Methicillin-resistant *Staphylococcus aureus* antibiotic resistance and virulence

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Summary

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most critical causes of healthcare-related or community-related infections. Resistance to most β-lactam antibiotics makes MRSA a big threat to clinical treatment. Utilization of low efficiency antibiotics such as vancomycin and teicoplanin makes new choices for therapies. Recently, much research has shed light on relevance between genetic mutations of MRSA and clinical characteristics such as antibiotic resistance, and virulence. These findings could contribute to development of novel antibiotics and vaccines.

*Keywords:* *Staphylococcus aureus*, antibiotic resistance, virulence, mutation, biofilm

1. Introduction

It has been fifty two years since the first methicillin-resistant *Staphylococcus aureus* (MRSA) was isolated by Patricia Jevons, only two years after the premier clinical utility of the antibiotic methicillin (1). MRSA is one of the most parlous pathogens, responsible for a great number of human infections all around the world (2-5) (Figure 1). From the 1980s, new strains of MRSA emerged which led to continuous pandemic infections of MRSA around the world. At present, many countries report that MRSA strains account for about 25-50% of infectious *Staphylococcus aureus* in hospitals (6). In contrast, some other countries such as some northern European countries have lower MRSA infection rate (often < 1%), most probably due to strict search-and-destroy and surveillance measures, as well as control in antibiotic prescriptions. Recently, a Japanese study suggests that antibiotic consumption without restraint leads to increased MRSA virulence with time (7).

MRSA can produce a series of toxins and present multiple resistance to antibiotics. Most of these functions are derived from mobile genetic elements (MGEs) on the genome (8,9). Resistance to methicillin primarily stems from acquisition of the mecA gene, not inherently existent in this strain, which produces a modified penicillin-binding protein (PBP2a) with lower affinity to β-lactams (10). Lately, MRSA which is negative for mecA has been discovered in human populations in the UK. The new diverse mecA was about 70% homologous to *Staphylococcus aureus* mecA (11). The continuous emergence of mutations of key genes makes it more difficult to prevent and control MRSA.

While for a long time MRSA infections were detected in hospitals (healthcare-acquired/associated-MRSA, HA-MRSA), however, in the recent decade infections have appeared in community (community-acquired/associated-MRSA, CA-MRSA) and also derived from livestock (livestock-associated-MRSA, LA-MRSA). Thus, MRSA can not only be taken as a hospital-associated problem, but also a society wide problem. This review will give an overview over the genome structure, pathogen and molecular biological characteristics of MRSA, and vaccines. Through this analysis, a light may be shed on the future prevention and control of MRSA.

2. Genome structure of MRSA

The *Staphylococcus aureus* genome was sequenced in detail recently (12). In the last period of time, there have been many related sequencing results released on
the NCBI website. Making a comprehensive survey of the great amount of sequencing data, three major points were revealed: i) there is a backbone of core genes which comprises about 97% of all the genes and is highly conserved; ii) except for backbone genes, there is a set of over 700 genes, named core variable (CV) genes which defines the Staphylococcus aureus lineage by various distribution patterns in the genome; iii) a group of mobile genetic elements (MGEs) genes exist discretely in the genome which can move around within the genome and play a critical role in the spread of virulence factors (13-16) (Table 1). CV genes have a major function in encoding surface proteins and structures which can interact with human genes and their regulators (14).

At present, Staphylococcus aureus is classified based on clonal complexes (CCs) via multilocus sequence typing (MLST), for example, CC1, CC5, and CC10. The MLST method is used to sequence seven conserved genes and allocate a sequence type (ST) number to a validated strain (17). One CC type can be subtyped into several different STs by single nucleotide polymorphisms (SNPs) on the seven house-keeping genes.

MGEs can be defined as a kind of fragments of DNA which transfer into the host cell to replicate or integrate into host DNA. The antibiotic resistance and virulence of Staphylococcus aureus are acquired from MGEs (18). Horizontal gene transfer (HGT) of these MGEs leads to higher invasiveness, virulence, antimicrobial resistance and host adaptation, but each MGE can only transfer into certain lineages not all lineages.

