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ORIGINAL RESEARCH

Genomic Characterization of Carbapenem-Resistant *Acinetobacter baumannii* in ICU Environments: Mobile Genetic Elements, Efflux Pumps, and Resistance Mechanism

Bing Wang[1,2], Weiran Wang[1,2], Min Lu[1,2], Hui Jin[1,2]

[1]Department of Disinfection Surveillance and Vector Control, Hangzhou Center of Disease Control and Prevention (Hangzhou Health Supervision Institution), Hangzhou, Zhejiang, People's Republic of China; [2]Zhejiang Key Laboratory of Multi-Omics in Infection and Immunity, Hangzhou Center of Disease Control and Prevention (Hangzhou Health Supervision Institution), Hangzhou, Zhejiang, People's Republic of China

Correspondence: Hui Jin, Hangzhou Center of Disease Control and Prevention (Hangzhou Health Supervision Institution), No. 568 Mingshi Road, Shangcheng District, Hangzhou, Zhejiang, 310021, People's Republic of China, Tel +86-571-85177602, Email jinhui1206@163.com

Purpose: To investigate the genomic resistance profile of carbapenem-resistant *Acinetobacter baumannii* (CRAB) isolates from ICU environments, with a focus on characterizing a representative CRAB strain I2 to elucidate its genomic determinants of resistance and assess their implications for infection control.

Methods: Between 2012 and 2015, a total of 24 *Acinetobacter baumannii* strains were isolated from high-touch surfaces ICUs of four hospitals. Antimicrobial susceptibility testing against 15 antibiotics was performed for all isolates using the VITEK® 2 system. One representative strain was selected for whole-genome sequencing. Resistance genes, virulence factors, and mobile genetic elements were systematically analyzed using bioinformatics tools and databases. In addition, the biofilm formation capacity of this strain was quantitatively assessed by crystal violet staining.

Results: Resistance rates to β-lactams ranged from 58.33% to 66.67%, while 95.83% of isolates remained susceptible to polymyxin. The representative CRAB strain I2 (sequence type 191) harbored three carbapenemase genes and 13 ade efflux pump genes, with 40 resistance genes identified (68.75% efflux-mediated). Genomic island GI16 (carrying transposase ISAba1) suggested horizontal gene transfer driving resistance dissemination. A total of 99 virulence genes and disinfectant resistance genes were detected. Biofilm formation capacity was moderate. Genomic analysis of strain I2 revealed a comprehensive resistance profile and potential mechanisms underlying environmental persistence and transmission.

Conclusion: The ICU environment constitutes an important reservoir for CRAB. The strain I2 harbored key resistance determinants, including efflux pump, and mobile genetic elements, which correlated with its carbapenem-resistant phenotype. Additionally, this strain harbors biofilm-associated genes and disinfectant efflux pump genes, and exhibits moderate biofilm-forming capacity, indicating strong environmental adaptability. The genomic characteristics of strain I2 provide a molecular basis for implementing targeted CRAB infection control strategies in high-risk healthcare settings.

Keywords: *Acinetobacter baumannii*, carbapenem resistance, genomic islands, biofilm, infection control

Introduction

Acinetobacter baumannii, a Gram-negative opportunistic pathogen, is a leading cause of hospital-acquired infections, frequently implicated in ventilator-associated pneumonia, bloodstream infections, and severe post-traumatic infections.[1] Over the past two decades, the global spread of multidrug-resistant (MDR) strains has elevated carbapenems (eg, imipenem, meropenem) to first-line therapies for *A. baumannii* infections.[2] However, the extensive use of these agents has exerted substantial evolutionary pressure, resulting in a dramatic rise in carbapenem resistance rates among clinical isolates. Data from the China Antimicrobial Surveillance Network (CHINET) reveal an alarming upward trend in

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carbapenem-resistant *A. baumannii* (CRAB) prevalence within Chinese ICUs, with the CRAB detection rate reaching 55.5% in 2023-a 2.1% increase from 2022 and the highest recorded level to date.[3] *A. baumannii* is one of the six ESKAPE pathogens. In recognition of its multidrug resistance and epidemic potential, the World Health Organization (WHO) designated CRAB as a critical priority pathogen in 2024, underscoring its grave threat to global public health.[4]

CRAB resistance to carbapenems primarily stems from the production of carbapenem-hydrolyzing class D β-lactamases (OXA-type enzymes), particularly OXA-23.[5] Furthermore, the horizontal transfer of resistance genes via mobile genetic elements (MGEs)-including insertion sequences (ISs), integrative conjugative elements, and resistance plasmids-accelerates the dissemination of carbapenem resistance across hospital environments.[6] The capacity for horizontal gene transfer (HGT) enables CRAB to rapidly acquire and disseminate antibiotic resistance genes (ARGs), facilitating adaptive evolution in response to environmental pressures.[7] The MDR phenotype of *A. baumannii* correlates with elevated clinical failure rates,[8] often leading to poor outcomes such as increased mortality, prolonged hospitalization, and heightened healthcare costs. Moreover, *Acinetobacter spp*. exhibit remarkable resistance to external stresses, including desiccation—a trait rare among Gram-negative bacteria—which confers a significant survival advantage. Some isolates remain viable on dry surfaces for up to three months.[9]

