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Genotyping and Molecular Analysis of Carbapenem-Resistant *Acinetobacter baumannii*: Correlations Between Antibiotic Resistance and Virulence Genes

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Abstract

Background: *Acinetobacter baumannii* is frequently implicated in infections that originate within hospitals and other medical facilities worldwide, with high mortality rates driven by multidrug resistance and virulence.

Objectives: The objective of this research is to investigate the patterns of antibiotic resistance, the presence of virulence-associated genes, and the genetic variability among clinical isolates of *A. baumannii*, focusing on carbapenem-resistant strains collected from healthcare settings in Tehran, Iran.

Methods: We collected 40 samples of *A. baumannii* from different types of infections in the hospital. We tested how these bacteria respond to various antibiotics using standard lab methods. We also looked for specific genes that make the bacteria resistant to carbapenems and those that might help them cause infections. To understand how related these bacteria are to each other, we used a genetic fingerprinting technique called enterobacterial repetitive intergenic consensus (ERIC)-PCR.

Results: Our results were concerning: 87.5% of isolates were resistant to multiple antibiotics. Each isolate had genes encoding carbapenemases, mainly *blaOXA*-51, *blaOXA*-24, and *blaOXA*-58. Additionally, 95% of isolates carried genes linked to their ability to cause disease, such as *recA*, *ompA*, and *lipA*. The genetic analysis showed that while some bacteria were closely related (suggesting spread within the hospital), others were more diverse, indicating multiple sources or ongoing evolution.

Conclusions: This study sheds light on the worrying presence of highly resistant and potentially dangerous *Acinetobacter* isolates in Tehran's hospitals. The mix of resistance and virulence genes, along with the genetic diversity, points to a need for stronger antibiotic stewardship, better infection control, and continuous monitoring to stop these bacteria from spreading and causing harm.

Keywords: *Acinetobacter baumannii*, Antibiotic Resistance, Carbapenemase Genes, Virulence Genes, ERIC-PCR

1. Background

Acinetobacter baumannii has emerged as a significant opportunistic pathogen in hospitals, particularly in the context of hospital-acquired infections. This gram-negative bacterium causes various clinical infections, including pneumonia, bloodstream infections, urinary tract infections (UTIs), and wound infections (1). The increasing prevalence of multidrug-resistant (MDR) strains of *A. baumannii* poses a significant challenge to healthcare providers, contributing to higher morbidity and mortality rates among infected patients (2). The alarming rise in extensively drug-resistant (XDR) strains underscores the urgent need for ongoing surveillance and research into resistance and virulence mechanisms.

Antibiotic resistance in *A. baumannii* is multifactorial. Key resistance determinants include various β-lactamases, such as OXA-type enzymes (e.g., *blaOXA*-24, *blaOXA*-51, *blaOXA*-58, *blaOXA*-143), which render β-lactam antibiotics ineffective (1). Additionally, metallo-β-

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lactamases like VIM and NDM further complicate treatment by hydrolyzing a wide range of β-lactams, including carbapenems (3). These resistance genes enable *A. baumannii* to thrive in environments with high antibiotic pressure, making it a significant challenge in clinical settings. The functions of these resistance genes are summarized in Appendix 1 in Supplementary File.

Beyond antibiotic resistance, *A. baumannii*'s virulence factors are key to its pathogenicity. Virulence genes like *ompA*, *bap*, *pbpG*, *basD*, *bfmR*, *recA*, *lipA*, *abeD*, *surA*, *csuA*, and *pld* enable the bacterium to adhere to host tissues and form biofilms (1). Biofilm formation is particularly relevant in nosocomial infections as it shields bacterial communities from the immune system and antimicrobial agents (4). The presence of these virulence factors is often associated with disease severity and treatment outcomes. The specific roles of these virulence genes are also detailed in Appendix 1 in Supplementary File.

Recent research has started exploring the relationship between specific virulence genes and antibiotic resistance profiles in clinical isolates of *A. baumannii*. Understanding these connections can shed light on the genetic factors influencing clinical outcomes. For example, certain virulence genes have been linked to higher levels of resistance to critical antibiotics (5). These findings emphasize the importance of studying resistance and virulence factors together. In Tehran, Iran, the growing prevalence of antibiotic-resistant *A. baumannii* is particularly concerning due to the increasing number of nosocomial infections. The healthcare system faces significant challenges in managing these infections because of limited treatment options and the high costs associated with extended hospital stays (1).

2. Objectives

This study aims to assess the relationship between patterns of antibiotic resistance and the occurrence of virulence genes in clinical isolates of *A. baumannii* from hospitals in Tehran. By using genotyping techniques like enterobacterial repetitive intergenic consensus (ERIC)-PCR and testing susceptibility to various antibiotics — including tigecycline (TIG), aztreonam (AZT), levofloxacin (LEV), tobramycin (TOB), imipenem (IMP), polymyxin B (PMB), piperacillin/tazobactam (TZP), ceftazidime (CAZ), ciprofloxacin (CIP), gentamicin (GEN), and amikacin — we aim to provide data that can guide effective treatment protocols and infection prevention measures. In conclusion, understanding the relationship between virulence factors and antibiotic resistance in *A. baumannii* is crucial to addressing the public health challenges posed by this pathogen. This research seeks to close knowledge gaps and offer valuable insights for clinicians and public health professionals in managing infections caused by carbapenemase-resistant *A. baumannii*.

3. Methods

3.1. Overview of Research Methodology and Ethics Approval Process

This study was adopted to explore the relationship between antibiotic resistance patterns, virulence genes, and genotypes in clinical isolates of *A. baumannii*. The research focused on samples collected from hospitalized patients in Tehran, Iran, between April and August 2024.

3.2. Sample Collection and Bacterial Identification

A total of 40 non-duplicative *Acinetobacter* isolates were collected from a range of specimen types, including blood, urine, and tracheal samples (Appendix 2 in Supplementary File). The collected specimens were streaked onto MacConkey and blood agar plates and incubated at 37°C overnight. After incubation, isolated colonies were identified using standard microbiological techniques, including gram staining and a series of biochemical tests, such as catalase, oxidase, Simmons citrate agar, TSI, MRVP, urease, and indole tests. To confirm the identity of the isolates at the molecular level, PCR amplification of the *blaOXA*-51 gene was performed (6, 7).

3.3. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility of the isolates was assessed using the Kirby-Bauer disk diffusion assay, following the 2021 Clinical and Laboratory Standards Institute (CLSI) recommendations. The antibiotics evaluated included CAZ (10 μg), IMP (10 μg), meropenem (MEM, 10 μg), AZT (30 μg), LEV (5 μg), TOB (10 μg), TZP (100/10 μg), CIP (5 μg), GEN (10 μg), amikacin (AK, 30 μg), TIG (15 μg), PMB (10 μg), and colistin (COL, 10 μg). Quality control procedures involved the use of *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 as standard reference strains. The MDR was defined as resistance to three or more distinct classes of antibiotics (6).

3.4. Detection of Carbapenemase, Virulence Factors, and blaOXA-51 Genes Using Multiplex PCR Technique

3.4.1. Bacterial DNA Extraction

DNA samples from the clinical strains under investigation were extracted using the Genomic Mini kit from A&A Biotechnology, Gdansk, Poland, according to the manufacturer's instructions. The extracted DNA was then stored at -20°C until further analysis.

