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Distribution of *lasI* and *rhlR* virulent gene among *Staphylococcus aureus* isolates in clinical samples from Ekiti state university teaching hospital, Ado Ekiti, Nigeria

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Abstract

Infection with *Staphylococcus aureus* has been considered a major problem in hospitals. The clinical importance of *S. aureus* is attributed to notable virulence factors and genetic diversity. The study aimed to investigate the distribution of *lasI* and *rhlR* virulent gene (a quorum sensing system reported in *Pseudomonas aeruginosa)* and different genotypes in some clinical isolates obtained from Ekiti State University Teaching Hospital, Ado Ekiti. A total of 100 clinical isolates were collected and screened out of which 75 samples with good *S. aureus* yield were selected for this study. 34 (45.3%) were from males and 41 (54.7%) were from females. The isolates were drawn from across several age groups and sample types. Result showed that most susceptible antibiotics was Ciprofloxacin (64.0 %), while the most resistant antibiotics was Ampiclox (88.0%). Using Polymerase Chain Reaction (PCR), virulent genes *lasL* and *rhiR* formed clusters in *S. aureus* isolates used in this study, *lasI* was absence in 22 of the isolates and only present in 53 (71%) of the isolates, *rhlR* was absent in 24 of the isolates and only present in 51 (68%) of the isolates. Virulence diversity was observed among isolates. Furthermore *S. aureus* was found to harbor *lasL* and *rhlR virulent gene***,** a quorum sensing system which was only reported in *Pseudomonas aeruginosa,* revealing the virulent mutants in the 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 OPH06, OPT06, OPT07 and OPHO7, primers. In conclusion there exist different genotypes which are mutants 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.

Keywords: *Staphylococcus aureus; lasI* and *rhlR* Virulent gene; Antibiotic resistance; Genetic diversity; Polymerase chain reaction

1. Introduction

The pathogenic organisms in the genus Staphylococcus encompass a number of species, with *Staphylococcus aureus* being one of the more significant.[1] In general, it is found on human skin and mucous membranes, but it can also be found in other places where people come into contact with each other, such as dirt, water, and food items.[2] The species is present as a short-term resident, short-lived contaminant, or long-term colony-forming organism and with the ability to cause a wide range of diseases, including septicemia, sepsis, wound sepsis, septic arthritis, osteomyelitis, food poisoning, and toxic shock syndrome.[3] Since a variety of factors are thought to have a role in the pathogenesis of *S. aureus* infections, it is currently impossible to determine the disease potential of staphylococcal strains solely based on the sequencing of their genome.[4]

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Staphylococcus aureus has the ability to express a variety of virulence factors and toxins that are crucial to host infection.[5] Global virulence regulators, such as a staphylococcal accessory regulator (sarA), an accessory gene regulatory system (agr ABCD), and a two component system (KdpDE), mainly control the gene expression and regulation of virulence components in human isolates of *S. aureus*.[6] Additionally, *S. aureus* global gene regulators also regulate a large number of virulence and resistance determinants found on mobile genetic elements (MGEs), including staphylococcal cassette chromosomes, pathogenicity islands, plasmids, bacteriophages, transposons, and insertion sequences.[5]

This behavior has been found in the *Staphylococcus aureus* pathogenicity islands (SaPIs), mobilizing two enterotoxin genes along with the toxin gene for toxic shock syndrome (tsst-1) and the adhesion protein Bap.[7] Another illustration is the catabolic mobile element of arginine, which has been linked to bacteria that carry particular subtypes of the methicillin resistance cassette (SCCmec) and is transported with it due to their proximity. In Staphylococcal species, the transfer of antibiotic resistance genes is frequent. Staphylococci have frequently been found to be resistant to methicillin, lincosamides, macrolides, aminoglycosides, and combinations of these antibiotics.[8]

It has been challenging to distinguish between a subset of commensal *S. aureus* clones and those with specific virulence characteristics that cause disease. Pulsed-field gel electrophoresis (PFGE) and multilocus sequencing typing (MLST) have thus been developed as molecular typing methods, with the PFGE method serving as the gold standard for the typing of *S. aureus*.[9] However, other molecular biology options have been suggested due to the high costs, such as PCR-RFLPs of the protein A gene (spA) or the coagulase gene (coa), which are genes that are conserved in genus and species, respectively. There was a 100% correlation between spA type and PFGE.[10] The limitation of these genes is still utilized today to characterize *S. aureus* strains from various origins. The fast evolution of this bacterium necessitates the investigation of virulence factors, which could shed light on how they contribute to the spread and environmental adaption of the same *S. aureus* clone.[11]

Animal models of staphylococcal infection can be useful for analyzing how potential virulence factors discovered in vitro contribute to pathogenesis, but their applicability to human disease is intrinsically limited and may not capture human-specific adaptive behavior. It is necessary to identify patients with *S. aureus* infections and describe bacterial gene expression profiles for various forms of infection directly in tissue specimens in order to address this. *S. aureus* isolates from Nigeria have been characterized by numerous investigations, but no one study has evaluated the virulence genes, morphologies, and genotypes of isolates from clinical samples. It is crucial to do this comparison since there is evidence that certain genotypes and virulence factors are more common in African isolates from infection compared to colonization.[12] this study was therefore carried out to genetically analyze virulent mutants among *Staphylococcus aureus* isolates from clinical samples.

2. Material and methods

2.1. Collections, isolation and purification of *Staphylococcus aureus* isolates from clinical samples and storage

This study was carried out in Ekiti State University Teaching Hospital Ado-Ekiti, Ekiti State, Nigeria. Ethical approval for collection of sample was obtained from the same institution. One hundred (100) different isolates of *S. aureus* from clinical samples ranging from urine, urethra, HVS, sputum and wound cultures was obtained from Ekiti State University Teaching Hospital Ado-Ekiti, Ekiti State, Nigeria. These isolates were identified by conventional microbiological methods, including gram staining, catalase and coagulase test. Seventy five (75) of these isolates were collected each into 1.5 ml Eppendorf tubes and store at 4 ^{0}C until genomic DNA extraction and purification.

2.2. Antimicrobial Susceptibility Testing

The Kirby-Bauer disc diffusion method, using Mueller-Hinton agar (Merck), was used following the guidelines of the Standard Clinical and Laboratory Institute (CLSI) to perform antimicrobial susceptibility tests.[13] each of the *S. aureus* isolate was inoculated into nutrient agar. Ten antibiotic discs were placed apart on the plated agar. After 24hrs of incubation at 37^{0}C, the diameter of inhibitory zone surrounding the discs were observed. The isolate sensitivity/resistance pattern was examined and documented. The antibiotics used were; Perfloxaxin (PER) 10 µg, Gentamycin (CN) 10 µg, Ampiclox (APX) 30 µg, Zinnacef (Z) 20 µg , Amoxacillin (AM) 30 µg, Rocephin (R) 25 µg, Ciprofloxacin (CPX) 10 µg, Streptomycin (S) 30 µg, Septrin (SXT) 30 µg, and Erythromycin (E) 10 µg.

2.3. Genomic DNA Isolations and Purification from *Staphylococcus aureus* isolates

Genomic DNA Isolations and Purification from *Staphylococcus aureus* isolates was done according to the method described by Onasanya *et al.*[14]. 0.3 g of washed bacterial cell was suspended in 200 µl of CTAB buffer (50mM Tris, pH 8.0); 0.7 mM NaCl; 10 mM EDTA; 2% hexadecyltrimethyl ammonium bromide and 0.1s% 2-mercaptoethanol), followed

by the addition of 100 µl of 20% SDS. It was incubated at 65 ^{0}C for 30 min. DNA was purified by two extractions with phenol: chloroform: isoamylalcohol (24:25:1) and centrifuged at 14,000 rpm at 4 ^{0}C for 10 mins. DNA was precipitated from supernatant with equal volume of absolute ethanol and centrifuged at 10,000 rpm at 4 ^{0}C for 10 mins. DNA pellet was washed with 70% ethanol, dried at room Temperature, re-suspended in 200 µl of sterile distilled water. DNA concentration was measured using a spectrophotometer at 260 nm. DNA degradation was checked by electrophoresis on a 1% agarose gel in TAE buffer (45 mM Tris-acetate, 1 mM EDTA, pH 8.0).

2.4. Genotyping and Genetic Virulence PCR Analysis

Genotyping PCR analysis of *Staphylococcus aureus* isolates was done using universal primer technology described by Mirnejad *et al*.[15]. The reaction mixture were; Genomic DNA 80 ng per reaction, Taq Reaction buffer, 100 mM dNTP (dATP; dCTP; dGTP; dTTP), 0.2 mM universal primer (15 primersS was screened), 2.5 mM $MgCl_2$, 5% Tween 20, 1U of Taq polymerase and Sterile distilled water.

Genetic virulence PCR analysis of *Staphylococcus aureus* isolates was also done using virulence factors primer technology described by Mirnejad *et al*. (2012). The reaction mixture (50 µL) were; Genomic DNA 80 ng per reaction, 2.5 µL Taq Reaction buffer, 5 µL 100 mM dNTP (dATP; dCTP; dGTP; dTTP), 5 µL 0.1 mM Reverse primer, 5 µL 0.1 mM Forward primer, 5 µL 2.5 mM $MgCl_2$, 5 µL 5% Tween 20, 1 µL 1U of Taq polymerase and 24 µL Sterile distilled water respectively. Amplification was carried out in a Bio-Rad thermal cycler, run for 45 cycles. The cycling programs for both procedures were**;** (I) 1 cycle of 94 ^{0}C for 3 min. (ii) 45 cycles of: 94 ^{0}C for 1 min for denaturation, 40 ^{0}C for 1 min for annealing of primer and 72 ^{0}C for 2 min for extension. (iii) Final extension at 72 ^{0}C for 7 min. (IV) Amplification products was maintained at 4 ^{0}C until electrophoresis.

2.5. Separation of PCR Products

The amplification products were resolved by electrophoresis in a 1.4% agarose gel using TAE buffer (45 mM Tris-acetate, 1 mM EDTA, pH 8.0) at 100 V for 2 hours. 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) was done using gel documentation system.

2.6. Statistical Analysis

Genomic Data using Genotyping Software was carried out to reveal different Genotypes and genetic virulence factors relationship among 75 *Staphylococcus aureus* isolates. The results were presented in tables, figures and charts.

3. Results

Table 1 showed the demographic characteristics of the subjects studied. Out of the 75 samples, 34 (45.3 %) were males and 41 (54.7%) were females. Furthermore, 14 (18.7%), 42 (56%), 11 (14.7%) and 8 (10.6%) belonged to age group 0-20 years, 21-40 years, 41-60 years and 61 years and above respectively. The nature of samples collected were; Abscess (4%), Aspirates (2.7%), CSF (1.3%), Ear swab (2.7%), HVS (20%), Pleural fluid (1.3%), Semen (24%), Sputum (4%), Urine (38.7%) and Blood (1.3%) respectively.

Table 2 showed the Genomic DNA concentration and purity of genomic DNA from 75 *S. aureus* isolates, table 3 showed the Identity of RAPD primers used for preliminary PCR amplification and discriminate screening, table 4 showed the identity of selected RAPD primers used for PCR analysis of all 75 *S aureus* genomic DNA, table 5 showed the identity of virulence target gene specific primers used for preliminary PCR amplification and screening of *S. aureus* isolates genomic DNA, Table 6 showed the Identity of selected virulence target gene specific primers used for PCR analysis detection of virulence target gene in all the 75 *S. aureus* isolates genomic DNA, and Table 7 showed the *rhlR* and *lasI.* virulent gene distribution and status in 75 *S. aureus* isolates.

Figure 1 showed the antibiotic susceptibility test of *Staphylococcus aureus* isolates. From the results obtained, the most susceptible antibiotics were Ciprofloxacin (64.0%), followed by Streptomycin (58.7%), Rocephin (54.7%), and Perfloxacin (52.0%). On the other hand, the most resistant antibiotics were Ampiclox (88.0%), followed by Zinnacef (78.7%), Amoxicilin (70.7%), Erythromycin (64.0%), Gentamycin (64.0%), and Septrin (56.0%).

Figure 2 showed the antibiotic susceptibility tests of *Staphylococcus aureus* isolates in relation to gender. From the results obtained, the susceptibility pattern of the antibiotics in male and female subjects were; Perfloxacin 17 (22.6%) and 22 (29.3%), Gentamycin 9 (12.0%) and 18 (24.0%), Ampiclox was 2 (2.7%) and 7 (9.3%), Zinnacef was 5 (6.7%)

and 11 (14.7%), Amoxicilin was 12 (16.0%) and 10 (13.3%), Rocephin was 18 (24.0%) and 23 (30.7%), Ciprofloxacin was 23 (30.7%) and 25 (33.3%), Streptomycin was 19 (25.3%) and 25 (33.3%), Septrin was 15 (20.0%) and 18 (24.0%), Erythromycin was 12 (16.0%) and 15 (20.0%) respectively.

Figure 3 showed the antibiotic susceptibility tests of *Staphylococcus aureus* isolates in relation to age. From the results obtained, the susceptibility pattern of Perfloxacin was 12%, 29.3%, 6.7% and 4% in age group 0–20 years, 21–40 years, 41–60 years and 61 years & above, Gentamicin was 8%, 22.7%, 4% and 3% respectively. Others are Ampiclox (1.3%, 8 %, 2.7% and 0%), Zinnacef (2.7%, 13.3%, 1.3% and 1.3%), Amoxicilin (5.3%, 20.0%, 1.3% and 2.7%), Rocephin (8%, 40%, 5.3% and 1.3%), Ciprofloxacin (10.7%, 44%, 6.7% and 2.7%), Streptomycin (5.3%, 37.3%, 10.7% and 5.3%), Septrin (4%, 32.0%, 5.3% and 2.7%) and Erythromycin (2.7%, 21.3%, 6.7% and 5.3%) in age group 0–20 years, 21–40 years, 41–60 years and 61 years & above respectively.

Figure 4 showed cluster of antibiotic response to 75 *S. aureus* isolates. Antibiotic response to 75 *S. aureus* isolates formed 3 main and 6 sub clusters among the ten antibiotics engaged. Only two of the antibiotic Zinacef and Amoxicilin had identical response. Figure 5 showed Cluster of 75 *S. aureus* grouping based on reactions with 10 commonly used antibiotics, Figure 6 showed *S. aureus* genomic DNA agarose gel electrophoretic pattern, Figure 7 showed the DNA standard curve using Sigma DNA Ladder, Figure 8 showed the PCR Profile of fifteen RAPD primers used to screen pooled *S. aureus* genomic DNA and Figure 9 showed the DNA fingerprint of 75 *S. aureus* genomic DNA as revealed by OPT06-PCR analysis.

Table 1 Demographic Characteristics of the Subjects

Variable	Number (n = 75)	Percentage (%)
Gender		
Male	34	45.3
Female	41	54.7
Age (years)		
0 – 20	14	18.7
21 – 40	42	56.0
41 – 60	11	14.7
61 & above	8	10.6
Nature of Sample		
Abscess	3	4.0
Aspirate	2	2.7
CSF	1	1.3
Ear Swab	2	2.7
HVS	15	20.0
Pleural fluid	1	1.3
Semen	18	24.0
Sputum	3	4.0
Urine	29	38.7
Blood	1	1.3

Figure 10 showed Cluster of genetic diversity among 75 *S. aureus* isolates amplified DNA OPT06 PCR-RAPD products analyzed by electrophoresis. DNA fingerprint of 75 *S. aureus* isolates using OPT06 primer revealed existence of different genotypes among the isolates; some isolates were identical. Figure 11 showed the DNA fingerprint of 75 *S. aureus* genomic DNA as revealed by OPH06-PCR analysis and Figure 12 showed Cluster of genetic diversity among 75 *S. aureus*

isolates amplified DNA OPH06 PCR-RAPD products analyzed by electrophoresis. DNA fingerprint of 75 *S. aureus* isolates using OPH06 primer revealed existence of different genotypes among the isolates; some isolates were identical. Figure 13 showed the DNA fingerprint of 75 *S. aureus* genomic DNA as revealed by OPT07-PCR analysis. Figure 14 showed Cluster of genetic diversity among 75 *S. aureus* isolates amplified DNA OPT07 PCR-RAPD products analyzed by electrophoresis. OPT07 primer revealed existence of different genotypes among the isolates; some isolates were identical. Figure 15 showed the DNA fingerprint of 75 *S. aureus* genomic DNA as revealed by OPH07-PCR analysis.

