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ORIGINAL ARTICLE / ÖZGÜN MAKALE

GENETIC DIVERSITY OF *ACINETOBACTER BAUMANNII* STRAINS ISOLATED FROM DIFFERENT HOSPITALS

FARKLI HASTANELERDEN İZOLE EDİLEN ACİNETOBACTER BAUMANNİİ SUŞLARININ GENETİK ÇEŞİTLİLİĞİ

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ABSTRACT

Objective: *The genotypic analysis of the strains can provide information to evaluate the genetic relationships among strains and epidemiological investigations, and it is crucial for monitoring their circulation in different geographic regions. This study was to aim to identify genetic similarities or dissimilarities among clinical Acinetobacter baumannii (A. baumannii) isolates from four different hospitals.*

Material and Method: *In this study, 78 non-duplicate clinical isolates of A. baumannii were received from patients in the critical care units. The colistin MIC values of 24 A. baumannii strains randomly selected from four different hospitals and known to have antibiotic susceptibility were determined. These strains were genetically characterized by the Enterobacterial repetitive intergenic consensus (ERIC)-PCR method.*

Result and Discussion: *The results of the study showed that the isolates were divided into 2 clusters (A1 and A2) and Cluster A2 was represented by a single genotype (C1) and 23 interrelated genotypes were in Cluster A1.*

Keywords: *Acinetobacter baumannii, ERIC-PCR, genetic diversity, resistance*

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ÖZ

Amaç: *Suşların genotipik analizi, suşlar arasındaki genetik ilişkileri ve epidemiyolojik araştırmaları değerlendirmek için bilgi sağlayabilir ve farklı coğrafi bölgelerdeki dolaşımlarının izlenmesi için önemlidir. Bu çalışma, dört farklı hastaneden elde edilen klinik Acinetobacter baumannii (A. baumannii) izolatları arasındaki genetik benzerlik veya farklılıkları belirlemeyi amaçlamıştır.*

Gereç ve Yöntem: *Bu çalışmada, yoğun bakım ünitelerindeki hastalardan 78 A. baumannii klinik izolatı alınmıştır. Dört farklı hastaneden rastgele seçilen ve antibiyotik duyarlılığı bilinen 24 A. baumannii suşunun kolistin MIK değeri belirlenmiştir. Bu suşlar, Enterobacterial repetitive intergenic consensus (ERIC)-PCR yöntemi ile genetik olarak karakterize edilmiştir.*

Sonuç ve Tartışma: *Çalışmanın sonucunda, izolatların 2 kümeye (A1 ve A2) ayrıldığı ve Küme A2'nin tek bir genotip (C1) ile temsil edildiği ve birbiriyle ilişkili 23 genotipin Küme A1'de olduğunu gösterilmiştir.*

Anahtar Kelimeler: *Acinetobacter baumannii, direnç, ERIC-PCR, genetik çeşitlilik*

INTRODUCTION

A. baumannii is an opportunistic pathogen that causes a wide range of serious nosocomial infections such as urinary tract infections, ventilator-associated pneumonia, and bloodstream infections [1]. The natural resistance of *A. baumannii* to dryness and disinfectants and the acquisition of a wide variety of resistance mechanisms through the gain of genetic elements such as plasmids, integrons, and transposons ensure its long-term survival in the environment, making it difficult to eradicate from the clinical environment and fight infection [2]. In recent years, multidrug-resistant strains of *A. baumannii* have spread worldwide. These resistant strains cause long hospital stays, increased costs, and high mortality rates. In addition, the emergence of pan-resistant species has become a serious clinical problem. Because a limited number of antibiotics are effective for such strains. *A. baumannii* is resistant to many antibacterial agents, with the inclusion of carbapenems and sometimes colistin, in most cases, optimal treatment is not available for nosocomial infections caused by this microorganism. Therefore, a great deal of research has been done recently to elucidate the characteristics of Acinetobacter infection, including nosocomial transmission, infection risk factors, drug-resistant strains, and effective treatments [3, 4]. In addition, the virulence characteristics and pathogenic potential of *A. baumannii* are often not fully elucidated. With the expansion of the *A. baumannii* sequence database over time, the development of a large array phylogenomic and phenotypic analyzes that provide insights into its evolution and adaptation as a human pathogen has therefore become important [5, 6].

