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Molecular Typing of Staphylococcus Aureus Isolated from Various Environmental Sources

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Abstract:- RAPD-PCR was employed for the characterization of *S. aureus* isolates from different sources such as soil, water, milk, meat and skin swab etc.,. Isolated pure cultures of *S.aureus* strains were subjected to genomic DNA isolation, purification, separation and quantification. Isolated DNA samples were distinguished by using 4 different random primers. Genome profile analysis obtained from the *S.aureus* demonstrated that it was possible to differentiate the *S. aureus* strains from different sources by RAPD technique. Results indicate possible relationships between host origin and genetic variation among *S. aureus* isolates from various sources. This RAPD method was thus useful for epidemiological studies of the *S. aureus* flora.

***Keywords*:-** *Staphylococcus aureus, Genomic DNA, Random Amplified Polymorphic DNA (RAPD) and Polymerase Chain Reaction (PCR).*

I. INTRODUCTION

Staphylococcus aureus is a facultative anaerobic gram-positive bacterium. It is frequently found as part of the normal skin flora on the skin and nasal passages. S.aureus is the most common species of staphylococci to cause staph infections [1]. S.aureus can be isolated from various samples majorly it can be found in meat, milk, soil, water [2]. S.aureus is an effective pathogen, the blend of host and bacterial immuno-shifty systems, one of these procedures the creation of carotenoid shade staphyloxanthin can be happens which is liable for the trademark brilliant shade of S.aureus and furthermore it causes some minor contaminations pimples, impetigo, bubbles, cellulites, folliculitis, and carbuncles. Every year 500,000 patients in American medical clinics contract a staphylococcal contamination [3].Methicillin-safe S.aureus (MRSA) is one of the no of extraordinarily dreaded strains of S.aureus which have gotten impervious to most anti-infection agents [4]. An ongoing report by the translational genomics research establishment indicated that half of the meat and poultry markets were sullied with S.aureus [5]. S.aureus is a Catalase positive, so able to convert hydrogen peroxide to water and oxygen [6]. S.aureus can be differentiated from most other staphylococci by the Coagulase test [7]. It can survive on domesticated animals, such as dogs, cats, horses and it can cause bumble foot in chickens [8]. It can have phages, for example, Panton-valentine leukocidin that expands its harmfulness. A few strains of S.aureus, which creates the exotoxin TSST-1, that are causative operator of poisonous stun condition. It duplicates abiogenetically [9]. The treatment of S.aureus infection is penicillin, gentamycin is used to treat serious infection such as endocarditic [10]. The present study was undertaken to determine the staphylococcus sp in meat, milk, soil, water and skin swab collected from Guntur district and followed by isolation of staphylococcus aureus, gram staining, Biochemical tests, DNA isolation, Purification and finally RAPD –PCR, This RAPD method was thus useful for epidemiological studies of the *S. aureus* flora.

II. MATERIALS AND METHODS

A. Collection of samples

The samples are collected from Guntur area located in Andhra Pradesh out of four samples Meat and Milk samples are collected from local market as well as soil and water samples are collected near vignan's university. All these samples are collected in sterile capped containers and transported to the laboratory within 6 hours of sample collection for further analysis by keeping them on ice. The microbial analysis process was done through isolation, characterization followed by slandered microbiological techniques like serial dilution, spread plate method and streak plate method. Through morphological and biochemical tests microbe characterization and identification was done.

B. Isolation of staphylococcus aureus

Isolation is carried out by traditional microbial technique serial dilution method.100ml of each samples are added on to the specific media (Mannitol salt agar) plates of staphylococcus aureus sp; and incubated at 37c for 24 hrs and observed the growth. After the formation of colonies pure culture was isolated by using streak plate method and confirmed by the confirmatory tests for the bacterial strains.

C. Preparation of staphylococcus aureus pure culture

A loopful of broth was streaked on to the mannitol salt agar medium and incubated at 37◦c for 24hrs and observed for the growth of single colonies.

D. Confirmatory tests for staphylococcus sp

Staphylococcus aureus morphological characterization was done by using gram's staining as well as biochemical tests like Catalase, Coagulase, indole production tests, methyl red test and mannitol tests were conducted according to the slandered microbiological techniques.

