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Application of RAPD-PCR for Determining the Clonality of Methicillin Resistant *Staphylococcus aureus* Isolated from Different Hospitals

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ABSTRACT

Randomly amplified polymorphic DNA (RAPD)-PCR was applied with ten random 10-mer primers to examine the molecular diversity among methicillin resistant Staphylococcus aureus *(MRSA) strains in the hospitals and to investigate the epidemiological spread of these strains between different hospitals. The main objective of the study was to identify appropriate primers, which successfully established the clonality of MRSA. Three of the primers yielded particularly discriminatory patterns and they were used to perform the RAPD analysis which revealed different bands ranging from 200 to 1500 bp. Dendogram was created by the un-weighted pair group method using arithmetic (UPGMA) average clustering and it was constructed based on the combination results of the new primers (S224, S232 and S395) which represented a novel approach for rapid screening of the strains and also provided the opportunity for monitoring the emergence and determining clonal dissemination of MRSA strains between the hospitals. Dendogram generated two main groups (Group I and II) with three clusters (A, B and C) and indicated that the strains isolated from the same hospital were closely related and they placed together in the same group. This technique could be of attractive use in controlling the sources and routes of transmission, tracking the spread of strains within hospital and between the hospitals, and especially preventing the nosocomial infections caused by the MRSA.*

Key words: MRSA, RAPD-PCR, clonal dissemination, UPGMA

INTRODUCTION

Methicillin resistant *Staphylococcus aureus* (MRSA), predominantly a nosocomial pathogen, first emerged in the beginning of the 1960s (Polyzou et al. 2001). Widespread emergence of MRSA strains with increasing resistance to a wide range of antibiotics other than methicillin has contributed to the development of clinically serious problems (Neela et al. 2005). Therefore, the analysis of the spread of these strains has become a great concern throughout the world (Cookson et al. 2011). Phenotypic and genotypic methods have been used to determine the polymorphism among these clinical isolates to follow up nosocomial infections (Singh et al. 2006). The shortcomings of phenotypical based typing methods have resulted in the improvement of genotypic typing methods based on DNA sequences. The point is that reliable and rapid typing is of primary importance for determining the clonally related strains (Neela et al. 2005). A large number of molecular methods that have been used to investigate the epidemiology of *S. aureus* strains include pulsed-field gel electrophoresis (Strandén et al. 2003), restriction fragment length polymorphism (Karakulska et al. 2011), multi-locus sequence analysis targeting (Lee et al. 2011)

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and PCR-based methods (Del Vecchio et al. 1995; van der Zee et al. 1999). Among these methods, although PFGE has been shown to be a gold standard as an accurate and reliable method (Strandén et al. 2003), it is very tedious and time-consuming, compared with ease and speed of performance of PCR-based techniques (Sabat et al. 2006).

Randomly amplified polymorphic DNA (RAPD)-PCR, a simple and rapid technique, has been extensively applied for the epidemiological analysis (Neela et al. 2005). On the other hand, this method has the ability of identifying genetic variation and establishing strain-specific fingerprints (Babalola 2003). This assay can be performed with low quantities of DNA (Rabouam et al. 1999) and short synthetic oligonucleotide primers with a random sequence about 9-10 bases in length (Olive and Bean 1999). These primers enable the amplification without requiring any genome information about the nucleotide sequence and anneal to template DNA sequences at lower temperatures (Rabouam et al. 1999). Synthesis of amplified fragments arises as a consequence of the differences in the distance between the primer binding sites.

MRSA is difficult to permanently eradicate when it arises in healthcare settings where it has not been previous. Specific measures for controlling the infections caused by these strains depend on hospital flora, contamination sources and also whether the infection control systems are sufficiently implemented or not. Providing eradication is important in terms of both reducing inter and intra-spread of MRSA and preventing epidemic infections. The objectives of present study were to examine the molecular diversity among the MRSA strains in the hospitals and to investigate the epidemiological spread of these strains between different hospitals with the use of RAPD-PCR along with selecting and identifying the optimal primers which could successfully establish the clonality of MRSA.

