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ORIGINAL ARTICLE

Detection of Pathogenicity Island-encoding Virulence Genes of *Staphylococcus aureus* Isolated from Various Clinical Sources

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ABSTRACT

Background*: Staphylococcus aureus is an important pathogen that causes a wide range of diseases in humans and animals. S. aureus pathogenicity islands (SaPIs) have a growing family of mobile genetic elements (MGEs) in Staphylococci. MGEs transferred Horizontally play an important part in the evolution of the pathogenic bacteria (S. aureus). Several SaPIs carry staphylococcal enterotoxin and many toxin genes.* ***Objective:*** *The goal of this study is to screen the pathogenicity islands-encoding virulence genes of S. aureus, determine antimicrobial resistance pattern and evaluate the distribution of virulence genes among these isolates.* ***Methodology:*** *A total of 108 S. aureus clinical isolates were identified and antimicrobial sensitivity pattern for twelve antimicrobial agents from different classes was assessed. In addition, Polymerase chain reaction (PCR) was performed for all isolates to detect virulence genes encoding on SaPI. PCR products were purified by The Thermo Scientific GeneJET Gel Extraction Kit. Nucleotide sequencing analysis by using 3500 Genetic Analyzer Version 6.0, (Applied Biosystems™). Using Sanger method, sequences data were assembled by GeneMapper™ secondary analysis software. The data analysis was done by using CLC Sequence Viewer 8 (clc-sequence-viewer.software.informer.com). Alignment of sequences by BLAST search of DNA Data Bank was performed. Identification of ORFs (Open Reading Frame) by using ORF Finder.* ***Results****: Out of the 108 S. aureus clinical isolates, 69 isolates (63.88%) were Methicillin-resistance Staphylococcus aureus (MRSA), 24 isolates (22.22%) were Vancomycin-resistance Staphylococcus aureus (VRSA), and 15 isolates (13.88%) were Methicillin-sensitive Staphylococcus aureus (MSSA). Eight hundred sixty-two nucleotides product aligned with groEL gene that encode Chaperonin GroEL protein, and with grol gene that encode Heat shock protein chaperone GroEL. The sequence 708 bp aligned with int gene encodes Integrase, groEL gene, grol gene and ktrB gene (encodes Potassium uptake protein). The sequence 493 bp aligned with hys gene encodes Hyaluronate lyase enzyme and pLys gene encodes Polysaccharide lyase enzyme. The sequence 366bp and 340bp aligned with metQ gene that encode with permease (MetQ/NIpA transporter protein).* ***Conclusion****: Identification of Pathogenicity Island (SaPI) virulence genes can provide useful information for understanding the pathogenicity, drug resistance and horizontal genetic transfer that play a vital part within the evolution of S. aureus.*

INTRODUCTION

S. aureus is a Gram- positive bacterium that has been considered as a major human pathogen. This pathogen is implicated in both community-acquired and nosocomial infections with considerable morbidity[1].

*S. aureus i*s a high-priority pathogen responsible for 80% of hospital infections. The impact on human health in community and healing center settings has driven to intensive investigation of this organism over recent years[2]. The infections are either minor as skin, soft tissues and UT infection or more severe and even lethal such as endocarditis, meningitis, sepsis and toxic shock syndrome[3,4]. *S. aureus* has the capacity for resistance to many anti-microbials. This capacity is further increased by consistent development of new clones, making *S. aureus* serve as a "superbug" [5].

S. aureus carries genes encoding a variety of virulence factors including enterotoxins and exfoliative toxins, often making it responsible for many toxin-mediated diseases, including toxic shock syndrome (TSS) and staphylococcal foodborne diseases (SFD). These factors and toxins of *S.* aureus also affect the human immune system[6,7].

S. aureus produces a plenty of pathogenicity factors which facilitate attachment, colonization, cell–cell interactions, and tissue damage. The development of resistance to penicillin, methicillin, and more recently, vancomycin diminishes the effect of efficient antibiotics[8,9].

Recently, *S. aureus* acquired many resistance genes for different antimicrobial targets that complicate antibiotic therapy; literally, an arms race took place between the new antibiotic compounds and additional resistance modes[10].

S. aureus harbors MGEs (Mobile Genetic Elements) of different classes. These elements include staphylococcal cassette chromosomes, plasmids, bacteriophages, transposons and SaPIs (*Staphylococcus aureus* Pathogenicity Islands). They can transmit genetic information of evolutionary benefit, and of clinical relevance, i.e., genes encoding virulence factors or antibiotic resistance properties[10,11].

Thus, these mobile elements have played and continue to play an integral part within the evolution of *Staphylococci* as a pathogen. Modern reports support that virulence traits are transmitted horizontally. The existence of accessory genetic elements within a strain may influence the other mobile genetic elements[12]. It will be recalled that PIs are accessory genetic elements that contain pathogenicity genes, are bordered by directly repeated sequences, can be deleted and may have integrase-like genes[13]

Several studies reported that SaPIs having a high diversity of genetic elements integrated at specific chromosomal sites[14]. Upon transduction to a recipient organism, SaPI1 integrates by the classical Campbell mechanism into the *att* site for which the SaPI1-coded integrase is necessary. Islands with different *att* sites have dissimilar integrases, which determines the integration site in the genome[15].

Thus, phages and SaPIs determine virulence and host specificity of *S. aureus*. *S. aureus* bacteriophages are the tailed bacteriophages with double-stranded DNA genomes[16]. Upon transducing their own genes, prophages are also capable of transducing additional bacterial DNA. Many strains of *S. aureus* carry more than one prophage[10].

Therefore, this study aims to detect pathogenicity island-encoding virulence genes in *S. aureus* isolates. PCR identification, sequence data analysis and determination of antimicrobial susceptibility pattern.

