Resistance and virulence factors of the *Staphylococcus aureus* - A brief review

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ABSTRACT

Introduction: *Staphylococcus aureus* is an opportunistic pathogen responsible for a wide range of infections in humans and able to rapidly adapt to anti-staphylococcal antibiotics and become resistant to several classes of antibiotics (multidrug-resistant). The biofilm- producing S. *aureus* has become notorious for causing several infections and chronic infections due to its ability to resist therapeutic treatment by forming biofilms on abiotic and biotic surfaces. This brief literature review discusses aspects of the antimicrobial resistance and virulence factors of the S. aureus. Methods: Literature searches were performed using PubMed indexed articles published between 2010 and 2023 to identify studies relevant to the review. Results: The success of MRSA is a consequence of the most virulence factors produced by S. *aureus* combined with β-lactam resistance and resistance to other antibiotic classes. S. *aureus* attachment to medical implants and host tissue, and the establishment of a mature biofilm, play an important role in the persistence of chronic infections. S. *aureus* has shown an increasing number of toxins and other virulence determinants produced by them, correlating with serious diseases. Conclusion: Antibiotic resistance and biofilm-forming capacity contribute to the success of S. *aureus* as a human pathogen in both healthcare and community settings.

Keywords: Biofilms, Methicillin-resistant *Staphylococcus aureus*, Multidrug-resistant pathogen, Review, Virulence.

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INTRODUCTION

Staphylococcus are Gram-positive cocci in clusters that produce catalase, nonmotile, nonspore-formation and facultative anaerobes. Is microbiologically characterized with a measurement of 0.5–1.5µm in diameter and is grouped as irregular cluster. The development of molecular techniques of research permitted to broaden nomenclature of species. The genus *Staphylococcus* numbers 72 species and 30 sub- species validated. Staphylococci are part of the physiological microbiota of the skin and the mucous membranes (nasal flora) of humans and animals. They are commonly associated with opportunistic infections, the impact of which is frequently enhanced by antimicrobial resistance [1,2,3,4].

Staphylococcus encodes for both the staphylococcal protein A and the coagulase enzyme; these two factors have diagnostic importance since they are used to differentiate coagulase-positive staphylococci (CoPS) from coagulase-negative staphylococci (CoNS). CoPS species, have a capacity to coagulate blood plasma of mammals [1,5,6]. CoPS including seven species: *Staphylococcus aureus*, *Staphylococcus delphini*, *Staphylococcus intermedius*, *Staphylococcus pseudintermedius*, *Staphylococcus lutrae*, *Staphylococcus schleiferi ssp*. coagulans and *Staphylococcus hyicus* [1,7].

In clinical practice, *S. aureus* is considered as the most virulent among Staphylococcus. Is an opportunistic pathogen responsible for a wide range of infections in humans, such as skin infections, pneumonia, food poisoning or sepsis. The natural reservoirs for *S. aureus* are humans. Historically, *S. aureus* was able to rapidly adapt to anti-staphylococcal antibiotics and become resistant to several classes of antibiotics. Methicillin-resistant *S. aureus* (MRSA) first emerged in 1961 and rapidly became a leading cause of nosocomial infections. Today, hospital-associated methicillin-resistant S. *aureus* (HA-MRSA) is a multidrug-resistant pathogen and is one of the most common bacteria responsible for hospital-acquired infections and outbreaks. The strain of MRSA found in hospitals are found in community settings as well (CA-MRSA) [1,7,8,9]. The biofilm-producing *S. aureus* has become notorious for causing several infections, such as endocarditis and sepsis, and chronic infections due to its ability to resist therapeutic treatment by forming biofilms on abiotic (indwelling medical devices) and biotic (cardiac tissue, cartilage and chronic wounds) surfaces. The biofilm-related antimicrobial resistance is partly due to the presence of some dormant *S. aureus* cells (also known as persister cells) encased by its biofilm. These cells maintain their dormancy during antimicrobial treatment and become active as soon as the treatment is withdrawn, thus causing a chronic recurrent infection. Biofilm-related infections are associated with increased morbidity and mortality (up to 66%), with infected medical devices often requiring surgical removal and increased durations of hospitalization. The challenge of developing therapeutics to treat staphylococcal biofilm infections is compounded by the existence of multiple biofilm mechanism in *S. aureus* [3,10,11,12,13].

