ISSN 2515-8260 Volume 08, Issue 03, 2021

Staphylococcus aureus: An Overview of Discovery, Characteristics, Epidemiology, Virulence Factors and Antimicrobial Sensitivity

Short Title: Methicillin Resistant *Staphylococcus aureus:* An overview

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Abstract

Staphylococcus aureus is an important infectious pathogen in health sector and communities. S. aureus was first described by the Scottish surgeon Alexander Ogaston in surgical abscess in 1881. It causes various infections ranged between simple to life threating infections. Owing potent toxins and other virulence factors enabled the bacteria to be very virulent. Additionally, acquisition of antimicrobial resistant genes increased the challenge in treating the infections caused by bacteria especially methicillin resistant S. aureus strains (MRSA) that are often multidrug resistant strains. The circulation of MRSA between health settings and communities resulted in changing even the genetic map for the strains in both places. Vancomycin was used for years and still acts as the drug of choice for treating MRSA infections but recently the resistance to vancomycin has risen and vancomycin resistant S. aureus were recorded. Consequently, different regimes were used like combination of antibiotics to reduce the resistance rate to antibiotics if they were used as a single drug and practiced the control measures at health settings to reduce the spread of MRSA strains. At last, global health organizations call for research and finding new antibiotics agents and put MRSA on the top list of infectious agents that need more antibiotics.

Key words: Staphylococcus aureus; MRSA; epidemiology; virulence factors; antimicrobial resistance

1. Introduction

Staphylococcus aureus (*S. aureus*) is a gram positive and commensal bacterium that colonizes 30% of healthy individuals from different body parts (1). It plays a significant role in causing infections in both hospitals and community ranging between simple to life threating infections (1-3). The organism causes infections by owing different virulent genes that encode different virulent factors such as toxins and enzymes, among others. Further, the virulence of *S. aureus* has risen with existence of antibiotics resistance strains such as

Methicillin resistant *S. aureus* (MRSA) and Vancomycin resistance *S. aureus* (VRSA) (1, 4). The resistance strains increased the challenge in treating the infections caused by them. The circulation of these strains in health care settings and community changed the epidemiology of their spread. Using preventive control measures are critical in controlling *S. aureus* infections (1). This review highlighted an overview of history of *S. aureus* discovery, epidemiology, virulent factors and antibiotics resistance.

2. History and taxonomy of *Staphylococcus aureus*

Staphylococci were first identified in 1880 and isolated from the pus of surgical abscesses by the Scottish surgeon Sir Alexander Ogston (5-9). Ogston was the first to describe pyogenic infections post-surgery that were related to a microorganism he described as "micrococci" (7, 8). The grape-like appearance of the bacteria, circular in shape and arranged in clusters, led Ogston to label it staphylococcus, derived from the Greek words "staphyle" (meaning "bunch of grapes") and "kokkos" (meaning "berry") (6, 9-11). Based on the arrangement description, staphylococci were differentiated from streptococci, which are arranged in chains and also cause post-surgical infections. In 1881, Ogston carried out experimental laboratory tests to investigate skin-associated infections caused by *S. aureus* by inoculating staphylococci into subcutaneous tissues of labratory animals such as guinea pigs and mice, which induced abscesses (7, 12). The German physician Friedrich Rosenbach isolated and cultured staphylococci from humans in 1884.(2, 11). He studied their characteristics and categorized them according to the production of golden or yellowish colonies, naming the species "aureus" from the Latin word meaning "golden, consequently, *S. aureus* was differentiated from *S. epidermidis* (previously known as *S. albus*) by their golden and white colonies, respectively (2, 6, 7, 11, 13, 14).

3. Bacteriology

3.1 Phenotypic characteristics

3.1.1 Colony morphology

Staphylococcus aureus colonies are characterized by their large, smooth, and elevated appearance with a golden yellow color (2, 11). The yellow color is the result of staphyloxanthin (a carotenoid) produced by the bacteria (7, 11), which covers and protects the microorganism from phagocytosis (7). Usually the microorganism causes hemolysis on enriched agar (blood agar with 5% sheep or horse blood), producing zones around the colonies (2, 11, 15). This hemolysis is due to the production of different kinds of enzymes called hemolysins (11). *S. aureus* is grown on selective media, such as mannitol salt agar containing 7.5-10% sodium chloride, as *S. aureus* is salt tolerant (3, 11). The pink color of medium shifts to yellow during the microorganism's fermentation of mannitol sugar, which yields an acid and changes the color of the medium. This can be utilized to distinguish *S. aureus* from *S. epidermidis*, a non-mannitol fermenter (16).

3.1.2 Cellular characteristics

Staphylococcus aureus is a gram-positive bacterium, spherical in shape, and sized between 0.5.-1.5 μm in diameter (2, 11, 14). It is non-motile and non-spore forming (14). *S. aureus*

appears bluish/purple by Gram staining (2, 3) and can be observed microscopically as a single coccus, in pairs, or in grape-like clusters (11). It is a facultative anaerobe that obtains energy for growth by aerobic respiration or fermentation. *S. aureus* divides by binary fission (14); its cell division occurs at different planes (2), and its optimum growth occurs at temperatures ranging between 18-40 °C (3).

