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Molecular Detection and Epidemiological Analysis of *Staphylococcus aureus* from Malaysian Hospitals Using Random Amplified Po lymorphic DNA-Typing

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Abstract: *Staphylococcus aureus* is among the most prominent pathogen in both community acquired and nosocomial infection. The epidemiology analysis of *Staphylococcus aureus* isolates will be required to ascertain the incidence, prevalence and diversity of strains. To investigate the epidemiological of *S. aureus* in Malaysia, a highly reliable typing method-Randomly amplified polymorphic DNA was applied to 50 *S. aureus* isolates obtained from 3 different hospitals in Malaysia namely Hospital Tunku Ampuan Afzan Kuantan, Hospital Besar Seremban and Hospital Miri Malaysia. The results obtained from this study showed that the isolates can be clustered into 8 different clones. All members of the respective clones are of the same origin. In addition, there were 2 clonal grouping of isolates for each hospital. However, the clonal groupings are not in accordance to the geographical distribution. To understand the epidemiology of these isolates in depth it is very important to have information about the patient's history. The Nei and Li's genetic distances obtained from this study ranged from 0.0803922-0.11111. Two genetic markers a band of size 500 bp when amplified with primer OPAE-14 and another marker band of size 750 bp amplified with primer OPAE-15 was identified and this band can be used as diagnostic marker for the rapid identification of *S. aureus*. Apart from the genetic markers, an epidemiological marker of 1200 bp was also identified for the Miri isolates. This marker can be used as the epidemiological marker for the identification of the isolates from Miri in the future outbreaks. From this study, RAPD has proved to be an useful aid to epidemiological investigations of *S. aureus*.

Key words: Random amplified polymorphic DNA, *Staphylococcus aureus*, primer, human pathogen, University Putra Malaysia

INTRODUCTION

Staphylococcus aureus has remained a primary pathogen of nosocomial and community-acquired infections. The group Staphylococci is frequently isolated as an etiologic agent of infectious processes, with the species *Staphylococcus aureus* being the most important human pathogen of this group. *S. aureus* causes superficial, deep-skin and soft-tissue infections, endocarditis and bacterimia with metastatic abscess formation. Other diseases are a variety of toxin-mediated diseases including gastroenteritis, staphylococcal scalded-skin syndrome and toxic shock syndrome (Fidalgo *et al*., 1990; Roberts *et al*., 1991). Worldwide, the increasing prevalence of multi-resistant *S. aureus* has become an additional problem. Epidemiology of *S. aureus* isolates will be required to ascertain the incidence, prevalence and diversity of strains. This epidemiological investigation requires the characterization of *S. aureus* isolates by typing systems that allow determination of isolate relatedness. It is common to use phenotypic techniques (conventional methods and antibiogram) and genotyping techniques (DNA based) for the characterization of organisms. The phenotypic methods are not accurate and time consuming. Majority of organism could be differentiated between its genera, species and strains based on polymorphisms in the genomic fingerprints produced by Randomly Amplified Polymorphic DNA technique (RAPD) (Welsh *et al*., 1991). Therefore, the epidemiology of *S. aureus* infections needs to be studied, for this purpose multiple typing technique based on the detection of DNA polymorphisms have been developed and optimized. A variety of typing techniques is available to help determine the source and transmission routes of *S. aureus* strains (Tambic *et al*., 1997). Accurate and rapid epidemiologic typing is crucial

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for the identification of the source and spread of infectious disease and thus could provide detailed information on the generation of *S. aureus* strains. RAPD is one of the genotypic methods used in epidemiological studies of *S. aureus*, a PCR-based method.

Randomly Amplified Polymorphic DNA (RAPD) assays use short primers with an arbitrary sequence to amplify genomic DNA. In this study is described, the use of RAPD analysis to investigate the epidemiology of clinical isolates of *S. aureus* obtained from different hospitals in Malaysia and identification of genetic markers and epidemiological marker.

