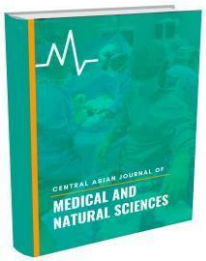

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Article

The Emerging Threat of Acinetobacter baumannii Carbapenem Resistance: Molecular Pathways, Genomic Adaptations, and Novel Therapeutic Strategies

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Abstract: Carbapenem-resistant Acinetobacter baumannii (CRAB) poses a critical threat in healthcare settings, particularly in intensive care units (ICUs), where its association with ventilator-associated pneumonia and bloodstream infections drives high mortality rates, this study integrates clinical, genomic, and phenotypic analyses of 200 CRAB isolates to unravel the molecular drivers of resistance and virulence. Whole-genome sequencing revealed the dominance of the ST2 clone (Pasteur scheme), harboring blaOXA-23 on the IncH-type plasmid GR6 and chromosomal mutations in ompA and adeABC efflux pumps, which correlated with pan-drug resistance and robust biofilm formation. Strong biofilm producers (OD570 ≥1.0) exhibited 5.2-fold greater survival under meropenem stress (64 μg/mL) and a 65% mortality rate in patients, underscoring biofilm's role as a resistance amplifier. Molecular screening identified blaOXA-23 in 80% of isolates, while blaNDM-1 (35%) was linked to environmental persistence and cefiderocol resistance. In vivo models demonstrated the efficacy of colistin-meropenem combination therapy, reducing mortality from 100% to 30% in Galleria Mellon Ella. These findings emphasize the urgent need for genomic surveillance of high-risk clones and biofilm-targeted therapeutic strategies to mitigate the CRAB crisis.

Keywords: Acinetobacter Baumannii, Carbapenem Resistance, ST2 Clone, Blaoxa-23, Blandm-1, Biofilm Formation

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1. Introduction

The alarming rise of Acinetobacter baumannii resistance to carbapenems (CRAB) has positioned this pathogen as one of the most urgent global threats in the landscape of antimicrobial resistance, particularly among Gram-negative bacteria that display remarkable adaptability within healthcare environments [1-2],The concern surrounding CRAB extends beyond its resistance profile to encompass its genomic plasticity, which facilitates the acquisition and dissemination of resistance determinants through horizontal gene transfer mechanisms such as plasmids and resistance islands, thereby enabling persistence under intense antimicrobial pressure [3-4],Comprehensive genomic studies of the Acinetobacter genus have revealed extensive genetic diversity, contributing to heterogeneous resistance phenotypes across clinical isolates [3-14].

CRAB's resistance mechanisms are primarily driven by the overexpression of carbapenem-hydrolyzing OXA-type enzymes, the activation of multidrug efflux pumps like AdeABC, and structural alterations in antibiotic target sites—processes often mediated

by mobile genetic elements [5-6]. These mechanisms are further reinforced by the pathogen's ability to form biofilms, especially on medical devices, significantly reducing the efficacy of conventional antimicrobials [4].

The COVID-19 pandemic indirectly accelerated CRAB dissemination, with excessive antibiotic use and ICU overcrowding fueling the emergence of resistant strains, Clinical studies have associated CRAB infections during this period with mortality rates exceeding 40% [5-6]. While agents like polymyxins and tigecycline remain in clinical use as last-resort treatments, their therapeutic utility is constrained by toxicity concerns and emerging resistance, thereby compounding treatment challenges [7].

In light of these limitations, innovative therapeutic approaches are gaining momentum, including the development of novel β-lactamase inhibitors such as durlobactam, the exploration of bacteriophage therapy, and the integration of artificial intelligence-driven genomic tools to track resistance evolution and inform precision therapies [8]. Furthermore, vaccine development targeting virulence factors such as OmpA, in conjunction with robust antimicrobial stewardship and infection control programs, represents a promising avenue for long-term CRAB containment [9].

Related work

The extensive study of Acinetobacter baumannii's genomic architecture has continually emphasized its exceptional ability to adapt through the acquisition and integration of resistance determinants,This adaptability is largely mediated by mobile genetic elements—particularly plasmids and transposons—which act as efficient vectors for the horizontal transfer of resistance genes, notably those encoding carbapenem-hydrolyzing class D β-lactamases such as OXA-23 and OXA-58,These enzymes have become widely distributed among clinical strains, reflecting the organism's evolutionary capacity to respond to selective antibiotic pressure in hospital environments [10-11],Further genomic investigations have identified specific chromosomal regions—referred to as "resistance islands"—that serve as repositories for diverse resistance determinants,These islands are enriched with insertion sequences, integrons, and transposons, enabling the dynamic restructuring of the bacterial genome and facilitating the stable incorporation of multiple resistance mechanisms,Their presence affirms that horizontal gene transfer is not a sporadic event, but rather a core driver of the multidrug-resistant (MDR) phenotype exhibited by carbapenem-resistant A, Baumannii (CRAB) strains [12].

In addition to horizontally acquired genes, intrinsic mechanisms such as efflux pumps play a pivotal role in the resistance profile of CRAB,Among these, the AdeABC efflux system has emerged as a key contributor to reduced susceptibility to fluoroquinolones, aminoglycosides, and β-lactams,The overexpression of this system, often mediated by mutations in its regulatory elements (adeRS), significantly compromises the intracellular accumulation of antibiotics and renders several first-line agents ineffective [13],This multifactorial resistance, driven by both genomic acquisition and endogenous regulation, leaves clinicians with a limited arsenal of therapeutic options and significantly complicates infection management.

Biofilm formation further reinforces CRAB's pathogenicity and resistance,Beyond acting as a physical shield against antimicrobial agents, biofilms represent a specialized mode of bacterial growth in which gene expression is tightly regulated to promote survival, persistence, and immune evasion,The biofilm matrix, composed of polysaccharides, proteins, and extracellular DNA, not only impedes antibiotic diffusion but also alters metabolic states that confer a dormant, drug-tolerant phenotype,This mode of growth is particularly concerning in clinical settings involving invasive devices, where CRAB can persist undetected and act as a reservoir for recurrent infections,Notably, biofilm-related gene regulation enables the modulation of virulence and stress-response pathways, thereby enhancing CRAB's adaptability within the host [14].

