RESEARCH ARTICLE

Genome Sequence of a Highly Virulent \( pvl \)-positive Vancomycin-intermediate-resistant \( S. aureus \) Sequence Type 30

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Abstract: Background: \( S. aureus \) isolates expressing the Panton-Valentine Leukocidin (PVL) have been related to a wide range of diseases. Recently, \( pvl \)-positive community-associated methicillin-resistant \( S. aureus \) belonging to USA1100 (ST30/CC30/SCCmec IV) lineage has emerged in Brazilian hospitals.

Objective: The aim of this work was to sequence the genome of a \( pvl \)-positive USA1100 Vancomycin-Intermediate-Resistant \( S. aureus \) (VISA) isolate from Rio de Janeiro, Brazil.

Methods: The 13420 genome was sequenced using the HiSeq 2500 platform. The draft genome, plasmids annotation, and genome analysis were performed using RAST. Comparison of the relative \( pvl \) gene expression of six \( S. aureus \) isolates was performed by qRT-PCR.

Results: The isolate presented the \( \phi \)PVL phage codifying for the H2b PVL protein isoform, and another prophage carrying a PVL variant named lukF and lukS-PV.2. The 13420 genome presented a high number of virulence determinants, such as genes codifying for serine-protease proteins, enterotoxins (egc), the immune evasion cluster (IEC), adhesion proteins, spermine/spermidine acetyltransferase gene (blt), superantigen-like proteins, as well as the ica operon. Point mutations at vrsA, icaA, and tcaB genes were detected. Moreover, the PVL mRNA relative expression of the 13420 isolate was five times higher than mRNA PVL levels of the USA300/ST8 reference strain.

Conclusion: We described for the first time the genome sequence of a VISA isolate harboring two \( pvl \)-associated genes and other virulence factors that may improve the USA1100/ST30 lineage fitness and impact its pathogenicity and spreading at Brazilian hospitals.

Keywords: \( S. aureus \), \( pvl \)-positive, MRSA, VISA, USA1100/ST30, virulence.

1. INTRODUCTION

\( S. aureus \) is an important cause of infections worldwide [1]. Methicillin resistance is usually associated with the presence of \( mecA \) or \( mecC \) genes, located at the Staphylococcal Cassette Chromosome \( mec \) (\( SCCmec \)), which codifies to an altered penicillin-binding protein (PBP) with reduced affinity for \( \beta \)-lactam antibiotics [1, 2]. Methicillin resistance may also be associated with the presence of the \( mecB \) gene, carried by plasmids [3].

Although vancomycin is used as a treatment for methicillin-resistant \( S. aureus \) (MRSA) invasive infections, full resistance to vancomycin (vancomycin-resistant \( S. aureus \), VRSA phenotype) has been reported [4]. The presence of vancomycin resistance genes (\( vanA, vanB \), and/or \( vanC \)) causes modification and/or elimination of vancomycin binding sites at the cell wall through enzymatic action [4]. Intermediate-resistance (vancomycin-intermediate resistant \( S. aureus \), VISA phenotype) is associated with increased cell wall thickness, reduced peptidoglycan cross-link or changes...
at penicillin-binding proteins levels [5, 6]. Moreover, some studies indicate that point mutations in vraS, msrR, graR, rpoB, fdh2, and este1 genes may lead to specific amino acid changes that play a role in this type of resistance [6-8].

*S. aureus* isolates present several virulence genes that contribute to its pathogenesis and dissemination. Panton-Valentine Leukocidin (PVL) is a bi-component and pore-forming toxin that is associated with a wide range of uncomplicated to severe diseases [9]. The *pvl* gene is composed of two co-transcribed open reading frames (ORFs), named as *lukS*-PV and *lukF*-PV, and is located on a lysogenized bacteriophage integrated into *S. aureus* chromosome [10]. Single nucleotide polymorphisms (SNPs) in the *pvl* genes have been reported and may generate different PVL isoforms [11]. For example, an SNP at position 527 leads to a histidine to arginine substitution at amino acid 176, generating the H and R isoforms [12]. Such modifications may increase the leukotoxicity of PVL protein [11].

