**In Silico Virulence and Resistance Profile Analysis of Staphylococcus species**

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**Abstract**

In silico studies of the genes of *Staphylococcus* spp. might establish some correlations with multiple pathological factors. Sixty isolates of *Staphylococcus* spp. have been studied here targeting virulence and antibiotic resistance genes through in silico tools. Here, in silico PCR (polymerase chain reaction) amplification detected both virulence and antibiotic resistance genes. Study revealed that most of the isolates harboured either cap5 (40%) or cap8 (31.67%) locus gene. Staphylococcal enterotoxin was detected in 63.33% of the isolates. The sea gene, responsible for food poisoning, was detected in 26.67% of the isolates. The tst positive isolates (5%), responsible for toxic shock syndrome, were present in only genotype 8. No exfoliative toxin was detected. The icad gene, responsible for intracellular adherence, appeared in 80% of the isolates. Alpha hemolysin gene, hla, was detected in 63.33% of the isolates. Sixty-five percent of the isolates harboured the mecA genes. Both β-lactamase (blaZ) and erythromycin resistance, ermA genes were available in 38.33% of the isolates. In silico pulsed field gel electrophoresis (PFGE) digestion was able to divide isolates into 23 genotypes. Genotype 8 and 11 harboured tetracycline resistance genes, tetM and tetK. The tetM gene (18.33%) was more prevalent than tetK gene (11.67%). Genotype 1 and 11 were considered more virulent than others. Genotype 11 also carried six antibiotic resistance genes but did not carry the genes msrA, msrB, ermB and ermC. The data generated here might aid in the prediction of the virulence and resistance profile based on genotyping as well as contribute in vaccine development.

**Key words:** *Staphylococcus*, Virulence genes, Antibiotic resistance genes, Pulse field gel electrophoresis, Genotype.

**Introduction**

*Staphylococcus* is a gram-positive commensal organism found in the skin, skin glands, hair, intestinal tract, genitourinary tract, upper respiratory tract and mucous membranes. The pathogenicity of bacteria depends on some virulence factors such as surface proteins, extracellular material, cellular proteins, toxins and protease. Capsular polysaccharide protects bacteria from phagocytic uptake and out of 11 capsular polysaccharides, only type 5 and 8 are predominant among clinical isolates (Hochkeppel et al., 1987). Enterotoxins are associated with the food poisoning outbreak (Hennekinne et al., 2012; Argudin et al., 2012). Fueyo et al. (2005) reported that toxic shock syndrome is caused by the exotoxin gene, tst. Kim et al. (2006) published that exfoliative toxins (ETs) are associated with skin infection. Epithelial layer disruption caused by hemolysin gene was reported earlier by Vandenesch et al. (2012). Multidrug resistance is a serious consequence of treatment and prevention of *Staphylococcus* infection. Duran et al. (2012) demonstrated that aminoglycoside nucleotidyltransferase (APHs) inactivates drug and confers resistance to aminoglycoside antibiotics. Clinical isolates carry ermA or ermC but the ermB gene is rather infrequent (Schmitz et al., 2000). Schmitz et al. (2000) and Torres et al. (1996) reported that tetracycline resistance in *Staphylococcus* spp. is acquired by...
ribosomal modification of widely disseminated \textit{tetM} or \textit{tetK} gene and \textit{tetK} is found most often in \textit{Staphylococcus aureus} (Trzcinski et al., 2000; Schmitz et al., 2001). \textit{In silico} analysis helps to extract useful information from vast amount of data. Recently, numerous \textit{in silico} gene analysis have been conducted by using numerous tools. In this regard, a throughout knowledge of molecular evaluation might assist to control bacterial dissemination (Bikandi et al., 2004; San Millan et al., 2013; Biswas et al., 2008; Zankari et al., 2012). Comparative genomics helps to improve knowledge on pathogenesis and drug resistance of microbial species (Feng et al., 2008).

The aim of the present study was to thorough \textit{in silico} investigation of 60 \textit{Staphylococcus} spp. and predict the virulence and resistance profile of this genus.

