

Original Article

Antibiotic Resistance Patterns and Molecular Typing of *Acinetobacter Baumannii* Strains Isolated from Burn Patients in Iran

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ABSTRACT

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Key words

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Background and Aims: *Acinetobacter baumannii* (*A. baumannii*) is an important multidrug-resistant opportunistic pathogen frequently causing various nosocomial infections and is a serious threat to burn patients. These infections are usually caused by the outbreak strains. The aim of this study was to show antibiotic resistance pattern and molecular typing of *A. baumannii* genes isolates collected from burn patients and also distribution of different types of burn patients.

Materials and Methods: In this study, 307 different strains were detected. Totally 100 *A. baumannii* strain was selected in burn center of Isfahan hospital. Antibiotic resistance pattern was determined by disk diffusion method (Kirby Bauer). The presence of genes coding in antibiotic resistance were analyzed by using multiplex-PCR method. The standard strains of *Escherichia coli* ATCC 25922 and *A. baumannii* ATCC 19606 were used as negative and positive controls.

Results: The antibiotic resistance pattern for *A. baumannii* showed high resistance for ciprofloxacin, ceftazidime, and tetracycline with frequency of 82.5%, 75.3%, 72%, respectively. Moreover, the most sensitive antibiotics were chloramphenicol, and nitrofurantoin with the resistance frequency of 3.9% and 2.8%. *CITM* (91.1%) was the highest detected gene.

Conclusions: High prevalence of antibiotic resistance pattern among *A. baumannii* isolated from burn center hospitals indicates the important role of multidrug resistant isolates.

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Introduction

Acinetobacter baumannii (*A. baumannii*) is a serious opportunistic and multidrug-resistant (MDR) pathogen that is responsible for nosocomial infections and frequently causes bacteremia and ventilator-related pneumonia in intensive care units (ICU) and health facilities [1, 2]. *A. baumannii* is a coccobacillus gram-negative bacterium, aerobic and non-fermented that belongs to the Moraxellaceae family. These species have a high prevalence in both community and hospital, especially among patients in ICU as well as high risk patients [3]. *A. baumannii* can be isolated from numerous sources such as soil, water, animals, and humans [4, 5]. Moreover, it has been implicated in different nosocomial infections, including endocarditis, meningitis, wounds, blood and infections of the skin, soft tissues, urinary tract, and those originating from other hospital environments [6].

The treatment of *A. baumannii* is difficult as it can be resistant to multiple classes of antimicrobials [7]. *A. baumannii* is naturally resistant to some antibiotics such as cephalosporin's. However, it has a great capacity to develop resistance against many antibiotics, including carbapenems, aminoglycosides, and fluoroquinolones [8]. The resistance is acquired through the transfer of mobile genetic elements like Integrons, plasmids or transposons which carry groups of genes encoding resistance to various antibiotic families [9]. *A. baumannii* can produce carbapenemases, as the principle mechanism is of responsibility for carbapenem resistance. This resistance is dramatically

being observed worldwide mediated by carbapenems-hydrolyzing β -lactamases, belonging to 3 classes; class A, class B (metallo- β -lactamases), and class D (oxacillinases) [10-12]. Antimicrobial resistance is acquired from enzymatic degradation, modification of targets, or bacterium in hospital environments that are responsible for epidemics of *A. baumannii* in hospital-acquired species [13]. Today there are global distributions of MDR in clinical isolates all over the world. In the clinical laboratory, *A. baumannii*, together with three other species (*Acinetobacter* genomic species 13TU, *Acinetobacter* genomic species 3 and *A. calcoaceticus*), comprise the *A. calcoaceticus*, *A. baumannii* complex. They show a similar phenotype and are difficult to distinguish. [14].

