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Original Article

Analysis of carbapenem-resistant *Acinetobacter baumannii* carbapenemase gene distribution and biofilm formation

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Abstract: Objective: In recent years, *Acinetobacter baumannii* has been appearing in hospitals with high drug resistance and strong vitality, which brings many difficulties to clinical treatment. In this study, 255 strains of *A. baumannii* were isolated from Youjiang Medical University for Nationalities Affiliated Hospital clinical samples and found to be highly resistant to carbapenems. The drug resistance, biofilm-forming ability, and carbapenase gene distribution of 145 carbapenem-resistant *A. baumannii* (CRAB) strains were analyzed statistically. Methods: The clinically isolated strains were detected using Vitek mass spectrometry and Vitek2-compact for bacterial identification and susceptibility testing, respectively. The biofilms of clinical isolates were quantitatively detected by microplate crystal violet staining, and qualitatively observed by confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM). And the common carbapenemase genes were detected by polymerase chain reaction (PCR). Results: The 255 clinical isolates from the Youjiang District of western Guangxi Province had a high resistance rate to carbapenems antibiotics. The main specimens were from the intensive care unit (49%), and the most important specimens were sputum specimens (80%). All 145 strains of CRAB produced different degrees of biofilm, and six carbapenenase genes were detected. We found that there were significant differences in biofilm formation between resistant and sensitive strains of tobramycin, levofloxacin, ciprofloxacin, tigecycline, and doxycycline ($P<0.05$). The distribution of bla_{OXA-23} and bla_{OXA51} genes was significantly different from CRAB biofilm formation ($P<0.05$). In addition, *AmpC*, bla_{OXA-23}, bla_{OXA-51}, and *TEM* genes were more distributed in antibiotic-resistant strains. Conclusion: The clinical strains have a high resistance rate to carbapenems, and the CRAB with bla_{OXA-51} and bla_{OXA-23} genes has a high resistance to antibiotics and a strong biofilm.

Keywords: Carbapenem-resistant *Acinetobacter baumannii*, biofilm, oxacillinases

Introduction

A. baumannii is a gram-negative bacterium, widely existing in nature, and an important pathogen of nosocomial infection. It is widely distributed and can survive for a long time. It can colonize the human body, skin, mouth, respiratory tract, gastrointestinal tract, and urogenital tract, causing respiratory tract infection, urinary tract infection, bacteremia, secondary meningitis, surgical site infection, etc. [1, 2]. In recent years, *A. baumannii* has become another drug-resistant pathogenic organism after *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterobacter species,* and is one of the important pathogens of "ESKAPE" [3].

The resistance mechanism of *A. baumannii* is closely related to the wide range of carbapenemase hydrolysis, and the inactivation of β-lactam drugs by carbapenemase production is the main mechanism of drug resistance of *A. baumannii*. OXA-type carbapenemases have emerged as the main resistance mechanism to carbapenems in *A. baumannii*, and the main reason why *A. baumannii* is resistant to carbapenemes [4, 5]. The drug resistance of *A. baumannii* is closely related to the generation of biofilm. *A. baumannii* with biofilm formation has a high resistance to antibiotics [6]. Biofilm enables *A. baumannii* to adapt to harsh environments and provide protection for submembrane bacteria, increasing resistance to various antibiotics [7], and more easily lead to chronic and persistent infection. Carbapenemase gene and biofilm formation greatly increased the resistance of *A. baumannii* to antibiotics, which brought great challenges to clinical control.

After *A. baumannii* developed resistance to antibiotics, polymyxin antibiotics have become the last effective means, and we urgently need to find an effective treatment plan for *A. baumannii*. Synergistic bactericidal and antibiofilm effects were observed when ceftazidime and tigecycline were combined with polymyxin B [8, 9]. Many studies have shown that natural compounds can inhibit the biofilm of *A. baumannii*. In Usmani's study, it was found that the amide derivatives of ursolic acid could inhibit the production of biofilm and reduce the expression of quorum-sensing effect genes [10]. Alves found that linalool from Coriandrum sativum could inhibit the formation of *A. baumannii*'s biofilm [11]. Meanwhile, in Sivaranjani's study, it was found that α-mangostin showed the potential of anti-biofilm against *A. baumannii* [12].

In this study, 255 strains of *A. baumannii* clinically collected from Guangxi province and tertiary hospitals from September 10, 2020 to April 28, 2021, were statistically found to be highly resistant to carbapenem antibiotics. The biofilm formation was quantitatively analyzed by crystal violet staining and the carbapenase gene of *A. baumannii* was detected by PCR. The relationship between the sensitivity of CRAB to antibiotics, the formation of biofilm, and the distribution of the carbapenicase gene in our hospital was analyzed.

Methods

Source and culture of the strain

A total of 255 strains of *A. baumannii* isolated from clinical specimens of the Youjiang Medical University for Nationalities Affiliated Hospital from September 2020 to April 2021 were collected. The standard strain used in this study was *A. baumannii* ATCC19606. All strains were isolated and cultured according to the 3rd edition of the National Operating Procedures for Clinical Examination. *A. baumannii* was cultured on China blue agar in a 37°C incubator for 12 to 24 h. 2~3 colonies were cultured overnight in a centrifugal tube containing 5 ml Luria-Bertani (LB) liquid medium. 4~5 colonies were stored in a 30% glycerol tube.

Identification and drug sensitivity test

The clinically isolated strains were detected using Vitek mass spectrometry and Vitek2-compact for bacterial identification and susceptibility testing, respectively. The evaluation criteria of drug sensitivity refer to the CLSI M100-S31 drug sensitivity Test guidelines.

Crystal violet staining measures biofilm formation

According to the method described by Zhao [13], after the bacterial solution was cultured overnight, the bacterial solution was adjusted to OD_{600} of 0.3, and the concentration of the bacterial solution was 1×10^8 CFU/ml and diluted to 1×10^6 CFU/ml. 200 μl bacterial solution was added to the 96-well plate, and the culture medium in the 96-well plate was removed after 48 h culture. The culture plate was rinsed with sterile water twice to remove free bacteria and then dried with 100 μl 0.1% crystal violet dye solution per well for 30 min. Then the dye solution was sucked out with a pipette. After rinsed twice, the dye solution was left for air drying at room temperature, and the crystal violet was dissolved with 200 μl 95% ethanol. The mean the of sterile medium was defined as the negative control. The negative control optical density cutoff value (OD) plus 3 times standard deviation (x±3s) was ODc, and the optical density cutoff value (OD) of the strain was compared. Strain biofilm forming ability was divided into four categories: OD≤ODc was negative (-), ODc

Table 1. Primer sequence and length

Primer name	Sequence (5'-3')	Amplicon size (bp)
TEM	F: AGGCACCTATCTCAGCGA	805
	R: CCGTGTCGCCCTTATTCC	
AmpC	F: TAAACACCACATATGTTCCG	663
	R: ACTTACTTCAACTCGCGACG	
bla_{OXA-23}	F: CCCCGAGTCAGATTGTTC	291
	R: GCTTCATGGCTTCTCCTAG	
bla_{OXA-51}	F: TAATGCTTTGATCGGCCTTG	353
	R: TGGATTGCACTTCATCTTGG	
NDM-1	F: CAGCACACTTCCTATCTC	292
	R: CCGCAACCATCCCCTCTT	
KPC	F: GCGGAACCATTCGCTAAACTC	340
	R: CGCCCAACTCCTTCAGCAACA	

Note: F: upstream primer, R: downstream primer.