This short literature review discusses aspects of the antimicrobial resistance and virulence factors of the *Staphylococcus aureus*.

METHODOLOGY

This is a narrative review study. Narrative reviews are informed by a broad analysis of the literature and make it possible to gain knowledge. Literature searches were performed using PubMed indexed articles published between 2010 and 2022 to identify studies relevant to the review. Search terms included: “Staphylococcus”, “Staphylococcus aureus”, “Staphylococcus aureus resistance” “Staphylococcus aureus virulence”, “Staphylococcus aureus biofilm”, “CA-MRSA” and “HA-MRSA”. The reference lists of all retrieved articles were checked for additional relevant references. Studies published in English was considered in this review.

RESULTS

STAPHYLOCOCCUS AUREUS

Staphylococcus aureus was first recognized as the etiological agent of suppurative abscesses more than 130 years ago. Today, is one of the most infamous and widespread bacterial pathogens, causing a hard-to-estimate number of uncomplicated skin infections and probably hundreds of thousands to millions of more severe, invasive infections globally per year. Is a common gram-positive human pathogen involved in both community-acquired and nosocomial infections, must array of virulence factors responsible for attaching, colonizing, invading, and avoiding host immune system [14,15,16].

S. aureus typical colonies are yellow in color – because of carotenoid pigments – smooth, slightly raised, and hemolytic (betahemolysis) from the hemolysin production on 5% sheep blood agar. Positive tests for catalase and coagulase and b mannitol deoxyribonuclease be used as identification method in laboratory. The selective culture medium commonly used is salty-mannitol agar, its hypertonicity helps to select *Staphylococcus* [3,17].

S. aureus stably colonizes human skin, nasopharynx and/or the perineum of approximately one-third of the human population, and it is estimated that around 15%– 30% of healthy adults are nasal carriers of S. aureus. However, *S. aureus* can also become an opportunistic pathogen, which replicates and disseminates to many different sites, responsible for a wide range of clinical diseases such as skin and soft tissue infections (impetigo, folliculitis or scalded skin syndrome), intravenous catheter-associated infections, food poisoning, toxic shock syndrome, osteomyelitis, pneumonia, endocarditis, deep-seated abscesses or bloodstream infections, which are associated with significant morbidity and mortality [7,14,18].

High levels of antibiotic use in healthcare settings selected strongly for HA- MRSA which reached levels of over 50% of all *S. aureus* isolated in some countries. HA- MRSA infections also were associated with higher mortality and prolonged lengths-of- stay compared with methicillin-sensitive *S. aureus* (MSSA) [19].

MRSA was initially recognized as a nosocomial pathogen. The first definite case of CA-MRSA was reported in 1993 in Western Australia. Subsequently, CA-MRSA was identified in the United States in children who died between 1997 and 1999. Currently, CA-MRSA strain USA300, a particularly successful PVL-positive CA-MRSA clone, is a major cause of CA-MRSA infections in the United States and Canada. In 2005, a variant of USA300 emerged in South America (Colombia) designated USA300 Latin American Variant and has spread rapidly. In Brazil, CA-MRSA is an emerging pathogen accounting for approximately one third of all *S. aureus* strains isolated from children with severe community-acquired infections, however these infections have been scarcely described. A study in Brazil has reported a 0.9% prevalence rate of CA-MRSA nasal colonization among healthy people living in the community [8,20,21].

STAPHYLOCOCCUS AUREUS RESISTANCE

The rapid acquisition, in few years, of antibiotic resistance by *S. aureus* is a significant problem for treatment of human infections caused by this organism (Figure 1). The resistance to penicillin, the first discovered beta-lactam antibiotic against *S. aureus* infection, was documented in 1942. Penicillin-resistant *S. aureus* (PRSA) began producing an extracellular beta-lactamase (penicillinase) enzyme, conferred by the blaZ gene, which inactivated the antibiotic through hydrolysis of the beta-lactam ring. The adaptability of the bacteria to fight antibiotics through mutations and other mechanisms led to penicillin resistance. Today, the vast majority of *S. aureus* isolates are resistant to penicillin. To counteract this resistance, in the late 1950s, new semisynthetic beta-lactam antibiotic was developed (methicillin). In 1961, the first report of resistance to methicillin, was already published in the United Kingdom. Since then, methicillin-resistant *S. aureus* – MRSA, has spread worldwide, and its prevalence has been increasing. By the 1970s, there was widespread resistance to this semi-synthetic group of penicillinase-resistant antimicrobial agents. MRSA has been isolated in community settings is characterized by multidrug resistance, being resistant, to varying degrees, to other antibiotics, such as macrolides, aminoglycosides, tetracyclines or fluoroquinolones [7,14,22,23,24].

