ACADEMIA JOURNAL OF BIOLOGY 2024, 46(2): 133–146
DOI: 10.15625/2615-9023/20722

GENOMIC INSIGHTS INTO MULTIDRUG-RESISTANCE AND VIRULENCE IN A VANCOMYCIN-RESISTANT *Staphylococcus aureus* STRAIN VR480

Nguyen Quang Huy*, Nguyen Thai Son, Nguyen Thai Uyen Thu, Nguyen Thuy Duong, Tran Thi Thanh Tam

University of Science and Technology of Hanoi, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Ha Noi, Vietnam

Received 4 May 2024; accepted 18 June 2024

ABSTRACT

Vancomycin-resistant *Staphylococcus aureus* (VRSA), a "high priority antibiotic-resistant pathogen", is emerging and threatening global health. Nevertheless, molecular mechanisms associated with virulence and multidrug resistance in VRSA are not fully investigated particularly in low- and middle-income countries. Here, for the first time, the complete genome of a VRSA strain VR480 isolated from a Vietnamese patient was generated by the combination of long- and short-read sequencing technologies. The VRSA strain VR480 was a multidrug-resistant phenotype with resistance to at least one antibiotic belonging to beta lactams, quinolones, lincomycin, oxazolidinones, tetracyclines, glycylcyclines, macrolide/lincosamide/streptogramin, nitrofurans, rifamycins, sulfonamides and glycopeptides. This strain was classified as vancomycin resistance (MIC $\geq$ 32 μg/mL). VRSA strain VR480 belonged to sequence type ST2779 and carried the SCCmec type II(2A). The VRSA strain VR480 genome contains five resistance mutations in genes. *gyr*A S84L, *par*C S80F, *par*E D432N, *fus*A L461K and *glp*T W355Stop, and eleven antibiotic resistant genes including *mec*A, *mec*R, *mec*I, *tet*M, *tet*38, *mep*A, *lmr*S, *ant*(9), *erm*A, *fos*B and *cat*A. This strain possesses various virulence factors associated with adherence, biofilm formation, colonization, invasion, anti-phagocytosis and toxicity that promote the infection and pathogenesis. Protein interaction network analysis revealed five clusters consisting of known and putative virulence proteins. Furthermore, epimerase, EssC, IcaA, SplA and Ssl1 were the key proteins within each cluster. This study raises a warning about the circulation and dissemination of VRSA in Vietnam. The key proteins would be potential targets for the development of anti-virulent agents to combat the VRSA infection.

Keywords: Antibiotic resistance, protein interaction network, *Staphylococcus aureus*, virulence, vancomycin resistance, whole genome sequencing.

Citation: Nguyen Quang Huy, Nguyen Thai Son, Nguyen Thai Uyen Thu, Nguyen Thuy Duong, Tran Thi Thanh Tam, 2024. Genomic insights into multidrug-resistance and virulence in a vancomycin-resistant *Staphylococcus aureus* strain VR480. *Academia Journal of Biology*, 46(2): 133–146. https://doi.org/10.15625/2615-9023/20722

Corresponding author email: nguyen-quang.huy@usth.edu.vn

INTRODUCTION

The massive use of antibiotics significantly contributes to the emergence and spread of drug-resistant bacteria worldwide. In 2017, the World Health Organization (WHO) published a priority pathogens list for research and development of new antibiotics, in which methicillin-resistant, vancomycin-intermediate, and vancomycin-resistant S*taphylococcus aureus* was in the category of priority 2 (high) (WHO, 2017). *Staphylococcus aureus* belongs to the ESKAPE group (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species) which are highly virulent and resistant to multiple antibiotics, and often associated nosocomial infections (Santajit & Indrawattana, 2016). *Staphylococcus aureus* is associated with skin infection, pneumonia, endocarditis, osteomyelitis, gastroenteritis, and severe cases of toxic shock syndrome (Tong et al., 2015). Methicillin-resistant *Staphylococcus aureus* (MRSA) strains/isolates have disseminated all over the world and are associated with both community- and hospital-acquired infections (Lee et al., 2018; Turner et al., 2019). Globally, the prevalence of MRSA was from 20 to > 70%, in Asia has the highest incidence of MRSA (Chen et al., 2014; Lee et al., 2018; Turner et al., 2019). Seriously, novel MRSA clones associated with human infection were found in livestock animals (Kock et al., 2010; Chen et al., 2014; Lee et al., 2018). Epidemiological data showed that mortality rates in patients with MRSA are significantly higher than in those with methicillin-susceptible *Staphylococcus aureus* infections (Lee et al., 2018; Turner et al., 2019). Patients with MRSA infections are treated with intensive antimicrobial therapy, unfortunately, few antibiotics are available. Vancomycin, a tricyclic glycopeptide antibiotic, is introduced as one of the last–resort drugs for the treatment of MRSA infections (Shariati et al., 2020). Unfortunately, vancomycin-intermediate *Staphylococcus aureus* (VISA) strains and vancomycin-resistant *Staphylococcus aureus* (VRSA) strains have been reported in many parts of the world, especially in South Asia where the health-care resources are limited (Shariati et al., 2020; Stogios et al., 2020; Cong et al., 2020). Molecular studies demonstrated that *van* gene clusters including *van*A, *van*B, *van*C, *van*D, *van*E, *van*F, *van*G, *van*I, *van*L, *van*M and *van*N are associated with vancomycin resistance (Gardete et al., 2014; McGuinness et al., 2017; Stogios et al., 2020). These genes are often found in plasmids rather than in chromosomes of vancomycin-resistant bacteria. Although genetic mutations could link to vancomycin resistance, nevertheless, the molecular mechanism associated with vancomycin resistance is still not fully understood (Gardete et al., 2014; McGuinness et al., 2017; Stogios et al., 2020). In addition, *Staphylococcus aureus* possesses various virulence factors including biofilms, adherence, exoenzymes, exotoxins promoting the infection, invasion and transmission (Tong et al., 2015). Nevertheless, the acquisition of virulence genes differs according to the genotypes of *Staphylococcus aureus* (Monistero et al., 2020). Identification of virulence genes helps to evaluate the disease development and transmission of the pathogen. Therefore, it is critical to conduct genomic surveillance of VRSA to get insight into the genetic determinants associated with multidrug-resistance and virulence of dominant clones circulating in each healthcare setting. Vietnam has a high burden of community- and hospital-acquired infections. The country is facing the rapid emergence and spread of antimicrobial-resistant bacteria (Phu et al., 2016; Le et al., 2016; Vu et al., 2021). According to a report on antimicrobial susceptibility testing results from 13 hospitals in Vietnam (VINARES, 2016–2017), the prevalence of MRSA was found to be as high as up to 74% (Vu et al., 2021). Notably, the proportion of VRSA was reported from 2% to 2.9% (Phu et al., 2016; Vu et al., 2021; Nguyen et al., 2024). Although the molecular mechanisms of *S. aureus* resistant to both methicillin and vancomycin have been

