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REVIEW

Methicillin-Resistant *Staphylococcus aureus*: Molecular Characterization, Evolution, and Epidemiology

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SUMMARY *Staphylococcus aureus*, a major human pathogen, has a collection of virulence factors and the ability to acquire resistance to most antibiotics. This ability is further augmented by constant emergence of new clones, making *S. aureus* a "superbug." Clinical use of methicillin has led to the appearance of methicillin-resistant *S. aureus* (MRSA). The past few decades have witnessed the existence of new MRSA clones. Unlike traditional MRSA residing in hospitals, the new clones can invade community settings and infect people without predisposing risk factors. This evolution continues with the buildup of the MRSA reservoir in companion and food animals. This review focuses on imparting a better understanding of MRSA evolution and its molecular characterization and epidemiology. We first describe the origin of MRSA, with emphasis on the diverse nature of staphylococcal cassette chromosome *mec* (SCC*mec*). *mecA* and its new homologues (*mecB*, *mecC*, and *mecD*), SCC*mec* types (13 SCC*mec* types have been discovered to date), and their classification criteria are discussed. The review then describes various typing methods applied to study the molecular epidemiology and evolutionary nature of MRSA. Starting with the historical methods and continuing to the advanced whole-genome approaches, typing of collections of MRSA has shed light on the origin, spread, and evolutionary pathways of MRSA clones.

KEYWORDS MRSA evolution, MRSA origin, MRSA typing, molecular epidemiology, SCC*mec*, *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), molecular characterization

INTRODUCTION

Staphylococcus aureus, a Gram-positive, coagulase-positive pathogen belonging to the family *Staphylococcaceae*, is a spherical bacterium of approximately 1 μm in diameter forming grape-like clusters. *S. aureus* is a commensal that is often present asymptomatically on parts of the human body such as skin, skin glands, and mucous membranes, including noses and guts of healthy individuals (1). Studies have shown that about 20% of individuals are persistent nasal carriers of *S. aureus* and around 30% are intermittent carriers, whereas about 50% are noncarriers (2). This colonization therefore significantly increases the chances of infections by providing a reservoir of the pathogen. The affected individuals in most cases are infected by the *S. aureus* strain that they usually carry as a commensal (3).

The history of knowledge of *S. aureus* dates back to 1880, when Alexander Ogston isolated *S. aureus* from a surgical wound infection. The isolated organism was able to produce abscesses when injected into guinea pigs and mice (4). Following this, Louis Pasteur injected pus from human staphylococcal infections, producing abscesses in animals. In 1882 Ogston coined the term *Staphylococcus* for the genus, and in 1884 Rosenbach divided the genus into the species *S. aureus* and *S. albus* (5, 6). These designations remained until 1939, when, based on coagulase testing, Cowan differentiated *S. epidermidis* as a separate species (7).

S. aureus is one of the first described pathogens. This is not surprising, however, as it was and still is one of the most common causes of infections in humans. It is of significant importance due to its ability to cause a plethora of infections as well as its capacity to adapt to diverse environmental conditions. *S. aureus* is one of the major causes of hospital and community-acquired infections, resulting in serious consequences. It can affect the bloodstream, skin and soft tissues, and lower respiratory tract and can cause infections related to medical instrumentation, such as central-line-associated bloodstream infection (CLABSI), as well as some serious deep-seated infections such as endocarditis and osteomyelitis (8–11). *S. aureus* is equipped with a repertoire of virulence factors and toxins, often making it responsible for many toxin-mediated diseases, including toxic shock syndrome, staphylococcal foodborne diseases (SFD), and scalded skin syndrome. These virulence factors and toxins also allow *S. aureus* to address challenges presented by the human immune system. Because it has such elaborate tools, one might think that humankind would be highly vulnerable to severe infections by *S. aureus*. Interestingly, however, *S. aureus* maintains a fine control of its virulence factors and for the most part rarely causes severe life-threatening infections in otherwise-healthy individuals.