METHODOLOGY

Isolation and identification of *S. aureus* isolates

A total of 140 non-duplicate Gram-positive *Staphylococcus* isolates were obtained from different Mansoura University Hospitals, Dakahlia governorate, Egypt with the approval of the Ethics committee in the Faculty of Pharmacy, Mansoura University**.** Identification of *S. aureus* isolates by using the biochemical standard methods[17,18]. The identified *S. aureus* isolates were collected from different clinical sources including wound, blood, urine, throat and burn. Isolates were subsequently stored in Mueller-Hinton broth (MHB) containing 20% (v/v) glycerol at -80°c.

Antimicrobial susceptibility testing

Susceptibility to different antimicrobials was done by Kirby-Bauer disc diffusion technique as recommended by the clinical laboratory standard institute (CLSI)[19]. Twelve antimicrobial discs of various categories were used including; imipenem (10 µg), Erythromycin (15 µg), Oxacillin (15µg), Cefepime (30µg) Gentamicin (10µg), Ceftriaxone (30µg), amoxicilin/Clavulanicacid (20/10µg), Cephalexin (30 µg), vancomycin (30µg), Ciprofloxacin (5µg), Levofloxacin (10µg), Piperacillin/tazobactam (110/10 µg); all discs were supplied from Thermo Scientific™ Oxoid™. Results were interpreted according to breakpoints of the clinical and laboratory standard[19].

Identification of MRSA by disc diffusion method

An overnight bacterial suspension was diluted to 0.5 MacFarland turbidity standards and streaked by swabbing on the surface of MHA supplemented with 4% NaCl. The plates were allowed to dry for few minutes and then Oxacillin discs were placed on the dried surface of the inoculated Mueller-Hinton agar (MHA). The plates were incubated for 24 hours at 37°C. Zone of inhibition around the disc was measured with the help of a scale and compared with CLSI zone size interpretative chart. It considered as sensitive, if zone size was ≥13 mm, intermediate if the zone size was 11-12 mm and resistant if the zone size was < 10mm (CLSI, 2005).

Detection the Minimum Inhibitory Concentration (MIC) of vancomycin

By standard agar dilution method, MIC of vancomycin was calculated and interpreted according to recommendations of CLSI[19]. Agar plates were prepared by serial dilution of vancomycin in MHA till reach the concentration of; 6µg/ml of vancomycin. Each tested isolate was inoculated in each MHA plate supplemented with different vancomycin concentration, Another MHA plate without antibiotic as a negative control. All plates were incubated overnight at 37 °C and examined for the bacterial growth. According to CLSI guidelines, the MICs of 2 µg/ml, 4–8 µg/ml and 16 µg/ml or more are identified as susceptible, intermediate and VRSA, respectively.

Molecular detection of some Staphylococcal Pathogenicity Island-encoding virulence genes

DNA extraction:

A rapid DNA extraction method (colony PCR) was performed. In PCR tubes, five fresh colonies were suspended in 100µl of sterile, nuclease-free water. The

product was boiled in the thermocycler at 95°C / 10 min, after which it was stored at -20°C[20].

Detection of some virulence genes:

The virulence genes of SaPIs were detected through PCR amplification, using specific primers listed in table (1). For a total volume of 25 µl containing 12.5 µl of PfuTurbo DNA Polymerase, 1 µl DNA extract, 1µl of forward primer (10 µM), 1 µl of reverse primer (10 µM) and 9.5 µl of nuclease free water. PCR was done by PCR thermal cycler (Model TC96K, AccuLab®, USA). The amplification includes; initial denaturation at 95 °C / 2 min, followed by 30 cycles of denaturation (95 °C / 30s), annealing according to temperature specified in (Table 1) for 30s and extension (72 °C / 6 min), and the reaction was ended by final extension step at 72 °C / 10 min. Negative control was included in all PCR assays.

Table 1: List of oligonucleotide primers and annealing temperature used in this study

Primers	Sequences (5' to 3')	Sites	Annealing temperature
SaPI4S	ATGGCAGGTGGACCAAGAAGAGGCGGACGTCGTCG	412747–412781	67 °C
SaPI4AS	CATTCTAGTAGACACCTCTGTCGTTTTAACATAAG	416278–416312	
SaPIbovS	GTTATGGGAGACTACCGTACGTATGATCACACAG	435843–435876	60 °C
SaPIbovAS	GTTGCTATTTATGGTGGCCTTTCAAGCAATATTAG	439465–439499	
SaPImw2S	CATCATGTTGGTTGTTTATCACTTTTCATGATGC	844043–844076	55 °C
SaPImw2AS	GAAAGAAAAAGAAAAGCAAAAAGATGACGACCACAG	845724–845759	
SaPI3externalS	GCTGTAGAAAGTGCGAAAGATAATCCTTATGC	877986–878017	56 °C
SaPI3externalAS	GCGAAATAGATTCATCATTAACTTGAGGGAG	878357–878387	
SaPI2S	AAATTACCGCTACACTAAAGGACAAATTTATC	2056106–2056137	52 °C
SaPI2AS	GGTATCGTTGATCCAACTAAAGTAACACGCTCAGC	2072459–2072493	
etdS	CGTTGTGAAAGATAATGAATCGGTTTGGGCTG	2275743–2275774	57 °C
etdAS	CTAAAGTGGCAGTAACAAATGATTTTAGTTTC	2278259–2278290	

Visualization of the amplicons by electrophoresis was done using 2% agarose gel stained with ethidium bromide and compared with a 100 bp DNA ladder. PCR products were purified by using The Thermo Scientific GeneJET Gel Extraction Kit.

