Infection and Drug Resistance

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ORIGINAL RESEARCH

Analysis of Genetic Characteristics, Biofilm Formation Ability, and Mortality Risk Factors in Patients Infected with Carbapenem-Resistant *Acinetobacter baumannii*

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Purpose: This study aims to investigate the prevalence of resistance genes, efflux pump genes, and biofilm-forming genes in carbapenem-resistant *Acinetobacter baumannii*, as well as the clinical characteristics of patients infected with carbapenem-resistant *Acinetobacter baumannii*, in order to provide guidance for rational clinical prevention and control.

Methods: This study included 244 patients with CRAB infections admitted to a tertiary hospital in Zhangjiakou, China, between June 2021 and December 2024. Statistical analysis was performed using SPSS 27.0 software to investigate potential risk factors for mortality associated with CRAB. Conventional PCR amplification was used to qualitatively detect resistance genes, efflux pump genes, and biofilm-related genes in isolated CRAB strains. Biofilm formation capacity was assessed using the crystal violet staining method. The study analyzed the association between the aforementioned genetic and phenotypic characteristics, biofilm formation capacity, and antibiotic resistance.

Results: A total of 244 CRAB isolates were collected in this study, primarily from sputum (55.33%), followed by bronchoalveolar lavage fluid (26.23%). The majority of isolates originated from the ICU (48.77%), followed by the Department of Respiratory Medicine (31.15%). PCR results showed that the predominant resistance genes in CRAB isolates from this hospital were *bla*OXA-51 and *bla*OXA-23, at 89.3% and 87.3%, respectively. The detection rates for *bla*NDM, *bla*OXA-58, and *bla*OXA-24 were 1.6%, 1.2%, and 0.4%, respectively. The efflux pump genes *adeB, adeR, adeS*, and *adeJ* all showed high detection rates, while the *adeG* gene was not detected. The biofilm-related genes with generally high detection rates were *bap* (240/244, 98.4%), *abaI* (227/244, 93.0%), and *ompA* (223/244, 91.4%); the detection rates of the remaining genes ranged between 84.8% and 90.6%. Of the 244 CRAB strains tested in this study, the vast majority (97.95%) possessed biofilm-forming ability. The OD values of the strains were determined using the crystal violet method, with weak biofilm-forming strains (47.54%) and moderate biofilm-forming strains (44.26%) predominating; strong biofilm-forming strains accounted for 6.15%, while strains incapable of biofilm formation accounted for only 2.05%. Antimicrobial susceptibility testing revealed that 99.58% of the 244 CRAB strains were resistant to piperacillin/tazobactam, and 99.15% were resistant to imipenem and meropenem. Resistance rates for amoxicillin/clavulanate, cefepime, ceftazidime, ceftiofur, ciprofloxacin, and levofloxacin, all exceeded 95%, while 3.08% were resistant to tigecycline. No resistance to polymyxin was observed. Results of multivariate logistic regression analysis showed that age (OR = 1.044, 95% CI: 1.009–1.079, $P < 0.05$) and white blood cell count (OR = 1.106, 95% CI: 1.029–1.188, $P < 0.05$) were independent risk factors for mortality in CRAB infections.

Conclusion: In this study, CRAB strains were primarily isolated from the lungs (81.56%) and mainly originated from the ICU and respiratory department. CRAB strains exhibited extremely high resistance rates to most commonly used antimicrobial agents;

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specifically, resistance rates to 9 antimicrobial agents, including piperacillin/tazobactam, imipenem, and meropenem, were all >90%. Only tigecycline showed a relatively low resistance rate (3.08%), and no resistance to polymyxin was detected. The CRAB strains in our hospital primarily harbor the *bla*OXA-23 and *bla*OXA-51 resistance genes and commonly carry efflux pump and biofilm formation genes, suggesting that their resistance may be closely associated with carbapenemases, efflux pump systems, and biofilm formation.

Keywords: *Acinetobacter baumannii*, carbapenem resistance, genes, efflux pumps, biofilms

Introduction

Antimicrobial resistance (AMR) refers to the ability of bacteria to resist drugs, resulting in reduced effectiveness of medications originally used to treat infections; its emergence and spread have become one of the key public health threats of the 21st century. According to statistics, antimicrobial resistance caused 1.27 million direct deaths and 4.95 million indirect deaths worldwide in 2019.[1] Ranked by number of deaths, these six pathogens—*Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii* (Ab), and *Pseudomonas aeruginosa*—each caused more than 250,000 deaths associated with antimicrobial resistance.[1] In recent years, the mortality rate among patients with *Acinetobacter baumannii* infections has ranged from 28% to 43% for bloodstream infections and from 40% to 70% for ventilator-associated pneumonia.[2] Consequently, in 2024, the World Health Organization (WHO) released a new list of priority bacterial pathogens; due to its resistance to multiple antibiotics, carbapenem-resistant *Acinetobacter baumannii* (CRAB) was designated a priority pathogen.[3]

Ab is a common non-fermentative, strictly aerobic, non-motile, catalase-positive, and oxidase-negative Gram-negative bacillus.[4] *A. baumannii* is a common colonizing bacterium among hospitalized patients and is widely found on the surfaces of medical devices. As an opportunistic pathogen, it frequently causes hospital-acquired infections, including ventilator-associated pneumonia, urinary tract infections, bacteremia, meningitis, and skin or soft tissue infections. It is one of the major pathogens responsible for infections in immunocompromised individuals.[5,6] In recent years, with the widespread clinical use of carbapenem antibiotics, there has been a steady increase in resistant Acinetobacter strains, including multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) strains. These strains are difficult to treat with currently available medications, exacerbating the problem of hospital-acquired infections caused by this bacterium.[7] CRAB exhibits multidrug resistance, has extremely limited treatment options, and has become widespread globally, posing a serious threat to global public health.[8] A nationwide survey of Acinetobacter isolates in the United States, covering hospitals, long-term care facilities, and outpatients, found that the rate of multidrug resistance reached 49%.[9] Since 2016, the overall incidence of carbapenem-resistant *Acinetobacter baumannii*-associated bloodstream infections at a tertiary hospital in South Korea has been estimated to exceed 87%.[10] In recent years, mortality rates associated with *Acinetobacter baumannii* infections have remained high, ranging from 28% to 43% among patients with bloodstream infections and as high as 40% to 70% among those with ventilator-associated pneumonia.[2] In China, Ab exhibits high resistance (>50%) to a range of antimicrobial agents, including carbapenems, and there is a trend toward decreasing susceptibility to bactericidal agents.[11,12]

The mechanisms underlying CRAB resistance to carbapenems are highly complex and primarily involve the expression of active efflux pumps; alterations in antibiotic binding sites; the production of carbapenemases; and biofilm formation.[13] Among these, the production of carbapenemases is the primary mechanism of resistance; this enzyme hydrolyzes and inactivates carbapenem antibiotics and is often encoded by plasmids, making it highly transmissible.[14] According to the Ambler classification, carbapenemases are divided into four classes: A, B, C, and D,[15] Class D OXA enzymes are the most common and exhibit remarkable genetic diversity. It is worth noting that active efflux mediated by multidrug efflux pumps of the resistance-nodulation-cell division (RND) superfamily is a key mechanism by which microorganisms defend against structurally diverse antimicrobial agents; overexpression of these pumps can significantly increase a strain's level of resistance.[16] The formation of biofilms enhances the environmental tolerance and antibiotic resistance of *Acinetobacter baumannii*, making it easier for the bacterium to colonize the surfaces of medical devices; this is a major factor contributing to hospital-acquired infections.[17–19] Biofilms interact synergistically with β-lactamases, further exacerbating the spread of multidrug-resistant strains, leading to prolonged and difficult-to-treat infections, and increasing the burden on public health.[20,21]

Although there have been numerous studies on the antimicrobial resistance profiles and clinical risk factors of *Acinetobacter baumannii* both domestically and internationally, there are significant regional variations in the epidemiological characteristics, resistance profiles, and molecular features of strains across different regions and healthcare institutions, making it difficult to generalize research findings across regions. Currently, the Zhangjiakou region lacks systematic epidemiological data on CRAB, and previous relevant studies are scarce. As a result, clear trends in antimicrobial resistance and local characteristics have not yet been established, limiting the development of precise antimicrobial therapy and strategies for the prevention and control of nosocomial infections in the region. Therefore, this study combines locally clinically isolated CRAB strains to comprehensively analyze their clinical characteristics, antimicrobial resistance phenotypes, and the distribution of genes related to efflux pumps and biofilms, while also exploring independent risk factors affecting patient prognosis. The aim is to establish epidemiological reference data for CRAB in this region, providing a scientific basis for personalized treatment of local CRAB infections and hospital infection control.