**Materials and Methods**

\textit{Strains used in the study}: Isolates used in this study are summarized in Table 1.

| Serial Number | Isolate Name |
|---------------|--------------|
| 1 | NC_017340 \textit{Staphylococcus aureus} 04-02981 |
| 2 | NC_018608 \textit{Staphylococcus aureus} 08BA02176 |
| 3 | NC_021670 \textit{Staphylococcus aureus} Bmb9393 |
| 4 | NC_021554 \textit{Staphylococcus aureus} CA-347 |
| 5 | NC_021059 \textit{Staphylococcus aureus} M1 |
| 6 | NC_007622 \textit{Staphylococcus aureus} RF122 |
| 7 | NC_002758 \textit{Staphylococcus aureus} strain Mu50 |
| 8 | NC_017451 \textit{Staphylococcus aureus} subsp. \textit{aureus} 11819-97 |
| 9 | NC_022113 \textit{Staphylococcus aureus} subsp. \textit{aureus} 55/2053 |
| 10 | NC_022222 \textit{Staphylococcus aureus} subsp. \textit{aureus} 6850 |
| 11 | NC_017673 \textit{Staphylococcus aureus} subsp. \textit{aureus} 71193 |
| 12 | NC_022226 \textit{Staphylococcus aureus} subsp. \textit{aureus} CN1 |
| 13 | NC_002951 \textit{Staphylococcus aureus} subsp. \textit{aureus} COL |
| 14 | NC_017343 \textit{Staphylococcus aureus} subsp. \textit{aureus} ECT-R 2 |
| 15 | NC_017337 \textit{Staphylococcus aureus} subsp. \textit{aureus} ED133 |
| 16 | NC_013450 \textit{Staphylococcus aureus} subsp. \textit{aureus} ED98 |
| 17 | NC_017763 \textit{Staphylococcus aureus} subsp. \textit{aureus} HO 5096 0412 |
| 18 | NC_009632 \textit{Staphylococcus aureus} subsp. \textit{aureus} JH1 |
| 19 | NC_009487 \textit{Staphylococcus aureus} subsp. \textit{aureus} JH9 |
| 20 | NC_017338 \textit{Staphylococcus aureus} subsp. \textit{aureus} JKD6159 |
| 21 | NC_017349 \textit{Staphylococcus aureus} subsp. \textit{aureus} LGA251 |
| 22 | NC_016928 \textit{Staphylococcus aureus} subsp. \textit{aureus} M013 |
| 23 | NC_002952 \textit{Staphylococcus aureus} subsp. \textit{aureus} MRSA252 |
| 24 | NC_016941 \textit{Staphylococcus aureus} subsp. \textit{aureus} MSHR1132 |
| 25 | NC_002953 \textit{Staphylococcus aureus} subsp. \textit{aureus} MSSA476 |
| 26 | NC_003923 \textit{Staphylococcus aureus} subsp. \textit{aureus} MW2 |
| 27 | NC_009782 \textit{Staphylococcus aureus} subsp. \textit{aureus} Mu3 |
| 28 | NC_002745 \textit{Staphylococcus aureus} subsp. \textit{aureus} N315 |
| 29 | NC_007795 \textit{Staphylococcus aureus} subsp. \textit{aureus} NCTC 8325 |
| 30 | NC_017333 \textit{Staphylococcus aureus} subsp. \textit{aureus} S0385 |
| 31 | NC_022443 \textit{Staphylococcus aureus} subsp. \textit{aureus} SA40 |
| 32 | NC_022443 \textit{Staphylococcus aureus} subsp. \textit{aureus} SA957 |
| 33 | NC_020529 \textit{Staphylococcus aureus} subsp. \textit{aureus} ST228 complete genome, isolate 10388 |
| 34 | NC_020564 \textit{Staphylococcus aureus} subsp. \textit{aureus} ST228 complete genome, isolate 10497 |
Table 1 contd.