3.1.3 Structure of the *S. aureus* cell wall

The *S. aureus* cell wall is composed of several layers, but is mainly composed of a thick and tough layer of peptidoglycan that comprises approximately 50% of the cell wall (2, 17). The cell wall also consists of a cytoplasmic membrane that encloses the cytoplasm and has a thin layer of lipids. Further, 40% of the cell wall composition is comprised of two types of teichoic acid (18): cell wall teichoic acid that is integrated in the cell wall, and cytoplasmic membrane lipoteichoic acid that is integrated in the lipid layer . Teichoic acids play a role in material transportation in bacterial cells. External proteins comprise the remainder of the cell wall, acting as virulence factors in infection pathogenesis (14). The majority of *S. aureus* strains cause diseases to humans by forming a capsule comprised of polysaccharides. The capsule protects bacteria from phagocytic cells, causing bacteremia *in vivo*. Furthermore, the capsule plays a major role in biofilm formation of microcolonies, which are resistant to antibiotics (19).

3.2 Biochemical characteristics

Various biochemical tests are utilized to identify and distinguish *S. aureus* from other gram-positive cocci microorganisms. *S. aureus* is catalase-positive, which can be used to differentiate it from catalase-negative streptococci species. *S. aureus* is also oxidase-negative (2, 11, 14). Staphylococci species can also be classified biochemically. *S. aureus,* which is coagulase-positive, produces a coagulase enzyme that agglutinates/clots blood or plasm (7, 11, 14), while other medically important species of staphylococci, such as *S. epidermidis* and *S. saprophyticus,* are coagulase-negative (7, 14, 20). It is worth noting that some other species of staphylococci are also coagulase-positive, such as *S. intermedius* and *S. hyicus* (2, 14), *S. pseudintermedius*, *S. lutrae*, *S. schleiferi*, and *S. delphini* (7, 20), but these mostly inhabit animals. *S. aureus* can be differentiated from *S. saprophyticus* by novambicin susceptibility, the latter being novambicin-resistant (21, 22). Additionally, the DNase test can be used to identify and differentiate *S. aureus* from other staphylococci species (16).

4. Epidemiology

Staphylococcus aureus is a commensal and opportunistic bacterium that colonizes different parts of the body, such as skin, nostrils, axilla, and inguinal areas (3, 23). Approximately 25-30% of healthy individuals are colonized with *S. aureus* (11, 24) and roughly 15% of the population persistently carry the bacteria (3). However, some groups of people are at higher risk of *S. aureus* colonization (up to 80%), including health care workers, diabetic persons, patients on intravenous drug individuals with weak immunity (25), patients with long hospital stays, recipients of surgical operations, indwelling catheter users, dialysis patients, individuals with ‘chronic metabolic diseases’ immune-compromised individuals, subjects

with previous methicillin-resistant *S. aureus* (MRSA) infection, and individuals with skin infections (11, 26). Carriers serve as sources of infection (25); transmission of *S. aureus* can take place from one person to another by close or direct contact, sharing personal items (3, 21, 27), food contamination, and fomite contamination such as doorknobs (25). *S. aureus* colonization in different parts of the body increases the risk of infection at the surgical site, as well as the infections of the lower respiratory and blood stream in hospitals (11). These infections are increased in healthcare facilities because the microorganism adapts quickly and effectively to the hospital environment (23, 28). Therefore, various measures are taken in hospitals to manage and reduce colonization and subsequently eliminate infections. Examples of these strategies include the use of different disinfectants, local application of antibiotics (such as mupirocin), and use of systemic antibiotics. Some hospitals deliberately colonize patient nostrils with harmless *S. aureus* strain 502A to reduce the colonization density of *S. aureus* through competition (11, 29-31).

4.1 Emergence of MRSA

The excessive use and misuse of antibiotics has led to the emergence of multidrug-resistant microorganisms (MDRM), such as MRSA (11), due to acquisition of the resistance gene *mec*A (32). The discovery of antibiotics began with penicillin in 1928 by Sir Alexander Fleming. Subsequent production and clinical use of pure penicillin to treat infections caused by *S. aureus* was achieved by Ernst Chain and Howard Florey in 1940. The first case of *S. aureus* resistance to penicillin *S. aureus* was recorded later the same year (14, 33, 34). A decade after reporting *S. aureus* penicillin resistance, most cases were restricted to hospital settings. The mechanism of penicillin resistance by beta-lactam penicillinase was elucidated in 1981, followed by identification of the *mec*A resistance gene (11, 35, 36). Clinical use of methicillin, a penicillin derivative, was introduced in 1961 to eliminate enzymatic degradation of penicillinase; its effectiveness lasted less than a year before methicillin resistance by *S. aureus* was recorded (37). Incidences of MRSA increased worldwide for the next decade in the form of hospital-acquired MRSA (HA-MRSA), especially in Europe (11, 14, 38). Additionally, management of MRSA-associated infections became more challenging due to the emergence of multidrug-resistant strains of *S. aureus* (6), which developed resistance to a range of antibiotics such as methicillin, cephalosporins, nafcillin, and oxacillin (21) due to their production of penicillin-binding protein (PBP)-2a. Consequently, treatment choices for life-threatening infections caused by multidrug-resistant staphylococci strains were limited. Hence, *S. aureus* was placed at the top of the list of bacterial resistant microorganisms by the World Health Organization (WHO) in 2017, exhorting the scientific community to explore new antibacterial agents (39).

