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ORIGINAL RESEARCH

Contribution of the AbaI/AbaR Quorum Sensing System to Resistance and Virulence of *Acinetobacter baumannii* Clinical Strains

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Background: *Acinetobacter baumannii* (*A. baumannii*) is one of the most important pathogens that cause serious nosocomial infections worldwide. However, there are few reports on the virulence of *A. baumannii* clinical isolates, and little is known about the mechanism regulating virulence and drug resistance. The aim of this study was to determine the prevalence of drug resistance and virulence profiles and explore features related to quorum sensing (QS).

Methods: A total of 80 clinical *A. baumannii* isolates were collected from Jilin province of China from 2012 to 2017. We investigated these clinical isolates with respect to biofilm formation, surface motility, adherence, invasion into A549 human alveolar epithelial cells, and virulence to *Galleria mellonella*. We also explored the prevalence of the AbaI/AbaR QS system and its correlation with bacterial virulence and drug resistance.

Results: The resistance rates of the isolates to 17 commonly used antibiotics were higher than 50%, and 75% of the isolates were multi-drug resistant. Approximately 95% (76/80) of the isolates showed the ability to form biofilms, of which 38 showed strong biofilm formation ability (+++). Only 5 strains showed strong surface-related motility. A high level of variability was found in adherence and invasion into A549 epithelial cells, and 16 isolates showed strong virulence to *Galleria mellonella* (none survived after 6 days of infection). Of the 61 isolates carrying *abaI* and *abaR* genes, 24 were found to produce N-acyl homoserine lactones (AHLs) detectable by biosensor bacteria. Correlation analysis revealed that *abaI* and *abaR* genes positively correlated with bacterial resistance rates. All strains showing obvious surface-related motility carried *abaI* and *abaR* genes and produced AHLs. The isolates with detectable QS systems also showed stronger invasiveness into A549 cells and pathogenicity toward *G. mellonella* than the QS-deficient isolates.

Conclusion: Our study demonstrates that the AbaI/AbaR QS system was widely distributed among the *A. baumannii* clinical isolates, was necessary for surface-related motility, and significantly correlated with drug resistance, invasion into epithelial cells, and virulence to *G. mellonella*.

Keywords: *Acinetobacter baumannii*, drug resistance, virulence, quorum sensing

Introduction

Acinetobacter baumannii (*A. baumannii*) is a clinically important, opportunistic pathogen that causes a wide range of clinical infections. Lately, many difficult-to-treat nosocomial infections caused by multidrug-resistant (MDR), extensive- or pan-drug-resistant (PDR) *A. baumannii* have been reported throughout the world, which often lead to morbidity due to the development of antimicrobial drug

resistance and the expression of virulence genes.[1–3] QS is a mechanism by which bacteria coordinate their group behavior by sensing their population density.[4] Small diffusible molecules termed autoinducers are produced constantly, including oligopeptides in Gram-positive bacteria and N acyl-homoserine lactones (AHLs) in some Gram-negative bacteria.[5] At a certain threshold concentration, the binding of the autoinducers and the cognate receptors will induce a cascade of reactions and modulate the expression of QS target genes in the organism.[6] It has been shown that the QS phenomenon exists widely in bacteria and links to various biological activities including motility, conjugation, biofilm formation, production of virulence factors, and pathogenic processes.[5,7] The QS system of *A. baumannii* has recently been reported to consist of AbaI/AbaR, a two-component system.[8] The *abaI* gene encodes the autoinducer synthases which catalyze the synthesis of AHL signals. The most predominant AHLs produced by *A. baumannii* is 3-hydroxy-C12-homoserine lactones.[9] The *abaR* gene encodes the receptor protein which binds to AHLs and behaves as transcriptional regulatory factors. Current studies showed that the mutation of *abaI* gene could result in a greatly reduction of biofilm formation.[8,10] Some strategies that inhibit quorum sensing also strongly inhibited *A. baumannii* motility and biofilm formation.[11,12] However, up to date, little is known about the association between the QS system and the resistance and virulence of *A. baumannii* clinical isolates. The objectives of this study were to determine the presence of the AbaI/AbaR QS system in *A. baumannii* clinical isolates and its effects on antimicrobial resistance and virulence-associated features. This will help to understand the potential roles of the QS system of *A. baumannii* in the regulation of various biological activities and to develop alternative strategies targeting the QS network to combat the infections caused by this notorious pathogen.

Methods
Bacterial Strains and Culture Conditions

Eighty clinical isolates of *A. baumannii* were collected from six hospitals representing three provinces in northeastern China extending from January 2012 until December 2017. The isolates were obtained from various departments including Respiration, Neurosurgery, Pediatrics, Intensive Care Units, and Emergencies. All clinical isolates were identified to the species level using the Vitek-2 system (bioMérieux, Marcyl'Etoile, France), and bla_{OXA-51} was amplified by

using PCR to confirm the presence of *A. baumannii*. Luria Bertani (LB) broth and LB agar plates (both from Merck, Darmstadt, Germany) were used to culture the bacterial isolates under aerobic conditions at 37 °C. *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853 were used as reference strains for susceptibility testing; *A. baumannii* ATCC17978 was used as a control strain for virulence tests.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of the isolated strains was performed according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2016) using the disc diffusion method. We tested the susceptibility of the isolates to piperacillin, ampicillin, ampicillin-sulbactam, piperacillin-tazobactam, ticarcillin-clavulanic acid, ceftazidime, cefepime, cefotaxime, ceftriaxone, imipenem, meropenem, gentamicin, tobramycin, amikacin, minocycline, ciprofloxacin, levofloxacin, and polymyxin B discs (Oxoid, UK). The drug susceptibility data for the *A. baumannii* isolates were analyzed using WHONET 5.6. Multi-drug resistance for *A. baumannii* isolates was defined as described previously.[1]

