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# Distribution of *Esp* 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 *(Enterococal Surface Protein) esp* virulent gene 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 used 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 of antibiotic susceptibility test against 75 S aureus isolate showed 64.0%, 58.7%, 54.7%, and 52.0% of the isolates were sensitive to ciprofloxacin, streptomycin, rocephin, and perfloxacin respectively. Conversely 88.0% of the isolate shows resistance to ampiclox 78.7% to zinnacef, 70.7% to amoxicillin, 64.0% to erythromycin, 64.0% to gentamycin and 56.0% to septrin. Using Polymerase Chain Reaction (PCR), virulent genes *esp* formed clusters in *S. aureus* isolates used in this study. Virulence diversity was observed among isolates. The genetic typing was carried out by Random Amplified Polymorphic DNA (RAPD-PCR) using OPT06 and OPHO6, primers. In conclusion 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.

**Keywords:** *Staphylococcus aureus*; Virulent gene; Antibiotic resistance; Genetic diversity; Polymerase chain reaction (PCR)

## 1. Introduction

The most common cause of hospital acquired infections is *Staphylococcus aureus*. The variety of illnesses that staphylococci can cause in people includes necrotizing pneumonia, toxic shock syndrome, osteomyelitis, endocarditis, and abscesses in organ tissues [1]. Specific strains of the adaptable pathogen *S. aureus* have been found in a variety of pathological-anatomical locales and disease entities. Numerous virulence factors, including cell wall-anchored proteins, secreted toxins, capsular- and exopolysaccharides, iron-transport systems, and modulators of host immunological activities, in addition to genes for antibiotic resistance, contribute to this versatility [2].

The bacterial chromosome contains the genetic information for the great majority of staphylococcal virulence factors, including surface proteins, sortase, *α*-haemolysin, exotoxins, and agr regulation. Adhesins, surface proteins such protein A, and, in the case of toxins, enterotoxins of *S. aureus* (SE) and β-Hemolysin (Hlb) are among the virulence factors that play a significant role in pathogenicity [30] Bacterial colonization and adhesion to host cells and extracellular components are aided by surface proteins. Toxic shock syndrome toxin-1 (TSST-1), Panton Valentine leukocidin (PVL), and other cellular proteins, proteases, and toxins that prevent phagocytosis and prevent the host from actively resisting

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infection through a particular immune response [4], these genes are present in all staphylococcal strains and are not connected to mobile DNA elements [5]

Through a global regulation system made up of the accessory regulatory gene (Agr) and the sigma factor (σB), *S. aureus* has the capacity to regulate the expression of virulence factors in accordance with the environmental conditions in which it is found [6]. The ability of *S. aureus* to adapt to various microenvironments with various environmental, nutritional, and stress conditions may result in the acquisition of genes coding for virulence factors that allow its survival in addition to gene regulation [7]. It has been shown that one mobile genetic element can mobilize multiple virulence factor-coding genes. For instance, in the bacteriophage *β*C-*φs*, the staphylokinase gene and complement inhibitor protein are mobilized alongside enterotoxin genes and Panton-Valentine leucocidin [8]

The human pathogen *Staphylococcus aureus* is persistent and causes a number of diseases with a wide range of clinical manifestations and severity [1]. In response to host signals, including as those found in target tissues and those associated to innate defenses triggered throughout the infectious phase, various networks of virulence genes are probably activated. This expectation shows up in vitro as a pattern of virulence determinant expression that is reliant on growth phase and is set up by global regulatory components [9]. The organism produces fibrinogen-binding, fibronectin-binding, and collagen-binding proteins as well as other cell-wall proteins with sticky properties during the exponential growth phase; this expression may facilitate colonization's early stages in the host. Cell-wall protein expression is suppressed as the growth cycle moves from the exponential to the stationary phase, while the production of extracellular toxins and enzymes takes center stage. These exotoxins may promote local invasion and spread of infection by their proteolytic activities and toxic effects on host cells [1].

Genetic study is crucial for understanding the genotype of *S. aureus* because it has been documented that it affects the complications, severity, and mortality of infection. A Comprehensive gel documentation of DNA fingerprint profile of *Staphylococcus aureus* isolates which is very important to show comparison of genetic diversity, virulence factors and degree of pathogenicity among isolates has never be carried out in the study area using clinical isolates. This study was therefore carried out to identify and differentiate the genetic diversity, antibiotic resistance and virulence factors of 75 *Staphylococcus aureus* isolates using their genomic DNA. The current study used a more sensitive genetic PCR and DNA sequencing analyses approaches to estimate virulence mutants in *Staphylococcus aureus*.

## 2. Material and method

### 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 $^{o}$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 [29]. 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) 30µg, 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.* (2003). 0.3g of washed bacterial cell was suspended in 200µl of CTAB buffer (50mM Tris, pH 8.0); 0.7 mm NaCl; 10 mm EDTA; 2% hexadecyltrimethyl ammonium bromide and 0.1% 2-mercaptoethanol), followed by the addition of 100 µl of 20% SDS. It was incubated at 65 $^{o}$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 $^{o}$C for 10 mins. DNA was precipitated from supernatant with equal volume of absolute ethanol and centrifuged at 10,000 rpm at 4 $^{o}$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 [10] The reaction mixture were; Genomic DNA 80 ng per reaction, Taq Reaction buffer, 100 mm dNTP (dATP; dCTP; dGTP; dTTP), 0.2 mm, Uuniversal primer (28 primers 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 [10]. 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**;**

- 1 cycle of 94 ℃ for 3 min.
- 45 cycles of 94 ℃ for 1 min for denaturation, 40 $^0$C for 1 min for annealing of primer and 72 ℃ for 2 min for extension.
- Final extension at 72 ℃ for 7 min.
- 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 (0.5µg/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, 41-60years 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 virulence 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 sensitive antibiotics were Ciprofloxacin (64.0%), followed by Streptomycin (58.7%), Rocephin (54.7%), and Perfloxacin (52.0%). On the other hand, the most insensitive 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 shows the antibiotic susceptibility tests of *S. aureus* isolates in relation to gender. From the results obtained, the susceptibility pattern of the antibiotics in male and female subjects was 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 shows the antibiotic susceptibility tests of *S. 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 and 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 shows 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 tested. Only two of the antibiotic zinacef and amoxicilin had identical response. Figure 5 shows the PCR Profile of fifteen RAPD primers used to screen pooled *S. aureus* genomic DNA. Figure 6 shows Cluster of 75 *S. aureus* grouping based on interactions with 10 commonly used antibiotics, Figure 7 shows *S. aureus* genomic DNA agarose gel electrophoretic pattern, Figure 8 shows the DNA standard curve using Sigma DNA Ladder, Figure 9 shows the PCR Profile of fifteen RAPD primers used to screen pooled *S. aureus* genomic DNA and Figure 10 shows the DNA fingerprint of 75 *S. aureus* genomic DNA as revealed by OPT06-PCR analysis.

Figure 11 shows 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 12 shows the DNA fingerprint of 75 *S. aureus* genomic DNA as revealed by OPH06-PCR analysis and Figure 13 shows 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 14 shows cluster of genetic diversity among 75 *S. aureus* isolates as revealed by 64 RAPD markers. The results obtained showed that 75 *S. aureus* isolates were 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 15 shows the PCR profile of virulence target gene detection by 16 specific primers used for preliminary PCR amplification and screening of *S. aureus* isolates genomic DNA. Figure 16 shows the PCR detection of *esp* virulence gene at 932 base pairs in 75 *S. aureus* isolates genomic DNA and Figure 17 shows the *esp v*irulence gene cluster in 75 *S. aureus* isolates genomes.