Resistance is due to modified penicillin binding protein (PBP2a) encoded by the mecA gene. The presence of PBP2a confers resistance towards all beta-lactam antibiotics. The mecA gene is a mobile genetic element (Staphylococcal Cassette Chromosome mec - SCCmec) that can carry resistance genes to other classes of drugs, configuring the multidrug-resistant microorganism [9,22].

Since the first reports of SCCmec I, II, and III in the early 2000s, various SCCmec elements have been reported by different researchers worldwide, and in addition to being adopted as a molecular epidemiology tool in healthcare settings, these elements have been utilized in the research of the evolution of Staphylococcus. SCCmec is characterized by terminal repeat regions, two essential genetic components (the ccr gene complex (ccr) and the mec gene complex) and the junkyard (J) regions have been described. Based on the nature of the mec and ccr gene complexes, and further classified into subtypes according to differences in their "J" region DNA, fourteen different SCCmec types have been identified (I, II, III, IV, V, VI, VII, VIII, IX, X, XI, XII, XIII, XIV). The SCCmec element is inserted in the orfX locus of the *S. aureus* genome. HA-MRSA is found in SCCmec I, II, and III, while CA-MRSA is found in SCCmec IV or V and are susceptible to antimicrobials other than beta-lactams [9,22,23,25,26,27,28].

These resistant *S. aureus* to beta-lactam drugs can occur in healthy people who do not have classic MRSA risk factors. MRSA is developed when MSSA acquires and inserts a SCCmec (mecA) into their genome (Figure 2) [9].

In 2011, a novel mec gene type was discovered in *S. aureus* which shares approximately 70% nucleotide sequence identity with mecA. This mec homologue was designated as mecC. *S. aureus* isolates harbouring the mecC gene have been isolated from humans in different countries. The mecC is located in a new SCCmec cassette type XI and exhibits 63% homology identity to the PBP2a encoded by mecA [2,26,27].

Resistance to beta-lactam antibiotics has been observed in some mecA- or mecC- negative *S. aureus* isolates. Early studies from the 1980s attributed this low-level oxacillin and/or methicillin resistance to hyperproduction of beta-lactamase. Beta-lactamase produce by activation of a gene named as blaZ gene. Beta-lactamase hyperproducers (BHP), termed borderline oxacillin-resistant *S. aureus* (BORSA), regain susceptibility upon introduction of a beta-lactamase inhibitor [9,29].

A new antibiotic was then needed to treat these infections that did not require attachment to the PBP2a site. In 1958, vancomycin, a glycopeptide, being approved for use in humans, but was first used to treat MRSA infections in a hospital setting in the late 1980s. Resistance to vancomycin was discovered in enterococci in the 1980s, and in the 1990s, in Japan, the first case of reduced susceptibility of *S. aureus* to vancomycin was reported (vancomycin-intermediate *S. aureus* – VISA). Further studies indicated that the VISA phenotype is frequently preceded by an intermediate phenotype known in the clinical laboratory as heterogenous VISA (hVISA). An hVISA phenotype refers to a mixed cell population, derived originally from a single colony of S. aureus, in which most cells have little or no resistance to vancomycin and a sub-population of cells is resistant to the antibiotic at the level of VISA. With the continued use of vancomycin, the first case of vancomycin-resistant *S. aureus* (VRSA) was reported in 2002, in the United States. Complete vancomycin

resistance in *S. aureus* is conferred by the vanA operon. Vancomycin interferes with late-stage cell wall peptidoglycan synthesis by forming non- covalent hydrogen bonds with the penultimate D-alanyl-D-alanine. Cell wall synthesis is inhibited and bound vancomycin-pentapeptide complexes accumulate within the cell. There were also cases of hVISA, VISA, and VRSA infections reported from every continent [14,23].