Studies have reported the circulation of MRSA in healthcare settings in Iraq (40-43). Besides, a study carried out by Tabana *et al.* revealed that MRSA strains were also prevalent among hospital workers in Syria (44). Worth mention, notable concerns have arisen over the spread of infectious diseases associated with the significant wave of immigration from countries such as Syria to the West (45, 46) as people flee their own countries to seek asylum or refuge due to continuous war and humanitarian crises in their countries of origin. Several countries have reported cases of MRSA and MDRM among refugees attending host community

hospitals such as Netherlands (47, 48), Germany (48-51) and Swiss (52). As a precaution, provinces in the Netherlands such as Weert, Drenthe, and Groningen implemented regulations in 2015 that all hospitalized asylum seekers needed to be quarantined for MRSA to eliminate its spread in healthcare settings (53). Separation of hospitalized refugee patients is also recommended in Germany (49). Refugees arriving in most European countries are screened for infectious diseases (48, 54).

4.2 Emergence of community- acquired MRSA

Most HA-MRSA strains circulated in hospitals and healthcare settings until the 1990s, becoming globally endemic and associated with high morbidity and mortality (55). In 1993, cases of infections caused by MRSA and MRSA colonization were reported worldwide in healthy individuals with no previous exposure to healthcare settings (56, 57). In the late 1990s, community-acquired MRSA **(**CA-MRSA) strains emerged that were not resistant to non-beta-lactam antibiotics, which was remarkable. In 1999, the first cases of life-threatening infections associated with MRSA were reported in America and later in India in individuals not exposed to risk factors (58-60). The origins of these strains were found to be nosocomial (58, 59). The epidemiology of CA-MRSA then became widespread in hospitals and other healthcare settings. The first case of acquired CA-MRSA circulating in hospitals was in an admitted patient exposed to risk factors in 2008 in the USA (61). Many countries have reported subsequent outbreaks caused by CA-MRSA in hospitals (62). With the dramatic spread of CA-MRSA in nosocomial settings, many reports have suggested that HA-MRSA might be replaced by CA-MRSA in hospitals (63). Despite the limited MRSA data available in Iraq, particularly in KRI, the first case of CA-MRSA was reported in 2015 (64). Further, studies have detailed the prevalence of MRSA in different provinces of Iraq (26, 41, 65-70) and among Syrian civilian (46, 69).

5. Comparison between HA-MRSA and CA-MRSA

HA-MRSA and CA-MSRA are distinguished according to their origin of infection, antimicrobial sensitivity profile, virulence factors, molecular characteristics, and clinical presentation (11, 27, 71). Based on their antibiotic susceptibility profile, CA-MRSA does not commonly exhibit resistance against non-beta-lactam antibiotics, whereas, HA-MRSA strains are usually resistant to several classes of non-beta-lactam antibiotics. Further, the two types of MRSA have different genetic features. *SCCmec* typing revealed that HA-MRSA usually harbors large *SCCmec* elements that are either type I, II, or III, while CA-MRSA has a smaller *SCCmec* sequence and usually belongs to type IV or V (11, 71). However, recent studies have reported that some HA-MRSA isolates carry *SCCmec* type IV, and *SCCmec* type III was identified in both categories (71). The other genotypic characteristics of the two types of MRSA include genes encoding virulence factors. For example, the *pvl* gene that encodes leucocidin toxin is predominant in CA-MRSA and is occasionally found in HA-MRSA (56, 71, 72). Other virulence genes, such as those encoding hemolysins and super-antigens that expose toxins, are expressed at higher levels in CA-MRSA (71). Furthermore, both types are distinguished based on their origin of infection; infections caused by CA-MRSA are usually related to skin and soft tissues, whereas, those caused by HA-MRSA are more invasive (56,

72). However, CA-MRSA-associated infections are fulminant and life-threatening, with more complicated clinical manifestations (57).

Different clones of MRSA are known to circulate in different countries. In China, for instance, MRSA-ST59-IV/V and MRSA-ST338-IV/V are the common CA-MRSA clones, while MRSA-ST-239-III and MRSA-ST5-II are the common HA-MRSA clones (71, 73). The dominant clones in Mediterranean countries are ST80-IV and ST5-IV/V (62, 74). In Iraq, the predominant CA-MRSA clones carry *SCCmec* type IV and USA-300 (66) or IVa (69), whereas the predominant HA-MRSA clones harbor *SCCmec* types I, II, and III (40). Furthermore, Sabri *et al.* (2013) revealed that HA-MRSA isolates in Iraq harbored *SCCmec* type III and *spa* type (t932). The prevalent of MRSA harboring *SCCmec* type in Syria community IVa (69)

6. Virulence factors

The virulence of *S. aureus* is related to the potency of virulence factors that the bacteria possess, and the host immune response to the infection. *S. aureus* causes various infections by producing numerous virulence agents that enable it to cause diseases. Many conditions caused by *S. aureus* are mediated by integration of more than one type of virulence factor, including elements that enhance bacterial attachment to cells and tissues of the host, act as antiphagocytic agents, and that are cell wall virulence factors (capsule, protein A, fibronectin-binding proteins, and clumping factors). In addition, other virulence factors include cytolytic exotoxins that attack immune cells and blood cells such as exotoxins (α, β, γ), and Panton-Valentine leucocidin. Furthermore, *S. aureus* expresses superantigen exotoxins such as enterotoxins (A-G), toxic shock syndrome toxin (TSST-1), and exfoliative toxins (ETA and ETB). These toxins cause toxin-mediated clinical conditions. (11, 25). In general, producing these proteins enhances the microorganism's ability to cause various infections, ranging from simple to life-threatening infections (75, 76).

