Acinetobacter baumannii quorum-sensing signalling molecule induces the expression of drug-resistance genes

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Abstract. Quorum-sensing signalling molecules such as N-acyl homoserine lactones (AHLs) enable certain Gram-negative bacteria to respond to environmental changes through behaviours, such as biofilm formation and flagellar movement. The present study aimed to identify Acinetobacter baumannii AHLs and assess their influence on antibiotic resistance. A clinical isolate of A. baumannii strain S (AbS) was collected from the wound of a burn patient and high-performance liquid chromatography and tandem quadrupole or quadrupole time-of-flight high-resolution mass spectrometry was used to identify AbS AHLs. Antibiotic sensitivity was assessed in an AHL-deficient AbS mutant (AbS-M), and the expression of drug-resistance genes in the presence of meropenem in AbS, AbS-M and AbS-M treated with the AHL N-3-hydroxy-dodecanoyl-homoserine lactone (N-3-OH-C₁₂-HSL). AbS-M was more sensitive to meropenem and piperacillin than wild-type AbS, but resistance was restored by supplementation with N-3-OH-C₁₂-HSL. In addition, meropenem-treated AbS-M expressed lower levels of the drug-resistance genes oxacillinase S₁, AmpC, AdeA and AdeB; treatment with N-3-OH-C₁₂-HSL also restored the expression of these genes. Overall, the results of the present study indicate that N-3-OH-C₁₂-HSL may be involved in regulating the expression of drug-resistance genes in A. baumannii. Therefore, this quorum-sensing signalling molecule may be an important target for treating multidrug-resistant A. baumannii infections.

Introduction

Staphylococcus aureus and Pseudomonas aeruginosa are the main bacteria that opportunistically infect patients with burns (1). However, recent reports (2-5) indicate that the proportion of infections caused by Acinetobacter baumannii is gradually increasing and, in some instances, already exceeds the number of infections caused by P. aeruginosa. A. baumannii is the most commonly detected Gram-negative organism infecting patients with burns (2-5). However, the emergence of multidrug-resistant A. baumannii complicates the clinical treatment of these infections (6-8).

Quorum sensing is a form of cell-cell communication that bacteria use to coordinate the expression of genes involved in certain behaviours, such as flagellar movement (9,10), virulence factor production (11,12), and secondary metabolite and biofilm production (9,13). Various quorum-sensing signalling molecules have been identified, including oligopeptides in Gram-positive bacteria and N-acyl homoserine lactones (AHLs) in some Gram-negative bacteria (14). Acinetobacter spp. also produce AHLs that possess quorum-sensing activity (15), and the A. baumannii AHL, N-3-hydroxy-dodecanoyl-homoserine lactone (N-3-OH-C₁₂-HSL), is known to affect its motility and biofilm formation (16,17).

Since quorum sensing allows bacteria to respond to environmental changes as a colony and thereby boosts survival, disrupting the quorum-sensing system may be a promising new strategy for the treatment of infections (18-20). It is important to investigate novel strategies for the inhibition of A. baumannii by targeting AHLs (16,17,21-23), but little is currently known about the types and functions of AHLs produced by A. baumannii.

All AHLs share a common homoserine moiety but can contain acyl side-chains of various lengths and degrees of saturation and with various groups at the third carbon position. AHLs generate characteristic fragment ions on electrospray ionization (ESI) at a mass-to-charge ratio (m/z) of 102, and the acyl side-chains generate the corresponding fragment ions at m/z [M+H-101]+ (15,24-33).

The most common methods for identifying AHLs involve a combination of thin-layer chromatography and biosensors (34,35). These methods are simple and inexpensive but are limited by the sensitivity of the biosensor and the use of...
standard substances as references. In the present study, a clinical isolate of \textit{A. baumannii} strain S (AbS) was collected from the wound of a burn patient and cultured. AHLs produced by AbS were subsequently analysed by high-performance liquid chromatography (HPLC) and either tandem quadrupole (TQ) or quadrupole time-of-flight (Q-TOF) high-resolution mass spectrometry (HRMS). The present study adds to the growing body of research on the quorum-sensing system of \textit{A. baumannii} and may contribute to the development of novel antibacterial therapies that target AHLs for treating multidrug-resistant \textit{A. baumannii} infection.

