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Why is *Staphylococcus aureus* Such a Successful Pathogen?

Mfonido A. Ukpanah[1*] and Peter U. Upla[2]

[1]Department of Molecular Medical Microbiology, School of Life of Sciences, University of Nottingham, UK.
[2]Department of Molecular Microbiology, Nottingham Trent University, Nottingham NG1 4FQ, UK.

Authors' contributions

This work was carried out in collaboration between both authors. Author MAU wrote the first draft of the manuscript. Both authors managed the literature searches. Both authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Staphylococcus aureus is a fast evolving and a well-adapted opportunistic pathogen that causes a variety of infections such as boils, abscesses, pneumonia, toxic shock syndrome, endocarditis, bacteraemia and food poisoning in humans. The colonisation of various body sites by *S. aureus* demonstrates the ability of this pathogen to disseminate at a fast pace in the hospital setting and in the community. The organism is endowed with a profusion of virulence factors that play a significant role in pathogenesis and disease severity. *S. aureus* infections are generally difficult to treat due to the evolution of strains with resistance to methicillin (methicillin-resistant *S. aureus*-MRSA) and vancomycin (vancomycin intermediate-resistant *S. aureus* [VISA] and vancomycin-resistant *S.* [VRSA]). The success of *S. aureus* as a human pathogen, therefore, relies largely on its capacity to produce an array of virulence factors that can evade or subvert the host immune responses and its resistance to a wide range of antibiotics. This review will focus largely on virulence determinants, immune evasion mechanisms and antibiotic resistance mechanism of *S. aureus* that makes it such a successful pathogen.

**Corresponding author: E-mail: mfonidoukpanah@uniuyo.edu.ng;*

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1. INTRODUCTION

Staphylococcus aureus is a Gram positive bacterial pathogen occurring in grape-like clusters. It is found predominantly and primarily in the nares of approximately 20% of healthy human population. [1] Colonisation of methicillin resistant *S. aureus* (MRSA) in the nose has strongly been attributed to subsequent disease outcome with MRSA than in those not colonised [2]. *S. aureus* is an opportunistic pathogen that causes a broad range of community-acquired and hospital associated infections (HAI). It also causes infections in both humans and animals, and can be transmitted efficiently in clustered human populations. Infections can range from mild and self-limiting diseases such as boils, to potentially life threatening severe complications such as toxic shock syndrome, endocarditis, bacteraemia, pneumonia, osteomyelitis, and sepsis [3]. Most importantly, invasive MRSA infection was shown to be responsible for approximately 20% bacteraemia deaths in the United States [4]. Indeed, death toll associated with *S. aureus* bacteraemia (SAB) in the United States now accounts for a higher percentage than mortalities caused by tuberculosis, viral hepatitis and AIDS combined, demonstrating its importance as a public health threat [4].

The ability of *S. aureus* to cause a massive range of diseases reflects its ability to express an array of virulence factors capable of subverting and disarming the immune system [5]. Virulence factors such as *S. aureus* protein A (SpA), haemolysins, exfoliative toxins, enterotoxins and many cell surface secreted factors have been linked to pathogenesis and disease severity [6]. One notorious virulence factor of *S aureus*, Panton Valentine Leukocidin (PVL) is associated with skin and soft tissue infections in the community settings and to a lesser extent necrotizing pneumonia [7].

One outstanding success of *S. aureus* is the evolution of strains with resistance to Methicillin known widely as MRSA [8]. Methicillin resistance is mediated by the acquisition of *mecA* gene. As newer antibiotics emerge, *S. aureus* finds a way to acquire genes for resistance to such antibiotics. The severity of *S. aureus* infections is therefore on the increase due to the acquisition of numerous antibiotic resistance genes, thus making infections caused by MRSA more difficult to treat [9].

2. HABITAT AND COLONISATION OF *S. aureus*

Nasal colonisation by *S. aureus* is a significant predisposing factor for numerous infections such as bacteraemia and surgical infections, and contributes tremendously to the dissemination of this pathogen in both the hospital and community environments [10]. It is also highly adapted to life on skin, perineum, axial and other body compartments. A recent investigation by Marshall and McBryde has also strongly demonstrated the role of *S. aureus* carriage in the pathogenesis of bloodstream infection [11]. The colonisation potential of *S. aureus* has been linked to a broad repertoire of cell wall-associated proteins broadly termed Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs) [12]. A recent study by Syed *et al*., has shown that triclosan, a biocide used in many personal care products and in medical equipment can promote the binding of *S. aureus* to host proteins such as fibronectin, fibrinogen, keratin and collagen, as well as inanimate surfaces and hence efficiently enhances *S. aureus* nasal colonisation [13].

3. VIRULENCE DETERMINANTS OF *S. aureus* AND ITS ROLE IN DISEASE

S. aureus produces a plethora of virulence factors which play diverse roles in immune subversion and pathogenesis. They are broadly divided into secreted and cell-surface associated factors (Table 1) [5], and others such as biofilm formation and overcoming iron depletion [14,15].

3.1 Virulence Gene Expression

S. aureus displays a highly coordinated expression of the different virulence factors during the different phases of infection to enable successful establishment of a disease. The array of virulence factors expression is tightly controlled by the different regulatory elements that respond to several conditions including population density (quorum sensing), availability of nutrients, pH and osmolarity [5]. The regulatory systems positively regulate the expression of genes involved in the early phase of infection such as genes encoding adhesion molecules and immune evasion factors. However, in the late phase of infections, genes involved in toxin production are upregulated to promote bacterial dissemination [5].

The accessory gene regulator (*agr*) system or quorum sensing system is the major regulatory element of virulence genes in *S. Aureus* and coordinates the expression of its effector molecule RNAIII which encodes the δ-haemolysin protein and modulates the expression of virulence factors at transcriptional and post-transcriptional levels [16,17]. However, a study by Oogai et al. [18] has revealed that iron depletion is also responsible for increased virulence factor (RNAIII) expression in serum.

Meanwhile, a recent report has demonstrated that the combined expression of RNAIII and small pathogenicity island RNA D (SprD) during the growth of the bacteria promote early and transient production of Staphylococcal binder of immunoglobulin G (Sbi) to evade host immune responses [19] Interestingly, *agr* system upregulates toxin gene expression during the late phase of growth and down-regulates cell surface factor by responding to accumulated auto-inducing peptides (AIP) produced by *S. aureus* [16] (Fig 1). Furthermore, a report by Ding et al. reveals that catabolite control protein E (CcpE) is a master regulator for virulence factor expression, iron acquisition, central metabolism and virulence of *S. aureus* [15].

Staphylococcal accessory element (*sae*) is another gene regulatory system of virulence gene expression. It positively regulates α-, β-, γ-haemolysins and negatively regulates SpA [20]. Other regulatory systems include Staphylococcal accessory regulator A (*sar*A) and its many homologues, primary sigma factor (σ^A) and alternative sigma factor (σ^B) [21].

3.2 Cell Surface Factors

S. aureus produces an exopolysaccharide capsule whose main function is to promote bacterial colonisation and persistence in blood stream of the infected host and on mucosal surfaces [23]. Importantly, it enables the organism to cause infections in those with implanted peritoneal catheter, intravenous catheters and pacemakers [24]. Interestingly, a group of MSCRAMMs anchored to the cell wall of *S. aureus* promote microbial adhesion to host proteins and are indispensable *in vivo* as they play a major role in the first step of the infection process [25]. In addition, clumping factor (Clf) and fibronectin binding proteins (FnBp) cause activation of platelets resulting in clotting. Other cell surface associated factors include; wall teichoic acid (WTA), SpA and staphyloxanthin (*S. aureus* golden pigment). Cell surface factors and their functions are summarised in Table 1.

3.3 Secreted Factors

To facilitate dissemination, *S. aureus* secretes various factors which disrupt host cells and tissues and interfere with the immune system of the host. These virulence factors are classified under four headings; exoenzymes, superantigens, cytotoxins (pore-forming toxins) and miscellaneous proteins [25].

3.3.1 Exoenzymes

Catalase, coagulase, lipases, hyaluronidases, fibrinolysin and phosphatidyl inositol - specific phospholipase C are the various enzymes secreted by *S. aureus* to combat host defense mechanisms and enhance severity of the disease [24]. Catalase neutralizes the toxic effect of hydrogen peroxide produced by phagocytic cells and has been found to promote *S. aureus* survival in the presence of *Streptococcus pneumoniae* [26]. Importantly, coagulase (Coa) and von Willebrand factor binding protein (vWbp) produced by *S. aureus* during infection can bind fibrinogen and prothrombin to enable the initiation of infection [26]. Lipase has been shown to promote spread of *S. aureus* through skin and subcutaneous tissue and has been implicated in causation of chronic furunculosis [24]. A study by Hu *et al.*, has also implicated lipase in enhanced biofilm formation, increased lethal effect and increased peritoneal abscesses in *S. aureus* infections [27].

Interestingly, fibrinolysin has the potential ability to degrade fibrin, whereas, hyaluronidase cleaves *β*-1, 4 glycosidic bond of glycosaminoglycans in intercellular tissue, all of which allows the pathogen to spread in the surroundings tissue [24]. Phosphatidyl inositol - specific phospholipase C (PI-PLC) produced by some strains of *S. aureus*, community-associated methicillin-resistant *S. aureus* (CA-MRSA) USA300, a genotype of MRSA, has been reported to promote the survival of this pathogen in whole human blood and in neutrophils but has no survival advantage in murine whole blood [28].

