Reemergence of antibiotic-resistant
Staphylococcus aureus in the genomics era

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***Staphylococcus aureus* is the leading cause of bacterial infections in developed countries and produces a wide spectrum of diseases, ranging from minor skin infections to fatal necrotizing pneumonia. Although *S. aureus* infections were historically treatable with common antibiotics, emergence of drug-resistant organisms is now a major concern. Methicillin-resistant *S. aureus* (MRSA) was endemic in hospitals by the late 1960s, but it appeared rapidly and unexpectedly in communities in the 1990s and is now prevalent worldwide. This Review focuses on progress made toward understanding the success of community-associated MRSA as a human pathogen, with an emphasis on genomewide approaches and virulence determinants.**

Introduction

Staphylococcus aureus is the leading cause of bacterial infections involving the bloodstream, lower respiratory tract, and skin and soft tissue in many developed countries, including the United States (1). Penicillin was initially highly effective against staphylococcal infections, but penicillinase-producing *S. aureus* emerged in the mid-1940s, and its prevalence increased dramatically within a few years (2, 3) (Figure 1A). This increase was entirely attributable to widespread use of penicillin, which selected for bacteria containing resistance genes. Several "epidemic waves" of antibiotic-resistant *S. aureus* have occurred since then (Figure 1A). Penicillin-resistant *S. aureus* was pandemic in the 1950s and early 1960s (4). These infections, both in hospitals and in the community, were caused primarily by one *S. aureus* clone known as phage-type 80/81 (4). Pandemic phage-type 80/81 *S. aureus* infections declined after the introduction of methicillin in 1959 (5). Within 2 years, however, Jevons and colleagues reported the emergence of methicillin-resistant *S. aureus* (MRSA) (Figure 1B) (6), and the first cluster of cases was reported shortly thereafter (7).

MRSA has since spread and is endemic in most hospitals worldwide. In the United States, *S. aureus* is the number one cause of hospital-associated infections, and a high percentage of these are caused by MRSA (8–10). The mortality rate associated with invasive MRSA infections is approximately 20% (11), and in the United States these infections are probably the leading cause of death by any single infectious agent; fatalities resulting from these infections are estimated to surpass those caused by HIV/AIDS (Figure 1C) (11, 12). Thus, it is clear that *S. aureus*, especially MRSA, remains a major problem for health care facilities.

The rapid emergence of community-associated MRSA (CA-MRSA) infections (i.e., those that occur outside of health care facilities in otherwise healthy people) is one of the most surprising events in infectious diseases in recent years (13–17). This is in part because community-associated *S. aureus* infections were traditionally caused by methicillin-susceptible *S. aureus* (MSSA), rather than the antibiotic-resistant strains so prevalent in hospitals (13). Thus, it remains a mystery why these antibiotic-resistant bacteria emerged in a niche (the community) not obviously under the relatively high selective pressure exerted by antibiotics in the hospital setting. CA-MRSA was first reported in Western Australia in the 1990s (18) and has since emerged worldwide (19) and become epidemic in the United States (16, 17). In addition to the increased prevalence of infections, CA-MRSA strains can cause severe or fatal disease. In this Review, we discuss key components of the recent emergence of CA-MRSA, including transmission and virulence, and we highlight some of the genome-wide approaches used to understand the success of this pathogen. Due to space constraints, we focus mainly, but not exclusively, on the epidemic in the United States.

Recent emergence of CA-MRSA

The molecular epidemiology and evolution of MRSA is a topic too expansive for this Review, but several key features of CA-MRSA strains merit mention. Based on molecular typing and microarray approaches, *S. aureus* can be grouped broadly into at least 10 major lineages, named clonal complexes (Table 1) (20–22). Pulsed-field gel electrophoresis is commonly used to identify *S. aureus* isolates in the United States, and for simplicity, here we use this typing system to describe *S. aureus* strains (Sidebar 1) (23). In 2003, pulsed-field gel electrophoresis types USA100, USA200, USA500, USA600, and USA800 were the predominant health care–associated strains, whereas USA300 and USA400 were the most abundant CA-MRSA strains (23). MRSA strains can be characterized further by at least five distinct staphylococcal cassette chromosome *mec* (SCC*mec*) types (I–V), mobile genetic elements that encode methicillin resistance. Health care–associated MRSA strains historically contain predominantly SCC*mec*I–III, whereas CA-MRSA typically carries SCC*mec*IV (24, 25). SCC*mec*IV, which is smaller than SCC*mec*I–III, lacks other antibiotic resistance elements and appears to impart little or no fitness cost to the organism (24). Consistent with this idea, CA-MRSA strains in general have faster growth rates than health care–associated MRSA strains (24). In addition to SCC-*mec*IV, CA-MRSA strains carry genes encoding a toxin known as Panton-Valentine leukocidin (PVL, discussed below), and both are often used as markers for CA-MRSA.

