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RESEARCH ARTICLE

Genome Sequence of a Highly Virulent *pvl*-positive Vancomycin-intermediate-resistant *Staphylococcus aureus* Sequence Type 30

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ARTICLE HISTORY

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Abstract: ***Background:*** *Staphylococcus 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 ϕPVL 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 *vraS*, *tcaA,* 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

Staphylococcus 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 Cassete Chromosome *mec* (*SCCmec*), which codifies to an altered penicillin-binding protein (PBP) with reduced affinity for β-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

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at penicillin-binding proteins levels [5, 6]. Moreover, some studies indicate that point mutations in *vraS*, *msrR*, *graR*, *rpoB*, *fdh2,* and *esle1* 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/*SCC mec* 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 lower 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 Ragout [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 *ϕ*SLT (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'Hara 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*, *vraS*, *graS, 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 37ºC. 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 37ºC. Then, bacterial turbidity was adjusted to OD_{600nm} = 0.05 in a 25 mL of BHI, following incubation in a shaker (180 rpm) for 4 to 5 h, until the OD_{600nm} = 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/*SCCmec*IV) 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 *rrsC-F* (5'- CATGCTGATCTACGATTACT-3'), *rrsC-R* (5' CCATAAAGTTGTTCTCAGTT-3') for *rrs* (16S rRNA gene) expression [28], and *lukS-PV F* (5'-AATAACGTATGGCAGAAATATGGATGT-3') and *lukS-PV R* (5' CAAATGCGTTGTGTATTCTAGATCCT-3') for *pvl* expression [28, 29]. Cycle thresholds (Ct) values were analyzed and the relative expression was identified using the $2^{-\Delta\Delta Ct}$ method [30]. The *pvl* 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 *pvl* 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 *blaR1* (β-lactam sensor) and *blaI* (penicilinase 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 *ϕ*PVL 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 *ϕ*SLT (**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 *egc* cluster encoding G, N, U, I, M, and O enterotoxins genes was also found at Phage #3. The complete Phage #4 presented 48 CDSs, 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. clfA*, *clfB*, *fnbA*, *fnbB*, *ebpS*, *cna,* and *bbp*) were also detected. Moreover, the genes encoding for SasH and SasD superantigen-like proteins, *sdrC* (serine aspartate repeat containing protein C), *sdrD* (serine aspartate repeat containing protein D), *hlgA* (alfa haemolysin), and *hlgB* (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 spermine/spermidine acetyltransferase gene (*blt*), 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-PV2*. gene (located between positions 2,078,729 and 2,077,674 bp) translated a protein named "uncharacterized leukocidin-like protein 2" (UniProt accession number **Q6GF49**). 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 **Q6GF50)**. 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 ortholog proteins 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 (ArlS) (UniProt accession number **Q6GGZ4)**, codified by the *arlS* 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.

