/L) and strain SDL40 (MIC = 64 mg/L) were resistant to Polymyxin B. Later, the biofilm formation ability of the samples was determined by crystal violet staining (Table 1 & Supplementary Table 1). It was observed that strains would form biofilm regardless of their biological sources; however, the *A. baumannii* isolates collected from the respiratory tract area were found to form more biomass than the other two samples collected from the urine or wound swab.
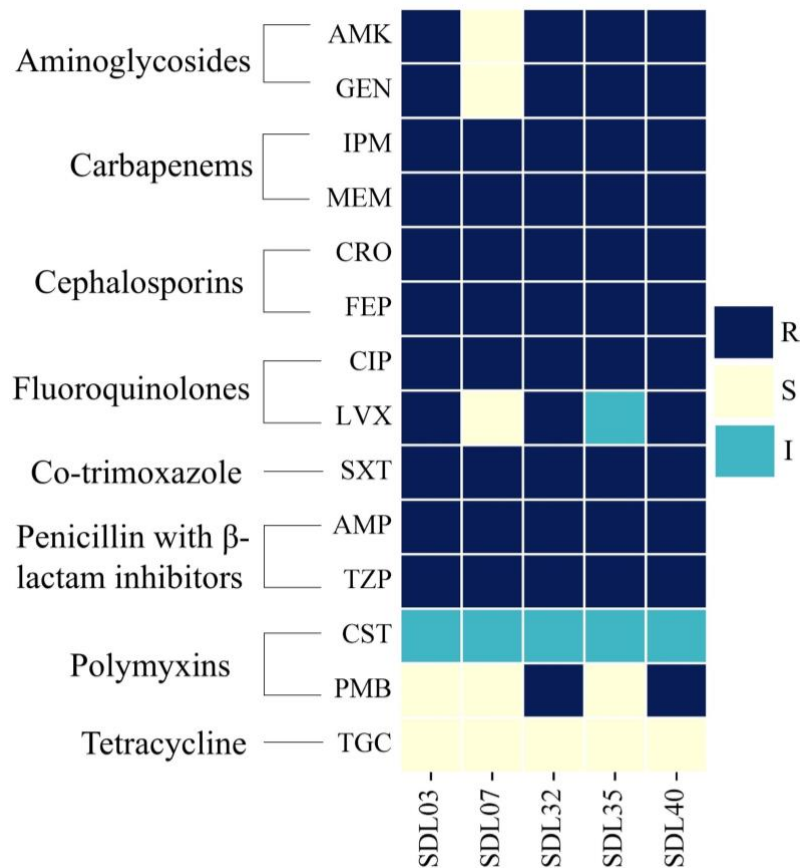
Figure 1. Antibiotic resistance phenotype in studied isolates. Here, blue, beige, and cyan boxes represent resistant, sensitive, and intermediately sensitive to a particular antibiotic. AMK: amikacin, GEN: gentamicin, IPM: imipenem, MEM: meropenem, CRO: ceftriaxone, FEP: cefepime, CIP: ciprofloxacin, LVX: levofloxacin, SXT: trimethoprim-sulfamethoxazole, AMP: ampicillin, TZP: piperacillin-tazobactam, CST: colistin, PMB: polymyxin B, and TGC: tigecycline.

Table 1. Biofilm formation ability of *A. baumannii*

Sample ID	Source	Biofilm presence
SDL03	Tracheal Aspirate	Strong
SDL07	Urine Sample	Weak
SDL32	Tracheal Aspirate	Very strong
SDL35	Endotracheal tube	Very strong
SDL40	Wound Swab	Very Weak

General features of the sequenced strains

In the WGS, the raw sequences were generated with coverage higher than the threshold 20X, with an average GC content of approximately 39%. The quality of the assembled genomes was assessed using *A. baumannii* K09-14 as a reference strain. Once assembled and annotated, the sequences were uploaded to the NCBI database to generate unique accession numbers for all the samples. The general features of the sequencing data are presented in Table 2 and Supplementary Figure 1.

Table 2. General characteristics of the sequence output of the studied strains and reference strain *A. baumannii* K09-14.

