Original Article

Identification of Genomic Species of *Acinetobacter* Isolated from Burns of ICU Patients

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Abstract

Background: The worldwide emergence of multi-drug resistant (MDR) bacteria in recent years has caused many problems for hospitals and patients, especially intensive care unit patients. Among these clinically important MDR bacteria are *Acinetobacter baumannii* complex species (*A. baumannii*, *Acinetobacter* genomic species 3 and *Acinetobacter* genomic species 13TU) that cause a wide range of infections.

Methods: The sequencing and bioinformatics analysis of a part of the Zone 1 of *rpoB* gene was performed for species identification of *Acinetobacter* isolates obtained from ICU patients with infected burns hospitalized in a hospital in Isfahan, Iran, over a 9-month period. Antibiotic sensitivity of *Acinetobacter* isolates was investigated using the disk diffusion method and different classes of antibiotics including amikacin, cefotaxime, ceftriaxone, ciprofloxacin, imipenem and piperacillin.

Results: *Acinetobacter* spp. were isolated from 10 of 80 (12.5%) investigated patients. All of the 10 *Acinetobacter* isolates were identified as *Acinetobacter baumannii* and multi-drug resistant according to antibiotic susceptibility tests.

Conclusion: Of the *Acinetobacter baumannii* complex members, only *A. baumannii* species was identified among the isolates obtained from patients with infected burns in an Isfahan hospital over a 9-month period.

Keywords: *Acinetobacter*, burn patients, genomic species, infection, multi-drug resistant

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Introduction

The worldwide emergence of multi-drug resistant (MDR) bacteria in recent years has caused many problems for hospitals and patients, especially intensive care unit (ICU) patients including burn and trauma patients and those requiring mechanical ventilation. One of these clinically important MDR bacteria is *Acinetobacter baumannii*. *A. baumannii* (gram-negative, nonmotile, obligate aerobic coccobacillus) is an opportunistic nosocomial pathogen that causes a wide range of infections including skin and soft-tissue, urinary tract, wound, blood stream and surgical site infections, ventilator-associated pneumonia, secondary meningitis, endocarditis, intra-abdominal abscess, and osteomyelitis due to its long-term survival, easy spread in the hospital environment and its remarkable ability to rapidly acquire resistance determinants against most of antimicrobial agents. Infections caused by it are sometimes life-threatening, especially in ICU patients.[1–4]

In addition to *A. baumannii*, *Acinetobacter* genomic species 3 (*A. pittii*) and *Acinetobacter* genomic species 13TU (*A. nosocamialis*) are other species of the *Acinetobacter* genus (consisting of more than 40 genomic species) with most clinical significance. These three species together are called the "*A. baumannii* complex" and are major causes of infections and nosocomial outbreaks caused by members of the *Acinetobacter* genus. These species are not distinguishable from each other by routine laboratory tests and are only differentiable by molecular methods.[1–5] Thus, accurate identification of MDR strains of the *Acinetobacter* genus in the hospital environment can be effective in identification of epidemic strains as well as planning for the prevention, control and treatment of infections caused by them.

The purpose of this study was identification of species of the *Acinetobacter* genus (*A. baumannii*, *Acinetobacter* genomic species 3, *Acinetobacter* genomic species 13TU or other species of *Acinetobacter* genus) causing infection in the burns of ICU burn patients.

Materials and Methods

Bacterial isolates

In order to isolate *Acinetobacter* species, 80 ICU infected burn patients hospitalized in one of the hospitals in Isfahan, Iran were randomly sampled over a 9-month period from July 2013 to February 2014. This study was approved by the ethics committee of the University of Isfahan and conformed to the provisions of the Declaration of Helsinki (as revised in Seoul, Republic of Korea, October 2008).

Identification of the isolates

Bacterial isolates were initially identified using biochemical

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Table 1. Primers used for identification of bacterial isolates.

