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ORIGINAL ARTICLE

Draft genome sequence of *Staphylococcus aureus* sequence type 5 SA01 isolated from bloodstream infection and comparative analysis with reference strains

Romulo Maia Ferreira[1] · Douglas Henrique dos Santos Silva[1] · Karinny Farias Silva[1] · Joveliane de Melo Monteiro[1] · Gabriella Freitas Ferreira[2] · Maria Raimunda Chagas Silva[3] · Luís Claudio Nascimento da Silva[4] · Letícia de Castro Oliveira[5] · Andrea Souza Monteiro[1]

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Abstract

A *Staphylococcus aureus* isolate (SA01) obtained from bloodstream infection exhibited a remarkable drug resistance profile. In this study, we report the draft genome sequence of *S. aureus* ST 5 SA01, a multidrug-resistant isolate, and analyzed the genes associated with drug resistance and virulence. The genome sketch of *S. aureus* ST5 SA01 was sequenced with Illumina and annotated using the Prokka software. Rapid Annotation Subsystem Technology (RAST) was used to verify the gene functions in the genome subsystems. The Comprehensive Antibiotic Resistance Database (CARD) and Virulence Factor Database (VFDB) were used in the analysis. The RAST indicated a contribution of 25 proteins to host adenine, fibronectin-binding protein A (FnbA), and biofilm formation as an intercellular polysaccharide adhesive system (PIA). The MLST indicated that *S. aureus* ST 5 SA01 belongs to ST5 (CC5). In silico analyses also showed an extensive repertoire of genes associated with toxins, such as LukGH leukocidin, enterotoxins, and superantigen staphylococcal classes (SSL). The 11 genes for antimicrobial resistance in *S. aureus* ST 5 SA01 showed similarity and identity above ≥ 99% with nucleotide sequences deposited in GenBank. Although studies on ST5 clones in Brazil are scarce, monitoring the clone of *S. aureus* ST 5 SA01 is essential, as it has become a problem in pediatrics in several countries.

Keywords *Staphylococcus aureus* · Genome · Antibiotic resistance · Virulence

Introduction

Staphylococcus aureus is an important Gram-positive bacterium, usually found in the human anterior nares and skin. As an opportunistic pathogen, it is involved in a range of clinical conditions, including sepsis, surgical wound infections, and severe pneumonia, and diabetic foot ulcers (Tong et al. 2015; Macedo et al. 2021; Buis et al. 2023). Moreover, this bacterium often acquires resistance to antibiotics routinely used in medical practice (Tong et al. 2015). The systemic dissemination of *S. aureus* is associated with various virulence determinants such as coagulase, lipases, adhesins, nucleases, hemolysin, and toxins (Cheung et al. 2021). The toxins harm many immune cells and are classified as gamma-hemolysin AB (HlgAB), gamma-hemolysin CB (HlgCB), leukotoxin GH (LukGH), leukotoxin ED (LukED), and Panton-Valentine leukocidin (PVL) (Ahmad-Mansour et al. 2021).

The α-, β-, γ-, and δ-hemolysin are responsible for erythrocyte lysis and may aggravate the clinical symptoms during

✉ Luís Claudio Nascimento da Silva
luiscn.silva@ceuma.br

[1] Laboratório de Microbiologia Aplicada, Universidade CEUMA, São Luís 65075-120, MA, Brasil

[2] Departamento de Farmácia, Universidade Federal de Juiz de Fora - Campus Governador Valadares, CEP 35010-180 Juiz de Fora, MG, Brasil

[3] Laboratório de Ciências Do Ambiente, Universidade CEUMA, São Luís 65075-120, MA, Brasil

[4] Laboratório de Patogenicidade Microbiana, Universidade CEUMA, São Luís 65075-120, MA, Brasil

[5] Departamento de Microbiologia, Universidade Federal Do Triângulo Mineiro, Imunologia E Parasitologia, 38025180 Uberaba, MG, Brasil

tissue and bloodstream infections (Vandenesch et al. 2012; Duan et al. 2018). In addition, α-hemolysin is more relevant for the pathogenicity of *S. aureus*, as it is responsible for the formation of pores in the plasma membrane of various host cells. Thus, α-hemolysin can modulate multiple cellular processes, including excessive production of cytokines and trigger cell death (Virreira Winter et al. 2016).

In a previous study, *S. aureus* strains were isolated from bloodstream infections in São Luís, a city in Northeast Brazil that is part of the Legal Amazon area. One isolate, denominated *S. aureus* ST 5 SA01 showed resistance against many antibiotics (including clindamycin, erythromycin, gentamicin, rifampicin, and tetracycline) being classified as multidrug-resistant (MDR) (Monteiro et al. 2019). The drug resistance profile of this isolate encouraged the genome analysis of this strain since the sequence data obtained allow the further characterization of the mechanisms involved in drug resistance and virulence of bacterial pathogens (Kumburu et al. 2018; McManus et al. 2020; Jesus et al. 2022; Kumari et al. 2023).

This study reports the draft genome sequence of *S. aureus* ST 5 SA01 and comparative analyzes of genes associated with drug resistance and virulence using standard strains. The genomic data reported in this study are useful for future in silico analysis providing more insights about the spread of genes related with virulence and drug resistance.

Materials and methods

Sample

The bacterium isolate (*S. aureus* SA01) used in the study belong to the culture bank of the Laboratory of Applied Microbiology at the CEUMA University. It was previously isolated as part of other study with positive blood culture of patients hospitalized in Intensive Care Units (ICUs) (Monteiro et al. 2019). The bacterium is kept in glycerol stocks preserved at − 20 °C.

Antimicrobial susceptibility test and time-kill curve for oxacillin

The antimicrobial susceptibility profile of *S. aureus* ST *5* SA01 isolate was determined using AST #105 and GP-ID cards from the VITEK® 2 Compact system (BioMérieux, Marcy l'Etoile, France), according to the Clinical Laboratory Standards Institute (CLSI 2017). The susceptibility was evaluated for the following antibiotics: oxacillin, erythromycin, clindamycin, gentamicin, rifampicin, teicoplanin, vancomycin, trimethoprim/sulfamethoxazole, ciprofloxacin, and linezolid.

For the time-kill assay, the bacterial suspension (100 µL at 1×10^6 CFU/mL) was added to 900 µL of Muller Hinton broth (MHB) containing different oxacillin concentrations (32, 64, 128, and 256 µg/mL). At specific periods (0, 6, 12, 18, and 24 h), aliquots were tenfold diluted and plated for colony-forming unit (CFU) enumeration. The results were expressed as Log CFU/mL.

Genome sequencing, annotation, and Multilocus sequence typing analysis

The partial genome was sequenced using the Illumina MiSeq paired library approach and prepared by the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA). Preassembled genomic DNA sequences were annotated using the Prokka software tool (Seemann 2014). The sequence readings were assembled with the A5 software and processed for adapter cutting, quality filtering, and error correction to generate the contigs and scaffolds. In addition, the CAP3 software was used to improve scaffolding assembly, cut low-quality regions, and correct erroneous links between contigs.

Multilocus sequence typing analysis (MLST) was used to confirm the type of sequence (ST) and clonal complex. The sequences of seven housekeeping genes were analyzed: (i) *arcC*, (ii) *aroE*, (iii) *glpF*, (iv) *gmk*, (v) *pta*, (vi) *tpi*, and (vii) *yqiL*. The STs were obtained through an online web tool http://saureus.mlst.net) (Enright et al. 2000).

