Original Research Article

Evaluation of Different Genotypes and Genetic Virulence in *Staphylococcus Aureus* Isolates from Clinical Samples

Abstract

Infection with Staphylococcus aureus has been considered a major problem in hospitals. The clinical importance of S. aureus isattributed to notable virulence factors and genetic diversity. The objective of this study therefore was to investigate the distribution of S, aureus virulence gene anddifferent genotypesin some isolates obtained from clinical samples from Ekiti State University Teaching Hospital, Ado Ekiti. A total of 25 isolates were screened for the presence of 16SrRNA,GeIE and asaIvirulence genes using Polymerase Chain Reaction (PCR).Virulence genes 16SrRNA, gelE, asaI) formed clusters in S. aureus isolates used in this study. 16SrRNA was absence in 5 of the isolates and only present in 21 of the isolates. GeIE virulence gene was absence in 2 of the isolates and present in 23 of the isolates. AsaI virulence gene was absence in 7 of the isolates and present in 18 of the isolates. Virulence diversity was observed among isolates. Which could be used as a guide to the pathogenicity of individual isolates and hence control spread of infection.The genetic typing was carried out by Random Amplified Polymorphic DNA (RAPD-PCR) using OPBO8, OPHO4, OPH03, OPBO5, and OPT12 Primers. Also there exist different genotypes among the S. aureus isolates used in this study revealing high level of genetic diversity occurrence among S. aureus isolates. The DNA fingerprint revealed high genetic diversity among isolates irrespective of their sources. Further work could be done considering the antibiotic resistant gene and also sequencing of virulence gene clusters peculiar to S. aureus pathogens.

Keywords: gene, virulence, staphyloccus aureus, invasiveness, genetic diversity

Introduction

The genus *Staphylococcus* includes several pathogenic organisms among which *Staphylococcus aureus* is one of the most important (Boyd *et al*, 2002). Generally it is found on human skin and mucous membranes. However it can also be found in other areas of human contact including soil, water, and food products (Chambers, 2001). The species is present as a short-term resident, short-lived contaminant, or long-term colony-forming organism and is capable of causing a wide variety of diseases, such as septicemia, sepsis, wound sepsis, septic arthritis, osteomyelitis, food poisoning, and toxic shock syndrome (Lowyls, 2000).

The genetic basis of pathogenicity depends on the number of virulence factors, including a variety of surface proteins that help in attachment and colonization of the bacteria within the cellular and extracellular material of the host, cellular proteins, protease, and toxins, which inhibit phagocytosis and interfere with the ability of the host to actively hinder infection by a specific immune response (Bhalakia, 2006.).

Hemolysin and other enzymes aid the bacterial population in the invasion of the host tissue (Boyd *et al.*, 2002). Genetic diversity is the total number of genetic characteristics in the genetic makeup of a species. It is distinguished from genetic variability, which describes the tendency of genetic characteristics to vary. Genotypic and phenotypic diversity have been found in all species at the protein, DNA, and organismal levels; in nature, this diversity is nonrandom, heavily structured, and correlated with environmental variation and stress (Nevo, 2001).

This study identified and differentiated the genetic diversity of 25 *Staphylococcus aureus* isolates using their genomic DNA, via genetic PCR analysis approach to estimate *Staphylococcus aureus* genotyping and virulence factors.

Study Area

The study was carried out in the city of Ado-Ekiti, Ekiti State. The State was created on October first, 1996 by the then head of state, the late Gen. Sanni Abacha. Ado-Ekiti functions both as the capital of Ekiti State, as well as the headquarters of Ado-Ekiti Local Government Area. And it is a municipal local government carved out of the old Ekiti Central Government Area in May, 1989. The city of Ado-Ekiti is located in the Central part of the state and it's bounded in the North and West by Irepodun/Ifelodun Local Government Area, while in the South and East it's bounded by Ikere and Gbonyin Local Government Areas respectively.

It's located on latitude $7^0 40^{'}$ North of the Equator and Longitude $5^0 16^{'}$ East of the Greenwich Meridian. Ado is about 200m above the sea level in the South but 500m in the North. It's longest North-East-West stretch is 20km. The landscape is characterized by rounded inselbergs and steep-sided volcanic hills such as Olota rock. The terrains are gently undulating. The major rivers in Ado-Ekiti are Amu, Awedele, Ureje and Ogbese.

Going by the 2006 national census conducted by the National Population Commission, Ado-Ekiti officially has a population of 308,321 (ADO-LEEDS, 2008).

Sample Collections and Preparation

Staphylococcus aureus isolates from 25 different clinical samples such as urine, sputum, semen and wound cultures were obtained from Ekiti State University Teaching Hospital Ado-Ekiti. The isolates were transferred into nutrient agar slant, incubated at 37^{o}C for 24hr. Each stab was sub-cultured on a nutrient agar plate, incubated and the pure culture was characterized and transferred into 1.5 ml Eppendorf tubes and stored at 4^{o}C for genomic DNA extraction and purification.

Genomic DNA Extraction

Genomic DNA was extracted and purified as done by Onasanya et l., (2003). DNA amplification was done using basic PCR technology, after which the amplicons were stored at 4 ^{o}C for electrophoresis. The amplicons were electrophoresed in a 1.4% agarose gel using TAE buffer (45 mMTris-acetate, 1mM EDTA, pH 8.0) at 100 V for 2 h . A 1 kb ladder was included as molecular size marker . Gels were visualized by staining with ethidium bromide solution (0.5 μg/ml). Banding patterns were photographed over UV light using UVP-computerized gel photo documentation system

Genotypes and Genetic Virulence Factors Relationship Determinant Analysis

Band Scoring**:** Using gel documentation system

Statistical Analysis

Statistical Analysis of Genomic Data UsingGenotyping Softwares: was carried out to reveal different Genotypes and genetic virulence factors relationship among 25 *Staphylococcus aureus* isolates

Results

Twenty-five isolates of *Staphylococcus aureus* obtained from different clinical samples were used for this study. The 25 clinical specimens were sourced from 16 males and 9 females,

while the age-brackets of the subjects respectively were; ≤ 1yr, 2; 2-21, 1; 22-41, 10; 42-61, 6 and 62-81, 6. (see Table 1).

Table: 1: Gender, Age and Specimen Distribution

Gender Distribution	
Male	16
Female	9
Total	25
Age Distribution	
≤ 1	2
2- 21	1
22-41	10
42-61	6
62-81	6
Specimen Distribution	
Ear	1
Eye	2
Penile Swab	1
Finger ulcer	2
Wound swab	2
Endocervical swab	3
Semen	5
Sputum	2
Urine	4
HVS(high vaginal swab)	2
Alveolar abscess	1
Total	25

Table 2: Identity of virulence genes detecting PCR primers used to screen pooled genomic DNA of *S. aureus* isolates.