Nucleotide sequence analysis:

Nucleotide sequencing of amplicons for both strands by 3500 Genetic Analyzer for Fragment Analysis (Applied Biosystems™) Version 6.0. It includes 3500 Series Data Collection Software, a Dell Workstation and monitor, reagent kits for system qualification. Sequences data were assembled by GeneMapper™ secondary analysis software. The data analysis was done by using CLC SequenceViewer8 (clc-sequence-viewer.software.informer.com). The data sequences were converted to fasta file format and compared with sequences of *S. aureus* standard strains to identify close relative. Alignment of sequences by BLAST search of DNA Data Bank (https://blast.ncbi.nlm.nih.gov/Blast.cgi.). Identification of ORFs (Open Reading Frame) was performed using ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder/).

Dendrogram and phylogenetic analysis:

A dendrogram shows the relationship of virulence genes among the isolates. Data have been clustered by the UPGMA (http://genomes.urv.cat/UPGMA/UPGMAboot_v12.cgi) Dice coefficient is the similarity index used to compare between the set of variables, the similarity at ≥ 70%.

Phylogenetic Neighbor-joining tree was constructed with DNA sequences of SaPI virulence genes using CLC Sequence Viewer 8.0. Neighbor-joining tree was constructed to analyze nucleotide sequence similarity and determine the relatedness between them.

Statistical analysis:

To collect descriptive results, data were tabulated, coded, and evaluated using the computer software. Statistical analysis was done: https://www.jmp.com/jmp-trial-win.

Ethical considerations:

The ethics committee of the Faculty of Pharmacy at EL-Mansoura University, Egypt, gave their clearance (2023-87). All of the participants in this study gave their informed permission.

RESULTS

Isolation and identification of isolates

In the present study, 108 isolates out of 140 specimens were identified as *S. aureus* by Gram staining, D-mannitol fermentation, catalase and coagulase production. The identified *S. aureus* isolates were collected from different clinical sources including wound 36.1% (39/108), blood 25% (27/108), sputum (11/108), urine 9.2% (10/108), throat swabs 4.6% (5/108), burn 3.7% (4/108), nose 3.7% (4/108), prostatic2.7% (3/108), chest 1.8% (2/ 108), oral swab 108% (2/ 108) and rectal swab 0.9% (1/108) as shown in figure 1.

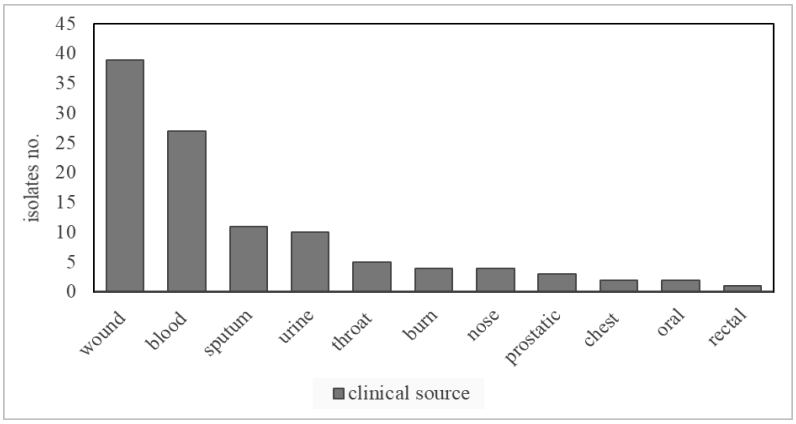

Fig. 1: Distribution of *S. aureus* isolates among various clinical sources.

Antimicrobial susceptibility testing:

Variable resistance patterns of 108 isolates were displayed against the used antibiotics (Figure 2). Cephalexin was inactive against all tested strains (108/108), followed by cefepime 90.74% (98/108), Oxacillin 86.11% (93/108), Piperacillin/tazobactam 82.41% (89/108), Ceftriaxone 79.63% (86/108), and Amoxicillin/Clavulanic acid 78.7% (85/108). A moderate resistance was observed towards Gentamycin (44.44%;48/108), Erythromycin (37.96%;41/108), and Ciproflaxacin 25.93% (28/108). Whereas, lower for vancomycin, levofloxacin, and imipenim as 23.14%, 19.44% and 13.88% respectively. Therefore, imipenem had considered as the furthermost effective antimicrobial agent against *S. aureus* isolates.

Concerning methicillin and vancomycin resistance; 69 isolates were MRSA (63.88%) as determined by disc diffusion method, 24 isolates were VRSA (22.22%) by vancomycin MIC Agar plates. The remaining 15 isolates were considered as MSSA (13.88%)

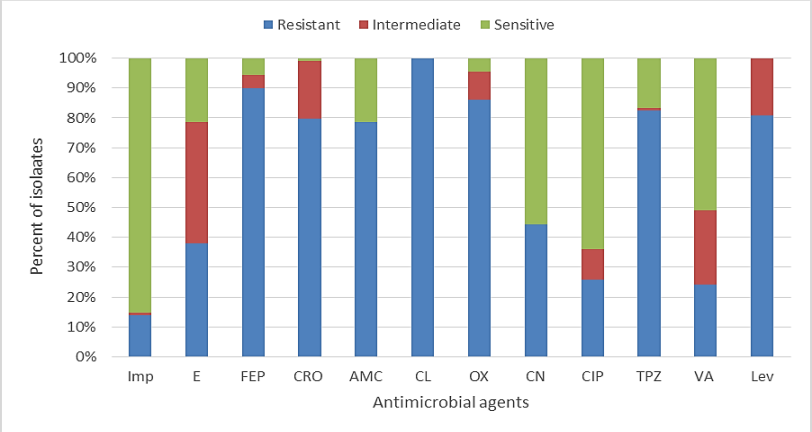

Fig. 2: Percentage of antibiotic resistance among S. aureus isolates.
IPM: Imipenem **E:** Erythromycin **OX:** Oxacillin **FEP:** Cefepime **CN:**Gentamicin **CRO:**Ceftrixone **AMC:**Amoxicilin/Clavulanic acid **CL:**Cephalexin **VA:**Vancomycin CIP:Ciprofloxacin **LEV:**Levofloxacin **TPZ:** Piperacillin/tazobactam