Partial analysis of Staphylococcus aureus SA01 genome

The annotated sequences of *S. aureus* ST 5 *SA01* genome were analyzed using Rapid Annotation using Subsystem Technology (RAST), available at https://rast.nmpdr.org/rast.cgi (Aziz et al. 2008). Virulence factor genes were identified by comparison with the Virulence Factor Database (VFDB) (availabe at http://www.mgc.ac.cn/cgi-bin/VFs/v5/main.cgi) (Chen 2004). The Comprehensive Antibiotic Resistance Database (CARD) (available at https://card.mcmaster.ca) was used to determine the antibiotic resistance profile or resistance of *S. aureus ST 5 SA01* (Alcock et al. 2019). A search for putative prophages in the *genome of S. aureus* ST 5 SA01 was performed with PHASTER (https://phaster.ca. (Arndt et al. 2016).

Mauve Contig Mover and BLAST Ring Image Generator (BRIG)

The Mauve Contig Mover was used to determine genome rearrangements after alignment. The genome *S. aureus* NCTC 8325 was selected as the reference in this analysis. The analysis was made for *S. aureus* SA01, *S. aureus* MS4, *S. aureus* Mu3, *S. aureus* Mu50, *S. aureus* N315, and *S. aureus* RF122. The comparison with other genomes was performed using Blast Ring Image Generator (BRIG) (Alikhan et al. 2011).

OrthoVenn and digital DNA-DNA hybridization analysis

A Venn diagram using OrthoVenn 2 (https://orthovenn2.bioinfotoolkits.net) (Xu et al. 2019) software allowed the visualization of genes encoding shared and unique proteins or pseudogenes between *S. aureus* ST 5 SA01 and *S. aureus* NCTC 8325 in each sequenced genome. Each lineage of *S. aureus* is represented by ovals of different colors with the number of groups of orthologs genes shared by the strains considered.

A phylogeny of the genome was generated using the TYGS server (http://tygs.dsmz.de.) (Meier-Kolthoff and Göker 2019). The generated genome sequence was used to determine the OGRI values about closely related *Staphylococcus* strains, including the digital DNA-DNA hybridization value (dDDH) calculated using GGDC web server formula two available at https://ggdc.dsmz.de/ggdc.php.

Results

Characterization of the Staphylococcus aureus SA01

The *S. aureus* ST 5 SA01 is resistant to oxacillin, erythromycin, gentamicin, and ciprofloxacin, and it is sensitive to teicoplanin, rifampicin, trimethoprim/sulfamethoxazole, vancomycin, and linezolid. In fact, *S. aureus* ST 5 *SA01* is MDR isolate. The MLST analyses confirmed that it belongs to clonal complex 5 (CC5) and sequence type 5 (ST5).

The growth of the *S. aureus ST 5 SA01* was evaluated in the presence of oxacillin at 32, 64, 128, and 256 μg/mL up to 24 h. The time-kill curve shows that *S. aureus* ST 5 *SA01* did not grow in the presence of the highest concentrations tested (128 μg/mL and 256 μg/mL). The reductions in CFU counting for oxacillin at 64 μg/mL were 2.44, 4.59, 2.34, and 0.24 for incubation during 6, 12, 18, and 24 h, respectively. For oxacillin at 32 μg/mL, the reduction was only observed after 6 h of incubation (1.98) (Fig. 1).

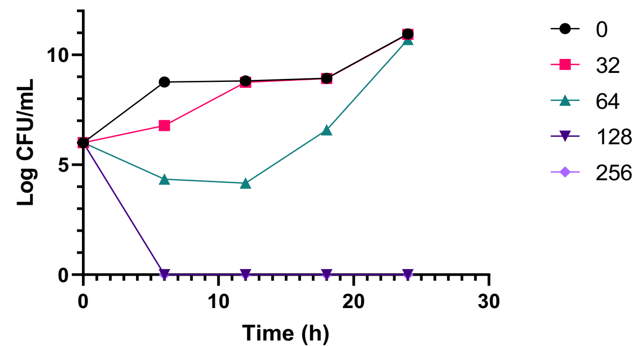


Fig. 1 Time-kill curve of *Staphylococcus aureus* SA01 grown in the presence of oxacillin at different concentrations (32, 64, 128, and 256 μg/mL)

Table 1 Genome assembly characteristics of the *Staphylococcus aureus* SA01 strain indicated by RAST

Characteristics	Data
Size	2.889,043
*G + C content	32.8
Number of coding sequences	2687
Number of operons tmRNA (RNA messenger-transporter)	1
Number of tRNA genes (transporter RNA)	60

[*] *G*, guanine; *C*, cytosin

Genome sequencing and annotation

The draft genome annotation data of *S. aureus* ST 5 SA01 are shown in (Table 1). Genomic sequencing data were deposited in the sequence read file database under Bio project prjna563016 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA563016), SAMN12661330 access biosample, Sequence Read Archive SRR10042834 under access (https://www.ncbi.nlm.nih.gov/search/all/?term=SRR10042834), and WGS was deposited with identification Access JANUHQ000000000 (https://www.ncbi.nlm.nih.gov/nuccore/JANUHQ000000000.1).

Identifying the sequences of coding genes (CDs) in the partial genome was 2687 CDs. The RAST analysis performed on several genes showed associations with several categories of subsystems (Fig. 2). However, this study highlights some genes that encode proteins associated with virulence and antibiotic resistance involved in iron capture. BLASTn compared these elements, and finally, the identities of the sequences were displayed.

Rapid Annotation Subsystem Technology (RAST)

Rapid Annotation Subsystem Technology (RAST) analysis revealed the pathogenicity potential of *S. aureus* ST 5 SA01 showing 25 proteins involved in adhesion, such as *sas*A protein anchored in the predicted cell wall (LPXTG reason), aggregation factors A and B (ClfA and ClfB) and protein A of binding to fibronectin (FnbA). Genes related to intercellular polysaccharide adhesin (IAP) and elastin-binding protein were also identified (Table 2).

Genomic inferences in *S. aureus* ST 5 SA01 also indicated the presence of 28 genes related to the iron acquisition system (Supplementary Table 1). Of the proteins found, 18 are related to the transport and metabolism of the heme ring. Its amino acid sequences are highly similar to the sequences deposited from other strains of *S. aureus*.

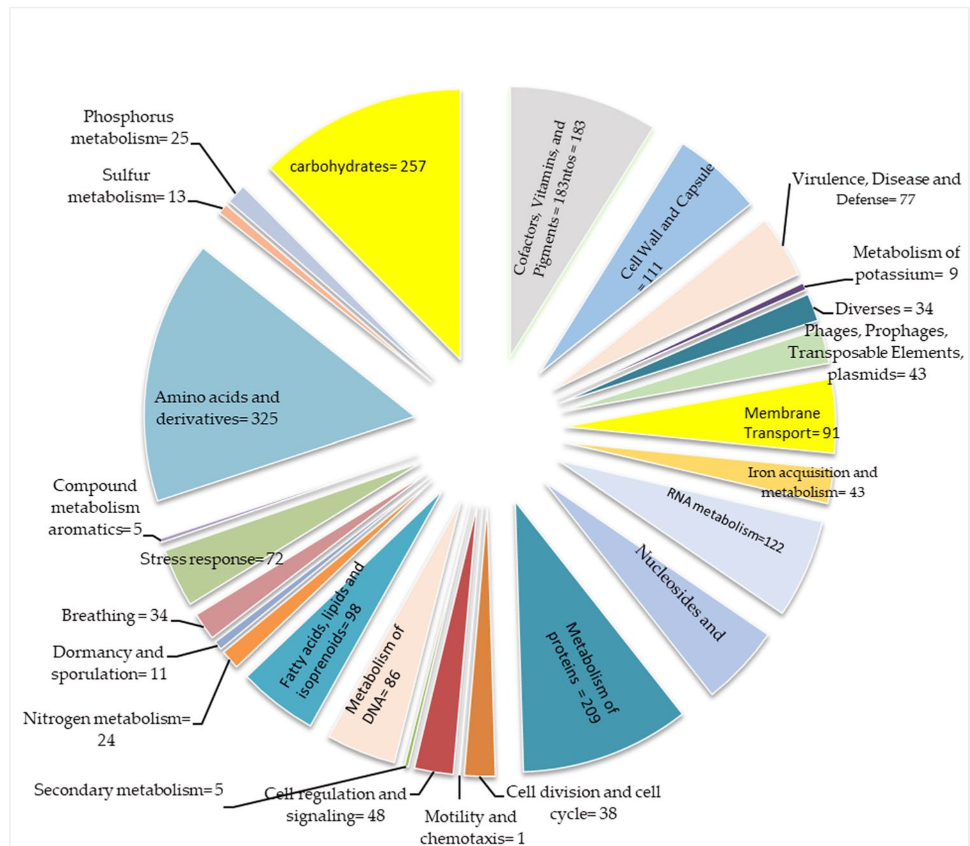


Fig. 2 Subsystem categories present non-genome of *Staphylococcus aureus* SA01 annotated by Rapid Annotation Subsystem Technology (RAST)