|   | Accession Number | Description | Length (bp) |
|---|------------------|-------------|-------------|
| 35 | NC_020532        | *Staphylococcus aureus* subsp. *aureus* ST228 complete genome, isolate 15532 |             |
| 36 | NC_020533        | *Staphylococcus aureus* subsp. *aureus* ST228 complete genome, isolate 16035 |             |
| 37 | NC_020566        | *Staphylococcus aureus* subsp. *aureus* ST228 complete genome, isolate 16125 |             |
| 38 | NC_020536        | *Staphylococcus aureus* subsp. *aureus* ST228 complete genome, isolate 18341 |             |
| 39 | NC_020537        | *Staphylococcus aureus* subsp. *aureus* ST228 complete genome, isolate 18412 |             |
| 40 | NC_020568        | *Staphylococcus aureus* subsp. *aureus* ST228 complete genome, isolate 18583 |             |
| 41 | NC_017342        | *Staphylococcus aureus* subsp. *aureus* T0131 |             |
| 42 | NC_017343        | *Staphylococcus aureus* subsp. *aureus* TCH60 |             |
| 43 | NC_017331        | *Staphylococcus aureus* subsp. *aureus* TW20 |             |
| 44 | NC_007793        | *Staphylococcus aureus* subsp. *aureus* USA300_FPR3757 |             |
| 45 | NC_010079        | *Staphylococcus aureus* subsp. *aureus* USA300_TCH1516 |             |
| 46 | NC_016912        | *Staphylococcus aureus* subsp. *aureus* VC40 |             |
| 47 | NC_022604        | *Staphylococcus aureus* subsp. *aureus* Z172 |             |
| 48 | NC_017341        | *Staphylococcus aureus* subsp. *aureus* strain JKD6008 |             |
| 49 | NC_009641        | *Staphylococcus aureus* subsp. *aureus* strain Newman |             |
| 50 | NC_012121        | *Staphylococcus carnosus* subsp. *carnosus* TM300 |             |
| 51 | NC_004461        | *Staphylococcus epidermidis* ATCC_12228 |             |
| 52 | NC_002976        | *Staphylococcus epidermidis* RP62A |             |
| 53 | NC_007168        | *Staphylococcus haemolyticus* JCSC1435 |             |
| 54 | NC_013893        | *Staphylococcus lugdunensis* HKU09-01 |             |
| 55 | NC_017353        | *Staphylococcus lugdunensis* N920143 |             |
| 56 | NC_022737        | *Staphylococcus pasteuri* SP1 |             |
| 57 | NC_017568        | *Staphylococcus pseudintermedius* ED99 |             |
| 58 | NC_014925        | *Staphylococcus pseudintermedius* HKU10-03 |             |
| 59 | NC_007350        | *Staphylococcus saprophyticus* subsp. *saprophyticus* |             |
| 60 | NC_020164        | *Staphylococcus warneri* subsp. *warneri* SG1 |             |

PCR primers: The primers used in the study are summarized in the table below:

Table 2. Primer used for detection of virulence genes.

| Virulence factor | Gene | Primer Sequence (5' to 3') | Amplicon size (bp) | Reference |
|------------------|------|---------------------------|-------------------|-----------|
| Intracellular adhesin | icaA | GATTATGTAATGTGCTTGGA ACTACTGCTGCGTTAATAAT | 770 | Peacock et al., 2002 |
| Putative adhesin | sdrE | AGTAAAATGTGTCAAAAGATTGGACTACCAGGCTATAT | 767 | Peacock et al., 2002 |
| Bone bound sialoprotein gene | bpo | AACTACATCTAGTACTCAACAACACG ATGTGCTTGAATAACACCATCATCT | 574 | Park et al., 2008 |
| *Staphylococcal enterotoxin A* | sea | GGTTATCAATGTGCGGGTGG CGGCACCTTTCTCTCTCGG | 102 | Saadati et al., 2011 |
| *Staphylococcal enterotoxin B* | seb | GTATGGGTGGTGAATCTGACGCCCAATAGTGCAGGTTAGG | 168 | Saadati et al., 2011 |
| *Staphylococcal enterotoxin C* | sec | CTCAGAAGACTGAACTCACAACCACTAGTTTGGATTTTACCTGCA | 276 | Saadati et al., 2011 |
| *Staphylococcal enterotoxin D* | sed | CCAATAGTAGAGAAAATAAAGGATTTTCGTTC | 278 | Saadati et al., 2011 |
| *Staphylococcal enterotoxin E* | see | CAGTACTCATAGATAAAGTTAAAACAAGC TAACTTACCGTGGACCTTCGC | 178 | Saadati et al., 2011 |
| *Staphylococcal enterotoxin Q* | seq | AATCTCTGGTCTGAACTGTAAGGATTTTCGTTC | 122 | Saadati et al., 2011 |
Table 2 contd.

