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Molecular characterization of *Staphylococcus aureus* - human pathogen from clinical samples by RAPD markers

Gaurav Kumar Singh[1], Beena Derajira Bopanna[1*] and Ganesh Rindhe[2]

[1]Post Graduation Department of Biotechnology, CMR Institute of ManagementStudies(Autonomous)

[2] Sangenomics Research Lab Pvt Ltd

E mail: beena2631@yahoo.com

ABSTRACT

Staphylococcus aureus is one of the most significant pathogens causing nosocomial and skin infection. It *is an opportunistic bacterium, frequently part of the human micro flora, causing disease when the immune system becomes compromised. Many genotypic variants and antibiotic strains have also been reported in the earlier studies. Therefore, the present study was carried out to examine the genotypic variations of S. aureus in different clinical samples.* Six different samples of *S. aureus isolated from Urinary tract infection (UTI), knee wound and sputum were subjected for various biochemical tests to confirm the identity of S. aureus as a pathogen. Molecular characterization of these isolates was carried out by using random amplified polymorphic DNA (RAPD). Out of five primers (D-18, D-20, T-7, W-2 and X-6) tested D-20 produced maximum number of bands. From the result obtained, it was seen that clinical isolates of S. aureus from knee wound (SA-1) and sputum sample (SA-6) are closely related. Close relation was also observed between sputum sample (SA-4) and UTI sample (SA-5). In conclusion, S. aureus strains are not specific for infection.*
Key words: RAPD, primers, clinical, dendrogram

Introduction

Staphylococcus aureus are Gram positive cocci occur in clusters belonging to *Micrococaceae* family. The pathogenicity of *S. aureus* is defined many years back (Stokes & Ridgway, 1980). It causes abscesses, boils, conjunctivitis especially in newborn, cross-infections in hospitals septicaemia, and mastitis (Olorunfemi *et al.*, 2005). *S. aureus* is described as a variable bacterium with many morphological variants (Kloos & Schleifer, 1981). Development of antibiotic resistance *S. aureus* strains which is a serious setback in many hospitals causing various hospital outbreaks has been reported in many studies (Allen *et al*., 1995; Francais, 1997) . Although, *S. aureus* is a prime pathogen of nosocomial and community infections, the rising prevalence of Methicillin and vancomycin resistant *S. aureus* globally has become a major clinical problem (Otter & French,

2006; O'Brien *et al.,* 2009; Abu Shady *et al*., 2012; Selma *et al*., 2013). In an effort to develop effective control strategies against the genotypically variated organisms, it is essential to characterize accurately the extent of genetic and phenotypic variation present in the pathogen population. There are various conventional methods **(**biotyping, antibiotyping and phagetyping) which can be used to identify and characterize these organisms (Jacques et al., 1994; DeLappe et al., 2003; Altun et al., 2013). But application of PCR based RAPD primers has been played a major tool in finding out the relationship between the various species of micro organisms (Fevzi, 2001; Deepika& Bhatnagar, 2006) . The technique effectively scans a genome for these small inverted repeats and amplifies intervening DNA segments of variable length. The profile of amplification products depends on the template primer combination and is reproducible for any given combination. Construction of dendrogram using similarity matrix gives the relationship between the isolates. Earlier report on similarity matrix of *S. aureus* by using RAPD primers has shown dissimilarity between the organisms present in the sputum of various infected persons (Sundar & Geeth 2011). The aim of the present study was to isolate, identify and analyze the genotypic variations among *S. aureus* collected from different clinical samples which help in further pharmacological investigations.