Primer Set	Virulence Target Gene	Sequence (5'- 3')	Position (bp)	Product (bp)	Size
P1	*16S rRNA*	TGGCATAAGAGTGAAAGGCGC	179	290	
		GGGGACGTTCAGTTACTAACGT	468		
P2	*Esp*	TTGCTAATGCTAGTCCACGACC	1217	932	
		GCGTCAACACTTGCATTGCCGA	2149		
P3	*gelE*	ACCCCGTATCATTGGTTT	762	405	
		ACGCATTGCTTTTCCATC	1163		
P4	*cylA*	GACTCGGGGATTGATAGGC	6656	688	
		GCTGCTAAAGCTGCGCTTAC	7344		
P5	*asaI*	CCAGCCAACTATGGCGGAATC	3122	529	
		CCTGTCGCAAGATCGACTGTA	3651		
P6	*Ace*	GGAATGACCGAGAACGATGGC	160	616	
		GCTTGATGTTGGCCTGCTTCCG	776		

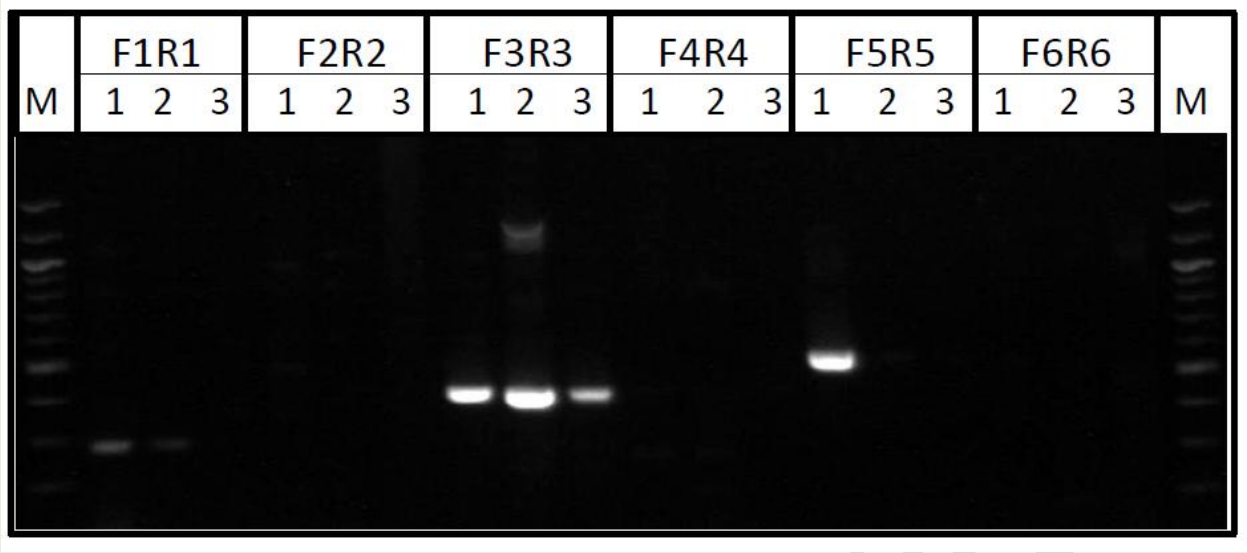

Figure 1. Virulence genes primer screening detection in three pooled genomic DNA of *S. aureus* isolates in PCR analysis.

Table 3: Identity of PCR primers that detected virulence genes in pooled genomic DNA of *S. aureus* isolates.

Primer Set	Virulence Target Gene	Sequence (5'- 3')	Position (bp)	Product Size (bp)
P1	*16S rRNA*	TGGCATAAGAGTGAAAGGCGC	179	290
		GGGGACGTTCAGTTACTAACGT	468	
P3	*gelE*	ACCCCGTATCATTGGTTT	762	405
		ACGCATTGCTTTTCCATC	1163	
P5	*asaI*	CCAGCCAACTATGGCGGAATC	3122	529
		CCTGTCGCAAGATCGACTGTA	3651	

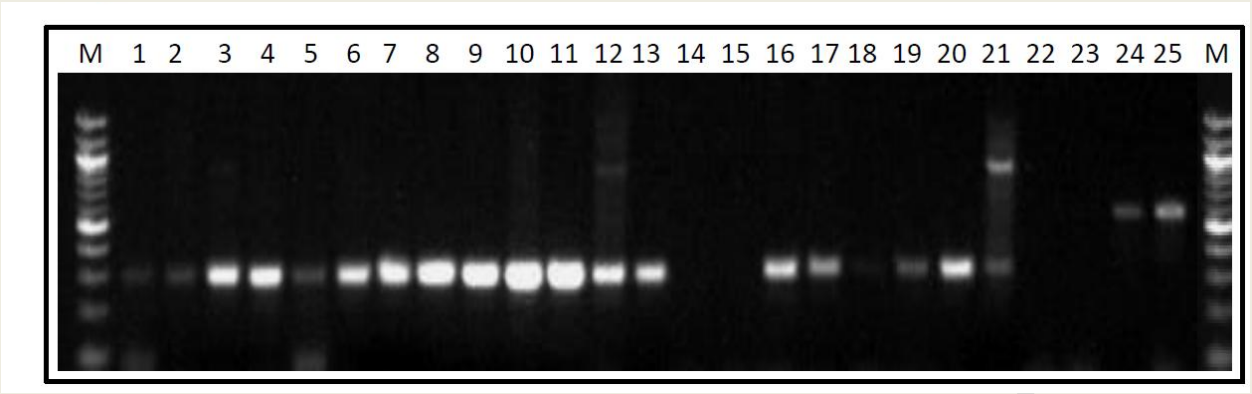

Figure 2. Virulence gene (*16S rRNA*) PCR detection in genomic DNA of 25 *S. aureus* isolates.

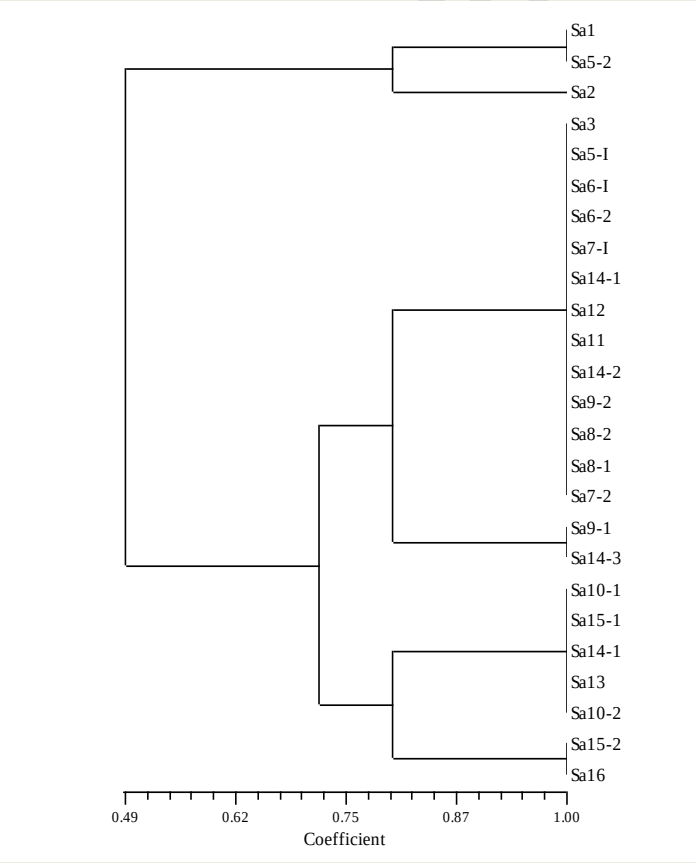

Figure 3: Virulence gene (*16S rRNA*) clusters identified in 25 *S. aureus* isolates genomic DNA.

Virulence gene (*16SrRNA*) detected in 20*S. aureus* isolates; The virulence gene (*16SrRNA*) formed 3 main and 6 sub clusters among the *S. aureus* isolates; Some isolates have identical virulence gene.