Table 1. Virulence factors of *S. aureus* and role in disease [5,22]

Virulence factors	Positive regulators	Role In disease	Proposed associated disease
Cell surface factors			
Capsular polysaccharides	Sig B, *agr*	Adherence to host cell and plastic materials	Biomaterial related infections. Serotype 5- Oxacillin resistance Serotype 8- Toxic shock like syndrome (TSLS)
Wall teichoic acid (WTA)	n.d	-	-
Staphylococcal protein A (SpA)	SarHI	Binds to IgG, interfering with opsonisation and phagocytosis	-
Staphyloxanthin	n.d	Resistance to neutrophil reactive oxidant-based phagocytosis	-
MSCRAMMs			
Fibronectin-binding proteins (FnbpA and FnbpB)	Sig B, *sar*A	Attachment to fibronectin and plasma clot	-
Clumping factor proteins (ClfA and ClfB)	n.d	Mediate clumping and adherence to fibrinogen in the presence of fibronectin	-
Collagen-binding protein	n.d	Adherence to collagenous tissues and cartilage	-
Secreted proteins ***Exoenzyme/proteins***			
Catalase	n.d	Break down hydrogen peroxide to water	-
Coagulase	*sae*	Bind fibrinogen, clots fibrin and enables development of infection	-
Hyaluronidases	Sig B	Degrade hyaluronic acid, potential for tissue dissemination	Invasive infection
Fibrinolysin	n.d	Degrades fibrin, pathogen spread to	-

Virulence factors	Positive regulators	Role In disease	Proposed associated disease
		surrounding tissue	
Phosphatidyl inositol - specific phospholipase C	*agr*	Increases survival in human blood and in neutrophils	
Lipases	*agr*	Inactivate fatty acid, enhance biofilm formation, increase lethal effect	Peritoneal abscesses
Nucleases	*agr*	Cleave nucleic acids	-
Staphylokinase (SAK)	*agr*	Activates plasminogen; inactivates antimicrobial peptides	-
Proteases			
Cysteine (e.g staphopain)	*agr*	Block neutrophil activation and chemotaxis	-
Aureolysin	*agr*	Inactivate antimicrobial peptides	-
Superantigens			
Toxic shock syndrome toxin-1 (TSST-1)	*agr, sar*A	Massive activation of T cells and antibody presenting cells	Toxic shock syndrome (menstrual and non-menstrual TSS.)
Staphylococcal enterotoxins (SEA, B, C, D, E, G and Q)	*agr, sar*A	Massive activation of T cells and antibody presenting cells	Staphylococcal food poisoning, some TSS
Exfoliative or epidermolytic toxin	*agr*	Induce the atypical, polyclonal proliferation of T cells separate glycosaminoglycan matrix of epidermis	Scalded Skin Syndrome (Ritter's disease)
Cytotoxins			
α-haemolysin	*agr, sar*A, *sae*	Forms β-barrel pores on a wide cell types, mainly platelets and monocyte	Furuncles, necrotizing pneumonia
β-haemolysin	*agr, sar*A, *sae*	Hydrolysis of sphingomyelin of the plasmatic membrane of monocytes, erythrocytes, neutrophils and lymphocytes; make cells susceptible to other lytic agents	-
γ-haemolysin	*agr, sar*A	Induce lysis on erythrocytes and	-

Virulence factors	**Positive regulators**	**Role In disease**	**Proposed associated disease**
		leukocytes	
ε-cytotoxin	n.d	Inhibits keratinocyte proliferation; prevents re-epithelialisation	-
δ-haemolysin	*agr*	Lysis human erythrocytes, neutrophils and other mammalian cells	-
Cytotoxins			
Leucocidin family			
Leukocidins E/D and M/F-PV	*agr, sar*A	Induce lysis on leukocytes	-
Panton Valentine Leukocidin (PVL)	*agr, sar*A	Induce lysis on leukocytes	Skin and soft tissue infections, CA-MRSA infections
Miscellaneous proteins			
Staphylococcal complement inhibitor (SCIN)	n.d	Inhibit complement activation	-
Extracellular fibrinogen binding protein (Efb)	n.d	Inhibit complement activation	-
Chemotaxis inhibitory protein of *S. aureus* (CHIPS)	n.d	Inhibit chemotaxis and activation of neutrophils	-
Formyl peptide receptor-like 1 inhibitory protein (FLIPr)	n.d	Inhibit chemotaxis of neutrophils	-
Extracellular adherence protein (Eap)	n.d	Inhibit neutrophil migration	-

n.d= not determined; (-)=unknown

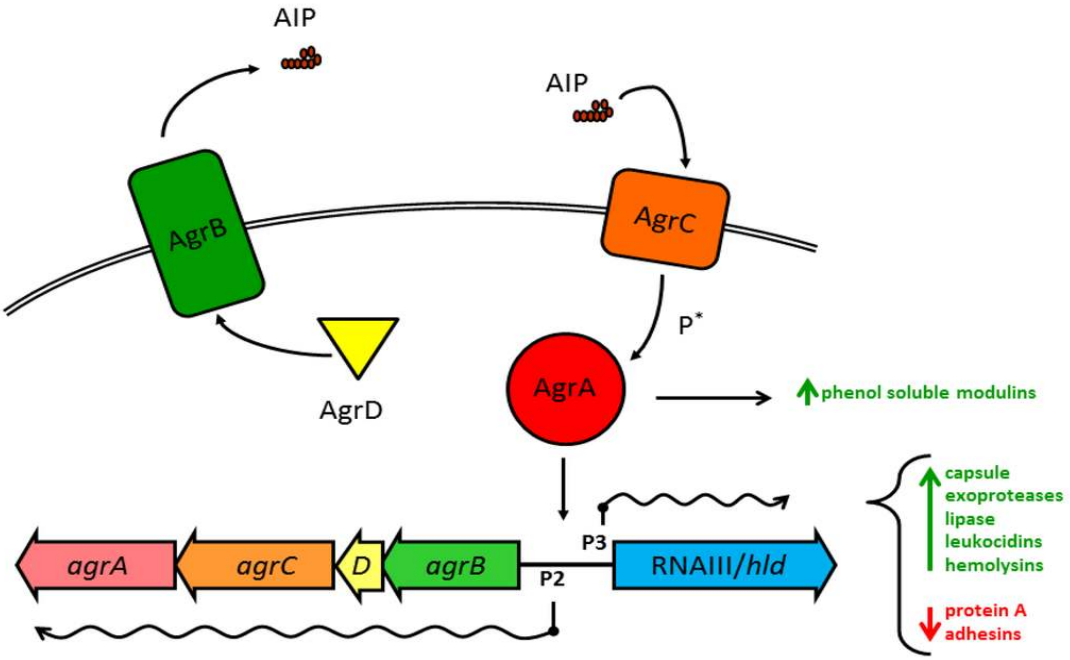

Fig. 1. The structure and function of the *agr* operon in *S. aureus* [16].

AgrB is a multifunctional endopeptidase and chaperone protein, and it has been suggested that AgrB is also involved in the export of AIP. AgrD is a propeptide processed by AgrB into the small thiolactone AIP. AgrC is the integral membrane sensor part of a two-component regulatory system. AgrA is the transcription factor response regulator companion to AgrC, and acts on the divergent P2/P3 promoter to upregulate agr and RNAIII expression, in addition to several other transcriptional targets. The regulatory RNA molecule RNAIII acts on numerous gene transcripts to modulate gene expression through post-transcriptional control.

3.3.2 Superantigens

Secreted factors of *S. aureus* which are classified under superantigens include; Toxic Shock Syndrome Toxin-1 (TSST-1), Staphylococcal Enterotoxins (SEs) and Enterotoxin-like proteins (SE-ls) and exfoliative or epidermolytic toxin and function potentially to activate T cells and antigen presenting cells such as dendritic cells or macrophages [24,25]. TSST-1 produced by *S. aureus* is responsible for all forms of menstrual TSS and half cases of all non-menstrual TSS. Staphylococcal enterotoxin B (SEB) has also been identified as the second leading cause of *S. aureus* TSS [29]. However, insufficient quantity or absence of neutralizing antibodies is also considered a risk factor for TSS [25]. Interestingly, a report by Salgado-Pabón et al., has demonstrated the role of superantigens in the establishment of sepsis, kidney infections and infective endocarditis [30].

SEs/SE-ls produced in food stuffs that have been improperly handled is a risk for food poisoning due to the contamination with *S. aureus*. SEs unlike SE-ls can also induce vomiting and diarrhoea. Of the many types of SE produced by *S. aureus*, SEA has been described as the commonest cause of *Staphylococcal* food poisoning globally [31]. Nevertheless, SEH has also been shown to be associated with staphylococcal food poisoning [32]. Some strains of *S. aureus* also produce two major types of exfoliative toxin; exfoliatoxin A (ETA) and ETB which have proteolytic activity and capable of causing exfoliation of the skin [33].

3.3.3 Cytotoxins

S. aureus secretes toxins which primarily target the cytoplasmic membrane of the host cell and are termed cytolytic toxins or cytotoxins due to their ability to form pores in the host membrane with subsequent leakage and lysis of the host cell. These toxins include; α-haemolysin or toxin, *β*-haemolysin, γ-haemolysin, leukocidins including Panton Valentine Leukocidin (Table 1) [34].

α-haemolysin is probably the best-studied cytolysin of *S. aureus*, with a broad spectrum of activities against many cell types such as leukocytes, erythrocytes, epithelial cells, platelets and fibroblasts [35,36]. Significantly, pore formation by α-haemolysin may be either receptor-dependent or receptor-independent depending on the concentrations of the toxins produced [34]. However, α-haemolysin is haemolytic, neurotoxic, dermonecrotic and lethal *in vivo*. Its dermonecrotic nature has a major role in the formation of furuncles [37]. Strikingly, pro-inflammatory response induced by *Staphylococcal* α-haemolysin from epithelial cells can promote the penetration of TSST-1 across vaginal mucosa in an *ex vivo* model [38].

