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Original Article

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

Samere Kiani[1] M.Sc., Hassan Momtaz[1*] Ph.D., Amir Arsalan Serajian[2] M.Sc., Elahe Tajbakhsh[1] Ph.D.

[1]Department of Microbiology, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.
[2]Health Education Research, Jahad University of Khuzestan, Ahvaz, Iran.

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A B S T R A C T

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_{CITM}, *vim*, *qnr*, bla_{SHV}, *sim*, *Oxa-24-like*, *Oxa-51-like*, *Oxa-58-like*, *Oxa-23-like*, *imp*, *cmlA* and *cat1* 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.

***Corresponding Author:** Department of Microbiology, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran, **Tel/Fax:** +98 3813361064**, Email**: hamomtaz@yahoo.com

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 proscription 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 polemerase (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
16S-23S ribosomal DNA	F- CATTATCACGGTAATTAGTG R- AGAGCACTGTGCACTTAAG	208	12
aadA1	(F) TATCCAGCTAAGCGCGAACT (R) ATTTGCCGACTACCTTGGTC	447	15
aac(3)-IV	(F) CTTCAGGATGGCAAGTTGGT (R) TCATCTCGTTCTCCGCTCAT	286	15
sul1	(F) TTCGGCATTCTGAATCTCAC (R) ATGATCTAACCCTCGGTCTC	822	15
bla$_{SHV}$	(F) TCGCCTGTGTATTATCTCCC (R) CGCAGATAAATCACCACAATG	768	15
CITM	(F) TGGCCAGAACTGACAGGCAAA (R) TTTCTCCTGAACGTGGCTGGC	462	15
cat1	(F) AGTTGCTCAATGTACCTATAACC (R) TTGTAATTCATTAAGCATTCTGCC	547	15
cmlA	(F) CCGCCACGGTGTTGTTGTTATC (R) CACCTTGCCTGCCCATCATTAG	698	15
tet(A)	(F) GGTTCACTCGAACGACGTCA (R) CTGTCCGACAAGTTGCATGA	577	16
tet(B)	(F) CCTCAGCTTCTCAACGCGTG (R) GCACCTTGCTGATGACTCTT	634	16
dfrA1	(F) GGAGTGCCAAAGGTGAACAGC (R) GAGGCGAAGTCTTGGGTAAAAAC	367	17
Qnr	(F) GGGTATGGATATTATTGATAAAG (R) CTAATCCGGCAGCACTATTTA	670	18
Imp	(F) GAATAGAATGGTTAACTCTC (R) CCAAACCACTAGGTTATC	188	19
Vim	(F) GTTTGGTCGCATATCGCAAC (R) AATGCGCAGCACCAGGATAG	382	19
Sim	(F) GTACAAGGGATTCGGCATCG (R) GTACAAGGGATTCGGCATCG	569	19
Oxa-51-like	(F) TAATGCTTTGATCGGCCTTG (R) TGGATTGCACTTCATCTTGG	353	20
Oxa-23-like	(F) GATCGGATTGGAGAACCAGA (R) ATTTCTGACCGCATTTCCAT	501	20
Oxa-24-like	(F) GGTTAGTTGGCCCCCTTAAA (R) AGTTGAGCGAAAAGGGGATT	246	20
Oxa-58-like	(F) AAGTATTGGGGCTTGTGCTG (R) CCCCTCTGCGCTCTACATAC	599	20
IntI	F: CAG TGG ACA TAA GCC TGT TC R: CCC GAC GCA TAG ACT GTA	160	21
IntII	F: TTG CGA GTA TCC ATA ACC TG R: TTA CCT GCA CTG GAT TAA GC	288	21
IntIII	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), sul1 (sulfonamide resistance), bla$_{SHV}$ and CITM (beta-lactam resistance), cat1 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 reveres 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]. Oxacyline 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 reveres 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 reveres 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 nitrofurantoine. 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.

