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# Determination of Genetic Diversity and Similarity Among Methicillin Resistant *Staphylococcus aureus* strains by RAPD-PCR

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**ABSTRACT:**
**Purpose:** RAPD PCR is a method used to determine genetic relatedness between bacteria. In this method, PCR is performed using a small amount of DNA and randomly selected primers at low annealing temperature. The aim of this study was to determine the genetic diversity and genetic similarity of Methicillin Resistant *Staphylococcus aureus* (MRSA) strains isolated from clinical samples.
**Material and Methods:** Thirty-two MRSA strains were identified by conventional methods. Methicillin resistance of strains were determined by PCR using the *mec*A gene primers. These strains were genetically typed by RAPD PCR using primers OLP-11 and OLP-13. Bionumerics V7.5 (Applied Maths) program was used for analysis and dendograms were generated by unweighted pair group method with arithmetic averages (UPGMA).
**Results:** All strains were confirmed as MRSA by PCR. Many different bands from 400 bp to 1000 bp were detected by RAPD PCR and five clusters (1-5) with OLP-11 and four clusters (1-4) were formed with OLP-13. In RAPD PCR performed with OLP-11 and OLP-13 primers, 80% (cluster 3-5) and 86% (cluster 1-4) similarities were found, respectively. MRSA strains isolated from wound samples were found to be more genetically similar to each other, with at least one in each cluster.
**Conclusion:** RAPD PCR was found to be an effective method for the evaluation of genetic similarity and genetic diversity of MRSA strains.
***Keywords:*** *MRSA, mecA, OLP-11, OLP-13, RAPD PCR*

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## INTRODUCTION

*Staphylococcus aureus* is a Gram-positive, coagulase-positive, spherical, forming grape-like clusters bacterium belonging to the Staphylococcaceae family, which is often present asymptomatically in the skin, skin glands, mucous membranes, nose and intestines of healthy individuals (Gould et al., 1995). *S. aureus* can cause skin and soft tissue infections, endocarditis, osteomyelitis, pneumonia, bacteremia (Ma et al., 2020). Although antibiotics such as penicillin and methicillin were effective against *S. aureus* in the mid-20$^{th}$ century, Methicillin-Resistant *S. aureus* (MRSA) strains that emerged in the following years remain a significant threat to human health today (McGuinness et al., 2017). Bacterial peptidoglycan stabilizes the intracellular pressure and maintains the cell shape. Peptidoglycan consists of glycan chains of N-acetylglucosamine and N-acetylmuramic acid cross-linked with peptides linked to N-acetylmuramic acid. Penicillin-binding proteins perform the polymerization (transglycosylation) of glycan chains and the transpeptidation between glycan chains (Sauvage et al., 2008). The transpeptidase activity of penicillin-binding proteins (PBPs), which are essential in peptidoglycan synthesis in the bacterial cell wall, is inhibited by β-

lactam antibiotics (Typas et al., 2011; Egan et al., 2017). Penicillin binding protein (PBP2a) has a weak affinity for β-lactams, so MRSA strains are resistant to all β-lactam antimicrobials (Ivbule et al., 2017). This protein is produced by the *mecA* gene. The *mecA* gene is located on *Staphylococcal cassette chromosome* (SCCmec) (Shoen et al., 2019). The *mecA* gene causes methicillin resistance (Fuda et al., 2004). The gold standard test used for MRSA identification is the detection of the *mec*A gene by PCR (Maes et al., 2002).

Many genotyping methods are used to identify MRSA strains, such as multilocus sequence typing (MLST), multiocus variable number tandem repeat analysis (MLVA), pulse field gel electrophoresis (PFGE), random amplified polymorphism DNA (RAPD) PCR, restriction fragment length polymorhic DNA (RFLP) PCR (Hookey et al., 1998; Rabello et al., 2007; Hennekinne et al., 2003; Sabat et al., 2003; Reinoso et al., 2004). RAPD PCR method is more useful than other methods with its easy application, fast results and cost-effectiveness. (Hakimi et al., 2017). RAPD PCR method is used for many purposes, identification of species and strains (Cocconcelli et al., 1995), analysis of genetic diversity (Koh et al., 1999), construction of genetic maps (Binelli et al., 1994), determination of genetic variation (Keshava et al., 1999), evaluation of genotoxicity of environmental pollutants (Rocco et al., 2011), etc. In this method, PCR is performed with a small amount of DNA using a randomly selected short single primer at low annealing temperature, amplicons are analyzed by agarose gel electrophoresis and the similarity among the strains is evaluated (Williams et al., 1990).