Compounding this challenge, CRAB exhibits environmental persistence through biofilm formation, enabling it to colonize hospital surfaces and serve as a reservoir for nosocomial outbreaks.[10] Current therapeutic options are largely restricted to tigecycline and colistin, both of which show limited efficacy against evolving CRAB strains.[11] With the pipeline for novel antibiotics stagnating, implementing robust infection prevention and control (IPC) strategies is imperative to mitigate CRAB-associated mortality.[12] A critical step toward this goal involves elucidating the genomic drivers of CRAB persistence, resistance, and transmission. Although whole-genome sequencing (WGS) has been increasingly integrated into outbreak investigations and public health surveillance of multidrug-resistant organisms,[13] its application to CRAB environmental reservoirs—particularly in ICU settings—remains limited. In contrast to traditional typing methods, WGS provides high-resolution insights into phylogenetic relationships, resistance gene carriage, mobile genetic elements, and virulence determinants without the need for prior species-specific assay design. The study by Hwang et al demonstrated the utility of WGS in elucidating transmission dynamics and genetic relatedness during clinical CRAB outbreaks.[14] However, such approaches have rarely been applied to environmental isolates. In this study, we employ WGS combined with comprehensive bioinformatic analyses to uncover genomic features associated with environmental persistence and resistance transmission in CRAB isolates recovered from high-touch surfaces in ICUs.

Despite advances in the understanding of CRAB epidemiology, current research remains predominantly focused on patient-derived isolates, while attention to environmental reservoirs in ICUs is still insufficient. In this study, *A. baumannii* isolates collected from high-touch surfaces in the ICUs of four tertiary hospitals were subjected to antimicrobial susceptibility testing. A representative CRAB strain was selected for whole-genome sequencing, virulence gene profiling, and biofilm formation assays. The aims of this study were: (1) to characterize the antimicrobial resistance profiles of *A. baumannii* in ICU environments; and (2) to elucidate the genomic features of a representative strain, thereby analyzing its resistance and adaptive mechanisms, and providing molecular insights for controlling CRAB transmission in healthcare settings.

Materials and Methods

Bacterial Isolates

A total of 24 *A. baumannii* isolates were obtained from high-touch surfaces (eg, nursing station countertops, bed rails) in six ICUs across four tertiary teaching hospitals between 2012 and 2015. Three hospitals were located in Hangzhou, Zhejiang Province, and one in Nanjing, Jiangsu Province. Environmental samples were collected using sterile swabs, and isolates were identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). Bacterial isolation and cultivation were performed using blood agar plates. Strains were cryopreserved at −80°C using ceramic bead preservation technology. For revival, a single bead was streaked onto blood agar plates with a sterile inoculation loop and incubated at 36°C for 48 hours. Third-passage subcultures were used for experiments to ensure stability and reproducibility.

Methods

Antimicrobial Susceptibility Testing

Minimum inhibitory concentrations (MICs) of 15 antibiotics from six classes were determined using the VITEK® 2 automated system (bioMérieux, Shanghai, China) with AST-N335 cards. Tested agents included β-lactams (piperacillin/tazobactam, ceftazidime, cefoperazone/sulbactam, cefepime, aztreonam, imipenem, meropenem), aminoglycosides (tobramycin), fluoroquinolones (ciprofloxacin, levofloxacin), tetracyclines (doxycycline, minocycline, tigecycline), polymyxins (colistin), and sulfonamides (trimethoprim/sulfamethoxazole). Susceptibility interpretations followed Clinical and Laboratory Standards Institute (CLSI) guidelines.[15]

Whole Genome Sequencing

Strain I2, which demonstrated the broadest antibiotic resistance profile among the 24 environmental CRAB isolates was selected as a representative for WGS to further explore its underlying resistance mechanisms. Genomic DNA was extracted and purified, and its concentration was initially quantified using a NanoDrop spectrophotometer. DNA quality was assessed based on the following criteria: an A_{260}/A_{280} ratio between 1.8 and 2.0, integrity confirmed by 1% agarose gel electrophoresis (5 V/cm for 20 min) showing no degradation, and a total yield of ≥10 μg. Purity and concentration were further accurately determined using a fluorometric method (Qubit dsDNA HS Assay Kit, Thermo Fisher Scientific).

The genome was sequenced using a combination of PacBio Sequel IIe and Illumina NovaSeq™ X Plus sequencing platforms.