Materials and Methods

Strain Source

The study included 244 patients diagnosed with CRAB infection at our hospital between June 2021 and December 2024, along with their bacterial isolates. Samples indicating CRAB colonization were excluded. For each patient, only the first isolate obtained during a single hospitalization was included, and duplicate isolates were excluded. All strains underwent species identification and antimicrobial susceptibility testing using the BD Phoenix™ 100 fully automated bacterial identification and susceptibility testing system (Becton, Dickinson and Company, Sparks, Maryland, USA), with CRAB identification based on CLSI standards. Escherichia coli ATCC 25922 was used as the internal quality control strain.

DNA Extraction

The strains were retrieved from the −80 °C stock library and streaked onto MacConkey agar medium for isolation and culture, followed by incubation at 37 °C for 24 h in a thermostatic incubator. An appropriate amount of colonies were scraped from the agar plate using an inoculation loop and transferred into a centrifuge tube containing 500 μL of sterile water, followed by thorough mixing. The tube was then heated at 100 °C for 15 min for inactivation and centrifuged at 12 000 r/min for 5 min. The supernatant was collected into a new centrifuge tube, and the precipitate was discarded. The obtained supernatant was used as the DNA template and stored at −20 °C until further use.

Common Resistance Genes Detected by PCR

The collected CRAB isolates were analyzed for drug resistance genes, efflux pump genes, and biofilm-related genes by conventional PCR amplification of gene sequences. (Table 1) Design primer sequences for the gene based on relevant literature.[22–25]The PCR reaction was performed in a total volume of 25 μL, containing 12.5 μL of 2× Taq Master Mix, 1 μL of forward primer, 1 μL of reverse primer, 1 μL of DNA template, and 9.5 μL of sterile enzyme-free water. Gene amplification was carried out under the following conditions: pre-denaturation at 95 °C for 5 min, denaturation at 95 °C for 30s, and annealing for 30s (See Table 1 for the annealing temperatures), Extension was performed at 72 °C for 30s, for a total of 35 cycles, followed by a final extension at 72 °C for 5 min. After completion of the reaction, the PCR products were stored at 4 °C or immediately subjected to agarose gel electrophoresis at 100 V for 45 min. Microscopic imaging and photographic documentation were performed after electrophoresis.

Detecting Biofilm Formation

Following revival, the strains were subcultured on MacConkey agar plates and incubated at 37 °C for 24 h overnight. Single colonies were picked and inoculated into broth medium, followed by shaking incubation at 37 °C for 24 h. The cultures were centrifuged at 3000 r/min at 4 °C for 5 min, and the supernatants were discarded. After washing with sterile

Table 1 CRAB Resistance Gene Primer Sequences

Gene	Primer	Primer Sequence (5′-3′)	Product Size (bp)	Annealing Temperature (°C)
*bla*OXA-23	F	ACCCCGAGTCAGATTGTTCAAGG	606	56
	R	CCAGCCCACTTGTGGTTTTATAT		
*bla*OXA-24	F	GTCCCTGCATCAACATTTAAGATGC	477	
	R	CACCCAACCAGTCAACCAACCTACCT		
*bla*OXA-51	F	GCTATGGTAATGATCTTGCTCGTGC	509	
	R	TCCAGTTAACCAGCCTACTTGTGGG		
*bla*OXA-58	F	ATATGGCACGCATTTAGACCGAG	515	
	R	CAACAAAACCCACATACCAACCC		
*bla*NDM	F	GGTTTGGGATCTGGTTTTC	621	58
	R	CGGAATGGCTCATCACGATC		
adeB	F	TTAACGATAGCGTTGTAACC	541	55
	R	TGAGCAGACAATGGAATAGT		
adeR	F	ACTACGATATTGGCGACATT	447	
	R	GCGTCAGATTAAGCAAGATT		
adeS	F	TTGGTTAGCCACTGTTATCT	544	
	R	AGTGGACGTTAGGTCAAGTT		
adeJ	F	ATTGCACCACCAACCGTAAC	453	
	R	TAGCTGGATCAAGCCAGATA		
adeG	F	TTCATCTAGCCAAGCAGAAG	468	
	R	GTGTAGTGCCACTGGTTACT		
bap	F	GAGGGAACTTCTGCAAAACTTTC	108	
	R	CAGACGTATGACTGCATTGGT		
ompA	F	GAGTCGTATTGCACTTGCTAC	594	
	R	GCAGGCTTCAAGTGACCACC		
csuA	F	GAGGGAAGATGATATTCAATCGTG	517	
	R	CCCTTAGATATACGACTACCATCAT		
csuB	F	GCAGCAGATCCTCAGCTCAATTCA	354	
	R	GTTTGTAGGTGTTGTAGCAGGC		
csuC	F	ATACGGGTAAGACCGATGC	466	
	R	GTTTTCAGTGCCGAAAGACG		
csuD	F	GCTGATTTTATTGTCGGGTGGA	464	
	R	GCCCTATACCAGACTGAGCGAC		
csuE	F	ACCAATGCTCAGACCGGAG	751	
	R	CTTGTACCGTGACCGTATCTTG		
abaI	F	CCGCTACAGGGTATTTGTTGAA	428	
	R	CACGATGGGCACGAAAACC		
bfmR	F	GAAGTTGGTGTAGAAACCGATG	557	
	R	GGATTTTCAGGATCATCGCC		
bfmS	F	CATTAGTGAAGGAGTCGCTCG	990	
	R	GGTGTACCCTGCTCTAGTTTT		

PBS, 3 mL of LB broth was added and mixed thoroughly. Bacterial suspensions equivalent to 0.5 McFarland standard were prepared in normal saline and inoculated into 96-well culture plates (200 μL of LB broth per well). Twelve wells in the first row were used as negative controls. A 20 μL aliquot of bacterial suspension was added to the second well of the first column, with seven biological replicates for each strain. The plates were incubated at 37 °C for 3 days, and the medium was replaced every 24 h. After incubation, the liquid in each well was discarded. A volume of 200 μL of 100% methanol was added to each well, and fixation was performed at 37 °C for 30 min. After removing the methanol, 1% crystal violet was added for staining for 30 min. The wells were rinsed thoroughly with PBS and dried at 37 °C for 30 min. Finally, 200 μL of 95% ethanol was added for elution. The optical density (OD) of each well was measured at a wavelength of 595 nm using a microplate reader. The cutoff OD value (ODc) was defined as the mean OD of negative

controls + 3SD. Biofilm formation ability was classified into four levels accordingly: Strong positive: 4×ODc < OD; Moderate positive: 2×ODc < OD ≤ 4×Odc; Weak positive: ODc < OD ≤ 2×Odc; Negative: OD ≤ Odc.[26]

Statistical Methods

Based on information from the CRAB pathogen database, we accessed electronic medical records to obtain various clinical data on patients and compiled this information into an electronic database (raw data). Statistical analysis was performed using SPSS 27.0 software. Continuous variables that followed a normal distribution were expressed as mean ± standard deviation (x ± s), and Student's t-test was used for comparisons between groups; Non-normally distributed quantitative data were described as M (P25, P75), and the Mann–Whitney U-test was used for comparisons between groups. Categorical data were expressed as numbers and percentages [n(%)], and comparisons were performed using Pearson's chi-square test or Fisher's exact test, with continuity correction applied when the frequency was less than 5. Variables with $P < 0.05$ in univariate analysis were subjected to multicollinearity testing. Multivariate logistic regression was used to analyze independent risk factors for mortality in patients with CRAB infection, and odds ratios (OR) and 95% confidence intervals (CI) were calculated; a P-value < 0.05 was considered statistically significant. Correlation heatmaps, pie charts, and bar charts were generated using ChiPlot (https://www.chiplot.online/).

