
***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

<http://dx.doi.org/10.5772/67338>

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 predominately 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 *mecA* 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

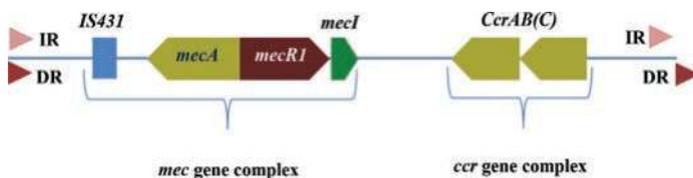


Figure 1. Basic structure of SCC*mec*. SCC*mec* constituted by *mec* gene and *ccr* gene complexes. The *mec* gene complex encodes PBP2a (*mecA*) and resistance regulators (*mecI* and *mecR1*). The *ccr* gene complex encodes the integration and excision of entire SCC element. The gene complexes are flanked 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 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 osoporus</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

References

- [1] Licitra G. Etymologia: *Staphylococcus*. *Emerg Infect Dis*. 2013;**19**:1553. DOI: 10.3201/eid1909.ET1909
- [2] Greenwood D, O'Grady F. Scanning electron microscopy of *Staphylococcus aureus* exposed to some common anti-staphylococcal agents. *J Gen Microbiol*. 1972;**70**:263–270. DOI: 10.1099/00221287-70-2-263
- [3] Foster T. Chapter 12: *Staphylococcus*. *Medical Microbiology*. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston, Galveston, Texas; 1996.
- [4] Touhami A, Jericho MH, Beveridge TJ. Atomic force microscopy of cell growth and division in *Staphylococcus aureus*. *J Bacteriol*. 2004;**186**:3286–3295. DOI: 10.1128/JB.186.11.3286-3295.2004 DOI: 10.1128/JB.186.11.3286-3295.2004
- [5] Liu GY, Essex A, Buchanan JT, Datta V, Hoffman HM, Bastian JF, Fierer J, Nizet V. *Staphylococcus aureus* golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity. *J Exp Med*. 2005;**202**:209–215. DOI: 10.1084/jem.20050846
- [6] Blair JE. Factors determining the pathogenicity of staphylococci. *Annu Rev Microbiol*. 1958;**12**:491–506. DOI: 10.1146/annurev.mi.12.100158.002423
- [7] Dinges MM, Orwin PM, Schlievert PM. Exotoxins of *Staphylococcus aureus*. *Clin Microbiol Rev*. 2000;**13**:16–34. DOI: 10.1128/CMR.13.1.16-34.2000
- [8] Brown DF, Edwards DI, Hawkey PM, Morrison D, Ridgway GL, Towner KJ, Wren MW; Joint Working Party of the British Society for Antimicrobial Chemotherapy; Hospital Infection Society; Infection Control Nurses Association. Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus* (MRSA). *J Antimicrob Chemother*. 2005;**56**:1000–1018. DOI: 10.1093/jac/dki372
- [9] Archer GL. *Staphylococcus aureus*: a well-armed pathogen. *Clin Infect Dis*. 1998;**26**: 1179–1181. PMID: 9597249

