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***Staphylococcus aureus*: Overview of Bacteriology, Clinical Diseases, Epidemiology, Antibiotic Resistance and Therapeutic Approach**

Arumugam Gnanamani, Periasamy Hariharan and
Maneesh Paul-Satyaseela

Additional information is available at the end of the chapter

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Abstract

Staphylococcus aureus is an important human pathogen that causes wide range of infectious conditions both in nosocomial and community settings. The Gram-positive pathogen is armed with battery of virulence factors that facilitate to establish infections in the hosts. The organism is well known for its ability to acquire resistance to various antibiotic classes. The emergence and spread of methicillin-resistant *S. aureus* (MRSA) strains which are often multi-drug resistant in hospitals and subsequently in community resulted in significant mortality and morbidity. The epidemiology of MRSA has been evolving since its initial outbreak which necessitates a comprehensive medical approach to tackle this pathogen. Vancomycin has been the drug of choice for years but its utility was challenged by the emergence of resistance. In the last 10 years or so, newer anti-MRSA antibiotics were approved for clinical use. However, being notorious for developing antibiotic resistance, there is a continuous need for exploring novel anti-MRSA agents from various sources including plants and evaluation of non-antibiotic approaches.

Keywords: *Staphylococcus aureus*, MRSA, CA-MRSA, HA-MRSA, anti-MRSA

1. Introduction

Staphylococcus aureus is a Gram-positive bacterium and causative agent of wide range of infectious diseases such as skin infections, bacteremia, endocarditis, pneumonia and food poisoning. The organism was originally a leading nosocomial pathogen and afterwards epidemiologically distinct clones emerged in community settings. *S. aureus* expresses number

of virulence factors which help to establish infection by facilitating tissue attachment, tissue invasion and evading from host immune response. The ability to acquire resistance to multiple antibiotics classes makes *S. aureus*, a challenging pathogen to treat. Emergence and spread of *S. aureus* strains which are resistant to methicillin, referred to as methicillin-resistant *S. aureus* (MRSA) resulted in high morbidity, high mortality and increased treatment costs. Vancomycin remained gold standard drug to tackle these strains for years but the emergence of resistance restricted its clinical utility. Newer anti-MRSA antibiotics which were approved by U.S. FDA came as respite for clinicians. However, new antibiotic discovery efforts and non-antibiotic approaches to tackle MRSA should not be diminished considering the ability of the pathogen to acquire resistance to newer drugs quickly after their introduction in clinics.

In this chapter, we present a comprehensive outlook of *S. aureus* with account on bacteriology, pathogenesis, epidemiology, antibiotic resistance and therapeutic approaches.

2. Bacteriology

2.1. Microscopic morphology

S. aureus cells are Gram-positive and appear in spherical shape. They are often in clusters resembling bunch of grapes when observed under light microscope after Gram staining. The name 'Staphylococcus' was derived from Greek, meaning bunch of grapes (*staphyle*) and berry (*kokkos*) [1]. The scanning electron microscopic observation reveals roughly spherical shaped cells with smooth surface [2]. The diameter of the cells ranges from 0.5 to 1.0 μM [3]. The transmission electron microscopy of cells shows thick cells wall, distinctive cytoplasmic membrane and amorphous cytoplasm [4].

2.2. General cultural and biochemical characteristics

S. aureus is an aerobic and facultative anaerobic organism that forms fairly large yellow or white colonies on nutrient rich agar media. The yellow colour of the colonies is imparted by carotenoids produced by the organism. The term 'aureus' is derived from Latin, which refers to the colour of gold [5]. The organism is often haemolytic in blood agar due to production of four types of haemolysins (alpha, beta, gamma and delta) [6, 7]. Nearly all isolates of *S. aureus* produce coagulase enzyme, a virulence factor that also helps in identification of the organism [6, 8]. The organism is salt tolerant, which is able to grow in mannitol-salt agar medium containing 7.5% sodium chloride [8]. The organism is catalase positive and oxidase negative.

2.3. Medical laboratory diagnosis

The primary objective in laboratory diagnosis is to identify whether the diagnosed *S. aureus* isolate is methicillin resistant. Since MRSA emerged as problematic pathogen, a systematic diagnostic approach is necessary for early diagnosis so that treatment with appropriate antibiotics can be initiated as early as possible. For the species identification, slide and tube

coagulase tests, latex agglutination tests and PCR-based tests are used. For detection of MRSA, determination of minimum inhibitory concentration (MIC) of methicillin or oxacillin or cefoxitin using broth micro-dilution method, cefoxitin disk screen, oxacillin agar screen and latex agglutination test for PBP2a and molecular methods for detection of *mecA* are employed [8].