CA-MRSA in the clinic

Clinical epidemiology and disease. CA-MRSA infections appear to have emerged first among indigenous people of the remote Kimberley region of Western Australia in the early 1990s (18, 26). The first
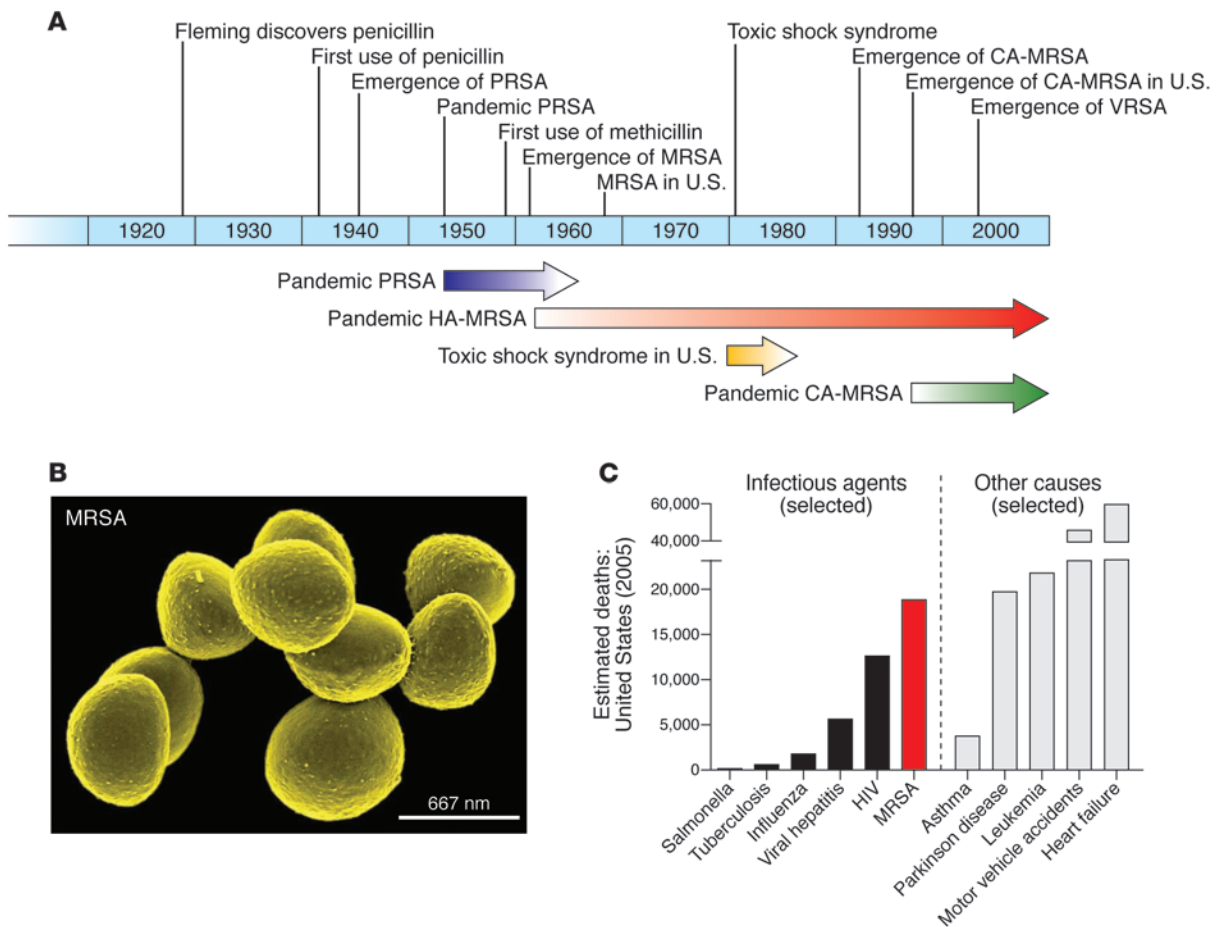
Figure 1
Emergence of antibiotic-resistant *S. aureus*, epidemic waves, and estimated deaths caused by MRSA in the United States. (**A**) Timeline indicates the year in which an event occurred or was reported. Arrows indicate approximate length of time for each pandemic/epidemic. (**B**) Scanning electron micrograph of strain COL, one of the first MRSA isolates from Colindale, United Kingdom. Original magnification, ×45,000. (**C**) Comparison of estimated deaths in the United States in 2005 due to individual infectious agents or other causes. Data are CDC estimates from National Vital Statistics Reports (12) and Klevens et al. (11). Deaths associated with MRSA infection are based on the estimated number of in-hospital deaths rather than attributable mortality, whereas data for all other causes of mortality are based on US Standard Certificate of Death. Note also that mortality due to MSSA is not included, and thus estimated mortality associated with all *S. aureus* infections is not shown.

well-documented cases of CA-MRSA infection in the United States occurred in four otherwise healthy children from rural Minnesota and North Dakota in 1997–1999 (15). Lacking any risk factors for MRSA, e.g., contact with a health care facility, they presented with severe infections, septic arthritis, bacteremia and septic shock, and necrotizing pneumonia. A retrospective study performed in Chicago suggested an earlier beginning (1993–1995) of the CA-MRSA epidemic in the United States (14). CA-MRSA has subsequently been identified in numerous populations, including high school, college, and professional athletes; prison inmates; men who have sex with men; military personnel; and children in day care centers (17, 27–32). CA-MRSA strains are so prevalent in the United States (17, 33) and abroad (19) (Table 1), and such an important cause of hospital infections (34, 35), that no group can be considered not at risk. This idea is underscored by the surprising finding that most MRSA clinical isolates in Denmark, a country of very low MRSA prevalence, were CA-MRSA isolates (36).

The CA-MRSA epidemic in the United States is actually two sequential, overlapping epidemics. USA400 — the original CA-

MRSA strain commonly known as MW2 — predominated prior to 2001 (15). This strain may be a descendant of an early Western Australian CA-MRSA strain (WA-1) (Table 1). Although USA400 is still an important cause of community-associated disease in some regions (e.g., Alaska and the Pacific Northwest), USA300 is now responsible for most CA-MRSA infections in the United States (17, 33). Importantly, the complete genomes of USA300 and USA400 have been sequenced, and these data have been fundamental for approaches directed toward better understanding the epidemiology and virulence of CA-MRSA infections and developing diagnostic tools (see below).

The emergence of CA-MRSA has led to an increase in the overall burden of staphylococcal disease (37, 38). Skin and soft tissue infections, the most common types of CA-MRSA infection, constitute approximately 90% of cases (Figure 2). However, CA-MRSA strains can cause virtually any infection, ranging from mild skin infections to fatal necrotizing pneumonia (Figure 2) (14, 16, 37, 39–48).

Approximately a third of healthy noninstitionalized humans are asymptomatically colonized by *S. aureus* (49, 50), and thus it can be

