Microreview

MRSA virulence and spread

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Summary

Methicillin-resistant Staphylococcus aureus (MRSA) is one of the most frequent causes of hospital- and community-associated infections. Resistance to the entire class of \( \beta \)-lactam antibiotics, such as methicillin and penicillin, makes MRSA infections difficult to treat. Hospital-associated MRSA strains are often multi-drug-resistant, leaving only lower efficiency drugs such as vancomycin as treatments options. Like many other \( S. \) aureus strains, MRSA strains produce a series of virulence factors, such as toxins and adhesion proteins. Recent findings have shed some new light on the molecular events that underlie MRSA epidemic waves. Newly emerging MRSA clones appear to have acquired phenotypic traits that render them more virulent or able to colonize better, either via mobile genetic elements or via adaptation of gene expression. Acquisition of Panton-Valentine leukocidin genes and increased expression of core genome-encoded toxins are being discussed as potentially contributing to the success of the recently emerged community-associated MRSA strains. However, the molecular factors underlying the spread of hospital- and community-associated MRSA strains are still far from being completely understood, a situation calling for enhanced research efforts in that area.

Introduction

Staphylococcus aureus is a dangerous pathogen, responsible for a multitude of human infections around the world (Lowy, 1998). Many \( S. \) aureus infections present as moderately severe infections of the skin or respiratory tract, but \( S. \) aureus may also cause more dramatic forms of disease that may be life-threatening, such as necrotizing fasciitis or necrotizing pneumonia. Considerable efforts have been undertaken to decipher the importance that specific molecular determinants have in defining \( S. \) aureus virulence and interaction with the host. From a clinical point of view, a major problem that physicians have to face when treating \( S. \) aureus infections is antibiotic resistance. Resistance to the first antibiotic, penicillin, emerged in the 1940s (Barber and Rozwadowska-Dowzenko, 1948). In 1942, penicillin-resistant \( S. \) aureus was detected (Rammelkamp and Maxon, 1942). Mechanistically, resistance to penicillin is due to an enzyme called penicillinase, which was found even before the introduction of penicillin into clinical use (Abraham and Chain, 1940). Penicillinase cleaves the \( \beta \)-lactam ring that is characteristic of \( \beta \)-lactam antibiotics such as penicillin and its derivatives. Already in the 1950s, penicillinase-containing strains of \( S. \) aureus were pandemic in hospitals and the community (Roundtree and Freeman, 1956). Nowadays, most infectious \( S. \) aureus isolates are resistant to penicillin.

To overcome the problem with penicillin-resistant \( S. \) aureus, the semisynthetic antibiotic methicillin was developed, which is derived from penicillin, but resistant to \( \beta \)-lactamase inactivation. Methicillin was introduced by Beecham in 1959; but already about 1 year later, methicillin-resistant \( S. \) aureus was detected in the UK (Jevons et al., 1963). Unlike in the case of resistance to penicillin, the mechanism underlying methicillin resistance protects the bacteria from the entire class of \( \beta \)-lactam antibiotics including penicillins, cephalosporins and carbapenems. Staphylococcus aureus epidemics occur in waves of antibiotic resistance (Chambers and DeLeo, 2009). The first epidemic penicillin-resistant strains were replaced by the so-called ‘archaic’ methicillin-resistant \( S. \) aureus (MRSA) strains first found in the UK. This epidemic was largely restricted to Europe. Starting in the 1980s, novel lineages of MRSA emerged, leading to a worldwide pandemic of MRSA that is still ongoing. Nowadays, many industrialized countries report that methicillin-resistant strains account for at least 25–50% of infectious
**S. aureus** isolates in hospitals (Diekema et al., 2001). In contrast, some countries such as The Netherlands and the Scandinavian countries historically have low MRSA infection rates (often < 1%), most likely owing to rigid search-and-destroy and surveillance policies, as well as restraint in antibiotic prescription. In fact, a recent Japanese study indicates that high antibiotic consumption rates lead to increased MRSA burden over time (Nakamura et al., 2012). While for a long time MRSA infections were limited to hospitalized patients, the most recent epidemic MRSA wave, beginning in the mid- to late 1990s, is characterized by the emergence of community-associated MRSA (CA-MRSA) with the capacity to infect otherwise healthy individuals.