MATERIALS AND METHODS

Bacterial isolates: Fifty isolates of *S. aureus* were obtained from Hospital Tunku Ampuan Afzan Kuantan, Hospital Besar Seremban and Hospital Miri Malaysia.

Bacterial growth: *Staphylococcus aureus* strains were cultured on blood agar plate that selects *S. aureus* by exhibiting large, yellowish, creamy and opaque colonies.

DNA extraction: Genomic DNA from each isolate was extracted from an overnight culture by using the Pure Gene bacterial Genomic DNA extraction (Biosyntech Inc.) protocol. The purity and quality of the DNA were determined by UV absorption with a UV spectrophotometer (Shimadzu UV-1601). An aliquot containing 100 ng of genomic DNA was used for RAPD analysis.

Primers: DNA primers OPAE- 14 and 15 were obtained from Operon 10-mer Kit (Kit AE, Operon Technologies Inc.) containing 10-base oligonucleotides.

RAPD typing: Amplification reaction were performed according to Neela *et al.* (2000) in 25 μL volume mixtures containing 80 mM MgCl2, PCR buffer, 3.75 mM DNTP mix (Fermentas), 15 picomole μL^{-1} of single 10-base primer (OPERON Technologies), 100-300 ng of template and 1 unit of Taq polymerase (BioSyntech Technologies). Amplifications were carried out by using a thermal cycler (Biometra-Trio Thermoblock) programmed for 1 min at 94°C followed by 30 cycles, each consisting of 1 min at 94°C, 1 min at 36°C and 2 min at 72°C and a final extension period of 7 min at 72°C. After amplification, 10 μL of the reaction mixture was loaded onto a 1.4% agarose gel and electrophoresed. The gel was then stained with ethidium bromide and photographed under UV illumination. The DNA from each isolate was subjected to the RAPD assay at least 3 times to ensure reproducibility of the results.

RESULTS

All the 50 isolates of *Staphylococcus aureus* were isolated from different samples from 3 different hospitals in Malaysia. RAPD revealed different DNA fingerprints when different primers were used on the same isolates. RAPD analysis with the 4 primers yielded 1-16 DNA markers ranging in size from 100-8000 bp. The representative profiles of the reproducible bands with primers OPAE- 14 and 15 for the isolates used in this study are presented in Fig. 1 and 2. The RAPD analysis based on the manual scoring for the presence or absence of bands were further used for similarity analysis to generate the dendrogram (Fig. 3) with the Computer assisted RAPDistance package. The dendrogram obtained with the combined data of the 4 primers gave 2 main groups I and II. Group I gave 2 clusters C1 and C2 and group 2 also gave 2 clusters C3 and C4. C3 of group II generated 2 sub clusters namely SC1 and SC2 whereas

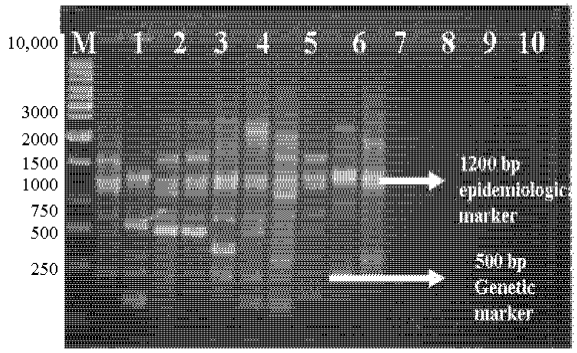

Fig. 1: RAPD genetic profiles of *Staphylococcus aureus* isolates obtained with primer OPAE-14. Lane M is the 1 kb molecular weight marker, while the rest of the lanes from 1-10 are the *S. aureus* isolates used in the genetic profiling