The increase of carbapenems and β -lactamases resistance in *A. baumannii* motivated us to study genotypic and phenotypic resistance pattern in detected specimens isolated from patients hospitalized in different wards. This study helps us to clarify different mode of *A. baumannii* antibiotic-resistance determinants in *A. baumannii* strains isolated from burn patients in Iran. This cross-sectional study was conducted to elucidate the antibiotic resistance in *A. baumannii* detected from hospitalized patients in Isfahan, a province of Iran. The aim of this study was to determine the resistance profiles and prevalence of β -lactamase genes in MDR *A. baumannii* clinical isolates by applying Multiplex-polymerase chain reaction (M-PCR) assays.

Materials and Methods

Bacterial isolates

The primary culture showed polymicrobial growth in 65.2% of the detected samples. The most frequently isolated pathogens were *Staphylococcus aureus* (n=45), *Acinetobacter* species (n=132), true fungi (n=27), *Staphylococcus epidermidis* (n=41), and *Pseudomonas aeruginosa* (n=62). Afterwards, 132 samples of *Acinetobacter* were collected from different burnt patients in internal section their of which distributions are shown in figure 1. 100 samples of isolate were identified as *A. baumannii*, 20 samples were *Acinetobacter Lwoffii* and 12 samples as other *Acinetobacter* species. All 100 isolates of *A. baumannii* were collected from individuals suffering from different types of burn in Imam Musa Kazem burn center hospital in Isfahan. *A. baumannii* was detected in sample wounds collected from

patients hospitalized in internal section. All the detected samples were directly cultured in 7% sheep blood agar (Merck, Darmstadt, Germany) and then incubated aerobically at 37°C for 48 h. After incubation, suspicious colonies were examined by using laboratory techniques appropriate for diagnosing *A. baumannii* species. These isolates were characterized and confirmed in the laboratories of the corresponding hospitals through routine microbiological and biochemical tests such as IMVIC, urease, triple sugar iron (TSI), OF, methyl Red–Voges-Proskauer (MRVP), sulfide-indole-motility (SIM), catalase, oxidase, and growth at 37°C and 42°C. The confirmed samples were stored at 30% glycerol at -70°C. The standard strains of *Escherichia coli* ATCC 25922 and *A. baumannii* ATCC 19606 were used as negative and positive controls (15-17). This cross-sectional study was in line with similar studies around the world during 2017.

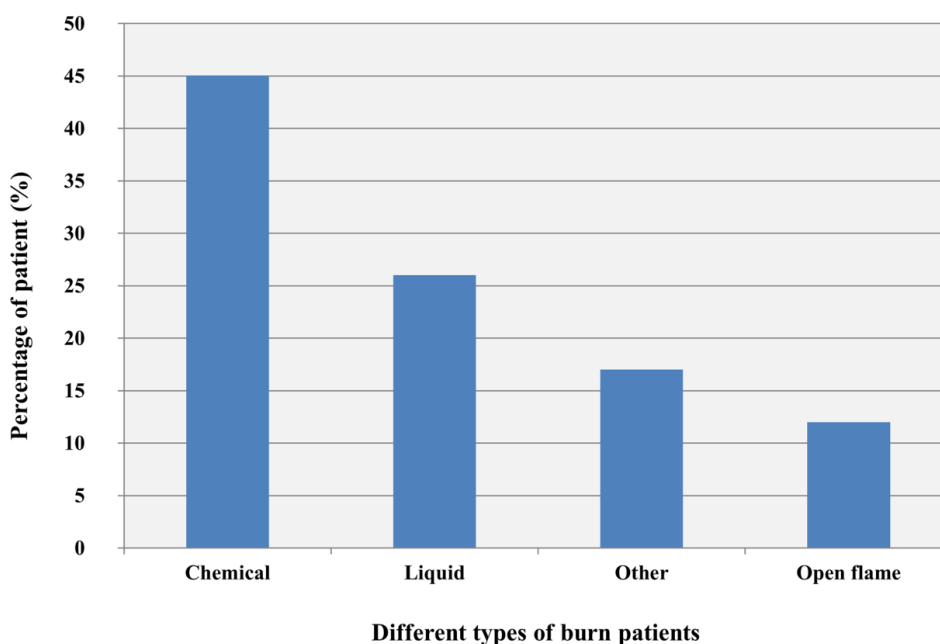


Fig. 1. The distribution of different types of burn patients

Antibiotic profiles

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), and erythromycin (15 µg), by the Kirby-Bauer disk diffusion method. MAST, Merseyside, England was used related to Clinical and Laboratory Standards Institute (CLSI), 2011. *A. baumannii* ATCC 19606 was used as the positive control strain [18-20].

