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Molecular Pathogenesis of *Staphylococcus aureus* Infection

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ABSTRACT: *S. aureus* has evolved a comprehensive strategy to address the challenges posed by the human immune system. The emergence of community-associated methicillin-resistant *S. aureus* (CA-MRSA) infections in individuals with no predisposing conditions suggests an increased pathogenicity of the bacterium, which may be related to acquisition of novel genetic elements. Remarkably, despite an abundance of research, the underlying cause of the epidemic is not known. Here, the various strategies used by *S. aureus* to evade obstacles laid out by the human host during colonization and infection were reviewed. The controversies surrounding MRSA research were described, and how acquisition of the novel genes could explain the increased incidence and severity of CA-MRSA diseases was described. **(*Pediatr Res* 65: 71R–77R, 2009)**

S aureus has an extraordinary repertoire of virulence factors that allows it to survive extreme conditions within the human host. Such an elaborate armamentarium might prompt one to speculate that human kind would be no match for this pathogen and could be highly vulnerable to severe *S. aureus* infection. Surprisingly, *S. aureus* maintains fine control of virulence expression, and for the most part, it rarely causes severe infection in previously healthy individuals.

The past 10 y however have witnessed the emergence of new clones of MRSA that have rapidly spread across continents, causing rampant skin and soft tissue infections and some unusually severe diseases. Unlike traditional MRSA clones which are largely confined to healthcare settings and prey on immunocompromised hosts or hosts with predisposing factors, these community-associated methicillin-resistant *S. aureus* (CA-MRSA) clones infect previously healthy hosts, particularly children, young and middle-aged adults.

This review is written with clinician scientists as the target audience. To impart a better appreciation of MRSA pathogenesis, I will first describe the obstacles that *S. aureus* needs to overcome to establish an infection and then highlight the aspects of pathogenesis that are unique to healthcare-associated MRSA (HA-MRSA) and CA-MRSA. Readers are referred to many excellent reviews of *S. aureus* colonization and pathogenesis (1–6) that describe more fully the mechanisms of virulence.

Colonization. *S. aureus* acquired from an external source could be the cause of an infection when inoculated into an open wound. More commonly, the human host is infected by bacteria that colonize her or his skin or mucosal surface (7,8). The mucosal surfaces that harbor *S. aureus* include the nose, throat, vaginal wall, and GI tract. Nasal carriage is probably most important because nose-picking could effectively disseminate the bacterium to other body surfaces and other hosts (9). Remarkably, 20% of individuals are persistently colonized in the nose and 30% are transiently colonized. The definition of persistent and transient carriage varies according to the study, but generally is described as a single positive culture on a nasal swab (transient) *versus* at least two consecutive positive cultures 1 wk apart (persistent). Colonization is also more frequent among younger children and patients with HIV and diabetes (4).

Although colonization predisposes an individual to *S. aureus* infection, one study shows that after nosocomial infection, colonized individuals have less severe *S. aureus* disease compared with noncolonized individuals (7). This begs the question whether colonization could induce low-level adaptive immunity so that subsequent infections become milder. In support of this view, a study showed that carriage of *S. aureus* harboring the Toxic Shock Syndrome Toxin (TSST) is associated with production and maintenance of antibody to the toxin (10). Conversely, most individuals who acquire *Staphylococcus* toxic shock syndrome do not have antibody to TSST.

For *S. aureus*, colonization of the human nose presents a significant challenge that requires not only adherence to nasal epithelial cells, but also an ability to cope with host defense and competing resident microorganisms. *S. aureus* adheres and invades host epithelial cells using a variety of molecules that are collectively termed microbial surface components recognizing adhesive matrix molecules (MSCRAMM). A number of bacterial products (including MSCRAMM) have been suggested to be important for adhesion and attachment to nasal epithelial cells, but two factors (clumping factor B and wall-associated teichoic acid) have so far proven roles in nasal colonization of humans and rats (11,12).

Host immune deterrents for bacterial nasal colonization include antimicrobial peptides, lysozyme, lactoferrin, and IgA

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Abbreviations: *agr*, accessory gene regulator; **ACME,** arginine catabolic mobile element; **CA-MRSA,** community-associated methicillin-resistant *S. aureus*; **HA-MRSA,** healthcare-associated methicillin-resistant *S. aureus*; **MSSA,** methicillin-sensitive *S. aureus*; **PSM,** phenol soluble modulin; **PVL,** *Panton-Valentine Leukocidin*; **ROS,** reactive oxygen species; **SCC*mec***, Staphylococcal cassette chromosome mec

(4). However, little is known of the critical host defenses against *S. aureus* colonization. A study in mice has identified the cystic fibrosis transmembrane conductance regulator and toll-like receptor 2, but not toll-like receptor 4 as important factors controlling *S. aureus* carriage (13).

Resident nasal flora present an equally formidable challenge for *S. aureus*. Studies of *S. aureus* carriers and noncarriers have shown that presence of certain bacteria, such as corynebacterium, *S.epidermidis*, or *S. pneumoniae*, could preclude carriage of *S. aureus* (14). Introduction of the *S. pneumoniae* vaccine, for example, has been shown in some studies (15), but not others (16), to lead to a significant increase in *S. aureus* colonization leading some to speculate that *S. pneumoniae* and *S. aureus* could compete for the same niche. The general mechanism of niche competition is proposed to be a bacterial competition for adhesion to the same host receptor. In addition, certain competitors such as *S. pneumoniae* secrete hydrogen peroxide, which at high concentration suppresses *S. aureus* growth (17). *S. aureus* could counter by secreting catalase and likely other antioxidants which neutralize hydrogen peroxide (18).

