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RESEARCH ARTICLE

Epidemiology of Acinetobacter baumannii isolates from patients with severe sepsis in anesthesia intensive care unit

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Epidemiology of Acinetobacter baumannii isolates from patients with severe sepsis in anesthesia intensive care unit

Summary

Aim: Acinetobacter baumannii is considered as an emerging nosocomial pathogen in intensive care units. The most frequent clinical manifestation is sepsis. The aim of this study was to make an epidemiological surveillance of A.baumannii blood isolates from severe sepsis patients.

Methods: Blood samples were collected from 34 patients with severe sepsis which has occured in anesthesia intensive care unit treatment conscutive three months period. In 11 of these blood samples A.baumannii was identified, DNA isolated and Randomly Amplified Polymorphic DNA (RAPD)-PCR fingerprinting was performed for genotyping.

Results: DNA fingerprints identified 5 distinct strains in 11 patients and the similarity level was at the 70% between the most distant strains.

Conclusions: This study gives an idea about the sources of the strains of A. baumannii in the studied intensive care unit. But more comprehensive studies are needed to prevent hospital-acquired infections via determining the sources of bacteria..

Key Words: *Acinetobacter baumannii, Sepsis, RAPD-PCR, Epidemiology.*

Anestezi Yoğun Bakım Ünitesinde Ağır Sepsisli Hastalardan İzole Edilen Acinetobacter baumannii İzolatlarının Epidemiyolojisi

Özet

Amaç: Acinetobacter baumannii yoğun bakım ünitelerinde ortaya çıkan bir nozokomiyal patojen olarak kabul edilmektedir. En sık klinik belirtisi sepsistir. Bu çalışmada, ağır sepsis hastalarının kanlarından elde edilen A. baumannii izolatlarının epidemiyolojik olarak incelenmesi amaçlanmıştır.

Metod: Anestezi yoğun bakım ünitesinde üç aylık ard arda bir tedavi sürecinde ağır sepsis gelişmiş 34 hastadan kan örnekleri toplanmıştır. Bu kan örneklerinin 11'inde A. baumannii belirlenmiş ve DNA izole edilerek genotipleme için Rastgele Arttırılmış Polimofik DNA (RAPD) PCR parmakizi yöntemi kullanılmıştır.

Sonuçlar: DNA parmak izleri 11 hastada 5 farklı genotip olduğunu ortaya koymuş ve benzerlik seviyesi en uzak suşlar arasında % 70 olarak belirlenmiştir.

Tartışma: Bu çalışma çalışmanın yürütüldüğü yoğun bakım ünitesinde A. baumannii suşlarının kaynakları hakkında bir fikir vermektedir. Ancak bakteri kaynaklarının belirlenmesi yolu ile hastane enfeksiyonlarının önlenmesi için daha kapsamlı çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: *Acinetobacter baumannii, Sepsis, RAPD-PCR, Epidemiyoloji.*

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INTRODUCTION

Acinetobacter are strictly aerobic gram negative coccobacilli that are widely distributed in soil and water, but also commonly found in the hospital environment (1). Genus *Acinetobacter baumannii* is considered as an emerging nosocomial pathogen in intensive care units. The most frequent clinical manifestation is sepsis and a fulminating course is observed when the patient presents with septic shock (2-5). Hospital-acquired infections caused by *A. baumannii* cause serious economic loss and a high mortality rate. In the control of the hospital-acquired infections, it is crucial that the clonal relationships between epidemic species and strains are determined and that the source is found for related strains (6). Discriminating and reproducible methods are required for the identification of pathogenic isolates to determine the origins of infection, the routes of transmission, and the duration of their persistence (7,8). A DNA fingerprinting analysis method based on randomly amplified polymorphic DNA (RAPD) is being used increasingly in many microbiology laboratories for epidemiological typing of an ever-increasing range of bacteria (9,10). The aim of this study was: revealing the source and mode of transmission of *A. baumannii* and emphasizing the significance of environmental contamination in our intensive care unit.

MATERIAL AND METHODS

Blood samples were collected from 34 patients with severe sepsis that has developed in intensive care unit treatment consecutive three months period (April-May-June 2011) from the Department of Anesthesiology and Reanimation, Medical Faculty, Eskisehir Osmangazi University. Informed consent in accordance with the study protocol, approved by the ethics committee of Medical Faculty, Eskisehir Osmangazi University, Eskisehir, was obtained from each patient.