Table 1
Major clonal complexes of *S. aureus*

Clonal complex[A]	Multilocus sequence type (selected)	Common names and/or PFGE types for specific MRSA clones[B]	Comment
CC1	ST1	USA400 and MW2	First US CA-MRSA strain,[c] SCC*mec*IV and PVL-positive (except in Canada)
	ST1	WA-1	Western Australian CA-MRSA, PVL-negative
CC5	ST5	USA100 and New York/Japan clone	Most common US health care–associated MRSA, SCC*mec*II
	ST5/	USA800 and pediatric clone	Among earliest recognized SCC*mec*IV-bearing strains
	ST5	EMRSA-3	
CC8	ST250	COL	First (archaic) MRSA clinical isolate, SCC*mec*I
	ST247/	Iberian clone and EMRSA-5	Descendant of COL
	ST239	Brazilian/Hungarian clone, EMRSA-1	SCC*mec*III
	ST239	AUS-2 and AUS-3	Eastern Australian epidemic clone of 1980s, SCC*mec*III
	ST8	Irish-1	SCC*mec*II
	ST8	EMRSA-2 and EMRSA-6	SCC*mec*IV
	ST254	EMRSA-10	SCC*mec*IV
	ST8	USA300, FPR3757, LAC, and SF8300	Predominant US CA-MRSA strain, SCC*mec*IV and PVL-positive, ACME-positive
	ST8	USA500	
CC9	ST9	Not applicable	MRSA strains are rare or not reported
CC15	ST15	Not applicable	MRSA strains are rare or not reported
CC22	ST22	EMRSA-15	International clone, prominent in Europe and Australia
CC25	ST25	Not applicable	MRSA strains are rare or not reported
CC30	ST30	USA1100	Uncommon US CA-MRSA, SCC*mec*IV and PVL-positive
	ST30	SWP	Southwest Pacific clone, CA-MRSA, SCC*mec*IV and PVL-positive
	ST36	USA200 and EMRSA-16	Single most abundant cause of MRSA infections in United Kingdom; second most common cause of MRSA infections in US hospitals in 2003
CC45	ST45	USA600	SCC*mec*II
	ST45	Berlin	
CC51	ST51	Not applicable	MRSA strains are rare or not reported
No defined CC	ST59	USA1000	Taiwan, East Asian CA-MRSA, SCC*mec*IV and PVL-positive
	ST72	USA700	
	ST80		European CA-MRSA, SCC*mec*IV and PVL-positive
	ST93	QLD	Queensland, Australia, CA-MRSA, SCC*mec*IV and PVL-positive

[A]Clonal complexes in which MRSA are almost exclusively found. [B]Clone, genetically identical bacteria that arose from a single common ancestor. [C]"Strain" refers to a group of bacteria of the same species that share certain features not characteristic of the entire species. CC, clonal complex; PFGE, pulsed-field gel electrophoresis; ST, sequence type.

considered normal flora. In this respect, CA-MRSA should be no different from other *S. aureus* strains. However, most individuals are colonized by MSSA rather than MRSA (28.6% were colonized with *S. aureus* in the United States in 2003–2004, of which 1.5% were colonized with MRSA), and only a fraction (15.6%) of these MRSA isolates are USA300 or USA400 (50). It should be noted, however, that this represents a doubling of the MRSA nasal colonization rate compared with 2001–2002 (before USA300 became so prevalent) and that much of this increase is attributable to USA300 and USA400 strains, which doubled in percentage of all isolates. Nevertheless, given the high prevalence of CA-MRSA infections in the United States, these findings suggest that CA-MRSA strains cause infection in the absence of nasal colonization, a notion that merits further investigation. CA-MRSA strains may be more easily transmitted than other stains for reasons that are not known (51). Skin-to-skin contact with a colonized or infected individual is the predominant mode of transmission, although contaminated fomites (e.g., towels and razors) may play a role in some settings

(33, 52, 53). The CDC has articulated "Five Cs" of transmission: first, contact with an infected or colonized person; second, cleanliness (actually, a lack thereof); third, compromised skin integrity; fourth, contaminated objects, surfaces, and items (e.g., towels and sauna benches); and fifth, crowded living conditions. Infection occurs when *S. aureus*, either as a component of the resident flora or when introduced from the outside by a contaminated agent, exploits a breach in host defenses, producing local infection, invasive disease, or both. Being from an ethnic minority, young age (in particular, less than 2 years of age), low socioeconomic status, and drug use (especially injection drug use) are risk factors for CA-MRSA infection (16, 27, 32, 42, 54–58).

Diagnosis, treatment, and prevention. Diagnosis of *S. aureus* infection is easily made by culturing organisms from a focal site of infection, blood, or other sterile body fluid. Methicillin resistance is determined by standard antimicrobial susceptibility test methods, including disk diffusion, broth dilution, and automated methods. Molecular typing to determine clonal type (23, 59–61) (Sidebar 1),

Sidebar 1

S. aureus typing methods and associated nomenclature

Multilocus sequence typing (MLST)

This bacterial classification method is based on the allelic variation of 7 housekeeping genes in *S. aureus*; it provides a discriminatory allelic profile for each bacterial isolate. The allelic profile is known as sequence type (ST) (60). MLST indexes variation that accumulates slowly and thus can be used to measure long periods of evolution among *S. aureus* strains. MLST results are also highly reproducible. A group of *S. aureus* strains that have identity at 5 or more of the 7 housekeeping genes/loci based on MLST are known as a clonal complex (CC). CCs are determined by an algorithm named BURST (21).

PFGE

PFGE is a bacterial classification method appropriate for evaluation of recent evolution among groups of strains. For *S. aureus*, the method relies on an analysis of fragments of *Sma*I-digested *S. aureus* genomic DNA. Fragments are compared by PFGE and typically clustered according to an 80% similarity coefficient (23). The CDC has developed a national PFGE database for *S. aureus*, which uses the "USA" designation, e.g., USA100, USA200, USA300, etc. (23).

***spa* typing**

spa typing is based upon sequence analysis of variable number tandem repeats in the gene encoding *S. aureus* protein A (*spa*) (61). Inasmuch as *spa* typing takes into account point mutations in the variable number tandem repeats region and the number of repeat variations, the method can be used to investigate local or global *S. aureus* outbreaks. Another advantage of the method is that epidemiological and phylogenetically robust data are provided by a single target locus.

SCC*mec* typing

SCC*mec* typing is used to define or differentiate *S. aureus* clones or closely related strains determined as such by MLST or PFGE. SCC*mec* is composed of *mec* and *ccr* gene complexes. The *mec* gene complex contains *mecA* (the methicillin resistance gene), associated regulatory genes, and insertion sequences that flank *mecA*. The *ccr* gene complex contains site-specific recombinases *ccrA* and *ccrB*, which give SCC*mec* mobility among staphylococci. SCC*mec* typing is based on different combinations of the *mec* and *ccr* gene complexes, which are identified by PCR (see ref. 140 for details).

presence of SCC*mec*IV (62), and/or presence of PVL genes (19) are useful for differentiating between CA-MRSA and classical health care–associated MRSA clones, but these methods are relatively labor intensive for routine use and of unproven clinical benefit.

