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Theoretical

Staphylococcus aureus: pathogenicity, antimicrobial resistance and clinical implications

Staphylococcus aureus: patogenicidade, resistência antimicrobiana e implicações clínicas.
Staphylococcus aureus: patogenicidad, resistencia a los antimicrobianos e implicaciones clínicas.

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ABSTRACT

Objective: To conduct a reflection about the pathogenicity, antimicrobial resistance, and clinical implications of the infections caused by *Staphylococcus aureus*. **Method:** Reflexive analysis, supported by theoretical references about the pathogenicity, antimicrobial resistance, and clinical implications of the staphylococcal infections. **Results:** The pathogenesis of *S. aureus* infections is complex and depends on the host characteristics, expression of virulence factors and ability to develop resistance to antimicrobials. Methicillin-resistant *S. aureus* (MRSA) is related to an advancement of healthcare- and community-acquired infections, being vancomycin the primary therapeutic option. Infections caused by *S. aureus* with reduced vancomycin susceptibility (hVISA/VISA) have been associated with treatment failures and increased mortality. **Conclusion:** With the evolution of *S. aureus* to MRSA, hVISA and VISA, the treatment of staphylococcal infections has become a major challenge for the medical clinic, and an adequate and early antibiotic therapy is essential for decreasing morbidity and mortality rates related to this microorganism.

Keywords: *Staphylococcus aureus*; Pathogenicity; Antimicrobial resistance; Reduced susceptibility to vancomycin.

RESUMO

Objetivo: Realizar uma reflexão sobre a patogenicidade, resistência antimicrobiana e implicações clínicas das infecções causadas por *Staphylococcus aureus*. **Método**: Análise reflexiva, apoiada em referências teóricas sobre a patogenicidade, resistência antimicrobiana e implicações clínicas das infecções estafilocócicas. **Resultados**: A patogênese das infecções por *S. aureus* é complexa e depende das características do hospedeiro, da expressão dos fatores de virulência e da capacidade de desenvolver resistência aos antimicrobianos. O *S. aureus* resistente à meticilina (MRSA) está relacionado ao avanço das infecções adquiridas na comunidade e nos serviços de saúde, sendo a vancomicina a principal opção terapêutica. Infecções causadas por *S. aureus* com sensibilidade reduzida à vancomicina (hVISA/VISA) foram associadas a falhas no tratamento e aumento da mortalidade. **Conclusão**: Com a evolução do *S. aureus* para MRSA, hVISA e VISA, o tratamento de infecções estafilocócicas tornou-se um grande desafio para a clínica médica, e uma antibioticoterapia adequada e precoce é essencial para diminuir as taxas de morbidade e mortalidade relacionadas a esse microrganismo.

Descritores: *Staphylococcus aureus*; Patogenicidade; Resistência antimicrobiana; Suscetibilidade reduzida à vancomicina.

RESUMÉN

Objetivo: realizar una reflexión sobre la patogenicidad, la resistencia a los antimicrobianos y las implicaciones clínicas de las infecciones causadas por *Staphylococcus aureus*. **Método**: Análisis reflexivo, respaldado por referencias teóricas sobre la patogenicidad, la resistencia a los antimicrobianos y las implicaciones clínicas de las infecciones estafilocócicas. **Resultados**: la patogenia de las infecciones por *S. aureus* es compleja y depende de las características del huésped, la expresión de los factores de virulencia y la capacidad para desarrollar resistencia a los antimicrobianos. *S. aureus* resistente a la meticilina (MRSA) se relaciona con un avance de las infecciones adquiridas en la comunidad y en la atención médica, siendo la vancomicina la opción terapéutica principal. Las infecciones causadas por *S. aureus* con sensibilidad reducida a la vancomicina (hVISA/VISA) se han asociado con fracasos del tratamiento y mayor mortalidad. **Conclusión**: con la evolución de *S. aureus* a MRSA, hVISA y VISA, el tratamiento de las infecciones estafilocócicas se ha convertido en un gran desafío para la clínica médica, y un tratamiento antibiótico adecuado y temprano es esencial para disminuir las tasas de morbilidad y mortalidad relacionadas con este microorganismo.

Descriptores: *Staphylococcus aureus*; Patogenicidad; Resistencia antimicrobiana; Susceptibilidad reducida a vancomicina.

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INTRODUÇÃO

S. aureus is one of the most frequent causes of healthcare-associated and community-associated infections, which present high mortality and morbidity rates[1,2]. Due to its pathogenic potential it is responsible for a large range of infections characterized for diverse clinical manifestations, including local conditions as much as high lethality systemic infections[3,4].

Infections caused by *S. aureus* are related to its large amount of virulence factors that contribute to the establishment and permanence of infectious processes[5-7]. The accessory gene regulator (*agr*) is the major quorum sensing system related with the control of virulence genes in *S. aureus*, being responsible for the most part of its virulence factors expression[8,9].

The increase of staphylococcal infections along with the irrational use of antibiotics has led to the emergence of *S. aureus* strains that present resistance mechanisms to different classes of antimicrobial agents, which makes treatment more difficult and aggravates the infectious process[10]. Methicillin-resistant *S. aureus* (MRSA) arose from the acquisition of genes that encode altered penicillin binding proteins (PBP2a), *mecA* or *mecC*, found in the mobile genetic element named *mec* staphylococcal cassette chromosome (SCC*mec*)[11].

With the advance of infections caused by MRSA, vancomycin has become the main therapeutic option. However, the constant use of this glycopeptide has increased the selective pressure among MRSA strains, leading to consequences, such as MRSA with reduced susceptibility to vancomycin (hVISA and VISA)[12,13]. These strains are related to failures in vancomycin treatments, persistent bacteremia, prolonged hospitalization and adverse clinical outcomes[12,14-16].