Molecular detection of Staphylococcal Pathogenicity Island-encoding genes

All 108 isolates were assessed for genes encoding SaPI (*Permease MetQ/NIpA, hyaluronate lyase(hys), polysaccharide lyase(pLys), heat shock protein chaperone GroEL, chaperonin GroEL and, integrase and ktrB* by PCR. Amplicons of 862,708, 493, 366, 340, 373 and 213bp were obtained (Figure 3)

The most predominant gene was *Permease MetQ/NIpA* transporter protein (n= 45, 31.9 %) followed by hyaluronate lyase(*hys*) and polysaccharide lyase(*pLys*) (n= 20, 14.2% each). While, chaperone *GroEL*, chaperonin *GroEL and*, integrase(*int*) and *ktrB* were the least common (n= 14, 9.9%) (Figure 4)

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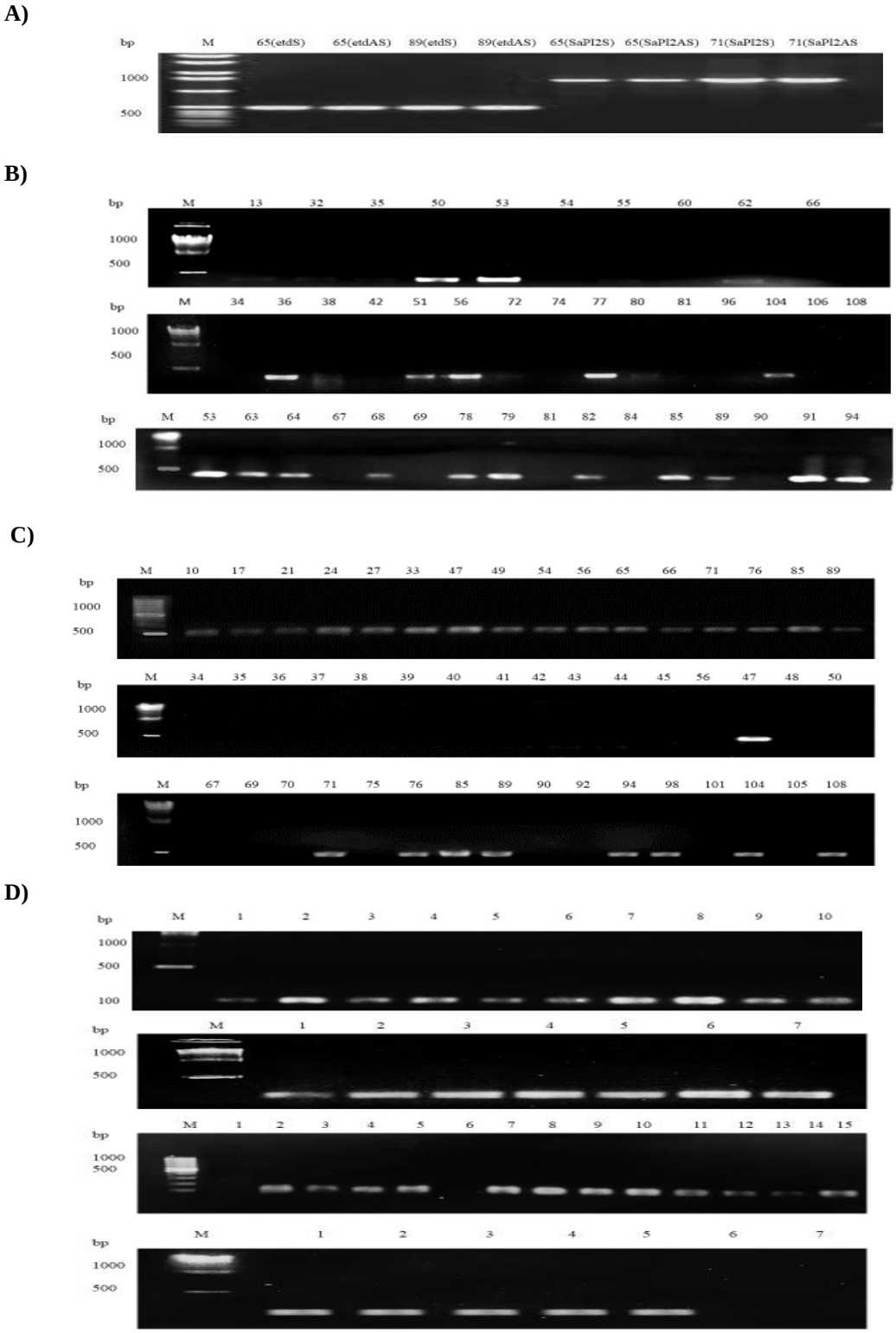

Fig. 3: Agarose gel electrophoresis of *Staphylococcus aureus* Pathogenicity Island (SaPI) virulence genes
A) Detection of virulence genes by using etd &SaPI2 primers respectively, where lane M: 100bp DNA ladder; bp: base pair.
B) Detection of virulence genes by using SaPIext primer, where lane M: 100bp DNA ladder; bp: base pair.
C) Detection of virulence genes by using etd primer, where lane M: 100bp DNA ladder; bp: base pair.
D) Detection of virulence genes by using SaPImw2, SaPI2, SaPIbov &SaPI4 primers respectively, where lane M: 100bp DNA ladder; bp: base pair.