3. General pathogenesis and clinical diseases

3.1. Pathogenesis

The process of *S. aureus* infections involves five stages. They are (1) colonization, (2) local infection, (3) systemic dissemination and/or sepsis, (4) metastatic infections and (5) toxinosis. The organism is in carrier state in the anterior nares and can remain so without causing infections for weeks or months. The colonization proceeds to infection under certain predisposing factors such as prolonged hospitalization, immune suppression, surgeries, use of invasive medical devices and chronic metabolic diseases. Localized skin abscess develop when the organism is inoculated into the skin from a site of carriage. This can further spread and results in various clinical manifestations of localized infections such as carbuncle, cellulitis, impetigo bullosa or wound infection. The organism can enter into blood and spread systemically to different organs causing sepsis. This haematogenous spread may result in endocarditis, osteomyelitis, renal carbuncle, septic arthritis and epidural abscess. Without a blood stream infection, specific syndromes can occur due to extra cellular toxins of *S. aureus*. These are toxic shock syndrome, scalded skin syndrome and foot borne gastroenteritis [9].

3.2. Hospital and community infections

S. aureus causes wide range of infections in human. The clinical infections of *S. aureus* are classified into community and nosocomial categories based on origin of infection. These two types are distinct in clinical manifestations of the infections, antibiotic susceptibility and the genetic background of the infecting *S. aureus* strains. For decades, *S. aureus* has been predominantly a nosocomial pathogen and is a leading cause of mortality and morbidity in hospitals. However, the community *S. aureus* infections are in rise. The important clinical *S. aureus* infections are bacteraemia, infective endocarditis, skin and soft tissue infections, osteoarticular infections and pleuropulmonary infections. Other clinical infections are epidural abscess, meningitis, toxic shock syndrome and urinary tract infections [9, 10].

3.3. Virulence factors

S. aureus possess battery of virulence factors. These factors enable the organism to be successful as pathogen that causes wide range of human and animal infections. Virulence factors help in attachment to host cells, breaking down the host immune shield, tissue invasion, causing sepsis and elicit toxin-mediated syndromes. This is the basis for persistent staphylococcal infections without strong host immune response [11]. Based on their mechanism of action and role in pathogenesis, staphylococcal virulence factors are classified as represented in **Table 1** [9, 12].

Factors	Characteristics
Helping attachment to host tissues	
<i>Microbial Surface Components Recognizing adhesive matrix molecules (MSCRAMM)</i>	Cell surface proteins which interact with host molecules such as collagen, fibronectin & fibrinogen, thus, facilitate the tissue attachment. Staphylococcal protein A, fibronectin-binding proteins A and B, collagen-binding protein & clumping factor A & B belong to this family. They are also involved in host immune evasion [13].
Breaking/evading the host immunity	
<i>Polysaccharide microcapsule</i>	Resist the phagocytosis & killing by polymorphonuclear phagocyte [14].
<i>Protein A</i>	It binds to Fc portion of immunoglobulin, prevents opsonization, functions as super antigen & limits the host immune response [15].
<i>Panton-Valentine leukocidin (PVL)</i>	PVL is found in most of community-associated MRSA (CA-MRSA) [16]. PVL belongs to group of membrane pores forming proteins. It consists of two protein components (LukS-PV and LukF-PV) which act together as subunits and form porins on cell membrane of host cells, leading to leakage of cell contents and cell death [17].
<i>Alpha-toxin (Alpha hemolysin)</i>	It was the first bacterial exotoxin to be identified as a cell membrane pore former which causes cell leakage & death [18].
<i>Chemotaxis-inhibitory protein of S. aureus (CHIPS):</i>	CHIPS is an extracellular protein which inhibits the chemotaxis functioning of neutrophil and monocytes [19].
Tissue invasion	
<i>Extracellular adherence protein (Eap)</i>	An exoprotein which binds to host cell matrix, plasma proteins & endothelial cell adhesion molecule ICAM-1. In addition to the roles of adhesion and invasion, it also has immune-modulatory activity [20].
<i>Proteases, lipases, nucleases, hyaluronatylase, phospholipase C, metalloproteases (elastase), & Staphylokinase</i>	These extracellular enzymes cause tissue destruction and, thereby, help in bacterial penetration into tissues.
Induces toxinosis	
Enterotoxins	<i>S. aureus</i> produces battery of enterotoxins which are potent gastrointestinal exotoxins. The Staphylococcal food poisoning is an intoxication which results from consumption of foods containing sufficient amount of preformed enterotoxins [21].
Toxic shock syndrome toxin -1 (TSST-1)	TSST-1 & some of enterotoxins are called as pyrogenic toxin super antigens. TSST-1 causes toxic shock syndrome especially in menstrual women [7].
<i>Exfoliative toxins A and B</i>	Serine proteases which selectively recognize and hydrolyze desmosomal proteins in the skin. ETs cause staphylococca-scalded skin syndrome, a disease predominantly affecting infants [22].