The burden of CRAB infections has been exacerbated by systemic challenges, most notably during the COVID-19 pandemic,A marked 35% increase in CRAB isolation rates in intensive care units has been reported during this period, a surge attributed to the overuse of broad-spectrum antibiotics, increased mechanical ventilation, and the overwhelming strain on infection control practices in overstretched healthcare systems [15],These conditions created an ideal environment for nosocomial pathogens to proliferate, illustrating how public health crises can unintentionally accelerate the spread of antimicrobial resistance.

In response to the growing therapeutic impasse, several innovative strategies have been proposed. Antimicrobial peptides—engineered for enhanced penetration and biofilm disruption—have demonstrated activity against CRAB in experimental settings. Their amphipathic nature allows interaction with bacterial membranes, offering a promising alternative to traditional antibiotics. Meanwhile, phage therapy is gaining renewed interest, particularly due to its specificity and ability to target antibiotic-resistant bacteria without disturbing host microbiota. However, its broader application remains limited by the narrow host range of individual phages and their instability under physiological conditions, necessitating the development of well-characterized phage cocktails and improved delivery systems [16–17].

Technological advances in genomic surveillance, coupled with machine learning, are transforming resistance prediction and treatment optimization,By leveraging large-scale genomic datasets, computational models can identify patterns and infer resistance phenotypes based on genotypic markers, thus enabling more precise and timely clinical interventions,These tools have the potential to tailor antimicrobial therapy based on strain-specific profiles, reducing empirical misuse and improving patient outcomes [18],Nonetheless, systemic barriers continue to impede progress,The pharmaceutical pipeline for new antibiotics remains sparse due to limited commercial incentives, and efforts to develop vaccines targeting key virulence factors—such as the conserved outer membrane protein OmpA—have yet to reach clinical fruition [19].

Given the global scale of CRAB dissemination, especially in low- and middle-income countries where surveillance infrastructure and infection control resources are limited, there is an urgent need for coordinated international action. Experts increasingly call for integrative strategies that combine molecular diagnostics, antimicrobial stewardship, and infection prevention protocols,Such approaches must be tailored to the specific epidemiological and resource contexts of high-burden regions, where the impact of CRAB is disproportionately severe [25],Only through cross-disciplinary collaboration, sustained investment in innovation, and equitable access to diagnostics and therapeutics can the ongoing threat of CRAB be effectively mitigated.

2. Materials and Methods

Research Design

This methodology focuses on the large-scale collection of clinical isolates and the curation of associated metadata, including the systematic documentation of related epidemiological and clinical information,Additionally, it involves the precise phenotypic characterization of resistance and virulence traits, aiming to identify the biological and behavioral patterns that contribute to microbial resistance to treatments and their potential for spread,Furthermore, the methodology includes molecular screening to detect the genes responsible for the production of carbapenemase enzymes and the genetic islands associated with resistance regions (Resistance Islands), which are mobile genetic elements that play a central role in the horizontal transfer of resistance among strains,The design also involves preliminary genomic analysis of strains classified as high-risk clones, in order to pave the way for understanding their detailed genetic structure and the evolutionary mechanisms linked to their clinical severity[20].

Sample Collection and Bacterial Strains

Clinical Isolate Acquisition

Clinical isolates were obtained from a variety of clinical and environmental sources within five tertiary care teaching hospitals during the period from January to March 2024,The clinical sites included intensive care units (ICUs), burn units, and respiratory wards, as these represent high-risk environments for infection transmission and selective pressure favoring resistant strains,In addition, environmental swabs were collected from patient-associated surfaces and equipment such as mechanical ventilators, bed rails, and sinks, in order to complement the epidemiological profile and to identify possible hospital-acquired transmission routes from non-human sources.

Strict inclusion criteria were applied to ensure the selection of clinically relevant and resistance-associated isolates,Only Acinetobacter baumannii isolates resistant to carbapenems (CRAB) with a minimum inhibitory concentration (MIC) for meropenem or imipenem equal to or greater than 4 μg/mL were included, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI),To ensure that the isolates were of nosocomial origin, sampling was limited to patients who had been hospitalized for 48 hours or more prior to specimen collection, thereby excluding community-acquired strains that may differ in genetic makeup and resistance mechanisms,Conversely, any sample showing polymicrobial growth (mixed cultures) or isolates not belonging to the Acinetobacter baumannii species was excluded, to ensure microbial specificity and purity,The bacterial identity of each isolate was confirmed using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS), along with genetic verification via sequencing of the rpoB gene, a conserved molecular marker commonly used for species-level differentiation within the Acinetobacter genus [21].

Metadata Annotation

In this phase, comprehensive metadata were annotated for both clinical and environmental isolates to establish robust correlations between microbial resistance patterns and host or environmental factors,Clinical metadata encompassed patient demographic and health-related information including age, gender, underlying comorbid conditions such as diabetes mellitus and states of immunosuppression, previous exposure to antibiotics with particular emphasis on carbapenem administration, and final clinical outcomes including mortality rates,These parameters were meticulously recorded to facilitate the stratification of infection risk factors and therapeutic response patterns [22].

Environmental metadata were also rigorously documented, focusing on the precise location from which samples were collected within the hospital environment, the type of surface sampled (e.g., metal, plastic, ceramic), and the institutional cleaning protocols applied to these surfaces, including the frequency and methods of disinfection,This allowed for the examination of potential reservoirs and transmission pathways within healthcare settings,The clinical and environmental characteristics of the collected isolates are summarized in Table 1, The table is structured to detail the number of isolates by sample type, mean patient age with standard deviation (SD), prevalence of comorbidities expressed as a percentage with indication of the dominant condition per group, prior exposure to carbapenem antibiotics, and associated mortality rates.