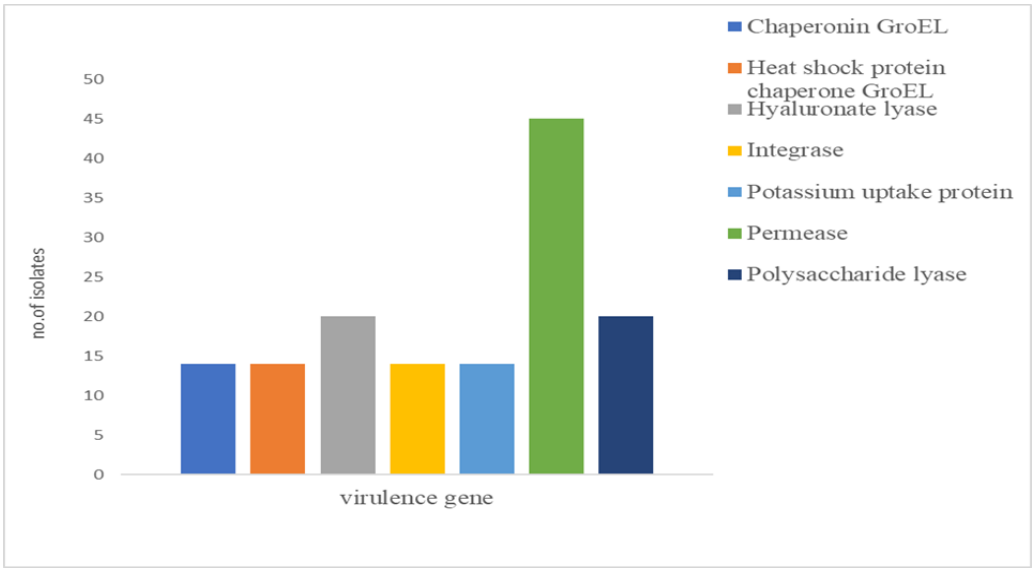

Fig. 4: Occurrence of Virulence genes among *S. aureus* isolates

The virulence genes were most common in wound (39.22%) followed by blood (21.57%) then urine (9.8%) and sputum (7.84%). While oral swab (3.92%), prostatic and respiratory tract (1.96%) were the least (Figure 5).

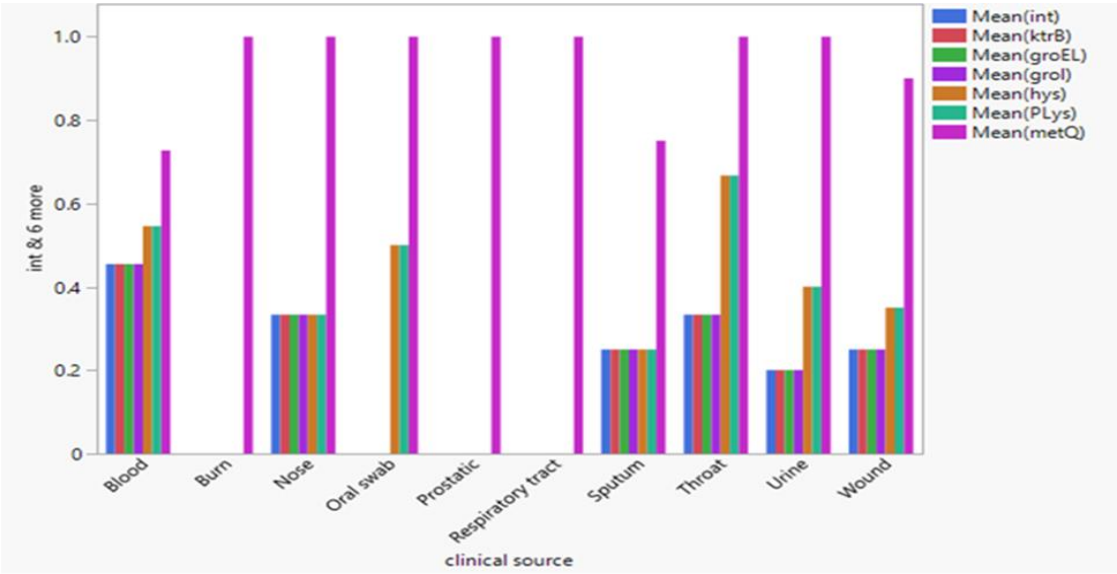

Fig. 5: The prevalence of virulence genes in *S. aureus* isolates from various clinical sources

Nucleotide sequencing analysis

We obtained PCR products of 862,708,493,366,340,373 and 213 bp. These nucleotide sequences aligned with other standard *S. aureus* strains in BLAST search of DNA Data Bank (https://blast.ncbi.nlm.nih.gov/Blast.cgi) table (2).The sequence 862 nucleotide product aligned with *groEL* gene that encode Chaperonin GroEL protein, and with *grol* gene that encode Heat shock protein chaperone GroEL. The sequence 708bp aligned with *int* gene encodes Integrase, *groEL* gene, *grol* gene and *ktrB* gene (encodes Potassium uptake protein). The sequence 493 bp aligned with *hys* gene encodes Hyaluronate lyase enzyme and *pLys* gene encodes Polysaccharide lyase enzyme. The sequence 366bp and 340bp aligned with *metQ* gene that encode with Permease enzyme (MetQ/NIpA transporter protein)

Table 2: The nucleotide sequences of the PCR products in BLAST search of DNA Data Bank.

