Investigation of mutations in adeR and adeS gene regions in gentamicine resistant Acinetobacter baumannii isolates

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ABSTRACT
This study evaluated the relationship between aminoglycoside resistance and the active efflux pump and investigated the role of the active efflux pump in resistance mechanisms. In addition, the mutations related to aminoglycoside resistance were investigated in Acinetobacter baumannii isolates obtained from different clinical specimens. The study included 32 A. baumannii isolates. They were identified and their susceptibilities were determined using conventional techniques and an automated system. Total genomic RNA, DNA and cDNA were obtained using commercial extraction kits. Primers for adeR and adeS were designed using sequences in GenBank. All isolates were subjected to polymerase chain reaction (PCR) to determine the presence of adeR and adeS. The PCR products were electrophoresed for the optimization study. Subsequently, real-time PCR was performed to determine the expression levels of the adeR and adeS genes. Sequence analysis of the two adeRS operons in our isolates showed five mutations differing from those of other isolates. Isolate A21 had three mutations: (Tyr31Phe), (Val136Ala) and (Leu142Ile); isolate A24 had two mutations: (Asn115His) and (Leu142Ile). In our study, the examined gene regions that play a role in the resistance mechanisms of A. baumannii were considered important. The results indicated that adeR and adeS expression clearly affect aminoglycoside resistance. However, gene expression alone does not seem sufficient to explain that. These results could help to design improved active efflux pump inhibitors.

Introduction
Acinetobacter baumannii has attracted much attention as one of the most important emerging bacterial pathogens of today.[1,2] Acinetobacter is a causative agent of nosocomial outbreaks and the epidemics caused by some strains have been able to spread in multiple hospitals within a city, in various regions in a country and even worldwide.[3,4] A. baumannii employs a wide array of antibiotic resistance mechanisms [5,6]: innate and acquired resistance, chromosomal and plasmid-borne antibiotic resistance genes. This allows this bacterium to develop extraordinary multiple resistance against many of the major classes of antibiotics, including broad spectrum β-lactams, third generation cephalosporins, carboxy- penicillins and increasingly to carbapenems.[7]

The AdeABC efflux pump system is the most well-characterized RND-type (resistance—nodulation—division) system in Acinetobacter.[8,9] Although it is chromosomally encoded, it has been identified only in clinical isolates.[8] The AdeABC pump is normally cryptic in wild-type A. baumannii isolates owing to stringent control by the AdeRS two-component system.[10] Innate expression of efflux pumps in Acinetobacter allows a broad range of substrates to be removed from the cell, conferring resistance to various antibiotic classes.[11] Increased expression of chromosomal efflux pumps and acquisition of additional efflux systems can then lead to multi-drug resistance (MDR).[11,12]

There are two regulatory genes, adeS and adeR, whose products are closely related to proteins of the two-component regulatory system. These genes are transcribed in the opposite direction and are localised upstream from adeA (reviewed in [13]). Two-component systems are signal transduction pathways in bacteria that respond to environmental conditions (the pump is dependent on the substrate).[8,10,14] The protein AdeR (regulator), which consists of 228 amino acids, is a typical transcriptional regulator and the AdeS protein (sensor kinase) is shorter and has bacterial histidine kinase activity. They work together to regulate target gene expression in response to stimuli.[8,10,13]
This study evaluated the relationship between aminoglycoside resistance and the active efflux pump and investigated the role of the active efflux pump in resistance mechanisms. In addition, the mutations related to aminoglycoside resistance were investigated in A. baumannii isolates from different clinical specimens.

Materials and methods

Isolates and antibiotic resistance testing

The study examined 32 A. baumannii isolates, the laboratory collection that had been isolated from samples sent from various clinics to the Ministry of Health Sakarya University Training and Research Hospital Microbiology Laboratory. Strains that had been re-isolated from the same patient were excluded.

