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Antibiotic Resistance and Carriage Integron Classes in Clinical Isolates of *Acinetobacter Baumannii* from Isfahan Hospitals, Iran

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Abstract

Background: *Acinetobacter baumannii* is a significant nosocomial pathogen around the world, especially in the intensive care unit that most *A. baumannii* infections are caused by the outbreak strains.
Objectives: This study has been performed in *Acinetobacter baumannii* isolates, aimed to detect integron classes I, II, III and molecular typing of *A. baumannii* genes.
Methods: In this Cross-sectional study, *Acinetobacter baumannii* isolated from 150 patients in Isfahan hospitals then antibiotic resistance pattern was determined by disk diffusion method (Kirby Bauer). The presence of genes coding in antibiotic resistance and integrons class I, II, III were analyzed by using of M-PCR method. The data were analyzed by Chi-square, Fischer's test and SPSS statistical software version 16.
Results: Antibiotic resistance pattern for *Acinetobacter baumannii* show that the high resistance was for ciprofloxacin with frequency of 98.3%, ceftazidime with 89.4%, and tetracycline with frequency of 87.3%. The most sensitive antibiotics were chloramphenicol, and nitrofurantoin with frequency of 3.5% and 3.2% resistance. The detection of *dfrA1* (63.7%), *sul1* (68.6%), *aac (3)-IV* (54.4%), *tet* (B) (22.4%), *tet* (A) (78.3%), *aadA1* (15.4%), *CITM* (17. %), *vim* (12.2%), *Qnr* (17.1%), *blaSHV* (19.8%), *sim* (7.8%), *Oxa-24-like* (13.2%), *Oxa-51-like* (11.9%), *Oxa-58-like* (39.4%), *Oxa-23-like* (12.6%), *imp* (9.2%), *cmlA* (19%) and *cat1* (8.6%) were respectively reported too. Also in this study Frequency of integrons class 1, 2, 3 were (100%), (28%), (6.6%) respectively.
Conclusions: High prevalence of integrons among *Acinetobater baumannii* isolated from Isfahan hospitals indicate the importance role of integron classes in multidrug resistance. Considering the increasing pattern of MDR infections is one of the important issues of treatment which can be effective strategy for curing.

Keywords: Antibiotic Resistance, Integron Classes, *Acinetobacter baumannii*

1. Introduction

Acinetobacter baumannii is a Gram-negative *coccobacillus* bacteria, aerobic and nonfermenting that belonging to the Moraxellaceae family these Species are opportunist pathogens with increasing relevance in both community-acquired and hospital-acquired, especially among patients in intensive care units and risk factor patient [1, 2]. *A. baumannii* have been implicated in different nosocomial infections, including ventilator-associated pneumonia, endocarditis, meningitis, wounds, blood and infections of the skin, soft tissues, urinary tract infections, and those originating from other hospital environment [3]. *A. baumannii* can be isolated from numerous sources such as soil, water, animals, and humans, where ever presented in health care institutions or nosocomial infections or even in environmental surfaces, it will be difficult to control it [4, 5].

Antimicrobial resistance owing from enzymatic degradation, modification of targets, or bacterium in hospital environments are responsible for epidemics of *A. baumannii* in hospital-acquired species [6]. Current study show resistance to nearly all major classes of antibiotics, especially including broad-spectrum penicillin's, cephalosporin's, carbapenems, aminoglycosides, fluoroquinolones, chloramphenicol, and tetracycline's. Today there are global distributions of multidrug resistant (MDR) in clinical isolates all over the word [7].

Increasing of carbapenem-resistance and β-lactamases resistance in *A. baumannii* tend us to study genotypic and phenotypic resistance pattern in detected specimens which isolated from patients hospitalized in different sections of hospital [8, 9].

This study helps us to clarify different mode of *A. baumannii* antibiotic resistance pattern and its epidemiology in different hospitals. The current review was conducted to elucidate of antibiotic resistance in *A. baumannii* that detected from hospitalized patients in Isfahan Iran.