MATERIALS AND METHODS

Bacterial strains and identification

Forty-nine strains of methicillin resistant *Staphylococcus aureus* were collected from three different hospitals in Ankara, Turkey. These strains were identified by the conventional methods (Kloos and Schleifer 1986). Resistance to methicillin was determined by Kirby-Bauer disk diffusion method using 1.0 µg oxacillin disc. The results were evaluated according to the Clinical and Laboratory Standards (CLSI 2005). The strains resistant to methicillin were designated as MRSA.

DNA extraction and RAPD fingerprinting

Genomic DNA was extracted using Genomic DNA Kit (BioBasic, Canada) and stored at -20°C. Amplification reaction was performed in 50 µl volume mixtures consisting of 1× PCR buffer (10 mm Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100), 2.5 mM dNTP (BioBasic, Canada), 5.0 µm of each RAPD primers (Table I), 50 ng of template DNA and 3U Taq DNA polymerase (Dr. Zeydanlı-Turkey). A single primer was used in each PCR reaction. Amplifications of DNA fragments were carried out by using a thermal cycler (Hamburg, Germany) with the following cycling profile: pre-denaturation at 95°C for 2 min, followed by 45 cycles of amplification (denaturation at 94°C for 1 min, annealing at 32°C or 34°C (Table 1) for 1 min, extension at 72°C for 2 min), ending with a final extension at 72°C for 5 min.

Amplification products were analyzed by 1.8% agarose gel electrophoresis (Scie-Plas; UK) using TBE 1× buffer (0.9 M Tris, 0.9 M Boric acid and 20mM EDTA, pH 8.3) at 100V for 4 h. A 1.5 kb molecular weight marker (BioBasic, Canada) was used as a DNA size standard. Amplified products were visualized staining ethidium bromide and using Gel Logic 200 Molecular Imaging System (Kodak, Rochester).

Table 1 - RAPD primers and their sequences used in this study.

Primers	Primer sequences 5′–3′	Tm [°C][a]	References
B0043-1 7	GCGATCCCCA	34	Pererira et al. 2002
B0043-1 10	TCACGCTGCG	34	Saulnier et al. 1993
S21	CAGGCCCTTC	34	Cenis 1992
S33	CAGCACCCAC	34	Byun et al. 1997
S63	GGGGGTCTTT	32	Danielle et al. 2005
S175	TCATCCGAGG	32	This study
S260	ACAGCCCCCA	34	This study
S224	CCCCTCACGA	34	This study
S232	ACCCCCCACT	34	This study
S395	AAGAGAGGGG	32	This study

[a] Tm temperature specified by the manufacturer

RAPD data analysis

The RAPD PCR banding patterns generated with each primer were analyzed using NTSYS-pc (version 2.1, USA). Each of the informative bands was scored: (1) for the presence or (0) for the absence. The data were submitted to the program to transform the polymorphic bands into Dice distance and dendogram was created by the unweighted pair group method using arithmetic (UPGMA) average clustering.

RESULTS AND DISCUSSION

All the 49 strains of MRSA obtained from different hospitals were examined by the RAPD-PCR technique with ten random 10-mer primers. Among these, three of primers were used in this study showed poor banding patterns. However, three of them yielded particularly discriminatory patterns and were used to perform the RAPD analysis which revealed different bands ranging from 200 to 1500 bp. These primers were individually able to distinguish the strains obtained from different hospitals. Figures 1 to 3 represent the representative profiles of banding patterns with primers S224, S232 and S395, respectively.

The RAPD-PCR, one of the most widely used genotyping methods, has been applied to investigate the spread of *S. aureus* in previous studies in which different RAPD primers were designated and discriminatory power of primers has been assessed (Hojo et al. 1995; van Belkum et al. 1995; Neela et al. 2005; Nikbakht et al. 2008).

