Chapter 5

Molecular Features of Virulence and Resistance Mechanisms in Nosocomial and Community-Acquired *Staphylococcus aureus*

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are known for their emergent multi-drug resistance phenotypes, implication in nosocomial infections and outbreaks worldwide, being commonly associated with hospital-acquired MRSA (HA-MRSA) and community-acquired MRSA (CA-MRSA) skin and soft tissue infections. *S. aureus* causes a wide spectrum of clinical symptoms, ranging from mild to life-threatening diseases; disease severity is determined by microorganism-related virulence factors and host condition. The ability of these strains to form microbial biofilms, one of the main pathogenicity factors, generates difficult medical problems, favored by the widespread use of large invasive medical procedures (probes, catheters, heart valves, prostheses). Contamination of these devices is associated with the risk of subsequent development of human infections. The knowledge of virulence and antibiotic resistance patterns of HA-MRSA and CA-MRSA and encoding genes are very important for supporting effective infection control measures and therapy of staphylococcal infections.

Keywords: *Staphylococcus aureus*, MRSA, resistance, virulence, biofilm

1. Introduction

Staphylococci are commensal bacteria that form part of microbiota of human and animal skin and mucous membranes. Among more than 40 species of the genus, *Staphylococcus aureus* is colonizing the nostrils and skin of ~30% of the population [1, 2]. *S. aureus* is an opportunistic pathogen,

causing infections when it crosses the barriers of natural defense and escapes the mechanisms of anti-infectious protection. Factors favoring staphylococcal infections include local (lesions of the skin, the presence of implants, catheters, etc.) and general (innate or acquired deficiencies of the immune system such as complement system deficiencies, granulocytopenia, agranulocytosis, AIDS, diabetes, immunosuppressive treatments, etc.). It is able to cause a plethora of community (CA) and health care (HA) infections, ranging from superficial skin infections to severe, and potentially fatal, invasive diseases [3–5] due to its ability to produce a spectrum of virulence factors and resistance to multiple antibiotics, frequently encoded by mobile genetic elements (MGEs) [6], which have eased the persistence of *S. aureus* in hospital environment [7]. *S. aureus* is causing skin and soft tissue infections (SSTIs), endovascular infections, pneumonia, septic arthritis, endocarditis, osteomyelitis, foreign-body infections, and sepsis [8]. *S. aureus* is the most commonly isolated bacteria from wound infections and studies involving patients with chronic venous leg ulcers found *S. aureus* positive cultures in 88–93.5% of infections [9]. Bacteremia caused by *S. aureus* is associated with higher morbidity and mortality, compared with bacteremia caused by other pathogens, with an annual incidence rate of 20–50 cases/100,000 population, and a mortality rate of 10 and 30%. The highest mortality rates occur in patients with primary bacteremic pulmonary infections and infective endocarditis, whereas the lowest rates occur in patients with central or peripheral venous catheter-related infections [10]. The major concern refers mainly to methicillin-resistant *S. aureus* (MRSA) isolates. Health care-associated MRSA (HA-MRSA) are represented by the *S. aureus* strains isolated from patients after a hospitalization of two or more days or with the MRSA risk factors (history of recent hospitalization, surgery, dialysis, catheters, etc.). Community-associated MRSA (CA-MRSA) are those *S. aureus* isolates obtained from patients within 2 days of hospitalization and without the above-mentioned MRSA risk factors [11]. Infections by CA-MRSA isolates are usually associated with children, young age, recurrent infections and the use of injectable drugs [12].

In this chapter, we review some aspects related to resistance and virulence features in CA-MRSA and HA-MRSA strains, underlying the evolution of the highly successful community- and health care-associated lineages and their plasticity in ability to adapt to environmental changes.

2. *Staphylococcus aureus* resistance to antibiotics

Antistaphylococcal antibiotics are mainly targeting cell wall synthesis, proteins and nucleic acid synthesis, and different metabolic pathways. The large use of antibiotics, not only in the medical field but also in the agriculture has facilitated the evolution and spread of resistance genes [13]. Bacterial resistance can be constitutive (mutations of the target genes, efflux pumps overexpression, etc.) or acquired by horizontal gene transfer via various mobile genetic elements like plasmids, transposons, bacteriophages, pathogenicity islands, and staphylococcal cassette chromosomes [14]. Plasmids and staphylococcal cassette chromosomes in particular have played a central role in conferring resistance to β-lactam antibiotics and vancomycin [15].