- [10] Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler Jr VG. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clin Microbiol Rev.* 2015;**28**:603–661. DOI: 10.1128/CMR.00134-14
- [11] Kim HK, Falugi F, Missiakas DM, Schneewinda O. Peptidoglycan-linked protein A promotes T cell-dependent antibody expansion during *Staphylococcus aureus* infection. *Proc Natl Acad Sci U S A.* 2016;**113**:5718–5723. DOI: 10.1073/pnas.1524267113
- [12] Gordon RJ, Lowy FD. Pathogenesis of methicillin-resistant *Staphylococcus aureus* infection. *Clin Infect Dis.* 2008;**46**:S350–S359. DOI: 10.1086/533591
- [13] Vazquez V, Liang X, Horndahl JK, Ganesh VK, Smeds E, Foster TJ, and Hook M.. Fibrinogen is a ligand for the *Staphylococcus aureus* microbial surface components recognizing adhesive matrix molecules (MSCRAMM) bone sialoprotein-binding protein (Bbp). *J Biol Chem.* 2011;**286**:29797–29805. DOI: 10.1074/jbc.M110.214981
- [14] Nilsson IM, Lee JC, Bremell T, Rydén C, Tarkowsk. The role of staphylococcal polysaccharide microcapsule expression in septicemia and septic arthritis. *Infect Immun.* 1997;**65**:4216–4221. PMID: PMC175605
- [15] Hong X, Qin J, Li T, Dai Y, Wang Y Liu Q, He L, Lu H, Gao Q, Lin Y, Li M. Staphylococcal Protein A promotes colonization and immune evasion of the epidemic healthcare-associated MRSA ST239. *Front Microbiol.* 2016;**7**:951. DOI: 10.3389/fmicb.2016.00951
- [16] Voyich JM, Otto M, Mathema B, Braughton KR, Whitney AR, Welty D, Long RD, Dorward DW, Gardner DJ, Lina G, Kreiswirth BN, De Leo FR. Is panton-valentine leukocidin the major virulence determinant in community-associated methicillin-resistant *Staphylococcus aureus* disease? *J Infect Dis.* 2006;**194**:1761–1770. DOI: 10.1086/509506
- [17] Genestier AL, Michallet MC, Prévost G, Bellot G, Chalabreysse L, Peyrol S, Thivolet F, Etienne J, Lina G, Vallette FM, Vandenesch F, Genestier L. *Staphylococcus aureus* Panton-Valentine leukocidin directly targets mitochondria and induces Bax-independent apoptosis of human neutrophils. *J Clin Invest.* 2005;**115**:3117–3127. DOI: 10.1172/JCI22684
- [18] Bhakdi S, Trantum-Jensen J. Alpha-toxin of *Staphylococcus aureus*. *Microbiol Rev.* 1991;**55**:733–751. PMID: PMC372845
- [19] Postma B, Poppelier MJ, van Galen JC, Prossnitz ER, van Strijp JA, de Haas CJ, van Kessel KP. Chemotaxis inhibitory protein of *Staphylococcus aureus* binds specifically to the C5a and formylated peptide receptor. *J Immunol.* 2004;**172**:6994–7001. PMID: 15153520
- [20] Edwards AM, Bowden MG, Brown EL, Laabei M, Massey RC. *Staphylococcus aureus* extracellular adherence protein triggers TNF α release, promoting attachment to endothelial cells via Protein A. *PLoS One.* 2012;**7**:e43046. DOI: 10.1371/journal.pone.0043046
- [21] Argudin MA, Mendoza MC, Rodico MR. Food Poisoning and *Staphylococcus aureus* Enterotoxins. *Toxins (Basel).* 2010;**2**:1751–1773. DOI: 10.3390/toxins2071751
- [22] Bukowski M, Wladyka B and Dubin G. Exfoliative Toxins of *Staphylococcus aureus*. *Toxins (Basel).* 2010;**2**:1148–1165. DOI: 10.3390/toxins2051148

- [23] Kluytmans JA, Wertheim HF. Nasal carriage of *Staphylococcus aureus* and prevention of nosocomial infections. *Infection*. 2005;**33**:3–8. DOI: 10.1007/s15010-005-4012-9
- [24] Williams REO. Healthy carriage of *Staphylococcus aureus*: its prevalence and importance. *Bacteriol Rev*. 1963;**27**:56–71. PMID: PMC441169
- [25] Wertheim HF, Verveer J, Boelens HA, van Belkum A, Verbrugh HA, Vos MC. Effect of mupirocin treatment on nasal, pharyngeal, and perineal carriage of *Staphylococcus aureus* in healthy adults. *Antimicrob Agents Chemother*. 2005;**49**:1465–1467. DOI: 10.1128/AAC.49.4.1465-1467.2005
- [26] Perl TM. Prevention of *Staphylococcus aureus* infections among surgical patients: beyond traditional perioperative prophylaxis. *Surgery*. 2003;**134**:S10–S17. DOI: 10.1016/S0039
- [27] 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. DOI: 10.1093/jac/dkp159
- [28] Kluytmans J. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. *Clin Microbiol Rev*. 1997;**10**:505–520. PMID: PMC172932
- [29] Fuda C, Suvorov M, Vakulenko SB, Mobashery S. The basis for resistance to β -lactam antibiotics by penicillin-binding protein 2a of methicillin-resistant *Staphylococcus aureus*. *J Biol Chem*. 2004;**279**:40802–40806. DOI: 10.1074/jbc.M403589200
- [30] Rammelkamp CH, Maxon T. Resistance of *Staphylococcus aureus* to the action of penicillin. *Exp Biol Med (Maywood)*. 1942;**51**:386–389. DOI: 10.3181/00379727-51-13986
- [31] Kirby, WM. Extraction of highly potent penicillin inactivator from penicillin resistant *Staphylococci*. *Science*. 1942;**99**:452–453. DOI: 10.1126/science.99.2579.452
- [32] Bondi JA, Dietz CC. Penicillin resistant *Staphylococci*. *Proc Royal Soc Exper Biol Med*. 1945;**60**:55–58. PMID: 21004029
- [33] Chambers HF. The changing epidemiology of *Staphylococcus aureus*? *Emerg Infect Dis*. 2001;**7**:178–182. DOI: 10.3201/eid0702.700178
- [34] Lowy FD. Antimicrobial resistance: the example of *Staphylococcus aureus*. *J Clin Invest*. 2003;**111**:1265–1273. DOI: 10.1172/JCI18535
- [35] Jevons PM. “Celbenin” – resistant *Staphylococci*. *Br Med J*. 1961;**1**:124–125. PMID: PMC1952889
- [36] Parker MT, Hewitt JH. Methicillin resistant *Staphylococcus aureus*. *Lancet*. 1970;**295**:800–804.
- [37] Ayliffe GAJ. The progressive intercontinental spread of methicillin-resistant *Staphylococcus aureus*. *Clin Infect Dis*. 1997;**24**:S74–S79. PMID: 8994782
- [38] Hartman A, Tomasz B. Altered penicillin-binding proteins in methicillin-resistant strains of *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 1981;**19**:726–735. PMID: PMC181513