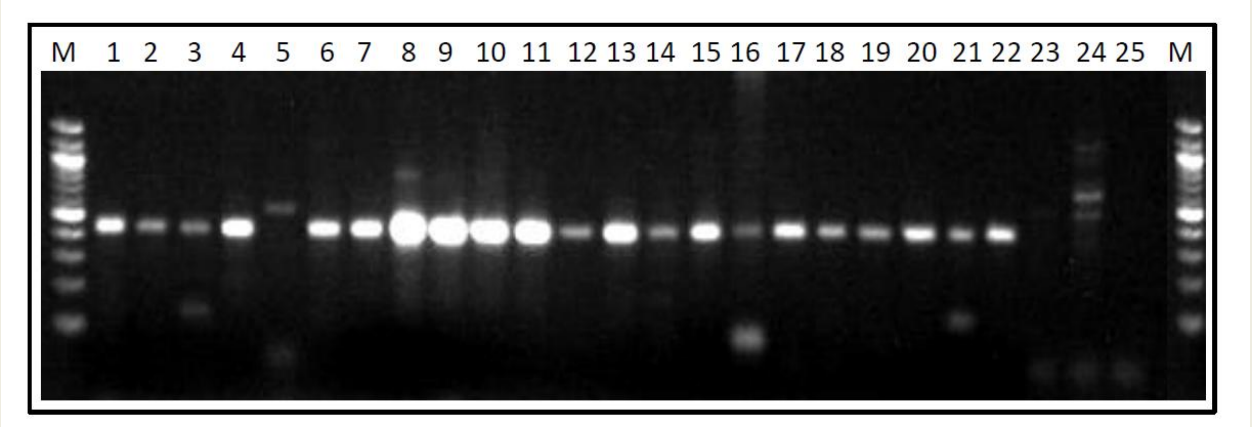

Figure 4: Virulence gene (*gelE*) PCR detection in genomic DNA of 25 *S. aureus* isolates.

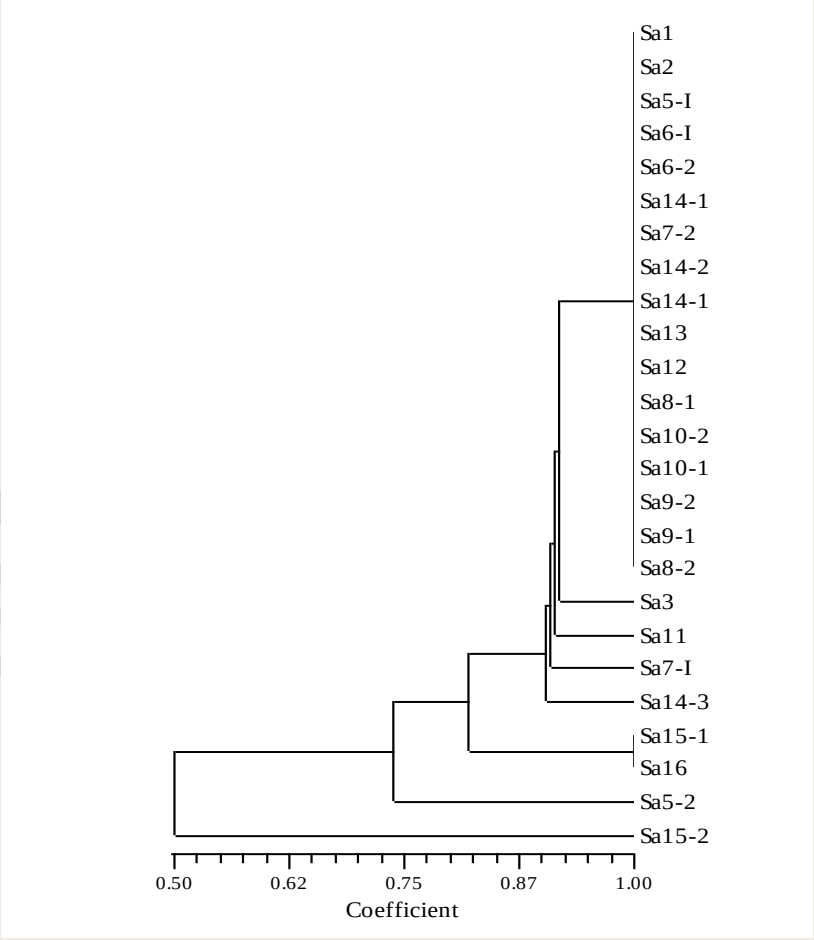

Figure 5: Virulence gene (*gelE*) clusters identified in 25 *S. aureus* isolates genomic DNA.

Virulence gene (gelE) detected in 23 S. aureus isolates; The virulence gene (geIE) formed 4 main and 8 sub clusters among the S. aureus isolates; Some isolates have identical virulence gene.

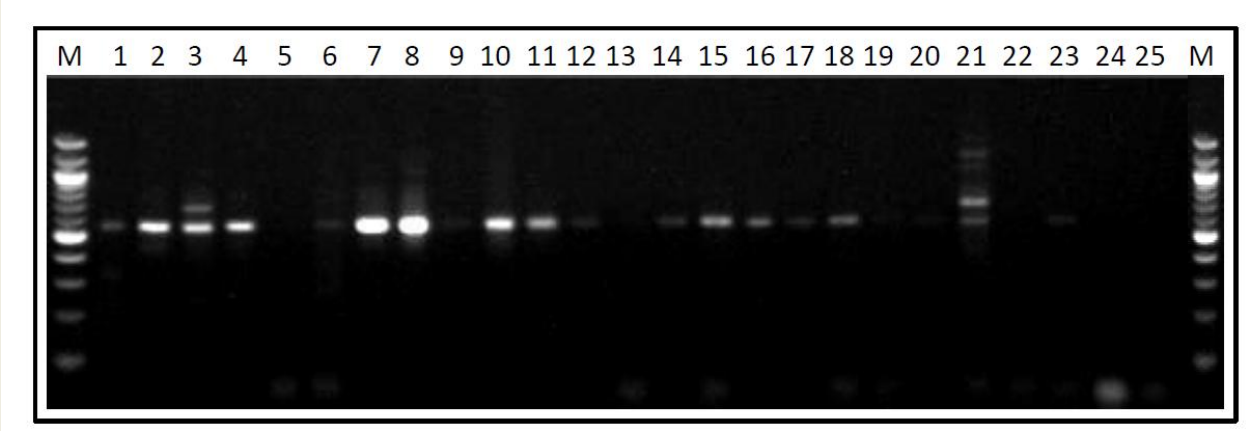

Figure 6: Virulence gene (*asaI*) PCR detection in genomic DNA of 25 *S. aureus* isolates.