**Materials and methods**

**Bacterial strains and growth conditions.** A single nosocomial specimen of AbS was collected from the wound surface exudates of a patient admitted to the Department of Burns and Plastic Surgery at Ruijin Hospital (Shanghai Jiaotong University School of Medicine, Shanghai, China) in 2008. Antibiotic sensitivity was assessed according to the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI) (36), which included using ATB test strips (BioMérieux, Marcy l’Etoile, France) and the Kirby-Bauer disk diffusion method with antibiotic discs from Oxoid, Ltd. (Thermo Fisher Scientific, Inc., Waltham, MA, USA). \textit{Agrobacterium tumefaciens} strain KYC55 was used as a biosensor, and was kindly provided by Professor Jun Zhu (College of Life Sciences, Nanjing Agricultural University, Nanjing, China). \textit{A. baumannii} was cultured statically in Luria-Bertani (LB) medium at 37°C, while \textit{A. tumefaciens} KYC55 was cultivated statically in LB medium at 28°C.

**Preparation of AHL extract.** \textit{A. baumannii} and \textit{A. tumefaciens} were stored at -80°C in bacteria stock solution (Beyotime Biotechnology, Shanghai, China). \textit{A. baumannii} were inoculated on LB agarose plates and incubated overnight at 37°C. Individual colonies (1x10^6 colony-forming units (CFU)/ml) were selected and cultured in 15 ml LB medium at 37°C. For HPLC-MS, bacteria were cultured in 500 ml LB medium from the overnight LB agarose plates, and 500 ml bacterial liquid was collected at 8 h, as determined by the AHL activity curve. Bacterial samples were centrifuged (4,500 x g for 20 min) and supernatants were passed through a 0.22 \( \mu \text{m} \) filter. An equal volume of 100% ethyl acetate was added to the filtrate, and the ethyl acetate phase was collected for AHL extraction and dried in a vacuum centrifuge. The residue was the AHL extract and was then re-dissolved in 50 \( \mu \text{L} \) ethyl acetate.

**Analysis of AHL activity.** AHL activity was measured at 4, 8, 16, 24, 32, 40 and 48 h after seeding. At each time point, 3 bacterial liquid were collected and the optical density (OD) 600 was measured. AHL extracts in 50 \( \mu \text{L} \) ethyl acetate from 4, 8, 16, 24, 32, 40 and 48 h were added to cultures of \textit{A. tumefaciens} KYC55 cultures, and \( \beta \)-galactosidase activity was measured to indirectly indicate AHL activity, as described previously (15,37). Following overnight incubation, the OD(590) was measured and 0.8 mL Z buffer (in each litre containing: 16.1 g (Na_2HPO_4) 7H_2O, 5.5 g (NaH_2PO_4) H_2O, 0.75 g KCl, 0.245 g (MgSO_4) 7H_2O, 2.7 mL 2-mercaptoethanol, adjusted to pH 7.0 with HCl), 10 \( \mu \text{L} \) 0.05% sodium dodecyl sulphate, 15 \( \mu \text{L} \) chloroform and 0.1 mL ortho-nitrophenyl-\( \beta \)-galactoside (4 mg/mL) were added, with a final sample volume of 0.2 mL. The time (T) taken for the solution to turn yellow was recorded, and 0.6 mL 1 M Na_2CO_3 was added to terminate the reaction. The OD(590) of supernatants was determined, and relative AHL activity was calculated as follows: Activity in Miller units = (1,000 x OD(590)/OD(590)×Tx0.2).

**Identification of AHLs using HPLC-MS.** AHLs of different structures contain the same homoserine lactone (HSL) ring, and this moiety generates characteristic fragment ions at m/z 102 (25). Based on this principle, AbS AHLs were identified by HPLC combined with either TQ or Q-TOF HRMS using a 1200 HPLC-6140 TQ MS or a 1260 HPLC-6538 Q-TOF HRMS (Agilent Technologies, Inc., Santa Clara, CA, USA), respectively. Resultant chromatograms were compared with those of standard substances to elucidate the structure of AbS AHLs. The test conditions were as follows: An Agilent Poroshell 120 SB-C18 chromatographic column (2.7 \( \mu \text{m} \), 2.1x100 mm; Agilent Technologies, Inc.) was used with acetonitrile and water as the mobile phase. The initial acetonitrile concentration was 40%, which was increased to 100% after 30 min, with a z-flow rate of 0.3 mL/min and sample injection volume of 10 \( \mu \text{L} \). Positive-ion ESI was conducted with the ion source at 350°C. The dry Nflow rate was 8 L/min, and the air pressure of atomizing N was 40 psi. The capillary voltage was 4,000 V. HPLC-TQ HRMS was performed with a precursor ion scan and daughter ion scan (collision energy, 15-30 units), while HPLC-Q-TOF HRMS involved MS1 and MS2 full scans (collision energy, 15-30 units).