The aim of this study is to determine the genetic diversity and genetic similarity of MRSA strains isolated from clinical samples by RAPD PCR method.

## MATERIAL AND METHODS

### Bacterial strains and identification

Thirty-two MRSA strains (wound, tracheal aspirate, joint fluid, tissue, prosthetic-tissue, catheter tip, abscess, bronchoalveolar lavage) isolated from clinical samples were included in this study. MRSA strains were obtained from Ankara University İbni Sina and Cebeci Hospital microbiology laboratories. Identification of strains were carried out by conventional methods (Kloos and Schleifer, 1986). The phenotypic determination of methicillin resistance was performed using 30 µg cefoxitin (Bioanalyse, Türkiye) by disk diffusion method, according to the European Committee on Antimicrobial Suscepti-bility Testing recommendations recommendations (EUCAST, 2021).

### DNA extraction of strains

Bacteria in the stock culture were thawed at room temperature and passaged into 5% sheep blood agar medium. After incubation at 35±1°C for 18±2 hours, DNA isolation was performed by using the DNA isolation kit (Thermo Scientific GeneJET Genomic DNA Purification Kit, Lithuania) in accordance with the manufacturer's recommendations.

### *mec*A gene amplification

Genotypic determination of methicillin resistance was performed as described by Radenoviç (Radenoviç et al., 2016). *mec*A-F (5'-AAA ATC GAT GGT AAA GGT TGG C-3'), *mec*A-R (5'-AGT TCT GCA GTA CCG GAT TTG C-3') primers were used for *mec*A gene amplification. The PCR reaction (50 µl) contains 5 µl of × 10X PCR buffer (added $MgCl_2$) , 0.4 µl of 25 mM dNTPs, 0.25 µl of 5U/µl of Taq DNA polymerase, 2 µl of 10 µM each of the primers, 35.35 µl $ddH_2O$ and 5 µl of template DNA. The amplification was carried out in a Thermal Cycler (LONGGENE A300, China). PCR amplification conditions: initial denaturation at 94 °C for 5 min, denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min (40 cycles) final extension at 72 °C for 5 minutes.

### RAPD-PCR

The RAPD-PCR assay was performed as described by Williams with some modifications (Williams et al., 1990). Primers OLP11 (5'-ACGATGAGCC-3') and OLP13 (5'-ACCGCCTGCT-3') were used in RAPD-PCR (Zare et al., 2019). The PCR reaction (50 µl) contains 5 µl of × 10X PCR buffer (added $MgCl_2$) , 0.4 µl of 25 mM dNTPs, 0.25 µl of 5U/µl of Taq DNA polymerase, 1,5 µl of 100 µM each of the primers, 37.85 µl $ddH_2O$ and 5 µl of template DNA. Ampilifications were

performed in a thermal cycle (LONGGENE A300, China). The amplification was performed as follows: initial denaturation at 94 °C for 5 mins, denaturation at 93 °C for 1 min, annealing at 45 °C for 90 s, extension at 72 °C for 1 min (40 cycles) and final extension at 72 °C for 7 minutes.

**Analysis of amplicons in electrophoresis**

Amplification products were analyzed by electrophoresis (RunVIEW, Cleaver Scientific, UK) on a 1.5% agarose gel (PRONA, Spain) containing ethidium bromide (0.5µg/ml) and visualized under UV light (Vılbert Lourmat Photodocumentation and Imaging Systems, France). A 100 bp DNA ladder (GeneDirex Inc., USA) was used to assess the size of the amplicons.

**Analysis of RAPD PCR data & statistics**

Bionumerics V7.5 (Applied Maths) program was used for analysis. Similarities of band profiles were determined by Dice Similarity coefficient and dendograms were created by unweighted pair group method with arithmetic averages (UPGMA). Groups with 95% similarity in dendograms are called clusters. Chi-square analysis was used to determine whether there was a relationship between the groups obtained and the origin of the bacteria.

**RESULT & DISCUSSION**

Thirty-two MRSA strains isolated from various clinical samples were identified by phenotypic methods. Two different methods were used to determine methicillin resistance of strains, the Kirby-Bauer disk diffusion method using cefoxitin disk and *mecA* gene was investigated by PCR. All strains resistant to cefoxitin and producing a 533 bp band by PCR were confirmed as MRSA (Figure 1).