β-haemolysin recognised as a neutral sphingomyelinase is one of the toxins with enzymatic activity [25] produced by *S. aureus* to hydrolyse sphingomyelin present in different cell types including monocytes, erythrocytes, lymphocytes and neutrophils [5]. Production of endothelial IL-8 is also highly suppressed due to *β*-haemolysin thus inhibiting transmigration of neutrophils [39]. In addition, *β*-haemolysin acts as a catalyst in *Staphylococcal* biofilm formation by directing the synthesis of nucleoprotein matrix [40]. γ-haemolysin and members of leucocidin family including PVL are known as bi-component toxins [25]. γ-haemolysins are divided into two namely; HlgAB and HlgCD. Intriguingly, γ-haemolysin AB (HlgAB) contributes predominantly to *S. aureus* bacteraemia infection in a CCR2-dependent manner [6].

Delta toxin (δ-haemolysin) is a surfactant that destabilizes cytoplasmic membranes with the ability to dissolve membranes of target cells at higher concentrations [41]. Interestingly, δ-haemolysin and phenol soluble modulins (PSMs) are responsible for CA-MRSA strains with virulence attributes. δ and α haemolysin gene expression and high prevalence have been reported to be associated with ready to eat foods in Malaysia [42]. A recent investigation by Merriman et al*.* has identified a novel exotoxin of *S. aureus* with no homologue to the existing exotoxins termed ε-cytotoxin from surgical site infection with potent biological effects on rabbit skin and keratinocytes. Importantly, this novel toxin can prevent the proliferation of keratinocytes and re-epithelialization [43].

Combined activity of different virulence factors of *S. aureus* has been described recently. Following a mouse model of bacterial endophthalmitis carried out by Kumar and Kumar, *S. aureus* virulence factors such as lipoteichoic acid, peptidoglycan, toxic-shock syndrome toxin 1, α-toxin and SpA were shown to induce significant amounts of inflammatory mediators like IL-6, IL-1β, TNF-α and MIP2. More so, loss of retinal function and intraoccular inflammation were strongly apparent in mice following injection with SpA and α-toxin. This unequivocally demonstrates a powerful link between these virulence factors and disease [44].

Leukocidins are so called because they kill host leucocytes. PVL is commonly associated with skin and soft tissue infection. Evidence of PVL association with deep-seated follicular infection arises from the report by Yamasaki *et al.*, who detected *pvl* genes in 16/40 strains isolated from furuncles demonstrating some involvement of PVL in the formation of furuncles with exacerbated erythema [45,46]. PVL of *S. aureus* can also cause necrotizing pneumonia both in healthy and in young children and is associated with community acquired methicillin resistant *S. aureus* (CA-MRSA) infection [47]. Nevertheless, hospital acquired MRSA (HA-MRSA) and methicillin-sensitive *S. aureus* (MSSA) strains have been found harbouring PVL [48].

3.4 Biofilm Formation and Small Colony Variants

Formation of biofilm confers survival advantage to *S. aureus* in otherwise harsh or adverse conditions. Biofilm is made up of exopolysaccharide with components such as poly-*β*(1-6)-Nacetylglucosamine shown to be regulated by *ica* gene locus, including protein components [14]. However, *S. aureus* can still produce biofilm in the absence of *ica* gene. Strikingly, SpA, surface proteins such as SasG, SasC, FnBpA and FnBpB can also promote biofilm formation [24]. Persistent infection is apparent with biofilm formation due to efficient immune evasion and resistance to antibiotic treatments.

Some strains of *S. aureus* produce small colony variants (SCV) with adaptable ability to resist the action of antibiotics [5]. These strains are isolated from patients with conditions like cystic fibrosis chronic or device-associated infections [49,50] and recently from osteomyelitis patients [51]. SCVs are metabolically inactive due to loss in the function of electron transport chain. ATP production by electron transport chain is vital to the synthesis of cell wall proteins and transport of

amino acids. Therefore, SCV formation by *S. aureus* is a major hurdle regarding management of the disease [5].

3.5 Overcoming Iron Depletion

Iron (Fe) is an essential nutrient for bacterial growth and multiplication and its limitation is an important barrier to *S. aureus* proliferation in the host because it is frequently being sequestered by host proteins. However, *S. aureus* has an effective and efficient mechanism for iron acquisition required for its survival and replication once inside the host [15]. One such Fe sequestration mechanism is by the production of siderophores which has a strong binding affinity for Fe thus ensuring the availability of iron for survival and replication [52]. Staphyloferrin A (SA) and Staphyloferrin B (SB) are two main citrate based siderophores produced by *S. aureus* which are not recognised by host protein lipocalin. More so, *S. aureus* utilizes siderophores (xenosiderophores) synthesized by other bacteria as well as norepinephrine, a host derived hormone with iron-binding potential [52]. Ferric hydroxamate uptake (Fhu) and Staphylococcal siderophore transporter (Sst) up-regulate the importation of xenosiderophores and norepinephrine during Fe-depletion [52,53]. The iron-regulated surface determinant protein IsdH of *S. aureus* can also be expressed under iron-limitation and acts essentially to sequester haem from haemoglobin [54].

4. IMMUNE EVASION STRATEGIES OF *S. aureus*

A wealth of information abounds for the immune evasion mechanism of *S. aureus* which is associated with the ability of the organism to cause persistent infections [55]. This will be discussed under specific headings.

4.1 Evasion of Phagocytosis

Immunoglobulin G plays an important role in the opsonisation of pathogens mediating phagocytosis by binding of its Fc region to receptors (FcγR) present on professional phagocytes. *S. aureus* is equipped with a profusion of factors that interfere with such opsonisation prior to phagocytosis [45]. One such factor is SpA. (See Fig. 2.)

SpA encoded by *spa* gene is a cell surface molecule and a super antigen that binds preferentially to the Fc region of immunoglobulin (Ig) and to the Fab region of the V_H3- type B cell receptors (IgM) [56]. These binding prevent IgG-dependent opsonophagocytosis and cause death of B cell *in vitro* [57]. More so, the binding of SpA to the Fc region of IgG hinders the activation of classical complement pathway [45]. Interestingly, there is sustained evidence that wild type *S. aureus* with *spa* gene evades immune surveillance of the host whereas *spa* variants elicit protective adaptive immune responses against subsequent infection, thus demonstrating the importance of SpA in immune escape [56].

Another immunoglobulin binding protein known as Sbi protein binds to the Fc domain of IgG in a similar way as SpA and binds C3 complement protein. It has been undoubtedly revealed that Sbi is an immune evasion factor that not only impedes neutrophil-mediated opsonophagocytosis but also enhances survival of the organisms in whole human blood [58].

Intriguingly, apart from utilisation or usurpation of host proteases, *S. aureus* produces proteases that prevent opsonisation by directly degrading opsonins. The protein staphylokinase, a plasminogen activator mediates the degradation of IgG and C3b by converting host plasminogen to serine protease plasmin [59]. Although a fraction of complement protein, C3b gets deposited on *S. aureus*, the organism is capable of recruiting fibrinogen by the secretion of extracellular fibrinogen binding protein (Efb) which shields the surface bound opsonin [60]. Thus, Efb is a bi-functional C3b and fibrinogen binding protein of *S. aureus* that hinders complement and antibody mediated phagocytosis both in plasma and in human whole blood [60]. Interestingly, a secreted polysaccharide polymer of *S. aureus*, capsular polysaccharide (cap) potentially encases the bacteria rendering it inaccessible to receptors of complement on neutrophils [58]. Other assaults launched by *S. aureus* on complement involve the degradation of complement and blockade of opsonisation. This attack is mediated by *S. aureus* secreted proteases (Aureolysin, Staphopain A/B and V8) [61,62].

It is well established that complement activation through any of the three pathways ultimately yield C3b which is a ligand for Mac-1 receptor on phagocytic cells. Additionally, activation of complement results in the production of pro-inflammatory anaphylatoxins C3a and C5a that potentially activate macrophages to secrete cytokines. Together, these acts to ensure that *S.*

aureus is engulfed and infection is subsequently cleared, but, not surprisingly, *S. aureus* encodes a host of secreted and cell surface associated factors that can potentially inhibit complement activation [45].

A cell surface associated factor, collagen-binding MSCRAMM Cna of *S. aureus* inhibits the activation of classical pathway of complement and occludes the production of a functional C3 convertase by binding directly to C1q protein of the host. Other *S. aureus* molecules capable of inhibiting complement activation include SCIN, SdrE, Sbi, ClfA and CHIPS. In summary, avoidance of phagocytosis and complement escape enables the persistence of *S. aureus* during infection [63]. The functions are summarised in Table 2.

4.2 Intoxication of Host Cell

One of the strategies employed by *S. aureus* to escape host immune response is the secretion of both pore-forming and membrane damaging toxins that promote loss of cell membrane integrity and lysis of the cells affected. A class of toxins collectively termed leukocidins represents one of the many noxious proteins secreted by *S. aureus* to kill leukocyte by forming membrane β-barrel pores. They are composed of five bi-component leukocidins, LukAB, LukED, HlgAB, HlgCB and LukSF-PV; α-hemolysin (Hla) and phenol soluble modulins (PSMs) whose individual functions are summarized in Table 2 [45].