3.5. Detection of Carbapenemase Genes

The detection of seven carbapenemase-encoding genes — *blaOXA*-24, *blaOXA*-51, *blaOXA*-58, SIM, VIM, NDM, and KPC — was conducted in 40 *Acinetobacter* isolates using multiplex PCR with eight pairs of primers (6). The first PCR reaction targeted the genes *blaOXA*-24, *blaOXA*-51, *blaOXA*-58, and *blaOXA*-143, while the second reaction focused on SIM, VIM, NDM, and KPC. The nucleotide sequences used as primers are listed in Table 1, while the configurations of the PCR assays are provided in Table 2. Table 3 summarizes the distinct thermal cycling parameters applied for each PCR protocol.

3.6. Detection of Virulence Genes

In this study, eleven virulence genes were identified in 40 *Acinetobacter* isolates using multiplex PCR with specific gene primers (10-12). These genes are associated with various functions, including biofilm formation (*ompA*, *csuA*, *bap*, *bfmR*), penicillin-binding protein G (*pbpG*), siderophore production (*basD*), stress response mechanisms (*recA*), toxin synthesis (*lipA*), host cell apoptosis (*abeD*), surface antigen protein A (*surA*), and phospholipase D (*pld*). Reaction III included primers for the genes *surA*, *pld*, *basD*, *csuA*, *lipA*, *bap*, *bfmR*, and *recA*, while Reaction IV focused on the genes *ompA*, *pbpG*, and *abeD*. The quantities and sequences of the forward and reverse primers are presented in Table 1, while Table 2 provides a description of the PCR setups used. Table 3 specifies the reaction parameters applied for each PCR assay.

3.7. Sequencing

The multiplex PCR products of identified virulence factors and carbapenemase genes in this study were purified for sequencing. These samples, along with the primers used, were sent to Niagenenoor Corporation in Tehran, Iran. The sequences were subsequently analyzed using the online BLAST tool available in the NCBI database (https://www.ncbi.nlm.nih.gov/BLAST/) and have been deposited under accession numbers PV275083-PV275091.

3.8. Enterobacterial Repetitive Intergenic Consensus PCR Genotyping

The ERIC-PCR analysis was performed on 40 clinical *Acinetobacter* isolates utilizing the ERIC-F (ATGTAAGCTCCTGGGGATTCAC) and ERIC-R (AGTAAGTGACTGGGGTGAGCG) primers (13). The specifics of the PCR setups and the reaction parameters are provided in Tables 2, and 3, respectively. Following amplification, samples were kept at -4°C. PCR products were resolved on a 1.0% (w/v) agarose gel, visualized under ultraviolet illumination, and images were captured. The resulting ERIC-PCR banding patterns were analyzed using Quantity One software (Bio-Rad, USA). For phylogenetic assessment, a UPGMA dendrogram was constructed with NTsys-pc version 2.1. Isolates exhibiting more than 55% similarity in their banding profiles were grouped within the same cluster.

3.9. Statistical Analysis

For statistical analysis, SPSS (version 25) and GraphPad Prism were employed. Descriptive statistics, including frequency and relative frequency, were used to characterize antibiotic resistance and susceptibility patterns. Since the outcome variable — resistant or sensitive — was categorical, the chi-square test was applied to examine the association between gene presence and antibiotic resistance, with a significance threshold set at 0.05. To further assess the relationship between specific gene profiles and resistance or sensitivity to different antibiotics, Spearman's correlation coefficient was calculated. The findings were visualized using a correlation matrix plot to provide an integrated view of these associations.

4. Results

4.1. Antibiotic Resistance of Isolates

Among the 40 isolates tested, 5 (12.5%) were susceptible to all antibiotics, while the remaining 35 (87.5%) isolates exhibited multiple antibiotic resistance profiles (Appendix 3 and 4 in Supplementary File). Of the tested isolates, 33 (82.5%) demonstrated MDR, 32 (80%) were resistant to six classes of antibiotics, and one (2.5%) was resistant to all tested antibiotics. Ten antibiotics, including ATM (80%), LVX (80%), TOB (80%), IMP (87.5%), TZP (87.5%), CAZ (87.5%), MEM (87.5%), CIP (87.5%), GEM (87.5%), and AMK (87.5%), showed resistance rates of 80% or higher. The TIG demonstrated a resistance rate of 30% among the isolates. In contrast, PMB and COL were found to be the most active agents, exhibiting the lowest resistance rates at 2.5% and 5%, respectively.

Table 1. Multiplex PCR Primer for Detection of the Carbapenemase Genes and Virulence Genes

Reaction; Primer Names	Sequence	Length	Reference
I			
blaOXA-51	F: 5'- TAATGCTTTGATCGGCCTTG-3'; R: 5'- TGGATTGCACTTCATCTTGG-3'	353	(8)
blaOXA-58	F: 5'- AAGTATTGGGGCTTGTGCTG-3'; R: 5'- CCCCTCTGCGCTCTACATAC-3'	599	(8)
blaOXA-24	F: 5'- GGTTAGTTGGCCCCCTTAAA-3'; R: 5'- AGTTGAGCGAAAAGGGGATT-3'	249	(8)
II			
VIM	F: 5'- GATGGTGTTTGGTCGCATA-3'; R: 5'- CGAATGCGCAGCACCAG-3'	390	(8)
KPC	F: 5'- CGTCTAGTTCTGCTGTCTTG-3'; R: 5'- CTTGTCATCCTTGTTAGGCG-3'	798	(9)
NDM	F: 5'- GGTTTGGCGATCTGGTTTTC-3'; R: 5'- CGGAATGGCTCATCACGATC-3'	621	(9)
SIM	F: 5'-TACAAGGGATTCGGCATCG-3'; R: 5'-TAATGGCCTGTTCCCATGTG-3'	271	(8)
III			
surA	F: 5'- GATGCGATTGCACCTGGAAC -3'; R: 5'- TTGACGTGCCATACGCTCTT -3'	822	(10)
pld	F: 5'- GCTGTGGCTTTGACAGGTTG -3'; R: 5'- TAGCGCAAACGGTGTTGTTG -3'	695	(10)
basD	F: 5'- TGCTGTTCGTTCTTTTGGCG -3'; R: 5'- GTTGAGTTTGAGCGCCGATG -3'	517	(10)
csuA	F: 5'- TGGTGAAGCTACCACAGGTT -3'; R: 5'- ACGACTACCATCATGGGCTG -3'	322	(10)
lipA	F: 5'- ATCTGAACCTGGACGCATCG -3'; R: 5'- TTGGACCCTGATGAACCACG -3'	300	This study
bap	F: 5'- TGAAAGTGGCTGCCAGTGAT -3'; R: 5'- TCTGCGTCAGCGTCACTATC -3'	223	(10)
bfmR	F: 5'- ACCGATGGTAACCGTGCAAT -3'; R: 5'- TCTGCACCCATTTCCAGACC -3'	194	(10)
recA	F: 5'- CACGCCCTAGACCCTCAATA -3'; R: 5'- CGATTAAATCAATTGCGCCT -3'	136	(11)
IV			
ompA	F: 5'- GCTGGTGTTGGTGCTTTCTG -3'; R: 5'- TCGGTTGATCCCAAGCGAAA -3'	490	(10)
abeD	F: 5'- TTGGCTTGCCAAATGACGTG -3'; R: 5'- TCTTGACGACTAACCGCACC -3'	247	(11)
pbpG	F: 5'- TGGATGCGCAAACAGGTGAA -3'; R: 5'- GGTCGGTGTGGTTGAGAACT -3'	467	(10)

Table 2. The PCR Systems

Reaction	Master Mix	Template DNA	Primer Forward	Primer Reverse	Total Volume
I - IV	2X (12.5 µL)	50 ng (25 µL)	10 pmol (3.3 µL)	10 pmol (3.3 µL)	25 µL
ERIC-PCR	2X (10 µL)	50 ng (3 µL)	10 pmol (0.5 µL)	10 pmol (0.5 µL)	20 µL

The clinical isolates showed the highest resistance rate (35 isolates, 87.5%) to IMP, TZP, CAZ, MEM, CIP, GEN, and amikacin, followed by ATM, LEV, and TOB (18 isolates, 29.0%). Among the MDR isolates, five unique antibiotic resistance patterns (RP2 - RP6) were identified (Figure 1), with each isolate showing resistance to at least seven out of the thirteen antibiotics tested in this investigation. The most prevalent profile, RP4, was found in 20 (50%) isolates.