Figure 16 showed Cluster of genetic diversity among 75 *S. aureus* isolates amplified DNA OPH07 PCR-RAPD products analyzed by electrophoresis. OPH07 primer revealed existence of different genotypes among the isolates; some isolates were identical. Figure 17 showed Cluster of genetic diversity among 75 *S. aureus* isolates as revealed by 64 RAPD markers. 75 *S. aureus* isolates are distinct with high level of genetic diversity as revealed by 64 RAPD PCR makers; only two sets of isolates Sa54, Sa55 and Sa66, Sa67 are identical respectively. Figure 18 showed the PCR Profile virulence target gene detection by 16 specific primers used for preliminary PCR amplification and screening of *S. aureus* isolates genomic DNA, and Figure 19 showed PCR detection of *lasI* virulence gene at 295 base pairs in 75 *S. aureus* isolates genomic DNA.

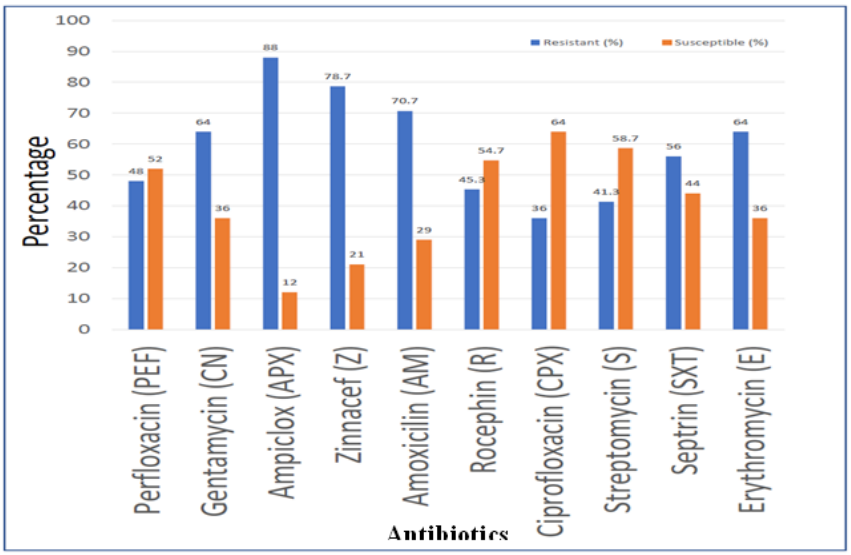


Figure 1 Response of 75 *S. aureus* isolates to 10 commonly used antibiotics

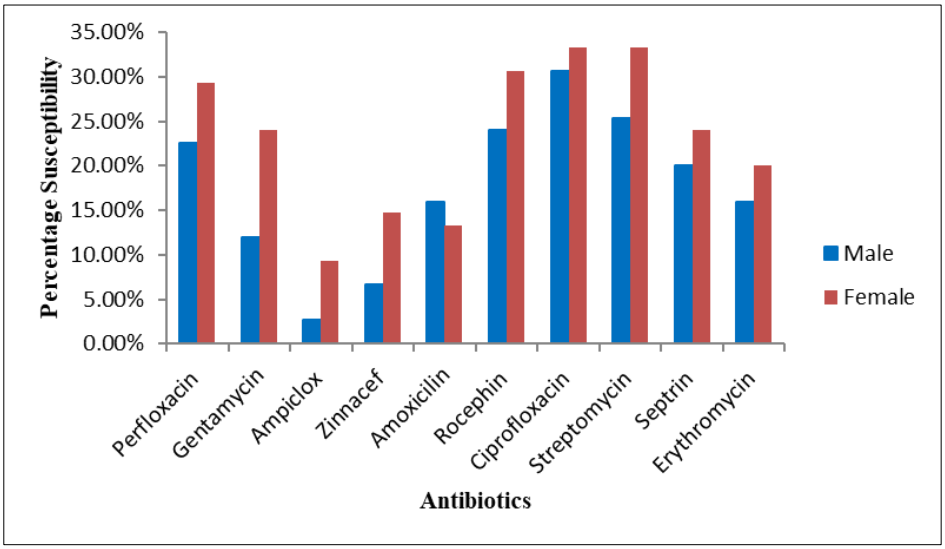


Figure 2 Antibiotic susceptibility test of 75 *Staphylococcus aureus* isolates in relation to gender

Figure 20 showed the *lasI v*irulent gene cluster in 75 *S. aureus* isolates genomes, Figure 21 showed the PCR detection of *rhlR* virulent gene at 1120 base pairs in 75 *S. aureus* isolates genomic DNA, Figure 22 showed *rhlR v*irulent gene cluster in 75 *S. aureus* isolates genomes, Figure 23 showed the *rhlR* and *lasI* virulent genes consensus cluster in 75 *S. aureus* isolates genomes and Figure 24 showed the Morphology and *rhlR, and lasI* virulent genes consensus cluster in 75 *S. aureus* isolates genomes.

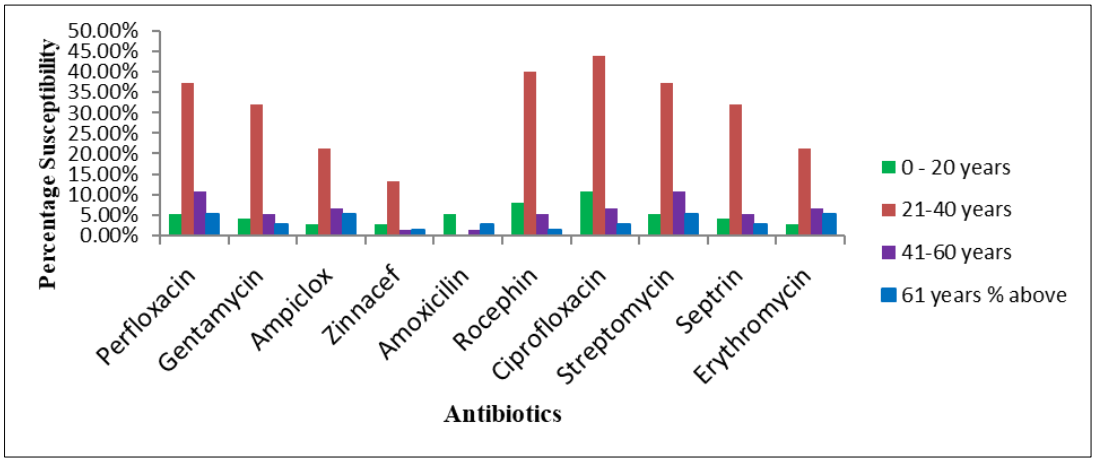


Figure 3 Antibiotic susceptibility test of 75 *Staphylococcus aureus* isolates in relation to age

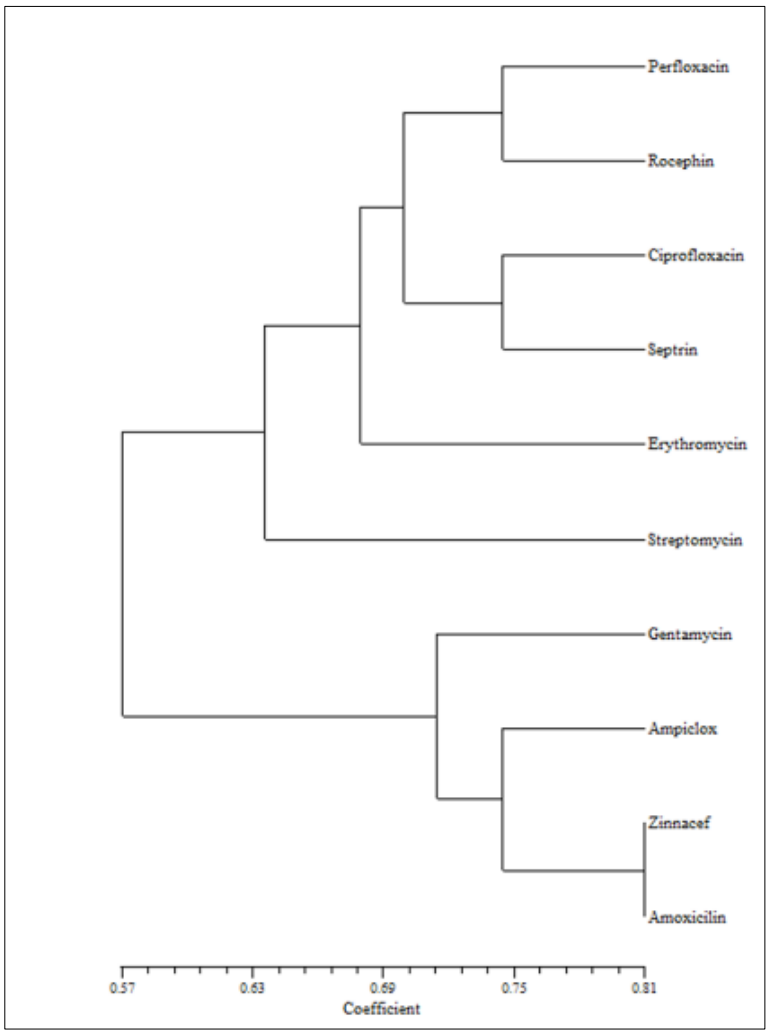


Figure 4 Cluster showing antibiotic response to 75 *S. aureus* isolates

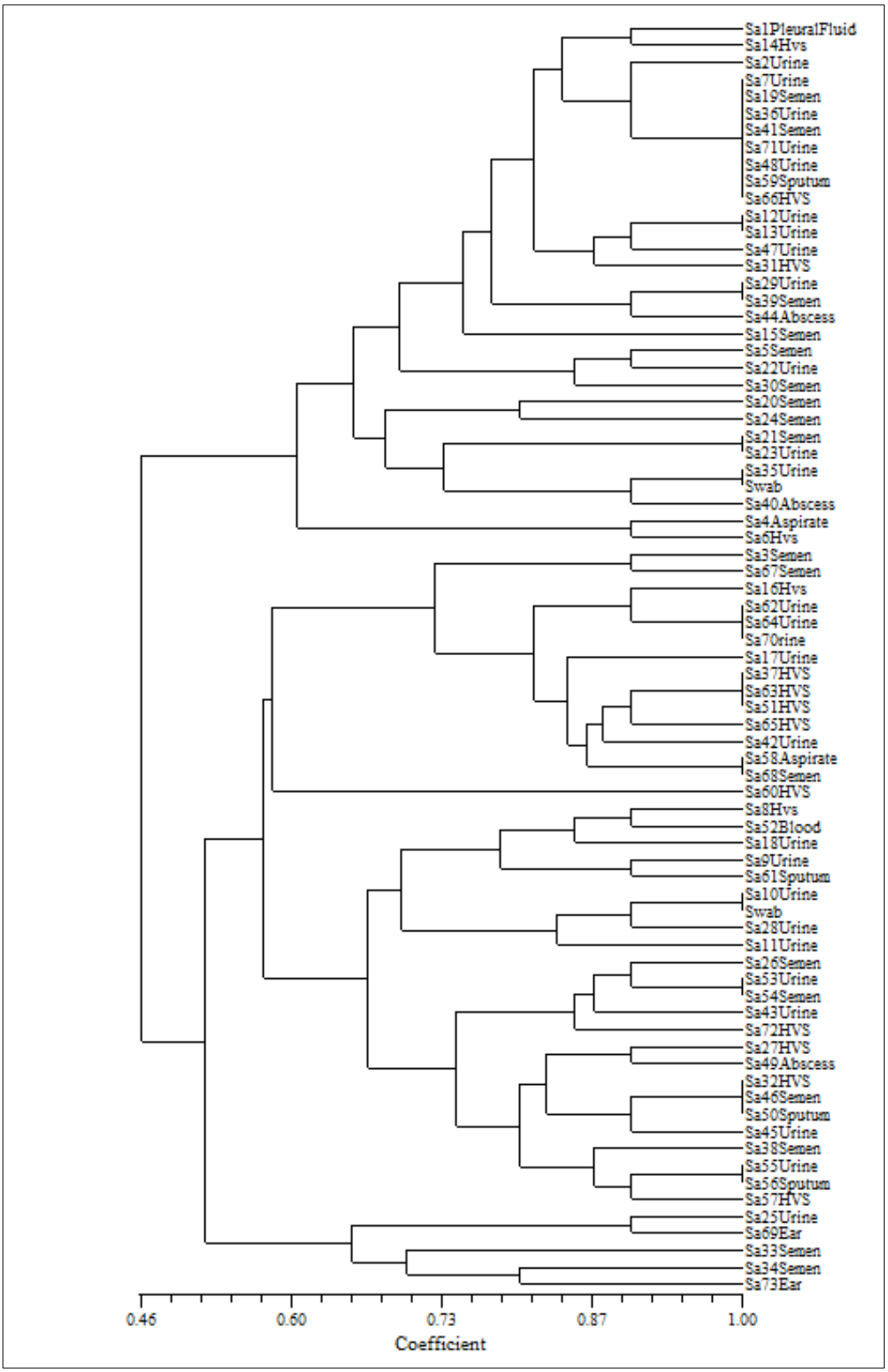


Figure 5 Cluster showing 75 *S. aureus* grouping based on reactions with 10 commonly used antibiotics

