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Determination of Genetic Relationship among some Methicillin Resistant *Staphylococcus aureus* isolated from some Iraqi Hospitals using Randomly Amplified Polymorphic DNA (RAPD)

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ABSTRACT

Methicillin-Resistant *Staphylococcus aureus* (MRSA) strains are resistant to methicillin and many other antibiotics. They are considered most of common nosocomial pathogens which cause diseases in humans. The aim of this study to investigate the genetic relationship among different Methicillin-Resistant *Staphylococcus aureus* strains by using the Random Amplified Polymorphic DNA (RAPD) technique. Isolates were collected from clinical specimens from different Iraqi hospitals, out of 90 isolated S. aureus, 10 strains (11.11%) were found to be Methicillin Resistant S. aureus. Polymerase chain reaction (PCR) technique was used with 10 random primers to analyze 10 clinical isolates of (MRSA); only 9 primers gave clear amplification. These primers produced 91 fragment lines across all isolates with an average of 10 fragment lines per primer, of these, 90 or (99%) were polymorphic. The size of the amplified bands ranged between 145-2109 bp. The genetic polymorphism value for all primers was (100%) except OP-X17 primer which gave (86%) polymorphism. The Dendogram obtained from the analysis of RAPD primers showed two major groups (Groups A and B), group B contained only one isolate (isolate number 1) while A group divided into two subgroups (A1 and A2), A2 subgroups contained isolates (3,4 and 5), while A1 subgroup divided into two subgroups (A1a and A1b), A1b subgroup included isolate number (10), while A1a divided into two subgroups (A1a1 and A1a2), A1a2 subgroup included isolate number (9), while A1a1 divided into two subgroups (A1a1b and A1a1c), A1a1c contained isolate number (8) while A1a1b included isolates (2,6 and 7). From the present study, it is clear that, RAPD-PCR assayed with combination of nine primers can be successfully applied to assess the genetic relationship between (MRSA) isolates.

Keywords: *Staphylococcus aureus*, MRSA, Randomly amplified polymorphic DNA, Genetic relationship, Dendogram.

1. INTRODUCTION

Staphylococcus aureus caused clinically considerable infections in humans, including endocarditis and deep-seated abscesses [1]. Methicillin-Resistant *Staphylococcus aureus* (MRSA) is antibiotic-resistant nosocomial pathogen, firstly emerged in 1960s in hospitals, improved in 1990s community acquired [2]. Widespread emergence of MRSA strains with increasing resistance to a wide range of antibiotics other than methicillin imposes a serious threat to patients and health care setups [3,4]. Recently, the increase in the frequency of methicillin-resistant *Staphylococcus aureus* MRSA as the fundamental agent of nosocomial infection and the possibility of development of resistance to multi-drug request a quick and reliable characterization of isolates and identification of clonal spread within hospitals [5].The typing techniques were used in tracking sources, pathways of spreading infections and studying the genetic relationship. In (MRSA) typing, both phenotypic and genotypic were used. Several techniques are

available for phenotypic differentiation of MRSA isolates such as ribotyping, bacteriophage typing, capsular typing and antibiotic resistance. Typing systems based only on phenotypic tests have restrictions such as relatively low discriminatory power and unassuming reproducibility because phenotypic traits are variably expressed [6,7], in addition, isolates may present the same antibiogram patterns although they differ in their genetic profiles [8]. Since this deficiency and weakness of phenotyping methods, this trigger to the gradual development of genotypic procedures like randomly amplified polymorphic DNA (RAPD) analysis. In this technique, single short primers with arbitrary nucleotide sequences are used in a polymerase chain reaction (PCR) to amplify genomic DNA. The profiles obtained after electrophoresis separation of the amplification products can be used to fingerprint strains of various prokaryotic and eukaryotic species as well as to establish the genetic relationship between them [9,10]. RAPD analysis considered a useful technique for strain-specific fingerprints like MRSA strains because of its simplicity and accuracy [11]. The aims of this study are (i) investigate the molecular diversity of MRSA isolates obtained from different Iraqi hospitals by generating a DNA fingerprint, (ii) estimation of the genetic relationship through studied isolates by using RAPD markers.