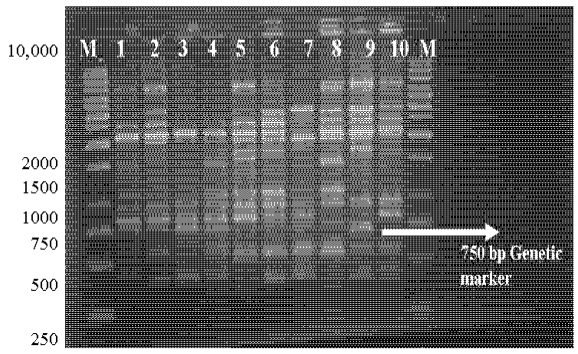

Fig. 2: RAPD genetic profiles of *Staphylococcus aureus* isolates obtained with primer OPAE-15. Lane M is the 1 kb molecular weight marker, while the rest of the lanes from 1-10 are the *S. aureus* isolates used in the genetic profiling

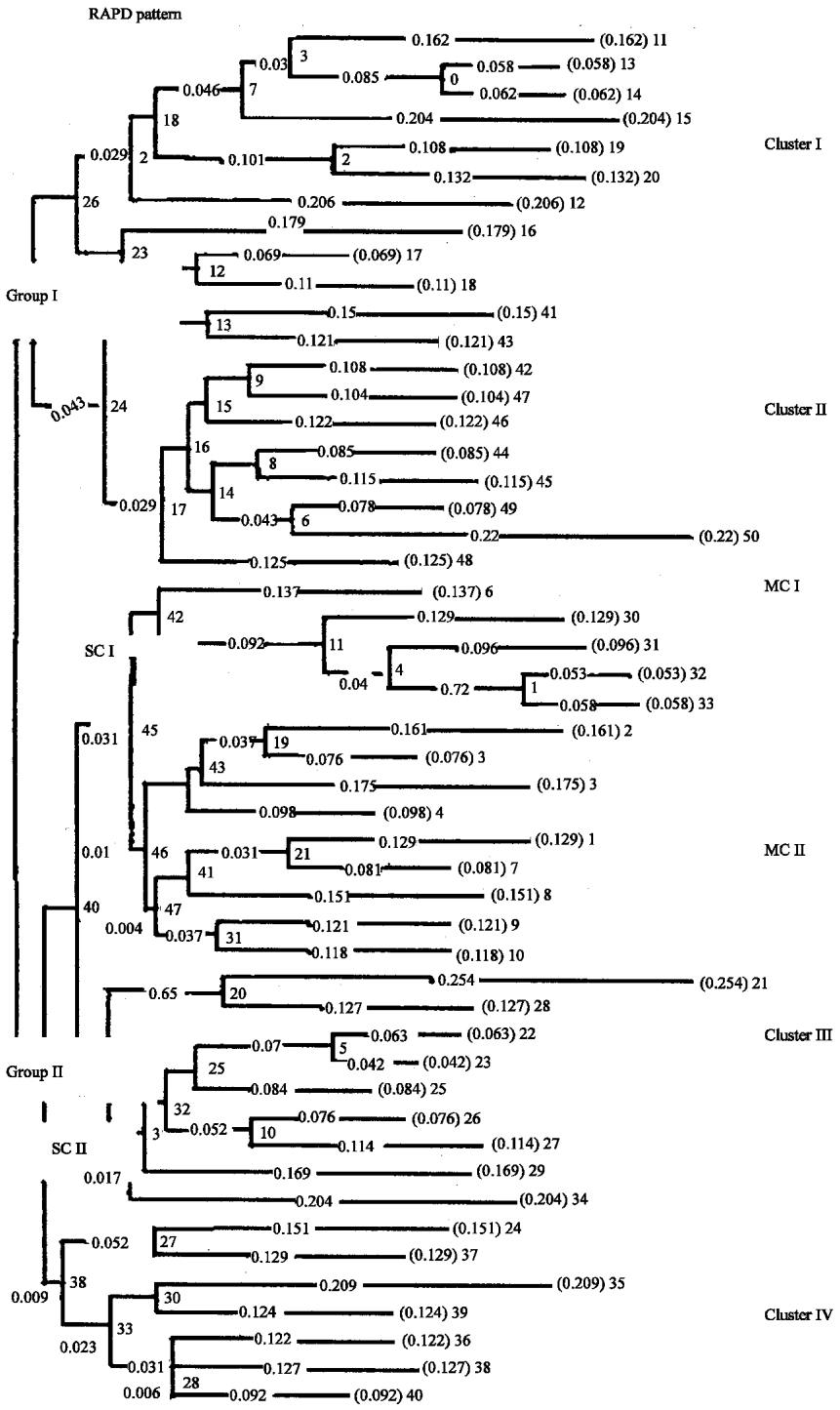

Fig. 3: Representing the cluster analysis of *Staphylococcus aureus* based on the RAPD pattern

C4 generated SC3 and SC4. Further more, the sub cluster SC1 generated two mini clusters MC1 And MC2. Out of the 20 isolates from Hospital Miri isolates numbers 1-5 and 7-10 belonged to MC2, whereas isolate number 6 was the member of MC1 and isolate numbers 11-20 to cluster C1. From the 17 isolates obtained from Hospital Kuantan isolate numbers 34, 21-29 were the members of SC2 and isolates 30, 31, 32, 33 represented MC1and other isolates 24, 35, 36, 37 belonged to C4. All the isolates obtained from Hospital Seremban belonged to the cluster C2 except

for isolate no: 38, 39 and 40, which were the members of C4. The Nei Li's genetic distances obtained from this study ranged from 0.0803922-0.11111. From the direct observation of the fingerprints 2 marker bands were identified in most of the isolates. A marker band of 500 bp when amplified with primer OPAE-14 and another marker band of size 750 bp amplified with primer OPAE-15 were identified in all 50 isolates. Apart from this common band another band of size 1200 bp was identified in all Miri isolates.

DISCUSSION

Amplification of DNA with arbitrary primers that involves the whole genome analysis is a powerful approach for the study of DNA polymorphisms. AP-PCR is widely used for the comparison of genomes from eucaryotes or bacteria (Ralph *et al*., 1993). From the results obtained in this investigation, it was found that different banding patterns of the amplified products generated by different primers, had allowed the genotyping of the *Staphylococcus aureus* isolates. A total of 4 primers out of 20 primers screened were selected to amplify the DNA of *Staphylococcus aureus*, thus producing finger print patterns that could be used to discriminate between isolates from different hospitals. The largest number of bands in one display for each isolate ranged from 1-16, respectively with markers ranging in size from 100-8000 bp in all isolates generated with 4 primers. The fingerprints generated by the 4 different primers revealed unique profiles for each strain in terms of number and position of RAPD bands. The current investigation carried out with 50 isolates of *S. aureus* from 3 different