Before testing, a suspension of each isolate was inoculated onto sheep blood agar plates and onto eosin methylene blue agar to ensure the purity and viability of the cultures. The inoculum suspensions for the VITEK®2 system (bioMérieux, France) were prepared in sterile saline at a turbidity equal to a 0.5 McFarland standard, as measured using DensiChek (bioMérieux, France). The VITEK®2 instrument automatically filled the individual test cards with the prepared culture suspension, sealed and incubated them at 35.5 °C for 18 h and optical density readings were taken automatically every 15 min. The antibiotic sensitivity of the 32 clinical A. baumannii isolates included in the study was tested using the VITEK 2 automated system and evaluated in terms of the recommendations of the Clinical and Laboratory Standards Institute.[15] The minimum inhibitory concentration (MIC) interpretive standards of the CLSI were used for the VITEK 2 automated system MIC values.

In our study, the 32 isolates were divided into four groups according to the susceptibility-testing results: (1) nine isolates that were resistant to gentamicin, imipenem and meropenem; (2) nine isolates that were sensitive to gentamicin and resistant to imipenem and meropenem; (3) six isolates that were sensitive to gentamicin, imipenem and meropenem; and (4) eight isolates that were resistant to gentamicin and sensitive to imipenem and meropenem (Table 1).

Table 1. Groups of isolates studied in this work.

| Group  | Gentamicin | Imipenem | Meropenem |
|--------|------------|----------|-----------|
| Group 1| R          | R        | R         |
| Group 2| S          | R        | R         |
| Group 3| S          | S        | S         |
| Group 4| R          | S        | S         |

Note: R: resistant, S: susceptible.

DNA and RNA extraction and cDNA synthesis

DNA was extracted from fresh culture of A. baumannii colonies, using a GeneJET Genomic DNA Purification Kit (Thermo Scientific, USA) according to the manufacturer’s instructions. RNA extraction was performed by the GeneJET RNA Purification Kit (Thermo Scientific, USA) extraction kit. In the first stage, the cell wall and membrane were split and then RNA was separated from other cellular macromolecules for centrifugation of the cell lysate. cDNA synthesis from mRNA was performed by Revertaid First Strand cDNA Synthesis Kit (Thermo Scientific, USA).

Primer design

Specific primers were designed for polymerase chain reaction (PCR) amplification of the target gene region for the active efflux pumps. Where possible, published primer pairs were used, otherwise primers were designed as part of the study. Database searches and multiple alignments were carried out using the BioManager CLUSTALW program (http://www.angis.org.au/), where there was sufficient homology, either the Primer 3 program was used to assist in primer selection (http://frodo.wi.mit.edu/cgibin/primer3/primer3_www.cgi) or choices were made manually and inserted into the Sigma program (http://www.sigma-genosys.com/calc/DNACalc.asp) to calculate suitability. The primers used in this study (Table 2) were supplied by Sentromer DNA Technologies (Turkey).

Conventional PCR

All PCR methods used the following concentrations of reagents unless otherwise indicated. The reaction volume was 25 µL and the reaction mixture contained 12.5 µL master mix, 1 µL forward (F) primer, 1 µL reverse (R) primer, 2 µL of sample and 8 µL of distilled DNA-free water. All PCR programmes followed a standard PCR protocol of a 5 min hot start. All had a 10 min final extension step at 72 °C. PCR reactions were performed on a Sensquest Labcycler cooled thermocycler.

Gradient PCR

Optimization studies were performed for all primers using gradient PCR (Labcycler SensoQuest, Germany).

Analysis of conventional PCR products

Electrophoresis was applied for the analysis of the amplicons by using ORTE (Salubris, Turkey) real-time electrophoresis. PCR products were separated in a 2% agarose gel and were visualised over a LED illuminator.
Quantitative RT-PCR (qPCR)

The transcription product levels of adeR, adeS, 16S rRNA, m13 and Oxa51 were identified by real-time PCR (qPCR) method using a Fluorion Instrument (Iontek, Turkey). Control cDNA was obtained from A. baumannii ATCC19606 standard strain. Amplification was carried out in triplicate from three different cDNA samples for all isolates.