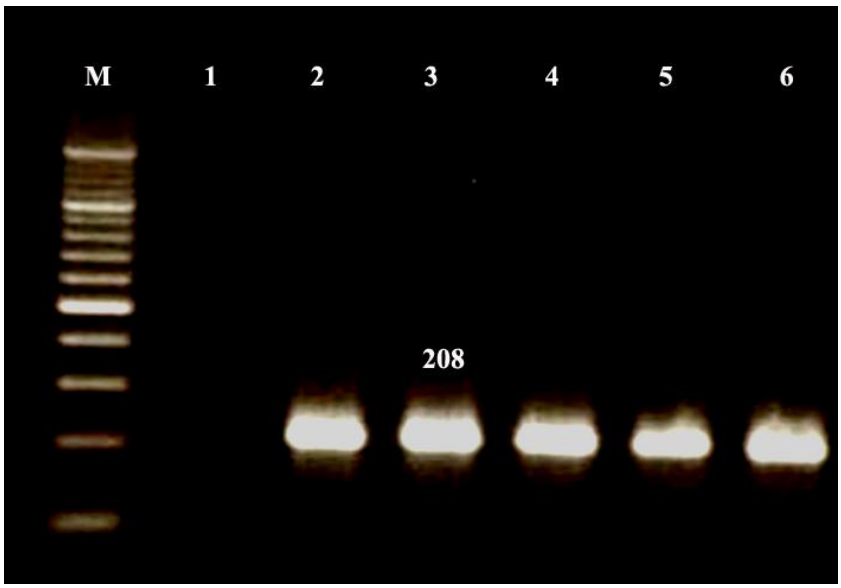

Fig.1. Agarose gel electrophoresis of PCR products of the amplification of 16S-23S ribosomal DNA in *A. baumannii* isolates
M) 100 bp DNA marker; 1) negative control; 2) positive control; 3-6) the studied samples containing the 208-bp fragment

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
lovofloxacin	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 *cat1* 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)
qnr	6	3	-	2	-	11
dfrA1	15	10	12	4	1	42
tetB	15	10	5	4	-	34
tetA	13	7	9	1	1	31
bla_{CITM}	7	5	2	2	1	17
bla_{SHV}	5	4	1	1	-	11
sul1	13	10	12	4	1	40
aac(3)-IV	12	9	10	4	1	36
aadA1	8	4	3	2	-	17
Oxa-58-like	1	1	-	2	-	4
Oxa-24-like	-	3	-	2	-	5
Oxa-23-like	1	-	3	-	-	4
Oxa-51-like	2	2	1	-	-	5
cmlA	1	1	-	-	-	2
cat1	-	-	1	-	-	1
Imp	1	2	1	-	-	4
sim	5	1	1	-	1	8
vim	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.

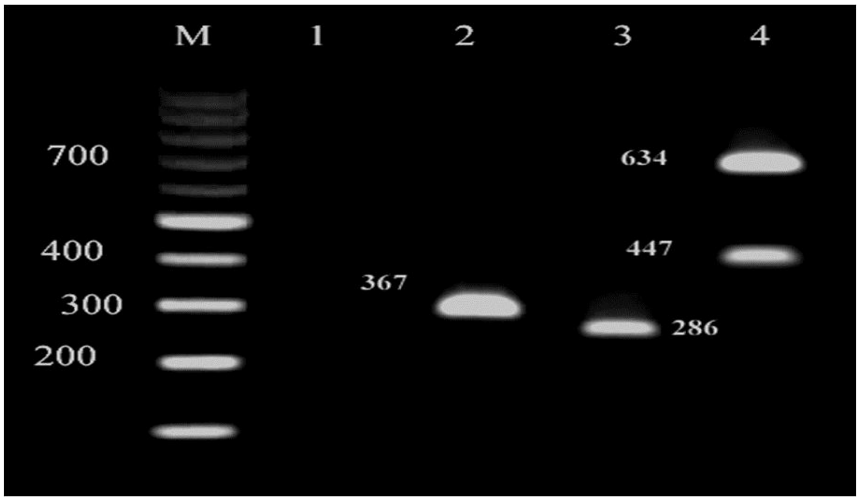

Fig. 2. Agarose gel electrophoresis of PCR products resulted from amplifying the antibiotic resistance genes in *A. baumannii* isolates.
M) 100 bp DNA marker; 1) negative control; 2-4) The studied samples containing the 286-bp fragment of aac(3)-IV gene, 447-bp fragment of dfrA1 gene, and 634-bp fragment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