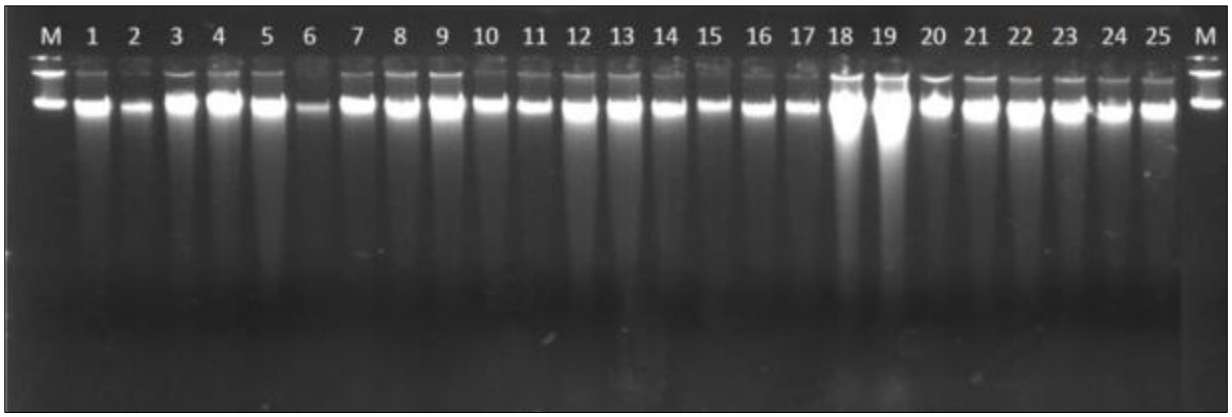


Figure 6a *S. aureus* genomic DNA agarose gel electrophoretic pattern

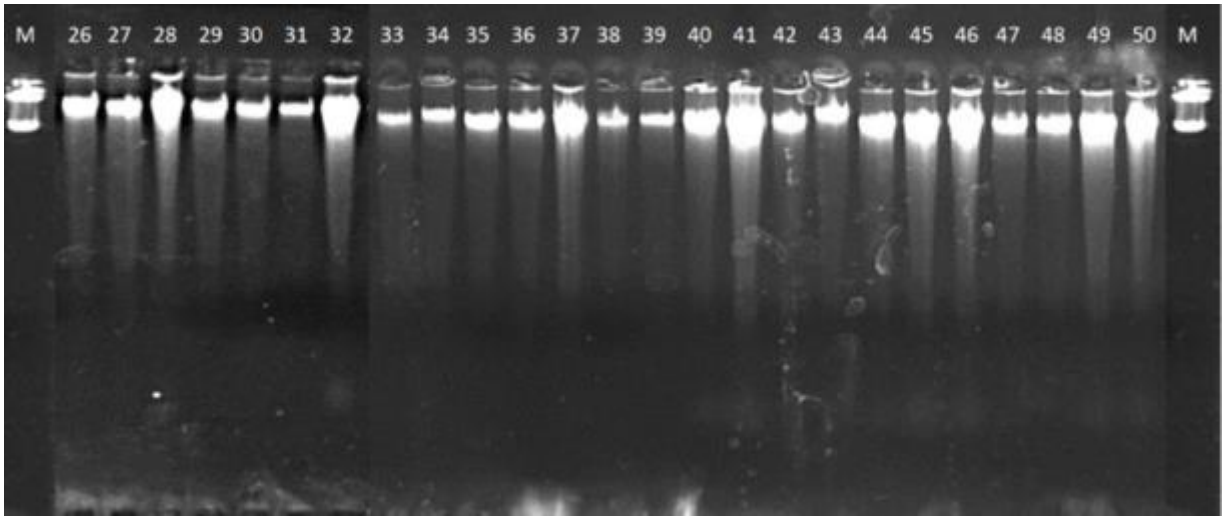


Figure 6b *S. aureus* genomic DNA agarose gel electrophoretic pattern

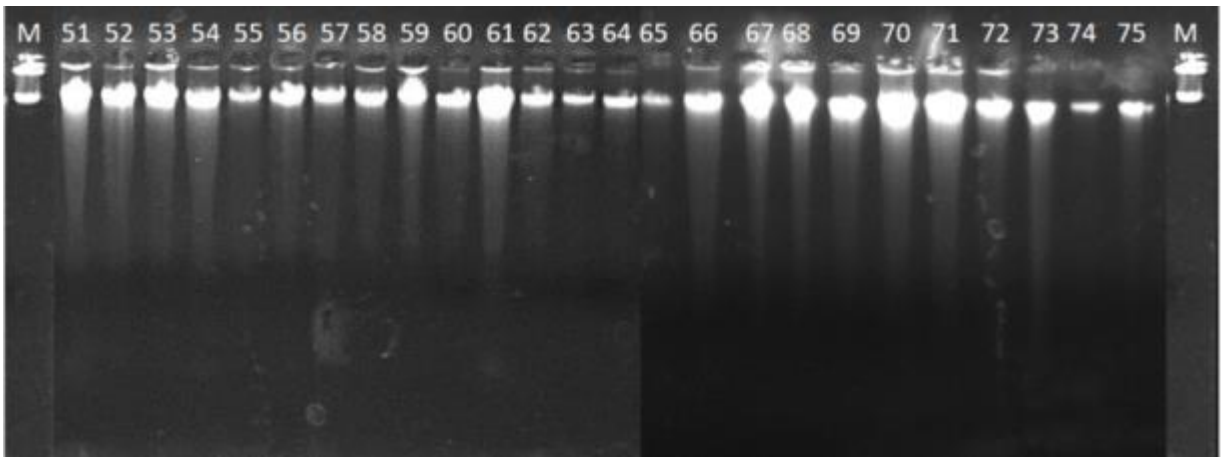


Figure 6c *S. aureus* genomic DNA agarose gel electrophoretic pattern

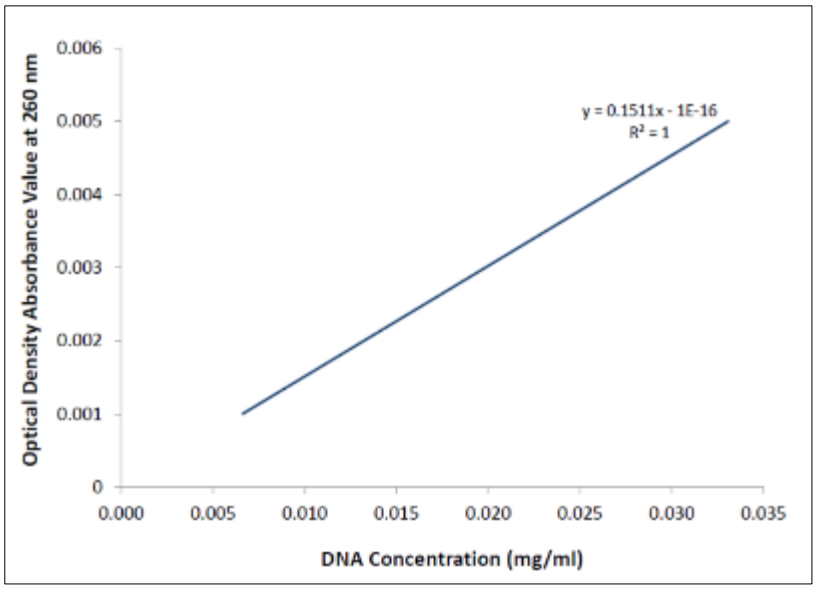


Figure 7 DNA standard curve using Sigma DNA Ladder. Standard linear curve equation: y = 0.1511x – 1E-16; R: Regression value.**=Highly significant (P≤0.01)

Table 2a Genomic DNA concentration and purity of genomic DNA from 75 *S. aureus* isolates

S/N	Isolate code	Genomic DNA Concentration (mg/ml)	DNA Purity Index OD260/OD280 (1.5-2)
1	Sa1	4.9	1.6
2	Sa2	2.6	1.9
3	Sa3	2.1	1.8
4	Sa4	2.1	1.8
5	Sa5	2.7	1.9
6	Sa6	2.9	1.9
7	Sa7	4.7	1.9
8	Sa8	1.8	1.9
9	Sa9	5.2	1.9
10	Sa10	2.2	1.8
11	Sa11	2.8	1.8
12	Sa12	3.5	1.9
13	Sa13	3.7	1.9
14	Sa14	3.7	1.9
15	Sa15	3.6	1.9
16	Sa16	2.7	1.9
17	Sa17	3.0	1.9
18	Sa18	2.8	1.9
19	Sa19	3.2	1.8
20	Sa20	2.8	1.8

21	Sa21	0.6	1.8
22	Sa22	2.8	1.9
23	Sa23	3.4	1.9
24	Sa24	1.6	1.8
25	Sa25	3.0	1.9
26	Sa26	2.9	1.9
27	Sa27	2.7	1.8
28	Sa28	2.2	1.8
29	Sa29	2.0	1.8
30	Sa30	3.1	1.9
31	Sa31	3.9	1.9
32	Sa32	2.4	1.8
33	Sa33	2.1	1.9
34	Sa34	3.5	1.9
35	Sa35	1.9	1.7
36	Sa36	4.4	1.9

Table 2b Genomic DNA concentration and purity of genomic DNA from 75 *S. aureus* isolates

+++	**Isolate code**	**Genomic DNA Concentration (mg/ml)**	**DNA Purity Index OD260/OD280 (1.5-2)**
37	Sa37	0.8	1.8
38	Sa38	3.3	1.8
39	Sa39	3.1	1.8
40	Sa40	1.3	1.8
41	Sa41	3.3	1.9
42	Sa42	4.2	1.9
43	Sa43	2.4	1.8
44	Sa44	4.5	1.8
45	Sa45	2.3	1.8
46	Sa46	3.5	1.9
47	Sa47	3.0	1.9
48	Sa48	2.9	1.9
49	Sa49	3.1	1.9
50	Sa50	1.6	1.8
51	Sa51	2.8	1.8
52	Sa52	7.8	1.8

53	Sa53	2.8	1.8
54	Sa54	2.5	1.8
55	Sa55	2.7	1.9
56	Sa56	3.2	1.9
57	Sa57	5.3	1.9
58	Sa58	2.1	1.8
59	Sa59	3.5	1.9
60	Sa60	5.1	1.9
61	Sa61	2.8	1.9
62	Sa62	3.7	1.9
63	Sa63	2.5	1.8
64	Sa64	4.0	1.9
65	Sa65	1.2	1.8
66	Sa66	1.8	1.8
67	Sa67	1.9	1.7
68	Sa68	2.3	1.8
69	Sa69	3.3	1.9
70	Sa70	1.3	1.8
71	Sa71	2.0	1.8
72	Sa72	1.6	1.8
73	Sa73	1.9	1.8
74	Sa74	2.9	1.8
75	Sa75	2.4	1.8

Table 3 Identity of RAPD primers used for preliminary PCR amplification and discriminate screening

S/N	Primer Name	Sequence
1	OPT01	5' – GGG CCA CTC A – 3'
2	OPH01	5' – GGT CGG ACA A – 3'
3	OPT02	5' – GGA GAG ACT C – 3'
4	OPH02	5' – TCG GAC GTG A – 3'
5	OPT03	5' – TCC ACT CCT G – 3'
6	OPH03	5' – AGA CGT CCA C – 3'
7	OPT04	5' – CAC AGA GGG A – 3'
8	OPH04	5' – GGA AGT CGC C – 3'
9	OPT05	5' – GGG TTT GGC A – 3'
10	OPH05	5' – AGT CGT CCC C – 3'
11	OPT06	5' – CAA GGG CAG A – 3'

12	OPH06	5' – ACG CAT CGC A – 3'
13	OPT07	5' – GGC AGG CTG T – 3'
14	OPH07	5' – CTG CAT CGT G – 3'
15	OPT08	5' – AAC GGC GAC A – 3'

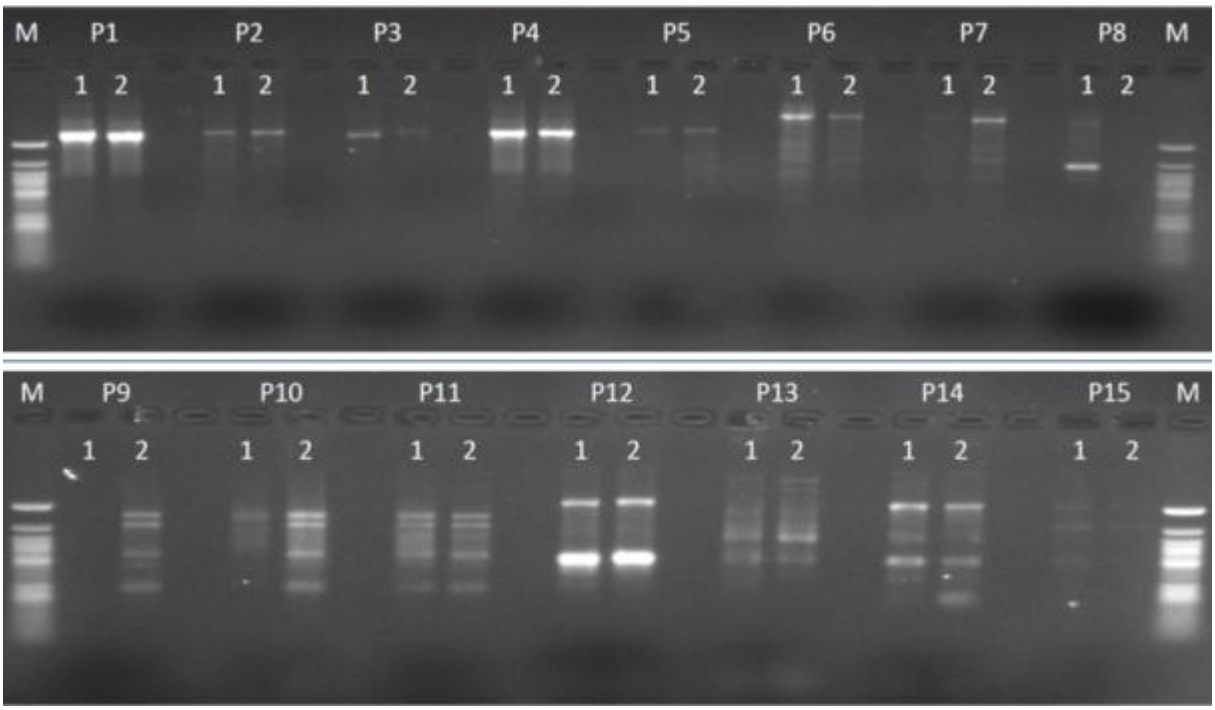


Figure 8 PCR Profile of fifteen RAPD primers used to screen pooled *S. aureus* genomic DNA

Table 4 Identity of selected RAPD primers used for PCR analysis of all 75 *S aureus* genomic DNA

S/N	Primer Name	Sequence
1	OPT06	5' – CAA GGG CAG A – 3'
2	OPH06	5' – ACG CAT CGC A – 3'
3	OPT07	5' – GGC AGG CTG T – 3'
4	OPH07	5' – CTG CAT CGT G – 3'

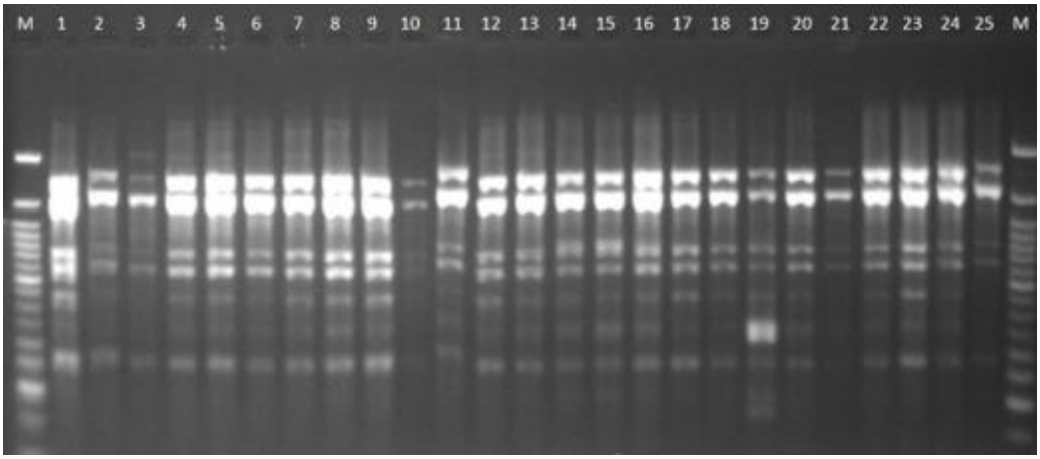


Figure 9a DNA fingerprint of 75 *S. aureus* genomic DNA as revealed by OPT06-PCR analysis

