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ORIGINAL ARTICLE

Frequency and Antimicrobial Susceptibility of *Acinetobacter baumannii* in Burn infections in Isfahan, Iran

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ABSTRACT
Bacterial infections and antibiotic resistance of causative agents are the most significant challenge faced by burn centers. Acinetobacter baumannii is a common and opportunistic pathogen causing nosocomial infections which its high antibiotic resistance has made it difficult to treat these infections. The purpose of this study is to determine the frequency of Acinetobacter baumannii strains in burn infections and antibiotic resistance pattern of isolates. Over a 5-month period, 227 samples were isolated from patients admitted in the burn center. Identification of isolates was performed using conventional biochemical tests. Acinetobacter baumannii strains were identified by amplifying bla$_{OXA-51}$ gene by PCR technique. Antibiotics sensitivity of isolates was evaluated by disc diffusion method. Among the samples, 65 samples were identified as Acinetobacter species. Based on the results of the antibiogram, 85% of isolates were resistant to two or more antibiotic (Multi Drug Resistance) and 15% were resistant to all used antibiotics (Pan Drug Resistance). In general, isolates showed the highest resistance to ciprofloxacin (100%) and the highest sensitivity to tobramycin (42%). The results of PCR confirmed the existence of bla $_{OXA-51}$ gene in 39 isolates. In this study, frequency of Acinetobacter sp. in burn wounds was 28.6% and the relative frequency of Acinetobacter baumannii also was determined by 17.1%. High frequency of Acinetobacter baumannii strains in burn wounds remembers the need to think about strategies required to control the rapid spread of these isolates in hospital environments, especially in burn centers. High antibiotic resistance in isolates necessitates revision of conventional treatment regimens and monitoring and controlling the spread of genes. Also, more attention to the development and use of new non-drug therapies such as phage therapy seems effective.
***Keywords**: Burn infections, Drug resistance, Acinetobacter baumannii*

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INTRODUCTION

Burn infections as a hospital-acquired infection, is an important cause of mortality and morbidity after burn. Burn patients are very sensitive to the infection due to loss of skin which is a barrier to prevent the penetration of microorganisms. On the other hand, burn units are suitable environment for the growth of bacteria such as *Pseudomonas sp., Enterobacter sp., Staphylococcus sp., Acinetobacter sp.* and others [1-2]. *Acinetobacter* is common among negative bacteria in nosocomial infections. *Acinetobacter* species have great potential for rapid spread in hospital settings, and nowadays, they are considered as one of the most important causes of nosocomial infections. The bacterial are considered infection factor in patients hospitalized in intensive care and burns [3].

The most important species of *Acinetobacter* being pathogenic to humans is *Acinetobacter baumannii*that is non-fermented, non-motile and catalase-positive and is found in soil, water, effluent and many therapeutic environments [4]. *Acinetobacter baumannii*causes a wide range of nosocomial infections such as pneumonia, urinary tract infections and infections in surgical ward and intensive care unit [5-6, 7]. This bacterium has simple growth requirements and can survive in dry conditions [5-8]. The items above, helps survival of the bacteria in the hospital environment which is its main reservoir. *Acinetobacter baumannii* is often resistant to antibiotics and shows multi-drug resistance. Currently, a number of *Acinetobacter baumannii* strains have become resistant to all available antimicrobial agents [9]. The

increasing prevalence of multi-drug resistant and pan- resistant *Acinetobacter baumannii* strains, have caused *Acinetobacter baumannii* become the most important pathogen after *Pseudomonas aeruginosa* among non-fermentative Gram-negative bacteria [5].
The most common resistance mechanism to carbapenem in *Acinetobacter* is Oxacillin production from Class D [9-10, 11]. Bla_{OXA-51} genes inherently exist in 97% of *Acinetobacter baumannii* isolates. The trace of this gene is a sensitive and accurate method for detection of bacteria compared to the commonly used biochemical tests [9]. The purpose of this study is to determine strains of *Acinetobacter baumannii* in burn samples by tracing bla_{OXA-51} gene using PCR.