6.1 Toxins

6.1.1 Pore-Forming Toxins

6.1.1.1 Panton-Valentine leukocidin genes

Panton-Valentine leukocidin (PVL) is a bicomponent leukotoxin (77) that was first studied and described by Woodin using a carboxymethyl cellulose column, running both components and using electrophoresis to separate them. The two proteins were categorized based on their elution speeds with two different molecular weights: slow, known as leucocidin S-PVL (LukS-PV), and fast, known as leucocidin F-PV (LukF-PV) (78). These two proteins are encoded by the *LukS-LukF* genes (1). The role of PVL toxin in disease pathogenicity has been studied, but its precise role in the clinical manifestations or virulence of diseases remains unclear (1, 79). PVL toxin is associated mostly with CA-MRSA strains (77, 80); approximately 85% of cases are skin and soft tissue infections and pneumonia; whereas, only 5% of cases are related to nosocomial isolates (77, 79). This relationship indicates that PVL has the ability to cause life-threatening infections in healthy individuals (1). PVL toxins are cytotoxic and damage immune cells, such as white blood cells and mostly phagocytic cells (1, 77), due to their ability to cause pores in the cell membrane of leukocytes (78). A study carried out by Gillet *et al.* (2002) determined that clinical cases of pneumonia caused by

PVL-carrying *S. aureus* were more fatal than *pvl*-negative staphylococci strains causing ulcers and hemorrhagic lesions due to damage to neutrophils and macrophages caused by PVL toxin. Other clinical conditions associated with PVL toxin include dermonecrosis furuncles, cutaneous abscesses, and severe necrotic skin infections (1, 81) while the toxin gene was not found in infections such as endocarditis, hospital-acquired pneumonia, mediastinitis, urinary tract infections, enterocolitis, and toxic-shock syndrome (1). However, experimental studies on animals have demonstrated that PVL toxin can cause invasive diseases (77). In Iraq, MRSA strains harboring the *pvl* gene were isolated in both community and healthcare settings (26, 40, 43, 64, 66, 68, 82). The prevalence of PVL toxin among Syrian refugees was studied in Western countries such as Netherlands (47, 83) and in Iraq (82)

6.1.1.2 LukE- LukD toxins

The LukE-LukD toxin is another type of pore-forming toxin (PFT) composed of bicomponent subunits LukE and LukD. The toxin has a leucocidal effect on rabbit blood cells and leukocytes, mouse phagocytes, and human neutrophils (1, 84, 85). PFTs use different pathways to cause death and lysis of host cells, including activation of the inflammation pathway of macrophages and monocytes to induce lytic and pro-inflammatory activities (86). According to a study carried out in Japan, approximately 87-93% of *S. aureus* isolates harbored this gene, which was found in 88– 99% of MRSA strains globally. This supports the hypothesis that this toxin has a significant role in *S. aureus* pathogenicity (1). The Iraqi community reportedly exhibits a high prevalence of MRSA carrying the *LukED* gene (82, 87) and among Syrian civilian in Iraq (82)

6.1.1.3 Hemolysin- α

Hemolysin- α (Hla) toxin is a major type of exotoxin produced by most clinical isolates (88, 89), encoded by the *hla* gene (89). The hla toxin is cytotoxic to erythrocytes; rabbit erythrocytes being especially highly susceptible (88, 89). This toxin is known to have an effect on different categories of human cells such as epithelial cells, macrophages, T cells, endothelial cells, and monocytes (1). The hla toxin plays an important role in the pathogenesis of staphylococcal diseases; *S. aureus* mutants without the *hla* gene demonstrated reduced virulence in invasive disease models (88) and in healthy people (1). *S. aureus* harboring the *hla* gene can cause pneumonia, sepsis, brain abscess, septic arthritis, and corneal infections (89). The toxin can cause host cell damage through two pathways: producing pores in the cell membrane of target cells, or inducing the release of cytokines and chemokines (88, 89). Pore formation can result in target cell death through the production of pores on susceptible host cell membranes, leading to changes in ion gradients, and consequently damaging membranes and cell death (88).

6.1.1.4 Hemolysin- β

Hemolysin-β (Hlb) toxin, encoded by the *hlb* gene, is another cytotoxic protein that causes pore formation in the membranes of host cells (59, 90). It is also called sphingomyelinase C because it hydrolyzes sphingomyelin in the cell membrane of the host, especially in eukaryotic cells (90, 91) forming phosphorylcholine and ceramide. The toxin is highly

specific to the type of cell and host species (90) which might be due to differing sphingomyelin compositions in erythrocyte cell membranes (92). Sphingomyelinase consists of phosphoric diester compounds, and its role in developing disease is not yet fully understood. It is toxic to human cells, including skin dermal cells, white blood cells (polymorphonuclear cells), T lymphocytes, and monocytes. Hlb toxin inhibits interleukin-8 (IL-8) secretion by endothelial cells, which protects *S. aureus* from phagocytic cells and promotes biofilm development (1, 93-95). Several studies have addressed the importance of Hlb toxin in the pathogenesis of diseases caused by *S. aureus*, such as pneumonia, endocarditis (1) and corneal infections (89).

Hlb toxin expression is reportedly predominant in most strains of MRSA (1). Pneumonia caused by *S. aureus* is characterized by an aggressive immune response, but the mechanism responsible for stimulating the immune system that causes lung damage remains unclear (90).