2. MATERIALS AND METHODS

2.1 Bacterial sample collection.

All the 10 strains of *Staphylococcus aureus* were isolated from Iraqi patients attending different hospitals in Baghdad city. Most of these isolates were obtained from nose and some from blood and wounds. *Staphylococcus aureus* isolates were identified by their phenotypic characterizations in different biochemical tests oxidase, coagulase, and catalase [12]. For genotypic identification, the obtained *Staphylococcus aureus* isolates were detected for the presence of mecA and nuc genes by polymerase chain reaction and all were confirmed to be MRSA isolates.

2.2 Genomic DNA extraction

Genomic DNA of the 10 MRSA isolates was extracted by using Promega DNA extraction kit, with 30 µg/ml lysozyme enzyme. Bacterial colonies were grown overnight in brain heart infusion broth at 37 ºC. 1 ml of overnight bacterial growth was centrifuged at 10000 rpm for 5 min. All the extraction steps were followed and the provided solutions were added according to the manufacturer´s recommendations with one additional step which is the 1-hour treatment of bacterial cells with lysozyme prior to extraction steps. Spectrophotometer was used to assess both concentration and purity of the extracted DNA samples, 0.8% agarose gel was used for checking DNA integrity using gel electrophoresis unit, subsequently, DNA bands were examined under UV light after staining with ethidium bromide [13].

2.3 Primer selection and RAPD- PCR analysis

In this study, 10 random primers from (Alpha DNA, USA/Canada) were examined. All primers produced results regarding to amplification and polymorphism including (OP-D18, OP-D20, OP-T07, OP-W02, OP-X12, OP-X17, OP-A03, OP-X06 and OP-Y13) except (OP-A06) which gave no amplified products (Table 1). Amplification was performed using thermal cycler (Labnet international. Inc - USA). PCR reaction was performed in 25 µl volume mixture containing 12.5 µl (1X) of Green Master Mix (Promega-USA) consisting of (10mM Tris-HCl (PH8), 50mM KCL, 1.5mM MgCl2, 200µM each deoxynucleotide triphosphate (dNTP) and 1U DNA polymerase). Thermocycling conditions were set at 94°C for 5 min as initial denaturation, and 45 cycles of 1 min at 94°C, 36°C for 1 min and 72°C for 2 min, and a final extension at 72°C for 10 min, followed by a hold at 4°C [14,15]. Each DNA sample was tested under the same conditions at least twice with the selected oligonucleotides. Twenty microliter of PCR amplicons were fractionated by electrophoresis in 2% agarose gel at a constant voltage of 5 volt/cm for 2 hour using 0.5X concentration of TBE buffer which consist of (10mM Tris-Borate,1 mM EDTA) and compared along with 100bp DNA ladder (promega-USA). The DNA bands were analyzed using UV transilluminator after staining with ethidium bromide.

2.4 Data analysis:

2.4.1 Molecular weight estimation

Molecular weight for the DNA bands generated from each primer was assessed by using Photo-Capture M.w. program version 1.0 [16]. A 100 bp ladder was run together with PCR products as a molecular weight marker.

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

No.	Primer name	Sequense '5--------3'
1	Op - D18	GAGAGCCAAC
2	Op - D20	ACCCGGTCAC
3	Op - T07	GGCAGGCTGT
4	Op - W02	ACCCCGCCAA
5	Op - X06	ACGCCAGAGG
6	Op - X17	GACACGGACC
7	Op - X12	TCGCCAGCCA
8	Op - Y13	CACAGCGACA
9	Op - A03	AGT CAG CCAC
10	Op - A06	GGT CCC TGAC

2.4.2 Primers parameters

Overall bands numbers were scored visually. Both polymorphic fragments and fragment lines were also scored. Polymorphism percentage, discrimination power and efficiency for each studied primer were determined using the following formula:

Polymorphism percentage= No. of polymorphic fragments\ No. of fragment lines amplified by the same primer.
Discrimination power= No. of polymorphic fragments\ total No. of polymorphic fragments obtained.
Efficiency= total No. of bands amplified by primer\ total No. of bands obtained.