Virulence Factor Database (VFDB)

The Virulence Factor Database (VFDB) system verified the broad spectrum of virulence genes. We expressed the results by comparing their absence and presence *to seven lines of S. aureus* Newman NCTC8325, N315, Mu3, MW2, JH1, and USA300 TCH1516 in the database (Fig. 3). The contribution of sa01 genome genes to virulence was quantified in 60.5% ($n = 76$) of VFDB, with most genes found (79%) related to enzymes, toxins, secretor systems, and adhesion. Comparatively, some genes for exotoxins detected in the genome of *S. aureus ST* 5 SA01 were the same as those seen in *S. aureus* NCTC 8325, *S. aureus* Mu3, *S. aureus* Mu50, and *S. aureus* N315.

Prediction of antibiotic resistance in *Staphylococcus aureus* SA01

The antimicrobial sensitivity profile of *S. aureus ST* 5 SA01 shows resistance to oxacillin, erythromycin, clindamycin, gentamicin, and ciprofloxacin, however sensitivity to teicoplanin, rifampicin, trimethoprim/sulfamethoxazole, vancomycin, and linezolid. The corroboration of these data in the genome of *S. aureus* ST 5 SA01 was possible to verify the presence of *gyrA*, *parC*, and *mecA* using the homologous protein model by the CARD software (Table 3). Other genes associated with antibiotic resistance were detected, such as efflux pumps, a superfamily of primary facilitators, and genes related to antibiotic target site modification (Table 3). The 11 genes showed similarity and identity above ≥ 99% with nucleotide.

Analysis of prophages

The presence of three distinct regions for bacteriophage sequences was verified through analysis of the genome of the *S. aureus* ST 5 SA01 scanned by PHASTER (Table 4, Supplementary Fig. 1). These were two incomplete sequences of 18.3 and 9.3 kb. In addition, the sequence of an entire region greater than 36.2 kb was verified, comprising 35 proteins, in which the most common phages were PHAGE_Staphy_phiMR25_NC_010808, PHAGE_Bacill_BtCS33_NC_018085, and PHAGE_Staphy_SPbeta_like_NC_029119.

In parallel to PHASTER, the VRprofile server was used to locate the homologs for gene ORFs conserved using an association of HMMer and BLASTp (using the value Ha 0.81). The analyses indicated nine regions with ORFs for prophages. The largest predicted region for phages was ORF

Table 2 Virulence, adherence, and biofilm profile *of the Staphylococcus aureus* SA01 strain indicated by Rapid Annotation Subsystem Technology with the search for similarity in *Genbank*

Gene	Protein/function	Size (pb)	Amino acids	Identity	Access	E. value
EbpS	Elastin binding protein	1461	486	100%	givel402,347,180l EJU82232.1	0.0
FnbA	Fibronectin binding protein	3048	1015	100%	givel1,236,594,387l WP_094970207.1	0.0
ClfA	Cvf agglutination factor, Fibrinogen binding protein	2952	983	100%	givel1,460,108,473l WP_116453838.1	0.0
ClfB	ClfB agglutination factor, Fibrinogen binding protein	2634	877	99%	givel446,668,545l WP_000745891.1	0.0
PIA /IcaA	Polysaccharide intercellular adhesin (PIA) and biosynthesis of N-glycosyltransferase IcaA (EC 2.4.)	1113	370	99%	givel1,003,114,435l AMO18184.1	0.0
PIA /IcaB	Intercellular polysaccharide adhesin (PIA) biosynthesis of deacetylase IcaB (EC 3.)	873	290	100%	givel446,800,113l WP_000877369.1	0.0
PIA/IcaC	Intercellular polysaccharide adhesin (PIA) biosynthesis of protein IcaC	1053	350	99%	givel486,342,046l WP_001587819.1	0.0
PIA/IcaD	Intercellular polysaccharide adhesin (IAP) biosynthesis of IcaD protein	306	101	99%	givel686,440,826l WP_031924131.1	1e—64
IcaR	Operon biofilm icaABCD type of regulator of negative icar type transcription	561	186	100%	givel1,365,302,832l AVO70163.1	5e -130
	Leucocidin LukGH subunit G	1017	338	100%	givel446,518,046l WP_000595392.1	0.0
	Leucocidin LukGH subunit H	1056	351	100%	givel446,714,080l WP_000791411.1	0.0
Hlb	Beta-hemolysin	825	274	99%	givel152,002,409l ABS19574.1	0.0
	Beta-hemolysin	201	66	100%	givel965,683,129l BAU03830.1	9e—35
	Hemolysin III	687	228	99%	givel1,029,620,557l SBB28019.1	1e—162
	gamma-hemolysin component A	966	321	99%	givel1,105,704,201l SGV67041.1	0.0
	gamma-hemolysin component B	978	325	100%	givel1,407,185,588l WP_111091585.1	0.0
	gamma-hemolysin C components	948	315	100%	givel446,839,439l WP_000916695.1	0.0

8, at 102 KB, with 149 ORFs and 44 hypothetical proteins for sequences traced in some genomes of *S. aureus*. In addition, the research indicated that many predicted sequences are related to prophages found in different strains of *S. aureus*. In addition, lines are associated with five types of enterotoxins or their precursors, such as enterotoxin Q, sec3-enterotoxin C1 precursor, entE-enterotoxin E precursor, speG-exotoxin G precursor, and seb-enterotoxin B, and also associated with hlgA precursor of hemolysin-gamma chain II.

Genomic analyses by MAUVE Contig mover and BLAST Ring Image Generator

In the analysis of genome similarities by MAUVE, preserved regions or blocks were observed, especially the total alignment of the seven strains of *S. aureus*, allowing the identification of 18 local choline blocks (LCBs), with between 6 and 9 LCBs presenting regions without a rearrangement of the homologous gene sequence (Fig. 4). The genome sketch of *S. aureus* ST 5 SA01 gives four LCBs in reverse orientation compared to the other isolates of *S. aureus*; however, the contigs of *S. aureus* NCTC 8325, MS4, Mu3, Mu50, N315, and RF122 had high similarity and homologous regions without rearrangements. This similarity is attributed to essential functions, such as virulence genes or determinants of antibiotic resistance, in the *selected S. aureus* strains.

Furthermore, MAUVE analyses showed synteny between the genome outline of *S. aureus* ST 5 SA01 with the complete genome of *S. aureus* NCTC 8325 (reference lineage) (Supplementary Fig. 2). MAUVE analysis revealed that a vast part of genetic information was conserved between the two strains.