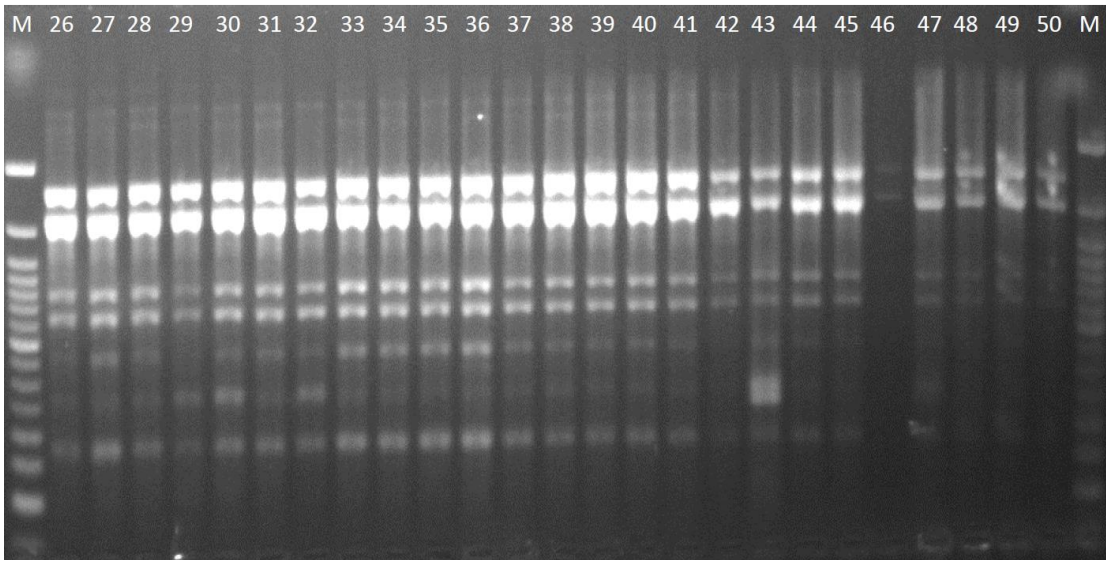


Figure 9b DNA fingerprint of 75 *S. aureus* genomic DNA as revealed by OPT06-PCR analysis

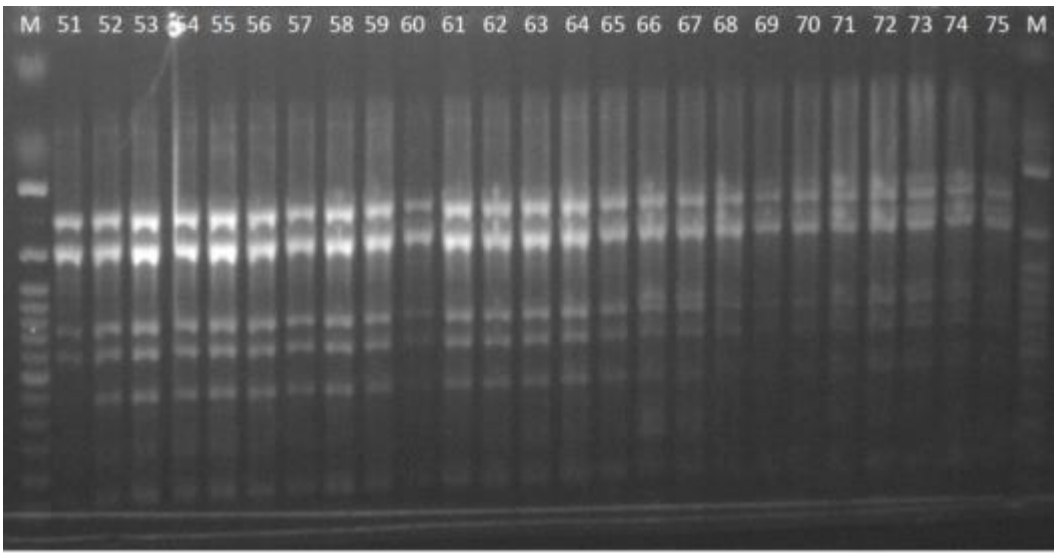


Figure 9c DNA fingerprint of 75 *S. aureus* genomic DNA as revealed by OPT06-PCR analysis

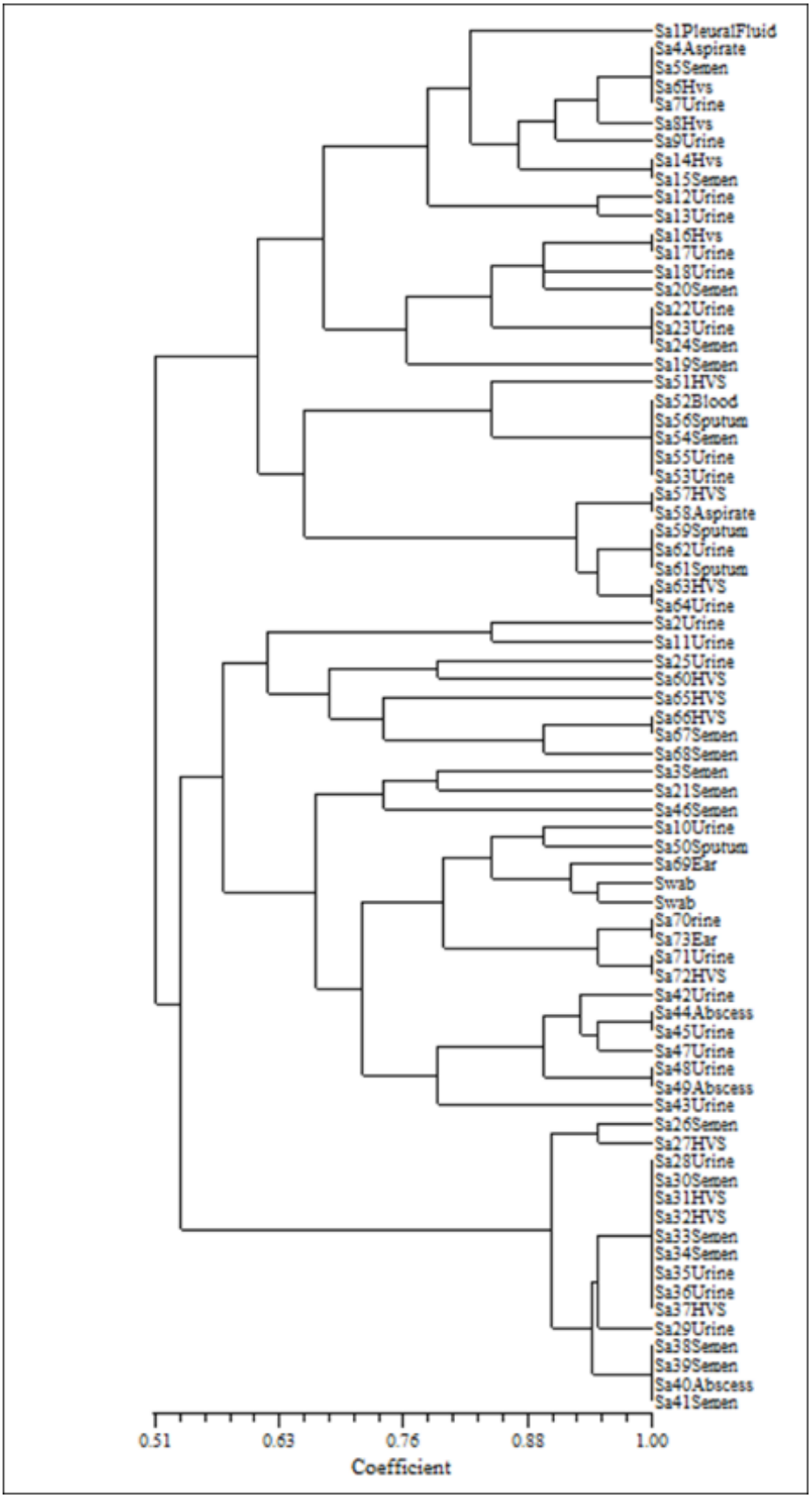


Figure 10 Cluster showing genetic diversity among 75 *S. aureus* isolates amplified DNA

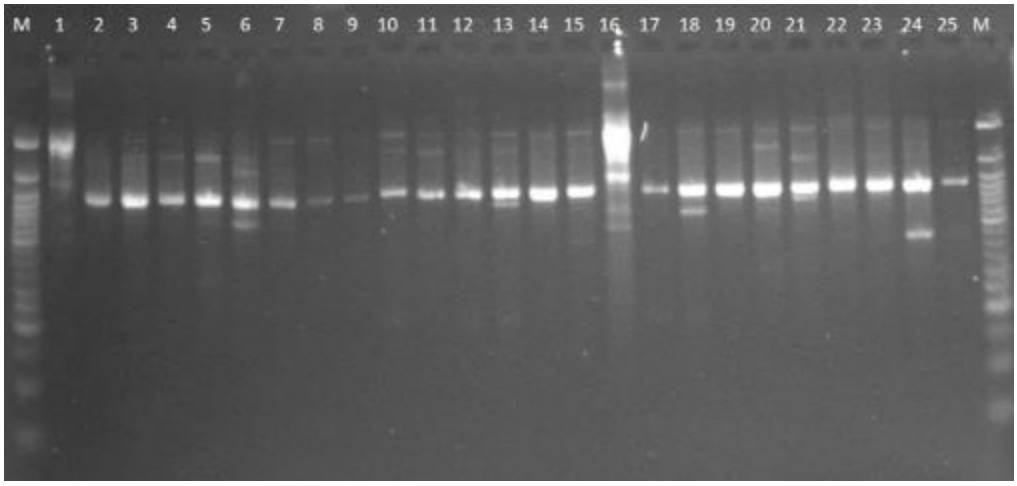


Figure 11a DNA fingerprint of 75 *S. aureus* genomic DNA as revealed by OPH06-PCR analysis

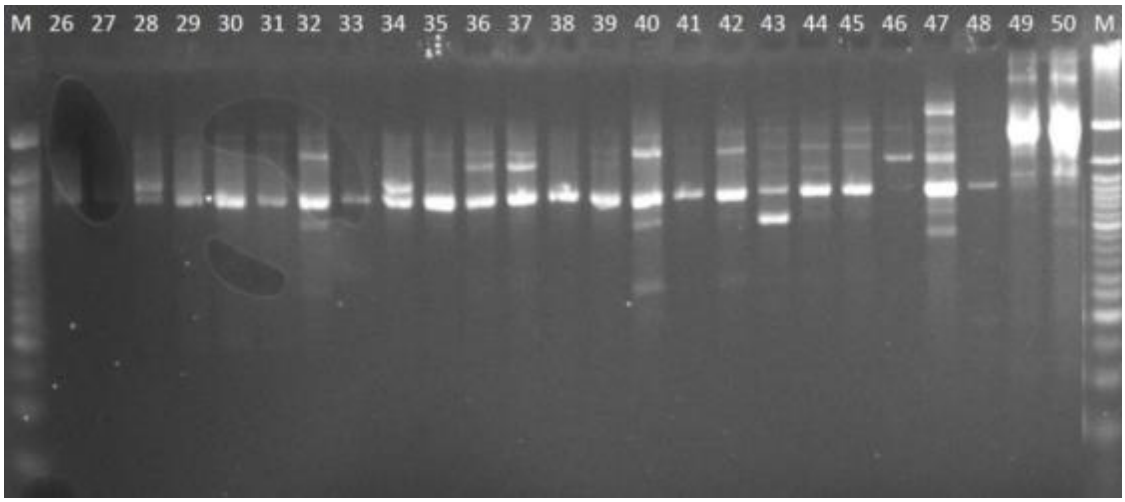


Figure 11b DNA fingerprint of 75 *S. aureus* genomic DNA as revealed by OPH06-PCR analysis

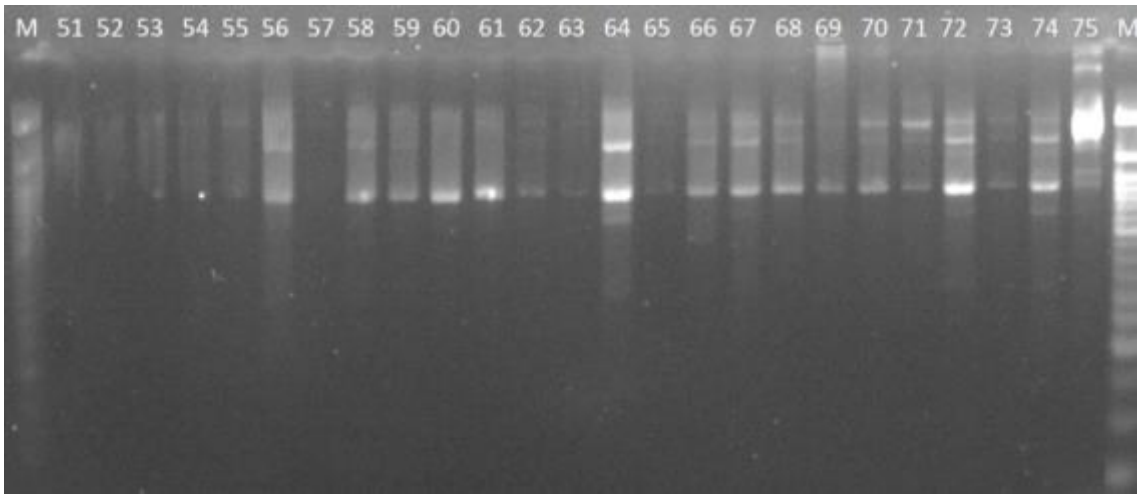


Figure 11c DNA fingerprint of 75 *S. aureus* genomic DNA as revealed by OPH06 RAPD-PCR analysis

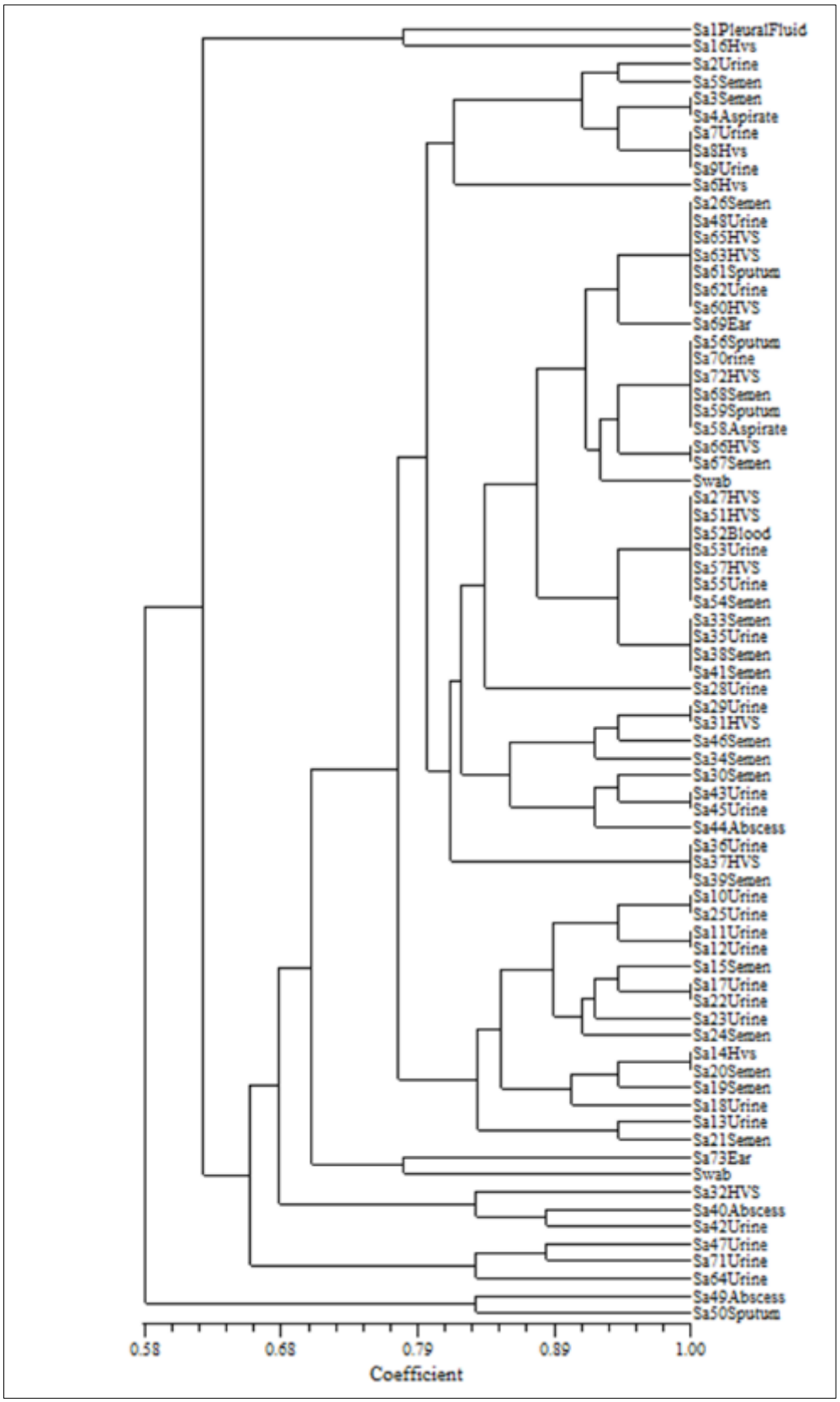


Figure 12 Cluster showing genetic diversity among 75 *S. aureus* isolates amplified DNA