This review will give an overview over virulence and colonization strategies of **S. aureus** with a focus on MRSA strains. While for the most part, these strategies do not appear to be different between methicillin-susceptible **S. aureus** (MSSA) and MRSA strains, there are some examples of virulence and colonization determinants that were found to play significant roles in the pathogenic success of specific MRSA clones. In particular, recent findings give first insight into the potential molecular underpinnings of the rise and disappearance of MRSA during epidemic waves. Furthermore, considerable efforts have been undertaken to understand the molecular factors defining the increased potential of CA-MRSA with the capacity to infect otherwise healthy individuals.

**Genetics of methicillin resistance in S. aureus**

Methicillin resistance in staphylococci is due to the acquisition of a mobile genetic element (MGE) called the staphylococcal cassette chromosome **mec** (SCCmec) (Katayama et al., 2000). SCCmec is a DNA fragment ranging from 21 to 67 kb in size, depending on the SCCmec type (Hiramatsu et al., 2001). The number of SCCmec types is constantly increasing with the discovery of new elements; currently, there are 11. All SCCmec types include the **mecA** gene, which codes for the low-affinity penicillin-binding protein PBP2a (Hartman and Tomasz, 1981), as factor necessary for methicillin resistance. Resistance is due to the fact that β-lactam antibiotics cannot inhibit PBP2a, in contrast to other **S. aureus** PBPs. SCCmec elements also include ccr genes for integration and excision from the chromosome (Hiramatsu et al., 2001). Other parts of SCCmec elements vary in composition. For example, they may contain additional antibiotic resistance genes. While it has been often stressed that SCCmec elements do not contain virulence genes, this view had to be reversed when it was found recently that a peptide toxin, PSM-mec, is encoded in close vicinity to the **mecA** gene in several SCCmec types (Queck et al., 2009).

**MRSA clones**

Different MRSA clones have emerged ever since the detection of the first MRSA strain in 1959 (DeLeo and Chambers, 2009). Notably, almost all MRSA clones detected worldwide belong to only five clonal complexes (CCs): 5, 8, 22, 30 and 45 (Fig. 1). The first (archaic) MRSA clone [strain COL, sequence type (ST) 250] harboured the SCCmec element of type 1 and belonged to CC 8. Many important MRSA clones that emerged later, during the MRSA pandemic in the 1980s, belong to the same CC, but had new SCCmec types (types 2 and 3).
These included the Iberian (EMRSA-5, ST247) clone, a descendant of COL, and the Brazilian/Hungarian (EMRSA-1, ST239) clone. Further major MRSA clones are the New York/Japan clone (ST5, USA100) and the ‘paediatric’ clone (ST5), both of which belong to CC5.

**Multi-drug resistance**

Many MRSA clones have acquired resistance to additional antibiotics, such as erythromycin, clindamycin, ciprofloxacin, tetracycline, etc. (Shorr, 2007). It is alarming that multi-drug-resistant strains of *S. aureus* are often only susceptible to vancomycin, an antibiotic with considerably lower efficiency compared for example with β-lactams. Furthermore, rare cases of highly vancomycin-resistant MRSA strains (VRSA) have been reported (CDC, 2002). Fortunately, these did not spread substantially, possibly owing to increased fitness cost associated with high-level resistance to vancomycin. The majority of CA-MRSA strains have not acquired resistance to additional antibiotics, with the exception of limited outbreaks of multi-drug-resistant CA-MRSA (Diep *et al.*, 2008).

