Infection and Drug Resistance

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REVIEW

Progress in the Prevalence, Classification and Drug Resistance Mechanisms of Methicillin-Resistant *Staphylococcus aureus*

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Abstract: *Staphylococcus aureus* is a common human pathogen with a variety of virulence factors, which can cause multiple infectious diseases. In recent decades, due to the constant evolution and the abuse of antibiotics, *Staphylococcus aureus* was becoming more resistant, the infection rate of MRSA remained high, and clinical treatment of MRSA became more difficult. The genetic diversity of MRSA was mainly represented by the continuous emergence of epidemic strains, resulting in the constant changes of epidemic clones. Different classes of MRSA resulted in different epidemics and resistance characteristics, which could affect the clinical symptoms and treatments. MRSA had also spread from traditional hospitals to community and livestock environments, and the new clones established a relationship between animals and humans, promoting further evolution of MRSA. Since the resistance mechanism of MRSA is very complex, it is important to clarify these resistance mechanisms at the molecular level for the treatment of infectious diseases. We firstly described the diversity of SCC*mec* elements, and discussed the types of SCC*mec*, its drug resistance mechanisms and expression regulations. Then, we described how the *vanA* operon makes *Staphylococcus aureus* resistant to vancomycin and its expression regulation. Finally, a brief introduction was given to the drug resistance mechanisms of biofilms and efflux pump systems. Analyzing the resistance mechanism of MRSA can help study new anti-infective drugs and alleviate the evolution of MRSA. At the end of the review, we summarized the treatment strategies for MRSA infection, including antibiotics, anti-biofilm agents and efflux pump inhibitors. To sum up, here we reviewed the epidemic characteristics of *Staphylococcus aureus*, summarized its classifications, drug resistance mechanisms of MRSA (SCC*mec* element, *vanA* operon, biofilm and active efflux pump system) and novel therapy strategies, so as to provide a theoretical basis for the treatment of MRSA infection.

Keywords: biofilm, efflux pump, MRSA, prevalence, resistance mechanism, SCC*mec*

Introduction

Staphylococcus aureus (*S. aureus*) is a kind of gram-positive conditional pathogen that widely exists in human living environment. It can asymptotically colonize the nasal cavity of normal humans. However, when the body's immune function is low, it can infect local skin and soft tissues, and even enter deep tissues and blood, causing systemic infections such as pneumonia, endocarditis, osteomyelitis and even bacteremia.[1] According to the molecular epidemiological evidences, methicillin-susceptible *Staphylococcus aureus* (MSSA) becomes methicillin-resistant *Staphylococcus aureus* (MRSA) after evolving several times. MRSA is one type of *S. aureus* carrying *mecA/mecC* gene or oxacillin MIC value≥4μg/mL,[2] which has become a multidrug-resistant bacterium that seriously threatens human health. With rapid spread and complex drug resistance mechanisms, case fatality rate in patients with clinical MRSA infection is high. Nearly 150,000 MRSA infections were reported annually in European Union countries, resulting in more than 7000

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deaths.[3] And in China, the infection rate of MRSA had been maintained at over 30% for the past five years according to data from CHINET surveillance system. Otherwise, bacteremia caused by MRSA infection is a common cause of global bloodstream infections, with a mortality rate of 32.4%, and even higher in developing countries.[4] Therefore, it is extremely important to understand the prevalence of MRSA and explore strategies for preventing and treating MRSA infection. In this paper, the prevalence status and resistance mechanisms of MRSA are reviewed below.

Epidemiology of MRSA

Biological Properties of *S. aureus*

S. aureus is a gram-positive bacterium belonging to the staphylococcal family. It is spherical in shape with 1μm in diameter and is named after the grape-like colony with gold pigmentation. *S. aureus* is positive for coagulase, mannitol ferment tests and DNAase tests, so it can not only decompose a variety of sugars to produce acid without gas, but also decompose mannitol and produce coagulase.[5] *S. aureus* has low requirements for the living environment, both aerobic and facultative anaerobic, and the optimal growth conditions are 37°C and pH 7.4. On ordinary plates, *S. aureus* can form thick, shiny, and round with 1~2mm in diameter colonies; on blood agar plates, there is a transparent hemolytic ring around each *S. aureus* colony.[6] The cell wall of *S. aureus* is a single lipid membrane, consisting of 50% peptidoglycan, 40% lipid membrane acid, and 10% surface proteins, exoproteins, and autolytic proteins.[7]

S. aureus can live symbiotic in the skin or mucous membranes of 30%~70% human bodies, especially in the anterior nasal cavity. When the skin or mucous membrane damages, it can infect wound to cause skin infection, and also can infect other organizations to cause pneumonia, bacteremia, endocarditis and so on.[1] In addition, *S. aureus* can produce a variety of virulence factors, mainly including pore-forming toxins, exfoliative toxins and superantigens. Pore-forming toxins include hemolysin-α, hemolysin-β, panton-valentine leukocidin and phenol-soluble modulins. These virulence factors evade the hosts' immune defense and cause different clinical manifestations. For example, panton-valentine leukocidin affects leukocytes and causes tissue necrosis and has been associated with furuncles, cutaneous abscesses and severe necrotic skin infections. Exfoliative toxins can induce skin peeling and blister formation. Superantigens can cause high fever, rash, desquamation, vomiting, diarrhea, hypotension, and can frequently result in multiple organ failure.[7]

Epidemiological Characteristic of MRSA

In 1959, methicillin, a semi-synthetic penicillin, was used clinically to treat *S. aureus* infections, and two years later, MRSA emerged in the United Kingdom.[8] Over the next decade, more and more MRSA strains were isolated in European countries such as Britain, Denmark, France and Switzerland. Some factors, such as unmanageable high-level colonization and infection, expensive preventive measures and overused antibiotics, lead to the increasing incidence of MRSA.[9] In the late 1980s, vancomycin was used to treat severe MRSA infections, and it was considered as the last line of defense against MRSA. In 1997, the first case of *S. aureus* with reduced vancomycin sensitivity was reported in Japan. And in 2002, the first vancomycin resistant *Staphylococcus aureus* (VRSA) strain was isolated in the United States.[10] The prevalence of VRSA increased from 2% before 2006 to 7% in 2015–2020 (Figure 1).[11]

Prevalence and Epidemic Typing of MRSA in China

According to the CHINET surveillance system (2013–2021), the detection rate of *S. aureus* has maintained high, but the infection rate of MRSA shows a downward trend (Figure 2A). The epidemic typing of MRSA has changed over time. In China, the dominant typing was ST239-t030-III before 2016.[12] Based on a national surveillance conducted in 2011, MRSA was mainly HA-MRSA, whose epidemic typing was ST239-t030-III (57.1%), ST239-t037-III (12.9%) and ST5-t002-II (8.1%).[13] But after 2016, it was mainly ST59-t437-IV.[12] A multicenter longitudinal study in 2022 showed that epidemic typing of MRSA was ST59-t437-IV (14.9%), ST239-t030-III (6.4%) and ST5-t2460-II (6.0%).[14] Besides, the epidemic typing varied in different administrative regions of China. Sichuan, Jiangxi, Fujian and Zhejiang were mainly ST59-t437-IV, Guangdong, Shanghai and Hubei were ST5-t2460-II, while in Inner Mongolia, mainly ST239-t030-III; in Hainan, ST45-IVa was dominant.[14–16] Figure 2B summarized the epidemic typing in the provinces of China over the past five years.

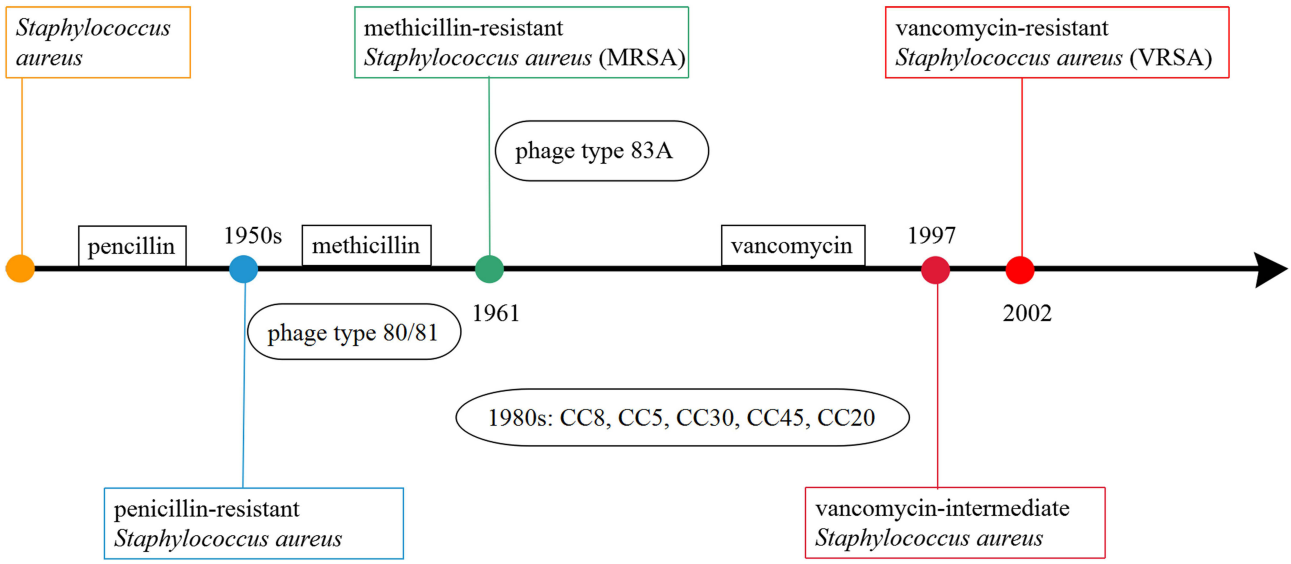

Figure 1 Evolution of drug resistance in *S. aureus*. As the antibiotic resistance of *S. aureus* evolved, so did epidemic typing. There had been several significant changes in epidemic typing around the whole world. In the 1950s, the epidemic typing was phage type 80/81; in 1960–1970s, it evolved into phage type 83A; in the 1980s, it evolved into five major epidemic typing CC8, CC5, CC30, CC45, and CC22.