Strain	Accession No	Coverage	Total length (Mb)	N50	GC (%)	No. of Coding genes	No. of Sub-systems	Total RNA	MLST type	No. of IS	No. of Pathogenicity islands	Human Pathogen probability
SDL03	JAMZEM000000000	122.4	3.92	104859	39.12	3903	315	51	ST1	70	25	0.852
SDL07	JAMOLG000000000	32.36	3.91	78650	39.12	4025	316	49	ST1	69	22	0.861
SDL32	JAMOLH000000000	88.80	3.98	72775	38.83	4137	307	51	ST2	31	25	0.851
SDL35	JAMOLI000000000	85.17	3.86	114833	39.00	3854	313	53	ST164	39	15	0.851
SDL40	JAMOLJ000000000	97.01	3.99	64518	38.91	4070	315	54	ST623	43	21	0.852
K09-14	NZ_CP043953.1	N/A	3.98	N/A	38.98	3592	310	94	ST46	02	18	0.857

Prediction of antibiotic resistance genes

The annotation of the genome sequences revealed the presence of many well-characterized ARGs, shown in Figure 2. All the Bangladeshi strains contain different ARGs from all the ARG groups, and these genes are widely distributed among the strains. There is no significant difference in ARG number and/or distribution among the Bangladeshi strains, except that the genes responsible for the inactivation of antibiotics are present unevenly among these strains. Efflux genes seem to be more predominant than other classes of genes across the strains. All the Bangladeshi strains except two harbor the *bla*$_{oxa-23}$ gene, whereas strain SDL07 harbored *bla*$_{NDM-9}$ and *bla*$_{NDM-23}$ genes. Other β-lactamase classes of ARGs are present infrequently in the phenotypically carbapenem-resistant strains.