Detection of *abaI* and *abaR* Genes

The presence of *abaI* and a*baR* genes in clinical isolates was detected by conventional PCR using the primers as following, abaI-F: 5′-AAAGTTACCGCTACAGGG-3′, abaI-R: 5′-CACGATGGGCACGAAA-3′; abaR-F: 5′-TCCTCGGGT CCCAATA-3′, abaR-R: 5′-TAAATCTACCGCATCAA-3′. The PCR program was as follows: 30 cycles of denaturation at 94 °C for 1 min, annealing at 52 °C for 30 s, and extension at 72 °C for 1.5 min, with a final elongation step at 72 °C for 5 min. The PCR products were detected by 1% agarose gel electrophoresis. The amplicon sizes of *abaI* and a*baR* genes were 435 bp and 310 bp, respectively.

N-Acyl Homoserine Lactone (AHL) Production by Clinical *A. baumannii* Isolates

AHL-producing bacteria were screened by using two AHL-sensing bacterial biosensors, *Chromobacterium violaceum* CV026[13] and *Agrobacterium tumefaciens* KYC55,[14] which respond to short-chain and long-chain AHLs, respectively, as described previously.[15,16] Briefly, fresh cultures of QS biosensor strains were inoculated on LB agar plates. KYC55 was grown in LB medium

supplemented with 100 μg/mL tetracycline and 100 μg/mL spectinomycin at 28 °C. CV026 was grown in LB medium supplemented with 20 μg/mL kanamycin at 28 °C. For both KYC55 and CV026, the agar was supplemented with 40 mg/mL X-gal (Sangon Biotech, Shanghai, China). Freshly cultured strains to be tested were streaked on the plates parallel to the biosensor strains. The plates were incubated overnight at 28 °C. Production of AHLs was indicated by the formation of the purple pigment, violacein, or by blue coloration due to β-galactosidase activity.[9,17]

Biofilm Formation Assay

The quantification of biofilm formation was performed using 96-well microtiter plates in accordance with the method of Stepanovic et al,[18] but with minor modifications. Briefly, an overnight culture of an *A. baumannii* isolate was suspended in sterile saline to achieve turbidity comparable to that of a 0.5 McFarland standard. Twenty microliters of the suspension were pipetted into each well of a 96-well microtiter plate (Corning, Corning, NY, USA) and mixed with 180 μL of LB medium. Negative control wells contained only 200 μL of sterile LB medium. The plates were incubated at 37 °C for 24 h. Then, the supernatant was carefully removed from the wells, and each well was washed three times with 200 μL of sterile saline. The plates were dried overnight at room temperature and then fixed with hot air at 65 °C for 1 h. The plates were stained with 150 μL of 1% crystal violet for 30 min. Excess dye was rinsed off with running tap water and dried at room temperature. Glacial acetic acid (150 μL, 33% (v/v)) was added to each well to resolubilize the dye bound to the adherent cells. The optical density (OD) of each well was measured at 600 nm using a spectrophotometer (Epoch, BioTek, CA, USA). Three independent experiments were performed, each in triplicate, and biofilms quantified as nonadherent, weakly adherent, moderately adherent, or strongly adherent.[19] The average value of three parallel test negative controls was considered as the ODC. Isolates were classified as follows: $OD \leq ODC$ = nonadherent (-), $ODC < OD \leq (2 \times ODC)$ = weakly adherent (+); $(2 \times ODC) < OD \leq (4 \times ODC)$ = moderately adherent (++), and $OD > (4 \times ODC)$ = strongly adherent (+++).

Measurement of Surface Motility

Surface motility of these clinical isolates was measured according to a previously described method,[20] but with minor modifications. Overnight cultures of each strain were adjusted to a concentration of 1×10^7 colony forming units (CFUs)/mL in LB broth. One microliter of the bacterial suspension was placed in the center of a motility assay plate containing 10 g/L tryptone, 5 g/L NaCl, and 0.3% Noble agar (Becton Dickinson, Sparks, MD, USA). Plates were allowed to dry at room temperature for 1–2 h and then incubated at 37 ºC for 24 h. The radii of surface extension were measured. All assays were performed in triplicate.