Treatment of staphylococcal infections has become a major challenge, due to *S. aureus* high virulence potential and the current narrow therapeutics options since penicillin, methicillin and recently vancomycin resistance rates are increasing. In this context, it is important to understand phenotypic and molecular characteristics of MRSA and strains with reduced vancomycin susceptibility to control its dissemination, as well as assist antibiotic therapy in cases related to this multiresistant microorganism.

The purpose of this review is to summarize the current knowledge on pathogenicity, antimicrobial resistance, and clinical implications of the infections caused by *Staphylococcus aureus*.

STAPHYLOCOCCUS AUREUS

S. aureus are gram-positive cocci, non-motile, non-spore forming bacteria with diameters of 0.5 – 1.5 µm, that microscopically are visualized in clusters[17,18]. These bacteria grows in non-selective culture media in optimal growing conditions at 37 ºC. It has high capacity of adaptation, being able to survive and multiply in hostile environments. They are facultative anaerobes, grow on mannitol salt agar, have a beta-hemolysis pattern in blood agar and produce catalase, coagulase and DNAse enzymes[17,19].

As an opportunistic microorganism, *S. aureus* behaves as both commensal and pathogen. They are normally found colonizing skin microbiota and sites, such as, nasopharynx, armpits, perineum and gastrointestinal tract; but due to its pathogenic potential it can cause a large variety of infections, mostly in immunity reduction cases or compromittment of the skin barrier[20].

Pathogenicity

S. aureus is one of the most frequent causes of healthcare-associated and community-associated infections, presenting a high mortality and morbidity rates[1,2]. Its transmission occurs mainly by direct contact with colonized or infected individuals and/or by contact with contaminated surfaces or objects[21,22].

The majority of infections caused by *S. aureus* are noticed to happen in asymptomatic individuals, colonized from short to long-term periods, resulting in disease when the immune system is compromised. Worldwide about 20 to 30 % of the population are persistent carriers and 60 % are periodical carriers of *S. aureus*[23,24].

Asymptomatic carriers are troubling due to the fact that even showing no clinical symptoms of an infectious disease they are potential sources of infection and can help to disseminate the pathogen on the environment[25]. Asymptomatic carrier status is even more worrying when it is a health professional. Due to the facility of transmission of *S. aureus*, the carrier is an important risk factor in the epidemiology and pathogenesis of the disease, since most part of healthcare-associated diseases are acquired after exposition to contaminated hands of professionals or through contact with colonized or infected patients[26].

S. aureus is a versatile pathogen, responsible for a large amount of infections characterized for diverse clinical manifestations, including from local conditions to systemic diseases presenting high lethality[3,27]. Among staphylococcal syndromes, the main types are listed: superficial syndromes, such as skin and soft tissue infections; systemic infections, such as bacteremia, osteomyelitis, pneumonia, endocarditis and meningitis; food-poisoning related to staphylococcal toxins, staphylococcal scalded skin syndrome and toxic shock syndrome[4,28].

The pathogenesis of *S. aureus* is complex and dependent of various factors associated to the clinical condition itself, including antimicrobial resistance, host susceptibility and virulence factors expression, which allow persistent colonization, escape from the immune system, tissue invasion and dissemination of the pathogen to other host sites. The set of necessary virulence factors to develop a staphylococcal infection depends on the site of infection, which can be determinant for its dissemination[7,8,29].

Virulence factors

Infections caused by *S. aureus* are related to its large amount of virulence factors which contributes for the adhesion to host cells, escape from the immune system, tissue invasion, toxins production and bacterial dissemination[5-7].

Staphylococcal infection initiates with surface adhesion, mediated by the set of adhesins named MSCRAMMs (Microbial Surface Components Recognizing Adhesive Matrix Molecules). MSCRAMMs integrants are A protein (SpA) which binds to the Fc region of G immunoglobulin inhibiting opsonization and phagocytosis processes; fibronectin binding proteins (Fnbp A e FnbpB); collagen binding protein (Cna); aggregation factors (ClfA e ClfB) which binds to fibrinogen and confers its anti-phagocytic properties[4,22,30-33].

Structural components are also determinant in *S. aureus* virulence, such as capsular polysaccharides that prevent phagocytosis and promote adhesion to host cells; peptidoglycan which is able to activate the complement system and increase chemotaxis of polymorphonuclear cells and teichoic acid stimulating interleukin-1 production[17].

Along with these factors, *S. aureus* also produces toxins as staphylococcal enterotoxins (SEs) associated to food poisoning; Panto-Valentine leucokidin (PVL) which induces leukocytosis through pore formation on leukocytes membranes; staphylococcal exfoliative toxin (Ets) that induces erythema and skin exfoliation observed in scalded skin syndrome; toxic shock syndrome toxin-1 (TSST-1) which stimulates lymphocyte-T proliferation resulting the toxic shock syndrome and alpha-, beta- and delta-hemolysins[9,28-29,33].

Extracellular enzymes are also among staphylococcal virulence factors as catalase, which inactivates hydrogen peroxide formed by the myeloperoxidase system inside phagocytic cells; coagulase, that catalyzes the conversion of fibrinogen into fibrin; fibrinolysin, that degrades fibrin clots facilitating pathogen dissemination; hyaluronidase which promotes pathogen dissemination through host tissues by degrading hyaluronic acid; lipase, that by degrading lipids also helps in pathogen dissemination through tissues; phospholipase, that promotes cell lysis by destruction of the phospholipids present in host cells cytoplasmatic membranes and nuclease, that cleaves DNA as well as RNA[17].

Great part of virulence factors in *S. aureus* is coordinated by the accessory gene regulator (*agr*), a set of genes with quorum sensing (QS) activity. QS is a cell-communication system which controls gene expression in response to population density by secreting auto inducers molecules[8,9].