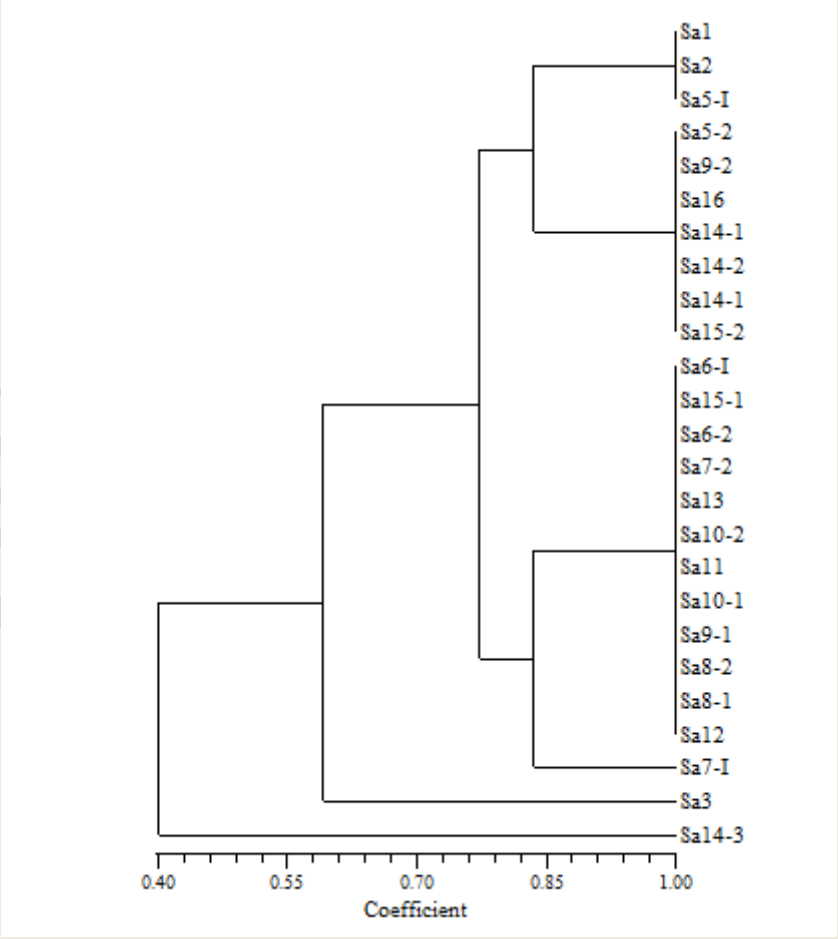

Figure 7: Virulence gene (*asaI*) clusters identified in 25 *S. aureus* isolates genomic DNA.

Virulence gene (asaI) detected in 18 S. aureus isolates; The virulence gene (geIE) formed 3 main clusters among the *S. aureus* isolates; Some isolates have identical virulence gene.

Table 4. Identity of RAPD primers used to screen for polymorphism in pooled genomic DNA of *S. aureus* isolates.

S/N	Primer	Sequence
1	OPB08	GTCCACACGG
2	OPH10	CCTACGTCAG
3	OPB17	AGGGAACGAG
4	OPH03	AGACGTCCAC
5	OPH04	GGAAGTCGCC
6	OPT16	GGTGAACGCT
7	OPB05	TGCGCCCTTC
8	OPT12	GGGTGTGTAG

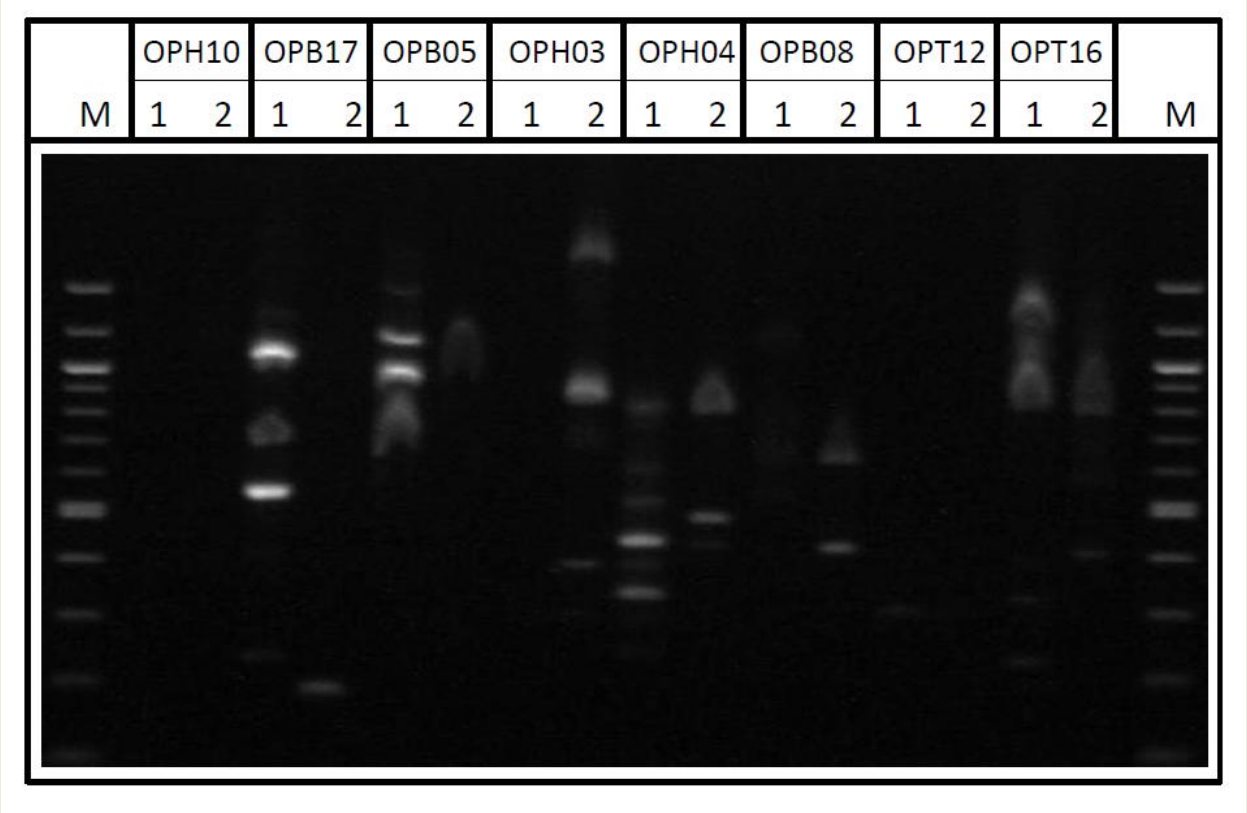

Figure 8: Detection of polymorphism in two pooled genomic DNA of *S. aureus* isolates using 8 RAPD primers in PCR analysis.

Table 5: Identity of five RAPD primers selected after polymorphism screening used for PCR analysis of genomic DNA of 25 *S. aureus* isolates.

S/N	Primer	Sequence
1	OPB08	GTCCACACGG
2	OPH03	AGACGTCCAC
3	OPH04	GGAAGTCGCC
4	OPB05	TGCGCCCTTC
5	OPT12	GGGTGTGTAG

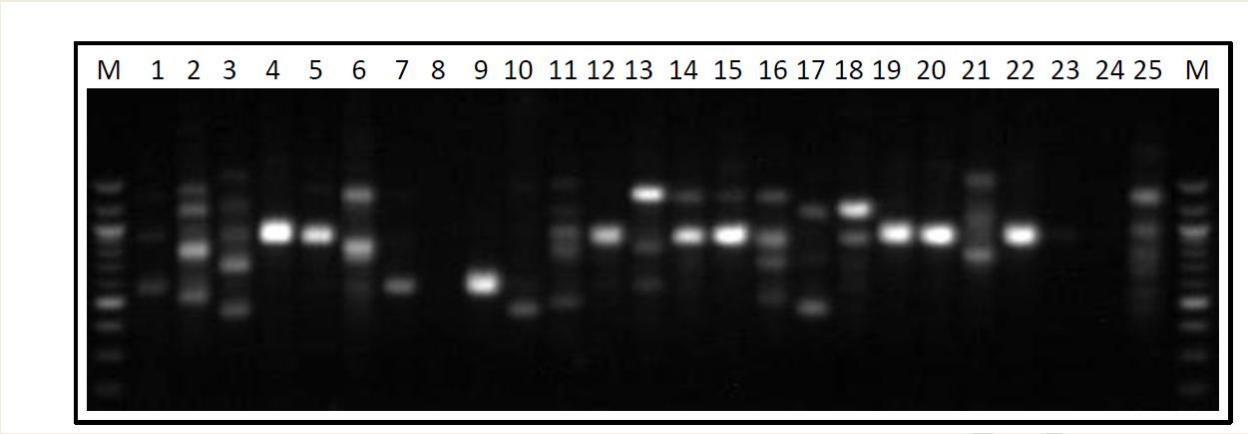

Figure 9: DNA fingerprints of 25 *S. aureus* isolates using OPB08 primers in PCR analysis.

