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THE DISTRIBUTION OF ANTIBIOTIC RESISTANCE GENES IN PLASMIDS AND GENOMIC ISLANDS OF *Acinetobacter baumannii* THROUGH WHOLE GENOME SEQUENCING

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ABSTRACT

Acinetobacter baumannii is a prominent pathogen in hospital environments, capable of causing various serious infections. The opportunistic pathogen *A. baumannii* contains variants of the AbaR genomic islands, which enhance its multidrug resistance capabilities. Whole genome sequencing (WGS) identified 36 plasmids across 30 samples, with 10 plasmids present in at least 5 samples; notably, 19 out of 36 plasmids contain antibiotic resistance genes. Plasmid AE271 was found in 18 samples that harbored important beta-lactam resistance genes, including *blaOXA-91, blaOXA-120, blaOXA-67*, and *blaOXA-66*. Both plasmids AC163 and AE689 have carried *blaOXA-23*, a commonly found resistance gene in *A. baumannii*. Plasmid AC715 was present in 10 samples, including a variety of resistance genes such as *blaOXA-23, aph(3')-VIb*, and *sul2*. Plasmid AC237, identified in 9 samples, also contains multiple resistance genes, including *sul1, armA*, and *msr(E),* reflecting its diversity in drug resistance. Some plasmids were classified as "novel," containing the *blaOXA-23* gene. Integrative antibiotic resistance elements (REIs) such as AbaR4 and Tn6167 confer carbapenem resistance, an essential antibiotic group for treating *A. baumannii* infections. Several REIs, including AbaR22, Tn6166, and delta-AbaR25, exhibit antibiotic resistance, including aminoglycosides, tetracyclines, sulfonamides, and beta-lactams. This finding underscores the complex genetics of plasmids in *A. baumannii* and their role in the spread of antibiotic resistance. The study also indicates that genomic islands may play a significant role in spreading antibiotic resistance genes, providing deeper insights into the diversity and genetic organization of RIs in *A. baumannii* populations that have yet to be explored.

Keywords: *Acinetobacter baumannii*, genomic island, plasmid, resistance island (REI), whole-genome sequencing

INTRODUCTION

Acinetobacter baumannii is one of the most serious threats to public health today due to its ability to resist multiple antibiotics. *A. baumannii* causes infections in hospital settings, particularly in Intensive Care Units

(ICUs), affecting individuals with compromised immune systems and becoming increasingly severe (Vázquez-López *et al.*, 2020). This bacterium is especially known for causing infections such as ventilator-associated pneumonia, bloodstream infections, meningitis, skin and soft tissue infections, endocarditis, osteomyelitis, and urinary tract infections (Papazachariou *et al.*, 2024). It carries various antibiotic resistance genes located on plasmids, transposons, and integrons, allowing transmission from one bacterium to another. Common genes include *bla_OXA-23*, *bla_OXA-58*, and *bla_NDM*-1 (Chan *et al.*, 2020; Sánchez-Urtaza *et al.*, 2023). Due to selective pressure from widespread antibiotic use, *A. baumannii* can persist and spread within hospitals, particularly in ICUs. In recent years, *A. baumannii* has shown resistance to multiple antibiotics, especially carbapenems, significantly increasing mortality rates and treatment costs for infected patients (Harding *et al*., 2018).