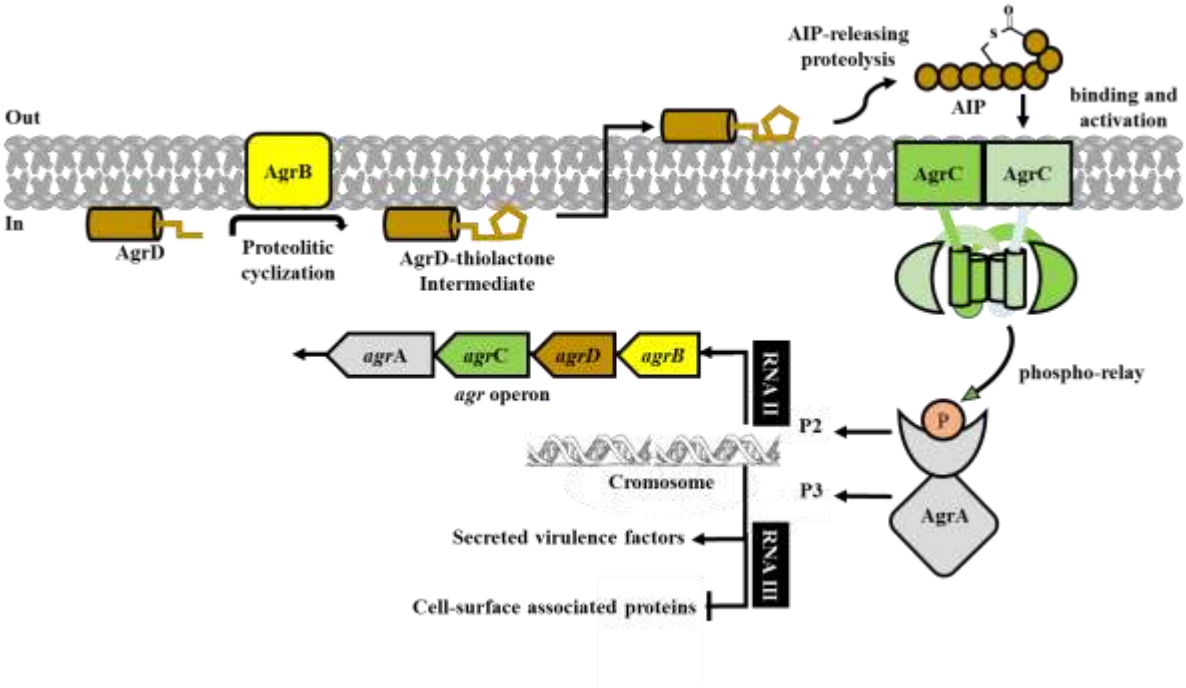
Accessory gene regulator

Accessory gene regulator controls the expression of virulence factors present in certain growing phases in *S. aureus*. During lag phase and the beginning of exponential phase, *S. aureus* produces cell wall associated virulence factors that aid tissue adhesion and immune system evasion. On the other hand, during post exponential phases exoproteins are secreted and the same time occurring diminution of cell wall associated factors, which facilitates infection dissemination[34].

In the *agr* locus (**Figure 1**), two operons are divergent transcribed from P2 and P3 promoters which produce RNA II and RNA III, respectively. The operon P2 that encodes RNA II is composed by *agrA*, *agrB*, *agrC* and *agrD* genes, while the operon P3 that encodes RNA III also

contain the *hld* gene responsible for delta-hemolysin synthesis. Polymorphism in agr results in four groups, agrI, agrII, agrIII and agrIV based on the specific AIP-AgrC receptor binding[34-40].

Figure 1. Structure of the *agr* system in *Staphylococcus aureus*.



agr = accessory gene regulator; AgrA = accessory gene regulator protein A; AgrB = accessory gene regulator protein B; AgrC = accessory gene regulator protein C; AgrD = accessory gene regulator protein D; *agr*I = *agr* type I polymorphism; *agr*II = *agr* type II polymorphism; *agr*III = *agr* type III polymorphism; *agr*IV = *agr* type IV polymorphism; AIP = Autoinducing Peptide.
Source: Adapted from Wang and Muir[34].

The products of *agrB* and *agrD* genes are, respectively, the AgrB and AgrD proteins, that bind to form an autoinducing peptide (AIP) which is released to extracellular environment. AgrD, the AIP precursor, is first proteolytically processed by a membrane bound peptidase, the AgrB, generating a thiolactone intermediate. This intermediate is exported across the membrane and then subjected to a second cleavage process to release the AIP into extracellular environment[34-36].

The AIP interacts with the AgrC sensor response, a transmembrane protein with AIP receptors, to cause phosphorylation and activation of the AgrA response regulator. The AgrA phosphorylated protein as an inducer of P2 promoter, regulates the RNA II transcription and consequently the synthesis of four Agr proteins fundamental to AIP synthesis. The AgrA as an inducer of P3 promoter, that regulates the RNA III transcription, a messenger RNA (mRNA) which works as an inducer or repressor of accessory genes. RNA III activates the gene expression that encodes virulence factors secreted by cells such as exoproteins, leukocidins, hemolysins, superantigens and enterotoxins and reduces the expression of cell associated virulence factors

such as coagulase enzyme, fibronectin, protein A and surface adhesins[8,34-36].

Polymorphism in *agr* from *S. aureus* results in four groups, *agrI*, *agrII*, *agrIII* and *agrIV* based on the specific AIP-AgrC receptor binding. Polymorphism in *agrC* and *agrD* genes result in AIP altered amino-acids sequences and on the AgrC corresponding receptor, being that AIP-receptor binding is specific for each allelic group. When an AIP from an allelic group binds to a AgrC receptor from another group it does not produce intrinsic factors, thus not producing a AgrC mediated sign, working as an antagonist. An AIP can only work as an agonist for its own allelic group, so that bacteria with different *agr* groups interfere in accessory proteins regulation of each other[34,36].