**Establishment of an AHL-deficient AbS mutant (AbS-M).** An AbS mutant that is unable to produce AHLs was established using pKNG101.abaI::Km, as previously described (38); the pKNG101.abaI::Km plasmid was provided by Professor Philip N. Rather (Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA, USA). This vector was transformed into \textit{Escherichia coli} strain SM10, and the resultant SM10/pKNG101.abaI::Km was cultured with AbS in a filter-mating system in LB medium at 37°C without antibiotics for 24 h, after which it was cultured and screened on LB agarose plates containing 10% sucrose without NaCl. Sucrose resistance indicates that the bacteria have lost the integrated pKNG101 plasmid and therefore streptomycin sensitivity. AbS-M was screened for kanamycin resistance, and Southern blotting was used to confirm that colonies with this phenotype had abaI::Km disruption, as previously described (38).

**Antibacterial sensitivity of AbS and AbS-M.** The minimum inhibitory concentration (MIC) of common antibacterial drugs (including, meropenem, piperacillin, ceftazidime, ciprofloxacin, sulfamethoxazole/trimethoprim and minocycline) against AbS, AbS-M and AbS-M supplemented with 10 \( \mu \text{mol} \) N-3-OH-C\(_{12}\)-HSL (AbS-M+HSL; #53727; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) was assessed using the broth-micro-dilution method, according to the CLSI protocol. Briefly, overnight bacterial cultures were inoculated at 5x10^5 CFU/ml in 1 ml MH medium containing...
Following 24 h incubation at 37°C, the MIC against bacterial growth was assessed by visual examination. Each antibiotic concentration was tested three times.

Expression of drug-resistance genes in AbS, AbS-M and AbS-M+HSL treated with 0.125 μg/ml meropenem or AbS untreated with meropenem (AbS-U) for 24 h.

A total of 45 μl 0.5 McFarland bacterial liquid (AbS, AbS-M and AbS-M+HSL) was added to 3 ml LB medium containing 0.125 μg/ml meropenem, with a final concentration of 10 μM AHL (N-3-OH-C₁₂-HSL). Alternatively, 45 μl 0.5 McFarland bacterial liquid was added into 3 ml LB medium without meropenem (AbS-U). The cultures were incubated at 37°C. After 24 h, 1 ml bacterial liquid was centrifuged (10,621 x g for 1 min) and supernatants were discarded. Total RNA was extracted using TRIzol Reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA), according to the manufacturer's protocol; concentration and purity were determined using a UV spectrophotometer. RNA was reverse transcribed into cDNA using the AMV First Strand cDNA Synthesis kit (New England Biolabs, Inc., Ipswich, MA, USA), according to the manufacturer's protocol. AbS-U, AbS, AbS-M and AbS-M+HSL cultures were incubated for 24 h and the expression levels of 16S rRNA, Oxacillinase (OXA)-51, AmpC type β-lactamase (AmpC), oxacillinase (OXA)-23, IMP type metallo-β-lactamase (IMP)-4, verona integron-mediated metallo-β-lactamase (VIM)-2, Acinetobacter drug efflux (Ade) A, AdeB and AdeC were assessed by quantitative polymerase chain reaction (qPCR) using a StepOnePlus Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.) and SYBR-Green Master Mix (Thermo Fisher Scientific, Inc.); primers used are listed in Table I. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression was measured as a reference, and gene expressions were calculated in terms of fold change using the comparative Cq method; relative mRNA expression was calculated using the 2^-ΔΔCq method (39). The experiments were repeated 3 times.

Statistical analyses. Data were presented as the mean ± standard deviation and analysed using Student's t-test, analysis of variance and least significant difference post hoc test with SPSS version 19.0 (IBM SPSS, Armonk, NY, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Changes in AHL activity of AbS. AbS growth rate and AHL activity were measured periodically between 4 and 48 h incubation (Fig. 1). AHL activity increased from 5.00±1.00 Miller units at 4 h culture to a maximum of 279.33±27.59 Miller units at 8 h. Subsequently, the activity decreased to 28.67±4.16 Miller units at 16 h and plateaued. The AbS growth curve did not correlate with AHL activity after bacterial growth reached the log phase; the OD₆₀₀ (bacterial growth) peaked at 0.90±0.01 after 24 h of culture and then plateaued.