Primer name	Sequence (5' to 3')	Target region	Product size (bp)	Reference
Ac696F	TAYCGYAAAGAYTTGAAAGAAG	Zone 1 of *rpoB*	397	7, 8
Ac1093R	CMACACCYTTGTTMCCRTGA			
16S rDNA-F	AGAGTTTGATCMTGGCTCAG	16S rDNA	1500	7, 9
16S rDNA-R	ACGGHTACCTTGTTACGACTT			
OXA-51-likeF	TAATGCTTTGATCGGCCTTG	$bla_{\text{OXA-51-like}}$	353	7, 10
OXA-51-likeR	TGGATTGCACTTCATCTTGG			

tests[6] and then the strains of the *Acinetobacter* genus were investigated by specific PCR test. Primers used in this study were Ac696F and Ac1093R designed for partial sequence of Zone 1 of RNA polymerase β-subunit (*rpoB*) gene, 16S rDNA-F and 16S rDNA-R designed for 16S rDNA and OXA-51-likeF and OXA-51-likeR designed for $bla_{\text{OXA-51-like}}$ gene (Table 1).[7–10] So, first, primers for 16srDNA and Zone 1 of *rpoB* were used for identification at genus level and then only PCR products of Zone 1 of *rpoB* gene were sequenced for identification at species level. The forward primer (Ac696F) was used for sequencing of 6 isolates, while the reverse primer (Ac1093R) was used for the rest only in one direction. The sequencing of PCR products was performed by Macrogen Company (Republic of Korea).

Analysis of sequences

Sequences were compared with sequences available in GenBank using BLAST, and the phylogenetic tree was constructed using MEGA software version 6.[11]

Antibiotic sensitivity

Antibiotic sensitivity of *Acinetobacter* isolates was investigated using the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) and different Antimicrobial categories of antibiotics[12] including amikacin (30 µg) (Aminoglycosides), cefotaxime (30 µg) (Extended-spectrum cephalosporins), ceftriaxone (30 µg) (Extended-spectrum cephalosporins), ciprofloxacin (5 µg) (Antipseudomonal fluoroquinolones), imipenem (10 µg) (Antipseudomonal carbapenems) and piperacillin (100 µg) (Antipseudomonal penicillins).

Results

During nine months in different times, 118 samples were collected randomly from patients with grade ≥ II burns hospitalized in the ICU. Of the tested samples, 38 were negative for any bacterium. So, these patients were excluded. Of 80 (67.8%) positive samples, only in ten patients (12.5%), ten bacterial isolates (ImdAb04, ImdAb06, ImdAb10, ImdAb12, ImdAb35, ImdAb47, ImdAb48, ImdAb68, ImdAb72, and ImdAb74) were identified as *Acinetobacter* species according to morphology (gram negative coccobacillus), growth on MacConkey agar and Simmons citrate agar, non-motility, alkaline over alkaline (K/K) in Triple sugar iron (TSI) agar and being negative in oxidase, Voges-Proskauer, methyl red, urease and indol tests. Identification of 10 isolates of *Acinetobacter* genus was confirmed by PCR using Ac696F and Ac1093R primers as well as 16S rDNA-F and 16S rDNA-R primers. The expected bands of ~ 400 and ~ 1500 bp in size were observed in the agarose gel using Ac696F_Ac1093R and 16S rDNA-F_16S rDNA-R primers, respectively. The characteristics of burn patients are shown in Table 2.

Sequencing of the yields of PCR with Ac696F and Ac1093R primers showed that the bacterial isolates were only strains of *Acinetobacter baumannii*, not others. Nucleotide sequences of isolates ImdAb04, ImdAb06, ImdAb10, ImdAb12, ImdAb35, ImdAb47, ImdAb48, ImdAb68, ImdAb72, and ImdAb74 are available in the GenBank under accession numbers KM077486, KM077487, KM077488, KM077489, KM077490, KM077491, KM077492, KM077493, KM077494, and KM077495, respectively. By blasting, the sequences of isolates ImdAb04 and ImdAb10 showed 100% identity with the sequences of *Acinetobacter baumannii* D1279779 (GenBank: CP003967.1) and *Acinetobacter baumannii* ATCC 17978 (GenBank: CP000521.1) and the sequences of the other 8 isolates had 100% identity with the *Acinetobacter baumannii* SDF sequence (GenBank: CU468230.2). Figure 1 shows the phylogenetic relationship among 10 *Acinetobacter baumannii* isolates obtained in this study and other strains of *Acinetobacter baumannii* as well as other species of *Acinetobacter* genus based on partial nucleotide sequence of Zone 1 of