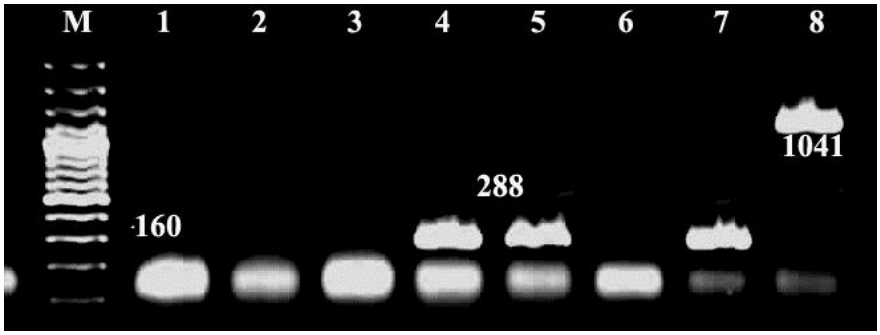

Fig. 3. Agarose gel electrophoresis of PCR products of integrons in *A. baumannii* isolates. M) 100 bp DNA marker; 1) negative control; 2-8) The studied samples containing 160-bp fragment of IntI, 288-bp fragment of IntII, and 1041-bp fragment of IntIII.

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 Aliakbarzafeh et al. in Tabriz, 94% of *A. baumannii* isolates were resistant to kanamycin, 86% to gentamycin, 81% to amicacin, 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 Kheltabadi 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 intgerons, 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

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References

[1]. Huang L, Sun L, Yan Y. Clonal Spread of Carbapenem Resistant *Acinetobacter baumannii* ST92 in a Chinese Hospital during a 6-Year Period. J Microbiol. 2013; 51(1): 113-17.

[2]. Ziglam H, Elahmer O, Amri S, Shareef F, Grera A, Labeeb M, et al. Antimicrobial resistance patterns among *Acinetobacter baumannii* isolated from burn intensive care unit in Tripoli, Libya. Int. Arab. J Antimicrob Agents. 2012; 2(3): 1-5.

[3]. Koczura M, Przyszlakowska B, Mokracka J, Kaznowski A. Class 1 Integrons and Antibiotic Resistance of Clinical *Acinetobacter calcoaceticus–baumannii* Complex in Poznan, Poland. Curr Microbiol. 2014; 69(3): 105-14.

[4]. Bergogne-Berezin E, Towner KJ. *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. Clin. Microbiol. Rev. 1996; 9(2):148-65.

[5]. Vahdani P, Yaghoubi T, Aminzadeh Z. Hospital acquired antibiotic-resistant *Acinetobacter baumannii* infections in a 400-Bed hospital in Tehran, Iran. Int J Prev Med. 2011; 2(3): 127-30.

[6]. Chiu CH, Lee HY, Tseng LY, Chen CL, Chia JH, Su LH, et al. Mechanisms of resistance to ciprofloxacin, ampicillin/sulbactam and imipenem in *Acinetobacter baumannii* clinical isolates in Taiwan. Int J Antimicrob Agents. 2012; 35(4): 382-86.

[7]. Kempf M, Rolain JM. Emergence of resistance to carbapenems in *Acinetobacter baumannii* in Europe: Clinical impact and therapeutic options. Int J Antimicrob Agents. 2012; 39(2): 105-14.

[8]. Nie L, Lv Y, Yuan M, Hu X, Nie T, Yang X, et al. Genetic basis of high level aminoglycoside Resistance in *Acinetobacter baumannii* from Beijing, China. J Acta Pharmsceutica Sinica B. 2014; 4(4): 259-300.

[9]. Irfan S, Turton JF, Mehraj J, Siddiqui SZ, Haider S, Zafar A, et al. Molecular and epidemiological characterisation of clinical isolates of carbapenem-resistant *Acinetobacter baumannii* from public and private sector intensive care units in Karachi, Pakistan. J Hosp Infect. 2011; 78(2): 143-48.