A relation between *agr* groups (I-IV) and the infection type has been described for *S. aureus*. Goudarzi et al. (2016)[41] report that *S. aureus* from *agr* group I was prevalent in non-invasive infections and those from *agr* group II in invasive infections. In according to these finds, Rasmussen et al. (2013)[42] had shown a significant association between *agr* group III and staphylococcal invasive infections. Cotar et al. (2012)[35] found a prevalence of *agr* group III in respiratory tract infections, while the *agr* group IV was related to staphylococcal scalded skin syndrome by Lamand et al. (2012)[43].

Despite *the* importance of *agr* for staphylococcal virulence, some studies have suggested that *agr* dysfunctions confer survival advantages for microorganism and worst clinical outcomes in patients infected by *S. aureus*. In study performed by Chong et al. (2013)[44] *agr* dysfunction was associated with persistent bacteremia caused by MRSA and MRSA with vancomycin heteroresistance. In 2014, Viedma et al. (2014)[45] evaluated the relation between *agr* dysfunction and vancomycin reduced susceptibility (VRS) and the results showed a significant association between *S. aureus* with VRS and dysfunctions in the *agr* locus. Corroborating with this study, Schweizer et al. (2011)[46] evaluated 814 patients with bacteremia caused by *S. aureus* and observed a significant association between *agr* dysfunctions and mortality in patients with staphylococcal infections.

Beyond *agr* importance for staphylococcal pathogenesis many studies have shown an important relation between *agr* groups polymorphisms and vancomycin reduced susceptibility in *S. aureus*. The findings show that *agrII* is often associated with vancomycin-reduced susceptibility and with treatment failures with vancomycin. Moreover, Cechinel et al. (2016)[47] reported that death risk increases by 12.6 times in patients with bacteremia caused by MRSA expressing *agrII* when compared to those that express other *agr* group type. In study conducted by Cázares-Domínguez et al. (2015b)[40] the *agr* type II polymorphism was the most prevalent among multidrug MRSA isolates. In 2015, Park et al.[48] evaluated 188 MRSA from blood culture and observed that among the isolates presenting MIC of 2 µg/mL the presence of *agrII* was significant when compared to other *agr* types.

ANTIMICROBIAL RESISTANCE

Methicillin-resistant *Staphylococcus aureus*

Penicillin was discovered in 1928 by Alexander Fleming, being the first choice for staphylococcal infections treatment in early 1940. However, in 1942 were reported the first cases of penicillin resistant *S. aureus* due production of a beta-lactamase enzyme (penicillinase) encoded by the *blaZ* gene. Penicillinase is able to degrade penicillin beta-lactam ring, inactivating its activity to inhibit the bacterial cell wall synthesis[48,50].

The introduction of methicillin, a semi-synthetic penicillin resistant to penicillinase, during the 1960s became an advance on anti-staphylococcal therapeutics. Beta-lactam antimicrobial agents act targeting penicillin binding proteins (PBPs) which are transpeptidases responsible for bacterial cell wall synthesis reactions. The binding between beta-lactam agents and PBPs prevent peptidoglycan to complete formation leading to cell death[51].

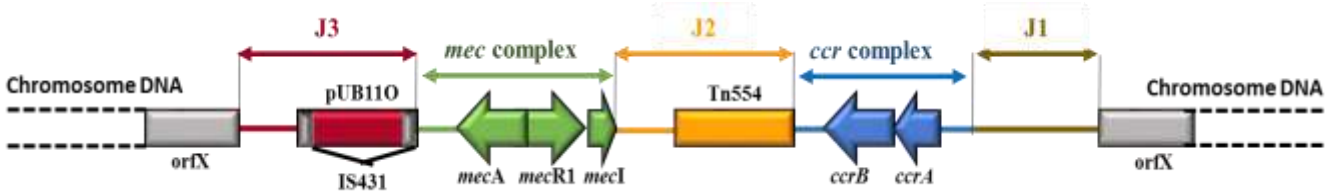
In 1961, the first report of a methicillin-resistant *S. aureus* (MRSA) was published. Methicillin resistance occurs due to acquisition of *mecA* gene (2.1 Kb), which encodes an altered penicillin binding protein (PBP2a or PBP2'; 77 kDa). This altered PBP presents low affinity with beta-lactam ring allowing a complete cell wall synthesis, thus becoming resistant to all beta-lactam agents except fifth generation cephalosporins. This gene is an important integrant of the mobile genetic element named *mec* staphylococcal cassette chromosome (SCC*mec*) which is found in a specific genome region named SCC*mec* attachment site (*attBscc*), in the 3' extremity of *orf*X gene. In addition to *mecA* gene, *mecC* gene, recently discovered, encodes a PBP2a homologue protein and has an identity of 70 % with *mecA* gene. The *mecC* gene is found in the SCC*mec* XI type and it is related to zoonotic transmission of MRSA[11,52-55].

MRSA identification can be realized by classical, automated or molecular methods which allows species characterization and antimicrobial susceptibility profile determination. Susceptibility testes according to CLSI[56] include disk-diffusion assay with cefoxitin or broth microdilution assay with oxacillin in order to determine beta-lactam resistance. Gene detection, *mecA*[57] ou *mec*C[58] can be performed by polymerase chain reaction (PCR) and it is considered the gold-standard method to confirm MRSA isolates.

Staphylococcal cassette chromosome *mec*

The *mecA* gene is a part of the *mec* complex present in the mobile genetic element called SCC*mec* (**Figure 2**). This region is bracketed by direct repeats, that contain integration site sequence recognized by cassete chromosome recombinases (*ccr*) and by a pair of inverted repeats. Also, it contains J regions (standing for junkyard), that are useful to classify the SCC*mec* in different subtypes[59-60].