Positions of scorable bands were transformed into a binary character matrix ("1" for the presence and "0" for the absence) [17].

2.4.3 Cluster analysis and Principal Components Analysis (PCA)

Dendogram was created by the unweighted pair group method using arithmetic (UPGMA) average clustering to construct genetic relationship tree diagrams among studied MRSA isolates [17]. Principal Component Analysis (PCA) was also carried out to show the multiple dimensions of the distribution of the accessions in a scatter-plot. The computer software Palaeontological Statistics (PAST) version 1.62 was used for generating both of cluster and PCA analysis [18].

3. RESULTS AND DISCUSSION

3.1 DNA amplification

Genetic relationships among ten MRSA isolates were analyzed using ten different RAPD primers. The ability for each primer for estimation diversity among MRSA isolates was varied. Among these ten decamer primers, there was one primer (OP-A06) that failed to generate PCR products with all MRSA isolates; hence, it was eliminated from the analysis. The remaining nine primers showed reliable banding patterns.

3.2 RAPD-PCR and Primers parameters

The RAPD-PCR consider one of the most extensively used genotyping method, it has been utilized to examine the spread of *S. aureus* in previous studies in which different RAPD primers were designated and discriminatory power of primers has been determined [19]. The RAPD primers used in this study generated 263 as total bands number with average of 29 bands per primer (Table 2). Each isolates were varied in term of generated bands; primer OP-X06 produced 4 bands showing the lowest primer efficiency (1.52%), while primer OP-D18 produced 53 bands showing the highest primer efficiency (20.15%). The molecular weight of the amplified bands was ranged from 145 bp (OP-T07) to 2109 bp (OP-D18). RAPD primers amplified 91 fragment lines across all isolates genomes with average of 10 fragment lines per primer. Among these fragment lines scored, 90 fragment lines (99.0%) were polymorphic with average of 10 polymorphic fragment lines per primer across the 10 MRSA isolates. Primer (OP-X12) amplified 15 fragment lines (100% polymorphism) representing the maximum discrimination power (17%), while primer OP-X06 amplified 2 polymorphic fragment lines (100% polymorphism) representing the minimum discrimination power (2.2 %).

Table 2: Number of amplified bands, polymorphic fragment lines, fragment lines, primer efficiency and discrimination power of the 10 RAPD primers.

Primer name	*Sequence (5 - 3)*	*Total bands (no.)*	*primer efficiency %*	*No. of fragment lines*	*Polymorphic fragments (no.)*	*% polymorphism*	*% discrimination power*
OP-A03	AGTCAGCCAC	52	19.77	14	14	100	15.5
OP-D18	GAGAGCCAAC	53	20.15	13	13	100	14.4
OP-D20	ACCCGGTCAC	24	5.32	9	9	100	10
OP-T07	GGCAGGCTGT	30	11.40	12	12	100	13.3
OP-W02	ACCCCGCCAA	33	12.54	13	13	100	14.4
OP-X06	ACGCCAGAGG	4	1.52	2	2	100	2.2
OP-X12	TCGCCAGCCA	27	10.26	15	15	100	17
OP-X17	GACACGGACC	22	8.36	7	6	86	7
OP-Y13	GGGTCTCGGT	18	6.84	6	6	100	7
Total		263		91	90		
Average		29		10	10		

The arbitrary primers (OP-X12, OP-A03, OP-D18 and OP-T07) were useful for discrimination MRSA isolates of distinct characteristics (Figure1 and 2).