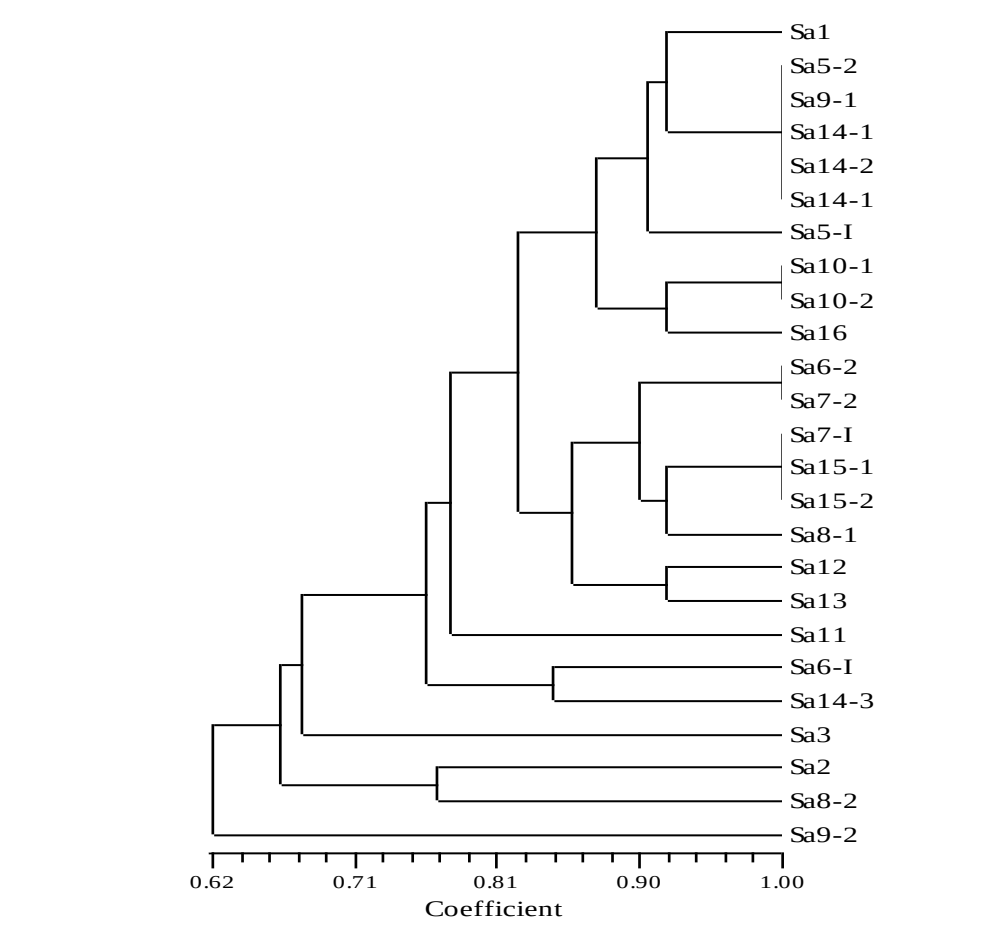

Figure 10: Genotypes of *S. aureus* isolates as revealed OPB08 PCR analysis.

DNA fingerprint of 25 *S. aureus* isolates using OPB08 primer revealed existence of different genotypes among the isolates; Some isolates were identical

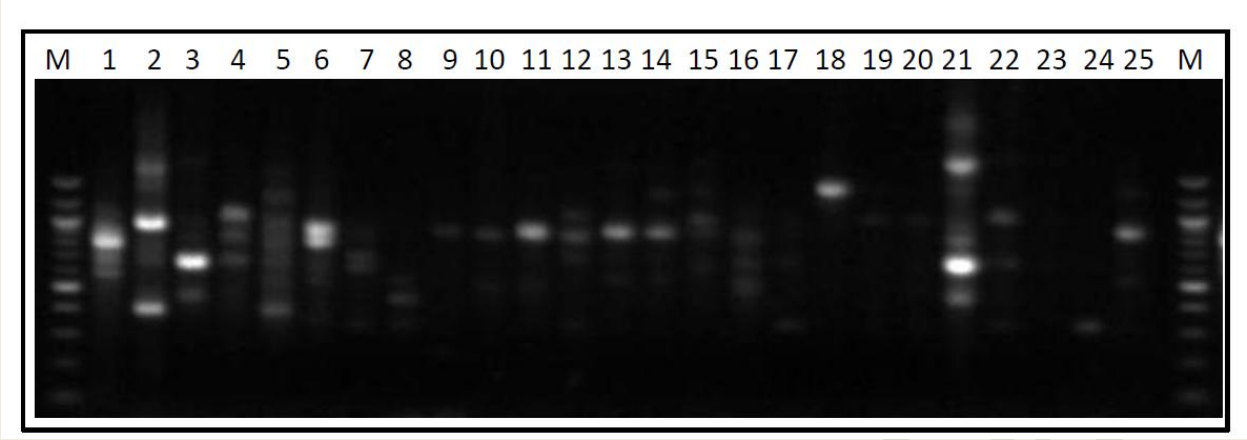

Figure 11: DNA fingerprints of 25 *S. aureus* isolates using OPH04 primers in PCR analysis.

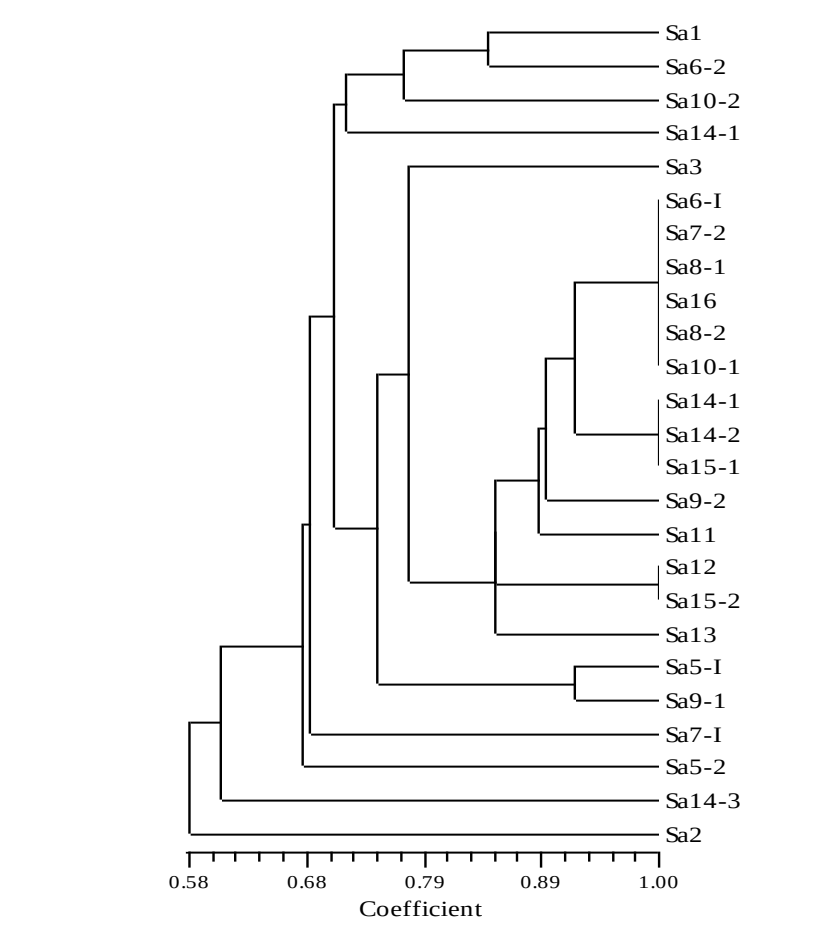

Figure 12: Genotypes of *S. aureus* isolates as revealed OPH04 PCR analysis.

