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Diversity among *Acinetobacter baumannii* isolates in intensive care units of a tertiary referral center in southern Iran

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ABSTRACT

Clinical significance and epidemic outbreaks of multi-drug resistant Acinetobacter baumannii (MDR-AB) in intensive care units (ICUs) are increasing. We provided a molecular epidemiology research of 85 A. baumannii *isolates of southern Iran using amplified restriction fragment polymorphism* (AFLP) *DNA* fingerprinting method. *Using a cut-off of 90% similarity, AFLP revealed 42 different AFLP types. This study approved evidence for prominent genotypic variations among MDR-AB population in southern Iran.*

Key words: *Acinetobacter baumannii*; AFLP

INTRODUCTION

Clinical significance and rapid expansion of multidrug resistant *Acinetobacter baumanii* (MDR-AB) is an increasing concern in the realm of healthcare, particularly in intensive care units (ICUs) [1-7]. Regarding to the rapid development of resistance against various antimicrobial agents due to high ability of genetic transformation and the potential for widespread dissemination because of its ability to survive on environmental surfaces, *A. baumanii* has now surpassed other bacteria as the second most commonly isolated glucose non-fermenter in clinical laboratories after *Pseudomonas aeruginosa* with high mortality rates of 41% [8]. However, control of infectious agents can be impaired in extensively drug-resistance (XDR) and pan drug-resistance (PDR) *A. baumanii* infections. Some therapeutic alternatives, such as Photodynamic therapy [9-12], nano-particle [13-16] organic compound such as sugar alcohol (e.g. Xylitol) [17] have been used as alternative antimicrobial agents to contemporary treatment of infections in cases not responding to conventional antibiotics treatments, although this therapy brings undesirable side effects.

Although reports from some areas of Iran have shown the distribution and/or frequency of bla_{OXA} genes among CR-AB [1-7]; the scarcity of local molecular epidemiologic data has been the most important reason why efforts were failed to control the spread of CR-AB infections. However, a global success to control the MDR-AB pandemic requires the incorporation of susceptibility profiles of MDR-AB combined with current epidemiologic data from all regions of the world. We presented here a molecular epidemiology study of 85 CR-AB isolates from a referral hospital in southern Iran.

MATERIALS AND METHODS

Isolates

A total of 85 non-repetitive isolates of *A. baumannii* were isolated from several clinical sources including the respiratory tract, postoperative wound, urine, blood, and *cerebrospinal fluid* (*CSF*) obtained from 7 ICUs of a referral hospital in the south (Shiraz) of Iran during February 2009 to March 2010. In the first isolates were

identified as *A. baumannii* using API-20NE system (bioMérieux, Marcy-l'Etoile, France), and were later confirmed by $bla_{\text{OXA-51-like}}$ gene PCR [18].

AFLP Genomic Fingerprint Analysis

Epidemiological types of all A. baumannii isolates were determined by amplified restriction fragment polymorphism (AFLP) as previously described [5]. Briefly, Semiquantitative PCR test for monitoring genomic DNA from isolates were performed as described previously [19]. Then genomic DNA from isolates were double digested with Mbo I and Mse I (Fermentas, Lithuania) and ligated to their corresponding adaptors. Ligation products served as templates for preliminary-PCR amplification. Optimization of PCR conditions carried out under previously set experiment conditions [20, 21] Diluted preliminary-PCR products were then used for selective PCR amplification to generate AFLPs profiles. To determine the AFLP genotypes, the selective PCR products were separated by agarose gel electrophoresis. Then, the AFLP results were interpreted as described previously [5].

RESULTS

Isolates

The 85 non-repetitive clinical *A. baumannii* isolated from hospitalized patiants: 27 isolates were separated from internal ICU (31%), 22 from surgical ICU (27%), 12 from central ICU (14%), 9 from neurosurgical ICU (11%), 7 from transplant ICU (8%), 5 from neonatal ICU (6%), 3 from pediatric ICU (3%). This clinical isolates were acquired from seven various specimens. Predominantly, nosocomial *A. baumannii* infections were involved in urinary (39-46%) and respiratory (21-25%) systems, blood-stream (11-13%), both wound and central nervous system (6% and 7% respectively), eye and nasal secretions (both were 1%) infections.