| Toxin                        | Gene | Primer Sequence (5' to 3')                                                                 | Amplicon size (bp) | Reference                 |
|------------------------------|------|-------------------------------------------------------------------------------------------|--------------------|---------------------------|
| Toxic shock syndrome toxin 1 | tst  | ACCCCCTGTCCCCCTATATCATC TTTTCAGTATTTTGAACGCC                                                                                              | 326                | Alfatemi et al., 2014     |
| Exfoliative toxin A          | eta  | GCAGGGTTGATGATTGCATTTGAGATGCCG AGATGTTTGTCTGTTTGTGGT                                                                                        | 93                 | Alfatemi et al., 2014     |
| Exfoliative toxin B          | etb  | ACAAGCAAAAAGATACAGGCG GTTTTTGCTCTCTCTG                                                                                                       | 226                | Alfatemi et al., 2014     |
| Alpha hemolysin              | hla  | CTGATTACATCAGAGAAATCGATTGCG TCTTCCAGGCGCTTATTTTGCG                                                                                           | 210                | Alfatemi et al., 2014     |
| Beta hemolysin               | hlb  | GTGCCATCTACTGCAATAGTCGGTGTAGTAGCTACCTTCG                                                                                                      | 310                | Jarraud et al., 2002      |
| Delta hemolysin              | hld  | AAGAATTTTCTATGAATTAGGAAGGAG TGGTATTGGAATTGTTCTGTCAG                                                                                           | 111                | Alfatemi et al., 2014     |
| Gamma hemolysin              | hlg  | GCCAAACGGGTATTAGAAAATGCC CCAATGCAACGCAAGG                                                                                                     | 938                | Peacock et al., 2002      |
| Capsular polysaccharide 5    | cap5 | ATGACGATGAGGATAGCG CACCTAACATAAGGCAAG                                                                                                         | 881                | Salasia et al., 2004      |
| Capsular polysaccharide 8    | cap8 | ATGACGATGAGGATAGCG CACCTAACATAAGGCAAG                                                                                                         | 1148               | Salasia et al., 2004      |

Table 3. Primer used for detection of antibiotic resistance genes.

| Antibiotic resistance gene   | Gene | Primer Sequence (5' to 3')                                                                 | Amplicon size (bp) | Reference                 |
|------------------------------|------|-------------------------------------------------------------------------------------------|--------------------|---------------------------|
| Penicillin resistance gene   | blaz | ACTTCAACACCTGCTGCTTTC TGGCCCTTTTATTGCAACCC                                                                                                   | 173                | Martineau et al., 2000    |
| Erythromycin resistance gene | ermA | TATCTTATCGTGAGAAGGATT CTACACTTGCTAGGATGAA                                                                                                    | 139                | Martineau et al., 2000    |
| Erythromycin resistance gene | ermB | CTATCTGATTGAGAAGGATT GTTACTTCTTTTAGAGTGAA                                                                                                     | 142                | Martineau et al., 2000    |
| Erythromycin resistance gene | ermC | CTGTGATTACGATATAATTTC AATCTCTGCAGTATTTC                                                                                                       | 190                | Martineau et al., 2000    |
| Oxacillin resistance gene    | meCA | AAACAGGTAATAATAGCATTGGTAAAG GTGCTGTTAATTTTTTTGATTGAGG                                                                                          | 114                | Martineau et al., 2000    |
| Erythromycin resistance gene | msrA | TCCAATCATTGCAAAAATCT AATTCCCTCATTTGGGTTG                                                                                                      | 163                | Martineau et al., 2000    |
| Aminoglycoside resistance gene | aac(6')-aph(2") | TGGGAAGATGAAAGTTTTAGA CCTTACTTCAAAATTGCTG                                                                                                    | 174                | Martineau et al., 2000    |
| Tetracycline resistance gene | tetK | GTAGCGCAATAGGTAATGTGATTG ATGAGTAGCTACCTCAA                                                                                                      | 361                | Duran et al., 2012        |
| Tetracycline resistance gene | tetM | AGTGGACACGTATTTGCTACAA CATATGCTTTGCGGTCG                                                                                                        | 159                | Duran et al., 2012        |
| Erythromycin resistance gene | msrB | TATGATATCAATAATTTGCAATACATGGAAGTTGAGTCTG                                                                                                       | 595                | Momtaz et al., 2013       |