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E. Genomic DNA extraction and purification of staphylococcus aureus

Grow the staphylococcus aureus over night at 37c for 12-24 hrs followed by centrifugation, the extraction was done by protinase k method, finally collect the supernatant stored at -20◦c, followed by slandered DNA purification protocol, and finally quantitative investigation was finished by spectrophotometer.

F. RAPD-PCR method

Prepare a cocktail of PCR reaction mix for the number of species to be tested. The variable component (control Genomic DNA and test DNA) is to be added separately. Add the following reagents to one PCR vial in the following order.DNA to be tested: 1µl (100ng/µl), Random primer (10pm/µl):1µl, PCR Master Mix: 22 µl, the random primers used in this method are OPA-01, OPB-01, OPD-07, OPC-12Mix the contents uniformly and gently. All the above additions to be done on ice. Centrifuge the samples briefly (6000rpm for 30 sec. at 4^0C) to bring down the contents of the tube.

III. RESULT AND DISCUSSION

In the present study, growth of *staphylococcus aureus* was observed in the collected samples. To identity the microbe, loopful of the broth culture was streaked on to the mannitol salt agar medium and incubated at 37c for 24 hours and observed the growth of single colonies. As shown in the fig.no.1 after 24 hours incubation, formation of single colonies was observed, to identify the species of staphylococcus, morphological characterization using gram's staining and biochemical tests were carried out following standard microbiological techniques. Gram's staining indicated that the microbe is Gram positive organism and it is present in pairs or as irregular grape like clustures.To further identify the organism, biochemical tests like Catalase, Coagulase,oxidase,indole production test, methyl red test and mannitol tests were conducted .the results are represented in table no.1 , 2 and 3.it was observed that Catalase, Coagulase ,methyl red test and mannitol tests are positive and citrate ,indole production tests are negative for all samples. After the confirmation of all these things isolation of DNA was done by using Agarose gel electrophoresis followed by RAPD-PCR. Isolated DNA samples were distinguished by using 4 different random primers. Genome profile analysis obtained from the *S.aureus* demonstrated that it was possible to differentiate the *S. aureus* strains from different sources by RAPD technique. Results indicate possible relationships between host origin and genetic variation among *S. aureus* isolates from various sources. This RAPD method was thus useful for epidemiological studies of the *S. aureus* flora.