The genome of *A. baumannii* includes both chromosomal and plasmid components, with plasmids playing a crucial role in disseminating virulence and antibiotic resistance genes (Partridge *et al.*, 2018). However, not all plasmids function equally in this capacity. Plasmids are capable of autonomous replication and can be transferred between bacteria, not only within the same species but also across different species, thereby increasing the spread of antibiotic resistance genes in the hospital environment (Cameranesi *et al.*, 2018; Hujer *et al.*, 2017; Mosqueda *et al.*, 2014; Wibberg *et al.*, 2018). These plasmids vary in size and characteristics, and many carry antibiotic resistance genes, particularly those encoding serine carbapenemases (OXA-type beta-lactamases), which are the main resistance mechanisms of this species. Surprisingly, some common plasmids lack genes associated with conjugation or mobile elements, indicating that other mechanisms or horizontal gene transfer play significant roles in the dissemination of plasmids in *A. baumannii* (Da Silva, Domingues, 2016). In Vietnam, a cross-sectional study on pneumonia patients in southern Vietnam revealed that over 80% to 90% of identified *A. baumannii* strains were carbapenem-resistant and multidrug-resistant (MDR) (Hoang Quoc *et al.*, 2019). According to a recent study by Hsu *et al*. (2017) summarizing carbapenem-resistant enterobacteria and *A. baumannii* in South Asia and Southeast Asia (Hsu *et al.*, 2016), international statistics published since 2010 indicate that the rate of carbapenem resistance among *A. baumannii* in Vietnamese hospitals is very high, ranging from 43% at a pediatric hospital in Ho Chi Minh City to 92% at a hospital in Hanoi. Notably, most studies were conducted in ICUs, where the rate of carbapenem resistance is typically elevated.

AbaR-type genomic islands were crucial factors responsible for antibiotic resistance in *A. baumannii* (Bi *et al.*, 2019). These mobile genetic elements (MGEs) are known for carrying multiple resistance genes and are associated with multidrug resistance phenomena in *A. baumannii* (Bi *et al.,* 2019; Li *et al.,* 2015). AbaR is prevalent in this species, with 2,091 genomic islands identified, but it is rare in other *Acinetobacter* species, because it is limited to this genus (Bi *et al.*, 2019). AbaR islands are highly diverse, containing various resistance genes with unique structural features depending on different epidemic lineages (Hamed *et al.*, 2022). For example, AbaR3 in global clone 1 (GC1) often carried

multiple resistance genes and was located within transposon Tn 6019 (Naderi *et al.*, 2023). In contrast, AbaR4, primarily found in GC2 and carrying the *blaOXA-23* gene, was differentiated from AbaR3 elements and had a backbone of Tn 6022 (Hamidian and Hall, 2017). Most AbaR disrupts the specific site of the *comM* gene on the chromosome, resulting in duplication of the target site with perfect direct repeats of 5 bp (DR). Resistance islands (RIs), containing various transposons, integrons, and specific resistance genes, are characterized in the genome of *A. baumannii*. REIs play a significant role in the horizontal gene transfer of resistance genes. Recently, AbGRI4 was identified in GC2 strains and non-GC2 strains, harboring *aadB, aadA2*, and *sul1* in an integron class I, targeting the α/β-hydrolase gene (Chan *et al.*, 2020). Furthermore, AbGIR factors are believed to originate from a plasmid segment containing Tn *6022* and Tn *6172*, which transposed into a single unit at the *comM* locus of GC2 strains; AbGRI5, the latest REI identified in *A. baumannii*, is similar to AbGRI3 in containing *armA, msrE - mphE, sul1*, and an integron class I harboring multiple resistance genes such as *blaPER−1, blaCARB−2, aadA2, cmlA1, aadA1*, along with the macrolide resistance gene *ere(B)* (Hua *et al.*, 2021). A diverse resistance region formed from two copies of Tn6018 was commonly found in AbaR3 elements belonging to GC1 (Hamidian *et al.*, 2021). In GC2, AbaR elements often contain *sul2, tet(B), strA, strB*, and/or *blaOXA-23*, with multidrug resistance also linked to factors AbGRI2 and/or AbGRI3 (Blackwell *et al.,* 2017).

MATERIALS AND METHODS

Samples

Thirty strains of *A. baumannii* isolated from clinical samples in various hospitals in Northern Vietnam were selected. These samples were identified as *A. baumannii* and exhibited resistance in antibiotic susceptibility tests against at least one antibiotic. The relationship between the genotypes and sequence types of the isolated *A. baumannii* strains was analyzed using WGS techniques. All the detailed information of antibiotics used in the study and the relationship between these resistant *A. baumannii* strains and antibiotic resistance genes in their genomes were also submitted in our previous publication (Nghiem *et al.*, 2024).