Table 1. Virulence factors of *S. aureus* and its characteristics.

4. Epidemiology of infections

4.1. Nasal carriage

S. aureus is a commensal and opportunistic pathogen. The anterior nares are the principal ecological niche, where the organism colonizes in humans. The nasal carriage of *S. aureus* increases the risk of infection especially in the hospital settings [23]. The average nasal carriage of *S. aureus* could be at 30% of human population [24]. Since, the nasal carriage increases the risk of development of surgical site, lower respiratory and blood stream infections in hospitals, efforts are made to eliminate the carriage using various strategies. Methods such as local application of antibiotics (eg. mupirocin) or disinfectants, administration of systemic antibiotics and use of a harmless *S. aureus* strain (type 502A) which competes for the colonization of nares with existing one are employed to decolonize the *S. aureus* from nares [25–28].

4.2. Emergence and evolution of MRSA

The MRSA are those *S. aureus* strains carrying a *mecA* gene, which codes for additional penicillin-binding protein, PBP2a. The beta-lactam antibiotics exert their antibacterial activity by inactivation of penicillin-binding proteins (PBPs), which are essential enzymes for bacterial cell wall synthesis. However, these antibiotics have only a low affinity towards PBP2a, thus this enzyme evades from inactivation and carry out the role of essential PBPs resulting in cell wall synthesis and survival of bacteria even in presence of beta-lactam antibiotics. Due to the presence of *mecA*, MRSA are resistant to nearly all beta-lactam antibiotics [29].

Penicillin is the first beta-lactam antibiotic discovered in 1928 and found to be effective weapon against *S. aureus* infections. In 1940s, sooner after its introduction into clinics, there were reports of *S. aureus* strains that were resistant to penicillin [30]. These strains produced plasmid-encoded beta-lactamase enzyme (penicillinase) which enzymatically cleaved the beta-lactam ring of penicillin rendering the antibiotic inactive [31, 32]. In 1950s, the penicillin resistance was restricted to hospital isolates of *S. aureus*. By late 1960s, more than 80% *S. aureus* isolates, irrespective of community and hospital origin, were resistant to penicillin due to plasmid transfer of penicillinase gene (*blaZ*) and clonal dissemination of resistant strains [33, 34].

Meanwhile, scientists who were challenged with penicillinase-mediated resistance in *S. aureus* discovered methicillin, a semi-synthetic penicillin that withstood the enzymatic degradation of penicillinase. Methicillin was introduced into clinics in 1961; however, in less than a year, resistance of *S. aureus* isolates to methicillin (MRSA) was reported [35]. Over the next 10 years, increasing number of MRSA outbreaks was reported in different parts of the world especially from the European countries [36, 37]. The notable feature of these reports is that, the incidences were from hospitals and thus MRSA emerged as a hospital-borne pathogen. The mechanism of resistance to beta-lactam antibiotics in these MRSA isolates was uncovered in 1981 [38].

As mentioned earlier, MRSA isolates carry a gene *mec A* which codes for PBP2a. The gene is part of a 21–60 kb mobile genetic element referred to as staphylococcal cassette chromosome *mecA* (SCC*mecA*). There are two hypotheses that explain the evolutionary origin of MRSA. The

single clone hypothesis suggests that the mobile genetic element entered the *S. aureus* population on one occasion and resulted in the formation of a single MRSA clone that has since spread around the world. The second and the most agreed hypothesis is that MRSA strains evolved number of times by means of the horizontal transfer of the mobile genetic element into phylogenetically distinct methicillin-susceptible *S. aureus* (MSSA) precursor strains [39, 40].

SCC*mec* elements are highly diverse in their structural organization and genetic content (**Figure 1**) and have been classified into types based on the combination of *mec* and *ccr*, which share variations (five classes in *mec* and eight in *ccr*). To date, at least 11 types of SCC*mec* elements have been identified [41–43].

4.3. Health care-associated and community MRSA

4.3.1. Health care-associated MRSA (HA-MRSA)

Health care-associated MRSA (HA-MRSA) are those *S. aureus* isolates obtained from patients 2 or more days after hospitalization or with the MRSA risk factors (history of recent hospitalization, surgery, dialysis, or residence in a long-term care facility within 1 year before the MRSA-culture date or presence of a permanent indwelling catheter or percutaneous medical device (e.g. tracheostomy tube, gastrostomy tube or Foley catheter) at the time of culture or previous isolation of MRSA [44, 45]. Community-associated MRSA (CA-MRSA) are those *S. aureus* isolates obtained from patients within 2 days of hospitalization and without the above-mentioned MRSA risk factors.