PCR amplification: In silico PCR amplification was done in the website http://insilico.ehu.es/PCR/ (San Millan et al., 2013; Bikandi et al., 2004).

PFGE digestion: PFGE digestion and construction of the dendrogram was done in the website http://insilico.ehu.es/digest/. The enzyme used for the digestion was SgrAl and recognition sequence was CR/CCGG_YG (San Millan et al., 2013; Bikandi et al., 2004).
Results and Discussion

In the present study, in silico PCR amplification detected eighteen virulence genes by using gene specific primer. Capsular polysaccharides are important virulence factors in the pathogenesis of staphylococcal infection. According to O'Riordan (2004), they persist on mucosal surface and promote bacterial colonization. In this study, it was found that 40% (n=24) isolates had the cap5 locus with 881 bp gene product, while 31.67% (n= 19) isolates had the cap8 locus with 1148 bp gene product (Figure 1). So, the cap5 locus was more prevalent than that of cap8. Na'was et al. (1998) reported that type 5 serotype was predominant among MRSA (Methicillin-resistant Staphylococcus aureus) isolates. Luong et al. (2002) demonstrated that capsular polysaccharides, type 5 and 8 are clinically more prevalent and have been used as targets for vaccine development.

Figure 1. Prevalence of Capsular polysaccharides.

Another investigation was also carried out to find the prevalence of staphylococcal enterotoxin, toxic shock toxin, exfoliative toxins, hemolysin, adhesion and bone bound sialoprotein genes. Staphylococcal enterotoxin is responsible for food poisoning and they disrupt water and electrolyte balance in the small intestine (Sheahan et al., 1970; Sullivan, 1969). Results revealed that (Figure 2) 26.67% (n=16) of the isolates were positive for sea, 20% (n=12) of the isolates were positive for seq, 6.67% (n=4) of the isolates were positive for both seb and sec. Only the isolate Staphylococcus aureus M1 was seen to harbour the sed gene (1.67%) and none was positive for see. Isolates harbouring the staphylococcal enterotoxin (SE) gene indicated the toxigenic and pathogenicity of the isolates (Push et al., 2016). Pinchuk et al. (2010) found that staphylococcal enterotoxins (SEA to SEE) were mainly responsible for staphylococcal food poisoning. Besides, Staphylococcus strains producing exfoliative toxin (ETs) or toxic shock syndrome toxin (TSST-1) has been shown to be an important clinical implication (Becker et al., 1998). Out of the 60 isolates analyzed, only 3 (Staphylococcus aureus strain Mu50, Staphylococcus aureus subsp. aureus Mu3, Staphylococcus aureus subsp. aureus N315) were positive for tst gene having the prevalence 5%. Alfatemi et al. (2014) reported earlier that the frequency of the tst gene was 11.64% in Staphylococcus spp. which is close the analyzed value. Study regarding eta or etb genes revealed that none of the isolates had these genes indicating no association with staphylococcal peeling skin syndrome.

Figure 2. Prevalence of Staphylococcal toxin genes.

Hemolysin gene helps bacteria to invade host tissue (Lowy, 2000). The alpha, beta, delta and gamma hemolysin toxins are coded by hla, hlb, hld, and hlg genes, respectively. Among 60 isolates, 38 (63.33%) harboured a 210 bp amplicon for hla gene. Forty-five isolates (75%) harboured 111 bp PCR amplicon for hld gene. Out of 60 isolates, 9 (15%) were positive for the PCR amplicon of 310 bp for hlb gene and 7 (11.67%) were positive for the amplicon of 938 bp for hlg (Figure 3). Li et al. (2015) reported that food poisoning outbreaks in China were caused by hla and hld genes.