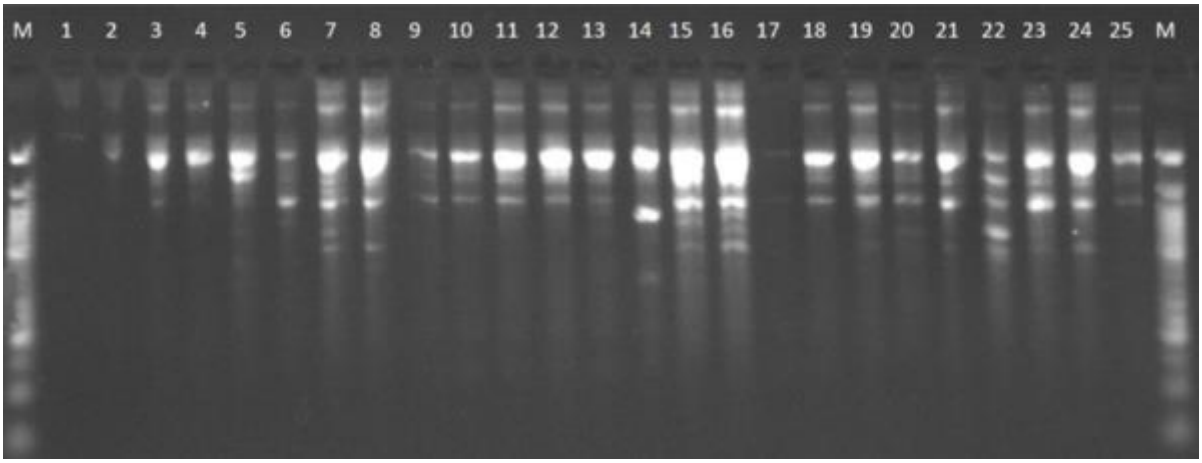


Figure 13a DNA fingerprint of 75 *S. aureus* genomic DNA as revealed by OPT07-PCR analysis

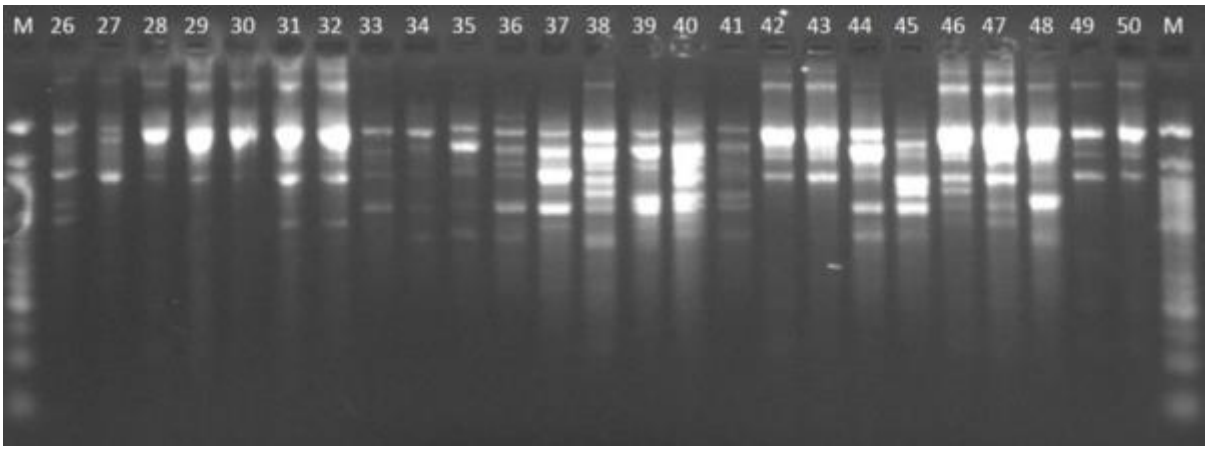


Figure 13b DNA fingerprint of 75 *S. aureus* genomic DNA as revealed by OPT07-PCR analysis

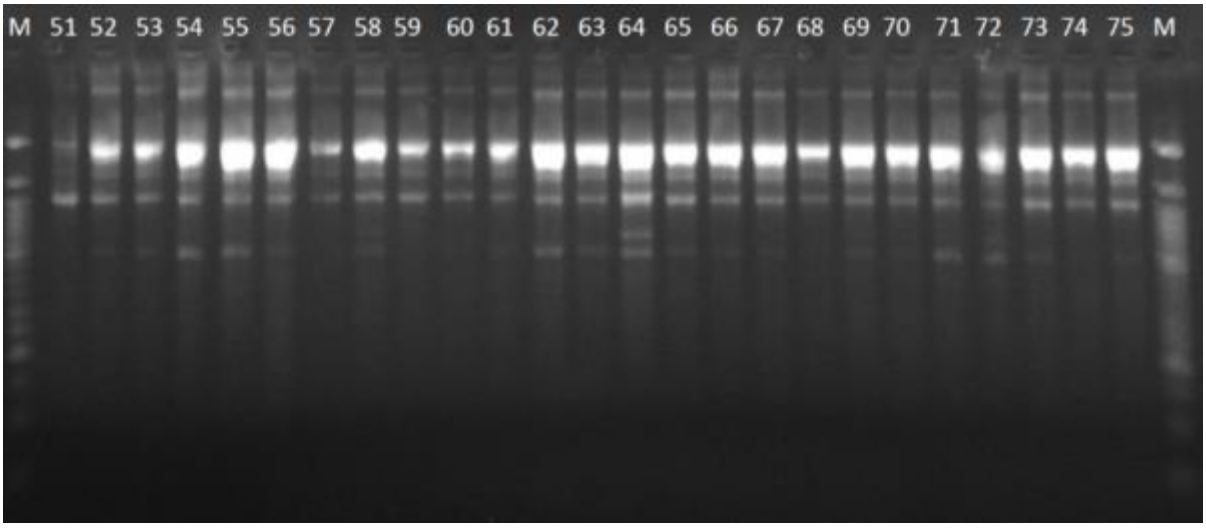


Figure 13c DNA fingerprint of 75 *S. aureus* genomic DNA as revealed by OPT07-PCR analysis

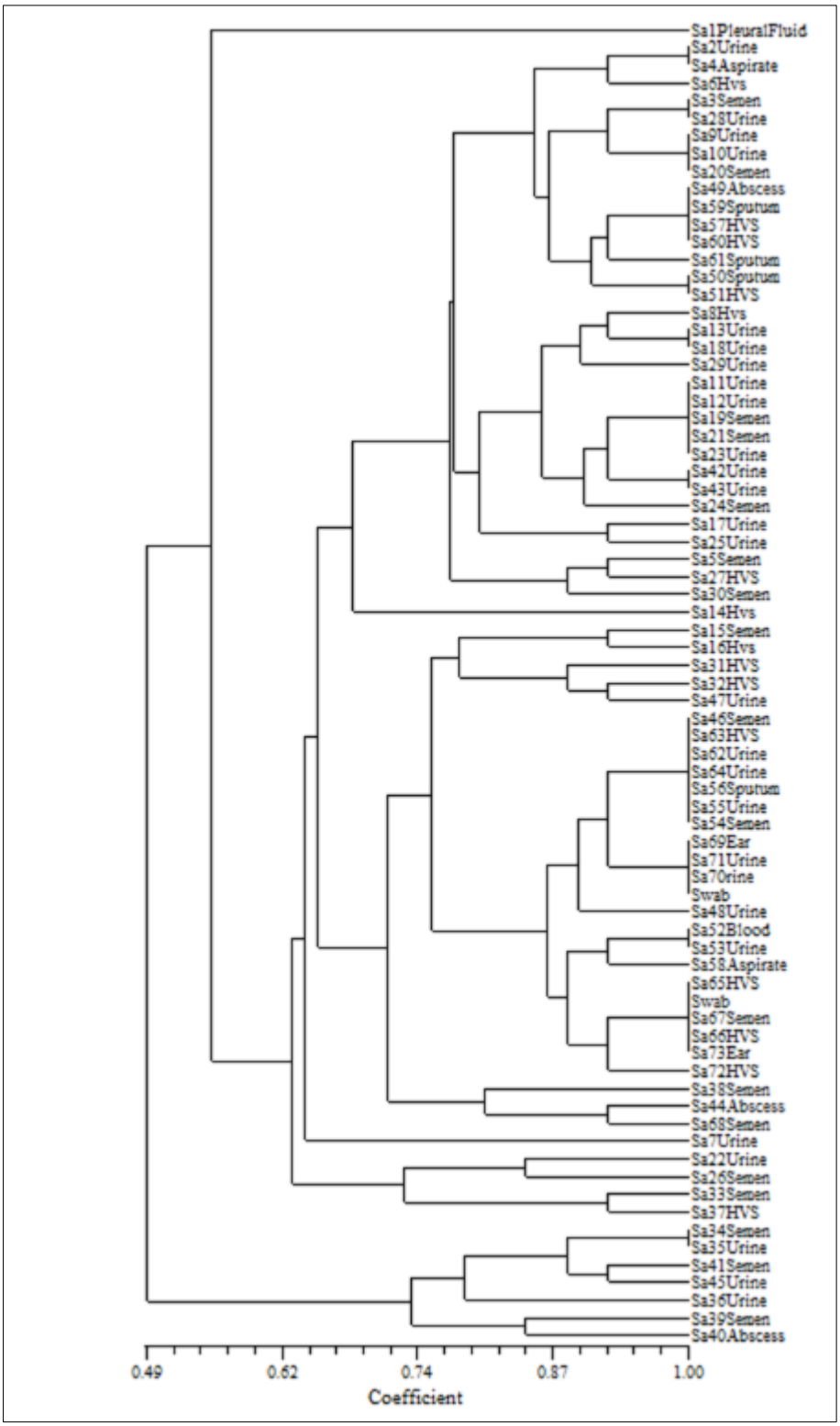


Figure 14 Cluster showing genetic diversity among 75 *S. aureus* isolates amplified DNA OPT07 PCR-RAPD products analyzed by electrophoresis

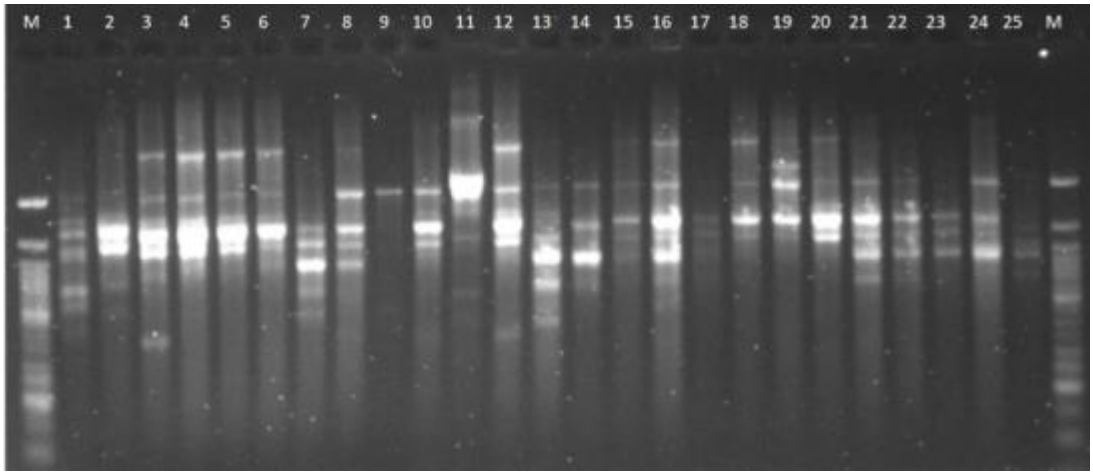


Figure 15a DNA fingerprint of 75 *S. aureus* genomic DNA as revealed by OPH07-PCR analysis

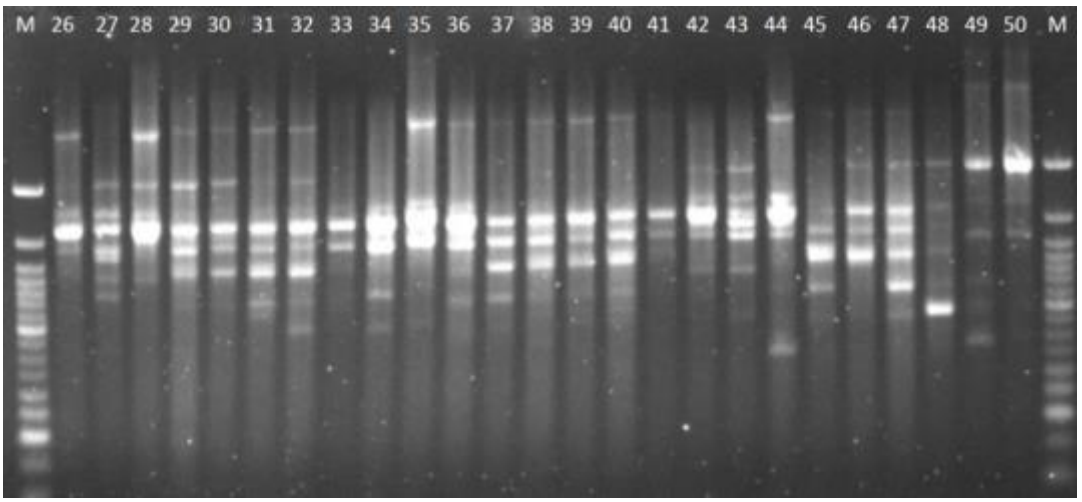


Figure 15b DNA fingerprint of 75 *S. aureus* genomic DNA as revealed by OPH07-PCR analysis

