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PCR- based Random Amplified Polymorphic DNA Fingerprinting of *Staphylococcus aureus* strains isolated from patients in Jizan Hospital, Saudi Arabia

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ABSTRACT: Characterization by RAPD-PCR using thee primers was performed to ten *Staphylococcus aureus* strains isolated from patients in Jizan Hospital during seven months period (November 2011 to January 2012) and the results were summarized in a dendogram to show the relationships between the test isolates. Fourty three RAPD fragments ranging in size from 100 to 1900 bp were amplified, 30 of which were polymorphic while the other were common among the ten *S. aureus* isolates. The mean percentage polymorphism shown by the three random primers was 88.24% .The polymorphic percentage of primers1, 2 and 3 were 83.33, 90.91, 90.91, respectively. UPGMA dendrogram showed two main clusters, the first included 7 isolates whereas, the second includes 9. The second cluster divided the 9 isolates into two sub-clusters, whereas the first contained isolate 2 with low similarity (36.7%) with isolate 7. The second contained 8 isolates and subsequently divided to two branches, the first branch containing isolates 5 and 4 (47.4%) and the second divided more to two sub-branches. One contained isolates 3 and 1 (60%) and the second showed four isolates. Two isolates (9 and 6) which revealed high similarity (78.3%).Isolate 6 revealed high similarity with isolate 9 (78%) followed by isolates 9 and 10 (76%). However, isolates 2 and 7 showed the lowest similarity percentage with 22%, followed by isolates 6 and 7 (25%). All the other remaining pairs showed intermediate similarity percentages. It should be noticed that most of isolates showed lower similarity percentages and this confirmed the different genetic backgrounds between ten *Staphylococcus aureus* clinical isolates under the present study.
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Key words: *Staphylococcus aureus*, RAPD analysis, patients, Jizan Hospital, Saudi Arabia

INTRODUCTION

Staphylococcus aureus is involved in a wide variety of infections found in human beings and animals, and some strains are also responsible for human food poisoning as they produce enterotoxins in foodstuffs (Rodreguez-Calleja et al., 2006).

Staphylococcus aureus is an important food-borne pathogen involved in a variety of invasive diseases and is a frequent cause of human infections which can become especially serious if induced by strains resistant to antimicrobial drugs (Schlegelova *et al.* 2003).

Several techniques are available for phenotypic differentiation of *S. aureus* isolates such as biotyping, bacteriophage typing and antibiotic resistance. Typing systems based solely on phenotypic tests have limitations such as relatively low discriminatory power and modest reproducibility because phenotypic traits are inconsistently expressed. The shortcomings of phenotypically-based typing methods have led to the progressive development of genotypic strategies like RAPD analysis, which has been proposed as the method of choice for typing *S. aureus* isolates and considered one of the most reproducible and discriminatory typing techniques (Tenover *et al.* 1994).

RAPD-PCR has been applied extensively as one of the most accurate ,rapid and effective genotyping methods to distinguish different isolates of *S. aureus* (Van Leeuwen *et al.* 2003). Accurate and rapid typing of *S. aureus* strains is crucial to the control of infectious strains. In this connection, Naffa *et al.* (2006) performed DNA fingerprinting by RAPD analysis of 100 clinical *Staphylococcus aureus* isolates to test whether isolates harboring the toxin genes. A total of 13 genotypes were identified with 47% similarity level. Genotypes I and V accounted for the largest number of enterotoxigenic isolates (19%). Their study demonstrated the genetic diversity of Jordanian clinical *S. aureus* isolates and

showed that the presence of the toxin genes is not genotype specific.

Reinoso *et al.* (2004) used RAPD-PCR to assess the genetic relationship between eighty *S. aureus* isolates from bovine and human hosts. Dendrogram obtained showed that all the isolates clustered into eleven groups (A-K) at a relative genetic similarity of less than 30% when analyzed with the three primers. Group A clustered 95% of the human host isolates and the remaining groups (B-K) clustered the bovine host isolates. The genetic diversity among the *S. aureus* isolates from bovine hosts is relatively low compared to that of isolates from human hosts. There were no statistically significant differences among isolates from bovine and human hosts. They suggested that RAPD-PCR assayed with their three primers can be successfully applied to assess the genetic relationship of *S. aureus* isolates from different hosts. Three well-known and frequently used staphylococcal typing methods (RFLP, RAPD-PCR and MLVA) were used for characterization,while the fermentative profile (Biolog GP2) was evaluated to characterize phenotypic diversity (Roach et al., 2006; Di Cagno et al., 2007).