Penicillin resistance is conferred by β-lactamase, an extracellular enzyme encoded by *blaZ* that is active when bacteria are exposed to β-lactam antibiotics. The enzyme acts on β-lactam ring by opening it through hydrolization [16].

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Methicillin resistance requires the presence of the chromosomally localized *mecA* gene. The *mecA* gene and its regulatory elements, form the *mec* complex: SCC*mec* elements carry the *mecR1* and *mecI* genes, which regulate the expression of *mecA*, with increased *mecA* translation induced by β-lactam antibiotic exposure. SCC*mec* elements can also carry resistance genes for other antibiotics and heavy metals as well as the psm-mec locus, which encodes cytolysin termed phenol-soluble modulin-mec (PSM-mec) [16].

PBPs are membrane-bound enzymes that are used in cross-linkage of peptidoglycan chains by catalyzing the transpeptidation reaction [17]. PBP2a has a low affinity for β-lactam antibiotics and thus methicillin resistance also grants resistance to all β-lactam antibiotics [16].

Eleven types of SCC*mec* have been described, distinguished by the type of *ccr* gene complex that mediates the site-specific excision and insertion of the SCC*mec* cassette out of or into the bacterial genome and the class of *mec* complex that they bear [18].

There have been revealed molecular differences between CA-MRSA and HA-MRSA strains regarding the types of SCC*mec*: HA-MRSA strains carry the large staphylococcal chromosomal cassette mec (SCC*mec*) belonging to type I–III and containing the *mecA* gene, mostly universal among MRSA isolates and usually are resistant to several classes of non-β-lactam antibiotics. It seems that the large SCC*mec* types I–III are present in HA-MRSA strains and were transferred to *S. aureus* from a commensal staphylococcal species [19]. Carriage of the psm-mec locus from type II SCC*mec* elements attenuates virulence, suppresses colony spreading activity, reduces expression of the chromosomally encoded PSMa, and promotes biofilm formation [20].

HA-MRSA strains seldom carry the genes for the Panton-Valentine leukocidin (PVL). CA-MRSA isolates carry smaller SCC*mec* elements, most commonly SCC*mec* type IV or type V [21]. CA-MRSA strains are resistant to fewer non-β-lactam classes of antibiotics and frequently carry PVL genes. There has been suggested that the smaller SCC*mec* types IV, V, VI, and VII have been transferred to methicillin-susceptible backgrounds [21, 22]. One study suggested that the type IV SCC*mec* element has been transferred to an MSSA strain [23]. The type IV SCC*mec* was originally associated with MRSA infections in patients with no HA-MRSA risk factors [21].

There have been revealed that the deletion of the gene encoding PBP 4 in two common CA-MRSA isolates, USA300 and USA400, resulted in a 16-fold reduction in oxacillin and nafcillin resistance in these particular stains. These studies suggest that PBP 4 is a significant target for the discovery of agents effective against CA-MRSA [24].

There have been also reported CA-MRSA strains positive for *mec*A and PBP 2a that were phenotypically oxacillin susceptible [25]. It was therefore suggested that *mec*A expression alone does not appear to be sufficient to guarantee phenotypic methicillin resistance, and that the existence of additional molecular targets could be associated with the susceptibility of oxacillin in certain strains and the involvement of genes different from the known effectors of methicillin resistance in CA-MRSA. The *vraS*/*vraR* two-component regulatory system is required for oxacillin resistance in CA-MRSA [26, 27].

The emergence/re-emergence of successful *S. aureus* clones suggests a rapid bacterial adaption and evolution, which includes the emergence of antibiotic resistance and increased virulence and/or transmissibility.

Most of the nosocomial MRSA infections are caused by five major lineages that circulate internationally: CC5, CC8, CC22, CC45 and CC30 [28].