Results

Clinical Characteristics

The 244 patients infected with CRAB were primarily from the ICU (119, 48.77%), followed by the Department of Respiratory Medicine (76, 31.15%), Neurosurgery (14, 5.74%), and Rehabilitation Medicine (9, 3.69%); the remainder were distributed across various other departments (Figure 1a). Specimens were primarily obtained from the respiratory tract, including sputum (139, 55.33%), bronchoalveolar lavage fluid (64, 26.23%), and blood (16, 6.56%); other specimens included a small number of wound secretions, among others (Figure 1b).

CRAB infections primarily affect the elderly, with men outnumbering women by a ratio of 2:1 (178 cases vs. 66 cases). To investigate risk factors associated with mortality following CRAB infection, patients were divided into a survival group and a death group based on survival outcomes, and univariate analysis was performed. The results showed statistically significant differences between the two groups in age, white blood cell count, neutrophil percentage, lymphocyte percentage, platelet count, albumin, and total length of hospital stay ($P < 0.05$). In the non-survivor group, the rates of procalcitonin, blood glucose, mechanical ventilation, endotracheal intubation, central venous catheterization, hemodialysis, bronchoscopy, and the use of carbapenem antibiotics and glucocorticoids were significantly higher in the non-survivor group, while the survivor group had significantly higher rates of surgery, drainage tube placement, use of cephalosporin and fluoroquinolone antibiotics, and incidence of trauma; all intergroup differences were statistically significant ($P < 0.05$) (Table 2). We performed a multicollinearity test on the variables in the table with $P < 0.05$ and found that the variance inflation factors for both the percentage of neutrophils and the percentage of lymphocytes were greater than 5, indicating

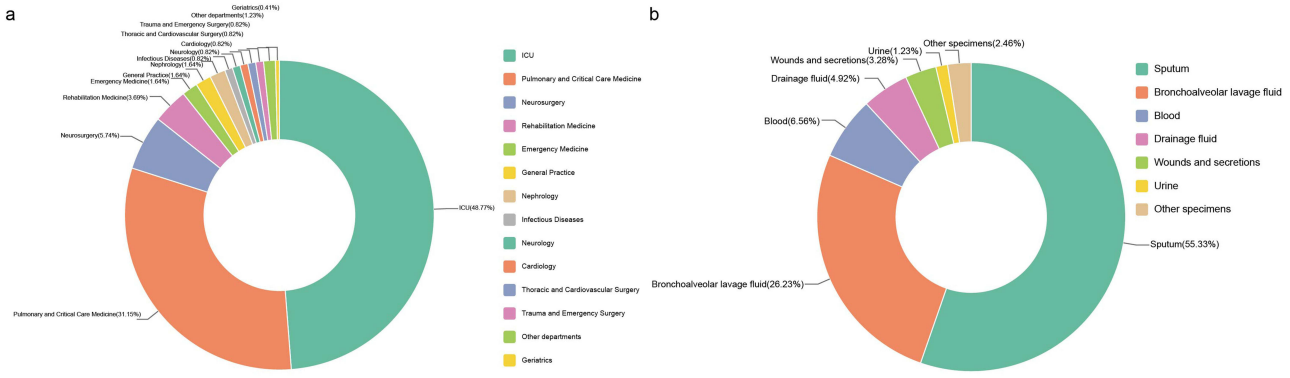

Figure 1 (**a**) Distribution of clinical departments. (**b**) Distribution of clinical specimens.

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Table 2 Univariate Analysis of the Survival and Mortality Groups Among Patients with CRAB Infection

Variables	Survival Group (n=164)	Non-Survival Group (n=80)	P Value
Age	67.5 (58, 75)	75.5 (69.25, 82)	<0.001
Male	118 (71.95)	60 (75)	0.615
WBC (10^9/L)	11.61 (8.49, 14.50)	14.56 (10.61, 17.98)	<0.001
Neu (%)	85.20 (80.25, 90.35)	91.55 (87.75, 94.80)	<0.001
Lym (%)	8.20 (4.90, 12.58)	4.35 (2.30, 6.88)	<0.001
Hb (g/L)	102.5 (88.25, 123.75)	100 (90, 123.75)	0.851
PLT (10^9/L)	227.5 (141.75, 306.25)	165.5 (96.5, 271.25)	<0.001
PCT (ng/mL)	0.32 (0.12, 1.12)	1.17 (0.61, 6.02)	<0.001
Alb (g/L)	31.25 (28.15, 33.8)	29 (26.58, 32.25)	0.005
BG (mmol/L)	7.57 (6.10, 10.78)	8.70 (7.10, 12.07)	0.017
Total Hospital Stay days	26 (16, 40.5)	16 (10.25, 21)	<0.001
ICU Stay days	11 (5, 20)	13.5 (8, 18)	0.214
COPD	21 (12.8)	11 (13.8)	0.837
Coronary Artery Disease	22 (13.4)	17 (21.3)	0.117
Hypertension	76 (46.3)	39 (48.8)	0.723
Diabetes Mellitus	30 (18.3)	22 (27.5)	0.099
Cerebral Infarction	21 (12.8)	12 (15)	0.638
Autoimmune Disease	7 (4.3)	2 (2.5)	0.744
Bronchiectasis	0	1 (1.3)	0.328
Asthma	0	1 (1.3)	0.328
Interstitial Lung Disease	1 (0.6)	0 (0)	1.000
Malignancy	7 (4.3)	7 (8.8)	0.263
Previous hospitalization	25 (15.2)	14 (17.5)	0.652
Mechanical Ventilation	121 (73.8)	76 (95)	<0.001
Tracheotomy	64 (39)	23 (28.7)	0.116
Endotracheal Intubation	115 (70.1)	72 (90)	<0.001
Central Venous Catheterization	120 (73.2)	74 (92.5)	<0.001
Hemodialysis	17 (10.4)	17 (21.3)	0.021
Bronchoscopy	62 (37.8)	42 (52.5)	0.029
Operation	62 (37.8)	10 (12.5)	<0.001
Drainage Tube Placement	72 (43.9)	16 (20)	<0.001
Cephalosporin Antibiotics	133 (81.1)	52 (65)	0.006
Fluoroquinolone Antibiotics	63 (38.4)	14 (17.5)	<0.001
Aminoglycoside Antibiotics	12 (7.3)	1 (1.3)	0.093
Carbapenem Antibiotics	91 (55.5)	61 (76.3)	0.002
Penicillin Antibiotics	88 (53.7)	33 (41.3)	0.069
Antifungal Agents	17 (10.4)	10 (12.5)	0.618
Glucocorticoids	76 (46.3)	57 (71.3)	<0.001
Septic Shock	16 (9.8)	29 (36.3)	<0.001
Trauma	28 (17.1)	3 (3.8)	0.003
Hypoalbuminemia	108 (65.9)	59 (73.8)	0.213

Notes: Data are expressed as n (%) or median (IQR).
Abbreviations: WBC, White Blood Cell; Neu, Neutrophil Percentage; Lym, Lymphocyte Percentage; Hb, Hemoglobin; PLT, platelet Count; PCT, Procalcitonin; Alb, Albumin; BG, Blood Glucose; COPD, Chronic Obstructive Pulmonary Disease.

significant multicollinearity between the two variables. After excluding the percentage of lymphocytes, the remaining variables were included in a multivariate logistic regression analysis. The results showed that age (OR = 1.044, 95% CI: 1.009–1.079, P < 0.05), white blood cell count (OR = 1.106, 95% CI: 1.029–1.188, P < 0.05) were independent risk factors for death from CRAB infection. Platelet count (OR = 0.995, 95% CI: 0.991–0.999, P < 0.05) and the use of cephalosporin antibiotics (OR = 0.398, 95% CI: 0.160–0.990, P < 0.05) were independent protective factors against death from CRAB

infection (Table 3). In addition, this study conducted a visual subgroup analysis of the survival and mortality groups by gender to compare differences in the distribution of age and various laboratory parameters across gender subgroups. This analysis is presented for descriptive purposes only and was not included in the multivariate regression adjustment. The results showed that in the mortality group, there were statistically significant differences in albumin levels ($P < 0.01$) (Figure 2a), age ($P < 0.05$) (Figure 2b), and hemoglobin levels ($P < 0.01$) (Figure 2c) between male and female patients. No significant statistical differences were observed for the remaining indicators between the two genders within the two groups.