The CA-MRSA epidemic has had a profound impact on empirical therapy of suspected staphylococcal infection. Given the prevalence of CA-MRSA throughout the United States, if antimicrobial therapy is indicated for treatment of staphylococcal infection, it should be active against MRSA strains. Unfortunately, this requires the use of second-line agents, such as trimethoprim-sulfamethoxazole and clindamycin, for which efficacy is not well defined, or vancomycin. Despite its being a drug of choice, there are major issues with the use of vancomycin, including problems with prolonged, persistent, and/or recurrent bacteremia during therapy (63), high rates of microbiological and clinical failure (64), nephrotoxicity (65), and increasing prevalence of nonsusceptible strains (66).

S. aureus genomics

CA-MRSA emerged coincident with completion of the first whole genome sequences of bacterial pathogens. There are at least 14 published *S. aureus* genomes, and reports of more are on the way. The first genomes of *S. aureus* to be sequenced were the genomes of MRSA strains N315 and Mu50, each of which is a pulsed-field gel electrophoresis type USA100 strain (67). Mu50 is also a vancomycin-resistant *S. aureus* (VRSA) strain. These studies, which were conducted by Kuroda et al. to better understand virulence and multidrug resistance, revealed that most of the antibiotic resistance genes and many of the virulence genes reside on mobile genetic elements, such as plasmids, transposons, and prophages, indicating substantial horizontal gene transfer from other bacteria (67). This work was followed by the completion of the genome sequences of several key *S. aureus* clinical isolates, including MW2 and MSSA476 (USA400 strains) (68, 69), MRSA252 (a USA200 strain) (69), and FPR3747 (a USA300 strain) (70). MW2 was found to be a distant relative of the previously sequenced USA100 strains and, surprisingly, to show PBP2a resistance only to β-lactam antibiotics (antibiotics that contain a β-lactam ring in their molecular structure) (68). This resistance was conferred by *blaZ* (which encodes a penicillinase) and *mecA* (which encodes PBP2a, a low-affinity penicillin-binding protein that mediates resistance to β-lactam antibiotics such as methicillin). The novel SCC*mec* element identified within MW2, SCC*mec*IVa, is much smaller than the SCC*mec* element found in USA100 strains and other hospital-associated MRSA clones (69) and appears to impart no fitness cost to the organism (24). Another major finding from the MW2 genome sequence was the presence of novel virulence determinants encoded by prophages, including the genes encoding PVL and the enterotoxin G and K homologs. However, the relative contribution of these molecules to human disease remains undetermined. In addition to identifying novel virulence and resistance determinants, the availability of complete *S. aureus* genome sequences served as a springboard for comparative genomics analyses and expression microarray studies.

Whole genome sequencing as a tool to understand the evolution of antibiotic resistance. VRSA strains are rare (there are 7 reported isolates from the United States) (71), but vancomycin-intermediate resistant *S. aureus* (VISA) are an emerging problem in the major *S. aureus* clones worldwide (66). A key difference between VRSA and VISA is the minimum inhibitory concentration of vancomycin required to inhibit growth in vitro (≥16 μg/ml for VRSA and 4–8 μg/ml

A

Skin and soft tissue
(87%–95.6%)

Respiratory
(1%–4%)

Bacteremia
(1%–4%)

Other
(1%–4.5%)

Bone
and joint
(1%–3%)
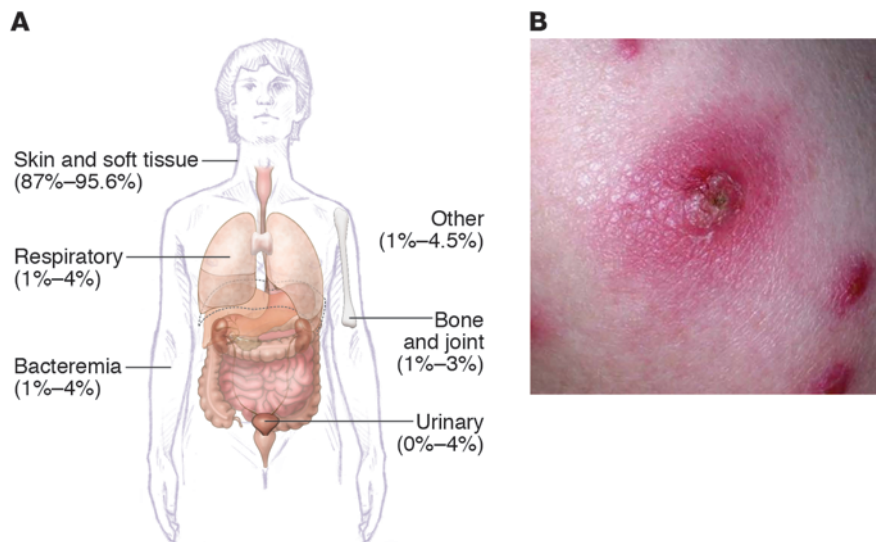
Urinary
(0%–4%)

B

Figure 2
CA-MRSA infections. (**A**) Distribution of all CA-MRSA infections (indicated as a percentage) (16, 34, 37, 139). Studies by Kaplan et al. (37) and Purcell et al. (139) reported distribution in children. (**B**) Human CA-MRSA skin infection with a typical "spider bite" appearance. Photo credit: Kelly Cardoro, Department of Dermatology, UCSF.

for VISA). The mechanisms for resistance are also quite distinct: VRSA isolates carry resistance on a specific mobile genetic element, whereas VISA isolates acquire resistance as a gradual adaptive process, and the molecular genetic basis for resistance was unknown until recently (72). Mwangi et al. used whole-genome sequencing to follow the evolution of the VISA phenotype in *S. aureus* isolates recovered from the same patient over time (72). These researchers sequenced an *S. aureus* isolate at the start of vancomycin therapy that was fully susceptible to vancomycin and then sequenced a nonsusceptible isolate recovered 3 months later. This interesting approach identified 35 point mutations in *S. aureus* that conferred a VISA phenotype and defined loci critical for multidrug resistance (72). These studies provide an illustration of how whole genome sequencing can be used to elucidate the molecular genetic basis of emerging antibiotic resistance.