In the United States, *pvl* genes have been commonly carried by the community-associated MRSA (CA-MRSA) USA300/ST8/SCCmec IV, a pandemic clone that can also be found in Europe and African countries [13]. In Brazil and other Latin American countries, the USA1100/ST30/SCCmec IV is the most prevalent CA-MRSA lineage that carries *pvl* genes [14, 15]. Recently, our group described for the first time a *pvl*-positive VISA *S. aureus* belonging to this lineage [14]. This isolate presented the Pulsed Field Gel Electrophoresis (PFGE) profile identical to other 17 *pvl*-positive *S. aureus* isolates from different hospitals in Rio de Janeiro, Brazil [14, 15].

Data regarding the MRSA isolates presenting *pvl* genes and reduced susceptibility to vancomycin have not been found. Moreover, most VISA isolates belong to the USA100/ST5/SCCmec II lineage in the United States [16] and Brazil [17]. Thus, the aim of this work was to present the genome sequence of a *pvl*-positive VISA isolate belonging to the USA1100/ST30/SCCmec IV lineage, highlighting the virulence factors that may impact on its pathogenicity and spreading at Brazilian hospitals.

2. MATERIALS AND METHODS

2.1. Microbiology Characteristics of *pvl*-positive *S. aureus* VISA Isolate

The isolate characterized as *pvl*-positive VISA (13420; original number 1342a) [14], was recovered from the blood of a neonate in March of 2009, and was selected for sequence analysis at the present study. The isolate was characterized as MRSA by the cefoxitin disk test and the *SCCmec* type IV was confirmed by Polymerase Chain Reaction (PCR) [18]. The isolate presented a Minimum Inhibitory Concentration (MIC) of 4 μg/mL for vancomycin confirmed by broth microdilution and population analysis tests [14].

2.2. Whole-genome Sequencing and Genomic Analyses

The 13420 DNA was extracted using the Qiagen DNeasy Kit (Qiagen, Hilden, Germany) and its concentration and purity were assessed using a NanoVue® spectrophotometer (GE Healthcare, Chicago, Illinois, EUA). The libraries were sequenced using HiSeq 2500 (Illumina San Diego, CA, USA) 2x100-bp paired-end sequence technology. The quality of the raw reads was assessed by FastQC 0.11.5. A total of 23,038,932 paired reads were generated, with an average size of 101 base pairs (bp). The reads were assembled using Spades [19] with the following attributes "-t 15 -k 21,33,55,77 --careful --cov-cutoff auto". Contigs longer than 200 bp were removed, resulting in 50 contigs with an average coverage of 366 and a total assemble length of 2,802,979 bp, with N50 of 173,959.

The contigs were ordered using MAUVE [20] with the *S. aureus* strain T0131 (higher homology using NCBI blast, GenBank accession number CP002643.1) as reference. The two larger contigs that did not align to the reference genome were used as input in NCBI Blast and identified as plasmids.

After plasmids removal, the contigs were assembled into one scaffold using Raygoat [21] with the *S. aureus* strains COL, T0131, and MRSA252 as references. The scaffold constructed only with *S. aureus* COL showed the best results and was chosen. Thereafter, genome comparisons were conducted with USA300 and Mu50 strains, using BLAST Ring Image Generator (BRIG) [22].

The draft genome and plasmids annotation were done using Rapid Annotation Subsystem Technology–RAST [23]. Prophages were identified using PHASTER [24].

The sequence of *lukS* and *lukF*-PV genes was compared with the phage φSLT (GenBank accession number ABO45978), to identify the PVL isoform carried by the 13420 isolate. The analysis was made using BioEdit Sequence Alignment Editor, according to O’Harra and coworkers [11].

To better characterize the *pvl*-positive VISA 13420 isolate, the orthologous gene comparisons were investigated using OrthoVenn [25].