Notably, γ-haemolysin AB and CB produced by *S. aureus* can invade human and murine leucocytes and erythrocytes by exploiting specific chemokine receptor such as CXCR1, CXCR2, CCR2 and complement receptors C5L2 and C5aR respectively. However, murine neutrophils appear to be resistant [6]. Interestingly, LukAB and LukED can launch prolonged attack on macrophages by engagement of specific receptors CD11b and CCR5 respectively on macrophages [64,65].

It is worthy to mention that at cellular level, inflammasome activation and IL-1β production is due to pore formation by Hla or leukocidins. For example, a recent study has revealed that LukAB engagement with CD11b potentially activates NLRP3 containing caspase-1 and inflammasomes resulting in the production of pro-inflammatory cytokine IL-1β when acting on the cell surface. Fascinatingly, *S. aureus* can kill macrophages in a caspase-1 independent activation. Hence, multiple cell death mechanisms coupled with toxin-mediated cell assaults can ultimately render macrophages incapable of combating *S. aureus* infection [66].

Surprisingly, *S. aureus* has been reported to usurp host molecules to execute death of macrophages. Adenosine is a molecule secreted by the host in response to neutrophil extracellular trap (NET) [67]. This molecule possesses both anti-inflammatory and immunosuppressive properties capable of inhibiting platelet aggregation, bursting neutrophil superoxide, activating T cells, de-granulating neutrophils and releasing cytokines IL-1α and IL-10 [68]. *S. aureus* in a fascinating way amplifies the extracellular concentration of adenosine by the expression of adenosine synthetase AdsA which catalyses the production of toxic deoxyadenosine molecules [67]. This in turn triggers macrophage death through the activation of caspase-3 [69].

4.3 Evasion of Phagolysosomal Killing and Host Antimicrobial Proteins

Although *S. aureus* unlike other intracellular pathogens is incapable of perturbing phagosome maturation, a recent study has demonstrated that even when *S. aureus* is ultimately phagocytosed by macrophages, some bacteria will survive phagolysosomal killing due to reduced phagosome acidification, reduced activation of cathepsin D and failure of phagolysosomal maturation. Thus, macrophage lysis or death is due to intracellular replication of phagocytosed *S. aureus* resulting in bacterial escape [70,71]. Several secreted proteins of *S. aureus* (α-PSM, α-hemolysin, δ-toxins, or LukAB) have been reported to contribute to phagosome escape [72, 73,74,75].

For this opportunistic pathogen to successfully replicate inside phagocytic cells, it must be able to overcome synthesized host cell antimicrobial protein known as lysozyme which can degrade peptidoglycan glycosidic linkages [45]. Undoubtedly, *S. aureus* through acetyl-transferase OatA catalysed synthesis of acetylated peptidoglycan can become resistant to lysozyme attack [76]. Several other molecules of *S. aureus* mediating resistance to host antimicrobial proteins with their functions are summarized in Table 2.

Table 2. *S. aureus* immune evasion factors and their immune evasion Strategy [45]

Evasion strategy	Factor	Functions
Avoidance of phagocytosis	***Opsonin interference***	
	Protein A and Sbi	Bind Fc region of IgG, occlude Fc region to prevent FcγR and C1q recognition
	Staphylokinase	Bacterial plasminogen activator; activates serine protease plasmin to promote degradation of complement and Ig
	Aureolysin	Secreted metalloprotease; degrades complement to prevent C3b opsonisation
	Staphopain A/B	Secreted cysteine proteases; degrade complement thereby preventing opsonisation
	V8	Secreted serine protease; degrades complement components and IgG
	Efb	Secreted bi-functional fibrinogen and C3b binding protein; Masks C3b on bacterial surface by promoting formation of a fibrinogen "shield"
	Capsule	Secreted polysaccharide polymer that encases polysaccharide the bacteria; shields bacterial surface from opsonins
Host cell Intoxication	***Leucocidins***	
	LukAB	Pore forming toxins, S subunit LukA engages CD11b subunit of Mac-1; targets macrophages and neutrophils of human origin
	LukED	Pore forming toxin; S subunit LukE engages CCR5, XCR1/2, and DARC; targets macrophages, neutrophils, T-lymphocytes and red blood cells from many animal species
	LukSF-PV	Pore forming toxin; S subunit LukS engages complement receptors C5aR and C5aR2 of human and rabbit origin, targets neutrophils, monocytes and macrophages
	HlgAB	Pore forming toxin; S subunit HlgA engages CXCR1, CXCR2 and CCR2; targets neutrophils, monocytes and macrophages of human and murine origin with the exception that murine neutrophils are resistant to lysis
	HlgCB	Pore forming toxin, S subunit HlgC engages C5aR1 and C5aR2 to target neutrophils, monocytes and macrophages; demonstrates broad species specificity excluding mouse
	α-hemolysisn	Pore forming toxin; Utilizes host protein ADAM10 as receptor; Targets many cell types including macrophages of many origins including mice and humans
	α-PSMs	Small amphipathic peptides; broad lytic activity *in vitro*; may function as intracellular lysins

Evasion strategy	Factor	Functions
	Complement inhibition	
	Can	A collagen binding surface expressed protein; binds complement protein C1q; blocks C1q-dependent complement activation
	SCIN	Small secreted molecule; binds directly C3 convertase required for processing of C3 to C3a and C3b to inhibit convertase function
	Sbi	A cell wall associated and secreted protein; can recruit human plasminogen that is converted to plasmin to degrade C3; Can bind C3 products with the complement regulatory factor H to promote Factor I cleavage of C3b to inactive iC3b
	SdrE	Cell surface associated protein; binds Factor H recruiting it to the bacterial surface where it can act as a co-factor with Factor I to promote cleavage of C3b to iC3b
	ClfA	Cell-wall associated fibrinogen binding protein; binds Factor I that mediates cleavage of C3b to its inactive form iC3b
	CHIPS	Blocks the neutrophil receptor for the major chemo-attractants
Evasion of macrophage anti-microbial defences	***Bacterial cell surface modification***	
	OatA	Acetylates peptidoglycan to confer resistance to lysozyme
	DltABCD	Catalyze the incorporation of D-alanine into wall teichoic acids to reduce the negative charge of the bacterial cell surface; decreases binding of cationic antimicrobial peptides
	MprF	Catalyzes modification of negatively charged cytoplasmic membrane lipids by incorporating lysine residues to make the membrane less anionic; decreases binding of cationic antimicrobial peptides
	Eap, EapH1, EapH2	Secreted proteins that selectively inhibit the serine proteases neutrophil elastase, proteinase 3, and cathepsin G that are expressed by neutrophils; may function to inhibit serine proteases expressed by macrophages
	Resistance to ROS and RNS	
	SodA and SodM	Superoxide dismutases that detoxify ROS by catalyzing the conversion of superoxide into hydrogen peroxide
	KatA	A catalase that detoxifies hydrogen peroxide by catalyzing its breakdown into water and oxygen

Evasion strategy	Factor	Functions
	Msr	Methionine sulfoxide reductase; catalyzes the repair of methionine residues damaged by oxidation.
	Staphyloxanthin	A carotenoid expressed by *S. aureus* giving the cocci its golden pigmentation but can also, because of its molecular structure, act as an antioxidant; May also promote resistance to antimicrobial peptides as staphyloxanthin production decreases membrane fluidity
	Ldh1	A *S. aureus* specific lactate dehydrogenase that catalyzes the reduction of pyruvate to L-lactate with the concomitant oxidation of NADH to NAD+; helps maintain cellular redox balance in cells that are unable to respire because of nitric oxide mediated damage to electron transport chain proteins
	Hmp	Flavohemoprotein that scavenges NO to minimize damage to other *S. aureus* cellular components; up-regulated in response to nitrosative stress

Abbreviations: DARC, Duffy antigen receptor of chemokines; CXCR, chemokine receptor; SCIN, staphylococcal inhibitor of complement; Sbi, Staphylococcal binding of IgG; IgG; gamma immunoglobulin; SOD; superoxide dismutase; ROS; reactive oxygen species; RNS, reactive nitrogen species.

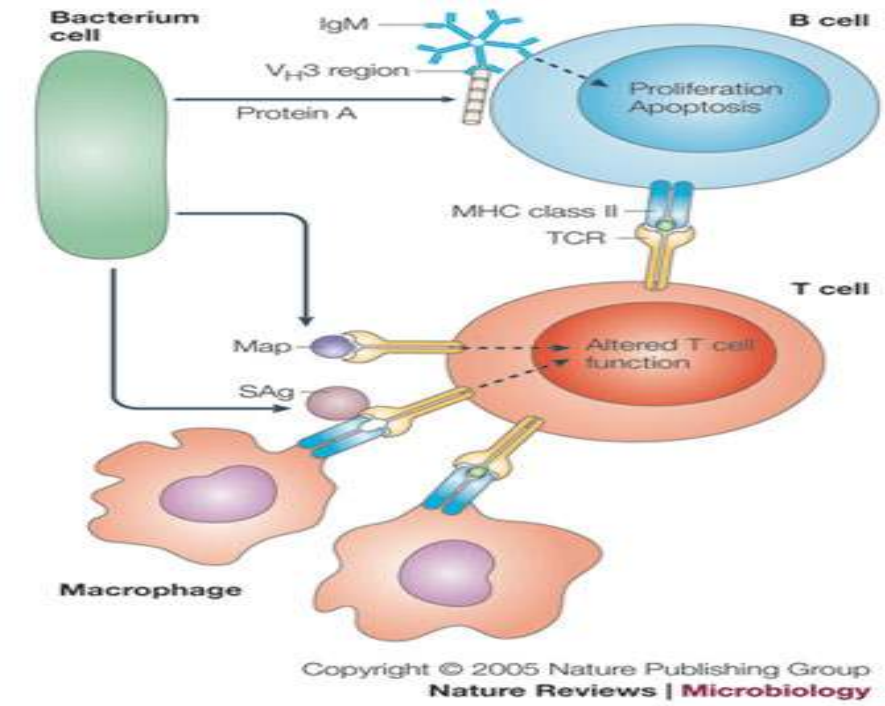

Fig. 2. Mechanisms of immunosuppression mediated by *S. aureus* [85].
This figure illustrates examples of immunomodulatory molecules used by S. aureus to alter the host immune response, including the superantigens (sAgs) enterotoxins and toxic shock syndrome toxin-1 that bind the MHC class II receptor to T-cell receptors; protein A, which binds immunoglobulin M (IgM) VH3 on B cells; and the MHC class II analogue protein Map, which binds the T-cell receptor (TCR)