A549 Adhesion and Invasion Assays

The A549 human alveolar epithelial cell line (ATCC CCL-185) was routinely cultured in Dulbecco's Modified Eagle Medium (DMEM, Hyclone) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco), 50 U/mL of penicillin, and 50 μg/mL of streptomycin. Cells were grown to 90% confluence in six well plates to get a monolayer of $\sim10^5$ cells per well. The monolayers were washed three times with PBS prewarmed to 37 °C before infection. Clinical isolates of *A. baumannii* were grown overnight in LB medium at 37 °C, washed in PBS, and adjusted to a multiplicity of infection of 10. Approximately 5×10^5 A549 cells were infected with 5×10^6 bacteria. The infected cells were incubated with DMEM without FBS and antibiotics in a 5% CO_2 atmosphere at 37 °C for 2 h. To determine bacterial adhesion, the infected monolayers were washed three times with PBS and then lysed in 500 μL of 0.1% Triton X-100. For invasion analysis, 500 μg/mL of gentamicin was added to each well for a 30-min incubation to eliminate all extracellular bacteria. Cells were washed three times with PBS and then lysed in 500 μL Triton X-100 to release the invaded bacteria from the infected A549 cells. After lysis in both cases, dilutions of the lysates were plated on LB agar and incubated at 37 °C for 24 h to measure the CFUs of bacteria. All invasion and adhesion assays were performed in three independent experiments, each in triplicates.

Galleria melonella Infection and Killing Assays

The virulence of the clinical strains of *A. baumannii* was evaluated using *Galleria melonella* as an in vivo infection model. Clinical strains of *A. baumannii* were cultured to the exponential (logarithmic) growth phase and washed 2–3 times with PBS. The concentration was adjusted to match the turbidity of 0.5 McFarland standard with PBS.

Ten randomly selected larvae of the same size were used to test the virulence of each bacterial strain. Twenty microliters of the culture of each strain were injected into the left or right hind leg of the larvae. Thus, the actual inoculum of each strain was about 3×10^6 CFU/larva. Ten larvae were injected with 20 μL of PBS as control, and ten untreated larvae were used as blank controls. After the injection, the larvae were incubated in a dark environment at 37 °C for 7 days. A larva was considered to be dead if it could not respond to gentle probing.

Statistical Analysis

The variance of mean values between the two groups was compared using Student's t-test. Qualitative data were analyzed using chi-square and Fisher's exact test. The resulting survival curves were plotted using the Kaplan-Meier method (Kaplan and Meier, 1958) and analyzed using a log-rank (Mantel-Cox) test. For all tests, the difference was considered to be statistically significant at $P < 0.05$.

Ethical Statement

All the clinical samples included in this study were part of the routine hospital laboratory procedure. Due to the anonymous nature of the study, no written informed consent was required.

Results

Detection of *abaI* and *abaR* Genes and AHL Production

All 80 isolates were investigated for the presence of QS genes (*abaI* and *abaR* genes), and the results showed that 83.75% (n=67) and 78.75% (n=63) of the isolates carried the *abaI* and the *abaR* genes, respectively (Table 1). Furthermore, the ability of these isolates to produce AHL signaling molecules was determined by using two AHL-sensing biosensor strains. A total of 24 isolates were determined to be AHL-producing strains based on color changes produced by the reporter strain, *A. tumefaciens* KYC55, which mainly responds to long-chain AHLs. No strain could induce the color change produced by CV026, which indicates that no short-chain AHLs were produced by any of the isolates.

Antimicrobial Susceptibility

A total of 80 clinical *A. baumannii* strains were tested for susceptibility to various antibiotics (Table 2). We found that

**Table 1** Distribution of *abaI* and *abaR* Genes in 80 Clinical Isolates of *A. baumannii*

Genes	Positive Numbers	Positive Rates (n=80)
abaI$^+$	67	83.75%
abaR$^+$	63	78.75%
abaI$^+$ *abaR*$^+$	61	76.25%
abaI$^+$ *abaR*$^-$	6	7.5%
abaI$^-$ *abaR*$^+$	2	2.5%
abaI$^-$ *abaR*$^-$	11	13.75%

Table 2 Antibiotic Susceptibility of *A. baumannii* Clinical Isolates

Antimicrobial Agent	Resistant n (%)	Intermediate n (%)	Susceptible n (%)
Piperacillin	61 (76.25)	8 (10)	11 (13.75)
Ampicillin	73 (91.25)	4 (5)	3 (3.75)
Ampicillin-sulbactam	51 (63.75)	5 (6.25)	24 (30)
Piperacillin-tazobactam	55 (68.75)	7 (8.75)	18 (22.5)
Ticarcillin-clavulanic acid	56 (70)	3 (3.75)	21 (26.25)
Ceftazidime	57 (71.25)	3 (3.75)	20 (25)
Cefepime	58 (72.5)	1 (1.25)	21 (26.25)
Cefotaxime	60 (75)	13 (16.25)	7 (8.75)
Ceftriaxone	57 (71.25)	15 (18.75)	7 (8.75)
Imipenem	54 (67.5)	0 (0)	26 (32.5)
Meropenem	52 (65)	2 (2.5)	26 (32.5)
Gentamicin	49 (61.25)	1 (1.25)	30 (37.5)
Tobramycin	45 (56.25)	2 (2.5)	33 (41.25)
Amikacin	42 (52.5)	2 (2.5)	36 (45)
Minocycline	9 (11.25)	8 (10)	63 (78.75)
Ciprofloxacin	54 (67.5)	0 (0)	26 (32.5)
Levofloxacin	41 (51.25)	8 (10)	31 (38.75)
Polymyxin B	0 (0)	0 (0)	80 (100)

