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Genetic Diversity of *Staphylococcus aureus* Isolated from Different Clinical Samples at Modibbo Adama University Teaching Hospital, Yola using RAPD-PCR

Ja'afar Nuhu Ja'afar[1,2*], Zailani Adamu[1], Tukur Baba Abdullahi[3] and Hayatu Raji[4]

Department of Biotechnology, Faculty of Life Sciences, Modibbo Adama University (MAU), P.M.B. 2076, Yola, Adamawa State, Nigeria [1], Chevron Biotechnology Centre, Modibbo Adama University (MAU), P.M.B. 2076, Yola, Adamawa State, Nigeria [2], Department of Science Laboratory Technology, Modibbo Adama University, PMB 2076, Yola, Adamawa State, Nigeria [3], Department of Natural and Environmental Sciences, School of Arts and Sciences, American University of Nigeria (AUN), P.M.B. 2250, Yola, Adamawa State, Nigeria [4].

Author for Correspondence*: jnjaafar@mau.edu.ng/+2348068442468. https://dx.doi.org/10.4314/sokjmls.v8i1.13

Abstract

Staphylococcus aureus (*S. aureus*) is a Gram-positive bacterium that causes a wide range of variable illnesses and can be either transmitted from one place to another through contact with patients or airborne. Because of the increased diversity of *S. aureus* globally, particularly those that are of clinical significance, and the rapid epidemiology of the bacteria, there is the need to explore the current pattern in our local settings. To achieve this target, there is the need for a genetic method that is fast, easy, cost- effective and able to genetically differentiate between microbes within a given species. Hence, this study utilized the simplicity of RAPD-PCR as a genetic fingerprinting tool in determining the genetic diversity of *S. aureus* isolated from clinical samples within a local setting. Eighteen isolates of *S. aureus* were RAPD-typed using a single primer and analyzed using NTSYSpc software. Of the 12 RAPD profiles identified, two major groups and two major clusters were deduced from the dendrogram. More so, ten subtypes were identified amongst which three were indistinguishable based on Jaccard's coefficient of similarity. The findings of this study reveals that most of the isolates are closely related genetically suggesting that may have emerged from closely related clones. Equally, the result of this study depicts the high-resolution power of RAPD-PCR in elucidating the genetic diversity of *S. aureus*. Therefore, this simple molecular technique could be employed as a routine technique for the molecular epidemiology studies of *S. aureus* in our local settings and the country as a whole.

Keywords: RAPD-PCR, Molecular epidemiology, *S. aureus*, Diversity, Fingerprinting

Introduction

Staphylococcus aureus (*S. aureus*) is a Gram-positive bacterium that exist as a normal skin flora as well as in the nostrils (Kluytmans *et al.*, 1997). It has also been isolated from the air, wounds and pus (Cimolai, 2008). The bacteria can cause a wide range of variable illnesses such as skin infections like pimples, boils, impetigo, cellulitis, carbuncles, scalded skin syndrome and abscesses to a highly life-threatening diseases like pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia and sepsis (Tong *et al*., 2015). Currently, there are 32 species and eight subspecies in the *Staphylococci* genus that preferentially colonize the human body (Kloos and Bannerman, 1994).

Epidemiological study of a pathogen is essential for public health management to monitor the transmission pattern of a disease (Bruisten and Schouls, 2010). Lately, the occurrence of *S. aureus* in Nigeria has been reported with varying incidences. Akortha and Ibadin (2008) recorded an incidence of *S. aureus* as 22.8 % among UTI patients in University of Benin Teaching Hospital. By 2011, Onwubiko and Sadiq reported an incidence of 30.7 % from clinical samples obtained at Aminu Kano Teaching Hospital. Nsofor *et al* (2016) recorded 24.5 % prevalence rate in Abia State University Teaching Hospital. Olowo-Okere *et al.* (2016) reported an incidence of 52.6 % from patients attending Specialist Hospital in Sokoto. Sampson *et al.* (2022) reported an incidence of 38.7 % among patients attending University of

Port Harcourt Teaching Hospital.