**Colonization**

In the hospital, contaminated fomites and medical devices may play a role as intermediate sources of MRSA infection, but ultimately these originate from patients or hospital personnel that carry MRSA. The anterior nares are the most frequent site of *S. aureus* carriage; and an association between *S. aureus* nasal carriage and disease has been noted already in 1931 (Wertheim *et al.*, 2005). Since then, several studies have confirmed the hypothesis that most *S. aureus* infections originate from strains that colonize the nose (von Eiff *et al.*, 2001; Wertheim *et al.*, 2004). While it has been emphasized that there are *S. aureus* colonization sites in the body other than the nose, such as the perineum and throat, the roles of these sites as sources of infection are poorly understood. Of note, it is often debated to eradicate MRSA carriage to minimize the potential for MRSA infection. However, studies evaluating the outcome of MRSA eradication produced widely divergent results. In a recent systematic survey it was concluded that mupirocin is effective in the short-term eradication of MRSA, such as for pre-surgical treatment in hospitals (Ammerlaan *et al.*, 2009).

Interestingly, only about 20% of individuals in the population are persistent nasal carriers of *S. aureus*, while 30% are intermittent carriers, and about 50% are never colonized (Wertheim *et al.*, 2005). The fact that carriage rates differ among ethnic groups and are higher in patients with certain diseases underlines the importance of host factors determining *S. aureus* colonization. However, the molecular underpinnings of these differences are not understood, and therefore, in the following there will be a focus on bacterial factors associated with colonization.

Surface-anchored *S. aureus*-binding proteins that interact with human matrix molecules (microbial surface proteins recognizing adhesive matrix molecules, MSCRAMMs) likely play a role in nasal colonization, particularly when the mucin layer is breached and matrix proteins are exposed. Clumping factor B and *S. aureus* surface proteins G and X (SasG, SasX) have been demonstrated to bind to nasal epithelial cells (O’Brien *et al.*, 2002; Roche *et al.*, 2003; Li *et al.*, 2012). The recently described SasX protein is of particular interest, because it has been linked to an MRSA epidemic wave (Li *et al.*, 2012). SasX is encoded on an MGE occurring predominantly in ST239 MRSA strains, which are the most frequent source of MRSA infections in Asia. The frequency of the sasX gene among invasive ST239 strains in Asia has increased significantly over the last decade. Given that it was shown to contribute to nasal colonization, biofilm formation, and immune evasion and virulence in animal infection models (see below), it is likely that sasX is a major factor responsible for the spread and pathogenic success of ST239. Notably, sasX and its distribution exemplify how the spread of a colonization and virulence factor by horizontal gene transfer may drive an MRSA epidemic wave.

Several surface polymers of *S. aureus*, such as teichoic acids, have been shown to determine the capacity of *S. aureus* to colonize the nose (Weidenmaier *et al.*, 2004), but it is not understood how this occurs on a molecular level. Furthermore, *S. aureus* has a series of mechanisms providing resistance to antimicrobial peptides, which form an important part of innate immune defence on epithelia (Peschel, 2002). Finally, it has been stressed that bacterial interference may have an influence on nasal colonization. However, there is no clear evidence yet to suggest that MRSA epidemic waves are caused by the direct interference of competing MRSA strains, differential expression of surface polymers involved in colonization, or mechanisms of immune evasion. Nevertheless, scenarios are easy to imagine, in which MRSA strains use such mechanisms to displace other strains.