<OD≤2ODc is weakly positive (+), 2ODc<OD≤4 ODc is positive (++), >4 ODc is strongly positive (+++).

Detection of biofilm formation by CLSM

Three strains of CRAB were selected to produce weakly positive, positive, and strongly positive biofilms. The bacterial solution was prepared as 1×10^6 CFU/ml. The cover piece was put into the 6-well plate with sterile forceps and stuck to the bottom of the 6-well plate. 2 ml bacterial solution was added into the 6-well cell culture plate and cultured in a 37°C incubator for 48 h. Separate the bacterial solution with a micropipette, gently wash off the planktonic bacteria with sterile water, add 300 μl 10 μg/ml FITC-ConA fluorescent staining solution, stain in the dark for 30 min, and then observe the thickness of the formed biofilm using CLSM.

The formation of biofilm was observed by SEM

The strains with weak positive, positive, and strong positive membrane-forming ability were selected and cultured to 1×10^6 CFU/ml. A diluted bacterial solution is added to the slide to allow the bacteria to grow attached to the surface of the slide Dehydrate with 30%, 50%, 70%, 90%, 100% ethanol, 100% ethanol twice, 15 minutes each time. The conductive adhesive was pasted on the loading platform, the specimen was placed on the loading platform, and the gold was sprayed for scanning electron microscope observation.

Detection of carbapenemase gene

Bacterial DNA was extracted by boiling method. The primers used in this experiment were all synthesized by Nanning Cody Biotechnology Limited, LTD. The length of the primer sequence and product is shown in **Table 1**. PRC reaction system: 1 μl upstream primer, downstream primers 1 μl, DNA template 5 μl, Taq Mix Pro 25 μl, sterile double steaming water supply system to 50 μl. The reaction conditions of PCR amplification of the carbapenemase gene were as follows: pre-denaturation at 94°C for 10 min, denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 6 s, and finally extension at 72°C for 10 min, 35 cycles.

Statistical analysis

The epidemiological analysis of drug resistance of 255 strains of *A. baumannii* in our hospital from September 2020 to April 2021 was carried out by whonet 5.6 and GraphPad Prism 9 software. One-way ANOVA was applied for the multiple-group comparisons, and Mann-Whitney test was conducted for the non-parametric data comparison between two groups. The significance level for all statistical tests was set at $P<0.05$.

Results

Epidemiological investigation of A. baumannii

The drug resistance of 255 strains of *A. baumannii* clinically isolated in our hospital was analyzed, and the detailed results of antibiotic resistance can be seen in **Figure 1A**. The resistance rate of clinical isolates to carbapenems was higher, of which 68.6% were resistant to imipenem, 71.2% to meropenem, and the resistance rates to polymyxin, tigecycline and amicacin were lower than other antibiotics (0.8%, 36.3%, 39.6%). The resistance to other antibiotics was more than 50%, cefepime (183, 71.8%), ceftazidine (186, 72.9%), ceftriaxone (11, 57.9%), tobramycin (172, 69.9%), genamicin (7, 70.0%), levofloxacin (178, 69.8%), ciprofloxacin (175, 71.1%), doxycycline (165, 68.8%), minocycline (152, 64.4%), cefoperazone-sulbactam (164, 66.9%), piperacillin-tazobactam (185, 75.5%), ticasillin-clavulanate (167, 70.8%). More than 80% of the strains were resistant to aztreonam (242, 98.8%) and ampi-

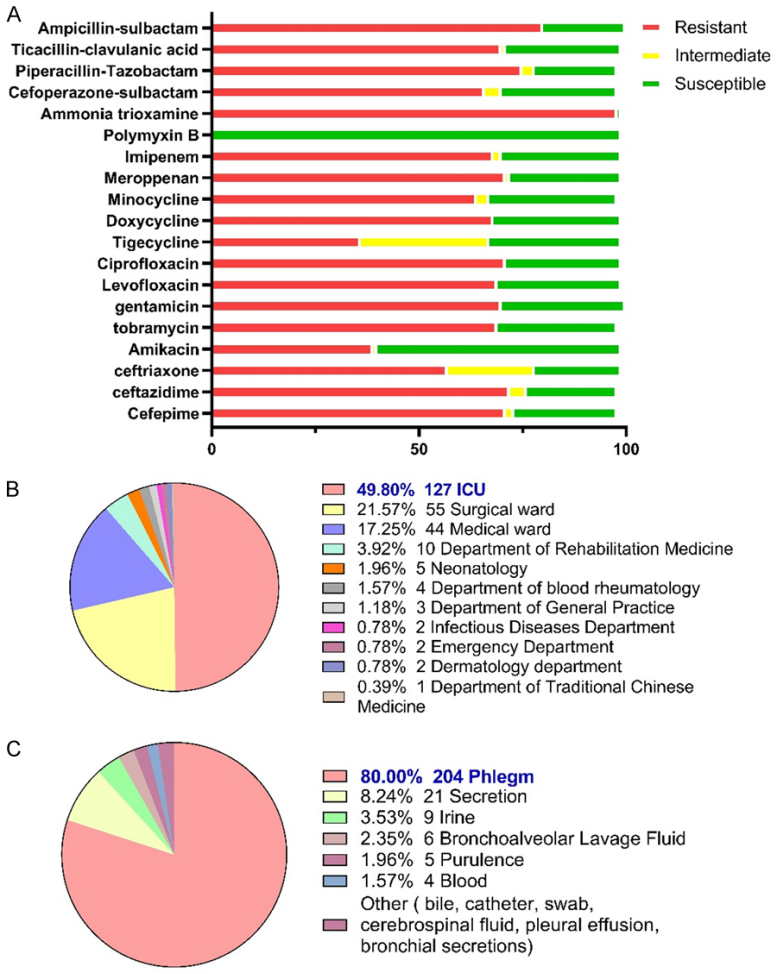

Figure 1. Epidemiological investigation of 255 clinically isolated *A. baumannii*. A. Antimicrobial susceptibility profiling. B. Distribution of clinical departments. C. Distribution of specimen sources.