DNA extraction of *A. baumannii* isolates

Stock cultures were maintained in both agar slant and 20% sterile buffered glycerin and were kept at -70°C. Genomic DNA was extracted from the bacterial isolates using the genomic DNA purification Kit (Merck, Germany) method, according to Kit protocol.

Detection of genes coding for virulence factors by using M-PCR assays

Detection of the main groups of genes which are effective in *A. baumannii* resistance was

performed by using M-PCR. The used primers are shown in Table 1. Amplification reaction was performed in a final volume of 50 µl. Each reaction mixture contained 5 µl of 10X PCR buffer; 1.6 IU of Taq DNA polymerase, and 2 µl of mixed deoxy ribonucleotide triphosphates, 2 mmol/L MgCl₂, 500 ng of primer and 10 ng of bacterial DNA. The conditions used for PCR were 94°C for 5 min, followed by 30 cycle of denaturation at 94°C for 25 seconds, annealing at 53°C for 40 seconds, extension at 72°C for 50 seconds and final extension at 72°C for 6 min. PCR products were separated by electrophoresis on a 1% agarose gel. Also 50 µl of M-PCR products were loaded on a 1% agarose gel containing 0.5 mg/ml of ethidium bromide in Tris-borate-EDTA buffer at 90 V for 1 h. Finally, the products were examined with ultraviolet illumination [21]. The Ethics Committee of Mashhad university of Medical Sciences, Mashhad, Iran approved this research.

Statistical Analysis

The data were analyzed by Chi-square, Fischer's test and SPSS statistical software version 16.

Table 1. Primers of polymerase chain reaction

Primers	Nucleotide sequences (5' → 3')	Amplicon size (bp)
<i>16S-23S ribosomal DNA</i>	(F) CATTATCACGGTAATTAGTG (R) AGAGCACTGTGCACTTAAG	208
<i>aadA1</i>	(F) TATCCAGCTAAGCGCGAACT (R) ATTTGCCGACTACCTTGGTC	447
<i>aac(3)-IV</i>	(F) CTTCAGGATGGCAAGTTGGT (R) TCATCTCGTTCTCCGCTCAT	286
<i>sul1</i>	(F) TTCGGCATTCTGAATCTCAC (R) ATGATCTAACCCTCGGTCTC	822
<i>bla_{SHV}</i>	(F) TCGCCTGTGTATTATCTCCC (R) CGCAGATAAATCACCACAATG	768
<i>CITM</i>	(F) TGGCCAGAACTGACAGGCAAA (R) TTTCTCCTGAACGTGGCTGGC	462
<i>cat1</i>	(F) AGTTGCTCAATGTACCTATAACC (R) TTGTAATTCATTAAGCATTCTGCC	547
<i>cmlA</i>	(F) CCGCCACGGTGTGTTGTTATC (R) CACCTTGCCTGCCCATCATTAG	698
<i>tet(A)</i>	(F) GGTTCACCTCGAACGACGTCA (R) CTGTCCGACAAGTTGCATGA	577
<i>tet(B)</i>	(F) CCTCAGCTTCTCAACGCGTG (R) GCACCTTGCTGATGACTCTT	634
<i>dfrA1</i>	(F) GGAGTGCCAAAGGTGAACAGC (R) GAGGCGAAGTCTTGGGTAAAAAC	367
<i>Qnr</i>	(F) GGGTATGGATATTATTGATAAAG (R) CTAATCCGGCAGCACTATTTA	670
<i>Imp</i>	(F) GAATAGAATGGTAACTCTC (R) CCAAACCACTAGGTTATC	188
<i>Vim</i>	(F) GTTTGGTTCGCATATCGCAAC (R) AATGCGCAGCACCAGGATAG	382
<i>Sim</i>	(F) GTACAAGGGATTTCGGCATCG (R) GTACAAGGGATTTCGGCATCG	569
<i>Oxa-51-like</i>	(F) TAATGCTTTGATCGGCCTTG (R) TGGATTGCACTTCATCTTGG	353
<i>Oxa-23-like</i>	(F) GATCGGATTGGAGAACCAGA (R) ATTTCTGACCCGATTTCCAT	501
<i>Oxa-24-like</i>	(F) GGTTAGTTGGCCCCCTTAAA (R) AGTTGAGCGAAAAGGGGATT	246
<i>Oxa-58-like</i>	(F) AAGTATTGGGGCTTGTGCTG (R) CCCCTCTGCGCTCTACATAC	599