Baksheeva *et al.* (2011) genotyped twenty *S. aureus* strains isolated from children nasal mucous membrane by RAPD-PCR. They detected the presence of six genotypes among the studied strains. The extent of genetic similarity between genotype 1 and 2 was established at 85%. Twenty of 63 (31.7%) of examined students were resident carriers of *S. aureus*. Three main genotypes of *S. aureus* that circulate in students were detected by RAPD analysis. This confirmed the necessity to use RAPD-PCR method for timely evaluation of epidemic situation in organized groups of children.

RAPD-PCR analysis using five primers was performed by Narmeen and Jubrael (2009) to detect the genetic identity of six isolates collected from Azadi General Hospital of Duhok City. Their results revealed the existence of genetic differences among these isolates in addition to the presence of DNA polymorphisms even within strains belonging to the same biotype.

Nikbakht *et al.* (2008) characterized eighty methicillin-resistant *Staphylococcus aureus* (MRSA) strains by RAPD-PCR using five primers and the results were summarized in a dendrogram to show the relationships between the test isolates. Forty-three RAPD-PCR profile were detected and the tested isolates were clustered into 18 groups with 50% similarity, indicating the heterogeneity of isolates. MRSA isolates fell into 41 antibiotic resistance patterns. They reported a correlation between antibiotic resistance patterns and the results of RAPD-PCR.

The objective of this study was to evaluate the genetic fingerprinting and phylogenetic diversity of *S. aureus* isolates from patients using RAPD-PCR .In addition, the detection of possible DNA polymorphisms even within strains belonging to the same biotype.

MATERIALS AND METHODS

Clinical samples

Ten *Staphylococcus aureus* strains isolated from patients in Jizan Hospital during the period from November 2011 to January 2012. Swabs of , skin, nose, blood and wounds samples were collected from each patient. They cultured in nutrient broth (composed of 5 g peptone, 1 g meat extract, 2 g yeast extract, 5 g sodium chloride and 15 g agar /L pH-7) and then incubated at 37^{o}C for 24 h. Ten of the 44 isolates were identified to be *Staphylococcus aureus* by biochemical tests such as gram staining, catalase production, bound and free coagulase and DNAase activity (Bannerman 2003). The ten *S. aureus* isolates were kept at -20^{o}C until use.

DNA extraction and RAPD analysis of *S. aureus* isolates

Genomic DNA was extracted from overnight cultures of *S. aureus* using the Wizard Genomic DNA Purification kit (Promega) as recommended by the manufacturer. RAPD analysis was performed using three 10-mer random primers (Metabion, Martinsried, Germany) as shown in Table (1). PCR amplification was carried out in a DNA thermocycler (Biometra, Germany) according to Nariman *et al.* (2008) for 30 cycles each. The PCR reaction was carried out in a final volume of 25 µl with 1X PCR buffer containing 10 mM Tris-HCl, 25 mM $MgCl_2$, 1 µl of template DNA, 0.2 mM dNTPs, 1 to 2 µM (each) primer and 0.5 U of *Taq* DNA polymerase (Promega).

PCR conditions consisted of an initial denaturation at 95°C for 2 min followed by 95°C for 1 min, annealing to primers at 35°C for 1 min and extension at 72°C for 1 min with a final extension step at 72°C for 5 min. PCR-amplified products were separated using agarose gel electrophoresis in 1% TBE buffer and stained with 0.2 µg/ml ethidium bromide according to (Salha *et al.* 2012). Amplified fragments were detected and photographed under UV light.

Table 1. Names and sequences of the three primers used for RAPD-PCR analysis.

RAPD primers		Primer sequences (5′-3′)
1	MN-45	AAGACGCCGT
2	KAY-1	AGCAGCCTGC
3	EPO-15	ACAACTGCTC

Genetic analysis

RAPD fragments were scored as present (+) or absent. The data was used for similarity-based analysis using the program MVSP (version 3.1b) from www.kovcomp.com. RAPD analysis was analyzed using the Nei genetic similarity index based on the equation: Similarity = $2N_{ab}/(N_a+ N_b)$, where N_{ab} = number of scored amplified fragments with the same molecular size shared between a and b, N_a and N_b = number of scored amplified fragments in a and b, respectively as discribed by Nei and Li (1979). A dendrogram was constructed based on the similarity matrix data by unweighted pair group method with average (UPGMA) cluster analysis.

RESULTS

Genetic characterization of clinical *S. aureus* isolates by RAPD analysis

A total of 34 amplified DNA fragments ranging in size from 100 to 1900 bp were detected by using three random primers; 1, 2 and 3, whereas 30 fragments were polymorphic and the other 4 amplified fragments were regularly identified among the ten *S. aureus* isolates (Table 2**)**.