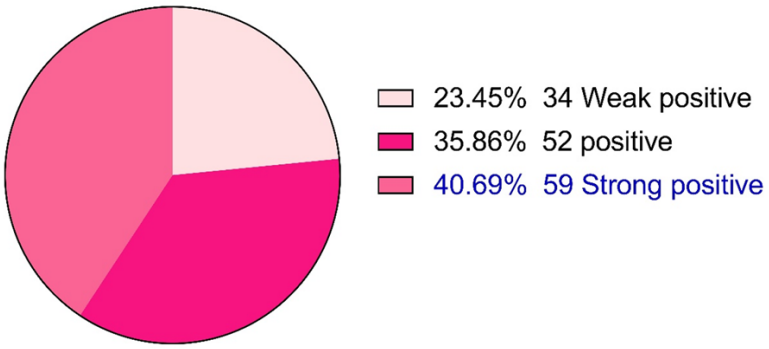

Figure 2. Proportion of biofilm formed by carbapenem-resistant *A. baumannii*.

cillin-sulbactam (8, 80%). As analyzed in **Figure 1B**, 49.80% of *A. baumannii* patients were distributed in intensive care units (ICU). It can be concluded from **Figure 1C** that the specimens in our hospital mainly come from sputum specimens, accounting for 80.00% of the total, followed by secretions and urine, accounting for 8.24% and 3.53% of the total.

Analysis of biofilm formation of CRAB and its sensitivity to antibiotics

The 145 clinical isolates of CRAB were all able to form biofilms. The OD_{570} value of the positive control strain ATCC19606 was 1.191± 0.012, and that of the negative control was 0.1010± 0.0050. The $OD_{570\ nm}$ values of 145 clinical drug-resistant *A. baumannii* strains ranged from 0.1047 to 1.2140. As described in **Figure 2**, 59 strains (40.69%) had strong biofilm forming ability, 52 strains (35.86%) had moderate biofilm forming ability, and 34 strains (23.45%) had weak biofilm forming ability. The formation of biofilms in different degrees can be seen in detail in **Figure 3**. The number of bacteria forming strong positive biofilms was higher than that under positive and weak positive biofilms. In the CLSM (**Figure 3A-C**) be seen with thin biofilms under weakly positive strains of bacteria are less sparse, but in positive and strong positive strains biofilms are thicker, and the number of bacterial biofilms under the greater. Under SEM (**Figure 3D-L**), positive biofilms adhered to each other and formed clouds, and the adhesion between bacteria was stronger.

To determine the relationship between biofilm formation and antibiotic resistance of strains, the susceptibility of strains to 16 kinds of antibiotics (cefepime, ceftazidime, amikacin, tobra-

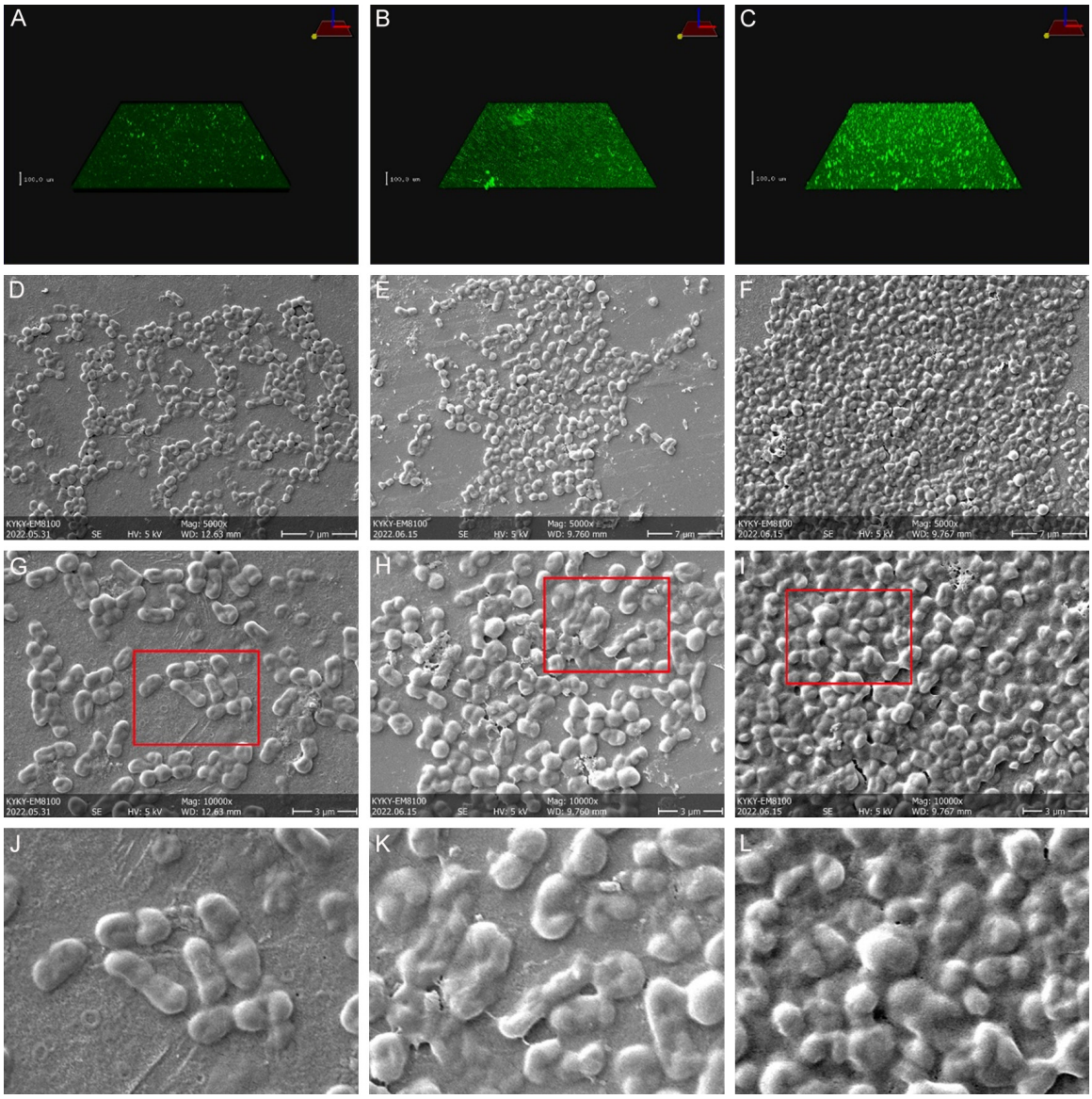

Figure 3. Different degrees of biofilm formed by carbapenem-resistant *A. baumannii.* Note: (A-C) is the confocal laser electron microscope. (D-L) is the scanning electron microscope. (A) Weak positive biofilm-producing strain. (B) Positive biofilm-producing strain. (C) Strong positive biofilm-producing strain. (D, G, J) Weak positive biofilm-producing strain. (E, H, K) Positive biofilm-producing strain. (F, I, L) Strong positive biofilm-producing strain. The scale bar in the second row is 7 μm. The third row is marked 3 μm.