ST1 pulsotype USA 400—represented the most frequently CA-MRSA clone in the United States after 1990s; was characterized by carrying SCC*mec* type IV that has been usually susceptible to most non-β-lactam antibiotics and cause SSTIs. This strain lacked PVL genes and circulates in the community in Australia (WA-MRSA-1) and England [18, 29]. ST80 is PVL+-bearing SCC*mec* type IV and have been reported in several Western European countries such as Austria, Norway, Denmark, Sweden, England, Switzerland, Greece and France [18]. ST30 corresponds to phage type 80/81 nosocomial strain of *S. aureus* from United States during the 1950s and 1960s. These strains were MSSA strains that carried the PVL genes [30] and the SCC*mec* type IV element. ST59 strains are PVL+, they have diverse spa types and several SCC*mec* types were isolated from different countries worldwide like: Taiwan, Australia, Denmark, Netherlands England, and the United States [18]. In Taiwan, ST59 clones with a multidrug-resistant phenotype are the most encountered, having a distinctive SCC*mec* DNA sequence [31]. ST93 was first identified in 2000 in Queensland and New South Wales, Australia. It spread rapidly to become the predominant PVL+ MRSA clone isolated from infections in those regions. USA300 Strains has the following characteristics: the carriage of SCC*mec* type IV, PVL genes, and, in most strains, the ACME element. USA300 is classified as ST8 by MLST and is usually classified as t008 by *spa* typing. It is frequently susceptible to several non-β-lactam antibiotics and became the dominant CA-MRSA strains in the United States, has been also identified in Western Europe [18], Japan [32] and Australia, where it has been called WA-MRSA-12 [18, 33].

MRSA infections in the community can also be caused by livestock-associated MRSA (LA-MRSA). LA-MRSA is initially associated with livestock and differs from genotypic HA-MRSA and genotypic CA-MRSA in its genomic traits. CC9 clonal complex (LA-MRSA) is most frequent among livestock in Asia [34]. In the USA, ST5 (MRSA) was isolated from pigs [35]. CC1 (MRSA) is prevalent in Romanian nosocomial infections and has low host specificity [36].

The quinolones act on DNA gyrase, which relieves DNA supercoiling, and topoisomerase IV, which separates concatenated DNA strands. Resistance to quinolones results from the stepwise acquisition of chromosomal mutations [16]. Quinolone affinity is reduced by changes of the amino acids in the enzyme-DNA complex (quinolone resistance-determining region [QRDR]). The most common sites of resistance mutations are GyrA subunit in gyrase and ParC (GrlA in *S. aureus*) of topoisomerase IV. Drugs primarily target topoisomerase IV and the mutations at this level are essential for resistance [37, 38]. The confluence of high bacterial density, especially inside biofilms, the likely preexistence of resistant subpopulations, and the sometimes limited quinolone concentrations achieved at sites of staphylococcal infections creates an environment that fosters selection of resistant mutants [37].

An additional mechanism of resistance in *S. aureus* is induction of the NorA multidrug-resistance efflux pump with increased expression can result in low-level quinolone resistance [39].

S. aureus response to vancomycin inhibitory activity divides the strains into sensitive, intermediate and resistant. Vancomycin intermediate-resistant *S. aureus* (VISA) have MICs to vancomycin of 8–16 μg/ml (MIC = 4–8 μg/ml). There has been identified the existence of a pre-VISA stage of resistance known as heterogeneous VISA (hVISA) [16]. An hVISA phenotype

refers to a mixed cell population—derived originally from a single colony of *S. aureus*—in which the majority of cells have little or no resistance to vancomycin (MIC ≤ 2 μg/ml) and a sub-population of cells is resistant to the antibiotic at the level of VISA (MIC ≥ 4 μg/ml) [40].

The molecular mechanisms that underlie development of hVISA are incompletely defined. Fundamental characteristics of the VISA phenotype include increased cell wall thickness, caused by differentially regulated cell wall biosynthesis and stimulatory pathways [15, 41], reduced cross-linking of peptidoglycan, decreased autolytic activity of the enzymes responsible to cell-wall turnover [15, 42], altered surface protein profile, dysfunction of the *agr* system and changes to growth characteristics [15, 43].

Molecular basis of the VISA phenotype is not fully understood but several genes/mutations are known to contribute to its development. The mutations within genes encoding two-component regulatory systems, such as *graRS* and *walKR* are of particular significance. GraRS differentially regulates transcription of cell wall biosynthesis genes and has been associated with a broad array of genes and regulators that play a role in the intermediate resistance phenotype [44]; GraRS also up-regulates genes in the capsule biosynthesis operon, leading to increased capsule production [44]. GraRS up-regulates the *dlt* operon and the *mprF/fmtC* genes, which are linked to teichoic acid alanylation and alteration of cell wall charge [15]. Point mutations within *graRS* reduced susceptibility to vancomycin [15, 45] and *graRS* mutations are linked to modified expression of global regulators, *rot* and *agr* [15, 44]. *rpoB* a gene encoding the DNA-dependent RNA polymerase β-subunit is commonly associated with increased resistance to vancomycin, prolonged propagation time and increased cell wall thickness [15, 46]. VISA isolates have been shown to have non-silent mutations in *vraSR*. Such mutations could lead to downstream up-regulation of over 40 cell wall synthesis genes, including genes required for producing cell wall derivatives such as D-Ala-D-Ala [15].