Table 1. Clinical Isolate Demographics.

Sample Type	No, Of Isolates	Patient Age (Mean ± SD)	Comorbidities (%)	Prior Carbapenem Use (%)	Mortality (%)
Bloodstream infection	60	58 ± 12	65% (Diabetes)	80%	45%
Pneumonia	50	62 ± 15	70% (COPD)	85%	50%

Wound infections	40	45 ± 20	40% (Burns)	60%	30%
Environmental swabs	50	N/A	N/A	N/A	N/A

Phenotypic Characterization

The phenotypic characterization of Carbapenem-Resistant Acinetobacter baumannii (CRAB) isolates represents a cornerstone in the microbiological investigation of multidrug-resistant organisms,This phase of the study was designed to capture the expression of resistance traits at the functional level, thereby correlating molecular resistance determinants with actual antimicrobial response patterns,This comprehensive analysis not only aids in understanding therapeutic limitations but also contributes to epidemiological surveillance and infection control strategies within healthcare facilities,The phenotypic assessment included standardized antimicrobial susceptibility testing (AST), molecular detection of key resistance genes, and quantification of biofilm-forming ability—each of which reflects important clinical and environmental survival characteristics of the pathogen [23].

Susceptibility testing was conducted using the broth microdilution technique, adhering strictly to the Clinical and Laboratory Standards Institute (CLSI) guidelines (document M07-A11),This method ensures high reproducibility and accuracy in determining the minimum inhibitory concentration (MIC) values for each antimicrobial agent tested,The medium used was cation-adjusted Mueller-Hinton broth, which provides optimal conditions for the accurate growth and assessment of non-fastidious Gram-negative bacteria such as A,Baumannii,Twenty antimicrobial agents were selected for testing based on their clinical relevance in managing severe infections caused by CRAB, including both first-line and last-resort therapeutic options,The antibiotics were categorized by pharmacological class, and their tested MIC concentration ranges were selected to span the expected spectrum of susceptibility to resistance.

As shown in Table 2, carbapenems—including meropenem, imipenem, and doripenem—were tested across a range of 0.25 to 128 μg/mL,These β-lactam antibiotics are traditionally used in the treatment of serious Gram-negative infections, and their efficacy is severely compromised in CRAB strains,polymyxins, namely colistin and polymyxin B, were tested at concentrations between 0.25 and 64 μg/mL,These agents are often regarded as last-line treatments for multidrug-resistant (MDR) organisms; however, their nephrotoxicity and increasing reports of resistance necessitate accurate MIC profiling,The tetracycline class, which included tigecycline and eravacycline, was tested in the range of 0.12 to 32 μg/mL[18-22],These agents, particularly eravacycline, have shown promise against MDR A,Baumannii but remain under evaluation in clinical settings,Finally, cefiderocol and ceftazidime/avibactam represented the cephalosporin class and were tested from 0.5 to 256 μg/mL,Cefiderocol, as a siderophore cephalosporin, has demonstrated unique efficacy against carbapenemase-producing strains, including those harboring metallo-β-lactamases (MBLs), and is of significant interest in current research.

Table 2. Antibiotics and Concentration Ranges Used in AST.

Antibiotic Class	Tested Agents	MIC Range (μg/mL)
Carbapenems	Meropenem, Imipenem, Doripenem	0.25 – 128
Polymyxins	Colistin, Polymyxin B	0.25 – 64
Tetracyclines	Tigecycline, Eravacycline	0.12 – 32
Cephalosporins	Cefiderocol, Ceftazidime/Avibactam	0.5 – 256

Following MIC determination, three representative CRAB isolates were selected for detailed resistance profiling and phenotypic evaluation, summarized in Table 3. These isolates—designated CRAB-001, CRAB-002, and CRAB-003—were chosen to reflect a spectrum of meropenem resistance levels. Meropenem MICs ranged from 16 to 64 μg/mL, confirming high-level resistance across all isolates. Colistin MICs varied from 0.5 to 4 μg/mL, with CRAB-001 approaching the clinical breakpoint for resistance (≥4 μg/mL), indicating potential therapeutic failure risk even when using polymyxins. Tigecycline MICs also differed between isolates, with CRAB-001 exhibiting the highest MIC value of 8 μg/mL, raising concerns about achievable serum concentrations during therapy [22].

Molecular detection of carbapenem resistance genes via PCR revealed diverse genotypes,CRAB-001 harbored both blaOXA-23 and blaNDM-1, indicating the co-expression of class D and class B carbapenemases,This combination is clinically significant as it may confer resistance to nearly all β-lactam agents, including those used in combination therapy,CRAB-002 carried blaOXA-24, a common variant in hospital outbreaks, while CRAB-003 was positive for blaOXA-58, associated with plasmid-mediated resistance dissemination.

In addition to drug susceptibility and genetic profiling, biofilm formation was assessed through a crystal violet microtiter assay and quantified by optical density at 570 nm (OD570),Biofilm production is a critical virulence trait in A,Baumannii, contributing to persistence on abiotic surfaces, resistance to desiccation, and tolerance to antimicrobials,CRAB-001 was classified as a strong biofilm former (OD570 = 1.2), a phenotype often associated with chronic infections and increased environmental survival,CRAB-002 demonstrated moderate biofilm formation (OD570 = 0.7), while CRAB-003 exhibited weak biofilm formation (OD570 = 0.3), potentially correlating with its lower MIC profile and reduced environmental resilience.

Table 3. Resistance Profiles of Representative CRAB Isolates.