In the analysis of BRIGs between *S. aureus* ST 5 SA01 and NCTC 8325, RF122, and Mu3, the region between 1600 and 1400 kbp showed abundant GC regions representing GC skew (−) (Fig. 5). The region presented 100% similarity between SA01 and NCTC8325, differentiating them from the other strains of *S. aureus*.

OrthoVenn and digital DNA-DNA hybridization analysis

Ortholog clusters in the *S. aureus* SA01, Mu50, Mu3, N315, and NCTC 8523 were analyzed using the OrthoVenn 2 software. The analysis of the strains indicated that they form 2873 orthologs clusters, which include 2211 central genome orthologs (Fig. 6A). *S. aureus* ST 5 *SA01* presented five

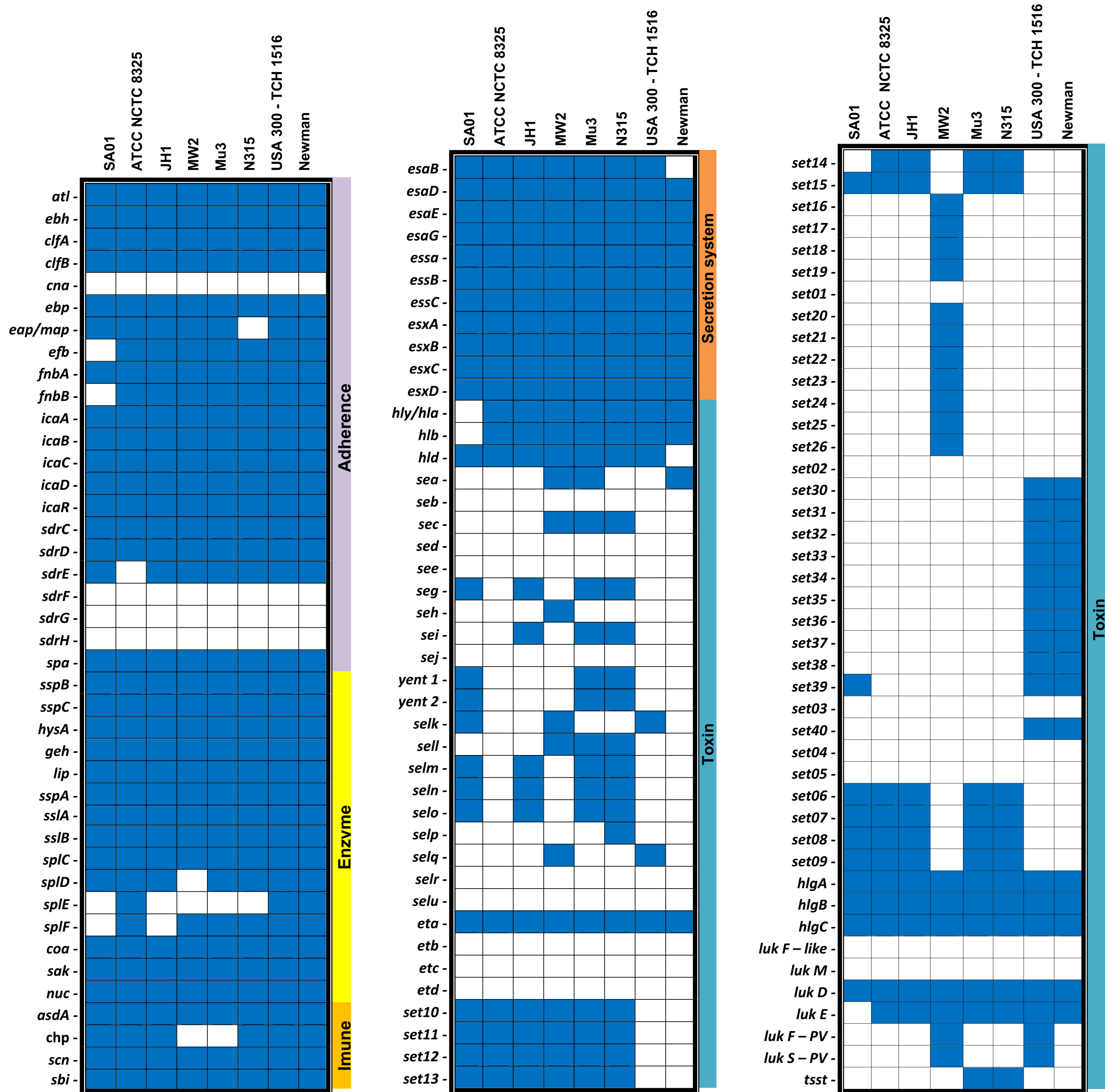


Fig. 3 Genes associated with virulence factors predicted in *Staphylococcus aureus* strains SA01 (1), Newman NCTC8325 (2), N315 (3), Mu3 (4), MW2 (5), JH1 (6), USA 300- TCH 1516 (7), and Mu50 (8). The blue network indicates the presence of virulence genes, while the empty network indicates their absence

unique (exclusive) clusters with proteins with no defined function. There were 24,955 gene families containing sequences of the four gene strains *S. aureus*, of which 2211 (76.96%), 289 (10.06%), 143 (5.08%), 216 (7.52%), and 14 (0.49%) were shared by five, four, three, two, and one of these species, respectively. The five strains of *S. aureus* formed 2873 clusters, 680 orthologs clusters (containing at least two species), and 2193 clusters of single-copy genes.

Figure 7 shows the functional classification of the proteins belonging to clusters the 2211 clusters shared by the five strains of *S. aureus*. Most proteins were classified as involved in biological, metabolic, and cellular metabolic processes. In this case, the biological processes represent a specific objective for which the organism is genetically programmed, such as cell division. On the other hand, metabolic processes involve chemical reactions and their pathways, through which

Table 3 Prediction of antibiotic resistance in *Staphylococcus aureus* SA01 using the Comprehensive Antibiotic Resistance Database (CARD)

Criterion	Term ARO	Detection criteria	Gene AMR family	Class of antibiotics and or substances	Resistance mechanism	% Region identity	% Length of the reference sequence
Perfect	*arlR*	Protein homologous model	Facilitating superfamily antibiotic outflow pump (MFS)	Fluoroquinolone, acridine dye	Efflux pump	100.0	100.00
Perfect	*mepR*	Protein homologous model	Multi-resist conveyor extrusion of toxic compounds (MATE)	Tetracyclines, glycylcycline	Efflux pump	100.0	100.00
Perfect	*mgrA*	Protein homologous model	Facilitating superfamily antibiotic flow pump (MFS), ATP binding cassette (ABC) pump of antibiotic efflux	Tetracycline, fluoroquinolone, glycopeptides, cephalosporin, acridine dye	Efflux pump	100.0	100.00
Perfect	*a(6)*	Protein homologous model	ANT(6)	Aminoglycosides	Inactivation of antibiotics	100.0	109.42
Perfect	*arlS*	Protein homologous model	Superfamily antibiotic outflow pump facilitator (MFS)	Fluoroquinolone, acridine dye	Efflux pump	100.0	100.00
Perfect	*APH(3′)-IIIa*	Protein homologous model	APH(3′)	Aminoglycosides	Inactivation of antibiotics	100.0	100.00
Perfect	*ErmA*	Protein homologous model	RNA metiltransferase ribossomal 23S de Er	Macrolide, lincosamide, streptogramin	Antibiotic target site change	100.0	100.00
Rigorous	*mecR1*	Protein homologous model	Methicillin-resistant P2	Monobactams, cephalosporin, gentamycin, carbapenem	Antibiotic target replacement	100.0	56.07
Rigorous	*mecca*	Protein homologous model	Methicillin-resistant P2	Monobactams, cephalosporin, cgentamycin carbapenem	Antibiotic target replacement	99.4	100.00
Rigorous	*SAT-4*	Protein homologous model	acetyltransferase of estreptotrichin (SAT)	Nucleosides	Inactivation of antibiotics	99.44	100.00
Rigorous	*tet(38)*	Protein homologous model	Superfamily antibiotic outflow pump facilitator (MFS)	Tetracyclines	Efflux pump	100.0	100.44
Rigorous	*Staphylococcus aureus gyrA*	Protein variant model	gyrA resistant to fluoroquinolones	Aminoglycosides, fluoroquinolones	Antibiotic target change	98.99	100.34
Rigorous	*AAC(6′)-Ie-APH(2″)-Ia*	Protein homologous model	APH(2″), AAC(6′)	Aminoglycosides	Inactivation of antibiotics	99.79	100.00
Rigorous	*Staphylococcus aureus parC*	Protein variant model	parC resistant to fluoroquinolones	Fluoroquinolone	Antibiotic target change	99.38	100.00
Rigorous	*mepA*	Protein homologous model	Multi-resist conveyor extrusion of toxic compounds (MATE)	Tetracyclines, glycylcycline	Efflux pump	99.56	100.00