mycin, levofloxacin, ciprofloxacin, tigecycline, doxycycline, minocycline, meropenem, imipenem, polymyxin B, amtriannan, cefoperazone-sulbactam, piperacillin-tazobactam, ticarcillin-clavulanate) was studied. We used one-way ANOVA of GraphPad Prism 9 to compare the mean values among multiple groups to analyze the relationship between CRAB biofilm formation and antibiotic resistance in **Figure 4**. As shown in **Figure 4**, it was found that for tobramycin (**Figure 4A**), levofloxacin (**Figure 4B**), ciprofloxacin (**Figure 4C**), tigecycline (**Figure 4D**), and doxycycline (**Figure 4E**), resistant strains tended to form better biofilms than sensitive strains ($P<0.05$). For tigacycline (**Figure 4D**), the intermediate strain formed a better biofilm than the sensitive strain ($P<0.005$). Resistance to minocycline (**Figure 4F**), and ticarcillin-clavulanic (**Figure 4G**) were not associated with biofilm formation. However, the results of cefepime,

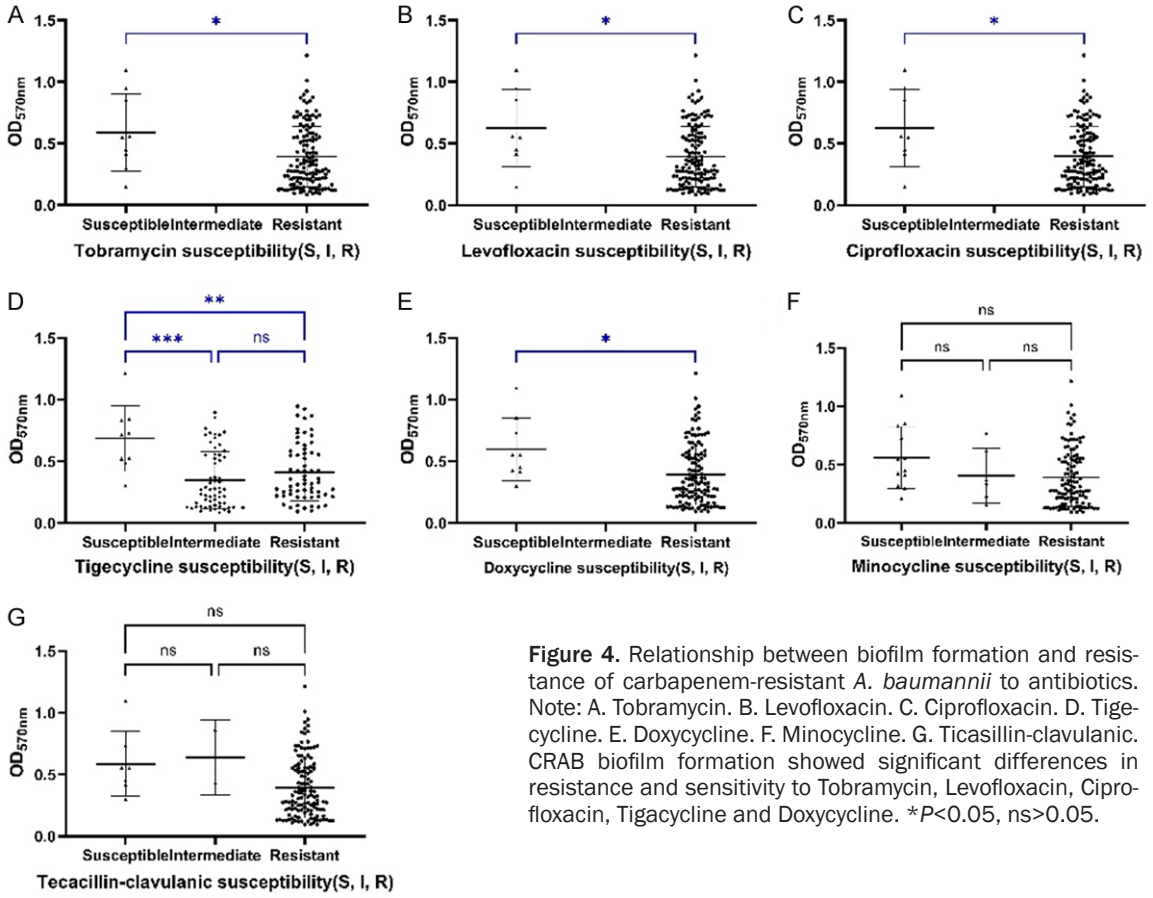

Figure 4. Relationship between biofilm formation and resistance of carbapenem-resistant *A. baumannii* to antibiotics. Note: A. Tobramycin. B. Levofloxacin. C. Ciprofloxacin. D. Tigecycline. E. Doxycycline. F. Minocycline. G. Ticasillin-clavulanic. CRAB biofilm formation showed significant differences in resistance and sensitivity to Tobramycin, Levofloxacin, Ciprofloxacin, Tigacycline and Doxycycline. *P<0.05, ns>0.05.

