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RAPD FINGERPRINTING FOR GENOTYPIC DIFFERENTIATION OF *S. aureus* CLINICAL ISOLATES FROM CAMEL (*Camelus dromedarius*)

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ABSTRACT

Randomly amplified polymorphic DNA (RAPD) typing was performed on 21 *S. aureus* isolates from clinical wounds and abscesses in camel. Out of ten primers chosen for screening to know discrimination ability for *S. aureus* isolates three primers namely opc 1,2 and 10 showed good discriminatory index and generated 151 polymorphic bands with size ranging from 200-2500 base pairs. All the isolates were classified into two groups. Group 1 contained only one isolate while rest 20 were in group 2. The study indicated that there is much more genetic variation than studied earlier and RAPD can successfully be applied for differentiation of *S. aureus* strains.

Key words: Camel, genotyping, RAPD, *S. aureus*

The skin infections due to staphylococci causing contagious skin necrosis, dermatitis, wounds, abscesses or similar lesions are constant problems inflicting camel. It is important in epidemiology and ecology to precisely identify bacterial species and strains involved in order to study the emergence of newer strains and containment of the infections through use of effective antibiotics.

Recently molecular DNA typing methods have been developed on the basis of genotypic characterisation. The amplification of discrete DNA fragments in the genome by the use of oligonucleotide primer with random sequences, have been largely and extensively applied to distinguish different isolates of *S. aureus* (Tenover *et al*, 1994; van Belkum *et al*, 1995). RAPD method when compared with biochemical method is cheap, simple, more sensitive and faster.

In present investigation RAPD-PCR technique was used to study genotypic differentiation and phylogenetic diversity of isolates of *S. aureus* from clinical cases of wounds and abscesses in camel. Such information will be useful in classification, epidemiological survey, ecology and diagnosis of *S. aureus* infections.

Materials and Methods

Preparation of *S.aureus* culture

The *S. aureus* culture was grown overnight in 25 ml nutrient broth in shaker incubator at 37°C and optical density of the overnight grown culture was adjusted to 0.4. The bacterial culture was then centrifuged at 5000 rpm in cooling centrifuge machine to obtain pellet and supernatant was discarded.

Isolation of DNA

DNA isolation was carried out as per the method of Nachimuthu *et al* (2001) with some modifications as described below :

(i) Bacterial cultures were pelleted and washed two times with PBS.

(ii) Final cell pellet was resuspended in 1 ml of Tris-EDTA solution (TE).

(iii) One hundred µl of lysozyme solution (conc. 3 mg/ml) was added and mixture was then incubated at 37°C in water bath for 15 minutes.

(iv) One hundred µl of 10% SDS solution and 2 µl of pronase (10 mg/ml) solution was added and incubated at 50°C in water bath overnight.

(v) Next day, sodium perchlorate (1M final concentration) was added and further incubated for one hour at 50°C in water bath.

(vi) Phenol - chloroform : isoamyl alcohol mixture (25:24:1) was prepared and equilibrated with 1/10th volume of TE buffer. Equal volume of this mixture was added to the DNA preparation and mixed gently for about 5-10 minutes.

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(vii) Above mixture was then centrifuged at 15000 rpm for 15 minute and upper aqueous phase containing DNA was transferred to another tube.

(viii) The aqueous phase was then subjected to ethanol precipitation and DNA was spooled out on a glass rod and redissolved in 200 µl TE buffer.

Purification and quantitation of DNA :

The proteins, including RNA were removed from isolated DNA by treating with DNAse free RNAse, integrity of purified DNA was checked by agarose gel electrophoresis in a horizontal, submarine electrophoresis unit in 0.8% agarose gel in TBE buffer containing ethidium bromide (0.5-1 µg) and concentration of DNA was estimated (Sambrook *et al*, 1989). The quantified DNA was diluted to a final concentration of 25 ng/µl in TE buffer.

RAPD - PCR Analysis :

RAPD analysis was carried out as per the method of Gerriston *et al* (1995) using ten primers of series C obtained from Operon Technologies, Alameda (California, USA).