Table I. Primer sequences used for quantitative polymerase chain reaction.

| Gene             | Primer sequence (5'-3') | Product length (bp) |
|------------------|-------------------------|---------------------|
| 16S rRNA         | F: ACGGTTCGCAAGACTAAAAACTCA R: GTATGTCAGGCCAGGTAAGGT | 108                 |
| OXA-51           | F: CTATGGTAATGATCTTGCTGTG R: TTGTTGTTGCTCTATGGTG   | 104                 |
| AmpC             | F: TTATGCGGGCAATACACCA R: CTGACAGAACCTAGCTCAAAATCG | 207                 |
| OXA-23           | F: AAGGCGGAGAAAAAGTCATT R: TCCCGATGACTGGGAAGCTGCA | 89                  |
| IMP-4            | F: ATCTCTACATCCTCCCCACAG R: CTTTTCAGGCGACCAACTAC | 185                 |
| VIM-2            | F: AACTCTTTTATCCTGGTGCTGC R: TGTCGGAACACTCTAAATCG | 105                 |
| AdeA             | F: AGTCCGGAGGTATCATGAAAAGG R: TGAACTTTGAGTCTGGCACCT | 162                 |
| AdeB             | F: ATGCCTGAATGGAAACAACTG R: CCAAGACAAGGAAGACAACTAACA | 145                 |
| AdeC             | F: GCCAATTTCACTCGTTTTTCTG R: GAGTTTATAGGGGCTGAGCTG | 117                 |
| GAPDH            | F: ACCACAGTCCATGCCCACAC R: TCCACACCGCTGTGTA         | 440                 |

bp, base pair; OXA, oxacillinase; F, forward; R, reverse; AmpC, AmpC type β-lactamase; IMP, IMP type metallo-β-lactamase; VIM, verona integron-mediated metallo-β-lactamase; Ade, Acinetobacter drug efflux; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
AHLs produced by AbS. AHLs that were extracted from AbS culture supernatants using ethyl acetate were screened using HPLC-TQ MS. As presented in Fig. 2, the precursor ion scan at m/z 102 detected 30 precursor ions, including those at m/z 282, 284 and 300 (each precursor ion represents one compound). MS2 spectrum analysis of these 30 ions confirmed that they could generate fragment ions at m/z 102, suggesting that they may be AHLs.

In the positive ionization mode of ESI, extracted AHLs generated a quasi-molecular ion at m/z 300 and major fragment ions at m/z 102 and m/z 74 (Fig. 3A). The ion at m/z 102 was the most abundant, and in order to determine its structure, HPLC-Q-TOF HRMS was used to examine the elemental composition of this ion and related daughter fragment ions. The elemental composition at m/z 300 was C_{16}H_{30}NO_4, representing the [M+H]^+ ions of N-3-OH-C_{12}-HSL. The two major fragment ions were C_{4}H_{8}NO (m/z 102) and C_{3}H_{8}NO (m/z 74), both of which were derived from the HSL ring of N-3-OH-C_{12}-HSL, which was the only AHL molecule identified (Fig. 3B). The major fragmentation pathway is shown in Fig. 4. In addition, some low-abundance ions were detected in the MS2 spectrum (m/z 121, 97 and 83), and they contained only two elements, H and C. We hypothesized that these were derived from the fragmentation of carbon chains near the acyl group.

Since components from the culture media may interfere with AHL detection, HPLC-Q-TOF HRMS and tandem MS were used to scan for AHL molecules identified in previous screens. The present study also determined the elemental compositions of the 30 precursor ions and their corresponding daughter ions at m/z 102. The results revealed that only ions detected at m/z 300≥102 met the structural requirement for AHLs. The daughter ions at m/z 102 were derived from 29 candidate molecules that contained C_{3}H_{5}NO and therefore could not be AHLs.

According to the composition and degree of unsaturation, the signal molecule at m/z 300 was inferred to be N-3-OH-C_{12}-HSL. HPLC-MS was then used to examine a commercially available N-3-OH-C_{12}-HSL, and the results confirmed that it was structurally identical to the N-3-OH-C_{12}-HSL detected in the present study.

Activity of mutant AHL. AHLs were isolated from the supernatant of AbS and AbS-M cultures incubated for 8 h at 37°C and the activity levels were analysed. AHL activity was significantly lower in AbS-M (12.67±1.53 Miller units) compared with wild-type AbS (255.67±16.01 Miller units) (P<0.01; Fig. 5).
AHLs produced by clinical isolates of Acinetobacter baumannii have been reported to vary depending on culture conditions (41). Therefore, the AHLs identified in the present study may differ from those identified previously from A. baumannii. The present study aimed to contribute to the growing body of literature on AHLs produced by clinical isolates of A. baumannii.

