

Short Conceptual Overview

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How colonization factors are linked to outbreaks of methicillin-resistant *Staphylococcus aureus*: the roles of SasX and ACME

Abstract: Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most frequent cause of hospital-associated morbidity and mortality. One reason why MRSA has remained a serious threat to public health is that new clones of MRSA constantly keep re-emerging. These new clones are better adapted to thrive in the hospital environment or even the community than their predecessors because they have developed increased and diversified antibiotic resistance and/or enhanced virulence. In addition, non-symptomatic colonization has been identified as a risk factor for subsequent MRSA infection; therefore, acquisition of factors promoting colonization has gained increased attention regarding the surge of MRSA outbreak clones. Two specific genes or genetic loci, namely *sasX* and the arginine catabolic mobile element (ACME), could recently be linked to the epidemiological success of MRSA clones, supporting the notion that colonization factors play a crucial role in MRSA outbreaks. SasX is a surface protein that enhances nasal colonization. ACME contains an *arc* arginine deiminase gene cluster promoting the survival of MRSA in the acidic skin environment, in addition to a polyamine resistance gene that deals with the increased production of toxic polyamines by the host that is prompted by *arc*. Notably, a better understanding of MRSA colonization on the molecular level may lead to eradication strategies based on vaccination or bacterial interference, with great promise to decrease MRSA infection rates.

Keywords: colonization; MRSA; *Staphylococcus aureus*.

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Introduction

Infectious diseases represent the second most frequent cause of death in the world, accounting for about one quarter of the 54 million deaths reported in 1998 by the World Health Organization. Many of those fatal infections are due to bacterial pathogens such as the tuberculosis bacillus *Mycobacterium tuberculosis* or bacteria causing respiratory tract infections, such as *Staphylococcus aureus* or *Streptococcus pneumoniae*. Antibiotic resistance in pathogenic bacteria severely complicates the treatment of bacterial infections and is especially common among hospital-associated (HA) germs, such as methicillin-resistant *S. aureus* (MRSA) (1). Often, infectious isolates are resistant to a wide variety of antibiotics, leaving only a few and frequently suboptimal antibiotics as treatment options. Notably, this situation is unlikely to change significantly within the foreseeable future, owing to the considerably reduced efforts of companies in antibiotic development. Given the predictable shortage of efficient antibiotics for treatment in the future, it is vital to understand how antibiotic-resistant bacteria such as MRSA manage to enhance pathogenicity and spread in the population, as the underlying mechanisms may possibly be a target for therapeutic interference aimed at keeping those strains in control.

Different strains of MRSA dominate in different regions of the world, and novel clones of MRSA keep emerging and spreading (2). However, the molecular factors responsible for the development of novel MRSA outbreak clones and the epidemic waves of MRSA appearance and disappearance are not well known. The acquisition of additional antibiotic resistance genes that provide protection from antibiotics in clinical use certainly is a major driving force for the occurrence of new epidemic MRSA clones. In addition, recent findings have highlighted that enhanced virulence is of immense importance, promoting the spread of MRSA infections in otherwise healthy people in the community (3). In contrast, the molecular underpinnings of the emergence of new HA clones of MRSA have remained

poorly understood. Here, recent research that sheds light on the role of colonization factors in the adaptation and spread of HA-MRSA will be discussed.

Methicillin resistance meets virulence: community-associated MRSA

Before discussing what drives HA-MRSA outbreaks, it is worth looking at the recent surge of community-associated (CA-) MRSA, because we have learned much about MRSA epidemiology in general from the extensive research efforts in that area. What underlies the success of CA-MRSA is the fact that antibiotic resistance in bacteria becomes particularly dangerous when it occurs in a highly virulent pathogen (4). In CA-MRSA strains, a low-fitness-cost mobile genetic element (MGE) conferring methicillin resistance was acquired by a highly virulent lineage, leading to a combination of virulence and resistance with high pathogenic potential (3). CA-MRSA clones thus became able to infect otherwise healthy individuals, overcoming the limitation of HA-MRSA clones, which need a predisposed or weakened patient to mount a successful infection. The main factors distinguishing CA- from HA-MRSA strains are enhanced production of core genome-encoded toxins, most notably α -toxin and phenol-soluble modulins, and, at least in some strains, acquisition of an MGE containing the Panton-Valentine leukocidin (3). Thus, toxins that kill human immune and other cells are primarily responsible for the high virulence potential of CA-MRSA.