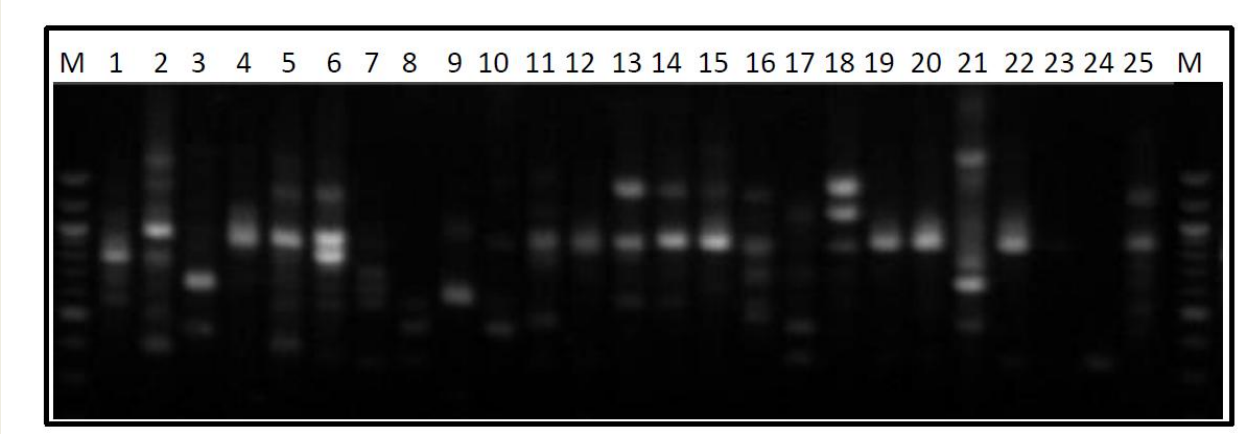

Figure 13: DNA fingerprints of 25 *S. aureus* isolates using OPH03 primers in PCR analysis.

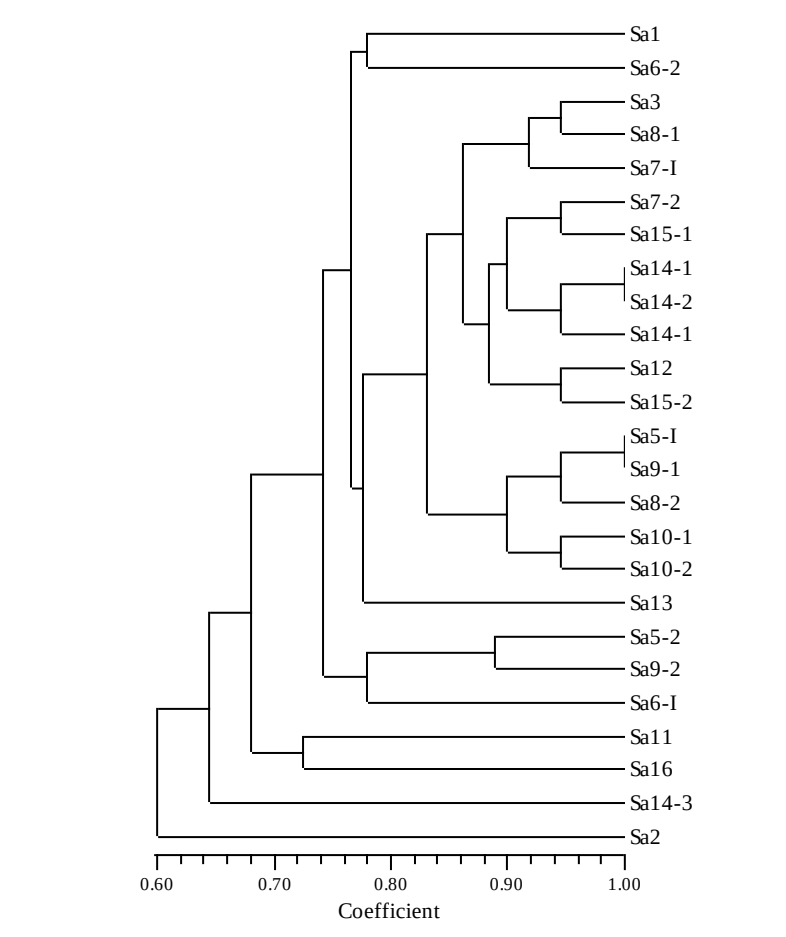

Figure 14: Genotypes of *S. aureus* isolates as revealed OPH03 PCR analysis.

DNA fingerprint of 25 S. aureus isolates using OPH03 primer revealed existence of difference genotypes among the isolates; Two isolates were identical

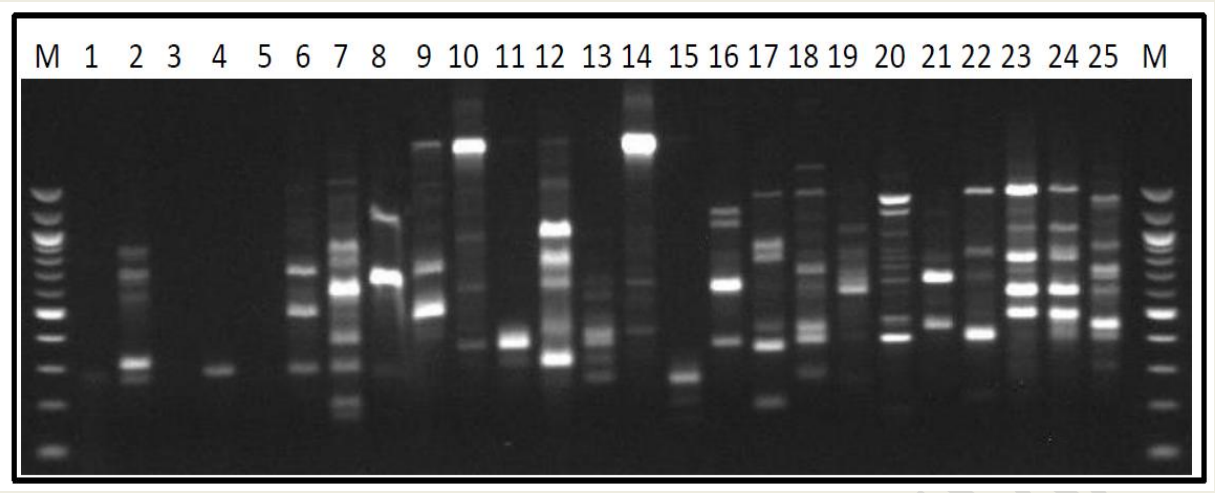

Figure 15: DNA fingerprints of 25 *S. aureus* isolates using OPB05 primers in PCR analysis.