While typing systems are either phenotypic or genotypic, phenotypic systems are capable of only grouping isolates into relatively large categories within a given species, while genotypic methods are more discriminative and better to detect subtle differences among strains and/or clones (Letellier *et al*., 1999). They are fast, sensitive, specific, reproducible, and less labour intensive. The results from pathogen detection are available within a 24 – 30 hour period (Whyte *et al*., 2002; Oliveira *et al*., 2003). On the contrary, phenotypic techniques are labour intensive, time consuming, expensive, and lack sensitivity and specificity (Oliveira *et al*., 2003; Bohaychuk *et al*., 2005). On average, an estimated time span of 4 – 7 days is required to obtain a positive result, excluding the time for serotyping (Seo *et al*., 2003; Jin *et al*., 2004).

Random amplified polymorphic DNA (RAPD) is a genotypic method that can be used to amplify a random DNA segment of bacteria (van Belkum *et al.*, 1995). It is easy to carry out because a single primer can be used which makes the protocol less laborious and cumbersome (Vila *et al.*, 1996). It has been used to distinguish between strains of many species such as *Hymenoptera* (Al-Rubaye *et al*., 2023); *Bacillus* (Rosic *et al*., 2023); *E. coli* (Mokhtari *et al*., 2022) and *Candida* (Rashid *et al*., 2022). However, the application of genotypic methods to determine the genetic diversity of *S. aureus* in Nigeria mostly targeted specific gene determinants rather than the whole genome. Shittu *et al.* (2011) targeted *S. aureus* protein A (*spa*) and methicillin resistant determinant gene (*mecA*); Raji *et al.* (2013), O'Malley *et al.* (2015) and Obasuyi *et al.* (2020) targeted *mecA* and Panton-Valentine leukocidin (*pvl*) genes. Others such as Okorie-Kanu *et al.* (2020) and Akinduti *et al.* (2021) targeted accessory gene regulators (*agr*), *spa*, *mecA* and *pvl* genes. However, Taiwo *et al.* (2005) studied the restriction pattern of its plasmid, whereas Onasanya *et al.* (2003), Esan *et al.* (2009) and Akobi *et al.* (2012) provided the true insight into the genome of the organism by using RAPD-PCR, PFGE and MLST, respectively.

Because of the increasing diversification of *S. aureus* particularly those of clinical significance and the rapid epidemiology of the bacteria, there is a need to exploit the genetic diversity and variants of the bacteria from different clinical samples. Hence, this study utilized the simplicity of RAPD-PCR as a molecular fingerprinting tool to determine the genetic diversity of *S. aureus* from different clinical samples.

Materials and Methods

Bacteria Isolates

Eighteen pure isolates of *Staphylococcus aureus* (*S. aureus*) from different clinical samples were collected from Modibbo Adama University Teaching Hospital (MAUTH), Yola, Adamawa State, Nigeria. The isolates were re-confirmed by standard microbiological procedures including Gram stain and catalase and coagulase tests as earlier described (Brown *et al.*, 2005). All samples were cultured on mannitol salt agar (Merck, Germany) and a single colony of each was picked with a sterile wire loop and sub-cultured in nutrient both (Merck, Germany) for genomic DNA extraction purpose. Ethical approval of the experiment was obtained from Adamawa State Ministry of Health.

Genomic DNA Extraction

DNA extraction was carried out using Wizard® Genomic DNA Purification Kit (Promega, USA) following the manufacturer's instructions. Briefly, 1 ml overnight culture was centrifuged at 13,000 x*g* to pellet the cells. To the re-suspended cells, 60 µl each of 10 mg/ml lysozymeand lysostaphin were added and incubated at 37 ^{O}C for 30 minutes. Following centrifugation, 600 µl Nuclei Lysis solution was added to re-suspend the pellet and incubated at 80 ^{O}C for 5 minutes. Additional 3 µl RNase solution was added and incubated at 37^{O}C for 30 minutes. Then, 200 µl Protein Precipitation solution was added and incubated on ice for 5 minutes before centrifuging. The supernatant was added to 600 µl isopropanol and centrifuged to pellet the DNA. Additional 600 µl of 70 % ethanol was used to wash the DNA pellet. After air-drying, 70 µl of DNA Rehydration solution was added and incubated overnight at 4 ^{O}C. Concentration and purity of DNA was checked using a Nanodrop spectrophotometer (Thermo Scientific, USA).