Bioinformatics Analysis

The HiFi reads generated from the PacBio platform were assembled to construct complete genomes using Unicycle v0.4.8 and uses Pilon v1.22 to polish the assembly using short-read alignments, reducing the rate of small errors. The coding sequences of chromosome and plasmid were predicted using Glimmer or Prodigal v2.6.3 and GeneMarkS.

Bioinformatic analyses were carried out using the following tools: GC-depth distribution was analyzed with Bowtie2 v2.5.1; tRNA genes were predicted using tRNAscan-SE v2.0.12; rRNA genes were identified with Barrnap v0.9; and a circular genome map was generated with CGView v2.0. Plasmid sequences were identified from the bacterial genome assembly using PLASMe (https://github.com/HubertTang/PLASMe). The obtained plasmid sequences were subsequently annotated via BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) against the PLSDB database (https://ccb-microbe.cs.uni-saarland.de/plsdb/). Functional annotation of genes was performed using the GO Database (release 2023–08-30). Antibiotic resistance genes were identified through annotation with the CARD database (v3.2.9). Virulence factor genes were annotated and statistically profiled by alignment against the VFDB core dataset (release 2024–03-01). Secretion systems were analyzed through comparative annotation using Diamond v0.8.35.

Biofilm Formation Assay

Biofilm production of the representative strain I2 was quantified using crystal violet staining as described by Lou et al.[16] Briefly, bacterial suspensions in tryptic soy broth (TSB) were incubated in 96-well plates for 24 hours at 36 °C. After removing planktonic cells via PBS washing (pH 7.4), adherent biofilms were fixed, stained with 1% crystal violet (20 min), and dissolved in 95% ethanol. Optical density (OD) was measured at 570 nm using a microplate reader. The cutoff value (ODc) was defined as the mean OD of negative controls (TSB only) plus three standard deviations. Biofilm-forming capacity was categorized: non-biofilm ($OD \leq ODc$), weak ($ODc < OD \leq 2 \times ODc$), moderate ($2 \times ODc < OD \leq 4 \times ODc$), or strong ($OD > 4 \times ODc$). Each isolate was tested in sextuplicate.

Results

Antimicrobial Resistance Profiles

Multidrug resistance was prevalent among the 24 *A. baumannii* isolates (Table 1). Resistance rates to β-lactams ranged from 58.33% (ceftazidime, imipenem, meropenem) to 66.67% (aztreonam). Polymyxins exhibited high efficacy, with 95.83% susceptibility to colistin (MIC ≤ 2 μg/mL) and 4.17% resistantto colistin (MIC ≥ 4 μg/mL). For tetracyclines,

Table 1 Antimicrobial Resistance and Susceptibility Rates of *Acinetobacter baumannii* I2

Class	Antimicrobial Agent	Resistance Rate (%)	Susceptibility Rate (%)	Intermediate Rate (%)
β-Lactams	Aztreonam	66.67	33.33	0.00
	Piperacillin/Tazobactam	62.50	37.50	0.00
	Ceftazidime	58.33	41.67	0.00
	Imipenem	58.33	41.67	0.00
	Meropenem	58.33	41.67	0.00
	Cefepime	41.67	45.83	12.50
	Cefoperazone/Sulbactam	29.17	45.83	25.00
Quinolones	Ciprofloxacin	58.33	41.67	0.00
	Levofloxacin	50.00	45.83	4.17
Sulfonamides	Sulfamethoxazole/Trimethoprim	54.17	45.83	0.00
Tetracyclines	Doxycycline	54.17	45.83	0.00
	Minocycline	20.83	58.33	20.83
	Tigecycline	0.00	66.67	33.33
Aminoglycosides	Tobramycin	45.83	50.00	4.17
Polymyxins	Colistin	4.17	95.83	0.00

Notes: Resistance rate, susceptibility rate, and intermediate rate represent the percentage of resistant, susceptible, and intermediately susceptible isolates among the total isolates, respectively.

58.33% of isolates were susceptible to minocycline (MIC ≤ 4 μg/mL), while 33.33% displayed intermediate susceptibility to tigecycline.

Genomic Features of *A. baumannii* I2

Antimicrobial susceptibility testing identified *A. baumannii* I2 as a multidrug-resistant CRAB strain, exhibiting resistance to carbapenems, aminoglycosides, fluoroquinolones, tetracyclines, and sulfonamides, with susceptibility retained only to polymyxins. Whole-genome sequencing revealed a genome size of 3.98 Mb (GenBank accession: SUB15337357) with an average GC content of 40.11%, encoding 73 tRNAs and 18 rRNAs. Multilocus sequence typing assigned the strain to ST-191.