Presence of different classes of virulence factor genes

The presence of virulence factors was also analyzed, and it was found that all the Bangladeshi strains contain different kinds of virulence factors compared to the reference strain (Figure 3). While virulence factors significant for adherence, effector delivery system, and biofilm formation were present in all strains, comparatively fewer immune modulation factors were present in the studied strains. Again, a sporadic pattern can be seen in the presence of the virulence factors responsible for a major iron uptake regulating siderophore and acinetobactin formation among the five strains of this study. Furthermore, most of the studied strains harbored a higher number of pathogenicity islands compared to the reference strain K09-14 (Table 2).
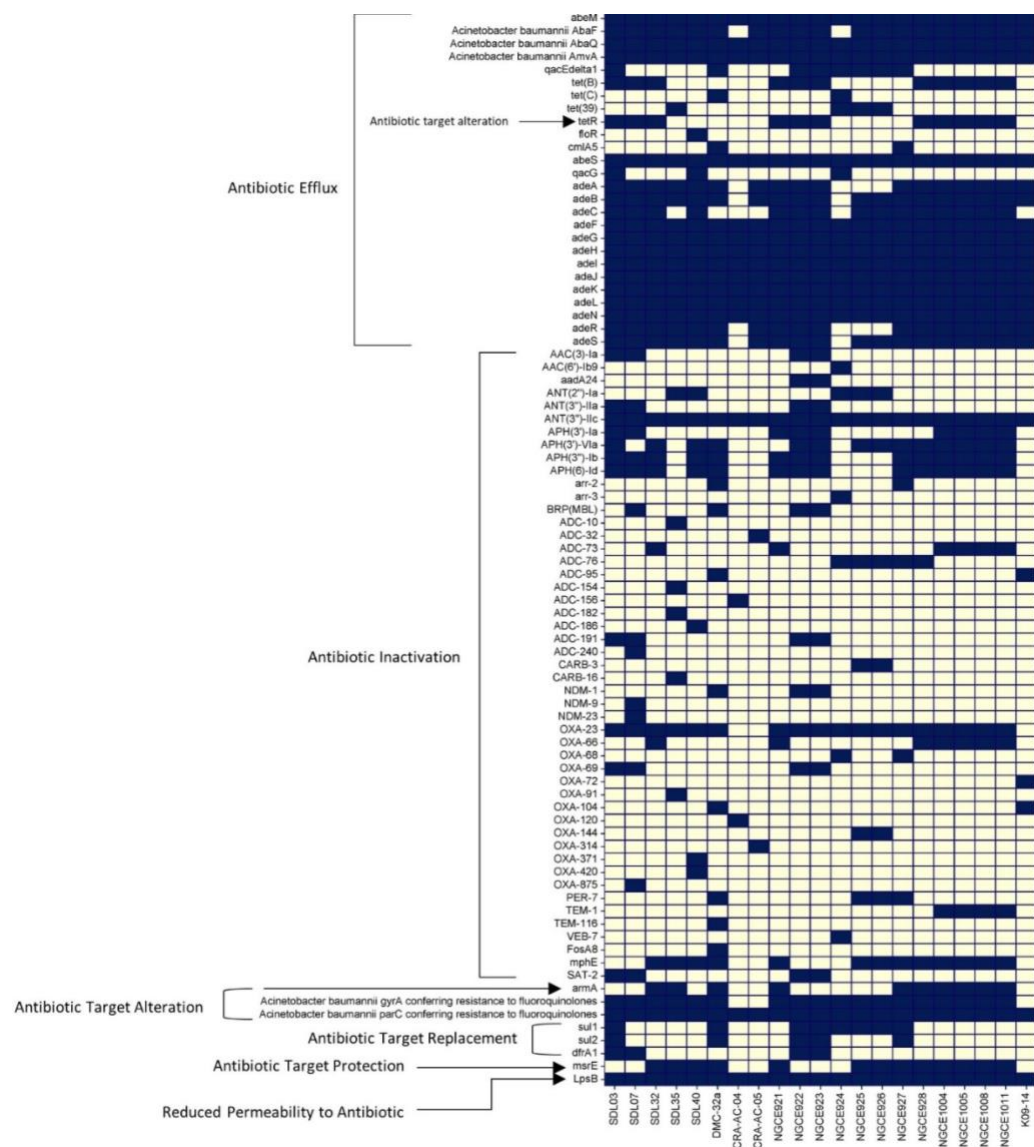
Figure 2. Distribution of predicted antibiotic resistance gene classes in the studied strains and the reference strain *A. baumannii* K09-14. Here, the blue box represents the presence, and the beige box represents the absence of a specific gene.