Once colonization is established, *S. aureus* is positioned in close proximity to the throat, ears, mouth, and sinus; yet, surprisingly nasal carriage rarely leads to overt infection of these sites. Studies of *S. aureus* regulation suggest that during colonization, many *S. aureus* virulence genes may be down-regulated (19). Among the genes that control *S. aureus* colonization and virulence, the best known global regulator is the accessory gene regulator *agr*, which has been described in details in many excellent reviews (19). Briefly, *agr* is a quorum sensing locus, which directly controls expression of a number of virulence and colonizing factors. Down-regulation of *agr* is associated with colonization and activation of *agr* with host invasion. A critical question then is what triggers activation of *S. aureus* virulence genes to initiate infection.

Pathogenesis. Infections occur frequently as a consequence of *S. aureus* inoculation into an open wound. Alternatively, in the upper airway, viral infection damages mucosal linings and predisposes the host to *S. aureus* pneumonia, which classically presents a week after onset of influenza infection.

Initial exposure of *S. aureus* to host tissues beyond the mucosal surface or skin is thought to trigger up-regulation of virulence genes (19). For the host, resident phagocytes and epithelial cells in the skin or mucosal tissue respond to either bacterial products or tissue injury by activation of the immune system. *S. aureus* peptidoglycan and lipoprotein are sensed by host pattern recognition molecules (20,21); hyaluronan breakdown products (22) and endogenous toll-like receptor ligands (RNA, DNA, HMGB1) released by necrotic tissues (23,24) during infection further augment proinflammatory signaling leading to local immune cell activation and neutrophil and macrophage recruitment.

S. aureus has been generally recognized to survive well both inside and outside of host cells. In the extracellular milieu, *S. aureus* must overcome opsonization by complement and antibodies, which directly or indirectly leads to killing of *S. aureus* or uptake by phagocytes through Fc or complement receptors. *S. aureus* avoids opsonophagocytosis by expressing on its surface a capsule, clumping factor A, protein A, and a number of complement inhibitors, all of which inactivate or prevent host opsonins from binding or targeting the bacterium for destruction (3,6) (Fig. 1).

S. aureus can shelter within epithelial cells, endothelial cells, and even macrophages (25). By contrast, neutrophils present a more formidable challenge to *S. aureus*, as is evidenced by increased incidence of invasive *S. aureus* infections in patients with neutrophil dysfunctions (*e.g.*, chronic granulomatous disease and leukocyte adhesion deficiency). *S. aureus* deploys a number of strategies to resist neutrophil killing. First, it secretes two molecules, chemotaxis inhibitory protein (CHIP) and extracellular adherence protein (Eap), which respectively block neutrophil recognition of chemotactic factors (26) and neutrophil binding to endothelial adhesion molecule ICAM-1 (27). Inhibition of ICAM-1 binding prevents leukocyte adhesion, diapadesis, and extravasation from the bloodstream to the site of infection.

Upon arriving at the infection site, neutrophils unleash a battery of antimicrobial substances, including antimicrobial peptides, reactive oxygen species (ROS), reactive nitrogen species, proteases, and lysozyme. Defense against ROS is mediated in *S. aureus* by deployment of a large number of antioxidant enzymes (*e.g.*, catalase, pigment, superoxide dismutase) that neutralize ROS and reactive nitrogen species (3). Antimicrobial peptides which rely partly on targeting of negatively charged bacteria are repelled by *S. aureus* strategies that alter its surface charges (28,29). Additionally, antimicrobial peptides are degraded (aureolysin) (30) and neutralized (staphylokinase) (31).

As a preemptive measure, *S. aureus* counters by secreting specific toxins, which lyse neutrophils. *S. aureus* expresses a large number of two-component toxins (32) many of which have specificity for human but not mouse cells; therefore, many of their functions have not been characterized. The recently identified phenol soluble modulin (PSM) is a group of bacterial peptides previously described in *S. epidermidis,*

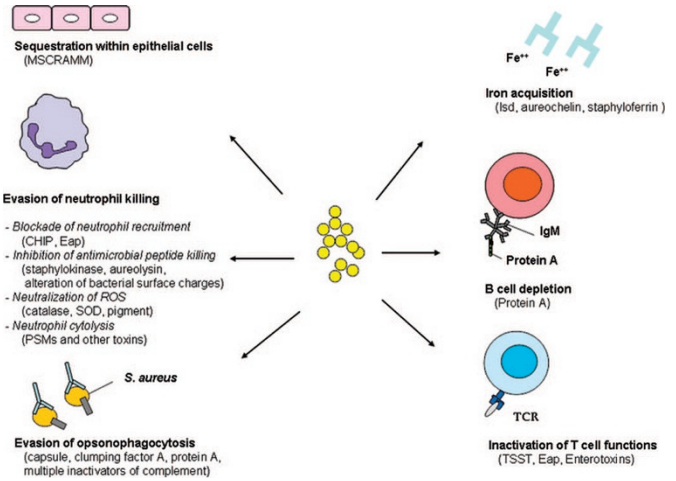

Figure 1. *S. aureus* survival strategies during infection. MSCRAMM, Microbial surface components recognizing adhesive matrix molecules; CHIP, chemotaxis inhibitory protein; Eap, extracellular adherence protein; SOD, superoxide dismutase; PSM, phenol soluble modulin; Isd, iron-regulated surface determinant; TCR, T cell receptor; TSST, toxic shock syndrome toxin.

which induce inflammation and neutrophil cytolysis. The virulence role of PSM peptides has been confirmed in a CA-MRSA skin infection model (33).

Apart from evasion of host immune defense, bacterial survival within the human host is dependent on successful acquisition of nutrients, particularly iron (34). During infection, 95% of iron is sequestered within host cells and serum iron is mostly bound to host proteins that are not easily accessed. *S. aureus* secretes high affinity iron-binding compounds (aureochelin and staphyloferrin) during iron starvation (35,36). Additionally, upon sensing low iron, *S. aureus* initiates transcription of an iron acquisition program (*isd*) that allows capture of heme and haptoglobin on the cell surface, transport of the iron complex across the plasma membrane and subsequent oxidative degradation of the heme within the cytoplasm (34).