The bacterial strain harbored a 0.03 Mb plasmid (designated PlasmidA), which carried multiple transposase genes (eg, pA_gene0014) belonging to the IS66 family. These mobile elements were co-localized with genomic island GI16. Within PlasmidA, we identified a BrnT-BrnA-type toxin-antitoxin (TA) system (pA_gene0011, pA_gene0029) and RelE/ParE family toxin genes (pA_gene0002, pA_gene0020). Notably, PlasmidA also encoded TonB-dependent receptor genes (pA_gene0013, pA_gene0031), such as *ZnuD2*, which mediate iron siderophore uptake. Additionally, conjugation-associated MobA/MobL family proteins (pA_gene0025, pA_gene0007) were detected, suggesting potential PlasmidA transferability. For detailed genomic annotations, refer to Figure 1 and Table S1.

Genomic analysis revealed the structural basis underlying CRAB I2's resistance and virulence traits, as evidenced by resistance gene clusters, mobile genetic elements, and biofilm-associated loci. PlasmidA carries conjugation-associated proteins (MobA/MobL), suggesting its potential to facilitate the horizontal transfer of resistance genes among strains via horizontal gene transfer.

Gene Ontology (GO) annotation classified 1723 genes into three categories: biological process (BP), cellular component (CC), and molecular function (MF). Membrane-associated (333 genes) and cytoplasmic (206 genes) components predominated (Figure 2).

Bioinformatics Analysis

Sixteen genomic islands (GIs) were predicted, with GI16 located on the PlasmidA and 15 on the chromosome. GI16 harbored 15 genes encoding transposases, insertion sequences, transcriptional regulators, toxin-antitoxin systems, and hypothetical proteins. Eighteen insertion sequences, predominantly ISAba1 and ISAba26, were identified.

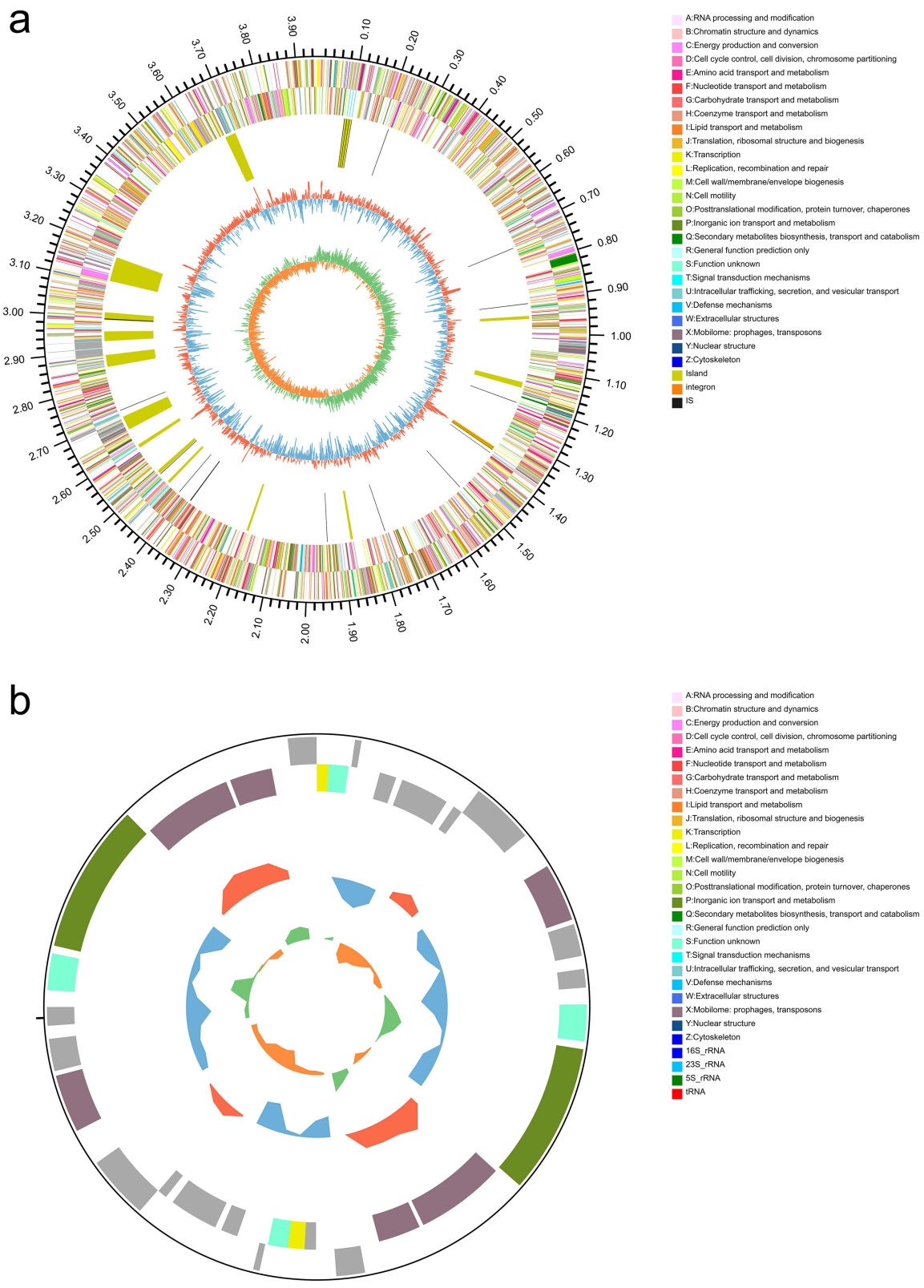

Figure 1 Genomic and PlasmidA CGView Maps of *Acinetobacter baumannii* 12.