Table 2. Characteristics of burn patients with infected burn sites caused by *A. baumannii.*

Patient	Age (year)	Sex	Type of burn	Extent of burn (%)	Depth of burn (degree)[a]	Length of hospital stay (day)	Outcome
04	21	Male	Electrical	35	II, III, IIII	34	Discharge
06	14	Male	Flame	47	II	41	Discharge
10	28	Male	Chemical (acid)	50	II, III	41	Discharge
12	45	Male	Flame	27	III	31	Discharge
35	42	Male	Hot liquid	50	II, III	23	Discharge
47	2	Male	Hot liquid	50	II	16	Death
48	31	Male	Flame	40	II	28	Discharge
68	33	Male	Flame	50	II	40	Discharge
72	46	Female	Flame	50	II	32	Discharge
74	47	Female	Flame	42	II, III	40	Discharge

[a] II = deep second degree burn (deep partial thickness burn): affects the epidermis and most of the dermis. III = third degree burn (full thickness burn): affects epidermis, and all layers of dermis, extending down to subcutaneous tissue. IIII = fourth degree burn (fourth degree burn): full-thickness burn extending to muscle or bone.

Figure 1. Maximum likelihood tree, based on partial nucleotide sequences of Zone 1 of *rpoB* gene of *Acinetobacter baumannii* strains isolated in this study (ImdAb04, ImdAb06, ImdAb10, ImdAb12, ImdAb35, ImdAb47, ImdAb48, ImdAb68, ImdAb72 and ImdAb74), other strains of *Acinetobacter baumannii*, other species of the *Acinetobacter* genus and strain of *Pseudomonas aeruginosa* as an out group (number of bootstrap replication is 1000). The numbers in parentheses are the GenBank accession numbers.

rpoB gene. It confirmed the blast results, as sequences with 100% identity were placed in the same clusters. All of our isolates were placed in the same cluster that also included *A. baumannii* species reported from elsewhere.

Also, by using OXA-51-like primers and observing ~ 350 bp segment in the agarose gel, $bla_{\text{OXA-51-like}}$ gene was detected in all 10 isolates. This also confirmed that our isolates were strains of *A. baumannii* species.

The results of antibiotic susceptibility tests are shown in Table 3. According to an international expert proposal for interim standard definitions for acquired resistance defining MDR as acquired non-susceptibility to at least one agent in three or more antimicrobial categories,[12] all of the *A. baumannii* isolates obtained in this study were multi-drug resistant. Isolates ImdAb72 and ImdAb74 were resistant to all of the tested antibiotics. Only amikacin and piperacillin were somewhat effective against some of the *A. baumannii* isolates.

Discussion

At present, MDR bacteria cause many problems for hospitals and patients, especially those in the ICU, and have imposed remarkable socioeconomic costs on the society. Multi-drug resistant bacteria are commonly found in clinical settings, especially in the

Table 3. The results of antibiotic susceptibility test.

A. baumannii isolates	Antibiotic susceptibility patterns					
	Amikacin	Cefotaxime	Ceftriaxone	Ciprofloxacin	Imipenem	Piperacillin
ImdAb04	S	R	R	R	R	I
ImdAb06	I	R	R	R	R	I
ImdAb10	S	R	I	R	R	I
ImdAb12	I	R	R	R	R	S
ImdAb35	I	R	R	R	R	R
ImdAb47	I	R	R	R	R	R
ImdAb48	R	R	R	R	R	I
ImdAb68	R	R	R	R	R	I
ImdAb72	R	R	R	R	R	R
ImdAb74	R	R	R	R	R	R

S = sensitive, R = resistant, I = intermediate

ICU where broad-spectrum antimicrobial agents are widely used. Burn is one of the most vulnerable environments to be rapidly infected. The consequences of burn infection can be delayed healing, failure of skin graft, progression of infection to the underlying tissues leading to systemic spread of bacteria and increased mortality of burn patients. In recent decades, gram-negative MDR bacteria have dominated as major infective agents in burn patients by harboring a set of virulence factors and antimicrobial resistance determinants.[13,14]