A severe bacterial infection normally induces the host to mount an adaptive immune response within 7–10 d to limit the ongoing infection and prevent future reinfections. However, one of the hallmarks of *S. aureus* biology is the ability of the pathogen to infect the human host repeatedly throughout life. The mechanism underlying evasion of adaptive immune response is poorly understood; however, studies have shown that staphylococcal enterotoxins, TSST, and Eap (a MHC class II analog) could all alter T cell functions by targeting the T cell receptor activation pathway (37,38). This has been construed as a tactic devised by *S. aureus* to prevent development of long-term memory. Likewise, protein A has been shown to deplete splenic marginal zone B cells, which are precursors to B cells (39). The results could be poor generation of specific B cell response. These mechanisms, coupled with strategies described earlier to block effective antibody binding to bacterial surface, could be important underlying reasons why we remain susceptible to *S. aureus* infections throughout our lives.

Other virulence mechanisms of clinical significance include biofilm formation which allows *S. aureus* to persist on plastics and resist host defenses or antibiotics (3), and small colony variants which help *S. aureus* survive in a metabolically inactive state under harsh conditions. Small colony variants have been implicated in chronic infections such as chronic osteomyelitis (40).

MRSA pathogenesis. MRSA deserves separate consideration in *S. aureus* pathogenesis because it is associated with distinct epidemiology, particularly morbidity and mortality. Remarkably, it is estimated that the number of invasive diseases and deaths attributable to MRSA in 2005 are 94,360 and 18,650 in the United States, eclipsing mortality attributed to HIV (41). MRSA can be divided into HA-MRSA and CA-MRSA, two genotypically dissimilar groups of bacteria that target different but overlapping populations and cause different diseases.

HA-MRSA. MRSA first emerged in the 1960s but became increasingly problematic in the 1990s especially in intensive care unit settings where it became a major cause of nosocomial infections (42). HA-MRSA harbors large staphylococcal chromosome cassettes (SCC*mec* types I-III), which encode one (SCC*mec* type I) or multiple antibiotic resistance genes (SCC*mec* type II and III). Resistance to antibiotics may have allowed the bacterium to survive an environment where antibiotic use is frequent.

Interestingly, when removed from the healthcare setting, HA-MRSA rarely causes diseases in individuals without predisposing conditions. It has therefore been suggested that HA-MRSA represents less robust strains of *S. aureus* that could only survive environments where bacterial competition is limited by antibiotic pressure (43). In support of this viewpoint, HA-MRSA shows a longer generation time compared with methicillin-sensitive *S. aureus* (MSSA) (30 min for HA-MRSA *versus* 23 min for MSSA) (44). In a small study, HA-MRSA strains showed increased susceptibility to killing by neutrophils and were less pathogenic when administered to mice systemically (45). Furthermore, direct comparison of CA-MRSA and HA-MRSA strains showed that HA-MRSA expressed lower levels of PSM peptides (33), thereby pointing to a possible defect in HA-MRSA virulence regulation. Consistent with the last finding, many clinical HA-MRSA isolates exhibit a agr^- or a mixed agr^+ and agr^- genotype (46). Although these genotypes could explain the relative nonpathogenic nature of HA-MRSA toward immunocompetent hosts, it is possible that an agr^- or a mixed agr^+ and agr^- genotype could be beneficial for HA-MRSA survival in the healthcare setting; agr^- genotype could for example facilitate biofilm formation (47)and proliferation on plastic tubings.

As physicians attempt to grapple with the antibiotic resistance problem posed by HA-MRSA, increasingly there are reports of the more virulent CA-MRSA infiltrating the healthcare setting (41,48). The impact of this migration bears more careful monitoring as it may demand more aggressive and different control and treatment strategies.

CA-MRSA. Until the late 1990s, MRSA infections were largely confined to immunocompromised individuals or individuals with healthcare exposure. In 1997, death of four healthy children from MRSA pneumonia and sepsis heralded the arrival of a new type of MRSA (49). Soon thereafter, MRSA cases burgeoned across continents; the majority of cases were confined to few clonal lineages that were markedly different from HA-MRSA, shared a small-sized Type IV SCC*mec* cassette, and encoded the genes for the *Panton-Valentine Leukocidin* (PVL) (50).

CA-MRSA strains were responsible for a dramatic increase in the incidence of infections, particularly of the skin and soft tissue (51,52) and were the cause of many unusually severe infections such as necrotizing pneumonia, necrotizing fasciitis, and myositis (53–55). The change in the clinical manifestations of *S. aureus* prompted speculation that CA-MRSA infections reflected infection by more virulent strains. Few comparative study of CA-MRSA *versus* MSSA virulence have been performed, and it is not clear whether all CA-MRSA clones are more virulent (56,57). However, one CA-MRSA clone, USA300 has proven to be particularly successful (58), spreading rapidly to become the dominant clone in most regions of the United States, and appearing in Canada and Europe. Many reports have linked USA300 to more severe infections of the bone, skin, and soft tissue (55,57). Therefore, studies of

USA300 could provide important information on pathogenesis of CA-MRSA.

The epidemiologic findings, though suggestive of a more virulent phenotype, need to be interpreted with caution. Specifically, increased CA-MRSA disease incidence could be attributed to 1) enhanced environmental survival (fomites, pets), 2) increased transmission, 3) more robust colonization, 4) lowered bacterial threshold to activate virulence genes, and 5) increased pathogenicity during infection. Multiple analyses of USA300 outbreaks suggest that the CA-MRSA clone may have enhanced transmission through skin-skin contact or skin-fomite contact (58,59). In a study of men who had sex with men, high rate of perineal, buttock, and genital infections with USA300 suggests this clone has higher transmission efficacy (59). Further, a comparison of skin colonization rate among HA-MRSA, CA-MRSA, and MSSA infected patients demonstrated that CA-MRSA infected individuals had significantly higher rate of skin colonization (58). Epidemiologic evidence to support greater pathogenicity of CA-MRSA compared with MSSA is available from a prospective review of osteomyelitis in children (57). In that study, children identified to have PVL^+ CA-MRSA infections had higher levels of inflammation markers (C reactive protein and sedimentation rate) on admission thereby excluding possible confounding effect of antibiotic treatment on disease outcome (57). Together, these studies suggest that clones such as USA300 are particularly successful because they are transmitted more easily, colonize better, and are more pathogenic.

