SUPPLEMENT ARTICLE

Pathogenesis of Methicillin-Resistant *Staphylococcus aureus* Infection

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Staphylococcus aureus **is a versatile pathogen capable of causing a wide range of human diseases. However, the role of different virulence factors in the development of staphylococcal infections remains incompletely understood. Some clonal types are well equipped to cause disease across the globe, whereas others are facile at causing disease among community members. In this review, general aspects of staphylococcal pathogenesis are addressed, with emphasis on methicillin-resistant strains. Although methicillin-resistant *S. aureus* (MRSA) strains are not necessarily more virulent than methicillin-sensitive *S. aureus* strains, some MRSA strains contain factors or genetic backgrounds that may enhance their virulence or may enable them to cause particular clinical syndromes. We examine these pathogenic factors.**

OVERVIEW OF THE PATHOGENESIS OF *STAPHYLOCOCCUS AUREUS*

This article summarizes the pathogenesis of *S. aureus* disease and specifically addresses the pathogenesis of infections caused by methicillin-resistant *S. aureus* (MRSA) strains originating in health care settings (hospital-acquired MRSA [HA-MRSA]) and in the community (community-acquired MRSA [CA-MRSA]). *S. aureus* pathogenesis is reviewed before the discussion of the pathogenesis of MRSA, because MRSA virulence factors are generally not unique to MRSA. Nonetheless, certain MRSA strains appear to contain particular factors or genetic backgrounds that enhance their virulence or enable them to cause particular clinical syndromes.

Colonization and disease. *S. aureus* is both a commensal organism and a pathogen. The anterior nares are the main ecological niche for *S. aureus*. Approximately 20% of individuals are persistently nasally colonized with *S. aureus*, and 30% are intermittently colonized. However, numerous other sites may be colonized, including the axillae, groin, and gastrointestinal tract. Colonization provides a reservoir from which bacteria can be introduced when host defenses are breached, whether by shaving, aspiration, insertion of an indwelling catheter, or surgery. Colonization clearly increases the risk for subsequent infection [1, 2]. Those with *S. aureus* infections are generally infected with their colonizing strain [3]. In a study of bacteremia, blood isolates were identical to nasal isolates in 82% of patients [4]. Colonization also allows *S. aureus* to be transmitted among individuals in both health care and community settings. The basis for *S. aureus* colonization is complex and incompletely understood but appears to involve the host's contact with *S. aureus* (e.g., other carriers) and the ability of *S. aureus* to adhere to host cells and to evade the immune response (reviewed by Wertheim et al. [1]).

Virulence factors and disease. The armamentarium of virulence factors of *S. aureus* is extensive, with both structural and secreted products playing a role in the pathogenesis of infection (figure 1). Selected examples of these factors are described in table 1. Two noteworthy features of staphylococci are that a virulence factor may have several functions in pathogenesis and that multiple virulence factors may perform the same function. In establishing an infection, *S. aureus* has numerous surface proteins, called "microbial sur-

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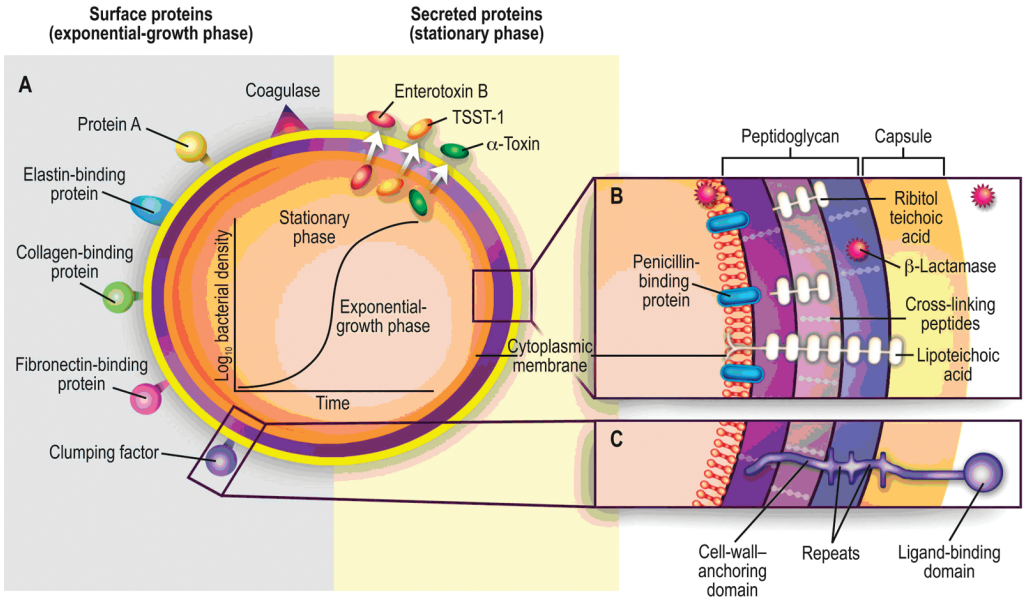