To identify point mutations at the isolate 13420, we analyzed the amino acid sequences encoded by the genes: *vraS*, *vraG*, *graR*, *tcaA*, *tcaB*, *msrR*, *fdh2*, and *rpoB* using the BioEdit Sequence Alignment Editor. The gene sequences of 13420 isolate were compared to genes sequences of the COL chromosome (GenBank accession number CP00046) [26].

2.3. Real-time Quantitative PCR (qRT-PCR) to Compare Relative *pvl* Gene Expression

Six *S. aureus* isolates, belonging to different *S. aureus* lineages, including the 13420 isolate, were cultured overnight (ON) on sheep blood Agar (Laborclin, Brasil) at 37°C. After this period, up to five colonies were transferred to 10 mL of brain heart infusion broth (BHI) (Difco, Becton, Dickinson and Company, USA) and cultured ON at 37°C. Then, bacterial turbidity was adjusted to OD600nm = 0.05 in a 25 mL of BHI, following incubation in a shaker (180 rpm) for 4 to 5 h, until the OD600nm = 0.8, which corresponds to approximately 1 × 10^8 CFU/mL. Then, 0.5 mL of the cultures were transferred to a microtube and treated with 1 mL of RNAprotect® Bacterial Reagent (Qiagen). The RNAs were extracted using RNeasy® Mini (Qiagen), in order to compare the relative *pvl* expression, according to the manufacturer’s instructions. The isolate 523a (USA300/ST8/SCCmecIV) was used as the reference strain for PVL mRNA expression [27]. Genomic DNA was eliminated by TURBO DNA-free™ DNase.
Treatment and Removal Reagents (Ambion®, Carlsbad, California, USA). Then, 500 ng of each RNA sample were reverse transcribed using the High Capacity cDNA Reverse Transcription (Applied Biosystem, Foster City, California, USA). The qRT-PCR were carried out using the GoTaq® qPCR Master Mix (Promega, Madison, Wisconsin, USA) at ABI7500 (Life Sciences, Carlsbad, California, USA) system, using the primers rrs F (5’-CATGTGATCTCAGATTAC-3’), rrs R (5’-CCATAAGTGTTGTCAGTT-3’) for rrs (165 rRNA gene) expression [28], and lukS-PV F (5’-AATAACGTATGCAAAATATGGAAGT-3’) and lukS- PV R (5’-CAATGCTGATCTACGA-3’) for pvI expression [28, 29]. Cycle thresholds (Ct) values were analyzed and the relative expression was identified using the 2^(-ΔΔCt) method [30]. The pvI Ct values were normalized to results obtained for rrs (endogenous control). The results were analyzed according to the MIQE guidelines [31].

2.4. Data Availability

Nucleotide sequence data obtained in this study have been submitted to the GenBank® under accession numbers CP021141 (https://www.ncbi.nlm.nih.gov/nuccore/CP021141), CP021142 (https://www.ncbi.nlm.nih.gov/nuccore/CP021142), and CP021143 (https://www.ncbi.nlm.nih.gov/nuccore/CP021143).

3. RESULTS

The genome of the pvI positive 13420 isolate (CP021141) consists of a circular 2,807,636 bp chromosome presenting 32.7% C+G content, 2606 Coding DNA Sequences (CDS), 62 RNA sequences, belonging to ST30, agr type 3, and spa-type t318. Besides the core genome, the isolate 13420 presented four prophages and six S. aureus genomic islands, characterizing its accessory genome (Fig. 1). The isolate 13420 also showed two plasmids, named as p24 and p29 (CP021142 and CP021143, respectively) (Fig. 2). The plasmid p24 presented 20,785 bp, 28% of C+G content, and contained the gene for β-lactamase A, blaZ, as well as the genes blalR (β-lactam sensor) and blal (penicillinase repressor). The plasmid p29 presented 8,110 bp and C+G content of 30.28% and did not codify for any previously annotated virulence or resistance genes.

According to the PHASTER tool, four phages were identified at the 13420 genome (Table 1). Phage classification as complete or incomplete was based on the detection of phage-formation proteins used for new phage particles. Therefore, two incomplete (Phages #1 and #3) and two complete phages (Phages #2 and #4) inserted at 13420 genome were detected. Phage #1, despite being related to an S. aureus Pathogenicity Island (SaPI) found at COL chromosome (CP00046), presented 20 CDS related to eight different phage species, with no relevant virulence gene associated to it.