Among putative virulence factors proposed to be the major determinant of the CA-MRSA epidemic, PVL has been most extensively studied (1,2,60). PVL was found in the phage type 80/81 epidemic *S. aureus* strain that caused high incidence of infections in the 1950s (61) and is found in most clones of CA-MRSA (50). It has been linked in many case series to severe necrotizing pneumonia (62), furunculosis (63), and severe osteomyelitis (57). The two component toxin, when injected into rabbits or mice, produced significant inflammation and necrosis (64,65), and has shown an ability to induce neutrophil cytolysis (66), apoptosis (67), or secretion of pro-inflammatory molecules depending on culture conditions (68). However, direct demonstration of a virulence role has been conflicting (69–71). Labandeira-Rey *et al.* (65) showed PVL is a major virulence determinant in a mouse necrotizing pneumonia model using laboratory strains into which a PVL expressing vector is introduced. By contrast, Voyich *et al.* (71) and Bubeck Wardenburg *et al.* (69) used PVL mutants in the background of USA300 and USA400 and found either no difference or a protective effect conferred by PVL. It is possible that mice represent a less sensitive model compared with the human host because mouse leukocytes, the target of PVL activity, show reduced sensitivity to PVL lysis compared with human leukocytes (66). We have recently tested this hypothesis by generating PVL mutants in the background of two USA300 necrotizing fasciitis isolates. In a model of severe soft tissue infection, we showed that PVL^+ necrotizing fasciitis USA300 strains caused more significant muscle injury compared with the PVL^- isogenic mutant strains (Tseng and Liu unpublished data). We speculate that use of higher inocula or more sensitive animal models could be the key for uncovering a PVL threshold effect.

The type I arginine catabolic mobile element (ACME) has many properties that make it an equally attractive candidate to explain the success of USA300 (72). ACME is believed to be horizontally transferred from ubiquitous skin commensal *S. epidermidis* (reviewed in Ref. 1). It encodes multiple genes, but two gene clusters, *arc* (arginine deiminase system) and *opp-3* (ABC-transporter), are of particular interest. The arginine deiminase system has been shown in certain bacteria to catabolize *L*-arginine to provide a source of ATP and could raise the pH of acidic human skin to one more suitable for bacterial colonization (1). Opp-3 is a member of the ABC transporter family implicated in multiple functions that could benefit bacterial survival on the skin surface, including peptide nutrient uptake, eukaryotic cell adhesion, and resistance to antimicrobial peptides. Thus, acquisition of ACME by *S. aureus*, a transient colonizer of the skin, may allow CA-MRSA to colonize the skin on a permanent basis, thereby enhancing the likelihood of a skin infection occurring upon any disruption of the skin barrier. The evidence for that CA-MRSA colonizes the skin better compared with MSSA and HA-MRSA has been provided by Miller and coworkers (58). So far, there has been no direct evidence that ACME contributes to skin colonization.

PSM peptides have been described previously to contribute to CA-MRSA skin infection in mice (33). Although not unique to CA-MRSA, PSM peptides are expressed at higher level in CA-MRSA compared with HA-MRSA, which prompts suggestion that differences in global virulence regulation could be an important factor in CA-MRSA virulence. Montgomery et al. (73) showed that among CA-MRSA, USA300 strains are more pathogenic than USA400 strains. The difference in virulence correlated with higher expression of multiple virulence genes by USA300 strains compared with USA400 strains.

Many other putative virulence factors uniquely expressed by CA-MRSA strains remain to be explored (50,72). How each product could add to the pathogenicity of the specific strain is not known. However, if Occam's razor, the principle of diagnostic parsimony frequently used in clinical decision making, is to guide the assessment of CA-MRSA pathogenesis, it is likely that one or very few factors are ultimately responsible for the simultaneous emergence of several CA-MRSA epidemic clones.

Future direction. The emergence of CA-MRSA heralded an era of uncertainty in public health and patient care as antibiotic resistance and virulence converged to create a major health crisis. As the epidemic evolved and expanded, research has strived to achieve the following goals: 1) identify the cause and mechanism underlying the epidemic; 2) develop antibiotics that do not promptly become obsolete; 3) develop an effective vaccine. So far, the goals have met with varying degrees of success.

Our understanding of CA-MRSA epidemic is still limited despite an abundance of epidemiologic and basic studies. Most fundamentally, we do not know what makes the bacterium more pathogenic. The study of *S. aureus* will require the development of animal models that more closely approximate

human infections. *S. aureus* is not a natural colonizer of mice; therefore, many of the virulence factors elaborated by *S. aureus* to evade the human immune system may prove more difficult to study in mice, with PVL being a prime example. Although there continues to be an important place for traditional mouse research which allows manipulation of the host immune factors using already generated knockout mice, a model that simulates human disease could be achieved by use of other animals or by development of partially humanized mouse models, in which the mouse innate or adaptive immune system is replaced by its human counterpart (74).

As discussed earlier, mechanisms other than virulence could explain the increased CA-MRSA disease incidence and severity. Therefore, study of bacterial factors must be expanded to assays beyond traditional virulence testing, including colonization, resistance to environmental stimuli, as guided by epidemiologic findings. These studies would optimally involve collaboration between epidemiologists and basic researchers.