Arbitrarily primed PCR (AP-PCR)

To be able to evaluate the similarity among the isolates, arbitrarily primed PCR (AP-PCR) was used. The M13 primer (5’-GAGGGTGGCGGTTCT-3’) was used for amplification of DNA in AP-PCR. The PCR programme included: two cycles of 5 min at 94 °C, 5 min at 40 °C and 5 min at 72 °C, followed by 40 cycles of 1 min at 94 °C, 1 min at 40 °C and 2 min at 72 °C. The amplification products were identified by agarose gel electrophoresis. The similarity among isolates was evaluated by comparing the band profiles.[16,17]

DNA sequence analysis

In our study, Primers 1 (F: AGATGACTAGCATATTGGCGNAC, R: AGCGANGACTTCAATTGGGT) and Primer 2 (F: ACCCAAATGAAGTCTGCT, R: GTTCGCTCTAGTGCACTGCT) were used to amplify DNA fragments. Analysis of the adeRS locus was performed by PCR and sequencing. The PCR products were analysed by Iontek (Turkey). The obtained sequence results were evaluated with basic local alignment search tool (BLAST) analysis. Database searches and multiple alignments were done using the Bio Manager CLUSTALW (SFI, Ireland) program, BLASTN 2.2.29+ National Center for Biotechnology Information (NCBI) and BLAST (UniProt).

Evaluation of gene expression

The expression of adeR versus adeS was examined using the above-mentioned primers designed for this work and another study. The A. baumannii ATCC 19606 16S rRNA gene was used as a housekeeping gene. Transcription data were analysed using the ∆∆Ct method and were used to calculate the relative expression ratio (R).[18]

Results and discussion

It is now known that chromosomally encoded multidrug efflux systems are also broadly distributed in Gram-negative bacteria (reviewed in [19]). Efflux systems capable of accommodating multiple antimicrobial agents fall into five main classes. In A. baumannii, an AdeABC efflux system belonging to the RND family has been identified, and its role in resistance to aminoglycosides and in decreased susceptibility to chloramphenicol, fluoroquinolones, trimethoprim and cefotaxime has been demonstrated clearly.[8,20] In our study, to investigate the relationship between the active efflux pump and aminoglycoside resistance and the role of the efflux pump in resistance mechanisms, expression and sequencing analysis was performed.

Gene expression analysis using qPCR

Expression of the adeR gene

Normally, in wild-type A. baumannii isolates, the AdeABC pump is cryptic as a result of strict control by the AdeRS system.[10] In our study, three primers were used for the adeR gene expression analysis. The expression levels were compared with that of the housekeeping gene and adeR/adeS gene expression could not be determined. In the absence of adeS gene expression, the adeR gene expression level could not be calculated.

Table 2. Primer sets used in this study.

| Primer sets | Nucleotide sequence (5’ → 3’) | Target DNA | GenBank accession number | Size (bp) of amplifier | Reference |
|-------------|--------------------------------|------------|--------------------------|------------------------|-----------|
| 1a | F: 5’-GGCATGAGTGTTATTCGG-3’
   R: 5’-CTCAGAGTGTTATATAAACCCG-3’ | adeR | HM440348.1 | 337 bp | This work |
| 1b | F: 5’-AGATGACTAGCATATTGGCGNAC-3’
   R: 5’-AGCGANGACTTCAATTGGGT-3’ | adeR | * | 307 bp | This work |
| 1c | F: 5’-ACCCAAATGAAGTCTGCT-3’
   R: 5’-GTTGCGCTCTAGTGCACTGCT-3’ | adeR | * | 274 bp | This work |
| 2a | F: 5’-TTCAACAAGAAGATGGACCC-3’
   R: 5’-GTTCGCTCTAGTGCACTGCT-3’ | adeS | HM440348.1 | 584 bp | This work |
| 2b | F: 5’-TCAGCAAGAGCCACAGTGG-3’
   R: 5’-GGCAAGACCCATTGCTT-3’ | adeS | * | 584 bp | This work |
| 3a | GAGGGTGGCGGTTCT-3’ | M13 | [5] |
| 4a | F: 5’-GAGCATCTGATGCGGGTCTG-3’
   R: 5’-CCCAACTCTACGACACCA-3’ | 16S rRNA | FJ855135.1 | 791 bp | This work |
| 5a | F: 5’-TCAGCAAGAGCCACAGTGG-3’
   R: 5’-GCTGAACAACCCATTGCTT-3’ | Oxa-51 | EU255296.1 | 188 bp | This work |

Note: F: forward primer; R: reverse primer. *Designed based on adeR and adeS gene region of 39 A. baumannii strains in the NCBI database.
In our study, gene expression was not detected with three different adeR primer sets and two adeS primer sets in four out of six strains (67%) in the group susceptible to all the three studied antibiotics and the other 28 strains (28/32, 88%) in the four groups showed similar levels of adeR and adeS gene expression.