- [39] Wielders CLC, Fluit AC, Brisse S, Verhoef J, Schmitz FJ. *mecA* Gene is widely disseminated in *Staphylococcus aureus* population. J Clin Microbiol. 2002;**40**:3970–3975. PMID: PMC139644
- [40] Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H, Spratt BG. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). Proc Natl Acad Sci U S A. 2002;**99**:7687–7692. DOI: 10.1073/pnas.122108599
- [41] Hiramatsu K, Katayama Y, Matsuo M, Sasaki T, Morimoto Y, Sekiguchi A, Baba T. Multi-drug-resistant *Staphylococcus aureus* and future chemotherapy. J Infect Chemother. 2014;**20**:593–601. DOI: 10.1016/j.jiac.2014.08.001
- [42] Ito T. Classification of Staphylococcal Cassette Chromosome *mec* (SCC*mec*): guidelines for reporting novel SCC*mec* elements. Antimicrob Agents Chemother. 2009;**53**(12):4961–4967. DOI: 10.1128/AAC.00579-09
- [43] Ito T, Kuwahara-Arai K, Katayama Y, Uehara Y, Han X, Kondo Y, Hiramatsu K. Staphylococcal Cassette Chromosome *mec* (SCC*mec*) analysis of MRSA. Methods Mol Biol. 2014;**1085**:131–148. DOI: 10.1007/978-1-62703-664-1_8
- [44] Naimi TS, LeDell KH, Como-Sabetti K, Borchardt SM, Boxrud DJ, Etienne J, Johnson SK, Vandenesch F, MD, Fridkin S, O'Boyle C, Danila RN, Lynfield R. Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. JAMA. 2003;**290**:2976–2984. DOI: 10.1001/jama.290.22.2976
- [45] Fridkin SK, Hageman JC, Morrison M, Sanza LT, Como-Sabetti K, Jernigan JA, Harriman K, Harrison LH, Lynfield R, Farley MM. Methicillin-resistant *Staphylococcus aureus* disease in three communities. N Engl J Med. 2005;**352**:1436–1444. DOI: 10.1056/NEJMoa043252
- [46] Hiramatsu K, Katayama Y, Yukawa H, Ito T. Molecular genetics of methicillin-resistant *Staphylococcus aureus*. Int J Med Microbiol. 2002;**292**:67–74. DOI: 10.1078/1438-4221-00192
- [47] Hiramatsu K, Cui L, Kuroda M, Ito T. The emergence and evolution of methicillin-resistant *Staphylococcus aureus*. Trends Microbiol. 2001;**9**:486–493. PMID: 11597450
- [48] Deresinski S. Methicillin-resistant *Staphylococcus aureus*: an evolutionary, epidemiologic, and therapeutic odyssey. Clin Infect Dis. 2005;**40**:562–573. DOI: 10.1086/427701
- [49] Chambers HF, DeLeo FR. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. Nat Rev Microbiol. 2009;**7**:629–641. DOI: 10.1038/nrmicro2200
- [50] Uhlemann AC, Otto M, Lowy FD, DeLeo FR. Evolution of community- and healthcare-associated methicillin-resistant *Staphylococcus aureus*. Infect Genet Evol. 2014;**21**:563–574. DOI: 10.1016/j.meegid.2013.04.030
- [51] David MZ, Daum RS. Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev. 2010;**23**:616–687. DOI: 10.1128/CMR.00081-09