Till 1990s, MRSA isolates were predominantly HA-MRSA and were also resistant to non-beta-lactam antibiotics. The multi-drug resistant phenotype of HA-MRSA was due to presence of non-beta-lactam antibiotic-resistant determinants in relatively large SCC*mec* [46]. During the period of 1960s to early 1990s, number of clones of HA-MRSA had spread widely across the world and HA-MRSA became endemic in hospitals and emerged as leading nosocomial pathogen [47]. The genetic background of these MRSA clones was characterized initially using phage typing subsequently by multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE), *spa* typing and SCC*mec* typing. The analysis of the genetic background of HR-MRSA

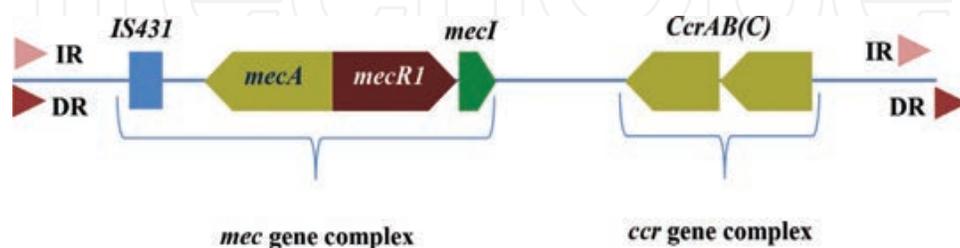


Figure 1. Basic structure of SCC*mec*. SCC*mec* constituted by *mec* gene and *ccr* gene complexes. The *mec* gene complex encodes PBP2a (*mecA*) and resistance regulators (*mecI* and *mecR1*). The *ccr* gene complex encodes the integration and excision of entire SCC element. The gene complexes are flanked by characteristic nucleotide sequences, inverted repeats (IR) and direct repeats (DR), at both ends. J (joining) regions are J1 (between right chromosomal junction and *ccr* complex), J2 (between *ccr* and *mec* complexes) and J3 (between *mec* complex and left chromosomal junctions). Adopted from Ref. [41].

isolates using these methods revealed the spread of early MRSA clone (Archaic clone) which contained type I *SCCmec* and sequence type 250 (ST250) in 1960s and extended into the 1970s in the form of Iberian clone. The Iberian clone was sequence type 247 (ST247) which evolved from ST250-MRSA by a single point mutation [48]. In the mid to late 1970s, Archaic and Iberian MRSA clones declined while, clones with novel *SCCmec* types II and III had emerged marking the on-going worldwide pandemic of HA-MRSA in hospitals and health care facilities [49, 50]. The lineages of common HA-MRSA clones are represented in **Table 2**. The rise in the prevalence of HA-MRSA throughout the world has been dramatic. In the United States, the proportion of MRSA among *S. aureus* isolates from the hospitalized patients was 2.4% in 1975, which increased to 51.6% (ICU patients) and 42% (non-ICU inpatients) by 1998–2003. Similar persistently high or increasing rates of MRSA among *S. aureus* isolates have also been observed for health care settings in many other regions of the world [51].

4.3.2. Community-associated MRSA (CA-MRSA)

MRSA isolates obtained from outpatients or from patients within 48 h of hospitalization and if they lack HA-MRSA risk factors mentioned earlier are referred to as CA-MRSA [52]. Scattered case reports of MRSA infections in healthy population whom had no exposure to health care facilities were published in the 1980s and mid-1990s. Beginning in 1993, case series of MRSA infection and colonization of patients lacking health care-associated risk factors were reported from six continents, in diverse states, nations and regions [51, 53]. The phenotypic and genotypic characterization of CA-MRSA isolates revealed the differences between CA-MRSA and HA-MRSA strains. While HA-MRSA strains carried a relatively large *SCCmec*, belonging to type I, II or III, CA-MRSA strains carried smaller *SCCmec* elements, most commonly type IV or type V. HA-MRSA strains were resistant to many classes of non-beta-lactam antibiotics, thus display multi-drug resistant phenotypes. CA-MRSA strains were often sensitive to non-beta-lactam antibiotics. Another notable feature of CA-MRSA strains was presence of genes for the PVL, which was rare among the HA-MRSAs. With respect to clinical cases, CA-MRSA infections were prevalent in previously healthy younger patients in contrast to HA-MRSA, which cause infections in hospitalized patients. CA-MRSA was often associated with skin and skin structure infections while HA-MRSA was implicated in wide range of infections such as pneumonia, bacteraemia, and invasive infections [48, 51]. Compared to infections caused by HA-MRSA, CA-MRSA infections had been associated with fulminant and lethal infections and worse clinical outcomes [49, 53].