Sequence	Gene	Protein	Standard strains	Accession No.
1(862bp)	*groEL*	Chaperonin GroEL	Staphylococcus aureus strain UP_620chromosome	CP047843.1
	Grol	Heat shock protein chaperone GroEL	Staphylococcus aureus strain E16SA093chromosome	CP031131.1
2(708bp)	*Int*	Integrase	Staphylococcus aureus	CAC6930705.1
	groEL	Chaperonin GroEL	Staphylococcus aureus strain Dresden-275757chromosome	CP054876.1
	Grol	Heat shock protein chaperone GroEL	Staphylococcus aureus strain E16SA093chromosome	LN831036.1
	KtrB	Potassium uptake protein	Staphylococcus aureus strain E16SA093chromosome	LN831036.1
3(493bp)	*Hys*	Hyaluronate lyase	Staphylococcus aureus strain MSSA476chromosome	BX571857.1
	pLys	Polysaccharide lyase	Staphylococcus aureus strain MSSA476chromosome	BX571857.1
4(493bp)	*Hys*	Hyaluronate lyase	Staphylococcus aureus strain MSSA476chromosome	BX571857.1
	pLys	Polysaccharide lyase	Staphylococcus aureus strain MSSA476chromosome	BX571857.1
5(366bp)	*metQ* (JF379_04290)	Permease (MetQ/NIpA transporter protein)	Staphylococcus aureus strain Newman NM-CQ chromosome	CP087593.1
6(340bp)	*metQ* (JF379_04290)	Permease (MetQ/NIpA transporter protein)	Staphylococcus aureus strain ncr_155_F133 chromosome	CP066488.1
7(373bp)	None	None	None	None
8(213bp)	None	None	None	None

The sequence of 340 bp chromosomal region containing attachment site for SaPI1(17-nucleotide sequences, was similar to the *att* site of SaPI1; 5'-TTATTTAGCAGGATAA-3'). Thus, might form the mechanism of pathogenicity island exclusion, has been deposited in GenBank, accession number U93687[30].

The 340 nucleotides product containing *S. aureus* attachment site for transposon 557 (Tn557) (figure 6). This shows significant homology with the integrases of Tn577.

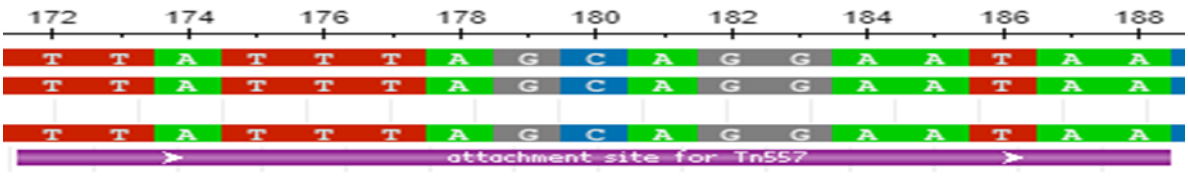

Fig. 6: *S. aureus* attachment site(*att*) for Tn55 found in 340bp nucleotide sequence

Identification of ORF that found in DNA sequences produced from PCR;

Sequence 862 bp has 14 ORFs (ORF1:ORF14), ORF4 and ORF12 encoded Chaperonin GroEL and groEL protein in *S. aureus* Newman (Accession No. A6QIM7.1)

Sequence 708bp has 9 ORFs, ORF1 that encoded Chaperonin GroEL and groEL protein in *S. aureus* Newman (Accession No. A6QIM7.1).

ORF2 encoded SCCmec chromosomal cassette elements in *S. aureus* MRSA252 (Accession No. Q6GKM5.1). Sequence3 (493bp) contains 9 ORFs, Hyaluronate lyase and Polysaccharide lyase (GAG Lyase) are encoded in ORF1 of *S. aureus* NCTC8325(Accession No. Q59801.1).

Sequence 4 (493bp) has 9 ORFs; ORF9 encoded Hyaluronate lyase and Polysaccharide lyase (GAG Lyase) in *S. aureus* NCTC8325 (Accession No. Q59801.1), Ligase *(Met)* in ORF6 in *S. aureus* JH9(Accession No. A51S12.1), Peptidase M *(Methionine amino peptidase)* in ORF7 in *S.aureus* Mu50 (P0A078.1). Sequence 5 (366bp) shows 4 ORFs; ORF1encoded Pyrophosphorylase (nucleotidetransferase) in *S. aureus* RF122 (Accession No.Q2YY49.1).

Sequence 6 (340bp) has 3 ORFs; ORF3 encoded Pyrophosphorylase (nucleotidetransferase) in *S. aureus* RF122 (Accession No. Q2YY49.1). Sequence 7 (373bp) contains 6 ORFs and Sequence 8 (213bp) contains 3 ORFs. But no significant similarity found in protein sequence (Table 3).

There is a smaller sequence: 'aaaaactattactaaaaatcaaaaatataaaaaaaacaaaaa' found in Sequence 3(493bp); By using ORF finder; three ORF, designed as ORF1, ORF2 and ORF3 were found.

ORF1, extending from nucleotide 342 to 380, encoding a protein

HTH LytTR –type transcriptional regulator SAB2242, LytR/AlgR family in *S. aureus* RF122 (Accession no. Q2YZ22.1)

ORF 2 from position 343 to 381, encoded a protein Integrase in Staphylococcus phage L54a (Accession no. P20709.1)

Table 3: ORF found in DNA sequences

DNA sequence	ORF identified	ORF Type	protein encoded	Organism	Accession No.
Sequence 1 (862bp)	14	ORF4 ORF12	-Chaperonin GroEL -groEL	Staphylococcus aureus Newman	A6QIM7.1
		ORF14	-SCCmec chromosomal cassette	Staphylococcus aureus MRSA252	Q6GKM5.1
Sequence 2 (708bp)	9	ORF1	-Chaperonin GroEL -groEL	Staphylococcus aureus Newman	A6QIM7.1
		ORF2	-SCCmec chromosomal cassette	Staphylococcus aureus MRSA252	Q6GKM5.1
Sequence 3 (493bp)	9	ORF1	-Hyaluronate lyase -Polysaccharide lyase (GAG Lyase)	Staphylococcus aureus NCTC8325	Q59801.1
		ORF3	-Ligase (Met)	Staphylococcus aureus JH9	A5IS12.1
Sequence 4 (493bp)	9	ORF9	-Hyaluronate lyase -Polysaccharide lyase (GAG Lyase)	Staphylococcus aureus NCTC8325	Q59801.1
		ORF6	-Ligase (Met)	Staphylococcus aureus JH9	A5IS12.1
		ORF7	Peptidase M (Methionine amino peptidase)	Staphylococcus aureus Mu50	P0A078.1
Sequence 5 (366bp)	4	ORF1	Pyrophosphorylase (nucleotidetransferase)	Staphylococcus aureus RF122	Q2YY49.1
Sequence 6 (340bp)	3	ORF3	Pyrophosphorylase (nucleotidetransferase)	Staphylococcus aureus RF122	Q2YY49.1
Sequence 7 (373bp)	6	ORF1:ORF6	NONE	NONE	NONE
Sequence 8 (213bp)	3	ORF1:ORF3	NONE	NONE	NONE

Moving forward with the sequencing results, phylogeny was used to reveal the relatedness between virulence gene on tested isolates.