**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 |

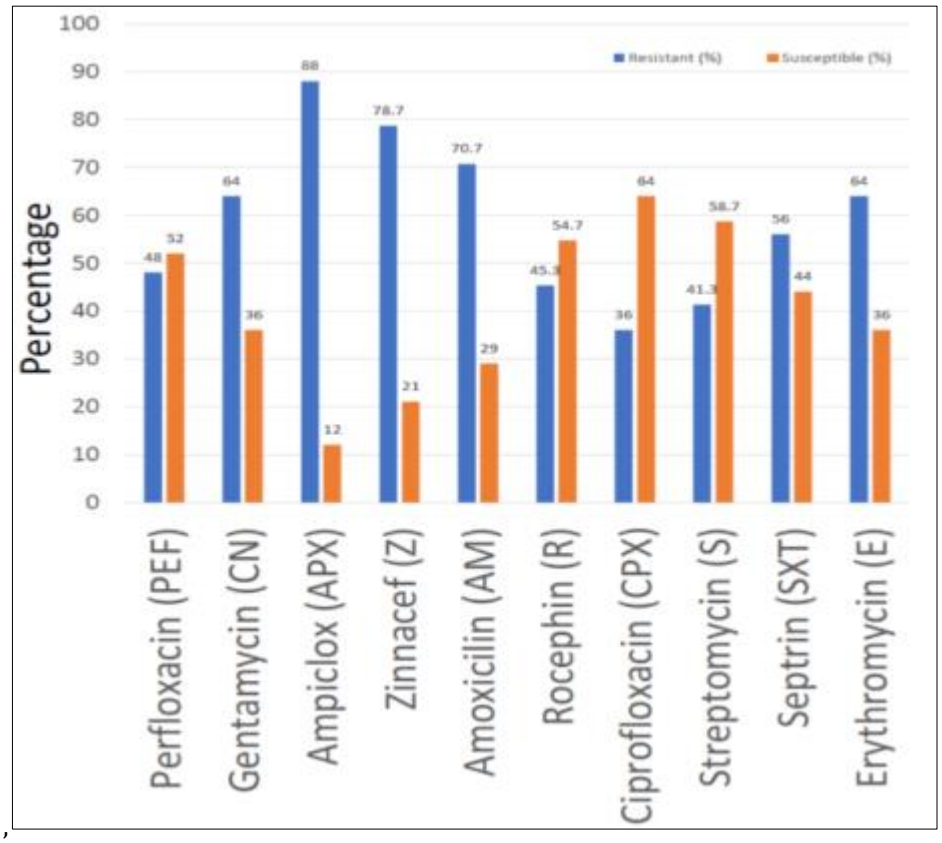



**Figure 1** Response of 75 *S. aureus* isolates to 10 commonly used antibiotics

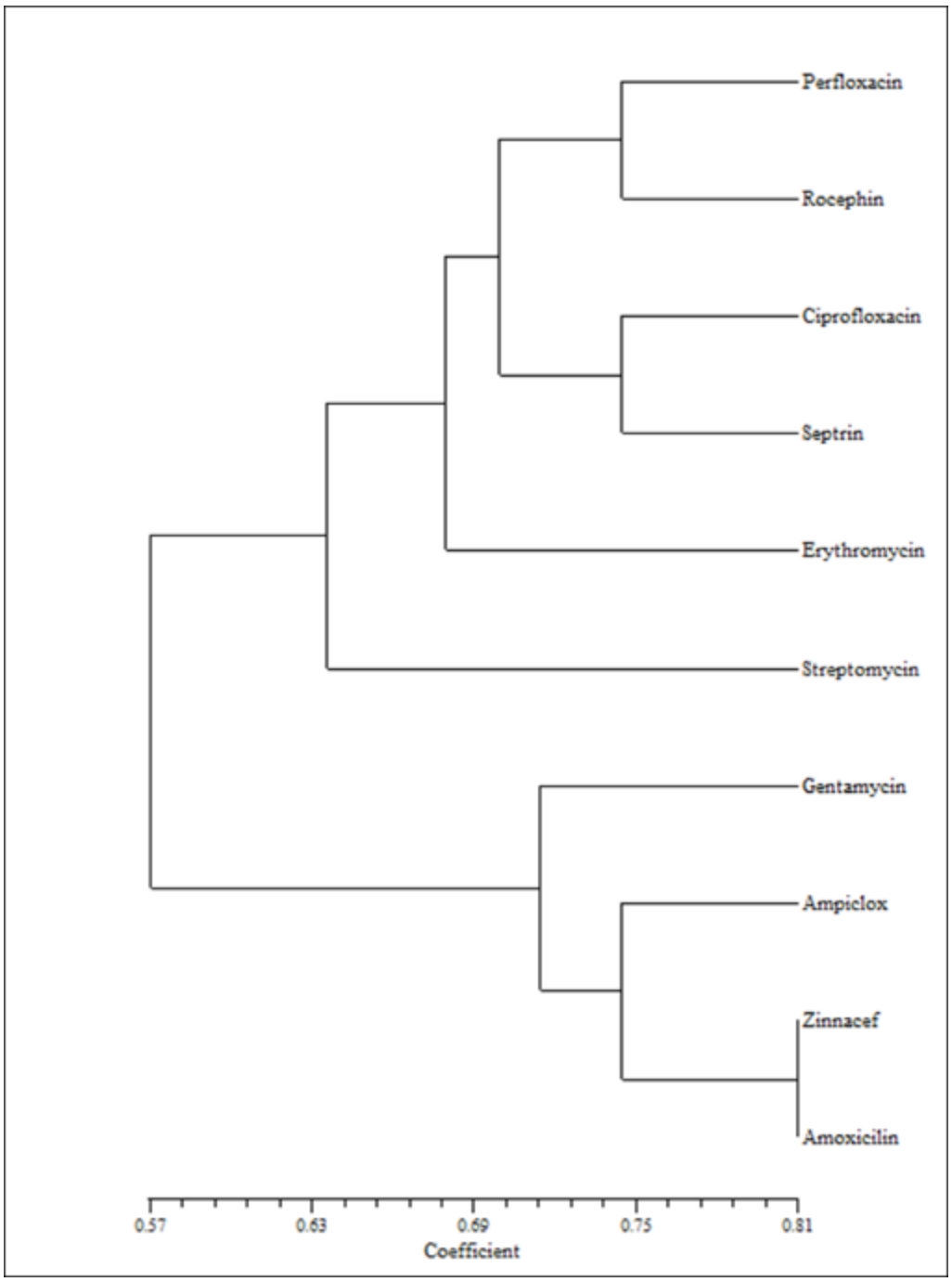



**Figure 2** Cluster showing antibiotic response to 75 *S. aureus* isolates

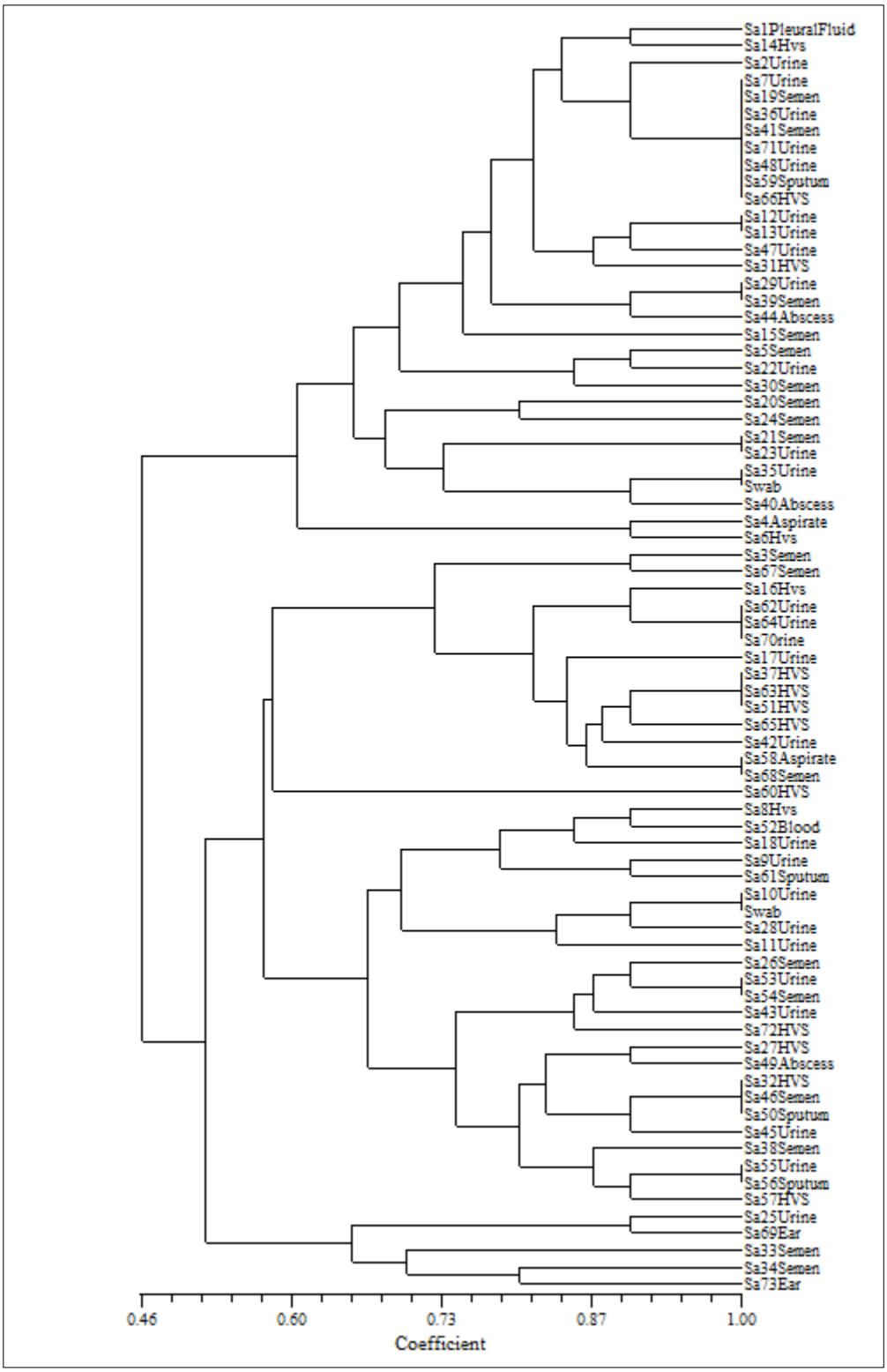



**Figure 3** Cluster showing 75 *S. aureus* grouping based on reactions with 10 commonly used antibiotics

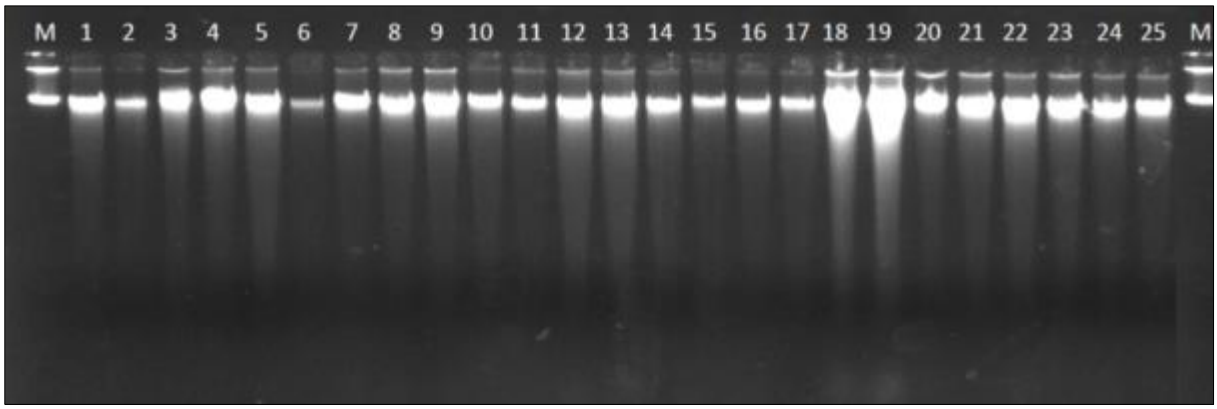