Whole genome sequencing

Raw sequencing data is assessed for quality using FastQC software and then cleaned to eliminate low-quality and short sequences. Subsequently, Trimmomatic is utilized to process the low-quality sequences. In this analysis, all sequences with a quality score below 20 (QC < 20) and any containing Illumina Nextera adapters are excluded. The cleaned data is then utilized for *de novo* genome assembly using SPAdes software, with the k-mer parameter chosen automatically. The assembled genome is evaluated for quality based on criteria such as total genome size, the longest contig length, and the N50 statistic, using QUAST software. The *A. baumannii* genome, post *de novo* assembly, undergoes gene prediction and functional annotation using Prokka. The output data from Prokka includes GFF3, GBK, FNA, and FAA files, all suitable for submission to the NCBI database. Subsequently, the genome is annotated for antibiotic resistance genes using ABRicate (https://github.com/tseemann/abricate). The ResFinder tool utilizes BLAST (Basic Local

Alignment Search Tool) to identify antibiotic resistance genes present in the genomic sequences.

Plasmid identification

Plasmids were identified using ABRicate software with the PlasmidFinder database and cross-validated on PlasmidFinder 2.1 (https://cge.cbs.dtu.dk/services/PlasmidFinder/). The minimum identity requirement was 95% with a minimum coverage of 60%. Additionally, the MOB-suite software was employed to search for plasmids in the assembled genome (https://github.com/phac-nml/mob-suite).

Genomic island identification

The presence of genomic islands (GIs) was identified using the Pathogenicity Island Database (PAIDB), which contains publicly available Pathogenicity Islands (PAIs) and antimicrobial resistance islands (REIs) for bacteria. Typing from the sequencing data of the 30 samples was conducted using SRST2 (Short Read Sequence Typing for Bacterial Pathogens). Furthermore, genomic islands were predicted using the IslandViewer tool (https://pathogenomics.sfu.ca/islandviewer).

RESULTS

Plasmid analysis in multidrug-resistant *A. baumannii* strains

The analysis of plasmids in multidrug resistant strains of *A. baumannii* was conducted based on the results of WGS. The study aimed to identify the quantity and types of plasmids present in the multidrug-resistant strains.

Table 1. Summary of predicted plasmids from MOB-suite.

Antibiotic resistance genes (considering all samples containing plasmids)	Number of samples present	Plasmid
blaOXA-23_1	9	AC163, AE689, AC715, AB082, novel_12a6c3e2aa896865d52105c3a512fa08, novel_207dfe76ce26e19a7b48fd531546ec09, novel_3bc7128461026ecd275d380a6d6c4e2e, novel_bbdf6f0e1a3b520c977560c2d8e4694e, novel_f19e0bb1c1d87ddd7be8c2c69cf27e78
mph(E)_1	5	AD096, AC237, AC716, AB114, AC861
msr(E)_1	5	AD096, AC237, AC716, AB114, AC861
armA_1	3	AD096, AC237, AC716
sul1_5	3	AC715, AC237, AC716
aph(6)-Id_1	3	AC715, AC716, AC861
aph(3'')-Ib_2	3	AC715, AC716, AB114
sul2_2	2	AC715, AC716
acc(3)-IIa_1	2	AC716, AG457
ant(3'')-Ia_1	2	AC715, AC237

Antibiotic resistance genes (considering all samples containing plasmids)	**Number of samples present**	**Plasmid**
tet(B)_1	2	AC715, AC716
floR_2	1	AB114
aph(3')-Ia_7	1	AC237
tet(39)_1	1	AB355
ant(2'')-Ia_13	1	AB702
aph(3')-VI_1	1	AE373
blaOXA-66_1	1	AE271
blaOXA-67_1	1	AE271
blaOXA-91_1	1	AE271
blaOXA-120_1	1	AE271
ARR-3_4	1	AC716
cmlA1_1	1	AC716
blaPER-7_1	1	AC716
aac(6')-Ian_1	1	AC716
blaTEM-1D_1	1	AC237
aph(3')-VIb_1	1	AC715