**Expression of the adeS gene**

Two primers were used for the adeS gene expression study. The results (Table 3) were compared with the expression of the housekeeping gene. In the first group, all of isolates were low positive. In the second group, all of the isolates were low positive, except A15 for adeS

In other words, in our study, gene expression was not detected with three different adeR primer sets and two adeS primer sets in four out of six strains (67%) in the group susceptible to all the three studied antibiotics and the other 28 strains (28/32, 88%) in the four groups showed similar levels of adeR and adeS gene expression.

**AP-PCR analysis**

The AP-PCR amplification products were subjected to agarose gel electrophoresis to examine the similarities among the 32 clinical isolates. The band profiles of the isolates showed 10 different patterns.

**Sequence analysis**

Primer 1 sequence analysis failed for isolates A19, A20, A22, A23 and A26. Primer 2 sequence analysis failed for isolates A2, A5, A6, A9, A18, A19, A20, A22, A23, A25 and A30.
All of the isolates, except A19, A20, A22, A23 and A26 for Primer 1 and A2, A5, A6, A9, A18, A19, A20, A22, A23, A25 and A30 for Primer 2, were evaluated using BLAST. These results are given in Tables 4 and 5. The sequences were compared against the GenBank database. The results include the E-value, identity, gaps and strain accession number. Strains KF147860.1, GU647217.1, EU290753.1, HM440348.1 and EF520292.1 were from GenBank (Tables 4 and 5).

The sequencing analysis of the PCR products that were obtained using two different primer sets associated with the adeR and adeS regions showed that the sequences of the aminoglycoside-sensitive isolates A21 and A24 were similar, with 97%–98% and 94%–96% identity, respectively. In addition, isolates A21 and A24 had 5–5 and 4–4 mutation sites, respectively.

According to the Primer 1 sequence analysis, A18 had no point mutations A6, A14, A16, A27, A28, A31, A32, ATCC1 and ATCC2 had one mutation each; A1, A3, A5, A7, A9, A13, A15 and A29 had two mutations; A2, A4, A12, A25 and A30 had three mutations; A11, A17, and A24 had four mutations; and A21 had five mutations. In the Primer 2 sequence analysis, A12, A14, A27, A28, A31, A32 and ATCC2 had no mutations; A1, A3, A7, A8, A10 and A15 had one mutation each; A4, A11, A13, A29 and ATCC1 had two mutations; A16 had three mutations; A24 had four mutations; and A21 had five mutations (Tables 6 and 7).

The sequence analysis of the adeRS operons of two isolates showed five mutations that differed from the other isolates. A21 had three mutations that were located in the (Tyr31Phe), (Val136Ala) and (Leu142Ile) positions. Isolate A24 had two mutations located in the (Asn115His) and (Leu142Ile) positions. These results may throw more light on some of the mechanisms underlying the increased pump activity, which still remain largely uncertain.[21] Several studies have proposed that point mutations in adeRS such as AdeR (Pro116Leu) and AdeS (Thr153Met), or insertion sequences such as ISABA-1 disrupting adeS, may be linked to adeABC overexpression and increased drug resistance.[10,22] However, others have suggested that mutations in adeRS have no correlation with adeB expression.[23,24] Sun et al. [25] demonstrated that the truncated AdeS protein was constitutively produced and stimulated the expression of AdeABC efflux pump via interaction with AdeR. Their findings suggest a mechanism of tigecycline resistance induced by an aberrant cytoplasmic sensor derived from an insertion element. [25] In another study, two substitutions, Gly186Val on