Figure 1. Pathogenic factors of *Staphylococcus aureus,* with structural and secreted products both playing roles as virulence factors. *A,* Surface and secreted proteins. *B* and *C,* Cross-sections of the cell envelope. TSST-1, toxic shock syndrome toxin 1. Reprinted from [32], with permission from the Massachusetts Medical Society. Copyright 1998 Massachusetts Medical Society. All rights reserved.

face components recognizing adhesive matrix molecules" (MSCRAMMs), that mediate adherence to host tissues. MSCRAMMs bind molecules such as collagen, fibronectin, and fibrinogen, and different MSCRAMMs may adhere to the same host-tissue component. MSCRAMMs appear to play a key role in initiation of endovascular infections, bone and joint infections, and prosthetic-device infections. Different *S. aureus* strains may have different constellations of MSCRAMMs and so may be predisposed to causing certain kinds of infections [5–8].

Once *S. aureus* adheres to host tissues or prosthetic materials, it is able to grow and persist in various ways. *S. aureus* can form biofilms (slime) on host and prosthetic surfaces, enabling it to persist by evading host defenses and antimicrobials [9]. The ability to form and reside in biofilms is one reason why prosthetic-device infections, for example, can be so difficult to eradicate without removal of the device. In vitro, *S. aureus* can also invade and survive inside epithelial cells, including endothelial cells, which theoretically may also allow it to escape host defenses, particularly in endocarditis [10–12, 30]. *S. aureus* is also able to form small-colony variants (SCVs), which may contribute to persistent and recurrent infection. In vitro, SCVs are able to "hide" in host cells without causing significant host-cell damage and are relatively protected from antibiotics and host defenses. They can later revert to the more virulent wild-type phenotype, possibly resulting in recurrent infection [13–15].

S. aureus has many other characteristics that help it evade the host immune system during an infection (reviewed by Foster [16]). Its main defense is production of an antiphagocytic microcapsule (most clinical isolates produce type 5 or 8). The zwitterionic capsule (both positively and negatively charged) can also induce abscess formation [17, 18]. The MSCRAMM protein A binds the Fc portion of immunoglobulin [31] and, as a result, may prevent opsonization. *S. aureus* may also secrete chemotaxis inhibitory protein of staphylococci or the extracellular adherence protein, which interfere with neutrophil extravasation and chemotaxis to the site of infection (reviewed by Foster [16]). In addition, *S. aureus* produces leukocidins that cause leukocyte destruction by the formation of pores in the cell membrane [19].

During infection, *S. aureus* produces numerous enzymes, such as proteases, lipases, and elastases, that enable it to invade and destroy host tissues and metastasize to other sites. *S. aureus* is also capable of producing septic shock. It does this by interacting with and activating the host immune system and coagulation pathways. Peptidoglycan, lipoteichoic acid, and α-toxin may all play a role [22–24] (reviewed by Lowy [32]). In addition to causing septic shock, some *S. aureus* strains produce superantigens, resulting in various toxinoses, such as food poisoning and toxic shock syndrome [25, 33]. Unlike the structural components noted earlier, these superantigens can produce a sepsis-like syndrome by initiating a "cytokine storm." Some strains also produce epidermolysins or exfoliative toxins capable of causing scalded skin syndrome or bullous impetigo [26].