Figure 2. General structure of the SCC*mec* element.



SCC*mec* in bacterial genome is flanked by terminal repeat sequences, specified by base pair complementarity. The *mec* complex harbor, the methicillin resistance gene *mecA* and its regulators *mecR1* and *mecI*, contains insertions sequences (IS431 or IS1272). In *S. aureus*, the *mec* complex is categorized by A, B, C and E classes. The *ccr* complex harbor recombinase genes responsible for integrate or excise SCC*mec* from genome, and contains open reading frames. The variable regions in SCC*mec* are the J regions (J1, J2 and J3) located between and around the complexes and may contain antimicrobials resistance determinants other than beta-lactam[59-65].

SCCmec mobility (integration and excision from chromosome) is mediated by recombinases belonging to invertase/resolvase family. Recombinases are encoded by three phylogenetically distinct *ccr* genes, *ccrA*, *ccrB* and *ccrC*, that present similarity below 50%. Generally, *ccr* genes with similarity over 85% are designed to the same allotype, while *ccr* genes with distinct allotypes have a nucleotide identity between 60% and 82%. Then, *ccrA*, *ccrB* and *ccrC* genes are categorized in four (*ccr*A1, *ccr*A2, *ccr*A3 e *ccr*A4), five (*ccr*B1, *ccr*B2, *ccr*B3, *ccr*B4 e *ccr*B6) and two (*ccr*C1 e *ccr*C2) allotypes, respectively. Based in the *ccr* allotypes differences and combinations, eight types of *ccr* complex were already described in *S. aureus*[59-61,66].

Besides *mec* and *ccr* complexes, *SCCmec* also harbor three regions J designated J1, J2 and J3, that constitute *SCCmec* non-essential components. J1 is placed between the direct junction and *ccr* complex, whereas J2 is located between *mec* and *ccr* complexes. J3 expands from *mec* complex to an open reading frame called *orfX*. Thereby, all *SCCmec* constitution is (*orf*X) J3-*mec*-J2-*ccr*-J1. Except *SCCmec* VII and IX, *ccr* complex is placed between J3 and J2 regions, and *mec* complex between J2 and J1 regions[60,61].

Despite J regions are considered less important regarding the *SCCmec* roles in the bacterial genome, they are epidemiologically significative, since may be target by plasmids (pUB110 and pT181) or transposons (Tn4001, Tn554 and ΨTn554) that could carry antimicrobials resistance determinants other that beta-lactam, and also heavy metals. pUB110 carries the *ant(4')* gene that encodes resistance to kanamycin and tobramycin, and the *ble* gene, that encodes resistance to bleomycin. These genes are frequently found in *SCCmec* II, and occasionally in *SCCmec* I and IV. pT181 carries *tetK* gene, responsible for resistance to tetracycline, found in the majority of *SCCmec* types III and V. The presence of gene *aacA-aphD* in Tn4001 transposon encodes resistance to aminoglycosides, and it is found in *SCCmec* IV. Tn554, found in *SCCmec* II and VIII, carries *ermA* and *spc* genes, that encode

resistance to erythromycin and spectinomycin, respectively. Finally, ΨTn554 carries *cad* gene, that encodes resistance to cadmium and is found mostly in *SCCmec* III[60,61].

The SCC*mec* types are defined by the combination of distinct classes from *mec* complex and diverse types of *ccr* complex and the subtypes are organized according to the differences in J regions[52,60,62,66]. Until now, there are 12 SCC*mec* types (I-XII) described, and this classification is extensively used in MRSA molecular typing[57,68]. The first SCC*mec* type was identified in 1999, Japan, using MRSA N315. In short time lapse, other two were described, SCC*mec* II and III[69]. Since then, various SCC*mec* were discovered around the world: SCC*mec* IV[70], SCC*mec* V[71], SCC*mec* VI[72], SCC*mec* VII[73], SCC*mec* VIII[74], SCC*mec* IX e X[75], SCC*mec* XI e SCC*mec* XII[66] (**Table 1**).

Table 1. Specifications of the SCC*mec* types[a].

SCC*mec*	*mec* complex	Structure of *mec* complex	*ccr* complex
I	B	IS*431*-*mec*A-Δ*mec*R1-IS*1272*	1 (A1B1)
II	A	IS*431*-*mec*A-*mec*R1-*mec*I	2 (A2B2)
III	A	IS*431*-*mec*A-*mec*R1-*mec*I	3 (A3B3)
IV	B	IS*431*-*mec*A-Δ*mec*R1-IS*1272*	2 (A2B2)
V	C2[c]	IS*431*-*mec*A-Δ*mec*R1-IS*431*	5 (C1)
VI	B	IS*431*-*mec*A-Δ*mec*R1-IS*1272*	4 (A4B4)
VII	C1[b]	IS*431*-*mec*A-Δ*mec*R1-IS*431*	5 (C1)
VIII	A	IS*431*-*mec*A-*mec*R1-*mec*I	4 (A4B4)
IX	C2[c]	IS*431*-*mec*A-Δ*mec*R1-IS*431*	1 (A1B1)
X	C1[b]	IS*431*-*mec*A-Δ*mec*R1-IS*431*	7 (A1B6)
XI	E[d]	blaZ-*mec*A_{LGA251}-*mec*$R1_{LGA251}$-*mec*I_{LGA251}	8 (A1B3)
XII	C2[c]	IS*431*-*mec*A-Δ*mec*R1-IS*431*	9 (C2)

[a] Table adapted from Wu et al.[66] and IWG-SCC[76].

[b] Class C1: IS*431* upstream and downstream of *mec*A are in the same direction.