Using microarray-based approaches to understand bacterial epidemics. There is not enough space here to highlight all the comparative genomics analyses of *S. aureus* performed to date (22, 73–75). However, the study by Fitzgerald et al. was the first to use a comparative microarray approach to study the evolution of MRSA (76). This work demonstrated that the *mecA* gene was acquired by distinct *S. aureus* genotypes multiple times, indicating that MRSA has evolved from nonresistant *S. aureus* strains independently on several occasions, a finding consistent with more recent work (77). Importantly, these studies revealed that the epidemic of toxic shock syndrome in the 1970s and 1980s (Figure 1A) was caused by a change in host environment rather than an emerging hypervirulent strain of *S. aureus* (76). Although the outbreak of toxic shock syndrome is unrelated to the current problem with CA-MRSA, the studies demonstrate how genome-wide approaches can be used to investigate *S. aureus* outbreaks/epidemics.

The first whole genome sequence of USA300. To better understand epidemic CA-MRSA in the United States, Diep et al. determined the first complete genome sequence of a USA300 strain in 2006 (70). It is much more closely related to *S. aureus* strain COL, one of the first MRSA clinical isolates, than it is to many of the other prominent contemporary MRSA strains, including the USA400 prototype CA-MRSA strain (70, 78). For example, the core genomes (excluding mobile genetic elements) of USA300 and strain COL differ by only

678 SNPs. By comparison, USA300 differs from the sequenced USA100 and USA200 strains by 21,313 and 44,964 SNPs, respectively (70). This finding is remarkable, because COL has limited virulence in mice (73) and is not a prominent cause of human disease, whereas USA300 is rapidly lethal in mice (73, 79–82) and is the leading cause of CA-MRSA infections in the United States (16, 17). However, although the core genomes of COL and USA300 are similar, there are differences in the mobile genetic elements that they harbor (70). COL carries SCC*mec*I, whereas USA300 carries SCC*mec*IV, the same SCC*mec* type as MW2. Immediately adjacent to SCC*mec*IV in USA300 is a novel mobile genetic element that is known as type I arginine catabolic mobile element (ACME) (70). ACME is present in most (more than 99%) PVL-positive USA300 clinical isolates but not in MW2 or the published genomes of any other *S. aureus* strains. Three other regions of difference between COL and USA300 correspond to prophages, and two of these mobile genetic elements are of interest here. Prophage φSa2 contains the PVL-encoding genes *lukS-PV* and *lukF-PV*, which are epidemiologically associated with emergence of CA-MRSA. Prophage φSa3 encodes staphylokinase and two modulators of the human innate immune system, staphylococcal complement inhibitor (SCIN) and *S. aureus* chemotaxis inhibitory protein (CHIPS) (83). Thus, both φSa3 and φSa2 carry genes that could contribute to the hypervirulence of USA300. *S. aureus* pathogenicity island (SaPI) 5 in USA300 is similar to SaPI3 of COL (84). Although SaPI5 lacks the toxic shock toxin gene (*tst*), it carries *seq* and *sek*, genes that encode two novel pyrogenic toxin superantigens, enterotoxin Q and K, respectively; these are also present in the MW2 genome. Acquisition of *seq* and *sek* is epidemiologically associated with emergence of USA300 and related strains (85), and therefore SaPI5 is of interest in our efforts to understand the success of USA300.

Clonal expansion of USA300: identifying the epidemic USA300 clone. Our understanding of emerging pathogens and bacterial epidemics has traditionally relied on approaches that index genomic variation at a relatively limited number of nucleotides. Although significant progress has been made using these methods, whole genome analysis of strains within a species is essential to fully understand key aspects of pathogen success, including emergence, virulence, and epidemics. To determine whether USA300 arose
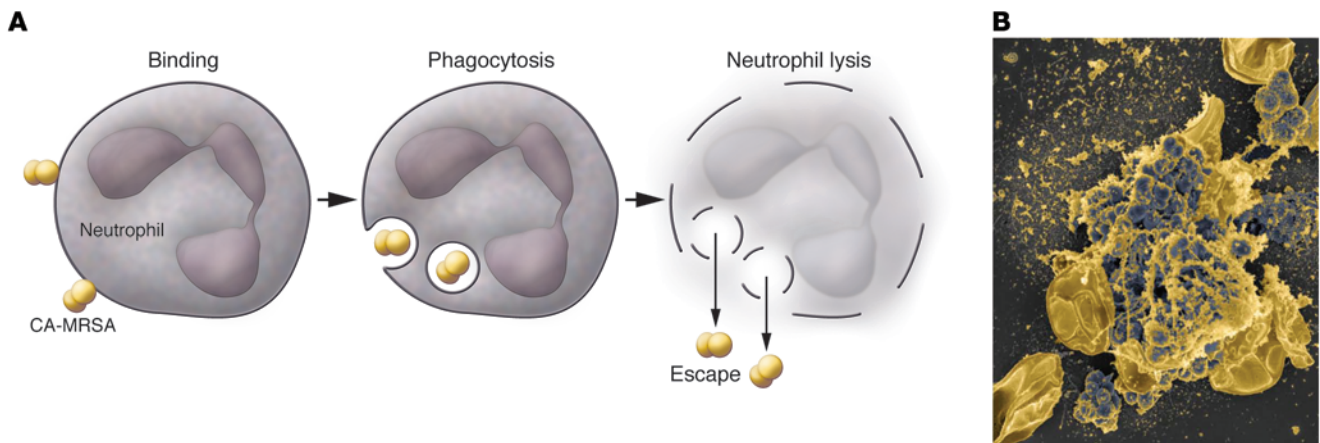
Figure 3
Destruction of neutrophils after phagocytosis of CA-MRSA. (**A**) A schematic model illustrating neutrophil lysis by CA-MRSA and subsequent escape of the pathogen. (**B**) Scanning electron micrograph of human neutrophils that have been destroyed after phagocytosis of USA400. Original magnification, ×5000.

from a recent common ancestor or by convergent evolution toward a hypervirulent phenotype, Kennedy et al. performed comparative whole genome sequencing of ten USA300 isolates recovered from patients hospitalized in several regions across the United States (80). Whole genome sequencing and comparative whole genome sequencing identifies all SNPs and non-SNP regions of difference within an organism and yields a much higher resolution of the genetic variability of strains that appear to be closely related by other methods. Although the ten USA300 isolates were relatively similar, as determined by analysis of SNPs, some were clearly much more closely related than others and had descended from a recent common ancestor. The studies demonstrated that there has been recent clonal expansion of a subset of USA300 isolates that are now known as the epidemic USA300 clone. This work (80) and that of Mwangi et al. (72) provide insight into the potential utility of whole genome sequencing on a much greater scale. As more whole genome sequences for *S. aureus* isolates become available, it may be possible to pinpoint precise evolutionary events in the emergence of new successful clones such as USA300. With such precise understanding of microbe evolution, it should be possible to alter the selective pressures (e.g., antibiotic use or specific environmental conditions) under which these microorganism evolve to become successful pathogens.