living organisms transform chemicals; while, cellular metabolic processes are chemical reactions and their pathways that occur within the cell to transform chemicals (Fig. 7A).

When analyzed from the point of view of molecular functions (Fig. 7B), 16% of the proteins present hydrolase activity, which is a class of enzymes responsible for breaking a chemical bond and dividing a large molecule into two smaller ones; 15% of the proteins are involved in a molecular process involving the action or activity of a gene product, and 13% are responsible for the movement of substances inside, outside, and between cells.

Table 4 Profane regions predicted by PHASTER in *Staphylococcus aureus* SA01

Region length	Integrity	Score	Total proteins	Position of the region	Phage	GC%
18.3 Kb	Incomplete	20	10	148,520–166829	PHAGE_Bacill_BtCS33_NC_018085	33.95
9.3 Kb	Incomplete	20	12	56,163–65,552	PHAGE_Staphy_Seta_like_NC_029119	32.14
36.2 Kb	Intact	120	35	52,353–88,626	PHAGE_Staphy_phiMR25_NC_010808	34.59

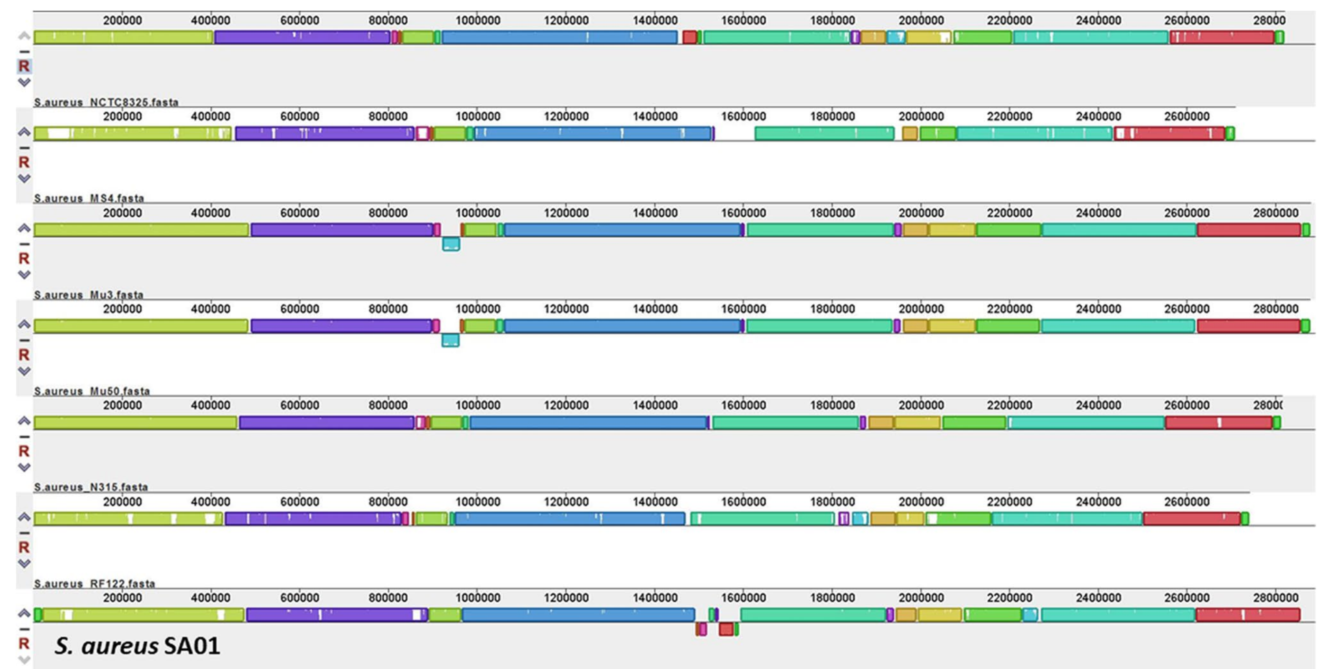


Fig. 4 Mauve comparison diagrams of the *S. aureus* NCTC8325, *S. aureus* MS4, *S. aureus* Mu3, *S. aureus* Mu50, *S. aureus* N315, and *S. aureus* RF122 genomes. Each colored region is a locally collinear block (LCB). The LCBs below the genome's center line are in reverse complement orientation compared with the *S. aureus* NCTC8325 genome

Regarding the cellular distribution, most proteins were classified as part of the cell and cell membrane (Fig. 7C).

The results of the TYGS database indicated high similarity *between S. aureus* ST 5 *SA01* and *S. aureus* N315, with 98.7% for dDhD; *S. aureus* ST 5 SA01 and *S. aureus* Mu50, with 98.1% for dDhD. However, we observed a value of 89.9% for DDDH *between S. aureus* ST 5 *SA01* and NCTC 8325 (reference lineage), with a difference of 0.05% in the content of G+C.

Phenotypic and genotypic differences and clonal relationships are supported by genomic relationships (ANI and dDDH). The phylogenetic analysis of the genome sequence sketch of *S. aureus* ST 5 *SA01* with other *Staphylococcus strains* revealed *a cluster of S. aureus* ST 5 *SA01 with S. aureus* N315 and *S. aureus* Mu50 are shown in Fig. 8. According to these results, genome analysis confirmed that *S. aureus* ST 5 SA01 actually belongs to *S. aureus* sub. *aureus* with ANI values of 98.76%; therefore, *S. aureus* sub. *aureus* N315, *S. aureus* sub. *aureus* Mu50, and *S. aureus* sub. *aureus* NCTC 8325 have ANI values of 98.81, 98.72, and 99.75%, respectively (Supplementary Table 2).

