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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 *icaA* 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

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ribosomal modification of widely disseminated *tetM* or *tetK* gene and *tetK* is found most often in *Staphylococcus aureus* (Trzcinski *et al.,* 2000; Schmitz *et al.,* 2001). *In silico* analysis helps to extract useful information from vast amount of data. Recently, numerous *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 *in silico* investigation of 60 *Staphylococcus* spp. and predict the virulence and resistance profile of this genus.

Materials and Methods

Strains used in the study: Isolates used in this study are summarized in Table 1.

Table 1. Name of the isolates.

Serial Number	Isolate Name
1	NC_017340 *Staphylococcus aureus* 04-02981
2	NC_018608 *Staphylococcus aureus* 08BA02176
3	NC-021670 *Staphylococcus aureus* Bmb9393
4	NC_021554 *Staphylococcus aureus* CA-347
5	NC_021059 *Staphylococcus aureus* M1
6	NC_007622 *Staphylococcus aureus* RF122
7	NC_002758 *Staphylococcus aureus* strain Mu50
8	NC_017451 *Staphylococcus aureus* subsp. *aureus* 11819-97
9	NC_ 022113 *Staphylococcus aureus* subsp. *aureus* 55/2053
10	NC_022222 *Staphylococcus aureus* subsp. *aureus* 6850
11	NC_017673 *Staphylococcus aureus* subsp. *aureus* 71193
12	NC_022226 *Staphylococcus aureus* subsp. *aureus* CN1
13	NC_002951 *Staphylococcus aureus* subsp. *aureus* COL
14	NC_017343 *Staphylococcus aureus* subsp. *aureus* ECT-R 2
15	NC_017337 *Staphylococcus aureus* subsp. *aureus* ED133
16	NC_013450 *Staphylococcus aureus* subsp. *aureus* ED98
17	NC_017763 *Staphylococcus aureus* subsp. *aureus* HO 5096 0412
18	NC_009632 *Staphylococcus aureus* subsp. *aureus* JH1
19	NC_009487 *Staphylococcus aureus* subsp. *aureus* JH9
20	NC_017338 *Staphylococcus aureus* subsp. *aureus* JKD6159
21	NC_017349 *Staphylococcus aureus* subsp. *aureus* LGA251
22	NC_016928 *Staphylococcus aureus* subsp. *aureus* M013
23	NC_002952 *Staphylococcus aureus* subsp. *aureus* MRSA252
24	NC_016941 *Staphylococcus aureus* subsp. *aureus* MSHR1132
25	NC_002953 *Staphylococcus aureus* subsp. *aureus* MSSA476
26	NC_003923 *Staphylococcus aureus* subsp. *aureus* MW2
27	NC_009782 *Staphylococcus aureus* subsp. *aureus* Mu3
28	NC_002745 *Staphylococcus aureus* subsp. *aureus* N315
29	NC_007795 *Staphylococcus aureus* subsp. *aureus* NCTC 8325
30	NC_017333 *Staphylococcus aureus* subsp. *aureus* S0385
31	NC_022443 *Staphylococcus aureus* subsp. *aureus* SA40
32	NC_022443 *Staphylococcusaureus* subsp. *aureus* SA957
33	NC_020529 *Staphylococcus aureus* subsp. *aureus* ST228 complete genome, isolate 10388
34	NC_020564 *Staphylococcus aureus* subsp. *aureus* ST228 complete genome, isolate 10497

Table 1 contd.