| Isolate | E-value | Identity   | Gaps          | Number of mutations/mismatches | Accession     |
|---------|---------|------------|---------------|-------------------------------|--------------|
| A1      | 7.00E-136 | 275/281(98%) | 4/281(1%)     | 2                             | KF147860.1   |
| A2      | 6.00E-137 | 274/279(98%) | 2/279(0%)     | 3                             | KF147860.1   |
| A3      | 2.00E-137 | 276/281(98%) | 3/281(1%)     | 2                             | KF147860.1   |
| A4      | 2.00E-135 | 274/280(98%) | 3/280(1%)     | 3                             | KF147860.1   |
| A5      | 1.00E-138 | 275/279(99%) | 2/279(0%)     | 2                             | KF147860.1   |
| A6      | 2.00E-138 | 276/280(99%) | 3/280(1%)     | 1                             | GU647217.1   |
| A7      | 9.00E-135 | 273/279(98%) | 4/279(1%)     | 2                             | GU647216.1   |
| A8      | 3.00E-139 | 277/281(99%) | 3/281(1%)     | 1                             | KF147860.1   |
| A9      | 7.00E-136 | 275/281(98%) | 4/281(1%)     | 2                             | KF147860.1   |
| A10     | 1.00E-137 | 276/281(98%) | 4/281(1%)     | 1                             | KF147860.1   |
| A11     | 6.00E-132 | 272/280(97%) | 4/280(1%)     | 4                             | EU290753.1   |
| A12     | 7.00E-136 | 275/281(98%) | 3/281(1%)     | 3                             | KF147860.1   |
| A13     | 2.00E-137 | 276/281(98%) | 3/281(1%)     | 2                             | KF147860.1   |
| A14     | 9.00E-140 | 278/282(99%) | 3/282(1%)     | 1                             | KF147860.1   |
| A15     | 3.00E-134 | 270/275(98%) | 3/275(1%)     | 2                             | HM440348.1   |
| A16     | 3.00E-140 | 276/279(99%) | 2/279(0%)     | 1                             | EU290753.1   |
| A17     | 2.00E-136 | 274/280(99%) | 2/280(0%)     | 1                             | EF520292.1   |
| A18     | 3.00E-139 | 277/281(99%) | 4/281(1%)     | 0                             | KF147860.1   |
| A21     | 3.00E-135 | 273/279(98%) | 1/279(0%)     | 5                             | KF147860.1   |
| A24     | 4.00E-104 | 259/278(92%) | 15/278(5%)    | 4                             | KF147860.1   |
| A25     | 1.00E-137 | 275/280(98%) | 2/280(0%)     | 3                             | KF147860.1   |
| A27     | 2.00E-137 | 271/274(99%) | 2/274(0%)     | 1                             | KF147860.1   |
| A28     | 2.00E-140 | 279/283(99%) | 3/283(1%)     | 1                             | KF147860.1   |
| A29     | 7.00E-136 | 275/281(98%) | 4/281(1%)     | 2                             | KF147860.1   |
| A30     | 2.00E-136 | 273/278(99%) | 2/278(0%)     | 3                             | KF147860.1   |
| A31     | 6.00E-141 | 277/280(99%) | 2/280(0%)     | 1                             | KF147860.1   |
| A32     | 9.00E-140 | 275/278(99%) | 2/278(0%)     | 1                             | KF147860.1   |
| ATCC1   | 4.00E-138 | 277/282(99%) | 4/282(1%)     | 1                             | EU290753.1   |
| ATCC2   | 7.00E-136 | 271/275(99%) | 3/275(1%)     | 1                             | EU290753.1   |

Note: Sequence analysis could not be made for isolates A19, A20, A22, A23 and A26.
the DHp (dimerization histidine phosphotransfer) domain of the AdeS protein and Ala136Val on the receiver domain of the AdeR protein, were conserved in all 81 studied isolates.\[26\]

In our study, sequence analysis of the adeRS operons in two antibiotic-susceptible isolates showed five mutations different from other isolates: three mutations in isolate A21 and two ones in isolate A24. Interestingly,

Table 5. Sequence reports for Sequence Primer 2.