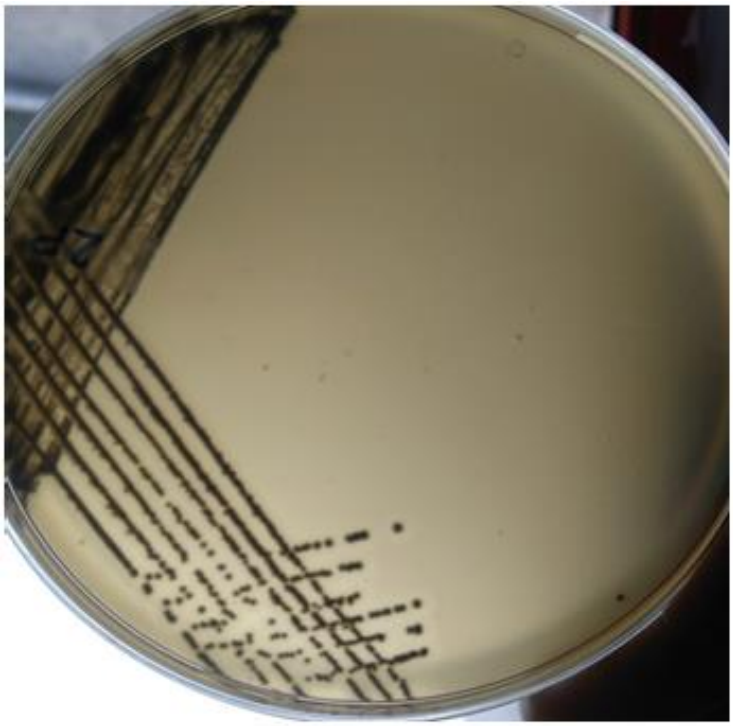

Fig 1:- staphylococcus aureus culture plate

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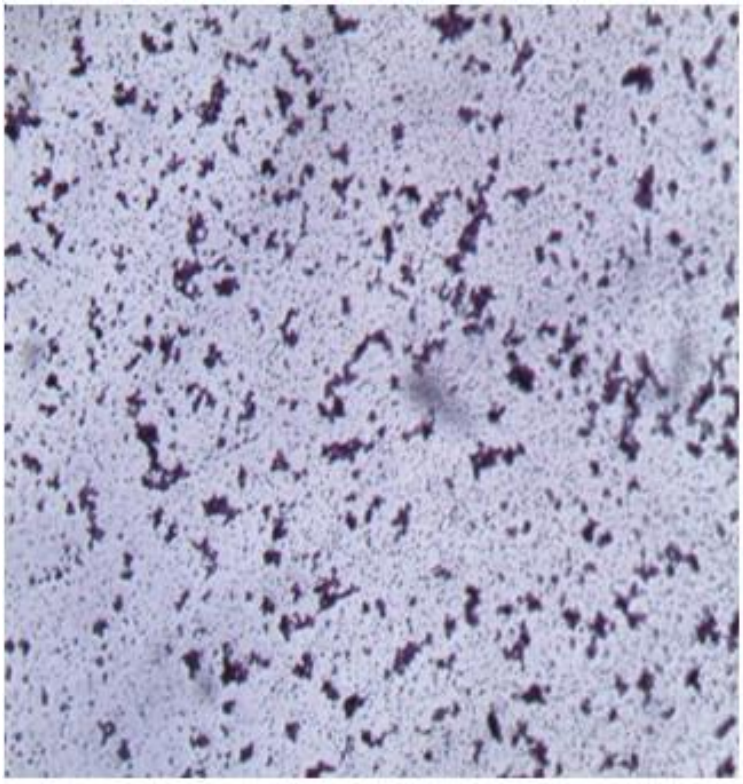

Fig 2:- Gram's staining

Microorganism source	*Colour*	*Margin*	*Temperature*	*Shape*	*Growth*
Milk	Yellow	Entire	Optimum	Cocci	Abundant
Meat	Yellow gold	Entire	Optimum	Cocci	Abundant
Water	Yellow	Entire	Optimum	Cocci	Abundant
Soli	Yellow	Entire	Optimum	Cocci	Abundant

Table 1:- Morphological characters of staphylococcus aureus

S.no	Sample	OD at 260nm	OD at 280nm	Conc. of DNA (ng/ml)	Purity :OD at 260nm/280nm
1	Milk	0.489	0.342	489	1.42
2	Meat	0.358	0.170	358	2.10
3	Water	0.407	0.234	407	1.73
4	Soil	0.413	0.250	413	1.65

Table 2:- Qualitative and Quantitative estimation of nucleic acids by UV visible spectrophotometer

S.no	Test	Milk	Meat	Water	Soil
1	Indole	_	_	_	_
2	MR	+	+	+	+
3	Mannitol	+	+	+	+
4	Coagulase	+	+	+	+
5	Catalase	+	+	+	+
6	Citrate	_	_	_	_