hospitals, shows the homology of these isolates corresponds to the geographical location. As in Fig. 3, the dendrogram generated showed distinct clustering between the isolates from three different hospitals. The dendrogram obtained with the combined data of the 4 primers gave 2 main groups, 4 main clusters, 2 sub clusters and 2 mini clusters. Out of the 20 isolates from Hospital Miri isolates numbers 1-5 and 7-10 belonged to MC2, whereas isolate number 6 was the member of MC1 and isolate numbers 11-20 to cluster C1. This shows that isolates 1-10 are very distantly related to isolates 11-20 although, they were obtained from the same hospital. This reason for this could be, the patients may have come from west Malaysia to east Malaysia. In respect to isolates obtained from Hospital Kuantan, although, the isolates are scattered to C4, SC2 and MC1, still all isolates are grouped together into one group (Group II). This shows that even though the isolates are not very closely related to each other, they are distantly related to the isolates obtained from other hospitals. This shows that isolates studied from Hospital Kuantan must have obtained from patients restricted to in and around Kuantan. All the isolates obtained from Hospital Seremban belonged to the cluster C2 except for isolate no: 38, 39 and 40, which were the members of cluster IV (C4), which indicates that these three isolates could have isolated from patients originated from Kuantan area. Ten isolates from Hospital Miri (1-10) and 10 isolates from Hospital Seremban (41-50) are grouped together in G1, which shows that there are high possibilities of these 20 isolates to be emerged from the same clone and disseminated to Miri or Seremban area. The direct visualization of the fingerprint for these 20 isolates also shows that they share most of the bands. From the overall results obtained from this study, it was found that all of the isolates are clustered into 8 different clones and it also suggest that all members of the respective clones are of the same origin. Furthermore, it was found that each hospital contained 2 clonal group of isolates and the clonal groups were not in accordance to the geographical distribution. Therefore, to understand the epidemiology of these isolates in depth it is very important to have information about the patient's history. The dendrogram illustrated the genetic diversity among isolates from different hospitals whereby the genetic distance values calculated by Nei and Li's pairwise genetic distance formula placed these isolates in different clusters and sub-clusters.