Various types of MGEs have been identified in Staphylococcus aureus: plasmids, Staphylococcus aureus pathogenicity islands, bacteriophages, transposons, staphylococcal cassette chromosome mec (SCCmec), and genomic islands (19). Among these, the most important MGEs for Staphylococcus aureus are the methicillin resistance gene mecA on the different SCCmec, the bacteriophages produced Panton-Valentine leukocidin (PVL) toxin, and many resistance elements encoded by plasmids and transposons (20). MGEs in Staphylococcus aureus can largely strengthen the pathogenic and resistance ability of this strain. It has been reported that MGEs are able to transfer, lose, and/or acquisition among different strains (14).

HGT is limited among various lineages by the hsdS gene. Different lineages have different hsdS genes which have different DNA modification and digestion sites. As a result, the lineage can recognize domestic DNA and destroy alien DNA (21). The hsdS genes could play a role of biomarker to differentiate different lineages (22).

3. Present pathogenic characteristic of MRSA

3.1. HA-MRSA

3.1.1. Antibiotic resistance

From a clinical standpoint, a critical situation that surgeons have to confront when treating Staphylococcus aureus infections is antibiotic resistance. Resistance to the first antibiotic, penicillin, occurred in the 1940s (23). In 1942, a penicillin-resistant Staphylococcus aureus strain was successfully found (24). Intrinsically, an enzyme called penicillinase caused the resistance to penicillin (25). Penicillinase cuts the β-lactam ring which is a core of β-lactam antibiotics such as penicillin and its derivatives. At present, most
infectious Staphylococcus aureus strains own resistance to penicillin and its derivatives. To resolve the dilemma with penicillin-resistant Staphylococcus aureus, methicillin was developed which stemmed from penicillin but can avoid penicillinase cleavage. Methicillin was used in the clinic in 1959; but just one year later, a methicillin-resistant strain was detected in the UK (26). Unfortunately, the mechanism of methicillin resistance protects Staphylococcus aureus from the whole group of β-lactam antibiotics including penicillins, cephalosporins and carbapenems. In recent times, many MRSA strains have acquired resistance to multiple antibiotics, such as ciprofloxacin, clindamycin, tetracycline, erythromycin, and so on (27). A recent research result from the CDC of the US confirms this and shows resistance to tetracycline and clindamycin in 9% and 6.2% of strains respectively among 823 infectious strains lately isolated (28). Furthermore, these strains contain transferable antibiotic-resistant plasmids.

Until now, the clinical therapy of MRSA infection mainly depends on utility of the glycopeptides vancomycin and teicoplanin, although at the same time many others are also employed, such as co-trimoxazole, tetracyclines, clindamycin, fusidic acid, linezolid, daptoycin, tigecycline, telavancin, and ceftaroline. For decades, glycopeptides, especially vancomycin, have been considered as the gold standard for therapy of MRSA infections until the appearance of resistance to these antibiotics in enterococci and subsequently in Staphylococcus aureus were found (29,30). In enterococci, glycopeptide resistance is due to mutation of the terminal alanine in the operons which promote the transferable cell wall-producing gene transcript and causes the glycopeptides to be off target. In the recent decade, the so-called VISA (vancomycin-intermediate Staphylococcus aureus) strains have attracted more attention in which minimum inhibitory concentrations (MICs) of vancomycin are ≥ 4 mg/L, and the strains, so-called hVISA strains, with vancomycin MICs are ≤ 2 mg/L that show heteroresistance. These strains always lead to a large amount of glycopeptide treatment failure in the clinic (31,32). Van Hal et al. summarizes that the treatment failure rate of hVISA infection is 2.5-fold higher than vancomycin-susceptible Staphylococcus aureus (33). These strains can cause prolonged bacteraemia for the mutations in the agr system which show decreased toxin generation and slow down virulence being examined in animal models (34,35). Moreover, a mutation in stp1 which encodes a serine/threonine phosphatase was reported to increase vancomycin resistance and decrease virulence (36). However, the relevance between vancomycin treatment failure and agr dysfunction is still in the dark, although a study indicated that agr dysfunction was related to a worse clinical treatment effect (37).

