African Scientist Vol. 23, No. 2 June 30, 2022
Printed in Nigeria

1595-6881/2021 $10.00 + 0.00

http://www.niseb.org/afs

AFS2022013/23205

Characterization of Hospital Associated Methicillin Resistant *Staphylococcus aureus* (HA-MRSA) Using Random Amplified Polymorphic DNA (RAPD) Marker

Ifediora, A.C.*[1] and Chikwendu, C.I.[2]

[1]Department of Microbiology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria
[2]Department of Microbiology, Federal University of Technology, Owerri, Imo State, Nigeria

*Corresponding author; Email: afoma20@yahoo.com Tel: +234 (0) 803 540 2258

(Received May 10, 2022; Accepted in revised form May 20, 2022)

ABSTRACT: *Staphylococcus aureus* is one of the most significant pathogens causing nosocomial infections. Twelve (12) methicilllin resistant *Staphylococcus aureus* (MRSA) strains obtained from clinical samples in Abia State, Nigeria was subjected to DNA fingerprinting by Random Amplified Polymorphic DNA (RAPD) analysis to examine their genotypic polymorphism and subsequently determine the degree of genetic relatedness among the various isolates. DNA extraction was carried out by boiling method and quantified using nanodrop1000 spectrophotometer. The amplification was carried out using three randomly selected oligonucleotide primers SaOLP6, SaOLP11 and SaOLP13. Amplification of isolates resulted in several polymorphic bands which ranged from 170-1000bp, Dendrogram analysis generated three main clusters (cluster A, B and C) and indicated that the strains isolated from the same hospital were closely related as they aligned together in the same cluster while two strains isolated from 2 different hospitals grouped together showing probable genetic relationship. This study shows that the simple and cost-effective RAPD fingerprinting could be effectively used in detection of polymorphism, tracking the spread of strains within and between hospitals, and thereby preventing the nosocomial infections caused by MRSA.

Keywords: Methicillin-resistant *Staphylococcus aureus*, Molecular typing, Oligonucleotide primers, RAPD.

Introduction

Meticillin-resistant *Staphylococcus aureus* (MRSA) strains are prevalent bacterial pathogens that cause both health care and community-associated infections. Increasing resistance to commonly prescribed antibiotics has made MRSA a serious threat to public health throughout the world (Watkin *et al*., 2012)

Consequently, the analysis of the spread of these strains has become a great concern and area of research throughout the world (Cookson *et al*., 2011). Phenotypic and genotypic methods have been instrumental in determining the polymorphism among these clinical isolates that cause nosocomial infections (Singh *et a*l., 2006). The shortcomings of phenotypical based typing methods have resulted in the improvement of genotypic typing methods based on DNA sequences, this has proved very helpful as the aim is to obtain reliable and rapid typing which is of primary importance for determining the clonally related strains (Neela *et al*., 2005).

The application of PCR based RAPD primers has been used to determine the relatedness between the various species of microorganisms (Fevzi, 2001; Deepika & Bhatnagar, 2006). The principle is that the RAPD technique effectively scans a genome for small inverted repeats and amplifies intervening DNA segments of variable length. The profile of amplification products depends on the template primer combination and is usually reproducible for

any given combination. Construction of dendrogram using similarity matrix gives the relationship between the isolates.

In other words, Random Amplified Polymorphic DNA (RAPD) is a technique that uses short primers of 10 base pairs (bp) with random sequences of nucleotides to randomly amplify DNA targets producing fragments, which serve as genetic markers. The amplification products are separated by agarose gel electrophoresis to generate a bacterial fingerprint and the banding patterns are used to compare the relatedness of bacterial strains. The method does not require prior special knowledge of specific DNA target sequences (Ranjbar *et al.,* 2013). With this technique, a DNA fingerprint may define an individual in a very fast and reliable way. RAPD-PCR method, when compared with biochemical methods is cheaper, simpler, more sensitive and faster. The RAPD technique has also been shown to be suitable for routine genotyping of hospital-acquired MRSA (Neslihan and Isil, 2014).