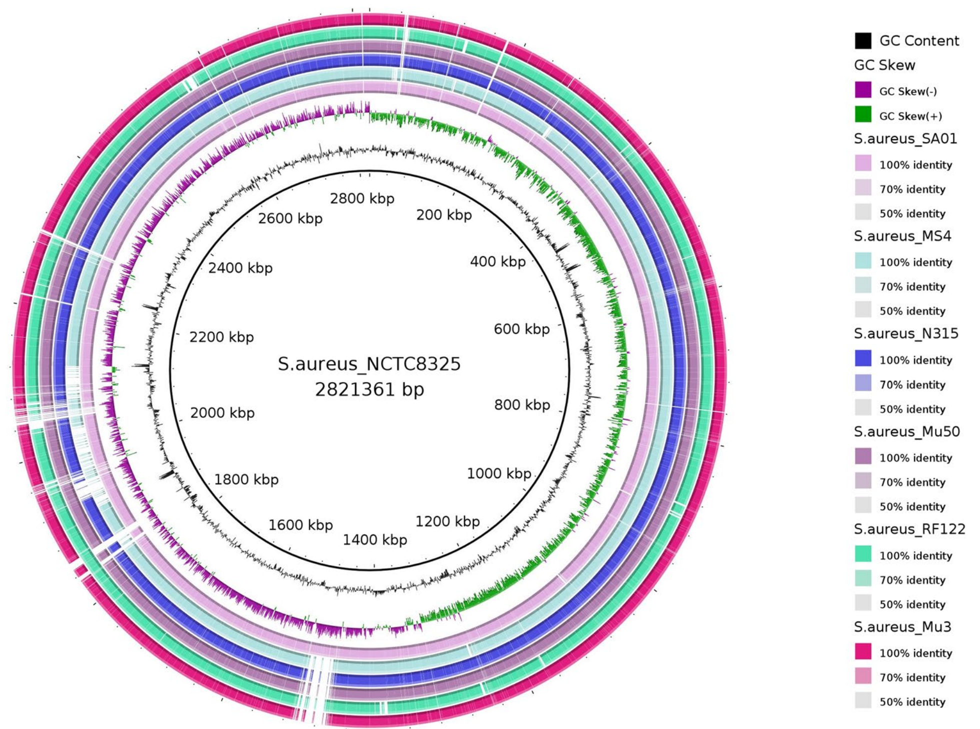
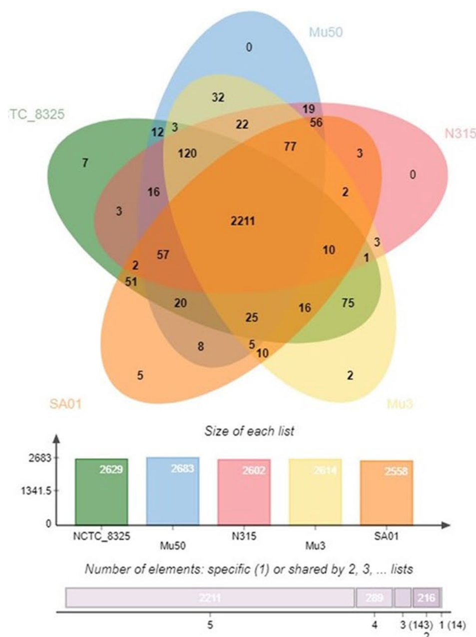


Fig. 5 Analysis of BRIGs among the strains of *Staphylococcus aureus* SA01, Mu50, Mu3, N315, RF122, and NCTC 8523. The innermost circle shows GC content. The cyan circle indicates the genome of NCTC 8523, and the lilac circle shows *P Staphylococcus aureus* SA01 genome. The white gaps indicate the absent sequences in the genomes



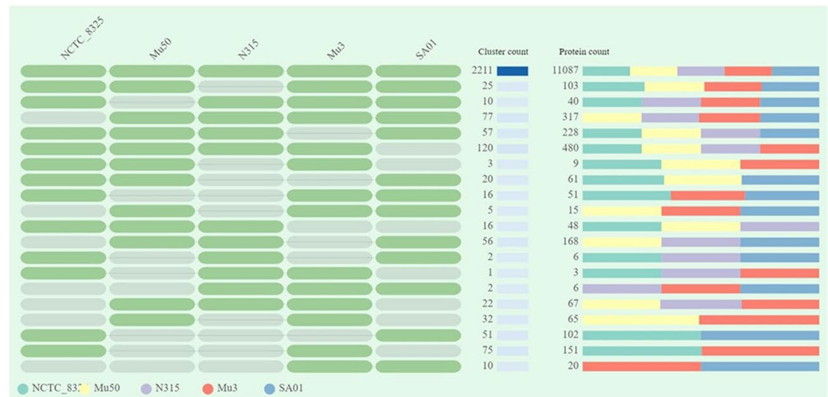


Fig. 6 Ortholog clusters in the *Staphylococcus aureus* strains SA01, Mu50, Mu3, N315, and NCTC 8523. **A** The table shows overlays identified by the OrthoVenn 2 analysis of *S. aureus* SA01, Mu50, Mu3, N315, and NCTC 8523. **B** The Venn diagram shows the distribution of ortholog groups shared among the *S. aureus* SA01, Mu50, Mu3, N315, and NCTC 8523

Discussion

S. aureus ST5 SA01 strains have become a problem in hospital environments and are spreading on several continents, and studies showing its dispersion are scarce in Brazil. In previous studies, it was observed that strains of *S. aureus* ST5 showed resistance rates against many antibiotics, including clindamycin, erythromycin, gentamicin,

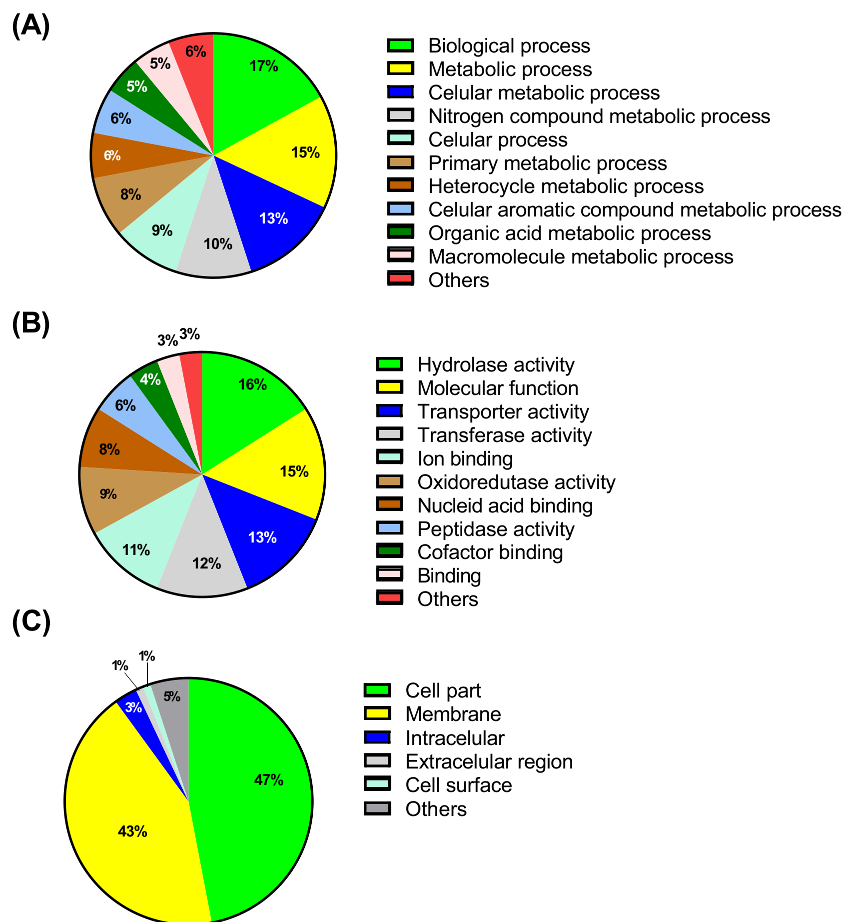


Fig. 7 Functional classification of proteins belonging to clusters in common among strains of *S. aureus*: SA01, NCTC 8325, Mu50, Mu3, N315. **A** Metabolic functions; **B** molecular functions; **C** cellular localization

rifampicin, and tetracycline (Kim et al. 2019; Monteiro et al. 2019). The success of *S. aureus* ST5 inside and outside a hospital environment is attributed to the ability of these lineages to acquire moving elements, such as transposons and prophages, which contain genes that encode virulence and resistance factors (Monecke et al. 2011).