Genetic relatedness of thirty-two MRSA strains were investigated by RAPD PCR using OLP-11 and OLP-13 primers. A large number of bands with sizes ranging from 400 bp to 1000 bp were produced by RAPD PCR (Figure 2, Figure 3). Based on 100% similarity, dendograms containing clusters were generated. As a result of RAPD PCR performed with OLP-11 primer, band formation was observed in all strains and the strains were divided into five clusters (1-5). Cluster 3 contains the most strains (11 isolates) and cluster 2 the least (2 isolates). Cluster 3 and cluster 4 consist of wound samples with 72.7% and 77.7% respectively. The dendogram showed 80% similarity between cluster 3 and cluster 5. RAPD PCR performed with OLP-13 showed band formation except for the 26$^{th}$ strain. The strains were divided into 4 clusters (1-4). Cluster 1, which contains 18 isolates, is the largest cluster. Cluster 4 has two isolates and 86% similar to cluster 1. The percentages of similarity in the clusters are variable (Figure 4, Figure 5). In RAPD PCR performed with OLP-11 and OLP-13, it was observed that the MRSA strains isolated from wound samples were genetically similar to each other and there was at least one wound sample in each cluster. It was found that there was a statistically significant relationship between the origin of the bacteria and the bacterial groups ($p<0,05$).

RAPD PCR is preferred for its easy application and fast results. In this method, there is no need for preliminary information about the genome of the organism to be examined (Williams et al., 1990).

In many studies, it has been stated that *S.aureus* is widely used for typing by RAPD PCR (Tambic et al., 1997; Onasanya et al., 2003; Morandi et al., 2010). Reinoso et al. (2004) divided eighty *S.aureus* isolates into eleven groups, one group being human isolates and the other groups bovine isolates. Zare et al. (2019) In their study, which included fifty *S.aureus* strains isolated from banknote, food, human infections and bovine mastitis, the largest number of bands were obtained from six bovine mastitis isolates with the OLP-13 primer, they observed no correlation between the RAPD pattern and the source of the isolate, except for clusters containing only strains from the same sources and host specificity. Debnath and Chikkaswamy, (2015) reported that three clusters were formed by the inter-location spread of ninety-seven clinical MRSA strains. Kurlenda et al. (2007) in the study, which included two hundred and thirty-four MRSA strains, they found no association between the strains and the hospital department or type of infection. Nikbakht et al. (2008) observed the same patterns in eighty MRSA strains isolated from inpatients and hospital staff, they stated that this result indicates in-

hospital transmission. Some studies have reported the transmission of MRSA strains from animals to humans (Nnachi et al., 2014; Juhasz Kaszanyitzky et al., 2007).

Lee (2003) determined that fifteen animal MRSA isolates were similar to six human isolates and reported that these isolates were transmitted by consuming foods of animal origin. In this study, genetic relatedness of MRSA strains were investigated by RAPD PCR using OLP-11 and OLP 13 primers. The similarity of MRSA strains isolated from wound samples and the presence of at least one in each cluster suggest that there may be in-hospital transmission.