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* str. JKD6008
49	NC_009641 *Staphylococcus aureus* subsp. *aureus* str. 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_0173533 *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* 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*	AGTAAAATGTGTCAAAAGA TTGACTACCAGGCTATAT	767	Peacock *et al*., 2002
Bone bound sialoprotein gene	*bbp*	AACTACATCTAGTACTCAACAACAG ATGTGCTTGAATAACACCATCATCT	574	Park *et al.,* 2008
Staphylococcal enterotoxin A	*sea*	GGTTATCAATGTGCGGGTGG CGGCACTTTTTTCTCTTCGG	102	Saadati *et al.,* 2011
Staphylococcal enterotoxin B	*seb*	GTATGGTGGTGTAACTGAGC CCAAATAGTGACGAGTTAGG	168	Saadati *et al.,* 2011
Staphylococcal enterotoxin C	*sec*	CTCAAGAACTAGACATAAAAGCTAGG TTATATCAAAATCGGATTAACATTATC	276	Saadati *et al.,* 2011
Staphylococcal enterotoxin D	*sed*	CCAATAATAGGAGAAAATAAAAG ATTGGTATTTTTTTTCGTTC	278	Saadati *et al.,* 2011
Staphylococcal enterotoxin E	*see*	CAGTACCTATAGATAAAGTTAAAACAAGC TAACTTACCGTGGACCCTTCAG	178	Saadati *et al.,* 2011
Staphylococcal enterotoxin Q	*seq*	AATCTCTGGGTCAATGGTAAGC TTGTATTCGTTTTGTAGGTATTTTCG	122	Saadati *et al.,* 2011

Table 2 contd.

Toxic shock syndrome toxin 1	*tst*	ACCCCTGTTCCCTTATCATC TTTTCAGTATTTGTAACGCC	326	Alfatemi *et al.*, 2014
Exfoliative toxin A	*eta*	GCAGGTGTTGATTTAGCATT AGATGTCCCTATTTTTGCTG	93	Alfatemi *et al.*, 2014
Exfoliative toxin B	*etb*	ACAAGCAAAAGAATACAGCG GTTTTTGGCTGCTTCTCTTG	226	Alfatemi *et al.*, 2014
Alpha hemolysin	*hla*	CTGATTACTATCCAAGAAATTCGATTG CTTTCCAGCCTACTTTTTTATCAGT	210	Alfatemi *et al.*, 2014
Beta hemolysin	*hlb*	GTGCACTTACTGACAATAGTGC GTTGATGAGTAGCTACCTTCAGT	310	Jarraud *et al.*, 2002
Delta hemolysin	*hld*	AAGAATTTTTATCTTAATTAAGGAAGGAG TGTTAGTGAATTTGTTCACTGTGTCGA	111	Alfatemi *et al.*, 2014
Gamma hemolysin	*hlg*	GCCAATCCGTTATTAGAAAATGC CCATAGACGTAGCAACGGAT	938	Peacock *et al.*, 2002
Capsular polysaccharide 5	*cap5*	ATG ACG ATG AGG ATA GCG CTC GGA TAA CAC CTG TTG C	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 TGACCACTTTTATCAGCAACC	173	Martineau *et al*., 2000
Erythromycin resistance gene	*ermA*	TATCTTATCGTTGAGAAGGGATT CTACACTTGGCTTAGGATGAAA	139	Martineau *et al*., 2000
Erythromycin resistance gene	*ermB*	CTATCTGATTGTTGAAGAAGGATT GTTTACTCTTGGTTTAGGATGAAA	142	Martineau *et al*., 2000
Erythromycin resistance gene	*ermC*	CTTGTTGATCACGATAATTTCC ATCTTTTAGCAAACCCGTATTC	190	Martineau *et al*., 2000
Oxacillin resistance gene	*mecA*	AACAGGTGAATTATTAGCACTTGTAAG ATTGCTGTTAATATTTTTTGAGTTGAA	174	Martineau *et al*., 2000
Erythromycin resistance gene	*msrA*	TCCAATCATTGCACAAAATC AATTCCCTCTATTTGGTGGT	163	Martineau *et al*., 2000
Aminoglycoside resistance gene	*aac(6')-aph(2")*	TTGGGAAGATGAAGTTTTTAGA CCTTTACTCCAATAATTTGGCT	174	Martineau *et al.*, 2000
Tetracycline resistance gene	*tetK*	GTAGCGACAATAGGTAATAGT GTAGTGACAATAAACCTCCTA	361	Duran *et al.*, 2012
Tetracycline resistance gene	*tetM*	AGTGGAGCGATTACAGAA CATATGTCCTGGCGTGTCTA	159	Duran *et al.*, 2012
Erythromycin resistance gene	*msrB*	TATGATATCCATAATAATTATCCAATC AAGTTATATCATGAATAGATTGTCCTGTT	595	Momtaz *et al.*, 2013

PCR amplification: *In silico* PCR amplification was done in the website http://insilico.ehu.eus/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 SgrAI 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.