The three primers showed a mean polymorphism of 88.24%, whereas the polymorphic percentage of primers 2 and 3 were higher (90.91); however primer 1 displayed lower percentage with 83.33.

Primer 1 revealed 12 fragments, 10 of which were polymorphic with sizes ranging from 100 to 1050 bp; two fragments with 200 and 300 bp were frequently observed among the ten isolates (Fig. 1and Table 2). The total number of amplified fragments of the isolates varied considerably. For instance, in isolates 8, 9 and 10, 10 fragments with different molecular sizes were amplified whereas, 8 and 9 isolates were similar in fragment sizes. Seven fragments were amplified in isolate 6, whereas 6 fragments were detected in three isolates (1, 3 and 4). Five fragments disappeared in isolates (5 and 7). Isolate number 2 revealed the lowest number with 3 fragments. On the other hand, one fragment with 600 bp was detected distinctively in isolate 10.As a result, RAPD analysis revealed that the amplified PCR products of most of the 10 isolates vary in molecular size patterns even with the equal total fragments.

Primer 2 revealed 11 fragments, 10 of which were polymorphic with sizes ranging from 150 to 1100 bp (Fig. 1and Table 2). The total fragment numbers of the ten isolates varied significantly in their fragments in a descending order from 9 to 3. Three isolates 6, 9 and 10 revealed the highest with 9 fragments, followed by isolates 3 and 5 with 8 fragments while isolate 8 revealed seven. The remaining fragments were descending decreased until 3 fragments in isolate 7.

Primer 3 revealed 11 fragments, 10 of which were polymorphic with sizes ranging from 180 to 1900 bp while the eleven one with 180 bp was commonly detected among all isolates (Fig. 1and Table 2). The total number of fragments of the ten isolates varied from two in isolates 3, 4 and 5 to nine in isolate 2, while three fragments were detected in three isolates (6, 7 and 9). Two distinctive amplified fragments with 1900 and 510 bp were *S. aureus* isolate-specific and were uniquely detected in isolates 7 and 2, respectively.