[10]. Zhenbo Xu, Lin Li, Mark E. Shirtliff, M. J. Alam, Shinji Yamasaki, Lei Shi. Occurrence and Characteristics of Class 1 and 2 Integrons in *Pseudomonas aeruginosa* Isolates from Patients in Southern China. J Clin Microbiol. 2009; 47(1): 230-34.

[11]. Yahaghi E, Imani Fooladi AA, Amin M, Mirnejad R, Nezamzade R, Amani J. Detection of Class I Integrons in Staphylococcus aureus Isolated From Clinical Samples. Iran Red Crescent Med J. 2014; 16(11):e16234.

[12]. Chiang MC, Kuo SC, Chen YC, Lee YT, Chen TL, Fung CP. Polymerase chain reaction assay for the detection of *Acinetobacter baumannii* in endotracheal aspirates from patients in the intensive care unit. J Microbiol Immunol Infect. 2011; 44(2): 106-10.

[13]. Hujer KM, Hujer AM, Hulten EA, Bajaksouzian S, Adams JM, Donskey CJ, et al. Analysis of antibiotic resistance genes in multidrug-resistant *Acinetobacter* sp. isolates from military and civilian patients treated at the Walter Reed Army Medical Center. Antimicrob Agents Chemother. 2006; 50(12): 4114-123.

[14]. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility testing; 20th informational supplement. CLSI/NCCLS M100-S20. Clinic Laboratory Standards Ins Wayne. Pa 2010.

[15]. Van TT, Chin J, Chapman T, Tran LT, Coloe PJ. Safety of raw meat and shellfish in Vietnam: an analysis of *Escherichia coli* isolations for antibiotic resistance and virulence genes. Int J Food Microbiol. 2008; 124(3): 217-23.

[16]. Randall LP, Cooles SW, Osborn MK, Piddock LJ, Woodward MJ. Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of *Salmonella* enterica isolated from humans and animals in the UK. J Antimicrob Chemother. 2004; 53(2): 208-16.

[17]. Toro CS, Farfán M, Contreras I, , Flores O, Navarro N, Mora GC, et al. Genetic analysis of antibiotic-resistance determinants in multidrug-resistant *Shigella* strains isolated from Chilean children. Epidemiol Infect. 2005; 133(1): 81-86.

[18]. Mammeri H, Van De Loo M, Poirel L, Martinez-Martinez L, Nordmann P. Emergence of plasmid-mediated quinolone resistance in Escherichia coli in Europe. Antimicrob Agents Chemother. 2005; 49(1): 71-6.

[19]. Mendes RE, Kiyota KA, Monteiro J, Castanheira M, Andrade SS, Gales AC, et al. Rapid detection and identification of metallo-beta-lactamase-encoding genes by multiplex real-time PCR assay and melt curve analysis. J Clin Microbiol. 2007; 45(2): 544-47.

[20]. Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. Int J Antimicrob Agents. 2006; 27(4): 351-53.

[21]. Koeleman JGM, Stoof J, Van Der Bijl MW, Vandenbroucke-Grauls CMJE, Savelkoul PHM. Identification of Epidemic Strains of *Acinetobacter baumannii* by Integrase Gene PCR. J Clin Microbiol. 2001; 39(1): 8-13.

[22]. Ploy MC, Denis F, Courvalin P, Lambert T. Molecular Characterization of Integrons in *Acinetobacter baumannii*: Description of a Hybrid Class 2 Integron. Antimicrob Agents Chemother. 2000; 44(10): 2684-688.

[23]. Fishbain J, Peleg AY. Treatment of *Acinetobacter* Infections. Clin Infect Dis. 2010; 51(1): 79-84.

[24]. Moammadi F, Arabestani M, Safari M, Roshanaii G, Alikhani M. Prevalence of class1, 2 and 3 integrons among extensive drug resistance *Acinetobacter baumanii* strains isolated from intensive care units in Hamadan, west province, Iran . Iran J Med Microbiol. 2014; 8(3): 8-14.

[25]. Adams MD, Goglin K, Molyneaux N, Hujer KM, Lavender H, Jamison JJ, et al. Comparative genome sequence analysis of multidrug-resistant *Acinetobacter baumannii*. J Bacteriol. 2008; 190(24): 8053-64.