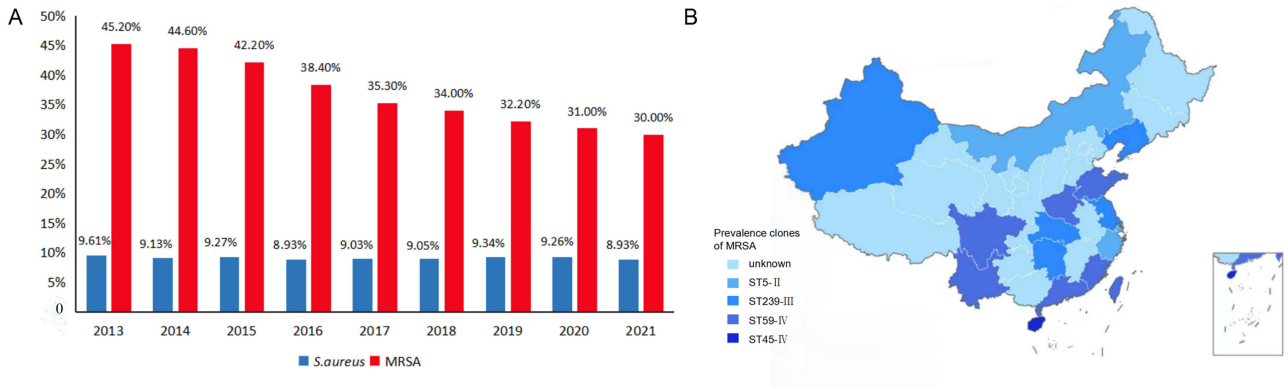

Figure 2 Prevalence and epidemic typing of MRSA in China. (**A**) The infection rate of MRSA in China (2014–2020). (**B**) The epidemic typing in some provinces of China over the past five years.

Classification of MRSA

According to the Epidemiological Classification

From the discovery in 1961 to the 1980s, MRSA was mainly transmitted in healthcares. Since 1990s, a new community-associated MRSA strain began to spread, and community-associated MRSA became an important infectious factor in the healthy population. In the early 2000s, livestock-associated MRSA was identified in domestic animals, and the food and production chains of livestock increased the spread of livestock-associated MRSA.

Healthcare-Associated MRSA (HA-MRSA)

Healthcare-associated MRSA is defined as a patient with MRSA infection found 48 hours after admission and one of the following three conditions: a history of surgery, hospitalization, or dialysis within one year; an indwelling catheter or percutaneous medical device; and a history of positive MRSA prior to this test.[17]

MRSA is prevalent in almost all healthcare facilities, and molecular epidemiology is commonly used to classify different clones and track the evolution and spread of MRSA across countries and healthcare facilities. Most HA-MRSA strains from different countries have the same genotype. In the 1950s, the epidemic typing was phage type 80/81; In

1960–1970s, it evolved into phage-type 83A; In the 1980s, it evolved into five major epidemic typing CC8, CC5, CC30, CC45, and CC22 (Figure 1).[5] The distribution of HA-MRSA clones varied with geographical location. In the United States, the most common clone type of HA-MRSA was USA100-spa t002-II, which was often multidrug-resistant, but it secreted lower levels of toxins making it less pathogenic.[18] However, in recent years, it has been reported that USA300 strain is gradually replacing USA100 as the main epidemic type of HA-MRSA.[19] Moreover, the epidemic type is mainly CC22-SCC*mec*IV (EMRSA-15) and CC30-SCC*mec*II (EMRSA-16) in the UK, CC5 and CC45-SCC*mec*IV in Germany and ST239-SCC*mec*III in South America and Asia.[20]

HA-MRSA is resistant to types of antibiotics and one symbol is resistant to fluoroquinolones in contrast to most CA-MRSA and LA-MRSA which are sensitive to fluoroquinolones. Fluoroquinolones are antibiotics that have a great influence on the incidence and clonal evolution of HA-MRSA. Varying fitness effects associated with high-level resistance to fluoroquinolones were demonstrated to confer an indirect growth advantage onto the international clone of HA-MRSA. All of the major international STs of HA-MRSA, such as ST5 and ST22, were shown to carry two typical quinolone-resistance determining regions (QRDR) mutations affecting the *gyrA* Ser84 and *grlA* Ser80 residues. Therefore, a decrease in the use of fluoroquinolones would result in a decline of these major clone strains yielding lower incidences.[21]

Community-Associated MRSA (CA-MRSA)

Community-associated MRSA is defined as a strain of MRSA isolated from an outpatient or inpatient within 48 hours of admission, who has not been exposed to the hospital environment within 6 months, has no history of *S. aureus* infection, has no central vascular catheter at the time of infection, and has not used antibiotics within 1 month.[22]

In the 1980s, Detroit reported the spread of CA-MRSA, and at that time, CA-MRSA was mainly confined to closed communities. By the late 1990s, CA-MRSA had emerged in the general healthy population. Most of these MRSA strains are monoclonal, which are susceptible to most non-β-lactam antibiotics, and generally infect healthy people with no risk factors.[23] In the early 2000s, USA300-SCC*mec*IV became the dominant CA-MRSA epidemic strains in the United States, and although USA300 had gained some resistance, its resistance was still lower than that of USA100 (the epidemic clone of HA-MRSA). In general, resistance to levofloxacin and clindamycin was considered to be a phenotypic symbol that can distinguish USA100 from USA300. Combination susceptibility to clindamycin and levofloxacin performed the best overall (sensitivity 80.7%, specificity 75.9%) to identify USA300.[24] In Asia, the infection rate of CA-MRSA could reach 2.5%~39%, and the main type was ST59.[25]

The main characteristic of CA-MRSA is the presence of panton-valentine leukocidin (PVL), which is associated with leukocyte toxins. PVL-positive CA-MRSA infection rates can reach 61.1%,[26] 70.4%,[27] and even 78.4%.[28] PVL induces the dissolution of monocytes and neutrophils, leading to leukocytosis and tissue necrosis, then causing skin and soft tissue infections, and even necrotizing pneumonia and necrotizing fasciitis, all of which increase the risk of sepsis.[26] Table 1 summarized the difference between HA-MRSA and CA-MRSA.

Table 1 The Difference Between HA-MRSA and LA-MRSA

Parameter	HA-MRSA	CA-MRSA
Risk population	Old individuals with prolonged hospital stay, poor immunity and other risk factors	Young and healthy individuals without risk factors
Main age	≥65years old	18–64 years old
Associated diseases	Bacteremia, pneumonia and other invasive infections	Skin and soft tissue infections, and lethal and severe infections such as necrotizing pneumonia and septicemia
PVL	PVL- (most common)	PVL+ (most common)
Resistance drug	Resistance to non-β-lactam antibiotics	Susceptible to most non-β-lactam antibiotics
Resistance genes	Many kinds of resistance genes	Usually not carry other antibiotic resistance genes
SCC*mec* element	SCC*mec*I, II, III	SCC*mec*IV, V
Common clone	China: **ST239**, ST5 America: USA100	China: **ST59**, ST8, ST30 America: USA300

Note: the bolded text, including ST239 and ST59, represents the most common clone.
Abbreviations: HA-MRSA, healthcare-associated MRSA; CA-MRSA, community-associated MRSA; SCC, staphylococcal cassette chromosome.