Phylogenomic tree analysis

A phylogenomic analysis was conducted to identify the evolutionary relationship among the strains isolated from Bangladesh and other MDR *A. baumannii* isolated worldwide, as shown in Figure 4. The phylogenomic tree demonstrates that Bangladeshi *Acinetobacter* strains are genetically diverse and distributed across different evolutionary branches. Such dispersion of these strains among non-Bangladeshi isolates implies that these *Acinetobacter* strains from Bangladesh are from multiple evolutionary origins, suggesting that they may have been introduced from different sources or evolved separately within distinct lineages. This finding also aligns with the concept that these strains may have acquired MDR independently or adapted to similar environmental pressures that drive their divergence.
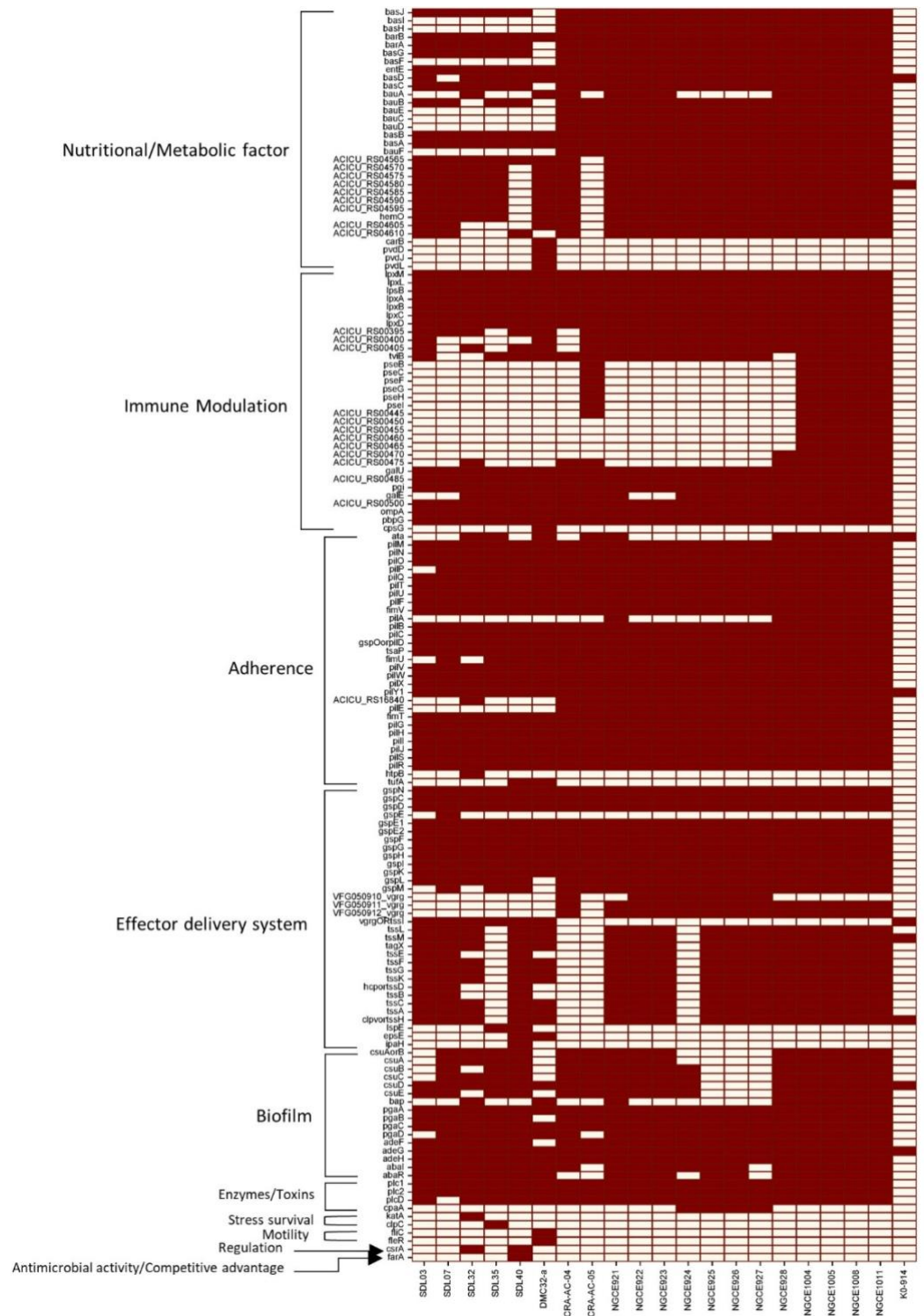
Figure 3. Distribution of predicted virulence factor genes across the studied strains and the reference strain *A. baumannii* K09-14. Here, the red box represents the presence, and the beige box represents the absence of a specific gene.
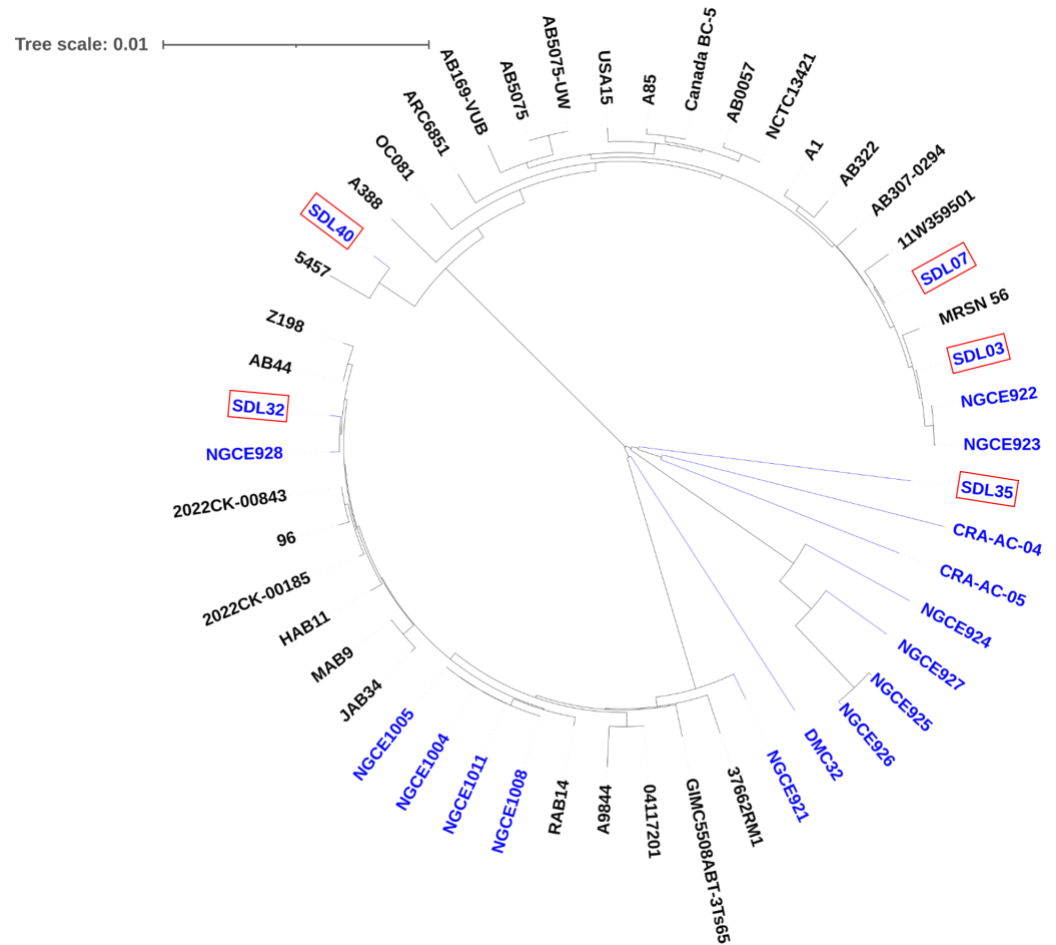
Figure 4. Phylogenomic tree of 50 *A. baumannii* strains collected from all over the world. Blue-colored strains are from Bangladesh. The labels in red boxes denote Bangladeshi strains collected in this study.