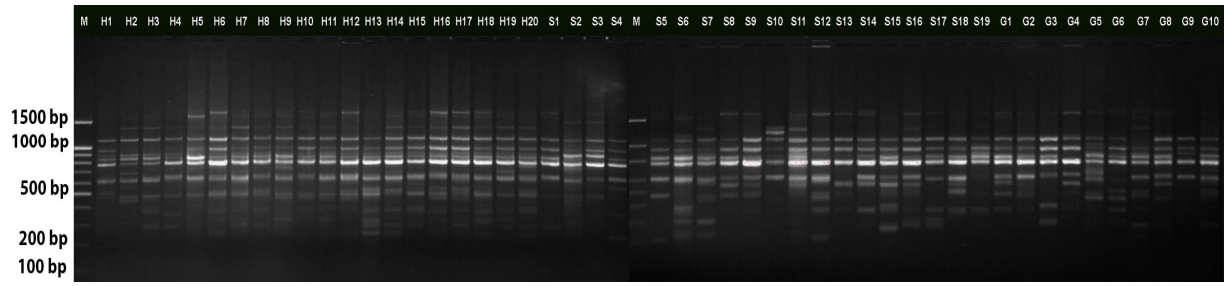

Figure 1 - RAPD banding patterns obtained with primer S224. Lanes H1-H20: Methicillin-resistant *Staphylococcus aureus* strains from Hospital-1, Lanes S1-S19: Methicillin-resistant *Staphylococcus aureus* strains from Hospital-2, Lanes G1-G10: Methicillin-resistant *Staphylococcus aureus* strains from Hospital-3, Lane M: 1.5 kb molecular weight marker.

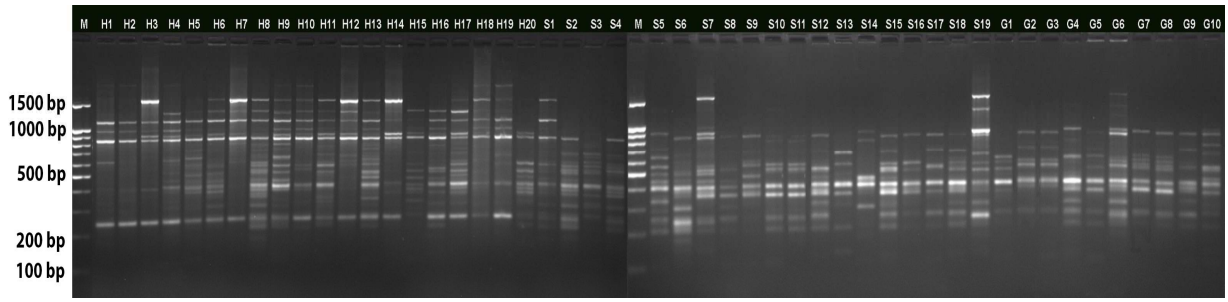

Figure 2 - RAPD banding patterns obtained with primer S232. Lanes H1-H20: Methicillin-resistant *Staphylococcus aureus* strains from Hospital-1, Lanes S1-S19: Methicillin-resistant *Staphylococcus aureus* strains from Hospital-2, Lanes G1-G10: Methicillin-resistant *Staphylococcus aureus* strains from Hospital-3, Lane M: 1.5 kb molecular weight marker.