The present study found that although the activity of AHLs produced by AbS was positively correlated with bacterial density in the log phase of growth, AHL activity reduced as growth plateaued; this trend has been previously reported for other bacteria (31,32). N-(3-oxohexanoyl)-L-HSL produced by Erwinia carotovora was revealed to be unstable at pH >7-8, which is the pH of the stationary phase of bacterial growth (40). Another study demonstrated that, during growth plateauing, A. tumefaciens produces abundant levels of acyl-homoserine lactonases, which reduce AHL activity (32). Thus, AHL activity seems to be regulated by the growth rate, which allows bacteria to respond to their changing density.

In the present study, HPLC-MS with TQ and Q-TOF was used to successfully identify AHLs. This method is advantageous because it does not depend on biosensor sensitivity and reference substances, and thus may be preferable to the conventional methods used for AHL identification, which combine thin-layer chromatography with biosensors.

AHLs produced by A. baumannii have been proposed to vary depending on culture conditions (41). One previous study identified 3-OH-C₁₇-HSL and other AHLs of unknown structure in cultures of A. baumannii strain M2 (38), whereas another study identified C₆-HSL and C₇-HSL in cultures of A. baumannii strain 4KT (15). Furthermore, P. aeruginosa infections in patients with cystic fibrosis have been reported to produce different AHLs in vivo and in vitro (42). Thus, the AHL identified in the present study may differ from those identified previously from A. baumannii, owing to the particular strain and culture conditions used. Additional experiments are required to identify the range of AHLs produced by this organism.

The present study established an AHL-deficient AbS mutant that was used to determine whether AHLs affected antibacterial drug sensitivity of AbS. Antibacterial drug-sensitivity assays...
Table II. Antibiotic sensitivity.

| Antibiotic                       | AbS          | AbS-M        | AbS-M + HSL |
|----------------------------------|--------------|--------------|-------------|
| Meropenem                        | 0.5          | 0.25         | 0.5         |
| Piperacillin                      | 2.0          | 1.0          | 2.0         |
| Ceftazidime                      | 0.25         | 0.25         | 0.25        |
| Cefpiroxacin                     | 0.5          | 0.5          | 0.5         |
| Sulfamethoxazole/trimethoprim    | 0.25/4.75    | 0.25/4.75    | 0.25/4.75   |
| Minocycline                      | 0.5          | 0.5          | 0.5         |

Abs, *Acinetobacter baumannii* strain S; AbS-M, AbS mutant; HSL, homoserine lactone.

Table III. mRNA expression levels of multidrug-resistance genes in meropenem-treated cultures or untreated cultures.

| Gene      | AbS-U | AbS   | AbS-M | AbS-M + HSL |
|-----------|-------|-------|-------|-------------|
| OXA-51    | 0.13±0.02 | 1.09±0.13 | 0.68±0.04* | 1.74±0.04 |
| AmpC      | 0.12±0.03 | 0.94±0.11 | 0.60±0.04* | 1.55±0.04 |
| OXA-23    | ND    | ND    | ND    | ND          |
| IMP-4     | ND    | ND    | ND    | ND          |
| VIM-2     | ND    | ND    | ND    | ND          |
| AdeA      | 0.08±0.04 | 1.17±0.17 | 0.59±0.08* | 1.66±0.25 |
| AdeB      | 0.09±0.08 | 1.08±0.16 | 0.51±0.09* | 1.31±0.11 |
| AdeC      | ND    | ND    | ND    | ND          |

*P<0.01 vs. Abs and AbS-M + AHHs. All data presented as the mean ± standard deviation. AbS-U, *Acinetobacter baumannii* strain S untreated; AbS, *Acinetobacter baumannii* strain S; AbS-M, AbS mutant; HSL, homoserine lactone; ND, not detected; OXA, oxacillinase; AmpC, AmpC type β-lactamase; IMP, IMP type metallo-β-lactamase; VIM, verona integron-mediated metallo-β-lactamase; Ade, *Acinetobacter* drug efflux.

revealed that the MICs of meropenem and piperacillin were lower in AbS-M compared with wild-type AbS; however, the MICs returned to wild-type AbS levels when AbS-M cultures were treated HSL. Although this AHL-mediated increase in MICs was not substantial, this finding is promising in that it confirms the association between AHLs and antibiotic resistance in *A. baumannii*.