A number of substitutions in genes including vraSR and graSR have been reported to enable susceptible MRSA strains to transform to hVISA and hVISA to VISA (38). The rpoB mutations selected by rifampicin were found to have multiple resistance to both vancomycin and daptoycin by a possible mechanism of increased cell wall thickness and these mutations always were found in VISA strains (39). Meanwhile, rifampicin-resistant strains were found to contain vancomycin resistance (40), and the researchers suggest the rpoB mutations play an important role in vancomycin resistance. These results may give a reason

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**Table 1. Mobile genetic elements (MGEs) validated in Staphylococcus aureus**

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<thead>
<tr>
<th>MGE</th>
<th>Description</th>
<th>Instances</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Bacterial phages toxins</td>
<td>Lysogenic phage carry toxin genes that can enhance the virulence of the bacterial host</td>
<td>Staphylococcal enterotoxin A (SEA), chemotaxis inhibitory protein (CHIP) staphylocinase , PVL Staphylococcal complement inhibitor (SCIN)</td>
<td>(13-15,19)</td>
</tr>
<tr>
<td>Pathogenicity islands</td>
<td>A distinct class of genomic islands acquired by microorganisms through horizontal gene transfer</td>
<td>Encode TSST, MDR transporters, superantigens (SEB, SEC), fusidic acid-resistant genes</td>
<td>(13,15,19)</td>
</tr>
<tr>
<td>Plasmids and transposons</td>
<td>Plasmids and transposons carry antibiotic, heavy metal and disinfectant resistance determinants, toxins, arginine metabolism</td>
<td>Plasmids: several resistance determinants such as resistance of blaZ, blaI, and blaR1 toβ-lactam antibiotics Transposons: Tn552 carries bla for penicillinase</td>
<td>(13,15,18,20)</td>
</tr>
<tr>
<td>Staphylococcal cassette chromosome mec (SCCmec)</td>
<td>A mobile genetic element that carries the central determinant for broad-spectrum beta-lactam resistance encoded by the mecA gene</td>
<td>SCCmec types I-XI</td>
<td>(13,15,16,19)</td>
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<th>Description</th>
<th>Instances</th>
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<tr>
<td>Three families: vSAα, vSAβ, vSAγ. Containing genes encoding phenol-soluble modulins (PSMs), responsible for pro-inflammatory activity, enterotoxins and bacteriocin production</td>
<td></td>
<td>(13,15,17,19)</td>
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for the worse outcomes in treatment of patients showing bacteraemia and endocarditis with both vancomycin and rifampicin (41-43). Some case reports suggested that employment of rifampicin in combination with other antibiotics can heal biofilm-related infections (44,45). Thus, rifampicin combination therapies can be utilized to treat biofilm-related infections although not for bacteraemia and endocarditis. The mechanisms of MRSA resistance to daptomycin are still poorly understood. There are certain possible explanations including an increased positive surface charge caused by mutations of \textit{mprF} and \textit{dltABCD}, increased cell wall thickness caused by mutations of \textit{rpoB} and mutations in \textit{cls} and \textit{pgsA} which change membrane lipids. Sometimes these factors work together (46-48).

Linezolid is another effective antibiotic and up until now has not encountered much resistance. Most existing resistance is derived from mutations on the target site on the 23S rRNA of the ribosome (49). However, MRSA has multiple (commonly 4 to 6) copies of the 23S rRNA genes, and resistance can just be induced by multiple mutations (50). Furthermore, MRSA strains with multiple mutations always show lower activity (51,52). As a result, linezolid-related MRSA infections are not a significant problem for healthcare although it has been utilized for more than one decade (53).

3.1.2. Colonization

Medical waste, contaminated devices, and patients or medical staffs who carry MRSA play a role as an infectious source of MRSA in hospitals. Nostrils are a major site for carrying MRSA, and the relativity between nasal carriage and infectious diseases has been reported eighty years ago (54). Then a theory emerged which suggested the nose colonization of MRSA led to infectious diseases (55,56). Besides the nose, perineum and throat are also colonization sites of MRSA, but there are few research studies on these sites. Obviously, prevention of MRSA carriage could decrease the infection probability. A recent study revealed that mupirocin is efficacious in short-term prevention of MRSA, such as administration before surgery in hospitals (57). It is reported that about 20% of people in the population are persistent nasal carriers of \textit{Staphylococcus aureus} (58), and carriage rates differentiate in different ethnic groups and are higher in patients with certain underlying medical conditions. These indicate that host factors are important for colonization of \textit{Staphylococcus aureus}. However, the molecular mechanisms of these phenomena are still unknown. Thus, focusing on molecular mechanism research will be a key to understand MRSA colonization.