**Figure 4** a. *S. aureus* genomic DNA agarose gel electrophoretic pattern

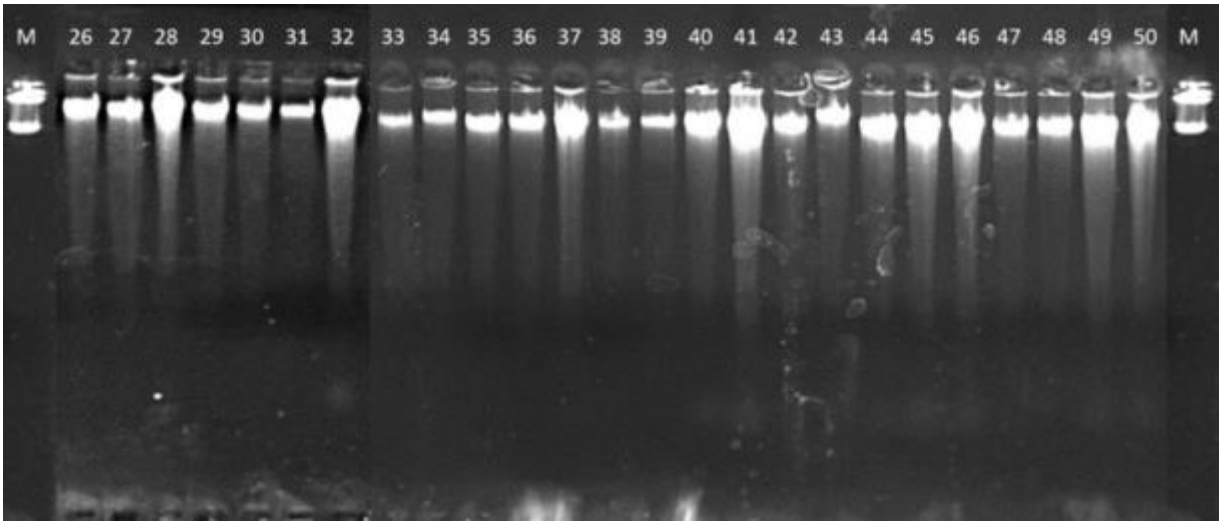



**Figure 4** b. *S. aureus* genomic DNA agarose gel electrophoretic pattern

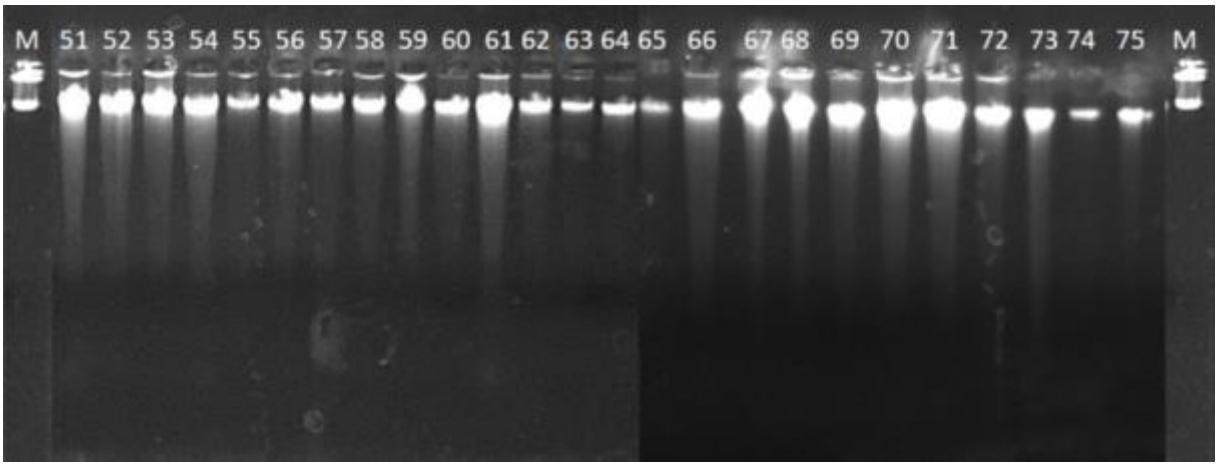



**Figure 4c.** *S. aureus* genomic DNA agarose gel electrophoretic pattern

**Table 2** 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' |

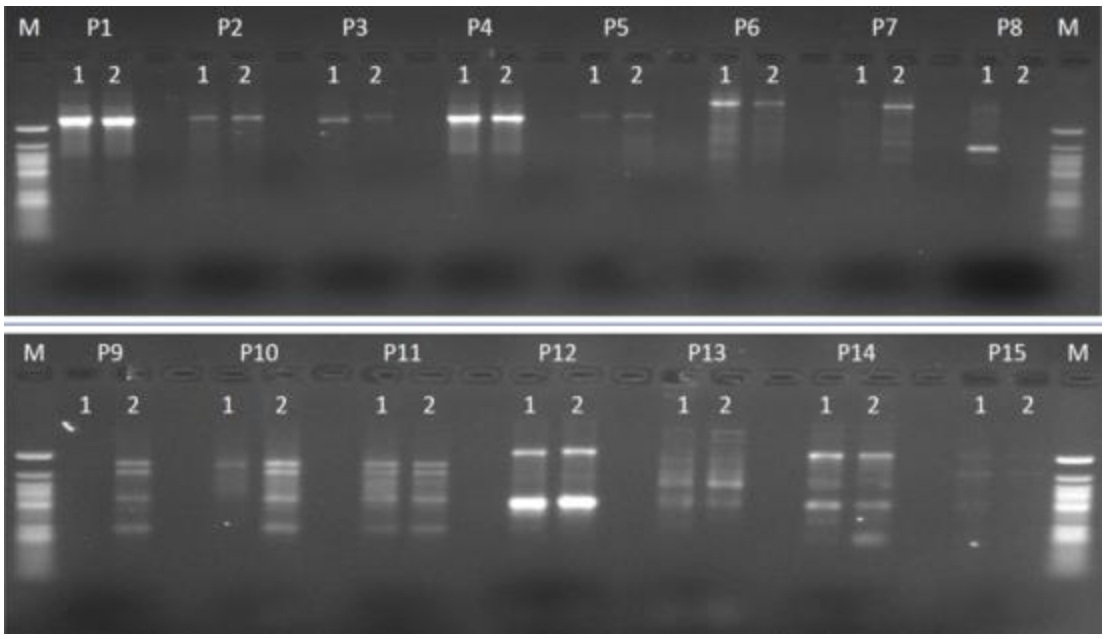