Table 3 Multivariate Logistic Regression Analysis of Risk Factors for Death Following CRAB Infection

Variables	P Value	OR	95% CI
Age	0.012	1.044	1.009–1.079
WBC (10^9/L)	0.006	1.106	1.029–1.188
Neu (%)	0.759	1.010	0.949–1.074
PLT (10^9/L)	0.014	0.995	0.991–0.999
Alb (g/L)	0.727	1.016	0.929–1.111
Total Hospital Stay	0.238	0.987	0.965–1.009
Mechanical Ventilation	0.899	1.150	0.132–10.034
Endotracheal Intubation	0.248	2.897	0.476–17.624
Central Venous Catheterization	0.062	3.704	0.935–14.669
Hemodialysis	0.969	0.979	0.339–2.827
Bronchoscopy	0.374	0.692	0.308–1.558
Operation	0.059	0.315	0.095–1.046
Drainage Tube Placement	0.861	0.910	0.317–2.617
Cephalosporin Antibiotics	0.048	0.398	0.160–0.990
Fluoroquinolone Antibiotics	0.123	0.481	0.190–1.219
Carbapenem Antibiotics	0.102	2.032	0.869–4.754
Glucocorticoids	0.123	1.933	0.836–4.471
Septic Shock	0.062	2.544	0.954–6.787
Trauma	0.797	0.814	0.170–3.900
Procalcitonin	0.341	1.029	0.970–1.092
Blood Glucose	0.191	1.065	0.969–1.171

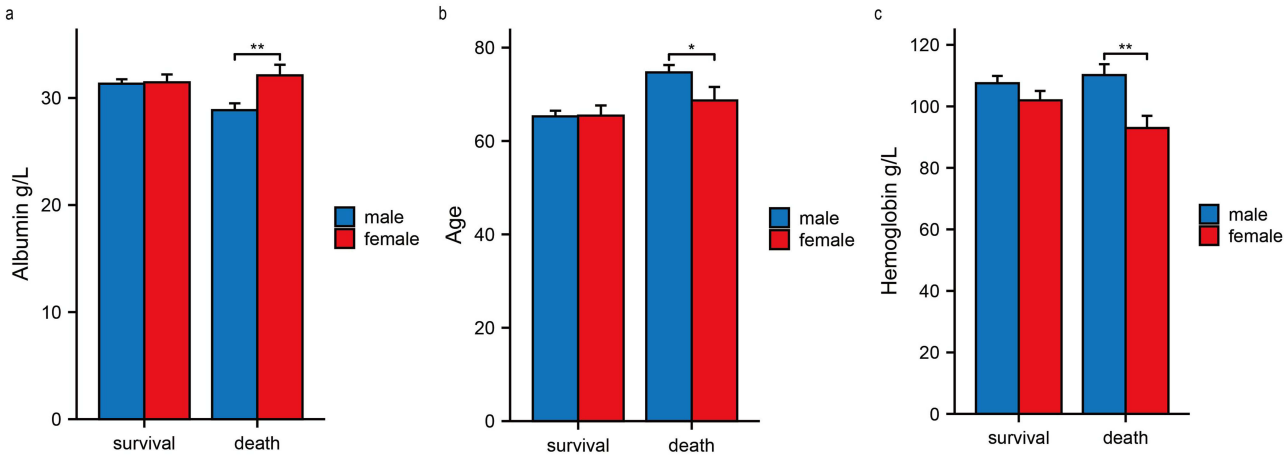

Figure 2 Differences in gender between the survival and non-survival groups of patients with CRAB infection.
Note: *p<0.05; **p<0.01.

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Antibiotic Susceptibility

The 244 clinically isolated CRAB strains collected in this study exhibited resistance rates as high as 90% to nine commonly used clinical antimicrobial agents. The resistance rate to piperacillin/tazobactam reached 99.58% (Figure 3a), while the resistance rates to imipenem (Figure 3b) and meropenem (Figure 3c) reached 99.15%. The resistance rates to amoxicillin/clavulanate (Figure 3d), cefepime (Figure 3e), ceftazidime (Figure 3f), ceftiofur (Figure 3g), ciprofloxacin (Figure 3h) and levofloxacin (Figure 3i) were both greater than 95%; resistance to gentamicin (Figure 3j) was 89.36%; resistance to ampicillin/ sulbactam (Figure 3k) was 79.57%, resistance to amikacin (Figure 3L) was 70.64%, resistance to co-trimoxazole (Figure 3m) was 50.64%, and resistance to tigecycline (Figure 3n) was 3.08%. However, all CRAB strains exhibited high susceptibility (100%) to polymyxin (Figure 3o).

CRAB Gene Carrier Status

The primary resistance genes identified in CRAB isolates from our hospital were *bla*OXA-51 and *bla*OXA-23, accounting for 89.3% and 87.3% of cases, respectively. The detection rates for *bla*NDM, *bla*OXA-58, and *bla*OXA-24 were 1.6%, 1.2%, and 0.4%, respectively. Concurrently, one strain carried *bla*OXA-23, *bla*OXA-51, and *bla*OXA-58; one strain carried *bla*OXA-51 and *bla*NDM; two strains carried *bla*OXA-23, *bla*OXA-51, and *bla*NDM; and 208 strains carried only *bla*OXA-23 and *bla*OXA-51 (Figure 4a). In this study, the detection rates of efflux pump genes were as follows: *adeB* (209/244, 85.7%), *adeR* (182/244, 74.6%), *adeS* (205/244, 84.0%), and *adeJ* (213/244, 87.3%); the *adeG* gene was not detected (Figure 4b). The