the resistance rates of *A. baumannii* to β-lactam drugs were as high as 63.75%–91.25%, among which the highest resistance rate of 91.25% was observed for ampicillin. Resistance rates for carbapenems were 67.5% (meropenem) and 65% (imipenem). *A. baumannii* also developed severe resistance to aminoglycoside antibiotics: 61.25% for gentamicin, 56.25% for tobramycin, and 52.5% for amikacin. The resistance rates to quinolone antibiotics also exceeded 50%: 67.5% for ciprofloxacin and 51.25% for levofloxacin. However, the resistance rate of *A. baumannii* to tetracyclines was relatively low (11.25%). All strains were sensitive to the polypeptide antibiotic, polymyxin B. Among these 80 strains, 64 were identified as MDR strains according to the definition of Magiorakos.[1] Figure 1 shows the numbers of sensitive and resistant isolates carrying the *abaI* and *abaR*
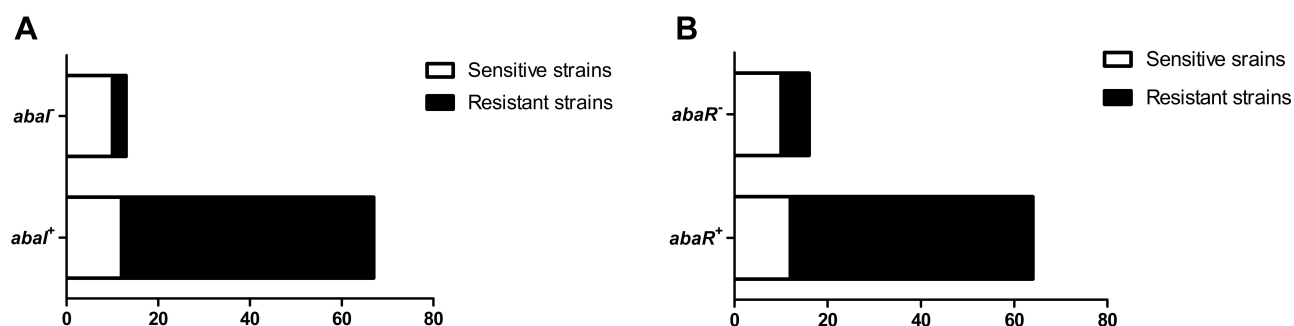
Figure 1 Correlation between drug resistance and carrier status of QS genes, (**A**) *abaI* and (**B**) *abaR* genes in clinical *A. baumannii* isolates.

genes. Among the strains carrying the *abaI* gene, 82.09% were drug-resistant, whereas only 23.07% were drug-resistant among the strains without the *abaI* gene. Among the strains carrying the *abaR* gene, drug-resistant strains accounted for 81.25%, whereas those without the *abaR* gene only accounted for 37.50%. Correlation analysis revealed that the carrying status of *abaI* and *abaR* genes was significantly correlated with the drug resistance of the isolates ($P < 0.01$).

Biofilm Formation

Based on the quantification data, the isolates were classified as strong, moderate, and weak biofilm producers or as non-biofilm producers. Among all 80 clinical isolates of *A. baumannii*, 76 strains were able to form biofilms.

Moreover, 45% of the isolates were strong biofilm producers, and 27.5% and 17.5% exhibited moderate and weak biofilm formation, respectively. There were 4 non-biofilm producers. We also analyzed the relationship between biofilm formation and the production of QS signaling molecules (AHLs). Strains producing AHLs showed a slightly higher ability to form biofilms; however, no significant difference was observed compared with the non-AHL-producing group.

Surface Motility

A total of 80 clinical isolates and ATCC 17978 were tested for surface motility on LB Noble agar plates (0.3%). As shown in Figure 2, the motility exhibited by these strains was highly variable. Five clinical strains, A41, A37, A38, A79, and A76, and ATCC17978 exhibited apparent surface
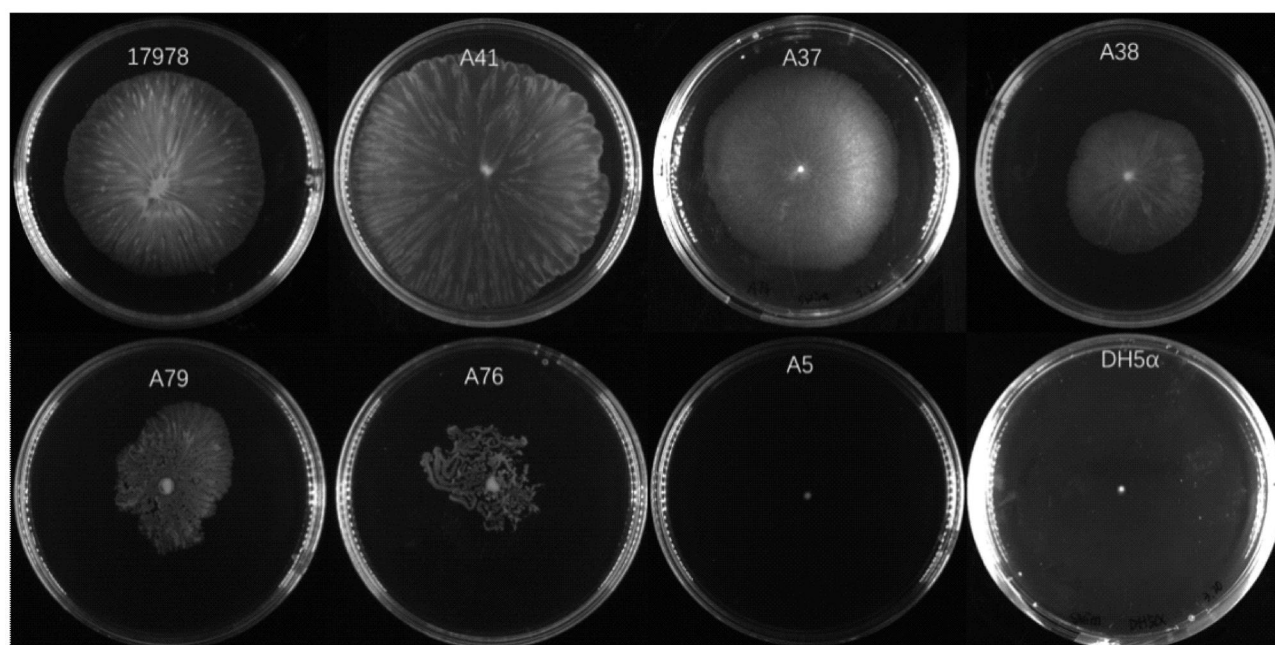
Figure 2 Surface-related motility of *A. baumannii* clinical isolates. ATCC17978 was used as a positive control to show surface motility. Five clinical strains A41, A37, A38, A79, and A76 showed apparent surface motility; A5 was used as a representative clinical strain without motility. DH5α was used as a negative control.