- [52] Morrison MA, Hageman JC, Klevens RM. Case definition for community-associated methicillin-resistant *Staphylococcus aureus*. J Hosp Infect. 2006;**62**:241. DOI: 10.1016/j.jhin.2005.07.011
- [53] DeLeo FR, Otto M, Kreiswirth BN, Chambers HF. Community-associated methicillin-resistant *Staphylococcus aureus*. Lancet. 2010;**375**:1557–1568. DOI: 10.1016/S0140-6736(09)61999-1
- [54] King MD, Humphrey BJ, Wang YF, Kourbatova EV, Ray SM, Blumberg HM. Emergence of community-acquired methicillin-resistant *Staphylococcus aureus* USA 300 clone as the predominant cause of skin and soft-tissue infections. Ann Intern Med. 2006;**144**:309–317. PMID: 16520471
- [55] Sowash MG, Uhlemann AC. Community-associated methicillin-resistant *Staphylococcus aureus* case studies. Methods Mol Biol. 2014;**1085**:25–69. DOI: 10.1007/978-1-62703-664-1_2
- [56] Tokajian S. New epidemiology of *Staphylococcus aureus* infections in the Middle East. Clin Microbiol Infect. 2014;**20**:624–628. DOI: 10.1111/1469-0691.12691
- [57] Hageman JC, Patel J, Franklin P, Miscavish K, McDougal L, Lonsway D, Khan FN. Occurrence of a USA300 vancomycin- intermediate *Staphylococcus aureus*. Diagn Microbiol Infect Dis. 2008;**62**:440–442. DOI: 10.1016/j.diagmicrobio.2008.08.005
- [58] Mediavilla JR, Chen L, Mathema B., Kreiswirth BN. Global epidemiology of community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA). Curr Opin Microbiol. 2012;**15**(5):588–595. DOI: 10.1016/j.mib.2012.08.003
- [59] Fletcher C. First clinical use of penicillin. Br Med J. 1984;**289**:1721–1723. PMCID: PMC1444782
- [60] Chain E, Florey HW, Adelaide MB, Gardner AD, Heatley NG, Jennings MA, Orr-Ewing J, Sanders AG. Penicillin as a chemotherapeutic agent. Lancet. 1940;**ii**:226–228. DOI: 10.1016/S0140-6736(01)08728-1
- [61] Klein E, Smith DL, Laxminarayanan R. Hospitalizations and deaths caused by methicillin-resistant *Staphylococcus aureus*, United States, 1999–2005. Emerg Infect Dis. 2007;**13**:1840–1846. DOI: 10.3201/eid1312.070629
- [62] Köck R, Becker K, Cookson B, van Gemert-Pijnen JE, Harbarth S, Kluytmans J, Mielke M, Peters G, Skov RL, Struelens MJ, Tacconelli E, Navarro Torné A, Witte W, Friedrich AW. Methicillin-resistant *Staphylococcus aureus* (MRSA): burden of disease and control challenges in Europe. Euro Surveill. 2010;**14**:15:19688. PMID: 20961515
- [63] Emmerson AM, Jones AM.. The quinolones: decades of development and use. J Antimicrob Chemother. 2003;**51**:13–20. DOI: 10.1093/jac/dkg208
- [64] King DE, Malone R, Lilley SH. New classification and update on the quinolone antibiotics. Am Fam Physician 2000;**61**:2741–2748. PMID: 10821154
- [65] Ball P. Quinolone generations: natural history or natural selection? J Antimicrob Chemother. 2000;**46**:17–24. PMID: 10997595

- [66] Hooper DC. Mode of action of fluoroquinolones. *Drugs*. 1999;**58**:6–10. PMID: 10553698
- [67] Hooper DC. Mechanisms of action and resistance of older and newer fluoroquinolones. *Clin Infect Dis*. 2000;**31**:S24–S28. DOI: 10.1086/314056
- [68] Raviglione MC, Boyle JF, Mariuz P, Pablos-Mendez A, Cortes H, Merlo A. Ciprofloxacin-resistant methicillin-resistant *Staphylococcus aureus* in an acute-care hospital. *Antimicrob Agents Chemother*. 1990;**34**:2050–2054. PMID: 171997
- [69] Weber SG, Gold HS, Hooper DC, Karchmer AW, Carmeli Y. Fluoroquinolones and the risk for methicillin-resistant *Staphylococcus aureus* in hospitalized patients. *Emerg Infect Diseases*. 2003;**9**:1415–1422. DOI: 10.3201/eid0911.030284
- [70] Dalhoff A, Schubert S. Dichotomous selection of high-level oxacillin resistance in *Staphylococcus aureus* by fluoroquinolones. *Int J Antimicrob Agents*. 2010;**36**:216–221. DOI: 10.1016/j.ijantimicag.2010.04.014
- [71] Jones RN, Mendes RE, Sader H. Ceftaroline activity against pathogens associated with complicated skin and skin structure infections: results from an international surveillance study. *J Antimicrob Chemother*. 2010;**65**:iv17–iv31. DOI: 10.1093/jac/dkq252
- [72] Dalhoff A. Global fluoroquinolone resistance epidemiology and implications for clinical use. *Interdiscip Perspect Infect Dis*. 2012;**2012**:976273. DOI: 10.1155/2012/976273
- [73] Levine DP. Vancomycin: a history. *Clin Infect Dis*. 2006;**42**:S5–S12. DOI: 10.1086/491709
- [74] Kirst HA. Historical yearly usage of vancomycin. *Antimicrob Agents Chemother*. 1998;**42**:1303–1304. PMID: 105816
- [75] Moellering Jr RC. Vancomycin: a 50-year reassessment. *Clin Infect Dis*. 2005;**42**(Suppl. 1):S3–S4. DOI: 10.1086/491708
- [76] Rodvold KA, McConeghy KW. Methicillin resistant *Staphylococcus aureus* therapy: past, present and future. *Clin Infect Dis*. 2014;**58**:S20–S27. DOI: 10.1093/cid/cit614
- [77] Courvalin P. Vancomycin resistance in Gram-positive cocci. *Clin Infect Dis*. 2006;**42**:S25–S34. DOI: 10.1086/491711
- [78] Howden BP, Davies JK, Johnson PDR, Stinear TP, Grayson ML. Reduced vancomycin susceptibility in *Staphylococcus aureus*, including vancomycin-intermediate and heterogeneous vancomycin intermediate strains: resistance mechanisms, laboratory, detection, and clinical implications. *Clin Microbiol Rev*. 2010;**23**:99–139. DOI: 10.1128/CMR.00042-09
- [79] Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC. Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. *J Antimicrob Chemother*. 1997;**40**:135–136. PMID: 9249217
- [80] Appelbaum PC. Reduced glycopeptide susceptibility in methicillin-resistant *Staphylococcus aureus* (MRSA). *Int J Antimicrob Agents*. 2007;**30**:398–408. DOI: 10.1016/j.ijantimicag.2007.07.011