MATERIALS AND METHODS

Over a 5 months period, 227 clinical specimens were collected from burn infected wounds of hospitalized patients in Imam Musa Kazim (AS) hospital of Isfahan. The samples were microbiologically evaluated. After inoculation of clinical specimens to the culture Mac Conkey agar and blood agar and incubation for 24 hours under 37^0C, standard bacteriological tests, including Gram staining, motility, catalase, fermentation and oxidation of sugars (OF) and growth on the Mac Conkey agar were conducted under 42^0C [12]. Bacterial drug susceptibility was determined by disk diffusion method or the Kirby – Bauer for antibiotics Piperacillin (100mcg), Ciprofloxacin (5mcg), Imipenem (10mcg), Amikacin (30mcg), Ceftazidime (30mcg), Ceftriaxone (30mcg), Cotrimoxazole (25mcg), Cefotaxime (30mcg) and Tobramycin (10mcg). The inhibition zone diameter was measured, and bacterial resistance and susceptibility to each antibiotic was determined after comparison to reference tables of CLSI 2010 (Clinical and Laboratory Standard Institute) [13]. In this study, the presence of 16S rDNA was investigated using PCR to prove the genus of and presence of bla $_{OXA-51}$ gene to prove *Acinetobacter baumannii* species. The sequence of primers for gene bla $_{OXA-51}$ including [10]:
Forward: 5 – TAATGGTTTGATCGGCCTTG- 3
Reverse: 5 – TGGATTGCACTTCATCTTGG- 3
The sequence of primers for gene 16S rDNA including [5]:
Forward : 5-AGAGTTTGATCCTGGCTCAG-3
Reverse : 5-GGT TAC CTT GTT ACG ACT T-3

RESULTS

Among 227 samples, 65 samples were identified as *Acinetobacter sp.* 45 samples (69.23%) were from males and 20samples (30.76%) were from the females. The results of separation of samples based on hospital wards are shown in Table 1. The results showed a high prevalence of *Acinetobacter* species in ICU.

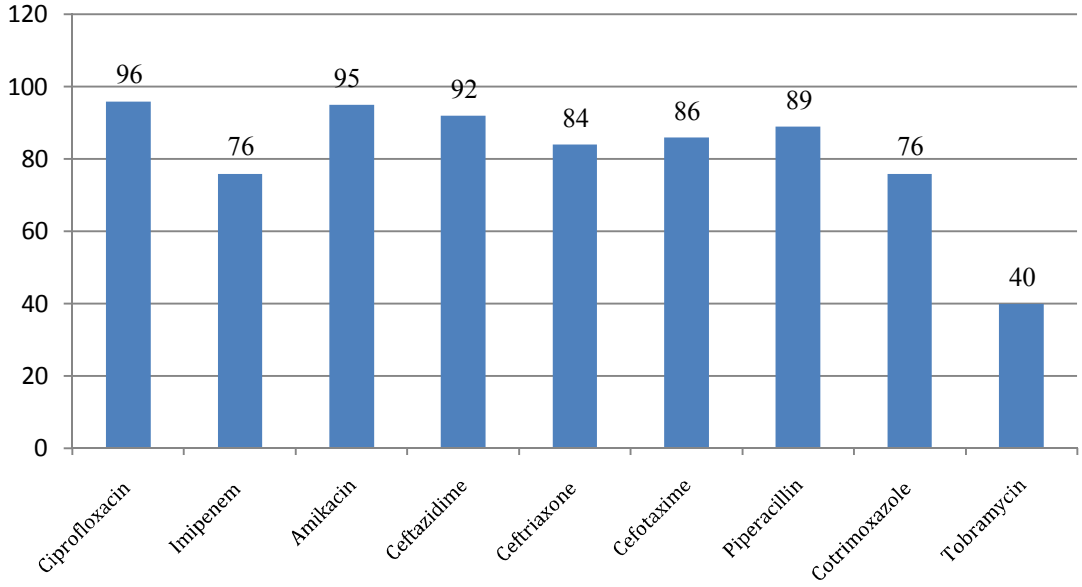

Figure 1: Resistance pattern of isolated *Acinetobacter* samples to different antibiotics

Based on the results of antibiotic susceptibility, among 65 samples of *Acinetobacter*, 10 samples were resistant to all nine selected antibiotics, in other words, Pan Drug Resistance (PDR) and 55 other samples

were resistant to three or more than three antibiotics, and in other words, they were Multi Drug Resistant (MDR).