The relation of all detected genes and phylogenic groups was analyzed using UPGAMA program (figure 7). Three different patterns (P1-P3) were detected. Isolates considered with the same pattern, upon the similarity level was at ≥ 70%.

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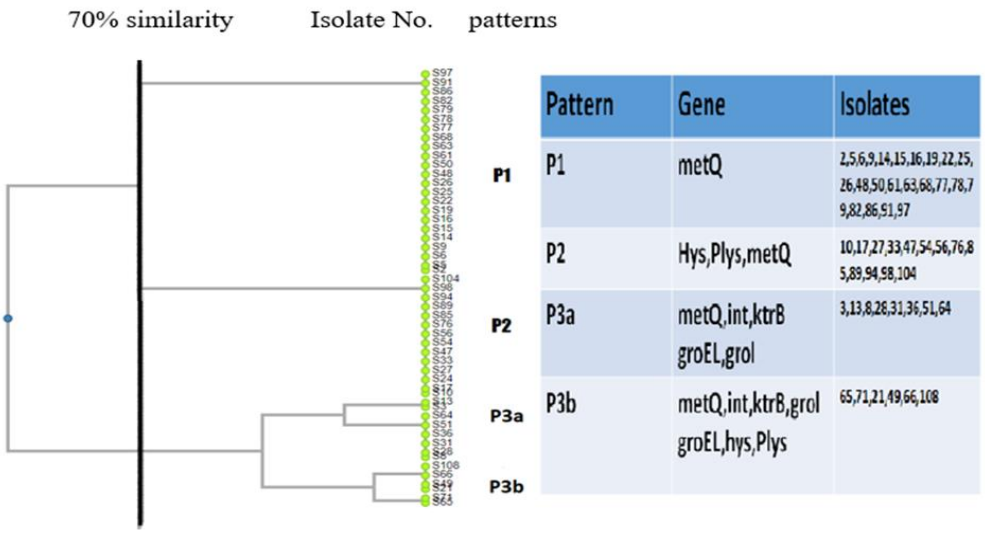
The most prevalent pattern was P1 (23 isolates) and P2 (13 isolates), that they harbored *metQ* and *Hys*, *pLys* respectively**.**

For Pattern P3, it was distributed in two groups (P3a & P3b). P3a represented by 8 isolates while P3b contained 6 isolates. Both patterns shared in harboring the same genes (*metQ, Int, grol, groEL, KtrB)* in addition to *Hys* and *pLys* genes in pattern P3b.



Pattern	Gene	Isolates
P1	metQ	2,5,6,9,14,15,16,19,22,25, 26,48,50,61,63,68,77,78,7 9,82,86,91,97
P2	Hys,Plys,metQ	10,17,27,33,47,54,56,76,8 5,89,94,98,104
P3a	metQ,int,ktrB groEL,grol	3,13,8,28,31,36,51,64
P3b	metQ,int,ktrB,grol groEL,hys,Plys	65,71,21,49,66,108

Fig.7: Dendrogram representing *S. aureus* isolates and virulence genes with similarity level ≥ 70% (http://genomes.urv.cat/UPGMA/UPGMAboot_v12.cgi.).

Phylogenetic Neighbor-joining tree was constructed using CLC Sequence Viewer 8.0 to create the relatedness between SaPI virulence genes. The distance of one sequence to the other indicates the level of relationship; that is closely related sequences on branches close to one another. Figure (8) represents the phylogenetic tree for sequences 862, 708, 493, 366, 340, 373 and 213 bp.

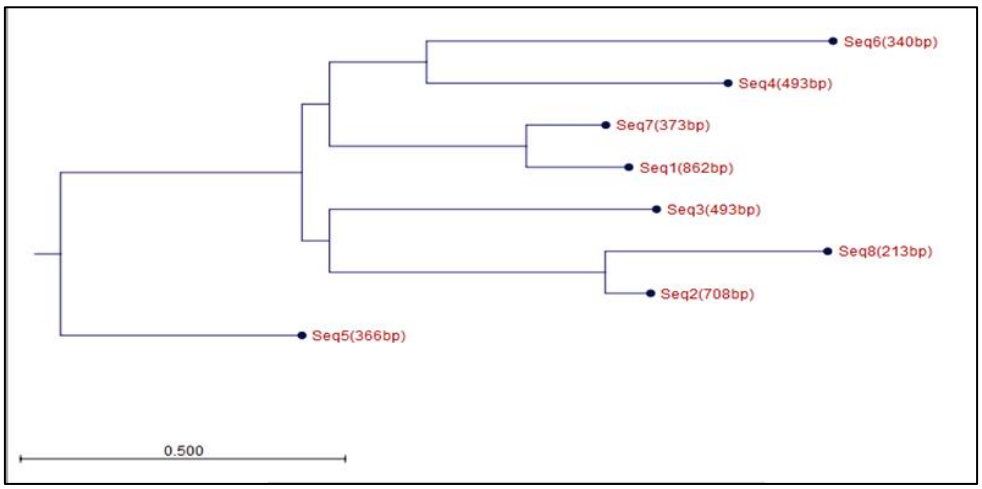


Fig. 8: Phylogenetic Neighbor-joining tree constructed with DNA sequences of *S. aureus* Pathogenicity Island (SaPI) virulence genes.