The icaA operon is essential for capsular polysaccharide synthesis and is a virulence marker of orthopedic infections (Arciola et al., 2003). The icaA gene is also required for biofilm formation (Cramton et al. 1999). Forty-eight isolates (80%) carried the icaA gene and showed the PCR amplification
product of 770 bp. The bbp gene was responsible for hematogenous tissue infection (Tristan et al., 2003). It had PCR amplification product of 574 bp and was available in only 3 isolates (Staphylococcus aureus subsp. aureus 55/2053, Staphylococcus aureus subsp. aureus MRSA252, Staphylococcus aureus subsp. aureus TCH60). The prevalence of bbp gene was 5%. The present study showed that icaA gene was detected at higher level than bbp gene. This gene enhances the adherence of staphylococci to the host cells. These findings are in line with Park et al. (2008). The sdrE genes are associated with bone infections and present study found no sdrE positive isolates (Figure 4).

Antibiotic resistance makes Staphylococcus spp. to survive in the hostile environment and contribute to the outbreak of staphylococcal infections (Kumar et al., 2009). β-lactamase production in staphylococci is encoded by blaZ gene. Twenty-three samples (38.33%) had the blaZ gene. The incidence of penicillin resistance found in the present study shows similar trend with Adwan et al. (2014). Erythromycin resistance is developed by alteration of 23S rRNA, which is a common binding site of macrolide, lincosamides and streptogramin B antibiotics. This modification is done by rRNA erm methylase (Sutcliffe et al., 1996). Twenty-three of the 60 samples had the ermA gene with the 139 bp amplicon. None of the isolates were positive for ermB gene. Out of the 60 isolates analyzed, only 2 (Staphylococcus aureus subsp. aureus CN1 and Staphylococcus carnosus subsp. carnosus TM300) were positive for ermC. The 190 bp gene product of ermC was present in 3.33% isolates. Nicola et al. (1998) and Westh et al. (1995) observed that erythromycin resistant S. aureus contained higher amount of ermA, no ermB and lower level of ermC. This is in agreement with the study of Martineau et al. (2000).

Lina et al. (1999) demonstrated that coagulase-negative staphylococci contained higher amount of msrA gene. Only isolates Staphylococcus aureus subsp. aureus 11819-97, Staphylococcus aureus subsp. aureus TW20 and Staphylococcus haemolyticus JCSC1435 harboured the msrA gene and one isolate (Staphylococcus haemolyticus JCSC1435) had the msrB gene. The mecA gene is responsible for resistance to methicillin and β-lactam antibiotics. It is usually expressed under antibiotic pressure. A total of 39 of the 60 samples had the mecA resistance gene with 174 bp amplicon product. Prevalence of aac(6')-aph(2') gene was 15%. The tetM gene and tetK genes were found in 18.33% and 11.67% isolates, respectively (Figure 5).
constructed in the website. Isolates were able to be grouped into 23 genotypes at 50% similarity coefficient (Figure 7). Onasanya et al. (2003) reported two major groups of *Staphylococcus aureus* at 50% similarity coefficient, while 12 different subgroups were obtained at 100% similarity coefficient. Genotype 7 was more prevalent (20%) followed by genotype 8 and 9 (10%) (Figure 6).

![Figure 6. Prevalence of Genotypes.](image)

![Figure 8. Distribution of cap5 and cap8 genes within genotypes.](image)

![Figure 9. Distribution of sea and seb genes within genotypes.](image)
Figure 7. Phylogenetic diversity of Staphylococcus spp. identified by PFGE.
Virulence genes mentioned in Table 2 had been analyzed in the present study. All genotypes were found to carry either cap5 or cap8 locus except genotype 3, 4, 5, 6, 22 and 23 (Figure 8). The cap5 locus was abundant in genotype 9, 10 and 18 (100%). On the other hand, the cap8 locus was prevalent in genotype 2, 11, 12, 13, 14, 17 and 20 (100%). Only genotype 16 and 7 carried both cap5 and cap8 locus. The presence of cap5 and cap8 locus in different genotypes indicates the increased chances of pathogenicity. From the graphical presentation of sea and seb gene (Figure 9), it was found that sea gene was more prevalent than seb gene among the genotypes. Both of them were not present in same genotype. Genotype 11 and 12 carried the highest number sea gene (100%). The seb gene was present in only genotype 7, 15 and 19.