RAPD-PCR Amplification and Analysis

RAPD-PCR optimization was carried out as

earlier described (Ja'afar *et. al*., 2016), hence not reported in this study. ZA RAPD primer, 5′GTGGATGCGA3′, synthesized at Inqaba Biotec™ West Africa Ltd was used for the study. To a 0.2 ml PCR reaction tube, 1X *Taq* 2X master mix (New England BioLabs™), 40 ng/µl genomic DNA, 0.6 µM ZA RAPD primer were added and made up to a final volume of 25 µl with distilled water. PCR thermal amplification was performed using SelectCycler™ II Thermal Cycler (Select Bioproducts, USA) with the following cycling profile: initial denaturation at 94 °C for 5 minutes; 40 cycles of denaturation at 94 °C for 30 seconds, annealing at 34.8 °C for 1 minute and extension at 72 °C for 1 minute. Final extension was at 72 °C for 10 minutes. PCR products were resolved using 1.2 % agarose gel in TAE buffer. Resolved products were visualized using a bench top UV transilluminator (UVP UV Transilluminator, USA). Two DNA samples did not produce any band during PCR reactions. A 1 Kbp DNA ladder (Promega, USA) was used as a DNA fragment size marker.

Fingerprint Pattern Analysis

RAPD-PCR bands generated were manually scored in binary format and used for fingerprinting analysis (Neela *et al.*, 2005). Presence of band was recorded as "1" while absence of band was recorded as "0" (Betancor *et al*., 2004). The RAPD-PCR banding patterns generated were analyzed using NTSYSpc software version 2.02j and NT edit version 1.1b (Rolhf, 1990). Similarly, dendrogram was created using the unweighted pair-group method with averaging (UPGMA) and DNA relatedness was estimated using the Jaccard similarity coefficient of the same software. Dendrogram was analyzed as earlier reported by Zare *et al.* (2019). *S. aureus* isolates with $\geq$ 80 % Jaccard's similarity coefficient refers to the probability of similar origin.

Results

To investigate the genetic relatedness of *S. aureus* from different clinical samples, RAPD-PCR was employed to type 18 different isolates using ZA RAPD primer and resolved by agarose gel electrophoresis (Fig. 1). Amplification of the isolates resulted in polymorphic bands that ranged from one to three clear bands and of 200 – 2,500 bp in size (Fig. 1).

A dendrogram showing the detailed relationship of the isolates generated two major groups from the RAPD profiles (Fig. 2). Group II aligned the isolates into two clusters, A and B. Sub-clusters of B, – BI and BII were also identified including genetically indistinguishable isolates (Jaccard's similarity coefficient = 100 %). Two isolates each were indistinguishable in sub-clusters BI*a* and BII*b*, while BII*a* had three indistinguishable isolates.

Furthermore, the two isolates that were not amplified were aligned between the two major groups. This suggests that the isolate in Group I is more genetically distant than the other isolates in Group II.