Genotypic techniques using specific molecular markers are widely used for typing Acinetobacter species. These techniques are Restriction Fragment Length Polymorphism (RFLP), Randomly amplified polymorphic DNA analysis (RAPD), Arbitrarily PCR (APPCR), Repetitive extragenic palindromic PCR (REP-PCR), Enterobacterial Repetitive Intergenic Consensus PCR (ERIC- PCR) and Pulse-field gel electrophoresis (PFGE) [7]. *A. baumanni* has sequences of repeats called symmetrical elements are present and are usually found in the non-coding part of the DNA. Enterobacterial recurrent intergenic consensus (ERIC)-PCR-based genotyping models can be particularly helpful to determine the circulation of epidemic clones and are used to differentiate bacterial types by conserved and repeated sequences in bacteria or some fungi. Compared to other methods, polymerase chain reaction (PCR)-based fingerprinting methods are easier and more cost-effective to implement [8]. In this study, 24 out of 78 *A. baumannii* isolates from four different hospitals were used to analyze the genetic variation using the ERIC-PCR method.

MATERIAL AND METHOD

Strain Collection

A total of 78 non-duplicate clinical isolates of *A. baumannii* were isolated from the samples received from patients in the critical care units (intensive care, burns, post-operative areas) of four different hospitals (Ankara Numune Training and Research Hospital Medical Microbiology Laboratory,

Ankara University Ibni Sina Hospital Central Microbiology Laboratory, Ankara University Cebeci Hospital Central Microbiology Laboratory, Dr. Sami Ulus Gynecology, Obstetrics and Child Health and Diseases Training and Research Hospital Microbiology Laboratories), Ankara, Turkey between July 2018 and June 2019. The isolates were collected from respiratory secretions sputum, pleural fluid, urine, wound, and blood. *A. baumannii* was identified in the clinical microbiology laboratory by standard biochemical tests as well as the Vitek 2 system (bioMérieux). Culture confirmation was done using MALDI-TOF MS (Bruker, ABD). The study was approved by the Ankara University Faculty of Medicine Clinical Research Ethics Committee. Antibiotic susceptibilities were determined by the standard disc diffusion method and interpreted according to the guidelines of the EUCAST (European Committee on Antimicrobial Susceptibility Testing) [9]. The colistin MIC (Minimal Inhibition Concentration) values of the strains were determined by the broth microdilution method with the recommendations of EUCAST 2020-01-01 [10].

Isolation of Bacterial DNA

The bacterial genomic DNA was prepared by using the lysis buffer (4 M Guanidium HCl, 1% Triton X-100, 25 mM Sodium citrate pH 7, 100 mM B-Mercaptoethanol). Briefly, colonies grown in fresh cultures of strains were collected with loops, suspended in a liquid medium, and incubated overnight at 37ºC.

The concentrations of the fresh bacterial suspensions prepared were adjusted to be similar in the spectrophotometer. Then, the bacterial suspensions were centrifuged at 10000 rpm in an Eppendorf tube and the supernatant was discarded. Lysis buffer was added to the precipitate, then centrifuged and treated with phenol-chloroform-alcohol.

Molecular Typing by ERIC-PCR Analysis

Molecular typing of randomly selecting 24 *A. baumannii* strains from four hospitals was performed by using the ERIC-PCR method [11]. For this purpose, the DNAs of all bacteria were obtained and quantified spectrophotometrically. Optimization studies were carried out to obtain suitable band profiles in PCR. Firstly, different primer annealing temperatures were tested. Afterwards, it was tried to increase the number of bands and move away from the smear image by increasing the primer concentrations and changing the Mg^{++} concentration by 1-7 mM. All strains were performed simultaneously after PCR optimization. The primer sequences, PCR mix, and temperature cycle used in the ERIC-PCR reaction were given in Table 1. After the reaction, amplicons were run on a 1.6% agarose gel at 65 volts for approximately 2 hours. The gel was then stained with ethidium bromide solution and recorded by photographing the bands under UV light on the VersaDoc (Bio-Rad) imaging system.