[c] Class C2: IS*431* upstream and downstream of *mec*A are in the opposite direction.

[d] Class E *mec* complex contain the gene *mec*C, a homologue of *mecA* gene.

CA-MRSA, HA-MRSA and LA-MRSA

MRSA emerged in the 1960s, after introduction of methicillin in clinical practice and rapidly spread in nosocomial environments. In the 1980s, specific MRSA lineages were found outside the hospital environment, furthermore called Community-Acquired MRSA (CA-MRSA). These strains present increased susceptibility to antimicrobials and increased virulence as major characteristics, when compared to nosocomial lineages (Hospital-Acquired MRSA; HA-MRSA)[63,77,78].

MRSA, according to Centers for Disease Control and Prevention (CDC) is considered CA-MRSA when recovered from patients coming from

community or up to 48h after hospital admission that do not present history of infections or colonization by MRSA, previous hospitalization or invasive procedures in past year. Normally, CA-MRSA are associated with skin infections in healthy young patients, resistance to beta-lactam and *SCCmec* types IV or V. Some cases are associated with the production of Panton-Valentine leukocidin, that confers more virulence to CA-MRSA[53,78-81].

HA-MRSA, on the other hand, are more prevalent in patients with severe illness, prolongated hospitalization and/or previous antimicrobial use. For example, elderly, newborn, immunocompromised and patients in dialysis, post-operatory and with invasive devices are the most affected patients. They mostly carry SCC*mec* types I, II or III and present multidrug resistance[53,63,78,82].

Recently, a new MRSA strain emerged from animals and was designated as Livestock-Associated MRSA (LA-MRSA). Originally, LA-MRSA appeared first in humans as MSSA and then spread to livestock animals, where acquired methicillin resistance. Data suggest that transference from humans to animals was accompanied by a decrease in the colonization ability, transmission and virulence in this host. However, LA-MRSA has been frequently identified in human infections[83,84].

Clinical relevance

MRSA arising is an important challenge in clinical practice, since they are prevalent in both nosocomial and communitary environments and have multidrug resistance, that limits therapeutic options[85]. In a study performed between 2004 and 2009 in 36 countries from Latin America, Asia, Africa and Europe by International Nosocomial Infection Control Consortium (INICC), methicillin resistance was observed in 71-84% of *S. aureus*[86]. Between 2009 and 2010, a total of 69475 healthcare-associated infections occurred in 2039 hospitals were reported to National Healthcare Safety Network (NHSN). Also, *S. aureus* was the most prevalent pathogen in healthcare-associated infections (15.6%) and in surgical sites (30.4%)[87].

According to CDC, 80461 patients were diagnosed with invasive infection by MRSA in 2011 in USA, with 11285 deaths[88]. Between 2011 and 2014, 365490 nosocomial infections that occurred in 4515 different hospitals were reported to National Health and Security Network which demonstrated that *S. aureus* was the second most frequent pathogen in healthcare-associated infections (11.8%) and the most prevalent pathogen in surgical sites (20.7%)[89]. Data obtained by World Health Organization (WHO) in the first report of antimicrobial resistance show that MRSA prevalence in all studied regions was higher than 20%, achieving even 80%. Moreover, there was a significative increase in mortality ($p<0.00001$), progression to septic shock ($p<0.0001$) and long-term hospitalization ($p<0.00001$) in patients with MRSA than MSSA[90].

The Active Bacterial Core Surveillance reported the national estimation of MRSA invasive disorders and mortality rates (in 100000 inhabitants per year) in nine states of USA at 2014. Cases were classified as hospital onset (HO), healthcare-associated community-onset

(HACO) and community-acquired (CA). National estimation and mortality rates by MRSA infections in HO, HACO and CA were 2106 and 0.66 (0.32-1.34); 5637 and1.77 (1.10-2.93); 1316 and 0.41 (0.18-0.87), respectively[91].

Staphylococcus aureus with reduced vancomycin susceptibility

Vancomycin is a glycopeptide that has been approved in 1958 by Food and Drug Administration (FDA) for treatment of infections caused by penicillin-resistant *S. aureus*. However, methicillin and cefoxitin were approved shortly after presenting lower toxicity. Because of vancomycin toxicity, its use was reserved for patients allergic to beta-lactam antimicrobials or with infections caused by microorganisms resistant to the new antimicrobials[92]. This antimicrobial acts by inhibiting the cell wall synthesis of gram-positive microorganisms by binding to the carboxyl terminus of D-alanine-D-alanine residues of the peptide precursors, forming a stable non-covalent complex, preventing the elongation of the peptidoglycan in the cell wall[93-95].

With the advancement of MRSA infections associated with the irrational use of antimicrobials, vancomycin has become the main therapeutic option[10]. The constant use of this glycopeptide and, consequently, the increase in selective pressure, resulted in the appearance of vancomycin intermediate *S. aureus* (VISA) in 1996 in Japan, called Mu50[96]. The next year also in Japan, the first *S. aureus* with heterogeneous vancomycin resistance (hVISA), known as Mu3, was isolated[97].

In 2002 in Michigan, USA, the first clinical infection with vancomycin-resistant *S. aureus* (VRSA) was described. In Brazil, the first isolate with this characteristic was reported in São Paulo in a 35-year-old patient with recurrence of skin and soft tissue infections[98]. The presence of VRSA has also been reported in other countries[99-101]. It is believed that this resistance is mediated by transposon *Tn1546*, acquired from vancomycin-resistant *Enterococcus faecalis*. *Tn1546* contains the *vanA* genes, which causes the D-alanyl-D-alanine (D-ala-D-ala) fragment to change to D-alanyl-D-lactate (D-ala-D-lac), preventing vancomycin binding and inhibiting its action on bacterial cell wall synthesis[99,101,102].

