RESEARCH ARTICLE / ARAŞTIRMA MAKALESI

Molecular Typing With PFGE Method of Methicillin-Resistant Staphylococcus Aureus Isolates From in A University Hospital

Bir Üniversite Hastanesinden Izole Edilen Metisilin Dirençli Stapylococcus Aureus Izolatlarının PFGE Yöntemi ile Moleküler Tiplendirilmesi

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Abstract

Objective Staphylococcus aureus is a pathogen that causes many infections in humans, and the emergence of methicillin-resistant (MRSA) isolates in the last fifty years poses a threat to society and hospitals. Preventing the spread of MRSA strains between and within hospitals can only be achieved with a correct typing. The PFGE method is considered to be the gold standard among molecular typing methods.

Materials and Methods In this study, a clonal relationship between nine methicillin-resistant S. aureus isolates isolated from Van Yüzüncü Yıl University hospital was determined .Pfge analysis was done with the Bionumerics program. The similarity index was created at a tolerance value of 1% using the "Dice" coefficient. The clustering relationship between isolates was demonstrated using the UPGMA (unweighted pair group method of arithmatic averages) method. The principles set out by Tenover et al. were adopted for the evaluation of genotypic association between isolates

Results As a result of the analysis 4 independent genotypes were identified. It was determined that there were 2 related isolates in 3 genotypes.

Conclusion In our study, it was determined that there was not a dominant MRSA clone in different years in the hospital studied, since samples from different years were studied. However, it is thought that it would be beneficial to increase the precautions in order to prevent the distribution of infection in all hospitals and to control the infection in all areas.

Keywords Methicillin-Resistant Staphyococcus aureus, Molecular Typing, Pulsed Field Gel Electrophoresis, Molecular Epidemiology

Öz

Amaç Staphylococcus aureus insanlarda birçok enfeksiyona neden olan bir patojen olup, son elli yılda metisiline dirençli (MRSA) izolatların ortaya çıkması toplum ve hastaneler için tehdit oluşturmaktadır. MRSA suşlarının hastaneler arasında ve hastaneler içinde yayılmasının önlenmesi ancak doğru bir tipleme ile sağlanabilir. PFGE yöntemi, moleküler tipleme yöntemleri arasında altın standart olarak kabul edilmektedir.

Gereç ve Yöntemle Bu çalışmada, Van Yüzüncü Yıl Üniversitesi hastanesinden izole edilen dokuz metisiline dirençli S. aureus izolatı arasında klonal bir ilişki belirlendi. Bionumerics programı ile PFGE analizi yapıldı. Benzerlik indeksi "Dice" katsayısı kullanılarak %1 tolerans değerinde oluşturuldu. İzolatlar arasındaki kümeleme ilişkisi, UPGMA (ağırlıksız çift grup aritmatik ortalamalar yöntemi) yöntemi kullanılarak gösterildi. Tenover ve arkadaşlarının kriterleri izolatlar arasındaki genotipik ilişkinin değerlendirilmesi için kabul edildi.

Bulgular Analiz sonucunda 4 bağımsız genotip tespit edilmiştir. 3 genotipte 2 ilişkili izolat olduğu belirlendi.

Sonuç Çalışmamızda, farklı yıllardan örnekler çalışıldığı için çalışılan hastanede farklı yıllarda baskın bir MRSA klonu olmadığı belirlendi. Ancak enfeksiyonun tüm hastanelerde yayılmasını önlemek ve enfeksiyonun her alanda kontrol altına alınması için tedbirlerin artırılmasının faydalı olacağı düşünülmektedir.

Anahtar Kelimeler Metisilin Dirençli Staphyococcus aureus, Moleküler Tiplendirme, Değişken Alanlı Jel Elektroforezi, Moleküler Epidemiyoloji

INTRODUCTION

Staphylococcus aureus (S. aureus) is one of the most important causes of community and hospital-acquired infections all over the world, and is frequently encountered among the causes of hospital-acquired surgical wound infections (1). Especially Methicillin-resistant *S. aureus* (MRSA) infection prolongs hospital stay and increases antibiotic use. MRSA is of great cross-community concern as this leads to increased costs and deaths (2).