Livestock-Associated MRSA (LA-MRSA)

In 2004, the Netherlands reported a case from the daughter of a pig farmer, who infected a new MRSA strain.[29] This is the first human case of pig-associated MRSA. Because this type of MRSA is primarily associated with livestock, it is called livestock-associated MRSA (LA-MRSA). LA-MRSA has no host specificity, and pigs, cattle, sheep and various poultries can be important hosts of LA-MRSA.[30] It can be transmitted not only among domestic animals but also among humans, and the increasing international trade in livestock has facilitated the spread of LA-MRSA between animals and humans.[31] Studies have shown that LA-MRSA is easy to colonize in people who get along closely with animals, but the infection rate caused by LA-MRSA is low and the disease is mild.[29]

The epidemic typing of LA-MRSA in European and North American countries is ST398, while in China, that is ST9-t899.[32] The ST9 LA-MRSA has typical multidrug resistance and exhibits a different virulence profile from other LA-MRSA clones. The clone can be colonized in animals and humans and can be transmitted between animals and humans, but human-to-human transmission is unknown.[33] Studies have shown that the ST9 LA-MRSA is transferred from humans to animals through the loss of *scn, chp, sak* and other immune escape genes, the acquisition of the *vwb* gene encoding SAPiBOV4-like elements and the acquisition of antibiotic resistance genes.[34] These also proved that ST9 LA-MRSA had different virulence profiles and drug resistance profiles compared to other clones. ST398 MRSA in China mainly comes from human infection, He et al[22] separated and detected ST398 CA-MRSA and proved that this type of CA-MRSA evolved from human MSSA by uptaking of SCC*mec* elements. In recent years, ST398 LA-MRSA has been gradually isolated from milk,[35] and pigs in farms[36] in China. ST398 LA-MRSA is closely related to ST398 HA-MRSA, and the detection rate of MRSA ST398 among slaughterhouse workers is much higher than that of community residents. At the meanwhile, ST398 MRSA has also been detected in fish ponds and in air dust near the farms. Therefore, it is speculated that ST398 MRSA may be transmitted to humans from the production chains of animals, namely infected slaughterhouse workers, transport vehicles, animals' bodies, and animal food chains. In China, the prevalence of ST398 LA-MRSA needs to be closely monitored to protect public safety.

Connection Between HA-MRSA, CA-MRSA and LA-MRSA

In most cases, HA-MRSA, CA-MRSA and LA-MRSA strains have different evolution origins and belong to different clonal lineages. However, in the era of whole-genome sequencing, the traditional epidemiological and molecular typing discriminated MRSA into HA-, CA- and LA-MRSA are constantly changing owing to the considerable overlaps of identical clones between these groups, such as CA-MRSA spreads in hospital settings, and LA-MRSA can be transmitted to humans through the animal production chains. So, this classification based on epidemiological populations becomes ambiguous and inaccurate.

The distinction between HA-MRSA and CA-MRSA is increasingly blurred. Gittens-St Hilaire et al[37] isolated a HA-MRSA strain, which has antibacterial properties similar to CA-MRSA; Preeja et al[27] isolated some HA-MRSA strains, all of which 31.4% (16/51) were SCC*mec*IV and 25.5% (13/51) were SCC*mec*V. It is inferred that CA-MRSA has infiltrated into the hospital environment, and the circulation of MRSA mainly comes from the community, while the true incidence rate of HA-MRSA is very low. Nichol et al[28] studied the infection rate of MRSA in Canada from 2007 to 2016, and found that the infection rate of HA-MRSA decreased from 79.2% to 43.8%, while that of CA-MRSA increased from 20.8% to 56.3%. Similarly, the prevalence of HA-MRSA in Finland decreased from 87% (2007) to 57% (2016), and that of CA-MRSA increased from 13% to 43% at the same time.[38] Besides, Chen et al[39] found that ST59 (the main clone of CA-MRSA) replaced ST239 (HA-MRSA) as the epidemic typing of MRSA in China by using the whole-genome sequencing. Therefore, CA-MRSA is gradually replacing HA-MRSA as the main category of MRSA infection.

In addition, further researches on different epidemic clones have found that different clonal complexes have different genetic characteristics, exhibiting different virulence and drug resistance characteristics. For example, ST59 and ST398. Compared with other lineages (such as ST5 and ST239), ST59 and ST398 had a higher prevalence of the protease-associated genes *VSaβ, paiB*, and *cfim*, which enhanced proteolytic activity, and showed a higher expression of *RNAIII* and *psmα*, resulting in greater proficiency at causing cell lysis. They were strongly recognized by human neutrophils and caused more cell apoptosis and neutrophil extracellular trap degradation.[40] Moreover, ST398 displayed higher adaptability to human epidermal keratinocytes and a unique genetic arrangement inside the oligopeptide ABC transport system. And all members of *S. aureus* CC398 can cause human bloodstream infection.

According to the report, there were two genes (SAPIG0966 and SAPIG1525) conditionally essential for CC398 MRSA survival in porcine blood. They were carried on two different mobile genetic elements, the Tn916 transposon and a phage element, and were associated with antibiotic resistance and host adaptation, respectively.[41] Differently, ST59 harbors two major clones: the Taiwan clone, which causes severe infections and carries a PVL-encoding prophage φSa2, and the Asian-Pacific clone, which is typically commensal and carries a staphylokinase-encoding prophage φSa3 that enhances the bacterium's capacity to colonize human hosts.[42] ST59 had a higher expression level for *hlb* than the other STs, an important virulence factor in skin colonization and chronic inflammatory diseases.[40] Of note, there were lower numbers of antimicrobial resistance genes in ST59 than in ST239 or ST5 MRSA isolates, which was related to that ST59 clones were more antimicrobial susceptible than others.[43] Futhermore, for ST30 and ST45, LukAB toxin derived from them is cytotoxic to CD11b (cluster of differentiation molecule 11B)-depleted human monocytes, although binding of LukAB to phagocytes is mediated by CD11b. For ST239, higher expression of secreted protein A in it may contribute to the colonization and immune evasion phenotypes observed clinically.[44]

PVL is generally considered as a marker of CA-MRSA, and MRSA strains isolated from hospitalized patients with PVL-negative are considered as true HA-MRSA. However, Abou Shady et al[45] separated and studied CA-MRSA carried in the nasal cavity in Saudi Arabia and Egypt, and found that the positive rate of PVL gene was only 15% and 11.5%. Moreover, PVL is mediated by the *lukS-PV* and *lukF-PV* genes carried by φSa2 of phage. When homologous *S. aureus* co-colonizes, phages transfer frequently and recombination occurs among different phages. Meanwhile, φSa2 can propagate vertically with chromosomes during the replication process, and it can enter the lysis cycle and spread horizontally to another cells.[46] Therefore, PVL cannot be a key factor in distinguishing HA-MRSA from CA-MRSA in that PVL can be transferred horizontally among different MRSA strains.

According to Oxacillin and *mecA*

With the continuous use of antibiotics and long-term evolution, *S. aureus* has gradually emerged as strains with induced resistance and strains with high allogenic resistance to oxacillin. Some strains differ in phenotype and genotype.

Oxacillin-susceptible MRSA (OS-MRSA) is susceptible to oxacillin (MICs≤2μg/mL) but *mecA*/*mecC*-positive.[47] Because OS-MRSA is sensitive to oxacillin, routine drug susceptibility tests are prone to misidentify MRSA, resulting in potential treatment failure. Therefore, the combination of drug resistance phenotype and PCR genotype is more appropriate to identify MRSA. OS-MRSA exhibits hetero-resistance to oxacillin and is sensitive to most non-β-lactam antibiotics, which can be treated with linezolid and vancomycin.[48] In China, the most common clinical OS-MRSA clone is ST338-t437-SCC*mec*V, and most of the OS-MRSA isolates are susceptible to the majority of antibacterial agents except macrolides, clindamycin and chloramphenicol.[49]

Borderline oxacillin-resistant *Staphylococcus aureus* (BORSA), without PBP2a/2c encoded by *mecA*/*mecC* genes, shows low critical resistance to penicillin and the MIC to oxacillin is usually 1–8μg/mL. The generation of BORSA may be related to the overproduction of β-lactamase encoded by the plasmid, or the modification of PBP gene caused by spontaneous amino acid substitution in the transpeptidase domain.[50,51] In general, BORSA does not contain PVL sites that express leukocyte toxins, but Zehra et al[52] detected PVL in BORSA isolated from community and animal-derived foods. BORSA is becoming more and more common, which may affect the therapeutic response of MRSA infection. However, there is a lack of surveillance for BORSA, and the prevalence, epidemic typing and infection control measures of BORSA are unknown (Table 2).[53]

Resistance Mechanisms of MRSA

With the emergence of multidrug resistance of MRSA, the resistance mechanism has become more complex, including chromosome DNA mediated intrinsic resistance, plasmid mediated acquired resistance and active efflux system.

Table 2 The Difference Among Three Types of *S. aureus*

Types	*mecA*/*mecC*	Oxacillin	Oxacillin MICs	Cefoxitin	PVL	Coagulase
MRSA	+	R	≥ 4μg/mL	R	LA-MRSA:– CA-MRSA:+	+
OS-MRSA	+	S	≤ 2μg/mL	S	Rare	+
BORSA	–	L~R	1–8μg/mL	S	–	–

Abbreviations: R, resistance; S, susceptible; L~R, mild resistance.