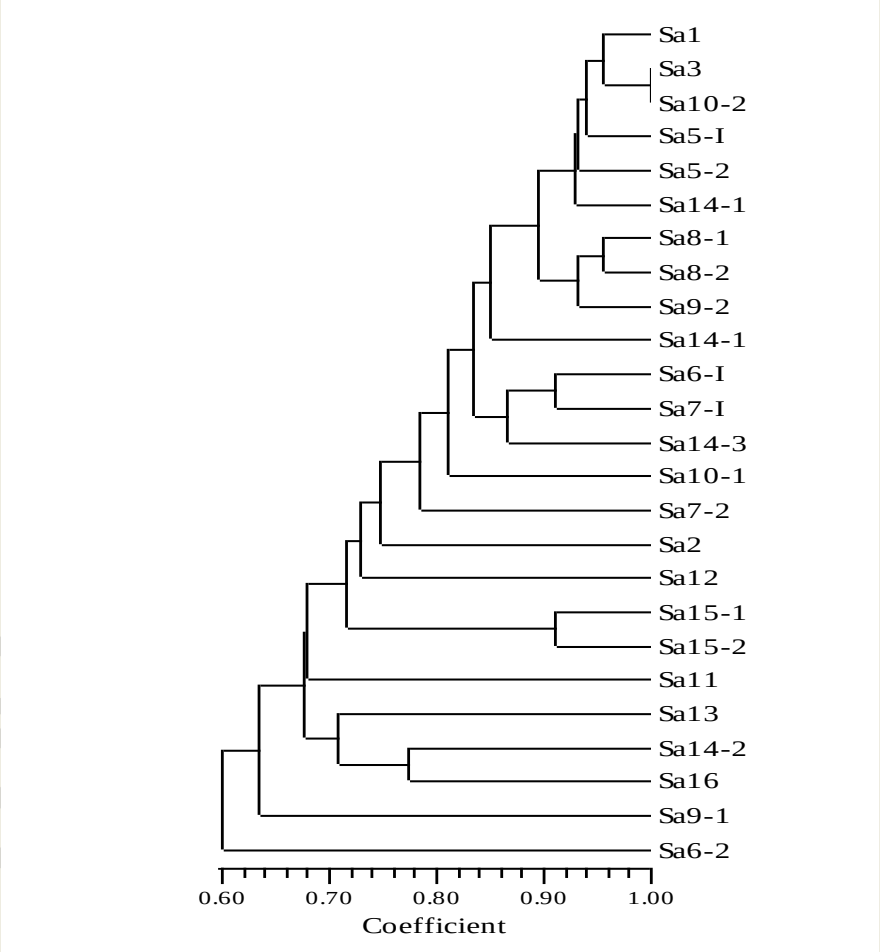

Figure 16: Genotypes of *S. aureus* isolates as revealed OPB05 PCR analysis.

DNA fingerprint of 25 *S. aureus* isolates using OPB05 primer revealed existence of difference genotypes among the isolates; two isolates were identical.

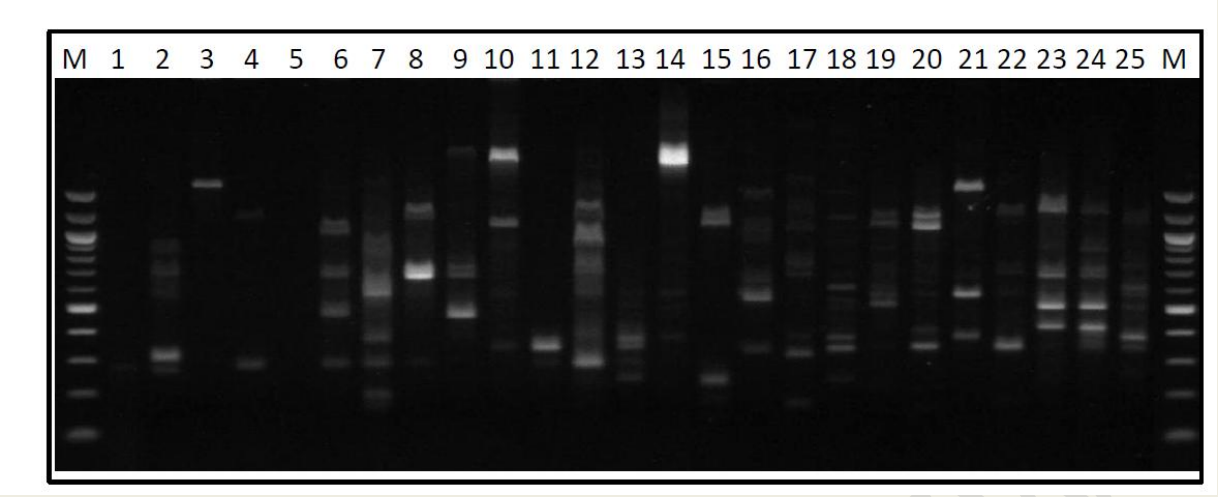

Figure 17: DNA fingerprints of 25 *S. aureus* isolates using OPT12 primers in PCR analysis.

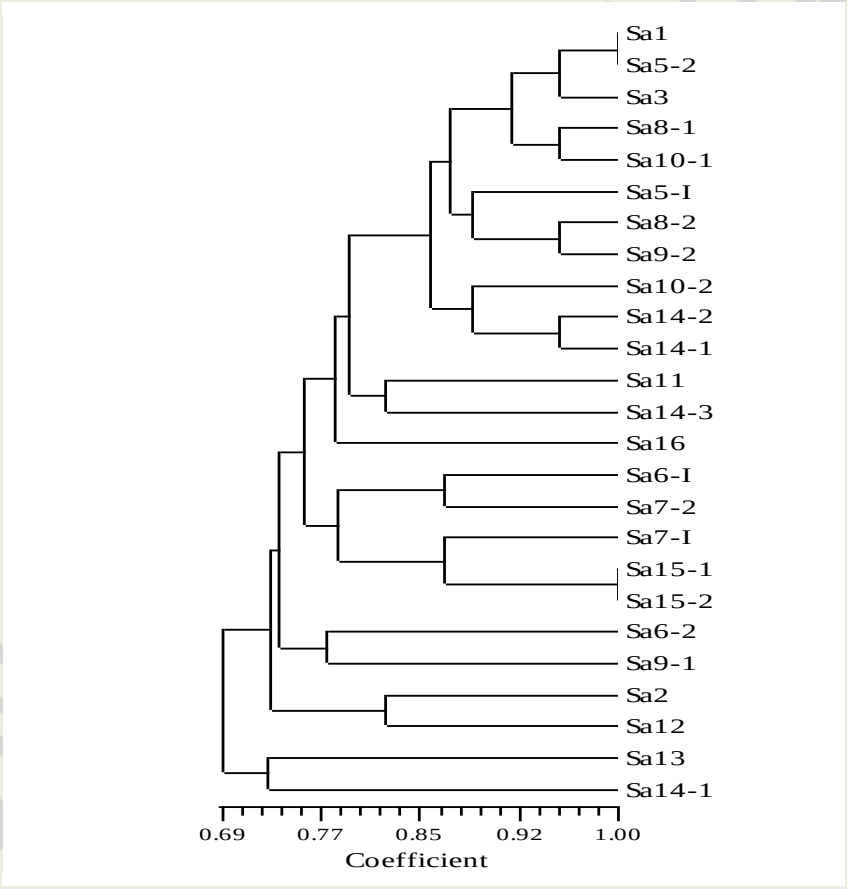

Figure 18: Genotypes of *S. aureus* isolates as revealed OPT12 PCR analysis.

DNA fingerprint of 25 *S. aureus* isolates using OPT12 primer revealed existence of difference genotypes among the isolates;Two isolates were identical.