Notes: (**a**) Genome Circular Map: The outermost ring denotes the genome size scale. The second and third rings represent coding sequences (CDS) on the forward and reverse strands, respectively, with color differentiation based on Clusters of Orthologous Groups (COG) functional categories. The fourth ring marks the distribution of rRNA and tRNA genes. The fifth ring illustrates GC content variations. The innermost ring displays GC-skew values. (**b**) PlasmidA Circular Map (outer to inner rings): Rings 1 and 4: CDS on forward/reverse strands with COG-based color coding. Rings 2 and 3: CDS, tRNA, and rRNA loci on forward/reverse strands. Ring 5: GC content profile (red: above average; blue: below average; peak height indicates deviation). Ring 6: GC-skew values, facilitating replication origin/terminus prediction. Innermost ring: PlasmidA size scale.

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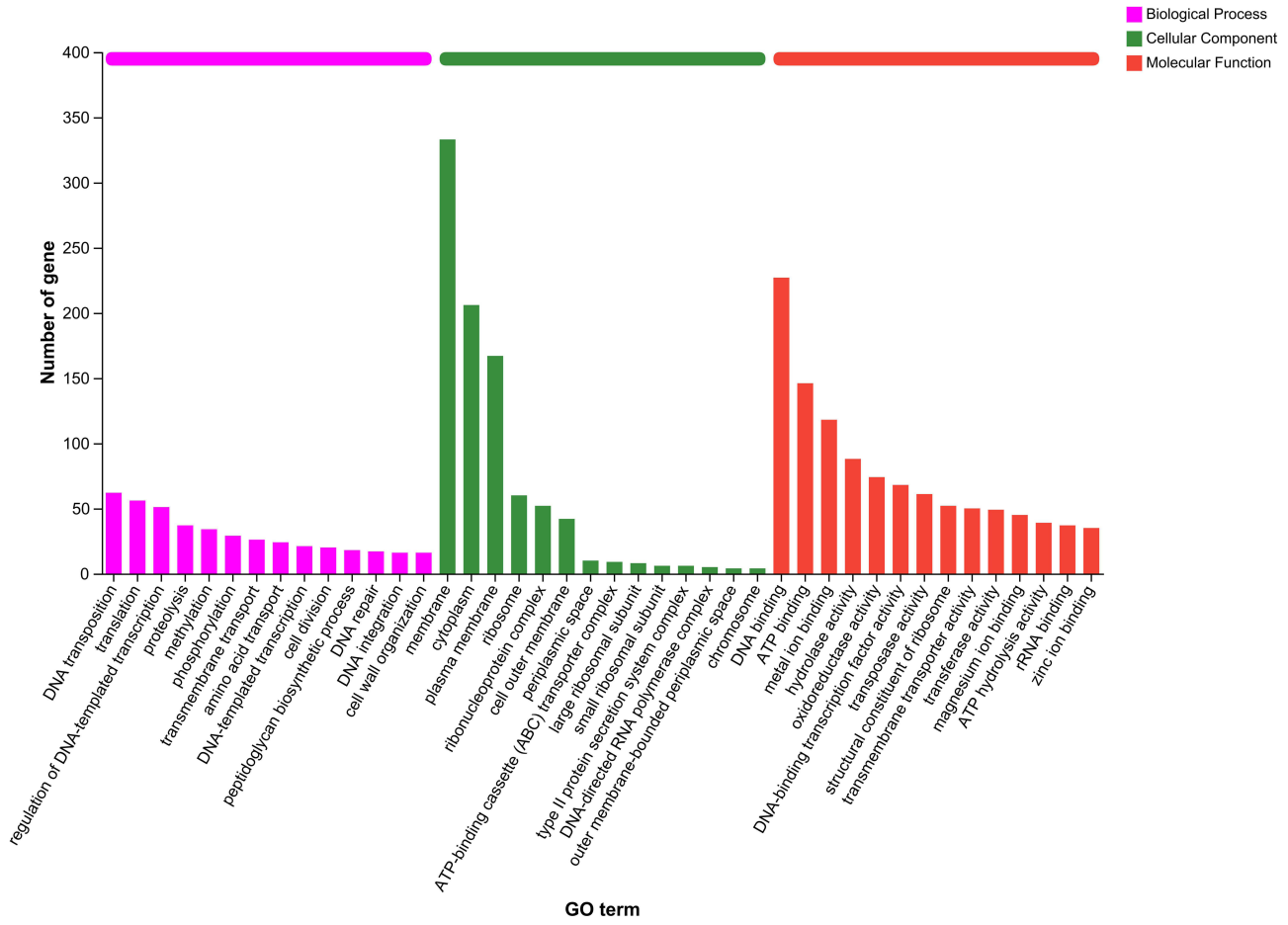

Figure 2 GO functional classification of *Acinetobacter baumannii* I2.
Notes: X-axis: GO categories (BP, CC, MF) and level 2 subcategories. Y-axis: Percentage of annotated genes.