Apart from the study of antibiotic resistance (Ikeh, 2003), little is known concerning the genetic diversity that exists in populations of *S. aureus* isolates from clinical samples in Nigeria thus prompting this research.

The aim of this study was to assess the genetic relationship of Hospital Acquired Methicillin Resistant *Staphylococcus aureus* (HA-MRSA) isolates using RAPD-PCR marker. Such information will be useful in the organism's classification, epidemiological survey, ecology and diagnosis**.**

Materials and methods

Sample collection: Methicillin resistant *S. aureus* isolates used in this study were obtained from three different hospitals in Aba, Umuahia and Isikwuato in Abia State. Their identity was phenotypically determined by conventional microbiological assay including cultural, biochemical tests and antimicrobial screening using cefoxitin discs and further genotypically characterized by PCR detection of *mecA*-encoding (*mecA*) genes as MRSA.

Genomic DNA extraction: DNA extraction was achieved by boiling method; 5ml of an overnight broth culture of the bacterial isolate in Luria Bertani (LB) was spun at 14,000rpm for 3 min. The cells were then re-suspended in 500µl of normal saline and further heated at 95^0C for 20 min. The heated bacterial suspension was cooled on ice and spun for 3 min at 14000rpm. The supernatant containing the DNA were transferred to a 1.5ml microcentrifuge tube and stored at -20ºC (Queipo-Ortuño *et al.,* 2008).

Random amplified polymorphic DNA (RAPD) for clonal relatedness: Random Amplified polymorphic DNA was carried out using three selected oligonucleotide primers: saOLP6: GAGGGAAGAG, saOLP11: ACGATGAGCC and saOLP13: ACCGCCTGCT primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 µl for 35 cycles. The PCR mix included: The X2 Dream Taq Master mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.2µM and 20ng of the extracted DNA as template. The PCR conditions was as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 40ºC for 30 seconds; extension, 72ºC for 40 seconds for 30 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 25 minutes and visualized on a UV transilluminator and clonal relatedness was shown among organisms with the same number of bands.

Phylogenetic analysis: Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN. These sequences were aligned using MAFFT. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 500 replicates (Felsenstein, 1985) was taken to represent the evolutionary history of the taxa analysed. The evolutionary distances were computed using the Jukes-Cantor method (Jukes & Cantor 1969).

Results

In the RAPD analysis with DNA from the clinical MRSA isolates, the primers saOLP6 and saOLP11 yielded less informative banding patterns. However, primers saOLP13 was more informative with regards to number, intensity and distribution of the bands. Consequently, it was thereby selected for typing analysis.

Dendrogram generated from the analysis of RAPD-PCR amplification patterns of the obtained isolates with the chosen primer generated 3 distinct but related clusters. These genotypes were assigned types A to C (Figure 1). The

isolates in cluster A (2,3,4 and 7) were highly related to one another with 91% similarity index. In a similar way the isolates in cluster C (1,8) had 95% similarity profile while those in cluster B were related with 89% similarity index. Genetic fingerprinting and phylogenetic diversity between different MRSA strains were determined by converting RAPD data into a Jaccard similarity matrix and analysed by UPGMA to produce a phylogenetic tree.

The RAPD profiles consisted of two to four amplicons ranging from 170 to 1000 base pairs in length (Table 1). Apart from two isolates, i.e., strain 5 and 6, all other isolates generated some conserved bands that could be detected in the patterns from all isolates. According to our results, RAPD types A with 4 isolates (40%), type B also with 4 1solates (40%) were the most frequently encountered, followed by RAPD type C with 2 isolates (20%) as the third (Table 1).