Surface-anchored \textit{Staphylococcus aureus} binding proteins which can bind to exposed human matrix molecules improve the nasal colonization of MRSA. Clumping factor B and \textit{Staphylococcus aureus} surface proteins G and X (SasG, SasX) have been shown to combine to nasal epithelial cells (59-60). Among them, SasX lately has attracted more attention because it was found to play an important role in an MRSA epidemic (60). SasX existed in a MGE mainly belonging to the ST239 MRSA strain which was a major ringleader of MRSA infections in Asia areas. It was discovered that SasX played a wide role in nasal colonization, biofilm generation, immune evasion and virulence in animal infection models. Thus, SasX may be a critical element promoting ST239 spread in Asia. The way SasX functions may providereference for doctors and researchers to understand how the spread of colonization and virulence elements through HGT drives an MRSA epidemic. Teichoic acids, a kind of surface polymer of \textit{Staphylococcus aureus}, helped make MRSA able to colonize the human nose (54). Moreover, MRSA has some mechanisms resistant to antibacterial peptides which cause the subsequent innate immune reaction (61).

3.1.3. Biofilm

Biofilms are a group of microorganisms in which cells stick to each other on a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS). Biofilm EPS is a polymeric conglomeration generally composed of extracellular DNA, proteins, and polysaccharides. Biofilms can form on living or non-living surfaces, such as medical settings (62,63). Biofilms protect MRSA from antibiotics and host immune defenses and then MRSA remains adherent on biotic or abiotic surfaces. Thus, biofilms can play a role in prolonging the duration of infection and promoting colonization. Whether \textit{Staphylococcus aureus} clones in the nose form biofilms is still an argument, but comparison of physiological situations between nasal colonization and in biofilms can bring certain hints. Nasal colonization and biofilms of \textit{Staphylococcus aureus} share the same trait of keeping relatively calm compared to the invasive situation of toxin-producing acute \textit{Staphylococcus aureus} disease. It was reported that many colonizing strains are deficient in global virulence regulator \textit{Agr} (64). Of note, there is a study which indicates that biofilmformation has been associated with the spread of some clones such as the Brazilian MRSA ST239 strain which is considered an ancestor of the Chinese SasX positive ST239 strains (65).

3.1.4. Virulence

Virulence of MRSA includes multiple elements such as toxins, immune system invasion and other factors. In different \textit{Staphylococcus aureus} strains, there are various toxin pools due to the reason that toxins are
encoded on MGEs which are variable in different strains. These MGE-derived toxins have various types including superantigens such as toxic shock syndrome toxin (TSST), some leukotoxins such as Panton-Valentine leukocidin which is typical factor in CA-MRSA, and exfoliative toxins. However, α-toxin, β-toxin, some leukotoxins and phenol-soluble modulins (PSMs) are synthesized in almost all strains. Different expression levels of toxin genes lead to different pathogenic activities. For example, obvious pathogenic differences are observed between Agr-positive strains and Agr-negative ones, and Agr is able to manage many toxin genes (66).

Surface proteins play many critical roles in MRSA pathogenesis. They have many functions including cell wall metabolism, immune evasion, bacterial aggregation and biofilm formation (67). Most surface genes are located on the core genome, so the virulence of MRSA may not be directly related to surface proteins. SasX on MGE promotes MRSA colonization by boosting bacterial aggregation which shares similar characteristics compared to aggregation caused by surface proteins (60).

Regulator systems, such as Agr, SaeRS, SarA, and so on, contribute to strengthen the virulence of MRSA. Agr, having been long acknowledged as a regulator of virulence, plays an important part in toxin production (68). Recent research suggested that Agr may increase surface protein expression in a strain-dependant way (69). Based on official guidelines, methicillin resistance of MRSA is generated by meca but the mechanism is poorly understood. Lately, some studies indicated that core genome-encoded regulators and the mec locus both can affect Agr (69,70).

3.2. CA-MRSA

3.2.1. Epidemiology

Before the 1990s MRSA was only known as a healthcare-associated disease in hospitals. At that time MRSA infection cases appeared in communities without any records of hospitalization. CA-MRSA is a moderately severe infection of the human skin and soft tissues. At present, CA-MRSA has emerged in most areas of the world. All of the *Staphylococcus aureus* species have appeared in CA-MRSA strains (71). The terrible spread of CA-MRSA is thought to be associated with strengthened virulence and increased transmissibility compared to former HA-MRSA. In the last decade, much research was performed to illuminate the molecular mechanism of virulence, but research on transmissibility did not make much progress (72).