In recent years, the threat posed by antibiotic resistant *S. aureus* has refueled research effort to discover novel classes of antibiotics. Because traditional drug library screens have been slow to identify new antibiotics, an alternative strategy has been the targeting of important virulence factors. As an example, we have demonstrated that the *S. aureus* golden pigment is a virulence factor because it shields the bacterium from host oxidant killing (75). Because *S. aureus* pigment and human cholesterol share synthesis of a common precursor, we have been able to identify a human cholesterol inhibitor that blocked *S. aureus* pigmentation and reduced *S. aureus* virulence in mice (76). Likewise, alpha toxin, which is elaborated by many but not all clinical *S. aureus* strains, has demonstrated virulence function in a CA-MRSA model of lung infection, and application of specific antibodies against alpha toxin has been shown to significantly ameliorate lung injury (77). These virulence-based strategies could prove to be useful adjuncts to traditional therapeutics.

Ultimately, an effective vaccine is needed to solve the MRSA health crisis. At the height of the penicillin resistant *S. pneumoniae* problem 8 y ago, introduction of an effective vaccine promptly decreased the incidence of invasive diseases and averted a major health crisis. A similar antibiotic resistance problem was solved by introduction of an effective vaccine against *H. influenzae*. However, the MRSA epidemic presents a different and more formidable challenge. For one, *S. aureus* is a more complex organism that is not dependent on a single major virulence factor to cause disease. Its selective up-regulation of virulence factors during different phases of infection could render vaccine against a single factor relatively ineffective. The recent failure of active or passive immunization trials against capsular polysaccharide (StaphVAX), ClfA, and SdrG (Veronate) (78) may be evidence of that principle. Therefore, experts have proposed that *S. aureus* vaccine would be more effective if multiple select factors are targeted (reviewed in Ref. 78).

A more fundamental issue, with direct implication on vaccine development, is why the human host is persistently susceptible to *S. aureus* infection throughout life. Research has indicated that bacterial products such as protein A and staphylococcal enterotoxins may have roles in modulation of T- and B-cell functions (38,39); however, adaptive immune evasion mechanisms after *S. aureus* infection remain largely unknown. Understanding of these mechanisms may hold the ultimate key to a successful vaccine.

In summary, *S. aureus* pathogenesis will remain an intensely important area of research for years to come. Most published studies estimate current CA-MRSA nasal colonization rate at less than 5% (79,80); therefore, if the rate of colonization continues to rise, the epidemic will likely expand. It is not clear whether over time the human host could develop an adaptive immune response to novel virulence factors expressed by CA-MRSA strains. If those virulence factors contribute significantly to the epidemic, neutralization of those factors may cause the epidemic to subside. If the human immune system is unable to adapt, human kind will need to address the problem through research, and success will depend on the concentration of research effort, funding, and well-coordinated multidisciplinary approaches directed at addressing select key questions.

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REFERENCES

1. Diep BA, Otto M 2008 The role of virulence determinants in community-associated MRSA pathogenesis. Trends Microbiol 16:361–369
2. Gordon RJ, Lowy FD 2008 Pathogenesis of methicillin-resistant *Staphylococcus aureus* infection. Clin Infect Dis 46:S350–S359
3. Foster TJ 2005 Immune evasion by staphylococci. Nat Rev Microbiol 3:948–958
4. Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, Nouwen JL 2005 The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis 5:751–762
5. van Belkum A, Emonts M, Wertheim H, de Jongh C, Nouwen J, Bartels H, Cole A, Hermans P, Boelens H, Toom NL, Snijders S, Verbrugh H, van Leeuwen W 2007 The role of human innate immune factors in nasal colonization by *Staphylococcus aureus*. Microbes Infect 9:1471–1477
6. Rooijakkers SH, van Kessel KP, van Strijp JA 2005 Staphylococcal innate immune evasion. Trends Microbiol 13:596–601
7. Wertheim HF, Vos MC, Ott A, van Belkum A, Voss A, Kluytmans JA, van Keulen PH, Vandenbroucke-Grauls CM, Meester MH, Verbrugh HA 2004 Risk and outcome of nosocomial *Staphylococcus aureus* bacteraemia in nasal carriers versus non-carriers. Lancet 364:703–705
8. von Eiff C, Becker K, Machka K, Stammer H, Peters G 2001 Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group. N Engl J Med 344:11–16
9. Wertheim HF, van Kleef M, Vos MC, Ott A, Verbrugh HA, Fokkens W 2006 Nose picking and nasal carriage of *Staphylococcus aureus*. Infect Control Hosp Epidemiol 27:863–867
10. Ritz HL, Kirkland JJ, Bond GG, Warner EK, Petty GP 1984 Association of high levels of serum antibody to staphylococcal toxic shock antigen with nasal carriage of toxic shock antigen-producing strains of *Staphylococcus aureus*. Infect Immun 43:954–958
11. Wertheim HF, Walsh E, Choudhurry R, Melles DC, Boelens HA, Miajlovic H, Verbrugh HA, Foster T, van Belkum 2008 A key role for clumping factor B in *Staphylococcus aureus* nasal colonization of humans. PLoS Med 5:e17
12. Weidenmaier C, Kokai-Kun JF, Kristian SA, Chanturiya T, Kalbacher H, Gross M, Nicholson G, Neumeister B, Mond JJ, Peschel A 2004 Role of teichoic acids in *Staphylococcus aureus* nasal colonization, a major risk factor in nosocomial infections. Nat Med 10:243–245
13. Gonzalez-Zorn B, Senna JP, Fiette L, Shorte S, Testard A, Chignard M, Courvalin P, Grillot-Courvalin C 2005 Bacterial and host factors implicated in nasal carriage of methicillin-resistant *Staphylococcus aureus* in mice. Infect Immun 73:1847–1851
14. Lina G, Boutite F, Tristan A, Bes M, Etienne J, Vandenesch F 2003 Bacterial competition for human nasal cavity colonization: role of Staphylococcal agr alleles. Appl Environ Microbiol 69:18–23
15. Bogaert D, van Belkum A, Sluijter M, Luijendijk A, de Groot R, Rumke HC, Verbrugh HA, Hermans PW 2004 Colonisation by *Streptococcus pneumoniae* and *Staphylococcus aureus* in healthy children. Lancet 363:1871–1872
16. Cohen R, Levy C, Thollot F, de La Rocque F, Koskas M, Bonnet E, Fritzell B, Varon E 2007 Pneumococcal conjugate vaccine does not influence *Staphylococcus aureus* carriage in young children with acute otitis media. Clin Infect Dis 45:1583–1587