The strain carried 99 virulence genes across 14 categories (Table S2), including nutrition/metabolism (28 genes), immune modulation (17), adhesion (28), secretion systems (15), biofilm formation (8), exotoxins (3), T6SS (3), and T2SS (12).

CARD database analysis identified 40 resistance genes conferring resistance to nine antibiotic classes. Efflux pumps (68.75% of mechanisms) dominated, followed by antibiotic inactivation (21.88%) and target alteration (9.38%). Key findings included efflux systems such as 13 ade family genes (*adeB/J/K/F* etc.) mediating resistance to β-lactams, tetracyclines, and fluoroquinolones; synergistic roles of *AbuO* and *abeM*. Carbapenem resistance includes three copies of $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-66}}$, encoding carbapenem-hydrolyzing enzymes. Polymyxin susceptibility such as *LpxC* (target alteration) and *LpsB* (reduced permeability) were detected, aligning with the observed 95.83% susceptibility rate. Disinfectant resistance such as genes *qacEdelta1* (efflux) and *abeM* (fluoroquinolone/disinfectant efflux) were present (Table 2).

Five secretion systems-T1SS, T2SS, T6SS, Sec-SRP, and Tat were identified (Table S3). T1SS secreted outer membrane proteins (eg, TolC), T2SS exported ATPase *GspE* and structural components (*GspL/F/D*), and T6SS delivered Vgr-family proteins and RHS-repeat domain-containing effectors.

Biofilm Formation Capacity

Crystal violet staining revealed moderate biofilm production (OD/ODc ratio), consistent with virulence gene predictions.

Discussion

The *A. baumannii* isolates in this study were recovered from high-touch surfaces in six ICUs across four tertiary teaching hospitals, indicating the presence of MDR in these environments and suggesting that ICU surfaces may act as reservoirs

Table 2 Antibiotic Resistance Genes in *Acinetobacter baumannii* I2

Gene ID	ARO Name	Drug Class	Resistance Mechanism
gene1779	*adeB*	Glycylcyclines; Tetracyclines	Antibiotic efflux
gene0750	*adeJ*	Carbapenems; Cephalosporins; Fluoroquinolones; Lincosamides; Macrolides; Tetracyclines	Antibiotic efflux
gene1198	*adeF*	Fluoroquinolones; Tetracyclines	Antibiotic efflux
gene0749	*adeK*	Carbapenems; Cephalosporins; Fluoroquinolones; Lincosamides; Macrolides; Tetracyclines	Antibiotic efflux
gene1197	*adeH*	Fluoroquinolones; Tetracyclines	Antibiotic efflux
gene1450	*AmvA*	Disinfectants and antiseptics; Macrolides	Antibiotic efflux
gene1780	*adeC*	Glycylcyclines; Tetracyclines	Antibiotic efflux
gene1654	*AbaQ*	Fluoroquinolones	Antibiotic efflux
gene3458	*AbuO*	Aminoglycosides; Carbapenems; Cephalosporins; Disinfectants and antiseptics; Glycylcyclines; Penicillins; Tetracyclines	Antibiotic efflux
gene3323	*abeM*	Disinfectants and antiseptics; Fluoroquinolones	Antibiotic efflux
gene2278	*AbaF*	Phosphonic acid antibiotics	Antibiotic efflux
gene0751	*adeI*	Carbapenems; Cephalosporins; Fluoroquinolones; Lincosamides; Macrolides; Tetracyclines	Antibiotic efflux
gene1199	*adeG*	Fluoroquinolones; Tetracyclines	Antibiotic efflux
gene1778	*adeA*	Glycylcyclines; Tetracyclines	Antibiotic efflux
gene1201	*adeL*	Fluoroquinolones; Tetracyclines	Antibiotic efflux
gene1776	*adeS*	Glycylcyclines; Tetracyclines	Antibiotic efflux
gene1777	*adeR*	Glycylcyclines; Tetracyclines	Antibiotic efflux
gene1504	*adeN*	Carbapenems; Cephalosporins; Fluoroquinolones; Lincosamides; Macrolides; Tetracyclines	Antibiotic efflux
gene1286	*qacEdelta1*	Disinfectants and antiseptics	Antibiotic efflux
gene1206	*abeS*	Aminocoumarins; Macrolides	Antibiotic efflux
gene1134	*ADC-30*	Cephalosporins	Antibiotic inactivation
gene1300	*mphE*	Macrolides	Antibiotic inactivation
gene1280	*APH(3')-Ia*	Aminoglycosides	Antibiotic inactivation
gene2442	*TEM-1*	Cephalosporins; Monobactams; Penicillins	Antibiotic inactivation
gene2045	*OXA-66*	Carbapenems; Cephalosporins; Penicillins	Antibiotic inactivation
gene0084	*OXA-23*	Carbapenems; Cephalosporins; Penicillins	Antibiotic inactivation
gene0093	*OXA-23*	Carbapenems; Cephalosporins; Penicillins	Antibiotic inactivation
gene0102	*OXA-23*	Carbapenems; Cephalosporins; Penicillins	Antibiotic inactivation
gene1285	*ANT(3'')-IIa*	Aminoglycosides	Antibiotic inactivation
gene1284	*catB8*	Amphenicols	Antibiotic inactivation
gene1283	*AAC(6')-Ib7*	Aminoglycosides	Antibiotic inactivation
gene3284	*LpsB*	Polymyxins	Reduced antibiotic permeability
gene1287	*sul1*	Sulfonamides; Sulfones	Antibiotic target replacement
gene3510	*sul2*	Sulfonamides; Sulfones	Antibiotic target replacement
gene3543	*parC*	Fluoroquinolones	Antibiotic target alteration
gene0861	*gyrA*	Fluoroquinolones; Nybomycin analogs	Antibiotic target alteration
gene0179	*LpxC*	Polymyxins	Antibiotic target alteration
gene1518	*LpxA*	Polymyxins	Antibiotic target alteration
gene1290	*armA*	Aminoglycosides	Antibiotic target alteration
gene1301	*msrE*	Macrolides; Streptogramins	Antibiotic target protection