Among the various clones of CA-MRSA, ST93, ST80 and ST8 are presently the predominant clones in Australia, Europe and the United States, respectively. In the United States, ST8-USA 300 is the most wide spread CA-MRSA clone [54], which harbour *SCCmec* type IV and genes encoding PVL. The concern about this clone is high virulence and increase in resistance to non-beta-lactam antibiotics [50, 53]. In United Kingdom, EMRSA-15 (ST22) and EMRSA-16 (ST36) are the dominant clones [49]. In Europe, ST80-IV, ST8-IV, ST398-V and ST152-V were commonly reported [55]. In Mediterranean countries, the dominant clones are ST80-IV and ST5-IV/V [55, 56].

In the last 10 years, there is a dramatic change in epidemiology of CA-MRSA as they invaded the health care settings. In 2008, first case of MRSA isolated from hospitalized patient turned out to

Clonal complex	Molecular sequence type	Common names for specific MRSA clones	Comment
CC5	ST5	USA100 and NewYork/Japan clone	Most common US health care-associated MRSA, SCCmecII
	ST5	EMRSA-3	SCCmecI
	ST5	USA800/Pediatric clone	Prevalent in Argentina, Colombia, United States, SCCmecIV
	ST5	HDE288/Pediatric clone	SCCmecVI
CC8	ST250	Archiac	First MRSA clone identified, COL strain as an example; SCCmecI
	ST247	Iberian clone and EMRSA-5	Descendant of COL-type strains, SCCmecIII
	ST239	Brazilian/Hungarian clone	SCCmecIII
	ST239	EMRSA-1	Eastern Australian epidemic clone of 1980s, SCCmecIII
	ST8	AUS-2 and Aus-3	SCCmecII
	ST8	Irish-1	Common nosocomial isolate in the 1990s in Europe and the United States
	ST8	USA500 and EMRSA-2-6	SCCmecIV
CC22	ST22	EMRSA-15	International clone, prominent in Europe and Australia, SCCmecIV
CC30	ST36	USA200 and EMRSA-16	Single most abundant cause of MRSA infections in UK; second most common cause of MRSA infections in US hospitals in 2003, SCCmecII
CC45	ST45	USA600 and Berlin	SCCmecII

Table 2. The lineages of common HA-MRSA (based on Ref. [49]).

be a CA-MRSA which marked the arrival of CA-MRSA into nosocomial settings [57]. Since then, hospital outbreaks of *S. aureus* strains which are phenotypically and genotypically CA-MRSA, have been reported many parts of the world [55]. Entry of CA-MRSA into hospitals blurred the differences between CA-MRSA and HA-MRSA. The increased reports of CA-MRSA outbreaks in hospital suggest that CA-MRSA may eventually displace HA-MRSA in hospitals [58].

5. Antibiotic resistance

5.1. Beta-lactam resistance

5.1.1. Penicillin resistance

The first beta-lactam antibiotic penicillin G was discovered in 1928 by Alexander Fleming and the drug was used in human as chemotherapeutic agent in 1941 [59]. The antibiotic was potent against Gram positive pathogens [60] and a power weapon against Staphylococcal infections. However, first reports of *S. aureus* strains that were resistant to penicillin appeared after a year of its clinical use [30]. Such penicillin-resistant isolates carried a plasmid gene, *blaZ* which encoded a beta-lactamase enzyme, referred to as penicillinase [33, 34]. The enzyme is capable of cleaving the beta-lactam ring of penicillin resulting inactivation of the antibiotic [31, 32].

The emergence and spread of penicillinase-mediated resistance in *S. aureus* is referred to as first wave of resistance. This has spread in alarm proportions and became pandemic in the 1960s. About 80% of both community and hospital acquired *S. aureus* isolates were resistant to penicillin by late 1960s [33, 49]. By early 2000s, more than 90% of Staphylococcal isolates produced penicillinase enzyme irrespective of their community or hospital origin [34].

5.1.2. Methicillin resistance

As discussed earlier, the penicillinase resistance in *S. aureus* was countered by the discovery of methicillin, penicillinase-stable semisynthetic penicillin. The drug was introduced into clinics in 1961 and subsequently strains showing methicillin resistance (MRSA) was reported in the same year [35]. After the initial report, MRSA clones spread rapidly across the world but restricted to nosocomial settings. This is referred to as second wave of beta-lactam resistance in *S. aureus* [40]. As discussed earlier, methicillin resistance was mediated by the presence of *mecA* gene. The therapeutic outcome of MRSA infections was worse than methicillin sensitive *S. aureus* (MSSA) due to the underlying comorbid factors such as old age, immune suppression and, importantly, lack of effective antibiotics to treat MRSA, which were often multi-drug resistant [34]. The rise in MRSA infections in hospitals resulted in high morbidity and mortality and increase in cost of health care [61, 62].

