

Molecular Analysis of the Isolates of *Acinetobacter baumannii* isolated from Tehran Hospitals Using ERIC-PCR Method

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Received:23 April 2018;Accepted:17 May 2018;

ABSTRACT

Background and Objective: *Acinetobacter* is a genus of non-fermenting Gram-negative cocci or coccobacilli, which have low nutritional requirements for growth and can survive for a long time in adverse conditions, on dry surfaces, and also in aqueous environments. The importance of the members of *Acinetobacter* genus as pathogens involved in nosocomial infections, is increasing. *Acinetobacter baumannii* is the most common species involved in a broad spectrum of nosocomial infections, including pneumonia, bacteremia, surgical wound infections, urinary tract infections, and meningitis. In this study, enterobacterial repetitive intergenic consensus sequence-based PCR (ERIC-PCR) technique was used for analysis and molecular typing of *Acinetobacter* strains, which has high discrimination power compared to phenotypic markers.

Methods: In the present study, a total of 40 *A. baumannii* strains were isolated from patients hospitalized in Tehran hospitals. After identification and confirmation of the isolates by serotyping and biochemical tests, a single colony of each isolate was cultured on liquid LB medium, and after DNA extraction, PCR was performed. After electrophoresis of PCR product, gel images were stored electronically for analysis and comparison of the isolates.

Results: In this study, 40 strains of *A. baumannii* were analyzed by ERIC-PCR method, of which 29 strains were typed into 10 groups and 11 other strains had no PCR bands or had a band that could not be assigned to any of the above groups.

Conclusion: In this study, it was found that *A. baumannii* strains could be typed using repetitive sequences. This extent of polymorphism shows that ERIC-PCR is a useful method for analysis of genetic variation of *A. baumannii* strains. High genetic variation of *A. baumannii* strains may be due to wide geographical distribution of this species in Iran

Keywords: *Acinetobacter baumannii*, ERIC-PCR, Molecular Typing.

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Introduction

Genus *Acinetobacter* is a heterogeneous group of bacteria, which is composed of 22 genomic species based on DNA-DNA hybridization (1). The importance of members of genus *Acinetobacter*, as pathogens involved in nosocomial infections,

is increasing (2). *Acinetobacter baumannii* is the most prevalent type involved in a broad spectrum of nosocomial infections, such as pneumonia, bacteremia, surgical wound infection, urinary tract infection, and meningitis (3-5). The long-term survival potential of this organism in hospital environment as well as its capability of

acquiring drug resistance genes has increased their role in nosocomial infections (6).

Phenotypic and genotypic techniques are used to design effective strategies for controlling infections caused by this bacterium (7). Phenotypic techniques include evaluation of biochemical characteristics, phage typing, bacteriocin typing, immunoblotting, protein analysis, and identification of antimicrobial resistance patterns (8). These techniques are not powerful enough to identify and differentiate *Acinetobacter* strains and cannot determine the source and ways of distribution and effective measures for controlling the spread of infection and prevalent isolates (9). In recent years, genotypic techniques using specific molecular markers are used for epidemiological studies and typing of *Acinetobacter* species. These techniques include ribotyping, plasmid analysis, PFGE, chromosomal RFLP, RAPD-PCR, AP-PCR, REP-PCR, and ERIC-PCR (10). These techniques are ribotyping, plasmid analysis, PFGE, chromosomal RFLP, RFLP-PCR, RAPD-PCR, AP-PCR, REP-PCR, and ERIC-PCR (11-13).

Repetitive sequences between genes, which are called symmetric elements, are often seen in non-coding sections of DNA (14). Given the variable number and length of these repetitive sequences, the number of bands obtained for each strain is different, and therefore the strains are classified based on diversity of bands (15). In the present study, genetic variation of *A. baumannii* isolates was evaluated using ERIC-PCR method.

Materials and Methods

Bacterial samples

In the present study, a total of 40 isolates of *A. baumannii* isolated from patients hospitalized in Tehran hospitals, were analyzed. Isolates were identified after purification on MacConkey agar using selective media, such as Simmons' citrate

agar and TSI.

DNA extraction

In order to extract DNA, first, one colony of the bacterium in LB media was incubated at 37°C for 18-24 h. After incubation, the bacterial suspension was centrifuged at 3000 rpm for 5 min and the sediment was used for DNA extraction. DNA extraction was performed using Diatom kit, which has been designed based on the use of GuSCN-Silica Gel.

Polymerase chain reaction

After DNA extraction, each sample was stored in -20°C. Specific ERIC primers were used for PCR and their sequence were as follows (15):

ERIC1: 5' -ATGTAAGCTCCTGGGGATTAC-3'

ERIC2: 5' -AAGTAAGTGACTGGGGTGAGCG-3'

In order to amplify the desired sequence, in a volume of 50 µl, 2.5 units of Taq polymerase, 50 pM of each primer, 200 µM of each four deoxynucleotides, 1 µl of template DNA, 1.5 µM of MgCl₂, 10 mM of Tris-Hcl (pH 8.3), and 50 mM of KCl, were used.