Regulation of expression of staphylococcal virulence factors plays a central role in pathogenesis. To reduce undue metabolic demands, expression occurs in a coordinated fashion—only when required by the bacterium. Expression of MSCRAMMs generally occurs during logarithmic growth (replication),

Table 1. Selected *Staphylococcus aureus* virulence factors.

Type of virulence factors	Selected factors[a]	Genes	Associated clinical syndromes	Reference(s)
Involved in attachment	MSCRAMMs (e.g., clumping factors, fibronectin-binding proteins, collagen, and bone sialoprotein-binding proteins)	*clfA, clfB, fnbA, fnbB, cna, sdr, bbp*	Endocarditis, osteomyelitis, septic arthritis, and prosthetic-device and catheter infections	[5–8]
Involved in persistence	Biofilm accumulation (e.g., polysaccharide intercellular adhesion), small-colony variants, and intracellular persistence	*ica* locus, *hemB* mutation	Relapsing infections, cystic fibrosis, and syndromes as described above for attachment	[9–15]
Involved in evading/destroying host defenses	Leukocidins (e.g., PVL and γ-toxin), capsular polysaccharides (e.g., 5 and 8), protein A, CHIPS, Eap, and phenol-soluble modulins	*lukS-PV, lukF-PV, hlg, cap5* and 8 gene clusters, *spa, chp, eap, psm-α* gene cluster	Invasive skin infections and necrotizing pneumonia (CA-MRSA strains that cause these are often associated with PVL) abscesses (associated with capsular polysaccharides)	[16–20]
Involved in tissue invasion/penetration	Proteases, lipases, nucleases, hyaluronate lyase, phospholipase C, and metalloproteases (elastase)	*V8, hysA, hla, plc, sepA*	Tissue destruction and metastatic infections	[21]
Involved in toxin-mediated disease and/or sepsis	Enterotoxins, toxic shock syndrome toxin-1, exfoliative toxins A and B, α-toxin, peptidoglycan, and lipoteichoic acid	*sea-q* (no *sef*), *tstH, eta, etb, hla*	Food poisoning, toxic shock syndrome, scalded skin syndrome, bullous impetigo, and sepsis syndrome	[22–26]
With poorly defined role in virulence	Coagulase, ACME, and bacteriocin	*arc* cluster, *opp-3* cluster, *bsa*		[27, 28]

NOTE. ACME, arginine catabolic mobile element; CA-MRSA, community-acquired methicillin-resistant *S. aureus*; CHIPS, chemotaxis inhibitory protein of staphylococci; Eap, extracellular adherence protein; MSCRAMMs, microbial surface components recognizing adhesive matrix molecules; PVL, Panton-Valentine leukocidin. Adapted from Projan and Novick [21] and Archer [29].

[a] Several factors may have >1 role in *S. aureus* pathogenesis.

whereas secreted proteins, such as toxins, are produced during the stationary phase. During infection, the early expression of the MSCRAMM proteins facilitates initial colonization of tissue sites, whereas the later elaboration of toxins facilitates spread. The accessory gene regulator (*agr*) is a quorum-sensing system that plays a critical role in the regulation of staphylococcal virulence. It has been studied extensively and has been reviewed by Yarwood and Schlievert [34] and Novick [35], among others. The *agr* mutants appear to have diminished virulence, and certain *agr* types are associated with particular clinical syndromes [36]. Other important regulators include the staphylococcal accessory regulator [37], ArlR and ArlS [38], SaeRS [39, 40], Rot [41], and *mgr* [42].

Host factors may also affect susceptibility to staphylococcal disease but, in general, are poorly characterized. In one large study, *S. aureus* nasal carriage and subsequent development of *S. aureus* bacteremia and mortality were assessed in nonsurgical, hospitalized patients. Among those who developed *S. aureus* bacteremia, noncarriers had mortality higher than that among carriers. Because most infections among carriers occurred with their colonizing strains, colonization may confer some protective immunity if staphylococcal infection develops [43]. Antibodies also appear to protect against the development of toxic shock syndrome, which occurs almost exclusively in those who lack antibodies to the implicated toxin at the time of acute illness [33].