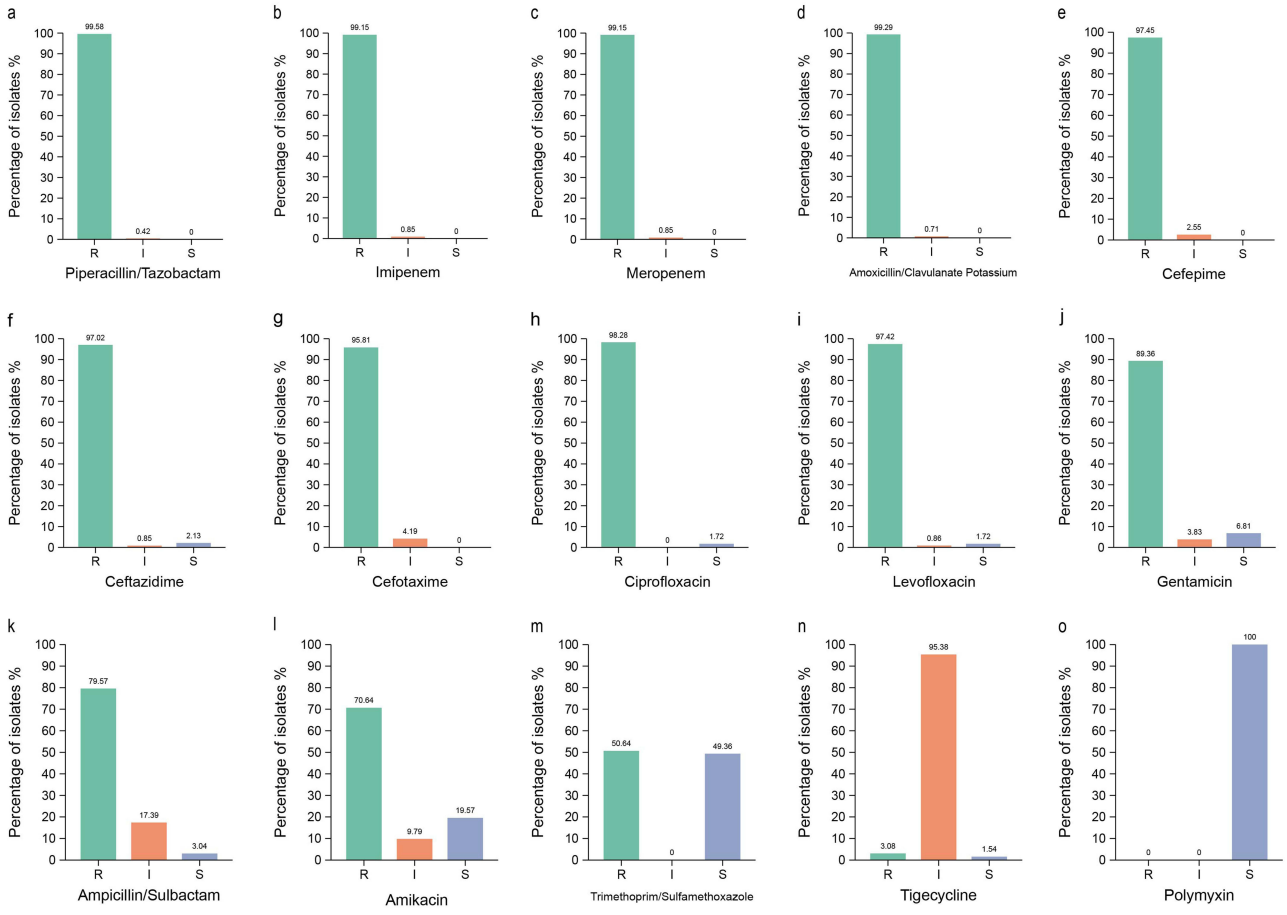

Figure 3 Antimicrobial resistance profiles of CRAB isolates.

Notes: Due to variations in the antibiotic testing panels used for some patients (differences in the types of antibiotics tested), the total number of strains detected for different antibiotics is not uniform. (**a**) Piperacillin/Tazobactam (n=237). (**b**) Imipenem (n=235). (**c**) Meropenem (n=233). (**d**) Amoxicillin/Clavulanate Potassium (n=140). (**e**) Cefepime (n=235). (**f**) Ciprofloxacin (n=233). (**g**) Levofloxacin (n=233). (**h**) Ceftazidime (n=235). (**i**) Cefotaxime (n=167). (**j**) Gentamicin (n=235). (**k**) Ampicillin/Sulbactam (n=230). (**l**) Amikacin (n=235). (**m**) Trimethoprim/Sulfamethoxazole (n=233). (**n**) Tigecycline (n=65). (**o**) Polymyxin (n=234).

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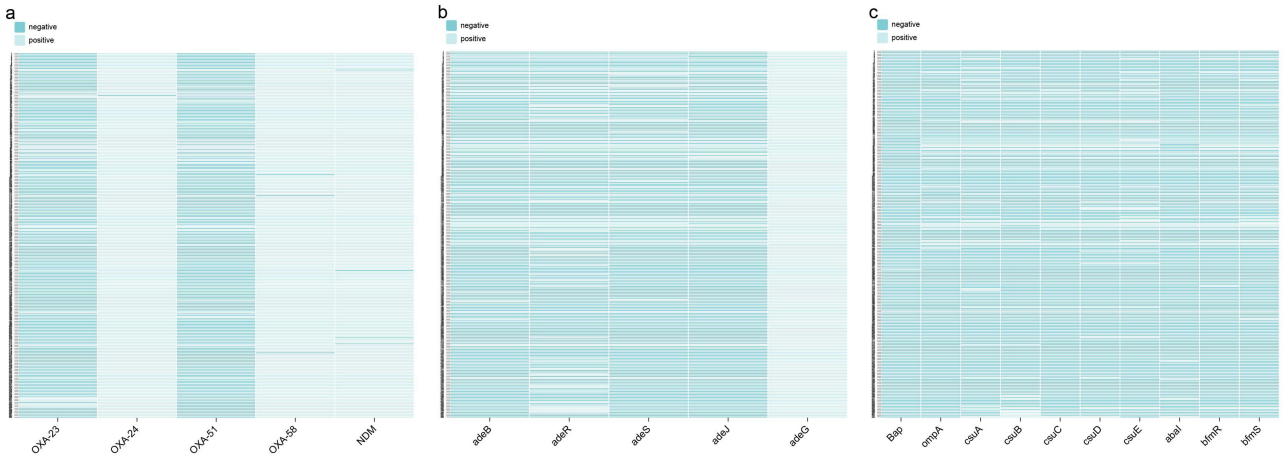

Figure 4 CRAB resistance gene, efflux pump gene, and biofilm gene detection results. (a) drug resistance gene. (b) efflux pump gene. (c) biofilm gene.

detection rates of biofilm-related genes were generally high for *bap* (240/244, 98.4%), *abaI* (227/244, 93.0%), and *ompA* (223/244, 91.4%); the detection rates of the remaining genes ranged from 84.8% to 90.6% (Figure 4c).

The Relationship Between CRAB's Biofilm-Forming Ability and Antibiotic Resistance

The results of strain detection in this study showed that only 2.05% of CRAB isolates exhibited no biofilm formation ability, 47.54% were weak biofilm producers, 44.26% were moderate biofilm producers, and 6.15% were strong biofilm producers. Overall, the vast majority of clinically isolated CRAB strains were able to form biofilms to varying degrees.To clarify the relationship between biofilm formation capacity and antimicrobial resistance, this study analyzed the correlation between CRAB biofilm formation and resistance phenotypes to amikacin and the compound sulfonamide-trimethoxypolymyxin (piperacillin-tazobactam, ampicillin-sulbactam, cefotaxime, ceftiofur, cefepime, ciprofloxacin, levofloxacin, gentamicin, imipenem, meropenem, tigecycline, amoxicillin-clavulanate, and polymyxin; due to the small number of resistant or susceptible strains, the sample sizes were severely unbalanced, and therefore no numerical comparisons or statistical analyses were performed). Amikacin and co-trimoxazole, which had relatively balanced sample distributions, were divided into resistant and susceptible groups, and Spearman correlation analysis was performed. The results of the two analyses showed that there were no statistically significant differences in biofilm levels between the amikacin-resistant group and the susceptible group, or between the co-trimoxazole-resistant group and the susceptible group. The correlation analysis indicated that there was no significant correlation between the amikacin resistance phenotype and biofilm formation ($r = -0.045$, $P = 0.491$), nor was there a significant correlation between the co-trimoxazole resistance phenotype and biofilm formation ($r = 0.09$, $P = 0.18$) (Table 4).

Discussion

The CRAB strains collected in this study were sourced from a variety of specimens, with sputum samples accounting for the largest proportion (55.33%), followed by bronchoalveolar lavage fluid (26.23%). In terms of clinical departments, the majority of samples were collected from the ICU (48.77%), followed by the respiratory department (31.15%). These distribution patterns may be highly correlated with the immune status of patients in these departments, the types of diagnostic and therapeutic procedures performed, and environmental exposure risks. For example, ICU patients primarily

Table 4 The Relationship Between Antibiotics and Biofilm Formation

Antibiotics	Resistance	Sensitive	r	p
Amikacin	0.29 (0.21, 0.40)	0.27 (0.19, 0.44)	−0.045	0.491
Trimethoprim-Sulfamethoxazole	0.26 (0.19, 0.40)	0.31 (0.21, 0.44)	0.09	0.18

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consist of those with severe trauma, multiple organ dysfunction syndrome (MODS), and postoperative critical care patients. Such patients generally exhibit immunosuppression and require frequent invasive procedures (such as mechanical ventilation, central venous catheterization, and blood purification), whereas *Acinetobacter baumannii* possesses a strong ability to form biofilms. By secreting extracellular polysaccharides, proteins, and other substances, it can form biofilms on the surface of respiratory mucosa or the inner walls of endotracheal tubes, enabling long-term colonization. When a patient's immune function declines or the local respiratory microecology becomes imbalanced, colonized CRAB can breach the mucosal barrier and trigger infection. This highlights the clinical need to strictly control the indications for invasive procedures and to tailor treatment plans to individual patient conditions. Whenever clinically feasible, invasive devices such as central venous catheters and endotracheal tubes should be removed as early as possible to reduce iatrogenic exposure, thereby lowering the risk of CRAB infection and nosocomial transmission.