SCC*mec*

The Structure and Function of SCC*mec*

MRSA is resistant to almost all β-lactam antibiotics, mainly because *S. aureus* acquired drug-resistant genomic island—staphylococcal cassette chromosome (SCC) elements, carring the *mecA*/*mecC* gene (SCCmec). The SCC*mec* element, a mobile genetic element, inserts into the chromosomes of sensitive strains and produces penicillin-binding protein (PBP2a/2c), which significantly reduces the binding affinity to β-lactam antibiotics, and thereby produces resistance to β-lactam antibiotics.[54]

SCC*mec* element is circular, generally 21–67 kb in size, and mainly includes *mec* gene complex, *ccr* gene complex and joining region (J region), that is orfX—J1 region—*mec* gene complex—J2 region—*ccr* gene complex—J3 region —direct repeats (DR).[55] Certified by International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC), SCC*mec* is divided into 14 types based on *mec* gene complex and *ccr* gene complex, SCC*mec*I—SCC*mec*XIV (Table 3).[54]

Table 3 SCC*mec* Element Type I to XIV

Types	*ccr* Gene Complex	*mec* Gene Complex	J Region	The First Strain	The Structure of First Strain	Other Characterizations
SCC*mec*I(1B)	1(A1B1)	B	J1, pls regulator; J3, plasmid pUB110	NCTC10442	34,359 nucleotides long—4 repeat regions, 2 mobile elements, and 41CDS	
SCC*mec*II(2A)	2(A2B2)	A	J1, *kdp* regulator; J3, plasmid pUB110	N315	53,017 nucleotides long—4 repeat regions, 3 mobile elements, and 51 CDS	
SCC*mec*III(3A)	3(A3B3)	A	J2, transposon ψTn554; J3, transposon Tn554, plasmid pT181	85/2082	66,896 nucleotides long—10 repeat regions, 6 mobile elements, and 97 CDS	The longest SCC*mec* element
SCC*mec*IV(2B)	2(A2B2)	B	J3, transposon Tn4001	CA05	24,244 nucleotides long—4 repeat regions, 2 mobile elements, and 22 CDS	The smallest SCC*mec* element
SCC*mec*V(5C2)	5(C2)	C2		JCSC3624	27,638 bp—6 repeat regions, 2 mobile elements, and 23 CDS	
SCC*mec*VI(4B)	4(A4B4)	B		HDE288	The only SCC*mec* element that has not complete sequence	The downstream region of the element is 99% similar to the corresponding part of SCC*mec*I
SCC*mec*VII(5C1)	5(C1)	C1		JCSC6082	26,753 nucleotides long—2 repeat regions and 29 CDS	
SCC*mec*VIII(4A)	4(A4B4)	A	J1, *copA* gene; J2, Tn554	C10628	32,184 bp—6 repeat regions, 1 mobile element, and 36 CDS	The structure of the *mec* gene complex from SCC*mec*VIII is similar to that of SCC*mec*II. The *ccr* genes of SCC*mec*VIII are similar to that of SCC*mec*VI
SCC*mec*IX(1C2)	1(A1B1)	C2	J1, *cadDX* operon, *copA* gene, *arsRBC* operon and *arsDARBC* operon	JCSC6943	43,710 nucleotides long—6 repeat regions, 2 mobile elements, and 42 CDS	The structure of the *mec* gene complex from SCC*mec*IX is similar to that of SCC*mec*VII
SCC*mec*X(7C1)	7(A1B6)	C1	J1, ISS*ha1*, *arsRBC* operon J3, *arsRBC* operon	JCSC6945	50,802 nucleotides long—6 repeat regions, 2 mobile elements, and 54 CDS	
SCC*mec*XI(8E)	8(A1B3)	E		LG251	29.4kb—29 CDS	
SCC*mec*XII(9C2)	9(C2)	C2		BA01611	24.3kb in length	
SCC*mec*XIII(9A)	9(C2)	A	J2, transposon Tn4001	55-99-44	32.3kb in length	
SCC*mec*XIV(5A)	5(C1)	A		SC792	41kb in length	

Abbreviations: CDS, coding sequences; *ccr*, cassette chromosome recombinase.

The circular SCC*mec* element is excised from a specific site (attSCC) under the mediation of *ccr* gene and inserts into the C terminal (attB) of open reading frame X (orfX). And then the two ends of the excised SCC*mec* element become attL and attR.[55] *orfX* gene encodes rRNA methyltransferase, a RlmH-type staphylococcal ribosome methyltransferase (The *orfX* gene is now called the *rlmH* gene), which could methylate N3 at the 1915 pseuduridine site (ψ1915) of 70S ribosome to form m3ψ1915, thereby playing a role in the termination of translation elongation and the ribosome recycling.[56]

mec gene complex, mainly consists of *mecA/mecC* (XI is *mecC*, all others are *mecA*), regulatory genes *mecR1* (encoding signal transduction protein), *mecI* (encoding inhibitory protein) and associated insertion sequence (IS). According to the different types, number and order of genes, *mec* gene complex can be divided into five types, class A, B, C1, C2 and E (Figure 3).[57,58]

ccr gene complex. SCC*mec* carries a group of unique cassette chromosome recombinase (ccr) genes including *ccrA, ccrB* and *ccrC*, which are specifically involved in the integration and excision of SCC*mec* and *S. aureus* chromosomes. The Ccr recombinases are a unique category of serine recombinases family, whose NH_2-terminus is homologous to the recombinases of the invertase/resolvase family, and it has a much larger COOH-terminal domain. When the COOH-terminal is absent, the integration and excision activity of the Ccr is greatly reduced; therefore, the C-terminal plays a greater role in the recombination events.[59] Currently, eight *ccrA* allotypes, nine *ccrB* allotypes and two *ccrC* allotypes have been described. CcrA and CcrB are parts of the dual gene operon. CcrA specifically binds to the attB and attL sites, and CcrB can bind to attB, attSCC, attR and attL. They always exist together and perform integration and excision tasks together. But in type V, VII, XII, XIII and XIV, only *ccrC* is carried, and integration and excision are performed by a single gene of *ccrC*. These *ccr* genes shared among *Staphylococcus* species, and antimicrobials can induce the expression of the *ccr* genes and initiate SCC*mec* transfer by inducing SOS responses. So, different *Staphylococcus* species can facilitate the rapid transfer of the SCC*mec* element under antimicrobial pressures.[60] *ccrAB* is only expressed in a small number of cells, and its expression level varies with environmental changes. Therefore, it is speculated that *ccrAB* expression is regulated by an unknown regulation mechanism. There is a highly conserved Inverted Repeat (IR) element in 55bp upstream of the *ccrA* translation initiation site, inhibiting *ccrAB* expression and thus stopping the

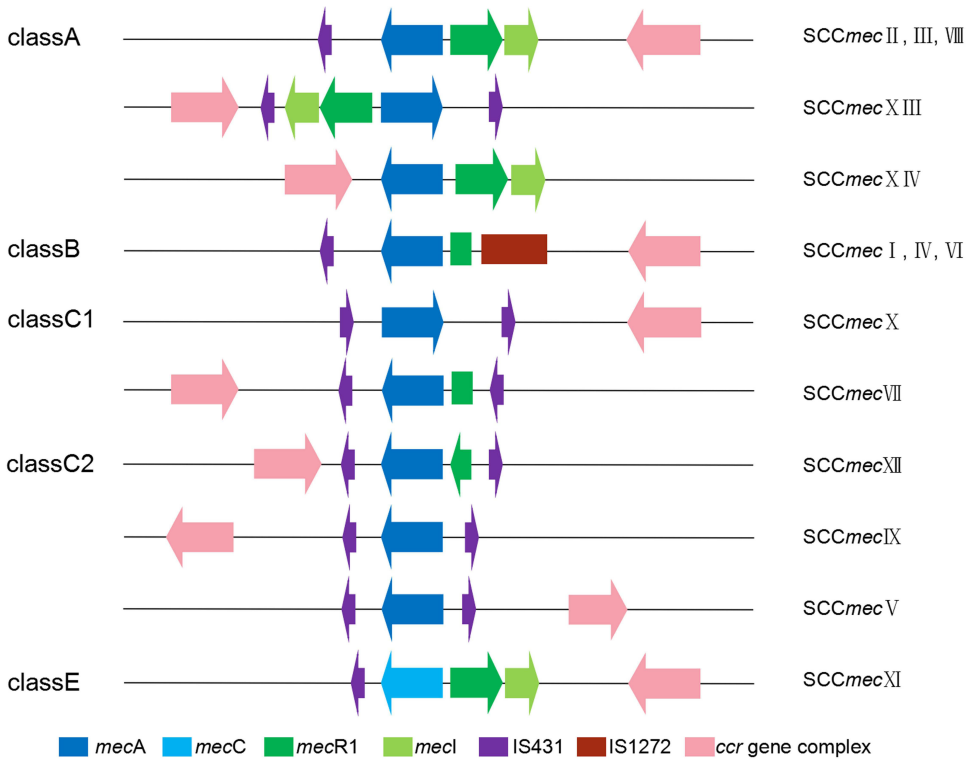

Figure 3 The structure of *mec* gene complex.

excision of SCC*mec*. However, the connection of transcription factor SarS and IR sequence can upregulate the expression of *ccrAB* and SCC*mec* excision.[61] Besides, sigB factor can bind to the *ccrAB* promoter to initiate *ccrAB* transcription, thereby upregulating *ccrAB* expression and SCC*mec* excision.[62] However, the specific regulation mechanism of *ccrAB* remains to be further explored.