Using the MOB-suite toolkit, 26 antibiotic resistance genes on plasmids were identified in a total of 30 samples, with 10 plasmids appearing in at least 5 samples. Among these, gene *blaOXA-23_1* was detected in 9 samples, indicating a high potential for this gene to confer resistance to beta-lactam antibiotics. Its presence in multiple new plasmids may suggest that it is spreading through plasmid transmission mechanisms, raising concerns about the increasing antibiotic resistance of *A. baumannii*. The genes *mph(E)_1, msr(E)_1*, and *armA_1* also appeared in 5 samples, reflecting not only a diverse resistance capability but also the adaptation of bacteria in environments under antibiotic pressure. The combination of these genes could lead to multidrug resistance (MDR), complicating treatment options. Additionally, the presence of genes like *floR_2* and *blaTEM-1D_1* in fewer samples indicates the emergence of specific resistance factors. There were 19 plasmids that contained antibiotic resistant genes, as annotated in Table 1. Plasmid AE271, found in 18 samples, carries multiple resistance genes crucial for β-lactam resistance, including *blaOXA-91, blaOXA-120, blaOXA-67*, and *blaOXA-66*. Plasmids AC163 and AE689 both contain *blaOXA-23*, a common resistance gene in *A. baumannii*. Plasmid AC715 present in 10 samples, harbors a range of resistance genes such as *blaOXA-23, aph(3')-VIb, sul2*, and *tet(B),* indicating diverse resistance capabilities. Plasmid AC237 found in 9 samples, also

contains several resistance genes, including *sul1*, *armA*, and *msr(E)*, demonstrating further diversity in drug resistance. Some plasmids labeled as "novel" contain the *blaOXA-23* gene, suggesting that these plasmids are new and not previously documented, highlighting the genetic diversity of this bacterium. *A. baumannii* exhibits high drug resistance, with multiple plasmids carrying various resistance genes. This indicates the bacterium's adaptation to antibiotic environments, potentially leading to challenges in treating infections. The presence of diverse resistance genes also suggests effective horizontal gene transfer among bacteria, increasing the risk of resistance within bacterial populations.