intensively investigated in the world (Jahanshahi et al., 2018; Kim et al., 2016; Muzammil et al., 2023), nevertheless, few such studies on MRSA circulating in Vietnam were conducted (Nguyen et al., 2019; Hoang et al., 2023), and no information on the genomic characteristics of VRSA strains is reported. Therefore, the present study aimed to investigate genetic determinants associated with multi-antibiotic resistance and virulence in a clinical vancomycin-resistant *Staphylococcus aureus* strain VR480 isolated from 108 Military Central Hospital, Hanoi, Vietnam. The genomic data may be an initial step in establishing an effective strategy for monitoring the emergence and spread of VRSA strains in healthcare settings.

MATERIALS AND METHODS

Bacterial strains

The clinical *Staphylococcus aureus* strain VR480 was kindly provided by the Department of Microbiology, 108 Military Central Hospital, Ha Noi, Vietnam in 2021. The strain *Staphylococcus aureus* VR480 was isolated from a sputum sample of a 65-year-old male patient with esophageal cancer hospitalized at the Department of Infectious Diseases, 108 Military Central Hospital, Ha Noi, Vietnam, in 2021. The patient has developed a clinical symptom with pneumonia and shock with a bacterial infection. The bacterial culture was conducted on a blood agar medium and subsequently identified as *Staphylococcus aureus* using the VITEK® MS system (Biomerieux, France).

Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MIC) of 16 drugs including Benzylpenicillin, Oxacillin, Gentamicin, Ciprofloxacin, Levofloxacin, Moxifloxacin, Erythromycin, Clindamycin, Quinupristin/Dalfopristin, Linezolid, Vancomycin, Tetracycline, Tigecycline, Nitrofurantoin, Rifampicin, Trimethoprim and Sulfamethoxazole were determined using antibiotic susceptibility testing (AST) cards for *Staphylococcus aureus* on the VITEK-2 compact system. The phenotypic results were interpreted to be susceptible, intermediate and resistant based on the manufacturer's instructions.

Screening of methicillin and vancomycin resistance genes

The total genomic DNA of *Staphylococcus aureus* strain VR480 was extracted using the Norgen Bacterial Genomic DNA Isolation kit (Norgen Biotek, Canada) according to the manufacturer's instructions. The quality and purity of DNA were examined by Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). The presence of *mec*A (methicillin resistance), *van*A and *van*B (vancomycin resistance) genes were screened by PCR using specific primers as follows: mecA_F (5'-TCCAGATTACAACTTCACCAGG-3') and mecA_R (5'-CCACTTCATATCTTGTAACG-3') (Ahmed et al., 2014); vanA_F (5'-CATGAATAGAATAAAAGTTGCAATA-3') and vanA_R (5'-CCCCTTTAACGCTAATACGATCAA-3') (Clark et al., 1993); vanB_F (5'-GATTTGATTGTCGGCGAAGTG-3'), vanB_R (5'-TCCTGATGGATGCGGAAGA-3') (He et al., 2020).

Short-read and long-read sequencing

The short-read sequencing of the genomic DNA of *Staphylococcus aureus* strain VR480 was conducted on the BGI platform (Beijing Genomics Institute, China). The long-read sequencing was implemented at the University of Science and Technology of Hanoi (USTH), using the ONT SQK-RBK004 rapid barcoding kit loaded on the R9.4.1 (FLO-MIN106D) flow cell and connected to the MinION M1kB device.

Bioinformatics analysis

Base-calling and de-multiplexing of long reads (ONT reads) were conducted with GuPPy v6.3.7 (Sherathiya et al., 2021). Short-read and long-read raw data were quality checked using FastQC and nanoQC (Wouter et al., 2018), respectively and then were assembled by Unicycler v0.5.0 in hybrid assembly mode (Wick et al., 2017). The assembled data were annotated according to the *baargin* Nextflow pipeline (Hayer et al.,

2023). Subsequently, MLST v2.22.0 (Jolley et al., 2018) and SCCmecFinder-1.2 (Kaya et al., 2018) were used to identify sequence type and staphylococcal cassette chromosome mec (SCCmec) elements. The Cluster of Orthologous Genes (COG) analysis was conducted using COGclassifier v.10.5 (https://github.com/moshi4/COGclassifier).

Antibiotic resistance genes, stress response and virulence genes were detected using AMRFinder v3.10.1 (Feldgarden et al., 2021). The assembled data were submitted to Prokka v1.14.6 for functional annotation (Seemann, 2014). Identification of plasmid among scaffolds was conducted using the BLASTn tool, NCBI. All scaffolds were aligned to a collection of plasmid sequences which were extracted from the NCBI nucleotide database (version 2024-01-15). Proksee was used to visualize chromosomes and plasmids (Grant et al., 2023). The genomic DNA sequences of VRSA strain VR480 was deposited on GenBank, NCBI under accession numbers: SRR28810672 and SRR28810674.

For protein-protein interaction network analysis, STRING and Cytoscape were used to generate and visualize protein networks for potential virulence mechanisms (Doncheva et al., 2019). MCODE (Molecular Complex Detection) was used to identify densely connected regions that may represent molecular complexes. CytoHubba was then used to score the nodes within a network to identify the most highly connected nodes for each network. The feature MCC (Maximal Clique Centrality) was used as a ranking method since it identified more essential proteins.