As described, *S. aureus* has numerous mechanisms to produce disease and to evade host defenses. However, it is important to note that not all *S. aureus* strains are created equal. Different strains may contain different adhesins or toxins or may differ in their ability to produce biofilms and resist phagocytosis. The distribution of some virulence factors is related to clonal type, whereas the presence of others is unrelated to genetic background [44]. In this regard, it is important to note that there is limited information on the expression of these genes during infection.

PATHOGENESIS OF HA-MRSA

History of MRSA. Methicillin was first introduced in 1959–1960, and, within a year, methicillin-resistant isolates were reported [45]. Methicillin resistance is conferred by the *mecA* gene, which encodes a penicillin-binding protein (PBP2A) with decreased affinity for β-lactam antibiotics. *mecA* is part of a mobile genetic element called the "staphylococcal cassette chromosome (SCC) *mec*." SCC*mec* is flanked by cassette chromosome recombinase genes (*ccrA*/*ccrB* or *ccrC*) that permit intra- and interspecies horizontal transmission of SCC*mec*. The initial reservoir of SCC*mec* is unclear but may have been a coagulase-negative staphylococcal species [46–48].

A limited number of MRSA lineages has emerged from the transfer of SCC*mec* into successful methicillin-susceptible *S. aureus* (MSSA) clones. Using multilocus sequence typing (comparing the internal sequences of 7 housekeeping genes), Enright et al. [49] demonstrated that MRSA clones evolved from 5 different groups of related genotypes or clonal complexes, each arising from a distinct ancestral genotype. The earliest MRSA isolates evolved from sequence type (ST) 8-MSSA, which, after a point mutation, evolved into ST250-MSSA. This MSSA was likely the first recipient of SCC*mec* (specifically, type I) to yield the first MRSA, labeled ST250-MRSA-I [49]. As in the work of Enright et al. [49], Crisóstomo et al. [50] identified probable recipient MSSA strains for early MRSA strains in another collection of isolates. Select MRSA clones are described in table 2.

HA-MRSA infections historically have been caused by internationally disseminated clones, including 5 major clones (the Iberian, Brazilian, Hungarian, New York/Japan, and Pediatric clones) that have been described in several ways (e.g., by multilocus sequence typing and PFGE) with the use of different nomenclature. Subsequently, these multidrug-resistant clones were disseminated globally and accounted for the majority of HA-MRSA infections in several regions. For example, the Brazilian clone spread to Portugal, Argentina, Uruguay, Chile, and the Czech Republic [55]. It remains unclear why particular clones are so transmissible and are able to become the "established" HA-MRSA strains in certain regions. Certainly, resistance to multiple antibiotics plays a role in establishing dominance in hospital settings. However, investigators have also postulated that these clones have enhanced virulence, as denoted by their increased transmissibility or ability to colonize hosts.

One example of a successful clonal type is phage type 80/81, which was responsible for pandemic *S. aureus* nosocomial and community-acquired infections throughout the 1950s. Its prevalence began to fade in the 1960s after methicillin became available. Phage type 80/81 is ST30 and contains the Panton-Valentine leukocidin (PVL) gene. This highly successful clone is related to the southwest Pacific (SWP) clone, a CA-MRSA clone that is also ST30 and contains SCC*mec* IV as well as PVL. Given the similar genetic backgrounds of these strains and the previous epidemicity of phage type 80/81, one would expect the SWP clone to have great potential to cause widespread disease. Of note, this clone has already appeared in the United Kingdom. Phage type 80/81 also is a likely close relative of the hospital-acquired, epidemic MRSA-16 strain (ST36-MRSA-II) [56].