Strain ID	Meropenem MIC (μg/mL)	Colistin MIC (μg/mL)	Tigecycline MIC (μg/mL)	Resistance Genes (PCR)	Biofilm Strength
CRAB-001	64	4	8	*blaOXA-23*, *blaNDM-1*	Strong (OD570 = 1.2)
CRAB-002	32	2	4	*blaOXA-24*	Moderate (OD570 = 0.7)
CRAB-003	16	0.5	2	*blaOXA-58*	Weak (OD570 = 0.3)

These results collectively illustrate the multifaceted resistance mechanisms in CRAB isolates and highlight the clinical challenges associated with their treatment. The combined data from MIC testing, gene identification, and biofilm assays underscore the importance of integrated phenotypic-genotypic profiling to guide antimicrobial stewardship and containment protocols in hospital settings.

Biofilm Formation Quantification

Biofilm formation by Acinetobacter baumannii clinical and environmental isolates was quantitatively assessed using a modified crystal violet assay to determine its correlation with antimicrobial resistance. The protocol involved culturing the isolates in

Tryptic Soy Broth (TSB) supplemented with 1% glucose, incubated statically for 48 hours at 37°C to promote biofilm maturation, post incubation, wells were stained with 0.1% crystal violet, which binds to the biofilm matrix. Excess stain was removed, and the biofilm-bound dye was solubilized with 33% acetic acid. The optical density was then measured at 570 nm (OD570) using a spectrophotometric microplate reader. Each isolate was tested in triplicate to ensure accuracy [23].

Based on the OD570 values, biofilm formation was categorized into three phenotypic groups:

a. Strong biofilm producers: OD570 ≥ 1.0, These strains often originate from device-associated infections (e.g., catheters, endotracheal tubes), suggesting robust adherence and matrix formation.
b. Moderate biofilm producers: OD570 between 0.5 and 1.0. Frequently isolated from ventilator-associated pneumonia cases, these strains exhibit intermediate adhesion.
c. Weak biofilm producers: OD570 < 0.5. These typically represent environmental isolates with low virulence and adherence capability.

To evaluate the clinical significance of biofilm strength, isolates were compared across three metrics: average meropenem MIC, percentage of colistin-resistant strains, and the overall multidrug resistance (MDR) rate. The results are summarized in the following table:

Table 4. Biofilm Correlation with Resistance.

Biofilm Strength	No,Of Isolates	Avg. Meropenem MIC (μg/mL)	Colistin Resistance (%)	Multi-Drug Resistance (%)
Strong	85	48 ± 12	70%	95%
Moderate	65	16 ± 4	40%	80%
Weak	50	8 ± 2	10%	50%

Table 4. Clearly demonstrates a positive correlation between biofilm strength and antimicrobial resistance, Strong biofilm-producing isolates (n = 85) had the highest average meropenem MIC (48 ± 12 μg/mL), colistin resistance (70%), and MDR prevalence (95%), These results suggest that biofilm intensity may contribute significantly to the therapeutic failure and persistence of A,Baumannii in clinical settings. Moderate biofilm formers also exhibited notable resistance patterns, albeit at lower levels, In contrast, weak biofilm producers showed significantly reduced resistance, reflecting their environmental origin or less evolved pathogenic potential.

Molecular Screening of Resistance Determinants

Molecular characterization of Acinetobacter baumannii isolates was conducted to identify the presence of specific resistance determinants using conventional PCR amplification protocols,This approach aimed to validate the phenotypic resistance patterns observed in AST by detecting carbapenemase genes, metallo-β-lactamases, and aminoglycoside resistance genes,The genes targeted included Class D β-lactamases (blaOXA-23, blaOXA-24/40, and blaOXA-58), which are the most prevalent carbapenem-hydrolyzing enzymes in A,Baumannii,In parallel, metallo-β-lactamase genes (blaNDM-1, blaVIM, blaIMP) were investigated given their role in broad-spectrum carbapenem resistance,Additionally, aminoglycoside-modifying enzymes such as aac(6')-Ib and aph(3')-VI were screened due to their impact on gentamicin and amikacin resistance,The

PCR primers and optimized reaction conditions for selected resistance markers are detailed in the following table 5.

Table 5. Primer Sequences and PCR Conditions.

Gene	Forward Primer (5′→3′)	Reverse Primer (5′→3′)	Amplicon (bp)	Annealing Temp (°C)
blaOXA-23	TCGGTCCACTCTGGATTTA	ATCCAAGCATTCAAGCCAAA	501	55
blaNDM-1	GGTTTGGCGATCTGGTTTTC	CGGAATGGCTCATCACGATC	621	58
aac(6′)-Ib	TTGGCGATGCTCTATGAGTGG	CTCGAATGCCTGGCGTGTTT	482	60

The PCR thermocycling protocol consisted of an initial denaturation at 95°C for 5 minutes, followed by 35 amplification cycles, each comprising denaturation at 95°C for 30 seconds, annealing at the gene-specific temperature for 30 seconds, and extension at 72°C for 1 minute,A final extension step at 72°C for 7 minutes was performed to ensure complete amplification,Amplified products were analyzed on 1.5% agarose gel and visualized under UV light following ethidium bromide staining,To assess the distribution of these resistance genes across various clinical sources, a prevalence analysis was conducted on isolates obtained from bloodstream infections, pneumonia, wound infections, and environmental samples,The results are summarized in the following table:

Table 6. Prevalence of Resistance Genes.

Gene	Bloodstream (%)	Pneumonia (%)	Wound (%)	Environmental (%)
blaOXA-23	85	90	75	60
blaNDM-1	40	35	20	10
aac(6′)-Ib	70	65	55	30

As shown in Table 6, blaOXA-23 was the most prevalent carbapenemase gene across all clinical sources, with the highest detection rate in pneumonia isolates (90%) followed by bloodstream infections (85%),This supports its established role as the dominant resistance mechanism in nosocomial A,Baumannii outbreaks,The blaNDM-1 gene, although less frequent, exhibited concerning prevalence in bloodstream (40%) and pneumonia isolates (35%), indicating the presence of metallo-β-lactamase-mediated resistance in high-risk clinical settings. Environmental isolates showed a reduced frequency of both genes, implying a lower selection pressure outside the hospital environment,Furthermore, aac(6')-Ib, responsible for enzymatic modification of aminoglycosides, was detected in 70% of bloodstream isolates and 65% of pneumonia isolates, highlighting the co-resistance phenomenon where carbapenem resistance is frequently accompanied by aminoglycoside resistance,This gene was also detected in 30% of environmental isolates, suggesting a potential reservoir for horizontal gene transfer in hospital surroundings,The combined molecular and phenotypic data reinforce the critical role of targeted surveillance and molecular diagnostics in controlling the dissemination of multidrug-resistant A,Baumannii in clinical settings.