The third wave of beta-lactam resistance in *S. aureus* began with reports of MRSA infections in community in early 1990s. As discussed earlier, these strains were phenotypically and genetically distinct from MRSA isolates from hospitalized patients, resulting in definitions of HA-MRSA and CA-MRSA [51, 53]. In the last decade, community MRSA strains invaded the hospital settings and the difference between HA and CA MRSA is now blurred [58].

5.2. Quinolones resistance

Nalidixic acid, the prototype quinolone and the second generation quinolones (e.g. ciprofloxacin and norfloxacin) are predominately active towards Gram negative bacteria while

third generation (e.g. levofloxacin) and fourth generation (e.g. moxifloxacin, gemifloxacin) quinolones exhibited improved and greater activity against Gram-positive bacteria [63–65]. Quinolones exert their antibacterial action by inhibiting bacterial topoisomerases (topoisomerase IV and DNA Gyrase), which are essential for relieving DNA super coiling and separation of concatenated DNA strands [66]. The resistance to quinolones in *S. aureus* arises in stepwise manner, due to point mutations primarily in GrlA subunit of topoisomerase IV and GyrA subunit of Gyrase. Additional mechanism by which *S. aureus* become resistant to quinolones is by expression of NorA efflux pumps [67].

The quinolone resistance in *S. aureus* is mostly associated with methicillin resistance though the mechanism of resistance and encoding genes are altogether different from each other. This could be due to higher usage of quinolones in hospital settings where the HA-MRSA prevalence is high resulting in selection of quinolone resistance [68–70]. In year 2008, the fluoroquinolone resistance among MRSA isolates implicated in acute bacterial skin and skin structure infections (ABSSSIs) in hospitals was at 70.3%. Due to such high level of quinolone resistance among MRSA in hospital settings, even third- and fourth-generation quinolones have not been considered for treatment of MRSA [71]. With respect to CA-MRSA, though they were susceptible to non-beta-lactam antibiotics including quinolones, the scenario has changed in recent years, with the rise in incidence of CA-MRSA infections which were multi-drug resistant [72].

5.3. Vancomycin resistance

Vancomycin, a glycopeptide antibiotic, was discovered from a microbial source (*Streptomyces orientalis*) in 1952. The drug was approved for clinical use in 1958; however, it was eclipsed by methicillin and other anti-staphylococcal penicillins which were considered less toxic than vancomycin and equally efficacious against penicillin-resistant Staphylococci [73]. Beginning early 1980s, there was sudden increase in vancomycin usage due to rise in HA-MRSA infections and emergence of pseudomembranous enterocolitis cause by *Clostridium difficile* in hospitalized patients [73–75]. Clinical efficacy of vancomycin efficacy in treatment of MRSA infections was well established over the period of time, thus the drug emerged as workhorse anti-MRSA drug [76].

5.3.1. Vancomycin intermediate *S. aureus*

The antibacterial activity of vancomycin is mediated by its binding to the C-terminal D-Ala-D-Ala residue of the peptidoglycan precursor, and formation of non-covalent complex, thereby, prevents the use of the precursor in bacterial cell wall synthesis [77, 78]. Three decades after its introduction into clinics, no clinical resistance to vancomycin was reported. The first report of a MRSA strain showing reduced susceptibility to vancomycin was reported in 1997. The vancomycin MIC against this strain (Mu50) was 8 mg/L, thus, designated as intermediate sensitive category. The strain had thickened cell wall when observed under electron microscopy and did not carry *vanA* or *vanB* genes as found in vancomycin-resistant enterococci (VRE) [79]. Subsequently, there were more reports of clinical infections due to MRSA strains with decreased vancomycin susceptibility similar to that of Mu50 strain. The *S. aureus* strains with a MIC range of 4–8 mg/L are referred to as

vancomycin intermediate *S. aureus* (VISA). There were strains, which showed vancomycin MIC of 2 mg/L but had subpopulation with vancomycin MIC of 4–8 mg/L. These strains are referred to as hetero VISA (hVISA) [80, 81].