17. Regev-Yochay G, Trzcinski K, Thompson CM, Malley R, Lipsitch M 2006 Interference between *Streptococcus pneumoniae* and *Staphylococcus aureus*: In vitro hydrogen peroxide-mediated killing by *Streptococcus pneumoniae*. J Bacteriol 188:4996–5001
18. Park B, Nizet V, Liu GY 2008 Role of *Staphylococcus aureus* catalase in niche competition against *Streptococcus pneumoniae*. J Bacteriol 190:2275–2278
19. Novick RP 2003 Autoinduction and signal transduction in the regulation of staphylococcal virulence. Mol Microbiol 48:1429–1449
20. Hashimoto M, Tawaratsumida K, Kariya H, Kiyohara A, Suda Y, Krikae F, Kirikae T, Gotz F 2006 Not lipoteichoic acid but lipoproteins appear to be the dominant immunobiologically active compounds in *Staphylococcus aureus*. J Immunol 177:3162–3169
21. Fournier B, Philpott DJ 2005 Recognition of *Staphylococcus aureus* by the innate immune system. Clin Microbiol Rev 18:521–540
22. Scheibner KA, Lutz MA, Boodoo S, Fenton MJ, Powell JD, Horton MR 2006 Hyaluronan fragments act as an endogenous danger signal by engaging TLR2. J Immunol 177:1272–1281
23. Pisetsky DS 2007 The role of nuclear macromolecules in innate immunity. Proc Am Thorac Soc 4:258–262
24. Cavassani KA, Ishii M, Wen H, Schaller MA, Lincoln PM, Lukacs NW, Hogaboam CM, Kunkel SL 2008 TLR3 is an endogenous sensor of tissue necrosis during acute inflammatory events. J Exp Med 205:2609–2621
25. Kubica M, Guzik K, Koziel J, Zarebski M, Richter W, Gajkowska B, Golda A, Maciag-Gudowska A, Brix K, Shaw L, Foster T, Potempa J 2008 A potential new pathway for *Staphylococcus aureus* dissemination: the silent survival of *S. aureus* phagocytosed by human monocyte-derived macrophages. PLoS One 3:e1409
26. de Haas CJ, Veldkamp KE, Peschel A, Weerkamp F, Van Wamel WJ, Heezius EC, Poppelier MJ, Van Kessel KP, van Strijp JA 2004 Chemotaxis inhibitory protein of *Staphylococcus aureus*, a bacterial antiinflammatory agent. J Exp Med 199:687–695
27. Chavakis T, Hussain M, Kanse SM, Peters G, Bretzel RG, Flock JI, Herrmann M, Preissner KT 2002 *Staphylococcus aureus* extracellular adherence protein serves as anti-inflammatory factor by inhibiting the recruitment of host leukocytes. Nat Med 8:687–693
28. Peschel A, Jack RW, Otto M, Collins LV, Staubitz P, Nicholson G, Kalbacher H, Nieuwenhuizen WF, Jung G, Tarkowski A, van Kessel KP, van Strijp JA 2001 *Staphylococcus aureus* resistance to human defensins and evasion of neutrophil killing via the novel virulence factor MprF is based on modification of membrane lipids with L-lysine. J Exp Med 193:1067–1076
29. Collins LV, Kristian SA, Weidenmaier C, Faigle M, Van Kessel KP, Van Strijp JA, Gotz F, Neumeister B, Peschel A 2002 *Staphylococcus aureus* strains lacking D-alanine modifications of teichoic acids are highly susceptible to human neutrophil killing and are virulence attenuated in mice. J Infect Dis 186:214–219
30. Sieprawska-Lupa M, Mydel P, Krawczyk K, Wojcik K, Puklo M, Lupa B, Suder P, Silberring J, Reed M, Pohl J, Shafer W, McAleese F, Foster T, Travis J, Potempa J 2004 Degradation of human antimicrobial peptide LL-37 by *Staphylococcus aureus*-derived proteinases. Antimicrob Agents Chemother 48:4673–4679
31. Jin T, Bokarewa M, Foster T, Mitchell J, Higgins J, Tarkowski A 2004 *Staphylococcus aureus* resists human defensins by production of staphylokinase, a novel bacterial evasion mechanism. J Immunol 172:1169–1176
32. Tomita T, Kamio Y 1997 Molecular biology of the pore-forming cytolysins from *Staphylococcus aureus*, alpha- and gamma-hemolysins and leukocidin. Biosci Biotechnol Biochem 61:565–572
33. Wang R, Braughton KR, Kretschmer D, Bach TH, Queck SY, Li M, Kennedy AD, Dorward DW, Klebanoff SJ, Peschel A, DeLeo FR, Otto M 2007 Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. Nat Med 13:1510–1514
34. Maresso AW, Schneewind O 2006 Iron acquisition and transport in *Staphylococcus aureus*. Biometals 19:193–203
35. Drechsel H, Freund S, Nicholson G, Haag H, Jung O, Zahner H, Jung G 1993 Purification and chemical characterization of staphyloferrin B, a hydrophilic siderophore from staphylococci. Biometals 6:185–192
36. Courcol RJ, Trivier D, Bissinger MC, Martin GR, Brown MR 1997 Siderophore production by *Staphylococcus aureus* and identification of iron-regulated proteins. Infect Immun 65:1944–1948
37. Lee LY, Miyamoto YJ, McIntyre BW, Hook M, McCrea KW, McDevitt D, Brown EL 2002 The *Staphylococcus aureus* Map protein is an immunomodulator that interferes with T cell-mediated responses. J Clin Invest 110:1461–1471
38. Llewelyn M, Cohen J 2002 Superantigens: microbial agents that corrupt immunity. Lancet Infect Dis 2:156–162
39. Goodyear CS, Silverman GJ 2004 Staphylococcal toxin induced preferential and prolonged in vivo deletion of innate-like B lymphocytes. Proc Natl Acad Sci USA 101:11392–11397
40. von Eiff C, Peters G, Becker K 2006 The small colony variant (SCV) concept—the role of staphylococcal SCVs in persistent infections. Injury 37:S26–S33
41. Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, Harrison LH, Lynfield R, Dumyati G, Townes JM, Craig AS, Zell ER, Fosheim GE, McDougal LK, Carey RB, Fridkin SK 2007 Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. JAMA 298:1763–1771
42. Klevens RM, Edwards JR, Tenover FC, McDonald LC, Horan T, Gaynes R 2006 Changes in the epidemiology of methicillin-resistant *Staphylococcus aureus* in intensive care units in US hospitals, 1992–2003. Clin Infect Dis 42:389–391
43. Eady EA, Cove JH 2003 Staphylococcal resistance revisited: community-acquired methicillin resistant *Staphylococcus aureus*—an emerging problem for the management of skin and soft tissue infections. Curr Opin Infect Dis 16:103–124
44. Laurent F, Lelievre H, Cornu M, Vandenesch F, Carret G, Etienne J, Flandrois JP 2001 Fitness and competitive growth advantage of new gentamicin-susceptible MRSA clones spreading in French hospitals. J Antimicrob Chemother 47:277–283