6.1.2 Staphylococcal Superantigens

6.1.2.1 Toxic shock syndrome toxin

Toxic shock syndrome toxin-1 (TSST-1) was discovered in the USA during a diagnosis of toxic shock syndrome in the 1980s. It causes multi-system infections alone or in combination with enterotoxins (9). TSST-1 is one of the superantigen toxins (SAgs); they were first known as staphylococcal enterotoxins (SEs) and renamed superantigen toxins in 2004 because the diseases they caused were not manifested by emesis and diarrhea (9, 96). TSST-1 causes disease by activating severe immune responses by T-lymphocytes (1, 9), leading to the release of a large amount of cytokines, such as IL-2, IFN-γ, and TNF, which link to mononuclear cells (1, 9). Thus, its infections involve multiple systems and are characterized by different clinical manifestations, such as fever, rash, skin exfoliation, hypotension, and regularly cause multi-organ dysfunction (1, 97). The role of SAgs remains unclear in some clinical conditions and is the subject of further studies in septicemia, skin, and airway allergy research (98, 99). Differing results have been obtained from studies detailing the prevalence of TSST-1 toxin in Iraq (82, 100, 101) and among Syrian civilian (82).

6.1.2.2 Epidermolytic toxins A and B

Epidermolytic toxins (ETs) are also known as exfoliative toxin types A and B (9, 102, 103). These two kinds of enzymes have high affinity for serine, which is secreted by *S. aureus*. These proteases cause intra-epidermal blisters at the granular cell layer (9) by destroying junctions between keratinocytes and epidermis cells, resulting in exfoliation of the skin and the formation of blisters (1). It is worth noting that these toxins destroy desmosome cadherins, substances located in the superficial part of the skin (102, 103). The severity of these blebs differs from the expanded blisters caused by *Permphigus neonatorum* (9). The clinical characteristics of epidermal exfoliation in newborn babies were described by Ritter Von Rittershain in 1878 (1), although the link between *S. aureus* and desquamate was only discovered by Lyell in 1967 (104). The time interval between observation and discovery of this relationship was because cultivation of samples from blisters and desquamate regions were free of *S. aureus* (102). The exact role of these toxins in the pathogenesis of exfoliation diseases in mice was described in 1972 (105).

Limited strains (5%) of *S. aureus* produce ETs. The epidemiology of ET types varies between different countries; ETB is predominant in Japan, while ETA is distributed more in the USA, Europe, and Africa (1). Excretion of ET leads to either localized (e.g., bullous impetigo) or systemic (e.g., staphylococcal scalded skin syndrome [SSSS]) epidermal clinical manifestations (102). Staphylococcal scalded skin syndrome is characterized by exfoliation of the skin, high fever, painful skin rash, blebs full of fluid, and detachment of skin (1, 9, 103). Both conditions, bullous impetigo and SSSS, are caused by the same etiology although their extended areas of infection differ. Bullous impetigo is restricted to limited areas of the body, whereas SSSS affects more extensive areas of the skin (9, 103). SSSS can occur in both infants and adults, but disease severity and mortality rates differ. The disease mostly occurs in infants and children; toxins are disseminated systematically in children who lack neutralizing antitoxin, but the mortality rate is relatively low (approximately 5%) with appropriate management of the condition. On the other hand, a higher mortality rate (approximately 59%) is observed in adults, especially in immunosuppressed individuals (1). A study in Iraq illustrated the presence of MRSA harboring ETA/ETB among clinical and food isolates (101). However, another study carried out among clinical samples in Iraqi hospitals revealed a higher rate of MRSA harboring the *eta* gene (100).

6.2 Arginine catabolic mobile element

Arginine catabolic mobile element (ACME) is originally present in *S. epidermidis*, which acts as a reservoir for ACME (106), and is then transferred to *S. aureus* through horizontal transfer (107). ACME is composed of two gene groups: arc (*arc*A, *arc*B, *arc*C, *arc*D) and *opp* cluster (*opp*3-A, *opp*3-B, *opp*3-C, *opp*3-D). ACME has three allotypes depending on the presence or absence of *opp-3* and *speG* genes. The common MRSA allotype to date is type I ACME (28). Carrying ACME by *S. aureus* enhances its ability to inhabit skin and different mucous membranes by changing the pH conditions (from acidic to alkaline) and its tolerance to polyamines (108, 109). AMCE is predominant among CA-MRSA strains (110). It was first identified in *S. aureus* strain USA300 (108) which is the predominant CA-MRSA clone in the USA (28). It is believed that this strain clone is disseminated in the USA and Europe (109). In addition, the first case of *S. aureus* harboring ACME in Asia was reported in Japan in 2007, with subsequent spread (111). Studies carried out in Australia and Singapore revealed that AMCE was associated with the ST-239 clone (MRSA-III) (112, 113). Another study showed that AMCE was present among MRSA sequence type (ST-8) and *SCCmec* type Iva (USA300) (ST8-MRSA-IVa isolates) (28). AMCE was isolated from Iraq and Syrian civilian volunteers in Iraq (82).