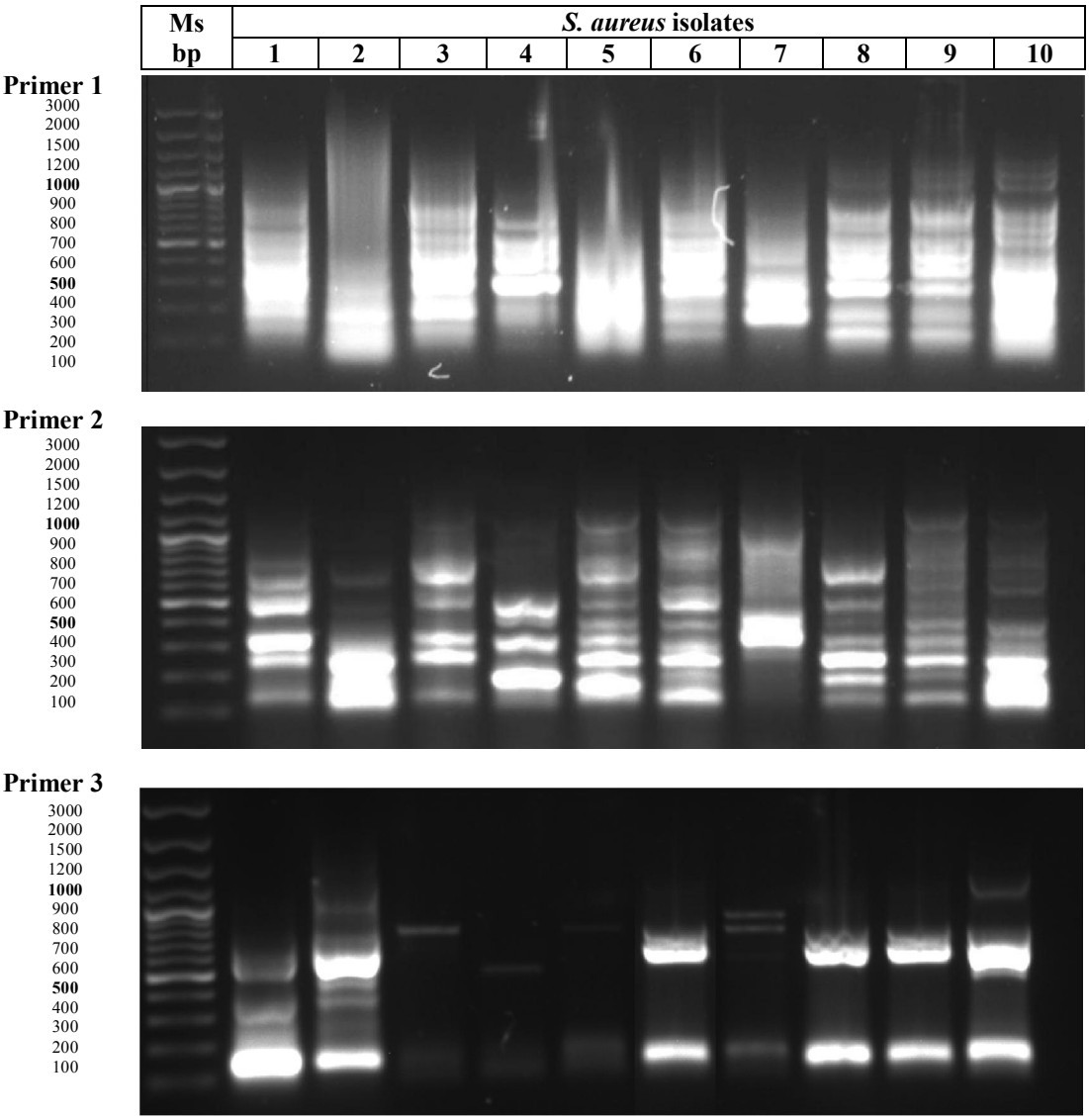

Fig. 1. RAPD amplification profiles of ten *S. aureus* isolates using three primers, Ms= 100 bp DNA ladder.

Table 2. RAPD analysis of the polymorphic fragments of ten *S. aureus* isolates using three primers.

Primer Name	*P%	Fragment №	Fragment Bs	*Staphylococcus aureus* isolates 1	2	3	4	5	6	7	8	9	10
		1	1050								+	+	+
		2	1000							+	+	+	+
		3	900	+				+		+	+	+	+
1		4	800	+		+	+		+		+	+	+
	83.33	5	700	+		+	+		+		+	+	+
		6	600										+
		7	500	+		+	+	+	+	+	+	+	+
		8	350						+		+	+	
		11	150			+			+		+	+	+
		12	100		+		+	+					
Total № = 12, Variable bands =10				**6**	**3**	**6**	**6**	**5**	**7**	**5**	**10**	**10**	**10**
		1	1100			+	+	+	+			+	+
		2	1000			+				+	+		
		3	870	+		+		+				+	+
2		4	790	+	+	+		+	+		+		+
		5	710						+			+	+
	90.91	6	550	+	+	+	+	+	+	+	+	+	
		7	460					+	+			+	+
		9	300	+	+	+		+	+		+	+	+
		10	220				+	+	+		+	+	+
		11	150	+	+	+			+		+	+	+
Total №= 11, Variable bands = 10				**6**	**5**	**8**	**4**	**8**	**9**	**3**	**7**	**9**	**9**
		1	1900							+			
		2	1500			+		+		+			
		3	1000		+		+		+		+	+	
		4	910	+	+				+		+	+	+
		5	740		+								+
3	90.91	6	600		+						+		+
		7	550		+						+		
		8	510		+								
		9	390	+	+								
		10	300	+	+								
Total №= 11, Variable bands = 10				**4**	**9**	**2**	**2**	**2**	**3**	**3**	**5**	**3**	**4**
Total variable bands = 30 **Overall total bands = 34**				Mean polymorphic percentage of the three primers= **88.24%**									

+ = Present of amplified bands, Bs = Molecular size by base pair, P% = Polymorphic percentage, Total = Total number of amplified bands.

Genetic similarity of the ten *S. aureus* isolates using RAPD analysis

Genetic similarity between each two pairs of the ten *S. aureus* was performed using the Nei similarity index on the basis of RAPD amplified fragments using the three random primers (Table 3). Genetic similarity percentages were calculated from the amplified fragment data using un-weighted pair group method with averages (UPGMA).

The constructed UPGMA dendrogram of the three primers showed two main clusters, the first included isolate 7 and the second includes 9 isolates. The second cluster divided the 9 isolates into two sub-clusters, whereas the first contained isolate 2 with low similarity (36.7%) with isolate 7. The second contained 8 isolates and subsequently divided to two branches, the first branch containing isolates 5 and 4 (47.4%) and the second divided more to two

sub-branches. One contained isolates 3 and 1 (60%) and the second showed four isolates. The isolates 9 and 6 revealed high similarity with 78.3% (Fig. 2).