Pan-core genome analysis

A pan-genome analysis of twenty *A. baumannii* strains was conducted, which comprised fifteen recently sequenced *A. baumannii* genomes originating from Bangladesh alongside our five studied strains. The pan-core plot (Figure 5) shows an increase in the total gene numbers, indicating the emergence of new genes by introducing a new genome to the pan-genome analysis. A total of 1944 genes were detected as core genes, shared by all the strains. There were 510 exclusively absent genes in the genome of *A. baumannii*; additionally, there were 1434 ± 182 accessory genes in the genome (Supplementary Table 4). Moreover, 1302 unique genes were detected in the pan-genome, where DMC32 was found to have acquired the highest number of unique genes (307), followed by SDL35 (296), whereas few strains lacked any unique genes. This observation is further supported by the fact that 82% of the unique genes identified in five specific *A. baumannii* strains: CRA-AC-04 (n=181), CRA-AC-05 (n=159), DMC32 (n=307), SDL35 (n=296), and SDL40 (n=128). In contrast, strains NGCE922, NGCE923, NGCE926, NGCE1004, NGCE1005, NGCE1008, and SDL07 did not harbor any unique genes. The functional adaptations of *A. baumannii* showed B_{pan} values of 0.183883 (i.e., <1) in the power-law regression model, suggesting that the pan-genome is open but may be closed soon [41].
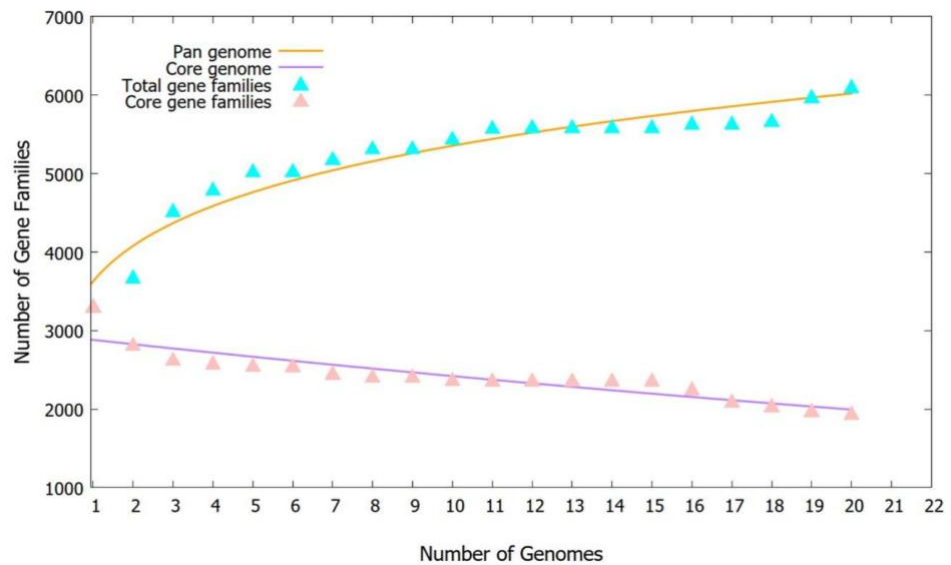
Figure 5. Distribution of core and pan-genome in *A. baumannii* genomes collected from Bangladesh.

DISCUSSION

A. baumannii strains pose a significant threat in Bangladesh, often exhibiting MDR or XDR traits [4,7,42]. Despite this, the genomic characteristics of Bangladeshi *A. baumannii* strains remained largely unexplored until recently [29], when they focused only on the distribution of *A. baumannii* strains in different clonal complexes. However, this gap limited our understanding of the antibiotic resistance mechanisms and virulence among Bangladeshi *A. baumannii* strains. We have sequenced the whole genomes of five clinical *A. baumannii* isolates and compared them to 15 additional *A. baumannii* strains from Dhaka to address this gap. WGS analysis showed that our five MDR strains harbored diverse resistance genes and virulence factors. The strains SDL03 and SDL07 belong to the clonal population of ST1 according to the Pasteur scheme of MLST typing (Table 2). SDL32 was found to be in ST2. The strains isolated from Bangladesh in different studies also belonged to ST1 and ST2 [10,29]. The remaining sequence types, ST164 and ST623 in strains SDL35 and SDL40, respectively, were reported globally but uncommon in Bangladesh [43,44]. Overall, this study sheds light on the unique genomic makeup of MDR *A. baumannii* strains in Bangladesh, highlighting their potential for diverse antibiotic resistance and virulence and paving the way for further investigation into their epidemiology and control strategies.

The studied strains are MDR in nature, exhibiting resistance to different classes of conventionally used antibiotics like β-lactams, carbapenems, aminoglycosides, and fluoroquinolones (Figure 1). Second-line antibiotics like polymyxin and tigecycline were effective against most of our isolates; however, two strains, SDL32 and SDL40, displayed resistance to polymyxin B. Only one strain, SDL07, was identified as susceptible to a third-generation fluoroquinolone, levofloxacin. This, coupled with non-susceptibility towards last-resort antibiotics, shows the emergence of XDR and further emphasizes the importance of understanding the molecular mechanism of drug resistance by analyzing the whole genome.