2. Methods

2.1. Bacterial Isolates

A total of 150 isolates of *A. baumannii* was collected from clinical specimens from 3 major hospitals in Isfahan, Iran. Finally 150 clinical samples from individuals suffering from different types of infections were selected.

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.

A. baumannii including blood (n = 38), wound (n = 33), tracheal secretion (n=10), meningitis (n=6) and urinary infection (n = 51) which were collected from patients hospitalized in ICU, orthopedic and internal section. All samples were directly cultured in to 7% sheep blood agar (Merck, Darmstadt, Germany) and incubated aerobically at 37°C for 48 hours. After incubation, suspicious colonies were examined by using laboratory techniques appropriate for diagnosing *A. baumannii* species [10-12].

2.2. Antibiotic Susceptibility Testing

A. baumannii isolates was selected and the antibiotic resistance pattern was performed by a disk diffusion method on Mueller-Hinton agar.

A. baumannii isolates were tested for sensitivity to tetracycline (30 μg), ceftazidime (30 μg), ciprofloxacin (30 μg), cotrimoxazole (25 μg), tobramycin (10 μg), Chloramphenicol (30 μg), Norfloxacin (10 μg), amikacin (30 μg), gentamycin (10 μg), Rifampin (5 μg), cefalotin (30 μg), Streptomycin (10 μg), Trimethoprim (5 μg), Levofloxacin (5 μg), Imipenem (10 μg), meropenem (10 μg), Nitrofurantoin (300 μg), azithromycin (15 μg), erythromycin (15 μg), by the Kirby-Bauer disk diffusion method. (MAST, Merseyside, England), according to clinical and laboratory standards institute (CLSI) 2011. *A. baumannii* ATCC 19606 was used as the positive control strain [13-15].

2.3. Molecular Typing and DNA Extraction

Detection of the main groups of genes which are effective in *A. baumannii* resistance were performed by using M-PCR. The primers used are shown in Table 1. DNA extraction was carried out using the phenol chloroform method.

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), *blaSHV* 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-*

Table 1. Primers of Polymerase Chain Reaction

Primers	Nucleotide Sequences (5' - 3')	Expected Amplicon Size, bp
16S-23S ribosomal DNA	F- CATTATCACGGTAATTAGTG	208
	R- AGAGCACTGTGCACTTAAG	
aadA1	(F) TATCCAGCTAAGCGCGAACT	447
	(R) ATTTGCCGACTACCTTGGTC	
aac(3)-IV	(F) CTTCAGGATGGCAAGTTGGT	286
	(R) TCATCTCGTTCTCCGCTCAT	
sul1	(F) TTCGGCATTCTGAATCTCAC	822
	(R) ATGATCTAACCCTCGGTCTC	
blaSHV	(F) TCGCCTGTGTATTATCTCCC	768
	(R) CGCAGATAAATCACCACAATG	
CITM	(F) TGGCCAGAACTGACAGGCAAA	462
	(R) TTTCTCCTGAACGTGGCTGGC	
cat1	(F) AGTTGCTCAATGTACCTATAACC	547
	(R) TTGTAATTCATTAAGCATTCTGCC	
cmlA	(F) CCGCCACGGTGTTGTTGTTATC	698
	(R) CACCTTGCCTGCCCATCATTAG	
tet(A)	(F) GGTTCACTCGAACGACGTCA	577
	(R) CTGTCCGACAAGTTGCATGA	
tet(B)	(F) CCTCAGCTTCTCAACGCGTG	634
	(R) GCACCTTGCTGATGACTCTT	
dfrA1	(F) GGAGTGCCAAAGGTGAACAGC	367
	(R) GAGGCGAAGTCTTGGGTAAAAAC	
Qnr	(F) GGGTATGGATATTATTGATAAAG	670
	(R) CTAATCCGGCAGCACTATTTA	
Imp	(F) GAATAGAATGGTTAACTCTC	188
	(R) CCAAACCACTAGGTTATC	
Vim	(F) GTTTGGTCGCATATCGCAAC	382
	(R) AATGCGCAGCACCAGGATAG	
Sim	(F) GTACAAGGGATTCGGCATCG	569
	(R) GTACAAGGGATTCGGCATCG	
Oxa-51-like	(F) TAATGCTTTGATCGGCCTTG	353
	(R) TGGATTGCACTTCATCTTGG	
Oxa-23-like	(F) GATCGGATTGGAGAACCAGA	501
	(R) ATTTCTGACCGCATTTCCAT	
Oxa-24-like	(F) GGTTAGTTGGCCCCCTTAAA	246
	(R) AGTTGAGCGAAAAGGGGATT	
Oxa-58-like	(F) AAGTATTGGGGCTTGTGCTG	599
	(R) CCCCTCTGCGCTCTACATAC	
IntI	F: CAG TGG ACA TAA GCC TGT TC	160
	R: CCC GAC GCA TAG ACT GTA	
IntII	F: TTG CGA GTA TCC ATA ACC TG	288
	R: TTA CCT GCA CTG GAT TAA GC	
IntIII	F: GCC TCC GGC AGC GAC TTT CAG	1041
	R: ACG GAT CTG CCA AAC CTG ACT	