4.4 Evasion of Oxidative and Nitrosative Killing

To overcome oxidative killing by reactive oxygen species, *S. aureus* possesses genes, *sod*A and *sod*M encoding superoxide dismutase that can potentially convert superoxide (O_2^-) to hydrogen peroxide (H_20_2). The H_20_2 can then be converted to H_20 and 0_2 by catalase enzyme encoded by *kat*A gene of *S. aureus* [77,78]. Also production of carotenoid pigment (staphyloxanthins) by *S. aureus* plays an important role in its resistance to killing by reactive oxygen species and promotes resistance to attack by antimicrobial peptides [79, 80]. Nevertheless, *S. aureus* may still be prone to reactive oxygen species (ROS) damage but surprisingly, this organism encodes genes encoding methionine sulfoxide reductases that can repair oxidized methionine molecules in proteins [81].

S. aureus that are phagocytosed must also demonstrate the ability to withstand the toxifying effects of Nitric oxide (NO^-) dependent killing [45]. To evade the killing effect of NO^- *S. aureus* carry *hmp* gene regulated by SsrAB which encodes flavohemoglobin that functions basically to scavenge Nitric oxide (NO^-) [82,83]. Interestingly, *S. aureus* can also metabolically adapt to nitrosative stress by encoding lactate dehydrogenase Ldhl induced mainly in the presence of Nitric oxide. Significantly, maintenance of redox homeostasis during oxidative stress is mediated via the production of L- lactate [84].

5. ANTIBIOTIC RESISTANCE MECHANISMS OF *S. aureus*

5.1 Brief History /Evolution of Resistant Strains

Prior to the development of penicillin to treat *S. aureus* infections in the early 1940s, death toll due to infections caused by *S. aureus* was almost about 80% [86]. With the production of penicillin, the medical community thought they have won the battle against infections caused by *S. aureus.* In 1942, just 2 years after penicillin introduction, a *S. aureus* strain resistant to penicillin was first isolated in the hospital and subsequently in the community. From 1960 to 2007, almost 80% of all strains of *S. aureus* were resistant to penicillin [87]. As a result of this, methicillin was manufactured to combat *S. aureus* infection and surprisingly, after two years strains with resistance to methicillin were isolated which were largely attributed to the acquisition of *mec*A gene [8]. Since then, various MRSA strains have been isolated in several hospitals worldwide and these stains are regarded as hospital acquired MRSA (HA-MRSA). However, these resistant strains widely known as community-associated MRSA (CA-MRSA) were later found circulating in the community from the late 1990s [88].

5.2 Resistance Mechanisms in *S. aureus*

Bacteria tend to employ four general antibiotic resistance mechanisms such as drug target modification, limiting drug uptake, drug inactivation and active efflux of the drug [89]. Undoubtedly, *S. aureus* has evolved over the years the capacity to resist the action of almost all antimicrobials available including resistance to antiseptics and disinfectants [90]. Interestingly, *S. aureus* has intrinsic resistance genes and is endowed with resistance genes acquired from other bacteria through plasmid, transposon or bacteriophage, that give it the great resistance adaptability. The most common *S. aureus* resistance is mediated by *mec*A gene element, found in the mobile genetic element, the SCC*mec.* Hence, *S. aureus* employs one or all the four mechanisms of resistance to occlude the action of antibiotics depending on the type of drug (Table 3) [89].

5.3 Resistance to β-lactams

Beta-lactam antibiotics are a group of antibiotics that function essentially to inhibit bacterial cell wall synthesis by binding specifically to its receptor, the penicillin-binding-proteins (PBPs) or transpeptidases, an enzyme involved in the synthesis of peptidoglycan cell wall in bacteria. This binding automatically impedes the synthesis of a functional cell predisposing the organism to further attack with subsequent lysis [89]. To overcome the attack by *β*-lactam group of antibiotics, *S. aureus* acquired *bla*Z gene carried by a plasmid encoding for the synthesis of *β*-lactamase enzymes. This enzyme otherwise called penicillinases hydrolyses the peptide bond of the *β*-lactam ring structure so that the drug cannot bind to PBPs. However, some *β*-lactam drugs are relatively resistant to these attacks for example cephalosporins [24].

Drugs with modification of *β*-lactam structure such as methicillin, ampicillin and oxacillin were developed to overcome such attack by *β*-

lactamase enzymes. Interestingly, *S. aureus* was found with a modified or altered PBP termed PBP"a or PBP2' which had a lower affinity for methicillin. Methicillin resistance is mediated by the acquisition of *mec*A gene through horizontal gene transfer of the mobile genetic element, SCCmec carrying *mec*A gene [89]. The *mec*A gene encodes a new PBP known as PBP2' or PBP2a that confers resistance to methicillin with cross resistance to many other *β*-lactam drugs [9]. However, the new PBP can still catalyse the synthesis of peptidoglycan.

The SCC*mec* carries the *mec* gene elements; *mec*A and its regulatory genes *mec*I and *mec*RI and the cassette chromosome recombinase complex *ccr* [91]. Presently, about 11 variants of SCC*mec* have been described based on the *mec* and *ccr* gene combination complexes [92]. However, SCC*mec* also contains insertion elements IS257, IS431, transposons, Tn554 and plasmids PUB100. Interestingly, SCC*mec* has been considered a resistance island since it encodes genes conferring resistance to other antibiotics such as tetracycline (*tet*), erythromycin (*erm*A), and tobramycin (*aad*D), in addition to genes for heavy metal detoxification [9,89].

5.4 Resistance to Other Cell Wall Antibiotics

With the circulation of strains with resistance to methicillin, the use of *β*-lactams gradually diminished. This led to the search for newer drugs to combat *S. aureus* infection. Vancomycin, a glycopeptide was one of such drugs introduced in the 1958 with significant use in mid-1980's and with activity against *S. aureus* cell wall synthesis [93]. As promising as vancomycin was after years of its discovery or development, two types of vancomycin resistance began to emerge. The first vancomycin resistance *S. aureus* (VISA) emerged in Japan (1996) after 40 years of its discovery, whose resistance is caused by multiple gene mutations for example *walkR, vraSR*, and *rpoB* genes [94,95]. In 2002, vancomycin-resistant *S. aureus* (VRSA) strains emerged with resistance mediated by *van*A gene acquisition from vancomycin-resistant enterococci (VRE) [96]. The *vanA* gene is carried by a plasmid-borne Tn1546 element and is acquired by *S. aureus* via conjugation [97]. Expression of *van*A gene results in modification of the precursors of peptidoglycan, decreasing vancomycin binding affinity [98]. Though only few strains have been reported, scientists worry that like MRSA, VRSA might become a serious medical issue in the coming years [89].

Daptomycin, a lipopeptide antibiotic was also developed in 2003 for the treatment of MRSA infections and again, strains with decreased susceptibility to daptomycin were later isolated [89,93,99]. Resistance to daptomycin is due to mutation in the *mpr*F gene that encodes an enzyme, lysylphosphatidylglycerol synthetase responsible for changes in cell membrane charge from negatively charged to positively charged thus decreasing the binding affinity of daptomycin to *S. aureus* cell membrane [100].

5.5 Resistance to Protein Synthesis Inhibitors, Nucleic Acid Synthesis and Metabolic Pathway Inhibitors

Antibiotics that inhibit the synthesis of protein function essentially by binding either to the 30S or 50S of the ribosomal subunit of the bacteria. Aminoglycosides and tetracycline are two classes of protein inhibitors that bind to 30S ribosomal subunit. Resistance to gentamicin and tobramycin, the two main aminoglycosides for the treatment of *S. aureus* emerged in the 1960's. *S. aureus* was later found with genes *acc, ant, aph* obtained from a plasmid encoding for aminoglycoside-modifying enzymes (AME) that basically modifies the drugs leading to decreased drug binding to 30Ssubunit [101]. *S. aureus* also developed resistance to tetracycline group of antibiotics by acquiring *tet*K gene from a plasmid responsible for active drug efflux from the organism. In addition, resistance is mediated by the production of ribosomal protection protein (RPP) and by acquisition of *tet*M gene from a plasmid that blocks the binding of the drug [102].