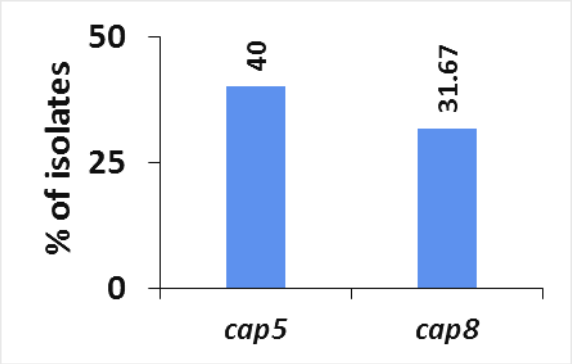

Figure 1. Prevalence of Capsular polysaccharides.

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 toxin 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.

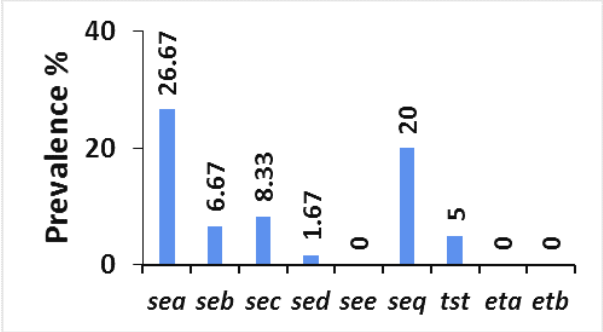

Figure 2. Prevalence of Staphylococcal toxin genes.

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).

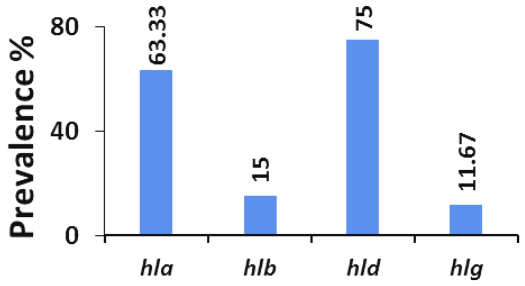

Figure 3. Prevalence of Hemolysin genes.

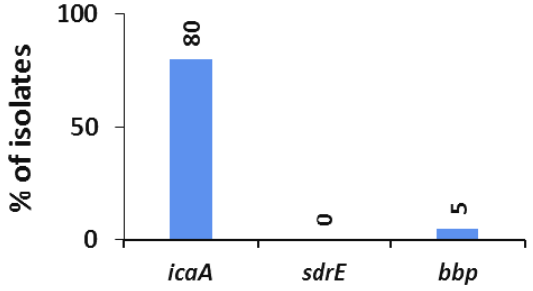

Figure 4. Prevalence of intracellular adhesin, putative adhesin and bone bound sialoprotein genes.

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).

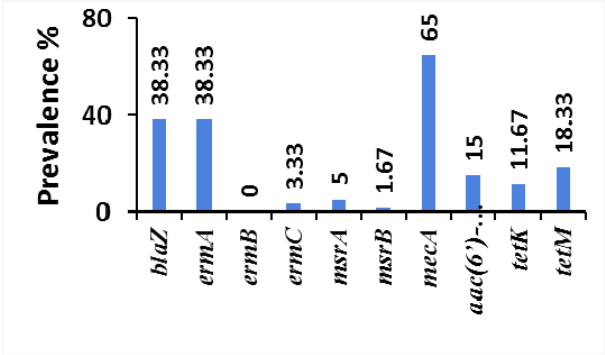

Figure 5. Prevalence of Antibiotic resistance genes.

The isolates were investigated by *in silico* pulsed field gel electrophoresis (PFGE), where fragments were obtained by SgrAI digestion. Dendrogram was

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).