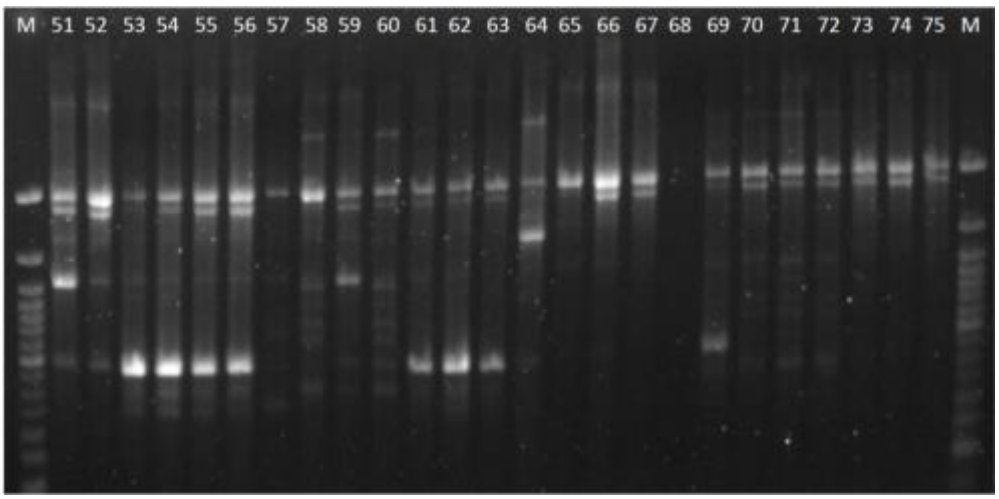


Figure 15c DNA fingerprint of 75 *S. aureus* genomic DNA as revealed by OPH07-PCR analysis

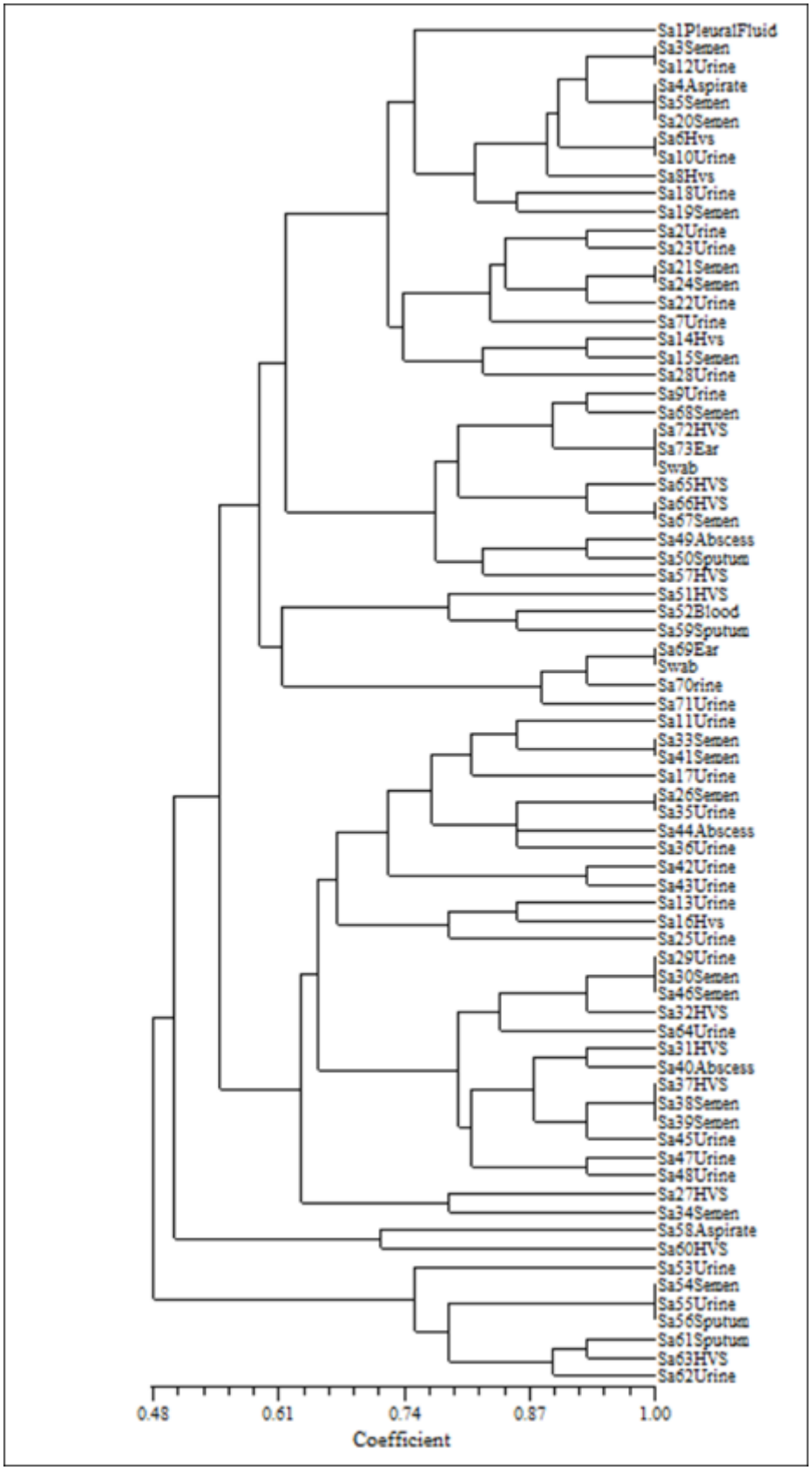


Figure 16 Cluster showing genetic diversity among 75 *S. aureus* isolates amplified DNA OPH07 PCR-RAPD products analyzed by electrophoresis

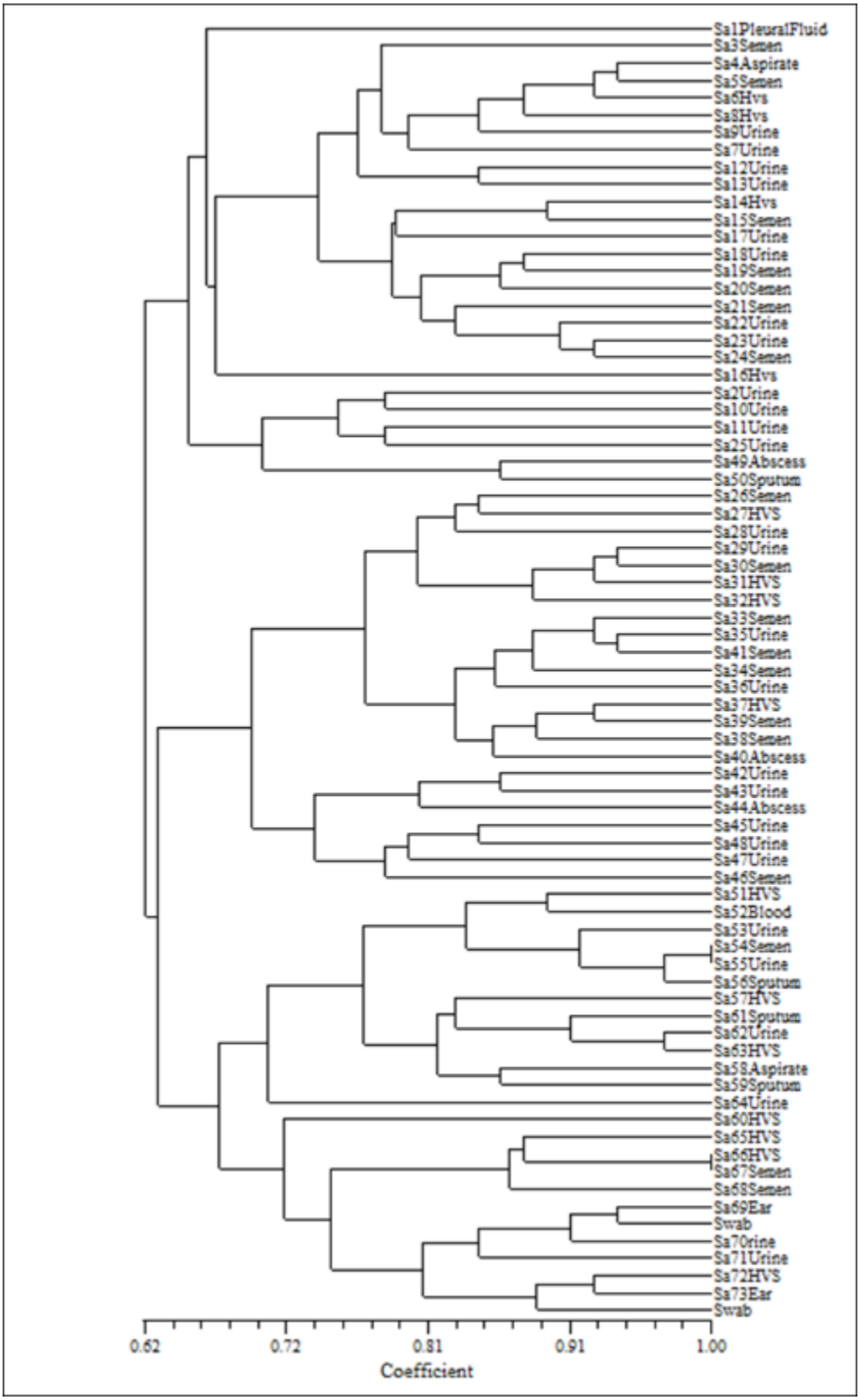


Figure 17 Cluster showing genetic diversity among 75 *S. aureus* isolates as revealed by 64

Table 5 Identity of virulence target gene specific primers used for preliminary PCR amplification and screening of *S. aureus* isolates genomic DNA

S/N	Primer Set	Virulence Target Gene	Sequence (5'- 3')	Product Size (bp)
1	P1	*16S rRNA*	TGGCATAAGAGTGAAAGGCGC	290
2			GGGGACGTTCAGTTACTAACGT	
3	P2	*Esp*	TTGCTAATGCTAGTCCACGACC	932
4			GCGTCAACACTTGCATTGCCGA	
5	P3	*gelE*	ACCCCGTATCATTGGTTT	405
6			ACGCATTGCTTTTCCATC	
7	P4	*cylA*	GACTCGGGGATTGATAGGC	688
8			GCTGCTAAAGCTGCGCTTAC	
9	P5	*asaI*	CCAGCCAACTATGGCGGAATC	529
10			CCTGTCGCAAGATCGACTGTA	
11	P6	*Ace*	GGAATGACCGAGAACGATGGC	616
12			GCTTGATGTTGGCCTGCTTCCG	
13	P7	*lasI*	CGTGCTCAAGTGTTCAAGG	295
14			TACAGTCGGAAAAGCCCAG	
15	P8	*lasR*	AAGTGGAAAATTGGAGTGGAG	130
16			GTAGTTGCCGACGACGATGAAG	
17	P9	*rhlI*	TTCATCCTCCTTTAGTCTTCCC	155
18			TTCCAGCGATTCAGAGAGC	
19	P10	*rhlR*	TGCATTTTATCGATCAGGGC	1120
20			CACTTCCTTTTCCAGGACG	
21	P11	*toxA*	GGAGCGCAACTATCCCACT	150
22			TGGTAGCCGACGAACACATA	
23	P12	*aprA*	GTCGACCAGGCGGCGGAGCAGATA	993
24			GCCGAGGCCGCCGTAGAGGATGTC	
25	P13	*rhlAB*	TCATGGAATTGTCACAACCGC	151
26			ATACGGCAAAATCATGGCAAC	
27	P14	*plcH*	GAAGCCATGGGCTACTTCAA	307
28			AGAGTGACGAGGAGCGGTAG	
29	P15	*lasB*	TTCTACCCGAAGGACTGATAC	153
30			AACACCCATGATCGCAAC	
31	P16	*fliC*	GGCAGCTGGTTNGCCTG	1250
32			GGCCTGCAGATCNCCAA	

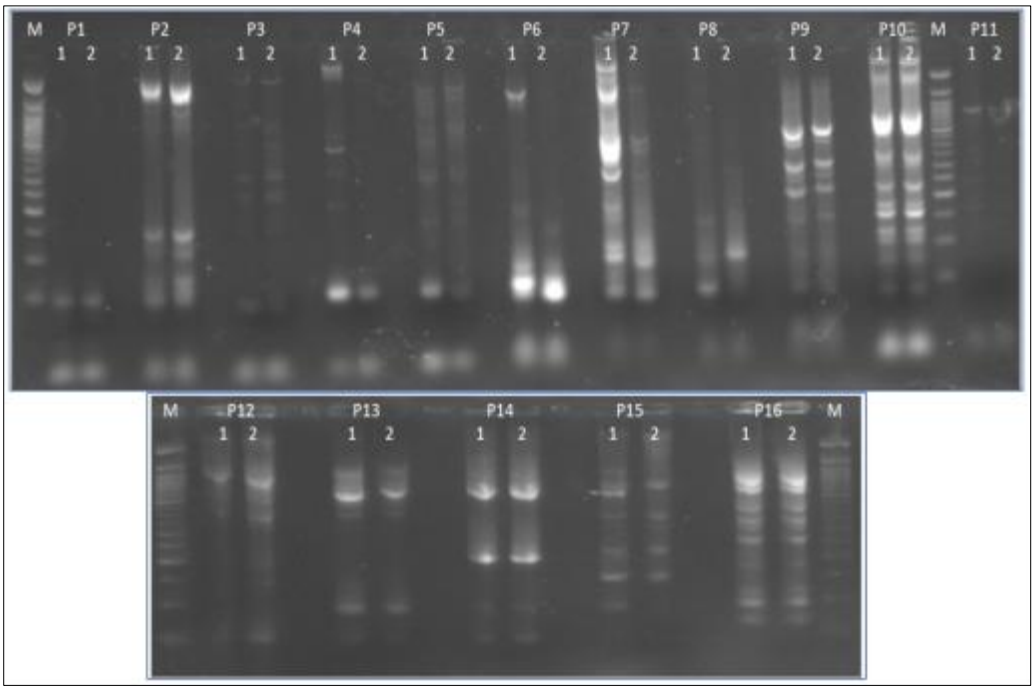
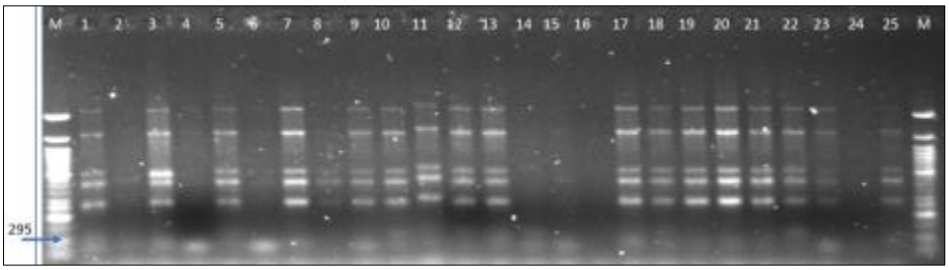
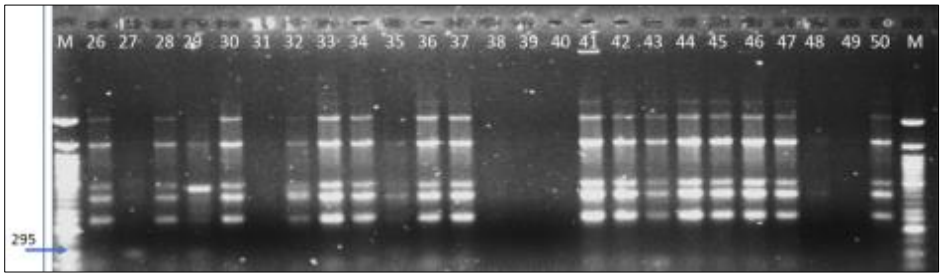


Figure 18 PCR Profile virulence target gene detection by 16 specific primers used for preliminary PCR amplification and screening of *S. aureus* isolates genomic DNA