for MDR *A. baumannii*. These findings support the importance of enhanced infection surveillance and preventive measures in healthcare settings.

Among 24 *A. baumannii* isolates, 58.33% demonstrated resistance to imipenem and meropenem, reflecting the high prevalence of CRAB in clinical settings. Polymyxins retained the highest efficacy, with only 4.17% of isolates exhibiting resistance-a finding consistent with global surveillance data. Historically regarded as last-resort antibiotics, carbapenems

show diminished effectiveness against CRAB, correlating with mortality rates up to 60% in CRAB-infected patients compared to carbapenem-susceptible strains. Globally, CRAB isolation rates vary regionally (30–80%), with Asia, Eastern Europe, and Latin America reporting the highest burdens.[17–19] For instance, a retrospective study at a tertiary hospital in Zhejiang Province (2015–2020) identified carbapenem resistance in 78.8% of 184 *A. baumannii* isolates.[20] The rise of CRAB is primarily driven by horizontal acquisition of oxacillinase genes ($bla_{\text{OXA-23}}$, $bla_{\text{OXA-24}}/_{40}$, and $bla_{\text{OXA-51}}$), with resistance patterns and mechanisms exhibiting marked geographic variability and temporal shifts in dominant clones.[21,22] While colistin and tigecycline remain frontline therapies for CRAB and MDR infections,[23] emerging resistance to ampicillin-sulbactam and colistin underscores the urgent need for novel antimicrobial strategies.[24]

The CRAB isolate I2 (ST-191) harbored three copies of $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-66}}$, encoding carbapenemases that hydrolyze imipenem and align with the observed 58.33% carbapenem resistance rate. Plasmid A-carrying transposases (IS66 family), toxin-antitoxin systems (*BrnT-BrnA, RelE/ParE*), and conjugation-associated *MobA/MobL* proteins-represents a highly mobile genetic platform capable of stabilizing and disseminating resistance determinants within ICU environments. The presence of genomic island GI-16 (harboring ISAba1 transposase) further supports horizontal gene transfer as a key driver of resistance gene acquisition, a mechanism implicated in CRAB outbreak dynamics. Given the global dominance of $bla_{\text{OXA-23}}$ positive clones,[25] genomic surveillance of these mobile elements is critical to curbing carbapenem resistance transmission.

Notably, 40.63% of resistance genes in isolate I2 belonged to the *ade* efflux pump family (*adeB, adeJ*), whose broad substrate specificity (β-lactams, tetracyclines, etc.) directly contributes to elevated resistance rates, including 58.33% resistance to ceftazidime and ciprofloxacin. The AdeABC efflux system has been mechanistically linked to carbapenem resistance, biofilm formation, bacterial motility, and host cell invasion, enabling persistent environmental colonization of high-touch ICU surfaces and amplifying cross-transmission risks.[26,27] Crystal violet staining confirmed moderate biofilm-forming capacity (OD/ODc = 2–4) in I2. Although the representative ST191 CRAB strain I2 exhibited only moderate biofilm-forming capacity in vitro, this trait—coupled with its multidrug resistance profile—may still facilitate its persistence on high-touch surfaces in ICUs. Previous studies have demonstrated that even weak biofilm-forming pathogens can survive for extended periods in food preservation environments, while strong biofilm producers exhibit even greater environmental endurance. This highlights biofilm formation as a key adaptive strategy for conditional pathogens to establish persistent contamination reservoirs. Biofilms are increasingly acknowledged as pivotal virulence determinants implicated in CRAB-associated ICU pneumonias.[28] Consequently, heightened attention should be directed towards CRAB strains with robust biofilm-forming capabilities to better understand and mitigate their clinical impact.