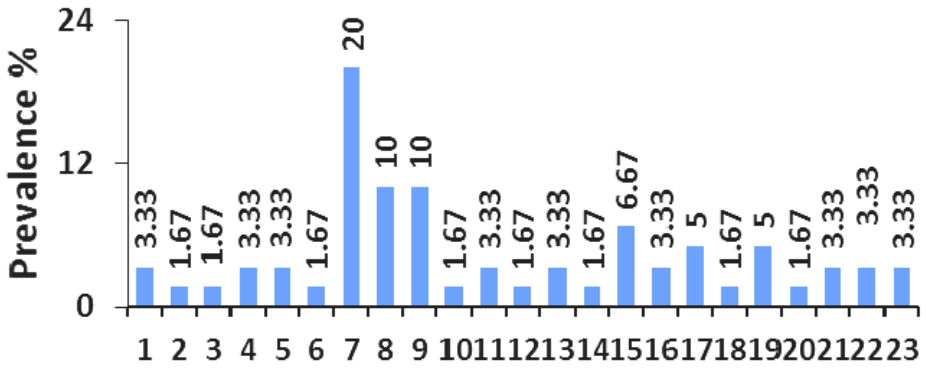

Figure 6. Prevalence of Genotypes.

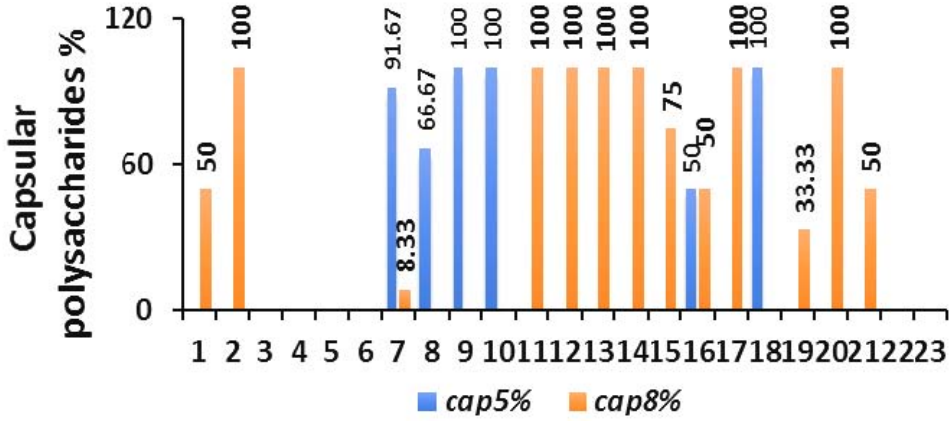

Figure 8. Distribution of *cap5* and *cap8* genes within genotypes.

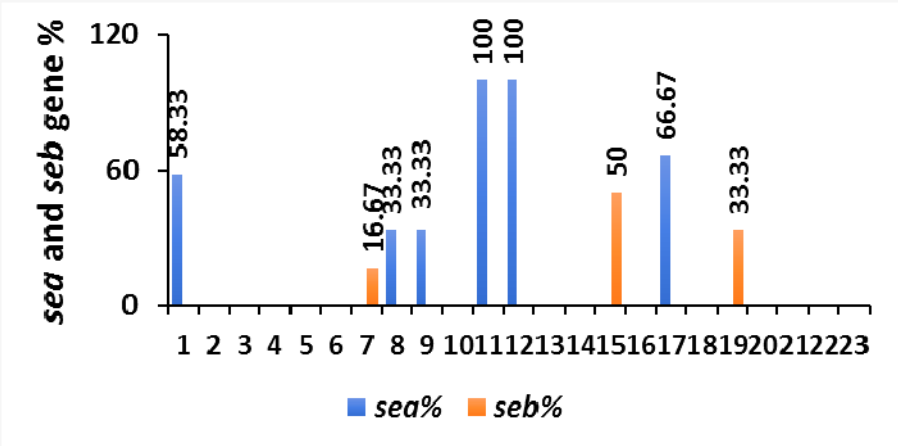

Figure 9. Distribution of *sea* and *seb* genes within genotypes.