4.2. The Presence of Carbapenemase and Virulence Genes

The PCR screening of the 40 clinical isolates demonstrated that all isolates (100%) carried carbapenemase genes, while virulence genes were detected in 38 isolates (95%), as shown in Figure 2 and Appendix 5, 6 in Supplementary File. Of the 40 isolates producing carbapenemases, three distinct carbapenemase genes were detected: The *blaOXA*-24 in 29 (72.5%) isolates, *blaOXA*-51 in 34 (85%) isolates, and *blaOXA*-58 in 28 (70%) isolates. Notably, the SIM, VIM, NDM, and KPC genes were undetected in any of the isolates. The classification of the 40 strains harboring carbapenemase genes revealed four distinct categories. Six isolates were identified as carrying either the *blaOXA*-24 or *blaOXA*-51 gene, while 23 (57.5%) isolates possessed both genes. Furthermore, 10 (25%) isolates were found to carry *blaOXA*-24, *blaOXA*-51, and *blaOXA*-58 simultaneously, and 2 (5%) isolates contained both *blaOXA*-51 and *blaOXA*-58.

Among the 38 isolates harboring virulence genes, eight different virulence determinants were detected. Each of these isolates possessed at least two virulence genes. However, none of the isolates demonstrated the presence of all three virulence genes, which are *abeD*, *pbpG*, and *basD*. Among the 38 clinical isolates, *recA* and *ompA* exhibited the highest detection rates, each at

Table 3. The PCR Conditions

Variables	Permutability	Denaturation	Annealing	Extension	Cycle Number	Re-extension
Reaction	94°C, 10 min	94°C, 30 s		72°C, 30 s	35	72°C, 10 min
I			59°C, 60 s			
II			57°C, 60 s			
III			59°C, 60 s			
IV			57°C, 60 s			
ERIC-PCR	94°C, 10 min	94°C, 60 s	56°C, 60 s	72°C, 60 s	35	72°C, 10 min

Abbreviation: ERIC, enterobacterial repetitive intergenic consensus.

97.36%. They were followed by lipA at 84.21%, bap at 78.94%, pld at 73.68%, and surA at 71.05%. The detection rate for *bfmR* was 55.26%, while *cusA* had the lowest rate at 42.10%. Among the 14 combinations of 11 positive genes, 'VR2' exhibited the highest detection rate at 40%. The genes *recA*, *ompA*, and *blaOXA*-51 were commonly detected together in 35 isolates, with a simultaneous detection rate of 87.5%, and were part of combinations like VR1, VR2, and others. Notably, *recA*, *ompA*, *lipA*, and *blaOXA*-51 were the most frequently co-occurring genes in these genetic patterns.

4.3. Statistical Analysis of Genes and Resistance Profiles

The findings of this study on antibiotic resistance revealed that for 76.92% (10 out of 13) of the tested antibiotics, resistance rates exceeded 80%. However, three antibiotics — TIG, COL, and PMB — demonstrated lower resistance rates of 30%, 5%, and 2.5%, respectively. A comprehensive summary of these results is provided in Table 4 and Figure 3. Furthermore, the study examined the relationship between gene presence and resistance to various antibiotics, as well as the correlation between gene presence and MDR (Appendix 6 - 8 in Supplementary File). Notably, isolates carrying the *lipA* gene exhibited significantly higher resistance to TIG compared with those lacking this gene ($P < 0.05$). While variations in resistance levels were observed concerning the presence or absence of other genes, these differences were not statistically significant ($P > 0.05$).

Additionally, correlation coefficients were calculated to assess the association between gene presence and antibiotic resistance. The results indicated that while certain genes showed a positive correlation with resistance, others displayed a negative correlation. Among these, only the association between the *lipA* gene and resistance to TIG was statistically significant ($P < 0.05$). A detailed presentation of these findings is available in Table 5, Figure 4, and Appendix 9 - 21 in Supplementary File.

4.4. Enterobacterial Repetitive Intergenic Consensus PCR Analysis

Analysis of ERIC-PCR banding patterns from 40 *Acinetobacter* isolates identified 14 unique clusters and 37 genotypes at a 55% similarity threshold (Figure 5). The diversity of these patterns, reflected by a Simpson's Index of Diversity (SID) of 0.50, indicates considerable genetic heterogeneity among the isolates. Of the clusters, nine were categorized as clonal types and five as monotypes. Clonal clusters (A5, A6, A7, A8, A10, A11, A12, A13, and A14) comprised 32 genotypes and 35 isolates, while each monotype cluster (A1, A2, A3, A4, and A9) contained a single genotype and isolate.

Among the clonal groups, cluster A10 exhibited the greatest diversity, encompassing seven genotypes and eight isolates. Cluster A8 included four genotypes and six isolates; clusters A12 and A14 each had four genotypes and four isolates; and clusters A5, A6, and A7 each consisted of three genotypes and three isolates. The remaining clonal clusters, A11 and A13, contained two genotypes and two isolates each.

5. Discussion

This study on the genotyping and analysis of antibiotic resistance and virulence gene correlations in clinical *Acinetobacter* isolates from Tehran, Iran, provides crucial insights into the intricate interplay between genetic diversity, antibiotic resistance patterns, and virulence factors in these pathogens. Recent research has underscored the emergence of carbapenem-resistant *Acinetobacter baumannii* (CRAB) as a significant global threat, with infections caused by these strains associated with high mortality rates (11, 14). Understanding these relationships is vital for developing effective infection control strategies.