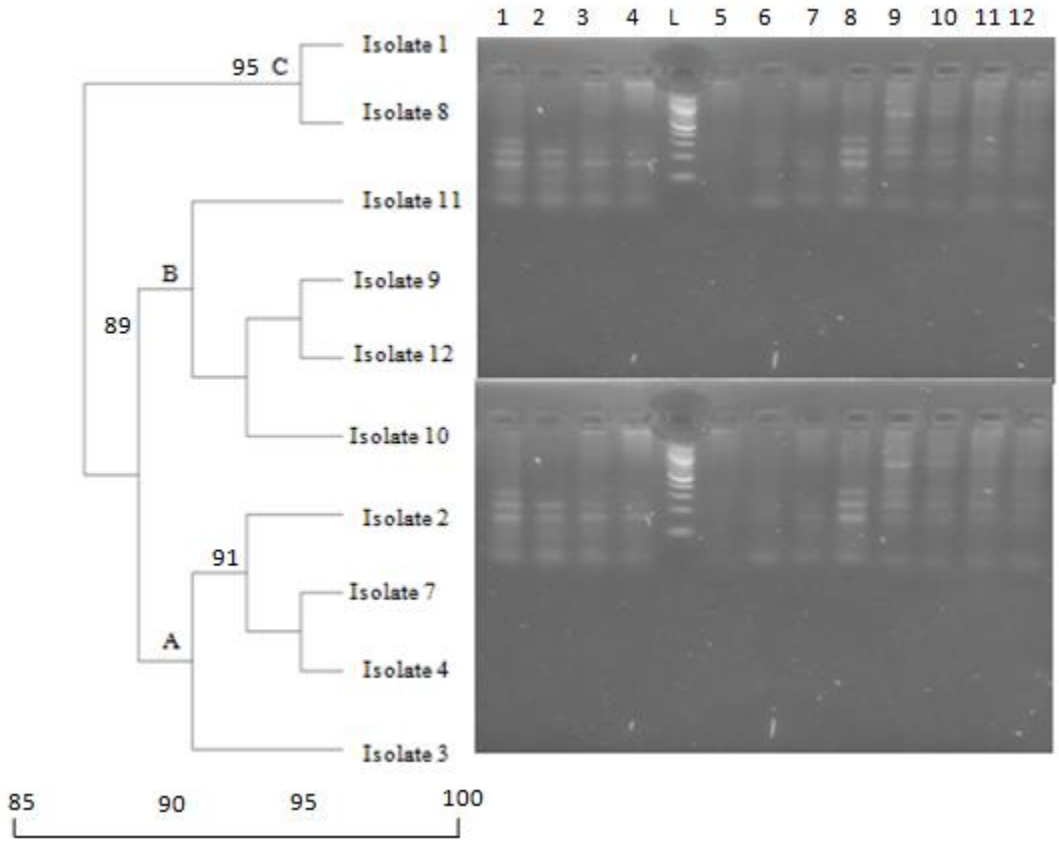

Fig 1: Dendrogram of genetic relationship of the isolates (1-12) revealing three clusters (A, B and C) between ten strains of methicillin-resistant *Staphylococcus aureus* obtained with saOLP13 RAPD primers.

Legend: L = DNA ladder of molecular weight 1000bp on lane 5; Isolates 1,8 on lanes 1 and 9 respectively belonging to RAPD type C; Isolates 2,3,4,7 on lanes 2,3,4 and 8 respectively belong to RAPD type A while isolates 9,10,11,12 on lanes 10,11,12 and 13 were of RAPD type B.

Table 1: Molecular Size of RAPD Amplicon (bp)

RAPD Type	No of Bands	100bp	170bp	220bp	300bp	500bp	700bp	900bp	1000bp	No of Isolates	Isolate Designation
A	2		1	1						4	2,3,4,7
B	4		1	1	1				1	4	9,10,11,12
C	3		1	1	1					2	1,8