RESULTS

The PCR results showed that, 16S rDNA sequence exists in all 65 collected samples, and this confirmed the accuracy of biochemical tests. The results of PCR of bla$_{OXA-51}$ gene showed that, this gene exists in 39 isolates which had been diagnosed as *Acinetobacter baumannii*. This difference may reflect the more accurate and more sensitive detection of molecular diagnosis in comparison with biochemical tests. Figure 2 shows the results of PCR of the 16S rDNA gene; Figure 3 shows the PCR results of the bla $_{OXA-51}$gene.

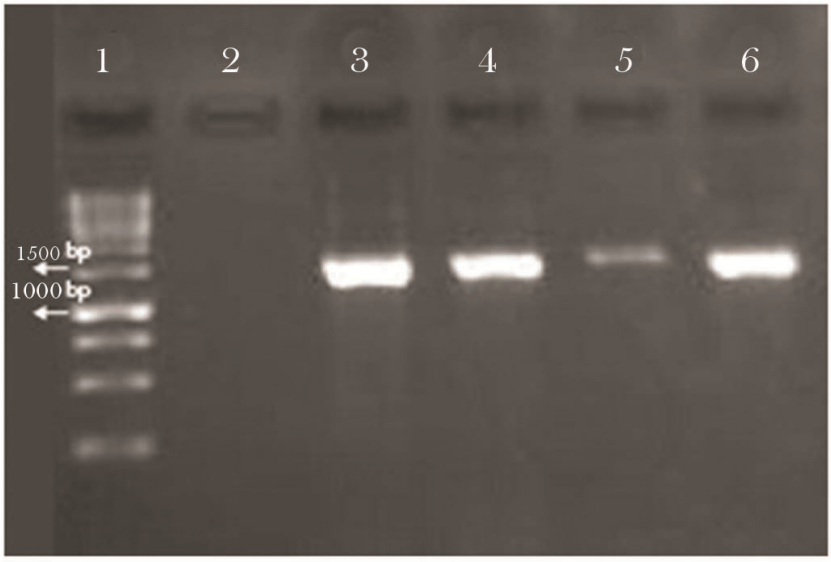

Figure 2. PCR Results of 16S rDNAgene. Lane 1: Molecular marker (Ladder 1 kb),Lane 2: negative control, Lane 3: positive control, Lane 4, 5, 6: clinical samples.

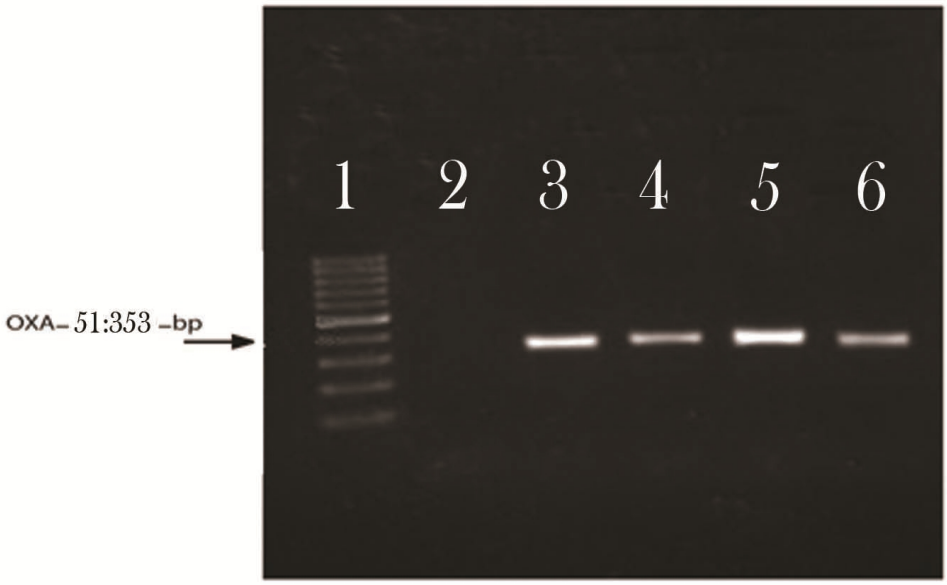

Figure3: Results of PCR of the *bla* $_{OXA-51}$ genein*Acinetobacter baumannii* 1: Molecular marker (Ladder 100 bp), Lane 2: negative control, Lane 3: positive control, Lane 4, 5, 6: clinical samples.