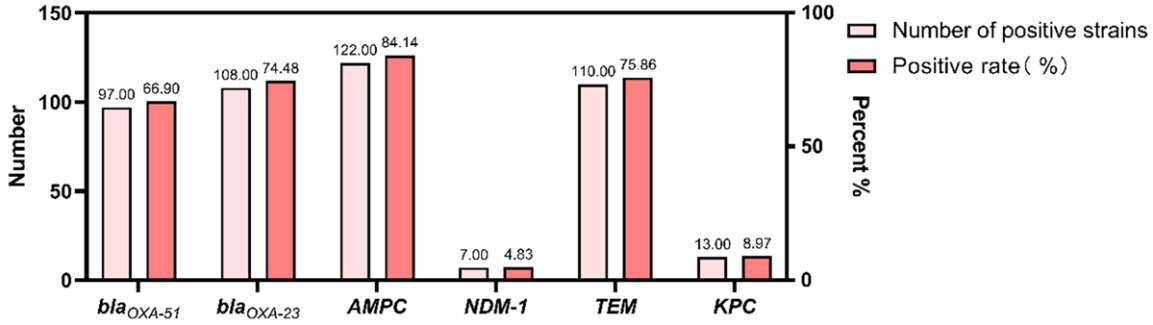

Figure 5. Distribution of carbapenemase gene in carbapenem-resistant *A. baumannii* strains (n=145).

ceftazidime, and amikacin antibiotics could not be confirmed by statistical analysis due to the small number of sensitive strains (Figure S1).

CRAB carbapenemase gene detection and its relationship with biofilm formation

Six carbapenemases were detected in 145 strains of CRAB. As shown in **Figure 5**, 97 (66.9%) were detected in bla_{OXA-51} gene, 108 (74.5%) in bla_{OXA-23} gene, 122 (84.1%) in *AMPC* gene, 110 (75.9%) in *TEM* gene, 7 (4.8%) in *NDM-1* gene, and 13 (9.0%) in *KPC* gene. The electrophoresis of six carbapenemase genes is shown in **Figure 7**. The carrying of *AmpC*, *NDM-1*, *TEM,* and *KPC* genes in CRAB was high. When sorting out the data, it was found that the antibiotic resistance rate of the strains carrying bla_{OXA-51}, bla_{OXA-23}, *AMPC*, and *TEM* genes was more than 70%, while the resistance rate of the strains carrying *NDM-1* and *KPC* genes was lower (**Figure 6**). The resistance rate of strains

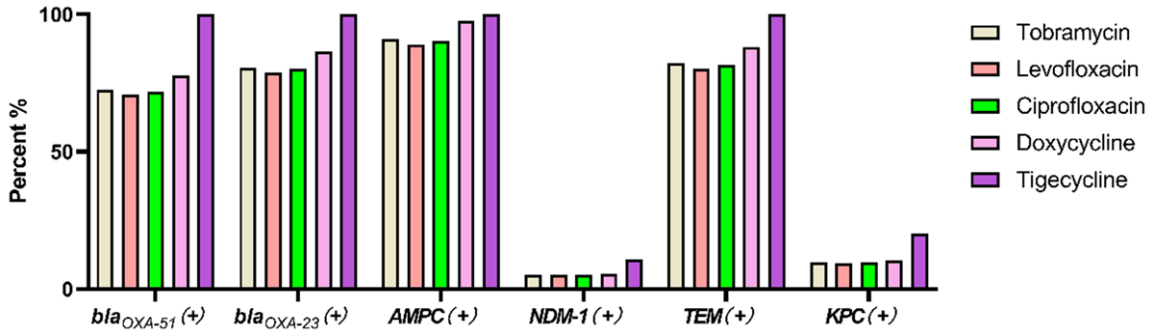

Figure 6. Antibiotic resistance rate of carbapenem-resistant *A. baumannii* carrying carbapenemase gene.