The main aim of this research was to investigate the genetic characteristics of clinical *Acinetobacter* isolates collected in Tehran, Iran, and to elucidate the

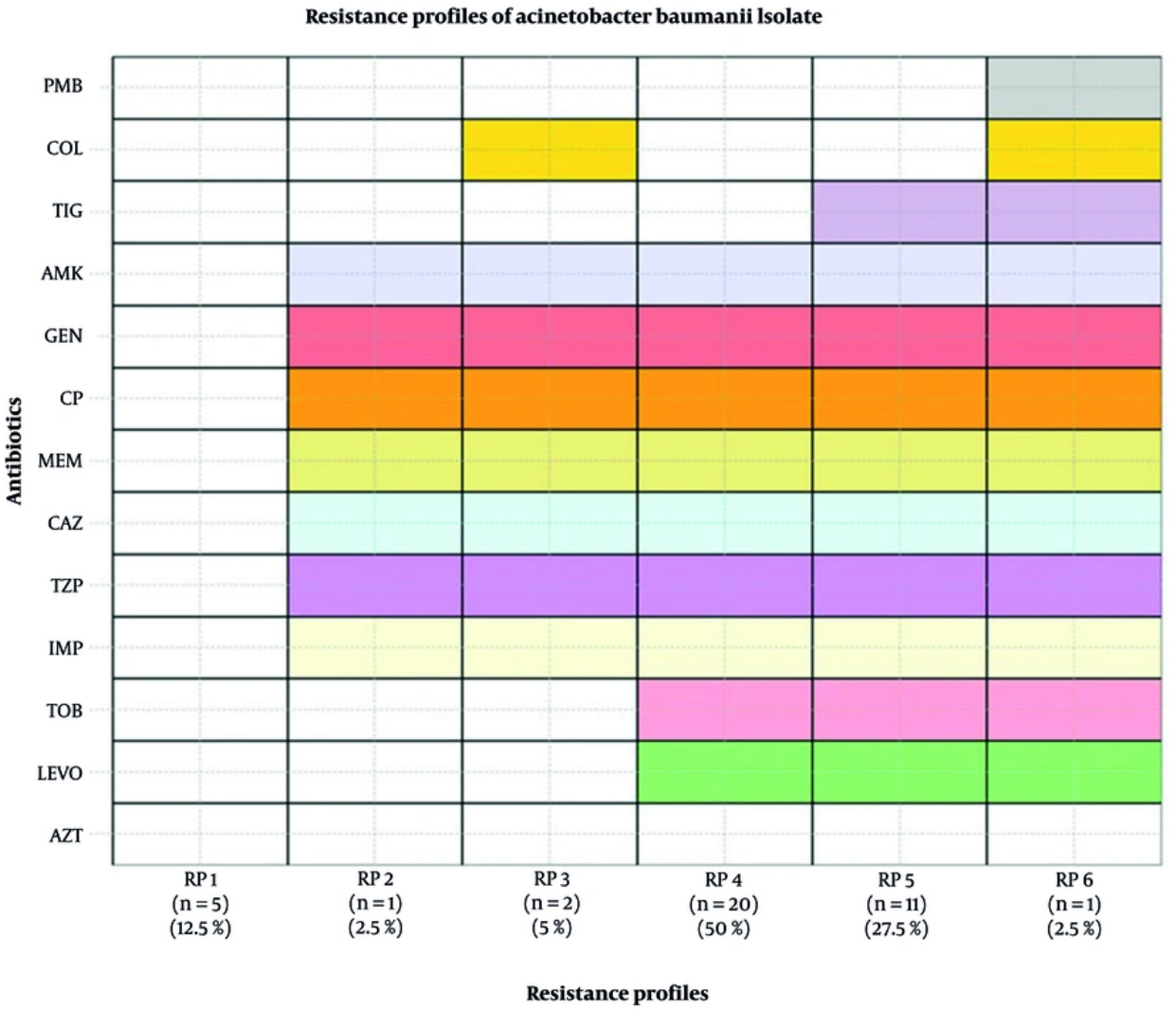

Figure 1. Resistance profiles of *Acinetobacter baumannii* isolates against tested antibiotics: The heatmap displays resistance patterns of 40 clinical *A. baumannii* isolates grouped into six distinct resistance profiles (RP1–RP6). Rows represent antibiotics tested: Polymyxin B (PMB), colistin (COL), tigecycline (TIG), amikacin (AMK), gentamicin (GEN), ciprofloxacin (CP), meropenem (MEM), ceftazidime (CAZ), piperacillin/tazobactam (TZP), imipenem (IMP), tobramycin (TOB), levofloxacin (LEV), and aztreonam (AZT). Columns correspond to resistance profiles, with the number of isolates (n) and percentage of total isolates shown below each profile. RP1 (n = 5, 12.5%): Isolates susceptible to all tested antibiotics. RP2 (n = 1, 2.5%): Resistant to IMP, TZP, CAZ, MEM, CP, GEN, and AMK. RP3 (n = 2, 5%): Resistant to IMP, TZP, CAZ, MEM, CP, GEN, AMK, and COL. RP4 (n = 20, 50%): Multidrug-resistant (MDR) isolates showing resistance to LEV, TOB, IMP, TZP, CAZ, MEM, CP, GEN, and AMK. RP5 (n = 11, 27.5%): Resistance to LEV, TOB, IMP, TZP, CAZ, MEM, CP, GEN, AMK, and TIG. RP6 (n = 1, 2.5%): Resistance to LEV, TOB, IMP, TZP, CAZ, MEM, CP, GEN, AMK, TIG, COL, and PMB. Colors indicate resistance status per antibiotic per profile; shaded cells represent resistance, and white cells indicate susceptibility (total isolates: N = 40).

correlations between antibiotic resistance patterns and virulence gene expression. By assessing these factors, we aimed to enhance the understanding of how genetic diversity influences both antimicrobial resistance mechanisms and virulence factor expression within this clinical context.

Recent studies have demonstrated that CRAB isolates exhibit extensive drug resistance, largely due to carbapenem-hydrolyzing enzymes such as *blaOXA*-23, which are widely distributed across different regions (15). Additionally, specific virulence genes — including *bap*, *ompA*, and others — have been identified as key contributors to enhanced pathogenicity (14). Our research sought to examine similar trends in Iranian