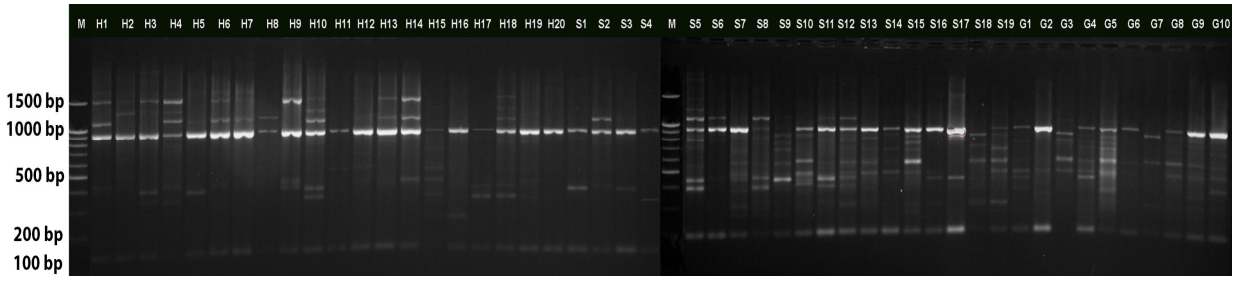

Figure 3 - RAPD banding patterns obtained with primer S395. Lanes H1-H20: Methicillin-resistant *Staphylococcus aureus* strains from Hospital-1, Lanes S1-S19: Methicillin-resistant *Staphylococcus aureus* strains from Hospital-2, Lanes G1-G10: Methicillin-resistant *Staphylococcus aureus* strains from Hospital-3, Lane M: 1.5 kb molecular weight marker.

Discriminatory powers of primers were evaluated according to the revealed banding profiles that provided genotyping of these strains. The choice of primers to be used in the RAPD analysis is the most critical factor and using many primers contributes to the occurrence of different banding patterns. In this study, some of the strains isolated from different hospitals could not be differentiated using one primer. High discriminatory power was accomplished with the combined fingerprinting using several primers. Therefore, dendogram was constructed based on the combination results of new three primers which represented a novel approach for rapid screening of the strains and also provided opportunity for monitoring the emergence and dissemination between the hospitals. Dendogram generated two main groups (Group I and II) with three clusters (A, B and C) (Fig. 4.). Cluster A consisted of 100% of Hospital-1 strains (H1-H20). Cluster B of Group II was divided into two sub-clusters (B1 and B2) and contained 57.9% of Hospital-2 strains. Sub-cluster B1 of the cluster B contained 36.8% of Hospital-2 strains. All the strains obtained from Hospital-3 were placed in the sub-cluster B2 with 21.1% of Hospital-2 strains. Cluster C was comprised of 42.1% of Hospital-2 strains. The strains of Hospital-2 were divided into two main clusters (B and C). The dendogram indicated that the strains isolated from the same hospital were closely related and placed together in the same group. Group I only included all of the Hospital-1 strains and this means that these strains might have emerged from one clone. On the other hand, all of the Hospital-2 and Hospital-3 strains formed Group II showing that Hospital-3 strains emerged from the same clone with that of Hospital-2. Although Hospital-2 and Hospital-3 strains were grouped together, Hospital-1 strains clustered separately. The reason for this situation could be that Hospital-1 patients admitted from other healthcare settings might have been infected by the introduction of strains from other institutions of many other towns. While considering the clones spreading in the city, it could be estimated that there were three main different clones. One clone from Hospital-1 and two from other hospitals enabled to understand monitoring the spread over either noticeable distances or a single hospital.

The percentage of similarity scale was represented above the dendogram (Fig. 4.). The dendogram showed that the highest percentage of similarity (96.9 %) was between G9 and G10 strain, whereas the lowest percentage of similarity (38.1%) was between H2 and S19 strain.

Similar observations have been reported in earlier studies also. Neela et al. (2005) reported that two major groups with three clusters each in one group revealed by the dendogram generated from the RAPD analysis of different *S.aureus* strains obtained from the hospitals. The strains isolated from the same hospital were genetically related and most of them were in the same cluster. Nikhbakt et al. (2008) found that MRSA strains obtained from two different hospitals with identical RAPD pattern suggested about the route of MRSA transmission. Although RAPD assay is a wide-spread method for the genetic fingerprinting, there is no specific primer for discrimination. The primers are sometimes insufficient to distinguish genetic differences among the related and unrelated strains. The primers used in this study to construct the dendogram provided an advantage for using the RAPD-PCR to potentially determine the clonal dissemination of MRSA strains.