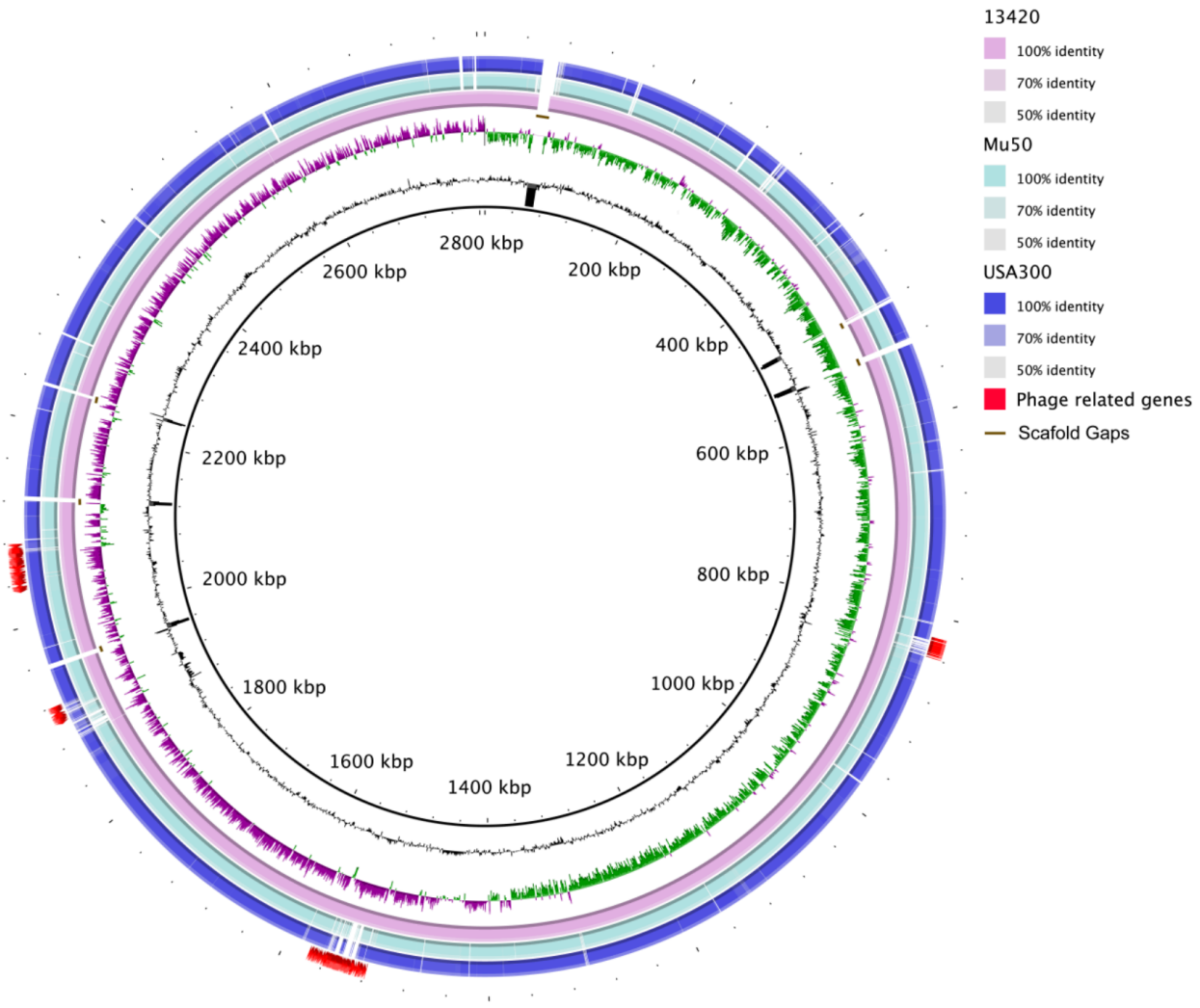

Fig. (1). Multiple genome comparison between *S. aureus* 13420, Mu50 and USA300 isolates, constructed using BLAST Ring Image Generator (BRIG). The dark blue, light blue, and pink circles represent USA300, Mu50, and 13420 genomes, respectively. The black line represents Scaffold gaps, while red lines phage related genes. Circular representation of the genome comparison of the three *S. aureus* strains 13420, Mu50, and USA300. Circle 1 (innermost circle) and circle 8 (outermost circle) indicate the distances from the putative origin of replication of the *S. aureus* 13420 chromosome. Circles 2 and 3 designate the G+C content (black) and G+C skew (green, plus strand; purple, minus strand). Circle 4, 5, and 6 show, respectively, the genome of 13420, Mu50, and USA300 chromosomes and their identities based on Blast using *S. aureus* 13420 as a reference. Circle 7 shows regions with higher amounts of phage related genes. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

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 *vrsA* 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*, *blaR1* (β-lactam sensor), and *blaI* (penicilinase repressor). Arêde *et al.*, 2013 [36] showed that the *bla* operon regulatory system could interfere with gene repression

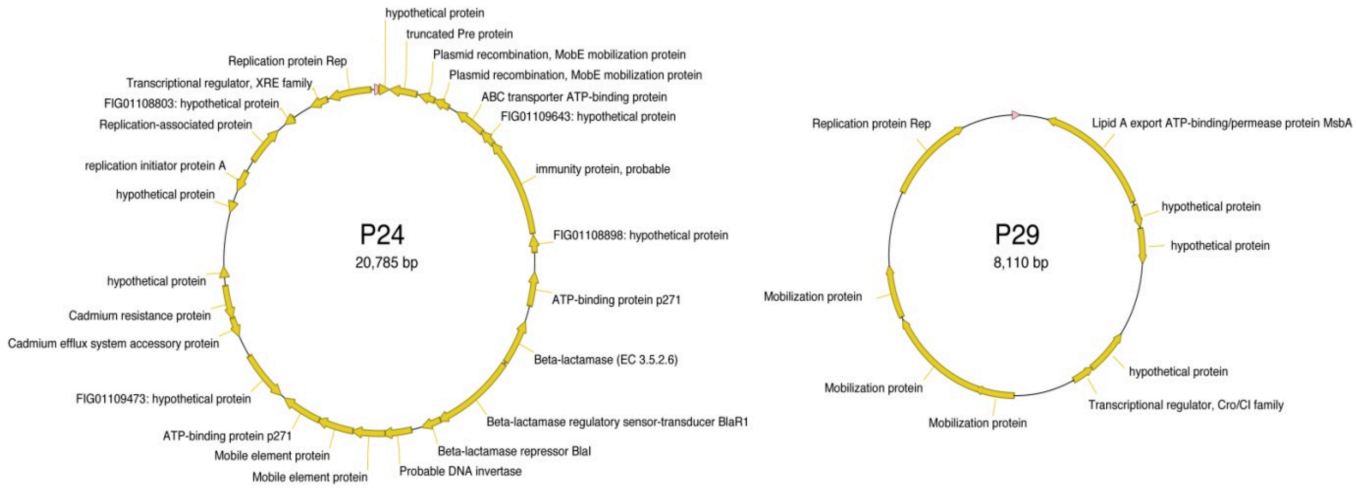

Fig. (2). Circular conformation and genes associated with the two plasmids from the *S. aureus* 13420 isolate sequenced in the present study. bp, base pairs. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 1. Characterization of four phages incorporated into the genome of the MRSA 13420 isolate according to the PHASTER tool.