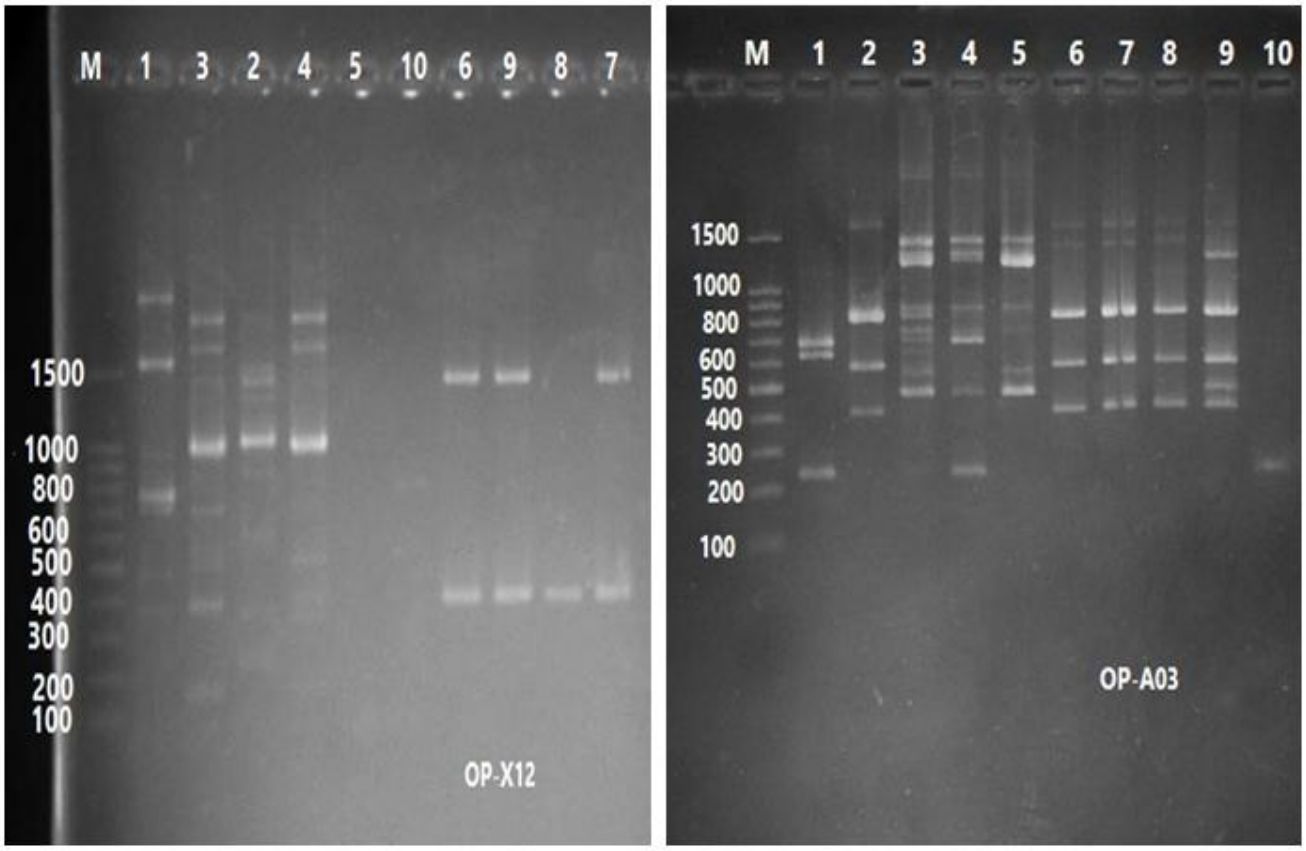

Figure 1: Agarose gel electrophoresis of primer OP-X12 and OP-A03 for DNA samples of the MRSA isolates. M: represent 100 bp ladder. Lanes: from 1-10 represent MRSA isolates.