The time-kill curve assay can be used to evaluate the bactericidal activity of antimicrobials against the most diverse microorganisms, among them *S. aureus* SA01, allowing observation of the interaction dynamics between antimicrobial organisms (Di Pilato et al. 2020). Treatment with concentrations of 32 and 64 µg/mL of oxacillin did not inhibit the cellular viability of *S. aureus* SA01. At the beginning of the exponential phase, the cells added in medium without oxacillin (control) showed rapid growth, and the treatment with a concentration of 32 µg/mL had the same tendency, demonstrating a latency phase observed compared to the rule, making clear the adaptation stage *of S. aureus* ST 5 SA01 to a new environment. Through macromolecular repair and cell growth synthesis through DNA replication and thus corroborating other studies (Zhou et al. 2017). According to the antimicrobial resistance profile of *S. aureus SA01*, the use of combination therapy has been a beneficial strategy for the treatment of certain infections with tolerant microorganisms or in a biofilm, such as those associated with devices such as catheters and prostheses, so the critical role of the in vitro synergism test stands out (Belley et al. 2008; Watson et al. 2020; Rieg et al. 2020).

Many of the factors found in the genome of *S. aureus* ST 5 SA01 are genes that encode proteins that may play an individual or additive role in the pathogenesis of bacteria in host tissues (Table 2). Adhesion to host cells is mediated by proteins associated with the bacterial cell wall, called microbial surface components that recognize adhesive matrix molecules (MSCRAMMs) (Alli et al. 2015). The binding protein—fibronectin (FnbA) found in the *genome S. aureus* SA01—is responsible for promoting tissue adhering to the extracellular matrix. In *S. aureus*, FnbA is essential in the early stage of infection and has been implicated in infectious endocarditis and osteomyelitis (Murai et al. 2016; Soltani et al. 2019).

In this study, cIfA and clfB, found in the genome *of S. aureus* SA01, are responsible for the transcription of aggregation factors A and B, respectively. The CIfA, which is present in all phases of bacterial growth, can connect to the complement factor system and facilitate immune system evasion. ClfB, present in the exponential phase of bacterial

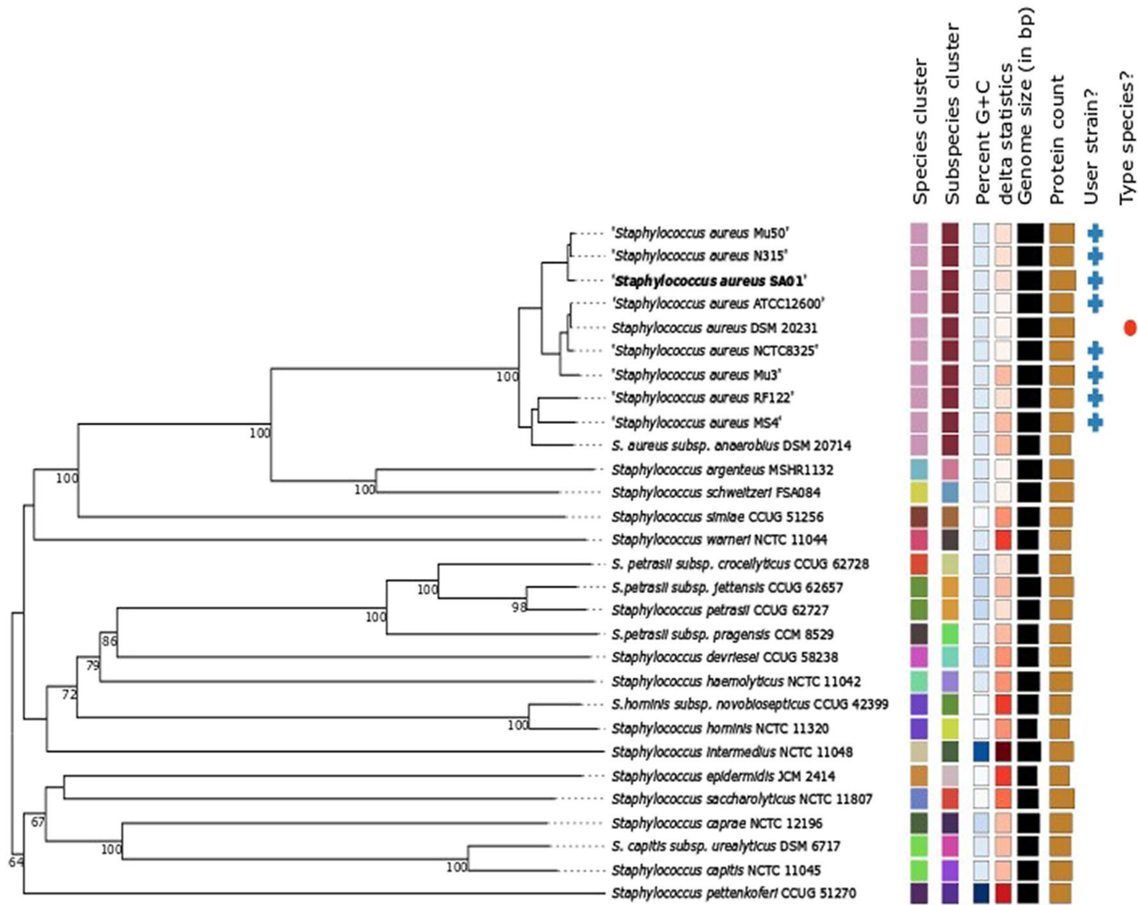


Fig. 8 The phylogenetic tree of *Staphylococcus aureus* SA01 and related *Staphylococcus* strains available in the TYGS database. The tree inferred with FastME 2.1.6.1 from the Genome BLAST Distance Phylogeny (GBDP) method shows the distances calculated from genome sequences. The lengths of the branches are scaled in terms of the distance formula d5 GBDP. The numbers above the branches are GBDP pseudo-bootstrap support values of 100 repetitions, with average agency support of 95.5%

growth, can bind to fibrin and cytokeratin (Haim et al. 2010; Liesenborghs et al. 2018). Previously, it was reported that aggregation factors A and B (ClfA and ClfB) are involved in the interactions of *S. aureus* with host-specific receptors, increasing the pathogen persistence at the infection site (Bonar et al. 2015; Hodille et al. 2017). In addition, the adhesivity and the ability of biofilm formation of *S. aureus* to abiotic surfaces are related to the production of polysaccharide intercellular adhesive (IAP) (You et al. 2014). In its genome, the *S. aureus* SA01 strain contains genes organized in the *icaABCD* operon, responsible for producing PIA, another host gene with regulatory function. The expression of *icaD* and *icaA* is associated with the initial phase of biofilm formation (Kot et al. 2018).

S. aureus produces a phospholipase specific to sphingomyelin called beta-hemolysin (HLB). HLB and gamma hemolysin (HLG) induce cellular lysis and are secreted by most *isolates of S. aureus* concerning chronic infections of human skin, systemic infections, and septic arthritis (Katayama et al. 2013; Soltani et al. 2019). We found that hlgA, hlgB, and hlgC in the genome of *S. aureus* ST 5 SA01 encode for hemolysin gamma. Interestingly, all strains of *S. aureus* used for comparison carried the genes for hemolysin gamma. HLG influences the pathogenicity of *S. aureus* and aggravates the symptoms of patients by being a potent leukotoxic and hemolytic stimulator (Koymans et al. 2015; Liesenborghs et al. 2018). After analysis by RAST BLASTn, the proteins LukG, and LukH presented 100% identity with other proteins from the GenBank database (Table 2).