The VISA strains produce considerable amounts of peptidoglycan and this generates thicker, irregularly shaped cell walls. They also expose more D-Ala-D-Ala residues available to bind and trap vancomycin which acts as a further impediment to drug molecules reaching their target on the cytoplasmic membrane [16].

Complete vancomycin resistance in *S. aureus* (MIC ≥16 μg/ml) is conferred by the vanA operon (containing *vanA*, *vanH*, *vanX*, *vanS*, *vanR*, *vanY* and *vanZ* genes) encoded on transposon Tn1546, originally a part of a vancomycin-resistant enterococci (VRE) conjugative plasmid [15, 47]. The *vanA* operon is controlled via a two-component sensor-regulator system encoded by *vanS* and *vanR* that sense vancomycin and activate transcription of the operon, respectively, VanA, VanH and VanX together are essential for the vancomycin resistance phenotype. *S. aureus* can acquire enterococcal plasmids during discrete conjugation events. Vancomycin resistance in *S. aureus* is maintained by retaining an original enterococcal plasmid or by a transposition of Tn1546 from the VRE plasmid into a staphylococcal resident plasmid [15, 48].

3. *Staphylococcus aureus* adhesion and biofilm development

The broad range of infections caused by *S. aureus* is related to a number of virulence factors that allow it to adhere to surface, invade or avoid the immune system and cause harmful toxic effects to the host [49].

The initialization of the colonization process is started by the attachment of *S. aureus* to the host cell surface through adhesins. Proteins covalently anchored to cell peptidoglycans represent one major class of *S. aureus* adhesins, which attach to the extracellular matrix or plasma components via a threonine residue and are known as the microbial surface component recognizing adhesive matrix molecules (MSCRAMMs) [49].

Staphylococcal cell wall-anchored (CWA) proteins are secreted by the Sec system and share a C-terminal cell wall anchoring motif, hydrophobic domain and positively charged domain [50]. Foster et al. [50] proposed to classify the Staphylococcal CWA proteins into four groups based on structural motifs: MSCRAMMs (microbial surface component recognizing adhesive matrix molecules), the NEAT motif family, the three-helical bundle family and the G5-E repeat family. All of these types of CWA proteins are involved in staphylococcal biofilm formation. MSCRAMMs are adhesins that contain at least two IgG-like folds and employ a ligand binding mechanism called dock, lock and latch [50]. MSCRAMMS are composed of a binding domain, a cell wall spanning domain and a domain for the covalent or non-covalent attachment. These adhesins can bind one or more human proteins (collagen—mostly via Cna, fibronectin—via FnbAB, fibrinogen—via ClfAB and Fib) [51, 52].

The Staphylococcal MSCRAMMs are the Clf-Sdr family proteins, including bone sialoprotein-binding protein (Bbp), the fibronectin-binding proteins (FnBPs) and collagen adhesion (CNA) [53]. The Clf-Sdr family consists of Clumping factor A (ClfA), clumping factor B (ClfB) and the Sdr proteins. ClfA and ClfB are fibrinogen-binding proteins in *S. aureus* [50, 53]. Rot and agr affect bacterial binding to fibrinogen by regulating clfB but not clfA [54]. *S. aureus* has two fibronectin-binding proteins, FnBPA and FnBPB, encoded by fnbA and fnbB, respectively. FnBP binding to fibronectin induces bacterial invasion into epithelial cells, endothelial cells and keratinocytes [53]. The FnBPs affect biofilm formation by a self-association mechanism that is distinct from ligand binding and virulence [55, 56].

The NEAT motif family consists of the iron-regulated surface determinant (Isd) proteins who bind heme or hemoglobin, facilitating its transport into the bacterial cell, and they are up-regulated in iron-limiting conditions [57]. *S. aureus* IsdA is the most abundant CWA protein in iron starvation conditions, and also decreases surface hydrophobicity, which makes *S. aureus* more resistant to bactericidal fatty acids and peptides in human skin. IsdA also is able to bind human fibrinogen and fibronectin [53].