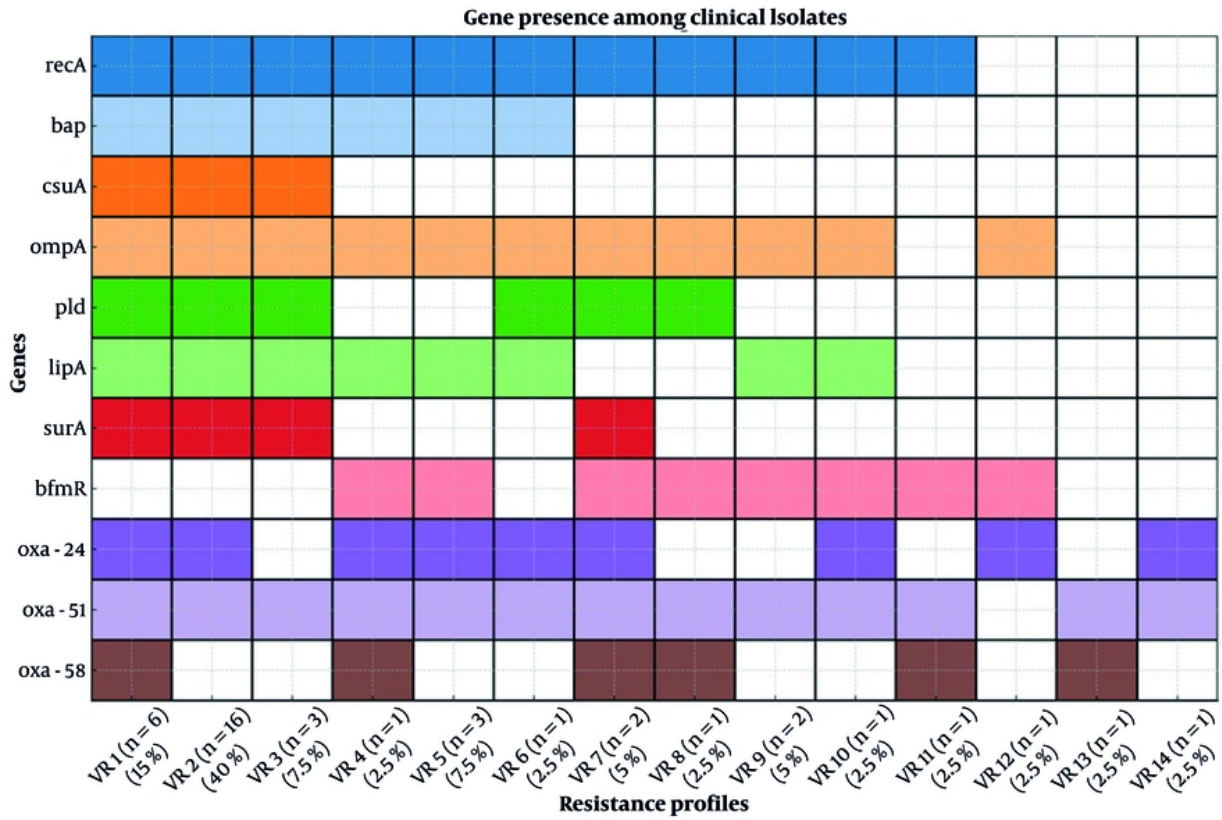

Figure 2. Combined virulence and resistance gene profiles (VR1–VR14) among clinical *Acinetobacter baumannii* isolates: This figure presents 14 distinct combined gene profiles (VR1 through VR14), each representing a unique pattern of presence or absence of selected virulence genes (*recA*, *bap*, *csuA*, *ompA*, *pld*, *lipA*, *surA*, and *bfmR*) along with key resistance genes (including *blaOXA*-58, *blaOXA*-51, and *blaOXA*-24). Each VR profile reflects a specific combination of these virulence and resistance determinants, highlighting the genetic diversity and potential pathogenicity variations among the isolates. The presence of each gene is indicated by colored squares in the heatmap, whereas absence is shown by blank cells.

healthcare settings by analyzing the genetic composition of local *Acinetobacter* isolates and assessing their potential implications for public health. The investigation into antibiotic resistance among clinical *Acinetobacter* isolates from Tehran, Iran, revealed an alarming prevalence of MDR strains. Of the 40 isolates analyzed, 87.5% displayed resistance to multiple antibiotics, 82.5% were classified as MDR, and one isolate exhibited resistance to all tested antimicrobial agents. These findings are consistent with recent studies highlighting *A. baumannii* as a critical global health threat due to its exceptional ability to develop resistance against multiple antibiotic classes (16, 17).

The high resistance rates observed against carbapenems (e.g., IMP and MEM), fluoroquinolones (e.g., CIP), and aminoglycosides (e.g., GEN and amikacin) align with reports from other regions (18, 19). For instance, a study conducted in Saudi Arabia found that nearly all *A. baumannii* isolates were MDR, exhibiting substantial resistance to β-lactams and fluoroquinolones (16). Similarly, an Iranian study reported high resistance rates among burn center isolates, particularly against CIP and CAZ (18). However, our findings also underscore the relative effectiveness of certain antibiotics, such as PMB and COL, which demonstrated lower resistance rates compared to other antimicrobial agents. The widespread distribution of MDR strains highlights the urgent need for robust antimicrobial stewardship programs in Iranian healthcare facilities. Implementing continuous surveillance that integrates molecular diagnostics with epidemiological data will be crucial in devising effective infection control strategies tailored to the local epidemiological landscape.

The analysis of carbapenemase and virulence genes among clinical *Acinetobacter* isolates provided

Table 4. Prevalence and Relative Frequency of Antibiotic Resistance/Susceptibility Status [a,b,c,d]

Variables	Values	Cumulative Percent
TIG		
Susceptible	28 (70.0)	70.0
Resistance	12 (30.0)	100.0
Total	40 (100.0)	-
AZT		
Susceptible	8 (20.0)	20.0
Resistance	32 (80.0)	100.0
Total	40 (100.0)	-
LEV		
Susceptible	8 (20.0)	20.0
Resistance	32 (80.0)	100.0
Total	40 (100.0)	-
TOB		
Susceptible	8 (20.0)	20.0
Resistance	32 (80.0)	100.0
Total	40 (100.0)	-
IMP		
Susceptible	5 (12.5)	12.5
Resistance	35 (87.5)	100.0
Total	40 (100.0)	-
PMB		
Susceptible	39 (97.5)	97.5
Resistance	1 (2.5)	100.0
Total	40 (100.0)	-
COL		
Susceptible	38 (95.0)	95.0
Resistance	2 (5.0)	100.0
Total	40 (100.0)	-
TZP		
Susceptible	5 (12.5)	12.5
Resistance	35 (87.5)	100.0
Total	40 (100.0)	-
CAZ		
Susceptible	5 (12.5)	12.5
Resistance	35 (87.5)	100.0
Total	40 (100.0)	-
MEM		
Susceptible	5 (12.5)	12.5
Resistance	35 (87.5)	100.0
Total	40 (100.0)	-
CIP		
Susceptible	5 (12.5)	12.5
Resistance	35 (87.5)	100.0
Total	40 (100.0)	-
GEN		
Susceptible	5 (12.5)	12.5
Resistance	35 (87.5)	100.0
Total	40 (100.0)	-
AMK		
Susceptible	5 (12.5)	12.5
Resistance	35 (87.5)	100.0
Total	40 (100.0)	-

Abbreviations: TIG, tigecycline; AZT, aztreonam; LEV, levofloxacin; TOB, tobramycin; IMP, imipenem; PMB, polymyxin B; COL, colistin; TZP, piperacillin/tazobactam; CAZ, ceftazidime; MEM, meropenem; CIP, ciprofloxacin; GEN, gentamicin; AMK, amikacin.

[a] Values are expressed as No. (%).

[b] Total number of isolates tested = 40.

[c] Susceptibility and resistance were determined according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

[d] Each antibiotic's total row confirms the number and percentage of isolates tested.

significant insights into the genetic determinants of antibiotic resistance and pathogenicity. All 40 isolates tested positive for carbapenemase genes, with *blaOXA*-51 being the most prevalent (85%), followed by *blaOXA*-24 (72.5%) and *blaOXA*-58 (70%). This finding aligns with previous studies reporting the predominance of OXA-type carbapenemases in various regions (20, 21). A particularly noteworthy finding in our study is the complete absence of metallo-β-lactamase (MBL) genes, including *blaSIM*, *blaVIM*, *blaNcorDM*, and *blaKPC*, in all isolates. This contrasts with reports from other regions where MBLs are increasingly implicated in carbapenem resistance (22, 23). The lack of MBL genes in our isolates suggests a distinct regional epidemiological pattern in Tehran, where carbapenem resistance appears to be predominantly driven by oxacillinase enzymes rather than MBLs.