Diverse resistance genes were identified using the CARD database, which can render resistance through antibiotic inactivation, target protection/modification, and efflux pumps. The phenotypic resistance profile observed (carbapenems, cephalosporins,

fluoroquinolones, aminoglycosides) is on par with the genotypic findings illustrated in Figure 2. A common thread emerged by comparing the data with a broader set of Bangladeshi strains: each isolate harbored at least one gene from each group of ARG families. The presence of Aminoglycoside Nucleotidyltransferase, ANT (3″) enzymes associated with resistance to streptomycin and spectinomycin [45], are present in all the strains used in the comparative analysis. Previous studies reported the frequent presence of genes like *aac(3)-I, aac(6′)-Ib, aph(3′)-I, aph(3′)-IIb, aph(3′)-VIa, aph6,* and *ant(3″)-I* in aminoglycoside-resistant strains found in Asia, often showcasing multiple coexisting genes associated with higher resistance levels [46,47]. Our analysis revealed a similar pattern, with diverse Aminoglycoside Phosphotransferases APH groups detected in our isolates and the other Bangladeshi strains (Figure 2). However, the aminoglycoside acetyltransferase AAC group of genes was less prevalent in Bangladeshi strains despite these strains being resistant to both amikacin and gentamicin. These findings are suggestive of the differences in resistance mechanisms prevalent among the strains of this region.

Another set of antibiotic-inactivating enzymes encoded by the different classes of β-lactamase can directly hydrolyze carbapenem antibiotics and are present in the studied five strains. OXA-23-like β-lactamase (bla_{oxa-23}) and OXA-51-like β-lactamase ($bla_{oxa-66,}$ $bla_{oxa-69,}$ $bla_{oxa-91,}$ $bla_{oxa-371,}$ and $bla_{oxa-875}$) are present in all five strains, aligned with reports linking them to increased carbapenem resistance in *A. baumannii* [48]. The presence of B, C, and D β-lactamase classes like bla_{NDM}, bla_{ADC}, and bla_{oxa-58}, especially NDM-9 and NDM-23, was predicted using the CARD database. The enzymes that break down carbapenems (β-lactamase genes) are one of the causes of resistance, and other factors, such as efflux pumps and porins, make the problem even worse [49]. These groups of carbapenem resistance contributing genes are present in the studied strains (Figure 2), accounting for the elevated resistance against both imipenem and meropenem.

Acquired resistance in bacteria can also be caused by polymorphisms in antibiotic target genes, resulting in target alteration. In this study, all five strains harbor *gyrA* and *parC* genes with mutations in the Quinolone-resistance determining region (QRDR) associated with fluoroquinolone resistance [50]. However, no known polymorphisms in the *pmrCAB* operon, responsible for mediating resistance towards the polymyxin group of antibiotics [51,52], were found in SDL32 and SDL40 (Figure 1).

Our strains displayed polymorphisms in the AdeS and AdeR regulatory proteins of the AdeABC efflux pump that belong to the resistance-nodulation-cell division family. Previously, AdeRS polymorphism has been linked to increased AdeABC expression in European clones [53], which are highly resistant to carbapenems, fluoroquinolones, and aminoglycosides [54,55]. In addition to the presence of different aminoglycosides modifying enzymes, β-lactamases, and mutations in the QRDR region, the polymorphic AdeRS system in the studied strains (Supplementary Table 5) can potentially be the reason for resistance towards both second and third-generation antibiotics of multiple classes.

Understanding the virulent potential of these isolates is equally crucial as deciphering the resistance mechanisms. The ability of these MDR *A. baumannii* strains to cause infections and potentially evade host defenses was assessed by looking into the specific virulence factors associated with these strains, as shown in Figure 3. Notably, three isolates from respiratory tracts displayed strong biofilm production, which is concerning due to their potential to contribute towards ventilator-associated pneumonia and ICU settings [56–58]. However, no correlation was found between the biofilm-producing ability of the studied strains and their AMR patterns, possibly due to a small number of strains (Supplementary Table 2).