6.3 Accessory gene regulator group

The vast majority of virulence-associated factor expression genes of *S. aureus* are controlled by three global regulators: accessory gene regulator (*agr*) (114, 115), staphylococcal accessory regulator (*sar*) (116) and *S. aureus* exoprotein expression (*sae*) (117). The *agr* locus contains four diverse transcription elements: *agr*A, *agr*C, *agr*D, and *agr*B. *Agr* is a quorum-sensing pathway that regulates and controls the expression of various virulence factor genes that are essential for *S. aureus* pathogenesis, including virulence factors such as hemolysin toxins (alpha and beta), TSST-1, and enterotoxins (B and C). However, it

represses exotoxins and surface proteins such as protein A, fibronectin-binding proteins, and fibrinogen-binding proteins (14, 118-120). The *agr* locus comprises two components of the signaling system encoded by promoter 2 (P2): *agr*A that functions as the response regulator, and *agr*C that acts as the signal receptor (114, 121). Further, *agr* P2 also encodes *agr* B and D, which bond together and produce autoinducing peptides (AIP). The *agr* P3 leads to transcription activation and production of RNAIII that acts as the effector *agr* response (114, 122). The *agr* locus is polymorphic and has highly variable regions; thus *S. aureus* can be categorized into four *agr* groups (*agr*I, *agr*II, *agr*III, and *agr*IV), which are located on *agr*C, *agr*D, and *agr*B (Figure 1) (118, 121). Some studies reported the possible association between *agr* group types and features of staphylococci strains such as resistance to glycopeptides with *agr* I and II, strains isolated from toxic shock syndrome and CA-MRSA with *agr* III, and SSSS isolates with *agr* IV (119). A study carried out in Iraq studying the prevelance of agr group among host community and Syrian civilian in Iraq (82).

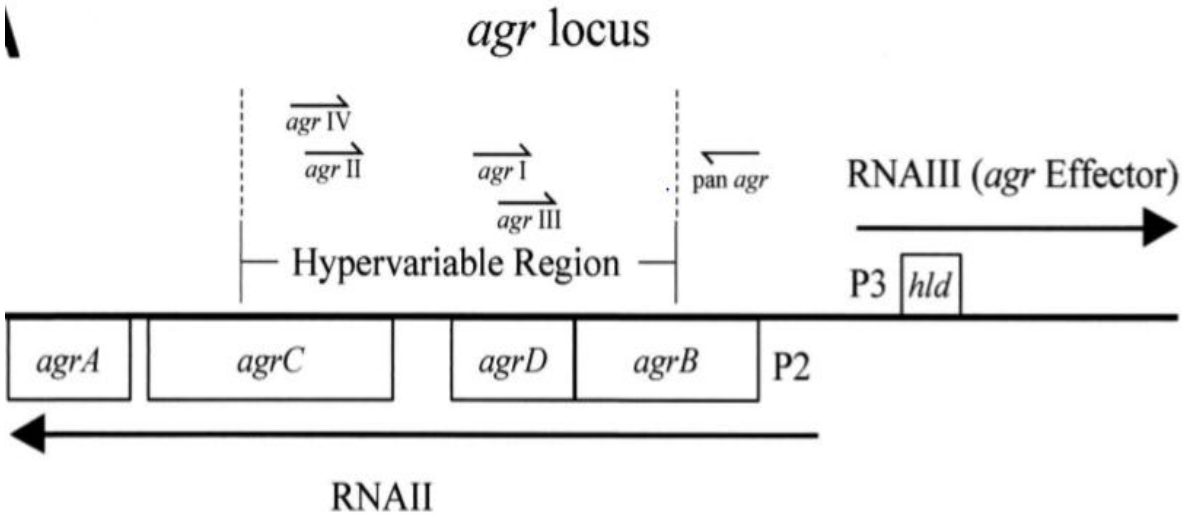

Figure 1. Schematic map of the *S. aureus agr* locus showing the locations of the different primers used for amplification of the hypervariable region. *agr*, accessory gene regulator (121).

7. Antimicrobial resistance

Staphylococcus aureus develops resistance to antimicrobial agents by different means, such as horizontal gene transfer of different mobile genetic elements (MGEs), including bacteriophages, plasmids, Staphylococcus cassette chromosomes (SCCs), transposons, and pathogenicity islands (PAIs) (2). All the aforementioned MGEs potentially carry antibiotic-resistant genes, which can be predicted based on the size of the plasmids possessed by bacteria. Small plasmids may carry genes resistant to tetracycline, erythromycin, and chloramphenicol, whereas large plasmids carry resistance genes against macrolides, beta-lactams, and aminoglycosides. On the other hand, larger plasmids carry genes that integrate with other MGEs and produce resistance to erythromycin, vancomycin, beta lactams, trimethoprim, and spectinomycin (2, 123). A study conducted by De Smalen illustrated the antimicrobial profile of MDRM and the role of immigration in its spread (124). A study by Taban addressed the antimicrobial profile of MRSA in Syria (44). The antimicrobial profile

of MRSA in Iraq among clinical isolates was detailed by Sultan and Al Meani (101). Further, the antimicrobial profile of *S. aureus* among healthy participants in Iraqi community (125) and Syrian community (126) were studied. The resistance of *S. aureus* to various antimicrobial classes is described in the following paragraphs.