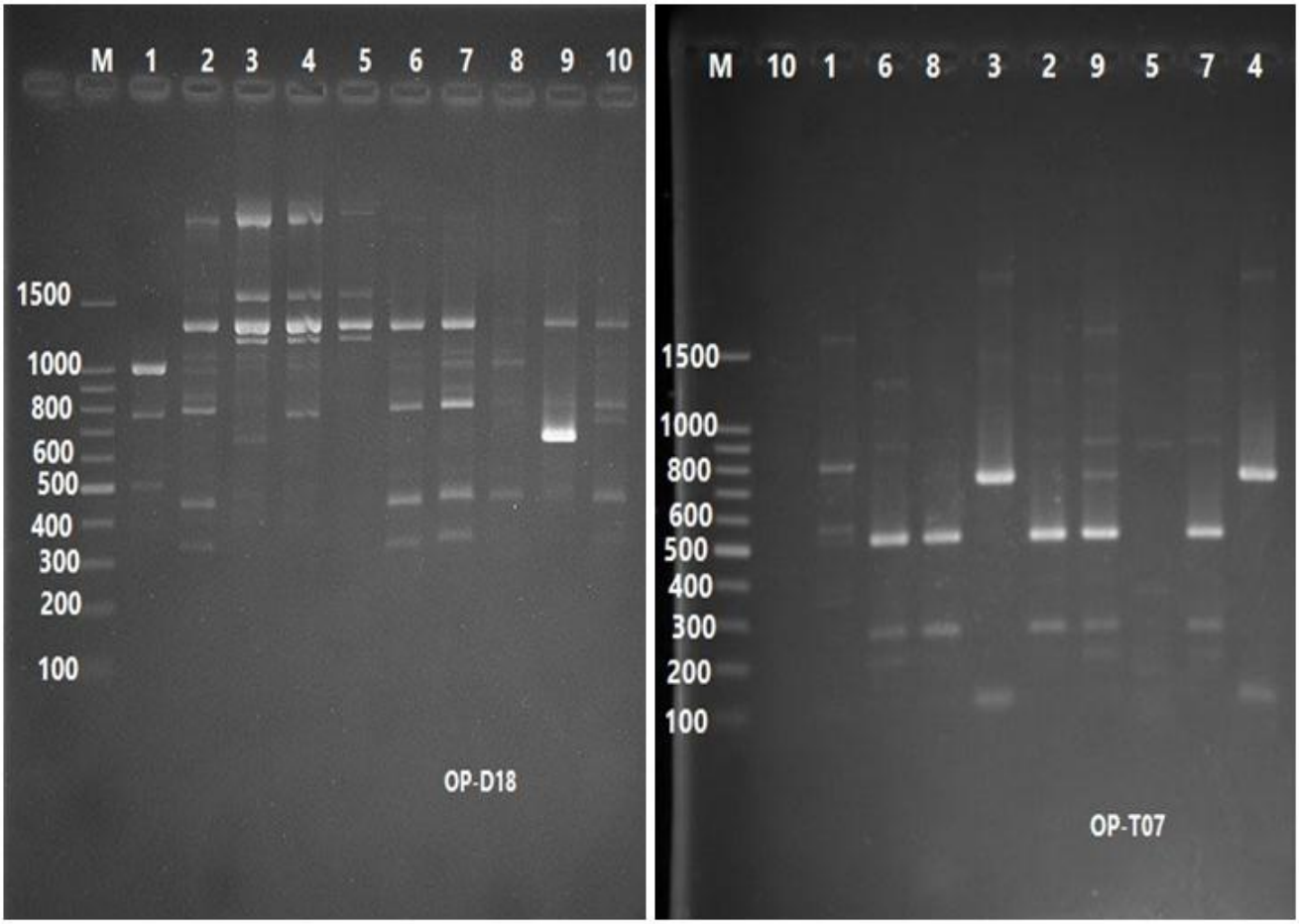

Figure 2: Agarose gel electrophoresis of primer OP-D18 and OP-T07 for DNA samples of the MRSA isolates. M: represent 100 bp ladder. Lanes: from 1-10 represent MRSA isolates.