Among the 244 patients with CRAB infection included in this study, the elderly constituted the largest group, and the male-to-female ratio was approximately 2:1. This distribution pattern is highly consistent with the results of previously reported epidemiological studies on CRAB infection both domestically and internationally, indicating its broad applicability. This study grouped patients with CRAB infection based on survival outcomes (survival/death) and conducted a multivariate analysis of risk factors. The results showed that age and white blood cell count ($P < 0.05$) were independent risk factors for death in patients with CRAB infection. Age, as an unmodifiable risk factor, poses a risk because the immune function of elderly patients declines with age, significantly weakening their resistance to pathogens and making them more susceptible to colonization and infection. Additionally, elderly individuals often have multiple comorbidities, such as chronic obstructive pulmonary disease (COPD), diabetes, and coronary heart disease, which impair the body's barrier functions and create conditions conducive to pathogen invasion and colonization. Elevated white blood cell (WBC) counts serve as a key indicator of the body's inflammatory response; abnormally high levels often signal an intensification of systemic inflammation caused by drug-resistant bacterial infections. As a core component of the human immune system, white blood cells participate in immune defense by phagocytosing pathogens. However, the drug-resistant nature of these bacteria makes infections difficult to control, stimulating the bone marrow to continuously release white blood cells. This further exacerbates multi-organ dysfunction and increases the risk of death. This study also found that the use of platelets and cephalosporin antibiotics was an independent protective factor against mortality in patients with CRAB infections. Platelets not only participate in coagulation but also play a role in innate immunity. By binding to, encapsulating, and clearing pathogens, they inhibit bacterial invasion and spread, mitigate excessive inflammatory responses, and thereby improve clinical outcomes. Clinically, for high-risk populations with CRAB infection who exhibit persistently low platelet counts, dynamic monitoring of complete blood counts should be strengthened, and coagulation disorders and immune imbalances should be actively corrected to reduce the risk of mortality in critically ill patients. Although CRAB is inherently resistant to most cephalosporin antibiotics, early empirical use of cephalosporins can effectively control early infections caused by susceptible bacteria, reduce the inflammatory response, and delay disease progression. Additionally, patients receiving cephalosporin therapy typically have relatively mild baseline infections and limited exposure to carbapenems, which provides a certain degree of protective effect.This study found that the length of hospital stay in the mortality group was significantly shorter than that in the survival group (16 days vs. 26 days, $P < 0.001$), suggesting a possible association with early mortality bias. It is likely that some patients in the mortality group were in critical condition early on, with their condition rapidly deteriorating and leading to death shortly after admission, resulting in a shorter overall length of stay; in contrast, surviving patients, whose conditions were relatively stable or gradually improved following treatment, had longer periods of observation and treatment during their hospital stay. This suggests that length of hospital stay is easily influenced by patient survival outcomes and the rate of disease progression, making it difficult to use solely as an indicator of disease severity or treatment efficacy. Therefore, selection bias resulting from early mortality constitutes a limitation of this study. Future studies could optimize their designs by establishing admission time screening criteria and increasing sample sizes to further enhance the reliability of their conclusions.

In terms of gender analysis, there were significant statistical differences in albumin levels, age, and hemoglobin levels between male and female CRAB patients in the mortality group, while no differences were observed in other indicators, suggesting that prognostic factors for CRAB infection exhibit gender-specific characteristics. In this study, the age and

hemoglobin levels at which female patients faced a risk of death were lower than those of male patients. This may be related to women's higher baseline risk of anemia and malnutrition. Additionally, the decline in estrogen levels after menopause weakens immune function, and combined with a higher prevalence of osteoporosis and autoimmune diseases among women, these factors collectively reduce their ability to recover from infection. Based on these gender-specific pathophysiological mechanisms, clinicians should develop individualized intervention strategies for female patients with CRAB infection.

The antimicrobial susceptibility test results from this study indicate that 99.15% of the strains exhibited high resistance to both imipenem and meropenem. This finding reflects the high prevalence of CRAB in clinical settings. Notably, no strains resistant to polymyxin were identified in this study, suggesting that polymyxin remains an effective treatment option for CRAB infections in current clinical practice. However, the widespread use of polymyxin has led to the emergence and prevalence of resistant isolates, a phenomenon that has been confirmed in Spain, South Korea, Iran, the United States, and Brazil,[27] It may also be co-regulated by genetic transcriptional characteristics (such as the transcriptional status of the *bap* gene) and environmental factors, including medium nutrient concentration, incubation temperature, and surface roughness of the carrier material.

This study systematically analyzed resistance genes, efflux pump genes, and biofilm genes in 244 CRAB isolates. The results showed that carbapenem resistance is primarily driven by OXA-type carbapenemases, particularly *bla*OXA-23 (87.3%), This is consistent with global trends in CRAB, where *bla*OXA-23 is the most common carbapenemase gene to date. This may be related to the fact that *bla*OXA-23 can be inserted into both chromosomes and plasmids and is typically transmitted via plasmids.[28] This study also showed that nearly all strains (89.3%) carried *bla*OXA-51, a gene inherent to the Ab chromosome. Notably, four CRAB strains (1.6%) were detected in this study that carried the *bla*NDM gene, of which 2 strains carried both the *bla*OXA-23 and *bla*OXA-51 genes, while 1 strain co-carried the *bla*OXA-51 gene alone. NDM-type metal β-lactamases can hydrolyze almost all β -lactam antibiotics (including carbapenems and cephalosporins) and can be horizontally transmitted between different strains via plasmids. Their detection in our hospital indicates that CRAB has developed a composite resistance mechanism combining OXA-type enzymes with metal-binding enzymes. Although the current detection rate is low, vigilance is required regarding the risk of widespread transmission due to plasmid-mediated spread.

The efflux pump system is another important resistance mechanism in Ab, aside from β-lactamases. This study showed that the *adeB* and *adeJ* genes had the highest detection rates (85.7% and 87.3%, respectively), followed by *adeS* (84.0%), while the detection rate for *adeR* was 74.6%. Accumulated genomic studies have confirmed that the *adeIJK* efflux pump operon is highly conserved and represents a core genetic component ubiquitously distributed across the genus *Acinetobacter*.[29] However, in the present study, *adeJ* was detected in only 87.3% of the isolates, while *adeG* was undetectable in all strains, which was inconsistent with previous literature. This discrepancy is primarily attributable to methodological limitations. In this study, routine PCR was used for gene screening, and subtle sequence variations within the primer-binding regions of clinical isolates may inhibit amplification and cause false-negative results. Meanwhile, the interpretation threshold for positivity, partial genomic recombination or fragment deletion could also reduce the detection efficiency of target genes. Furthermore, geographical genetic characteristics of isolates from this single-center cohort may contribute to such deviations. Whole-genome sequencing will be adopted in future studies to achieve accurate verification.In clinical isolates of *Acinetobacter baumannii*, studies have shown that overexpression of any of the three major RND family efflux pumps (AdeABC, AdeFGH, and AdeIJK) is associated with antibiotic resistance in these strains. The expression of AdeABC is strictly regulated by the two-component regulatory system AdeR-AdeS.[30] When associated with various class D carbapenemase The correlation between *adeB* gene overexpression and the level of resistance in clinical isolates further supports the evidence that carbapenem efflux is mediated by the AdeABC system. Overexpression of AdeABC leads to significantly higher levels of carbapenem resistance, particularly resistance to imipenem and meropenem.[31] The AdeIJK efflux pump is encoded by the AdeIJK operon and mediates the intrinsic resistance of *Acinetobacter baumannii* to β-lactam antibiotics.[32] Studies have shown that the AdeIJK and AdeABC efflux pumps exhibit functional synergy and jointly participate in the active efflux of antimicrobial compounds such as tigecycline: Inactivating AdeIJK alone or overexpressing AdeABC reduces the minimum inhibitory concentration (MIC) of tigecycline by 3-fold and 8-fold, respectively; however, when both efflux pumps are inactivated simultaneously,

the tigecycline MIC decreases significantly by 85-fold, fully confirming their synergistic effect in mediating tigecycline resistance.[32]