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 $MgCl_2$, 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*, *blaSHV*, *CITM*, *cat1*, *cmlA*, *tet (a)*, *tet (B)*, *dfrA1*, and *qnr*. The thermal program was a cycle of 94°C for 6 minutes, 33 repetitive cycles of 95°C for 70 seconds, 55°C for 65 seconds, and 72°C for 90 seconds, and a final cycle of 72°C for 8 minutes [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 $MgCl_2$, 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 minutes, 30 repetitive cycles of 94°C for 45 seconds, 58°C for 60 seconds, and 72°C for 40 seconds, and a final cycle of 72°C for 5 minutes [19]. Oxacillin 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 $MgCl_2$, 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 minutes, 32 repetitive cycles of 95°C for 50 seconds, 60 °C for 60 seconds, and 72°C for 70 seconds, as well as a final cycle of 72°C for 10 minutes [16-20].

50 microliters of M-PCR products were resolved on a 1.5% agarose gel containing 0.5 mg/mL of ethidium bromide in Tris-borate-EDTA buffer at 90 V for 1 hour. Finally the products were examined with ultraviolet illumination [20].

2.4. Analyzing the Presence of Integrons

Classes 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 $MgCl_2$, 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 minutes, 35 repetitive cycles of 94°C for 1 minutes, 56°C for 1 minutes, and 72°C for 45 seconds, as well as a final cycle of 72°C for 6 minutes. 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 (fermentas-Lithuania), on a UV transluminator (UV itech, England) after electrophoresis at 90 V for approximately 45 minutes.

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 $MgCl_2$, 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, *blaSHV*, *CITM*, *cat1*, *cmlA*, *tet (a)*, *tet (B)*, *dfrA1*, and *qnr*. The thermal program was a cycle of 94°C for 6 minutes, 33 repetitive cycles of 95°C for 70 seconds, 55°C for 65 seconds, and 72°C for 90 seconds, and a final cycle of 72°C for 8 minutes [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 $MgCl_2$, 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 minutes, 30 repetitive cycles of 94°C for 45 seconds, 58°C for 60 seconds, and 72°C for 40 seconds, and a final cycle of 72°C for 5 minutes [19]. Oxacillin 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 $MgCl_2$, 200 μmol of dNTPs mix, 0.5 μmol of forward and reveres primer pairs (for each gene), 1.5 unit of ribosomal DNA) through simple disc diffusion method. All the studied isolates demonstrated the multidrug resistance (MDR).

2.5. 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.