Table 6 Identity of selected virulence target gene specific primers used for PCR analysis detection of virulence target gene in all the 75 *S. aureus* isolates genomic DNA

Primer Set	Virulence Target Gene	Sequence (5'- 3')	Product Size (bp)
P7	lasI	CGTGCTCAAGTGTTCAAGG	295
		TACAGTCGGAAAAGCCCAG	
P10	rhlR	TGCATTTTATCGATCAGGGC	1120
		CACTTCCTTTTCCAGGACG	





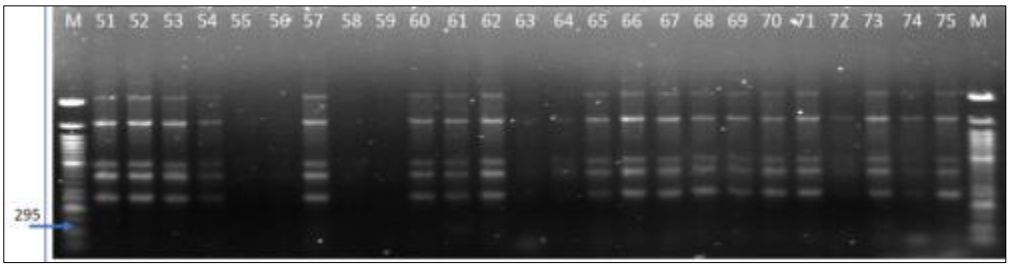


Figure 19 PCR detection of *lasI* virulent gene at 295 base pairs in 75 *S. aureus* isolates genomic DNA

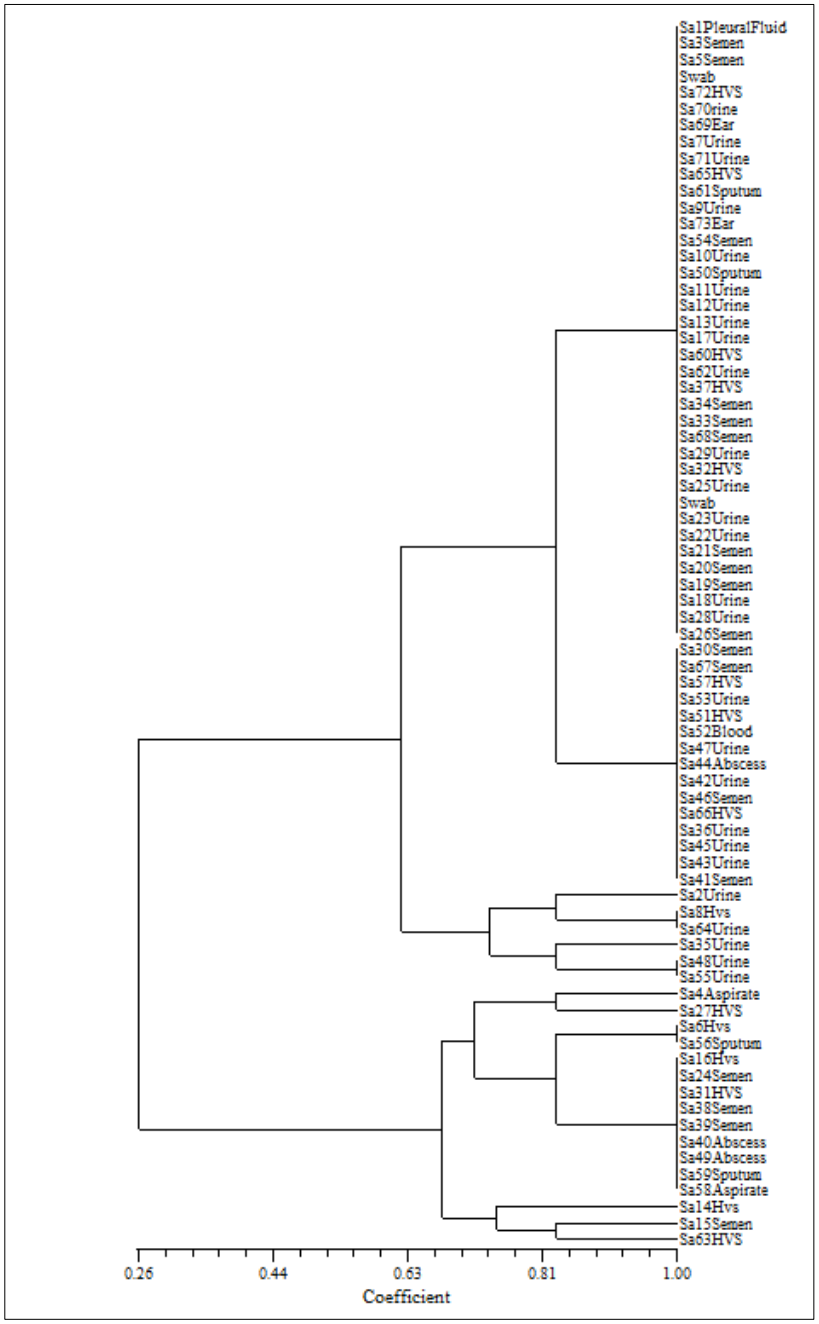


Figure 20 *lasI* virulent gene cluster in 75 *S. aureus* isolates genomes

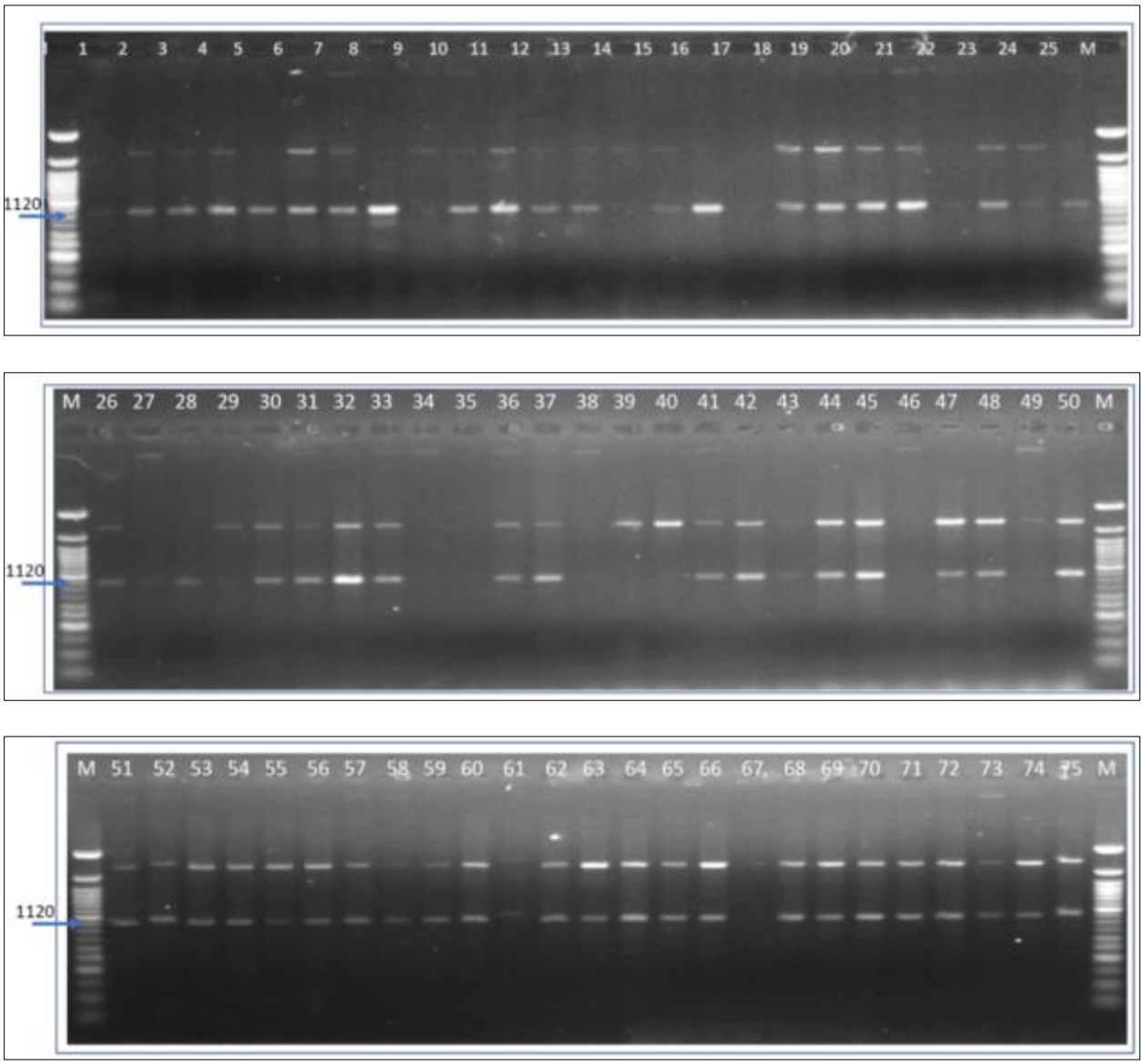


Figure 21 PCR detection of *rhlR* virulent gene at 1120 base pairs in 75 *S. aureus* isolates genomic DNA

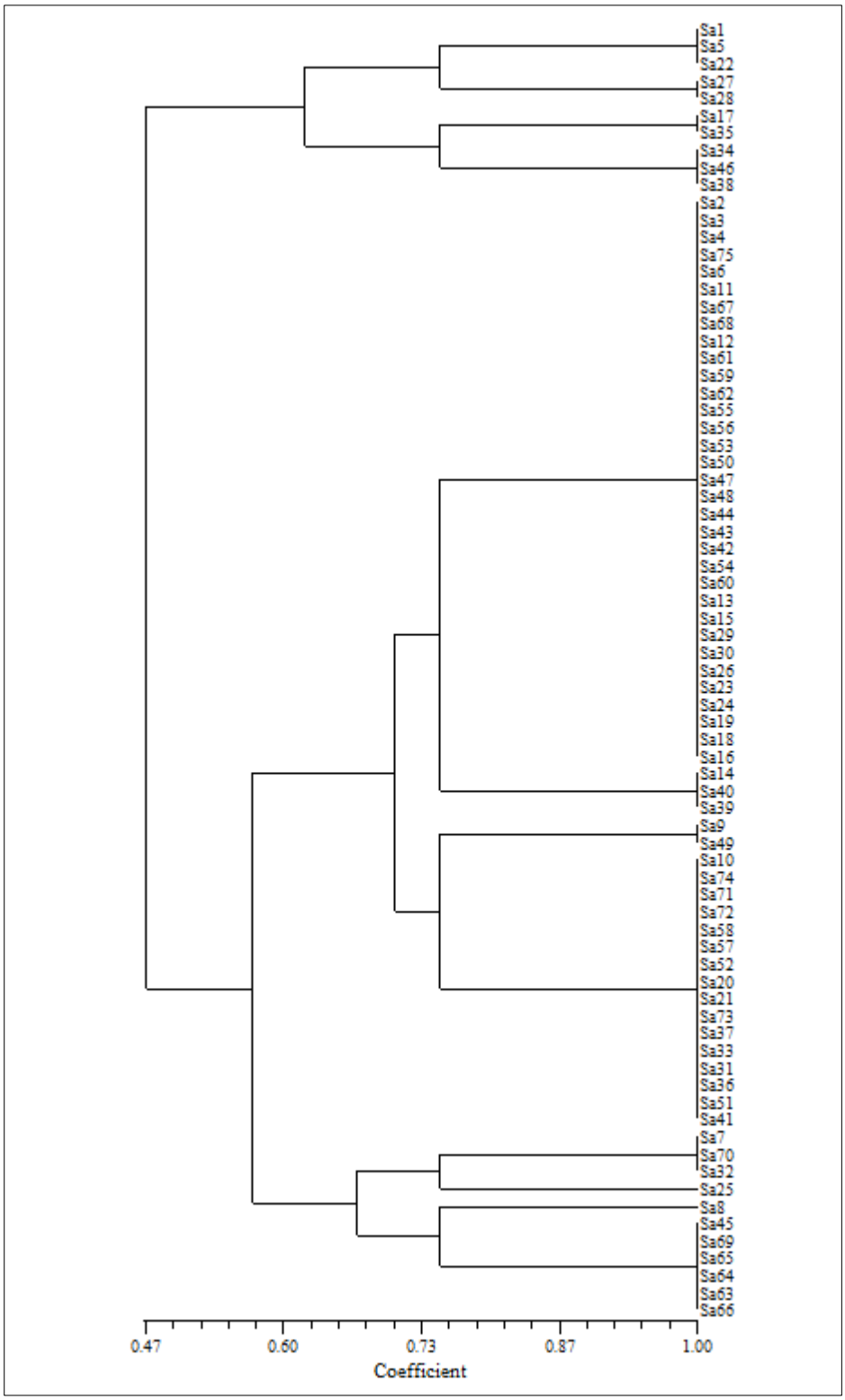


Figure 22 *rhlR v*irulent gene cluster in 75 *S. aureus* isolates genomes

Table 7a Virulent gene *rhlR* and *lasI* distribution and status in 75 *S. aureus* isolates.

S/N	Isolate Code	Virulent Gene Distribution		Virulent Status (100%)
		***rhlR* (68%)**	***lasI* (68%)**	
1	Sa1PleuralFluid	+	-	+
2	Sa2Urine	+	-	+
3	Sa3Semen	+	-	+
4	Sa4Aspirate	+	+	+
5	Sa5Semen	+	+	+
6	Sa6Hvs	+	+	+
7	Sa7Urine	+	+	+
8	Sa8Hvs	+	+	+
9	Sa9Urine	+	+	+
10	Sa10Urine	+	-	+
11	Sa11Urine	+	-	+
12	Sa12Urine	+	-	+
13	Sa13Urine	+	-	+
14	Sa14Hvs	+	+	+
15	Sa15Semen	+	+	+
16	Sa16Hvs	-	+	+
17	Sa17Urine	-	+	+
18	Sa18Urine	+	+	+
19	Sa19Semen	+	+	+
20	Sa20Semen	+	-	+
21	Sa21Semen	+	-	+
22	Sa22Urine	+	+	+
23	Sa23Urine	-	+	+
24	Sa24Semen	-	+	+
25	Sa25Urine	-	-	+
26	Sa26Semen	+	-	+
27	Sa27HVS	+	-	+
28	Sa28Urine	+	-	+
29	Sa29Urine	+	-	+
30	Sa30Semen	+	+	+
31	Sa31HVS	+	+	+
32	Sa32HVS	+	+	+
33	Sa33Semen	-	+	+
34	Sa34Semen	-	+	+
35	Sa35Urine	-	+	+
36	Sa36Urine	+	+	+
37	Sa37HVS	+	+	+

38	Sa38Semen	+	+	+
39	Sa39Semen	+	+	+
40	Sa40Abscess	+	-	+

Table 7b Virulence gene distribution and status in 75 *S. aureus* isolates

S/N	Isolate Code	Virulence Gene Distribution		Virulence Status (100%)
		***rhlR* (68%)**	***lasI* (68%)**	
41	Sa41Semen	+	-	+
42	Sa42Urine	+	+	+
43	Sa43Urine	-	+	+
44	Sa44Abscess	-	+	+
45	Sa45Urine	+	+	+
46	Sa46Semen	+	+	+
47	Sa47Urine	-	+	+
48	Sa48Urine	+	+	+
49	Sa49Abscess	+	+	+
50	Sa50Sputum	-	+	+
51	Sa51HVS	-	+	+
52	Sa52Blood	+	+	+
53	Sa53Urine	+	-	+
54	Sa54Semen	+	-	+
55	Sa55Urine	+	+	+
56	Sa56Sputum	-	+	+
57	Sa57HVS	-	+	+
58	Sa58Aspirate	-	+	+
59	Sa59Sputum	-	+	+
60	Sa60HVS	+	+	+
61	Sa61Sputum	+	+	+
62	Sa62Urine	-	+	+
63	Sa63HVS	-	+	+
64	Sa64Urine	-	-	+
65	Sa65HVS	-	-	+
66	Sa66HVS	-	+	+
67	Sa67Semen	+	+	+
68	Sa68Semen	+	+	+
69	Sa69Ear Swab	+	+	+
70	Sa70rine	+	+	+
71	Sa71Urine	+	+	+
72	Sa72HVS	+	-	+

73	Sa73Ear Swab	-	-	+
74	Sa74HVS	-	+	+
75	Sa75Urine	+	+	+

Note: (+) Present, (-) Absent.