Blood samples in Bactec Plus (30 ml) blood culture bottles were placed appropriately in Bactec 9240 automated blood culture device. Samples with breeding signal as a result of fluorometric meauserement of Bactec 9240 automated blood culture device during the incubation process were inoculated to mediums with blood, Eosin Methylene-blue (EMB) and chocolate. Breeding colonies in the mediums were purely passaged and processed for identification and antibiotic sensitivity. In 11 of blood samples *A. baumannii* was identified. Reasons of patients for being in intensive care unit are given in Table 1. Identified strains were stored purely in skim milk at -70 °C until DNA isolation.

Table 1. Reasons of patients for being in intensive care unit

Patient Number	Reason
1	Multiple Trauma
2	Postoperative Respiratory Failure
3	Multiple Trauma
4	Multiple Trauma
5	Post Resuscitation Syndrome
6	Multiple Trauma
7	Anterior Myocardial Infarction
8	Multiple Trauma
9	Post Resuscitation Syndrome
10	Postoperative Respiratory Failure
11	Multiple Trauma

Bacterial DNA isolation was carried out according to kit procedure (Vivantis, Malesia). RAPD-PCR was performed in a 50 µl reaction mixture containing 1-2 µl of template DNA, 10X PCR buffer, 2 mM dNTP, 5 U of Taq DNA polymerase, 25 mM $MgCl_2$ and 100 pmol of each primer. These primers were for M13 5′-GAGGGTGGCGGTTCT-3′ and for DAF4 5′-CGGCAGCGCC-3′ (Biomers, Germany). DNA amplification fingerprinting (DAF) and core region of bacteriophage M13 is a strategy for genetic typing and mapping that uses one or more very short (≥5 nt) arbitrary oligonucleotides to direct the enzymatic amplification of discrete portions of a DNA template resulting in a spectrum of products characteristic of the DNA starting material (11). These patterns are strain specific and useful for epidemiological typing (12). Thus, RAPD analysis is a useful approach to resolve urgent questions regarding the possible epidemiological relatedness of small sets of strains at the hospital

level. However, it is not useful for longitudinal or interlaboratory studies with large numbers of strains (13). The PCR reaction was performed in a thermo cycler (Corbett Palm Cycler, Australia) using the following cyclic conditions: 2 cycles with denaturation at 94°C for 5 min, annealing at 40°C for 5 min, extension at 72 °C for 5 min and 40 cyles with denaturation at 94°C for 1 min, annealing at 40 °C for 1 min, extension at 72 °C for 2 min adapted to Vila et al (14). The PCR products were separated in 2% agarose gel with 4 µL of ethidium bromide (10mg/ml), and then were visualized using a CCD camera (Wealtec Dolphin-DOC). Gel photograph of RAPD-PCR fingerprinting patterns were loaded to TotalLab TL120 program (Shimadzu Biotech). DNA bands of patterns were signed and base pair (bp) lengths were calculated by programme according to 100 bp DNA marker (Vivantis, Malesia) which was used as a control (Because of the feature of the programme DNA marker and bp lengths of DNA bands are not shown in Figure of dendrogram). After calculation of bp lengths, dendrogram of RAPD-PCR patterns derived by the programme. Patterns which showed 100% similarity considered to be the same genotype.

RESULTS

A dendrogram is a branching diagram representing a hierarchy of categories based on degree of similarity or number of shared characteristics especially in biological taxonomy. In this study dendrogram of the RAPD-PCR fingerprinting patterns yielded 5 genotypes in 11 patients. The numbers on horizontal of the dendrogram are percentages of similarity and the numbers on vertical of the dendrogram are patient numbers (PN). Genotype 1, 4 and 5 were found in one patient PN 5, 2 and 9 respectively. Genotype 2 was found in four patient (PN: 1,3,6,7) and genotype 3 was found in four patients (PN: 4,8,10,11) at the 100 % similarity level. The similarity level was at the 70% between the most distant genotypes 4 an 5. The similarity level was at the 94 % between the most nearest genotypes 1 an 2 (Figure 1).