Table 3:- Biochemical tests

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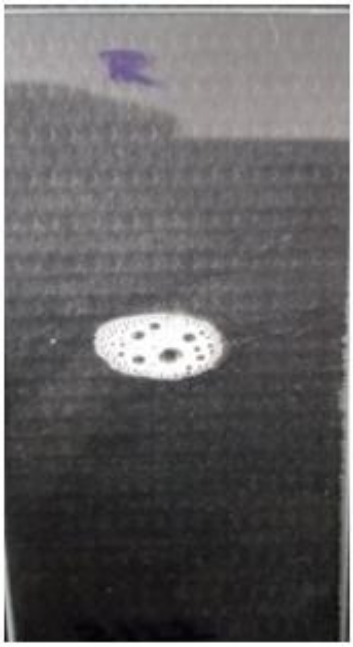
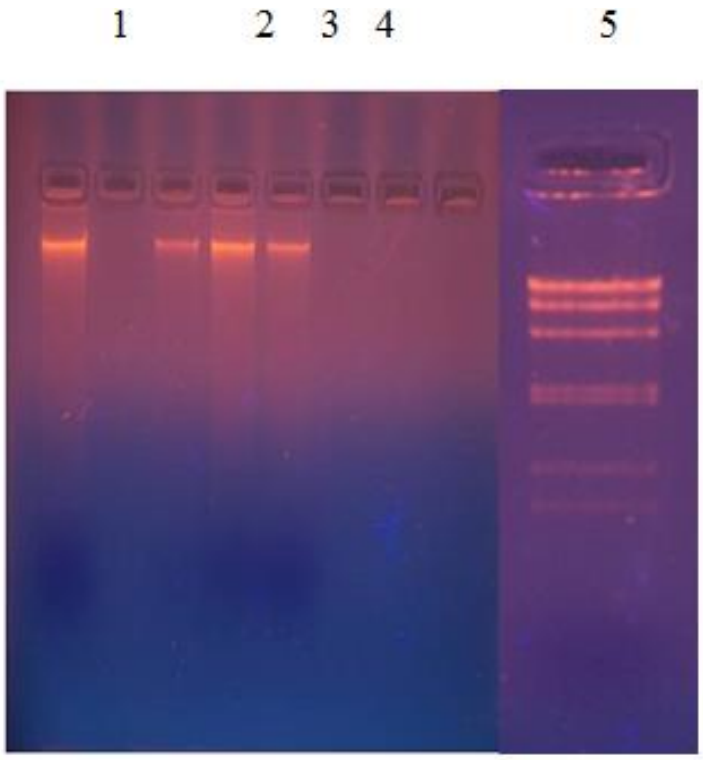


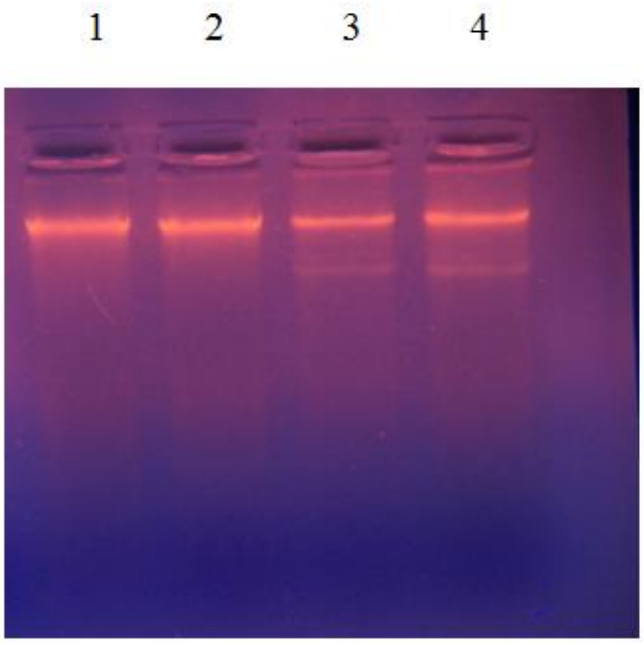
Fig 3:- Catalase Test

Fig 4:- Mannitol Test



Lane 1: genomic DNA isolated from milk, Lane 2: genomic DNA isolated from meat
Lane 3: genomic DNA isolated from water, Lane 4: genomic DNA isolated from soil
Lane 5:0.5-3 kb marker

Fig 5:- Isolated DNA samples by Agarose gel electrophoresis



Lane 1: PCR product from milk, Lane 2: PCR product from meat,
Lane 3: PCR product from water, Lane 4: PCR product from soil
Fig 6:- RAPD PCR

IV. CONCLUSION

The polymerase chain reaction was used to obtain randomly amplified polymorphic DNA profiles for genetic fingerprinting of different isolates of *Staphylococcus aureus* using RAPD-PCR. RAPD markers revealed possible relationship between host origin, mutation and genetic variation among *S. aureus* isolates, and this demonstrated its fingerprinting and diagnostic potential. Obviously, for these DNA bands patterns to have a practical meaning in the areas of medicine, population biology and epidemiology.

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