Figure 1 - Timeline delineating the advent of antibiotic therapies and subsequent emergence of antibiotic-resistant *S. aureus.*

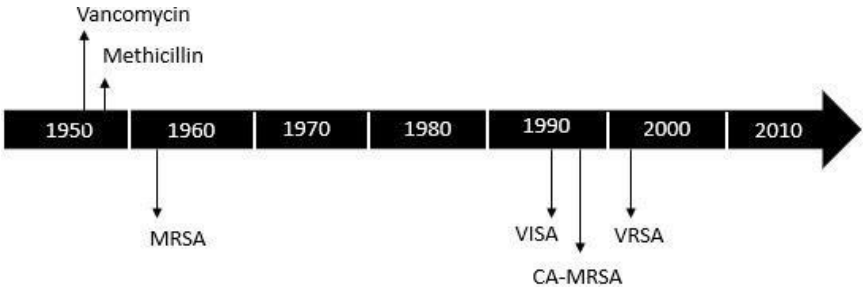
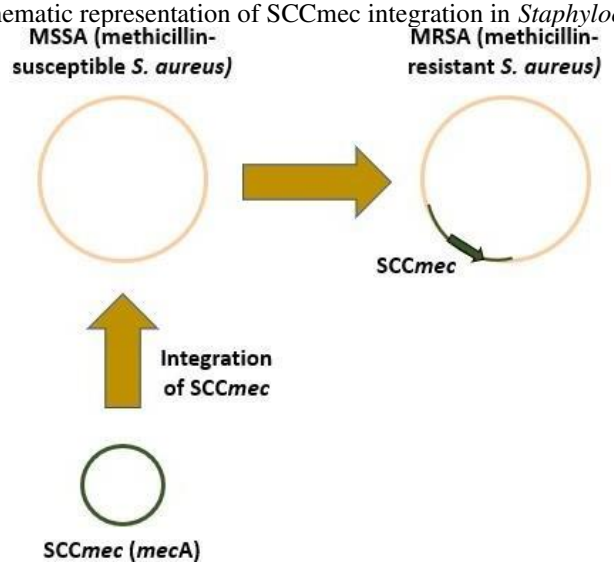


Figure 2 - Schematic representation of SCCmec integration in *Staphylococcus aureus*.



BIOFILM-PRODUCING ABILITY AND OTHERS VIRULENCE FACTORS

Bacterial biofilms are complex communities of organisms containing layers of bacteria within a glycocalyx. A mature biofilm contains specific three-dimensional structures referred to as towers or mushrooms separated by fluid filled channel. Biofilm- forming capacity contribute to the success of *S. aureus* as a human pathogen in both healthcare and community settings. The ability of *S. aureus* to form biofilms on implanted medical devices or damaged host tissue is also a key virulence factor for this pathogen, especially in healthcare settings where antibiotic usage is high and such biofilm formation represents a survival mechanism for the bacteria [30,31,32].

Similar to any other bacterial biofilm, *S. aureus* biofilm also has two distinct components – water (about 97%) and the organic matter which includes EPS and microcolonies. The EPS

constitutes about 50 to 90% of the total organic matter of a biofilm and is a complex of different polymeric substances, such as extracellular DNA (eDNA), proteins and polysaccharides. The major component of EPS is the polysaccharide intercellular adhesin (PIA), also known as poly-N-acetyl-glucosamine (PNAG). PIA are cationic in nature and play a significant role in colonization, biofilm formation and biofilm-related infections, immune evasion, resistance to antimicrobials and phagocytosis. *S. aureus* EPS also contains a range of proteins including accumulation associated proteins (Aap), surface binding protein A (Spa), fibrinogen binding protein (FnBP) A and B, extracellular matrix binding protein (Embp), amyloid fibers and *S. aureus* surface binding protein (SasG). In S. aureus, biofilm formation is mainly encoded by different genes, fibrinogen-binding proteins (fib) gene, fibronectin-binding proteins (fnbA and fnbB) genes, intercellular adhesion (icaA, icaB, icaC and icaD) genes, clumping factor (clfA and clfB), elastin binding protein (ebps), laminin binding protein (eno), collagen binding protein (cna) and bone sialoprotein genes. They are covalently linked to peptidoglycans of bacteria cell wall such as MSCRAMMs (microbial surface components recognizing adhesive matrix molecules), an important factor of biofilm production [3,12].