The emergence of isolates of *S. aureus* that are resistant to many antibiotics has become a major problem for most hospitals today. It is important to investigate the resistance of isolates to various antibiotics, especially methicillin, in determining the prevalence of MRSA, which varies both between geographical regions and in the same region. Because; MRSA isolates cause serious and difficult-to-treat infections. Therefore, typing of S. aureus isolates isolated from different sources is important in terms of monitoring the spread (3). Various molecular typing methods are used for this purpose. These include restriction fragment length polymorphism (RFLP), multi locus variable tandem repeat (MLVA), multi locus sequancing typing (MLST), fluorescent amplified-fragment length polymorphism (AFLP), ribotyping and Pulsed Field Gel Electrophoresis (PFGE) (4–7).

Pulsed-field gel electrophoresis (PFGE) is a power discriminative molecular typing technique that is generally used in epidemiological investigations for many bacterial pathogens all over the world. *S. aureus* is one of them. It plays a significant role epidemic control and tracking the sources of infection. Among many molecular methods, the PFGE method is currently known the "gold standard" of molecular typing methods for bacterial pathogens and nosocomial infections (8,9). In an epidemic case, rapid clustering can be performed with PFGE and epidemiologically related cases can be easily distinguished from sporadic cases by this method. However, the PFGE method, has disadvantages such as being time-consuming, expensive and technically difficult (10). The general principle of PFGE is to create large DNA fragments from an intact whole bacterial chromosome with restriction endonuclease cutting enzymes then size-fractionated on an agarose gel. The resultant banding patterns are analyzed and compared to other isolates (11).

In this study, in order to understand whether *S. aureus* isolates which isolated from different parts of the hospitals which are the continuation of an epidemic or unrelated samples, molecular typing was performed with the PFGE method and it was tried to determine whether there was a clonal relationship between the samples.

MATERIAL AND METHODS

1.Bacterial isolates

Nine different *S. aureus* isolates were included in the study. The samples are clinical strains isolated on different dates, strain 1, 2, 5,6 and 7 are known to be isolated in 2015, strain 3 in 2018, strain 4 in 2017 and strain 8 and 9 in 2016. Isolates were identified by conventional methods -and stored at -80° degrees. Accuracy and methicillin resistance of the regenerated strains were determined by the conventional method. After the strains were resuscitated on Braid-Parker agar medium, gram staining was performed to identify the species. Afterwards, coagulase, thermostable nuclease production test, glucose and mannitol anaerobic utilization test were applied. Methicillin resistance was tested a second time using a 1 µg oxacillin disc on Mueller Hilton agar and interpreted according to Clinical and Laboratory Standard Institute (CLSI) standards and according to CLSI standards, a zone diameter of ≥14 mm was considered susceptible, a zone diameter of 10-13 mm was considered moderately sensitive, and a zone diameter of ≤9 mm was considered resistant (12,13).

2.PFGE Analysis

PFGE (Pulsed Field Gel Electrophoresis) was applied by modifying the method applied by Maslow et al. (14). In the study, 1 mg/mL lysostaphin (Sigma) enzyme was used for lysozyme and plugs (12,14) were obtained using SmaI restriction endonuclease (Promega). DNA patterns were shown on a 1.2% agarose gel by running in a CHEF DR II (Bio-Rad Hercules, USA) PFGE device with 6 V/cm current, 14°C temperature and 0.5X TBE for 24 hours with start and end times of 5 and 34. The gel formed after PFGE was treated with ethidium bromide and DNA patterns were photographed under UV light. The similarity index was created at a tolerance value of 1% using the "Dice" coefficient. The clustering relationship between isolates was demonstrated using the UPGMA (unweighted pair group method of arithmatic averages) method. The principles set out by Tenover et al. were adopted for the evaluation of genotypic association between isolates (15) According to the interpretative criteria of Tenover et al. the isolates were classified as indistinguishable, related or different (16).