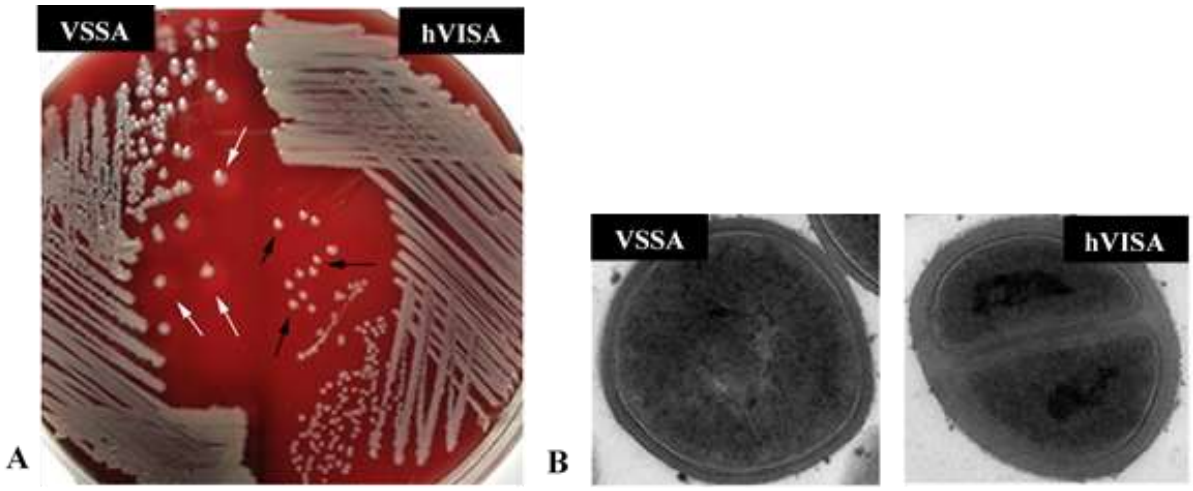
The American Clinical and Laboratory Standards Institute (CLSI)[56] manual ranks as susceptible to vancomycin isolates with MICs less than or equal to 2 µg/mL (VSSA), intermediate MIC between 4 and 8 µg/mL (VISA), and resistant MIC higher than or equal to 16 µg/mL (VRSA). The hVISA phenotype is characterized by the presence of a subpopulation with reduced sensitivity to vancomycin. In general, they are vancomycin-sensitive MRSA (MIC ≤ 2 µg/mL), with a subpopulation of approximately 10^{-5} to 10^{-6} cells, which has MIC ≥ 4 µg/Ml[103-105].

Staphylococcus aureus with heterogeneous vancomycin resistance

Isolates of hVISA are characterized by the presence of a subpopulation with reduced susceptibility to vancomycin. In general, they are isolated from vancomycin-sensitive MRSA when analyzed by conventional methods, ie, with MIC lower or equal to 2 µg/mL, but with a

subpopulation of approximately 10^{-5} to 10^{-6} cells exhibiting intermediate levels of resistance to vancomycin, with MIC higher than or equal to 4 µg/mL[103-106]. hVISA strains have heterogeneous morphology, presence of small colonies, low growth rate, reduced autolysis and hemolysis, thick cell wall and with reduced susceptibility to vancomycin (**Figure 3**)[107-109].

Figure 1. Characteristics of *Staphylococcus aureus* with heterogeneous vancomycin resistance[a,b]



Source: Adapted from Zhu[109].

[a] Low growth rate and reduced hemolytic activity (**Figure 3A**).

[b] Thick cell wall (**Figure 3B**).

The mechanism of acquisition of hVISA and VISA phenotypes is not fully elucidated but is mainly related to mutations in two component regulatory systems (TCRS), *vraRS* and *walKR*, and in the gene encoding the beta subunit of RNA polymerase, *rpoB*. It causes the thickening of the bacterial cell wall that entails the trapping of the vancomycin molecules and, consequently, hinders its action at the binding site[95,102,108,110-113].

After its first description in 1997 in Japan[97], the presence of hVISA among MRSA isolates has been reported in the world with variable frequency: 1.2-18.8% in the United States[1,114,115]; 2.2-4.7% in Malaysia[116,117]; 2.9% in Thailand[118]; 3.3% in Argentina[105]; 3.4% in the United Kingdom[119]; 5.3% in Canada[120]; 5.6-22.1% in China[16,121,122]; 6.51% in Japan[123]; 6.9-25.9% in India[124,125]; 9.7% in Brazil[126]; 13.7% in Turkish[127] and 37.7% in Coreia[128].

In the systematic review published in 2015, Zhang et al.[2] analyzed the prevalence of hVISA and VISA among MRSA isolates from different study periods, geographic regions, clinical samples, and genetic backgrounds. Regarding different periods of study, the prevalence of hVISA increased considerably from 4.68% before 2006 to 5.38% between 2006 and 2009, reaching 7.01% between 2010 and 2014. Likewise, the prevalence of VISA was 2.05% before 2006, 2.63% between 2006 and 2009, and 7.93% between 2010 and 2014. With regard to the different geographic regions, the prevalence of hVISA was 6.81% in Asia and 5.60% in Europe and America, and VISA was 3.42% in Asia and 2.75% in Europe and America. Regarding the

clinical samples, the prevalence of hVISA and VISA was higher in blood culture samples with 9.81% and 2.00%, respectively. With regard to the genetic background, most hVISA presented *SCCmec* II (48.16%), followed by *SCCmec* IV (18.07%), *SCCmec* III (17.99%) and *SCCmec* I (2.12%). *SCCmec* II (37.74%) was predominant, followed by *SCCmec* III (32.72%), *SCCmec* I (11.79%) and *SCCmec* IV (10.08%).