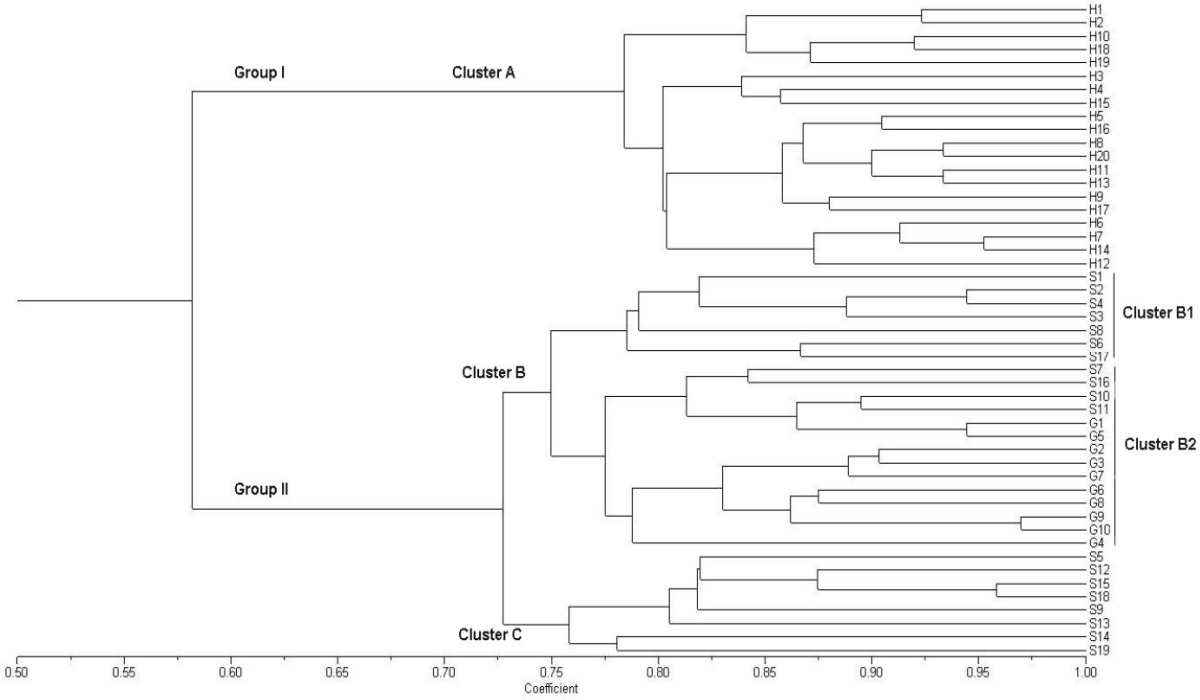

Figure 4 - Dendogram of genetic relationship between forty-nine strains of methicillin-resistant *Staphylococcus aureus* obtained with three RAPD primers. H1-H20: Methicillin-resistant *Staphylococcus aureus* strains from Hospital-1, S1-S19: Methicillin-resistant *Staphylococcus aureus* strains from Hospital-2, G1-G10: Methicillin-resistant *Staphylococcus aureus* strains from Hospital-3.

CONCLUSIONS

Rapid and accurate epidemiological typing systems are required to monitor the inter or intra-spread of MRSA strains. In the present study, RAPD-PCR was applied with many primers and three of them were chosen for the analysis. The dendogram obtained clearly showed distinct clustering that most of the strains were clustered corresponding to the hospitals from which they were isolated. In the RAPD analysis, all the strains tested were type-strains with each of the primers used and there was no unique MRSA strain. This technique could be of attractive use in controlling the sources and routes of transmission, tracking the spread of strains within hospital, and between the hospitals, and especially preventing the nosocomial infections caused by the MRSA.

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