Materials and methods

Samples of Urinary tract infection (UTI), knee wound and sputum of six different individuals were collected from Doctor diagnostic Laboratory Kammanahalli, Bangalore. Collected samples were serially diluted and spread on sterile Mannitol Salt Agar medium and incubated at 37^0 C for 24 hrs. Yellow colour colonies obtained were screened for the conformation of *S. aureus* by Gram staining' and biochemical (Mannitol fermentation, Citrate utilization and catalase) tests (Cappuccino & Sherman, 1996). Conformations was done comparing the characteristics with Bergey's mannuanl (Bergey's Manual, 1985). The confirmed *S. aureus* were labeled with numerals 1-6 (SA-1 & SA-2- knee wound sample; SA-3 & SA-5 –UTI sample; SA-4 & SA-6- sputum sample). Cultures were further multiplied in Luria-Bertani broth and genomic DNA was isolated by phenol - chloroform method (Sambrook *et al.,* 1989**)**. Quantification was done by nanodrop ND-1000 UV-vis spectrophotometer (Nanodrop ScientificTechnologies). For RAPD analysis, each strain was tested with five primers as described previously (Hadrys *et al., 1992).* The DNA amplification reaction was carried out in a 25-µl volume containing 1µl DNA (1 µg), 2.5µl PCR buffer (10X), 1.5µl deoxynucleoside triphosphate (10mM), 1µl of primer (100pMol), 2.0µl of *Taq* DNA polymerase (1U/ µl) and 17µl of sterile water. For amplification, five different primers viz; D-18 (5'-GAGAGCCAAC-3'), D-20 (5'- ACCCGGTCAC-3'), T7 (5'GGCAGGCTGT-3'), W-2 (5'-ACCCCGCCAA-3') and X-6 (5'-ACGCCAGAGG-3') were used. Amplification was carried out in thermal cycler (CR Corbett PCR) which was programmed as for four cycle as follows:

Cycle1: Initial one cycle denaturation at 94°C for 5 minutes;

Cycle2: Ten cycles of denaturation at 94°C for 45 seconds, annealing at 32°C for 1 minute, extension at 72°C for 1 minute

Cycle 3: Final denaturation of 30 cycles at 94°C for 45 seconds, annealing at 34°C for 1 minute, extension at 72°C for 1 minute

Cycle 4: One cycle of final extension at 72°C for 5 minutes. The amplified DNA fragments were analyzed by electrophoresis at 100 volt for 1 hour on a 2% agarose gel in TBE buffer (0.89 M Tris, 0.89 M boric acid, 0.02 M disodium EDTA, pH 8.4) stained with ethidium bromide (0.5 µg/ml). RAPD profiles were defined by bands

that were present in different amplification reactions by using Gel Documentation system (alpha imager HP software, alpha Innotech Gel Doc). All the visible bands on the gel were counted and data were scored for the presence or absence of amplification products. RAPD assay was repeated at least three times under the same conditions to confirm the reproducibility of the method. Phylogenetic variation were determined by converting RAPD data into a frequency similarity and analyzed by Un-weighted pair Group Method with Arithmetic mean (UPGMA) cluster analysis to produce a phylogenetic tree.

Result and Discussion

All the samples SA-1 to SA-6 collected from six different individuals were found to be gram positive cocci in clusters. Positive response of all the samples for biochemical tests (mannitol fermentation, citrate utilization and catalase test) confirmed *S. aureus* (Table1). Though these isolates were confirmed with biochemical tests, there may be minor genetic variations which changes in their drug susceptibility pattern. Emergence of the drug resistance strains of *S. aureus* has already reported in animals, poultry and human beings (Andrew *et al*., 2011; Chambers, 1997). These changes were confirmed with genetic variability test in the present study. Purity of genomic DNA isolated from clinical samples was analyzed by running in agarose gel (0.8%) electrophoresis. Quantity of DNA varied from 188 ng/µl to 1590 ng/µl. Application of nanodrop for quantification of genomic DNA was also reported for *E. coli (*Bilgin *et al*., 2011*).* The present study revealed polymorphic DNA bands in six clinical samples. Maximum amplification was obtained in primer D-20 which produced 8 bands in samples SA-5 and SA-3 followed by 6 bands in SA-4. This result is similar to the earlier report (Sundar & Geethu, 2011) *where, RAPD* primer was successfully applied to assess the genetic relationship and produced polymorphic bands in four clinical sputum samples of *S. aureus* isolated from various sputum samples of suspected hosts. The present study on RAPD analysis revealed that, there were some bands which are common in all the samples and some were not evident. Molecular variation within the *S. aureus* collected from various clinical samples had 47% similarity, cut-off value gave 13 major clusters and seven subclusters including genetically related isolates in microbiology, laboratory of Jordan University Hospital, Amman, Jordan (Randa et al., 2006). In the present exploration also, the differences in the polymorphic bands of DNA were visualized, scored and further clarified by similarity matrix. The distance matrix similarity ranged from 3.00-4.80 (Table2). At the linkage distance of 4.35, there were two clusters formed. Cluster 1 comprising of SA1- knee wound sample and SA6- sputum sample showed close linkage distance. Cluster 2 consisted four samples (SA-2, SA-4, SA-5 and SA-3) among which SA-4- sputum sample and SA-5 -UTI sample were closely related at linkage distance of 3.3 (Figure 1). So, the present study reveals that, *S. aureus* is not specific for infections. This clearly shows that, one can't use the same drug for a particular infection caused by *S. aureus* because of their variation in DNA polymorphism. Therefore further study on antibiotic sensitivity and sequence analysis would help to devise and prescribe a better drug for the future.