RESULTS

Antibiotic susceptibility profile

Table 1. Antibiotic susceptibility profile of *Staphylococcus aureus* VR480

No.	Antibiotic group	Antibiotic	*Staphylococcus aureus* VR480	
			MIC (µg/mL)	Interpretation
1	Aminoglycoside	Gentamicin	≥ 16	R
2	Beta lactams	Benzylpenicillin	≥ 0.5	R
3		Oxacillin	≥ 4	R
4		Cefoxitin	4	R
5	Quinolones	Ciprofloxacin	≥ 8	R
6		Levofloxacin	≥ 8	R
7		Moxifloxacin	≥ 8	R
8	Lincomycin	Clindamycin	≥ 8	R
9	Oxazolidinones	Linezolid	≥ 8	R
10	Tetracyclines	Tetracycline	≥ 8	R
11	Glycylcycline	Tigecycline	1	R
12	Macrolide-lincosamide-streptogramin	Quinupristin/Dalfopristin	4	R
13		Erythromycin	≥ 8	R
14	Nitrofuran	Nitrofurantoin	256	R
15	Rifamycin	Rifampicin	16	R
16	Sulfonamide	Trimethoprim and Sulfamethoxazole	80	R
17	Glycopeptide	Vancomycin	≥ 32	R

Note: R: resistant.

The MIC results revealed that *Staphylococcus aureus* strain VR480 was a multidrug resistant phenotype, with resistance to at least one antibiotic belonging to 12 different antibiotic groups (Table 1). In addition, this strain was positive with the *mec*A gene by a *mec*A PCR-based amplification, therefore was also resistant to methicillin.

According to the WHO guideline, *Staphylococcus aureus* strain VR480 is classified as VRSA with the MIC of vancomycin ≥ 32 µg/mL. Nevertheless, this strain was negative with the two most common vancomycin resistant-associated genes *van*A and v*an*B, underling other molecular mechanisms of resistance involved.

Genomic characteristics of *Staphylococcus aureus* strain VR480

The short read and long read sequence hybrid assembly results in a fully completed genome of *Staphylococcus aureus* strainVR480 with a chromosome and a plasmid. The total genome size was 2,859,064 bp with a GC content of 32.9%. This genome had 2635 coding DNA sequences, 16 rRNA and 61 tRNA genes (Table 2). *Staphylococcus aureus* strainVR480 belonged to the sequence type 2779 and possessed the SCC*mec* type II(2A), a mobile genetic element carrying the *mec*A gene responsible for methicillin resistance. COG functional analysis detected 79.60% (2111/2652) sequences classified into 4 categories including cellular processes and signalling (n = 431, types of D, M, N, O, T, U and V), information storage and processing (n = 502, types of J, K, X and L), metabolisms (n = 947, types of C, E, F, G, H, I, P and Q) and poorly characterized (n = 231, types of R and S) (Fig. 1).

Table 2. Sequencing data and general features of the genome sequence of *Staphylococcus aureus* VR480

Total reads	Read coverage (X)	Genome size (bp)	Genome	Completeness	GC content (%)	Number of CDSs	Number of tRNA
15.456.774	772	2.859.064	1 chromosome; 1 plasmid	100%	32,9	2.635	61

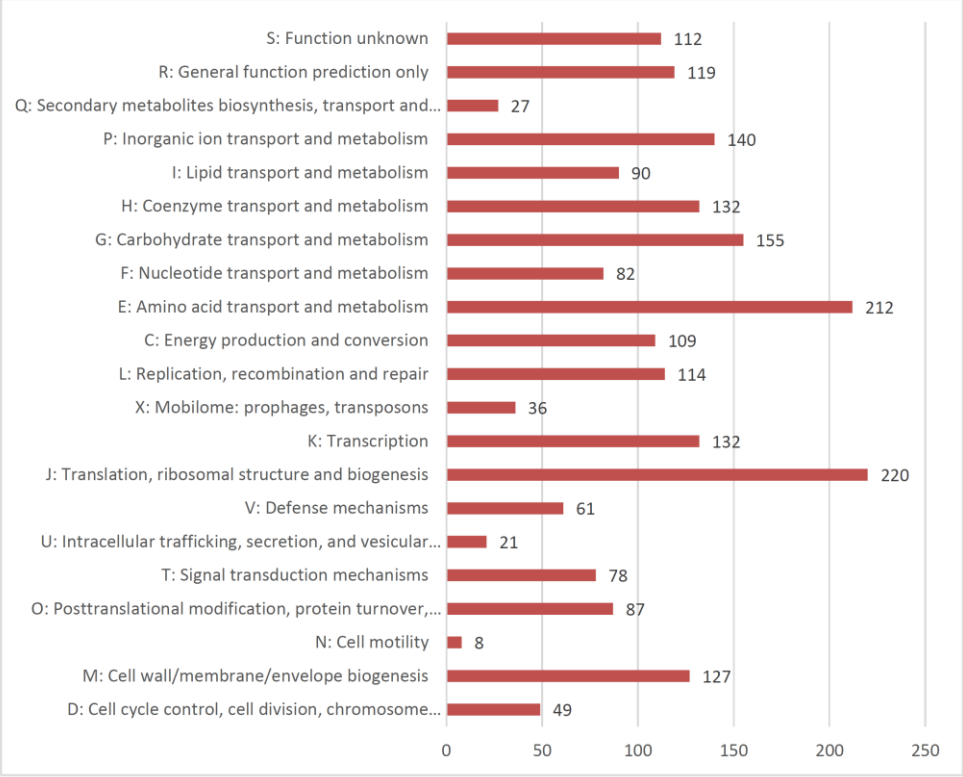

Figure 1. COG Functional categories of the genome of *Staphylococcus aureus* VR480

Detection of antibiotic-resistant genetic determinants

Genomic analysis of the strain *Staphylococcus aureus* VR480 revealed 5 chromosomal antibiotic-resistant mutations and 11 antibiotic-resistant genes (Table 3). These genetic determinants are associated with resistance to eight given antibiotic groups including beta lactams, aminoglycosides, quinolones, tetracyclines, chloramphenicol, fusidane, phosphonic and macrolide-lincosamide-streptogramin. In addition, *mep*A and *lmr*S encoding for multidrug resistant efflux pumps were also detected. Among 11 antibiotic-resistant genes detected, 10 genes were located in the chromosome (Fig. 2A) and only one gene *cat*A associated with chloramphenicol resistance was found in the plasmid (Fig. 2B). Eight genes including *mec*A, *mec*R1, *mec*I, *tet*(M), *tet*(38), *mep*A, *lmr*S, *fos*B and *cat*A had only one copy, while two genes *ant*(9)-Ia and *erm*(A) were detected two copy numbers. None of either *van* genes or genetic mutations associated with vancomycin resistance was detected in the genome of *Staphylococcus aureus* VR480. The location of antibiotic-resistant mutations and genes on the chromosome of *Staphylococcus aureus* VR480 and its plasmid was shown in Figure 2.