PCR was performed using an Eppendorf gradient thermocycler with the following conditions: 5 min of initial denaturation at 95°C, then 40 cycles at 95°C for 30 s, 51°C for 1 min, 65°C for 8 min, and finally 72°C for 10 min for amplification of incomplete segments.

Observation of PCR products

About 10 µl of each PCR product was loaded into each well of the gel, and electrophoresis was carried out for 1 h at 90 V. The electrophoresis gel was placed on a UV transilluminator and picture was taken from the visible bands. The gel images were saved electronically for further analysis and typing of the isolates.

Results

After biochemical and serotyping confirmation, 40 *Acinetobacter* isolates were identified as *A. baumannii* and then analyzed by ERIC-PCR method.

After analysis of the results, 29 strains were typed into 10 groups (all from Shariati hospital) and 11 other strains had no PCR bands or had a band that could not be assigned to any of above groups. The frequency of genetic patterns of *A. baumannii* determined by ERIC-PCR is shown in the Table 1. Most of the isolates with the same pattern were placed in group 1.

Table 1
The frequency of genetic patterns of *A. baumannii* by ERIC-PCR method

No.	Strain group
1	2, 20, 23, 26, 27, 28, 32
2	4, 14
3	17, 18
4	21, 22
5	16, 19
6	3, 15
7	8, 9, 10, 11
8	37, 38, 39, 40
9	12, 13
10	35, 36

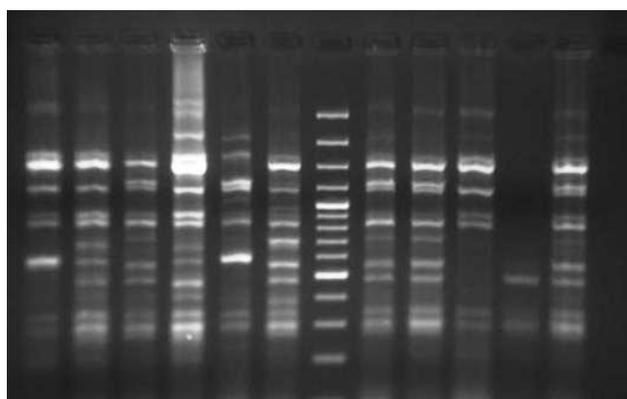


Fig. 1
An example of electrophoresis gel image, a product of PCR strains of *A. baumannii* by ERIC-PCR method. M is molecular marker and columns 1 to 11 are isolates 14, 20, 23, 24, 25, 26, 27, 28, 30, 31, and 32, respectively.

Discussion

Up to now, many studies have been conducted on typing of *A. baumannii* strain by different genotypic and phenotypic methods, and different genetic profiles have been reported in various countries (16-19). All profiles identified in this study (conventionally named as numbers 1 to 10), were classified into 10 patterns that were observed in 29 isolates. Also, a number of isolates had a special pattern and were not classified into any of the 10 groups. ERIC-PCR results showed that although there were no band patterns or similar profiles among the Iranian *Acinetobacter* strains, the existence of 10 different genetic profiles indicated genotypic variability and distribution of the Iranian strains. The diversity of genetic pattern among the isolates is due to wide variety of hospitals and hospital wards and no epidemic outbreak occurrence by this bacterium in the studied hospitals during the study period, otherwise several strains with identical genetic patterns and common origin, were being definitely observed. In the present study, most of the isolates with identical genetic pattern were placed in the group 1, in which 7 isolates had identical genetic profiles. all strains of this group had been isolated from Shariati Hospital.

Investigation of genetic similarity and diversity among different strains of a bacterial species by DNA fingerprinting is a useful method for detection of strains involved in outbreaks as well as determination of epidemiological relationship among the isolates.

Identification of an acceptable level of genetic diversity among the isolates by this technique, indicated that this method is useful for studying and typing *A. baumannii* isolates, and isolates with different origins can be classified into different groups using this method.

From the results of this study, it is concluded that repetitive sequences can be used for typing of *A. baumannii* strains and this level of

polymorphism shows that this technique is useful for analysis of genetic variation in *A. baumannii* strains. It was also found that relatively high genetic diversity in *A. baumannii* populations may be attributed to wide geographic distribution of this species in Iran.

Acknowledgement

Thanks to Bahar Tashkhis Teb Inc. for free translation and scientific editing services.

Conflict of interest

The authors declare that there is no conflict of interests.

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How to cite this article

Maleki A, Vandyousefi J, Mirzaie Z, Ghafourian S, Kazemian H, Sadeghifard N. Molecular Analysis of the Isolates of *Acinetobacter baumannii* Isolated from Tehran Hospitals Using ERIC-PCR Method. Mod Med Lab J. 2018;1(1):12-16.