- [81] Appelbaum PC. The emergence of vancomycin-intermediate and vancomycin-resistant *Staphylococcus aureus*. *Clin Microbiol Infect*. 2006;**12**:16–23. DOI:10.1111/j.1469-0691.2006.01344.x
- [82] Cui L, Ma X, Sato K, Okuma K, Tenover FC, Mamizuka EM, Gemmell CG, Kim MN, Ploy MC, El-Solh N, Ferraz V, Hiramatsu K. Cell wall thickening is a common feature of vancomycin resistance in *Staphylococcus aureus*. *J Clin Microbiol*. 2003;**41**:5–14. PMID: PMC149586
- [83] Gardete S and Tomasz A. Mechanisms of vancomycin resistance in *Staphylococcus aureus*. *J Clin Invest*. 2014;**124**:2836–2840. PMID: PMC149586
- [84] Satola SW, Farley MM, Anderson KF, Patel JB. Comparison of detection methods for heteroresistant vancomycin-intermediate *Staphylococcus aureus*, with the population analysis profile method as the reference method. *J Clin Microbiol*. 2011;**49**:177–183. DOI: 10.1128/JCM.01128-10
- [85] Riederer K, Shemes S, Chase P, Musta A, Mar A. Detection of intermediately vancomycin-susceptible and heterogeneous *Staphylococcus aureus* isolates: comparison of Etest and Agar screening methods. *J Clin Microbiol*. 2011;**49**(6):2147–2150. DOI: 10.1128/JCM.01435-10
- [86] Ford BA. Identification of low-level vancomycin resistance in *Staphylococcus aureus* in the era of informatics. *J Clin Microbiol*. 2016;**54**:836–839. DOI: 10.1128/JCM.00071-16
- [87] Zhang S, Sun X, Chang W, Dai Y, Ma X. Systematic review and meta-analysis of the epidemiology of vancomycin-intermediate and heterogeneous vancomycin-intermediate *Staphylococcus aureus* isolates. *PLoS One*. 2015;**10**:e0136082. DOI: 10.1371/journal.pone.0136082
- [88] Pai MP, Neely M, Rodvold KA, Lodise TP. Innovative approaches to optimizing the delivery of vancomycin in individual patients. *Adv Drug Deliv Rev*. 2014;**77**:50–57. DOI: 10.1016/j.addr.2014.05.016
- [89] Álvarez R, López Cortés LE, Molina J, Cisneros JM, Pachón J. Optimizing the clinical use of vancomycin. *Antimicrob Agents Chemother*. 2016;**60**:2601–2609. DOI: 10.1128/AAC.03147-14
- [90] Sievert DM *et al.*, CDC. *Staphylococcus aureus* Resistant to vancomycin – United States, MMWR. 2002;**51**:565–567. PMID: 12139181
- [91] Sievert DM, Rudrik JT, Patel JB, McDonald LC, Wilkins MJ, Hageman JC. Vancomycin-resistant *Staphylococcus aureus* in the United States, 2002–2006. *Clin Infect Dis*. 2008;**46**:668–674. DOI: 10.1086/527392
- [92] Depardieu F, Podglajen I, Leclercq R, Collatz E, Courvalin P. Modes and modulations of antibiotic resistance gene expression. *Clin Microbiol Rev*. 2007;**20**:79–114. DOI: 10.1128/CMR.00015-06
- [93] Périchon B, Courvalin P. VanA-type Vancomycin-resistant *Staphylococcus aureus*^v. *Antimicrob Agents Chemother*. 2009;**53**:4580–4587. DOI: 10.1128/AAC.00346-09