Legend: bp = base-pair

Discussion

Molecular typing methods have been evaluated not only for their ability to discriminate among strains for epidemiologic purposes but also for their potential of having a taxonomic value (Mobasherizadeh *et al*., 2016). Previous studies have shown that *S. aureus* is a polymorphic species and has a clonal population structure implying that it is not naturally transformable quite unlike some other pathogenic microorganisms with ability to recombine (Feil *et al*., 2003). As a result, several molecular methods have been used for epidemiological surveillance of MRSA isolates in order to track the distribution, infection source and transmission routes. These methods include multilocus sequence typing (MLST) (Enright *et al*., 2000), pulsed-field gel electrophoresis (PFGE) and SCC mec typing. Most of these methods suffer from the disadvantages that they require high expertise, complicated laboratory settings and complex procedures to clearly differentiate the various MRSA. Among all these methods, PFGE has been shown to be a gold standard as an accurate and reliable method (Strandén *et al*. 2003). However, it is also very tedious and time- consuming, compared with ease and speed of performance of PCR-RAPD techniques (Sabat *et al*. 2006). In the present study we endeavored to find the molecular variation of MRSA isolates using a rather simple and cost-effective molecular typing method, namely, Random Amplified Polymorphic DNA (RAPD) analysis. Compared with other typing methods for *S. aureus* strains such as PFGE, this procedure generates greater polymorphism, is technically friendly and faster, and requires no radioactive materials (Reinoso *et al.,* 2004).

Our results indicated that RAPD fingerprinting can classify isolates of MRSA into different clusters through which the relationship of strains can be evaluated. In our investigation the RAPD application in HA-MRSA genotype analysis resulted in 3 clusters. Out of the 12 HA-MRSA isolates, 8 isolates (66.7%) were grouped into two clusters, i.e., cluster A and B, and 2 isolates (16.7%) classified into a small cluster termed cluster C, while two failed to be amplified. The failure may be as a result of degeneration or the loss of DNA during transfer to the PCR tube (Piyamongkol *et al.,* 2003). Cluster A comprised of isolates which originated from the same hospital while cluster B from another hospital. The dendrogram indicated that the strains from the same hospital were closely related and this means that these strains might have evolved from one clone. Remarkably, the small cluster C which had an isolate from Isiukwuato and Umuahia showed similar band pattern. The reason for this situation could be that patients admitted from other healthcare settings might have introduced these strains from other institutions in the other town. The dendrogram showed that the highest percentage of similarity was 95% among the strains.

Similar observations have been reported in earlier studies carried out by Neela *et al*. (2005) who discovered that the RAPD analysis of different *S. aureus* strains showed that the strains isolated from the same hospital were all genetically related and mostly in the same cluster. Nikbakht *et al.* (2008) found that MRSA strains isolated from two different hospitals having similar RAPD pattern suggested about the route of MRSA transmission as being from cross infection between the hospitals. Although RAPD assay is a widely used method for genetic fingerprinting, there is no known specific primer for discrimination. The primers sometimes are insufficient to differentiate the genetic differences among related and unrelated strains (Neslihan & Isil, 2014). While considering the genetic clones spreading in the state, we can imply based on our study population there are 3 different clones in circulation among the hospitals as all strains analyzed aligned in these groups. From this finding it can be inferred that group A and B are the major prevalent strains circulating in Abia State. The genetic diversity observed in the clusters may also suggest possible and frequent occurrence of mutants among the strains. Whether these strains have a particular feature that facilitates their colonization and adaptation capacity within the tested population is a question that remains to be answered by further studies including more advance molecular typing assay such as multilocus sequence typing (MLST).

Conclusion

RAPD-PCR can be successfully utilized to find out the distribution and epidemiological relationship of MRSA, and also monitor the inter or intra- spread of MRSA strains within hospital, and between different hospitals, provided that the technique is implemented under careful reproducibility condition and importantly duplication of PCR runs to obtain valid results.

This will be useful especially in preventing nosocomial infections caused by MRSA.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

Cookson B, Bonten MJM, MacKenzie FM., Skov RL, Verbrugh, HA, Tacconelli, E: Meticillin-resistant *Staphylococcus aureus* (MRSA): screening and decolonization. Int J Antimicrob Agents 37:195-201. 2011.

Deepika A, Bhatnagar SK: Biodiversity of few Indian charophyte taxa based on molecular characterization and construction of phylogenetic tree. Afr J Biotechnol. 5 (17): 1511-1518. 2006.

Enright MC, Day NPJ, Davies CE, Peacock SJ, Spratt BG: Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol, 38(3):1008-1015. 2000.