The PCR reaction was performed in a final volume of 25 µl containing 1 unit of Taq polymerase in 10x assay buffer, 200 µM of each dNTPs, 10 picomole of random primer and 50 ng of total genomic DNA. The PCR was performed in Biometra Thermocycler using the following cycling parameters :

Cycle-1	Denaturation (94°C)	5 min
	Primer annealing (37°C)	1 min
	Primer extension (72°C)	2 min
Cycle 2-43	Denaturation (94°C)	1 min
	Primer annealing (37°C)	1 min
	Primer extension (72°C)	2 min
Cycle-44	Denaturation (94°C)	1 min
	Primer annealing (37°C)	1 min
	Primer extension (72°C)	5 min

The PCR products, after addition of 2 µl trekking dye were resolved in 1.2% agarose gels prepared in 1x TBE buffer containing 0.5 µg/ml of ethidium bromide and λ DNA Eco Rl + Hind III double digest and λ DNA Hind III digest were used as molecular marker. The amplified products were electrophoresed for 5 hours at 50 Volts. The gel was then visualised under U.V. trans-illumintor.

Scoring of RAPD Products :

RAPD bands were designated on the basis of their molecular sizes based on length of DNA fragment amplified. The molecular size was estimated by molecular weight markers loaded simultaneously with each primer products in the gel. The distance run by amplified fragments from the well was translated to molecular sizes with reference to molecular weight marker using computer programme DNA OPT ver. 1.0 (Ragava, 1995). The presence of each band was scored as 1 and its absence as 0. Faintly visible bands were not considered for scoring but a major band corresponding to faint band was scored.

Statistical Analysis :

The score (0 or 1) for each band obtained from photographs remained in the form of a rectangular data matrix (qualitative data matrix). Massing data represented by a code score-Pairwise association coefficients were calculated from qualitative data matrix using Jaccard's similarity coefficient (Jaccard, 1908) using the following equation :

f = nxy / (nt-nz) where

nxy = number of bands common to sample A and B

nt = total number of bands present in all samples

nz = number of bands not present in sample A or B but found in other samples

Cluster analysis for the genetic distance was then carried out using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clustering method. The genetic distances obtained from cluster analysis through UPGMA were used to construct the dendrogram, depicting the relationship with clones using computer programme NTSYS pc version 1.7 (Rohlf, 1987).

The discriminatory ability of primers was identified by discriminatory index (D) by following formula given by Hunter and Gatson (1988) :

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{S} nj\,(nj-1)$$

Where ;

