

Original Article

Detection of Integrons in *Acinetobacter Baumannii* Strains Isolated from the Nosocomial Infections of Ahvaz City and their Relation with the Resistance Pattern

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Key words

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Background and Aims: *Acinetobacter baumannii* is regarded as an important nosocomial pathogen around the world, especially in the intensive care unit that today seems to be resistant to the most antibiotics. Therefore, this study aimed to trace classes 1, 2, and 3 integrin in the isolates resistant to *Acinetobacter baumannii*.

Materials and Methods: In this descriptive study, *Acinetobacter baumannii* of 67 patients in Ahvaz hospitals were all isolated and their antibiotic resistance pattern was determined by the disk diffusion. The presence of genes coding for antibiotic resistance as well as integrons (class 1, 2 and 3) were analyzed using polymerase chain reaction method.

Results: Out of 67 isolates, the most resistance was observed for the antibiotic tetracycline (89.5%) and the most sensitivity to antibiotic was reported for chloramphenicol, meropenem and nitrofurantoin (2.9%). The distribution of *dfrA1*, *sul1*, *aac(3)-IV*, *tet(B)*, *tet(A)*, *aadA1*, *bla_{CTM}*, *vim*, *qnr*, *bla_{SHV}*, *sim*, *Oxa-24-like*, *Oxa-51-like*, *Oxa-58-like*, *Oxa-23-like*, *imp*, *cmlA* and *catI* were 42 (62.6%), 40 (59.7%), 36 (53.7%), 34 (50.7%), 31 (46.2%), 17 (25.3%), 17 (25.3%), 16 (23.8%), 11 (16.4%), 11 (16.4%), 8 (11.9%), 5 (7.4%), 5 (7.4%), 4 (5.9%), (5.9%), 4 (5.9%), 2 (2.9%), and 1 (1.4%) ,respectively. Moreover, frequency of class 1, 2 and 3 integrons was 67 (100%), 22 (32.8%), and 3 (4.4%) ,respectively.

Conclusions: High prevalence of integrons among *Acinetobater baumannii* isolated strains in Ahvaz hospitals indicate the importance role of integrons in multidrug resistance in this bacteria. Therefore, unnecessary use of antibiotics are recommended to be avoided.

Introduction

Acinetobacter baumannii (*A. baumannii*) is introduced as an important worldwide nosocomial pathogen, specifically in intensive care units (ICUs), which is able to produce various infections such as septicemia, urinary tract infection, nosocomial meningitis, wound infection, bacteremia, infection of skin and soft tissue, and high-mortal pneumonia [1, 2]. This bacterium is a gram negative, non-motile, obligate aerobic, oxidase-negative, capsule-forming, non-spore-forming, and non-fermenting coccobacillus [3]. *A. baumannii* prefers a humid environment and has minimal nutritional needs being widely spread in the nature [4], which is abundantly found in soil, food, vegetables, meat, and fish [5]. As a matter of fact, *A. baumannii* is able to survive on human skin, equipment, and objects in different sections, especially burn centers and ICUs for a long time [6]. This bacterium can rarely develop infections in people with normal immune system status and thus, has hardly ever been mentioned as the normal flora of a healthy body [5]. The potent inducers of *A. baumannii* infections include previous antibiotic treatments, major surgeries, burns, suppression of immune system, and presence of mechanical ventilators [7]. Today, *A. baumannii* has been reported to be resistant to the majority of antimicrobial agents including aminoglycosides, fluoroquinolones, betalactams, and cephalosporins [8]. Hence, choosing the appropriate antibiotics has become a major matter of concern in regard with this bacterium treatment [7]. The excessive antibiotic proscriptioin in former treatments has been

recently believed to be the main reason of the emergence of resistance genes [9]. Several motile genetic elements such as plasmids, transposons, phages, and integrons are responsible for the transmission of antibiotic resistance genes. Recently, integrons have been demonstrated to play a major role in antibiotic genes transfer in the clinical environments. In fact, integrons are genetic elements including a number of genes and the specific sites for recombination system fusion which enables them to retain the motile genetic cassettes. Regarding the general structure of integrons, the resistance genes are found on specific gene cassettes which mediate the resistance gene transfer by their ability to fuse to the “integron sets” through a site-specific recombination process. There are only three classes of integrons with clinical importance which have been extensively scrutinized, namely classes 1, 2, and 3 [10, 11].