This absence is significant for several reasons. First, MBL-producing *A. baumannii* strains often exhibit broader resistance profiles and pose greater therapeutic challenges (24). Their absence could imply that, despite high resistance rates, the carbapenem resistance mechanisms circulating in Tehran may be more predictable and potentially more amenable to targeted

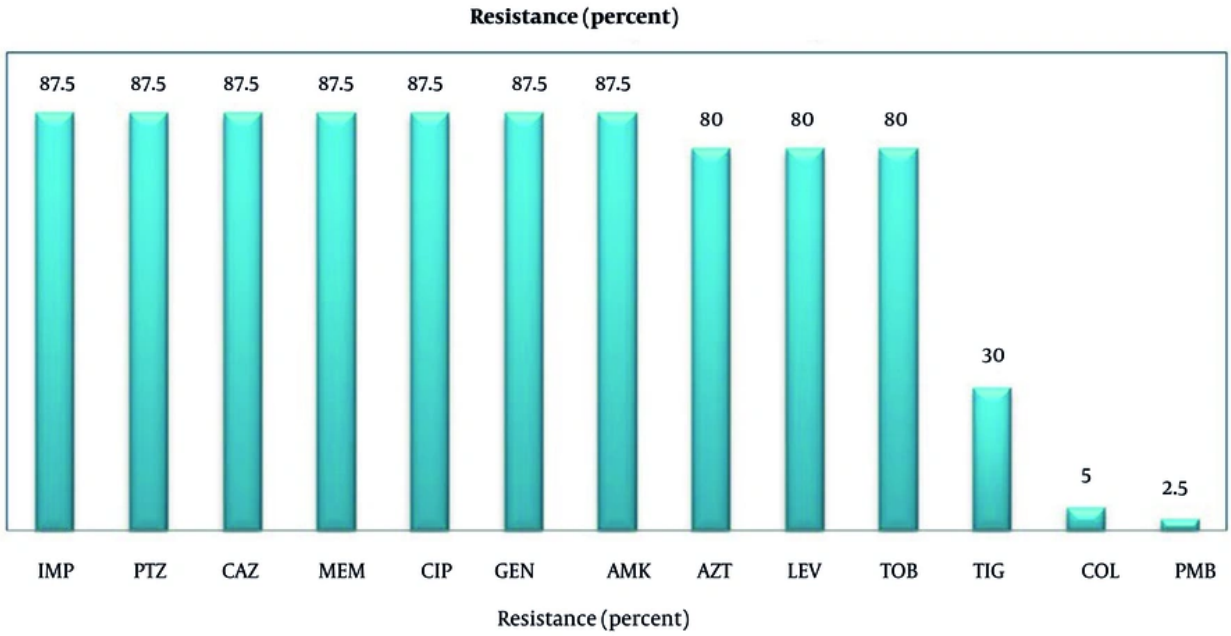

Figure 3. Relative frequency of antibiotic resistance among 40 clinical *Acinetobacter* isolates: This bar chart illustrates the proportion (%) of isolates categorized as resistant to each of the 14 antibiotics tested. Antibiotics are listed along the x-axis with their full names and standard abbreviations: Imipenem (IPM), piperacillin/tazobactam (TZP), ceftazidime (CAZ), meropenem (MEM), ciprofloxacin (CIP), gentamicin (GEN), amikacin (AMK), aztreonam (AZT), levofloxacin (LEV), tobramycin (TOB), tigecycline (TIG), colistin (COL), and polymyxin B (PMB). The y-axis represents the percentage of isolates. Resistance was determined using the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines (the total number of isolates tested was 40).

Table 5. Spearman Correlation Coefficient Between the Presence of Resistance/Virulence Genes and Antibiotic Resistance/Susceptibility in 40 Clinical *Acinetobacter baumannii* Isolates

Variables	*bla OXA-58*	*bla OXA-51*	*bla OXA-24*	*recA*	*bap*	*csuA*	*ompA*	*pld*	*abeD*	*pbpG*	*basD*	*bfmR*	*lipA*	*surA*
TIG	-0.19	-	0.18	0.18	0.12	-0.08	0.18	-0.16	-	-	-	0.07	0.32	-0.12
AZT	0.05	-	-0.14	-0.14	-0.14	-0.22	-0.14	-0.19	-	-	-	0.15	-0.25	-0.21
LEV	0.05	-	-0.14	-0.14	-0.14	-0.22	-0.14	-0.19	-	-	-	0.15	-0.25	-0.21
TOB	0.05	-	-0.14	-0.14	-0.14	-0.22	-0.14	-0.19	-	-	-	0.15	-0.25	-0.21
IMP	0.24	-	-0.10	-0.10	-0.04	-0.15	-0.10	-0.08	-	-	-	0.09	-0.18	-0.10
PMB	-0.10	-	0.04	0.04	0.09	-0.13	0.04	0.10	-	-	-	0.15	0.08	0.11
COL	0.10	-	0.06	0.06	0.13	0.04	0.06	0.15	-	-	-	-0.01	0.11	0.15
TZP	0.24	-	-0.10	-0.10	-0.04	-0.15	-0.10	-0.08	-	-	-	0.09	-0.18	-0.10
CAZ	0.24	-	-0.10	-0.10	-0.04	-0.15	-0.10	-0.08	-	-	-	0.09	-0.18	-0.10
MEM	0.24	-	-0.10	-0.10	-0.04	-0.15	-0.10	-0.08	-	-	-	0.09	-0.18	-0.10
CIP	0.24	-	-0.10	-0.10	-0.04	-0.15	-0.10	-0.08	-	-	-	0.09	-0.18	-0.10
GEN	0.24	-	-0.10	-0.10	-0.04	-0.15	-0.10	-0.08	-	-	-	0.09	-0.18	-0.10
AMK	0.24	-	-0.10	-0.10	-0.04	-0.15	-0.10	-0.08	-	-	-	0.09	-0.18	-0.10

Abbreviations: TIG, tigecycline; AZT, aztreonam; LEV, levofloxacin; TOB, tobramycin; IMP, imipenem; PMB, polymyxin B; COL, colistin; TZP, piperacillin/tazobactam; CAZ, ceftazidime; MEM, meropenem; CIP, ciprofloxacin; GEN, gentamicin; AMK, amikacin.

antibiotic stewardship and infection control measures. Second, the predominance of *blaOXA*-51, *blaOXA*-24, and *blaOXA*-58 genes, often found in combination within the same isolates, indicates the circulation of successful clonal lineages adapted to local selective pressures (25). The co-occurrence of multiple OXA-type carbapenemase