DISCUSSION

S. aureus, a normal flora of mucosa, seizes the opportunity to cause opportunistic infections, from superficial to invasive infections like bacteremia or pneumonia, etc., either as nosocomial or community infections[21]. *S. aureus* found in commensal microbiota of humans, that included in multiple human infections and food poisoning[22]. The drug-resistant *S. aureus*, methicillin-resistant *S. aureus* (MRSA) reported in 1990s[23] and VRSA in 2002[24].

S. aureus adapted to environments and hosts efficiently by the help of various factors that play a great effect on pathogenicity. Exchange and transfer of genes encoding virulence factors among staphylococcal strains has a great part in *S. aureus* pathogenicity[25]. SaPIs are clinically important because they encode and disseminate toxins[26]. Toxins constitute an important group of *S. aureus* virulence factors[27].

Investigation of virulence genes revealed that the highest prevalence was found in Wound (39.22%), this raises a serious public health alarm. Many enterotoxins,

including TSST and food poisoning are produced by *S. aureus*[28].

17-nucleotide sequence, *TTATTTAGCAGGAATAA*, in a 340 bp nucleotide product, represented the chromosomal attachment site; *att* $_{SaPI1}$.The obtained result is similar to a previous study that showed identical a 17-nucleotide sequence in an 828bp. So that the integration sites in these strains contain SaPI1 has a specific insertion site[29].

This attachment site: *5'ggcatttttatttaattgataTTATTTAGCAGGAATAAttagccag attatcaagga-3'*, emphasis that the excision and the integration of SaPI1 through the classical Campbell mechanism[30].

The 340 bp also contains attachment site for transposon attTn557.This indicate that Tn557 plays a role in the mobilization of virulence genes to several bacterial species. A previous study by MURPHY, ELLEN estimated similar result; SaPI1 is highly integrated and transferred by transduction at the specific site of transposon, Tn554[31].

The 493bp aligned with Hyaluronate lyase enzyme and Polysaccharide lyase enzyme. Both are virulence factor of *S. aureus*, produced by enzyme capable of degrading the hyaluronic acid (HA)[32] and cleave glycosidic linkages in acidic polysaccharides[33].

The 708 bp alignment with integrase (*int*). Gene *int* is able to integrate SaPIs at site-specific on *S. aureus* genome[28]. Otherwise it is similar also to SaPI1 as described by Novick & Ram[34] in carrying homologs of phage integrase (*int*). This SaPI has no an excisionase gene (*xis*). Just like the SaPI1, coding for extracellular β-lactamase homolog, this result resembles to that reported by Burgold-Voigt[10]. It hypothesizes that the SaPI1 int-like gene is capable for the mobility of SaPI1 involves the classic Campbell mechanism that excise, circulate and integrate in a specific target genome[35].

The demonstrated mobility of SaPI1 and functional integrases of these islands support that they stay a noteworthy developmental scheme of *S. aureus*. So, it will produce new enterotoxins and pathogenicity islands[36].

SaPIs are also capable of mediating generalized transduction. Like phages, homologs of SaPI sites are scattered throughout the host genome and have highly variable efficiency, resulting in genes being transduced at different frequencies[37].

Smaller sequence: aaaaactattactaaaaatcaaaaatataaaaaaaacaaaaa in sequence 3 (493bp), ORF1 encoding a protein LytTR–type transcriptional regulator SAB2242, LytR/AlgR family regulate the cell autolysis (IrgAB). Thus, although LytTR- containing transcriptional regulators perform significant roles in regulating virulence factors.

ORR2 encode, Integrase [Staphylococcus phage L54a]. Bacteriophage L54a is a temperate phage originally carried as a prophage in *S. aureus* PS54. Integrase is able to integrate the phage into the host genome by site-specific recombination, belongs to the 'phage' integrase family. Similar findings reported by Lindsay, Jodi A., *et al.* stated that SaPI1 included an ORF whose predicted product is integrase (Int) family of recombinases, so SaPI1 has a mechanism of functional integration.

Our results revealed that sequence1 (862bp) and Sequence2 (708bp) aligned with *groEL* gene that encode Chaperonin GroEL protein, and with *grol* gene that encode Heat shock protein chaperone GroEL. Also have ORFs that encoded the same proteins. (Chaperonin GroEL and groEL protein). Sequence 3(493bp) and sequence 4(493bp) aligned with Hyaluronate lyase and Polysaccharide lyase, contain ORFs that encoded the same enzymes (Hyaluronate lyase and Polysaccharide lyase in *S. aureus*).

Therefore, understanding the importance and of PAIs in *S. aureus* is crucial for developing new strategies to combat this pathogen. Further research is required to elucidate the mechanisms of virulence and resistance to find potential targets for new therapies.

Conclusion

The present study highlights the detection of pathogenicity islands-encoding virulence genes. SaPI is necessary for understanding the virulence of *S. aureus*. The occurrence of the *att* site of SaPI1 and *att* Tn557 in a 340 bp facilitate the transmission and incorporation of SaPI1**,** meaning that they can be easily transferred between bacterial cells. So, it has a critical part in the spread of its severity.

Furthermore, the highest prevalence of virulence genes found in Wound (39.22%), raises a serious public health alarm. These urge the government implementing for effectively control in all health care settings. Additionally, there is ongoing research to develop vaccines and immunotherapies that target *S. aureus* virulence factors. Overall, studying of pathogenicity islands in *S. aureus* has significant potential to develop new therapeutic strategies for preventing and treating infections.

Consent for publication

Not applicable

Availability of data and material

Data are available upon request

Competing interests

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