Since nosocomial *A. baumannii* infection has generated an abundance of therapeutic obstacles for the treatment of hospitalized patients in Iran, as well as other countries, the knowledge regarding the prevalence level of antibiotic resistance genes and the resistance pattern of this bacterium to the initial antibiotics is required in order to control, prevent, and cure *A. baumannii*-driven infections. Thus, the present study has addressed the detection of classes 1, 2, and 3 integrons in *A. baumannii* strains isolated from the nosocomial infections of Ahvaz and their relation with the resistance pattern of this bacterium.

Material and Methods

Isolation and characterization of bacteria

In the present descriptive study, 67 strains of *A. baumannii* were isolated from various infections of humans (suppurative wounds (n=6), blood infections [n=28], urinary tract infections (n=14), respiratory infections (n=18), and meningitis (n=1) in a 3-month period (January to March, 2014) from patients of Imam Khomeini and Talegani hospitals in Ahvaz, Khoozestan, Iran. These isolates were characterized and confirmed in the laboratories of the corresponding hospitals through routine microbiological and biochemical tests such as IMVIC, urease, TSI, OF, MRVP, SIM, catalase, oxidase, and growth at 37°C and 42°C. The confirmed samples were stored at 30% glycerol at -70°C.

The presence of *A. baumannii* in the isolates was confirmed via amplification of 16S-23S ribosomal DNA through polymerase chain reaction (PCR) using the primer pairs shown in table 1. PCRs were carried out in 25 µl and the ingredients consisted of 2.5 µl of 10X PCR buffer, 1.5 mmol of MgCl₂, 100 µmol of dNTPs mix, one unit of Taq DNA polymerase (fermentas-Lithuania), 1.0 µmol of reverse and forward primers, and 2 µl of template DNA (being the DNA of the isolates). PCR program was set to a cycle of 94°C for 3 min., 30 repetitive cycles of, respectively, 95°C for 40 s, 59°C for 55 s, and 72°C for 60 s, as well as a

final cycle of 72°C for 6 min. Amplification of a fragment with a size of 208 bp indicates the presence of *A. baumannii* in the samples [12].

Determination of resistance and sensitivity to antibiotics

The resistance to antibiotics was evaluated using antibiotic discs (Iran Padtan Teb Company, Iran) according to the protocols of Clinical and Laboratory Standards Institute (CLSI) through disc diffusion (Kirby-Bauer) method in Mueller-Hinton agar medium [13, 14]. The standard strains of *Escherichia coli* ATCC 25922 and *A. baumannii* ATCC 19606 were implemented as, respectively, negative and positive controls in the antibiogram tests. The examined antibiotics were tetracycline (30 µg), ceftazidime (30 mg), ciprofloxacin (30 mg), co-trimoxazole (25/1/75/23 µg), tobramycin (10 mg), chloramphenicol (30 mg), norfloxacin (10 mg), amikacin (30 mg), gentamicin (10 mg), rifampin (5 µg), cephalothin (30 µg), streptomycin (10 mg), trimethoprim (5 µg), levofloxacin (5 µg), imipenem (10 µg), meropenem (10 mg), nitrofurantoin (300 mg), azithromycin (15 mg) and erythromycin (15 µg). Mueller-Hinton agar media were inoculated with the suspension of bacteria by an optical density of 0.5 McFarland. The inhibition halo was measured for each antibiotic, and sensitivities were calculated according to the protocol.