Using the three primers, isolate 6 revealed the highest similarity with isolate 9 (78%) followed by isolates 9 and 10 (76%). However, isolates 2 and 7 showed the lowest similarity percentage with 22%, followed by isolates 6 and 7 (25%) as shown in Table (3). All the other remaining pairs showed intermediate similarity percentages between the highest and lowest percentages. It should be noticed that most of isolates showed lower similarity percentages and this confirmed the different genetic backgrounds between ten *Staphylococcus aureus* clinical isolates under the present study.

Table 3. Genetic similarity percentages of *S. aureus* isolates based on RAPD amplified products.

Isolates	1	2	3	4	5	6	7	8	9
2	50								
3	60	32							
4	40	32	47						
5	46	32	52	47					
6	52	39	59	55	46				
7	35	**22**	42	35	42	**25**			
8	52	44	52	42	36	64	38		
9	52	30	52	48	46	**78**	32	69	
10	52	35	52	36	46	64	**27**	63	**76**

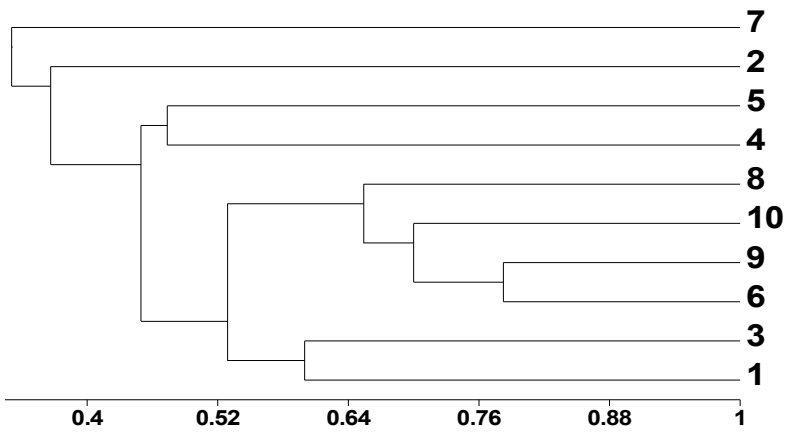

Fig. 2. Dendrogram represented the genetic relationships among ten *S. aureus* isolates using UPGMA cluster analysis of Nei genetic similarity coefficients generated from three RAPD primers.

Discussion

In the present study, the three RAPD primers showed DNA polymorphism among *Staphylococcus aureus* isolated from clinical specimens,, either in the occurrence of amplified fragments or in the variable genetic similarities of each isolate with the others. Despite the fact that they should display narrow and low variation due to the genomic structure of the *Staphylococcus aureus* species and the structure of the 10 mer-RAPD primers. Eventually, the fluctuation of genetic similarity values each of the ten isolates with others using the three primers evidently revealed the divergent genetic backgrounds of such isolates with their DNA polymorphism patterns. The results revealed that the 10 isolates were genetically

different ,furthermore, primers 2 and 3 showed high polymorphism. In accordance with our results, several reports stated polymorphism among isolates using RAPD fingerprinting. For instance, ten out of 100 primers showed polymorphism among 18 different isolates of *S. aureus* from Nigeria generating 88 bands, 51 of which were polymorphic (Onasanya *et al.* 2003). Also, Singh *et al*. (2006) detected polymorphisms between isolates of *S. aureus* and they suggested that biotyping often lacks discriminatory power because of variations in gene expression and random mutations that may alter biologic properties of microorganisms. RAPD-PCR grouped 35 *S. aureus* isolates from food raw materials and workers' hands into five clusters (A-E) showing 19 RAPD types with discrimination indexes (D) of 0.949 (Ye *et al.,* 2012).

It was found that identification of genetic diversity in *S. aureus* depends on sources of isolates, different host cells and occurrence of mutants (Onasanya *et al*.,2003; Neela *et al.*, 2005 and Tenover *et al*., 2007). In this connection, Morandi *et al*. (2010) revealed marked genetic variability among 130 *S. aureus* strains isolated from different raw-milk dairy products (122 isolates) and human samples (eight isolates). Nikbakht *et al.* (2008) detected 43 RAPD profiles of MRSA isolates collected from clinical specimens (noses of 460 staff and patients). The isolates clustered into 18 taxa with 50% similarity, indicating the heterogeneity of their test isolates.).

The DNA fingerprint defined for each race of *S.aureus* should be useful for epidemiological surveys, medical diagnosis, and in the identification of new virulent strains and isolates and their origin

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