Table 3 Characteristics of Five Clinical Isolates with Apparent Surface Motility

Strains	IC	Drug Sensitivity	AHLs	*abaI* Gene	*abaR* Gene	Biofilm	Motility Diameter (mm)*
A37	II	MDR	+	+	+	+++	58 ± 2.48
A38	II	MDR	+	+	+	++	39.51 ± 2.32
A41	II	MDR	+	+	+	+	71.18 ± 2.63
A76	5	MDR	+	+	+	+	30.98 ±11.20
A79	4	MDR	+	+	+	++	37.57 ± 5.93

Note: *values are mean ± S.D. (standard deviation).
Abbreviations: IC, international clone; MDR, multiple drug resistant; AHLs, N-acyl-homoserine lactones.

motility. The motility diameters are shown in Table 3. The other isolates were either noticeably less motile than the above strains or did not exhibit any motility. It is noteworthy that all five clinical strains exhibiting surface-associated motility carried the *abaI* and *abaR* genes and produced AHLs.

Adherence and Invasion into A549 Cells

A549 human type 2 alveolar epithelial cells were used to examine the adherence and invasiveness of *A. baumannii* clinical isolates. All 80 isolates exhibited different degrees of adherence and invasion into the A549 cells. Interestingly, strains carrying *abaI* and *abaR* genes or producing AHLs displayed significantly higher levels of invasion into A549 cells compared with strains without the QS genes or without the ability to produce signaling molecules (Figure 3).

Virulence to *Galleria mellonella*

Galleria mellonella was used as an infection model to evaluate the virulence of 80 isolates in vivo. Injection of *A. baumannii* isolates resulted in larval death at 24 or 48 h post-inoculation. No larva death occurred within 7 days in the PBS and blank groups. We compared the survival of *G. mellonella* infected with AHL-producing (AHL$^+$) and non-producing isolates (AHL$^-$) and found that the AHL$^+$ group induced significantly greater mortality of *G. mellonella* than did the AHL$^-$ group (Figure 4). This suggested that the virulence of AHL-producing *A. baumannii* isolates was significantly higher than that of non-producing isolates.