[26]. Stokes HW, Gillings MR. Gene flow, mobile genetic elements and the recruitment of antibiotic resistance genes into Gram-negative pathogens. FEMS Microbiol Rev. 2011; 35(5): 790-19.

[27]. Vikas M, Sanchaita S, Singh NP. Multi drug Resistant *Acinetobacter*. J Glob Infect Dis. 2010; 2(3): 291-304.

[28]. Momtaz H, Khamesipour F, Tavakol M, Awosile B. Determination of antimicrobial resistance and resistant genes in *Acinetobacter baumannii* from human clinical samples. West India Med J. 2015.

[29]. Shakibaie MR, Adeli S, Salehi MH. Antibiotic resistance patterns and extended-spectrum β-lactamase production among *Acinetobacter* spp. isolated from an intensive care Unit of a hospital in Kerman, Iran Antimicrob Resist Infect Control. 2012; 1(1): 1-8.

[30]. Aliakbarzade K, Farajnia S, KarimiNik A, Zarei F, Tanomand A. Prevalence of Aminoglycoside Resistance Genes in *Acinetobacter baumannii* Isolates from patients in city Tabriz. J Microb World 2013; 6(3); 219-27.

[31]. Azhari F, Farajnia S, Rahnema M. Investigation of prevalence of ESBL types VEB-1 and PER-2 genes and INT-1 in *Acinetobacter bumannii* strains isolated from patients of Imam Reza hospital in Tabriz. Quarter J Biologic Sci. 2010; 44(11): 1-10.

[32]. Huang L, Sun L, Xu G, Xia T. Differential susceptibility to carbapenems due to the AdeABC efflux pump among nosocomial outbreak isolates of *Acinetobacter baumannii* in a Chinese hospital. Diagn Microbiol Infect Dis. 2008; 62(3): 326-32.

[33]. D'Arezzo S, Principe L, Capone A, Petrosillo N, Petrucca A, Visca P. Changing carbapenemase gene pattern in an epidemic multidrug-resistant *Acinetobacter baumannii* lineage causing multiple outbreaks in central Italy. J Antimicrob Chemother. 2011; 66(1): 54-61.

[34]. Farahani Kheltabadi R, Moniri R, Shajari GhR, Nazem Shirazi MH, MusavI GhA, et al. Antimicrobial Susceptibility patterns and the distribution of resistance genes among *Acinetobacter* species isolated from patients in Shahid Beheshti hospital, Kashan. KAUMS J. 2009; 12(4): 61-67.

[35]. Mirnejad R, Mostofi S, Masjedian F. Role of class 2 integron in antibiotic susceptibility pattern of *Acinetobacter baumannii* strains isolated from hospitals in Tehran. Sci J Hamdan Uni Med Sci. 2012; 18(4): 22-8.

[36]. Peymani A, Farajnia S, Nahaei MR, Sohrabi N, Abbasi L, Ansarin K, et al. Prevalence of class 1 integron among multidrug-resistant *Acinetobacter baumannii* in Tabriz, northwest of Iran. Pol J Microbiol. 2012; 61(1): 57-60.

[37]. Japoni S, Japoni A, Farshad S, Ali AA, Jamalidoust M. Association between existence of integrons and multidrug resistance in *Acinetobacter* isolated from patients in southern Iran. Pol J Microbiol. 2011; 60(2): 163-68.

[38]. Mirnejad R, Mostofi S, Masjedian F. Antibiotic resistance and carriage class 1 and 2 integrons in clinical isolates of *Acinetobacter baumannii* from Tehran, Iran. Asia Pac J Trop Biomed. 2013; 3(2): 140-45.

[39]. Lin MF, Liou ML, Tu CC, Yeh HW, Lan CY. Molecular epidemiology of integron-associated antimicrobial gene cassettes in the clinical isolates of *Acinetobacter baumannii* from northern Taiwan. Ann Lab Med. 2013; 33(4): 242-47.

[40]. Mengeloğlu FZ, Copur Çiçek A, Koçoğlu E, Sandallı C, Budak EE, Ozgümüş OB. Carriage of class 1 and 2 integrons in *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolated from clinical specimens and a novel gene cassette array: blaOXA-11-cmlA7. Mikrobiyol Bul. 2014; 48(1): 48-58.