HA-MRSA virulence: the Brazilian clone. The Brazilian clone (also known as Brazilian epidemic clonal complex [BECC]), PFGE type A_1, became the major cause of invasive staphylococcal infections at João Barros Barreto University Hospital (Belém, Brazil) in the 1990s. In 1995, it accounted for 38% of *S. aureus* isolates and, by 1998, 79% of isolates. Investigators compared BECC A_1 strains to MSSA and sporadic MRSA strains (rarely detected in hospitals) in several in vitro

Table 2. Details of select important methicillin-resistant *Staphylococcus aureus* (MRSA) clones and their clonal complexes.

Clone name[a]	Clonal complex	Other names of clone[b]
ST1-MRSA-IV	1	USA400, MW2
ST5-MRSA-I	5	UK EMRSA-3
ST5-MRSA-II	5	New York/Japanese, GISA, and USA100
ST5-MRSA-IV	5	USA800 and Pediatric
ST228-MRSA-I	5	Southern Germany
ST8-MRSA-II	8	Irish-1
ST8-MRSA-IV	8	UK EMRSA-2, -6, USA300, and USA500
ST239-MRSA-III	8	UK EMRSA-1, -4, -11, Portuguese, Brazilian, and Viennese
ST247-MRSA-I	8	UK EMRSA-5, -17, and Iberian
ST250-MRSA-I	8	First MRSA and Archaic
ST22-MRSA-IV	22	UK EMRSA-15 and Barnim
ST36-MRSA-II	30	UK EMRSA-16 and USA200
ST30-MRSA-IV	30	Southwest Pacific
ST45-MRSA-IV	45	Berlin and USA600
ST72-MRSA-IV	…	USA700

NOTE. EMRSA, epidemic MRSA; GISA, glycopeptide-intermediate *S. aureus*. Adapted from [51], with permission from Elsevier.

[a] The clone name is comprised of the sequence type (ST), which is the multilocus sequence type based on the sequences of 7 housekeeping genes, and the MRSA staphylococcal cassette chromosome (SCC) *mec* type.

[b] Only select "other names" are included. Additional sources: Enright et al. [49], McDougal et al. [52], Tenover et al. [53], and Melles et al. [54].

experiments. BECC A_1 strains produced significantly more biofilm than did the other strains. They also had higher adhesion to polystyrene, as well as to bronchial epithelial cells, and were more likely to invade these cells. The presence of accessible fibronectin-binding domains appeared to be necessary for a high level of invasion. These in vitro studies suggest that this particular clone may be successful because it has an enhanced ability to bind, persist, and invade [57]. Whether these attributes are present in other HA-MRSA epidemic clones is unknown.

PATHOGENESIS OF CA-MRSA INFECTION

Until the 1990s, MRSA rarely caused infections among community members without exposure to the health care setting (one exception is injection drug users). An outbreak of CA-MRSA infections occurred between 1989 and 1991 among indigenous Australians in western Australia without health care contact [58]. CA-MRSA infections were also reported in people from neighboring regions [59]. In the late 1990s, several cases of aggressive MRSA infection also occurred among individuals in the United States without established risk factors for MRSA. Four children died of CA-MRSA infections in Minnesota and North Dakota from 1997 to 1999. All the cases were rapidly fatal and were associated with necrotizing pneumonia or pulmonary abscesses and sepsis [60]. The strain responsible for these infections was ST1 and PFGE type USA400 (also known as the MW2 strain) [52]. Subsequently, clonal outbreaks of skin and soft-tissue infection caused by CA-MRSA were also reported among prison inmates, men who have sex with men, soldiers, and athletes, particularly football players [61–64]. The strain responsible for these infections was ST8 and PFGE type USA300 [53]. Cases of CA-MRSA skin infection and necrotizing pneumonia were reported internationally as well [65, 66].

In addition to causing necrotizing pneumonia, CA-MRSA has recently been reported to cause infections or infectious complications in situations in which *S. aureus* or MRSA is an unusual pathogen. These have included cases of necrotizing fasciitis caused by PFGE type USA300 [67], as well as cases of pyomyositis [68, 69], purpura fulminans with toxic shock syndrome [70], and Waterhouse-Friderichsen syndrome [71].