45. Voyich JM, Braughton KR, Sturdevant DE, Whitney AR, Said-Salim B, Porcella SF, Long RD, Dorward DW, Gardner DJ, Kreiswirth BN, Musser JM, DeLeo FR 2005 Insights into mechanisms used by *Staphylococcus aureus* to avoid destruction by human neutrophils. J Immunol 175:3907–3919
46. Shopsin B, Drlica-Wagner A, Mathema B, Adhikari RP, Kreiswirth BN, Novick RP 2008 Prevalence of agr dysfunction among colonizing *Staphylococcus aureus* strains. J Infect Dis 198:1171–1174
47. Vuong C, Saenz HL, Gotz F, Otto M 2000 Impact of the agr quorum-sensing system on adherence to polystyrene in *Staphylococcus aureus*. J Infect Dis 182:1688–1693
48. Liu C, Graber CJ, Karr M, Diep BA, Basuino L, Schwartz BS, Enright MC, O'Hanlon SJ, Thomas JC, Perdreau-Remington F, Gordon S, Gunthorpe H, Jacobs R, Jensen P, Leoung G, Rumack JS, Chambers HF 2008 A population-based study of the incidence and molecular epidemiology of methicillin-resistant *Staphylococcus aureus* disease in San Francisco, 2004–2005. Clin Infect Dis 46:1637–1646
49. 1999 Four Pediatric Deaths from Community-Acquired Methicillin-Resistant *Staphylococcus aureus*—Minnesota and North Dakota, 1997–1999. MMWR Morb Mortal Wkly Rep 48:707–710
50. Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, Liassine N, Bes M, Greenland T, Reverdy ME, Etienne J 2003 Community-acquired methicillin-resistant *Staphylococcus aureus* carrying six genes: worldwide emergence. Emerg Infect Dis 9:978–984
51. Moran GJ, Krishnadasan A, Gorwitz RJ, Fosheim GE, McDougal LK, Carey RB, Talan DA 2006 Methicillin-resistant *S. aureus* infections among patients in the emergency department. N Engl J Med 355:666–674
52. Stryjewski ME, Chambers HF 2008 Skin and soft-tissue infections caused by community-acquired methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis 46:S368–S377
53. Bradley SF 2005 *Staphylococcus aureus* pneumonia: emergence of MRSA in the community. Semin Respir Crit Care Med 26:643–649
54. Pannaraj PS, Hulten KG, Gonzalez BE, Mason EO, Jr., Kaplan SL 2006 Infective pyomyositis and myositis in children in the era of community-acquired, methicillin-resistant *Staphylococcus aureus* infection. Clin Infect Dis 43:953–960
55. Miller LG, Perdreau-Remington F, Rieg G, Mehdi S, Perlroth J, Bayer AS, Tang AW, Phung TO, Spellberg B 2005 Necrotizing fasciitis caused by community-associated methicillin-resistant *Staphylococcus aureus* in Los Angeles. N Engl J Med 352:1445–1453
56. Miller LG, Quan C, Shay A, Mostafaie K, Bharadwa K, Tan N, Matayoshi K, Cronin J, Tan J, Tagudar G, Bayer AS 2007 A prospective investigation of outcomes after hospital discharge for endemic, community-acquired methicillin-resistant and -susceptible *Staphylococcus aureus* skin infection. Clin Infect Dis 44:483–492
57. Bocchini CE, Hulten KG, Mason EO, Jr., Gonzalez BE, Hammerman WA, Kaplan SL 2006 *Panton-Valentine leukocidin* genes are associated with enhanced inflammatory response and local disease in acute hematogenous *Staphylococcus aureus* osteomyelitis in children. Pediatrics 117:433–440
58. Miller LG, Diep BA 2008 Clinical practice: colonization, fomites, and virulence: rethinking the pathogenesis of community-associated methicillin-resistant *Staphylococcus aureus* infection. Clin Infect Dis 46:752–760
59. Diep BA, Chambers HF, Graber CJ, Szumowski JD, Miller LG, Han LL, Chen JH, Lin F, Lin J, Phan TH, Carleton HA, McDougal LK, Tenover FC, Cohen DE, Mayer KH, Sensabaugh GF, Perdreau-Remington F 2008 Emergence of multidrug-resistant, community-associated, methicillin-resistant *Staphylococcus aureus* clone USA300 in men who have sex with men. Ann Intern Med 148:249–257
60. Boyle-Vavra S, Daum RS 2007 Community-acquired methicillin-resistant *Staphylococcus aureus*: the role of *Panton-Valentine leukocidin*. Lab Invest 87:3–9
61. Robinson DA, Kearns AM, Holmes A, Morrison D, Grundmann H, Edwards G, O'Brien FG, Tenover FC, McDougal LK, Monk AB, Enright MC 2005 Re-emergence of early pandemic *Staphylococcus aureus* as a community-acquired meticillin-resistant clone. Lancet 365:1256–1258
62. Gillet Y, Etienne J, Lina G, Vandenesch F 2008 Association of necrotizing pneumonia with *panton-valentine leukocidin*-producing *Staphylococcus aureus*, regardless of methicillin resistance. Clin Infect Dis 47:985–986
63. Yamasaki O, Kaneko J, Morizane S, Akiyama H, Arata J, Narita S, Chiba J, Kamio Y, Iwatsuki K 2005 The association between *Staphylococcus aureus* strains carrying *panton-valentine leukocidin* genes and the development of deep-seated follicular infection. Clin Infect Dis 40:381–385
64. Cribier B, Prevost G, Couppie P, Finck-Barbancon V, Grosshans E, Piemont Y 1992 *Staphylococcus aureus* leukocidin: a new virulence factor in cutaneous infections? An epidemiological and experimental study. Dermatology 185:175–180
65. Labandeira-Rey M, Couzon F, Boisset S, Brown EL, Bes M, Benito Y, Barbu EM, Vazquez V, Hook M, Etienne J, Vandenesch F, Bowden MG 2007 *Staphylococcus aureus Panton-Valentine leukocidin* causes necrotizing pneumonia. Science 315:1130–1133
66. Szmigielski S, Prevost G, Monteil H, Colin DA, Jeljaszewicz J 1999 Leukocidal toxins of staphylococci. Zentralbl Bakteriol 289:185–201
67. Genestier AL, Michallet MC, Prevost G, Bellot G, Chalabreysse L, Peyrol S, Thivolet F, Etienne J, Lina G, Vallette FM, Vandenesch F, Genestier L 2005 *Staphylococcus aureus Panton-Valentine leukocidin* directly targets mitochondria and induces Bax-independent apoptosis of human neutrophils. J Clin Invest 115:3117–3127
68. Konig B, Koller M, Prevost G, Piemont Y, Alouf JE, Schreiner A, Konig W 1994 Activation of human effector cells by different bacterial toxins (leukocidin, alveolysin, and erythrogenic toxin A): generation of interleukin-8. Infect Immun 62:4831–4837