Table 1. Primer sequences, PCR mix, and temperature cycling were used in the ERIC-PCR reaction.

Primers	
ERIC 1	5' ATGT AAG CTC CTG GGG ATT CAC 3'
ERIC 2	5' AAG TAA GTG ACT GGG GTG AGC G 3'
PCR Mix	
dNTP (dA+dT+dG+dC)	0.2 mM
Primer 1: ERIC1	0.8 µM
Primer 2: ERIC2	0.8 µM
$MgCl_2$	4.5 mM

Table 1 (*continue*). Primer sequences, PCR mix, and temperature cycling were used in the ERIC-PCR reaction.

PCR Mix			
DreamTaq Buffer	1x		
DreamTaq	0.5 u		
Template DNA	100 ng		
H_2O			
Temperature cycle	**Temperature (°C)**	**Time (second)**	**Cycles**
Initial denaturation	94	120	1
Denaturation	94	15	40
Annealing	52	30	
Extension	72	120	
Final extension	72	300	1

RESULT AND DISCUSSION

Bacterial Isolates and Antimicrobial Susceptibility Pattern

A. baumannii causes important hospital infections, especially in intensive care units [12]. Long hospitalization period, stay in the intensive care unit, parenteral nutrition, invasive interventions, mechanical ventilation, and device use (permanent catheter, endotracheal tube, etc.) are among the known risk factors for *A. baumannii* colonization and infection [13]. In treating *A. baumannii* infections, it is important to consider the different treatment options available when considering resistance profiles. *A. baumannii* is currently resistant to many antibacterial agents. Although sulbactam was used to treat carbapenem-resistant clinical isolates against *A. baumannii* ten years ago, today the percentage of sulbactam resistance has also increased and it has been used in combination with β-lactam antibiotics. Similarly, in the treatment of nosocomial infections caused by *A. baumannii*, it is recommended to use combined regimens with other antimicrobials to prevent the possible development of resistance during treatment with tigecycline [14,15]. According to antimicrobial susceptibility test results, all strains were determined as multi-drug resistant. The colistin MIC values of *A. baumannii* isolates found ranged between 0.125-64 mg/l. It was determined that 8 (10.2%) of these strains were resistant to colistin. The distribution of MIC values of the isolates was given in Figure 1.