**Role of biofilms**

Biofilms are surface-attached bacterial agglomerations embedded in extracellular matrix. Staphylococci are known as especially good biofilm formers, which is due primarily to the production of a series of surface molecules that promote extracellular matrix formation (Otto, 2008). Biofilms provide significant protection from antibiotics and host defences, in addition to enabling the bacteria to remain attached to biotic or abiotic surfaces. Thus,
biofilms may contribute to prolonged infection and colonization, and the spread of MRSA in hospital and community settings. Whether *S. aureus* colonies in the nose can be considered biofilms is debatable, but the physiological status during colonization may be comparable in many aspects to that in biofilms. The strategy of colonizing or biofilm-forming *S. aureus* to remain in or on human epithelia in relative ‘silence’ is opposed to an aggressive status of active toxin production during acute *S. aureus* disease. It is noteworthy in that regard that many colonizing *S. aureus* isolates were shown to be defective in the global virulence regulator Agr (Shopsin et al., 2008). Interestingly, there is evidence to suggest that increased capacity to form biofilms and adhere to epithelial cells may have been linked for example to the spread of the so-called Brazilian MRSA clone (ST239) (Amaral et al., 2005), the likely predecessor of the Chinese sasX-positive ST239 strains.

**Diseases caused by MRSA**

The most common diseases caused by MRSA in the hospital are not different from those caused by MSSA and mostly include skin, soft-tissue, respiratory, bone, joint and endovascular infections, and sepsis (Lowy, 1998). Furthermore, together with coagulase-negative staphylococci, *S. aureus* is the most common cause of infections on indwelling medical devices. The mortality rate of severe, invasive MRSA infections is about 20% and it has been estimated that MRSA infections are the leading cause of death by a single infectious agent in the USA, exceeding deaths caused by HIV/AIDS (Klevens et al., 2007). Notably, MRSA as opposed to MSSA infection is associated with significantly higher costs, due to prolonged stay in the hospital, and mortality (Hanberger et al., 2011).

**Virulence**

Virulence of *S. aureus* is multi-factorial, dependent on a series of toxins, adhesion, immune evasion and other virulence determinants; the same is true for MRSA strains. In the following, determinants that have been linked to the virulence of specific MRSA strains will be highlighted; and it will be discussed which factors are responsible for the pathogenic success of CA-MRSA strains in particular.

**Toxins**

Differences in the toxin repertoire between *S. aureus* clones are common and due to the fact that many *S. aureus* toxins and other virulence determinants are encoded on MGEs, whose presence varies considerably between strains. Such MGE-encoded toxins include superantigens such as toxic shock syndrome toxin (TSST), some leukotoxins such as Panton-Valentine leukocidin, and exfoliative toxins. In contrast, α-toxin, γ-toxin, some leukotoxins and phenol-soluble modulins (PSMs) are produced by most strains. Nevertheless, differential expression of such core-genome encoded toxin genes may also cause significant differences in the pathogenic potential between *S. aureus* strains. A particularly dramatic difference is seen in mutants that are functionally defective in Agr, which controls the expression of many *S. aureus* toxin genes (Novick et al., 1993).

Among HA-MRSA strains, there are a few examples of lineages that are characterized by harbouring specific toxin genes. For example, TSST is associated with the CC30/USA200 lineage, but its role in CC30 pathogenicity awaits detailed investigation. It is interesting that present isolates of the CC30 lineage carry mutations in the agrC and hla (α-toxin) loci, which significantly reduce acute virulence (DeLeo et al., 2011). As CC30 is the second most predominant HA-MRSA lineage in the USA, it may be concluded that sustained establishment of an MRSA clone in hospital settings involves reduced aggressive toxicity. Nevertheless, the agrC mutation in contemporary CC30 clones (such as EMRSA-16, representative clone Sanger 252) only slightly reduces Agr activity; and EMRSA-16 still produces toxins such as δ-toxin at considerable levels (Cheung et al., 2011). Notably, EMRSA-16 also produces significant amounts of PSM-mec.

PSM-mec is a member of the PSM class of *S. aureus* peptide toxins, but in contrast to all other known PSMs it is encoded on MGEs, namely SCCmec elements of types 2, 3 and 8, and thus correlated with specific MRSA lineages (Queck et al., 2009; Chatterjee et al., 2011). PSM-mec has cytolytic activity towards neutrophils and erythrocytes. It is a significant determinant of *S. aureus* infection, at least in strains that produce high relative amounts of PSM-mec compared with other potent PSM cytolsins.