Feil SJ, Smith JM, Murphy M, Spratt BG, Moore CE, Day NP: How clonal is *Staphylococcus aureus*? J Bacteriol. 185(11):3307–3316. 2003.

Felsenstein J: Confidence Limits on Phylogenies: An Approach Using the Bootstrap. Evol 39(4): 783–791. 1985.

Fevzi B: Random Amplified Polymorphic DNA (RAPD) Markers. Turk J Biol, 25: 185-196. 2001.

Ikeh EI: (2003). A twelve-month surveillance of MRSA at Jos University Teaching Hospital. Afr J Clin Exp 4(1): 52-55. 2003.

Jukes TH, Cantor CR. Evolution of protein molecules. In Munro HN, editor, Mammalian Protein Metabolism, pp. 21-132, Academic Press, New York. 1969.

Mobasherizadeh S, Shojaei H, Havaei SA, Mostafavizadeh K, Davoodabadi F, Khorvash F, Ataei B, Daei-Naser A: Application of the Random Amplified Polymorphic DNA (RAPD) fingerprinting to analyze genetic variation in Community Associated-Methicillin Resistant *Staphylococcus aureus* (CA-MRSA) isolates in Iran. Glob J Health Sci 8(8):185-191. 2016.

Neela V, Mariana NS, Radu S, Zamberi S, Raha AR, Rosli R: Use of RAPD to investigate the epidemiology of *Staphylococcus aureus* infection in Malaysian Hospitals. World J Microbiol Biotechnol 21:245-251. 2005.

Neslihan I, Isil SB: Application of RAPD-PCR for determining the clonality of methicillin resistant *Staphylococcus aureus* isolated from different hospitals. Braz Arch Biol Technol, 57(4):548-553. 2014.

Nikbakht M, Nahaei MR, Akhi MT, Asgharzadeh M, Nikvash S: Molecular fingerprinting of methicillin resistant *Staphylococcus aureus* strains isolated from patients and staff of two Iranian hospitals. J Hosp Infect 69:46-55. 2008

Piyamongkol W, Bermúdez MG, Harper JC, Wells D: Detailed investigation of factors influencing amplification efficiency and allele drop-out in single cell PCR: implications for preimplantation genetic diagnosis. Mol Hum Reprod 97: 411-420. 2003.

Queipo-Ortuño MI., De Dios Colmenero J, Macias M, Bravo MJ, Morata P: Preparation of bacterial DNA template by boiling and effect of immunoglobulin G as an inhibitor in real-time PCR for serum samples from patients with brucellosis. Clin Vaccine Immunol 15(2), 293–296. 2008. https://doi.org/10.1128/CVI.00270-07

Ranjbar R, Naphoni A, Yousefi S, Ahmadi A, Jonaidi N, Panahi Y: The study of genetic relationship among 3rd generation cephalosporin-resistant *Salmonella enteric* strains by ERII-PCR. Open Microbiol J 7; 142 -145. 2013.

Reinoso E, Bettera S, Frigerio C, DiRenzo M, Calzolari A, Bogni C: RAPD-PCR analysis of *Staphylococcus aureus* strains isolated from bovine and human hosts. Microbiol Res 159(3):245–255. 2004.

Sabat A, Malachowa N, Miedzobrodzki J, Hryniewicz W: Comparison of PCR-based methods for typing *Staphylococcus aureus* isolates. J Clin Microbiol 44: 3804-3807. 2006.

Saitou N, Nei M: The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406-425. 1987.

Strandén A, Frei R, Widmer AF: Molecular typing of methicillin-resistant *Staphylococcus aureus*: can PCR replace pulsed-field gel electrophoresis? J Clin Microbiol 41: 3181-3186. 2003.

Singh A, Goering RV, Simjee S, Foley SL, Zervos MJ: Application of molecular techniques to the study of hospital infection. Clin Microbiol Rev, 19: 512-530.2006.

Watkins RR, David MZ, Salata RA: Current concepts on the virulence mechanisms of meticillin-resistant *Staphylococcus aureus*. J Med Microbiol 61(Pt 9): 1179–1193. 2012 https://doi.org/10.1099/jmm.0.043513-0.