The number of CA-MRSA infections appears to be increasing, and the strains responsible for these infections have now entered the health care setting, blurring the line between "community" and "hospital" strains [72, 73]. The strains that cause these virulent infections carry SCC*mec*IV (sometimes SCC*mec*V), the smallest of the SCCs that confer methicillin resistance, and are generally susceptible to several non–β-lactam antibiotics. This is in contrast to the multidrug-resistant nosocomial MRSA strains that carry larger SCC*mec* types [74, 75]. CA-MRSA strains may also have a growth advantage over HA-MRSA strains [27, 76].

Although SCC*mec*IV has appeared in several different genetic

backgrounds [55], PFGE types USA300 (ST8) and USA400 (ST1)—both *agr* type III—accounted for the vast majority of CA-MRSA infections in individuals without the usual MRSA risk factors or health care contact in the United States [52, 77]. USA300 is now the predominant strain. Of interest, some of these USA300 isolates that cause infections are PVL positive but methicillin susceptible [78].

Worldwide, there are other prevalent CA-MRSA strains, such as ST80 (France-Switzerland), ST30 (SWP clone), and ST93 (Australia Queensland clone) [65]. Said-Salim et al. [77] identified additional "community-acquired strains" (CA-MRSA strains defined as containing SCC*mec*IV); however, these were in individuals with MRSA risk factors or health care contact.

The basis for the apparent increased virulence of CA-MRSA strains is incompletely understood. Numerous factors have been proposed, such as increased fitness, improved evasion of the host immune system, and unique toxin production. The genes and mechanisms by which CA-MRSA strains may cause aggressive disease are discussed in the sections that follow. Because these strains usually contain PVL, which is usually absent in HA-MRSA strains, some researchers postulate that this protein, with leukocytolytic and dermonecrotic activity, is responsible.

The role of PVL versus other virulence determinants. There is a strong epidemiological association between PVL and the emergence of CA-MRSA infections. PVL is uncommonly found in MSSA and HA-MRSA isolates [79–83]. In a study of 593 *S. aureus* isolates in France, PVL was absent in HA-MRSA isolates but was associated with all CA-MRSA strains [83]. In another study, PVL was ubiquitous in a large sample of CA-MRSA isolates collected from across the globe [65]. It is usually present in USA300 and USA400 [27, 53, 77] and is often harbored by other SCC*mec*IV-containing strains [77]. The outbreaks of skin and soft-tissue infections and necrotizing pneumonia mentioned above were caused by PVL-positive strains.

Lina et al. [66] determined the presence of *lukS-PV* and *lukF-PV* (the cotranscribed genes for PVL) in 172 *S. aureus* strains collected from patients with a variety of clinical syndromes. PVL was significantly associated with community-acquired pneumonia (85% of strains), compared with hospital-acquired pneumonia (0%). PVL was also significantly associated with strains causing invasive skin infections such as furunculosis (93%) and cutaneous abscess (50%), compared with superficial folliculitis (0%). PVL was not observed in strains associated with infective endocarditis, urinary tract infections, toxic shock syndrome, or mediastinitis, although few strains were tested [66]. Diep et al. [80] reported a similar association of PVL and skin and soft-tissue infections caused by MRSA isolated from inpatients and outpatients from San Francisco General Hospital and inmates in county jails.

In addition to the epidemiological evidence suggesting that PVL may be a virulence factor in CA-MRSA, there is a scientific rationale for this association. Staphylococcal leukotoxins, including PVL, are secreted as bicomponent toxins consisting of S and F proteins [16, 84]. Depending on the combination of particular S and F proteins, a toxin is formed with varying leukocytolytic, erythrocytolytic, and dermonecrotic properties [84, 85]. PVL consists of LukS-PV and LukF-PV and 4 units of each form of octameric β-barrel pores in leukocyte membranes in vitro, resulting in cell lysis [19, 86–88]. This may cause cells such as neutrophils to release inflammatory enzymes and cytokines (sublytic concentrations of PVL also appear to induce the release of these substances) [88–90]. PVL also appears to induce apoptosis of neutrophils via a mitrochondrial pathway at lower concentrations, whereas, at higher concentrations, PVL induces necrosis [91]. In vivo, PVL causes dermonecrosis when injected intradermally in rabbits [92].