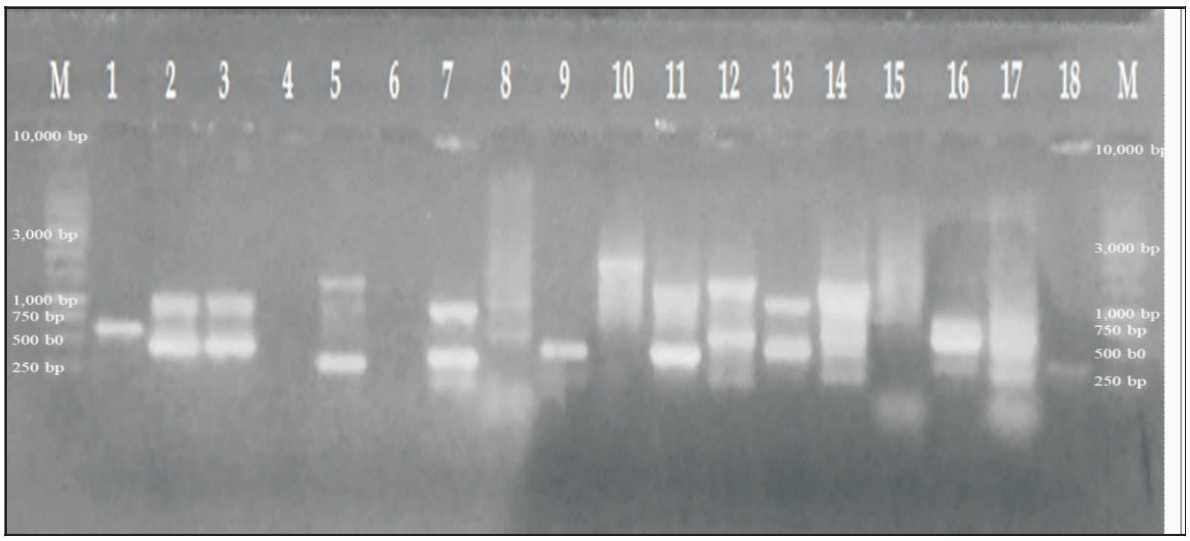

Figure 1: Agarose gel electrophoresis image showing RAPD-PCR band profile of 18 *S. aureus* isolates with ZA RAPD primer. DNA in lanes 4 and 6 were not PCR-amplified. Lanes M are 1 kbp DNA ladders.

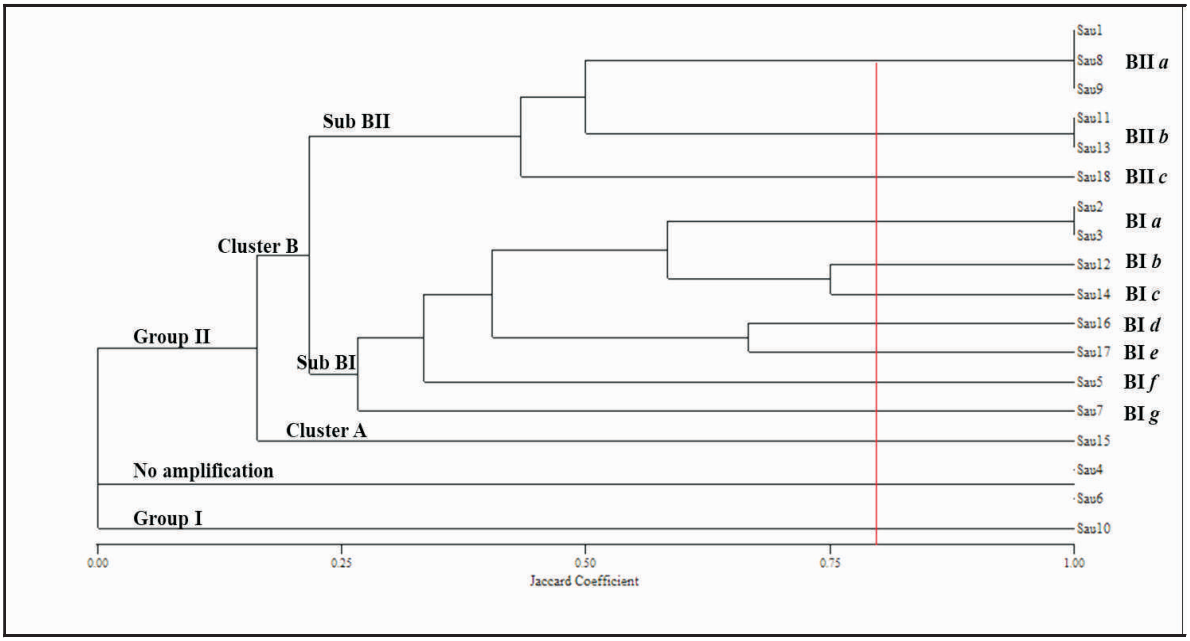

Figure 2: Dendrogram of genetic relationship between 18 isolates of *S. aureus* obtained with ZA RAPD primer. Two main groups and cluster were generated. Cluster B had two sub-clusters that were further divided into various subtypes.