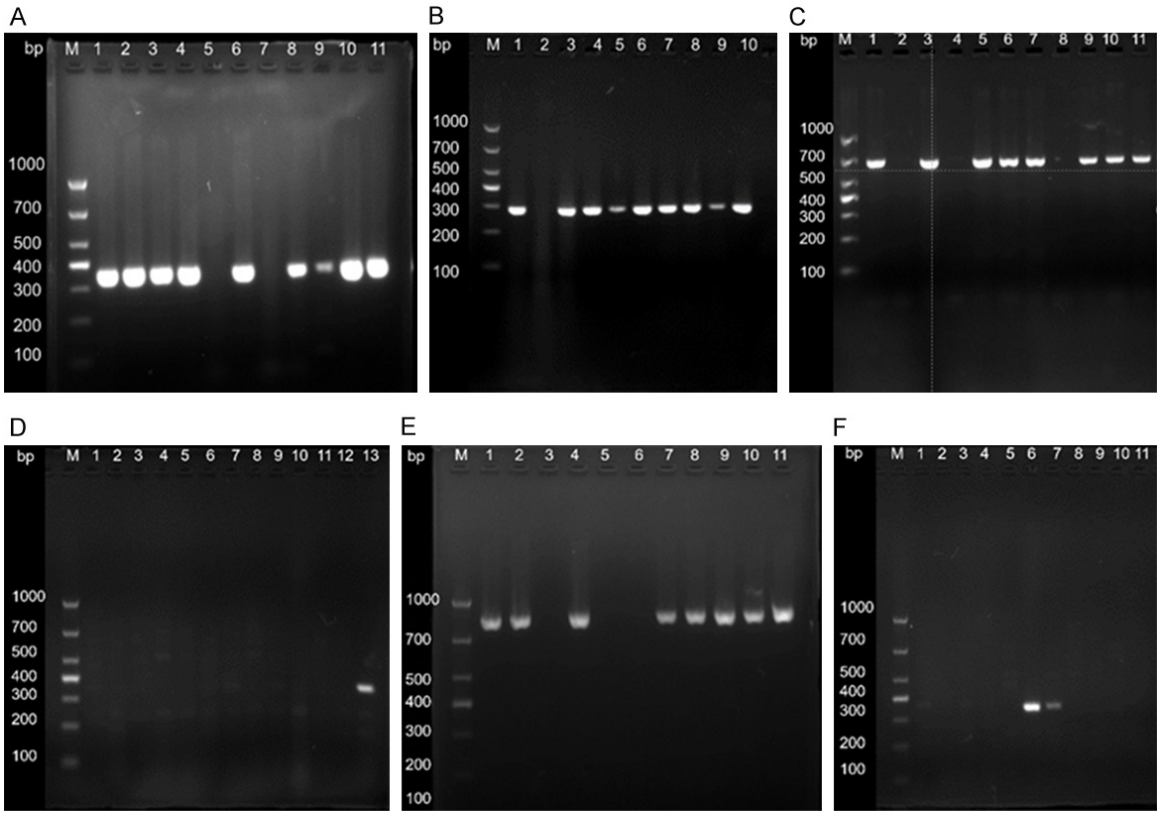

Figure 7. Electrophoresis of carbapenemase genes. Note: bp is the Marker of different fragment lengths, and DNA Marker are added to the point loading holes in column M. A. The DNA of strains numbered 8H4, 8H5, 8H6, 8H7, 5A2, 5B5, 5B6, 5B7, 5B8, 5B9 and 5B10 were added to the loading holes in columns 1-11, respectively. Among them, 5H4, 5H5, 5H6, 5H7, 5B5, 5B7, 5B8, 5B9, and 5B10 strains carried bla_{OXA-51} gene. B. The DNA of strains numbered 5C9, 5C8, 5C7, 5C6, 5C5, 5C4, 5C3, 5C2, 5C1 and 5B10 were added to the loading holes in columns 1-10, respectively. Among them, 5C9, 5C7, 5C6, 5C5, 5C4, 5C3, 5C2, 5C1 and 5B10 strains carried bla_{OXA-23} gene. C. The DNA of strains numbered 5E3, 5E2, 5E1, 5D10, 5D8, 5D7, 5D6, 5D5, 5D4, 5D3 and 5D2 were added to loading holes in columns 1-11, respectively. Among them, 5E3, 5E1, 5D8, 5D7, 5D6, 5D4, 5D3 and 5D2 strains carried *AmpC* gene. D. The DNA of strains numbered 8D6, 8D7, 8D8, 8D9, 8D10, 8E1, 8E2, 8E3, 8E4, 8E5, 8E6, 8E7 and 8F1 were added to the loading holes in columns 1-13. Among them, 8F1 strain carried *NDM-1* gene. E. The DNA of strains numbered 5F2, 5F3, 5F4, 5F5, 5F6, 5F7, 5F9, 5F10, 5G1, 5G2 and 5G4 were added to loading holes in columns 1-11, respectively. Among them, 5F2, 5F3, 5F5, 5F9, 5F10, 5G1, 5G2 and 5G4 strains carried *TEM* gene. F. The DNA of strains numbered 8F6, 8F8, 8F9, 8F10, 8G1, 8G2, 8G3, 8G4, 8G5, 8G6 and 8G7 were added to the loading holes in columns 1-11, respectively. 8G1 and 8G2 strains carried *KPC* gene.

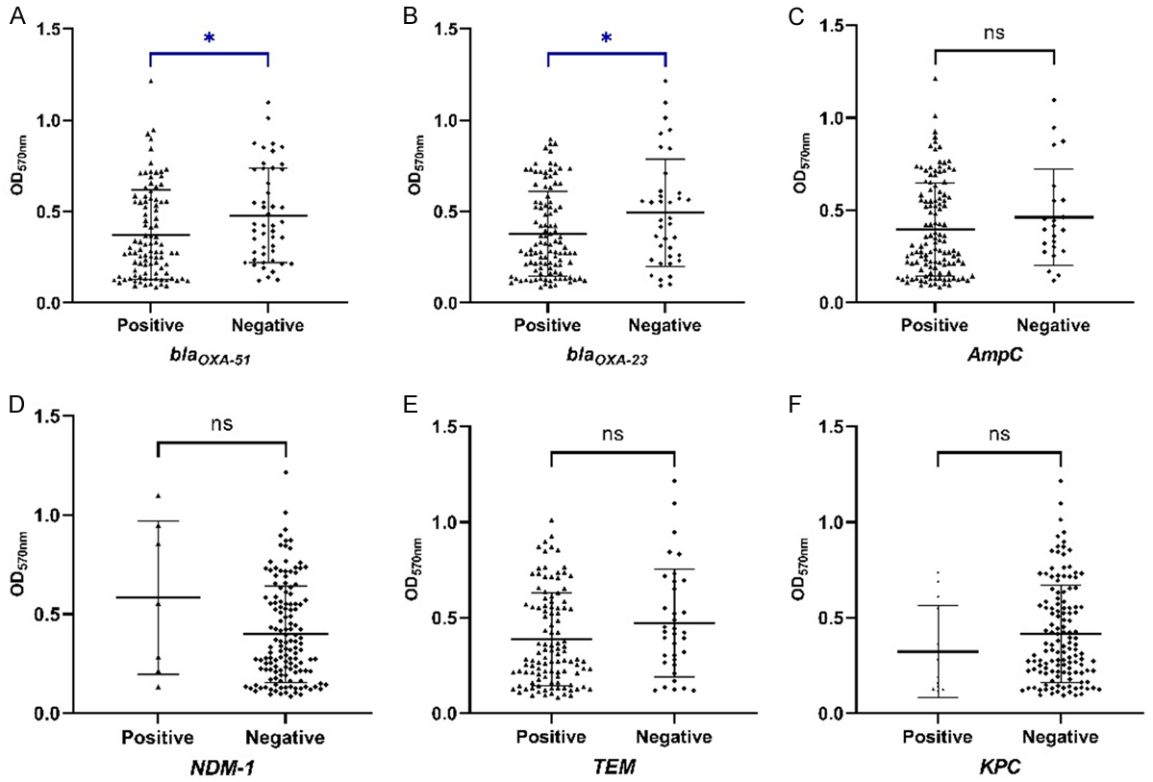

Figure 8. Correlation between carbapenemase gene and biofilm formation. Note: A. *Bla*$_{OXA-51}$ gene. B. *Bla*$_{OXA-23}$ gene. C. *AmpC* gene. D. *NDM-1* gene. E. *TEM* gene. F. *KPC* gene. Non-parametric Mann-Whitney U assay was used to compare the formation of CRAB biofilms with the carbapenemase genes *bla*$_{OXA-51}$. There were significant differences in the distribution of *bla*$_{OXA-23}$. *P<0.05, ns>0.05.

carrying carbapenemase gene to all antibiotics is shown in Figure S2. This further suggests that *bla*$_{OXA-51}$, *bla*$_{OXA-23}$, *AMPC*, and *TEM* genes are associated with antibiotic resistance. In this study, in the T-test with 95% confidence interval, we used the Mann-Whitney test to conduct a non-parametric test to analyze the relationship between carbapenase genes with biofilm formation, and the results are shown in **Figure 8**. It was found that *bla*$_{OXA-51}$ gene, *bla*$_{OXA-23}$ gene, and CRAB biofilm formation had significant differences (*P*<0.05). Other genes *AmpC*, *NDM-1*, *TEM,* and *KPC* did not correlate with biofilm formation.