Notably, despite the higher aggressiveness of CA- as opposed to HA-MRSA strains, typical HA-MRSA lineages are still predominant in hospitals worldwide and have only been replaced to a limited extent by CA-MRSA lineages (5–7). The latter is seen especially in the US, where the CA-MRSA clone USA300 is now predominant (8). While we might not yet have reached the end of this replacement process on a global scale, this situation seems to indicate that HA-MRSA strains contain specific adaptations to the hospital environment and the development of HA infections. It has been stressed that aggressive virulence may be counterproductive for HA-MRSA clones to spread sustainably in the hospital setting, such as exemplified by the success of strains belonging to clonal complex 30, which are defective in the virulence regulator Agr and produce the abovementioned toxins only at low levels (9). However, it is largely unknown which determinants are responsible for the sustained success of HA-MRSA strains

to thrive in the hospital environment and infect patients in that setting.

Deciphering the molecular underpinnings of MRSA outbreaks in hospitals

Staphylococcus aureus outbreak clones usually have acquired a new factor that makes them less susceptible to current methods of antibiotic treatment, such as when MRSA developed as a bacterial response to the widespread use of methicillin (10). As discussed above, such new clones may have an altered composition of further genetic factors that promote their pathogenic success, such as toxins in the case of CA-MRSA (3). As for HA-MRSA, the spread of specific HA-MRSA clones is certainly due to some extent to the acquisition of additional antibiotic resistance genes by horizontal gene transfer. However, there are not many clear examples of such scenarios. This may be due to the variety of alternative antibiotics used to treat MRSA and/or the fact that the acquisition of an antibiotic resistance gene may come with a pronounced fitness cost, such as in the case of vancomycin-resistant *S. aureus* (11). However, recently, it was shown by comparative genome sequencing that the acquisition of fluoroquinolone resistance led to the spread of a new fluoroquinolone-resistant subclone of EMRSA-15 (12), underscoring the importance of antibiotic resistance gene transfer in the development of new epidemic HA-MRSA clones.

SasX: a colonization factor promoting the spread of MRSA clones

In addition to antibiotic resistance genes, a series of other factors may in theory promote the spread of a new epidemic clone, such as virulence factors in the case of CA-MRSA clones. Furthermore, in *S. aureus*, factors facilitating colonization also need to be considered as potentially crucial for the spread of epidemic clones. This is because *S. aureus* is a colonizer in about one third of the population, with permanent colonization occurring mostly in the nose (13), and there is strong evidence indicating that *S. aureus* infections originate from colonizing strains (14). Therefore, any factor that facilitates and prolongs colonization can be considered a risk factor for

S. aureus infection. The capacity of *S. aureus* and MRSA strains to colonize patients and health-care personnel in a hospital setting is of immense importance because these colonizers represent a reservoir of clones that may become infectious to susceptible patients.

Many factors were shown to enhance *S. aureus* nasal colonization, such as teichoic acids (15) or cell surface proteins (16); however, they were not epidemiologically linked to an MRSA outbreak, mostly because these factors are present among all or in a wide majority of *S. aureus* strains. In a recent study, Li et al. (17) provided evidence that a factor promoting nasal colonization spread widely among MRSA strains in China, exemplifying for the first time how a non-resistance-related gene may contribute to an MRSA outbreak. This gene, *sasX*, was first identified by whole-genome sequencing of an outbreak strain of sequence type (ST) 239 from London (18), a lineage that is particularly frequent in Asian countries. Li et al. showed that the frequency of *sasX* in HA-MRSA strains in China increased significantly over the last decade. Notably, while *sasX* was first found predominantly in ST239 isolates, it began spreading to other STs within the last years. This fast spread is likely due to the fact that *sasX* is encoded on an MGE, namely a prophage, and horizontal gene transfer may thus occur by phage mobilization and transduction.