Discussion

QS has been described as a general mechanism for regulating many biological processes including virulence, resistance, motility, and biofilm formation in many gram-negative pathogens.[7] At present, there are few research reports on the presence of the QS system in *A. baumannii* clinical isolates and its contribution to antibiotic resistance and virulence. In this study, we determined the carrier status of *abaI* and *abaR* genes in *A. baumannii* clinical isolates and found that 76.25% of the *A. baumannii* strains carried both *abaI* and *abaR* genes, among which 24 strains were found to produce AHLs, based on responses by biosensor strains. Some studies have proved that the presence of QS systems significantly correlates with drug resistance of isolates. Dou et al,[21] found that N-3-hydroxy-dodecanoyl-homoserine lactone (N-3-OH-C12-HSL) produced by *A. baumannii* could induce the expression of drug-resistance genes such as bla_{OXA-51}, bla_{AmpC}, $adeA$, and $adeB$. Our study revealed that the presence of *abaI* and *abaR* genes significantly correlated with multidrug resistance rates, which means that isolates with a QS system were more likely to be multidrug-resistant strains. This suggests that AbaI/
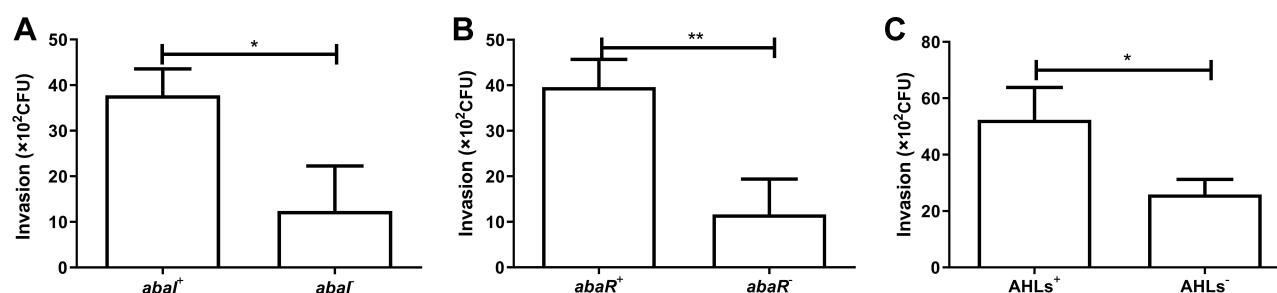
Figure 3 Correlation of invasiveness into A549 cells with carrier status of QS genes, (**A**) *abaI* and (**B**) *abaR*, and with (**C**) AHLs production in clinical *A. baumannii* isolates. *Means statistically significant and p <0.05; **Means statistically significant and p <0.01.
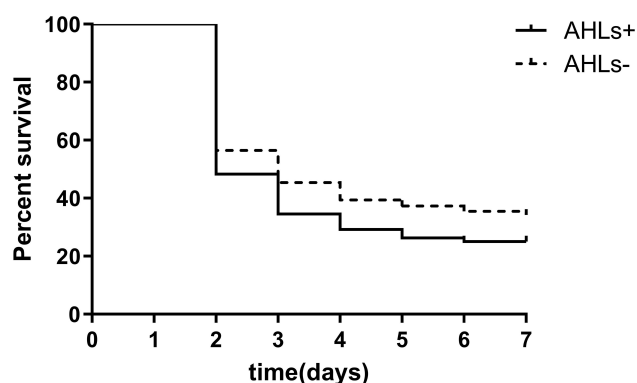
Figure 4 Kaplan–Meier survival curves of *G. mellonella* infected with *A. baumannii* clinical isolates. AHL-producing *A. baumannii* induced significantly greater mortality than did non-AHL-producing *A. baumannii* (P<0.01).

AbaR QS systems are involved in antibiotic resistance and may be an important target for treating multidrug-resistant *A. baumannii* infections.

Biofilm formation has been considered to be important for the colonization of *A. baumannii* on biological and abiotic surfaces and closely related to the multidrug-resistant phenotypes of microorganisms through the obstruction of antibiotic effects on the bacteria. Our study found that most of the *A. baumannii* strains analyzed herein showed an impressive ability to form biofilms on abiotic surfaces. However, the data obtained in this study did not reveal a strong correlation between biofilm formation and the QS system. This result based on clinical strains was not consistent with the results of previous studies using mutant strains. Niu et al constructed an *abaI* mutant strain and found that biofilm formation by the mutant strain was significantly reduced.[8] Phenomenon of biofilm formation is not determined by any single factor or single genotype, but is a complex biological process that is regulated by several factors and these factors seem to be strain-dependent.[22] Similar inconsistent or contradictory results also appeared in the study of the correlation between biofilm formation and drug resistance phenotype.[23] Further in vitro and in vivo studies are required to clarify the contribution of QS systems to biofilm formation by *A. baumannii*.

Previously, *A. baumannii* was thought to be a non-motile bacterium because it has no flagellum structure. But in recent years, many studies have found that some clinical and environmental *A. baumannii* isolates exhibit a robust surface motility on low-percentage agar plates.[24,25] In this study, we analyzed the motility of 80

clinical isolates and found that five strains exhibited apparent surface-related motility, whereas others were less motile or non-motile. In addition, all five strains carried *abaI* and *abaR* genes and produced AHL signaling molecules. A previous study demonstrated that a null allele in the *abaI* decreased motility dramatically, and the addition of exogenous N-(3-hydroxy)-dodecanoyl homoserine lactone (N-3-OH-C12-HSL) restored the motility of the *abaI* mutant.[26] To further analyze the roles of these genes, we knocked out *abaI* or *abaR* or both of them and found that the mutant strains were non-motile (data not shown). Currently, the surface-associated motility of *A. baumannii* appears to rely on the synthesis of 1.3-diaminopropane (DAP),[25] the production of core lipopolysaccharide[27] and response to blue light.[28] Some genes required for motility has been shown strongly activated through quorum sensing,[26,29] which suggests that the AbaI/AbaR QS system is important for the surface motility of *A. baumannii*.

The adhesion and invasion of *A. baumannii* into epithelial cells are key factors in the colonization and nosocomial infection of *A. baumannii*. In this study, 80 *A. baumannii* isolates displayed different abilities to adhere to epithelial cells and subsequently invade them. In particular, those strains carrying the QS system demonstrated stronger invasiveness with respect to epithelial cells. Nesse et al,[30] reported that the addition of AHLs increased the invasiveness of wild *Salmonella typhimurium* strain into epithelial cells. In *P. aeruginosa*, it was found that 3O-C12-HSL could alter the integrity of the cell barrier,[31] disrupt cell junction associations,[32] further trigger multiple signaling pathways, and regulate various functions and behaviors of eukaryotic host cells.[33]

Galleria mellonella has been used to assess the virulence of isolates and compare the pathogenicity between strains and has proved to have good correlation with mammalian models.[34] In this study, the pathogenicity of all 80 clinical isolates was assessed using *G. mellonella* as an infection model; marked differences were displayed between isolates, which reflected the different levels of pathogenicity of clinical isolates to the host. Furthermore, we found that AHL-positive isolates were more virulent than AHL-negative isolates. This result suggests that the QS system may be involved in the pathogenic process of *A. baumannii*. The virulence of QS mutants, including the lasI mutant, rhlI mutant, or lasI rhlI double mutant, decreased significantly compared to that of the parent in *P. aeruginosa*[35,36] in many

different animal models. However, the exact role of the AbaI/AbaR QS system in the pathogenicity of *A. baumannii* requires further investigation.