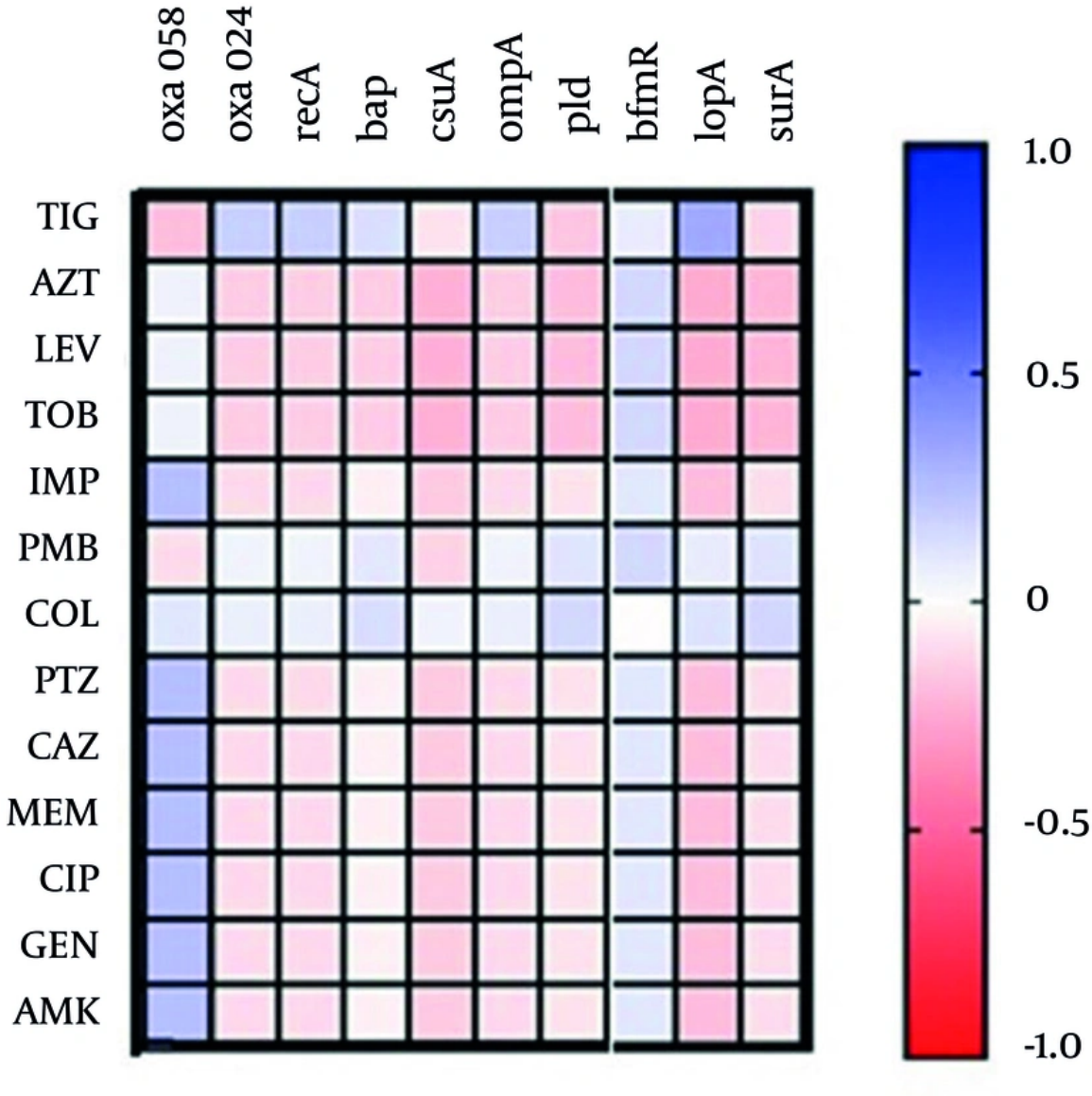

Figure 4. Heatmap illustrating correlations between antibiotic resistance and the presence of carbapenemase and virulence genes in 40 clinical *Acinetobacter* isolates: The heatmap shows the strength and direction of associations between resistance to different antibiotics and the presence of specific carbapenemase (*blaOXA*-58 and *blaOXA*-24) and virulence genes (*recA*, *bap*, *csuA*, *ompA*, *pld*, *lipA*, *surA*, and *bfmR*). Blue cells indicate a statistically significant positive correlation, while pink cells indicate a significant negative correlation. Non-colored or white cells represent no significant correlation. Antibiotic abbreviations and gene names are defined in the Methods section (the total number of isolates analyzed was 40).

genes in 25% of isolates (*blaOXA*-24, *blaOXA*-51, and *blaOXA*-58 simultaneously) is a distinctive pattern that may enhance resistance levels and contribute to the persistence and spread of these strains in hospital settings. Such combinations have been less frequently reported in the Iranian context, underscoring the importance of continuous molecular surveillance to detect emerging resistance trends (26).

Virulence genes were detected in 95% of the isolates, with each strain harboring at least two virulence factors. The most prevalent genes were *recA* and *ompA*, each identified in 97.36% of the isolates, highlighting their

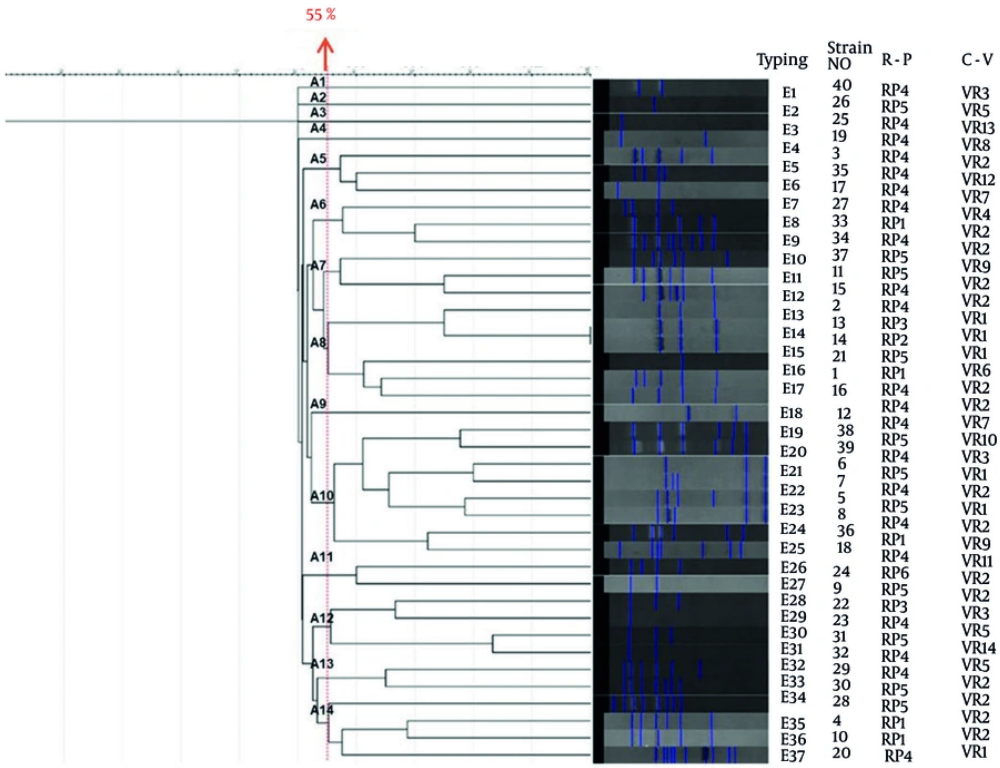

Figure 5. Enterobacterial repetitive intergenic consensus (ERIC)-PCR fingerprint profiles and UPGMA dendrogram of 40 clinical *Acinetobacter* isolates: The dendrogram (left) displays genetic relationships among 40 isolates based on ERIC-PCR banding patterns, analyzed by the unweighted pair group method with arithmetic mean (UPGMA). The Similarity Scale is shown at the top, and clusters (A1 - A14) are defined at a 55% similarity threshold (red dashed line). The central panel shows ERIC-PCR electropherograms, with blue bands representing DNA fragments. The right panel presents isolate information: Typing (ERIC type), strain No. (1 - 40), R-P (resistance profile; RP1 - RP5, refer to Figure 1), and C-V (combined virulence gene profile; VR1 - VR14, refer to Figure 2).

significant roles in the pathogenicity of *A. baumannii*. The *recA* gene is vital for DNA repair and homologous recombination, which enhances bacterial survival under stressful conditions, whereas *ompA* contributes to bacterial adhesion, biofilm formation, and evasion of host immune defenses (27).