The sole three-helical bundle cell wall-anchored protein is Staphylococcal Protein A (SpA), which is present in all strains of *S. aureus*. SpA allows immune evasion by binding to the conserved Fc region of immunoglobulin IgG, and contributes to disruption of the host immune response by promoting bacterial survival in human blood after being released from the cell wall [53, 58].

G5-E Repeat Family: Aap/SasG—G5-E repeats are found in cell wall-anchored adhesins in Gram-positive organisms (are named after the five conserved glycine residues in each repeat). *S. aureus* SasG promotes attachment to human desquamated nasal epithelial cells via it's A domain [59]. Multiple studies have shown that the G5-E repeats of SasG and Aap are able to dimerize by binding to Zn^{2+}, forming a "twisted rope" structure [53, 60]. This property is thought to enable intercellular adhesion when adjacent SasG or Aap proteins dimerize via their G5-E domains.

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Uncategorized CWA Proteins—the remaining uncategorized cell wall-anchored proteins are Bap and several Sas proteins, including SasA/SraP. SasX is another cell wall-anchored adhesin that has been shown to play an important role in virulence [53].

Surface-associated proteinaceous adhesins—autolysins AtlA and AtlE are found in *S. aureus* involved in cell wall turnover, cell division and cell lysis [53], they attach to extracellular matrix materials and can augment the biofilm matrix with eDNA by inducing cell lysis.

Non-proteinaceous surface-associated adhesins—wall teichoic acids and lipoteichoic acids have been shown to play a role in adhesion, colonization of host cells and biofilm formation. Wall teichoic acids are covalently linked to the peptidoglycan and consist of alternating phosphate and ribitol, while lipoteichoic acids attach to the outer leaflet of the cell membrane and have alternating phosphate and glycerol [61].

The polysaccharide intercellular adhesion (PIA) is a secreted polysaccharide that is synthesized by the *ica* operon and has been thoroughly studied in the context of biofilm formation, immune evasion, and pathogenesis. PIA is a glycan of β-1,6-linked 2-acetamido-2-deoxy-D-glucopyranosyl residues with a net positive charge that promotes intercellular aggregation and attachment of cells to inert surfaces. The *ica* operon consists of four biosynthesis genes such as *icaA*, *icaD*, *icaB* and *icaC* and a divergently transcribed repressor, *icaR*. Carriage of the *ica* locus is a characteristic of most clinical *S. aureus* strains [62].

One of the reasons the staphylococcal infections are difficult to eradicate is the bacteria's ability to develop community structures known as biofilms by attaching to different surfaces (tissues, catheters and medical devices), and often occur in areas of the body that are not easily accessible for treatment [63].

Biofilm is an assemblage of microbial cells that are irreversibly associated to a surface and embedded in a protective extracellular polymeric matrix. Biofilm-associated organisms have proteins production and genes expression modification compared to their planktonic counterparts [64].

Biofilm formation can be divided into three major stages: initial attachment, development/maturation of the biofilm and dispersion.

During initial attachment, bacteria adhere to the available surface and in case of abiotic one its conditioning is important through various physiochemical parameters: chemical composition of the material, hydrophobicity, electrostatic charges, surface energy and surface roughness and in the case of biotic adhesion: serum and tissue protein adsorption [65].

The final stage of biofilm development is the detachment of cells from the biofilm colony and their dispersal into the environment, which contributes to biological dispersal, bacterial survival and disease transmission. Like other stages of biofilm development, dispersal is a complex process that involves numerous environmental signals, signal transduction pathways and effectors [66].

The biofilm matrix is a complex structure that contains extracellular DNA (eDNA), both from lysed bacteria and potentially from host neutrophil cell death [53], proteinaceous adhesins directly associated with bacteria in the biofilm or free in the biofilm matrix [67], recycled

cytoplasmic proteins that moonlight as components of the extracellular matrix [68], the extracellular polysaccharide intercellular adhesin (PIA), teichoic acids [53]. The matrix can impede the access of certain types of immune defenses, such as macrophages [69].

Cell adhesion and subsequently biofilm formation are processes mediated by covalently and non-covalently cell wall proteins and non-protein factors. For *S. aureus* more than 20 adhesins were identified [53].