The complete Phage #2 presented 78 CDS related to the φPVL that codifies for lukS-PV (978 bp) and lukF-PV (939 bp) genes. Comparison of the sequence of both genes, lukS and lukF-PV, with the phage φSLT (ABO45978), showed that the 13420 isolate presented the H2b PVL protein isoform, with SNVs at 470, 527, 633, and 1729 nucleotide positions, resulting in amino acid changes at 157 (Phe > Tyr), 176 (Arg > Hist), 222 (Ser > Ala), and 577 (Arg > Gln), respectively.

Phage #3 showed the presence of serine protease-like proteins (SPL) genes, such as splC, splE, and splF. In addition, the eeg cluster encoding G, N, U, I, M, and O enterotoxins genes was also found at Phage #3. The complete Phage #4 presented 48 CDS, some of them related to genes encoding for the staphylococcal complement inhibitor (SCIN), the chemotaxis inhibitory protein (CHIPS), and the staphylokinase (SAK).

Virulence factors of the core genome (e.g. cflA, cflB, fnbA, fnbB, ebpS, cna, and bhp) were also detected. Moreover, the genes encoding for SasH and SasD superantigen-like proteins, sdCR (serine aspartate repeat containing protein C), sdrD (serine aspartate repeat containing protein D), hlaG (alpha haemolysin), and hlaB (beta haemolysin) were found, as well as the ica operon, responsible for biofilm production. Moreover, it is noteworthy to mention that the 13420 isolate presented a sperrmidine/serine acetyltransferase gene (bta), with 100% of identity with S. aureus subsp. aureus Z172 (UniProt accession number A0A0E1ANJ6 - unreviewed).

Two new uncharacterized leukocidin-like proteins with 1056 and 1020 bp were detected at Phage #4 and were named as lukS-PV.2 and lukF-PV.2, respectively. BLAST analysis revealed that lukS-PV.2 and lukF-PV.2 presented 100% of identity with 12 and 27 S. aureus sequenced genomes, respectively.

According to the UniProt database, the lukS-PV.2 gene (located between positions 2,078,729 and 2,077,674 bp) translated a protein named "uncharacterized leukocidin-like protein 2" (UniProt accession number Q6G6F9). The lukF-PV.2 gene (located between 2,077,652 and 2,076,633 bp) translated a protein named "uncharacterized leukocidin-like protein 1" (UniProt accession number Q6G6F0). Both proteins were reviewed by the Swiss-Prot database, and 3D structures were elucidated at the Protein Modal Portal, with its model structure provided by the Swissmodel portal. Both proteins are similar to the crystal structure of LukGH from S. aureus USA300 isolate [32].

Since the 13420 isolate was the first USA1100/ST30/SCCmec IV presenting a VISA phenotype recovered from the community environment in Rio de Janeiro/Brazil [14], we compared its genome with the Mu50 (USA100/ST5/SCCmec II/VISA) (BA000017) [7] and USA300 (ST8/SCCmec IV) (CP000255) [33] genomes (Fig. 1). The Venn diagram (Fig. 3) revealed 1097 orthologs protein clusters shared by the 3 isolates, plus 166 clusters were shared at least by two isolates, and 3 clusters were found exclusively at only one of the genomes, being two clusters at 13420 and one at Mu50.

One of the protein clusters exclusively found in 13420 isolate was related to a signal transduction histidine-protein kinase (ArtL2) (UniProt accession number Q6GGZ4), codified by the artL2 gene and also found at S. aureus MRSA252 (E-MRSA 16/ST36/CC30) isolate (BX571856) [34]. Another protein found on the 13420 genome was the sensor SrrB (UniProt accession number Q6GGK7), also found at the MRSA252 genome (UniProt accession number BX571856). However, only the srrB gene, but not the srrA, was found at the 13420 genome.
The sequenced S. aureus 13420 isolate presented amino acid substitutions at vraS, tcaA, and tcaB genes, associated with vancomycin intermediate-resistance (Table 2). The mutation substitution of a serine residue to a leucine at amino acid position 239 of the vraS gene was observed. For the tcaA gene, point mutations at F31L, L218P, Y237H, and S448P were observed, while for the tcaB gene, mutations L173M and V360I were detected (Table 2).