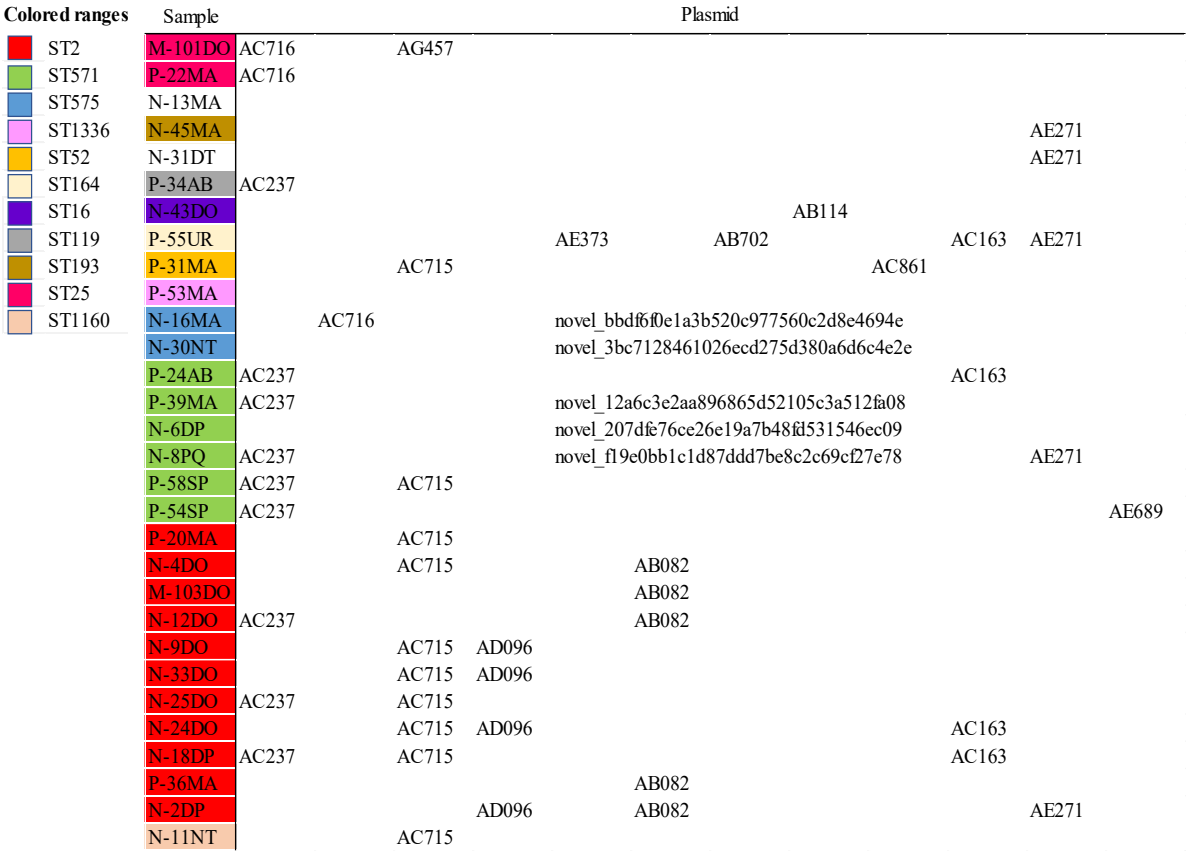

Figure 1. Distribution of plasmids found in each of the 30 samples.

The diversity of antibiotic resistance genes in the *A. baumannii* samples was demonstrated by the data in Figure 1, showing varying numbers of genes present in different samples, ranging from none to multiple genes simultaneously. Notably, *blaOXA-23* was found in many samples, indicating that it is one of the most common resistance genes. Two other widely recognized antibiotic resistance genes are *msr(E)* and *mph(E)*, suggesting a broad distribution of these genes within the bacterial population. Other genes, such as *aph(3')-Ia, sul1_S*, and *tet(B),* were also present in many samples, demonstrating the diverse resistance capabilities of *A. baumannii*. Several samples contained plasmids AC237 and AC716, indicating that

these plasmids can carry multiple resistance genes and contribute to drug resistance. The presence of novel plasmids (such as novel_bb and novel_3c) may also suggest the emergence or discovery of new resistance genes not previously known. Some genes, like *blaOXA-91, blaOXA-120*, and *blaOXA-67*, were less frequently observed and only appeared in the same sample belonging to genotype ST164, which may indicate their association with specific bacterial groups.

Genomic island search from the PAIDB database

The PAIDB is a database that includes publicly available PAIs and REIs of bacteria. Within this database, *A. baumannii* contains 39 REIs. The results indicate that 15 REIs had been identified across the 30 strains. Information about the REIs is presented in Table 2.

Table 2. Information on the 15 REIs found in 30 strains.

REIs	Host strain	Function	GenBank Accession	Length
AbaR3	*Acinetobacter baumannii* RUH3247	European clone I	JF262177	705
AbaR4	*Acinetobacter baumannii* D36	Resistance to carbapenem	JN107991_R1	16812
AbaR14	*Acinetobacter baumannii* LUH5881	European clone I	JF262170	3846
AbaR17	*Acinetobacter baumannii* LUH8592	European clone I, gentamycin	JF262173	1830
AbaR22	*Acinetobacter baumannii* MDR-ZJ06	Tetracycline efflux pump, streptomyces resistance, sul1 resistance gene	NC_017171_R1	38950
AbaR25 (BJAB07104)	*Acinetobacter baumannii* BJAB07104	blaOXA-23 (Beta-lactamase class D), tra system, tetA(B) island	NC_021726_R1	122327
AbaR25 (K51-65)	*Acinetobacter baumannii* K51-65	blaoxa-33 amikacin, gentamicin, tobramycin	JX481978	47230
delta-AbaR25	*Acinetobacter baumannii* K51-74	str, tet resistance	JX481979	41408
AbaR26	*Acinetobacter baumannii* BJAB0868	blaOXA-23 (Beta-lactamase class D), tra system, tetA(B) island	NC_021729_R1	121136
AbaR4a	*Acinetobacter baumannii* LT-3	MEM, IPM, TZP, CAZ, AN, CIP, PIP, blaoxa-66, EC2	JN129845	17996