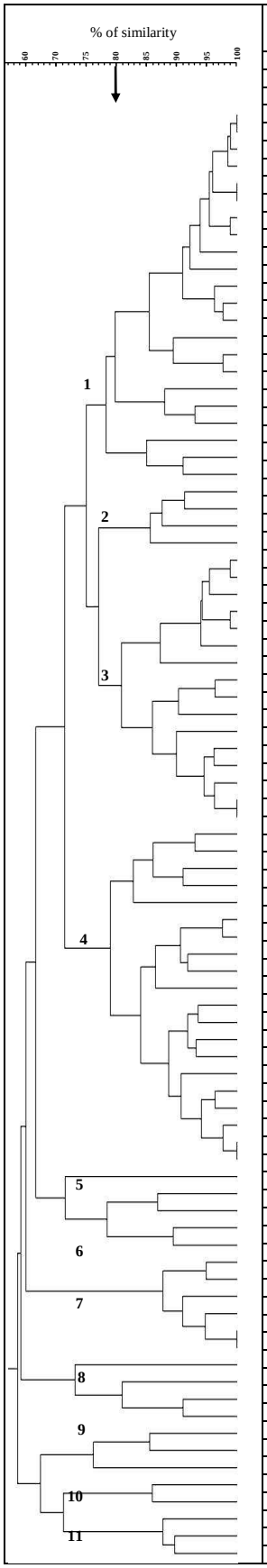
Genotyping; AFLP

Dendrogram constructed following AFLP patterns preparation and results of cluster analysis of all *A. baumannii* isolates are shown in figure 1. Using a cut-off of 90% similarity, corresponding to type level of strains, AFLP revealed 42 different AFLP types; one major type with 13 isolates, two types with 6 isolates each, one type with 5 isolates, three types with 4 isolates, 9 pairs and 25 single isolates (isolates with a unique AFLP profile).

AFLP categorized 73% of the isolates in one large and heterogeneous cluster (A) at the similarity threshold index of ≥ 75% (Fig. 1). Cluster 5 also displayed a very low level of similarity (56%) compared to others and was clearly far less related to any of the other clusters. AFLP revealed 10 isolates with 100% genetic similarity (94, 36; 21, 15; 1, 2; 54, 104; 42, 17) with different antimicrobial patterns. Further, despite a close relationship between the isolates pairs with 100% genetic similarity, these isolates were collected from diverse wards. Variation in isolates regarding both ICs and AFLP types in antibio-dendrogram (Fig. 1) implies the lack of correlation between the genotypic (whether various ICs or AFLP clusters) and antimicrobial *susceptibility profiles*. Two PDR *A. baumannii* isolates with an extended resistance profile were belonged to cluster A; however, one of these isolates remained susceptible only to tigecycline. The $bla_{\text{OXA-23-like}}$ gene was detected in all clusters, while the $bla_{\text{OXA-24-like}}$ gene was detected in clusters 1, 4 and 5 (Fig 1).

DISCUSSION

Due to the rapid spread of resistance against the common antibiotics used to treat *A. baumannii* infections as a global problem, it is important to know the regional molecular epidemiology in order to prevent the spread of antibiotic-resistant *A. baumannii* strains. In the current study, AFLP was used to differentiate *A. baumannii* genotypes and to explore their genetic relationship. AFLP has been widely admitted as an excellent genotyping tool because of its typability and discriminatory power, and sensible reproducibility; likewise, it's simple to perform and to draw interpretations [22]. AFLP pattern analysis identified 3 genotypes (1, 3 and 4) as the most common genotypes of clinical isolates obtained from ICUs of a referral hospital in the south (Shiraz) of Iran. Differences between AFLP-denrogram, particularly in strains with genetic similarity value of 100%, indicated no phenotypic similarity in genotypes and the emergence of resistance to antimicrobial agents in *A. baumannii* has been influenced by horizontal gene transfer in the related ICUs. This result was approved by spread of different AFLP types compared to the previous studies in Iran. On the other hand, small clusters scattered throughout the AFLP-dendrogram suggests cross– transmission of *A. baumannii* strains from different reservoir in the ICUs.