Table 1. The sequences of reverse and forward primers used in this study

| Gene locus | Primer sequence (5'- 3') | Product size (bp) | Reference |
|------------------------------|--|-------------------|-----------|
| <i>16S-23S ribosomal DNA</i> | F- CATTATCACGGAATTAGTG R- AGAGCACTGTGCACTTAAG | 208 | 12 |
| <i>aadA1</i> | (F) TATCCAGCTAAGCGGAACT (R) ATTTGCCGACTACCTTGCTC | 447 | 15 |
| <i>aac(3)-IV</i> | (F) CTCAGGATGGCAAGTTGGT (R) TCATCTCGTTCTCCGCTCAT | 286 | 15 |
| <i>sulI</i> | (F) TTCGGCATTCTGAATCTCAC (R) ATGATCTAACCCCTCGGTCTC | 822 | 15 |
| <i>bla_{SHV}</i> | (F) TCGCCTGTGTATTATCTCCC (R) CGCAGATAAATCACCACAATG | 768 | 15 |
| <i>CITM</i> | (F) TGGCCAGAAGTACAGGCAAA (R) TTTCTCTGAACGTGGCTGGC | 462 | 15 |
| <i>catI</i> | (F) AGTTGCTCAATGTACCTATAACC (R) TTGTAATTCATTAAGCATTCTGCC | 547 | 15 |
| <i>cmlA</i> | (F) CCGCCACGGTGTGTTGTTATC (R) CACCTTGCCTGCCCATCATTAG | 698 | 15 |
| <i>tet(A)</i> | (F) GGTTCACTCGAACGACGTCA (R) CTGTCCGACAAGTTGCATGA | 577 | 16 |
| <i>tet(B)</i> | (F) CCTCAGCTTCTCAACGCGTG (R) GCACCTTGCTGATGACTCTT | 634 | 16 |
| <i>dfrA1</i> | (F) GGAGTGCCAAAGGTGAACAGC (R) GAGGCGAAGTCTTGGGTAAAAAC | 367 | 17 |
| <i>Qnr</i> | (F) GGGTATGGATATTATTGATAAAG (R) CTAATCCGGCAGCACTATTTA | 670 | 18 |
| <i>Imp</i> | (F) GAATAGAATGGTTAACTCTC (R) CCAAACCACTAGGTTATC | 188 | 19 |
| <i>Vim</i> | (F) GTTTGGTCGCATATCGCAAC (R) AATGCGCAGCACCAGGATAG | 382 | 19 |
| <i>Sim</i> | (F) GTACAAGGGATTTCGGCATCG (R) GTACAAGGGATTTCGGCATCG | 569 | 19 |
| <i>Oxa-51-like</i> | (F) TAATGCTTTGATCGGCCTTG (R) TGGATTGCACTTCATCTTGG | 353 | 20 |
| <i>Oxa-23-like</i> | (F) GATCGGATTGGAGAACCAGA (R) ATTTCTGACCGCATTTCAT | 501 | 20 |
| <i>Oxa-24-like</i> | (F) GGTTAGTTGGCCCCCTTAAA (R) AGTTGAGCGAAAAGGGGATT | 246 | 20 |
| <i>Oxa-58-like</i> | (F) AAGTATTGGGGCTTGTGCTG (R) CCCCTCTGCGCTCTACATAC | 599 | 20 |
| <i>IntI</i> | F: CAG TGG ACA TAA GCC TGT TC R: CCC GAC GCA TAG ACT GTA | 160 | 21 |
| <i>IntII</i> | F: TTG CGA GTA TCC ATA ACC TG R: TTA CCT GCA CTG GAT TAA GC | 288 | 21 |
| <i>IntIII</i> | F: GCC TCC GGC AGC GAC TTT CAG R: ACG GAT CTG CCA AAC CTG ACT | 1041 | 22 |

Investigating the presence of antibiotic resistance coding genes

Primer pairs shown in table 1 were used to detect antibiotic resistance genes, including *aadA1* (streptomycin resistance), *aac(3)-IV* (gentamycin resistance), *sulI* (sulfonamide

resistance), *bla_{SHV}* and *CITM* (beta-lactam resistance), *catI* and *cmlA* (chloramphenicol resistance), *tet(A)* and *tet(B)* (tetracycline resistance), *dfrA1* (trimethoprim resistance), *qnr* (quinolone resistance), *imp*, *vim*, and *sim* (carbenicillin resistance), and *Oxa-23-like*, *Oxa-*

24-like, Oxa-51-like, and Oxa-58-like (oxacillin resistance). Genomic DNAs of *A. baumannii* isolates were extracted using Genomic DNA Purification Kit (fermentas Lithuania) and implemented as a template DNA in PCR amplification of the intended fragments. The PCR programs were set according to the size of the fragments as follows:

A multiplex PCR containing 50 µl of ingredients (5 µl of 10X PCR buffer, 2 mmol of MgCl₂, 150 µmol of dNTPs mix, 0.5 µmol of F and R primer pairs (for each gene), 1.5 unit of Taq DNA polymerase, and 2 µl of each DNA sample) was used to detect *aadA1*, *aac(3)-IV*, *sul1*, *bla_{SHV}*, *CITM*, *cat1*, *cmlA*, *tet(a)*, *tet(B)*, *dfrA1*, and *qnr*. The thermal program was a cycle of 94°C for 6 min., 33 repetitive cycles of 95°C for 70 s, 55°C for 65 s, and 72°C for 90 s, and a final cycle of 72°C for 8 min. [15-18]. Strains of *E. coli* O157:K88ac:H19, CAPM 5933 and *E. coli* O159:H20, CAPM 6006 were used as the positive controls. The amplification of *imp*, *vim*, and *sim* genes was carried out in a multiplex PCR in a volume of 50 µl containing 5 µl of 10X PCR buffer, 1.5 mmol of MgCl₂, 100 µmol of dNTPs mix, 1.0 µmol of forward and revers primer pairs (for each gene), 1.0 unit of Taq DNA polymerase, and 2.5 µl of each DNA. The program was set to a cycle of 95°C for 4 min., 30 repetitive cycles of 94°C for 45 s, 58°C for 60 s, and 72°C for 40 s, and a final cycle of 72°C for 5 min. [19]. Oxacycline resistance genes (*Oxa-23-like*, *Oxa-24-like*, *Oxa-51-like*, *Oxa-58-like*) were detected in a 50 µl multiplex PCR with 5 µl of 10X PCR buffer, 2.5 mmol of MgCl₂, 200 µmol of dNTPs mix, 0.5 µmol of forward and revers primer pairs (for each gene), 1.5 unit of

Taq DNA polymerase, and 2.0 µl of each DNA of each isolate. The program included a cycle of 94°C for 5 min., 32 repetitive cycles of 95°C for 50 s, 60 °C for 60 s, and 72°C for 70 s, as well as a final cycle of 72°C for 10 min. [20].

Analyzing the presence of integrons

Classe 1, 2, and 3 integrons were detected using the primers shown in table 1 in the multiplex PCR. The reaction volume was set to 25 µl containing 2.5 µl of 10X PCR buffer, 1.5 mmol of MgCl₂, 200 µmol of dNTPs mix, 0.5 µmol of forward and revers primer pairs, 1.0 unit of Taq DNA polymerase, and 2.5 µl of each DNA sample. Thermal program was set to a cycle of 94°C for 6 min., 35 repetitive cycles of 94°C for 1 min., 56°C for 1 min., and 72°C for 45 s, as well as a final cycle of 72°C for 6 min. Since the positive control sample was not used in regard with detection of some genes, to confirm or reject the PCR results, the PCR products of the primary positive samples were purified using a PCR product purification kit (Roche Applied Science, Germany) and sent to the Macrogen Co. (South Korea) for sequencing. The PCR amplifications were carried out in an Eppendorf, Mastercycler® 5330 (Eppendorf-Netheler-Hinz GmbH, Hamburg Germany). The amplified fragments were visualized on ethidium bromide containing 1.5% of agarose gels, in which 15 µl of each product was loaded, in addition to 100 bp DNA marker (fermentase-Lithuania), on a UV transluminator (UVitech, England) after electrophoresis at 90 V for approximately 45 min.

Statistical analysis

The collected data were analyzed by SPSS statistical software (ver.16) using Chi-square and Fischer's exact statistical tests at the confidence level of 95 percent.

Results

A total of 67 *A. baumannii* strains isolated from nosocomial infections in two hospitals of Ahvaz, Iran were selected and analyzed in regard with their antibiotic resistance. These isolates were confirmed by the biochemical test and 16S-23S ribosomal DNA gene detected all of them. The patterns of the isolates resistance to 18 common antibiotics, used in treatments of infections in humans, were evaluated after their identity was confirmed (through sequencing their 16S-23S

ribosomal DNA) (Fig. 1) through simple disc diffusion method. All the studied isolates demonstrated the multidrug resistance (MDR) (Table 2), among which the most antibiotic resistance was observed for tetracycline (89.5%), while the minimum resistance (2.9%) accompanied chloramphenicol and nitrofurantoin. The statistical analysis demonstrated a statistically significant difference between tetracycline and other antibiotics. In addition, the resistance to cotrimoxazole, trimethoprim, and gentamicin was significantly different from the resistance to other antibiotics except for tetracycline.