- [94] Then RL, Kohl I, Burdeska A.. Frequency and transferability of trimethoprim and sulfonamide resistance in methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. J Chemother. 1992;4(2):67–71. PMID: 1629749
- [95] 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(6):763–770. PMID: 10837427
- [96] Schmitz FJ, Krey A, Sadurski R, Verhoef J, Milatovic D, Fluit AC; European SENTRY Participants. Resistance to tetracycline and distribution of tetracycline resistance genes in European *Staphylococcus aureus* isolates. J Antimicrob Chemother. 2001;47:239–240. DOI: 10.1093/jac/47.2.239
- [97] Fluit AC, Florijn A, Verhoef J, Milatovic D. Presence of tetracycline resistance determinants and susceptibility to tigecycline and minocycline. Antimicrob Agents Chemother. 2005;49:1636–1638. DOI: 10.1128/AAC.49.4.1636-1638.2005
- [98] Storrs MJ, Courvalin P, Foster TJ. Genetic analysis of gentamicin resistance in methicillin- and gentamicin-resistant strains of *Staphylococcus aureus* isolated in Dublin hospitals. Antimicrob Agents Chemother. 1988;32:1174–1181. PMCID: PMC172372
- [99] Freitas FI, Guedes-Stehling E, Siqueira-Júnior JP. Resistance to gentamicin and related aminoglycosides in *Staphylococcus aureus* isolated in Brazil. Lett Appl Microbiol. 1999;29:197–201. PMID: 10530041
- [100] Schmitz FJ, Fluit AC, Gondolf M, Beyrau R, Lindenlauf E, Verhoef J, Heinz HP, Jones ME. The prevalence of aminoglycoside resistance and corresponding resistance genes in clinical isolates of staphylococci from 19 European hospitals. J Antimicrob Chemother. 1999;43:253–259. PMID: 11252331
- [101] Fayyaz M, Mirza IA, Ahmed Z, Abbasi SA, Hussain A, Ali S. In vitro susceptibility of chloramphenicol against methicillin-resistant *Staphylococcus aureus*. J Coll Physicians Surg Pak. 2013;23:637–640. DOI: 09.2013/JCPSP.637640
- [102] Frank AL, Marcinak JF, Mangat PD, Tjho JT, Kelkar S, Schreckenberger PC, Quinn JP. Clindamycin treatment of methicillin-resistant *Staphylococcus aureus* infections in children. Pediatr Infect Dis J. 2002;21:530–534. PMID: 12182377
- [103] Putnam CD, Reynolds MS. Mupirocin: a new topical therapy for impetigo. J Pediatr Health Care. 1989;3:224–227. PMID: 2502615
- [104] Parenti MA, Hatfield SM, Leyden JJ. Mupirocin: a topical antibiotic with a unique structure and mechanism of action. Clin Pharm. 1987;6:761–770. PMID: 3146455
- [105] Ward A, Campoli-Richards DM. Mupirocin. A review of its antibacterial activity, pharmacokinetic properties and therapeutic use. Drugs. 1986;32:425–444. PMID: 3098541
- [106] Villiger JW, Robertson WD, Kanji K, Ah Chan M, Fetherston J, Hague IK, Haycock D, Hunter P. A comparison of the new topical antibiotic mupirocin ('Bactroban') with oral

- antibiotics in the treatment of skin infections in general practice. *Curr Med Res Opin.* 1986;**10**:339–345. DOI: 10.1185/03007998609111100
- [107] Bork K, Brauers J, Kresken M. Efficacy and safety of 2% mupirocin ointment in the treatment of primary and secondary skin infections—an open multicentre trial. *Br J Clin Pract.* 1989;**43**:284–288. PMID: 2516463
- [108] Rode H, Hanslo D, de Wet PM, Millar AJ, Cywes S. Efficacy of mupirocin in methicillin-resistant *Staphylococcus aureus* burn wound infection. *Antimicrob Agents Chemother.* 1989;**33**:1358–1361. PMID: 172654
- [109] Gaspar MC, Uribe P, Sánchez P, Coello R, Cruzet F. Hospital personnel who are nasal carriers of methicillin-resistant *Staphylococcus aureus*. Usefulness of treatment with mupirocin. *Enferm Infecc Microbiol Clin.* 1992;**10**:107–110. PMID: 1643130.
- [110] van Rijen M, Bonten M, Wenzel R, Kluytmans J. Mupirocin ointment for preventing *Staphylococcus aureus* infections in nasal carriers. *Cochrane Database Syst Rev.* 2008;**8**:CD006216. DOI: 10.1002/14651858.CD006216.pub2.
- [111] Godtfredsen W, Roholt K, Tybring L. Fucidin: a new orally active antibiotic. *Lancet.* 1962;**1**:928–931. PMID: 13899434
- [112] Dobie D, Gray J. Fusidic acid resistance in *Staphylococcus aureus*. *Arch Dis Child.* 2004;**89**:74–77. DOI: 10.1136/adc.2003.019695
- [113] Wilkinson JD. Fusidic acid in dermatology. *Br J Dermatol.* 1998;**139**:37–40. PMID: 9990411
- [114] Morley PA, Munot LD. A comparison of sodium fusidate ointment and mupirocin ointment in superficial skin sepsis. *Curr Med Res Opin.* 1988;**11**:142–148. DOI: 10.1185/03007998809110457.
- [115] White DG, Collins PO, Rowsell RB. Topical antibiotics in the treatment of superficial skin infections in general practice—a comparison of mupirocin with sodium fusidate. *J Infect.* 1989;**18**:221–229. PMID: 2501394
- [116] Wood MJ. The comparative efficacy and safety of teicoplanin and vancomycin. *J Antimicrob Chemother.* 1996;**37**:209–222. DOI: 10.1093/jac/37.2.209
- [117] Svetitsky S, Leibovici L, Paul M. Comparative efficacy and safety of vancomycin versus teicoplanin: systematic review and meta-analysis. *Antimicrob Agents Chemother.* 2009;**53**:4069–4079. DOI: 10.1128/AAC.00341-09
- [118] Dodds TJ, Hawke CI. Linezolid versus vancomycin for MRSA skin and soft tissue infections (systematic review and meta-analysis). *ANZ J Surg.* 2009;**79**:629–635. DOI: 10.1111/j.1445-2197.2009.05018.x
- [119] Kalil AC, Murthy MH, Hermsen ED, Neto FK, Sun J, Rupp ME. Linezolid versus vancomycin or teicoplanin for nosocomial pneumonia: a systematic review and meta-analysis. *Crit Care Med.* 2010;**38**:1802–1808. DOI: 10.1097/CCM.0b013e3181eb3b96