In addition, the sed gene was present only in genotype 9 displaying the prevalence 16.67% (Figure 10). The availability of sec gene was 50% in genotype 8, 16 and 17 and rest of the genotypes contained no sec or sed gene. In the same time, the tst positive isolates were present in genotype 8 (50%) (Figure 11). The seq gene was more prevalent in genotype 11 and 12 followed by genotype 15, 17, 13, 9 and 7. Both hla and hlb genes were present in genotype 7, 9, 15, 16 and 17 (Figure 12). The hlb gene was abundant (100%) in genotype 16. The hla genes were more prevalent in genotype 1, 7, 8, 9, 11, 13, 14, 16 and 18 (100%).

![Figure 10. Distribution of sec and sed genes within genotypes.](image1)

![Figure 11. Distribution of seq and tst genes within genotypes.](image2)
It was also observed that genotype 1, 2 and 19 carried both hld and hlg genes (Figure 13). Lower level of hld gene prevalence was encountered in genotype 13 and 21 (50%). Seventy five percent isolates in genotype 15 harboured the hld gene. Besides, icaA gene was found more prevalent than bhp gene among the genotypes (Figure 14). Only the genotype 1 and 2 carried both icaA and bhp genes.

Study regarding the antibiotic resistance genes mentioned in Table 3 revealed that mecA gene was much more prevalent (Figure 15) and only absent in genotype 3, 6, 16, 22 and 23. The present study was also found that blaZ gene was present in 38.33% of the isolates. The blaZ gene was more abundant in genotype 1, 2, 3, 11, 12, 14 and 22 (100%). Out of 23 genotypes, 10 genotypes harboured no blaZ gene (Figure 16). Besides, the ermA gene was prevalent in higher level in genotype 11, 12, 14 and 20 (100%) (Figure 17). Genotype 5 contained both ermA and ermC genes and their prevalence within the genotypes was 50%. In case of msrA and msrB genes (Figure 18), genotype 21 harboured both of these genes and their prevalence within the genotypes were 50%. Other genotypes harboured no msrB gene.
Figure 15. Distribution of meca genes within genotypes.

Figure 16. Distribution of blaz genes within genotypes.

Figure 17. Distribution of ermA and ermC genes within genotypes.

Figure 18. Distribution of msrA and msrB genes within genotypes.
Figure 19 presents the distribution of *tetK* and *tetM* genes within genotypes. Genotype 8, 11 and 19 harboured both *tetM* and *tetK* genes. Genotype 11 carried same number of *tetM* and *tetK* genes (100%). The prevalence of *tetM* genes in genotype 12, 14 and 22 was 100%. Besides, the distribution of *aac (6')-aph (2")* genes within genotypes (Figure 20) found that genotype 11 contained the highest number of this gene. The prevalence of *aac(6')-aph (2")* gene in genotype 5, 13, 21 and 22 was 50%.

**Conclusion**

The *icaA* gene, accountable for intracellular adherence, was detected in 80% of the isolates. Hemolysin gene (*hla*) was also found in 63.33% of the isolates. The *cap5* locus was detected in 40% of the isolates. Sixty five percent isolates harboured the *mecA* resistance gene. Both *blaZ* and *ermA* gene were detected in 38.88% of the isolates. No virulence genes were detected in genotype 3, 4, 5, 6, 22 and 23. Genotype 1 was considered more virulent followed by genotype 11. Genotype 1 harboured six virulent genes and all hemolysin genes were present except *hla* gene. Genotype 11 harboured six antibiotic resistance genes except *msrA*, *msrB*, *ermB* and *ermC*. Genotype 6 and 16 carried no antibiotic resistance gene. Thus, this study has provided epidemiological data to study the characteristics of *Staphylococcus* strains and the virulence factors associated with infection.

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