**Figure 5** PCR Profile of fifteen RAPD primers used to screen pooled *S. aureus* genomic DNA

**Table 3** 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' |

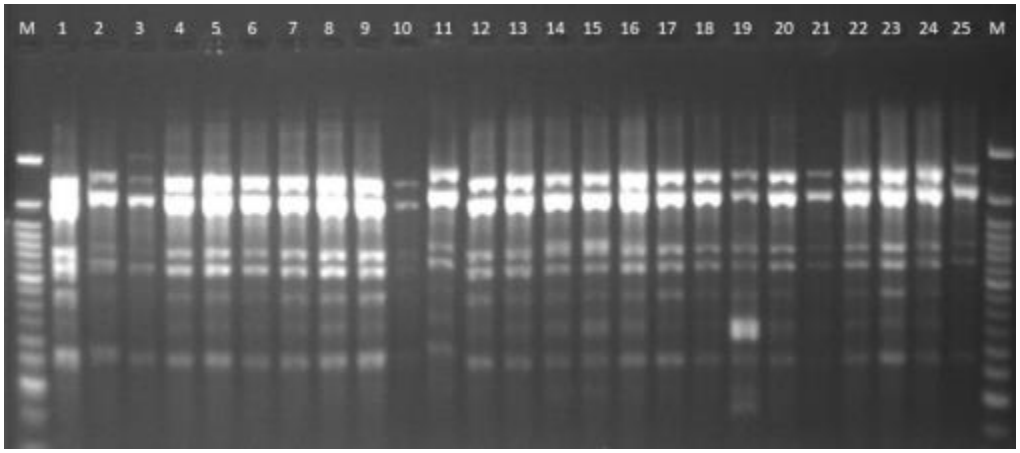



**Figure 6a** DNA fingerprint of 75 *S. aureus* genomic DNA as revealed by OPT06-PCR analysis

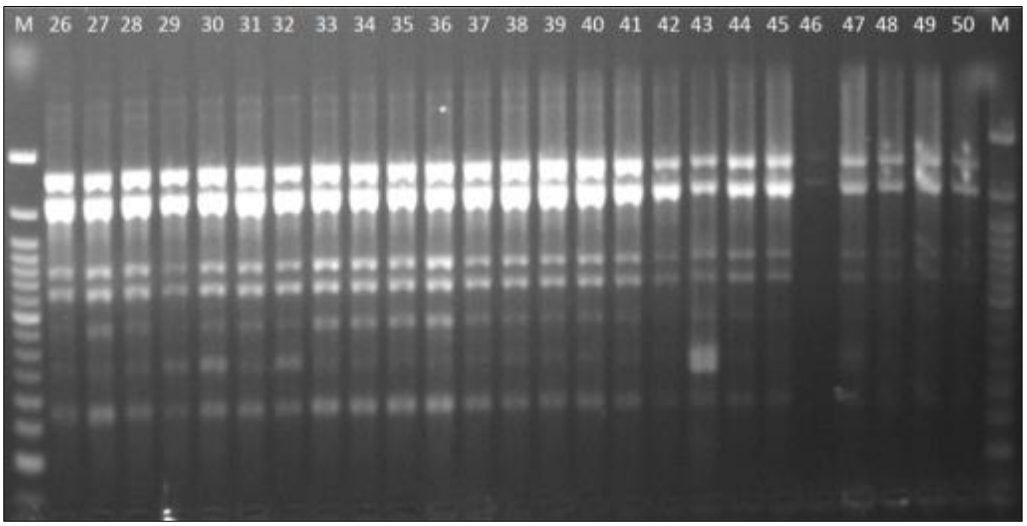



**Figure 6b** DNA fingerprint of 75 *S. aureus* genomic DNA as revealed by OPT06-PCR analysis

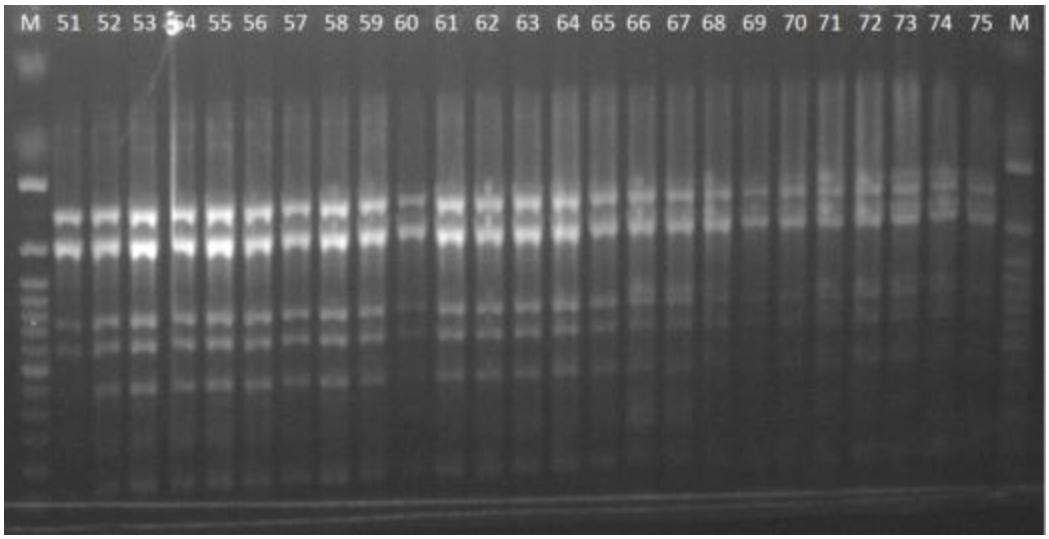