The level of polymorphism among MRSA isolates was 100% identified through RAPD markers. This considered high level percentage of polymorphism produced by random primers when it is compared to other RAPD studies in MRSA which were 93.9% [20] and 88.24% [6].

3.3 Cluster analysis

A Dendogram was constructed based on Jaccard genetic similarity using UPGMA clustering to investigate the genetic relationships among 10 MRSA isolates (Figure 3). The Dendogram obtained from the analysis of RAPD primers showed two major groups (Groups A and B), group B contained only one isolate (isolate number 1) while A group divided into two subgroups (A1 and A2), A2 subgroups contained isolates (3,4 and 5), while A1 subgroup divided into two subgroups (A1a and A1b), A1b subgroup included isolate number (10), while A1a divided into two subgroups (A1a1 and A1a2), A1a2 subgroup included isolate number (9), while A1a1 divided into two subgroups (A1a1b and A1a1c), A1a1c contained isolate number (8) while A1a1b included isolates (2,6 and 7).In this study, most of the studied MRSA isolates (3,4 and 5) isolates and (2,6 and 7) isolates represent approximately the same banding patterns with (OP-A03, OP-D18, OP-T07 and OP-X12) primers. Therefore, banding patterns obtained by these primers had their impact somehow on the grouping of these isolates. We can expect that the studied primers may be linked or closely genetic region. According to all the results we explained above, we found that these results were closely related to the results obtained by [21].

3.4 Principal Component Analysis (PCA)

In order to confirm the results of genetic relationships among MRSA isolates, the data were analyzed by Multivariate Principal Component Analysis (PCA) as shown in (Figure 4). Each scattered diagram of the first two (PC1 and PC2) based on 90 polymorphic fragment lines of RAPD exhibited the same clusters of isolates as in dendrogram.