While our five strains contained lesser virulence factors responsible for immune modulation and metabolic factors, adherence and biofilm formation factors were uniformly present. The presence of these genes shows the potential for increased pathogenicity in the future. Therefore, further investigation into their evolutionary origins and spread is crucial. Our phylogenetic analysis offered valuable insights into this aspect. The comparative analysis in Figure 4 illustrates that despite being small in sample number, our studied strains are not grouped in a single cluster. Instead, they are present along with global strains in three different clades. The diverse distribution of strains across different clades suggests a complex global origin, further evident from the presence of one Global Clone 1 Lineage 3 strain (SDL40), originally tracked back to Indian strains [59]. The Bangladeshi strains, on the other hand, were found to be very closely related to each other even when in different clades, probably due to the fact that all of these were collected from the hospitals of Dhaka city. However, this does not imply the lack of diversity among the Bangladeshi strains. For example, SDL07 harbors two β-lactamase genes, bla_{NDM-23} and $bla_{NDM-9,}$ which are usually associated with *K. pneumoniae*; gene transfer might have occurred between these two bacterial species [60,61]. The pangenome analysis in Figure 5 shows the pangenome is still open, suggestive of ongoing evolution and gene gains, pointing towards a possibility of unique evolutionary trajectories.

Previous studies [29,30] and whole genome analysis of our five strains revealed that β-lactamase genes represent a key contributor to resistance in carbapenem-resistant *A. baumannii*; however, antibiotic resistance in this pathogen is not mediated by a single factor. Instead, multiple factors synergistically increase the resistance toward different classes of antibiotics. These observations emphasize the complex interplay of various resistance genes in mediating antibiotic resistance among Bangladeshi *A. baumannii* strains. While the overall pattern aligns with the previous report [29], our findings suggest differences in specific gene prevalence (Figure 2) and potential polymorphisms (Supplementary Table 5) within Bangladeshi strains. Due to the small number of strains, the observations present merely association, not definite causation of resistance. However, this is one of the first genomic studies on *A. baumannii* in Bangladesh. Thus, it will provide comprehensive genomic information and insights into common factors in drug-resistant *A. baumannii*.

CONCLUSIONS

This study aimed to look deep into the genetic makeup of different elements of five MDR *A. baumannii* with the help of whole genome sequencing. Here, 5 Bangladeshi strains were compared with 15 other Bangladeshi *A. baumannii* strains. While a larger, more diverse strain collection could further enhance the depth and generalizability of findings, even limited sample sizes can reveal meaningful trends and foundational knowledge. It was revealed that the bacteria possess both ARGs and virulence factors. The phenotypical observation of high resistance could be explained by the numerous antibiotic-resistant genes of all groups present alongside several SNPs/polymorphic genes. The presence of biofilm as a virulent factor indicates this nosocomial pathogen's dissemination in healthcare setup by persisting on different abiotic medical devices. This comprehensive genomic characterization provides valuable insights into the local prevalence and evolution of MDR *A. baumannii* in Bangladesh, reiterating the need for vigilant surveillance to measure the potential spread within the region along with targeted treatment strategies.

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AUTHOR CONTRIBUTIONS

SF: Investigation; formal analysis; writing – original draft. KMSA: Investigation; formal analysis. AA: Formal analysis; visualization; writing – review & editing. SRS: Formal analysis; writing – review & editing. IJ: Formal analysis; writing – review & editing. KPH: Investigation; formal analysis. AKH: Validation; data curation; formal analysis; visualization. MIK: Data generation. MFAR: Resources; Data generation. MH: writing – review & editing. KNH: writing – review & editing. SI: Conceptualization; methodology; resources; writing – review & editing; supervision; funding acquisition. All authors contributed to the article and approved the submitted version of the manuscript.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

SUPPLEMENTARY MATERIALS

Supplementary Table 1. Biofilm formation data, Supplementary Table 2. Pearson correlations between biofilm formation capacity and antibiotic resistance pattern, Supplementary Table 3. The accession number of 50 *A. baumannii* strains used to construct phylogenomic trees, Supplementary Table 4. The number of accessories, unique, and exclusively absent genes resulted from pan-genome analysis of the twenty different strains of *A. baumannii*, Supplementary Table 5. Presence of polymorphisms in different antibiotic resistance gene encoding proteins, Supplementary Figure 1. Sequence alignment of five *A. baumannii* strains, Supplementary Figure 2. Matrix heatmap of the pairwise Average Nucleotide Identity (ANI), and Supplementary Figure 3. Sample figures showing disk diffusion test results for five studied *A. baumannii* strains against various antibiotics (Supplementary materials).

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