3. Results

During this study, 220 samples of *Acinetobacter* were collected from different patients in internal section, orthopedic and ICU. One hundred and fifty samples of isolate were identified as *A. baumannii* (68.8%), 50 samples were *Acinetobacter Lwoffii* (27.7%) and 20 samples (9.09%) were identified as other *Acinetobacter* species. Among of isolated *A. baumannii* strains, all samples (100%) were multidrug-resistant. Results of this study showed that 79 samples of *A. baumannii* (52.6%) were resistant to three or more than three antibiotics and 97 samples (64.6%) showed resistance to more than two antibiotics. Also, none of resistant strains were showed complete resistance to all antibiotics. According to antibiotic resistance pattern, it was mentioned that, approximately 98.3% of samples were resistant to ciprofloxacin, 89.4% of isolates were resistance to ceftazidime and there were 87.3% resistance to tetracycline. Other antimicrobial resistance profiles of *A. baumannii* is shown in Table 2.

Table 2. Antibiotic Resistance Pattern in *A. baumannii* Isolates

Antibiotic	Sensitive Percent	Intermediate Percent	Resistance Percent
Tetracycline	87.3	10.8	1.9
Ceftazidime	89.4	5.2	5.4
Ciprofloxacin	98.3	1.7	0
Cotrimoxazole	65	25.1	9.9
Tobramycin	53	35.4	11.6
Chloramphenico	3.5	11.5	85
Norfloxacin	78	12.5	9.5
Amikacin	55.7	30.3	14
Gentamycin	79.5	18.3	2.2
Rifampin	45.8	40.8	13.4
Cefalotin	56.1	27.3	16.6
Streptomycin	34.3	7	58.7
Trimethoprim	73	13.6	13.4
Levofloxacin	55	20.5	24.5
Imipenem	43	38.8	18.2
Meropenem	66.5	29.4	4.1
Nitrofurantoin	3.2	13.5	83.3
Azithromycin	74	14.5	11.5
Erythromycin	65	17.9	17.1

Molecular typing of genes that related to *A. baumannii* antibiotic resistance pattern show the frequency of genes as *dfrA1* (63.7%), *sul1* (68.6%), *aac(3)-IV* (54.4%), *tet(B)* (22.4%), *tet(A)* (78.3%), *aadA1* (15.4%), *CITM* (17. %), *vim* (12.2%), *Qnr* (17.1%), *blaSHV* (19.8%), *sim* (7.8%), *Oxa-24-like* (13.2%), *Oxa-51-like* (11.9%), *Oxa-58-like* (39.4%), *Oxa-23-like* (12.6%), *imp* (9.2%), *cmlA* (19%) and *cat1* (8.6%) respectively. Frequency of integrons class I, II, III was (100%), (28%), (6.6%) respectively. There were a significant relationship between integron I gene and detected specimens. All of the specimens were contains integron class I. Frequency of other integron classes and its relationship with different groups of specimens are show in Table 3.

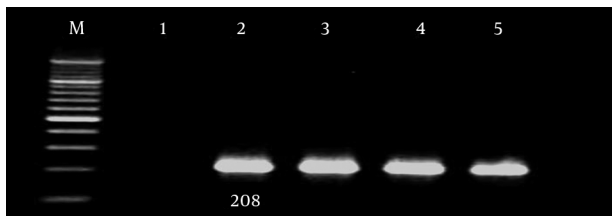
Table 3. Frequency of Integron Classes with Different Groups of Specimens

Frequency Sample Type	Integron I	Integron II	Integron III
38/Blood	38	9	1
33/Wound	33	10	2
51/UTI	51	16	1
6/meningitis	6	2	1
22/T. secretion	22	5	5
Total	150	42	10

A. baumannii isolates which detected from different Patients in Prevalence of age show that the most frequent age range of the patients were between 61 - 70 age (57.9%) who were hospitalized in different section of hospitals. Also 69% of patients were women in this study.