RT-qPCR comparison studies of pvl relative expression revealed that the 13420 isolate expressed up to five times higher PVL mRNA levels when compared to the USA300 isolate 526a [27], used as a PVL-positive reference strain (Table 3). The relative expression of PVL mRNA was also high for the 1155 isolate, belonging to the same lineage. Notably, the highest relative pvl expression was observed at USA1100/ST30/CC30 lineages (Table 3).

4. DISCUSSION

In order to better understand the genetic features of the 13420 pvl-positive isolate, its genome has been sequenced and showed a G+C content, CDS and RNA genes similar to other S. aureus genomes previously published [7, 26, 33, 34]. However, the 13420 genome presented exclusive characteristics, probably due to the presence of mobile genetic elements (MGEs), acquired by horizontal gene transfer (HGT) [7, 35], genomic islands, and other MGEs such as phages, transposons, and chromosomal cassettes that constitute the auxiliary or accessory genome of S. aureus [35].

Two plasmids, named as p24 and p29, were found among the accessory genome. The plasmid p24 presented the genes blaZ, blaK1 (β-lactam sensor), and blal (penicillinase repressor). Arédé et al., 2013 [36] showed that the bla operon regulatory system could interfere with gene repression
mediated by mecI at mec gene, allowing a higher PBP2a expression. Besides, some authors have suggested that the development of the VISA phenotype could be associated with modifications at the peptidoglycan metabolism, generally due to PBP2a super expression [5, 37]. Therefore, the presence of this plasmid could contribute to the VISA phenotype of 13420 isolate. Expression analysis studies are needed to determine the correlation between the bla operon and the low vancomycin susceptibility of 13420 isolate. Although the plasmid p29 did not present any annotated virulence or resistance genes, additional analysis is necessary to understand the functional role of this plasmid among USA1100/ST30 isolates.

The 13420 isolate presented the ϕPVL phage and an H2b-PVL protein isoform. Some PVL isoforms, carrying non-synonymous mutations (NSM), have been reported and are able to alter the functionality of the PVL protein [11]. Despite a previous work that described the association of H2b-PVL isoform with a methicillin-susceptible S. aureus (MSSA), this is the first report describing this isoform in an MRSA USA1100/ST30 isolate, highlighting the occurrence of a new PVL isoform in S. aureus isolated at Brazilian hospitals. Besides, two extra PVL variations were also detected at the 13420 genome (lukS-PV.2 and lukF-PV.2), urging the need for studies to elucidate the role of different PVL isoforms on infection severity caused by pvl positive S. aureus.
Table 2. Comparison of amino acid substitutions at genes associated with vancomycin intermediate resistance among Staphylococcus aureus 13420 and hVISA, VISA and VSSA isolates previously sequenced.

| Genes | 13420* | hVISA/VISA (n = 101) | VSSA (n=32) | References |
|-------|--------|----------------------|-------------|------------|
| vraS  | S239L  | 15N<sup>a</sup>, G88D, L123H, S167N, S239L<sup>d</sup>, F243S, K272I, L315M, I317T, F321L | nd | [6, 50, 51, 53] |
| vraS  | nd     | E59D, A1113V, S164P | S26R, E59D, F85L, I86L, E87K, A1113V, R117H, R121S, S164P | [50, 51] |
| graS  | nd     | R14L, L26F, M29R, L59L, D148Q, A153P, T224I, N289Y, Y301E, V304E, N332K | L26F, I59L, D148Q, T224I, S303R, R325K, N332K, V676I | [50, 51] |
| graR  | nd     | D148Q, F151L, N197S<sup>d</sup> | M90N, D147E, D148Q, S197G, V135I, V136I | [50, 51] |
| tcaA  | F31L, L1218P, Y237H, S448P | M202T, L218P, Y237H, T262S, T279I, R283H, G312D, N371I | K2E, N133I, M202T, L218P, Y237H, Y262S, R283H, G312D, I431V | [50, 51] |
| tcaB  | L173M, V360I | A91P, I232L, W308G | H6Y, V145F, F207L, S341N, V360I, K396R | [50, 51] |