**Figure 6c** DNA fingerprint of 75 *S. aureus* genomic DNA as revealed by OPT06-PCR analysis

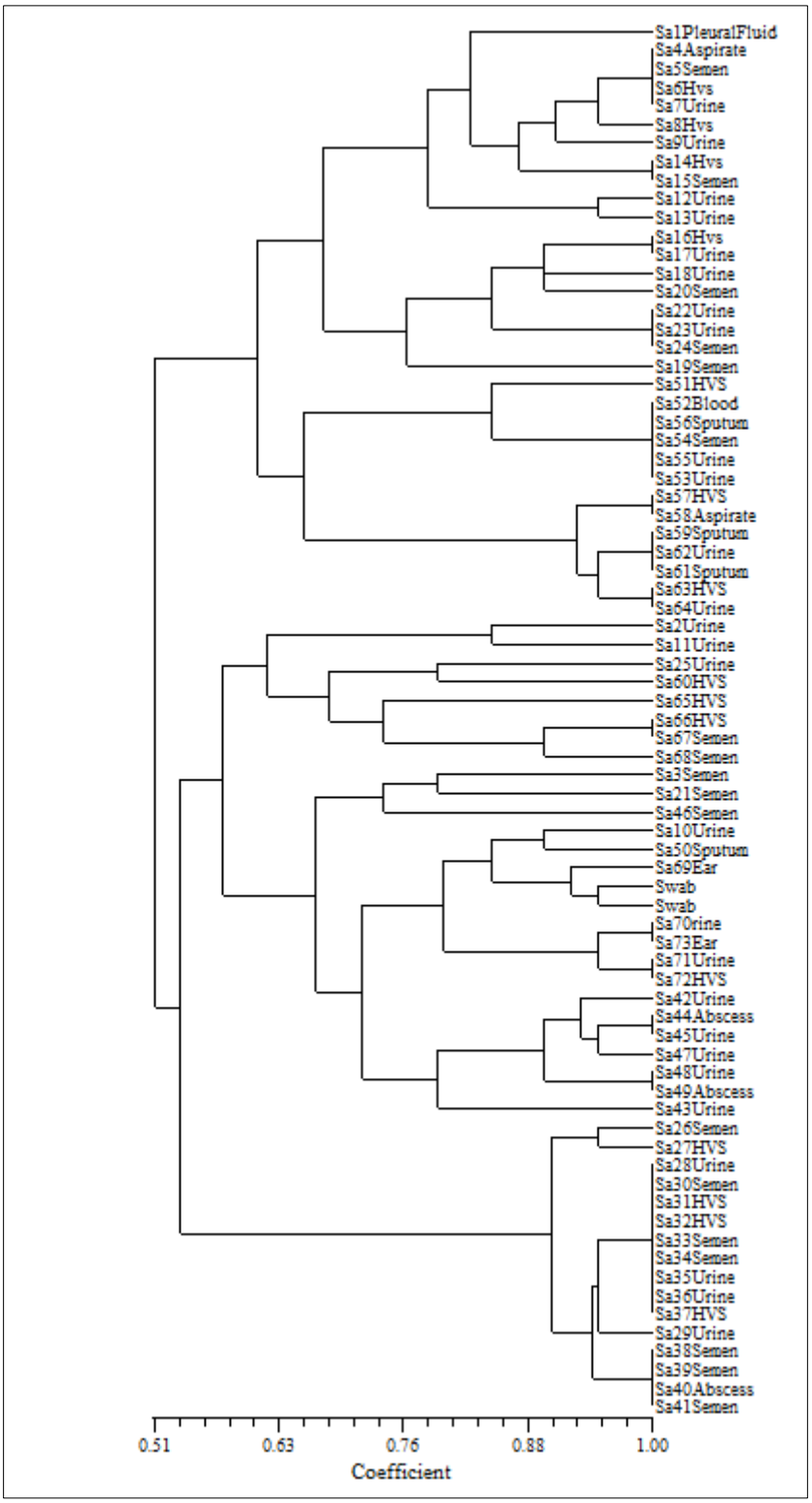



**Figure 7** Cluster showing genetic diversity among 75 *S. aureus* isolates amplified DNA

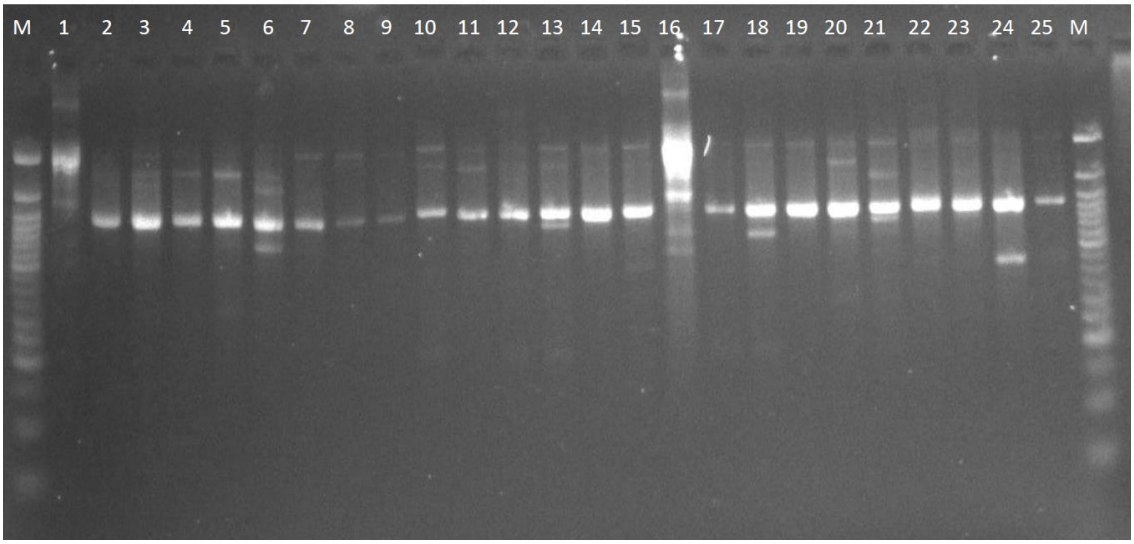



**Figure 8a** DNA fingerprint of 75 *S. aureus* genomic DNA as revealed by OPH06-PCR analysis

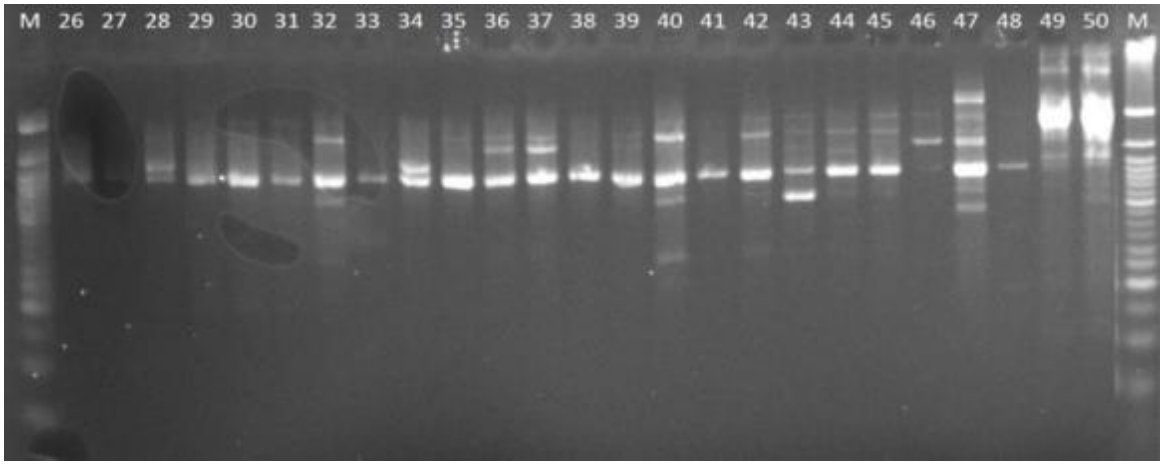



**Figure 8b** DNA fingerprint of 75 *S. aureus* genomic DNA as revealed by OPH06-PCR analysis

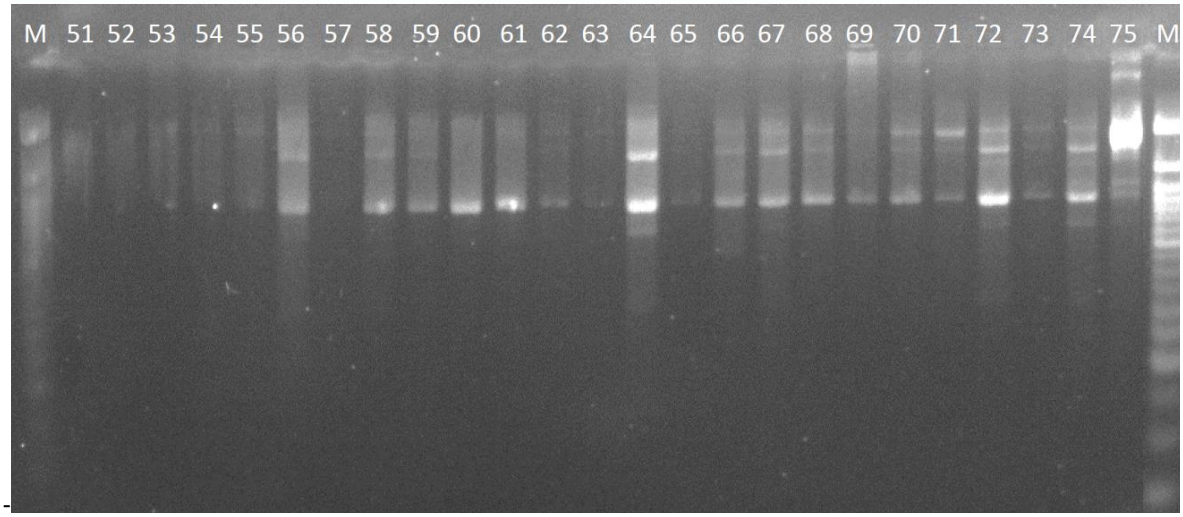