Given this evidence and the strong epidemiological association between PVL-containing CA-MRSA strains and necrotizing pneumonia and skin and soft-tissue infections, it is plausible that PVL is partly responsible for the enhanced virulence of CA-MRSA (other leukocidins may also play a role). However, recent studies comparing the virulence of PVL-positive and PVL-negative strains have had conflicting results.

Saïd-Salim et al. [77] compared human polymorphonuclear cell lysis among PVL-positive and PVL-negative CA-MRSA strains with similar genetic backgrounds and found no difference in polymorphonuclear lysis. Voyich et al. [93] compared PVL-positive strains and PVL-negative strains with similar genetic backgrounds in mouse sepsis and abscess models, as well as PVL knockouts created for the USA300 and USA400 strains. There was no difference in survival in the mouse sepsis model. In the abscess model, PVL-negative strains unexpectedly caused slightly larger abscesses than did the PVL-positive strains. Isogenic *pvl* strains of USA300 and USA400 showed no difference in the ability to cause polymorphonuclear lysis in vitro. The authors concluded that the PVL "…toxin is not the major determinant of disease caused by these prominent CA-MRSA strains" [93, p. 1769]. It is possible that the mouse models used in this study were not optimal to assess the in vivo effects of PVL, or, as the authors suggested, that PVL either is a marker for other virulence factors present in these strains or is one of many factors causing the enhanced virulence of particular CA-MRSA strains.

PVL was investigated in a mouse pneumonia model by Labandeira-Rey et al. [94]. Mice were infected with isogenic PVL-positive and PVL-negative (non–CA-MRSA) strains. PVL-positive strains caused necrotizing pneumonia similar to that seen in humans, whereas PVL-negative strains showed only some leukocytic invasion. When PVL-negative mutants were complemented with plasmids containing the PVL operon, massive tissue damage and mortality resulted. In mice, exposure to LukS-PV and LukF-PV toxin was sufficient to cause lung dam-

age, weight loss, and increased mortality in a concentration-dependent fashion [94]. In these studies, however, a single non–CA-MRSA strain was used.

In contrast, Bubeck Wardenburg et al. [95] recently reported conflicting results. They demonstrated that α-hemolysin and not PVL was responsible for mortality in a mouse pneumonia model, using USA300 and USA400 CA-MRSA strains.

These studies suggest that the association of PVL with enhanced *S. aureus* virulence is complex and controversial and warrants further investigation. Furthermore, Wang et al. [20] recently discovered that phenol-soluble modulins, a previously unrecognized class of secreted *S. aureus* peptides, are up-regulated in CA-MRSA strains, compared with the level in HA-MRSA strains; cause inflammation; destroy neutrophils; and are responsible for virulence in mouse abscess and bacteremia models. Other toxins, such as the enterotoxins, may also play an important role in these infections.

Virulence of USA400. USA400 (or MW2) is a highly virulent CA-MRSA strain. This is apparent not only in human disease but also in animal models [27, 93]. Initially, its only resistance genes were *mec* and *blaZ*, which encodes penicillinase. Researchers sequenced USA400 and compared its sequence with the sequences of 5 other strains (N315, a Japanese MRSA; Mu50, a vancomycin-resistant MRSA; E-MRSA-16, an epidemic MRSA in the United Kingdom; COL, a MRSA strain; and NCTC8325, a widely used reference strain) to identify potential virulence factors associated with this strain. USA400 was the only strain to contain the PVL operon. In addition, it contained 16 unique superantigen genes, including 11 exotoxin genes and 5 enterotoxin genes. These genes had at least a 2% difference in their amino acids, compared with their homologues. One exception was staphylococcal enterotoxin H (*seh*), which was unique to USA400 [27] and can cause a toxic-shock–like syndrome [96]. USA400 also contained a novel gene cluster dubbed "bacteriocin of *S. aureus*" (*bsa*). *bsa* encodes a potential bacteriocin, or antibacterial agent. This bacteriocin could help USA400 compete with other colonizing flora and increase the chance of infection with this strain [27]. These data suggest that there are several factors that may contribute to the virulence of USA400 and that these factors are ripe for future investigation.