<sup>a</sup> MIC of 4 mg/L for vancomycin; hVISA – S. aureus with heterogeneous intermediate resistance to vancomycin; VISA – S. aureus with intermediate resistance to vancomycin; VSSA – Vancomycin susceptible S. aureus; <sup>b</sup>Mu50 - VISA clinical strain with vraS (15N) and msrR (E146K), graR (N197S), rpoB (H481Y), and fih2 (A297V) mutations and MIC of 12 mg/L for vancomycin; <sup>c</sup>Mu3- hVISA clinical strain with vraS (15N) and msrR (E146K) mutation and MIC of 3 mg/L for vancomycin; <sup>d</sup>H14 - hVISA strain with vraS (S329L) mutation and MIC of 2 mg/L for vancomycin; A Alanine; D Aspartic acid; E Glutamic acid; F Phenylalanine; G Glycine; H Histidine; I Isoleucine; K Lysine; L Leucine; M Methionine; N Asparagine; P Proline; G Glutamine; R Arginine; S Serine; T Threonine; V Valine; Y Tyrosine; nd - not detected; n - number of isolates; In bold: mutations found among 13420 and hVISA/VISA and VSSA isolates.

Table 3. Molecular, clinical characteristics and relative pvl expression of six pvl-positive S. aureus isolates.

| Isolate Number | Clonality ST | SCCmec Type | Clinical Source | Relative Expression (pvl)<sup>+</sup> | SD |
|----------------|--------------|-------------|-----------------|----------------------------------|----|
| 526            | USA300/ST8   | IV          | renal abscess secretion | 1                                | 0.07 |
| 559            | USA800/ST5   | IV          | peritoneal liquid   | 0.903                            | 0.04 |
| 945            | USA400/ST1   | IV          | nares             | 0.002                            | 0.01 |
| 1155           | USA1100/ST30 | IV          | bone secretion    | 4.597                            | 0.10 |
| 13420<sup>†</sup> | USA1100/ST30 | IV          | blood             | 5.378                            | 0.01 |
| 1348           | USA400/ST1   | na          | blood             | 1.146                            | 0.03 |

<sup>+</sup> relative pvl expression in comparison with 526 (USA300/ST8) isolate; †isolate sequenced at the present study; ST - Sequence type; SCCmec - Staphylococcal Cassette Chromosome mec; SD - standard deviation; na - not applicable (methicillin-sensitive isolate). The experiments were performed in three independent triplicates.

Regarding toxin genes, the sequenced isolate showed genes codifying for spl, enterotoxins, CHIPS, SCIN, and adhesion proteins. It has been suggested that SPLs could trigger the T<sub>1</sub>2-related immune response in asthmatic patients that were colonized by S. aureus [38], while the egc cluster, is associated with foodborne intoxication [39]. Moreover, studies suggest that the SCIN protein is able to block the C3 convertase protein, which triggers the complement system, inhibiting its function at C3b deposition, phagocytosis and C5a generation, preventing bacterial lyses by complement system activation [40]. On the other hand, the role of CHIPS in staphylococcal infection is due to inhibition of activation of neutrophils and monocytes. Moreover, its interaction with the human complement receptor C5aR and formylated peptide receptor could lead to specific phagocyte responses inhibition [41]. Curiously, C5a-R and C5L2 are host targets for PVL, mediating both toxin binding and cytotoxicity [9, 42]. Jamrozy and coworkers [43] described that nearly USA300 isolates evaluated at their study carried the immune evasion cluster (IEC) composed of s<sub>c</sub>n, chp, and sak genes, which was also detected amongst some non-USA300 isolates. In addition, the presence of blt, the spm/spermidine acetyltransferase gene, is noteworthy, as spermidine modulates host immune response and its produced in high levels by keratinocytes at inflammation sites [44]. USA300 isolates are more resistant to the spermidine activity, due to the presence of speG gene, which codifies for N-acetyltransferase protein, responsible for the acetylation and inactivation of spermidine [45]. A metabolic evaluation study of 64 S. aureus isolates cultured in 300 different culture media showed that only the two USA300/ST8 isolates were able to use spermidine as the only source of carbon and nitrogen, due to the presence of speG [35]. Therefore, the presence of spermine/spermidine acetyltransferase gene in the 13420 CA-MRSA genome, as well as the IEC could confer to the ability of USA1100/ST30 isolates to
cause skin and soft tissue infections in our environment, similar to what is observed for USA300 CA-MRSA isolates in the USA.