Resistance to drugs that bind to 50S ribosomal subunit by *S. aureus* is not an exception. Chloramphenicol resistance is due to acquisition of chloramphenicol acetyltransferase (*cat*) gene. This gene acetylates the drug leading to a reduced drug binding affinity to 50S subunit [103]. Clindamycin, erythromycin and quinupristin/dalfopristin is via methylation of the ribosome (See Table 3). Linezolid is a new antibiotic in the class oxazolidinone developed in 2000, with potent activity against protein synthesis. Surprisingly, in 2001 *S. aureus* strains with resistance to linezolid emerged due to ribosomal RNA (*rrn* gene) mutation and ribosomal RNA (*cfr* gene) methylation [104,105]. Interestingly, *cfr* gene has been proven to mediate resistance to other antibiotics such as

Table 3. Antimicrobial resistance genes and mechanisms in *Staphylococcus aureus* [89]

Antimicrobial agents	Mechanisms of resistance	Genetic basis
β-lactams		
Penicillins	β-lactamases-inactivate drugs	*blasZ*- plasmid
Cephalosporins		
Monobactams	Altered penicillin-binding protein	*mecA*- acquired?
Carbapenems	(PBP2a) targets	
Glycopeptides	VISA- cell wall thickens	Multiple gene mutation
Vancomycin	VRSA- modified target	*vanA*- from enterococci
Lipopeptides	Change in cell membrane charge-	*mprF* gene mutation
Daptomycin	Decreased drug binding	
Protein synthesis inhibitors		
Aminoglycosides	Aminoglycoside modifying	*aac*- plasmid
Amikacin	enzymes-	*ant*- plasmid
Gentamycin	Modify target	*aph*-plasmid
Tobramycin		
Tetracyclines		
Tetracycline	Active efflux	*tetK*- plasmid
Minocycline	Ribosomal protection-	*tetM*- plasmid
Tigecycline	Competitive binding	
Chloramphenicol	Acetylation of drug-inactivation	*cat*- plasmid
Macrolides and		*ermA, ermB, ermC*- plasmid
Lincosamides		
Erythromycin	Methylation ribosome-	
Clindamycin	Decreased binding	*rrn*
Oxazolidinones	Mutation of ribosomes	*cfr*- plasmid
Linezolid	Methylation of ribosome	
Streptogramins		
Quinupristin/Dalfopristin	Methylation of ribosomes	*ermA, ermB, ermC*
Fluoroquinolones/Nucleic acid inhibitors		
Ciprofloxacin	Modified target- gyrase	*gyrA*
Norfloxacin	Modified target- topoisomerase	*grlA*
Gatifloxacin	IV Active efflux	*norA*
Moxifloxacin		
Metabolic Pathway Inhibitors		
Trimethoprim/Sulfamethoxazole	Target enzymes modification	TMP- *dhfr* SMZ- *dhps*

SMZ- sulfamethoxazole, TMP- trimethoprim, VISA- vancomycin-intermediate Staphylococcus aureus, VRSA- vancomycin-resistant Staphylococcus aureus

clindamycin, chloramphenicol and streptogramins [103,106]. Furthermore, *S. aureus* has become resistant to drugs that inhibit nucleic acid synthesis and metabolic pathway inhibitors. In summary, *S. aureus* can be regarded as a multi-drug resistant organism with survival advantage in the presence of these drugs [107]. See Table 3.

6. SUMMARY

It is apparently clear that *S. aureus* encodes an array of virulence factors that enables it to cause a massive range of diseases. These factors strongly correlate with the organisms' ability to supress the host innate and adaptive immune responses and are indispensable *in vivo* in the pathogenesis of *S. aureus.* Initially, *S. aureus* infections were only restricted to infections associated with prolonged hospital stay but now the organism as evolved rapidly causing even more serious infections in the community and in immunocompetent individuals. The organism is also highly adaptable to live on different ecological niches. However, a greater percentage of *S. aureus* strains are now

resistance to methicillin with such strains circulating in the hospital (HA-MRSA), community (CA-MRSA) and in livestock (LA-MRSA). *S. aureus* has also developed resistance to antibiotic of last resort (vancomycin) and newer antimicrobials like linezolid and daptomycin due to its efficient mechanism of resistance gene acquisition from other bacteria. There is, therefore, a need for the development of vaccines and effective newer antimicrobials with a high barrier to resistance to circumvent this alarming nature of *S. aureus* evolutionary trend if infections caused by this organism is to be effectively managed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Weidenmaier C, Goerke C, Wolz C. *Staphylococcus aureus* determinants for nasal colonisation. Trends Microbiol. 2012;20(5):243-250.
   DOI: 10.1016/j.tim.2012.03.004
2. Kao K, Chen C, Hu H, Chang H, Huang C, Huang Y. Risk factors of methicillin-resistant *Staphylococcus aureus* infection and correlation with nasal colonization based on molecular genotyping in medical intensive care units. Medicine. 2015; 94(28):e1100.
3. Saavedra-Lozano J, Mejías A, Ahmad N, Peromingo E, Ardura MI, Guillen S, et al. Changing trends in acute osteomyelitis in children: Impact of methicillin resistant *Staphylococcus aureus* infections. J Pediatr Orthop. 2015;28:569-575.
4. Van Hal SJ, Jensen SO, Vaska VL, Espedido BA, Paterson DL, Gosbell IB. Predictors of mortality in *Staphylococcus aureus* bacteremia. Clin Microbiol Rev. 2012;25:362-386.
5. Costa AR, Deivid, WF, Batistão RM, Ribas AM, Sousa MO, Botelho CM. *Staphylococcus aureus* virulence factors and disease. Formatex. 2013;702-710.
6. Spaan AN, Vrieling M, Wallet P, Badiou C, Reyes-Robles T, Ohneck EA, et al. The staphylococcal toxins γ-haemolysin AB and CB differentially target phagocytes by employing specific chemokine receptors. Nat Commun. 2014;11(5):5438.
7. Cheung AS, Aboltins CA, Daffy JR, Stanley PA. Necrotising pneumonia due to Panton-Valentine leukocidin-positive methicillin-sensitive *Staphylococcus aureus*. Med J Aust. 2008;188(6):373.
8. Jevons MP. Celbenin-resistant staphylococci. Br Med. 1961;1:124-125.
9. Sá Figueiredo AM, Ferreira FA. The multifaceted resources and microevolution of the successful human and animal pathogen methicillin-resistant *Staphylococcus aureus.* Mem Inst Oswaldo Cruz. 2014;3:265-278.
10. Coates T, Bax R, Coates A. Nasal decolonization of *Staphylococcus aureus* with mupirocin: Strengths, weaknesses and future prospects. J Antimicrob Chemother. 2009;64:9-15.
11. Marshall C, McBryde E. The role of *Staphylococcus aureus* carriage in the pathogenesis of bloodstream infection. BMC Res Notes. 2014;7:428.
12. Speziale P, Pietrocola G, Rindi S, Provenzano M, Provenza G, Di Poto A, et al. Structural and functional role of *Staphylococcus aureus* surface components recognizing adhesive matrix molecules of the host. Future Microbiol. 2009;4(10):1337-1352.
13. Syed AK, Ghosh S, Love NG, Boles BR. Triclosan promotes *Staphylococcus aureus* nasal colonization. M. Bio. 2014;5(2): e01015-13.
14. O'Gara JP. *ica* and beyond: Biofilm mechanisms and regulation in *Staphylococcus epidermidis* and *Staphylococcus aureus.* FEMS Microbiol Lett. 2007;270:179-88.
15. Ding Y, Liu X, Chen F, Di H, Xu, B, Zhou L, et al. Metabolic sensor governing bacterial virulence in *Staphylococcus aureus.* PNAS. 2014;E4981-E4990.
16. Gray B, Hall P, Gresham H. Targeting agr- and agr-like quorum sensing systems for development of common therapeutics to treat multiple Gram-positive bacterial infections. Sensors. 2013;13(4):5130-5166.
17. Novick RP, Geisinger E. Quorum sensing in staphylococci. Annu Rev Genet. 2008;42:541–564.
18. Oogai Y, Matsuo M, Hashimoto M, Kato F, Sugai M, Komatsuzawa H. Expression of virulence factors by *Staphylococcus aureus* grown in serum. Appl Environ Microbiol. 2011;22:8097-8105.