Clinically, a major issue associated with *S. aureus* is the remarkable level of acquisition of resistance against multiple antibiotic classes, complicating treatment. Historically, *S. aureus* resistance emerged within 2 years of the introduction of penicillin (12). In 1942, the first penicillin-resistant *S. aureus* strain was detected (13). The semisynthetic antibiotic methicillin was then developed in the late 1950s, and methicillin-resistant *S. aureus* (MRSA) was clinically identified in 1960 (14). The outbreaks associated with resistance to different antibiotics in *S. aureus* occur in waves (15). Penicillin-resistant epidemic *S. aureus* strains were followed by the so-called "archaic" MRSA strains first found in the United Kingdom. This epidemic was initially largely restricted to Europe. However, beginning in the 1980s, novel lineages emerged, leading to a worldwide calamity that is still going on.

Infections due to methicillin-resistant strains of *S. aureus* are associated with higher mortality rates than infections caused by methicillin-susceptible strains. In addition, they result in increased lengths of hospital stays as well as associated health care costs (16–20). As discussed below, MRSA strains produce an altered penicillin-binding protein (PBP) associated with decreased affinity for most semisynthetic penicillins. The protein is encoded by an acquired gene, *mecA* (21–24). This methicillin-resistant genetic component is carried on a mobile genetic element (MGE) designated staphylococcal cassette chromosome *mec* (SCC*mec*) (25, 26). Hence, the emergence of methicillin-

resistant strains of staphylococci is due to the acquisition and insertion of these mobile genetic elements into the chromosomes of susceptible strains. This acquisition of antimicrobial resistance has presented a challenge to the medical world in terms of treatment and control of staphylococcal infections. MRSA in most cases accounts for at least 25 to 50% of *S. aureus* infections in hospital settings (8). They are of major concern due to the high morbidity and mortality as well as their resistance to all available penicillins and most of the other β-lactam drugs, except ceftaroline and ceftobiprole.

MRSA was once associated only with health care settings, including hospitals and other health care environments, as well as people associated with these environments. However, it have now also emerged as a major cause of community-associated infections and has created reservoirs in both settings. Hence, MRSA is no longer only a nosocomial pathogen. Community-acquired MRSA infections have been rising in frequency since they was first described in 1980s (27–29). This suggests that the epidemiology of MRSA has changed with the global emergence of community-associated MRSA (CA-MRSA) strains. These strains were connected principally with skin and soft tissue infections (SSTIs) but now cause health care-associated infections as well (30–33). CA-MRSA is genetically distinct from hospital-associated MRSA (HA-MRSA), being resistant to fewer non-β-lactam antibiotics, carrying a smaller version of SCC*mec*, and often producing a cytotoxin, Panton-Valentine leukocidin (PVL) (15, 34–46). As mentioned, CA-MRSA strains were traditionally limited to populations outside health care settings. At the time of their emergence, they were mostly responsible for causing only mild illnesses which were limited to uncomplicated skin and soft tissue infections. This, therefore, was once the basis to differentiate CA-MRSA from HA-MRSA. However, recently blurring of this definition has been observed, and the prevalence of CA-MRSA strains has increased. The epidemiological as well as molecular distinction between these two types of strains has become less well defined, as numerous reports of CA-MRSA invading health care settings have identified CA-MRSA as the etiological agent of nosocomial outbreaks (47–55).

Apart from humans, MRSA has also been known in animals for a long time (56). MRSA colonization and infection have been reported in a number of animals, from domesticated livestock to companion animals to captive or free-living wild terrestrial and/or aquatic species (57–61). The indiscriminate use of antimicrobial agents in animal husbandry and other agricultural activities has largely contributed to the wide distribution of MRSA among livestock. It has affected more than 40% of pigs, 20% of cattle, and 20 to 90% of turkey farms in Germany (62, 63). Numerous studies have indicated that humans in contact with livestock are also at high risk of becoming colonized and infected with livestock-associated MRSA (LA-MRSA). Studies have reported that around 23 to 32% of pig farmers are colonized with MRSA from swine farms in the Netherlands (64, 65). In North America, this rate has been found to be about 20% (66). These findings suggest that livestock and other animals may become a permanent reservoir for human MRSA infections.