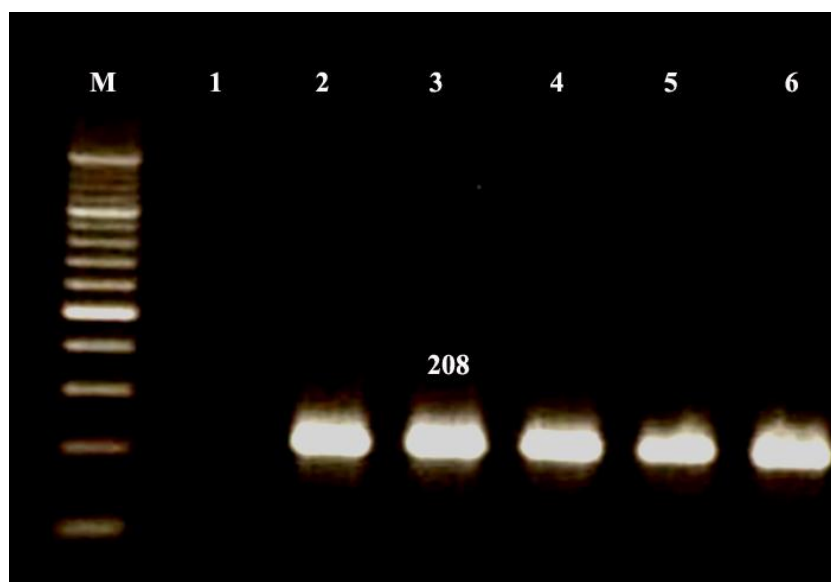


Fig.1. Agarose gel electrophoresis of PCR products of the amplification of 16S-23S ribosomal DNA in *A. baumannii* isolates
M) 100 bp DNA marker; 1) negative control; 2) positive control; 3-6) the studied samples containing the 208-bp fragment

Table 2. The antibiotic resistance patterns of *A. baumannii* strains isolated from nosocomial infections in Ahvaz

| | Blood (n=28) | Sputum (n=18) | Urine (n=14) | Pus (n=6) | CSF (n=1) | Total (n=67) |
|-----------------------|--------------|---------------|--------------|-----------|-----------|--------------|
| Trimetoprim | 13 | 8 | 10 | 2 | 1 | 34 |
| Tetracycline | 27 | 17 | 12 | 3 | 1 | 60 |
| ceftazidime | 1 | 1 | 1 | - | - | 3 |
| cephalotin | 6 | 4 | 2 | 2 | - | 14 |
| co-trimoxazole | 13 | 10 | 11 | 3 | 1 | 38 |
| tobramycin | - | 2 | 3 | - | - | 5 |
| amikacin | 2 | - | 2 | - | - | 4 |
| gentamicin | 11 | 9 | 8 | 3 | 1 | 32 |
| streptomycin | 7 | 4 | 2 | 1 | 1 | 14 |
| erythromycin | 3 | 4 | 2 | 1 | - | 10 |
| rifampin | 1 | 2 | - | - | - | 3 |
| nitrofurantoin | 1 | - | 1 | - | - | 2 |
| cloramphenicol | - | 1 | 1 | - | - | 2 |
| Meropenem | 1 | 1 | - | - | - | 2 |
| imipenem | - | 1 | 1 | 1 | - | 3 |
| lovofoxacin | 1 | - | 2 | - | - | 3 |
| ciprofloxacin | 2 | 1 | - | 1 | - | 4 |
| Azithromycin | 2 | 1 | 1 | - | - | 4 |

The PCR analysis of the presence of 18 antibiotic resistance genes demonstrated that 97.01% of isolates had *tetA* and *tetB* (tetracycline

resistance), 41.79% had *vim*, *sim*, and *imp* (carbenicillin resistance), and 4.47% had *catI* and *cmlA* (chloramphenicol resistance) (Table 3).

Table 3. Distribution of genes responsible for antibiotic resistance in *A. baumannii* strains isolated from nosocomial infections in Ahvaz

| | Blood (n=28) | Sputum (n=18) | Urine (n=14) | Pus (n=6) | CSF (n=1) | Total (n=67) |
|---------------------------|--------------|---------------|--------------|-----------|-----------|--------------|
| <i>qnr</i> | 6 | 3 | - | 2 | - | 11 |
| <i>dfrA1</i> | 15 | 10 | 12 | 4 | 1 | 42 |
| <i>tetB</i> | 15 | 10 | 5 | 4 | - | 34 |
| <i>tetA</i> | 13 | 7 | 9 | 1 | 1 | 31 |
| <i>bla_{CITM}</i> | 7 | 5 | 2 | 2 | 1 | 17 |
| <i>bla_{SHV}</i> | 5 | 4 | 1 | 1 | - | 11 |
| <i>sulI</i> | 13 | 10 | 12 | 4 | 1 | 40 |
| <i>aac(3)-IV</i> | 12 | 9 | 10 | 4 | 1 | 36 |
| <i>aadA1</i> | 8 | 4 | 3 | 2 | - | 17 |
| <i>Oxa-58-like</i> | 1 | 1 | - | 2 | - | 4 |
| <i>Oxa-24-like</i> | - | 3 | - | 2 | - | 5 |
| <i>Oxa-23-like</i> | 1 | - | 3 | - | - | 4 |
| <i>Oxa-51-like</i> | 2 | 2 | 1 | - | - | 5 |
| <i>cmlA</i> | 1 | 1 | - | - | - | 2 |
| <i>catI</i> | - | - | 1 | - | - | 1 |
| <i>Imp</i> | 1 | 2 | 1 | - | - | 4 |
| <i>sim</i> | 5 | 1 | 1 | - | 1 | 8 |
| <i>vim</i> | 7 | 3 | 5 | 1 | - | 16 |