**Surface proteins**

Surface proteins have multiple important roles in *S. aureus* pathogenesis. In addition to key functions in bacterial cell wall metabolism, they serve to bind to host tissue, facilitate internalization and immune evasion, and are involved in bacterial aggregation and biofilm formation (Foster and Hook, 1998). Most surface proteins are encoded on the core genome. Therefore, the specific success of an MRSA clone has not been linked to a specific surface protein. The SasX protein already mentioned above is an exception, inasmuch as it is encoded on an MGE and in addition to its role in colonization, has...
CA-MRSA transmissibility

The sustained spread of CA-MRSA may in principle be due only to the shear number of infections and direct transmission from infected patients, and thus to infectivity or virulence. However, CA-MRSA may also show increased transmissibility and colonization characteristics. CA-MRSA isolates carry SCCmec elements of type 4 or 5, which as a result of their smaller sizes compared with other SCCmec elements may be associated with lower fitness costs. Otherwise, there are only limited data assigning potentially increased colonization capacity to a molecular factor uniquely present in CA-MRSA. The arginine catabolic mobile genetic element (ACME) of USA300 harbours a spermidin acetyltransferase gene (speG) that transfers resistance to spermidin and other polyamines, molecules, which are produced by most living cells except for \( S. aureus \) (Joshi et al., 2011). The exceptional sensitivity that \( S. aureus \) has to polyamines is only abolished in ACME-containing USA300 strains. Thus, speG may serve to explain augmented colonization capacity in USA300. However, other CA-MRSA, including some South American clones of USA300, lack ACME. Moreover, ACME harbours an arginine deiminase and an oligopeptide gene cluster, which have been implicated in colonization capacity, but there are no clear experimental data supporting this hypothesis (Otto, 2010). Finally, CA-MRSA strains may express and regulate surface adhesins in a manner different from other strains, but whether this translates to increased colonization capacity is now known (Cheung et al., 2011).

CA-MRSA virulence

The theory that increased virulence, as compared with HA-MRSA strains, is accountable for the ability of CA-MRSA strains to infect healthy people was confirmed in animal infection models and is generally accepted. Increased virulence of CA-MRSA strains may be due to a considerable extent to the fact that they exhibit high capacity to circumvent killing by human neutrophils, the first line of defence of the human body against staphylococcal infections. However, there has been much debate about which mechanisms are responsible for the exceptional ability of CA-MRSA strains to evade human immune defences and lead to infection in healthy hosts. Two main hypotheses have been discussed. The first hypothesis attributes increased CA-MRSA virulence to the acquisition of MGEs, namely that containing Panton-Valentine leukocidin (PVL) (Vandenesch et al., 2003), while the other explains this by increased expression of core genome-encoded virulence genes, such as PSM cytolysins, \( \alpha \)-toxin and other virulence determinants (Li et al., 2009). Notably, these two explanations are not mutually exclusive.
Panton-Valentine leukocidin

Panton-Valentine leukocidin is a member of the bi-component family of staphylococcal leukocidins. It has been associated early with specific forms of skin infections, such as furunculosis. At the beginning of the CA-MRSA epidemic, the PVL genes lukS and lukF were found in virtually all CA-MRSA clones, while PVL is typically absent from HA-MRSA (Vandenesch et al., 2003). Thus, researchers focused on PVL as a potential cause of increased CA-MRSA virulence. However, two main findings have cast severe doubt on a significant role of PVL as a virulence factor in CA-MRSA disease (Otto, 2010). First, CA-MRSA clones have emerged in the meantime that lack PVL genes and still show significant virulence. Second, analysis of isogenic PVL gene deletion mutants did not confirm a substantial impact of PVL on CA-MRSA virulence in several animal models.