Despite being characterized as a CA-MRSA, the 13420 genome shared more ortholog proteins with a Mu50 *S. aureus* hospital isolate (51 proteins), than with USA300 (31 proteins), a classical CA-MRSA found in the USA [46] (Fig. 3). Besides these similarities, the ArlS protein, a member of the two-component regulatory system (TCS) ArlS/ArlR involved in *S. aureus* adhesion regulation, autolysis, multidrug resistance [47], was detected exclusively at the 13420 genome. Mutations in both genes increased protein production, such as toxins, hemolysins, lipases, coagulase, and serine proteases and protein A (Spa), indicating that the arl operon down-regulates the production of virulence factors by decreasing gene transcription [47].

A recent study showed that the ArlS/ArlR TCS plays a role in biofilm formation on implanted catheters by activating PNAG exopolysaccharide production [48]. Another member of TCSR protein found only at 13420 genome was the sensor SrrB, an SrrA/SrrB TCS member which regulates agr RNAIII, protein A, SrrAB, and TSST-1 levels [49]. Although only the srrB gene was found at the 13420 genome, the presence of different TCS among USA1100/ST30 isolates could be related to a highly virulent profile, the prevalence of this pvl-positive *S. aureus* community lineage in our environment.

Genetic alterations in TCSR genes are strongly associated with *S. aureus* glycopeptide-resistance [8, 50]. Reduced bacterial susceptibility to vancomycin is related to point mutations at cell wall synthesis genes [50], as well as antimicrobial resistance genes [6, 8]. Point mutations at Mu50 (VISA) strain at vraS (IS5), msrR (E146K), graR (N197S), rpoB (H481Y), and fdh2 (A297V) genes, and at Mu3 (heterogeneous VISA, hVISA) strain at vraS (IS5) and msrR (E146K) genes could lead to an intermediate-resistance phenotype [6, 8]. Plus, it was shown that both vraS (S329L) and msrR (E146K) mutations generated the hVISA phenotype, and the introduction of two more mutations, into graR (N197S) and rpoB (H481Y) converted the hVISA phenotype into the same vancomycin resistance level as the Mu50 VISA isolate [6]. Here, we detected the vraS (S329L) mutation at the 13420 (Table 2). Other authors suggested that this point mutation leads to the constitutive activation of vraSR operon leading to an up-regulation of cell wall synthesis genes [6]. Further studies are necessary to clarify the role of such point mutations in the VISA phenotype since previous studies also showed some of these SNVs, including L218P and Y237H at tcaA and V360I at tcaB genes on vancomycin-susceptible isolates [50, 51] (Table 2).

Although pvl gene expression is usually investigated using USA300/ST8/SCCmec IV isolates [29], a study conducted by Boakes and coworkers [52] showed the PVL expression by 142 MRSA from different lineages (CC1, 5, 8, 22, 30, 59, 80, and 88), isolated in Wales and England, from 2005 to 2008. Different levels of PVL expression were observed between the isolates with a higher expression in CC8 isolates, usually related to USA300 clone, when compared to CC5, 22, 30, 80, and 88 isolates [52].