A significant difference was observed between the presence of chloramphenicol resistance genes and others ($P < 0.05$). Since integrons are among the major causes of antibiotic

resistance in bacteria, the presence of classes 1, 2, and 3 integrons in *A. baumannii* isolates were also examined in this study which the results are shown in table 4.

Table 4. The frequencies of three main classes of integrons in *A. baumannii* strains isolated from nosocomial infections in Ahvaz

| Sample | Count | Class 1 integrons | Class 2 integrons | Class 3 integrons |
|------------|-------|-------------------|-------------------|-------------------|
| Blood | 28 | 28 | 10 | 1 |
| Sputum | 18 | 18 | 7 | 2 |
| Urine | 14 | 14 | 3 | - |
| Pus | 6 | 6 | 2 | - |
| CSF liquid | 1 | 1 | - | - |
| Total | 67 | 67 | 22 | 3 |

According to the study results, class 1 integrons were present in all 67 isolates and classes 2 and 3 were observed in 32.83% and

4.4% isolates, respectively. The difference between the presence of class 3 with the other two sorts of integrons was found to be

statistically significant ($p < 0.05$). The presence of class 3 integrons in blood and phlegm was also significantly different compared to other

isolates ($p = 0.03$). The PCR products of amplifying antibiotic resistance genes and integrons are shown in figures 2 and 3.

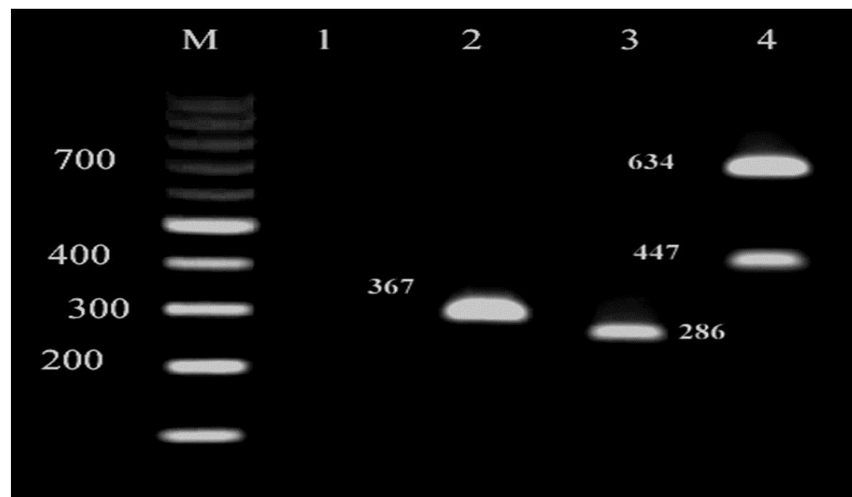


Fig. 2. Agarose gel electrophoresis of PCR products resulted from amplifying the antibiotic resistance genes in *A. baumannii* isolates. M) 100 bp DNA marker; 1) negative control; 2-4) The studied samples containing the 286-bp fragment of *aac(3)-IV* gene, 447-bp fragment of *dfrA1* gene, and 634-bp fragment of *tetB* gene.

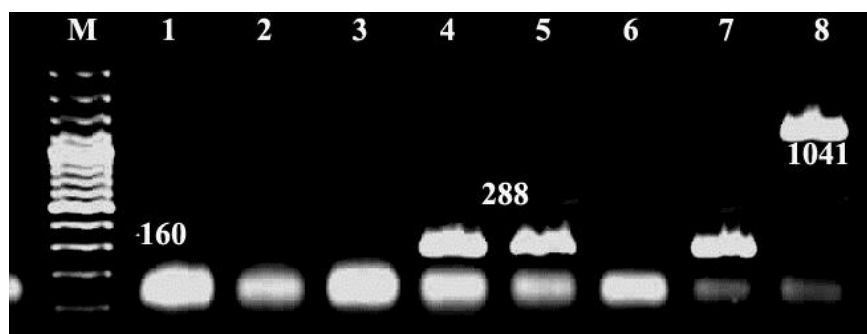


Fig. 3. Agarose gel electrophoresis of PCR products of integrons in *A. baumannii* isolates. M) 100 bp DNA marker; 1) negative control; 2-8) The studied samples containing 160-bp fragment of *IntI*, 288-bp fragment of *IntII*, and 1041-bp fragment of *IntIII*.