REIs	Host strain	Function	GenBank Accession	Length
AbaR4b	*Acinetobacter baumannii* LT-11	MEM, IPM, TZP, SAM, CAZ, GM, AN, CIP, PIP, blaoxa-66, EC2	JN129846	7090
AbaR4c	*Acinetobacter baumannii* LT-V1	MEM, IPM, TZP, SAM, CAZ, GM, AN, CIP, PIP, blaoxa-66, EC2	JN129847	4052
AbaR4d	*Acinetobacter baumannii* 1656-2	beta lactamase (blaPER-1), aminoglycoside, sulfonamides	NC_017162_R1	34541
Tn6166	*Acinetobacter baumannii* RUH134	Resistance to streptomycin, tetracycline, sulfonamide piperacillin, gentamicin, co-trimoxazole, spectinomycin, kanamycin, and neomycin; KL9 (O antigen)	JN247441_R1	17630
Tn6167	*Acinetobacter baumannii* A91	Resistance to carbapenem, sulfonamide, streptomycin, and tetracycline; KL2 (O antigen)	JN968483_R1	37068

Some REIs, such as AbaR4 and Tn6167, confer resistance to carbapenems, a critical group of antibiotics for treating infections caused by *A. baumannii*. Several REIs, including AbaR22, Tn6166, and delta-AbaR25, exhibit antibiotic resistance to aminoglycosides, tetracyclines, sulfonamides, and beta-lactams. The lengths of the REIs vary, ranging from 705 bp (AbaR3) to 122,327 bp [AbaR25 (BJAB07104)]. The larger size may be related to the ability to carry multiple resistance genes. The REIs were identified across various *A. baumannii* strains, highlighting the diversity and potential spread of resistance factors among strains. Many REIs (AbaR3, AbaR14, and AbaR17) belong to "European Clone I", indicating the prevalence of these clones in antibiotic resistance transmission.

Genomic island identification using IslandViewer

Samples such as E-31AS (30 GIs) and E-43PH (28 GIs) exhibit the highest number of genomic islands in Figure 2, indicating a richness in genetic factors that may be related to antibiotic resistance and genetic diversity. In contrast, samples N-34BE and N-55UR have only 12 GIs, suggesting a lower capacity to accumulate diverse genetic factors. Samples like E-31AS, E-43PH, and E-25PH show significant GI counts, potentially reflecting greater adaptability and higher antibiotic resistance in these bacterial strains. Several other samples (S-101PH, E-16BL, and N-31BL) had GI counts ranging from 18 to 24, indicating that diversity and adaptability continue to exist within the bacterial population.

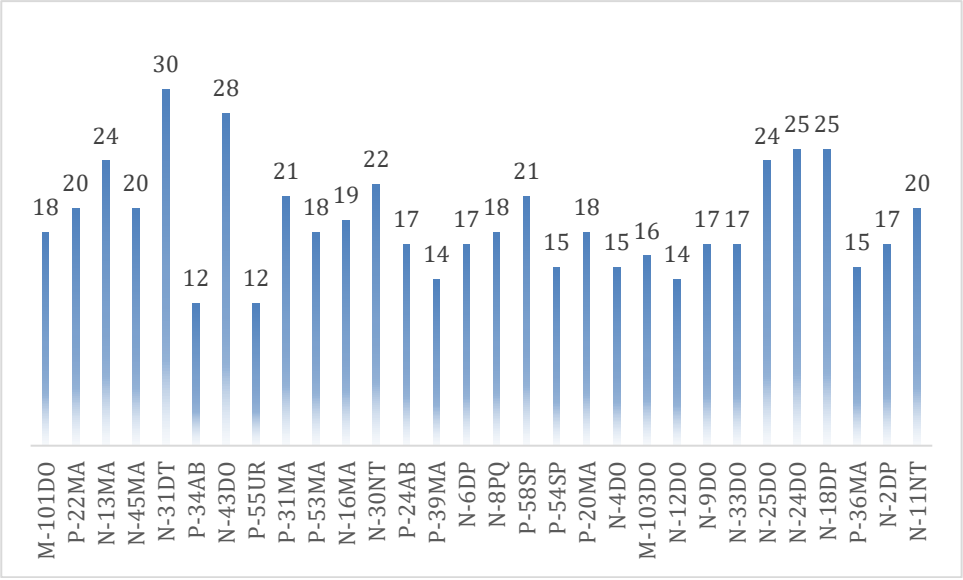

Figure 2. Summary of predicted Genomic islands by IslandViewer.