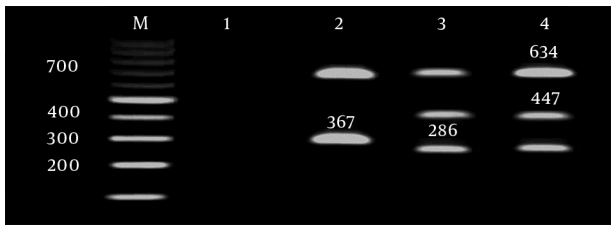
In current study most of the integron class I and integron classes II were found among multidrug resistant *A. baumannii* strains. The results of our study were in concordance with similar studies around the world. Due to increased drug resistance, wider search and further study is necessary and can be useful to control hospital infections [21]. Gel electrophoresis of samples are show in Figures 1 - 3.

Figure 1. PCR Products of 16S-23S Ribosomal DNA



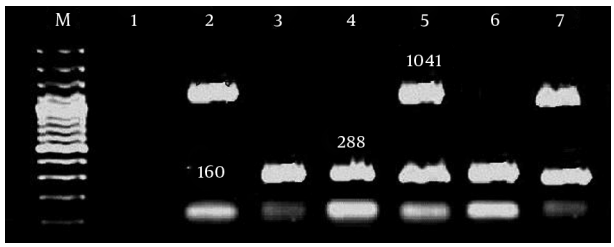
M, marker; 1, negative; 2 - 5, positive.

Figure 2. PCR Products of Genes



M, marker; 1, negative; 286, *aac (3)-IV*; 367, *dfrA1*; 447, *aadA1*; 634, *tetB*.

Figure 3. PCR Products of Genes



M, marker; 1, 2 - 7, *IntI*; 288, *IntII*; 1041, *IntIII*.

4. Discussion

Molecular typing of genes that related to *A. baumannii* antibiotic resistance pattern show the frequency of genes as *dfrA1* (63.7%), *sul1* (68.6%), *aac(3)-IV* (54.4%), *tet(B)* (22.4%), *tet(A)* (78.3%), *aadA1* (15.4%), *CITM* (17. %), vim (12.2%), Qnr (17.1%), *blaSHV* (19.8%), *sim* (7.8%), *Oxa-24-like* (13.2%), *Oxa-51-like* (11.9%), *Oxa-58-like* (39.4%), *Oxa-23-like* (12.6%), *imp* (9.2%), *cmlA* (19%) and *cat1* (8.6%) respectively. Frequency of integrons class I, II, III was (100%), (28%), (6.6%) respectively. There was a significant relationship between integron I gene and detected specimens. All of the specimens were contains integron class I. Frequency of other integron classes and its relationship with different groups of specimens were show in Table 3.

Different species of Acinetobacter have strong tendency to become resistant to antibiotics and are inherently resistant to some broad antibiotics, and on the other hand have a great ability to gain new mechanisms of resistance. Plasmids, transposons and integrons are important factors in the acquisition and transfer the mechanisms of antibiotic resistance [22].

Today, the spread of antibiotic resistance through integron structures with multiple antibiotic resistance has become a serious problem in treatment of infections caused by *Acinetobacter baumannii*. Due to the spread of antibiotic resistance in *Acinetobacter baumannii*, and since the city had not done a comprehensive study on antibiotic resistance of these bacteria, this study was conducted to show pattern of antibiotic resistance, genetic pattern of antibiotic resistance and tracking Integrons classes. All isolates examined in this study were MDR [23].

The highest rate of drug resistance related to tetracycline (87.3%), ciprofloxacin (98.3%), and ceftazidime (89.4). Similar studies have been conducted in different regions of Iran too. In a similar study conducted by Momtaz et al. (2015) in two large hospitals in Tehran, almost similar pattern was observed that 90.90 % of *Acinetobacter baumannii* isolated from human infection were resistance to tetracycline, 61.98% to trimethoprim, 51.23% were resistance to cotrimoxazole and between 9.91% to 31.40% of isolates were resistant to aminoglycoside compounds [24].