The main biofilm component, PIA, is synthesized by four genes, icaA, icaD, icaB, and icaC, encoded by the icaADBC operon (Figure 3) and mediates cell-to-cell adhesion and slime production. The transmembrane proteins, IcaA, and IcaD, work in concert as an N-acetylglucosaminyltransferase to synthesize PNAG oligomers that are less than 20 residues in length. IcaC is a membrane protein believed to transport IcaAD-synthesized oligomers across the cell membrane. IcaC is also involved in the formation of long oligomers of PIA/PNAG. The IcaB protein, which can be found in association with the bacterial cell surface and culture supernatants, deacetylates PIA/PNAG resulting in a positively charged polymer. Deacetylation is believed to promote the interaction of PIA/PNAG with the negatively charged cell surface. A fifth gene, icaR, is a negative regulator of icaADBC [12,30,32].

Using clinical isolates of S. aureus, was reported that MRSA strains express an icaADBC-independent biofilm phenotype *in vitro*, which is instead dependent on the fibronectin binding proteins (A and B) and the major autolysin (Atl) [10].

The pathogenic lifestyle of *S. aureus* is facilitated by a wide array of virulence factors, including toxins, proteases, adhesins, and immune-modulatory factors. *S. aureus* produces an arsenal of pore-forming toxins (PFTs) that kill host cells, thereby combatting immune responses and liberating nutrients from the host. The three major PFTs are: alpha-hemolysin (or Hla), Panton-Valentine leukocidin (PVL), and leukocidin AB. *S. aureus* exhibits several other virulence factors, such as catalase, coagulase, protein A, and many other toxins that contribute to its pathogenicity and enable it to escape from the host's immune system [25,33,34].

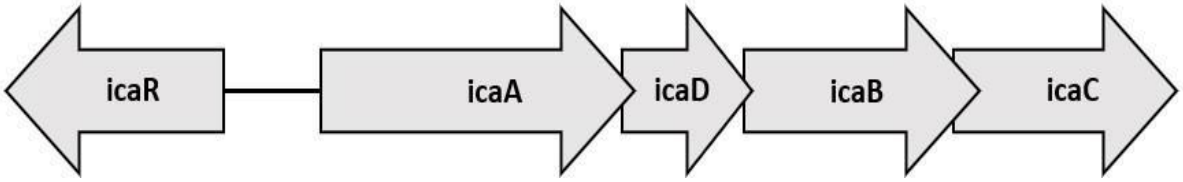
Alpha-hemolysin – Hla, was the first recognized PFT and is regarded as a key virulence factor of S. aureus. Is a toxin extremely conserved (99%), core genome-encoded cytotoxin that assembles into a homo-heptameric pore. Alpha-toxin contributes to *S. aureus* pathogenesis of skin infection and pneumonia, inhibits macrophage phagocytosis, and promotes death of these phagocytes in concert with secreted LukAB. Alpha-toxin also upregulates host autophagy, allowing *S. aureus* to become tolerated by the host by downregulating expression of the toxin receptor [33,35].

PVL is an exotoxin shows strong lytic activity against host defense cells such as human polymorphonuclear neutrophils, monocytes, macrophages, and rabbit neutrophils but not murine neutrophils *in vitro*. Pore formation requires the presence of the two components of the toxin, LukS-PV and LukF-PV, encoded by lukS-PV and lukF-PV genes. PVL aggravates many infections, such as skin and soft tissue infection, necrotizing pneumonia, bone joint infections, and even bacteremia. The prevalence of the pvl gene has been less common in MSSA isolates than in MRSA. The pvl gene locus represents a stable genetic marker of CA-MRSA strains [36,37].