Results

A. baumannii antibiotic susceptibility tests

Among the detected *A. baumannii* strains, all samples (100%) were MDR. On the basis of antibiotic resistance pattern, it was mentioned that approximately 82.5% of samples were resistant to ciprofloxacin, 75.3% to ceftazidime and 72% to tetracycline. In addition, none of resistant strains showed complete resistance to all antibiotics. Other antimicrobial resistance profiles of *A. baumannii* are shown in figure 2. Antibiotic susceptibility pattern of *A. baumannii* is shown in Table 2.

The result of M-PCR in *A.baumannii*

Molecular typing of genes related to *A. baumannii* antibiotic resistance pattern

shows the frequency of genes (Fig. 3). *CITM* (91.1%) was the highest detected genes. The frequency of the other genes were *qnr* (91.3%), *imp* (80.2%), *tet(B)* (76.2%), *Oxa-58-like* (73.4%), *sul1* (67.3%), *sim* (62.9%), *dfrA1* (62%), *Oxa-51-like* (61.5%), *Oxa-24-like* (61.2%), *Oxa-23-like* (53.5%), *blaSHV* (51.2%), *tet(A)* (43.1%), *vim* (33.3%), *aadA1* (25.6%), *cmlA* (18%), *cat1* (6.8%). *A. baumannii* isolates detected from different burnt patients showed that the most frequent age range of the patients were between 61-70 (60.2%) who were hospitalized in different wards of the hospitals. Also 59% of patients were women in this study.

Table 2. Antibiotic susceptibility pattern of *A.baumannii*

	Resistance	Intermediate	Sensitive
Ciprofloxacin	97.2	2.8	0
Ceftazidime	88.3	6.5	5.2
Tetracycline	86.2	10.2	3.6
Trimethoprim	74	12.6	13.4
Norfloxacin	73.2	15	11.8
Gentamicin	69.6	22	8.4
Azithromycin	69	14	17
Meropenem	67.2	20.2	12.6
Erythromycin	64.8	32.9	2.3
Sulfamethoxazole trimethoprim	59	26.1	14.9
Tobramycin	58.2	21.2	20.6
Cephalothin	57.2	30.9	11.9
Levofloxacin	56.2	23.8	20
Amikacin	47.2	33	19.8
Rifampin	46.1	36.2	17.7
Imipenem	44	36.2	19.8
Streptomycin	35.2	9.5	55.3
Chloramphenicol	3.9	7.2	88.9
Nitrofurantoin	2.8	9.2	88