Discussion

Phenotypic markers for bacterial typing might show considerable variability due to environmental changes, such as culture conditions, subculture and storage. By contrast, nucleic acid-based markers for bacterial typing can generate a robust read-out from the bacterial sample, as the genome remains largely stable over time, and it is resistant to environmental changes (Sauer and Kliem, 2010). In this study, RAPD-PCR explained the genetic diversity of *S. aureus* isolated from clinical samples. Based on the 12 RAPD profiles identified and the constructed dendrogram, the isolates were broadly divided into two groups, I (one isolate) and II (15 isolates) at 80 % cut off of Jaccard's similarity coefficient. More so, Group II was divided into two clusters, A and B where Cluster B was subdivided into two major sub-clusters, BI and BII. Furthermore, BI had seven sub types while BII had three sub types suggesting that the isolates could have come from different sources. Amongst the sub types, three (BI*a*, BII*a* and BII*b*) were observed to have 100 % Jaccard's similarity coefficient. This could mean that the isolates were from similar source. The finding of this study corroborates the work of Onasanya *et al.* (2003) who also classified the 18 isolates of *S. aureus* in their study into two broad groups. Other RAPD-PCR studies that typed *S. aureus* into various groups and clusters include Zare *et al.* (2019), who utilized three RAPD primers to type 50 isolates of *S. aureus* from different sources and identified four, five and seven groups, respectively with the primers. Similarly, Alni *et al.* (2017) investigated the genetic relatedness of 208 *S. aureus* strains isolated from different sources and identified 47 RAPD profiles and nine clusters. More so, Idil and Bilkay (2014) applied RAPD-PCR to investigate the clonality of methicillin resistant *S. aureus* (MRSA) from different hospitals. Their study identified two major groups and three clusters. Other reports earlier published on the utilization of RAPD-PCR in typing *S. aureus* include Kurlenda *et al.* (2007); Reinoso *et al.* (2004) and Pereira *et al.* (2002).

Notably, two DNA samples did not amplify during the study. This sort of inconsistency such as no amplification, difficult to interpret complex patterns, and primer artefacts has been reported previously (Demeke *et al*., 1992; Echt *et al*., 1992). Another possible reason for lack of amplification is denaturation of DNA. However, in this study, the DNA samples were not denatured.

To improve the genotypic characterization of some organisms, other researchers employed other genotypic methods in addition to RAPD-PCR. Chiang *et al.* (2014) utilized PFGE in addition to RAPD-PCR to discriminate *S. aureus* isolates from clinical samples and food poisoning cases. However, Saulnier *et al.* (1993) did not find such combination with PFGE discriminatory. It is noteworthy to mention that to improve the discriminatory index (DI) of RAPD-PCR it requires appropriate primer selection and optimized PCR conditions (Ellsworth *et al*. 1993).

Although a consensus standard typing method has not been specified for every organism, most genotypic techniques have demonstrated useful insights into the epidemiological relationships of strains of several pathogens (Betancor *et al.,* 2004). RAPD-PCR has proved to be a powerful tool for discriminating different species and for genetic analysis among populations for a variety of microorganisms, plants, and mammals (Caetano-Anolles *et al*., 1991; Rafalski *et al*., 1991). RAPD-PCR has been used in typing *S.* Typhi (Nath *et al.*, 2010), *S.* Enteritidis (Mare *et al.*, 2001) and *C. jejuni* (Nielsen *et al.*, 2000).

Conclusion

The ability to discern the genetic diversity of clinical isolates with a simple technique like RAPD-PCR calls for its adoption within the hospital settings as a routine molecular epidemiology tool to monitor microbial genetic events within any given period of time.

Recommendation

For RAPD-PCR to be successful as a routine laboratory practice, there is the need to increase the number of RAPD primers and work towards standardization of protocols so that information can be shared amongst different laboratories within the country. More so, interpretation of genetic analysis should be done in conjunction with classical epidemiological knowledge.

Limitation

The major limitation of the present study was lack of proper documentation of isolates so that original source could be properly linked to the diversity identified in the dendrogram.

Conflict of Interest

The authors declare no any conflict of interest.

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