While others leukocidins are secreted toxins, Leukocidin AB (LukAB) is found both secreted into the extracelular milieu and associated with the bacterial cell envelope. The sorting of LukAB follows a multistep process controlled by the cell envelope, resulting in differential deposition of the toxin on the bacterial cell or into the extracellular milieu, dependent on growth conditions. LukAB, appears to be the major toxin responsible for primary human polymorphonuclear leukocyte (PMN) cell death during tissue culture infection and impairs function of and kills antigen presenting cells thus potentially reducing the host defense and immunological memory needed to combat current and subsequent infections [34,38,39].

CA-MRSA usually differ from HA-MRSA by the fact that they carry a phage encoded toxin called Panton-Valentine Leucocidin (PVL) [8,9].

Figure 3 - Organization of the intercellular adhesion (ica) operon in *Staphylococcus aureus*.



CONCLUSION

A high prevalence of antimicrobial resistance not only to methicillin but also to other antimicrobials was thought to have correlations with the inappropriate use of antimicrobials, so the prevalence of MRSA is still used as an indicator of good infection control and prevention practices and appropriateness of antimicrobial practice. The rapid and accurate diagnosis of antimicrobial resistance in *S. aureus* is crucial to the early initiation of directed antibiotic therapy and to improve

clinical outcomes for patients. *S.* aureus is an important pathogen that causes a variety of infections. The ability to develop biofilms varies among different *S. aureus* isolates. The formation of biofilms on indwelling medical devices enables *S. aureus* to evade host immune responses and establish chronic infections. Multiple environmental factors, including nutrients, antibacterial agents, pH, temperature, and so on can induce stress responses and can profoundly affect the stages of biofilm formation, including initial attachment, maturation, and detachment. The formation of a biofilm decreases the susceptibility to antimicrobials and immune defenses, making these infections difficult to eradicate. An understanding of colonization, transmission, risk factors for progression to infection and conditions that promote the emergence of resistance will enable optimization of strategies to effectively control MRSA.

DISCLOSURE STATEMENT

The authors disclose any financial and personal relationships with other people or organizations that could inappropriately influence their work. All authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

All the authors of this review wrote the manuscript.

REFERENCES

1. Balbustkaya, A. A., Dmitrenko, O. A., & Skvortsov, V. N. (2017). The modern characteristics of species identification of coagulase-positive bacteria of Staphylococcus genus. *Klin Lab Diagn*, 62(8), 497-502.

2. Loncaric, I., Kübber-Heiss, B., Posautz, A., Ruppitsch, W., Lepuschitz, S., Schauer, B., et al. (2019). Characterization of mecC gene-carrying coagulase-negative Staphylococcus spp. isolated from various animals. *Vet Microbiol*, 230, 138-144.

3. Silva-Santana, G., Cabral-Oliveira, G. G., Oliveira, D. R., Nogueira, B. A., Pereira-Ribeiro, P. M. A., & Mattos-Guaraldi, A. L. (2021). Staphylococcus aureus biofilms: an opportunistic pathogen with multidrug resistance. *R Med Microbiol*, 32(1), 12-21.

4. List of Prokaryotic names with Standing in Nomenclature (LPSN). (2023). Staphylococcus. Disponível em: https://lpsn.dsmz.de/search?word=staphylococcus.

5. Beça, N., Bessa, L. J., Mendes, A., Santos, J., Leite-Martins, L., Matos, A. J. F., & da Costa, P. M. (2015). Coagulase-positive Staphylococcus: Prevalence and antimicrobial resistance. *J Am Anim Hosp Assoc*, 51(6), 365-71.

6. Sued-Karam, B. R., & Pereira-Ribeiro, P. M. A. (2022). Staphylococcus warneri: brief literature review. *Braz J Health Review*, 5(2), 4423-4429.

7. Sanchini, A. (2022). Recent developments in phenotypic and molecular diagnostic methods for antimicrobial resistance detection in Staphylococcus aureus: A narrative review. *Diagnostics*, 12, 208.

8. Argudín, M. A., Deplano, A., Nonhoff, C., Yin, N., Michek, C., Martiny, D., et al. (2021). Epidemiology of the Staphylococcus aureus CA-MRSA USA300 in Belgium. *Eur J Clin Microbiol Infect Dis*, 40(11), 2335-2347.

9. Tara, T., Khan, Z., Ali, H., Sardar, S., Samad, A., Ullah, K., et al. (2021). A review of global epidemiology and antibiotic resistance of Staphylococcus aureus. *Pakistan J Med Health Sci*, 15(12), 3921-3925.