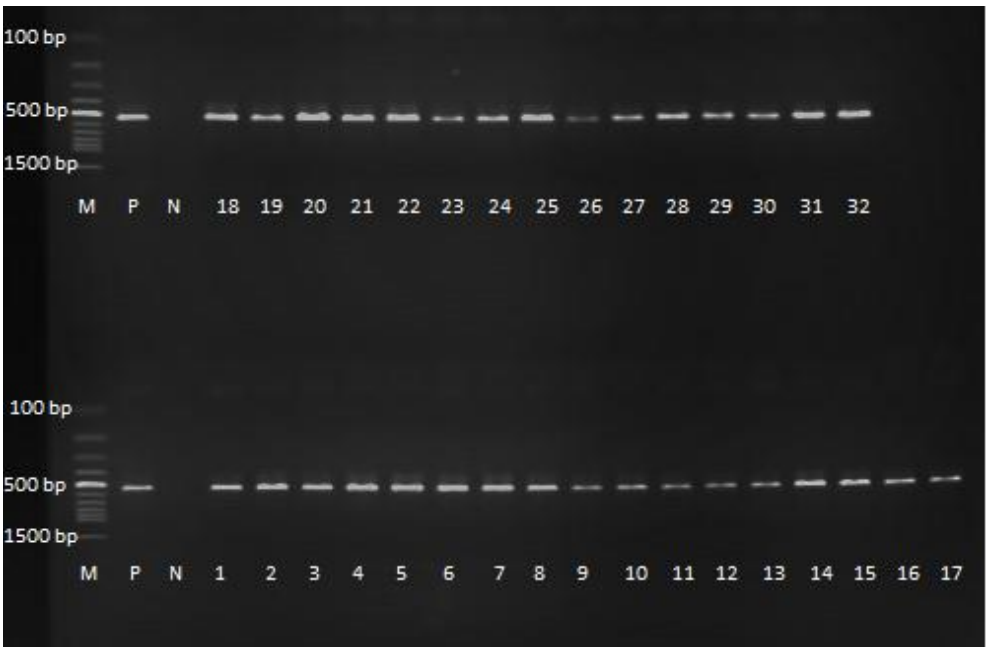


**Figure 1.** Agarose gel electrophoresis of *mec*A gene. M: 100 bp DNA Ladder, P: Positive control (*S.aureus* ATCC 43300), N: Negatif control (sterile distilled water), 1-32; *S.aureus* positive isolates (533 bp)