The LukGH system is one of the most potent two-component leukocidin systems (Trstenjak et al. 2020). It can be compared with the strength of LukSF-PV in damaging human phagocytic cells, with action similar to PVL, contributing to immune evasion and cell lysis (Yanai et al. 2014). LukGH genes are an essential part of the Genome of *S. aureus*, while the lukSF/PVL gene, encoded by prophages, is expressed by only 5–10% of the clinical isolates *of S. aureus* (Vandenesch et al. 2012). After genomic analysis of *S. aureus* ST 5 SA01 proteins, staphylococcal superantigen classes (SSL) (SSL1, SSL2, SSL3, SSL4, SSL5, SSL7,

SSL8, SSL9, and SSL10) were found. The SSL protein class is one of the prominent proteins secreted by *S. aureus* strains related to the invasion and colonization of host tissues (Thammavongsa et al. 2015; Lewis and Surewaard 2018; Bretl et al. 2019). SSLs are immune avoidance molecules that directly interfere with a range of innate and adaptive immune defense responses, which may be associated with the blockade, degradation, cell lysis, and modulation of immune function (Koymans et al. 2015). The genes of the SSL class detected in the genome of *S. aureus* ST 5 SA01 have structural similarities and distinct roles. These genes are located in the nucleus of the *genome*, corroborating other studies (Katayama et al. 2013; Zhao et al. 2018).

S. aureus is a species of bacteria that needs iron for critical biochemical reactions. This requirement is linked to the hemolytic capacity of the bacterium, leading to the depletion of host erythrocytes, mainly associated with infectious conditions such as sepsis (Roetzer et al. 2016). Two transport systems regulated by ISD and HTS involved in iron heme acquisition were detected in S. aureus SA01. These genes express proteins anchored to the cell wall, such as IsdA, IsdB, IsdC, and IsdH, and membrane transporters, such as IsdDEF (Liu et al. 2008). The ISD components allow *S. aureus* to extract the heme from hemoglobin (Hb), transport it to the bacterial cytoplasm and release iron from the porphyrin ring, thus, contributing to microbial pathogenesis (Conroy et al. 2019; Gianquinto et al. 2019; Mikkelsen et al. 2020). However, the role of these systems in the physiology of *S. aureus* is controversial (Mason and Skaar 2009; Sheldon and Heinrichs 2015).

The percentage of enterotoxin-related genes in S. aureus varies according to the strain and the types of phages found in the genome. In *S. aureus* SA01, genes related to staphylococcal enterotoxins, such as sec, yent1, yent2, selk, selm, seln, and seal, were found. These represented a rate of 35% (7/20) of the same genes detected in the Mu50 and N315 strains, at a rate of 50% (Kuroda et al. 2001; Collery et al. 2009; Arabestani et al. 2018) (Fig. 3 A–C, Supplementary Table 2).

The resistance of *S. aureus* ST 5 SA01 to ciprofloxacin (a fluoroquinolone) is due to mutations present in *gyrA*, *gyrB*, *parE*, and *parC* that encode the subunits of DNA gyrase and topoisomerase IV, the primary sites of action for these drugs. These mutations occur mainly in quinolone resistance determinant regions (QRDRs) (Röderova et al. 2017). Earlier studies have shown that ciprofloxacin-resistant *S. aureus isolates* showed *gyrA and parC mutations*, causing a reduction in quinoline binding affinity to enzymes gyrase and topoisomerase (Fuzi 2016; de Oliveira et al. 2019).

However, other mechanisms may be associated with quinolone resistance, such as changes in antibiotic flow systems; changes in the target action site of these drugs were also seen in the genome of *S. aureus* ST 5 SA01 (Ardebili et al. 2014). The presence of *mecA*, which encodes a penicillin-binding protein, altered in the genome *of S. aureus ST 5* SA01 is closely related to oxacillin resistance. Resistance to methicillin and oxacillin occurs due to a mutation that leads to a change in penicillin-binding protein 2 (PBP2) that *confers resistance to S. aureus* SA01. The molecular pattern observed for *S. aureus* ST 5 SA01 is similar to that found for other strains of *S. aureus*, such as methicillin-resistant *S. aureus* (MRSA), isolated from the bloodstream (Chen et al. 2014).

S. aureus strains classified as ST5 are essential pathogens *of community-associated methicillin-resistant S. aureus* (CA-MRSA), epidemiologically relevant with worldwide distribution, which has been associated with severe invasive diseases in humans, especially in children (Sola et al. 2012; Rokney et al. 2019). Genome sketch analyses of *S. aureus* ST 5 SA01 indicated virulence factors relevant to bacterial pathogenesis in humans associated with phages. In addition, the contribution of phages is relevant to the pathogenesis with the acquisition of leukocidin genes and other aggression factors (Coombs et al. 2020).

The region of similarity between *S. aureus* ST 5 *SA01* and *S. aureus* NCTC 8325, located in the ring (BRIG), about the part of the slope GC (−), between 1600 and 1400 kbp, is represented by the imbalance of nucleotide concentration as the frequency of the four DNA bases, which was not observed in the other lines (Lobry 1996). This similarity with *the S. aureus* NCTC 8325 lineage, dated as a *strain of S. aureus* containing a high concentration of nucleotide exchange (mutation) in the gene composition, reflects selective pressure during the host infectious process (Kumburu et al. 2018). However, the presence of mobile genetic elements in the *S. aureus* ST 5 SA01 strains assumes the necessary advantages, capable of promoting the cellular adaptation of microorganisms to infected hosts, where this characteristic is due to the performance potential of strains of *S. aureus* in the horizontal transfer of genes associated with IGs (Moon et al. 2016; Turner et al. 2019; Kläui et al. 2019).

The high similarity between the sequences of strains *S. aureus* SA01, *S. aureus* N315, *S. aureus* Mu50, and *S. aureus* NCTC 8325 obtained by TYGS is congruent with the results obtained by the OrthoVenn Web server, and *S. aureus* strains may perform similar biological functions (Liang et al. 2019; Chamon et al. 2020). These proteins found in the genome of *S. aureus ST 5* SA01 are related to necrosis, hemolysins GO-0001906, regulation of symbiosis in the host GO-0009405, enterotoxins GO-0090729 and go-07155 adhering to being a characteristic of the species, and may be related to a highly virulent profile, allowing the prevalence of this lineage in our environment, previous corroborating studies (School et al. 2016). In addition, the strains *of S. aureus* SA01, *S. aureus* N315, and *S. aureus* Mu50 belong to the sequence type group, classified as ST5 (CC5), unlike the *S. aureus* NCTC 8325 lineage, classified as ST8 (CC8).

Although *S. aureus* N315 is a methicillin-resistant *S. aureus* (MRSA) strain that was isolated in 1982, and Mu50 is

a vancomycin-*resistant S. aureus* (VRSA) lineage that was isolated in 1997, its genomes were characterized in 2001 (Kuroda et al. 2001; Roetzer et al. 2016). Interestingly, they contain two copies of Tn554 in a site-specific transposon related to resistance to macrolide-lincosamide-streptogramin B (Kuroda et al. 2001). Coincidentally, none of the *three strains of S. aureus*—SA01, N315, and Mu50—carries lukS-PV, and lukF-PV encoding for PVL, although some reports indicate that ST5 can express this leukocidin (Rokney et al. 2019).

Conclusions

Here, the findings of the genomic characterization of *S. aureus* ST 5 SA01, a lineage belonging to the ST5 group, demonstrate the extensive repertoire of genes involved in antimicrobial resistance and virulence, such as leukocidin GH, a potent pore-forming toxin in human leukocytes. Although studies on ST5 clones in Brazil are scarce, it is essential to monitor this clone, as it has become a problem in pediatrics in some countries.

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Data availability All data generated or analyzed during this study are included in this article [and its supplementary information files].

Declarations

Competing interests The authors declare no competing interests.

Ethics approval Our study did not involve human or human tissue samples. The bacterium isolate used in the study was previously isolated as part of other published study and now is part of our Microbiological collection.

Conflict of interest The authors declare no competing interests.

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