In this study, the AbaI/AbaR QS system was found to be widely distributed among the *A. baumannii* clinical isolates, which was evidently correlated with bacterial resistance, invasiveness into epithelial cells, and pathogenicity to *G. mellonella*. This study highlights the promise of a new strategy of interfering with the AbaI/AbaR signaling system, which can help to control the infections caused by MDR *A. baumannii*. However, elucidation of the QS network for the regulation of antimicrobial resistance and virulence of *A. baumannii* at the molecular and cellular levels is necessary for the application of this new strategy.

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Disclosure

The authors report no conflicts of interest for this work and declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiol Infection*. 2012;18(3):268–281. doi:10.1111/j.1469-0691.2011.03570.x
2. Gajdacs M, Batori Z, Abrok M, Lazar A, Burian K. Characterization of resistance in gram-negative urinary isolates using existing and novel indicators of clinical relevance: a 10-year data analysis. *Life*. 2020;10:2. doi:10.3390/life10020016
3. Gajdacs M, Burian K, Terhes G. Resistance levels and epidemiology of non-fermenting gram-negative bacteria in urinary tract infections of inpatients and outpatients (RENFUTI): A 10-Year epidemiological snapshot. *Antibiotics*. 2019;8:3.
4. Gajdacs M, Spengler G. The role of drug repurposing in the development of novel antimicrobial drugs: non-antibiotic pharmacological agents as quorum sensing-inhibitors. *Antibiotics*. 2019;8:4. doi:10.3390/antibiotics8040270
5. Miller MB, Bassler BL. Quorum sensing in bacteria. *Annu Rev Microbiol*. 2001;55:165–199. doi:10.1146/annurev.micro.55.1.165
6. Waters CM, Bassler BL. Quorum sensing: cell-to-cell communication in bacteria. *Annu Rev Cell Dev Biol*. 2005;21:319–346. doi:10.1146/annurev.cellbio.21.012704.131001
7. Papenfort K, Bassler BL. Quorum sensing signal-response systems in Gram-negative bacteria. *Nat Rev Microbiol*. 2016;14(9):576–588. doi:10.1038/nrmicro.2016.89
8. Niu C, Clemmer KM, Bonomo RA, Rather PN. Isolation and characterization of an autoinducer synthase from Acinetobacter baumannii. *J Bacteriol*. 2008;190(9):3386–3392. doi:10.1128/JB.01929-07
9. Erdonmez D, Rad AY, Aksoz N. Quorum sensing molecules production by nosocomial and soil isolates Acinetobacter baumannii. *Arch Microbiol*. 2017;199(10):1325–1334. doi:10.1007/s00203-017-1408-8
10. Gaddy JA, Actis LA. Regulation of Acinetobacter baumannii biofilm formation. *Future Microbiol*. 2009;4(3):273–278. doi:10.2217/fmb.09.5
11. Stacy DM, Welsh MA, Rather PN, Blackwell HE. Attenuation of quorum sensing in the pathogen Acinetobacter baumannii using non-native N-Acyl homoserine lactones. *ACS Chem Biol*. 2012;7 (10):1719–1728. doi:10.1021/cb300351x
12. Castillo-Juarez I, Lopez-Jacome LE, Soberon-Chavez G, et al. Exploiting quorum sensing inhibition for the control of pseudomonas aeruginosa and acinetobacter baumannii biofilms. *Curr Top Med Chem*. 2017;17:1915–1927. doi:10.2174/1568026617666170105144104
13. McClean KH, Winson MK, Fish L, et al. Quorum sensing and Chromobacterium violaceum: exploitation of violacein production and inhibition for the detection of N-acylhomoserine lactones. *Microbiology*. 1997;143(Pt 12):3703–3711. doi:10.1099/00221287-143-12-3703
14. Zhu J, Chai Y, Zhong Z, Li S, Winans SC. Agrobacterium bioassay strain for ultrasensitive detection of N-acylhomoserine lactone-type quorum-sensing molecules: detection of autoinducers in Mesorhizobium huakuii. *Appl Environ Microbiol*. 2003;69 (11):6949–6953. doi:10.1128/AEM.69.11.6949-6953.2003
15. Ravn L, Christensen AB, Molin S, Givskov M, Gram L. Methods for detecting acylated homoserine lactones produced by Gram-negative bacteria and their application in studies of AHL-production kinetics. *J Microbiol Methods*. 2001;44(3):239–251. doi:10.1016/S0167-7012(01)00217-2
16. Gajdacs M, Spengler G. Standard operating procedure (SOP) for disk diffusion-based quorum sensing inhibition assays. *Acta Pharm Hung*. 2019;89(4):117–125. doi:10.33892/aph.2019.89.117-125
17. Hou HM, Zhu YL, Wang JY, et al. Characteristics of N-Acylhomoserine lactones produced by hafnia alvei H4 isolated from spoiled instant sea cucumber. *Sensors*. 2017;17:4. doi:10.3390/s17040772
18. Stepanovic S, Vukovic D, Dakic I, Savic B, Svabic-Vlahovic M. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. *J Microbiol Methods*. 2000;40(2):175–179. doi:10.1016/S0167-7012(00)00122-6
19. Basson A, Flemming LA, Chenia HY. Evaluation of adherence, hydrophobicity, aggregation, and biofilm development of Flavobacterium johnsoniae-like isolates. *Microb Ecol*. 2008;55 (1):1–14. doi:10.1007/s00248-007-9245-y
20. Carretero-Ledesma M, Garcia-Quintanilla M, Martin-Pena R, Pulido MR, Pachon J, McConnell MJ. Phenotypic changes associated with Colistin resistance due to Lipopolysaccharide loss in Acinetobacter baumannii. *Virulence*. 2018;9(1):930–942. doi:10.1080/21505594.2018.1460187