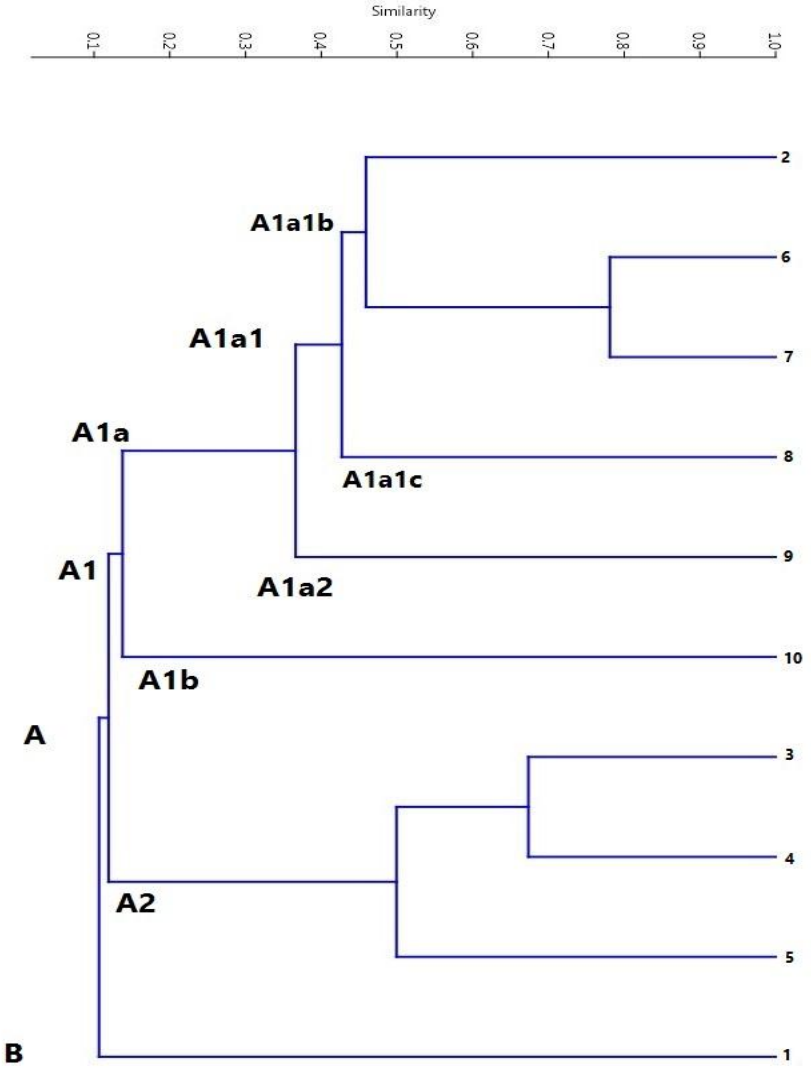

Figure 3: Genetic relationships among the 10 MRSA isolates estimated by RAPD analysis.

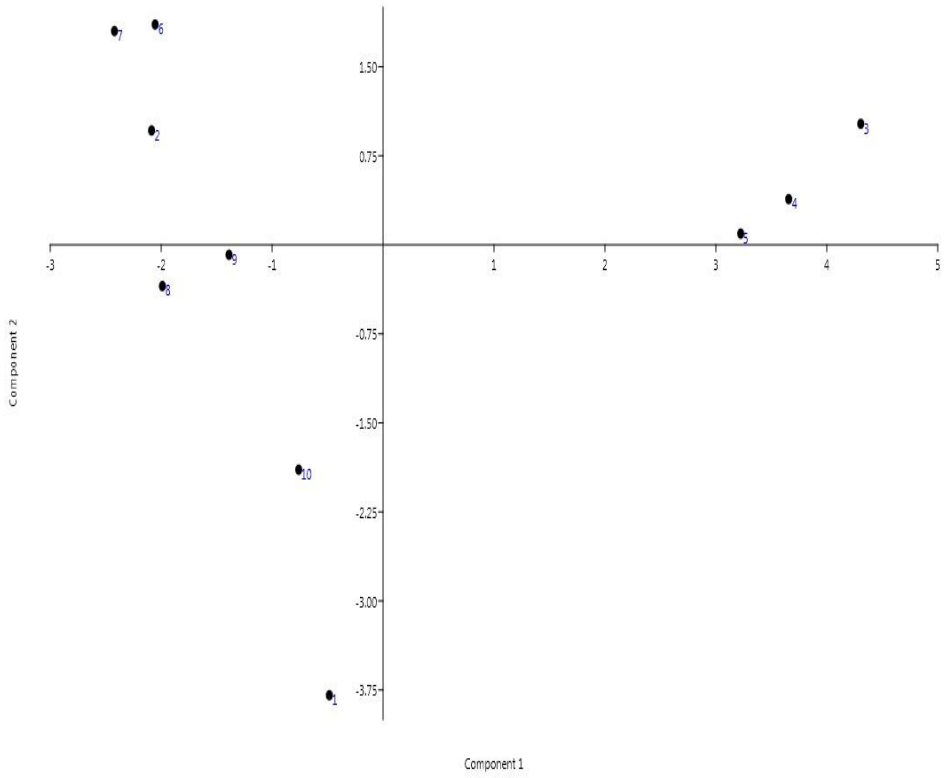

Figure 4: Principal component analysis of the 10 MRSA isolates estimated by RAPD markers.

4. CONCLUSION

It is important to have rapid and reliable epidemiological typing method to monitor the spreading of multi-drug resistant MRSA strains. Although the fact that the advances in the improvement of more accurate and reproducible molecular techniques have offered new typing choices for *S.aureus* strains, such as Pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST) and restriction fragment length polymorphism (RFLP), our results demonstrate that RAPD-PCR technique can be successfully applied to assess the genetic relationship of MRSA isolates from different Iraqi hospitals. 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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