Table 3. Antibiotic-resistant genetic determinants presented in the genome of *Staphylococcus aureus* VR480

Genetic determinants	Functional protein	Antibiotic Resistance	Number of gene(s)
Mutations associated with antibiotic resistance			
S84L	GyrA	Quinolones	1
S80F	ParC		1
D432N	ParE		1
L461K	FusA	Fusidic acid	1
W355STOP	GlpT	Fosfomycin	1
Antibiotic resistant gene			
*mec*A	PBP2a family beta-lactam-resistant peptidoglycan transpeptidase MecA	Beta-lactams	1
*mec*R1	beta-lactam sensor/signal transducer MecR1		1
*mec*I	mecA-type methicillin resistance repressor MecI		1
tet(M)	tetracycline resistance ribosomal protection protein Tet(M)	Tetracyclines	1
tet(38)	tetracycline efflux MFS transporter Tet(38)		1
*mep*A	multidrug efflux MATE transporter MepA	Multidrug	1
*lmr*S	multidrug efflux MFS transporter LmrS		1
ant(9)-Ia	aminoglycoside nucleotidyltransferase ANT(9)-Ia	Aminoglycosides	2
erm(A)	23S rRNA (adenine(2058)-N(6))-methyltransferase Erm(A)	Macrolide-lincosamide-streptogramin	2
*fos*B	FosB1/FosB3 family fosfomycin resistance bacillithiol transferase	Fosfomycin	1
*cat*A	type A-7 chloramphenicol O-acetyltransferase	Phenicols	1*

Note: *: Genes were detected on plasmid.

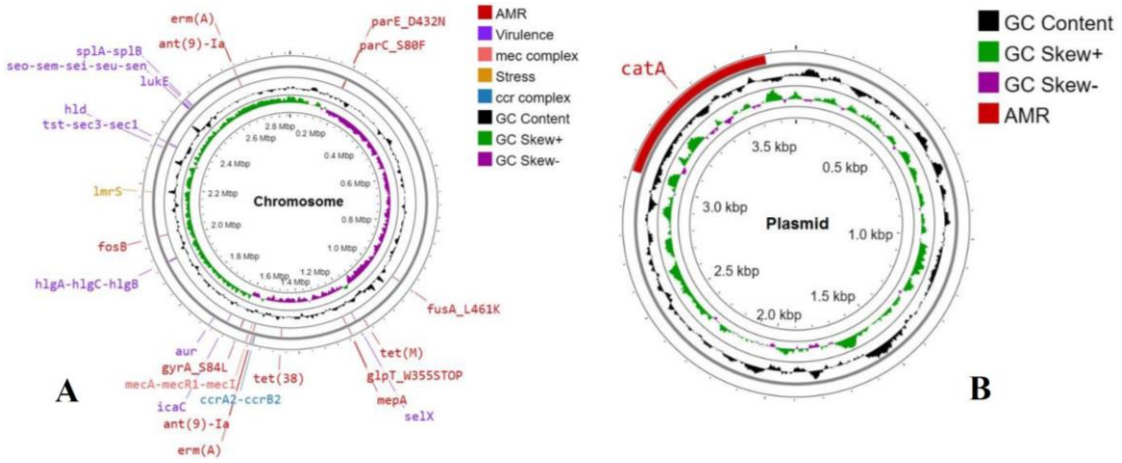

Figure 2. The distribution of antibiotic-resistant and virulent genes on the chromosome (A) and plasmid (B) of *Staphylococcus aureus* VR480

Detection of virulence genes

Whole genome sequence analysis revealed that *Staphylococcus aureus* VR480 carried various virulence genes responsible for infection, biofilm formation, toxin production, host invasion and anti-phagocytosis (Fig. 2A). Specifically, this strain possessed a gene *ica*C involved in polysaccharide intercellular adhesin biosynthesis, cell aggregation and biofilm formation which plays an important role in infection and colonization in the host (Arciola et al., 2015). Genes encoding protease were detected including *spl*A and *spl*B encoding for serine protease type A and B played a role in infection and immune escape and *aur* encoding for zinc metalloproteinase aureolysin functioned as thermolysin (Dasari et al., 2022). A gene *luk*E is responsible for the production of leukocidin LukED subunit E, an important virulence factor of *Staphylococcus aureus*, which lyses host cells and promotes infection (Marilyn et al., 2020). Like many other *Staphylococcus aureus* strains, *Staphylococcus aureus* VR480 carried a gene cluster of *hlg*A, *hlg*B, *hlg*C and *hld* encoding for bi-component gamma-hemolysins HlgAB subunit A, HlgAB/HlgCB subunit B, HlgCB subunit C and delta-hemolysin (Tam & Torres, 2019). Notably, this strain possesses nine genes encoding for different types of toxins including enterotoxin type C3 (*sec*3), type L (*sel),* type O *(seo),* type M *(sem*), type I *(sei),* type U *(seu),* type N *(sen),* enterotoxin-like toxin X (*sel*X) and toxic shock syndrome toxin TSST-1 (*tst*).

Protein interaction network

Protein interaction network analysis was conducted to investigate potential virulence mechanisms in *Staphylococcus aureus* VR480. The virulent protein network was generated in 70 nodes with 353 edges, in which 5 protein interaction clusters were detected (Fig. 3). The full virulent protein network is presented in the supplemental document. The cluster I consisted of 15 proteins with 104 interactive edges responsible for biosynthesis of cellular surface components including capsular polysaccharides, sugars and proteins. Among 15 proteins, UDP-N-acetylglucosamine 2-epimerase was recognized as the seed protein which catalyzes the conversion of UDP-N-acetylglucosamine into UDP-N-acetylmannosamine, a precursor of the teichoic acid linkage unit on the cell surface. Cluster II contained 11 proteins which are important for the establishment of infection in the host. In this cluster, EssC, a component of the type VII secretion system, was identified as the seed protein. Cluster III consisted of 8 genes which play an important role in adhesion, clumping factor, colonization and pathogenesis. The IcaA, an intercellular adhesion protein A (biofilm adhesion polysaccharide) was the seed protein within this cluster. The cluster IV

consisted of 13 genes involving biofilm formation, toxin production, and anti-phagocytosis. The SplA, one of the six serine proteases unique to *Staphylococcus aureus*, was the seed protein. Finally, cluster V included only 3 genes encoding for protease and secreted proteins that play an essential role in immune innate response inhibition, cleaving host proteins. The Ssl1 mediates virulence by proteolytically cleaving host proteins, including collagens types I and IV as well as human cytokines IL8, IL17A, and IFN-gamma, which plays the role of seed protein within this cluster (Dasari et al., 2022; Tam & Torres, 2019). The 20 remaining proteins were not clustered in the protein interaction network.