A previous report regarding the influence of AHLs on bacterial drug resistance mainly focused on their influence on biofilm formation (9). Previous studies have also described multiple mechanisms of drug resistance in *A. baumannii*, including the production of β-lactamases (43), which can be divided into four categories: Extended spectrum β-lactamases (44-46), metallo-β-lactamases (47,48), AmpC enzyme (49) and oxacillinases (50,51). However, the present study sought to determine the influence of AHLs on the expression of drug-resistance genes and revealed that in the presence of meropenem AbS expressed OXA-51 and AmpC, but not OXA-23, IMP-4, or VIM-2. OXA-51 was previously demonstrated to be strongly expressed in *Acinetobacter* spp. and may be the main drug-resistance gene (52,53), whereas AmpC is often found in *A. baumannii* strains from China (54,55). The present study found that the mRNA expression levels of OXA-51 and AmpC were significantly lower in AbS-M compared with wild-type AbS, but the levels recovered upon supplementation of the AbS-M culture with an AHL extract. These results indicate that AHLs may strengthen drug resistance by moderating the expression of drug-resistance genes.

In addition to producing β-lactamases, *A. baumannii* expresses efflux pump genes AdeA, AdeB and AdeC, which confer resistance to β-lactam antibiotics, aminoglycosides, erythromycins, quinolones, tetracyclines, chloramphenicol and trimethoprim (43,56-62). The present study found that AdeA and AdeB were expressed by AbS in the presence of meropenem. It was not unexpected that AdeC was not detected, since this gene is not essential for efflux pump activity (59). The mRNA expression levels of AdeA and AdeB were significantly lower in AbS-M than in wild-type AbS, and the expression of both AdeA and AdeB was recovered with AHL supplementation. Results from the present study indicated that AbS AHLs promote the expression of OXA-51, AmpC, AdeA and AdeB in the presence of meropenem, suggesting that AbS produces AHLs to enhance antibiotic resistance. Furthermore, upregulation of AdeB expression has been reported to be associated with the emergence of pan-resistant *A. baumannii* (57). Thus, AHLs may promote the emergence of meropenem-induced multidrug- and pan-resistance.

The present study has some limitations that should be noted. Although a mutant strain AbS was designed to be deficient in AHL, subsequent experiments with this mutant may have been influenced by the presence of abaI homologues; AbaI is similar to the LuxI family of autoinducer synthases (37). In addition, it is well known that AHLs can be degraded by N-acylhomoserine lactone-lactonase (32). Therefore, we cannot rule out the possibility of AHL degradation due to lactonolysis. Lastly, the present study did not determine whether the mutation in AbS-M specifically reduces the transcription of abaI or whether it causes a generalized reduction in transcription.

In Gram-negative bacteria, AHL receptor systems include the cytoplasmic LuxR receptor and the transmembrane LuxN receptor (19). Inactivation of suppressor of division inhibition (SdiA), a bacterial homolog of LuxR, hampers the expression of the efflux pump drug-resistance genes acrA and acrB, which are responsible for bacterial multidrug resistance, and AHLs may interact with SdiA to enhance the expression of acrA and acrB (63). Similar systems may exist in *A. baumannii*, and the interaction of AHLs with such systems may be able to induce the expression of drug-resistance genes. However, the MIC of the antibiotics ceftazidime, cefpiroxacin, sulfamethoxazole/trimethoprim and minocycline did not differ between the presence and absence of AHLs, suggesting that drug-resistant phenotypes may be produced by a diverse range of factors and genes. Conversely, exposure to meropenem for 24 h was perhaps insufficient to induce significant phenotypic alterations, and additional experiments are required to rule out longer-term
changes to genes encoding resistance to these antibiotics. However, results from the present study are notable, since to the best of our knowledge no previous study has addressed the mechanisms underlying the influence of AbS AHLs on the expression of drug-resistance genes.

In the present study, the quorum-sensing system of AbS was demonstrated to involve N-3-OH-C_{12}-HSL, which induced the expression of drug-resistance genes OXA-51, AmpC, AdeA and AdeB in the presence of meropenem. Loss of AHL production in AbS-M resulted in reduced mRNA expression of these four drug-resistance genes, while treatment with N-3-OH-C_{12}-HSL restored their expression. Thus, AHL-mediated induction of AdeA and AdeB expression could in turn lead to multidrug resistance in A. baumannii. These results highlight a new direction for the development of drugs targeting A. baumannii, particularly pan-resistant strains.

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