J region. Except *mec* gene complex and *ccr* gene complex, there are some fragments in SCC*mec* elements called junction regions, which are divided into J1, J2 and J3 according to their locations. J region usually includes some regulation genes, transposons and plasmid-encoded antibiotic resistance determinants.[5] The J1 region usually contains several open reading frames and regulation genes. *copA* gene, encoding the transmembrane P1 copper transport ATPase, forms *copAZ* operon with *copZ* gene. The strains use *copAZ* gene products to prevent them from copper poisoning.[63] *pls* gene encodes Plasmin-Sensitive proteins to increase the biofilm formation.[64] The J2 region contains some genetic elements, transposon Tn554, ψTn554, etc. Transposons can carry plenty of resistance genes. Tn554 carries *ermA* (encoding erythromycin resistance) and *spc* (encoding spectacular mycin resistance),[65] and ψTn554 encodes a determinant of cadmium resistance. The J3 region includes some antibiotic resistance genes encoded by plasmids. Plasmid pUB110 encodes kanamycin, tobramycin and bleomycin resistance, plasmid pT181 encodes tetracycline and mercury resistance, and *kdp* operon encodes key enzymes of potassium transport system.[66] In recent years, the application of whole-genome sequencing has enriched the transposon family, and more drug-resistant genes and their variants have been discovered. For example, transposons Tn554-like all include *tnpA, tnpB* and *tnpC* genes encoding transposable function, and most of them contain antibiotic resistance genes *ant(9)-Ia, ermA* and *spc*. Furthermore, Tn6133 contains *vgaE*, which is a novel streptomycin A, truncated praline and lincosamide resistance gene;[67] Tn6188 contains biocide resistance gene *qacH*, Tn6674 and Tn6823 contain *fexA* and *optrA*, Tn558 contains *fexA*, Tn6260 contains *lnu(G)*, Tn5406 contains *vga(A)*, Tn559 contains *dfrK* and Tn553 contains *blaZ*;[68,69] Tn560 contains *spc* gene variant and *lsa (E), lnu (B)* genes.[70]

In addition, there are some special nucleotide sequences, reverse repeats, or direct repeats at either end of the SCC*mec* elements. These insertion sequences and transposons are the channels transferring informations between chromosomes and plasmids. The mobile plasmids are transferred along this channel, and the drug-resistant genes spread along the transfer of mobile plasmids.[71]

SCC*mec* elements are relatively stable and conservative. The upstream of elements' recombinase operon is a single operon that encodes a large ATPase, Cch or Cch2, and one or two additional proteins. As a self-loading helicase, Cch is an MCM-like helicase encoded by SCC*mec* elements. LP1413, a conserved protein encoded by the SCC family of staphylococcal genomic islands, coordinates with Cch to maintain the replication of the elements themselves.[72] And, the operons in the SCC*mec* elements encode some proteins such as CCPol and MP to maintain the replication of SCC*mec* element.[73] The precise replication of SCC*mec* elements is beneficial to the stability of re-insertion after excision and the efficiency of horizontal transfer, which facilitates the spread of SCC*mec* elements among different strains. Although the transfer of SCC*mec* components has been debated for more than 50 years, there are no clear conclusions so far.[74] Yet the replication and transfer of SCC*mec* elements, as well as gene mutation, can produce new clones of MRSA, which may affect the prevalence of multidrug resistant MRSA strains.

Resistance Mechanism of *mecA* Gene

The important mechanism of antibiotic resistance in *S. aureus* is the acquisition of the *mecA* gene, which encodes a high molecular weight penicillin-binding protein PBP2a with a low affinity for β-lactam. The precursor of *mecA* is the *mecA1* gene, widely found in *S. sciuri*. The *mecA* homologs encode PBPs, which are involved in the synthesis of peptidoglycan, the cell wall component. After β-lactam binds to PBP, the break of β-lactam cyclic amide bond and the acylation of PBP occurs, thus preventing the growth of bacteria.[75] But the changes in the structure of PBPs active sites and the evolution of the promoter region of *mecA1* gene lead to resistance to β-lactam.[76] PBP2a has the activity of transglycosylase and transpeptidase, a transglycosylase domain at the N-terminal and a transpeptidase domain at the C-terminal. Its acylation efficiency is low, and the serine S403 site is not easy to be covalently modified. Therefore, in the presence of β-lactam antibiotics, PBP2a can catalyze the cross-linking reaction between two adjacent peptides in the process of peptidoglycan biosynthesis, so that MRSA can still synthesize cell walls and survive in the antibiotic environment.[77] Corrêa Argondizzo et al[78] evaluated the

immunogenicity of the transglycosylase domains of PBP2a. The transglycosylase domain can be used as a specific target for immunotherapy, and the transglycosylase inhibitor is less affected by the development of drug resistance. So, immunotherapy targeting PBP2a is a promising therapeutic approach in the future.

The study found that the expression level of *mecA* gene and the expression amount of PBP2a had no relationship with the level of MRSA's resistance to β-lactam antibiotics, and there are other factors involved in the regulation of methicillin resistance, such as *fem* (factor essential for methicillin resistance) gene cluster and auxiliary factors (*aux*). FemX, FemA and FemB participate in the synthesis of peptidoglycans in the cell wall by adding the 1st, 2nd and 3rd, 4th and 5th glycine to the pentaglytic peptide bridge respectively;[79] AuxA and AuxB stabilize the lipid acids in the cell wall.[80] These accessory factors participate in the biosynthesis of bacterial cell wall, thereby improving the expression process of MRSA resistance to methicillin. Furthermore, under stress conditions, the growth rate of MRSA is low, but the transcription and translation of *mecA* increase, suggesting that the strict stress response plays a key role in the level of β-lactam resistance in MRSA strains.[81]

Resistance Mechanism of *mecC* Gene

In 2011, *mecA*-negative MRSA was found in the United Kingdom and Denmark, containing $mecA_{LGA251}$ drug resistance gene (later renamed *mecC*), which is located in the SCC*mec* XI element and shares 70% homology with *mecA* at the DNA level.[5] Since then, several clones of *mecC*-positive MRSA have been collected in different countries and regions, most of which are derived from LA-MRSA, and dairy cows are important hosts and sources.[82,83] The *mecC* gene encodes penicillin-binding protein PBP2c, and both PBP2a and PBP2c are associated with β-lactam antibiotic resistance, but they have different properties. Different from PBP2a's high affinity for cefoxitin, PBP2c's affinity for oxacillin is higher than cefoxitin, which may be related to the extensive use of cephalosporins in farms.[84] PBP2c has the highest activity and stability at 25°C, and the activity decreases with the increase of temperature after 25°C. At 37°C, the conformation of PBP2c changes and is less stable than that of PBP2a, which results in the decreased sensitivity of *mecC*-positive MRSA to methicillin.[84] The different biochemical properties of PBP2c may be the reason why *mecC*-positive MRSA strains have not been detected in humans.

Regulation System of Resistance Gene Expression

MRSA is resistant to β-lactam antibiotics due to the acquisition of *mecA* gene and the production of β-lactamase. Their expression is mainly regulated by the *mecA* regulation system (*mecR1-mecI* system) and the β-lactamase regulation system (*blaR1-blaI* system). *mec* and *bla* genes exist in one operon with different regulatory genes *mecR1*/*blaR1* and *mecI*/*blaI*. MecR1/BlaR1 are signal transduction proteins, and MecI/BlaI are transcription suppressor proteins. The β-lactam binds to the domains of extracellular penicillin-binding proteins, and MecR1/BlaR1 is activated, transmitting the signals to the cytoplasmic domains, which results in the hydrolysis of metalloproteinases. Then, MecI/BlaI proteins are inactivated and lose the ability to bind the promoter-operator sequence of the β-lactam operon, thereby inducing the expression of the *mec* and *bla* genes.[85]

The *mecA* gene encodes PBP2a, which is regulated by a three-component system. In addition to the *mecR1-mecI* gene, it also contains the *mecR2* gene, which is co-transcribed with *mecR1-mecI* from the *mecR2* promoter. The *mecR2* encodes the anti-inhibitory factor MecR2, which directly interacts with MecI to destroy the binding of MecI to *mecA* promoter and compensate for the inefficient induction of MecR1 to *mecA*, so that MRSA strains with functional *mecR1-mecI* sequences can optimally express β-lactam resistance.[86]

The β-lactamase is encoded by the *blaZ* gene, which is regulated by *blaR1* and *blaI*. Most of these genes are located on plasmids but are also present on chromosomes.[87] When β-lactam antibiotics lacks, BlaI binds to the conserved sequence TACA/TGTA of *blaZ* promoter, which inhibits *blaZ* transcription and thus inhibits the production of β-lactamase. However, when β-lactam antibiotics exists, the antibiotics can bind with *blaR1* to remove the inhibitory effect of *blaI-blaZ* and then produce β-lactamase.[88]