The frequent co-occurrence of multiple virulence determinants in MDR strains suggests that these bacteria may possess enhanced pathogenic potential compared to less resistant strains (25, 26). Notably, the simultaneous presence of *recA*, *ompA*, *lipA*, and the carbapenemase gene *blaOXA*-51 in 87.5% of isolates indicates a possible genetic linkage between resistance and virulence factors, potentially increasing the fitness and virulence of local CRAB strains. This finding aligns with emerging evidence that successful *A. baumannii* clones often harbor both resistance and virulence factors, facilitating their adaptation and persistence in healthcare environments (28). Additionally, recent studies have highlighted the role of plasmids and mobile genetic elements in spreading carbapenemase genes among bacterial populations, contributing to the genetic diversity of OXA-type enzymes observed in clinical isolates (20). Our findings support this notion, highlighting the diverse combinations of OXA-type enzymes present within clinical isolates. Moreover, while MBLs such as VIM are frequently reported in certain regions, our data suggest a distinct pattern in Tehran, where OXA-type enzymes predominate (22).

A detailed analysis of our clinical *A. baumannii* isolates revealed a noteworthy link between the presence of specific virulence genes and resistance to antibiotics. In particular, strains harboring the *lipA* gene showed a markedly higher rate of TIG resistance compared to those without this gene. This observation points to a possible interplay between certain virulence

factors and antibiotic susceptibility, reflecting the complex genetic landscape that shapes drug resistance. The *lipA* gene encodes a lipase that participates in lipid metabolism, which could influence the architecture and permeability of the bacterial cell membrane. Since TIG must traverse the outer membrane to exert its effect inside the cell, any changes in membrane composition brought about by lipase activity might limit antibiotic entry and reduce its effectiveness (29).

Furthermore, TIG resistance in *A. baumannii* is frequently associated with the action of efflux pumps, such as the AdeABC system. The function of these pumps can be affected by alterations in membrane structure or by regulatory mechanisms that control their expression (30). Although our research did not directly evaluate efflux pump activity or biofilm formation, it is plausible that virulence factors like *lipA* contribute to antibiotic tolerance by supporting biofilm development and maintaining membrane integrity (31). Taken together, these results reinforce the idea that virulence and resistance determinants may be closely linked, collectively enhancing the ability of *A. baumannii* to withstand antimicrobial treatment. Elucidating these relationships is essential for devising more effective therapeutic strategies against MDR infections.

The ERIC-PCR analysis of 40 clinical *A. baumannii* isolates revealed substantial genetic heterogeneity, with 14 distinct clusters (A1 - A14) and 37 unique genotypes identified at a 55% similarity threshold (Figure 5). The calculated SID was 0.50, reflecting a moderate level of genetic diversity among the isolates. This finding suggests a balance between clonal expansion and the introduction or persistence of genetically diverse strains within the hospital population. Notably, nine clonal clusters comprised 32 isolates, indicating that certain lineages have achieved successful transmission and persistence in the hospital environment. In contrast, five clusters were monotypic, each containing a single isolate, likely representing sporadic introductions or less successful strains in terms of nosocomial spread (32).

A moderate SID, as observed here, implies that while clonal transmission is occurring — potentially leading to localized outbreaks — there is also ongoing importation or emergence of unrelated strains. This pattern is clinically significant, as it highlights the dual challenges faced by infection control teams: Preventing both the spread of dominant, high-risk clones and the establishment of new, unrelated strains with distinct resistance or virulence profiles.

Comparison with international datasets, such as the World Health Organization's Global Antimicrobial Resistance Surveillance System (WHO GLASS), further contextualizes our findings. The most recent WHO GLASS report identifies *A. baumannii* as a leading cause of healthcare-associated infections globally, with high rates of resistance to carbapenems and other critical antibiotics reported across multiple regions. In our study, the predominance of MDR and XDR profiles among the isolates aligns with global trends documented by WHO GLASS, particularly in the Eastern Mediterranean and European regions, where carbapenem resistance rates in *A. baumannii* often exceed 50%.

The moderate genetic diversity observed in our ERIC-PCR analysis (SID = 0.50) is also consistent with international surveillance data, which frequently report the co-circulation of both clonal and genetically diverse *A. baumannii* strains within hospital settings. These parallels underscore the ongoing challenge of controlling the spread of MDR *A. baumannii* in healthcare environments and highlight the importance of harmonized surveillance, molecular typing, and infection control interventions as recommended by WHO GLASS (33). Our results are in agreement with previous studies from the region and internationally. For instance, ERIC-PCR-based investigations in Saudi Arabia and other countries have demonstrated similar clustering patterns, highlighting the method's utility in tracking nosocomial transmission and identifying outbreak-related strains (34).

Furthermore, combining ERIC-PCR with other molecular typing methods, such as multilocus variable-number tandem-repeat analysis (MLVA), has been shown to enhance the resolution of epidemiological investigations and provide a more comprehensive understanding of *A. baumannii* population structure and transmission dynamics (35). The identification of multiple clonal types, alongside singletons, in our study emphasizes the ongoing risk of hospital-acquired infections caused by both endemic and newly introduced *A. baumannii* strains. These findings highlight the importance of continuous molecular surveillance and robust infection prevention strategies to limit the spread of MDR clones and to detect the emergence of novel strains.

Ultimately, understanding the genetic diversity and transmission patterns of *A. baumannii* within healthcare settings is essential for informing targeted interventions and reducing the burden of nosocomial infections. While this study offers valuable insights into the genetic determinants of antibiotic resistance in clinical *Acinetobacter* isolates, its limitations should be acknowledged. The study's focus on Tehran exclusively

means that broader national comparisons could provide additional insights into the epidemiology and genetic diversity of *Acinetobacter* isolates.

Future research should prioritize the implementation of robust antimicrobial stewardship programs, enhanced surveillance systems that integrate molecular diagnostics with epidemiological data, and the exploration of novel therapeutic strategies targeting the specific resistance mechanisms identified in this study. Expanding investigations to include broader geographical regions and employing diverse molecular typing approaches will be essential for developing targeted infection control strategies tailored to local and global epidemiological profiles.

5.1. Conclusions

This study provides critical insights into the genetic diversity, antibiotic resistance patterns, and virulence gene correlations among *A. baumannii* isolates from Tehran, Iran. The findings reveal a high prevalence of MDR strains, with significant rates of carbapenemase production and virulence gene expression, underscoring major public health concerns related to limited treatment options and increased pathogenicity. The observed moderate genetic diversity suggests both clonal dissemination and the potential for nosocomial transmission within healthcare settings.

These results highlight the urgent need for comprehensive antimicrobial stewardship programs and continuous molecular surveillance to guide targeted infection control measures based on local epidemiological profiles. Ultimately, a deeper understanding of the complex interactions between genetic determinants, antimicrobial resistance, and virulence factors is essential for mitigating the risks associated with infections caused by highly resistant *A. baumannii* in Iranian healthcare settings and beyond.

Supplementary Material

Supplementary material(s) is available here [To read supplementary materials, please refer to the journal website and open PDF/HTML].

Footnotes

Authors' Contribution: S. M., M. F., A. S. T. B., and Z. H. contributed to the design of the study. Material preparation and data collection were performed by S. M. and M. F. Data analysis was carried out by A. S. T. B. and Z. H. The manuscript was drafted by A. S. T. B. All authors (S. M., M. F., A. S. T. B., and Z. H.) read and approved the final version of the manuscript.

Conflict of Interests Statement: The authors declare no conflict of interest.

Data Availability: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval: To ensure the study's integrity and ethical standards, the research received approval from the Ethics Committee of the East Tehran Branch, Islamic Azad University, Tehran, Iran (IR.IAU.ET.REC.1400.013).

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Informed Consent: Informed consent to participate was obtained from all participants involved in the study. For participants under the age of 16, informed consent was obtained from their parents.

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