A significant part of the Li et al. (17) study comprised the functional characterization of the SasX protein, delineating how it may enhance the pathogenic success of *sasX*-positive clones. SasX is a member of the family of surface-located proteins that are attached to the bacterial cell wall by the sortase enzyme (19). Many of these proteins facilitate attachment to human tissues (20). Importantly, it was found that SasX mediates adhesion to nasal epithelial cells harvested from human volunteers and promoted nasal colonization in a murine colonization model (17). Furthermore, SasX enhanced virulence in mouse models of lung and skin infection. Of note, these effects were shown in two different strain backgrounds, ST239 and ST5, demonstrating that SasX contributes to colonization capacity and virulence in different lineages. This may explain the fast spread of SasX in and beyond the ST239 lineage. Mechanistically, the contribution to virulence is likely due to the fact that SasX promotes biofilm formation and aggregation, leading to increased resistance to neutrophil phagocytosis and survival in human blood (17). The precise molecular interactions underlying the aggregation and adhesion behavior mediated by SasX are currently unknown. It is possible that SasX increases cell-cell adhesion between bacterial cells and adhesion to epithelial cells by a similar mechanism or, alternatively,

uses a different mechanism to bind to epithelial cells via a specific receptor.

The arginine catabolic mobile element (ACME)

The ACME element was first described in the CA-MRSA strain USA300 and likely originates from *S. epidermidis*, where it is found frequently (21). It primarily contains an arginine deiminase operon (*arc* genes), an oligopeptide permease operon (*opp* genes), and a gene (*speG*) encoding a spermidine (polyamine) acetyltransferase. It has been suggested early that ACME may enhance the colonization capacities of USA300 (22). One possible mechanism underlying the enhanced ability of USA300 strains to colonize and cause skin infections that was suggested was that of pH adjustment of the acid environment on the skin by the ammonia produced by the catalytic activity of arginine deiminase. Recently, Thurlow et al. (23) provided evidence that the ACME-encoded Arc system in fact contributes to the success of USA300 to thrive in acidic environments as present on the human skin. However, arginine deiminase activity also leads to enhanced production of host polyamines, probably because it competes for arginine use with the host's inducible nitric oxide (NO) synthase (iNOS) system. This necessitates the presence of *speG*, which detoxifies polyamines (24). Other *S. aureus* strains lack *speG*. This situation is quite unique among bacteria, which commonly all have *speG* and are thus polyamine resistant. Notably, Thurlow et al. (23) showed that *speG*-mediated polyamine detoxification is required for the pathogenic success of *S. aureus* in skin abscesses, a form of disease that is particularly frequently caused by USA300 strains (2). It also needs to be stressed that the decreased production of NO by diminished function of iNOS is not the reason for enhanced survival of ACME-containing *S. aureus* on the skin because *S. aureus* is inherently resistant to NO.

The ACME element thus represents another factor, which – similar to *sasX* – enhances colonization and could be linked to an MRSA outbreak. In further similarity to *sasX*, it also contributes to pathogen success in certain infection types. While USA300 strains are primarily linked to CA infections and the enhanced skin colonization capacities certainly play a considerable role in the sustainable spread of USA300 in the community, the rising additional success of USA300 as an HA-MRSA clone may very likely also be due to the promotion of colonization by ACME. In strong support of this notion, the ACME element

has recently been reported to spread to other HA sequence types, such as ST5 in Japan (25), in further resemblance with the situation seen with *sasX*.

Conclusions and outlook

In conclusion, there is increasing evidence that enhancement of colonization capacities is an important factor underlying the success of newly arising MRSA outbreak clones. The fast spread of such factors by horizontal gene transfer of MGEs appears to play a crucial role in that success. Colonization factors thus join antibiotic resistance and virulence factors, which have an already more established and more widely discussed role in contributing to MRSA outbreaks. Given that the role of colonization as a risk factor for *S. aureus* disease is becoming more and more appreciated, it is likely that more colonization-dependent MRSA outbreak factors such as ACME and *sasX* will be found in the future. Furthermore, there is increasing evidence that rectal and intestinal colonization plays a yet underestimated role as a source for *S. aureus* disease (26), necessitating more intensive research into

the molecular factors promoting *S. aureus* colonization in these areas as well.

Ultimately, a better understanding of MRSA colonization on the molecular level may lead to therapeutic strategies preventing *S. aureus* colonization in the hospital, and possibly in the community. From all that we know about colonization as the source of infection, such strategies have great potential to considerably decrease MRSA infection rates in hospitals. These strategies may include, for example, vaccination against predominant colonization factors. In addition, there is increasing appreciation of the role of the commensal human microflora in balancing colonization with potentially pathogenic germs such as *S. aureus* (27, 28). Bacterial interference with commensal species may thus be employed to limit colonization by MRSA and other harmful bacteria.

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