The *mecR1-mecI* system and *blaR1-blaI* system have similarities and commonalities in genetic regulation level. However, the *blaR1-blaI* system is the main β-lactam resistance mechanism of MSSA, and the *mecR1-mecI* system is the main resistance mechanism of MRSA. The regulatory effect of *mecR1-mecI* system is stronger than that of *blaR1-blaI*

system. Clinically, most strains can induce multidrug resistance of MRSA by controlling the expression of *mecA* gene (PBP2a) through BlaI.[89] Moreover, the expression level of BlaI is the main regulator of drug-resistant phenotype in OS-MRSA, and the initial amount of BlaR1 plays a decisive role in the rate of phenotypic transformation under β-lactam exposure. The *bla* system played a crucial role in regulating oxacillin susceptibility in OS-MRSA isolates.[90]

vanA Operon

vanA operon, a vancomycin resistance gene encoded by transposon Tn1546, is located on conjugated plasmids (eg, Inc18-like, pRUM-like, pMG1-like and pHT-like), including *vanA, vanH, vanX, vanS, vanR, vanY* and *vanZ*.[91] The *vanA* operon is more common in enterococcus and can be transferred to MRSA. It can be horizontally transferred through two different processes, one is the transmission of the Tn1546 variant plasmids among strains with different clonal backgrounds, and the other is the translocation of Tn1546 among different plasmids.[92] VanA is a ligase that catalyzes the synthesis of D-Ala-D-Lac ester bonds, and VanH is a dehydrogenase that reduces pyruvate to form D-Lac. VanA and VanH produce a low affinity for glycopeptide antibiotics through the synthesis of D-Ala-D-Lac. VanX is an aminopeptidase, which can eliminate the ester bond of wild-type D-Ala-D-Ala by hydrolysis, so as to ensure the binding of newly formed D-Ala-D-Lac to UDP. VanY is a D, D-carboxypeptidase, playing a role in teicoplanin resistance. VanZ is an accessory protein that protects bacteria from glycopeptide antibiotics by affecting their binding to cell surfaces (Figure 4).[93,94]

The expression of *vanA* operon is mainly regulated by the VanSR two-component transduction system. VanS, as a sensor, is a membrane-bound histidine kinase involved in signal transduction. VanR is a transcription factor that acts as a response regulator. When vancomycin exists, VanS detects the stimulation of vancomycin and self phosphorylates at histidine residues. Then, the phosphate groups on VanS transfers to the aspartic acid residues of VanR, thus activating VanR. Phosphorylated VanR binds to the promoter within the *vanA* operon, activating the transcription of resistance genes and leading to the resistance to vancomycin and teicoplanin. However, when vancomycin does not exist, VanS dephosphorylates VanR, thus keeping VanR in a transcriptionally inactive state.[95] Nevertheless, how VanS perceives vancomycin is still uncertain, and there are two main models to explain this process. One is that vancomycin induces the conformation changes of VanS through molecular interaction with VanS, leading to VanS autophosphorylation. The other is the cellular changes in response to the VanS's perception to vancomycin, such as the vancomycin-lipid II complex.[96,97]

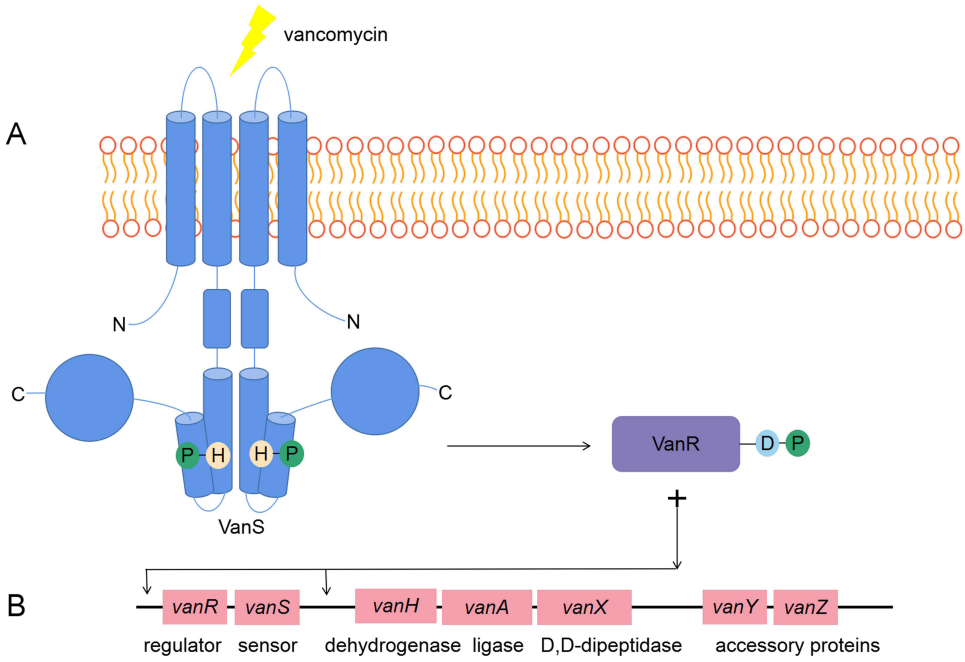

Figure 4 *vanA* operon and VanSR two-component transduction system. (**A**) When vancomycin exists, VanS autophosphorylates and activates VanR, thereby activates the expression of *vanA* operon. (**B**) *vanA* operon.

Resistance Mechanism Mediated by Biofilm

Biofilms are microbial cell groups composed of extracellular matrix (ECM) containing polysaccharides, teichoic acid, extracellular DNA (eDNA) and surface proteins. They can attach to the surface of biological materials such as human tissues or retained catheters, facilitating bacteria to quickly adapt to physical, chemical and biological pressures.[98] The development of *S. aureus* biofilm is dynamic and cyclic, mainly including five processes: attachment, multiplication, exodus, maturation, and dispersion (Table 4).[99] Study has showed that the biofilm forming ability of MRSA was significantly higher than that of MSSA.[100]

The mechanism of biofilm-mediated drug resistance is very complex, mainly because the components in biofilms reduce the permeability of antibiotics, the bacteria in biofilms reduce the growth rate to escape the stimulation of antibiotics, and there are some specific resistance genes in biofilms.[101] The unique physiological properties of biofilms reduce the effectiveness of antibiotics against biofilms,[102] allowing bacteria to better adapt to rapidly changing environments. First of all, the biofilms contain a lot of persistent cells, which is a kind of dormant state of cells. The presence of a large number of persistent cells enables bacteria to maintain a low metabolic level and close the targets on the surface of bacteria, thus protecting bacteria from the damage of antibiotics and producing the resistance to antibiotics.[103] After leaving the antibiotic environment, the biofilm cells resume their growth and infectivity.[104] Secondly, the extracellular polymeric substance (EPS) matrix of biofilms prevents the diffusion of antibiotics, and its barrier function can significantly reduce the penetration of drugs.[105] Biofilms can attach to different biological materials and be sealed in polymer substrates. Studies have shown that lysostaphin resistance protein A (LyrA) and methicillin-resistant FemA/B and FmtA were detected in biofilm matrix on polystyrene, borosilicate glass and plexiglass materials. These materials can cause protein-dependent antibiotic resistance.[106] In addition, the chemical bonds among eDNA molecules lead to the tight connection of cells in biofilms, which increases the plasmid transfer through coupling and mobilization, and promotes the horizontal transfer of drug-resistant genes;[107] eDNA, negatively charged, acts as a chelating agent for cationic antibacterials and has been proved to participate in the resistance to cationic peptides.[108] Moreover, different concentrations of antibiotics are associated with drug resistance in biofilms. At subinhibitory concentrations, some antibiotics can

Table 4 Model of *S. Aureus* Biofilm Development

Stage	Description	Biofilm Matrix	Mode	Determinants
Attachment	*S. aureus* cells attach to a surface to initiate biofilm formation.	Protein-mediated	Abiotic surface: electrostatic and hydrophobic interactions Biotic surface: bind with a variety of CWA proteins	Abiotic surface: AtlA, δ-toxin, teichoic acids Biotic surface: MSCRAMMs
Multiplication	Adherent *S. aureus* cells begin to divide and accumulate	Proteins and eDNA	Cell division and accumulation are mediated by some CWA proteins, such as SasG, Protein A, SasC, Bap.	
Exodus	The cells release approximately six hours after the initiation of the multiplication stage	eDNA degradation	Coincide with microcolony formation	Agr independent, Sae-regulated nuclease degradation
Maturation	The time of microcolony formation	eDNA, proteins, PSMs	Rapid growing microcolony: exhibit constitutive *lrgAB* expression Slow growing microcolony: exhibit constitutive *cidABC* expression	Two microcolony types associated with *cid* and *lrg* expression
Dispersal	Planktonic bacteria are released from the biofilm matrix	eDNA, proteins, PSMs	Molecular interactions within the biofilm matrix disrupt→ cells disperse→ return to planktonic cells	Agr-mediated proteases, PSM production

Notes: MSCRAMMs in *S. aureus* are Spa (*Staphylococcus* protein A), FnbpA and FnbpB (fibronectin binding proteins), ClfA and ClfB (clumping factors), Cna (collagen-binding protein), SdrC, SdrD and SdrE (serine aspartate repeat proteins), which play key roles in adhesion, colonization and evasion of innate immune defences. Alt is involved in the initial bacterial adhesion to the surface and in the lysis of bacterial cells that determines the eDNA release in the biofilm matrix. eDNA derives from the autolysis of sessile and planktonic cells, which mediates the intracellular adhesion resulting in autoaggregation. The *agr* operon play a role in the modulation of biofilm formation, down-regulating genes involved in host colonization including those encoding for the MSCRAMMs, FnBPAB and Spa, and upregulating those encoding for some proteins involved in tissue damage and autolysis.