CA-MRSA virulence and pathogenesis
Is CA-MRSA more virulent than traditional health care–associated MRSA? Klevens et al. found that 85% of severe invasive MRSA infections either occur in hospitals or can be traced to the health care system (11). The death rate was approximately ten times higher for health care–associated MRSA infections than for CA-MRSA infections, 5.7 versus 0.5 deaths per 100,000 infections, respectively (11). Bacteremia and pneumonia accounted for 75.2% and 13.3% of these health care–associated infections, respectively. In contrast, Fridkin and colleagues found that 77% of CA-MRSA infections were of skin and soft tissue (mainly abscesses and cellulitis), with only 6% causing invasive disease (16). In the United States, USA100, USA200, and USA500 constitute the most abundant isolates from health care–associated infections, whereas USA300 and USA400

account for the greatest number of CA-MRSA isolates (11, 17, 23, 47, 86). These differences in invasive disease and mortality for health care–associated MRSA versus CA-MRSA infections could suggest that health care–associated strains are more virulent than those causing community infections. However, there is strong evidence to suggest the contrary.

First and foremost is the host. Health care–associated infections occur in individuals with predisposing risk factors — including surgery, dialysis, history of hospitalization, substance abuse, indwelling devices, and/or residence in a long-term care facility — that make them susceptible to invasive disease (11, 43). In contrast, CA-MRSA infections occur in otherwise healthy individuals lacking such risk factors. Notably, USA300 and USA400 have the capacity to cause severe and fatal infections in healthy subjects, albeit relatively infrequently (15, 40).

Although transmission and spread of the organism are not a component of disease pathogenesis per se, they contribute to the overall burden of disease and thus are an important component of virulence. USA300 has an extraordinary ability to spread among groups of individuals (53), and thus it disseminated rapidly throughout the United States (11, 17, 47). USA300 has invaded the health care system and is an abundant cause of bloodstream infections in hospitals (11, 35, 87, 88). In contrast, typical health care–associated MRSA strains (e.g., USA100 and USA200), although highly prevalent among health care facilities, are an infrequent cause of community-associated infections (11, 23, 55). This indirect evidence suggests that prominent CA-MRSA strains have enhanced virulence compared with traditional health care–associated MRSA.

There is also direct evidence that prominent CA-MRSA strains (i.e., USA300 and USA400) are more virulent than representative health care–associated strains (73). For example, MRSA252, a USA200 strain representative of the EMRSA-16 epidemic hospital clone, is essentially avirulent in a mouse model of sepsis, whereas USA300 and USA400 cause rapid death (73, 79). There is also considerable variance in virulence among CA-MRSA strains in mouse models of disease (79, 80, 82). For example, although USA300 and USA400 each cause fatal sepsis in a mouse bactere-

mia model, USA300 is substantially more virulent than USA400 in mouse and rat models of pneumonia (79, 82, 89). This observation is interesting because MW2 was notorious for causing fatal necrotizing pneumonia (15) and sepsis (40). Therefore, the mouse or rat lung infection models may not fully mimic human CA-MRSA necrotizing pneumonia, and/or the human disease may occur under specific preexisting conditions, such as antecedent infection with influenza.

The success of *S. aureus* as a human pathogen depends largely on its ability to circumvent destruction by neutrophils. Compared with representative health care–associated MRSA strains, CA-MRSA strains have substantially enhanced ability to evade killing by human neutrophils (73). This ultimately results in lysis of the neutrophils, which occurs more rapidly with CA-MRSA strains than with prominent health care–associated MRSA strains (73). Early studies by Rogers and colleagues demonstrated that *S. aureus* is readily phagocytosed by neutrophils and that a substantial number of these bacteria remain viable and either serve as a potential reservoir for infection or ultimately cause neutrophil lysis (90). Consistent with these early studies, most *S. aureus* strains we have tested cause eventual neutrophil lysis after phagocytosis, albeit with marked variance among strains (ref. 73 and our unpublished observations). The important point here is that the most prominent CA-MRSA strains excel at killing neutrophils after phagocytosis (Figure 3). Thus, there is little doubt that the most prominent CA-MRSA strains are more virulent than traditional health care–associated MRSA strains.

As a first step toward understanding this differential virulence, Voyich et al. used a microarray-based approach to elucidate changes in the transcriptomes of *S. aureus* strains COL, USA200, USA300, and USA400 during phagocytosis by human neutrophils (73). The studies revealed changes common among all *S. aureus* strains during phagocytosis and those specific to USA300 and USA400, including many genes encoding proteins of unknown or uncharacterized function (73). Although progress has been made, understanding the molecular basis for the enhanced virulence of USA300 remains an area of intense research.

Is CA-MRSA more virulent than CA-MSSA? Historically, community-associated *S. aureus* infections were almost always caused by MSSA. However, USA300 isolates are now the most abundant cause of community-associated *S. aureus* infections in many regions of the United States (17, 47). CA-MRSA and CA-MSSA each can cause disease in otherwise healthy individuals. The ability of CA-MRSA, and USA300 strains in particular, to displace CA-MSSA suggests no loss, and perhaps enhancement, of virulence or fitness. Indeed, one retrospective study found higher rates of hospitalization, higher rates of recurrent infection, and higher rates of infections in household contacts for patients with CA-MRSA compared with CA-MSSA infection (91). In other studies, clinical outcomes for CA-MRSA and CA-MSSA infections were similar (56, 92, 93). There is no a priori reason to expect that methicillin resistance, although an issue for treatment, would enhance virulence per se. Consistent with this idea was the demonstration that deletion of SCC*mec*IV in the USA300 epidemic clone had no impact on the ability of bacteria to colonize vital organs in a rabbit model of bacteremia (94). Infections caused by community-associated methicillin-resistant USA300 strains (CA-MRSA) or methicillin-susceptible variants of USA300 (CA-MSSA), which account for approximately 4.2%–31.0% of the CA-MSSA clinical isolates (17, 56, 95), have similar clinical outcomes. Thus, if CA-MRSA strains are more virulent than those that are CA-MSSA, factors other than antibiotic resistance must account for this difference.