- [120] Eckmann C, Dryden M. Treatment of complicated skin and soft-tissue infections caused by resistant bacteria: value of linezolid, tigecycline, daptomycin and vancomycin. *Eur J Med Res.* 2010;15:554–563. DOI: 10.1186/2047-783X-15-12-554
- [121] Deresinski S. Counterpoint: vancomycin and *Staphylococcus aureus* – an antibiotic enters obsolescence. *Clin Infect Dis.* 2007;44:1543–1548. DOI: 10.1086/518452
- [122] Dhand A, Sakoulas G. Reduced vancomycin susceptibility among clinical *Staphylococcus aureus* isolates ('the MIC Creep'): implications for therapy. *F1000 Med Rep.* 2012;4:4. DOI: 10.3410/M4-4
- [123] Micek ST. Alternatives to vancomycin for the treatment of methicillin-resistant *Staphylococcus aureus* Infections. *Clin Infect Dis.* 2007;45:S184–S190. DOI: 10.1086/519471.
- [124] Ohlsen K. Novel Antibiotics for the Treatment of *Staphylococcus aureus*. *Expert Rev Clin Pharmacol.* 2009;2:661–672. DOI: 10.1586/ecp.09.26
- [125] Kurosu M, Siricilla S, Mitachi K. Advances in MRSA drug discovery: where are we and where do we need to be? *Expert Opin Drug Discov.* 2013;8:1095–1116. DOI: 10.1517/17460441.2013.807246
- [126] Stevens DL, Dotter B, Madaras-Kelly K. A review of linezolid: the first oxazolidinone antibiotic. *Expert Rev Anti Infect Ther.* 2004;2:51–59. PMID: 15482171
- [127] Watkins RR, Lemonovich TL, File TM. An evidence-based review of linezolid for the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA): place in therapy. *Core Evidence.* 2012;7:131–143. DOI: 10.2147/CE.S33430
- [128] Alder JD. Daptomycin: a new drug class for the treatment of Gram-positive infections. *Drugs Today (Barc).* 2005;41:81–90. DOI: 10.1358/dot.2005.41.2.882660
- [129] Steenbergen JN, Alder J, Thorne GM, Tally FP. Daptomycin: a lipopeptide antibiotic for the treatment of serious Gram-positive infections. *J Antimicrob Chemother.* 2005;55:283–288. DOI: 10.1093/jac/dkh546
- [130] Doan TL, Fung HB, Mehta D, Riska PF. Tigecycline: a glycyclin antimicrobial agent. *Clin Ther.* 2006;28:1079–1106. DOI: 10.1016/j.clinthera.2006.08.011
- [131] Stein GE, Craig WA. Tigecycline: a critical analysis. *Clin Infect Dis.* 2006;43:518–524. DOI: 10.1086/505494
- [132] Laudano JB. Ceftaroline fosamil: a new broad-spectrum cephalosporin. *J Antimicrob Chemother.* 2011;66:iii11–iii18. DOI: 10.1093/jac/dkr095
- [133] Shirley DA, Heil EL, Johnson JK. Ceftaroline fosamil: a brief clinical review. *Infect Dis Ther.* 2013;2:95–110. DOI: 10.1007/s40121-013-0010-x
- [134] Scott LJ. Telavancin: a review of its use in patients with nosocomial pneumonia. *Drugs.* 2013;73:1829–1839. DOI: 10.1007/s40265-013-0144-x.