| Isolate | E-value | Identity | Gaps | Number of mutations/mismatches | Accession |
|---------|---------|----------|------|--------------------------------|-----------|
| A1      | 1.00E-117 | 238/242(98%) | 3/242(1%) | 1 | GU647217.1 |
| A3      | 3.00E-119 | 238/241(99%) | 2/241(0%) | 1 | GU647217.1 |
| A4      | 3.00E-115 | 233/237(98%) | 2/237(0%) | 2 | GU647217.1 |
| A7      | 5.00E-117 | 237/241(98%) | 3/241(1%) | 1 | GU647217.1 |
| A8      | 2.00E-119 | 238/241(99%) | 2/241(0%) | 1 | GU647217.1 |
| A10     | 1.00E-118 | 237/240(99%) | 2/240(0%) | 1 | GU647217.1 |
| A11     | 1.00E-106 | 226/243(97%) | 3/243(1%) | 2 | GU647217.1 |
| A12     | 4.00E-118 | 237/240(99%) | 3/240(1%) | 0 | GU647217.1 |
| A13     | 7.00E-81  | 216/238(91%) | 20/238(8%) | 2 | GU647217.1 |
| A14     | 1.00E-113 | 231/235(98%) | 4/235(1%) | 0 | GU647217.1 |
| A15     | 1.00E-112 | 226/229(99%) | 2/229(0%) | 1 | HM440348.1 |
| A16     | 8.00E-115 | 226/234(97%) | 3/234(1%) | 2 | EU290752.1 |
| A17     | 1.00E-107 | 231/241(96%) | 2/241(0%) | 8 | HM440348.1 |
| A21     | 1.00E-112 | 234/241(97%) | 2/241(0%) | 5 | GU647217.1 |
| A24     | 8.00E-90  | 189/194(97%) | 1/194(0%) | 4 | GU647217.1 |
| A26     | 2.00E-83  | 189/199(95%) | 3/199(1%) | 7 | GU647217.1 |
| A27     | 2.00E-120 | 241/244(99%) | 3/244(1%) | 0 | GU647217.1 |
| A28     | 1.00E-118 | 237/241(98%) | 3/241(1%) | 2 | GU647217.1 |
| A29     | 5.00E-117 | 230/231(99%) | 1/231(0%) | 2 | GU647217.1 |
| A31     | 1.00E-112 | 226/229(99%) | 2/229(0%) | 1 | HM440348.1 |
| A32     | 4.00E-108 | 226/232(97%) | 6/232(2%) | 0 | GU647217.1 |
| ATCC1   | 4.00E-113 | 227/230(99%) | 1/230(0%) | 4 | EU290753.1 |
| ATCC2   | 2.00E-101 | 212/217(99%) | 5/217(2%) | 0 | EU290753.1 |

Note: Sequence analysis could not be made for isolates A2, A5, A6, A9, A18, A19, A20, A22, A23, A25 and A30.

Table 6. Point mutations identified using Sequence Primer 1.

| Isolate | Mutations |
|---------|-----------|
| A1      | UUA(L)→UUU(F) AUC(I)→CUC(L) |
| A2      | AUC(I)→CUC(L) AAA(K)→UAA(STOP) UAU(Y)→UCU(S) |
| A3      | UUA(L)→UUU(F) AUC(I)→CUC(L) |
| A4      | UUA(L)→UUU(F) AUC(I)→CUC(L) GAA(E)→GAG(E) |
| A5      | UUA(L)→UUU(F) AUC(I)→CUC(L) |
| A6      | AUC(I)→CUC(L) |
| A7      | AUC(I)→CUC(L) AAA(K)→AAC(N) |
| A8      | AUC(I)→CUC(L) AUC(I)→CUC(L) |
| A9      | UUA(L)→UUU(F) AUC(I)→CUC(L) |
| A10     | AUC(I)→CUC(L) |
| A11     | GUC(V)→CUC(L) CGU(R)→CUU(L) UUA(L)→UAA(STOP) AUG(START)→UUG(L) |
| A12     | UUA(L)→UUU(F) AUC(I)→CUC(L) GAA(E)→GUA(V) |
| A13     | AUC(I)→CUC(L) UAU(Y)→AAU(N) |
| A14     | AUC(I)→CUC(L) |
| A15     | GUC(V)→CUC(L) GGC(G)→GGG(G) |
| A16     | GUC(V)→CUC(L) |
| A17     | GAA(E)→GAG(E) GCU(A)→GCC(A) GUA(V)→GUG(V) |
| A21     | UUA(L)→UUU(F) AUC(I)→CUC(L) GGU(G)→GGC(G) UUG(L)→CUG(L) UAU(Y)→UUU(F) |
| A24     | AAA(K)→AAG(K) CAU(H)→CAC(H) GGC(G)→GCU(A) AAC(N)→CAC(H) |
| A25     | UUA(L)→UUU(F) AUC(I)→CUC(L) UAU(Y)→UUU(F) |
| A27     | AUC(I)→CUC(L) |
| A28     | UUA(L)→UUU(F) AUA(E)→GAG(E) |
| A29     | UUA(L)→UUU(F) GAA(E)→GAG(E) |
| A30     | UUA(L)→UUU(F) AUA(E)→GAG(E) GUC(V)→AUC(I) |
| A31     | AUC(I)→CUC(L) |
| A32     | UUA(L)→UUU(F) |
| ATCC1   | GUC(V)→CUC(L) |
| ATCC2   | GUC(V)→CUC(L) |