As among neutrophils of commonly used model animals, only those of rabbits show considerable sensitivity to PVL, rabbit models are now used to investigate the contribution of PVL to virulence. Results from rabbit models of severe lung infection and osteomyelitis indicated a significant role of PVL in the pathogenesis of these disease types (Cremieux et al., 2009; Diep et al., 2010). Contrastingly, two studies failed to detect an impact of PVL to CA-MRSA skin infection in rabbits. In the first study, which used isogenic USA300 deletion mutants, PVL failed to show an impact on virulence, whereas other toxins (PSMα, α-toxin) showed strong effects (Kobayashi et al., 2011). In the second study, PVL-negative CA-MRSA strains showed virulence characteristics similar to those of PVL-positive strains (Li et al., 2010). Then again, another group reported a moderate yet significant impact of PVL on virulence in rabbit skin infection, but they did not compare with the effects of other toxins (Lipinska et al., 2011).

Overall, PVL does not appear to exert the dramatic effects on CA-MRSA virulence that it was first believed to have. Furthermore, given the only minor or absent impact it has on skin infections, it is hard to imagine how PVL could be a significant factor driving the CA-MRSA epidemic. Nevertheless, because different groups have achieved contrasting results until very recently, the role of PVL in CA-MRSA infection is still a matter of debate.

PSMs, α-toxin and other core-genome encoded toxins

Phenol-soluble modulins are a family of amphipathic peptides, some of which show pronounced cytolytic capacities, in particular to human neutrophils (Wang et al., 2007). The PSMα peptides of S. aureus have a considerable impact on virulence of CA-MRSA strains in mouse and rabbit skin infection models. While psm genes are present in all S. aureus strains, CA-MRSA strains in average show increased PSM production compared with HA-MRSA strains (Li et al., 2009). Further, the cytolytin α-toxin significantly affects CA-MRSA virulence in skin and lung infection models (Bubeck Wardenburg et al., 2007; Kobayashi et al., 2011). While not cytolytically towards human neutrophils, α-toxin lyses a series of other cells, such as erythrocytes and macrophages, and facilitates breach of the epithelial barrier, at least in part mediated by interaction with the ADAM10 receptor (Hanberger et al., 2011). Finally, a core genome-encoded superantigen, SEIX, was recently described, which contributes to CA-MRSA necrotizing pneumonia, as demonstrated in a rabbit lung infection model (Wilson et al., 2011).

As most S. aureus strains carry genes for these toxins, increased virulence that is dependent on those factors must be mediated by increased expression. High expression of PSMs, α-toxin and additional virulence determinants such as proteases has been shown to occur in the lineage that leads to the USA300 clone, which includes the USA300 predecessor USA500 (Li et al., 2009). Likely, one reason for increased virulence factor expression in that lineage is increased activity of Agr. However, the exceptional virulence potential of USA300 and other CA-MRSA clones can certainly not be explained by increased activity of Agr alone. Presumably, genetic rearrangements that are yet poorly understood have led to clones that are highly virulent and exceptionally good colonizers. Most research has been performed on strain USA300, but it is possible that other CA-MRSA clones underwent different changes to obtain these phenotypic features. Additionally, at least some CA-MRSA clones such as USA300 may have been optimized in virulence and colonization capacities by acquiring molecular determinants on MGEs, such as ACME and the genes coding for PVL (Fig. 2).

Concluding remarks

Novel MRSA clones keep occurring in hospitals and more recently in the community, often causing sustained epidemics. We are now beginning to understand how S. aureus optimizes gene content and expression to create such new strains with optimized virulence and colonization capacities. In addition, MRSA strains are constantly accumulating further antibiotic resistance genes, creating highly virulent and hardly treatable ‘superbugs’. It is therefore an important task of current S. aureus pathogenesis research to delineate factors that define virulence of the entire range of infectious MRSA strains, in order to generate drugs with as broad and long-lasting an anti-staphylococcal potential as possible. This should happen simultaneously to maintained efforts aiming to find new, efficient antibiotics.
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