**Figure 8c** DNA fingerprint of 75 *S. aureus* genomic DNA as revealed by OPH06 RAPD-PCR analysis.

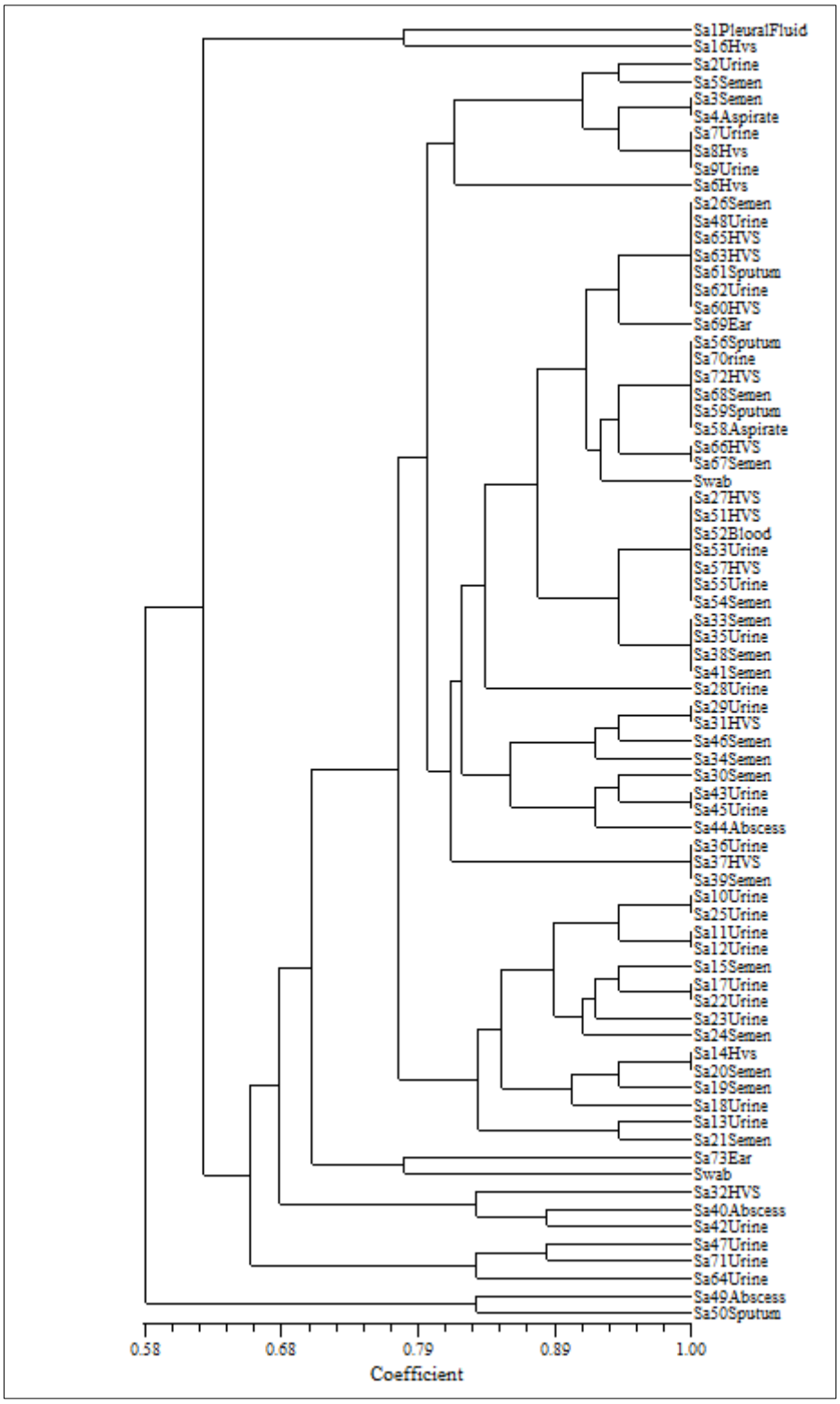



**Figure 9** Cluster showing genetic diversity among 75 *S. aureus* isolates amplified DNA

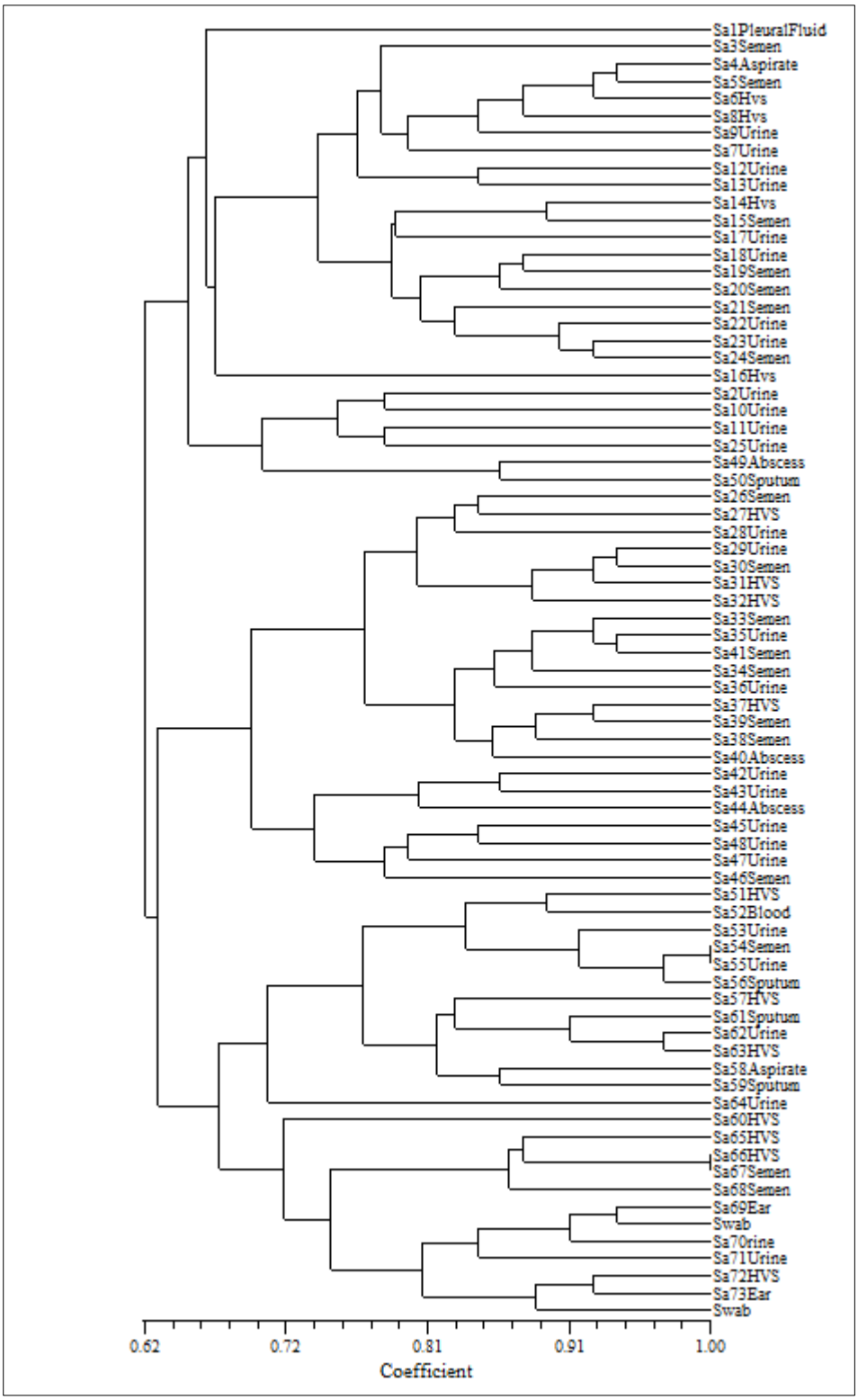



**Figure 10** Cluster showing genetic diversity among 75 *S. aureus* isolates as revealed by 64 RAPD markers

**Table 4** 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 | |

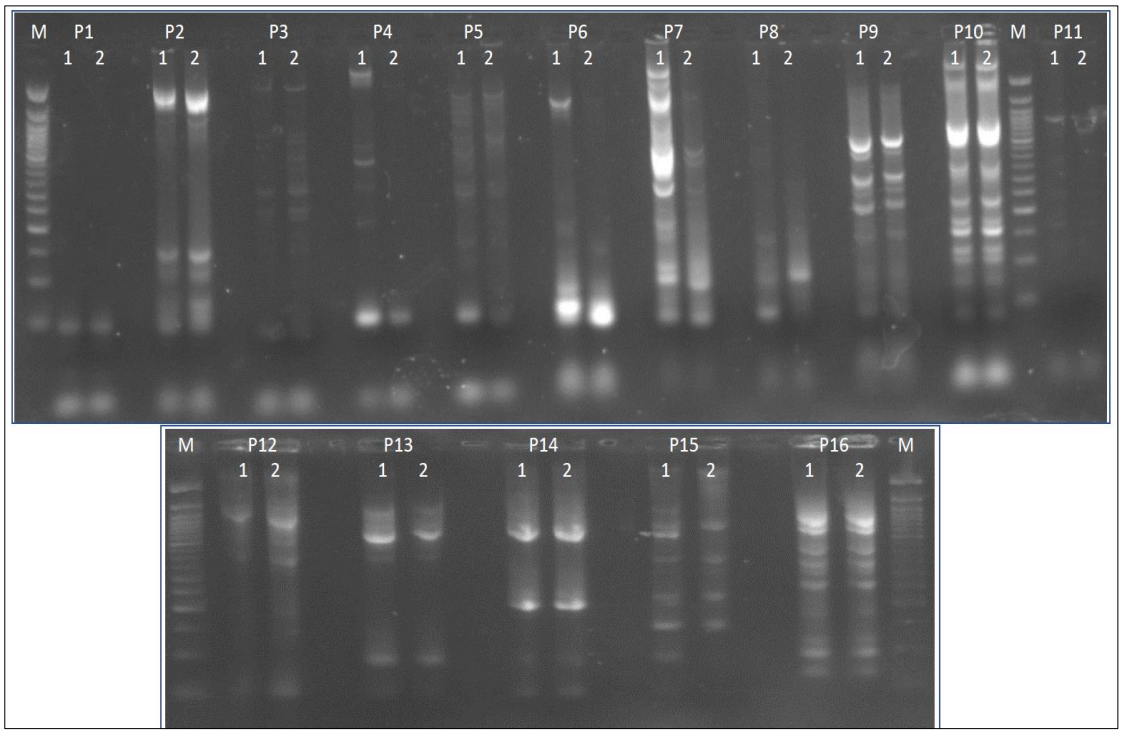