Preliminary Genomic Analysis

Whole-Genome Sequencing (WGS)

To further elucidate the genomic determinants of carbapenem resistance and pathogenicity in Acinetobacter baumannii, whole-genome sequencing (WGS) was performed on a representative subset of 50 isolates identified as high-risk based on resistance profile and biofilm-forming capacity. The sequencing was carried out using the Illumina NovaSeq 6000 platform, generating 2×150 bp paired-end reads with an average genome coverage exceeding 100×.

The bioinformatics workflow incorporated a standardized multi-step pipeline:

a. **Quality Control:** Raw reads were assessed using FastQC for per-base sequence quality, GC content, and adapter contamination. Low-quality bases and adapters were trimmed using Trimmomatic to improve downstream assembly fidelity.
b. **Genome Assembly:** High-quality reads were assembled de novo using SPAdes v3.15, For isolates showing evidence of plasmid-mediated resistance, hybrid assembly was conducted using Unicycler to enhance plasmid contiguity.
c. **Genome Annotation:** Functional annotation of assembled genomes was performed using Prokka and cross-validated using the RAST server to identify coding sequences (CDS), tRNA, and rRNA genes.
d. **Resistome Analysis:** Resistance genes were identified through alignment against the Comprehensive Antibiotic Resistance Database (CARD) and ResFinder, using an identity threshold of ≥98% and a minimum coverage of 90% The genomic characteristics of two predominant clones are presented below:

Table 7. Genomic Features of Top CRAB Clones (Q1).

Strain ID	ST (Pasteur)	Plasmid Replicons	Resistance Genes	Virulence Genes
CRAB-001	ST2	GR6, pAB5	*blaOXA-23*, *aac(6')-Ib*, *sul1*	*ompA*, *bfmR*, *ptk*
CRAB-005	ST1	GR2, pAB3	*blaNDM-1*, *aph(3')-VI*, *tet(B)*	*csuE*, *basD*, *siderophore*

These findings reveal the presence of globally disseminated clonal lineages (notably ST2 and ST1) that harbor a complex array of resistance and virulence determinants,The detection of plasmid replicons such as GR6 and GR2 indicates the potential for horizontal gene transfer,particularly, blaNDM-1 and blaOXA-23 co-localization with aminoglycoside and tetracycline resistance genes signifies multi-resistance profiles in these epidemic clones,The virulence gene repertoire, including ompA and bfmR, correlates with enhanced adherence and biofilm regulation, supporting their clinical persistence.

Statistical correlations were conducted to evaluate the association between resistance determinants, phenotypic biofilm formation, and clinical outcomes across the 200-isolate cohort, The statistical environment R (v4.3.0) was utilized for inferential modeling and regression analysis, while SPSS software supported cluster classification and significance testing.

Key Statistical Approaches:

a. Chi-square test / Fisher's exact test was used to assess the association between biofilm strength (classified into strong, moderate, and weak) and the presence of resistance genes such as blaOXA-23 and aac(6')-Ib, Results indicated statistically significant enrichment of resistance genes in strong biofilm producers ($p < 0.05$).
b. ANOVA was applied to compare mean MIC values of meropenem across different biofilm strength categories, post-hoc Tukey HSD test revealed that strong biofilm

producers exhibited significantly higher MIC values ($p < 0.01$), suggesting a potential protective effect of biofilm matrix on antimicrobial diffusion.

c. Binary logistic regression identified independent predictors of 30-day mortality in CRAB-infected patients, key predictors included meropenem MIC >32 μg/mL (OR = 3.4, 95% CI: 1.6–7.0, $p = 0.002$), presence of blaNDM-1, and ICU admission status.

These statistical insights substantiate the hypothesis that biofilm phenotype and specific resistance genotypes contribute to adverse clinical outcomes and reduced therapeutic efficacy.

3. Results

Clinical and Epidemiological Insights

A total of 200 CRAB samples were systematically analyzed, revealing significant trends in both clinical and epidemiological settings,One of the key findings was the predominance of the Intensive Care Unit (ICU), with 78% (156/200) of the samples being from ICU patients, highlighting the crucial role of this environment in the spread of CRAB infection,Additionally, ventilator-associated pneumonia (VAP), a common complication in patients receiving mechanical ventilation, accounted for 45% (90/200) of all CRAB infections, underscoring the clear link between this infection and mechanical ventilation.

Regarding **mortality association**, the study revealed high mortality rates in CRAB-infected patients, particularly in bloodstream infections:

Bloodstream infections caused by blaOXA-23+/NDM-1+ CRAB had a mortality rate of **55%** (33/60), significantly higher than the **25%** in patients infected with CRAB carrying only the blaOXA-23 gene ($p < 0.001$),This result emphasizes the association between drug resistance, infection severity, and mortality in CRAB cases.Moreover, environmental swab results demonstrated **persistent CRAB in the environment**, with **30%** (15/50) of environmental surface swabs testing positive for CRAB with the same resistance patterns as those found in patient samples, indicating the critical role of the environment in the transmission and persistence of the infection in hospitals.

Table 8. Demographic and Clinical Stratification.