Phage	Classification	*Score**	Initial Position	Final Position	Size (Kb)	G+C (%)	CDS	Reference Genome †
#1	Incomplete	20	819741	838155	18.4	31.5	20	NC_007045
#2	Complete	100	1518148	1574122	55.9	33.1	78	NC_012784
#3	Incomplete	20	1901925	1918694	16.7	28.9	19	NC_031125
#4	Complete	100	2043339	2078729	35.3	33	48	NC_008617

*Percentage of identity with the reference genome; †Genome Accessing Number; Kb Kilobases; CDS Coding DNA Sequence.

mediated by *mec*I 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*.

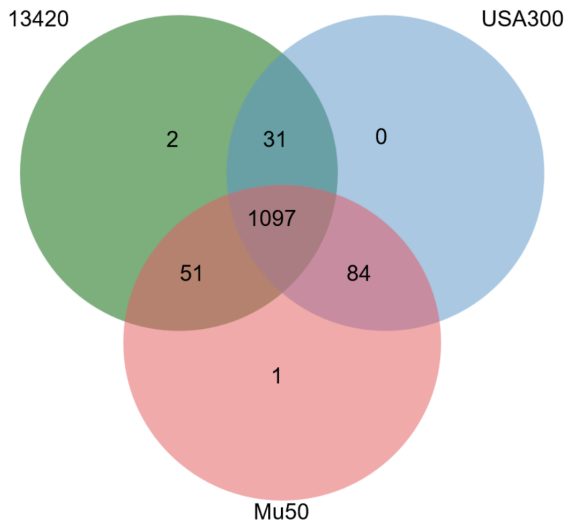

Fig. (3). Venn diagram plotted by the OrthoVenn program shows shared orthologous protein clusters among the genomes of *S. aureus* 13420 (in green), Mu50 (pink), and USA300 (blue) isolates. The numbers indicate the unique genes in each strain or the shared genes between groups of strains, satisfying the criteria of more than 50% amino acid identity on 50% of the total length of a protein. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

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[a]	hVISA/VISA (n = 101)	VSSA (n=32)	References
vraS	**S239L**	I5N[b,c], G88D, L123H, S167N, **S239L**[d], F243S, K272I, L315M, I317T, F321L	nd	[6, 50, 51, 53]
vraS	nd	E59D, A113V, S164P	S26R, E59D, F85L, I86L, E87K, A113V, R117H, R121S, S164P	[50, 51]
graS	nd	R14L, L26F, M29R, I59L, D148Q, A153P, T224I, N289Y, V301E, V304E, N332K	L26F, I59L, D148Q, T224I, S303R, R325K, N332K, V676I	[50, 51]
graR	nd	D148Q, F151L, N197S[b]	M90N, D147E, D148Q, S197G, V135I, V136I	[50, 51]
tcaA	F31L, **L218P**, **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]

[a] 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*; [b]Mu50 - VISA clinical strain with *vraS* (I5N), *msrR* (E146K),*graR* (N197S),*rpoB* (H481Y), and *fdh2* (A297V) mutations and MIC of 12 mg/L for vancomycin; [c] Mu3- hVISA clinical strain with *vraS* (I5N) and *msrR* (E146K) mutation and MIC of 3 mg/L for vancomycin; [d] 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; Q 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*)*	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†	USA1100/ST30	IV	blood	5.378	0.01
1348	USA400/ST1	na	blood	1.146	0.03

*relative *pvl* expression in comparison with 526 (USA300/ST8) isolate; †isolate sequenced at the present study; ST - Sequence type; *SCCmec* - Staphylococcal Cassete 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_H2-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 C5a-(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 *scn*, *chp,* and *sak* genes, which was also detected amongst some non-USA300 isolates. In addition, the presence of *blt*, the spermine/spermidine acetyltransferase gene, is noteworthy, as spermidine modulates host immunity 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 the *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 (TCRS) 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 TCRS plays a role in biofilm formation on implanted catheters by activating PNAG exopolysaccharide production [48]. Another member of TCRS protein found only at 13420 genome was the sensor SrrB, a SrrA/SrrB TCRS 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 TCRS among USA1100/ST30 isolates could be related to a highly virulent profile, allowing the prevalence of this *pvl*-positive *S. aureus* community lineage in our environment.

Genetic alterations in TCRS 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* (I5N), *msrR* (E146K), *graR* (N197S), *rpoB* (H481Y), and *fdh2* (A297V) genes, and at Mu3 (heterogeneous VISA, hVISA) strain at *vraS* (I5N) 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 *lukS-PV.2* and *lukF-PV.2* genes, indicating that the relative mRNA expression of *pvl* was related only to the ϕPVL 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 *lukS-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, *egc,* 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

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 Cassete 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 Cívil, 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 genomic 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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