10. Pozzi, C., Waters, E. M., Rudkin, J. K., Schaeffer, C. R., Lohan, M. J., Tong, P., et al. (2012). Methicillin resistance alters the biofilm phenotype and attenuates virulence in Staphylococcus aureus device-associated infections. *PLoS Pathog*, 8(4), e1002626.

11. Moormeier, D. E., & Bayles, K. W. (2017). Staphylococcus aureus biofilm: A complex developmental organism. *Mol Microbiol*, 104(3), 365–376.

12. Idrees, M., Sawant, S., Karodia, N., & Rahman, A. (2021). Staphylococcus aureus biofilm: Morphology, genetics, pathogenesis and treatment strategies. *Int J Environ Res Public Health*, 18, 7602.

13. Sultan, A. R., Lattwein, K. R., Toom, N. A. L., Snijders, S. V., Kooiman, K., Verbon, A., & van Wamel, W. J. B. (2021). Paracetamol modulates biofilm formation in Staphylococcus aureus clonal complex 8 strains. *Sci Rep*, 11(1), 5114.

14. McGuinness, W. A., Malachowa, N., & DeLeo, F. R. (2017). Vancomycin Resistance in Staphylococcus aureus. *Yale J Biol Med*, 90(2), 269-281.

15. Cheung, G. Y. C., Bae, J. S., & Otto, M. (2021). Pathogenicity and virulence of Staphylococcus aureus. *Virulence*, 12(1), 547-569.

16. Tasneem, U., Mejmood, K., Majid, M., Ullah, S. R., & Andleeb, S. (2022). Methicillin-resistant Staphylococcus aureus: a brief review of virulence and resistance. *J Pak Med Assoc*, 72(3), 509-515.

17. Yeagley, A. A., Su, Z., McCullough, K. D., Worthington, R. J., & Melander, C. (2013). N-substituted 2-aminoimidazole inhibitors of MRSA biofilm formation accessed through direct 1,3-bis (tert-butoxycarbonyl) guanidine cyclization. *Org Biomol Chem*, 11, 130-137.

18. Thomer, L., Schneewind, O., & Missiakas, D. (2016). Pathogenesis of Staphylococcus aureus bloodstream infections. *Annu Rev Pathol*, 11, 343-364.

19. Henderson, A., & Nimmo, G. R. (2017). Control of healthcare- and community-associated MRSA: recent progress and persisting challenges. *Br Med Bull*, 125(1), 25-41.

20. Ossa, R. P., Prado, S. I., Cervi, M. C., Lima, D. A. F. S., Martinez, R., & Bellissimo-Rodrigues, F. (2018). Is community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA) an emerging pathogen among children in Brazil? *Braz J Infect Dis*, 22(5), 371-376.

21. Santimaleeworagun, W., Preechachuawong, P., Samret, W., & Jitwasinkul, T. (2021). The first report of a methicillin-resistant Staphylococcus aureus isolate harboring type iv sccmec in Thailand. *Pathogens*, 10(4), 430.

22. Monecke, S., Coombs, G., Shore, A. C., Coleman, D. C., Akpaka, P., Borg, M., et al. (2011). A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant Staphylococcus aureus. *PLoS One*, 6(4), e17936.

23. Unni, S., Siddiqui, T. J., & Bidaisee, S. (2021). Reduced susceptibility and resistance to vancomycin of Staphylococcus aureus: A review of global incidence patterns and related genetic mechanisms. *Cureus*, 13(10), e18925.

24. Aragonés, L. G., Sancho, J. J. B., Luque, J. C. S., Rodriguez, F. M., Alfaro, E. M., & Del Pozo, J. S. G. (2022). What do beta-lactams add to vancomycin or daptomycin in the treatment of patients with methicillin-resistant Staphylococcus aureus bacteraemia? A review. *Postgrad Med J*, 98(1155), 48-56.

25. Martins, K. B., Olmedo, D. W. V., Paz, M. M., & Ramos, D. F. (2020). Staphylococcus aureus and its effects on the prognosis of bronchiectasis. *Microb Drug Resist*, 27(6), 823-834.