DISCUSSION

We detected 5 different *A. baumannii* genotypes in 11 blood isolates in our study within a period of 3 months. But we did not obtain cultures from the hands of healthcare professionals and the materials in intensive care unit. So emergency of genotype 2 and genotype 3 in four patients should be

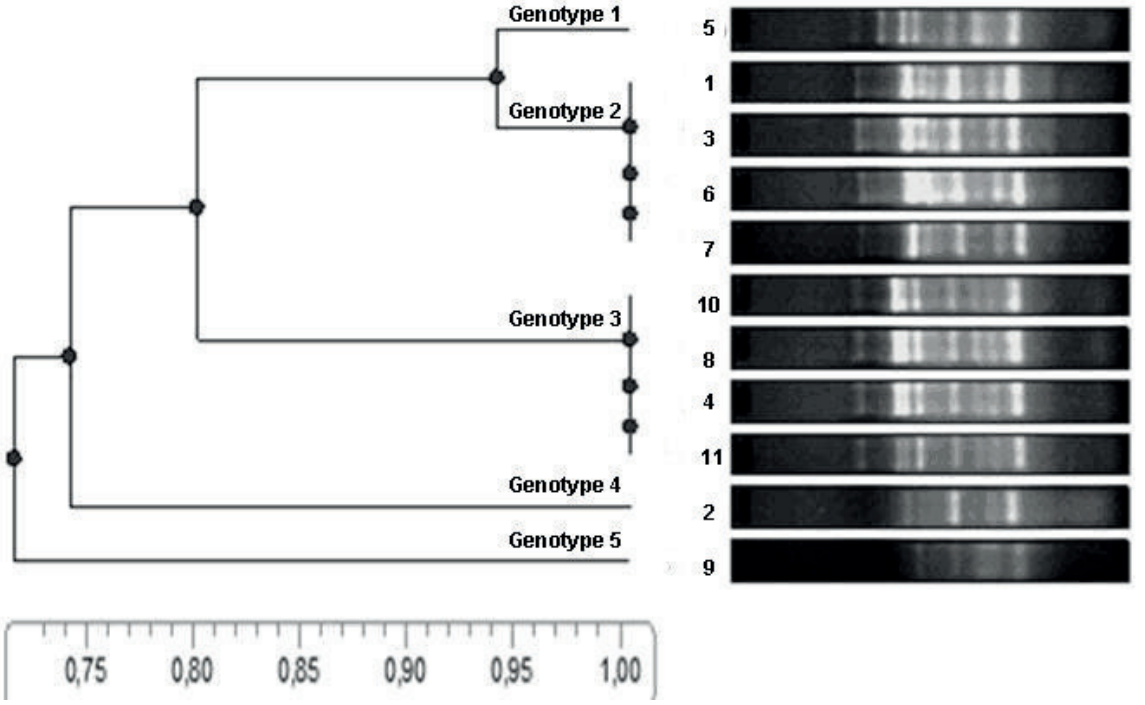

Figure 1. Dendrogram derived from analysis of the RAPD-PCR patterns of *A. baumannii*

explained with cross-contamination, environmental contamination, and airborne transmission in our intensive care unit. We also did not obtain cultures from other departments of our hospital. Genotype 1, 4 and 5 were found in one patient and this situation should be explained with patient transfer from other departments. There have been various studies reporting that cross-contamination, patient transfer, environmental contamination, and airborne transmission play important roles in epidemics of *A. baumannii* infection.(6,15-17). In further studies to identifiy sources of *A. Baumannii*, samples from the hospital and intensive care unit environment and from the hands of staff may be isolated for RAPD-PCR fingerprinting. Thus after the disinfection of the sources of *A. Baumannii*, spread of *A. baumannii* and infections like sepsis in intensive care unit due to *A. baumannii* could be prevented. Akalın et al. also reported antibiotic susceptibility profiles of the detected genotypes of *A. baumannii* during 3 consecutive study periods (6). Antibiotic susceptibility profiles and long term studies should also be considered for futher studies.

When we compared the RAPD-PCR fingerprinting method used in our study with other molecular methods; there have been other molecular epidemiological studies of *A. baumannii* performed by both Pulsed field gel electrophoresis (PFGE) and RAPD-PCR fingerprinting. Bou et al. showed that PFGE is superior to RAPD-PCR fingerprinting RAPD-PCR fingerprinting (18). But in another study, it was shown that the separating potential of PFGE and RAPD-PCR fingerprinting is similar (19).

As a conclusion this study gives an idea about the source of the strains of *A. baumannii* in the studied intensive care unit. But more comprehensive studies are needed to prevent hospital-acquired infections via determining the sources of bacteria.

Conflict of Interest

The authors declare that they have no conflict of interest.

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