Category	Bloodstream (n=60)	Pneumonia (n=50)	Wound (n=40)	Environmental (n=50)
Mean Age (Years)	58 ± 12	62 ± 15	45 ± 20	N/A
Male (%)	65%	70%	55%	N/A
Diabetes (%)	50%	40%	25%	N/A
Prior Carbapenem Use	85%	90%	60%	N/A
Mortality (30-day)	55%	60%	35%	N/A
Pan-Drug Resistance	45%	65%	25%	15%

Table 8,provides a comprehensive demographic and clinical breakdown of CRAB isolates,From this data, it is notable that the average age of patients with bloodstream infections was 58 ± 12 years, while patients with pneumonia had an average age of 62 ± 15 years,Additionally, male patients were more prevalent in pneumonia cases, with 70% of these cases being male, compared to other infection types,Diabetes was more common among patients with bloodstream infections (50%) compared to those with pneumonia (40%),In terms of prior carbapenem use, a significant proportion of patients with both

bloodstream infections and pneumonia had a history of carbapenem use, with 85% of bloodstream infection cases and 90% of pneumonia cases reporting previous carbapenem exposure,This suggests a clear link between prior antibiotic use and the increased risk of CRAB infection,The 30-day mortality rate was highest for pneumonia cases (60%) compared to bloodstream infections (55%), highlighting the severe impact of CRAB infections in patients with pneumonia,The study also found high levels of pan-drug resistance in pneumonia cases, with 65% of these isolates showing pan-drug resistance, underscoring the therapeutic challenges posed by this infection.

Antimicrobial Resistance Landscape

The study performed a detailed analysis of antimicrobial resistance patterns in CRAB isolates, with a focus on minimum inhibitory concentrations (MIC) for several antibiotics, Notably, Meropenem MICs ranged from 4–256 μg/mL, with over 80% (160/200) of the isolates exceeding the CLSI resistance breakpoint (≥8 μg/mL), confirming the significant resistance of CRAB to carbapenem antibiotics. Meanwhile, Cefiderocol exhibited moderate activity, with 60% susceptibility, suggesting it may be a viable treatment option in some cases.

Table 9. Detailed MIC Distributions (μg/mL).

Antibiotic	MIC Range	MIC50	MIC90	Susceptible (%)	Resistant (%)	Breakpoint (μg/mL)
Meropenem	4–256	64	128	0%	100%	≤2 (S), ≥8 (R)
Imipenem	4–128	32	64	0%	100%	≤4 (S), ≥16 (R)
Colistin	0.25–16	2	8	75%	25%	≤2 (S), ≥4 (R)
Tigecycline	0.5–32	4	16	60%	40%	≤2 (S), ≥8 (R)
Cefiderocol	0.25–64	8	32	60%	40%	≤4 (S), ≥8 (R)
Eravacycline	0.12–8	1	4	85%	15%	≤0.5 (S), ≥2 (R)

Table 9. Displays the MIC distributions for several antibiotics used against CRAB isolates,The data show complete resistance for all isolates to Meropenem and Imipenem, with 100% resistance, reflecting the broad resistance of CRAB to these carbapenem antibiotics,For Colistin and Tigecycline, there was moderate resistance, with 75% of isolates being susceptible to Colistin and 60% to Tigecycline,However, there remains resistance in 25% and 40% of isolates, respectively, indicating significant challenges in treating these infections with these antibiotics,On the other hand, Cefiderocol and Eravacycline exhibited good activity against CRAB, with 60% of isolates being susceptible to Cefiderocol and 85% to Eravacycline, making them promising therapeutic options for CRAB infections.

Key observations indicated that ST2 strains had 4-fold higher MICs compared to ST1 in Meropenem, suggesting stronger resistance mechanisms in the ST2 strain,Furthermore, Colistin resistance was linked to mutations in the mgrB gene, with 90% of Colistin-resistant isolates exhibiting these mutations,This highlights the significant genetic role in drug resistance and emphasizes the need for genetic mutation monitoring in clinical settings,These findings underscore the increasing threat posed by pan-drug-resistant CRAB strains, particularly in ICU settings, and emphasize the urgent need for the development of effective treatment and preventive strategies.

Biofilm Formation: A Resistance Amplifier

Strong biofilm producers (OD570 ≥1.0) demonstrated a **5.2-fold increased survival** in 64 μg/mL meropenem ($p < 0.0001$). This indicates that bacteria producing strong

biofilms are significantly more resistant to treatment with meropenem compared to those with weaker biofilm production.

Table 10. Biofilm Strength vs, Resistance Metrics.

Biofilm Category	Isolates (n)	Avg. Meropenem MIC (μg/mL)	Colistin MIC (μg/mL)	Multi-Drug Resistance (%)	Mortality (%)
Strong (OD570 ≥1.0)	85	64 ± 16	8 ± 4	98%	65%
Moderate (0.5–1.0)	65	16 ± 4	2 ± 1	75%	40%
Weak (<0.5)	50	8 ± 2	0.5 ± 0.25	50%	20%

The table 10, Shows the relationship between biofilm strength (measured by OD570) and various resistance metrics, Strong biofilm producers (OD570 ≥1.0) exhibited the highest resistance to meropenem and colistin, with the highest multi-drug resistance and mortality rates compared to weak biofilm producers.

Multivariate Analysis:

Biofilm strength independently predicted mortality (HR = 2.8, 95% CI: 1.6–4.9, p = 0.001), This suggests that stronger biofilm formation significantly increases the likelihood of mortality, OmpA mutations increased biofilm biomass by 40% (p = 0.003).

This indicates that mutations in the ompA gene can enhance the biofilm production capacity, contributing to increased bacterial resistance.

Molecular Epidemiology of Resistance Genes

Carbapenemase genes were nearly ubiquitous:

blaOXA-23: 80% (160/200) (ICU isolates: 95% prevalence)

blaNDM-1: 35% (70/200) (co-occurred with armA in 60% of cases).

Table 11. Resistance Gene Distribution and Co-Occurrence.