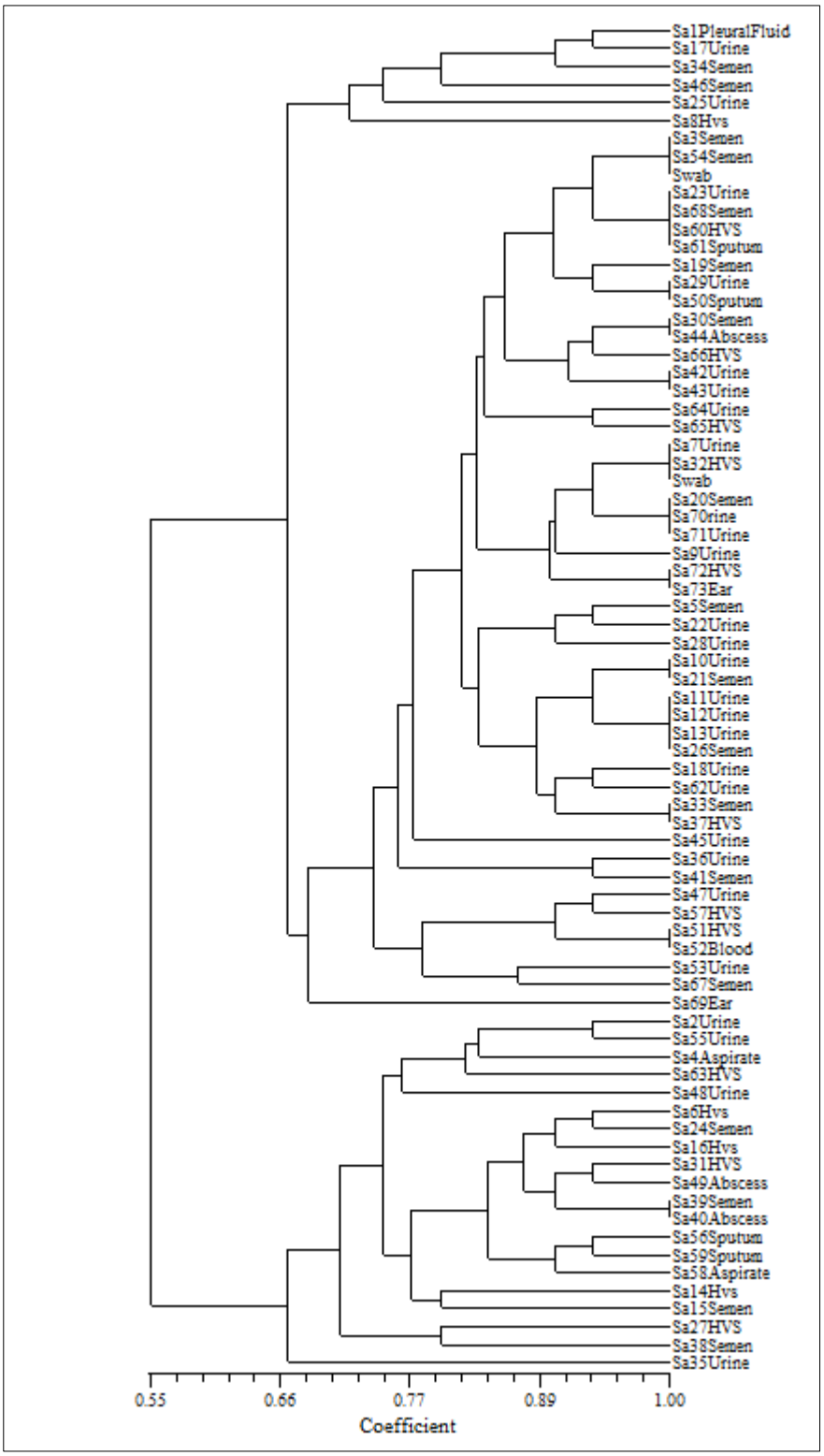
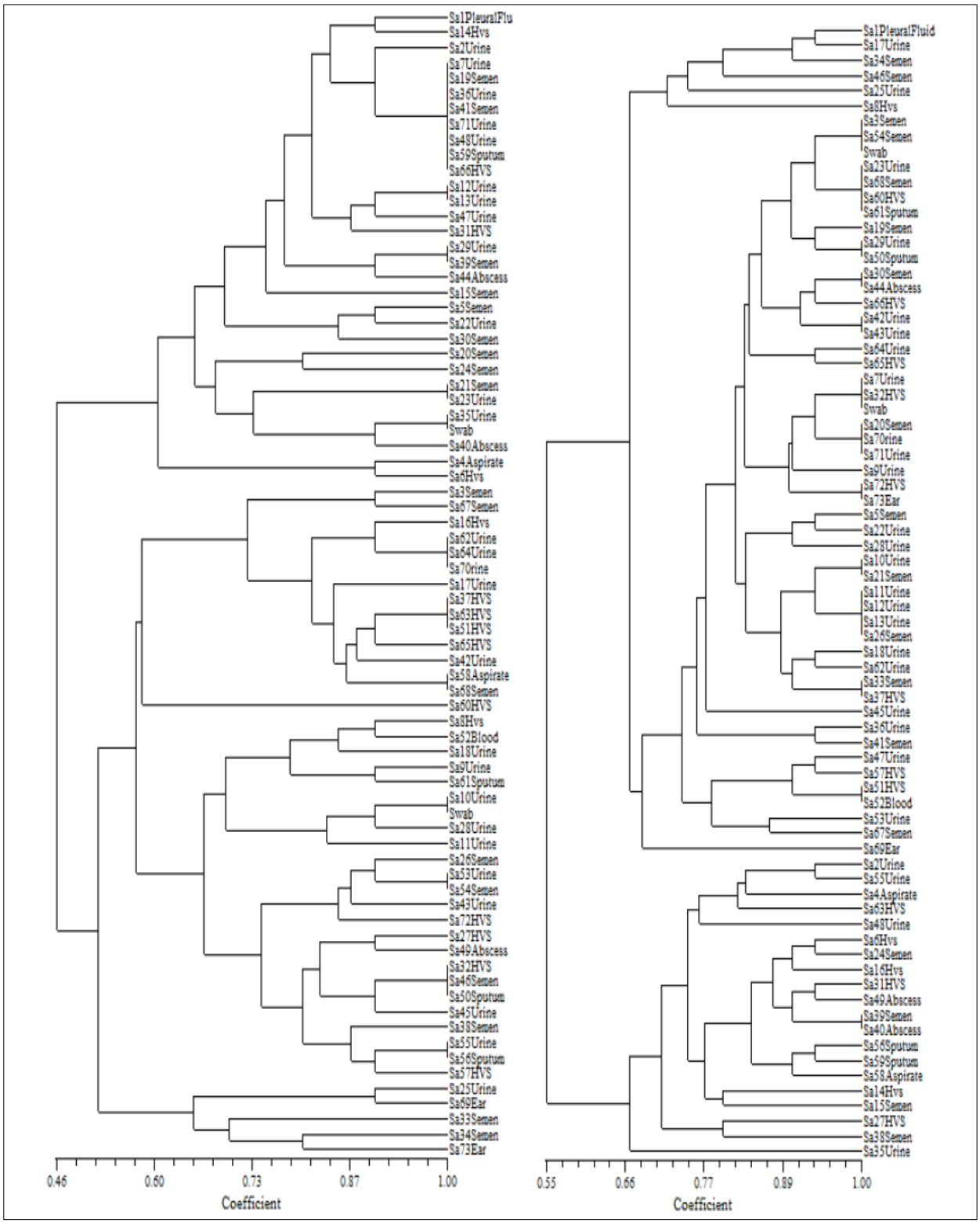


Figure 23 *rhlR* and *lasI* virulent genes consensus cluster in 75 *S. aureus* isolates genomes



Morphology ; B. rhlR, and lasI virulence genes consensus cluster

Figure 24 Morphology and *rhlR, and lasI* virulent genes consensus cluster in 75 *S. aureus* isolates genomes

4. Discussion

Staphylococcus aureus is a very prevalent source of infection in hospitals, and it is especially likely to infect newborns, people recovering from surgery, elderly people who are undernourished, people with diabetes, and people with other

chronic disorders[16]. This study was carried out to determine the distribution of *rhlR* and *lasI* virulent gene among *Staphylococcus aureus* isolates from clinical samples in Ekiti State University Teaching Hospital, Ado-Ekiti, Ekiti State, Nigeria. The highest frequency of isolates of *Staphylococcus aureus* (56%) in the present study was observed in the 21-40 years age group, followed by 0-20 years age group in which neonates and infants were included. This finding is similar to a previous study by Nwankwo and Nasiru[17]. Neonatal immunity is thought to be underdeveloped during this time, making newborns prone to bacterial illnesses and extremely contagious, especially when hospitalized.

From the study, male subjects were more infected with S. aureus (54.7%) compare with female subjects (45.3%). This finding is in agreement with previous report[17-18]. However, it is not clearly understood why males were more infected than females in the present study. *Staphylococcus aureus* was found to more associated with Urine (38.7%), Semen (24%) and HVS (20%) respectively. A study by Ndip et al.[19] at Ilorin, Nigeria reported wound infections of 38% as the highest frequency of S. aureus isolates. This contradicts the finding of the present study where ear swab had the highest isolate of 30.7%.

Over time, Staphylococcus aureus develops highly quick and effective resistance to several antibiotics. The highest frequency of S. aureus occurred with susceptibility to antimicrobial agent Ciprofloxacin (64%) followed by Streptomycin (58.7%) while the least was Ampliclox (12%). The low percentage sensitivity of S. aureus observed in the present study against the following drugs; Zinnacef, Amoxicillin, Erythromycin and Gentamicin was in agreement with the reports published by previous authors[19-22] from Nigeria.

The greater level of resistance may be related to the medications' earlier exposure to isolates that may have a higher propensity to acquire resistance. Antibiotic abuse is rampant in this setting due to self-medication, which is frequently accompanied by insufficient dosage, noncompliance with therapy, and consumer access to antibiotics over the counter with or without a prescription[23]. In the current investigation, the sensitivity of *Staphylococcus aureus* to Gentamicin was 36%. This contrasts well with studies that some authors have published[18]. In underdeveloped nations like Nigeria, where there are no regulatory laws in this regard, it has been noted that the indiscriminate use of antibiotics without prescription has rendered the routinely used antibiotics utterly ineffective in the treatment of *Staphylococcus aureus* infections[23].

The identity of sixteen virulence gene primer set used in this study for pooled genomic DNA is shown in Table 2. P1 (*16SrRNA*), P2 *(Esp*), P3 *(geIE*), P4 (*cylA*), P5 (*asal*), P6 (*Ace)*, P7 *Lasl*, P8 *LasR,* P9 *rhil,* P10 *rhir*, P11 *toxA,* P12 *aprA,* P13 *rhiAB*, P14 *picH*, P15 *lasB*, and P16 *FliC* respectively. The virulence screening of the sixteen primer set reveals that lasl and rhiR were significantly present, so they were selected for the screening of the genomic DNA of the 75 S. aureus isolates as reported by this study. Furthermore, the identity of RAPD primers used in this study for polymorphism screening of pooled genomic DNA is shown in table 3 (OPT01, OPH01, OPT02, OPH02, OPT03, OPH03, OPT04, OPH04, OPT05, OPH05, OPT06, OPH06, OPT07, OPH07 and OPT08). Detection of polymorphism in two pooled genomic DNA of S. aureus isolates using 15 RAPD primers in PCR analysis. Figure 10 reveals OPT06, OPH06, OPT07 and OPH07 as sets

The genetic typing was carried out by Random Amplified Polymorphic DNA (RAPD-PCR) using OPT06, OPH06, OPT07 and OPH07 Primers. DNA fingerprint of 75 S. aureus isolates using OPT06 primer revealed existence of different genotypes among the isolates; some isolates were identical figure 14 and figure 15. DNA fingerprint of 75 S. aureus isolates using OPH06 primer revealed existence of difference genotypes among the isolates; two isolates were identical Genotypes of S. aureus isolates as revealed by 64 RAPD PCR markers showed75 S. aureus isolates with distinct high level of genetic diversity as revealed by 64 RAPD PCR makers and there was 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.

Among the 75 S. aureus isolates screened in this study for *lasl* and *rhiR* virulence gene, result shows 71% for lasl and 68% for *rhlR*. 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. Virulence genes (*lasl* and *rhiR)* and their clusters detected in S. aureus isolates used in this study formed the genetic basis for pathogenicity of the pathogen in human. The existence of different genotypes among the S. aureus isolates was due to the presence of virulence genes (*lasl* and *rhiR*) and their clusters in S. aureus revealed inherent relationship between genotypes and genetic virulence factors in S. aureus isolates genome.

Staphylococcus aureus has historically been characterized as a versatile bacterium with a wide range of pathogenic and antibiotic-resistant variations[24-25]. The characterization of the pathogen was complicated and ambiguous due to the small number of morphological and cultural characteristics of S. aureus and the absence of standardization of cultural conditions and virulence tests among various laboratories[14,25]. The (*lasl* and *rhiR*) virulence genes, which are typically

found in *Enterococcal faecalis* and Pseudomonas aeruginosa, respectively, were discovered to be present in *Staphylococcus aureus* in the current study.

Pseudomonas aeruginosa two quorum-sensing systems, *lasl* and *rhlR*, control the expression of the virulence genes. The las system includes the transcriptional activator *LasR* and the auto-inducer N-(3-oxododecanoyl) homoserine lactone's synthesis regulator *LasI* (PAI-1). *LasR* and *PAI-1* are necessary for the induction of the *lasB* (encoding elastase) and other virulent genes. *RhlR,* a potential transcriptional activator, and RhlI, which controls the production of N-butyryl homoserine lactone, make up the *rhl* system (*PAI-2*). The *rhl* system and *rhlAB* have both been reported to be necessary for rhamnolipid synthesis in P. aeruginosa (encoding a rhamnosyl transferase)[26]. The mechanism of action of lasl and *rhiR* in S. aureus is yet to be fully elucidated and calls for further research.

5. Conclusion

In conclusion, the DNA fingerprint revealed high genetic diversity among isolates irrespective of their sources. Virulence genotypes were highly diverse in these isolates. Our research indicates that focusing on *rhlR* and *lasl* with small molecule inhibitors may offer an interesting direction for the creation of novel antimicrobials. To disclose the level of pathogenicity and stop the spread of infection, thorough gel documenting of DNA fingerprint profiles of clinical laboratory isolates, not just *Staphylococcus aureus* isolates should be carried out on a regular basis. It is vitally necessary to implement good drug policies in Nigeria and to educate the public about the ineffectiveness of these antibiotics against *S. aureus* infections in order to minimize treatment failures in the absence of data on antibiotic susceptibility testing.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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