The low genetic diversity of isolates from the Hospital Kuantan, three isolates from Hospital Seremban (38-40) and 10 isolates (1-10) obtained form Hospital Miri would enhance the ease of treatment using similar antibiotics, while the high genetic diversity need different antibiotics to treat different strains of the same species. These results demonstrated the practical value of RAPD for studying the genetic profile of *S. aureus* isolates for molecular epidemiology purposes, as typing by RAPD is technically simple and highly reliable. In addition, the RAPD technique utilizing genotypic analysis can give accurate differences or similarity between strains in comparison to the biochemical analysis, which is based on phenotype characterization. This technique can be applied to the analysis of other clinical isolates, marine isolates, food isolates at species or genus level etc., Okazaki *et al*. (1999) reported, RAPD provides a discriminatory potential and reproducibility compared to Pulse Field Gel Electrophoresis (PFGE) assay with less complexity in a shorter time. It was also suggested that the introduction of the RAPD assay as a routine technique into clinical microbiology laboratories might help prevent nosocomial outbreaks. The 2 genetic markers a band of size 500 bp when amplified with primer OPAE-14 and another marker band of size 750 bp amplified with primer OPAE-15 identified in this study can be used as

diagnostic marker for the rapid identification of *S. aureus*. The development of species-specific non-radioactive DNA probes derived from the highly conserved amplified fragment produced by RAPD can simplify the identification at species or subspecies level (Letocart *et al*., 1997). Byun *et al*. (1997) reported that optimal combination of RAPD primers would make RAPD assay as one of the powerful tools for the epidemiological study of *S. aureus*. Apart from the 2 genetic markers an epidemiological marker of size 1200 bp amplified with primer OPAE-14 was identified in all Miri isolates. The presence of this marker band only in Miri isolates and not in other isolates clearly indicates that this marker could be used as epidemiological marker for the epidemiological investigation of isolates from Miri. Results from our investigations confirmed the possibility of applying the RAPD analysis to any sample whose DNA could be extracted to study the relatedness of the samples. Based on the dendrogram obtained (Fig. 3), the clonal relationship between the isolates, genetic distances and differences among the 50 isolates of *S. aureus* can be visualized.

CONCLUSION

This study gives us the clear indication that RAPD analysis is a rapid, accurate and a highly reliable tool that can give the genetic relatedness among the *S. aureus* strains and can group the isolates according to the geographical area and determine genetic diversity of strains that are otherwise impossible by biochemical analysis. RAPD improves the understanding of the epidemiology of *S. aureus* isolates and thus, aids the formulation of effective control measures. As RAPD analysis can correctly type *S. aureus* isolates, this technique would be of great use in preventing nosocomial *S. aureus* infections and thus could be applied in hospitals.

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