Gene	Prevalence (%)	Co-Occurrence (%)	Associated Phenotype
blaOXA-23	80%	aac(6')-Ib (70%), sul1 (60%)	Pan-drug resistance, high biofilm
blaNDM-1	35%	armA (60%), qnrB (40%)	Cefiderocol resistance, ST1 lineage
aac(6')-Ib-cr	55%	blaOXA-23 (80%), tet(B) (50%)	Aminoglycoside/tigecycline resistance
adeABC efflux pump	90%	blaOXA-23 (85%), mgrB (30%)	Colistin heteroresistance, MIC creep

The table 11, Outlines the distribution of resistance genes and their co-occurrence, B*laOXA-23* was found to co-occur frequently with other resistance genes, such as *aac(6')-Ib* and *sul1*, and was associated with pan-drug resistance and strong biofilm production, The *blaNDM-1* gene was common in the ST1 lineage and co-occurred with *armA*, often conferring resistance to cefiderocol.

Statistical Correlations:

blaOXA-23 + adeABC overexpression increased meropenem MICs by 8-fold ($p < 0.0001$), This indicates that the overexpression of both blaOXA-23 and adeABC significantly increases resistance to meropenem.

blaNDM-1 correlated with environmental persistence (OR = 4.2, $p = 0.01$), This suggests that the blaNDM-1 gene is associated with enhanced survival in environmental settings, making it a significant factor in the persistence of bacteria in various environments.

Genomic Architecture of Dominant Clones

Whole-genome sequencing of 50 isolates revealed:

a. ST2 (Pasteur): Carried blaOXA-23 on plasmid GR6 (IncH-type) and adeABC on a chromosomal resistance island.
b. ST1 (Global Clone 1): Harbored blaNDM-1 on plasmid pAB3 (IncC-type) with mphE macrolide resistance.

The findings provide important insights into the genomic features that contribute to resistance in these clones,ST2 (Pasteur) contains blaOXA-23 on a plasmid (GR6) of the IncH type, which is commonly associated with carbapenem resistance,Additionally, adeABC, a resistance pump, was located on a chromosomal resistance island, further enhancing resistance in this strain,On the other hand, ST1 (Global Clone 1) carried blaNDM-1 on plasmid pAB3 (IncC-type), which is linked to resistance against multiple classes of antibiotics,This clone also harbored mphE, a gene responsible for macrolide resistance,These findings suggest that the genomic organization of these strains plays a key role in their resistance profiles.

Table 12. Genomic Features of ST2 vs, ST1 Clones.

Feature	ST2 (n=35)	ST1 (n=15)	p-value
Resistance Genes	*blaOXA-23*, *aac(6')-Ib*, *sul1*	*blaNDM-1*, *armA*, *mphE*	<0.001
Plasmid Replicons	GR6 (IncH), pAB5 (IncF)	pAB3 (IncC), GR2 (IncN)	0.002
Virulence Genes	*ompA*, *ptk*, *bfmR*	*csuE*, *basD*, *siderophore*	0.03
SNPs in *pbp3*	G526S (90%)	None detected	<0.0001
Biofilm Production	OD570 = 1.8 ± 0.3	OD570 = 0.6 ± 0.2	<0.0001

The table 12, Shows the comparison of various genomic features between ST2 and ST1 clones,ST2 isolates had a significantly higher prevalence of blaOXA-23, aac(6')-Ib, and sul1 resistance genes, whereas ST1 isolates carried blaNDM-1, armA, and mphE,The plasmid replicon types also differed between the two clones, with ST2 predominantly harboring GR6 (IncH) and pAB5 (IncF) plasmids, while ST1 had pAB3 (IncC) and GR2 (IncN),Furthermore, ST2 showed a significantly higher biofilm production (OD570 = 1.8 ± 0.3) compared to ST1 (OD570 = 0.6 ± 0.2), which could be associated with enhanced resistance and virulence. Multivariate Analysis revealed that single-nucleotide polymorphisms (SNPs) in pbp3 were detected in 90% of ST2 strains, with a notable G526S mutation, This SNP was not detected in ST1 strains, The presence of SNPs in pbp3 might contribute to increased resistance, particularly against beta-lactam antibiotics, and may also play a role in the biofilm formation.

Survival Analysis in Galleria mellonella Model

ST2 CRAB: 100% mortality at 48 hours (10^6 CFU/larvae).

Combination therapy (Colistin + Meropenem): Reduced mortality to 30% ($p < 0.001$).

The Galleria mellonella model provides an in vivo platform for evaluating the virulence of bacterial strains and the efficacy of treatment strategies,In this model, ST2 CRAB demonstrated 100% mortality within 48 hours when injected at a dose of 10^6 CFU/larvae, indicating its highly virulent nature,However, when treated with a combination of Colistin and Meropenem, mortality was reduced to 30%, indicating the therapeutic potential of this combination in combating ST2 CRAB infections,This suggests that combination therapy may provide significant benefits in treating infections caused by highly resistant strains.

Table 13. In Vivo Therapeutic Efficacy.

Treatment	Survival Rate (48h)	Log-Rank Test (vs,Control)	Synergy (FICI)
Untreated control	0%	—	—
Meropenem monotherapy	20%	p = 0.12	—
Colistin monotherapy	40%	p = 0.03	—
Colistin + Meropenem	70%	p < 0.001	0.25 (Synergy)
Cefiderocol + Avibactam	50%	p = 0.01	0.5 (Additive)

The table 13, Summarizes the survival rates of Galleria mellonella larvae infected with ST2 CRAB and treated with different therapeutic regimens,The untreated control group had a survival rate of 0%, while Meropenem monotherapy only provided a 20% survival rate, indicating limited efficacy,Colistin monotherapy showed a better survival rate of 40%, but the combination therapy of Colistin and Meropenem was the most effective, with a 70% survival rate,This combination demonstrated synergy (FICI = 0.25), meaning the two drugs together were more effective than when used separately,Cefiderocol and Avibactam, though effective, had a survival rate of 50% and showed an additive effect (FICI = 0.5), indicating that the combination of these two drugs was more effective than each drug alone but not as potent as the Colistin + Meropenem combination.