DISCUSSION

The results of the study show that many samples contain resistance genes such as *blaOXA-23, blaOXA-91*, and *msr(E)* at high levels. Similarly, the study by Ibrahim *et al.* on multidrug-resistant *A. baumannii* indicated the presence of MDR strains within the bacterial community, suggesting that resistance genes could be spread among bacteria via plasmids (Ibrahim *et al.,* 2021). This aligns with the simultaneous presence of multiple resistance genes in many samples, indicating the potential for gene transmission and accumulation among strains. Furthermore, Hamidian *et al.* have reported an outbreak of carbapenem-sensitive *A. baumannii* in Australia caused by plasmids carrying resistance genes, highlighting the rise of multidrug-resistant strains, particularly those with *blaOXA* and *msr(E)*, and suggesting that this outbreak may be related to environmental factors in hospitals (Hamidian *et al*., 2021). These findings emphasize the urgency of monitoring and controlling antibiotic-resistant infections in clinical settings.

In the study by Wang *et al.,* it was found that isolates carrying the *tet(Y)* gene were susceptible to tigecycline pressure and exhibited reduced sensitivity to tigecycline. A plasmid carrying *tet(Y)* was stably maintained in host strains (Wang *et al*., 2022). This finding is crucial since tigecycline is one of the last treatment options for infections caused by resistant bacteria. The presence of *tet(Y)* could diminish treatment efficacy and increase the risk of uncontrolled infections. The location of this gene on a plasmid suggests an easy transmission to other bacterial strains, potentially leading to a rapid increase in resistance within communities and hospitals. Many samples in the dataset (e.g., N-53BL with 1,336 resistance genes) demonstrated the presence of various resistance genes, including *blaOXA*, *msr(E),* and *sul*. This suggests that *A. baumannii* can develop and maintain multiple resistance mechanisms. The study also indicates that some strains

harboring antibiotic resistance genes may be plasmid-encoded, with the presence of *ant(2'')-Ia, tet(B)*, and *tet(39)* potentially conferring resistance to tigecycline. This is consistent with findings by Ha *et al*. (2021) regarding *ant(2'')-Ia* and *tet(39)*, indicating that resistance genes may be disseminated via plasmids, enhancing the resistance capabilities of strains.

Resistance islands such as AbaR25 and AbaR4 contain resistance genes *blaOXA-23* and *blaOXA-66*, which are associated with beta-lactam resistance. Additionally, high-resistance *A. baumannii* strains show the presence of resistance genes like *strA* and *tetA(B)* in AbaR22. The resistance element AbaR4 indicates resistance to multiple antibiotics, including carbapenems. These results align with resistance islands reported in the study by Naderi *et al.* (2023), suggesting that *A. baumannii* can harbor a significant number of resistance genes, leading to robust and challenging treatment resistance. This highlights that the spread of these resistant strains often occurs through genetic islands like AbGRI, contributing to increased resistance. AbGRI3 is the most frequently identified resistance island, present in all 19 isolates, followed by AbGRI1 (15 isolates; 78.9%) and AbGRI2 (three isolates; 15.8%). Notably, AbGRI was identified in one A. *baumannii* strain isolated from a medical device used in an ICU treating COVID-19 patients. Furthermore, novel structures of resistance islands AbGRI1 and AbGRI3 were discovered in this study, marking the first report of these structures (Naderi *et al*., 2023).

CONCLUSION

The study not only has the identification of antibiotic resistance genes underlying MDR and XDR in *A. baumannii* been conducted, but WGS has also been utilized in the current study to identify resistance islands carried by clinical isolates of *A. baumannii*. Further analysis of the mechanisms and pathways of gene transmission is essential to enhance management strategies and effective treatment options.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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