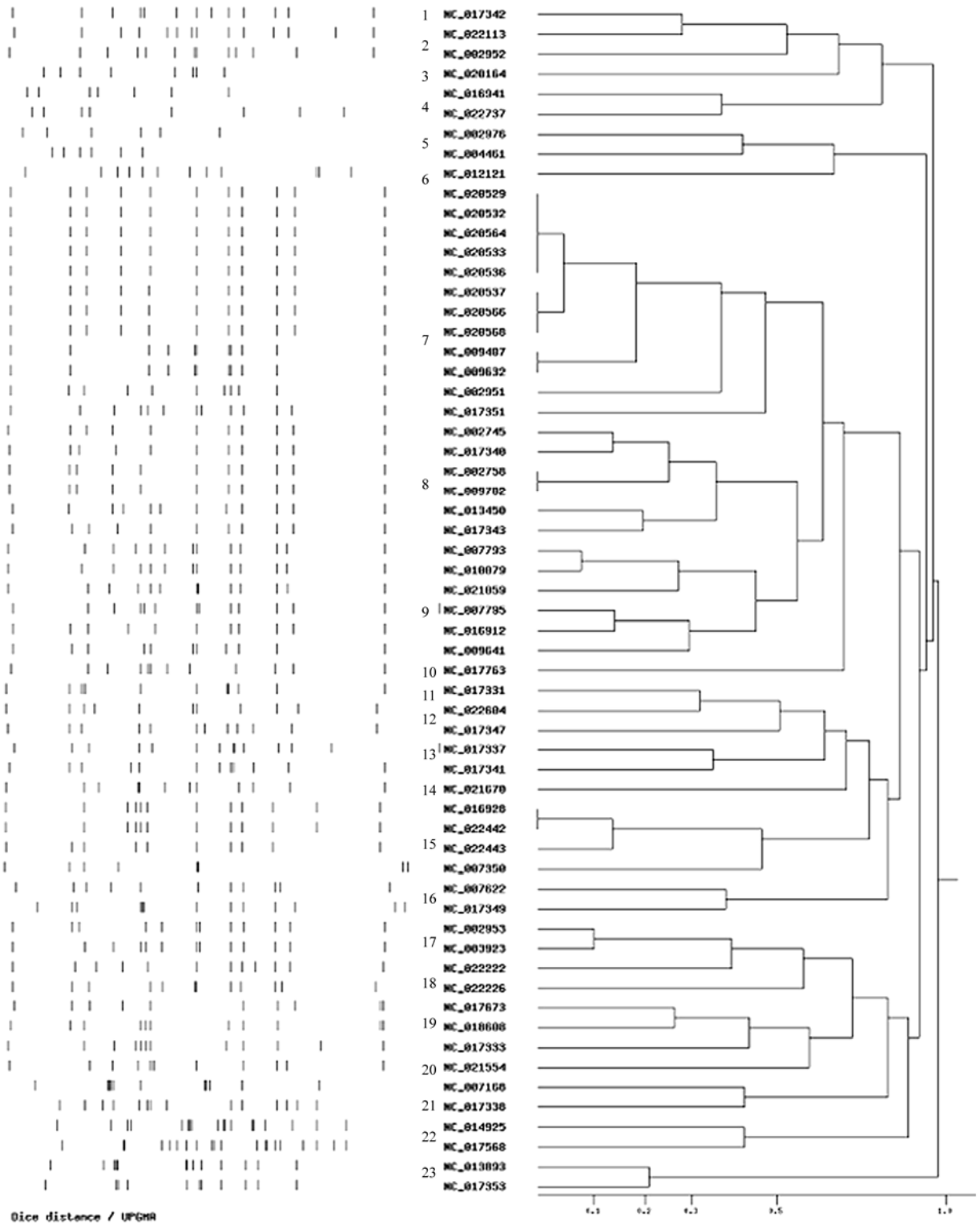

Figure 7. Phylogenetic diversity of *Staphylococcus* spp. identified by PFGE.

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%).