Here we show that the relative expression of PVL mRNA was higher in USA1100/ST30/CC30 isolates in comparison to other lineages (Table 3). However, it is important to notice that the primers used to access pvl expression did not align with lukF-PV.2 and lukF-PV.2 genes, indicating that the relative mRNA expression of pvl was related only to the PVL phage. Although the role of lukSF-PV.2 as leukocidin has not yet been addressed, the concomitant expression of both lukSF-PV and lukSF-PV.2 genes could increase 13420 isolate virulence. Further analysis is needed to better understand the role of the proteins codified by lukSF-PV.2 and lukF-PV.2 genes.

**CONCLUSION**

In conclusion, the low vancomycin susceptibility of an MRSA isolate belonging to a commonly community found lineage, but isolated in a hospital in Rio de Janeiro, highlights the ability of such isolates to adapt and spread to different environments. Moreover, the high relative expression of pvl in combination with other virulence determinants, such as new leukocidin-like proteins, SPLs, ebc, and immune evasion cluster, alongside to a spermine/spermidine acetyltransferase genes, could improve the fitness of such lineage, impacting on its pathogenicity, allowing its entrance and establishment at Brazilian hospitals.

**LIST OF ABBREVIATIONS**

| Abbreviation | Definition |
|--------------|------------|
| BHI          | Brain Heart Infusion Broth |
| BLAST        | Basic Local Alignment Search Tool |
| bp           | Base pairs |
| BRIG         | BLAST Ring Image Generator |
| CA-MRSA      | Community-Associated MRSA |
| CC           | Clonal Complex |
| CDS          | Coding DNA Sequences |
| CFU          | Colony Forming Units |
| CHIPS        | Chemotaxis Inhibitory Protein |
| Ct           | Cycle thresholds |
| HGT          | Horizontal Gene Transfer |
| hVISA        | Heterogenous VISA |
| IEC          | Immune Evasion Cluster |
| MGE          | Mobile Genetic Elements |
| MIC          | Minimum Inhibitory Concentration |
| MRSA         | Methicillin-Resistant *S. aureus* |
| MSSA         | Methicillin-Susceptible *S. aureus* |
| NCBI         | National Center for Biotechnology Information |
| NSM          | Non-Synonymous Mutations |
| ON           | Overnight |
| ORF          | Open Reading Frames |
| PBP          | Penicillin Binding Protein |
| PCR          | Polymerase Chain Reaction |
| PFGE         | Pulsed Field Gel Electrophoresis |
| PVL          | Panton-Valentine Leukocidin |
| qRT-PCR      | Real Time Quantitative PCR |
| RAST         | Rapid Annotation Subsystem Technology |
| *S. aureus*  | *Staphylococcus aureus* |
| SAK          | Staphylokinase |
| SaPI         | *Staphylococcus aureus* Pathogenicity Island |
| SCCmec       | Staphylococcal Cassette Chromosome mec |
SCIN = Staphylococcal Complement Inhibitor
SNP = Single Nucleotide Polymorphisms
SPL = Serine Protease-like Protein
ST = Sequence Type
TCRS = Two-Component Regulatory System
USA = United States of America
VISA = Vancomycin-Intermediate Resistant S. aureus
VRSA = Vancomycin-Resistant S. aureus

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study uses strains obtained from a public Hospital in Rio de Janeiro. This study was approved by the Comitê de Ética em Pesquisa da Secretaria Municipal de Saúde e Defesa Civil, Rio de Janeiro, Brazil, (reference number SMSDC-RJ 0205.0.314.000-10).

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Nucleotide sequence data obtained in this study have been submitted to the GenBank® under accession numbers CP021141 (https://www.ncbi.nlm.nih.gov/nuccore/CP021141), CP021142 (https://www.ncbi.nlm.nih.gov/nuccore/CP021142) and CP021143 (https://www.ncbi.nlm.nih.gov/nuccore/CP021143).

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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RC designed the study, performed, and contributed to all the experiments and analyses and wrote the manuscript draft; TO and TG extracted genonic DNA and RNA and helped at qRT-PCR experiments; RF helped at qRT-PCR analysis; LMM and JT performed the Illumina sequencing; CR performed genome annotation and genomic analysis; LOM and KS contributed to the study design and edited the manuscript.

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