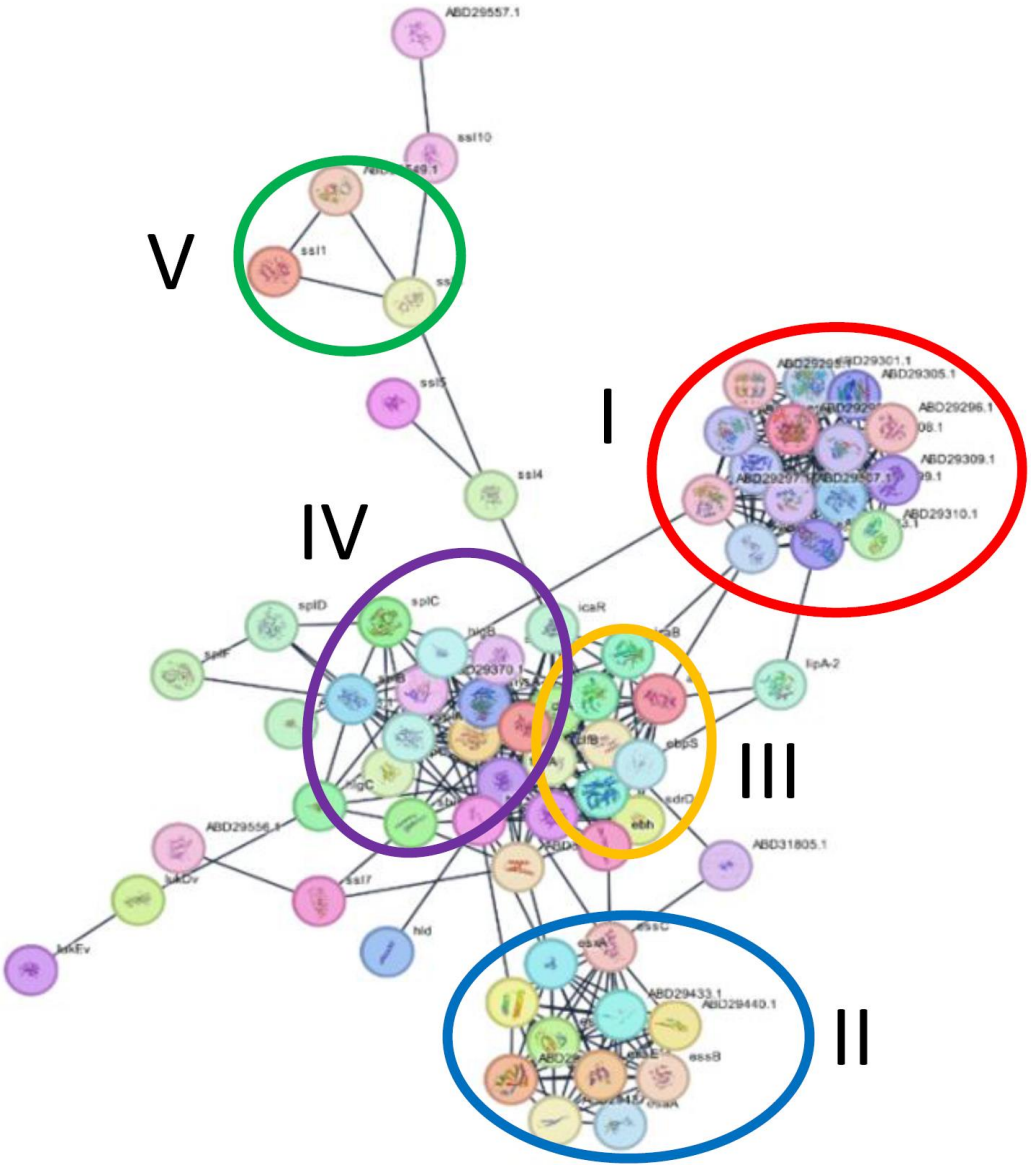

Figure 3. Protein-protein interaction networks in *Staphylococcus aureus* VR480. The nodes correspond to proteins and the edges correspond to known or predicted protein interactions. Cluster I: red circular; cluster II: green circular; cluster III: orange circular; cluster IV: purple circular; cluster V: blue circular

DISCUSSION

Staphylococcus aureus, a common opportunistic pathogen, is the leading cause of lethality in hospital- and community-acquired infections (Tong et al., 2015; Lee et al., 2018; Turner et al., 2019). This pathogen possesses various virulence factors and quickly develops resistance to antibiotics. Recently, the emergence and spread of MRSA, followed by VRSA is threatening healthcare settings worldwide (Shariati et al., 2020; Cong et al., 2020). The emergence of *Staphylococcus aureus* strains with resistance to multiple antibiotics requires the identification of bacterial virulence genes and the development of novel therapeutic strategies. The present study analyzed the complete genome of a clinical VRSA strain VR480 to identify genes responsible for antibiotic resistance and virulence. Overall, the prevalence of VRSA is reported < 3% in Vietnam (Vu et al., 2021; Nguyen et al., 2024), nevertheless, it is unknown the prevalence of VRSA in the 108 Military Central Hospital. The VRSA strain VR480 belonged to the genotype ST2779 with the SCC*mec* II(2A) carrying *mec*A, which was recently found in MRSA from China (Zhao et al., 2021) and Brazil (Monteiro et al., 2019), but this genotype was not reported in other countries and in Vietnam. Therefore, the presence of VRSA VR480 ST2779 in this hospital warns of the necessity to conduct genomic surveillance research to get insight into molecular mechanisms associated with antibiotic resistance and virulence. This knowledge is essential for controlling the emergence and spread of VRSA because to our knowledge this is the first study that reported the complete genome and its characteristics of the clinical VRSA strain in Vietnam.