RESULTS

As a result of the control tests, it was confirmed that all of the strains were methicilline resistant *S. aureus.* All of the nine MRSA strains were genotyped with PFGE 4 different clusters were detected and named as cluster 1-4. Two of the strains (ST1, ST2) are clonally related and located in cluster 1, strain 3 is in cluster 1A since the similarity rate with strain 1 and 2 is below 90%, and strain 3 is located in strain 1 and 2 were found to be related. It was seen that ST5 and ST6 were in the same cluster with each other,

and ST8 and ST9 were in the same cluster with 95% similarity rates. It was determined that ST7 was associated with ST5 and ST6 with a similarity rate of 88%. Strains that were in the same cluster but showed less than 90% similarity and had 3 or more bands were considered related. Looking at the isolation dates, it was determined that strain 1 and 2 belonged to the same year, the infection was from the same clone, but the other 3 strains isolated in the same year were clonally close to each other, but were unrelated to strains 1 and 2 (Figure 1).

DISCUSSION

S.aureus is one of the most important factors causing hospital and community-acquired infections in both healthy people and people with weakened immune systems for various reasons. Our study findings showed that there was no dominant clone between 2015 and 2018 in the hospital where the study samples were detected. The small number of MRSA isolates detected and the fact that they are from different clones have shown us that a single clone outbreak is not a potential hazard in the hospital. In the study, the PFGE method, which is still the gold standard among molecular techniques, was used. Compared to many other sequence-based methods, it was determined that the PFGE method revealed the distinction between strains even with a single band difference. This suggests that the change in a single band between strains may be too discriminatory with correct interpretations (17,18). In a study comparing the multi locus sequencing typing (MLST) method, which is one of the sequence-based molecular typing methods, and the PFGE method, 1, 2 or 3 band differences were detected in the PFGE method between strains that looked exactly the same with MLST typing, and it was thought that there was a possibility that these strains might be related, not the same (18). However, in comparative studies conducted by many different researchers, in which the PFGE method and other PCR-based molecular techniques were used together, the PFGE method still has high discriminatory power for S. aureus between spa, MLST or MLVA methods due to its high discriminatory power and high inter-laboratory and intra-laboratory reproducibility. It has been stated that the highest method (13, 19–22). In our study, it was determined that there was not a dominant MRSA clone in different years in the hospital studied, since samples from different years were studied. However, it is thought that it would be beneficial to increase the precautions in order to prevent the distribution of infection in all hospitals and to control the infection in all areas.

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Conflict Of Interest

There is no conflict of interest relevant to this article was reported.

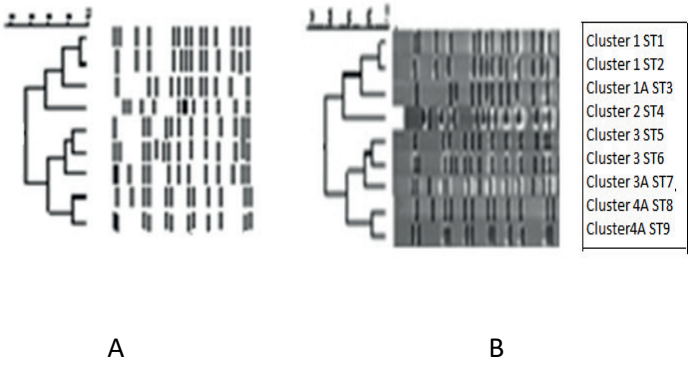

Figure 1: Dendogram of the PFGE typing results. Nine strains (st1-st9) were clustered in four groups. Four strains in three subgroups and one strain in the unique profile.

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