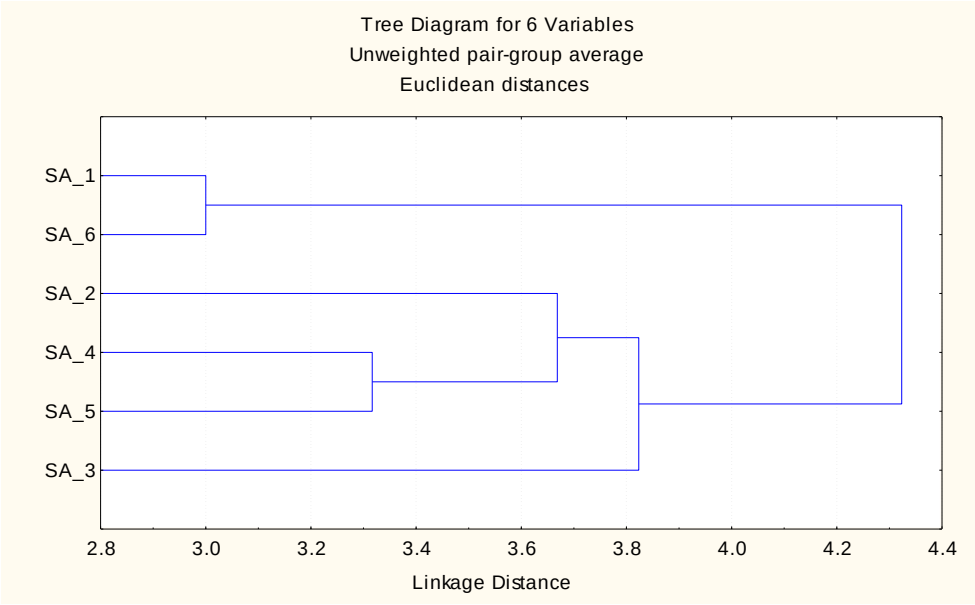

Figure 1: Dendrogram developed from UPGMA cluster analysis showing relationship between *S. aureus* species. SA-1& SA-2, knee wound samples; SA-3& SA-5, Urinary Tract Infection samples and SA-4& SA-6, sputum samples collected from 6 different individuals.

Morphological/Biochemical test	SA-1	SA-2	SA-3	SA-4	SA-5	SA-6
Gram staining	+ve cocci in cluster	+ve cocci in cluster	+ve cocci in cluster	+ve cocci in cluster	+ve cocci in cluster	+ve cocci in cluster
Mannitol fermentation test	+	+	+	+	+	+
Citrate utilization test	+	+	+	+	+	+
Catalase test	+	+	+	+	+	+

Table 1: Gram training and biochemical tests for confirmation of *S. aureus*

	SA-1	SA-2	SA-3	SA-4	SA-5	SA-6
SA-1	00					
SA-2	4.12	00				
SA-3	4.47	4.12	00			
SA-4	4.24	3.87	3.74	00		
SA-5	4.80	3.46	3.61	3.32	00	
SA-6	3.00	4.00	4.12	4.36	4.47	00

Table 2: Distance similarity matrix for S. aureus generated using Eucledian distances

Conclusion

In conclusion, the data acquired in the present work confirm the wide genotypic diversity of *S. aureus* from various clinical samples. It is interesting to note that, there was no evident correlation between the observed strain variability and the sample from which the isolates originated. So, it is a challenge for future researchers to screen all the genotipically variant *S. aureus* strains and design the drugs based on their drug susceptibility.

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