Genomic analysis classified I2 as ST-191, a predominant clone in China alongside ST-195 and ST-208.[29] ST-191 is strongly associated with ICU-acquired infections and carries an arsenal of resistance genes alongside secretion systems (T2SS, T6SS) that may enhance colonization through effector protein delivery. Co-detection of disinfectant resistance genes (*qacEdelta1, abeM*) raises concerns about the efficacy of chlorine-based decontamination protocols, as efflux-mediated resistance may compromise disinfection outcomes. Rostami et al analyzed the transcription levels of four efflux pump genes (belonging to distinct families: *qacEdelta1* of the SMR family, adeB of the RND family, amvA of the MFS family, and abeM of the MATE family) in both clinical and environmental isolates of *A. baumannii*.[30] The study further evaluated the association between efflux pump activity and susceptibility/resistance to commonly used disinfectants. It was found that the expression level of the *qacEdelta1* gene significantly differed between disinfectant-resistant and disinfectant-sensitive isolates exposed to MICROZED ID-MAX. Additionally, the abeM gene was specifically associated with resistance to hydrogen peroxide-based disinfectants in environmental isolates.

Implementing rotating disinfectant regimens may help counteract disinfectant resistance in opportunistic pathogens and effectively eradicate CRAB from ICU environments.

Although environmental (non-clinical) isolates exhibited 95.83% susceptibility to colistin, the presence of *lpxC* (lipopolysaccharide modification) and *lpsB* (outer membrane permeability reduction) genes signals adaptive evolution under prolonged antimicrobial exposure. Moreover, 33.33% of isolates exhibited intermediate susceptibility to tigecycline (MIC $\geq$ 4 μg/mL), approaching resistance breakpoints and underscoring a potential risk of treatment failure. Genomic surveillance, particularly utilizing whole-genome sequencing, is crucial for tracing the dissemination routes of resistance

genes and enabling early outbreak detection. Although this study focuses on the genomic characteristics of strain I2 and cannot directly infer evolutionary patterns of environmental adaptation, the mobile genetic elements (eg, ISAba1 within GI16) and disinfectant resistance genes carried by this strain provide important targets for future multi-strain comparative studies. Previous research by Niu et al indicated that ST191/195/208 strains are associated with more severe bloodstream infections, demonstrating enhanced multidrug resistance and excess mortality.[31] Similarly, a study by Hwang et al on a hospital CRAB outbreak revealed that clinical isolates (such as ST191, ST369, and ST451) carried $bla_{\text{OXA-23}}$, $bla_{\text{OXA-66}}$, and *armA* genes, which collectively confer a survival advantage in healthcare environments.[14] The environmental ST191 strain obtained from the ICU in this study carried an identical core resistance gene cluster ($bla_{\text{OXA-23}}$, $bla_{\text{OXA-66}}$), suggesting potential shared environmental adaptive strategies between environmental and clinical strains.

Conclusion

This study revealed that high-touch surfaces in ICU environments serve as significant reservoirs for CRAB, with a high detection rate of 58.33%. Polymyxins remain a viable empirical therapeutic option, demonstrating a susceptibility rate of 95.83%. Furthermore, a predominant ST-191 CRAB strain I2 was identified and its genomic characteristics were thoroughly characterized, providing potential molecular targets for the development of precision infection control strategies.

This study has several limitations that should be considered. First, the collection of samples from four tertiary teaching hospitals may limit the generalizability of the findings to other regions or healthcare settings. Additionally, the relatively small number of environmental isolates included, along with the selection of only one *A. baumannii* strain for whole-genome sequencing, constrains the comprehensive understanding of the molecular epidemiology of antimicrobial resistance genes.

To overcome these limitations, future studies should employ larger and more diverse sample sets encompassing both clinical and environmental isolates. This will enhance representativeness and facilitate comparative genomic analyses of matched clinical–environmental pairs, in conjunction with phenotypic assessments of disinfectant susceptibility. Moreover, integrating multi-omics approaches—such as transcriptomics (RNA-seq) and proteomics—will be critical to dynamically profile the expression and regulation of key resistance genes under varying environmental stressors, including subinhibitory antibiotic concentrations and disinfectant exposure. These investigations will provide a systematic understanding of the molecular mechanisms driving environmental adaptation and resistance evolution in CRAB.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Disclosure

The authors report no conflicts of interest in this work.

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