Data are presented as percent

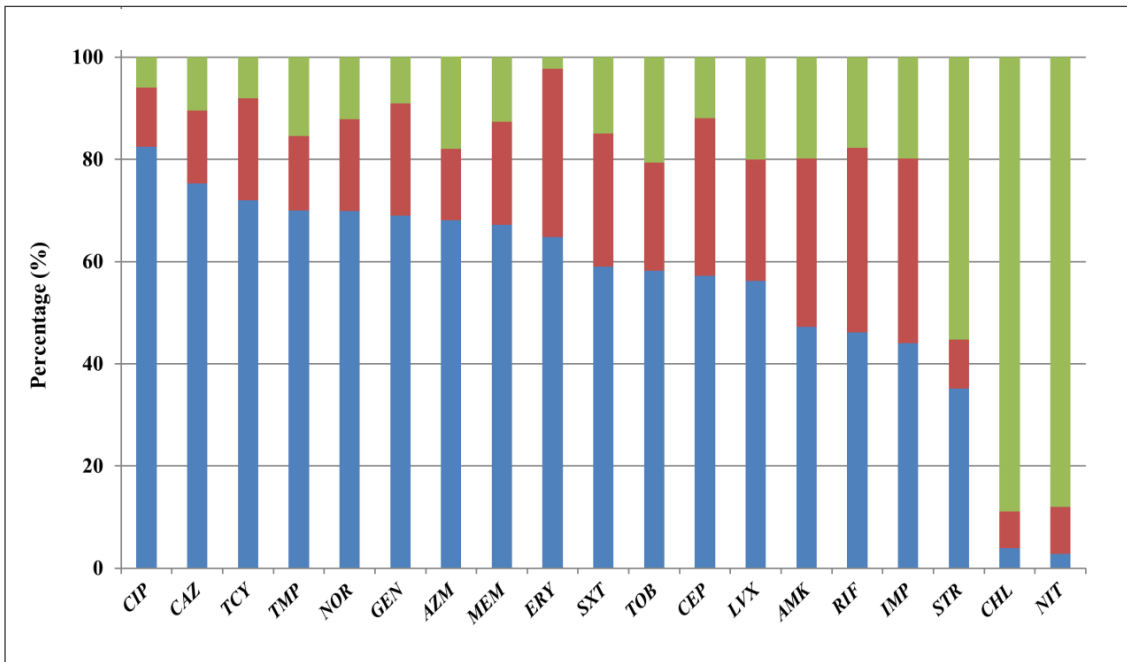


Fig. 2. Antimicrobial resistance pattern in *A. baumannii* burn isolates. Blue for resistant, red for intermediate and green for sensitive. CIP=Ciprofloxacin; CAZ=Ceftazidime; TCY=Tetracycline; TMP=Trimethoprim; NOR=Norfloxacin; GEN=Gentamicin; AZM=Azithromycin; MEM=Meropenem; ERY=Erythromycin; SXT=Sulfamethoxazole trimethoprim; TOB=Tobramycin; CEP=Cephalothin; LVX=Levofloxacin; AMK=Amikacin; RIF=Rifampin; IMP=Imipenem; STR=Streptomycin; CHL=Chloramphenicol; NIT=Nitrofurantoin.