Discussion

The rise of drug resistance of *A. baumannii* is global. Surveillance studies show that *A. baumannii* in Morocco [14], Austria [15], Inner Mongolia Autonomous Region in North China [16], and Guangdong Province in South China [17] has a high resistance rate to carbapenem antibiotics, which makes the treatment of *A. baumannii* infection a challenge. The 255 strains of *A. baumannii* treated in our hospital showed high drug resistance to carbapenem-resistant antibiotics commonly used to treat *A. baumannii*. Among them, in many regions, the resistance rate of *A. baumannii* to carbapenem antibiotics exceeded 90% [15, 16] and was only sensitive to polymyxin B. The reason may be that some doctors' empirical drug use leads to the abuse of carbapenem antibiotics such as meropenem and imipenem. *A. baumannii* is widely distributed in clinical departments of hospitals, especially in ICU [17]. Due to the complex condition of ICU patients, the need for more invasive surgery leads to the easier invasion of patients' bacteria. Among the 255 specimens, 49.80% were from the ICU, which was also demonstrated in the investigation with Mona [18]. As the respiratory system is the main route of *A. baumannii* infection [19], 255 specimens in our hospital were mainly from sputum (80.00%) and 8.24% from secretions, which was similar to the findings of Chang, Yang [19, 20].

It has been found that the generation of biofilms plays an important role in the horizontal transfer of bacterial drug-resistance genes under biofilms [21]. CRAB isolated in our hospital all produced biofilms, and 40.69% of them could produce stronger biofilms, which was similar to Hazhirkamal's investigation [22]. In Hazhirkamal's study, it was also found that the pattern of antimicrobial resistance was significantly correlated with the generation of strong biofilms. In Hassan's experiments, however, *A. baumannii* isolated from soil was found to be more sensitive to antibiotics than the clinical isolates, and the soil isolates produced stronger biofilms than the clinical isolates. Strains isolated from soil need to survive in a dry environment for a long time, and the capsules in the biofilm have the potential to maintain the viability of the isolates under water scarcity conditions [23]. In our study, bla_{OXA-51} (66.9%) and bla_{OXA-23} (74.48%) were highly carried, and the genes with higher carrying rates were *AmpC* (84.14%) and *TEM* (75.86%), while the carrying rates of *NDM-1* (4.83%) and *KPC* (8.97%) were lower. Zhang also made similar findings with us when studying carbapenase genes, with high carrying rates of $bla_{OXA-51-like}$ (100.0%) and bla_{OXA-23} (93.4%), while bla_{NDM-1} (8.8%), bla_{OXA-24} (2.2%), and bla_{OXA-58} (2.2%) had lower carrying rates [24]. The *TEM* gene was detectable in 60% of strains isolated at the Children's Medical Center in Tehran, Iran [25]. However, the *TEM* gene of isolates from Turkish university hospitals accounted for only 2% [26]. The distribution of TEM gene has great regional differences.

In this study, the correlation between antibiotic resistance, carbapenemase gene carrying, and biofilm formation of CRAB was statistically analyzed. In our analysis, biofilm formation was associated with tobramycin, levofloxacin, ciprofloxacin, tegacycline, and doxycycline, which belong to the aminoglycoside, quinolone, and tetracycline classes of antibiotics. Yang's study found a relationship between antibiotics and biofilms, and different antibiotics have different relationships with biofilms [27]. Bla_{OXA-23} and bla_{OXA-51} are the main causes of resistance of *A. baumannii* to carbapenems [19, 28]. Clinical research data show that bla_{OXA-23} and bla_{OXA-51} have high detection rates in some areas [26, 29, 30]. We tested the relationship between six carbapenase genes and biofilm formation and found that bla_{OXA-23} or bla_{OXA-51} strains formed better biofilms. Ozkul found that bla_{OXA-23} + bla_{OXA-51} strain could produce stronger biofilm when studying carbapenase and biofilm [31]. More findings were made that *AmpC*, bla_{OXA-23}, bla_{OXA-51}, and *TEM* had higher gene-carrying rates in antibiotic-resistant strains. We can conclude that strains carrying bla_{OXA-23} and bla_{OXA-51} genes, in addition to being more resistant to antibiotics, also tend to form better biofilms, which is rarely seen in other studies.

In previous studies, we have learned that *A. baumannii* has many drug resistance mechanisms, including β-lactamase production, decreased membrane permeability, overexpression of efflux pump, and biofilm formation [32]. In this study, it was investigated that the production of carbapenenase genes and the formation of biofilms develop stronger resistance to strains. CRAB can develop drug resistance from multiple aspects, which makes us extremely afraid. To avoid the further spread of drug-resistant *A. baumannii* bacteria in the hospital, the laboratory also needs to provide accurate identification and drug sensitivity results, real-time and dynamic monitoring of drug resistance changes of *A. baumannii* strains, and provide a laboratory basis for accurate anti-infection. Clinical pharmacists and clinicians need to make rational medication plans according to the drug sensitivity results, and precise medication to delay the generation of drug-resistant strains.

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Disclosure of conflict of interest

None.