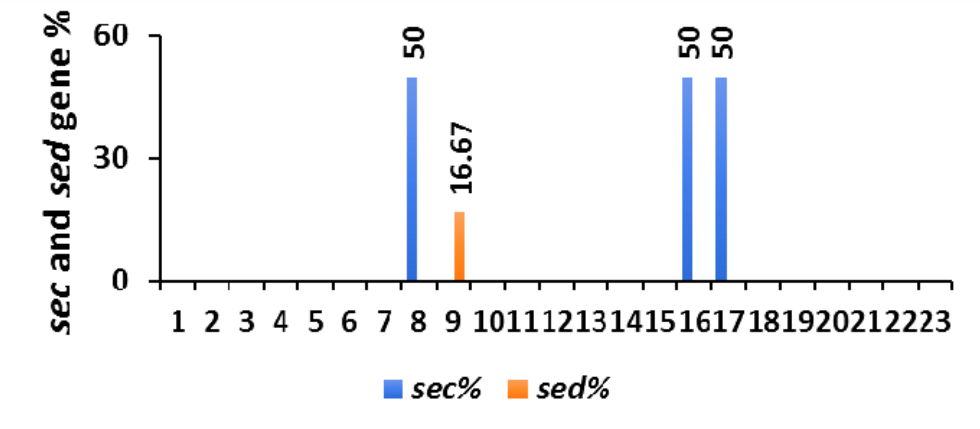

Figure 10. Distribution of *sec* and *sed* genes within genotypes.

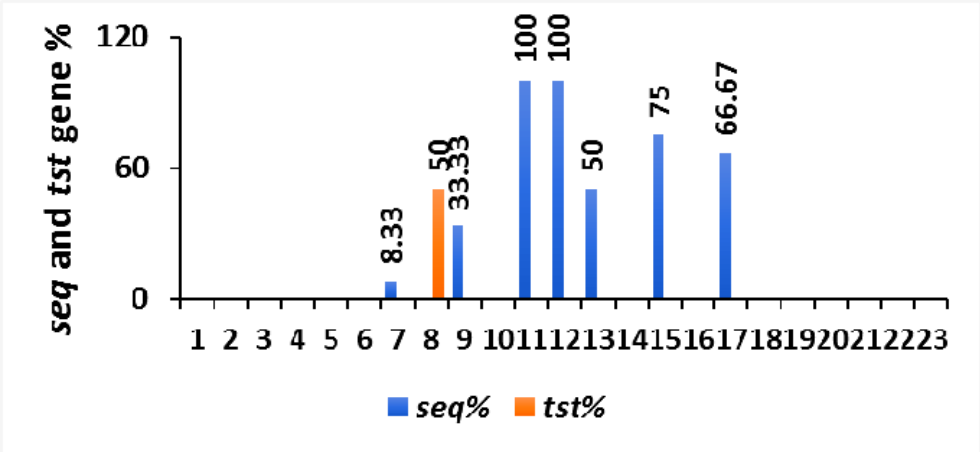

Figure 11. Distribution of *seq* and *tst* genes within genotypes.

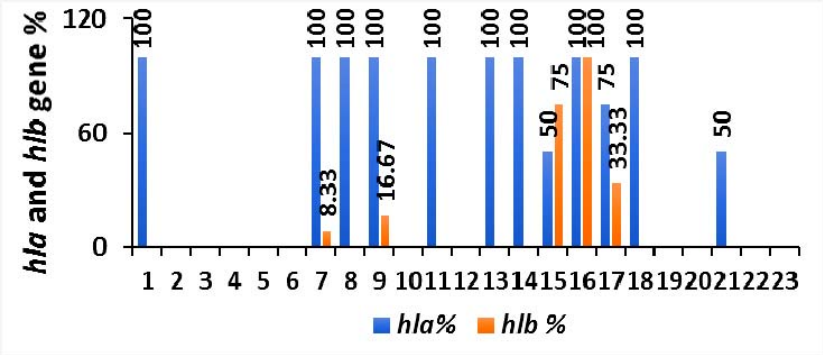

Figure 12. Distribution of *hla* and *hlb* genes within genotypes.

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 *bbp* gene among the genotypes (Figure 14). Only the genotype 1 and 2 carried both *icaA* and *bbp* 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.

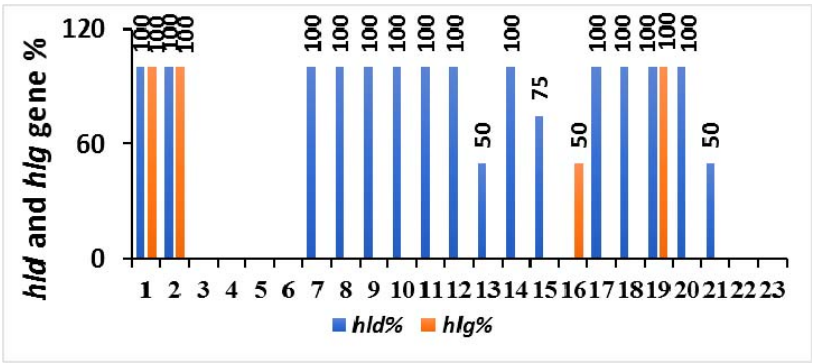

Figure 13. Distribution of *hld* and *hlg* genes within genotypes.