Virulence determinants

S. aureus has an extensive repertoire of virulence factors, which includes both freely secreted and surface-bound factors (96). These molecules promote host colonization, alter leukocyte recruitment or function (81, 97), inhibit complement (97) and antimicrobial peptides (98–101), and cause destruction of leukocytes (81, 102–104). Also, the enhanced growth rate of prominent CA-MRSA strains could play a role in establishing infection or contribute to disease severity (24). Although many types of host cells and molecular processes are involved in *S. aureus*–host interaction, for the purposes of this Review we focus on those for which there is information in the context of CA-MRSA (19, 68, 70, 79, 81, 82, 85, 94, 105, 106).

One hundred years of PVL. The role of PVL in the emergence of CA-MRSA is hotly debated, and the relative contribution of PVL to severe invasive CA-MRSA syndromes remains to be determined. The ability of *S. aureus* to produce molecules cytolytic for leukocytes has been known for more than a century. In 1894, van de Velde identified such a substance, which he named "substance leucocide" or leukocidin (107). Panton and Valentine then discovered a leukocidin that was nonlethal for rabbits and distinct from the hemolytic activity associated with a lethal toxin (103). However, Panton and Valentine showed a correlation between presence of the leukocidin and severe skin infections, especially carbuncles (large abscesses), some of which were ultimately fatal in the absence of antibiotics (103). PVL has been studied extensively over the past 70 years. This leukocidin consists of two subunits, LukS-PV and LukF-PV (108), which are encoded by *lukS-PV* and *lukF-PV*, respectively, within specific bacteriophages (68, 70, 109). Thus, acquisition of PVL by *S. aureus* may occur by horizontal gene transfer. PVL assembles as a pore-forming octamer containing LukS-PV/LukF-PV heterodimers on the surface of susceptible host cells (110), although the plasma membrane binding site and/or receptor is unknown (111). PVL is highly specific for myeloid cells, especially granulocytes, and there are differences in susceptibility among granulocytes and macrophages from various animal species and humans (112). In general, PVL damages the plasma membrane of myeloid cells; this may ultimately cause cell lysis depending on the concentration of toxin. Sublytic concentrations of PVL trigger neutrophil apoptosis within 6 hours of treatment, whereas higher toxin concentrations cause cell lysis within 1 hour (113). Woodin and colleagues made the unexpected discovery that PVL induces immediate changes in neutrophils that mimic priming or activation, including degranulation (114). PVL causes release of granulocyte leukotriene B4 and IL-8 (115), prominent mediators of inflammation. In addition, sublytic concentrations of PVL prime neutrophils for enhanced release of ROS after stimulation with *N*-formylpeptide (fMLP) (116). Therefore, it is reasonable to speculate that PVL might contribute to pathogenesis by recruiting and activating neutrophils, thereby eliciting an exaggerated inflammatory response.

Notably, PVL is nonlethal even at high concentrations. Jeljaszewicz and colleagues found that although intravenous injection of partially or highly purified PVL in rabbits or mice caused transient granulocytopenia followed by marked granulocytosis, animal health remained unaffected (117, 118). This transient granulocytopenia probably was caused by redistribution of granulocytes from blood into tissues, rather than direct lysis in the bloodstream, which

would dramatically affect animal health. Subsequent work demonstrated that purified PVL causes dermonecrosis in rabbits (119), and these findings were confirmed by Cribier et al., who described the ability of the toxin to cause edema, erythema, and tissue necrosis (120). This effect was limited to administration of purified PVL, as there was no difference in skin lesions between animals infected with PVL-producing and -nonproducing bacteria (120). Even with these caveats, there is no question that PVL is an *S. aureus* virulence factor. The prevailing question is to what degree does PVL contribute to emergence of CA-MRSA and to its pathogenesis?

Early epidemiologic studies were at variance in correlating PVL-producing *S. aureus* strains and severe skin infections (120, 121). However, Lina and colleagues subsequently provided strong evidence that PVL is associated with severe furunculosis and severe necrotic hemorrhagic pneumonia (122). In 2002, Gillet et al. reported that PVL-producing *S. aureus* strains, mostly MSSA strains, were associated with fatal necrotizing pneumonia in otherwise healthy individuals (123). These reports coincided roughly with the emergence of CA-MRSA (14, 15, 19). CA-MRSA strains, including USA300 and USA400, contain the genes that encode PVL (19, 68, 70). Given that USA300 is a leading cause of skin and soft tissue infections and that PVL is associated with severe skin infections, PVL is often assumed to be the driving force behind the current CA-MRSA epidemic in the United States. However, PVL-encoding genes are found in less than 5% of all *S. aureus* clinical isolates (124–127), and studies of *S. aureus* nasal carriage isolates reveal a relatively low prevalence of those that are PVL-positive (0.6% in one study) (50, 128). If PVL were critically important for the success of *S. aureus* as a human pathogen, including the success of CA-MRSA, it should be found in higher abundance overall. Moreover, PVL has been reported in *S. aureus* since at least the early 1900s, yet CA-MRSA emerged only recently, suggesting that emergence of PVL is not driving the CA-MRSA epidemic. In support of this notion, comparative genome sequencing studies suggest that association of PVL with USA300 is not sufficient to account for the current epidemic (80), findings consistent with the coexistence of PVL-positive and PVL-negative USA400 strains in Canada (129). As mentioned above, two recent studies found no difference in clinical outcomes or mortality between patients with CA-MRSA and CA-MSSA skin and soft tissue or bloodstream infections (92, 93) (although an independent report found otherwise; ref. 91), and Kaplan and colleagues reported that CA-MSSA was associated with more invasive disease compared with CA-MRSA (37). These observations are important because PVL is infrequently associated with CA-MSSA strains other than USA300 (95).