In a study done by Shakibaii on 50 isolates of *A. baumannii* which detected from the intensive care unit in Kerman hospitals, the antibiotic-resistant pattern show 73.3% resistance to imipenem, 66% to ciprofloxacin, 93.3% to piperacillin-tazobactam, 53.3% to amikacin, 93.3% to Cefepime and 100% reported to piperacillin [25].

In a study conducted by Aliakbarzade et al. (2014) in Tabriz hospitals, antibiotic resistance pattern of *Acinetobacter baumannii* isolated from patients were at a rate of 94% to kanamycin, 86% to gentamicin, 81% were resistant to amikacin and 63% were resistant to tobramycin. Different antibiotic resistance in various studies can be related to clinical sample types, or geographic region of the test [26].

Distribution genes encoding resistance to antibiotics was one of the goals of this study. The presence of this genes coding in antibiotic resistance was show in this 150 isolates. Molecular typing of genes that related to A. *baumannii* antibiotic resistance pattern show the frequency of genes as *dfrA1* (63.7%), *sul1* (68.6%), *aac(3)-IV* (54.4%), *tet(B)* (22.4%), *tet(A)* (78.3%), *aadA1* (15.4%), *CITM* (17. %), *vim* (12.2%), *Qnr* (17.1%), *blaSHV* (19.8%), *sim* (7.8%), *Oxa-24-like* (13.2%), *Oxa-51-like* (11.9%), *Oxa-58-like* (39.4%), *Oxa-23-like* (12.6%), *imp* (9.2%), *cmlA* (19%) and *cat1* (8.6%) respectively and integrons class I, II, III was (100%), (28%), (6.6%) respectively. Compared with this study, Huang et al. (2008) in China, reported distribution of β-lactamase genes that were evaluated in 21 isolates of *A. baumannii*. In this study all of the isolates contain $bla_{OXA\text{-}51\text{-}Like}$ and just 8 strains contains $bla_{OXA\text{-}23\text{-}Like}$ gene [27].

Another study by Arezzo in Italy (2011) show that 93.8% of 114 isolates were resistance to imipenem and 71.1% of isolates contains $bla_{OXA\text{-}23\text{-}Like}$ genes and 22.8% of isolates contains $bla_{OXA\text{-}58\text{-}Like}$, Compared with our study they didn't detect *Oxa-24-like*, *Oxa-51-like*, *vim*, *sim* and *imp* in their isolates [28].

Frequency of integrons class I, II, III in our study was

(100%), (28%), (6.6%) respectively. There was a significant relationship between integron I gene and detected specimens. All of the specimens were contains integron class I.

Unlike these results in the study of Mirnejad et al. (2012) 82% of *Acinetobacter baumannii* isolated from hospitals in Tehran, carried Integrons Class II. There was a significant correlation between integrons and antibiotics resistance pattern to Cefepime, aztreonam, amikacin, ciprofloxacin, norfloxacin, ofloxacin and ceftazidime [29].

Also in a survey conducted by Mohammadi et al. presence of integrase gene I, II and III in XDR of *Acinetobacter baumannii* which detected from patients in Hamedan hospitals, was 97%, 31% and 0% respectively [30]. In a study in Taiwan, 72% of *Acinetobacter baumannii* just contains integrase gene class I [31].

In Koczura reviews (2014) in Poland only 63.5% of *Acinetobacter baumannii* contains integron class I. Similar to this study in Turkey (2014) only 33% of the isolates of the bacteria carry Integrons class I, and class II [32].

Because of the differences in the prevalence of class I integrons in two recent studies with this study and also similar studies in Iran, so it can probably relate to the verities of geographical area , the proper use and control of antibiotics in out of Iran [33].

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Footnotes

Authors' Contribution: Fahimeh Nourbakhsh provided conception and design of the study and revised the article. Majid Rajai and Fahimeh Nourbakhsh participated in data collection and drafting the manuscript, also the data analysis and interpretation performed by DR. Hassan Momtaz.

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