Biofilm formation is one of the major characteristics underlying the colonization of *Acinetobacter baumannii* in healthcare settings and the development of chronic infections. It acts as a physical barrier to reduce antibiotic penetration, ultimately leading to treatment failure. The process of biofilm formation is complex and involves multiple key factors, including bacterial aggregation, collagen adhesion, fimbrial expression, and iron acquisition.[33] Among the 244 carbapenem-resistant *Acinetobacter baumannii* isolates in this study, biofilm-associated genes exhibited high detection rates, predominated by the *bap, abaI, ompA*, and *csu* gene families. These findings indicate that biofilm formation-related systems are widely distributed and highly prevalent among local CRAB strains, and may play an important role in bacterial adhesion, colonization, biofilm maturation, and the development of antimicrobial resistance. Most *Acinetobacter baumannii* strains encode and express the CsuA/BABCDE usher-chaperone fimbrial assembly system, which mediates biofilm formation of *A. baumannii* on abiotic surfaces.[34] Its expression is regulated by the BfmRS two-component regulatory system and is crucial for the formation and maintenance of biofilms.[35] The fimbriae encoded by the *csuA/BABCDE* gene cluster consist of two structural subassemblies. The short, slender tip fimbrial fiber encoded by *csuE* is anchored to the helical rod subassembly encoded by *csuA/B*. These components serve as surface recognition and adhesion sites and support fimbrial biogenesis, and their phenotypic expression is highly regulated by environmental conditions. Studies have demonstrated that the absence of CsuA/BABCDE-mediated fimbrial biosynthesis results in impaired adherence to abiotic plastic surfaces and defective biofilm formation in mutant strains.[8] *Acinetobacter baumannii* also produces biofilm-associated protein, a class of large surface-exposed macromolecular proteins that share homology with the Bap protein initially identified in *Staphylococcus aureus*.[36] It participates in intercellular adhesion and supports the development and maturation of biofilms on various substrates. Combined with the biofilm formation assay results of CRAB isolates, most strains exhibited biofilm-producing ability. Weakly and moderately biofilm-forming strains accounted for up to 91.8% (224/244), and the detection rates of biofilm-associated genes in these strains all exceeded 85%. This indicates that the carriage of biofilm-associated genes may serve as a necessary prerequisite for biofilm formation. However, the magnitude of biofilm production is not determined merely by the detection of these genes; instead, it is co-regulated by transcriptional profiles (such as the mRNA expression level of the *bap* gene) and environmental factors, including nutrient concentration in the medium, incubation temperature, and surface roughness of substrates.

The present study showed that there was no statistically significant difference in the level of biofilm formation between the amikacin- and co-trimoxazole-resistant groups and the corresponding susceptible groups. Additionally, no significant correlation was observed between the resistance phenotypes of these two agents and biofilm production. These findings suggest that biofilm-forming capacity is not a major factor mediating resistance to amikacin and co-trimoxazole among the CRAB isolates in this cohort. It is traditionally believed that biofilms reduce bacterial susceptibility to antimicrobial agents by acting as a physical barrier to block drug penetration and by reducing bacterial metabolic activity, thereby making it easier for bacteria to develop resistance. However, this study did not observe such an association. This may be attributed to the high baseline level of multidrug resistance among the strains in this study, as they commonly carried multiple resistance elements, thereby weakening the additional effect of biofilms on resistance and making it difficult to detect a correlation. On the other hand, the fact that biofilm levels were similar across most strains and showed little variation may also have resulted in low correlation coefficients, making it difficult to achieve statistical significance. It should be noted that, given the prevalence of CRAB in this hospital, relying solely on anti-biofilm interventions is unlikely to reverse resistance to amikacin and co-trimoxazole. Clinical prevention and control efforts should not focus excessively on inhibiting biofilms; instead, they should prioritize strengthening hospital-acquired infection control, standardizing antimicrobial use, monitoring the spread of resistant clones, and rationally selecting susceptible drugs for combination therapy, thereby reducing the risk of CRAB colonization and infection. However, as this study was limited to genetic qualitative screening and did not further assess gene expression levels, the underlying mechanisms linking these factors remain unclear. Consequently, it is difficult to determine whether high biofilm-forming capacity is more likely to induce the development of resistance, nor can we rule out inherent differences in biofilm formation among resistant strains themselves.

This study has certain limitations. As a single-center retrospective study, its findings may not fully reflect the epidemiological characteristics of the strain across different regions and hospitals of varying levels. Due to study constraints, this study only used PCR to detect the presence of target genes; it did not perform mRNA expression analysis, MLST typing, or whole-genome sequencing. Consequently, it was unable to assess transcriptional activity, and the analysis of resistance mechanisms was relatively limited. Future studies could adopt a multicenter design to expand the sample size and combine gene expression analysis with molecular typing to further elucidate the epidemiological characteristics and mechanisms of local CRAB.

Conclusion

This study confirms that CRAB exhibits extremely high levels of resistance, which is primarily mediated by carbapenemases and may be synergized by factors such as the overexpression of efflux pumps and biofilm formation. Clinically, it is essential to prioritize the prevention of CRAB infections to avoid the development of multidrug resistance caused by the widespread use of antibiotics. At the same time, strict adherence to antimicrobial stewardship guidelines, enhanced resistance monitoring, and the implementation of standardized prevention and control measures are crucial for controlling CRAB-associated nosocomial infections.

Data Sharing Statement

The raw data supporting the conclusions of this study are available from the first corresponding author on reasonable request.

Ethics Statement

This study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Hebei North University (Ethics Approval No.: K2024287) and conducted in strict accordance with the ethical principles of the Declaration of Helsinki. Given the retrospective design of this study, which only analyzed routine stored bacterial isolates, and with strict measures implemented to protect personal privacy and confidential information throughout the research, a waiver of informed consent was granted by the ethics committee.

Author Contributions

All authors made substantial contributions to this study, including one or more of the following: conception of the study, experimental design, implementation of the study, data collection, data analysis, and interpretation; participated in the drafting, revision, or rigorous proofreading of the manuscript; approved the final version to be published; agreed on the target journal; and agreed to take responsibility for all aspects of this study.

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Disclosure

The authors declare that they have no competing interests in this work.

References

1. Murray CJL, Ikuta KS, Sharara F, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet*. 2022;399 (10325):629–655. doi:10.1016/S0140-6736(21)02724-0
2. Oh MH, Islam MM, Kim N, et al. AbOmpA in Acinetobacter baumannii: exploring virulence mechanisms of outer membrane-integrated and outer membrane vesicle-associated AbOmpA and developing anti-infective agents targeting AbOmpA. *J Biomed Sci*. 2025;32(1):53. doi:10.1186/s12929-025-01147-5
3. WHO. Bacterial priority pathogens list, 2024: bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance. Available from: https://www.who.int/publications/i/item/9789240093461. Accessed November 19, 2025.