7.1 Resistance to beta-lactams antibiotics

Penicillin belongs to the beta-lactam group of antibiotics and has a beta-lactam ring in its structure, which binds to PBP on the cell wall of bacteria, inactivates it, and prevents bacterial cell wall synthesis. The resistance mechanism of *S. aureus* against beta-lactam agents occurs by two means: beta-lactam penicillinase and the *mec*A gene. The first mechanism requires the production of penicillinase enzyme (11, 14) or beta-lactamase enzymes (127), which are located on plasmids and encoded by *bla*Z. This enzyme breaks down the beta-lactam ring in the beta-lactam antibiotic structure, thus inactivating the antibiotic (11). The second defense mechanism requires the acquisition of the *mec*A gene, which encodes PBP2a protein, and assists in bacterial cell wall synthesis even in the presence of beta-lactam antibiotics (3, 14, 32, 127). MRSA carries the *mec*A gene, which confers resistance to most beta-lactam products (32). The *mec*A gene is located on a mobile chromosomal DNA fragment, chromosomal cassette type *SCCmecA*, and is only present in MRSA strains (3, 11, 14, 128-130). Evolution of the second mechanism of MRSA resistance has two different theories. The single clone hypothesis suggests the vertical transfer of *mec*A gene elements into *S. aureus* on one occasion, followed by formation of the MRSA clone, and subsequent global distribution of the resistant genes (11, 131-133). Molecular analysis of the *mec* gene revealed that *mec*A originally belonged to the coagulase-negative staphylococci species known as *sciuri* species group, which includes *S. fleurettii*, *S. sciuri*, and *S. vitulinus.* The *mec*A gene nucleotides in MRSA are 99.8% identical to that of *S. fleurettii* (131, 133).

The second and the most accepted hypothesis, is that MRSA strain resistance developed through horizontal gene transfer to the chromosome of methicillin-sensitive *S. aureus* (MSSA) (127, 132, 134). This hypothesis was solidified by cloning and sequencing the genetic elements that carry the acquired gene (*mec*A) with other genes that control its expression, such as *mecR*1 signal transducer protein *MecR*1, and *mecI*, which encodes the repressor protein *MecI*, supporting the idea of gene exchange between various *S. aureus* strains (127). The DNA fragment that carried the *mec*A gene was previously studied and found to carry site-specific recombinases designated as cassette chromosome recombinases (*ccr*), later called *SCCmec* (127, 135, 136). *SCCmec* are classified into different elements; five classes of *ccr* gene complexes and eight *SCCmec* types, based on their combination (11, 133) (Figure 2). These elements vary in structure but share numerous characteristics (127): (a) carrying the *mec* gene complex (*mec*A and its regulators), (b) carrying the *ccr* gene complex (*ccr*A, *ccr*B, *and ccr*C), (c) flanked regions at sequence ends by both inverted and direct repeats, and (d) integrated staphylococcal chromosome at a specific site called the integration site sequence (ISS) at the 3'end of *orfX* (127, 133). The *SCCmec* gene can be classified into various subtypes for epidemiological purposes, depending on specific regions on the staphylococcal chromosome called junkyard regions (J-regions) (133).

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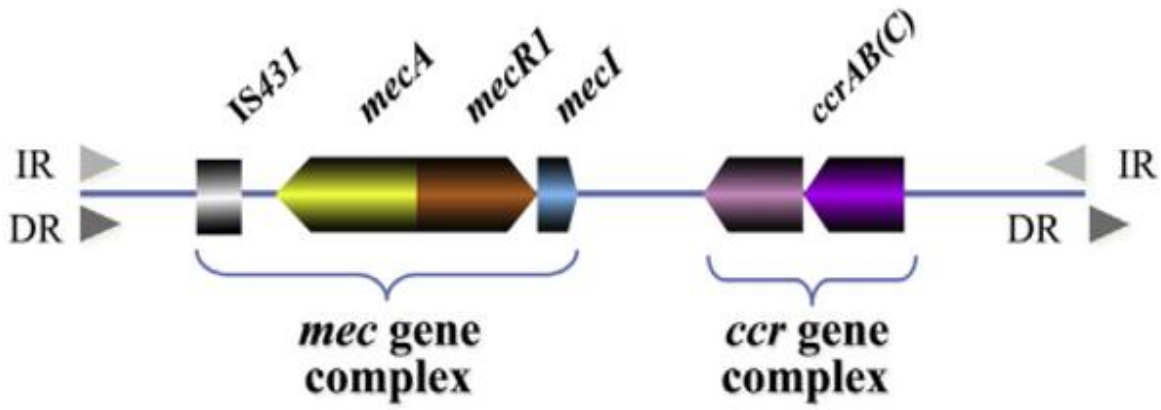

Figure 2 The Staphylococcal cassette mec (*SCCmec*) composition consisting of two necessary complexes: *mec* gene complex and *ccr* gene complex. The *mec* gene complex comprises the *mec*A gene and two regulators (*mec*RI and *mec*I). The *ccr* gene complex encodes integration and excision of the entire *SCC* element. IR, inverted repeats; DR, direct repeats. Adopted from ref. (133).

7.2 Resistance to Glycopeptides

Vancomycin, discovered by Edmund Kornfeld in 1953 (6), belongs to the glycopeptide antibiotic class and is considered the drug of choice to treat infections caused by MRSA. The first case of vancomycin resistance was documented in Japan in 1996. The strains were characterized by intermediate resistance to vancomycin; therefore, these strains were called vancomycin intermediate *S. aureus* (VISA) (6, 14, 137). Additional resistant strains were observed in many other countries. In 2002, the first case of vancomycin-resistant *S. aureus* (VRSA) was recorded in a clinical isolate in the USA (138). Vancomycin resistance is caused by two pathways. The first pathway was elucidated in VISA, and is due to the thickening of the bacterial cell wall and production of additional peptidoglycan targets that require more antibiotic to inhibit bacterial cell growth (139). The second mechanism of resistance, observed in VRSA, is due to the acquisition of the *van*A gene. It is hypothesized that *S. aureus* acquires this gene from vancomycin-resistant enterococci isolated through gene transfer (14). The second mechanism is characterized by changing the target side of PBP and its substrate by changing D-ala-D-ala to D-ala-D-lac by binding to the C-terminal of the pentapeptide (2). With increased global dissemination of vancomycin resistance, detection of new antimicrobial agents or vaccine development are urgently required (14).