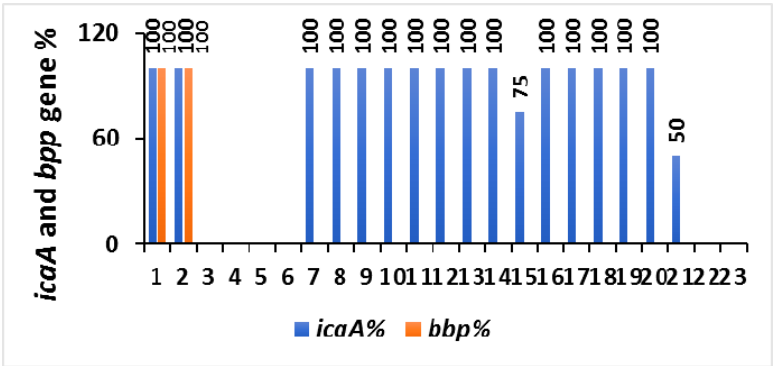

Figure 14. Distribution of *icaA* and *bbp* genes within genotypes.

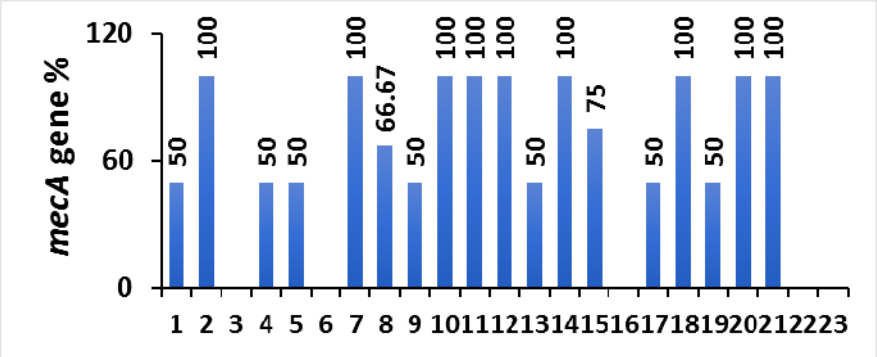

Figure 15. Distribution of *mecA* genes within genotypes.

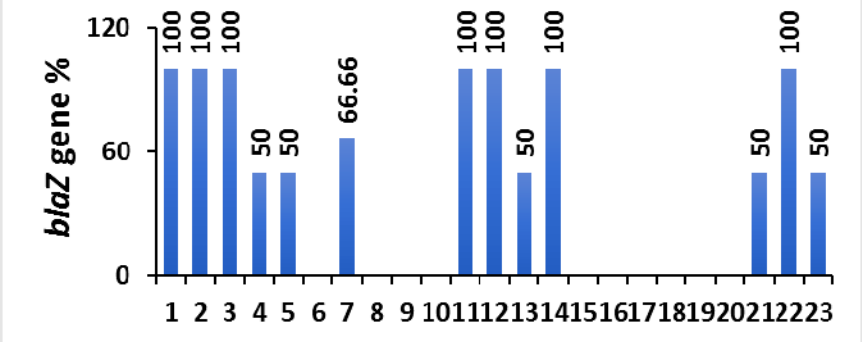

Figure 16. Distribution of *blaZ* genes within genotypes.