Abbreviations: AtlA, autolysin A; MSCRAMM, microbial surface components recognizing adhesive matrix molecules; eDNA, extracellular DNA; PSM, phenol soluble modulins; Agr, accessory gene regulator; CWA proteins, cell wall-anchored proteins.

act as signaling molecules to induce biofilm formation and increase the biomass of biofilms; at low concentration, antibiotics can accelerate the horizontal transfer of drug-resistant genes in the biofilms and promote the spread of drug-resistant genes.[109]

Based on the properties of *S. aureus* biofilms, the removal or inhibition of *S. aureus* biofilms is an increasingly concerned topic in the field of global public health. Therefore, it is necessary to constantly explore the mechanism of biofilm-mediated drug resistance and find new anti-biofilm agents and new drug delivery routes.

Active Efflux Mechanism

Antibiotics can effectively reduce the infectivity of bacteria, and multidrug-resistant bacteria can develop resistance through efflux of antibiotics. Therefore, the overexpression of efflux pump is the main cause of multidrug resistance. The efflux systems of MRSA fall into five categories, major facilitator superfamily (MFS), small multidrug resistance family (SMR), multidrug and toxin extrusion family (MATE), ATP-binding cassette superfamily (ABC), resistance nodulation division superfamily (RND).[110] According to the energy source of drug transports, efflux pump can be divided into primary transporters and secondary transporters. The primary transporters are directly powered by ATP hydrolysis, such as ABC; the secondary transporters are powered by the concentration difference formed by protons/ions, including MFS, SMR, RND, and MATE. And it has reported that secondary active transporters are highly substrate specific and their recognition sites are often antimicrobial drug targets (Figure 5 and Table 5).[111]

MFS is the largest and most diverse membrane protein transport family, as well as the most well-studied efflux pump, which mainly includes *norA, norB, norC, tet(K), tetL, mdeA, sdrM, qacA/B* and other genes. Members of MFS have 12/14 monomeric proteins with transmembrane-spanning (TMS) helices ranging from 388 to 600 amino acids in length.[116] The drug resistance determinant of *nor* gene is located on chromosome. NorA is the first found efflux pump in *S. aureus*, with a molecular weight of 42.32 kDa. It is a transporter composed of 12 TMS and 388 amino acids, which is resistant to hydrophilic fluoroquinolones (ciprofloxacin, norfloxacin); NorB and NorC are, respectively, composed of 12 TMS, 464 amino acids and 14 TMS, 462 amino acids, and are resistant to hydrophobic fluoroquinolones (moxifloxacin and sparfloxacin).[115] The resistance determinants of *tet* are primarily present on small transmissible plasmids, which are occasionally integrated into the chromosomes of staphylococci and thereby promote acquired resistance in bacteria. The *tet* gene encodes the efflux protein Tet, which is a membrane-bound efflux protein with 46kDa in size and 12 hydrophobic membrane spanning regions.[117] QacA, composed of 14 TMS and 514 amino acids, can mediate resistance

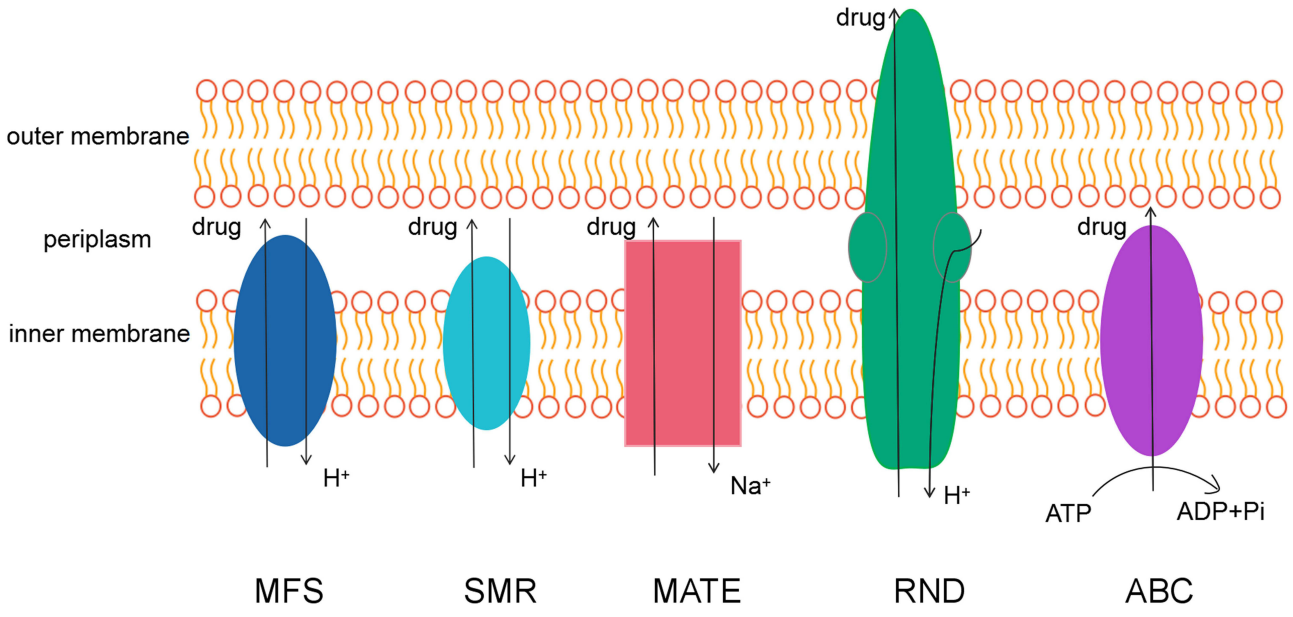

Figure 5 The model of efflux pump family.
Abbreviations: MFS, major facilitator superfamily; SMR, small multidrug resistance family; MATE, multidrug and toxin extrusion family; ABC, ATP-binding cassette superfamily; RND, resistance nodulation division superfamily.

Table 5 Common Proteins and Their Substrates in Various Efflux Pump Systems

Family	Efflux Pump	Location	Substrates	Ref
MFS	QacA	Plasmid	Quaternary ammonium compounds, guanyl hydrazones, biguanidines, diamidines	[112]
	QacB	Plasmid	Quaternary ammonium compounds, Tetraphenylphosphonium, ethidium bromide, acriflavine, rhodamine	[112]
	LmrS	Chromosome	Linezolid, chloramphenicol, florfenicol, trimethoprim, erythromycin, kanamycin, fusidic acid, lincomycin, Streptomycin, tetraphenylphosphonium, ethidium bromide	[112]
	NorA	Chromosome	Fluoroquinolones, biocides and dyes	[112]
	NorB	Chromosome	Fluoroquinolones, tetracycline, biocides and dyes	[113]
	NorC	Chromosome	Fluoroquinolones	[113]
	MdeA	Chromosome	Ciprofloxacin, macrolides, piperine	[113,114]
	SdrM	Chromosome	Norfloxacin, biocides and dyes	[113]
	Tet(K)	Plasmid	Tetracyclines	[113]
	Tet38	Chromosome	Tetracycline, fosfomycin, palmitoleic acid, tunicamycin, and Congo red	[113]
	FexA	Transposon	All phenicols	[115]
SMR	QacC	Plasmid	Benzalkonium, cetrimide and dyes	[115]
	QacJ	Plasmid	Benzalkonium, cetyltrimethylammonium bromide	[115]
	QacG	Plasmid	Benzalkonium, ethidium	[115]
	QacH	Plasmid	Benzalkonium, ethidium, proflavine	[115]
	SepA	Chromosome	Benzalkonium, chlorhexidine, acriflavine	[115]
MATE	MepA	Chromosome	Fluoroquinolones, glycylcyclines, quaternary ammonium compounds and dyes	[115]
RND	FarE	Chromosome	Linoleic and arachidonic acids, rhodomyrtone	[115]
ABC	AbcA	Chromosome	Hydrophobic β-lactams	[115]
	MsrA	Plasmid	Macrolides, type B streptogramins, erythromycin	[115]
	Sav1866	Chromosome	Vinblastine, doxorubicin, dyes	[115]
	VgaA	Plasmid	Lincosamides, streptogramin A, pleuromutilins	[115]
	VgaB	Plasmid	Pristinamycin, streptogramin A, streptogramin B virginiamycin, mikamycin, synergistin, dalfopristin	[115]

to different chemical classes of cationic lipophilic antibacterial compounds, especially to divalent cationic compounds. QacB is a paratrogeneic homologue of QacA. Compared with QacA, position 323 of QacB is replaced by alanine, resulting in the inability to transport bivalent substrates.[118]

For multidrug-resistant bacteria mainly mediated by efflux mechanism, it is alternative to combine efflux pump inhibitors (EPIs) and antibiotics. EPIs inhibit the efflux pump capacity of bacteria and increase the concentration of antibiotics in bacterial cells. At present, several EPIs have been identified but have not been clinically approved due to their low potency, uncertainty in pharmacokinetics, and high toxicity. Therefore, available EPIs could be screened from already approved drugs in the future.

Other Resistance Mechanisms

In addition to above resistance mechanisms, the change in temperature can change the drug resistance of MRSA. MacFadden et al[119] studied the relationship between temperature and regional patterns of antibiotic resistance across the United States. They found that the resistance of *S. aureus* increased by 2.7% when the temperature increased by 10°C in each region. Temperature can affect the growth of bacteria in vitro and regulate the transfer of genes encoding antibiotic resistance. Therefore, as global climate changes dramatically, we should pay more attention to the effect of temperature on MRSA resistance.