To directly address the role of PVL in CA-MRSA pathogenesis, Voyich et al. compared USA300 and USA400 wild-type and isogenic PVL deletion (Δ*pvl*) strains in mouse models of abscess and sepsis (79). PVL did not contribute to disease in these models, and survival of wild-type and Δ*pvl* strains was equivalent following phagocytosis by human neutrophils (79). Moreover, PVL did not contribute to neutrophil lysis after phagocytosis, despite the observation that there is substantial upregulation of *lukS-PV* and *lukF-PV* transcripts under those conditions (79). USA300 and USA400 encode several two-component toxins that have up to 82% amino acid identity with PVL subunits and therefore possess redundant mechanisms to kill leukocytes.

Labandeira-Rey et al. studied laboratory *S. aureus* strains expressing PVL in a mouse model of pneumonia (130). An intriguing idea suggested by this study was that PVL functions as a regulator of global gene expression, promoting not only lung injury but also colonization and transmission (130). Unfortunately, this proved to be incorrect, as microarray- and proteomics-based studies demonstrated that PVL has no impact on either USA300 or USA400 gene or protein expression (131). Subsequently, Bubeck Wardenburg et al. evaluated USA300 and USA400 wild-type and isogenic Δ*pvl* strains in both C57BL/6J and BALB/c models of *S. aureus*–induced pneumonia (82, 105) and skin infection (105). In contrast to the observations of Labandeira-Rey et al., but consistent with the findings of Voyich et al., wild-type and isogenic CA-MRSA Δ*pvl* strains were equally virulent in these mouse models (82, 105). Recent follow-up studies by Bowden and colleagues used the same USA300 wild-type and Δ*pvl* mutant strains in similar mouse infection models and reported that the wild-type strain was more virulent than the mutant (132). The reason for the discrepancy between studies is unclear, but collectively the observations indicate that PVL is not a major virulence determinant in mouse models of CA-MRSA infection. Consistent with this notion, Daum and Montgomery found comparable virulence of USA300 wild-type and Δ*pvl* mutant strains in a rat pneumonia model (133). As a next step toward addressing the role of PVL in CA-MRSA virulence, Diep et al. used two rabbit bacteremia models to compare hematogenous dissemination of wild-type and Δ*pvl* CA-MRSA strains to major organs (131). PVL did not promote seeding of lungs, spleen, or blood by USA300; there was a modest and transient effect of PVL in the kidney (131).

Data therefore suggest that if PVL contributes to CA-MRSA pathogenesis in humans, its overall role is relatively minor. For the rare cases of severe CA-MRSA disease, such as necrotizing pneumonia, there is likely a host susceptibility component, which could involve PVL, but this topic is not discussed here due to space limitations.

ACME. ACME was discovered by whole genome sequencing of USA300. It is juxtaposed to SCC*mec*IV and can be mobilized by recombinases encoded within it (94). ACME contains genes encoding a complete arginine deiminase pathway; these genes are within an *arc* cluster and an oligopeptide permease operon (Opp-3) (70). Arginine deiminase is a virulence factor of *Streptococcus pyogenes* (134), and other chromosomally encoded oligopeptide permease operons appear to be important for *S. aureus* virulence (135); thus, ACME, as a newly described component of USA300, might contribute to fitness and epidemic spread. This hypothesis was tested in follow-up studies, which demonstrated that deletion of ACME but not SCC*mec* decreased the fitness of USA300 (94). Specifically, ACME promoted hematogenous dissemination to major organs in a rabbit bacteremia model of USA300 infection. Studies by Diep and colleagues also demonstrated a strong epidemiological link between ACME and the epidemic USA300 clone (94). Collectively, these findings indicate that ACME has contributed to the success (and perhaps to the emergence) of the epidemic USA300 clone.

α-Type phenol-soluble modulins. Wang et al. discovered a group of peptides in *S. aureus* that have similarity with the phenol-soluble modulins (PSMs) of *Staphylococcus epidermidis* (81). These PSMs were produced at much higher levels in vitro by prominent CA-MRSA strains, including USA300 and USA400 (81), than by health care–associated strains, leading to the hypothesis that PSMs of USA300 and USA400 might contribute to the enhanced virulence of CA-MRSA. Wild-type and isogenic PSM deletion strains of USA300 and USA400, when tested in mouse models of skin infection and sepsis, demonstrated that four of the *S. aureus* PSMs, which are named α-type PSMs (PSMα), contribute significantly to

USA300 and USA400 virulence (81). PSMα peptides were shown to recruit, activate, and ultimately lyse human neutrophils, thus promoting *S. aureus* pathogenesis. This study was the first to identify molecules encoded by CA-MRSA that account at least in part for the enhanced virulence of USA300 and USA400.

α-*Hemolysin.* α-hemolysin (Hla; also known as α-toxin), which probably is the most widely studied *S. aureus* toxin, is lethal in animal models of *S. aureus* bacteremia (136, 137). This pore-forming toxin destroys a wide range of host cells, including epithelial cells, erythrocytes, fibroblasts, and monocytes. Hla is not unique to CA-MRSA strains and for years has been known to play a major role in *S. aureus* pathogenesis (137). However, Bubeck Wardenburg et al. demonstrated recently that Hla was essential for the ability of USA300 and USA400 to cause pneumonia in a mouse model of infection (82). Moreover, the level of Hla produced by these strains in vitro correlates with severity of lung disease in mice and rats (82, 89, 138). It is likely that enhanced in vivo expression of selected virulence factors, many of which are regulated by the accessory gene regulator (Agr) system of *S. aureus*, is a critical component of the success of CA-MRSA, as proposed by Wang et al. (81) and Daum and colleagues (89).

Concluding remarks

Several questions must ultimately be addressed in order to make significant advances toward reducing disease caused by antibiotic-resistant strains of *S. aureus*, including those that cause CA-MRSA infections. For example, what is the driving force behind the recent emergence of CA-MRSA? Antibiotics, although highly effective, have been overused and misused for decades, inevitably contributing to the growing problem of resistance (13) (Figure 1). A better understanding of the basis of pathogen emergence is essential to developing alternative approaches for controlling the current CA-MRSA epidemic. New technologies, especially high-throughput whole genome sequencing, will be a major asset in our quest to fully understand, control, and prevent emergence and reemergence of drug-resistant *S. aureus*. Notably, the question of how new, more virulent strains emerge would be more readily addressed if we had available several hundred *S. aureus* genome sequences that span the current CA-MRSA epidemic.

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