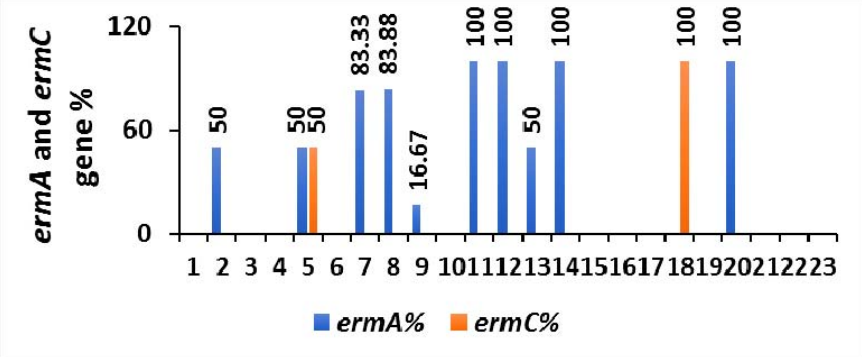

Figure 17. Distribution of *ermA* and *ermC* genes within genotypes.

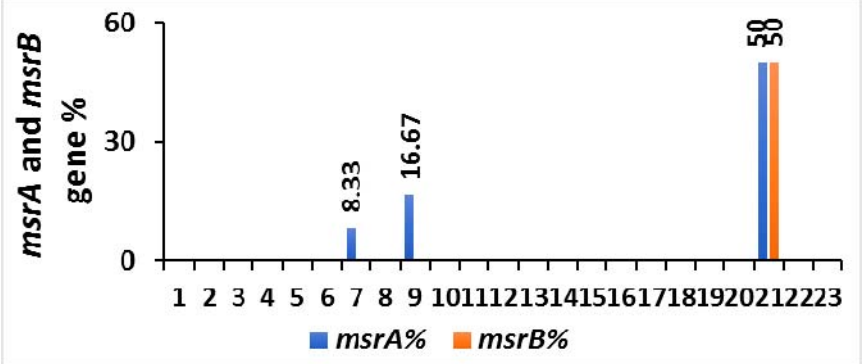

Figure 18. Distribution of *msrA* and *msrB* genes within genotypes.

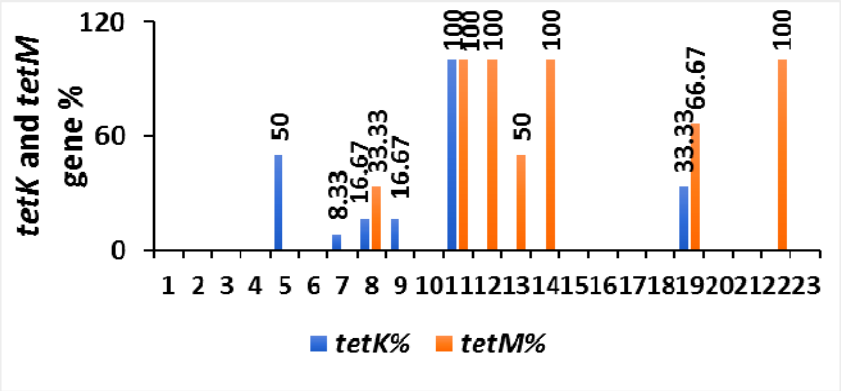

Figure 19. Distribution of *tetK* and *tetM* genes within genotypes.

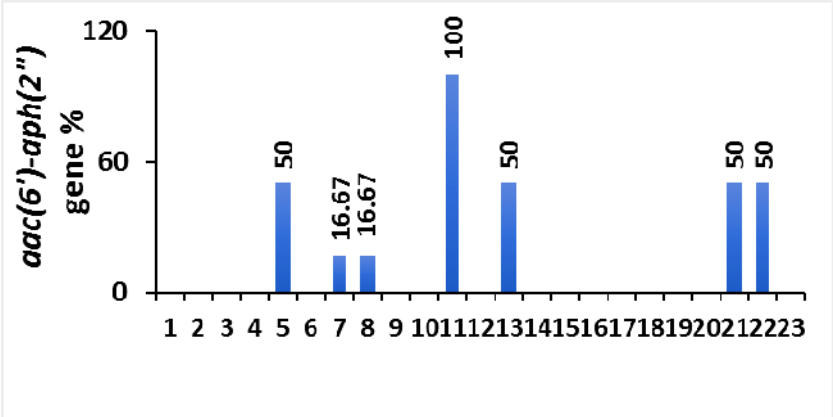

Figure 20. Distribution of *aac (6')-aph (2")* genes within genotypes.

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 *hlb* 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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