19. Chabelskaya S, Bordeau V, Felden B. Dual RNA regulatory control of a virulence factor. Nucleic Acids Res. 2014;42:4847-58.
20. Liang X, Yu C, Sun J, Liu H, Landwehr C, Holmes D. and Ji Y. Inactivation of a two-Component signal transduction system, SaeRS, eliminates adherence and attenuates virulence of *Staphylococcus aureus*. Infect Immun. 2006;8:4655-4665.
21. Ziebandt AK, Becher D, Ohlsen K, Hacker J, Hecker M, Engelmann S. The influence of *agr* and óB in growth phase dependent regulation of virulence factors in *Staphylococcus aureus.* Proteomics. 2004; 4(10): 3034-3047.
22. Bronner S, Monteil H, Prèvost G. Regulation of virulence determinants in *Staphylococcus aureus*: Complexity and applications. FEMS Microbiol Rev. 2004;28:183-200.
23. O'Riordan K, Lee JC. *Staphylococcus aureus* capsular polysaccharides. Clin Microbiol. Rev. 2004;17(1):218–234.
24. Miljković-Selimović B, Dinić M, Orlović J, Babić T. *Staphylococcus aureus*: Immunopathogenesis and human immunity. Acta facultatis medicae Naissensis. 2015;4:243-257.
25. Lin Y, Peterson ML. New insights into the prevention infections and toxic shock syndrome staphylococcal. Expert Rev Clin Pharmacol. 2010;3(6):753-767.
26. Park B, Nizet V, Liu GY. Role of *Staphylococcus aureus* catalase in niche competition against *Streptococcus pneumoniae*. J Bacteriol. 2008;90:2275-8.
27. Hu C, Xiong N, Zhang Y, Rayner S, Chen S. Functional characterization of lipase in the pathogenesis *of Staphylococcus aureus*. Biochem Biophys Res Commun 2012;419:617-620.
28. White MJ, Boyd JM, Horswill AR, Nauseef WM. Phosphatidylinositol-specific phospholipase C contributes to survival of *Staphylococcus aureus* USA300 in human blood and neutrophils. Infect Immun. 2014;82:1559-71.
29. Venkataraman R, Sharma S, Talavera F. Toxic shock syndrome: Background, Pathophysiology, Epidemiology; 2015. Available:http://emedicine.medscape.com/article/169177-overview
30. Salgado-Pabón W, Breshears L, Spaulding AR, Merriman JA, Stach CS, Horswill AR, et al. Superantigens are critical for *Staphylococcus aureus* infective endocarditis, sepsis, and acute kidney injury. Mbio. 2013;4(4):e00494-13.
31. Argudín MA, Mendoza MC, Rodicio MR. Food poisoning and *Staphylococcus aureus* enterotoxins*.* Toxins 2010;2:1751-1773.
32. Ikeda T, Tamate N, Yamaguchi K, Makino S. Mass outbreak of food poisoning disease caused by small amounts of staphylococcal enterotoxins A and H. Appl Environ Microbiol. 2005;71:2793-2795.
33. Bukowski M, Wladyka B, Dubin G. Exfoliative Toxins of *Staphylococcus aureus.* Toxins. 2010;2:1148-1165.
34. Otto M. *Staphylococcus aureus* toxins. Curr Opin Microbiol. 2014;17:32-37.
35. Berube BJ, Bubeck WJ. *Staphylococcus aureus* alpha-toxin: nearly a century of intrigue. Toxins. 2013;5:1140–1166.
36. Menestrina G, Dalla SM, Comai M, Coraiola M, Viero G, Werner S, et al. Ion channels and bacterial infection: the case of β-barrel pore-forming protein toxins of *Staphylococcus aureus.* FEBS Lett. 2003; 552(1):54-60.
37. Dingles MM, Orwin PM Schlievert, PM. Exotoxins of *Staphylococcus aureus*. Clin Microbiol Rev. 2000;13(1):16-34.
38. Brosnahan AJ, Mantz MJ, Squier CA, Peterson ML, Schlievert PM. Cytolysins augment superantigen penetration of stratified mucosa. J Immunol. 2009;182(4): 2364-2373.
39. Tajima A, Iwase T, Shinji H, Seki K, Mizunoe Y. Inhibition of endothelial interleukin-8 production and neutrophil transmigration by *Staphylococcus aureus* β-hemolysin. Infect Immun. 2009;77(1): 327-334.
40. Huseby M, Kruse AC, Digre J, Kohler PL, Vocke JA, Mannc EE, et al. Beta toxin catalyzes formation of nucleoprotein matrix in staphylococcal biofilms. Proc Natl Acad Sci USA. 2010;107(32):14407-14412.
41. Vandenesch F, Lina G. Henry T. *Staphylococcus aureus* hemolysins, bi-component leukocidins, and cytolytic peptides: A redundant arsenal of membrane-damaging virulence Factors? Front Cell Infect Microbiol. 2012;16:2-12.
42. Puah SM, Chua KH, Tan JI. Virulence factors and antibiotic susceptibility of *Staphylococcus aureus* isolates in ready-to-eat foods: Detection of *S. aureus* contamination and a high prevalence of

virulence genes. Int J Environ Res Public Health. 2016;13:199.
DOI: 10.3390/ijerph13020199.

43. Merriman JA, Klingelhutz AJ, Diekema DJ, Leung DY, Schlievert PM. Novel *Staphylococcus aureus* secreted protein alters keratinocyte proliferation and elicits a proinflammatory response *in vitro* and in vivo. Biochemistry. 2015;54:4855−4862.
44. Kumar A, Kumar A. Role of *Staphylococcus aureus* virulence factors in inducing inflammation and vascular permeability in a mouse model of bacterial endophthalmitis. PLoS One. 2015;10(6): e0128423.
DOI: 10.1371/journal.pone.0128423
45. Flannagan RS, Heit B, Heinrichs DE. Antimicrobial mechanisms of macrophages and immune evasion of *Staphylococcus aureus*. Pathogens. 2015;4:826-862.
46. Yamasaki O, Kaneko J, Morizane S, Akiyama H, Arata J, Narita S, et al. The association between *Staphylococcus aureus* strains carrying Panton-Valentine leukocidin genes and the development of deep-seated follicular infection. Clin Infect Dis. 2005;40:381-385.
47. Löffler B, Niemann S, Ehrhardt C, Horn D, Lanckohr C, Lina G, et al. Pathogenesis of *Staphylococcus aureus* necrotizing pneumonia: The role of PVL and an influenza coinfection. Expert Rev Anti infect Ther. 2014;11(10):1041-1051.
48. Özekinci T, Dal T, Yanık K, Ozcan N, Cand S, Tekina A, Yıldırıme HI, Kandemir I. Panton-Valentine leukocidin in community and hospital-acquired *Staphylococcus aureus* strains. Biotechno Biotechnol Equip. 2014;28 (6):1089-1094.
49. Neut D, van der Mei HC, Bulstra SK, Busscher HJ. The role of small-colony variants in failure to diagnose and treat biofilm infections in orthopedics. Acta Orthop. 2007;78:299-308.
50. Proctor RA, von Eiff C, Kahl BC, Becker K, McNamara P, Herrmann M, et al. Small colony variants: A pathogenic form of bacteria that facilitates persistent and recurrent infections. Nat Rev Microbiol. 2006;4(4):295-305.
51. Tuchscherr L, Kreis CA, Hoerr V, Flint L, Hachmeister M, Geraci J, et al. *Staphylococcus aureus* develops increased resistance to antibiotics by forming dynamic small colony variants during chronic osteomyelitis. J Antimicrob Chemother. 2016;71(2):438-48.
52. Beasley FC, Marolda CL, Cheung J, Buac S, Heinrichs DE. *Staphylococcus aureus* transporters Hts, Sir, and Sst capture iron liberated from human transferrin by staphyloferrin A, staphyloferrin B, and catecholamine stress hormones, respectively and contribute to virulence. Infect Immun. 2011;79:2345-2355.
53. Sebulsky MT, Heinrichs DE. Identification and characterization of *fhuD1* and *fhuD2*, two genes involved in iron-hydroxamate uptake in *Staphylococcus aureus*. J Bacteriol. 2001;183:4994-5000.
54. Pishchany G, Sheldon JR, Dickson CF, Alam MT, Read TD, Gell DA, et al. IsdB-dependent hemoglobin binding is required for acquisition of heme by *Staphylococcus aureus*. J Infect Dis. 2014;209:1764-1772.
55. Richards RL, Haigh RD, Pascoe B, Sheppard SK, Price F, Jenkins D, et al. Persistent *Staphylococcus aureus* isolates from two independent cases of Bacteremia display increased bacterial fitness and novel immune evasion phenotypes. Infect Immun. 2015;83:3311-3324.
56. Falugi F, Kim HK, Missiaka DM, Schneewind O. Role of protein A in the evasion of host adaptive immune responses by *Staphylococcus aureus*. MBio. 20134;(5):e000575-13.
57. Kobayashi SD, Deleo FR. *Staphylococcus aureus* protein A promotes immune suppression. MBio. 2013;4(5):e00764-13.
58. Smith EJ, Visai L, Kerrigan SW, Speziale P, Foster TJ. The Sbi protein is a multifunctional immune evasion factor of *Staphylococcus aureus*. Infect Immun. 2011;79(9):3801-3809.
59. Rooijakkers SH, van Wamel WJ, Ruyken M, van Kessel KP, van Strijp JA. Anti-opsonic properties of Staphylokinase. Microbes Infect. 2005;7:476-484.
60. Ko YP, Kuipers A, Freitag CM, Jongerius I, Medina E, van Rooijen WJ, et al. Phagocytosis escape by a *Staphylococcus aureus* protein that connects complement and coagulation proteins at the bacterial surface. PLoS Pathog. 2013;9;e1003816.
61. Laarman AJ, Ruyken M, Malone CL, van Strijp JA, Horswill AR and Rooijakkers SM. *Staphylococcus aureus* metalloprotease aureolysin cleaves complement C3 to mediate immune evasion. J Immunol. 2011;186:6445-6453.
62. Kolar SL, Ibarra JA, Rivera FE, Mootz JM, Davenport JE, Stevens SM, et al. Extracellular proteases are key mediators

of *Staphylococcus aureus* virulence via the global modulation of virulence-determinant stability. Microbiologyopen. 2013;2:18-34.