21. Dou Y, Song F, Guo F, et al. Acinetobacter baumannii quorum-sensing signalling molecule induces the expression of drug-resistance genes. *Mol Med Rep*. 2017;15(6):4061–4068. doi:10.3892/mmr.2017.6528

22. 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

23. Behzadi P, Urban E, Gajdacs M. Association between biofilm-production and antibiotic resistance in uropathogenic Escherichia coli (UPEC): an in vitro study. *Diseases*. 2020;8:2. doi:10.3390/diseases8020017

24. Harding CM, Tracy EN, Carruthers MD, Rather PN, Actis LA, Munson RS Jr. Acinetobacter baumannii strain M2 produces type IV pili which play a role in natural transformation and twitching motility but not surface-associated motility. *mBio*. 2013;4:4. doi:10.1128/mBio.00360-13

25. Skiebe E, de Berardinis V, Morczinek P, et al. Surface-associated motility, a common trait of clinical isolates of Acinetobacter baumannii, depends on 1,3-diaminopropane. *Int J Med Microbiol*. 2012;302(3):117–128. doi:10.1016/j.ijmm.2012.03.003

26. Clemmer KM, Bonomo RA, Rather PN. Genetic analysis of surface motility in Acinetobacter baumannii. *Microbiology*. 2011;157(Pt 9):2534–2544. doi:10.1099/mic.0.049791-0

27. McQueary CN, Kirkup BC, Si Y, et al. Extracellular stress and lipopolysaccharide modulate Acinetobacter baumannii surface-associated motility. *J Microbiol*. 2012;50(3):434–443. doi:10.1007/s12275-012-1555-1

28. Mussi MA, Gaddy JA, Cabruja M, et al. The opportunistic human pathogen Acinetobacter baumannii senses and responds to light. *J Bacteriol*. 2010;192(24):6336–6345. doi:10.1128/JB.00917-10

29. Perez-Varela M, Corral J, Aranda J, Barbe J. Roles of efflux pumps from different superfamilies in the surface-associated motility and virulence of acinetobacter baumannii ATCC 17978. *Antimicrob Agents Chemother*. 2019;63:3. doi:10.1128/AAC.02190-18

30. Nesse LL, Berg K, Vestby LK, Olsaker I, Djonne B. Salmonella Typhimurium invasion of HEp-2 epithelial cells in vitro is increased by N-acylhomoserine lactone quorum sensing signals. *Acta Vet Scand*. 2011;53:44. doi:10.1186/1751-0147-53-44

31. Vikstrom E, Bui L, Konradsson P, Magnusson KE. The junctional integrity of epithelial cells is modulated by Pseudomonas aeruginosa quorum sensing molecule through phosphorylation-dependent mechanisms. *Exp Cell Res*. 2009;315(2):313–326. doi:10.1016/j.yexcr.2008.10.044

32. Vikstrom E, Bui L, Konradsson P, Magnusson KE. Role of calcium signalling and phosphorylations in disruption of the epithelial junctions by Pseudomonas aeruginosa quorum sensing molecule. *Eur J Cell Biol*. 2010;89(8):584–597. doi:10.1016/j.ejcb.2010.03.002

33. Holm A, Vikstrom E. Quorum sensing communication between bacteria and human cells: signals, targets, and functions. *Front Plant Sci*. 2014;5:309. doi:10.3389/fpls.2014.00309

34. Peleg AY, Jara S, Monga D, Eliopoulos GM, Moellering RC Jr, Mylonakis E. Galleria mellonella as a model system to study Acinetobacter baumannii pathogenesis and therapeutics. *Antimicrob Agents Chemother*. 2009;53(6):2605–2609. doi:10.1128/AAC.01533-08

35. Pearson JP, Feldman M, Iglewski BH, Prince A. Pseudomonas aeruginosa cell-to-cell signaling is required for virulence in a model of acute pulmonary infection. *Infect Immun*. 2000;68(7):4331–4334. doi:10.1128/IAI.68.7.4331-4334.2000

36. Tang HB, DiMango E, Bryan R, et al. Contribution of specific Pseudomonas aeruginosa virulence factors to pathogenesis of pneumonia in a neonatal mouse model of infection. *Infect Immun*. 1996;64 (1):37–43. doi:10.1128/IAI.64.1.37-43.1996