The genetic basis of emergence of VISA appears complex. The genetic analysis of VISA strains identified mutations in determinants that control the biosynthesis of bacterial cell wall and/or mutations in the ribosomal gene *rpoB* [82]. The increased MRSA infection in hospitals has led to extensive use of vancomycin resulting in the selection of MRSA strains with reduced vancomycin susceptibility [83]. The study on prevalence of hVISA and VISA has met with the problem of accurate detection of decreased susceptibility to vancomycin. Different diagnostic methods showed variable sensitivity and specificity leading to contradictory reports in prevalence [80, 84–86]. During 2010–2014, the prevalence rates of hVISA and VISA among MRSA strain were at 7.01% and 7.93%, respectively [87]. The emergence and increased incidence of hVISA and VISA has limited the therapeutic use of vancomycin in the treatment of MRSA infections in hospital. However, by optimizing the dose regimen and drug delivery, thereby, achieving the desired blood plasma concentration which would give the clinical efficacy is the way forward in preserving the clinical utility of vancomycin [88, 89].

5.3.2. Vancomycin-resistant *S. aureus*

S. aureus strains which are referred to as hVISA and VISA are not considered resistant based on vancomycin susceptibility breakpoint (vancomycin MIC of 8 mg/L) defined by clinical laboratory standards institute (CLSI). Unlike VRE, these strains do not carry *vanA* or *vanB* type of genes to confer resistance to vancomycin. In 2002, first report of a *S. aureus* strain showing vancomycin MIC of >128 mg/L was published. The strain was methicillin resistant and carried *vanA* gene which was responsible for high-level resistance to vancomycin [90]. This report was followed by sporadic incidences of isolation of *S. aureus* strains with resistance to vancomycin [91]. All these strains showed high vancomycin MIC (>8 mg/L) and are referred to as vancomycin-resistant *S. aureus* (VRSA).

VRSA strains carried copies of the transposon Tn1546, which was acquired from vancomycin-resistant *Enterococcus faecalis*. The transposon which mediates the VanA-type resistance, encodes a dehydrogenase (VanH), which reduces pyruvate to D-Lac, and the VanA ligase, which catalyzes the formation of an ester bond between D-Ala and D-Lac. The resulting D-Ala-D-Lac depsipeptide replaces the D-Ala-D-Ala dipeptide in peptidoglycan synthesis, a substitution that decreases the affinity of the molecule for vancomycin and other glycopeptide antibiotic, teicoplanin, considerably [92, 93].

5.4. Resistance to other antibiotics

Since HA-MRSA strains are often MDR phenotype, drugs such as sulphonamides, tetracyclines, aminoglycosides, chloramphenicol and clindamycin were sidelined due to lack of activity, while vancomycin remained the mainstay of therapy. Resistance to sulphonamides and trimethoprim [94], tetracyclines [95–97], aminoglycosides [98–100], chloramphenicol [101] and clindamycin [102], occurring in *S. aureus* especially among MRSA was widely reported.

6. Therapeutic approach

Therapeutic approach to *S. aureus* infections depends on the type of infection, patient age, clinical manifestation of the disease, co-morbidity, antibacterial susceptibility of infecting organism and hospitalization. Various drugs as single agent and drug combinations have been used to treat *S. aureus* infection. In general, management of infections due to MRSA is difficult compared to that of MSSA. There are guidelines and reviews to help in the treatment of community and hospital infections of MRSA.

6.1. Topical anti-MRSA drugs

6.1.1. Mupirocin

Mupirocin is used as topical antibiotic to treat impetigo due to *S. aureus* and *S. pyogenes* [103]. The drug is also used for nasal decolonization of *S. aureus* [27]. Mupirocin belongs to monoxycarboxylic acid class and it exerts antibacterial action by binding to isoleucyl t-RNA synthetase, thereby, inhibiting the protein synthesis [104]. The antibiotic shows excellent activity against Staphylococci and most Streptococci [105]. Clinical efficacy of mupirocin ointment in treating *S. aureus* superficial skin infections and wound infections was established [106–108]. Various reports also demonstrated effectiveness of mupirocin in nasal decolonization of *S. aureus* [25, 109, 110] that is a risk factor for MRSA infections in nosocomial settings.

6.1.2. Fusidic acid

Fusidic acid is an antibiotic, which belongs to a class referred to as fusidanes. Chemically it is a tetracyclic triterpenoid [111] and it binds to bacterial elongation factor G (EF-G), which results in impaired translocation process and inhibition of protein synthesis [112]. It has potent activity against *S. aureus* and clinically used in treatment of mild to moderately severe skin and soft-tissue infections, for example, impetigo, folliculitis, erythrasma, furunculosis, abscesses and infected traumatic wounds [113]. The efficacy of fusidic acid ointment in treatment of *S. aureus* infections is widely reported [114, 115]. The drug has also been used systemically to treat invasive *S. aureus* infections but its efficacy was questioned [116].