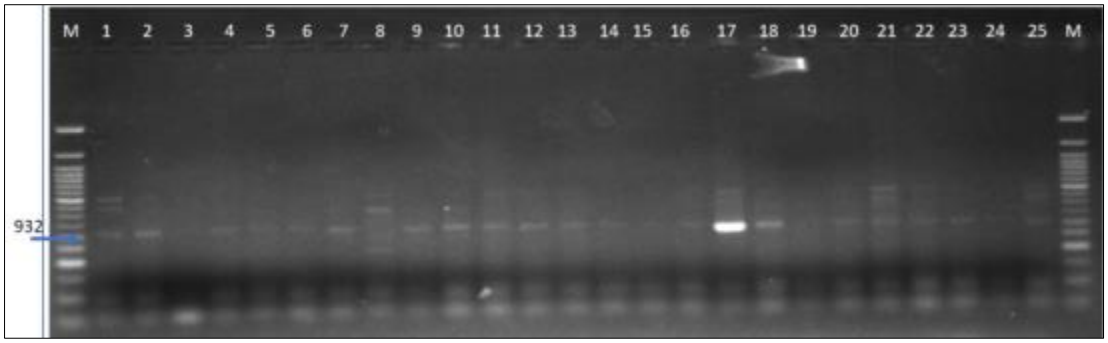
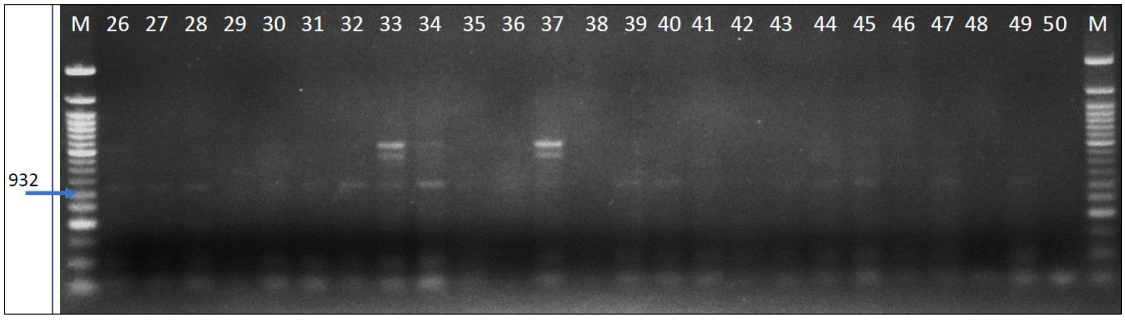



**Figure 11** PCR Profile virulence target gene detection by 16 specific primers used for preliminary PCR amplification and screening of *S. aureus* isolates genomic DNA

**Table 5** 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) |
|---|---|---|---|
| P2 | Esp | TTGCTAATGCTAGTCCACGACC | 932 |
| | | GCGTCAACACTTGCATTGCCGA | |