Strain ID	ICU	Specimen
94	Neu	B
36	Neo	N
38	Neo	B
75	Sur	U
21	Int	U
15	Sur	U
89	Tra	U
93	Int	W
90	Sur	S
50	Int	U
10	Int	U
11	Sur	U
14	Tra	W
69	Sur	C
85	Neu	C
86	Int	S
97	Int	U
91	Int	S
27	Sur	U
28	Tra	U
55	Cen	S
7	Int	U
5	Neu	C
13	Sur	C
100	Neo	B
102	Int	U
98	Sur	U
43	Ped	U
107	Sur	S
88	Int	U
20	Int	S
44	Sur	S
76	Tra	U
62	Cen	S
70	Tra	S
65	Int	U
99	Int	U
29	Cen	S
19	Ped	U
108	Int	W
1	Sur	U
2	Int	S
51	Cen	C
109	Int	U
53	Int	U
59	Neu	U
57	Int	S
72	Sur	U
111	Tra	S
78	Cen	B
4	Cen	U
103	Cen	B
77	Sur	U
83	Int	U
80	Int	B
6	Int	U
74	Neu	C
45	Sur	W
87	Int	U
67	Neu	U
54	Cen	U
104	Int	U
56	Cen	S
33	Sur	U
71	Int	U
41	Ped	S
3	Int	S
30	Neu	W
79	Int	B
46	Neu	B
24	Cen	U
42	Sur	S
17	Tra	U
52	Neu	W
58	Neo	U
8	Sur	S
66	Cen	U
9	Sur	S
105	Sur	S
84	Int	B
60	Sur	B
61	Sur	B
35	Neo	E
40	Sur	E
110	Cen	S

Figure 1. Dendrogram constructed following AFLP patterns preparation and results of cluster analysis of all *A. baumannii* isolates
Int: Internal ICU, Sur: Surgical ICU, Cen: Central ICU, Neo: neonatal ICU, Neu: Neurosurgical ICU, Tra: Transplant ICU, Ped: Pediatric ICU, N: Nose secretion, E: Eye secretion, B: Blood, U: Urine, W: Wound, S: Sputum, C: CerebroSpinal Fluid

In agreement with the other studies [23, 24], our AFLP data demonstrated that respiratory and urinary tracts are considered important sources for spread of *A. baumannii* in ICUs of a referral hospital in the south (Shiraz) of Iran. It is the probable reason for spreading of clusters 1, 3 and 4 compared to small clusters such as 8-13, in which, clusters 1, 3 and 4 were isolated more from urine and sputum samples than from the other specimen, while clusters 8-13 are further isolated from blood. *A. baumannii* isolated from urine and sputum could cause the widespread outbreaks (such as clusters 1, 3 and 4) by creating persistent environmental contamination because of their easier dissemination and high resistance of their isolates to antibiotics compared to isolates from other clinical samples. Evaluating AFLP-dendrogram pattern outlined the probable cross-transmission of *A. baumannii* strains among the various ICUs. In this study the majorities of isolates from internal and surgical ICUs were found in frequent clusters especially large clusters 1, 3 and 4; therefore internal and surgical ICUs consider as a potential reservoir for distribution of *A. baumannii* in this hospital.

In conclusion, in the present study, respiratory and urinary tract of patients hospitalized in the internal and surgical ICUs have been suggested as potential reservoirs for strains of *A. baumannii*. Cross transmission of these isolates amongst ICUs/wards have contributed in development and emergence of drug resistance and extensive outbreaks in this hospital. Our findings emphasize necessity of applying appropriate strategies to control of *A. baumannii*.

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