6.2. Systemic anti-MRSA drugs

6.2.1. Vancomycin

As discussed earlier, vancomycin remained the mainstay of therapy against MRSA infections in hospitalized patients for decades. Though the antibiotic was available for clinical use since 1958, it gained prominence among clinicians only after the surge in nosocomial MRSA infections in 1980s [73, 75]. Numerous reports documented the clinical efficacy of vancomycin in treating various MRSA infections in hospitalized patients [116–120]. The emergence and spread of hVISA and VISA strains has threatened the clinical utility of vancomycin. In addition, over the years, the mean MIC of vancomycin against susceptible MRSA

Newer-MRSA drug	Year of approval	Class	Source	Mode of action	Route of administration	References
Linezolid	2000	Oxazolidinone	Synthetic	Inhibition of protein synthesis	Oral & intra-venous	[126, 127]
Daptomycin	2003	Cyclic lipopeptide	<i>Streptomyces oseosporus</i>	Cell membrane depolarization	Intra-venous	[128, 129]
Tigecycline	2005	Glycylcyclines (Tetracyclines)	Semisynthetic	Inhibition of protein synthesis	Intra-venous	[130, 131]
Ceftaroline	2010	Cephalosporin (Beta-lactam)	Semisynthetic	Inhibition of cell wall synthesis	Intra-venous	[132, 133]
Telavancin	2013	Lipoglycopeptide	Semisynthetic	Inhibition of cell wall synthesis & cell membrane depolarization	Intra-venous	[134, 135]
Tedizolid	2014	Oxazolidinone	Synthetic	Inhibition of protein synthesis	Oral & intra-venous	[136, 137]
Dalbavancin	2014	Lipoglycopeptide	Semisynthetic	Inhibition of cell wall synthesis	Intra-venous	[138, 139]
Oritavancin	2014	Lipoglycopeptide	Semisynthetic	Inhibition of cell wall synthesis & cell membrane depolarization	Intra-venous	[140, 141]

Table 3. Newer anti-MRSA drugs.

populations has increased but within the susceptible range. This phenomenon is referred to as vancomycin MIC creep. There has been poor response to vancomycin therapy in patients infected with vancomycin-susceptible MRSA isolates which had vancomycin MIC at the higher end of susceptible range (2 mg/L) [121, 122]. Optimizing the dose regimen and drug delivery, in order to achieve the desired blood plasma concentration which would give the clinical efficacy is the way forward in preserving the clinical utility of vancomycin [91, 92].

6.2.2. Newer anti-MRSA drugs

The problem of MRSA infections in hospitals and lack of effective antibiotics other than vancomycin to treat them necessitated the discovery of novel anti-MRSA drugs. The continued efforts of researchers in discovering novel anti-MRSA drugs fructified resulting in arrival of number of newer anti-MRSA drugs for clinical use in the last 15 years [78, 123–125]. The following **Table 3** lists the newer anti-MRSA drugs that were approved by U.S. FDA for clinical use.

7. Alternative therapeutic approach

Apart from chemotherapeutic approach to tackle the *S. aureus* infection, alternatives such as agents which inhibit the virulent factors expression and vaccines have been investigated. Various phytochemical are also found to have anti-MRSA activity. All these are at investigational stages and more research is necessary to bring promising candidates for clinical usage.

7.1. Anti-virulence agents

Clinical use of agents which are not conventional antibiotics but able to inhibit the expression or function of the virulence factors, rendering the bacteria non-pathogenic is considered an alternative approach to tackle MRSA. Stripping microorganisms of their virulence properties without threatening their existence may offer a reduced selection pressure for drug-resistant mutations. Virulence-specific therapeutics would also avoid the undesirable dramatic alterations of the host microbiota that are associated with current antibiotics [142, 143].

Accessory gene regulator (*agr*)-mediated quorum sensing system of *S. aureus* plays a central role in pathogenesis of Staphylococci. Scientists identified small molecules which inhibited the *agr* system [144–146]. Active and passive immunization strategies targeting the virulence factors of *S. aureus* have also been explored [147].

7.2. Plants

Plants have immune system and other defensive mechanisms against microorganisms that cause plant diseases. Hence, the plants with huge diversity provide a vast source for exploration of anti-MRSA phytochemicals. *In vitro* Anti-MRSA activity of crude extracts of medicinal plants has been extensively reported [148]. Various phytochemicals such as β -asarone, Mansonone F, prenylated flavonoids and thymoquinone showed *in vitro* anti-MRSA activity [149–152].

Author details

Arumugam Gnanamani^{1*}, Periasamy Hariharan² and Maneesh Paul-Satyaseela^{2,3}

*Address all correspondence to: gnanamani3@gmail.com

1 Microbiology Division, CSIR-CLRI, Adyar, Chennai, India

2 Orchid Pharma Ltd., Chennai, India

3 St Martha's Hospital, Bangalore, India

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