26. Goudarzi, M., Navidinia, M., Dadashi, M., Hashemi, A., & Pouriran, R. (2021). First report of methicillin-resistant Staphylococcus aureus carrying the mecC gene in human samples from Iran: prevalence and molecular characteristics. *New Microbe and New Infect*, 39, 100832.

27. Uehara, Y. (2022). Current status of Staphylococcal Cassette Chromosome mec (SCCmec). *Antibiotics (Basel)*, 11(1), 86.

28. McClure, J., Conly, J. M., Obasuyi, O., Ward, L., Ugarte-Torres, A., Louie, T., & Zhang, K. (2020). Novel assay for detection of methicillin-resistant Staphylococcus aureus directly from clinical samples. *Front Microbiol*, 11, 1295.

29. Sawhney, S. S., Ransom, E. M., Wallace, M. A., Reich, P. J., Dantas, G., & Burnham, C. D. (2022). Comparative genomics of borderline oxacillin-resistant Staphylococcus aureus detected during a pseudo-outbreak of methicillin resistant S. aureus in a neonatal intensive care unit. *mBio*, 13(1), e0319621.

30. Cue, D., Lei, M. G., & Lee, C. Y. (2012). Genetic regulation of the intercellular adhesion locus in staphylococci. *Front Cell Infect Microbiol*, 2, 38.

31. McCarthy, H., Rudkin, J. K., Black, N. S., Gallagher, L., O'Neill, E., & O'Gara, J. P. (2015). Methicillin resistance and the biofilm phenotype in Staphylococcus aureus. *Frontiers in Cellular and Infection Microbiology*, 5, 1. doi:10.3389/fcimb.2015.00001

32. Pereira-Ribeiro, P. M. A., Cabral-Oliveira, G. G., Olivella, J. G. B., Ribeiro, F. C., Nogueira, B. A., Sued-Karam, B. R., & Mattos-Guaraldi, A. N. (2022). Biofilm-producing ability of Staphylococcus spp. multidrug-resistance isolated from hospitalized patients with osteomyelitis. *International Journal of Scientific Research in Multidisciplinary Studies*, 20(4).

33. Ford, C. A., Hunford, I. M., & Cassat, J. E. (2020). Antivirulence strategies for the treatment of Staphylococcus aureus infections: A mini review. *Frontiers in Microbiology*, 11, 632706. doi:10.3389/fmicb.2020.632706

34. Zheng, X., Marsman, G., Lacey, K. A., Chapman, J. R., Goosmann, C., Ueberheide, B. M., & Torres, V. J. (2021). The cell envelope of Staphylococcus aureus selectively controls the sorting of virulence factors. *Nature Communications*, 12(1), 6193. doi:10.1038/s41467-021-26258-3

35. Reyes-Robles, T., & Torres, V. J. (2017). Staphylococcus aureus pore-forming toxins. *Current Topics in Microbiology and Immunology*, 409, 121-144. doi:10.1007/82_2017_27

36. Parvez, A. K., Ferdous, R. N., Rahman, S., & Islam, S. (2018). Healthcare-associated (HA) and community-associated (CA) methicillin resistant Staphylococcus aureus (MRSA) in Bangladesh – Source, diagnosis and treatment. *Journal of Genetic Engineering and Biotechnology*, 16(2), 473-478.

37. Gao, M., Sang, R., Wang, G., & Xu, Y. (2019). Association of pvl gene with incomplete hemolytic phenotype in clinical Staphylococcus aureus. *Infection and Drug Resistance*, 12, 1649-1656. doi:10.2147/IDR.S204207

38. Fernandez, J., Sanders, H., Henn, J., Wilson, J. M., Malone, D., Buoninfante, A., et al. (2022). Vaccination with detoxified leukocidin ab reduces bacterial load in a Staphylococcus aureus minipig deep surgical wound infection model. *Journal of Infectious Diseases*, 225(8), 1460-1470. doi:10.1093/infdis/jiab556

39. Zheng, X., Ma, S. X., John, A., & Torres, V. J. (2022). The major autolysin Atl regulates the virulence of Staphylococcus aureus by controlling the sorting of LukAB. *Infection and Immunity*, 90(4), e0005622. doi:10.1128/IAI.00056-22