Discussion

Members of *Acinetobacter* genus have a strong tendency to become resistant to antibiotics in addition to a great ability in developing new mechanisms of resistance [23, 24]. Plasmids, transposons, and integrons are considered as major factors in acquisition and transmission

of antibiotic resistance genes [25, 26]. The spread of antibiotic resistance through integron structures have nowadays been accompanied by the emergence of multidrug resistance which has become a serious threat in treatment of *Acinetobacter*-derived infections [10, 27].

According to the accumulating resistance of *A. baumannii* to antibiotics as well as scant

number of studies in this field in Ahvaz, the present study was designed to address three objectives:

a) Analysis of antibiotic resistance patterns: All 67 isolates showed multidrug resistance. The resistance to tetracycline, cotrimoxazole, trimethoprim, and gentamycin was 89.55%, 74.71%, 50.56%, and 47.76%, respectively, and only 2.98% of isolates showed to be resistant to meropenem, chloramphenicol, and nitrofurantoin. In a similar study by Momtaz et al. (2015), in two grand hospitals of Tehran, a consistent pattern was also observed in a way that 90.90% of *A. baumannii* isolates were resistant to tetracycline, 61.98% to methoprim, 51.23% to cotrimoxazole, and 9.91-31.40% to aminoglycoside compounds [28]. Shakibaei et al. (2012), in their study conducted on 50 strains of *A. baumannii* isolated from ICUs in Kerman province, reported these isolates' resistance to be 73.3% to imipenem, 66% to ciprofloxacin, 93.3% to piperacillin tazobactam, 53.3% to amikacin, 93.3% to cefepime, and 100% to piperacillin [29]. In another study carried out by Aliakbarzafteh et al. in Tabriz, 94% of *A. baumannii* isolates were resistant to kanamycin, 86% to gentamycin, 81% to amikacin, and 63% to tobramycin [30]. The differences in antibiotic resistance patterns could be due to variations in the types of clinical samples as well as the geographical regions.

b) Genetic pattern of antibiotic resistance: Addressing the distribution of antibiotic resistance genes was also stated as one of the objectives of this study. The presence of 18 genes responsible for antibiotic resistance was

examined in 67 isolates. Tetracycline resistant genes (tetA and tetB) were present in 97.01% of isolates, and the distribution of other genes was as follows: trimethoprim resistance gene (dfrA1) was detected in 62.68% of isolates, sulfonamide resistance gene (sul1) in 59.70%, gentamycine resistance gene (aac(3)-IV) in 53.73%, beta-lactam (bla_{SHV} and bla_{CITM}) and carbonicilin (imp, vim, sim) resistance genes in 41.79%, oxacilin resistance genes (Oxa-like) in 26.86%, streptomycin resistance gene (aadA1) in 25.37%, quinolone resistance gene (qnr) in 16.41%, and chloramphenicol resistance genes (cmlA, cat1) in 4.47%. In an investigation by Azhari et al., the production levels of ESBL and the presence of PER-2, VEB-1, and INT-1 genes were studied in *A. baumannii* strains isolated from Imam Reza hospital in Tabriz. The ESBL production was positive in 60% of isolates in the phenotypical examinations and VEB-1 gene was detected in 10% of ESBL-positive isolates [31]. In another study in China, Huang et al. (2008) analyzed the distribution of beta-lactamase genes in 21 isolates of *A. baumannii*. The presence of bla_{OXA-51-Like} and bla_{OXA-23-Like} genes was confirmed in all and 8 isolates, respectively [32]. Arezzo et al. reported a 93.8% resistance of *A. baumannii* to imipenem in 114 isolates in Italy. Bla_{OXA-23-Like} and bla_{OXA-58-Like} genes were detected in 71.1% and 22.8% of isolates, respectively, while Oxa-24-like, Oxa-51-like, vim, sim, and imp genes were not present in any isolates [33]. In Nie et al.'s (2014) study carried out on the genetic basis of resistance of *A. baumannii* to aminoglycosides, it was demonstrated that 7 and 5 genes, from a total