4. Giamarellou H, Antoniadou A, Kanellakopoulou K. Acinetobacter baumannii: a universal threat to public health? *Int J Antimicrob Agents*. 2008;32(2):106–119. doi:10.1016/j.ijantimicag.2008.02.013
5. Cureño-Díaz MA, Plascencia-Nieto ES, Loyola-Cruz MÁ, et al. Gram-negative ESKAPE bacteria surveillance in COVID-19 pandemic exposes high-risk sequence types of Acinetobacter baumannii MDR in a tertiary care hospital. *Pathog*. 2024;13(1):50. doi:10.3390/pathogens13010050
6. Dijkshoorn L, Nemec A, Seifert H. An increasing threat in hospitals: multidrug-resistant Acinetobacter baumannii. *Nat Rev Microbiol*. 2007;5(12):939–951. doi:10.1038/nrmicro1789
7. Ramirez MS, Bonomo RA, Tolmasky ME. Carbapenemases: transforming Acinetobacter baumannii into a yet more dangerous menace. *Biomolecules*. 2020;10(5):720. doi:10.3390/biom10050720
8. Mea HJ, Yong PVC, Wong EH. An overview of Acinetobacter baumannii pathogenesis: motility, adherence and biofilm formation. *Microbiol Res*. 2021;247:126722. doi:10.1016/j.micres.2021.126722
9. Appaneal HJ, O'Neill E, Lopes VV, LaPlante KL, Caffrey AR. National trends in hospital, long-term care and outpatient Acinetobacter baumannii resistance rates. *J Med Microbiol*. 2021;70(12). doi:10.1099/jmm.0.001473
10. Jun SH, Hwang HR, Kim N, Kwon KT, Kim YK, Lee JC. Clonal evolution and antimicrobial resistance of Acinetobacter baumannii isolates from Korean hospitals over the last decade. *Infect Genet Evol*. 2023;108:105404. doi:10.1016/j.meegid.2023.105404
11. Qin X, Ding L, Hao M, Li P, Hu F, Wang M. Antimicrobial resistance of clinical bacterial isolates in China: current status and trends. *Jac-Antimicrob Resist*. 2024;6(2):dlae052. doi:10.1093/jacamr/dlae052
12. Lin F, Xu Y, Chang Y, Liu C, Jia X, Ling B. Molecular characterization of reduced susceptibility to biocides in clinical isolates of Acinetobacter baumannii. *Front Microbiol*. 2017;8:1836. doi:10.3389/fmicb.2017.01836
13. Lee CR, Lee JH, Park M, et al. Biology of Acinetobacter baumannii: pathogenesis, antibiotic resistance mechanisms, and prospective treatment options. *Front Cell Infect Microbiol*. 2017;7:55. doi:10.3389/fcimb.2017.00055
14. Nordmann P, Poirel L. Epidemiology and diagnostics of carbapenem resistance in gram-negative bacteria. *Clin Infect Dis*. 2019;69(Suppl 7):S521–S528. doi:10.1093/cid/ciz824
15. Ambler RP. The structure of beta-lactamases. *Philos Trans R Soc Lond B Biol Sci*. 1980;289(1036):321–331. doi:10.1098/rstb.1980.0049
16. Xie L, Li J, Peng Q, et al. Contribution of RND superfamily multidrug efflux pumps AdeABC, AdeFGH, and AdeIJK to antimicrobial resistance and virulence factors in multidrug-resistant Acinetobacter baumannii AYE. *Antimicrob Agents Chemother*. 2025;69(7):e01858–24. doi:10.1128/aac.01858-24
17. Longo F, Vuotto C, Donelli G. Biofilm formation in Acinetobacter baumannii. *New Microbiol*. 2014;37(2):119–127.
18. Smani Y, McConnell MJ, Pachón J. Role of fibronectin in the adhesion of Acinetobacter baumannii to host cells. *PLoS One*. 2012;7(4):e33073. doi:10.1371/journal.pone.0033073
19. Gaddy JA, Actis LA. Regulation of Acinetobacter baumannii biofilm formation. *Future Microbiol*. 2009;4:273–278. doi:10.2217/fmb.09.5
20. Sanchez CJ, Mende K, Beckius ML, et al. Biofilm formation by clinical isolates and the implications in chronic infections. *BMC Infect Dis*. 2013;13:47. doi:10.1186/1471-2334-13-47
21. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Sci*. 1999;284(5418):1318–1322. doi:10.1126/science.284.5418.1318
22. Yang Z, Wang P, Song P, Li X. Carbapenemase OXA-423: a novel OXA-23 variant in Acinetobacter baumannii. *Infect Drug Resist*. 2020;13:4069–4075. doi:10.2147/IDR.S277364
23. Nordmann P, Poirel L, Carrër A, Toleman MA, Walsh TR. How to detect NDM-1 producers. *J Clin Microbiol*. 2011;49(2):718–721. doi:10.1128/JCM.01773-10
24. Lari AR, Ardebili A, Hashemi A. AdeR-AdeS mutations & overexpression of the AdeABC efflux system in ciprofloxacin-resistant Acinetobacter baumannii clinical isolates. *Indian J Med Res*. 2018;147(4):413–421. doi:10.4103/ijmr.IJMR_644_16
25. Wang J, Feng L, Zheng YL, et al. Study on correlation between multidrug resistance of Acinetobacter baumannii and efflux pumps and biofilm formation. *J Kunming Med Univ*. 2024;45(12).
26. Yang CH, Su PW, Moi SH, Chuang LY. Biofilm formation in Acinetobacter baumannii: genotype-phenotype correlation. *Molecules*. 2019;24(10):1849. doi:10.3390/molecules24101849
27. Genteluci GL, Gomes DBC, Pereira D, et al. Multidrug-resistant Acinetobacter baumannii: differential adherence to HEp-2 and a-549 cells. *Braz J Microbiol*. 2020;51(2):657–664. doi:10.1007/s42770-020-00252-x
28. Karampatakis T, Tsergouli K, Behzadi P. Carbapenem-resistant Acinetobacter baumannii: virulence factors, molecular epidemiology, and latest updates in treatment options. *Microorganisms*. 2025;13(9):1983. doi:10.3390/microorganisms13091983
29. Naidu V, Bartczak A, Brzoska AJ, et al. Evolution of RND efflux pumps in the development of a successful pathogen. *Drug Resist Updates*. 2023;66:100911. doi:10.1016/j.drup.2022.100911
30. Marchand I, Damier-Piolle L, Courvalin P, Lambert T. Expression of the RND-type efflux pump AdeABC in Acinetobacter baumannii is regulated by the AdeRS two-component system. *Antimicrob Agents Chemother*. 2004;48(9):3298–3304. doi:10.1128/AAC.48.9.3298-3304.2004
31. Coyne S, Courvalin P, Périchon B. Efflux-mediated antibiotic resistance in Acinetobacter spp. *Antimicrob Agents Chemother*. 2011;55(3):947–953. doi:10.1128/AAC.01388-10
32. Damier-Piolle L, Magnet S, Brémont S, Lambert T, Courvalin P. AdeIJK, a resistance-nodulation-cell division pump effluxing multiple antibiotics in Acinetobacter baumannii. *Antimicrob Agents Chemother*. 2008;52(2):557–562. doi:10.1128/AAC.00732-07
33. Rajangam SL, Narasimhan MK. Current treatment strategies for targeting virulence factors and biofilm formation in Acinetobacter baumannii. *Future Microbiol*. 2024;19(10):941–961. doi:10.2217/fmb-2023-0263
34. Nowak P, Paluchowska P. Acinetobacter baumannii: biology and drug resistance - role of carbapenemases. *Folia Histochem Cytobiol*. 2016;54(2):61–74. doi:10.5603/FHC.a2016.0009
35. Harding CM, Hennon SW, Feldman MF. Uncovering the mechanisms of Acinetobacter baumannii virulence. *Nat Rev Microbiol*. 2018;16(2):91–102. doi:10.1038/nrmicro.2017.148
36. Cucarella C, Solano C, Valle J, Amorena B, Lasa I, Penadés JR. Bap, a Staphylococcus aureus surface protein involved in biofilm formation. *J Bacteriol*. 2001;183(9):2888–2896. doi:10.1128/JB.183.9.2888-2896.2001