DISCUSSION AND CONCLUSION

The most important concern facing burn team is the infection operating in more than 50% mortality after burn injury [14]. Various studies over the last 20 years have shown the increase in incidence of nosocomial outbreak of *Acinetobacter* resistant to several antibiotics in patients admitted to hospitals in different countries [3].

In this study, frequency of *Acinetobacter* strains in burn wounds was determined by 28.6 and relative frequency of *Acinetobacter baumannii* was determined by 17.1%.

Norouzi et al. investigated 863 samples from patients with burns in Motahari Burn Center in three periods of years 1993, 2000 and 2001. *Acinetobacter sp.*was not observed in 1993, but, its frequency in 2000 reached 15.5% and in 2001 reached 20.5% [1]. Saadatian Farivar et al. (2004) determined the frequency of *Acinetobactersp*.by 21% in surgical intensive care unit of Rasoul Akram complex [15].

Also, in a study conducted by Ghazvini et al (2007) in Imam Reze (AS) hospital of Mashhad, the frequency of *Acinetobacter sp.* in burn wounds were determined about 10% [16].

In a similar study conducted by Mamani et al. (2009) in Hamedan, frequency of *Acinetobacter* in burn wounds were determined about 16.6% [17].

A comparison between the results of present study and the recent studies suggested an ascending trend in relative frequency of *Acinetobacter sp.* in burn wounds over the recent years. Difference in these findings can reflect the increase in relative frequency of *Acinetobacter* in burn wounds.

In the present research, 15% of the isolates were resistant to the used antibiotics and 85% had multi-drug resistance. In an investigation conducted by Heydari and Karbasizadeh (2012), 50 *Acinetobacter baumannii* strains were isolated among 456 samples in intensive care ward. Antimicrobial pattern of the isolates showed that, 85% had multiple antibiotic resistances. In this study that is closer in terms of time to the present study, the number of strains with multiple drugresistances is the same [18].

Observed antibiotic resistance in this research is much higher than the similar researches in other parts of Iran [3-15-18-19, 20,29]. Difference in the findings is probably due to variability in clinical samples, time of the study, and treatment strategies for each geographic region. When comparing this study with other studies, the difference in the findings could indicate increasing resistance to antibiotics in these strains, this means that the resistance increases with time. Surveys conducted in Asia and the Middle East also represents *Acinetobacter baumannii*prevalence with multi-drug resistance in these areas [21].

bla_{OXA-51}genes are one of the most important factors of resistance to carbapenem antibiotics in *Acinetobacter* [22-23] which exists inherently in all *Acinetobacter baumannii* strains and is chromosomal [23-24]. In the present study also the existence of the bla $_{OXA-51}$genewas investigated to prove *Acinetobacter baumannii* strains. The results indicated the presence of this gene in all the strains of *Acinetobacter baumannii* and consistent with previous research in this field [21-25].

Important in this study was high incidence of *Acinetobacter* strains in burn wounds. A comparison between the present study and the recent studies suggests an increasing trend in the relative abundance of *Acinetobacter sp.* in burn wounds in recent years [1-15-26-27-28]. This problem remembers the need to think about strategies required to control the rapid spread of the bacteria in hospital environments, especially in burn centers.

In the present study, a high percentage of isolates had multi drug resistance. Some strategies should be designed and developed to control the spread of multidrug-resistant strains of *Acinetobacter sp..* Correct management of dealing with hospital infections, especially burn infections seems very essential. More attention to the development and use of new non-drug therapies such as phage therapy seems more effective.

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