- [135] Sandrock CE, Shorr AF. The role of telavancin in hospital-acquired pneumonia and ventilator-associated pneumonia. *Clin Infect Dis*. 2015;**61**:S79–S86. DOI: 10.1093/cid/civ535
- [136] Wong E, Rab S. Tedizolid phosphate (sivextro): a second-generation oxazolidinone to treat acute bacterial skin and skin structure infections. *P T*. 2014;**39**:555–579. DOI: PMCID: PMC4123804
- [137] Rybak JM, Roberts K.. Tedizolid Phosphate: a next-generation oxazolidinone. *Infect Dis Ther*. 2015;**4**:1–14. DOI: 10.1007/s40121-015-0060-3
- [138] Juul JJ, Mullins CF, Peppard WJ, Huang AM. New developments in the treatment of acute bacterial skin and skin structure infections: considerations for the effective use of dalbavancin. *Ther Clin Risk Manag*. 2016;**12**:225–232. DOI: 10.2147/TCRM.S71855
- [139] Leuthner KD, Buechler KA, Kogan D, Saguros A, Lee HS. Clinical efficacy of dalbavancin for the treatment of acute bacterial skin and skin structure infections (ABSSSI). *Ther Clin Risk Manag*. 2016;**12**:931–940. DOI: 10.2147/TCRM.S86330
- [140] Markham A. Oritavancin: first global approval. *Drugs*. 2014;**74**:1823–1828. DOI: 10.1007/s40265-014-0295-4
- [141] Mattox J, Belliveau P, Durand C. Oritavancin: a novel lipoglycopeptide. *Consult Pharm*. 2016;**31**(2):86–95. DOI: 10.4140/TCP.n.2016.86
- [142] Cegelski L, Marshall GR, Eldridge GR, Hultgren SJ. The biology and future prospects of antivirulence therapies. *Nat. Rev. Microbiol*. 2008;**6**:17–27. DOI: 10.1038/nrmicro1818
- [143] Rasko DA, Sperandio V. Anti-virulence strategies to combat bacteria-mediated disease. *Nat Rev Drug Discov*. 2010;**9**:117–128. DOI: 10.1038/nrd3013
- [144] Queck SY, Jameson-Lee M, Villaruz AE, Bach TH, Khan BA, Sturdevant DE, Ricklefs SM, Li M, Otto M.. RNAlII-independent target gene control by the agr quorum-sensing system: insight into the evolution of virulence regulation in *Staphylococcus aureus*. *Mol Cell*. 2008;**32**:150–158. DOI: 10.1016/j.molcel.2008.08.005
- [145] Singh R, Ray P. Quorum sensing-mediated regulation of staphylococcal virulence and antibiotic resistance. *Future Microbiol*. 2014;**9**:669–681. DOI: 10.2217/fmb.14.31.
- [146] Sully EK, Malachowa N, Elmore BO, Alexander SM, Femling JK, Gray BM, DeLeo FR, Otto M, Cheung AL, Edwards BS, Sklar LA, Horswill AR, Hall PR, Gresham HD. Selective chemical inhibition of agr quorum sensing in *Staphylococcus aureus* promotes host defense with minimal impact on resistance. *PLoS Pathogens*. 2014;**10**:e1004174. 10.1371/journal.ppat.1004174
- [147] Giersing BK, Dastghey SS, Modjarrad K, Moorthy V. Status of vaccine research and development of vaccines for *Staphylococcus aureus*. *Vaccine*. 2016;**34**:2962–2966. DOI: 10.1016/j.vaccine.2016.03.110

- [148] Kali A. Antibiotics and bioactive natural products in treatment of methicillin resistant *Staphylococcus aureus*: a brief review. *Pharmacogn Rev.* 2015;**9**:29–34. DOI: 10.4103/0973-7847.156329
- [149] Sujina I, Prabhu V, Hemlal H, Ravi S. Essential oil composition, isolation of β -asarone and its antibacterial and MRSA activity from the rhizome of *Acorus calamus*. *J Pharm Res.* 2012;**5**:3437–3440.
- [150] Shin DY, Kim HS, Min KH, Hyun SS, Kim SA, Huh H, Choi EC, Choi YH, Kim J, Choi SH, Kim WB, Suh YG. Isolation of a potent anti-MRSA sesquiterpenoid quinone from *Ulmus davidiana* var. *japonica*. *Chem Pharm Bull (Tokyo).* 2000;**48**:1805–1806. PMID: 11086922
- [151] Sasaki H, Kashiwada Y, Shibata H, Takaishi Y. Prenylated flavonoids from *Desmodium caudatum* and evaluation of their anti-MRSA activity. *Phytochemistry.* 2012;**82**:136–142. DOI: 10.1016/j.phytochem.2012.06.007
- [152] Hariharan P, Paul-Satyaseela M, Gnanamani A. In vitro profiling of antimethicillin-resistant *Staphylococcus aureus* activity of thymoquinone against selected type and clinical strains. *Lett Appl Microbiol.* 2016;**62**:283–289. DOI: 10.1111/lam.12544