Switching between planktonic and biofilm-forming modes represents a major life style change for microbes, and has been shown to be a tightly regulated process [70] through quorum sensing (QS) which is a cell-cell communication mechanism in which bacteria secrete and sense small diffusible molecules called autoinducers (AIs) to coordinate social activities, such as bioluminescence, biofilm formation, swarming behavior, antibiotic production and virulence factor secretion [71].

Staphylococcal biofilm formation is affected by growth conditions (e.g., NaCl, glucose, human plasma, etc.) and is controlled by multiple global regulators such as SarA, Agr, SigB and Sae. The Sae-regulon includes both the factors promoting biofilm formation (i.e., Coa, Emp, Eap, FnBPA, FnBPB, Hla and Hlb) and biofilm dispersal factors (nuclease and proteases) and depending on growth conditions and strain backgrounds, the Sae system could affect biofilm formation either positively or negatively [72].

Bacteria in biofilms can tolerate ten to thousand fold higher levels of antibiotics than the genetically equivalent planktonic bacteria. Staphylococcal biofilms cause biomaterial-associated infections which do not respond to antimicrobial treatment often requiring removal of the same leading to substantial morbidity and mortality. It has also been observed that biofilms harbour persister cells and small colony variants [73], whereas planktonic persisters are eliminated by the immune system *in vivo*, persisters in biofilms serve as a shield evading the immune response and a reservoir of such shielded persisters is a potential source for the emergence of heritable antibiotic resistance [14].

4. *Staphylococcus aureus* soluble virulence factors

S. aureus secretes numerous exotoxins, including polypeptides that destroy the integrity of the host cell plasma membrane. These polypeptides are pore-forming toxins (PFT): α-hemolysin and the bi-component leukocidins γ-hemolysin, β-hemolysin, the Panton Valentine leukocidin (PVL), LukED, and LukGH/AB and phenol soluble modulins (PSMs) [74].

α-Hemolysin is the most characterized virulence factor of *S. aureus*. Upon binding to the cell surface, α-hemolysin forms pores that allow the transport of molecules such as K+ and Ca^{2+} ions, leading to necrotic death of the target cell.

S. aureus possesses several other PFTs in addition to α-hemolysin that for pore formation involves two polypeptides that have been named S (slow) and F (fast) based on their electrophoretic mobility. The PFTs include (i) γ-hemolysin corresponding to two combinations of a

S component (HlgA or HlgC) with a F component (HlgB); (ii) the PVL made of LukS-PV and LukF-PV; (iii) LukED and (iv) LukGH, also known as LukAB [74]

The γ hemolysin variant and leukocidin E-D gene, as well as other genes encoding exotoxins, were detected evenly in HA and CA-MRSA strains, while sec and sek genes were found only in CA-MRSA strains [75].

β-Hemolysin is a neutral sphingomyelinase, it hydrolyses a plasma membrane lipid—sphingomyelin and does not form pores in the plasma cell. β-hemolysin's enzymatic activity is required for its hemolytic activity [74].

δ-Hemolysin is a small amphipathic α-helix-structured peptide (26 AA). Its hemolytic activity can be realized by forming transmembrane pores, affecting the membrane curvature or acting as detergent to solubilize the membrane [76].

This family of cytotoxic peptides includes new peptides termed PSM. PSMs represent a secreted α-helical peptides produced by different *Staphylococcus* species. PSMs encoding genes are able to activate and lyse human neutrophils and are generated at high concentrations by CA-MRSA strains [77]. The *psm-mec*, was the first PSMs gene found within an SCCmec MGE and was linked to the class A mec gene complex present in SCCmec types II, III and VIII, with a conserved location next to the *mecI* gene [74, 78].

Protein A prevents the opsonization and phagocytosis by ineffectually binding the Fc region of IgG. It also initiates a proinflammatory cascade in the airway by activating tumor necrosis factor receptor 1 (TNFR1) and B cells in concert with other ligands. MRSA strains with certain *spa* types have a decreased ability to invade human cells in vitro, revealing an association with certain *spa* types and virulence [18].

S. aureus produces a group of toxins called the toxic shock syndrome toxin-1 (TSST-1) and enterotoxins (SEA, SEB, SECn, SED, SEE, SEG, SEH and SEI) and the exfoliative toxins A and B (involved in staphylococcal scalded skin syndrome). Cytolytic toxins form pores of holes called β-barrel pores in the plasma membrane. This leads to leakage of the cell's content and lysis of the target cell [79].