of 15 genes responsible for alterations in aminoglycoside enzymes, were present in 102 aminoglycoside and 5 carbapenem resistant isolates, respectively [8]. Furthermore, 66.7% of *A. baumannii* isolates from Shahid Beheshti hospital in Kashan were found to possess multi-drug resistance (MDR) in a study by Khelatabadi Farahani et al. In addition, the frequencies of *aphA6*, *aacC*, *ADC-7*, *OXA-SET-C*, *aadA1*, and *aadB* genes were reported 65%, 63.2%, 56.7%, 53.3%, 41.7%, and 3.3%, respectively [34]. In a study by Momtaz et al. (2015), *qnr* gene was not detected in any of the studied *A. baumannii* isolates [28], while 11 isolates showed to have this gene in the present study suggesting the spread of antibiotic resistance and acquisition of various antimicrobial agent resistance genes in *A. baumannii*.

c) Detection of integrons: The presence of three major classes of integrons were examined in 67 *A. baumannii* isolates and the results indicated that class 1 was present in all, class 2 in 32.83%, and integrase 3 gene in only 4.47% of isolates. In Mirnejad et al.'s study (2012), class 2 integron was present in 82% of *A. baumannii* strains isolated from hospitals in Tehran. Moreover, a significant relationship was observed between the integron presence and isolates resistance to cefepime, aztreonam, amikacin, ciprofloxacin, norfloxacin, ofloxacin, and ceftazidime [35]. Furthermore, the frequencies of integrase I, II, and III genes were estimated to be 97%, 31%, and 0%, respectively in XDR isolates of *A. baumannii* from hospitalized patients in Hamedan [24]. The results of the present study demonstrated a

higher frequency of integrons in *A. baumannii* isolates compared to the similar studies so as class 3 integron was even detected in the strains isolated from bacteremia and respiratory infections. In another study, Pymani et al. (2012) proposed that class 1 integron was detected in 92.5% of MDR *A. baumannii* isolates [36]. Japoni et al. also revealed that the frequencies of classes I, II, and III integrons were 47.7%, 3.4%, and 0%, respectively [37]. In addition, in an investigation by Mirnejad et al. in Tehran (2013), 42% and 82% of *A. baumannii* isolates had classes 1 and 2 integrons, respectively [38], in which the frequency of class 2 integron was reported to be higher than class 1 in contrary to the results of other studies in the country as well as those of the present study. However, as mentioned earlier, this can partly be explained by the effect of type and the geographical region (even sub-country regions) of the clinical samples on the prevalence of integrons. In an investigation in Taiwan, 72% of *A. baumannii* isolates had integrase I gene while class 2 integron was absent [39]. Koczura et al. showed that only class 1 integron was present in 63.5% of *A. baumannii* isolates in Poland [3]. Similarly, in a study in Turkey, the class 1 integron was detected in only 33% of isolates of this bacterium, while class 2 was not observed in any [40]. The reason behind the differences in the results of the two latter studies as well as the present and other studies conducted in Iran could be related to the difference in the geographical region which demonstrates the

appropriate and controlled prescription of antibiotics in the foreign countries.

Conclusion

The findings of the present study revealed that similar to other parts of the country, *A. baumannii* isolates are highly resistant to antibiotics in Ahwaz, as well and all isolates presented the multi-drug resistance. On the other hand, all the isolates and approximately 30% of them possessed classes 1 and 2 integrons as one of the major factors in antibiotic resistance spread. Furthermore, in spite of the majority of studies in the country, the presence of quinolone resistance gene (qnr) and integrase 3 gene was demonstrated in *A. baumannii* for the first time. As a result, regardless of whether resistance genes were present on integrons, these structures (integrons) were correlated with the reduced sensitivity to many antibiotic categories which seem to be threatening, since they can easily

transmit the antibiotic resistance genes in different strains of a bacterium and make resistant strains to newer and stronger antibiotics such as imipenem and meropenem. Therefore, it is recommended to add the detection of integrase genes to routine antibiogram examinations, analyzing the antibiotic resistance genes, and monitoring integron-derived antibiotic resistance in management programs as well as *A. baumannii* infection control in hospitals in Iran, especially when dealing with MDR strains. This can be accomplished as a national plan to elucidate the drug resistance patterns of this bacterium throughout the country via detection of all factors involved in the development and spread of the antibiotic resistance.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgment

There is no acknowledgment to declare.

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