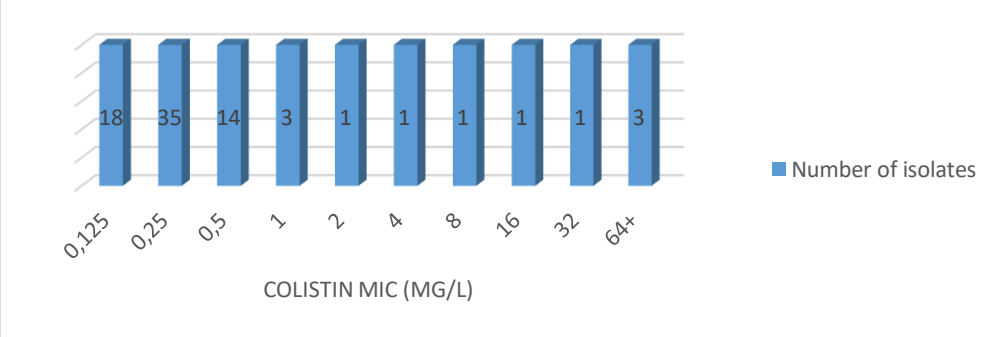

Figure 1. MIC values of *A. baumannii* isolates

ERIC-PCR Analysis

Post-reaction amplicons indicated in Figure 2 were run on a 1.6% agarose gel at 65 volts for approximately 2 hours. They were stained with ethidium bromide solution and images were taken in a UV transilluminator (VersaDoc, Bio-Rad). Band profiles were analyzed using the GelAnalyzer2010a program. By using the UPGMA (Unweighted pair group method with mathematical averaging) method and the Dice similarity coefficient, the dendrogram of the band profiles was created and cluster analysis was performed. Cophenetic Correlation Coefficient (CP) = 0.84. FigTree v1.4.4. the phylogenetic tree was created with the program and given in Figure 3. It appears that the strains were collected in 2 clusters (A1 and A2) and cluster A2 was represented by a single genotype (C1). It was observed that there were 23 interrelated genotypes in cluster A1. It was observed that the genotypes of C7 and C3, SU3 and A7, M1 and C10, M24 and M20, M34 and M27, M6 and M2, SU1 and A4 were 99% identical to each other in Cluster A1. The SU3 and A7 genotypes were 98% identical to the C5 genotype. M1 and C10 genotypes were 98% identical to the A1 genotype. These two groups were 97% linked. There was a 96% similarity between these groups and the A8 genotype. It was observed that this group was associated with the genotype C7 and C3 at a rate of 95%. M2 and M6 genotypes were 98% identical to the M7 genotype. It was observed that M20, M24, M27, and M34 genotypes isolated from the same hospital had a 98% correlation with each other. They were 97% identical to the C19 genotype. It was found that the group with M2, M6, and M7 was 96% identical to this group. And SU1 and A6 genotypes were 98% identical to the C22 genotype.

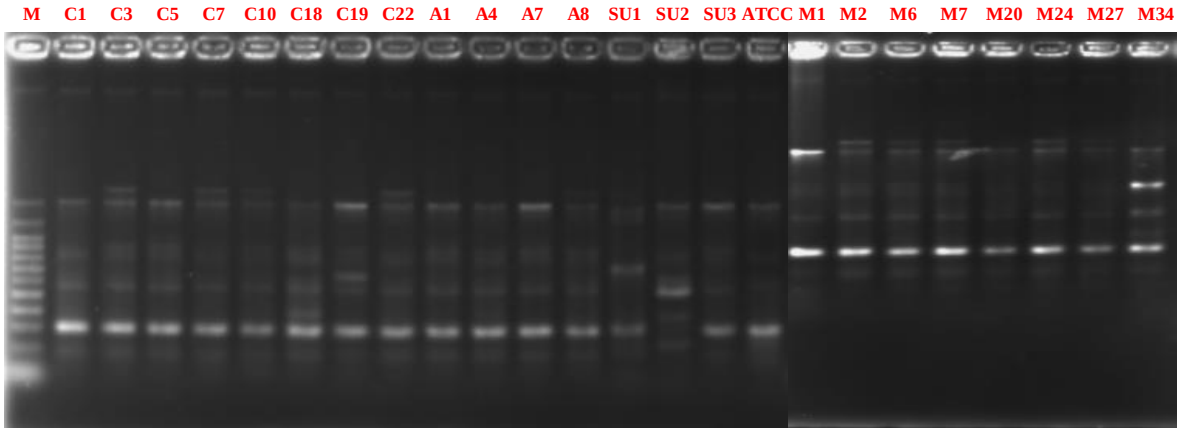

Figure 2. ERIC-PCR gel images of *A. baumannii* isolates (M. Marker)

Among the PCR-based fingerprint techniques, ERIC-PCR is preferred as an economical, simple, and fast method used to identify and distinguish Acinetobacter species [16]. Therefore, it is important to control infections in hospitals and periodically monitor antimicrobial resistance patterns in different regions for infection control [17]. The study conducted by Fallah et al. investigated the genetic relationships of 82 *A. baumannii* isolates from a burn hospital with ERIC-PCR and revealed 14 different ERIC models, including 11 common types and three unique types [18]. Gautam et al. determined the clonal diversity of 22 *A. baumannii* from different clinics of a hospital and obtained 15 different patterns, 13 of which were unique and 2 were common, from the ERIC-PCR analysis [19]. Lotfi et al. examined the genetic relationship of 127 *A. baumannii* isolates from two teaching hospitals by ERIC-PCR and found that 88 isolates were divided into seven groups, while the remaining 21 isolates were single [20]. In our study, clonal diversity was determined in ERIC-PCR analysis, and according to the results of this study, for 24 *A. baumannii* and 24 different patterns were obtained, one unique and 23 common ERIC patterns.