69. Bubeck Wardenburg J, Palazzolo-Ballance AM, Otto M, Schneewind O, DeLeo FR 2008 *Panton-Valentine leukocidin* is not a virulence determinant in murine models of community-associated methicillin-resistant *Staphylococcus aureus* disease. J Infect Dis 198:1166–1170
70. Bubeck Wardenburg J, Bae T, Otto M, Deleo FR, Schneewind O 2007 Poring over pores: alpha-hemolysin and *Panton-Valentine leukocidin* in *Staphylococcus aureus* pneumonia. Nat Med 13:1405–1406
71. Voyich JM, Otto M, Mathema B, Braughton KR, Whitney AR, Welty D, Long RD, Dorward DW, Gardner DJ, Lina G, Kreiswirth BN, DeLeo FR 2006 Is *Panton-Valentine leukocidin* the major virulence determinant in community-associated methicillin-resistant *Staphylococcus aureus* disease?. J Infect Dis 194:1761–1770
72. Diep BA, Gill SR, Chang RF, Phan TH, Chen JH, Davidson MG, Lin F, Lin J, Carleton HA, Mongodin EF, Sensabaugh GF, Perdreau-Remington F 2006 Complete genome sequence of USA300, an epidemic clone of community-acquired meticillin-resistant *Staphylococcus aureus*. Lancet 367:731–739
73. Montgomery CP, Boyle-Vavra S, Adem PV, Lee JC, Husain AN, Clasen J, Daum RS 2008 Comparison of virulence in community-associated methicillin-resistant *Staphylococcus aureus* pulsotypes USA300 and USA400 in a rat model of pneumonia. J Infect Dis 198:561–570
74. Shultz LD, Ishikawa F, Greiner DL 2007 Humanized mice in translational biomedical research. Nat Rev Immunol 7:118–130
75. Liu GY, Essex A, Buchanan JT, Datta V, Hoffman HM, Bastian JF, Fierer J, Nizet V 2005 *Staphylococcus aureus* golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity. J Exp Med 202:209–215
76. Liu CI, Liu GY, Song Y, Yin F, Hensler ME, Jeng WY, Nizet V, Wang AH, Oldfield E 2008 A cholesterol biosynthesis inhibitor blocks *Staphylococcus aureus* virulence. Science 319:1391–1394
77. Bubeck Wardenburg J, Schneewind O 2008 Vaccine protection against *Staphylococcus aureus* pneumonia. J Exp Med 205:287–294
78. Schaffer AC, Lee JC 2008 Vaccination and passive immunisation against *Staphylococcus aureus*. Int J Antimicrob Agents 32:S71–S78
79. Ellis MW, Griffith ME, Dooley DP, McLean JC, Jorgensen JH, Patterson JE, Davis KA, Hawley JS, Regules JA, Rivard RG, Gray PJ, Ceremuga JM, Dejoseph MA, Hospenthal DR 2007 Targeted intranasal mupirocin to prevent colonization and infection by community-associated methicillin-resistant *Staphylococcus aureus* strains in soldiers: a cluster randomized controlled trial. Antimicrob Agents Chemother 51:3591–3598
80. Gorwitz RJ, Kruszon-Moran D, McAllister SK, McQuillan G, McDougal LK, Fosheim GE, Jensen BJ, Killgore G, Tenover FC, Kuehnert MJ 2001 Changes in the prevalence of nasal colonization with *Staphylococcus aureus* in the United States-2004. J Infect Dis 197:1226–1234, 2008