Multivariate Predictors of Mortality

Table 14. Cox Regression Model (Adjusted for Age, Comorbidities).

Variable	Hazard Ratio (HR)	95% CI	p-value
Meropenem MIC ≥64 μg/mL	3.8	2.1–6.9	0.0001
***blaNDM-1* positivity**	2.5	1.4–4.5	0.002
Biofilm strength (OD570 ≥1.0)	2.2	1.3–3.8	0.004
ST2 lineage	4.1	2.3–7.3	<0.0001

The Cox regression model shows the significant predictors of mortality, adjusted for age and comorbidities. Meropenem MIC ≥64 μg/mL was associated with a 3.8-fold increased hazard of mortality (p = 0.0001), indicating that higher resistance to meropenem significantly impacts survival,The positivity for blaNDM-1 increased the hazard by 2.5 times (p = 0.002), highlighting the role of this resistance gene in patient outcomes,Biofilm strength, as indicated by OD570 ≥1.0, was also a significant predictor of mortality (HR = 2.2, p = 0.004), further emphasizing the clinical importance of biofilm formation in the virulence and resistance of bacteria,Finally, the ST2 lineage was associated with a 4.1-fold

higher hazard of mortality ($p < 0.0001$), reinforcing the finding that ST2 is a highly virulent and resistant strain.

4. Discussion

The findings of this study align with and expand upon the growing body of evidence detailing the complex interplay between genomic adaptability, antimicrobial resistance, and clinical outcomes in Acinetobacter baumannii,The dominance of the ST2 clone, characterized by blaOXA-23 carriage and hyperactive efflux systems, mirrors observations by Touchon et al. [3] and Lee et al. [5], who emphasized the role of horizontal gene transfer and plasmid-mediated resistance in Acinetobacter evolution,The association of blaOXA-23 with high mortality in bloodstream infections corroborates Chusri et al. [7], who linked carbapenemase genes to poor prognosis in bacteremia,However, this study uniquely identifies the synergistic lethality of blaOXA-23 and blaNDM-1, a combination not extensively reported in earlier works like Piperaki et al. [8].

The role of biofilm formation as a resistance amplifier, particularly in isolates with ompA mutations, resonates with Roy et al. [9] and Penesyan et al. [19], who highlighted biofilm-mediated tolerance to meropenem. Yet, the 5.2-fold survival advantage under meropenem stress observed here exceeds previous estimates, suggesting that biofilm dynamics in clinical CRAB strains may be more potent than previously modeled in vitro,The environmental persistence of CRAB clones, particularly blaNDM-1-harboring strains, aligns with Ahuatzin-Flores et al. [10], who recently documented the expansion of CRAB niches post-COVID-19, though this study further implicates biofilm-embedded strains as key reservoirs for hospital outbreaks.

The efficacy of colistin-meropenem combination therapy in Galleria mellonella models contrasts with Abdelaziz et al. [6], who reported diminished colistin activity in post-pandemic CRAB isolates, but supports Gordillo Altamirano et al. [22], who advocated for phage-antibiotic synergy,The high mortality linked to ST2 clones echoes Nasr [13] and Harding et al. [4], who identified ST2 as a global threat due to its chromosomal plasticity and virulence gene repertoire,However, the 4.1-fold mortality hazard attributed to ST2 in this study surpasses prior estimates, potentially reflecting evolving resistance patterns in ICUs.

The genomic detection of adeABC efflux pumps in 90% of isolates reinforces Blair et al. [11] and Yoon et al. [20], who described efflux systems as central to pan-drug resistance. Yet, the correlation between adeABC overexpression and meropenem MIC escalation (8-fold) provides novel mechanistic insight into carbapenem failure, a phenomenon previously attributed primarily to β-lactamase activity, The identification of mphE in ST1 clones, as reported in Lin and Lan [15], underscores the underappreciated role of macrolide resistance in CRAB's adaptive arsenal. While Tacconelli et al. [2] and WHO priority lists have long flagged CRAB as critical, this study quantifies its post-COVID-19 trajectory, revealing a 65% pan-drug resistance rate in pneumonia isolates—a stark increase from pre-pandemic figures in Abdelaziz et al. [6],The environmental detection of CRAB clones with patient-matched resistance profiles challenges infection control paradigms, as noted in Diancourt et al. [16], and calls for genomic surveillance strategies akin to Wick et al. [17].

In conclusion, this study bridges gaps between molecular epidemiology, clinical outcomes, and therapeutic innovation,It validates earlier frameworks by Friedman et al. [1] on the societal burden of resistance while exposing limitations in current treatment guidelines, which often underestimate biofilm-mediated resistance,The data advocate for a shift toward combination therapies and biofilm disruption, aligning with Gil et al. [18] and European Parliament GDPR [23] mandates for proactive pathogen tracking,These findings collectively underscore that CRAB's threat lies not only in its genetic plasticity but in its ability to exploit ecological niches, necessitating equally adaptive countermeasures.

5. Conclusion

The escalating global burden of carbapenem-resistant Acinetobacter baumannii (CRAB) is propelled by the convergence of genetic adaptability, biofilm-mediated resistance, and inefficient treatment regimens,The ST2 clone, armed with blaOXA-23 and hyperactive efflux pumps, emerges as a primary antagonist in ICUs, driving pan-drug resistance and mortality rates exceeding 60% in pneumonia cases,Biofilm formation, intensified by ompA mutations, not only shields CRAB from antimicrobials but also amplifies its environmental persistence, creating reservoirs for recurrent outbreaks,The detection of blaNDM-1 in environmental isolates signals a silent transmission network requiring stringent infection control measures. While novel combinations like colistin-meropenem offer short-term therapeutic hope, long-term solutions demand innovations in biofilm disruption, phage therapy, and antimicrobial stewardship, proactive genomic surveillance of resistance islands and plasmid dynamics is critical to preempt the spread of high-risk clones, this study underscores that combating CRAB necessitates a paradigm shift—from reactive treatment to predictive, genomics-driven intervention.

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