The VRSA strain VR480 possessed 17 genetic determinants associated with resistance to antibiotic groups of beta lactams, aminoglycosides, quinolones, tetracyclines, chloramphenicol, fusidane, phosphonic and macrolide-lincosamide-streptogramin. Overall, this result is concordant with the phenotypic-resistant profile, except for vancomycin, none of the genetic determinants associated with vancomycin resistance was detected in the genome of *S. aureus* VR480. It has been reported in the literatures that the acquisition of genetic determinants associated with vancomycin resistance is incompletely demonstrated in *Staphylococcus aureus* (Gardete et al., 2014; McGuinness et al., 2017; Stogios et al., 2020). Nevertheless, *Staphylococcus aureus* could develop vancomycin resistance via the decreased permeability of the cell wall to prevent the vancomycin intracellular diffusion (Shariati et al., 2020). Our findings indicate that other molecular mechanisms contributing to vancomycin resistance in this strain need to be further investigated. It has been demonstrated that *van*A, *van*B, *van*D, *van*F, *van*I, and *van*M, encoding for d-Ala:d-Lac ligases, are often associated with high-level vancomycin resistance (MICs > 256 mg/mL), while *van*C, *van*E, *van*G, *van*L, and *van*N, encoding for d-Ala:d-Ser ligases, are generally linked to low-level resistance (MICs of 8–16 mg/mL) (Gardete et al., 2014; McGuinness et al., 2017; Stogios & Savchenko, 2020). In addition, several studies have found mutations in genes such as *vra*S, *vra*R, *yvq*F, *gra*R, *gra*S, *wal*R, *wal*K, and *rpo*B in VRSA strains (Hafer et al., 2012; Hiramatsu et al., 2014), therefore these mutations were proposed to be responsible for vancomycin resistance. Nevertheless, these findings were not well verified and thus the molecular mechanisms underlying VRSA development thought the acquisition of chromosomal mutations are incompletely demonstrated. Thus, the use of vancomycin properly as the frontline antibiotic against MRSA in treatment therapy would reduce the selection pressure of vancomycin-resistant strains.

Staphylococcus aureus possesses various virulence factors that facilitate the adaptation and survival of this pathogen in different tissues and environmental conditions, and significantly influence its clinical manifestations (Cheung et al., 2021). Nevertheless, different *Staphylococcus aureus* strains carry different virulence factors and

cause varying pathogenic characteristics, resulting in different diseases (Cheung et al., 2021; Shettigar & Murali., 2020). Here, we found various genes encoding for virulence factors in VRSA strain VR480 exhibited including tissue adherence and biofilm formation (*ica*C), invasive proteases (*spl*A and *spl*B), hemolysin (*hlg*A, *hlg*B, *hlg*C and *hld*), leukocidin (*luk*E), enterotoxins (*sec*3, *sel, seo, sem, sei, seu,* and *sen),* enterotoxin-like toxin X (*sel*X) and particularly toxic shock syndrome toxin TSST-1 (*tst*), which is one of the most dangerous superantigens (Arciola et al., 2015; Dasari et al., 2022; Marilyn et al., 2020; Tam & Torres, 2019). The presence of many virulence factors underlines the ability of this strain to cause different infections. All these virulence factors allow this pathogen to attach to tissues, causing pathogenesis, and to penetrate the immune system, causing toxicity (Cheung et al., 2021). Thus, the circulation of this strain in the hospital can increase the potential risk of hospital-acquired infections and high mortality. In addition, analysis of the protein interaction network revealed 5 clusters consisting of known and putative virulence proteins responsible for infection and pathogenesis in the host. To our best knowledge, this is the first report on the protein interaction network conducted in VRSA. For each cluster, a key protein has been previously demonstrated to be associated with virulence in *Staphylococcus aureus*: epimerase, epimerase is involved in the synthesis of the capsule precursor UDP-ManNAcA that contributes to the adhesion, colonization and anti-phagocytosis (Cress et al., 2014); EssC is an essential component of protein secretion system type VII (Jäger et al., 2018); SplA, a serine protease, plays the role in the invasive stage of the infection (Paharik et al., 2016); IcaA is involved in biofilm formation (Kadkhoda et al., 2020); Ssl1 is a protease with activity on host structural proteins and immunity system (Tang et al., 2019). These key proteins would be potential molecular targets for the development of anti-virulence agents to reduce infection and invasion in hosts because they are key proteins in the combined network. Although the prevalence of VRSA infection is low, VRSA is still a potential threat to public health. Intensive surveillance of vancomycin-resistance, proper use of antibiotics along prompt notification to infection prevention and control authorities upon VRSA determination in clinical settings are essential for preventing outbreaks and dissemination of VRSA strains.

CONCLUSION

VRSA is a deadly and costly opportunistic pathogen that is difficult to control and a significant concern for hospital-acquired and community-acquired infections. Our study provides insight into the genetic determinants associated with virulence and multidrug resistance in a VRSA strain VR480 genotype ST2279 by using the combination of short read and long read NGS technology. This knowledge is crucial for better controlling and managing the transmission of VRSA within hospitals and from them to the community in Vietnam.

Acknowledgements: The authors are grateful to LMI DRISA, USTH and the Department of Microbiology, 108 Military Central Hospital for their support. This work was funded by the Vietnam Academy of Science and Technology under the grant number: THTETN.01/21–23.

REFERENCES

WHO, 2017. WHO publishes list of bacteria for which new antibiotics are urgently needed (https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed). Accessed: 20/03/2024.

Santajit S. and Indrawattana N., 2016. Mechanisms of Antimicrobial Resistance in ESKAPE Pathogens. *Biomed Res Int*, 2016: 2475067.

Tong S. Y., Davis J. S., Eichenberger E., Holland T. L., Fowler V. G., 2015. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical

manifestations, and management. *Clin Microbiol Rev*, 28(3): 603−61.

Lee A., Lencastre H. D., Garau H., Kluytmans J., Malhotra-Kumar S., Peschel A., Harbarth S, 2018. Methicillin-resistant *Staphylococcus aureus*. *Nat Rev Dis Primers*, 4: 18033.

Turner N. A., Sharma-Kuinkel B. K., Maskarinec S. A., Eichenberger E. M., Shah P. P., Carugati M., Holland T. L., Fowler V. G., 2019. Methicillin-resistant *Staphylococcus aureus*: an overview of basic and clinical research. *Nat Rev Microbiol*, 17: 203–218.

Chen C. J. and Huang Y. C., 2014. New epidemiology of *Staphylococcus aureus* infection in Asia. *Clin Microbiol Infect*, 20(7): 605−23.

Köck R., Becker K., Cookson B., Gemert-Pijnen J. E., Harbarth S., Kluytmans J., Mielke M., Peters G., Skov R. L., Struelens M. J., Tacconelli E., Navarro T. A., Witte W., Friedrich A. W., 2010. Methicillin-resistant *Staphylococcus aureus* (MRSA): burden of disease and control challenges in Europe. *Euro Surveill*, 15(41): 19688.