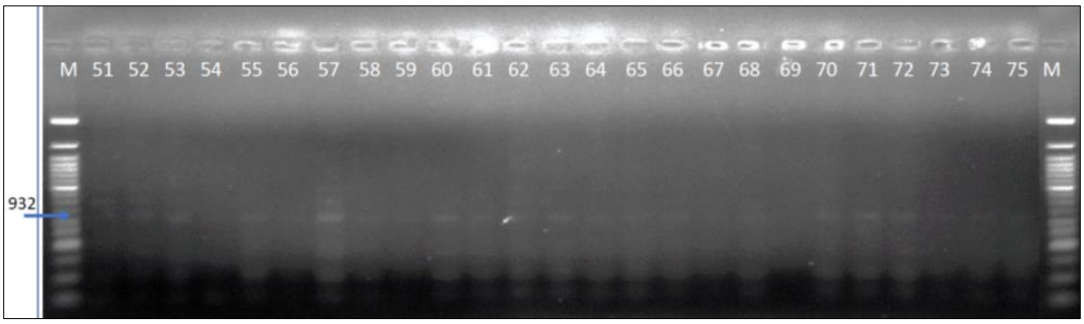



**Figure 12** PCR detection of *Esp* virulence gene at 932 base pairs in 75 *S. aureus* isolates genomic DNA

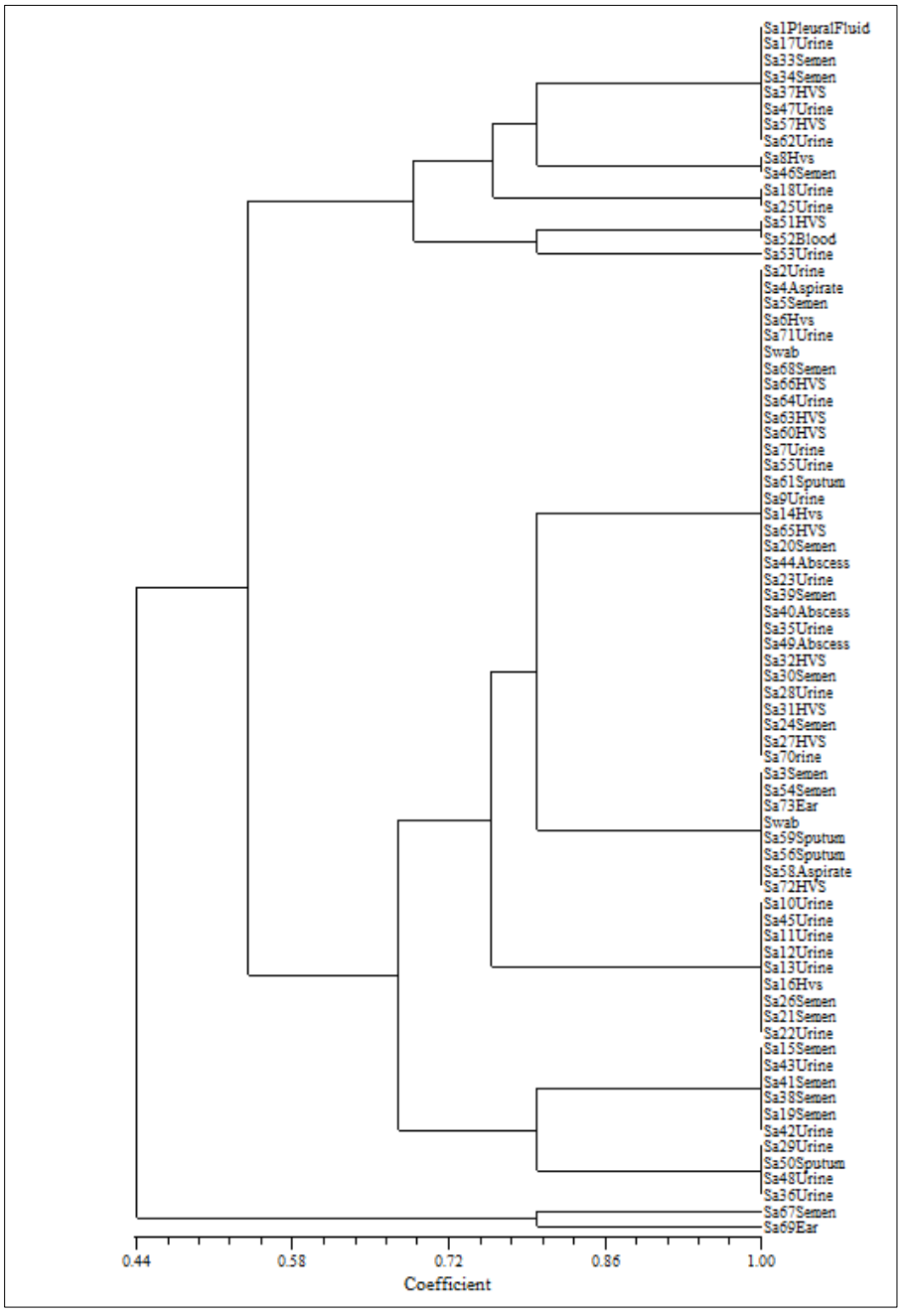



**Figure 13** *Esp v*irulence gene cluster in 75 *S. aureus* isolates genomes

## 4. Discussion

*Staphylococcus aureus* is a gram-positive bacterium that can occasionally cause pus and abscesses, sepsis, and even fatal septicemia in humans. It can be found in the axillae, nose, groin, perineal area (in males), mucous membranes, mouth, mammary glands, hair, and intestinal, genitourinary, and upper respiratory tracts. Numerous virulence factors that promote colonization and disease in the host influence *S. aureus* capacity to cause disease. [11] This study was carried out to determine the distribution of *esp* 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 [12] 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 [12]; [13] However, it is not clearly understood why males were more infected than females in the present study. *Staphylococcus aureus* was found to be more associated with Urine (38.7 %%), Semen (24%) and HVS (20%) respectively. A study by [15] 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 were in agreement with the reports published by previous authors from Nigeria [12]; 14]; [15]; [16]; [17]

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 [18] in the current investigation, the sensitivity of *Staphylococcus aureus* to Gentamicin was 36%. This contrasts well with studies that some authors have published. 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 [18]

The enterococcal surface protein *(esp),* a high-molecular-weight surface protein with no known function, is much more common in isolates of *Enterococcus faecalis* that are the result of infections [19] In this study, the *esp* virulent gene was detected by PCR at 932 base pairs in *S. aureus* isolates genomic DNA (figure 10 and 11). According to [12], there exist different genotypes among the *S. aureus* isolates used in the 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 12

A clinical strain of *E. faecalis* genome underwent random sequencing, and the e*sp* gene was discovered there [20]. *esp* strongly suggests a function in the pathogenesis of *E. faecalis* because it has been preferentially found in infection-derived *E. faecalis* strains but not in other less pathogenic enterococcal species [20]. *Staphylococcus epidermidis*, a non-pathogenic bacterium typically found in human nose and oral cavities, secretes *esp*, a type of serine protease. *Staphylococcus aureus*, a bacterium that can be found on the skin and in the nasal passages of up to 25% of healthy individuals and animals, is closely related to *S. epidermis*. Some *S. aureus* strains produce a heat-stable toxin that causes disease and is challenging to eradicate because it can build a biofilm. In this study, the *esp* gene thought only to be present in *Staphylococcus epidermidis, E. faecalis* and enterococcal species, was found to be present in *Staphylococcus aureus* isolated from clinical isolates. The *esp* virulent gene was detected by PCR at 932 base pairs in *S. aureus* isolates genomic DNA (figure 10 and 11).

The e*sp* was originally isolated and biochemically studied by [21], who found that it can break down a number of proteins with a high predilection for cleavage following glutamic acid residues, including fibrinogen and complement protein C5. Therefore, it was asserted that this process allowed *S. epidermidis* to sidestep the complement defense system. In actuality, another team of scientists backed up this assertion [21].[22] reported that the gene structure of *Esp* and the amino acid sequence of its mature form showed close resemblance with one of the *S. aureus* serine

proteases, which have been long regarded as crucial virulence factors that obliterate blood proteins and tissues, thus contributing to increased invasiveness [22].

*Esp,* as it turned out, prevented *S. aureus* colonization and infection by preventing the development of biofilms, according to [23]. *Esp* was found to degrade *S. aureus* biofilm by causing *S. aureus* to change from the sessile to the planktonic form, even though the exact mechanism by which *Esp* breaks the *S. aureus* biofilm is still unknown and does not resemble any bacterial interference mechanisms like growth inhibition and bactericidal activity. *Esp*-containing supernatant incubation for 16 hours produced a startling four-fold reduction in *S. aureus* biofilm. *Esp* differs from other biofilm breakers in that it releases bacteria from the biofilm rather than killing the bacteria inside it. This is one way in which *esp* differs from other biofilm breakers. As a result*, S. aureus* won't be able to develop a resistance to *esp* [23].

*Esp* demonstrates traits of surface protein receptors identified as microbial surface components, identifying adhesive matrix molecules, and attaching to host components to begin colonization [24]. Many of these proteins have a modular structure and contain a number of tandem repeat domains, which are most likely the result of numerous instances of duplication and/or recombination. *esp* may have a direct ligand-binding activity to the extracellular matrix under in vivo settings or may indirectly play a function in influencing the ligand-binding activity of other molecules, even though we have no evidence that it contains domains for binding to host factors. If this is the case, *esp*'s major function in the host-bacterium interaction would not be to facilitate *E. faecalis* adhesion to plastic biomaterials*. Esp* has been suggested to improve the hydrophobicity of cell surfaces and promote hydrophobic interactions [20]. Our results demonstrate that the presence of *Esp* in the cell surface could increase hydrophobicity, adherence to abiotic surfaces, and biofilm formation.

It is recognized that *S. aureus* surface proteins play a significant role in transmitting infectious illnesses to people, ranging from mild skin infections to life-threatening infections [25]. SdrD, Spa, IsdA, and Emp are required for the development of an abscess [26]. Because of its significance in several aspects of infection (such as anti-inflammatory and antiangiogenic characteristics and abscess formation), *esp* has attracted the attention of researchers [27]. By forming complexes with pathogen-specific antibodies, complement factor 3d, or VH3-type IgM on the surface of B cells, immunoglobulin-binding proteins Spa and Sbi aid in immune evasion [27]. Our results unequivocally show that the newly discovered *esp* in *S. aureus* breaks down various surface proteins and toxins, including coagulase and -toxin, indicating that Esp might be useful for the prevention or treatment of infectious illnesses brought on by this pathogen. *esp* should only be used therapeutically, since its safety has not yet been shown, therefore this should be taken into consideration. An alternate strategy could be the creation of a vaccination that specifically targets proteins that are broken down by *esp* and also aid in the biofilm formation and colonization of human skin and the human nasal cavity by *S. aureus*.

## 5. Conclusion

In this study, the *esp* virulent gene was detected by PCR at 932 base pairs in *S. aureus* isolates genomic DNA (figure 14 and 15). DNA fingerprint revealed high genetic diversity among isolates irrespective of their sources. These isolates had a wide range of virulence genotypes. According to estimates, biofilm-related infections account for about 65% of nosocomial infections in the developed world. Since the usage of medical implants is projected to rise in the near future, this serves as a warning flag. In this setting, bacterial molecules involved in host protein-mediated attachment as well as others, like *esp,* involved in adhesion to inorganic surfaces and biofilm formation could become promising therapeutic targets in control programs for eliminating persistent enterococcal infections linked to the presence of biofilms.

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