A. baumannii is one of the clinically important gram negative MDR bacteria and an important and most common cause of burn infections. Due to its extraordinary survival and the poor penetration of antibiotics into burn sites, fighting against *A. baumannii* in the burn patients is one of the most important challenges for clinicians and burn treatment centers.[14] Also, the infection can increase the burn depth.[15] In this study, different degrees of burn depth were observed (Table 2); this, alongside the presence of multidrug resistance of *A. baumannii*, may have delayed the healing process.

Using biochemical tests, *A. baumannii* infection in burn units are reported, although its prevalence in the burn site infections is significantly different depending on different geographic locations. Infection and colonization of up to 48 cases per 100 admission in burn intensive care units have been reported for *A. baumannii*.[16] In a study by Simor, *et al.* (2002) multi-drug resistant *A. baumannii* was acquired by 31 out of 247 (13%) of acute burn patients in a regional burn unit in Canada.[17] In another study in Singapore, multi-drug resistant *A. baumannii* was isolated (either infected or colonized) from 77 out of 517 (14.9%) burn patients.[18] Twenty seven multi-drug resistant *A. baumannii* were isolated from wounds of 217 burn patients (12.4%) in the USA.[19] In a retrospective cohort study by Albrecht, *et al.* (2006), 52 (6.5%) and 59 (7.3%) out of 802 burn patients were respectively colonized and infected with *A. baumannii* complex (overall 14%) in a USA military tertiary burn center.[20] The most prevalent bacterium isolated from burn patients (burns with total body surface area of < 60%) in the US Army Institute of Surgical Research burn center was *A. baumannii* (from 22% of the cases tested)[21,22] while this was 23.4% in a tropical area.[23] In Iran, also *A. baumannii* isolates were isolated from burn sites of burn patients at a teaching hospital.[24] So, the range of incidence in different regions and times varies from 12.4% to 48%.

Accurate detection of MDR bacteria can be very useful in investigation of outbreaks, epidemiological studies and application of proper procedures for prevention, control and treatment of infections caused by them. Among the clinically important MDR bacteria are some species of the *Acinetobacter* genus. It has been found that, compared to other species of the *Acinetobacter* genus, *A. baumannii*, *Acinetobacter* genomic species 3 and *Acinetobacter* genomic species 13TU are clinically more important and comprise causes of the most nosocomial outbreaks associated with the *Acinetobacter* genus. These three species are not distinguishable from each other by biochemical tests and are only differentiable using molecular methods.[1–4] As the identification of the three species from each other is not routinely performed by molecular methods in clinical settings, the prevalence and clinical significance of each of these three species are not well studied, especially in burn patients.

The nucleotide sequences of Zone 1 of *rpoB* gene are more accurate for the discrimination and taxonomic classification of *Acinetobacter* species.[7,8] For this reason, in this study, the sequencing and bioinformatics analysis of a part of the Zone 1 of *rpoB* gene were performed to identify *Acinetobacter* genomic species. In this study, of the three clinically important species of *A. baumannii* complex (*A. baumannii*, *Acinetobacter* genomic species 3 and *Acinetobacter* genomic species 13TU) only *A. baumannii* was isolated from 10 out of 80 burn patients (12.5%) in an Isfahan hospital over a 9-month period. As, to the best knowledge of the authors, there is no previous report on the incidence of different genomic species of the *Acinetobacter* in burn patients, comparison with other data was not possible. To elucidate the information about prevalence and different clinical outcomes of different genomic species of *Acinetobacter* in the burn patients, more studies are suggested with higher numbers of cases.

The bacteria infecting ICU patients, including burn patients, may originate from the patient her/himself, contaminated hospital equipment and environment, staff and other patients. Also, transfer of the bacteria from one hospital to another may occur with staff circulation. On the other hand, resistance may be obtained during long treatment periods.[2,4,13,25] The observation of different antibiotic resistance patterns for the *A. baumannii* isolates in this study may indicate different sources of infection in different patients.

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