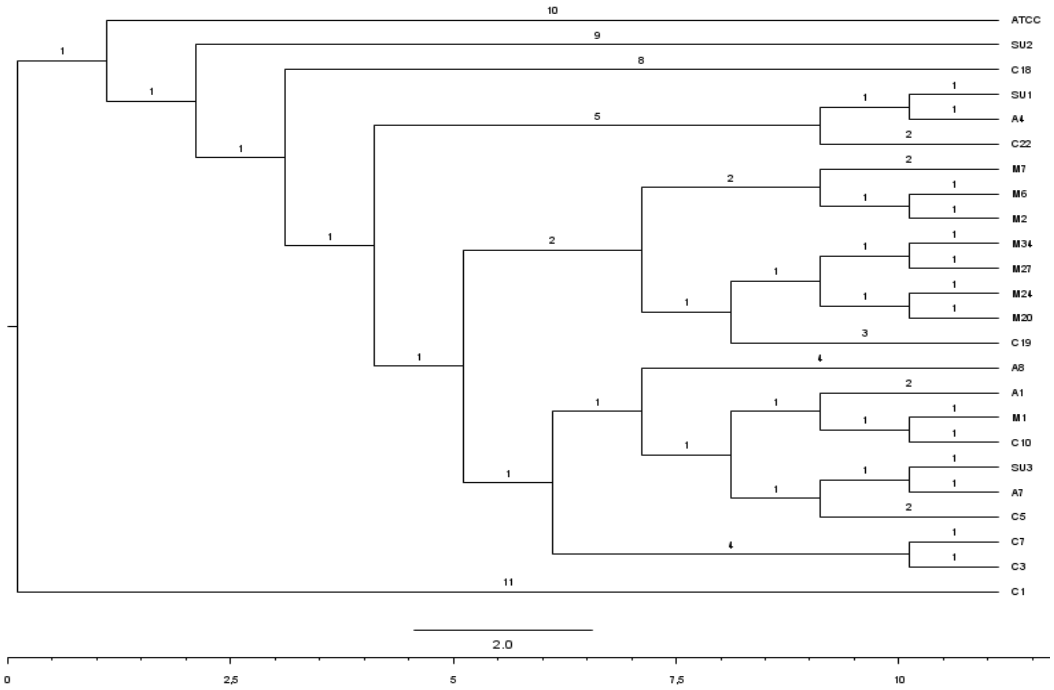

Figure 3. ERIC-PCR dendrogram diagram of *A. baumannii* isolates

In this study, the ERIC-PCR technique was used to see the genetic distribution of *A. baumannii* strains which cause common nosocomial infections. Overall, the findings of this study showed that the ERIC-PCR method is a viable method to reveal the genetic diversity of different *A. baumannii* strains. The results are important in that the samples were taken from different hospitals and that the samples were closely related to each other. It is recommended that the study be performed with a larger profile bacterial group and the results should be compared with newer molecular methods such as Pulsed Field Gel Electrophoresis (PFGE).

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AUTHOR CONTRIBUTIONS

Concept: H.B.E.; Design: H.B.E., B.K., N.A., S.Y.; Control: H.B.E., B.K., N.A., S.Y., SY.; Sources: H.B.E., B.K.; Materials: H.B.E., B.K., N.A., S.Y., SY.; Data Collection and/or Processing: H.B.E., B.K., N.A., S.Y., SY.; Analysis and/or Interpretation: H.B.E., B.K., N.A., S.Y., SY.; Literature Review: H.B.E., B.K., N.A., S.Y., SY.; Manuscript Writing: H.B.E., B.K., N.A., S.Y., SY.; Critical Review: H.B.E., B.K., N.A., S.Y., SY.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

This study was approved by the Ankara University Faculty of Medicine Clinical Research Ethics Committee (2018). Ethics Committee approval was obtained with decision no. 11-748-18 and dated 25 June 2018.

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