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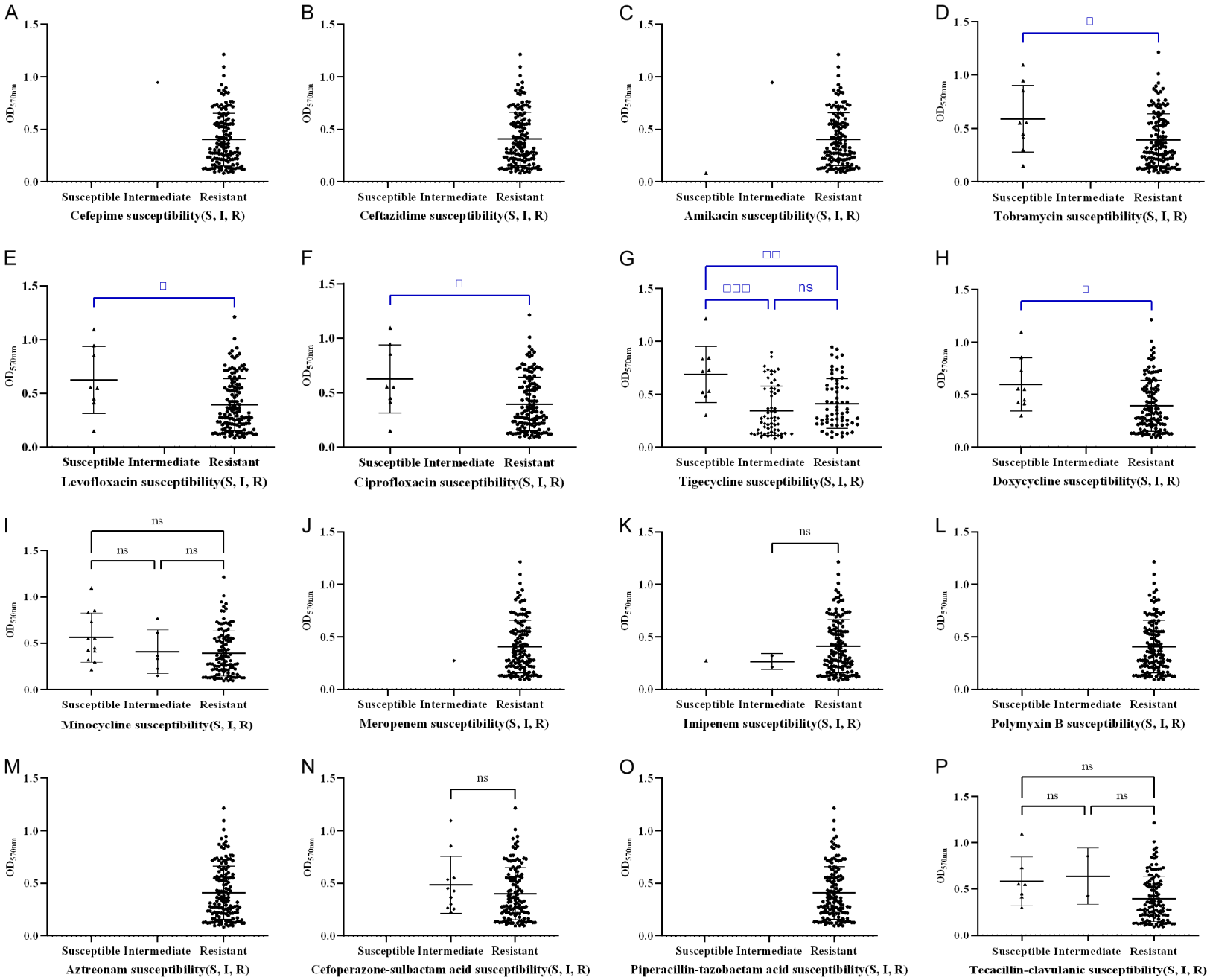
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Susceptible Intermediate Resistant
Cefepime susceptibility(S, I, R)
Ceftazidime susceptibility(S, I, R)
Amikacin susceptibility(S, I, R)
Tobramycin susceptibility(S, I, R)
Levofloxacin susceptibility(S, I, R)
Ciprofloxacin susceptibility(S, I, R)
Tigecycline susceptibility(S, I, R)
Doxycycline susceptibility(S, I, R)
Minocycline susceptibility(S, I, R)
Meropenem susceptibility(S, I, R)
Imipenem susceptibility(S, I, R)
Polymyxin B susceptibility(S, I, R)
Aztreonam susceptibility(S, I, R)
Cefoperazone-sulbactam acid susceptibility(S, I, R)
Piperacillin-tazobactam acid susceptibility(S, I, R)
Tecacillin-clavulanic susceptibility(S, I, R)
ns

Figure S1. Relationship between biofilm formation and resistance of carbapenem-resistant *A. baumannii* to 16 antibiotics. Note: A. Cefepime. B. Ceftazidime. C. Amikacin. D. Tobramycin. E. Levofloxacin. F. Ciprofloxacin. G. Tigecycline. H. Doxycycline. I. Minocycline. J. Melopenem. K. Imipenem. L. Polymyxin B. M. Amtriannan. N. Cefoperazone-sulbactam. O. Piperacillin-tazobactam. P. Ticasillin-clavulanic. CRAB biofilm formation showed significant differences in resistance and sensitivity to Tobramycin, Levofloxacin, Ciprofloxacin, Tigacycline, and Doxycycline. **P*<0.05, ns>0.05.

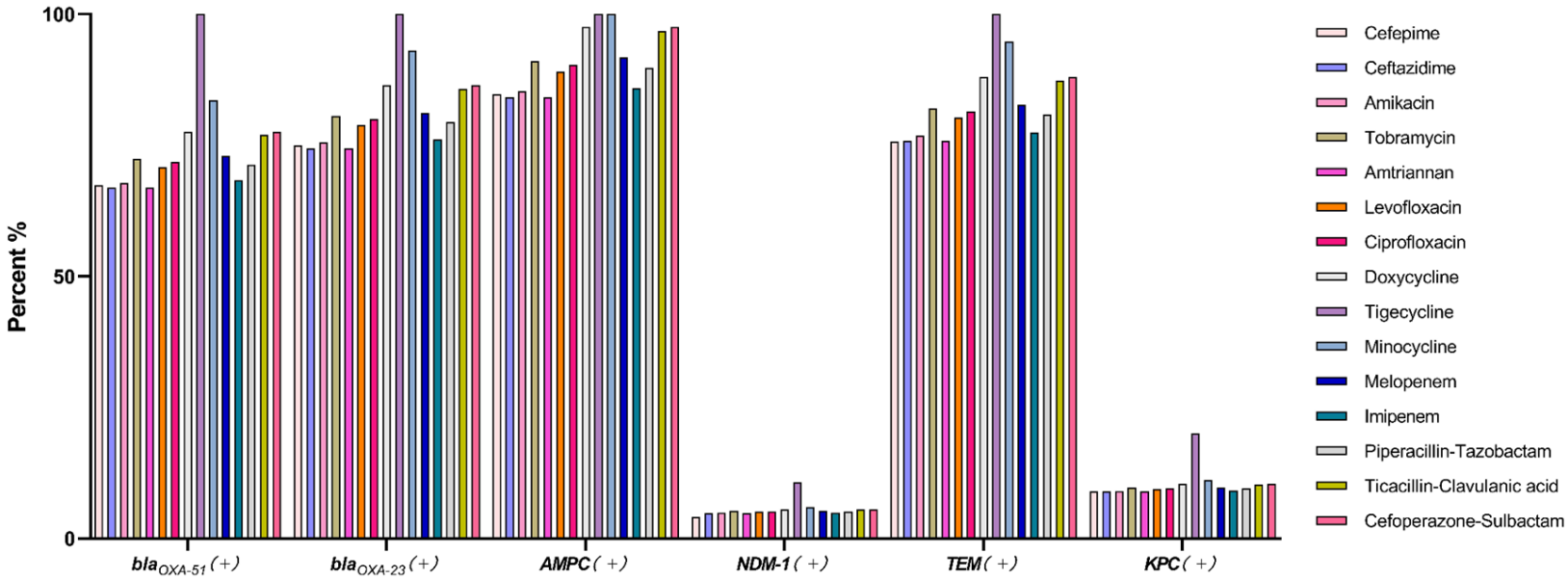

Figure S2. Antibiotic resistance rate of carbapenem-resistant *A. baumannii* carrying carbapenemase gene.