Note: L: leucine; F: phenylalanine; I: isoleucine; K: lysine; S: serine; STOP: stop codon; E: glutamic acid; N: asparagine; V: valine; START: start codon; G: glycine; A: alanine; H: histidine.
Despite the expression of efflux pumps, these isolates are susceptible to aminoglycosides. It could, therefore, be concluded that these mutations affect the pump expression patterns of the two isolates. Mutations leading to constitutive expression of the pump would lead to multidrug resistance. These results indicated that although adeR and adeS expression affects aminoglycoside resistance, gene expression alone does not seem sufficient to explain this. Further studies need to be performed country-wide to specify guidelines to reveal the effectiveness and prevalence of the active efflux pump systems in the multi-drug resistance of A. baumannii strains which may cause morbidity and mortality and even epidemics.

Conclusions

This study focused on evaluation of the antibiotic sensitivity patterns of multi-resistant A. baumannii isolates and analysis of the effect of the active efflux pumps on the formation of multi-resistance in antibiotic resistant isolates. The obtained results show that aminoglycoside resistance has multiple causes. There is a relationship between aminoglycoside resistance and the efflux pump. The gene regions examined that play a role in the resistance mechanisms of A. baumannii are important. The results indicate that adeR and adeS expression affects aminoglycoside resistance, but gene expression alone is insufficient to explain that. These results could help to design improved active efflux pump inhibitors.

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Disclosure statement

There are no conflicts of interest in connection with this paper.

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Table 7. Point mutations identified using Sequence Primer 2.

| Isolate | Mutation |
|---------|----------|
| A1      | AGA(R) → GGA(G) |
| A2      | UUA(L) → UUU(F) |
| A3      | CUA(L) → GAA(E) |
| A4      | AGA(R) → GGA(G) |
| A5      | UUA(L) → UUU(F) |
| A10     | CGU(R) → AGU(S) |
| A11     | GAG(E) → GAA(E) |
| A12     | AAA(N) → ACC(T) |
| A13     | CUA(L) → CUC(L) |
| A14     | GGA(G) → GAC(D) |
| A15     | GAG(E) → GAA(E) |
| A16     | AAA(N) → GCA(A) |
| A17     | CUC(L) → CUC(L) |
| A18     | AUG(START) → UUG(L) |
| A19     | AAG(K) → AAA(K) |
| A20     | CUC(L) → AUC(L) |
| A21     | CUC(L) → AUU(I) |
| A22     | CUG(L) → CUA(L) |
| A23     | GAA(E) → GCG(G) |
| A24     | GAA(E) → GCA(A) |
| A25     | GAA(E) → GCA(A) |
| A26     | AUG(START) → UUG(L) |
| A27     | GAA(E) → GAG(G) |
| A28     | GCA(A) → CAC(H) |
| A29     | GCA(A) → CAC(H) |
| ATCC1   | GCA(A) → GGC(G) |
| ATCC2   | GCA(A) → GGC(G) |

Note: L: leucine; F: phenylalanine; I: isoleucine; K: lysine; S: serine; STOP: stop codon; E: glutamic acid; N: asparagine; V: valine; START: start codon; G: glycine; A: alanine; H: histidine; R: arginine; Q: glutamine; P: proline; D: aspartic acid.
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