Virulence of USA300. Like USA400, USA300 is associated with virulent disease [93]; however, USA300 causes far more incident cases of CA-MRSA infection and is becoming resistant to several non–β-lactam antibiotics [28]. The genome of USA300 was sequenced by Diep et al. [28] and compared with 10 previously sequenced *S. aureus* strains as well as 4 coagulase-negative strains to identify factors potentially associated with its high virulence. Of interest, there were minimal differences between the core sequences of USA300 and COL, an early MRSA. In addition to harboring SCC*mec*IV and the PVL operon, USA300 contained homologues closely related to staphylococcal enterotoxins Q and K, designated SEQ2 and SEK2. Like COL and USA400, USA300 also has a genome that includes a bacteriocin gene cluster. Most notably, USA300 contains a genomic island, termed "arginine catabolic mobile element" (ACME), which encodes an arginine deaminase pathway that converts L-arginine to carbon dioxide, adenosine triphosphate, and ammonia. Arginine deaminase, a known virulence factor in other pathogens, may enhance the virulence of USA300 by enabling it to (1) survive more easily on acidic, human skin; (2) proliferate more easily in conditions low in oxygen, such as abscesses; and (3) evade host defenses by inhibiting production of nitric oxide and mononuclear cell proliferation as in *Streptococcus pyogenes* [28, 97]. Further investigation of ACME may help elucidate the remarkable success and virulence of the USA300 strain.

Colonization and CA-MRSA. As discussed above, the anterior nares are the classic reservoir for nosocomial *S. aureus* infections, including HA-MRSA. However, data suggest that other sites of colonization or modes of transmission play an important and underappreciated role in the development of CA-MRSA infection. Heterosexual contact was recently identified as a mode of transmission of CA-MRSA. Most cases had genital CA-MRSA colonization without nasal colonization [98]. In an outbreak investigation of CA-MRSA abscesses among St. Louis Rams football players, no MRSA was isolated from nasal or environmental samples. Perhaps other sites of colonization, shared items, or an unsampled environmental site played a role in transmission [64]. Future epidemiological investigations of CA-MRSA should include sampling of several environmental and body sites in addition to the anterior nares.

IS MRSA MORE VIRULENT THAN MSSA?

There is an active debate as to whether MRSA is more virulent than MSSA. Some epidemiologic studies, including a meta-analysis, found increased morbidity and/or mortality from nosocomial MRSA (e.g., bloodstream infections, surgical-site infections, and pneumonia), compared with those from MSSA [99–102]; however, these studies may be confounded because not all accounted for important factors such as time to initiation of appropriate therapy or patient comorbidities. A recent retrospective review found increased mortality for MRSA bacteremia but not MRSA pneumonia [103]. Other studies did not demonstrate increased mortality associated with nosocomial MRSA bacteremia [104] or ventilator-associated pneumonia [105], compared with MSSA infections. An investigation that compared CA-MRSA skin infections and CA-MSSA skin infections did not find more serious outcomes for the CA-MRSA infections [106]. To date, there is no compelling evidence that MRSA, in general, is more virulent than MSSA. Although this issue remains unresolved, invasive MRSA infection is as-

sociated with greater costs [101, 102, 104] and limited treatment options.

UNANSWERED QUESTIONS

Although considerable progress has been made in understanding the pathogenesis of *S. aureus* infection, numerous questions remain unanswered. The role of many virulence factors in the pathogenesis of staphylococcal disease is unclear. This is a result, in part, of the redundancy of function and/or the ubiquitous nature of many virulence factors in addition to the complex nature of virulence factor regulation. In particular, the role of PVL in staphylococcal virulence remains uncertain. Also, as discussed above, particular clonal strains have the ability to persist for years and to establish themselves globally. Why certain clonal types have this ability remains unknown. Other clonal types have become established among otherwise healthy community members. Understanding what enables these strains to do this, what their reservoirs are, and what their means of transmission are requires further investigation. We hope that, in the future, a better understanding of the pathogenesis of staphylococcal disease will lead to improved prevention and treatment strategies.

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