Shariati A., Dadashi M., Moghadam M. T., Belkum V. A., Yaslianifard S., Darban-Sarokhalil D., 2020. Global prevalence and distribution of vancomycin resistant, vancomycin intermediate and heterogeneously vancomycin intermediate *Staphylococcus aureus* clinical isolates: a systematic review and meta-analysis. *Sci Rep*, 10(1): 12689.

Gardete S., Tomasz A., 2014. Mechanisms of vancomycin resistance in *Staphylococcus aureus*. *J Clin Invest*, 124(7): 2836–2840.

McGuinness W. A., Malachowa N., DeLeo F. R., 2017. Vancomycin Resistance *in Staphylococcus aureus*. *Yale J Biol Med*, 90(2): 269–281.

Stogios P. J. and Savchenko A., 2020. Molecular mechanisms of vancomycin resistance. *Protein Sci*, 29(3): 654−669.

Monistero V., Barberio A., Biscarini F., Cremonesi P., Castiglioni B., Graber H. U., Bottini E., Ceballos-Marquez A., Kroemker V., Petzer I. M., Pollera C., Santisteban C., Dantos M. V., Bronzo V., Piccinini R., Re G., Cocchi M., Moroni P., 2020. Different distribution of antimicrobial resistance genes and virulence profiles of *Staphylococcus aureus* strains isolated from clinical mastitis in six countries, *J Dai Sci*, 103(4): 3431−3446.

Cong Y., Yang S., Rao X., 2020. Vancomycin resistant *Staphylococcus aureus* infections: A review of case updating and clinical features. *J Adv Res*, 21: 169−176.

Phu V. D, Wertheim H. F., Larsson M., Nadjm B., Dinh Q. D., Nilsson L. E., Rydell U., Le T. T., Trinh S. H., Pham H. M., Tran C. T., Doan H. T., Tran N. T., Le N. D., Huynh N. V., Tran T. P., Tran B. D., Nguyen S. T., Pham T. T., Dang T. Q., Nguyen C. V., Lam Y. M., Thwaites G., Nguyen V. K, Hanberger H., 2016. Burden of Hospital Acquired Infections and Antimicrobial Use in Vietnamese Adult Intensive Care Units. *PLoS One*, 11(1): e0147544.

Le N. K., Wertheim H. F., Vu P. D., Khu D. T. K., Le H. T., Hoang B. T. N., Vo V. T., Lam Y. M., Vu D. T. V., Nguyen T. H., Thai T. Q., Nilsson L. E., Rydell U., Nguyen K. V., Nadjm B., Clarkson L., Hanberger H., Larsson M., 2016. High prevalence of hospital-acquired infections caused by gram-negative carbapenem resistant strains in Vietnamese pediatric ICUs: A multi-centre point prevalence survey. *Medicine (Baltimore)*, 95(27): e4099.

Vu T. V. D., Choisy M., Do T. T. N., Nguyen V. M. H., Campbell J. I., Le T. H., Nguyen V. T., Wertheim H. F. L., Pham N. T., Nguyen V. K., Doorn H. R., the VINARES consortium., 2021. Antimicrobial susceptibility testing results from 13 hospitals in Viet Nam: VINARES 2016–2017. *Antimicrob Resist Infect Control*, 10(78).

Nguyen V. A., Le H. L. H., Vu H. L., Nguyen T. H. V, Pham Q. H., Le V. H., Nguyen T. S., Le T. H., Dinh V. H., Hoang T. K., Le N. M., Nguyen H. V., Nguyen D. H., Pham N. V., Ta B. T., Tran V. T., Le H. H., 2024. Antimicrobial resistance patterns of *Staphylococcus aureus* isolated at a general hospital in Vietnam between 2014 and 2021, *Dovepress*, 2024: 259−273.

Jahanshahi A., Zeighami H., Haghi F., 2018. Molecular Characterization of Methicillin and Vancomycin Resistant *Staphylococcus aureus* Strains Isolated from Hospitalized Patients. *Microb Drug Resist*, 24(10): 1529−1536.

Kim E. S., Bae I. G., Cho J. E., Choi Y. J., Kim I. H., Kang G. S., Sin H. Y., Song K. H., Park C., Lee D. G., Kim M., Park K. U., Kim H. B., 2016. Clinical and Molecular Characterization of Invasive Heteroresistant Vancomycin-Intermediate *Staphylococcus aureus* Infections in Korean Hospitals. *J Clin Microbiol*, 54(3): 760−763.

Muzammil I., Ljaz M., Saleem M. H., Ali M. M., 2023. Molecular characterization of vancomycin-resistant *Staphylococcus aureus* isolated from bovine milk. *Zoonoses Public Health*, 70(5): 420−433.

Nguyen T. S., Vu T. T. H., Vu T. K. L., Do T. Q. N., Tran T. H. A., Tang T. N., Le N. M. H., Tran Q. B., 2019. First Report on Multidrug-Resistant Methicillin-Resistant *Staphylococcus aureus* Isolates in Children Admitted to Tertiary Hospitals in Vietnam. *J Microbiol Biotechnol*, 29(9): 1460−1469.

Hoang D. P., Tran T. T. T., Le T. T. H., Nguyen T. K. O., Vu D. H. L., Kieu D. H., Banuls A-L, Nguyen Q. H., 2023. Molecular characterization of methicillin-resistant *Staphylococcus aureus* strain BM85 isolated from a Vietnamese patient with blood stream infection. *Vietnam Journal of Biotechnology*, 21(1): 35−44.

Sherathiya V. N., Schaid M. D., Seiler J. L., Lopez G. C., Lerner T. N., 2021. GuPPy, a Python toolbox for the analysis of fiber photometry data. *Sci Rep*, 11(1): 24212.

Ahmed B. O., Elmekki A. M., Omer E. E., Elhassan M., 2014. Molecular detection of methicillin-resistant *Staphylococcus aureus* in patients with urinary tract infections in Khartoum State. *J. Sci. Technol*, 15: 1–8.

Clark N., Cooksey R. C., Hill B. C., Swenson J. M., Tenover F. C., 1993. Characterization of glycopeptide-resistant enterococci from U.S. hospitals. *Antimicrob Agents Chemother*. 37(11): 2311−2317.