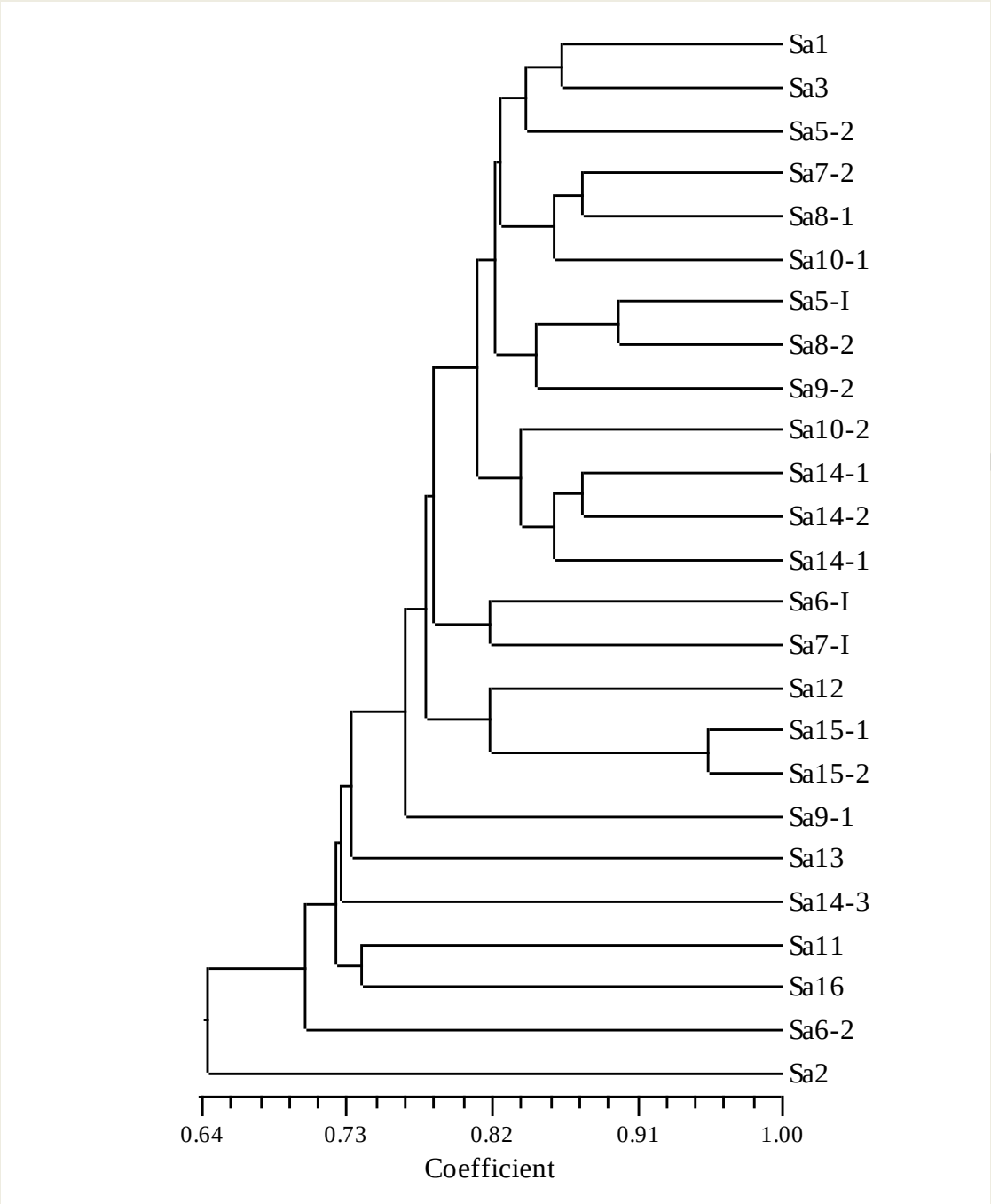

Figure 19: Genotypes of *S. aureus* isolates as revealed 88 RAPD PCR markers.

25 *S. aureus* isolates are distinct with high level of genetic diversity as revealed by 88 RAPD PCR makers; No identical isolates.

Discussion

The ability of *S aureus* to cause disease depends on a wide range of virulence factors that contribute to colonization and disease in the host. (Voyich, *et al.*, 2006).

Results showed in figure 2 and figure 3 that from the 25 *S. aureus* isolates screened for 16SrRNA virulence gene, three main and six sub clusters were formed. Five of the isolates, Sa 10-1 (from semen), Sa 10-2 (from the same semen), Sa 13 (from sputum, Sa 14-1 (from urine) and Sa 15-1(High viginal swab, show complete lack of 16SrRNA virulence gene. 16SrRNA *gene is used for identification of a broad range of clinically relevant bacteria pathogen).*Figure 4 and figure 5 showed that from the 25 *S. aureus* isolates screened for gelE virulence gene, four main and eight sub clusters were formed. Two of the isolates, Sa 15-1(High viginal swab) and Sa 16 (endocervical swab), show complete lack of gelE virulence gene.AsaI virulence gene showed in figure 6 and figure 7 three main and six sub clusters and was absence in 7 of the isolates, (Sa5-2 from eye swab of a neonate, Sa9-2 from semen, Sa14-1 from urine, Sa14-2, Sa14-4, Sa15-2 and Sa16), and present in 18 of the isolates. Virulence diversity was observed among isolates, which could be used as a guide to the pathogenicity of individual isolates and hence control spread of infection.

The identity of RAPD primers used in this study for polymorphism screening of pooled genomic DNA is shown in table 4. Detection of polymorphism in two pooled genomic DNA of *S. aureus* isolates using 8 RAPD primers in PCR analysis. Figure 14 reveals OPB08, OPH03, OPH04, OPB05 and OPT12 as sets with significant polymorphism.

The genetic typing was carried out by Random Amplified Polymorphic DNA (RAPD-PCR) using OPBO8, OPHO4, OPH03, OPBO5, and OPT12 Primers.DNA fingerprint of 25 *S. aureus* isolates using OPB08 primer revealed existence of different genotypes among the isolates; Some isolates were identical figure 11 and figure 12.DNA fingerprint of 25 S. aureus isolates using OPH03 primer revealed existence of difference genotypes among the isolates; Two isolates were identicalGenotypes of *S. aureus* isolates as revealed by 88 RAPD PCR markers showed

25 *S. aureus* isolates with distinct with high level of genetic diversity as revealed by 88 RAPD PCR makers; No identical isolates. There exist different genotypes among the *S. aureus* isolates used in this study revealing high level of genetic diversity occurrence among

S. aureus isolates. The DNA fingerprint revealed high genetic diversity among isolates irrespective of their sources.

Virulence genes (*16SrRNA, gelE, asaI*) and their clusters detected in *S. aureus* isolates used in this study formed the genetic basis for pathogenicity of the pathogen in human. Different genotypes among the *S. aureus* isolates used in this study revealing high level of genetic diversity occurrence among *S. aureus* isolates.

The existence of different genotypes among the *S. aureus* isolates was due to the presence of virulence genes (*16SrRNA, gelE, asaI*) and their clusters in*S. aureus* revealed inherent relationship between genotypes and genetic virulence factors in *S. aureus* isolates genome. Historically, S aureus has been described as a variable bacterium with many pathogenic and antibiotic resistance variants (Coltman, 1979, Kioos and Schleifer, 1981).

Conclusion and Recommendation

DNA fingerprint revealed high genetic diversity among isolates irrespective of their sources. Virulence genotype were highly diverse in these isolates. Further work could be done considering the antibiotic resistant gene and also sequencing of virulence gene clusters peculiar to *S. aureus* pathogens.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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