The significant discrepancy of the epidemiological data, despite reflecting the geographic variation, is largely due to the methodological inconsistency of the detection process of this phenotype; the absence of standardization and, also, due to the biological characteristics and the mechanism of resistance of these isolates. Factors such as the site of the clinical sample, the population of patients tested and the number of samples analyzed can also interfere at the rates found[2,129].

Currently, the most commonly used screening methods for the detection of the hVISA phenotype are: (a) Macro Etest, which associates dense inoculum, prolonged incubation and nutrient medium with vancomycin Etest strips; (b) Glycopeptide Resistance Detection (GRD), which uses a vancomycin and teicoplanin double-sided gradient in a single strip for detection of hVISA and VISA; and (c) vancomycin-supplemented agar, where more resistant colonies are selected from the growth in BHI agar containing 6 µg/mL vancomycin (BHIA-6V)[12,108,125].

The confirmatory method, considered gold standard for the detection of the hVISA phenotype, is the Population Analysis Profile - Area Under the Curve (PAP-AUC). However, this is a time-consuming, laborious and expensive method to be applied in routine clinical microbiology laboratory [104,105,130-132].

This methodology is based on calculating the area under the curve (AUC) generated after the growth of different cell densities (10^{-1} and 10^{-7} UFC/mL) in BHI agar containing various concentrations of vancomycin. When the ratio of the AUC of the isolate to the hVISA control (Mu3) is 0.9 to 1.3, the isolate is reported as hVISA[131].

Clinical relevance

Despite of clinical impact, hVISA phenotype is not definitively enlightened[134,135], some studies suggest that the presence of hVISA is commonly associated with failure in vancomycin treatment, persistent bacteremia, prolonged hospitalization, and adverse clinical outcomes[14-16,138,139].

In the cohort study published in 2013, Casapao et al.[137] analyzed the outcomes of patients with bloodstream infections (BSI) caused by hVISA and vancomycin-susceptible MRSA (VS-MRSA). The results showed that vancomycin treatment failure rates were 11-fold higher in patients with BSI caused by hVISA (82%) than by VS-MRSA (32.8%; $p < 0.001$). Patients with BSI by hVISA were also more likely to have persistent bacteremia (59% vs. 21.3%, respectively, $p < 0.001$), recurrent infections (25.5% vs. 1.9%, respectively, $p < 0.001$), and prolonged hospital stay (183 days vs. 16 days, respectively, $p = 0.022$). Mortality related to 30-day MRSA infection, although twice as high in patients with hVISA infection, was not statistically significant compared to VS-MRSA

mortality (21.3% vs. 9.8%, respectively; p=0.081).

In 2016, Koh et al. (2016)[13] analyzed the clinical impact of hVISA in patients with *S. aureus* bacteremia (SAB) or pneumonia (SAP). The results showed that hVISA isolates were not associated with failures in vancomycin treatment in patients with SAB (p=0.054) and were significantly associated with treatment failures in patients with SAP (p=0.014). The presence of hVISA in patients with SAB and SAP was not associated with the 30-day mortality related to these infections. On the other hand, in the retrospective study conducted by Hu et al. (2015)[14], mortality was significantly higher in patients with BSI caused by hVISA than by VSSA (92.9% vs. 72.9%, p=0.046).

Other studies have reported increased vancomycin treatment failures and mortality from VS-MRSA isolates, particularly those with MICs of 1.5 or 2 μg/mL[129,134]. Takesue et al.[140], when analyzing 128 MRSA from bacteremia, found that the efficacy of vancomycin was 78.8% in MRSA-infected patients with MIC of 1 μg/ml, whereas efficacy was only 30% in patients infected by MRSA presenting MIC of 2 μg/mL. In a meta-analysis performed by Jacob et al.[134], when evaluating clinical outcomes in patients with MRSA infections with low MIC for vancomycin (< 1.5 μg/mL) and high MIC for vancomycin (≥ 1.5 μg/mL), verified that the risk of treatment failures and mortality increases in MRSA infections with high MIC when compared to those of low MIC.

In a systematic review published in 2014, Kalil et al. (2014)[135] evaluated the association between vancomycin minimal inhibitory concentration and mortality among patients with SAB. The mortality rate was 30.7% among patients with SAB by hVISA with high MIC for vancomycin (≥ 1.5 μg/mL) compared to 35.4% among patients with SAB by hVISA with low MIC for vancomycin (<1.5 μg/ml). In 2011, Chen et al. (2011)[121] evaluated 554 MRSA and observed a growth in hVISA incidence when the MIC for vancomycin increased from 1 to 2 μg/mL, with 40% being hVISA in the isolates with MIC of 2 μg/mL. In 2015, Hu et al. (2015)[14], when analyzing patients with SAB by hVISA, found that high MIC for vancomycin is statistically associated with the development of hVISA (p<0.001). Other studies have also suggested that the proportion of hVISA is directly related to the increase of the minimal inhibitory concentration for vancomycin[115,121,137].

CONCLUSION

With the evolution of *S. aureus* to MRSA, hVISA and VISA, the treatment of staphylococcal infections has become a major challenge for the medical clinic, because of the antimicrobial options have been reduced since the appearance of these resistances. An adequate and early antibiotic therapy is essential for decreasing morbidity and mortality rates related to the infectious processes caused by MRSA, hVISA and VISA. Proper detection methods and complete understanding of infections implications, associated with clinical information of patients, are essential for clinical decision making, as well as guiding the physician to choose the appropriate antimicrobial for the treatment of

infections caused by these multiresistant microorganisms.

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CONTRIBUTION OF AUTHORS

Rossato AM collaborated in the analysis and interpretation of data, writing and critical review. Reiter KC collaborated in writing and critical review, while Azevedo PA collaborated in the critical review of the manuscript. All authors approved the final version of the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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