PVL is a bi-component exotoxin transmitted by bacteriophages, encoded by two genes, *lukF-PV* and *lukSPV*. PVL genes are carried by CA-MRSA strains and in a small proportion by clinical MSSA strains. Therefore PVL plays an important role in fitness, transmissibility and virulence, but the role of PVL in the pathogenesis of CA-MRSA infections is not fully understood [80]. PVL genes are spreading among *S. aureus* strains by clonal expansion and horizontal transfer. There have been demonstrated that β-lactam antibiotics increased the production of PVL in vitro through transcriptional activation [81, 82]. PVL inserts itself into the host's plasma membrane and forms a pore of a hole. PVL exhibits a high affinity toward leukocytes. PVL can inactivate mitochondria and induce apoptosis. In animal models, PVL has been shown to be dermonecrotic [18], perhaps explaining the pathobiology of the characteristic skin lesions associated with CA-MRSA SSTIs. That the presence/absence of PVL genes in MRSA strains did not interfere with strain virulence in mouse models of sepsis and SSTI, and their presence did not decrease neutrophil survival in in vitro assays [18].

α-Toxin lyses immune cells like macrophages and lymphocytes, alters platelet morphology, which increased thrombotic events associated with *S. aureus* sepsis [83]. The nucleases, proteases, lipases, hyaluronidase and collagenase convert local host tissue into nutrients required for bacterial growth.

Arginine catabolic mobile element (ACME) is a large MGE that plays an important role in the growth, transmission and pathogenesis of CA-MRSA. Two main gene clusters identified as *arc* genes (*arcA*, *arcB*, *arcC* and *arcD*) and the *opp* genes (*opp-3A*, *opp-3B*, *opp-3C*, *opp3-D* and *opp3-E*) are recognized to be virulence factors [84].

5. Regulation of virulence factors expression in *Staphylococcus aureus*

In *S. aureus*, virulence factors production is coordinated by different regulators, for example, DNA binding proteins (e.g., SarA and its homologues), two-component signaling systems (e.g., ArlRS, AgrAC, SaeRS and SrrAB) and alternative sigma factor B [72].

Among the regulatory elements, the Agr (the accessory gene regulator) system is the only characterized QS system in *S. aureus* and controls the expression of approximately 150 genes [85]. Agr system regulation depends on cell density. During initial stage of colonization, when there is a low cell density, the Agr QS system is expressed low, but when the biofilm reach maturation and the cell density is high, Agr activity increases and upregulates secreted virulence factors. The *agr* regulation of the proteases is via Rot, whose transcriptional repression of the proteases is relieved when *agr* is induced [65, 86].

S. aureus produces AI-2 through the functional *luxS* gene it has. Due to the dual function of LuxS and the absence of genomic evidence of established AI-2 receptors, the AI-2 quorum-sensing function in *S. aureus* has been intangible, until now [71].

Sigma B (SigB) is an alternative sigma factor of RNA polymerase that is activated in stress response and modifies gene expression. SigB upregulates the expression of different factors involved in initial stages of biofilm formation like coagulase, FnBPA and clumping factor [87]. It also controls negative factors that are associated with a planktonic phenotype and seeding dispersal, including enterotoxin B, cysteine protease (SplB), serine protease (SplA), the metalloprotease Aur, staphopain, leukotoxin D and β-hemolysin [53].

The *sar* (Staphylococcal accessory regulator) locus produces three transcripts from three separate promoters, all of which contain the ORF for the DNA-binding protein SarA [53]. SarA also directly regulates several genes that affect biofilm formation. There have been demonstrated that the mutation of *sarA* gene in the USA300 clone limits accumulation of α-toxin and PSMs through the increased production of extracellular proteases rather than from transcription of the *hla* or *agr* genes [88].

6. Conclusions

Staphylococcus spp. are common residents of the normal human and animal microbiota, but in certain favoring conditions, they can surpass the anti-infectious defense barriers and become

opportunistic pathogens. Staphylococci can cause numerous types of infections some of them potentially fatal due to their numerous virulence factors, biofilm formation capacity and antibiotic resistance mechanisms. Taking into account the increased incidence and spread of MRSA and multiple-drug resistant strains, a better knowledge of the virulence and pathogenicity mechanisms and of their relationships with resistance, as well as of the quorum sensing mechanisms is essential for the development of novel anti-staphylococcal strategies, targeting the expression of virulence factors or of their regulatory mechanisms.

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Conflict of interest

There is no conflict of interest.

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