3.2.2. Transmissibility

Spread of CA-MRSA was attributed to direct transmission from patients and/or hospital staff. But, in fact, CA-MRSA also showed transmissibility activity. CA-MRSA commonly contains SCCmec elements of type 4 or type 5 which have stronger transmissibility as a result of a smaller size than other elements. The arginine catabolic mobile genetic element (ACME) of certain strains contains a spermidin acetyltransferase gene (*speG*) which transfers resistance to spermidin and other polyamines (73). Furthermore, there is an arginine deiminase and an oligopeptide gene cluster located on ACME, which can promote colonization of CA-MRSA, but there are still no unimpugnable experimental results to support this theory (72). Meanwhile, CA-MRSA utilizes surface adhesions in a different way from other strains and mechanism studies are still in progress (69).

3.2.3. Virulence

The hypothesis that CA-MRSA has higher virulence than HA-MRSA to infect humans has been validated in animal infection models and has gradually become common sense. Evidence of increased virulence of CA-MRSA is that strains show considerable ability to evade attack from neutrophils which are the frontline defense against bacteria in the human body. There are two hypotheses to explain this evasion capacity of CA-MRSA. One is that CA-MRSA acquired MGEs containing Panton-Valentine leukocidin (PVL) (74). The other is that CA-MRSA promotes expression of core genome-encoded virulence genes, such as PSM cytolysins, α-toxin and so on (75). Actually, these two hypotheses can work together to increase the virulence of CA-MRSA.

Panton-Valentine leukocidin (PVL), which is associated with *staphylococcal* skin and pulmonary infections, is a member of the bi-component family of *staphylococcal* leukocidins. In the CA-MRSA epidemic, PVL genes *lukS* and *lukF* were discovered in CA-MRSA strains, and interestingly, PVL is typically absent in HA-MRSA (74). Thus, PVL is supposed to play an important role in CA-MRSA virulence. However, two experimental results cast a damper over the assumption. The first is that even in strains without PVL genes, virulence is still strong (76). The second is that isogenic PVL gene deletion mutants did not decrease the CA-MRSA virulence in a few animal models (77,78).

Phenol-soluble modulins (PSMs) are amphipathic peptides produced by staphylococci that have multiple functions in pathogenesis (79). PSMs have showed virulence increasing capacity in several animal models. Although PSMs exist in all *Staphylococcus aureus* strains, the expression level in CA-MRSA is obviously higher than HA-MRSA (75).

Cytolysin α-toxin greatly increases virulence of CA-MRSA in some animal models (76,80). The α-toxin was proven to significantly increase virulence
by lysing a series of cells, such as macrophages and erythrocytes, and cause collapse of the epithelial barrier (81). Recently, a core genome-encoded toxin, SEIX, was reported to lead to CA-MRSA pneumonia in a lung infection animal model (82).

4. Vaccines

In consideration of research results until now, a vaccine strategy would be an economical measure to prevent and control MRSA infections, but this will be a serious challenge for researchers to develop effective vaccines. A vaccine which targets two surface antigen clusters of MRSA was reported to fail in a Phase III trial (83). A few vaccines are still in their early stage of development, and no one has gotten close to authorization (84). This is a long road for investigators to walk.

5. Conclusion

For decades, doctors and researchers have been fighting with MRSA continuously and every time when new antibiotic weapons were developed, MRSA could raise novel shields of resistance accordingly. In the war with MRSA, although humans have obtained partial success, the challenges from antibiotic-resistant *Staphylococcus aureus* are still severe. Especially during recent years, the appearance of CA-MRSA brought humans to a novel battle field. The hard work of many laboratories shed a light on the relevance between genetic mutations and MRSA phenomena, such as antibiotic resistance, virulence, and biofilms. The mutations of MRSA could be ideal targets for sequential development of novel antibiotics and vaccines.

Acknowledgements

This study was supported by Grants-in-Aid from the Ministry of Education, Science, Sports, and Culture of Japan.

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(Received May 6, 2013; Revised June 23, 2013; Accepted June 25, 2013)