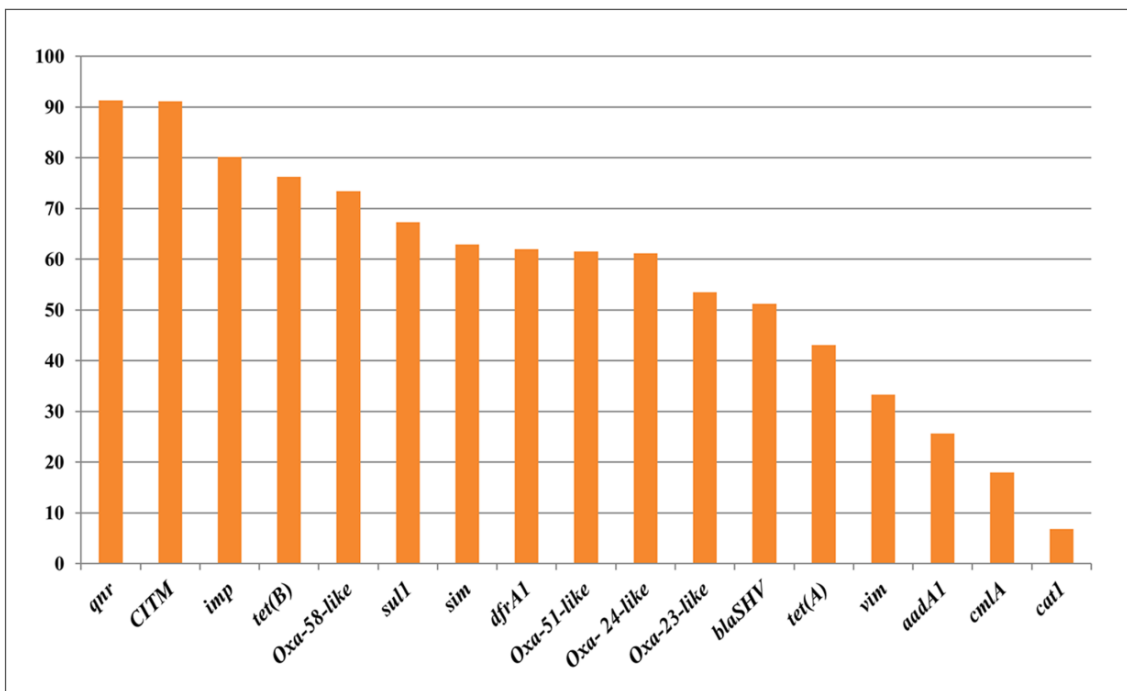


Fig. 3. The distribution of genes related to antibiotic resistance in *A. baumannii* burn isolates

Discussion

Different species of *Acinetobacter* have either strong tendency to become resistant to antibiotics or inherently resistant to some broad antibiotics. On the basis of, they have a great ability to gain new mechanisms of resistance [22, 23]. According to the spread of antibiotic resistance in *A.baumannii*, and since burn part of Isfahan had not conducted a comprehensive study on antibiotic resistance pattern and gene expression of these bacteria, this study was carried out to show frequency of genetic pattern in antibiotic resistance isolated in burn hospital in Isfahan. The majority of isolates examined in this study were MDR. The highest rate of drug resistance related to tetracycline (72%), ciprofloxacin (82.5%), and ceftazidime (75.3%). In line with our research, some studies have been conducted in different part of hospitals in Iran. In a similar study, Momtaz et al. observed almost the same pattern that *A. baumannii* are resistance to tetracycline (90.9%), trimethoprim (61.9%), cotrimoxazole (51.2%) and aminoglycoside compounds in a range between 9.9-31.4% [24].

In a study performed by Shakibai et al. on 50 samples of *A. baumannii* isolates selected from the ICU in Kerman hospitals, the antibiotic-resistant pattern showed that the percentage of resistance to imipenem, ciprofloxacin, piperacillin-tazobactam, amikacin, cefepime and piperacillin have been 73.3%, 66%, 93.3%, 53.3%, 93.3%, and 100%, respectively [25].

In another study, the result of antibiotic resistance pattern of *A. baumannii* isolated

from patients was partially different from the results of ours. It was shown the resistance rate of 94% to kanamycin, 86% to gentamicin, 81% to amikacin and 63% to tobramycin. Different antibiotic resistance in various studies can be related to clinical sample types, or geographic region of the test [26].

The distribution of genes encoding resistance to antibiotics was one of the important goals of our study. The presence of these genes was shown in these 100 isolates. Compared with this study, the distribution of β -lactamase genes was reported in 21 nosocomial outbreak isolates of *A. baumannii* in a Chinese hospital. In this study, all of the isolates contain *bla*_{OXA-51-Like} and just eight strains contains *bla*_{OXA-23-Like} gene [27]. Another study identified that the isolates were resistant to imipenem (93.8%), and contained *bla*_{OXA-23-Like} (71.1%), and *bla*_{OXA-58-Like} genes (22.8%). Compared with our study, they did not detect *Oxa-24-like*, *Oxa-51-like*, *vim*, *sim* and *imp* in their isolates [28]. Unlike these results Mirnejad et al. demonstrated that 82% of *A. baumannii* 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].

Conclusion

The results of this cross-sectional study were in agreement with similar studies around the world. Due to increased drug resistance, wider

research and further studies are necessary to control hospital infections.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgment

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