7.3 Resistance to Tetracyclines

Tetracyclines are bacteriostatic (2) and broad-spectrum antibiotics (140). They inhibit protein synthesis by working precisely on 30s ribosomal subunits and blocking tRNA (2). Resistance to tetracycline is developed by two methods: protection of the ribosome, which is encoded by *tet*M (2, 140) and *tetO* (140) *genes*, and the efflux pump system, which is encoded by *tet*K (2, 140) and *tetL* genes carried by plasmid (140).

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7.4 Resistance to Fluoroquinolone

The fluoroquinolone antibiotic class inhibits DNA synthesis by attacking DNA gyrase enzymes, encoded by *gyr*A and *gyr*B genes, and topoisomerase IV, encoded by *ParC* and *ParE* genes. The mechanism of fluoroquinolone resistance arises from mutations in the target gyrase or topoisomerase IV, or by changing antibiotic permeability into the bacterial cell. Additionally, resistance evolution to fluoroquinolone occurs due to the multidrug efflux pump system and is mediated by the *nor*A gene (2, 141).

7.5 Resistance to Aminoglycosides

Aminoglycosides are used to treat different bacterial infections, including infections caused by *S. aureus* (142, 143). This antibiotic class interrupts protein synthesis and binds to 30S ribosomal subunits (2, 142). Resistance to aminoglycosides occurs via three pathways, including mutations in the ribosomal binding site to antibiotics, modifications to aminoglycoside-modifying enzymes (AMEs) that result in drug inactivation (2, 142, 143), and the efflux pump system (142). Acquisition of these enzymes is important for aminoglycoside resistance acquired by staphylococci (142). Examples of the most clinically important AMEs are aminoglycoside acetyl transferases (AACs), aminoglycoside-nucleotidyltransferases (ANTs), and aminoglycoside phosphotransferases (APHs), which are encoded by genetic elements (142, 143). Staphylococci bacteria develop resistance to different aminoglycoside antibiotics, such as gentamycin and kanamycin, by involving enzymes AAC (6') and APH (2') (143).

7.6 Resistance to ansamycins

The excessive use and misuse of antibiotics leads to resistance in *S. aureus* and the emergence of MRSA strains. As mentioned earlier, vancomycin is the target drug of choice to treat clinical conditions caused by MRSA, but incidences of VRSA have increased recently. Thus, rifampicin, a member of the ansamycin class of antibiotics, is used in combination with vancomycin to treat MRSA conditions. It is worth noting that rifampicin is the only working antibiotic in cases of multidrug-resistant tuberculosis. Thus, caution should be taken when using rifampicin for non-tuberculosis infections (144, 145) as a number of rifampicin-resistant MRSA (RIF-R-MRSA) cases have been recorded. For example, a study conducted in China demonstrated that the incidence of RIF-R-MRSA was 15.5% in 2004, however, it reached 50.2% four years later (145). Rifampicin interrupts protein synthesis by inhibiting transcription, which is achieved by blocking RNA polymerase (2, 146). On the other hand, rifampicin resistance is mediated by a mutation in the *rpo*B gene, which encodes a beta distinct unit of RNA polymerase (2).

7.7 Resistance to clindamycin and fusidic acid

Clindamycin belongs to the lincosamide class of antibiotics. It disrupts protein synthesis in the bacterial cell by binding to the 50S ribosomal subunit (147). Resistance to lincosamides occurs through methylation of its receptor binding site on the ribosome, consequently altering the target cell. Methylation is mediated by an enzyme called methylase and is encoded by *erm* genes (2).

On the other hand, fusidic acid is a miscellaneous agent that belongs to the fusidane group. It is used to treat infections caused by MRSA, such as skin, soft tissue, and joint infections. It is usually used in combination with other antibiotics, most often rifampicin, thus reducing the resistance rate compared with a single drug. Fusidic acid inhibits protein synthesis by blocking elongation factor G (EF-G), which prevents elongation of the peptide chain by impeding peptidyl tRNA translocation. By reducing the protein synthesis rate, staphylococci are more susceptible to phagosomes (148). Additionally, resistance occurs due to chromosomal mutation in the *fus*A gene, which encodes EF-G, and inhibits or blocks antibiotic attachment to the peptidyl chain, thereby preventing protein synthesis (149).

8. Conclusions

Staphylococcus aureus is a commensal gram-positive bacterium that has captured the attention of the medical community for more than a century. It possesses many virulence factors, including toxins, superantigens, and exoproteins that are cell wall-associated. Furthermore, the emergence of MRSA strains in both healthcare settings and the community has increased the risk of infection caused by these strains, as they usually express multidrug resistance. The challenge of treating infections caused by MRSA has been increased by MRSA acquiring antibiotic resistance genes by different means, especially after recording strains resistant to vancomycin, which was the drug of choice for MRSA treatment. Many hospitals employ measures to control/reduce the spread of MRSA stains, but more education is needed regarding how the bacteria spreads and its control in the community and in hospitals as MRSA is prevalent worldwide.

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