He Y-H., Ruan G-J., Hao H. H., Xue F. F., Ma Y-K., Zhu S-N., Zheng B., 2020. Real-time PCR for the rapid detection of vanA, vanB and vanM genes. *Journal of Microbiology, Immunology and Infection*, 53(5): 746−750.

Wouter D. C., Svenn D., Darrin T. S., Marc C., Christine V. B., 2018, NanoPack: visualizing and processing long-read sequencing data. *Bioinformatics*, 34(15): 2666–2669.

Wick R. R., Judd L. M., Gorrie C. L., Holt K. E., 2017. Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 13(6): e1005595.

Hayer J., Dainat J., Marcy E., Banuls A-L., 2023. Baargin: a Nextflow workflow for the automatic analysis of bacterial genomics data with a focus on Antimicrobial Resistance. *Journal of Open Source Software*, 8(90): 5397.

Jolley K. A., Bray J. E., Maiden M. C. J., 2018. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *Wellcome Open Res*., 24(3): 124.

Kaya H., Hasman H., Larsen J., Stegger M., Johannesen T. B., Allesøe R. L., Lemvigh C. K., Aarestrup F. M., Lund O., Larsen A. R., 2018. SCCmecFinder, a Web-Based Tool for Typing of Staphylococcal Cassette Chromosome mec in

Staphylococcus aureus Using Whole-Genome Sequence Data. *mSphere*, 3(1): e00612–17.

Feldgarden M., Brover V., Gonzalez-Escalona N., Frye J. G., Haendiges J., Haft D. H., Hofmann M., Pettengill J. B., Klimke W., 2021. AMRFinderPlus and the Reference Gene Catalog facilitate examination of the genomic links among antimicrobial resistance, stress response, and virulence. *Sci Rep*, 11: 12728.

Seemann T., 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics*, 30(14): 2068−2069.

Grant J. R., Enns E., Marinier E., Mandal A., Herman E. K., Chen C., Graham M., Domselaar G. V., Stothard P., 2023. Proksee: in-depth characterization and visualization of bacterial genomes. *Nucleic Acids Research*, 51(W1): W484–W492.

Doncheva N. T., Morris J. H., Gorodkin J., Jensen L. J., 2019. Cytoscape StringApp: Network Analysis and Visualization of Proteomics Data. *J Proteome Res*, 18(2): 623−632.

Arciola C. R., Campoccia D., Ravaioli S., Montanaro L., 2015. Polysaccharide intercellular adhesin in biofilm: structural and regulatory aspects. *Front Cell Infect Microbiol*, 10(5): 7.

Dasari P., Nordengrün M., Vilhena C., Steil L., Abdurrahman G., Surmann K., Dhople V., Lahrberg J., Bachert C., Skerka C., Völker U., Bröker B. M., Zipfel P. F., 2020. The Protease SplB of *Staphylococcus aureus* Targets Host Complement Components and Inhibits Complement-Mediated Bacterial Opsonophagocytosis. *J Bacteriol*, 204(1): e0018421.

Vasquez M. T., Lubkin A., Tamara R. R., Christopher J. D., Keenan A. L., Michael P. J., Victor J. T., 2020. Identification of a domain critical for *Staphylococcus aureus* LukED receptor targeting and lysis of erythrocytes, *J Biologic Chemis*, 295(50): 17241–17250.

Tam K., Torres V.J., 2019. Staphylococcus aureus Secreted Toxins and Extracellular Enzymes, *Microbiol Spectr*, 7(2): 10.

Zhao X., Yuan X., Hu M., Zhang Y., Li L., Zhang Q., Yuan X., Wang W., Liu Y., 2021. Prevalence and characterization of Staphylococcus aureus and methicillin-resistant *Staphylococcus aureus* isolated from bulk tank milk in Shandong dairy farms. *Food Control*, 125: 107836.

Monteiro A. S., Pinto B. L. S., Monteiro J. M., Ferreira R. M., Ribeiro P. C. S., Bando S. Y., Marques S. G., Silva L. C. N., Neto W. R. N., Ferreira G. F., 2019. Phylogenetic and Molecular Profile of *Staphylococcus aureus* Isolated from Bloodstream Infections in Northeast Brazil. *Microorganisms*, 7: 210.

Hafer C., Lin Y., Kornblum J., Lowy F. D., Uhlemann A. C., 2012. Contribution of selected gene mutations to resistance in clinical isolates of vancomycin-intermediate *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 56(11): 5845–51.

Hiramatsu K., Kayayama Y., Matsuo M., Aiba Y., Saito M., Hishinuma T., Iwamoto A., 2014. Vancomycin-intermediate resistance in *Staphylococcus aureus*. *Journal of Global Antimicrobial Resistance*, 2(4): 213–224.

Cheung G. Y. C., Bae J. S., Otto M., 2021. Pathogenicity and virulence of *Staphylococcus aureus*. *Virulence*, 12(1): 547–569.

Shettigar K., Murali T. S., 2020. Virulence factors and clonal diversity of *Staphylococcus aureus* in colonization and wound infection with emphasis on diabetic foot infection. *Eur J Clin Microbiol Infect Dis*, 39: 2235–2246.

Cress B. F., Englaender J. A., He W., Kasper D., Linhardt R. J., Koffas M. A. G., 2014. Masquerading microbial pathogens: capsular polysaccharides mimic host-tissue molecules, *FEMS Microbiology Reviews*, 38(4): 660–697.

Jäger F., Kneuper H., Palmer T., 2018. EssC is a specificity determinant for *Staphylococcus aureus* type VII secretion. *Microbiology*, 164(5): 816–820.

Tang A., Caballero A. R., Bierdeman M. A., Marquart M. E., Foster T. J., Monk I. R., O'Callaghan R. J., 2019. *Staphylococcus aureus* Superantigen-Like Protein SSL1: A Toxic Protease. *Pathogens*, 8(1): 2.

Kadkhoda H., Ghalavand Z., Nikmanesh B., Kodori M., Houri H., Taghizadeh M. D., Karimi B. A., Eslami G., 2020. Characterization of biofilm formation and virulence factors of *Staphylococcus aureus* isolates from paediatric patients in Tehran, Iran. *Iran J Basic Med Sci*, 23(5): 691–698.

Paharik A. E., Salgado-Pabon W., Meyerholz D. K., White M. J., Schlievert P. M., Horswill A. R., 2016. The Spl Serine Proteases Modulate *Staphylococcus aureus* Protein Production and Virulence in a Rabbit Model of Pneumonia. *mSphere*, 1(5): e00208–16.