The above summary has shown that *mecA* and *vanA* operon can promote the synthesis of *S. aureus* cell wall to mediate its antibiotic resistance. According to research reports, the reconstruction and autolysis of cell walls also affected the antibiotic resistance. A defect in cell wall recycling may confer antibiotic resistance in *S. aureus* by reduced autolysis and a thickened cell wall.[120] And changes in some cell wall components, such as β-glycosylated wall teichoic acids, reduced the binding affinity between *S. aureus* autolysin and cell wall, and reduced cell wall autolysis to result in antibiotic resistance.[121] In *S. aureus*, phosphatases could dephosphorylate teichoic acid, a molecule that plays a key role for bacterial colonization on artificial surfaces,

and they expressed on strains' surface and caused dephosphorylation of different proteins. Among these, alkaline phosphatase plays an indispensable role in phosphate metabolism and biofilm formation. Alkaline phosphatase may promote aerobic pathways to regulate biofilm formation, yet the impact of aerobic pathways on biofilm formation needs further study. Alkaline phosphatase inhibition may be a novel target for anti-biofilm therapy.[122] Phosphatase Stp also impacted antibiotic resistance because Stp deletion strains are more susceptible to cell wall-acting antibiotics like tunicamycin, fosfomycin and β-lactam antibiotics, and Stp contributes to reduced susceptibility to vancomycin.[123]

Therapeutic Strategies

MRSA is a "super bacterium" that is resistant to various drugs such as penicillin, aminoglycosides, tetracyclines, macrolides, quinolones and so on. The resistance mechanism involves in gene mutations, biofilm effects, and drug efflux pump effects, which poses a great challenge to the treatment of MRSA infection. Therefore, there is an urgent need to develop new drugs and methods to treat the infection of MRSA. Table 6 summarized the indications, advantages and

Table 6 New Therapeutic Strategies for MRSA Infection

Therapeutic Strategies		Indications	Advantages	Disadvantages	Ref
Antibiotics	Vancomycin	The first choice for severe MRSA infection.	The bactericidal effect is relatively strong and it is not easy to cause allergies.	Many adverse reactions, such as ototoxicity and nephrotoxicity. Poor tissue infiltration and slow killing time. Reduced efficacy for MSSA.	[6]
	Teicoplanin	Severe MRSA infection, and patients who are resistant/allergic to penicillin, cephalosporins and β-lactam antibiotics.	A much longer half-life than vancomycin.	Adverse reactions, such as renal toxicity, allergic reactions, fever, liver and kidney dysfunction.	[6]
	Daptomycin	Skin and soft tissue infections and bloodstream infections caused by MRSA, but not applicable to pneumonia caused by MRSA.	Faster bactericidal effect than vancomycin, linezolid or quinuputin/daloforpine.	Adverse reactions, such as gastrointestinal reactions, injection site reactions, fever, headache, insomnia, dizziness, rash and so on.	[6]
	Linezolid	Systemic infection caused by MRSA infection.	The survival rate and clinical cure rate of MRSA infected patients treated with linezolid were significantly higher than those treated with vancomycin.	Adverse reactions, such as diarrhea, headache, nausea, bone marrow suppression, blindness and even lactic acidosis.	[6]
	Ceftaroline	Community-acquired pneumonia, acute bacterial skin infections (including infections complicated with bacteremia).	Combined with daptomycin may reduce hospital mortality rate (especially when starting treatment early in the course of the disease).	Adverse reactions, such as some allergic reactions.	[6]
	Dalbavancin	*S. aureus* bacterial skin infections.	Have a uniquely long half-life.	The potential role of dalbavancin in endovascular infections has not been established.	[125]

(*Continued*)

Table 6 (Continued).

Therapeutic strategies		Characteristics	Ref
Antibiotics combination therapies	Vancomycin + β-lactams	Reduce the duration of bacteremia or mortality, with an increase in the speed of bacterial killing.	[126]
	Daptomycin + β-lactams	This synergistic effect is linked to enhanced daptomycin binding and cidality, resulting in rapid clearance of bacteria and infection resolution, even in daptomycin-resistant MRSA.	[127]
	Daptomycin + ceftaroline	It clears persistent bacteremia and reverts the MRSA back to a daptomycin-susceptible phenotype. This combination can be used in high-risk MRSA bacteremia patients.	[127]
	Daptomycin + fosfomycin	This combination can slow daptomycin resistance development. It can be used to treat patients with left-sided staphylococcal endocarditis.	[127]
Anti-biofilm agents	Nisin A	Affect the membrane potential of MRSA biofilm cells, form stable pores, and lead to ATP leakage. Eradicate the MRSA biofilm matrix as well as kill all the sessile bacteria.	[128]
	BacteriocnXJS01	Disrupt the biofilm established on the skin wound surface and reduce the biofilm-isolated bacteria, thereby decreasing the release of pro-inflammatory cytokines and the proliferation of alternatively activated macrophages.	[129]
	Bacteriophage	Phages have many advantages such as high specificity, low toxicity, strong reproductive ability, and no cross-resistance with antibiotics. Phage can mediate the transfer of antibiotic resistance genes and virulence factors, and there are concerns that the host will produce an immune response due to the entry of phage, especially for intravenous administration of phage.	[6]
	Nanoparticles	Carry other antibacterial agents by various molecular interactions, to make a multifunctional antibacterial platform. Penetrate the pores of biofilms and deliver active agents to destroy the biofilms effectively.	[6,130]
	Quorum sensing inhibitors	These inhibitors could block the quorum sensing system of MRSA and inhibit the expression of bacterial virulence genes without affecting the growth and proliferation of bacteria. So the application of quorum sensing inhibitors can prevent bacteria from developing resistance due to growth stress.	[6]
	Combination therapy	It is a more effective therapy when anti-biofilm agents and antibiotics are used in amalgamation. Because anti-biofilm agents can sensitize antibiotics.	[131]
Efflux pump inhibitors	Piperine	Lower the ciprofloxacin MIC in ciprofloxacin-resistant *S. aureus* mutants.	[132]
	INF240	Enhance antibacterial activity against NorA overproducing *S. aureus* strains.	[132]
	Reserpine	Eradicate the resistance conferred by the NorA of *S. aureus* and demonstrate a fourfold decrease in tetracycline MIC values. But the concentrations needed for effective NorA inhibition are neurotoxic.	[132]
	INF271	One of the most potent NorA EPIs reported to date.	[132]
	Boronic acids	Be considered as NorA inhibitors and β-lactamase inhibitors.	[132]

disadvantages of the new generation of antibiotics and antibiotic synergistic approaches, which had been used in clinical practice. And then, the characteristics of some new anti-biofilm agents and efflux pump inhibitors were summarized, which could be combined with antibiotics to combat multiple drug resistance of MRSA. But these drugs were still in the experimental stage and could not be used in clinical practice. In addition to the treatment methods summarized here, other alternative methods for treating MRSA infection, including hemolysin therapy and vaccination, were being studied,[124] and the number of alternative methods for treating MRSA infection was constantly increasing.

Conclusions and Future Perspectives

As a superbacterium, MRSA is rapidly evolving, highly toxic and MRSA infection is difficult to treat. It makes a major threat to human health due to the genetic adaptability and the emergence of a series of successful epidemic strains. MRSA has evolved to optimize its gene contents, creating strains that are super virulent and resistant to multiple drugs.

At present, we have gained a full understanding of the resistance mechanisms of *S. aureus*: SCC*mec* elements, *vanA* operon, biofilm formation, and efflux pumps. Among these, SCC*mec* elements and *vanA* operon are not unique to *S. aureus*. They have also been found in other staphylococcus or enterococcus and can transfer into *S. aureus* by horizontal transfer. Understanding drug resistance is fundamental to the development of new drugs and treatment regimens. We can better formulate drug delivery plans for any new drugs, thereby minimizing the emergence of drug resistance. In addition, there is an urgent need for new antibiotics or novel alternative treatment options, such as anti-biofilm agents and EPIs. However, none of these drugs are currently clinically approved. So, it is necessary to construct animal models of different diseases, gradually evaluate the efficacy of drugs, and ultimately select clinically defined patients with MRSA for evaluation. And then these drugs can be used in clinical treatment. In the coming years, the prevention and treatment of MRSA remain an area that needs to be continuously overcome.

Abbreviations

ABC, ATP-binding cassette superfamily; BORSA, borderline oxacillin-resistant *Staphylococcus aureus*; CA-MRSA, community-associated methicillin-resistant *Staphylococcus aureus; ccr*, cassette chromosome recombinase; eDNA, extracellular DNA; EPIs, efflux pump inhibitors; HA-MRSA, healthcare-associated methicillin-resistant *Staphylococcus aureus*; LA-MRSA, livestock-associated methicillin-resistant *Staphylococcus aureus*; MATE, multidrug and toxin extrusion family; MFS, major facilitator superfamily; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; orfX, open reading frame X; OS-MRSA, oxacillin-susceptible methicillin-resistant *Staphylococcus aureus*; RND, resistance nodulation division superfamily; *S. aureus, Staphylococcus aureus*; SCC, staphylococcal cassette chromosome; SMR, small multidrug resistance family; VRSA, vancomycin resistant *Staphylococcus aureus.*

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Disclosure

The authors declare no conflicts of interest in this work.

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