63. Kang M, Ko YP, Liang X, Ross CL, Liu Q, Murray BE, et al. Collagen-binding microbial surface components recognizing adhesive matrix molecule (MSCRAMM) of Gram-positive bacteria inhibit complement activation via the classical pathway. J Biol Chem. 2013;288:20520-20531.
64. DuMont AL, Yoong P, Day CJ, Alonzo F, McDonald WH, Jennings, MP, et al. *Staphylococcus aureus* LukAB cytotoxin kills human neutrophils by targeting the CD11b subunit of the integrin Mac-1. Proc Natl Acad Sci. 2013a;110:10794-10799.
65. Reyes-Robles T, Alonzo F, Kozhaya L, Lacy DB, Unutmaz D, Torres VJ. *Staphylococcus aureus* leukotoxin ED targets the chemokine receptors CXCR1 and CXCR2 to kill leukocytes and promote infection. Cell Host Microbe. 2013;14:453-459.
66. Melehani JH, James DB, DuMont AL, Torres VJ, Duncan JA. *Staphylococcus aureus* Leukocidin A/B (LukAB) kills human monocytes via host NLRP3 and ASC when extracellular, but not intracellular. PLoS Pathog. 2015;11, e1004970.
67. Thammavongsa V, Kern JW, Missiakas DM, Schneewind O. *Staphylococcus aureus* synthesizes adenosine to escape host immune responses. J. Exp Med. 2009;206:2417-2427.
68. Csóka B, Nèmeth ZH, Virág L, Gergely P. A2A adenosine receptors and C/EBP beta are crucially required for IL-10 production by macrophages exposed to *Escherichia coli*. Blood. 2007;110:2685-2695.
69. Thammavongsa V, Missiakas DM. Schneewind O. *Staphylococcus aureus* degrades neutrophil extracellular traps to promote immune cell death. Science. 2013;342:863–866.
70. Flannagan RS, Heit B, Heinrichs DE. Intracellular replication of *Staphylococcus aureus* in mature phagolysosomes in macrophages precedes host cell death, and bacterial escape and dissemination. Cell Microbiol. 2016;4:514-535.
71. Jubrail J, Morris P, Bewley MA, Stoneham S, Johnston SA, Foster SJ, et al. Inability to sustain intraphagolysosomal killing of *Staphylococcus aureus* predisposes to bacterial persistence in macrophages. Cell Microbiol. 2016;1:80-96.
72. Jarry TM, Memmi G, Cheung AL. The expression of alpha-haemolysin is required for *Staphylococcus aureus* phagosomal escape after internalization in CFT-1 cells. Cell Microbiol. 2008;10:1801-1814.
73. Giese B, Glowinski F, Paprotka K, Dittmann S, Steiner T, Sinha B, et al. Expression of δ-toxin by *Staphylococcus aureus* mediates escape from phago-endosomes of human epithelial and endothelial cells in the presence of β-toxin. Cell Microbiol. 2011;13:316-329.
74. DuMont AL, Yoong P, Surewaard BG, Benson MA, Nijland R, van Strijp JA, et al. *Staphylococcus aureus* elaborates leukocidin AB to mediate escape from within human neutrophils. Infect Immun. 2013b;81:1830-1841.
75. Grosz M, Kolter J, Paprotka K, Winkler AC, Schäfer D, Chatterjee SS, et al. Cytoplasmic replication of *Staphylococcus aureus* upon phagosomal escape triggered by phenol-soluble modulin α. Cell Microbiol. 2014;16:451-465.
76. Bera A, Biswas R, Herbert S, Götz F. The presence of peptidoglycan O-acetyltransferase in various staphylococcal species correlates with lysozyme resistance and pathogenicity. Infect Immun. 2006;74:4598-4604.
77. Cosgrove K, Coutts G, Jonsson IM, Tarkowski A, Kokai-Kun JF, Mond JJ, et al. Catalase (KatA) and alkyl hydroperoxide reductase (AhpC) have compensatory roles in peroxide stress resistance and are required for survival, persistence, and nasal colonization in *Staphylococcus aureus*. J Bacteriol. 2007;189:1025-1035.
78. Ballal A, Manna AC. Regulation of superoxide dismutase (*sod*) genes by SarA in *Staphylococcus aureus*. J Bacteriol. 2009;191:3301-3310.
79. Liu GY, Essex A, Buchanan JT, Datta V, Hoffman HM, Bastian J, et al. *Staphylococcus aureus* golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity. J Exp Med. 2005;202:209-215.
80. Mishra NN, Liu GY, Yeaman MR, Nast CC, Proctor RA, McKinnell J. et al. Carotenoid-related alteration of cell membrane fluidity impacts *Staphylococcus aureus* Susceptibility to Host Defense Peptides. Antimicrob Agents Chemother. 2011;55, 526–531.

81. Pang YY, Schwartz J, Bloomberg S, Boyd JM, Horswill AR, Nauseef WM. Methionine sulfoxide reductases protect against oxidative stress in *Staphylococcus aureus* encountering exogenous oxidants and human neutrophils. J Innate Immun. 2014;6:353–364.
82. Gonçalves VL, Nobre LS, Vicente JB, Teixeira M, Saraiva LM. Flavohemoglobin requires microaerophilic conditions for nitrosative protection of *Staphylococcus aureus*. FEBS Lett. 2006;580:1817-1821.
83. Richardson AR, Dunman PM, Fang FC. The nitrosative stress response of *Staphylococcus aureus* is required for resistance to innate immunity. Mol Microbiol. 2006;61:927-939.
84. Richardson AR, Libby SJ, Fang FC. A nitric oxide-inducible lactate dehydrogenase enables *Staphylococcus aureus* to resist innate immunity. Science. 2008;319:1672-1676.
85. Foster TJ. Immune evasion by staphylococci. Nat Rev Microbiol. 2005;3:948-958.
86. Skinner D, Keefer CS. Significance of bacteraemia caused by *Staphylococcus aureus*. Arch Intern Med.1941;68:851-875.
87. Deurenberg RH, Vink C, Kalenic S, Friedrich AW, Bruggeman CA, Stobberingh EE. The molecular evolution of methicillin-resistant *Staphylococcus aureus.* Clinical Microbiol Infect. 2007;13:222-235.
88. Deurenberg RH, Stobberingh EE. The evolution of *Staphylococcus aureus*. Infect Genet Evol. 2008;8:747-768.
89. Reygaert WC. Antimicrobial mechanisms of *Staphylococcus aureus*. FORMATEX. 2013;297-305.
90. Nakipoğlu Y, Iğnak S, Gürler N, Gürler B. The prevalence of antiseptic resistance genes (*qac*A/B and *smr*) and antibiotic resistance in clinical *Staphylococcus aureus* strains. Mikrobiyol Bul. 2012; 46(2):180-189.
91. Holden MT, Feil EJ, Lindsay JA, Peacock SJ, Day NP, Enright MC, et al. Complete genomes of two clinical *Staphylococcus aureus* strains: Evidence for the rapid evolution of virulence and drug resistance. Proc Natl Acad Sci USA. 2004;101:9786–9791.
92. Turlej A, Hryniewicz W, Empel J. Staphylococcal cassette chromosome *mec* (SCC*mec*) classification and typing methods: An overview. Pol J Microbiol. 2011;60:95-103.
93. Boucher HW, Sakoulas G. Perspectives on daptomycin resistance, with emphasis on resistance in *Staphylococcus aureus.* Clini Infect Dis. 2007;45:601-608.
94. Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC. Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced van-comycin susceptibility. J Antimicrob Chemother. 1997;40:135-6.
95. Gardete S, Tomasz A. Mechanisms of vancomycin resistance in *Staphylococcus aureus*. J Clin Invest. 2014;124(7):2836-2840.
96. Kacica M, McDonald LC. Vancomycin-resistant *Staphylococcus aureus*-New York. MMWR Morb Mortal Wkly Rep. 2004;53:322-3.
97. Pèrichon B, Courvalin P. VanA-Type Vancomycin-Resistant *Staphylococcus aureus.* Antimicrob Agents Chemother. 2009;53(11):4580-4587.
98. Zhu W, Murray PR, Huskins WC, Jernigan JA, McDonald LC, Clark NC, et al. Dissemination of an enterococcus Inc18-like vanA plasmid associated with vancomycin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother. 2010;54:4314-4320.
99. Mangili M, Bica I, Snydman DR, Hamera DH. Daptomycin-resistant, methicillin-resistant *Staphylococcus aureus* bacteraemia. Clin Infect Dis. 2005;40: 1058-1060.
100. Friedman L, Alder JD, Silverman JA. Genetic changes that correlate with reduced susceptibility to daptomycin in *Staphylococcus aureus*. Antimicrob Agents Chemother. 2006;50:2137-2145.
101. Chandrakanth RK, Raju S, Patil SA. Aminoglycoside-resistance mechanisms in multidrug-resistant *Staphylococcus aureus* clinical isolates. Curr Microbiol. 2008; 56:558-562.
102. Trzcinski K, Cooper BS, Hryniewicz W, Dowson CG. Expression of resistance to tetracyclines in strains of methicillin-resistant *Staphylococcus aureus*. J Antimicrob Chemother. 2000;45:763-770.
103. Schwarz S, Kehrenberg C, Doublet B, Cloeckaert A. Molecular basis of bacterial resistance to chloramphenicol and florfenicol. FEMS Microbiol Rev. 2004;28: 519-542.
104. Tsiodras S, Gold HS, Sakoulas G, Eliopoulos GM, Wennersten C, Venkataraman L, et al. Linezolid resistance

in a clinical isolate of *Staphylococcus aureus.* Lancet. 2001;358(9277):207-208.
105. Arias CA, Vallejo M, Reyes J, Panesso D, Moreno JC, Castañeda E, et al. Clinical and microbiological aspects of linezolid resistance mediated by the *cfr* gene encoding a 23S rRNA methyltransferase. J Clini Microbiol. 2008;46:892-896.
106. Long KS, Poehlsgaard J, Kehrenberg C, Schwarz S, Vester B. The Cfr rRNA methyltransferase confers resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A antibiotics. Antimicrob Agents Chemother. 2006;50:2500-2505.
107. Winston LG, Chambers HF. Antimicrobial resistance in staphylococci: Mechanisms of resistance and clinical implications. In: Mayers DL, ed. Antimicrobial drug resistance, Volume 2, Clinical and Epidemiological Aspects. New York, NY: Humana Press. 2009;735-748.

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