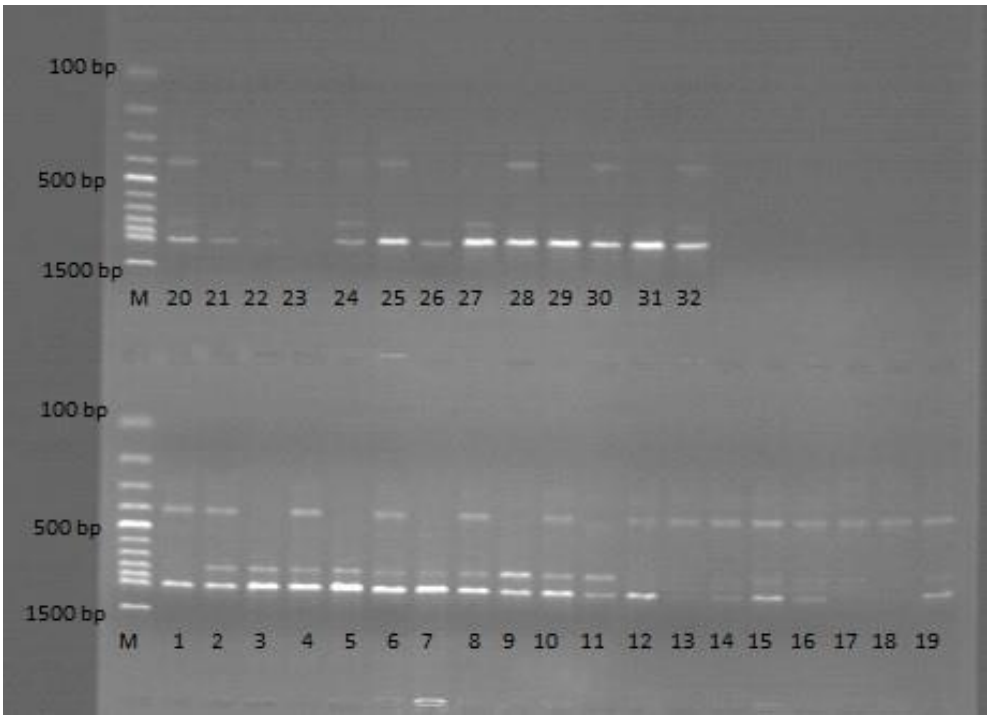


**Figure 2.** RAPD PCR (OLP-11)

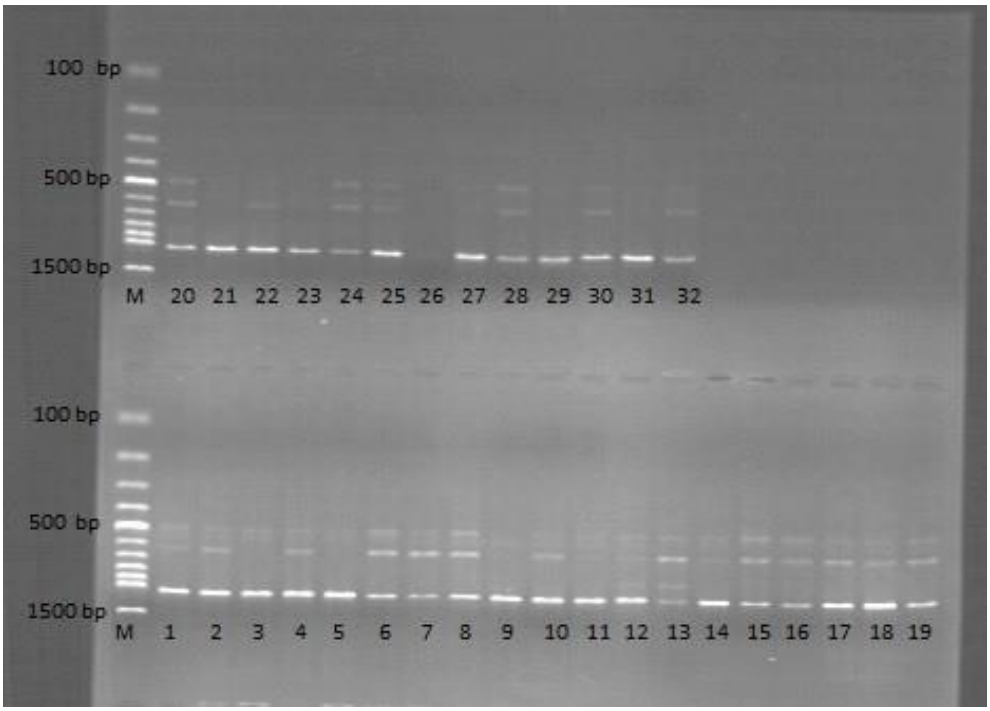


**Figure 3.** RAPD PCR (OLP-13)

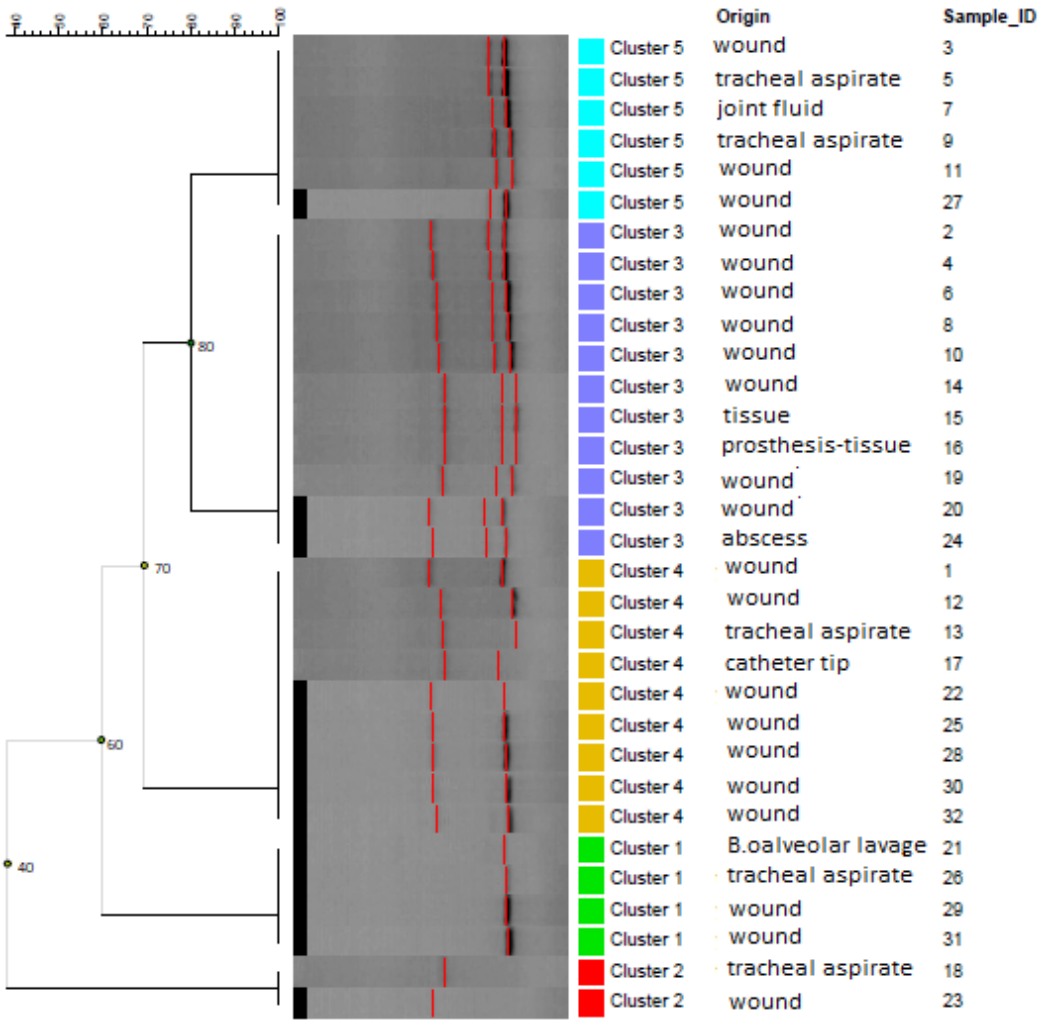


**Figure 4.** Dendogram of MRSA strains (OLP-11)

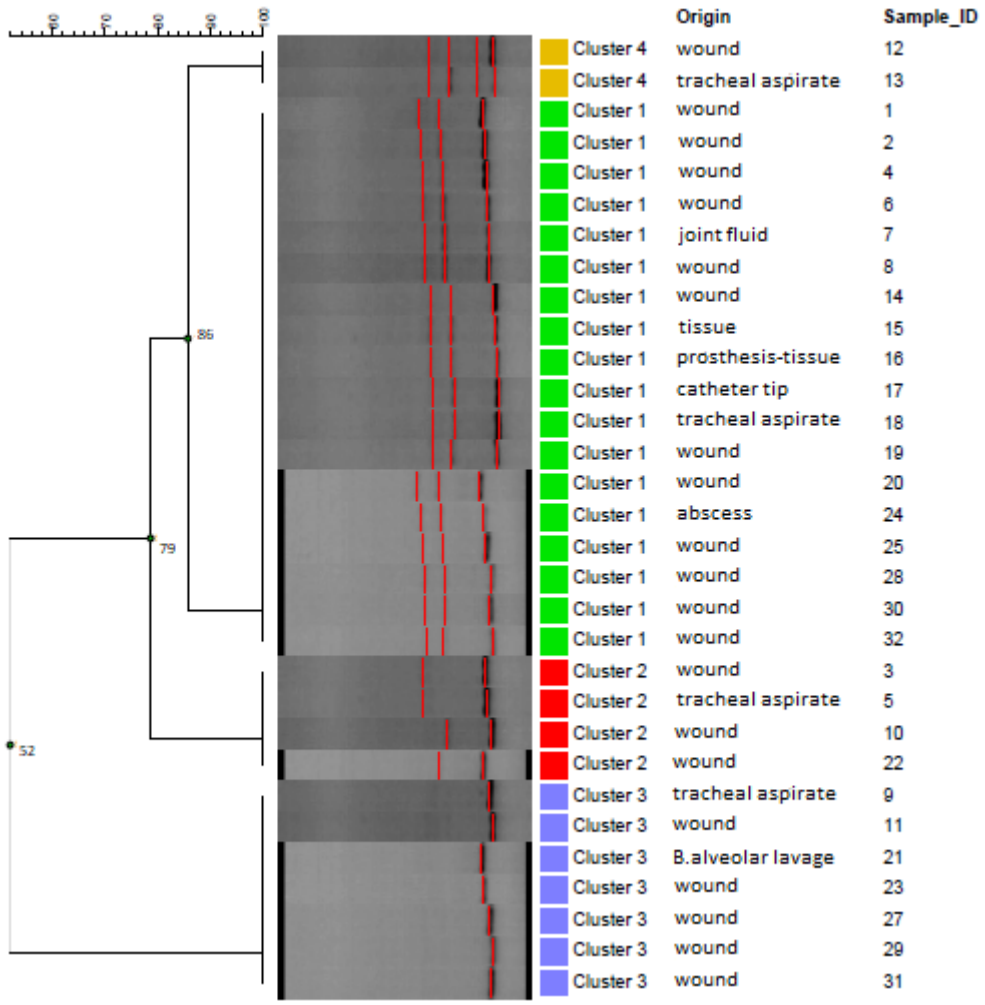


**Figure 5.** Dendogram of MRSA strains (OLP-13)

## CONCLUSION

MRSA continues to cause both community and hospital-acquired infections. Finding the source of MRSA infections and monitoring the spread are important for the treatment of infections. RAPD PCR is a method used to determine the genetic diversity and clonal relationship in MRSA strains. In this study, RAPD PCR analysis of strains were performed with two different primers and dendograms were generated. It was observed that RAPD PCR is an effective method for determining the genetic relatedness of MRSA strains isolated from clinicalsamples.

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