D = Discriminatory index

S = total no. of type used

nj = No. of strains belonging to jth type

N = total no. of strains

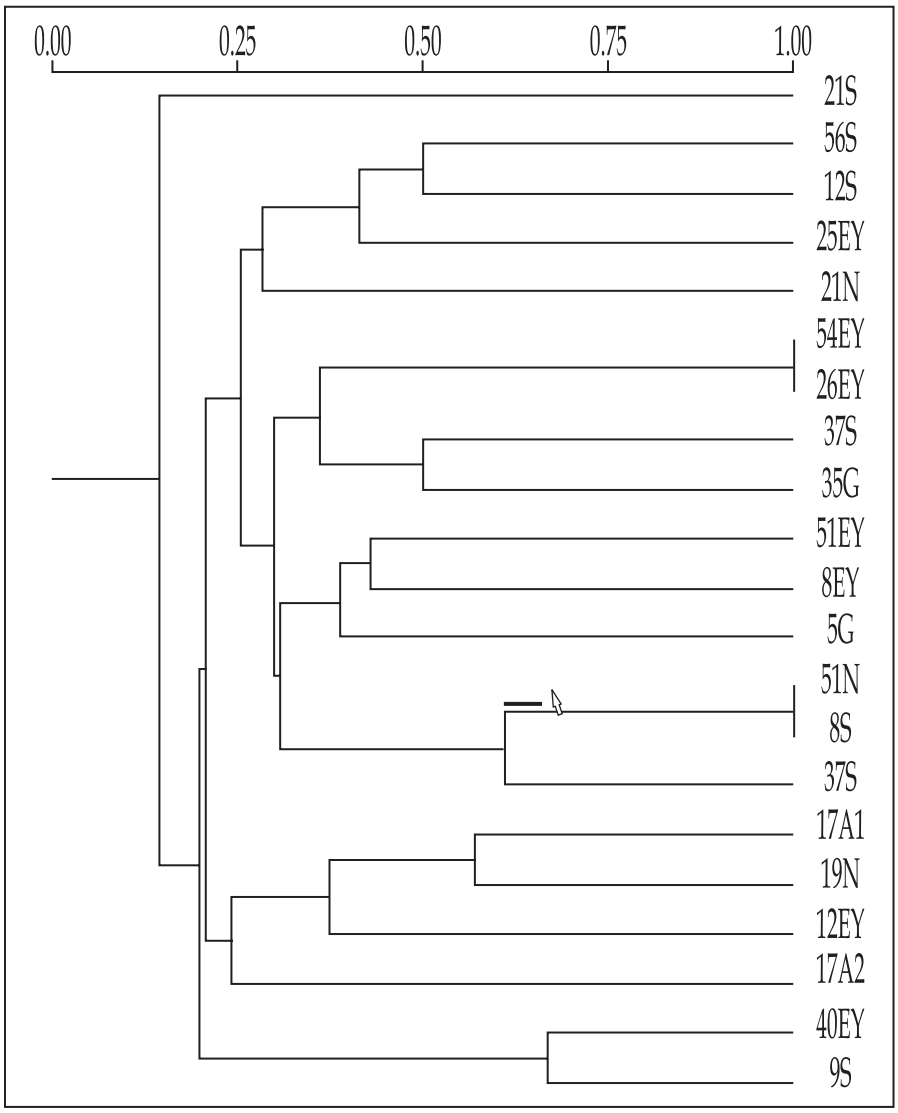

Fig 1. Dandrogram showing relationship among *S. aureus* isolates using UPGMA generated by RAPD analysis with primer group OPC1-OPC10.

RAPD - PCR Profile :

Three primers OPC1 (TTC GAG CCA G), OPC2 (GTG AGG CGT C) and OPC10 (TGT CGT GGT G) showed polymorphism among individual isolates out of 10 primers tested. The amplification reaction with primers generated 151 polymorphic bands with size ranging between 200 and 2500 base pairs. On the basis of dendrogram the isolates were phylogenetically divisible into two groups at about 25 % similarity coefficient. Out of 21 isolates, there was only one isolate (21S) in group 1, whereas other twenty isolates clustered in group 2 generating 18 sub groups. The levels of dissimilarity of *S. aureus* strains ranged between 25 to 100 %. Two isolates each from subgroup 2 showed 100% similarity level and were put in same group.

RAPD has been found to be a simple and rapid method with practical value in molecular typing of *S. aureus* (Onasanya *et al*, 2003). The usefulness of RAPD fingerprinting to distinguish between different subtypes of *S. aureus* has also been demonstrated by Gillespie *et al* (1999).

Results and Discussion

The present study was carried out with the aim to characterise *S. aureus* isolated from wounds and abscesses in camel skin present on different parts of the body viz. eye, nostrils, groin region, back and shoulders. Out of the total isolates obtained in the investigation 21 *S. aureus* were subjected to randomly amplified polymorphic DNA (RAPD) analysis in order to study the variation in the genomes of these isolates. The selection of bacterial isolates which were put to RAPD analysis was made on the basis of two different localities of animals, difference in the property of coagulase production, variation in the types of toxins produced, difference in biochemical properties and differences in the antibiogram properties.

The results show 100% genetic diversity among *S. aureus* isolates included in the study except isolates 54 Ey and 26 Ey both of which were isolated from eye wounds and isolates 51 N and 8S isolated from nostril and skin wounds (Fig 1). The results in the present study are in complete agreement to the finding of Grundmann *et al* (2001) who measured the genetic diversity among *S. aureus* isolates obtained from the individuals of same age and environment using RAPD typing method. They reported genetic diversity of 97.6%. Similarly van Belkum *et al* (1997) also recorded a great heterogeneity in *S. aureus* strains.

Martinez *et al* (2000) during characterisation of *S. agalactae* isolates from bovine and human origin by

RAPD analysis also observed no clustering of bovine isolates originating from the same region and none of the human isolates shared identical RAPD patterns with three primers used. They reported a high genetic diversity for this organism also.

The division of 21 isolates in the present study is similar to findings of Onasanya *et al* (2003) who carried out genetic fingerprinting using RAPD markers to study phylogenetic diversity of 18 different *S. aureus* isolated from different sources and found the *S. aureus* to fall in two groups and 12 sub groups but all the 7 animal isolates were in a same group.

All the isolates in the present study which were from different animals, various anatomical positions, two different places and from normal skin and wounds and abscesses showing wide variation in genotype may be suggestive of adaptation of *S. aureus* to different host cells, environment or to different anatomical positions in a single host and occurrence of mutants. The use of primers with high discriminatory power may also be an important factor for higher genetic diversity in the present investigation.

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