Poor infection control measures as well as continued indiscriminate exposure of humans and animals to antibiotics have resulted in this huge problem of acquisition and dissemination of MRSA. This in turn limits the choice of treatment for MRSA infections. MRSA has been reported to have developed resistance to most antibiotics used for the treatment of its infections (67). However, compared to HA-MRSA, CA-MRSA is resistant to fewer antibiotics. Therefore, most patients infected with CA-MRSA can be treated using mostly aminoglycosides, erythromycin, clindamycin, fluoroquinolones, etc. However, patients who are seriously ill and hospitalized with HA-MRSA require antibiotics active against the infecting strain. Vancomycin has historically been the drug of choice and sometimes the last resort for the treatment of serious MRSA infections, providing empirical coverage and definitive therapy. However, its increased use has now become questionable as it is a less effective antistaphylococcal agent then penicillin. In addition, its increased use has already led to vancomycin-intermediate *S. aureus* (VISA) as well as vancomycin-resistant *S. aureus* (VRSA) in certain parts of the world (68–70).

The menace of MRSA colonization and infection has increased from hospitals to the community and further to animals. MRSA lineages and strains can be identified using various typing methods, such as pulse-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), SCC*mec* typing, and *spa* typing. The information hence obtained can be epidemiologically useful for tracing outbreaks, identifying the likely source of colonization (such as livestock or human associated), and distinguishing between community and hospital strains. Some isolates (such as CC398) may not be identifiable via certain methods (such as SmaI-PFGE) (71). Therefore, sometimes a combination of methods may be needed to identify some strains.

The prevalence and the epidemiology of MRSA are constantly changing, with novel MRSA clones appearing in different geographical regions. Therefore, continuous vigilance for MRSA through monitoring the characteristics, host specificity, and transmission routes of newer strains in each setting is required. Understating the molecular epidemiology of MRSA is therefore critical in assessing existing precautionary measures and planning appropriate prophylaxis. In this review, we first describe the origin of MRSA, with emphasis on the diverse nature of SCC*mec*. *mecA* and its new homologues (*mecB*, *mecC*, and *mecD*), SCC*mec* types (13 SCC*mec* types have been discovered to date), and their classification criteria are discussed. We then describe various typing methods applied to study the molecular epidemiology and evolutionary nature of MRSA, starting with the historical methods and continuing to the advanced whole-genome approaches. We then discuss the evolutionary nature of MRSA by highlighting the constant change of MRSA clones appearing in various geographical regions around the world, followed by a description of the epidemiology of the MRSA worldwide spread and prevalence, examining first the traditional HA-MRSA, then the global emergence of CA-MRSA, and finally LA-MRSA.

MRSA ORIGIN AND SCC*mec* ELEMENTS

History of MRSA

Alexander Flaming's research and report on the bactericidal effects of a fungal contaminant producing penicillin against *Staphylococcus aureus* led to the discovery and eventual mass production of penicillin (72, 73). This discovery consequently decreased the death toll due to bacterial pneumonia and meningitis during World War II. Penicillin is a bactericidal antibiotic compound, directly interfering with bacterial cell wall biosynthesis through peptidoglycan assembly and thereby promoting osmotic fragility in cell wall-deficient bacteria (74). Before the introduction of penicillin and the treatment of staphylococcal infections with this antibiotic in the early 1940s, the mortality rate from systemic *S. aureus* infections was nearly 80% (11, 75). However, the introduction of penicillin led to the appearance of resistant *S. aureus* strains (12). Only 2 years after the clinical introduction of penicillin, these strains became predominant: 80% of clinical isolates were resistant by 1945 (13, 75). They produced a plasmid-encoded β-lactamase capable of hydrolyzing the β-lactam ring of this antibiotic, thereby inactivating it (75, 76). This plasmid-encoded enzyme is readily transferable via transduction or conjugation, now causing nearly 90 to 95% of *S. aureus* strains worldwide to be resistant to penicillin (75, 77).

A penicillin-resistant *S. aureus* clone called phage type 80/81 was among the most remarkable clone responsible for causing epidemics during the 1950s. This clone rapidly emerged, becoming predominant in Australia, the United Kingdom, the United States, and Canada and causing severe skin infections, sepsis, and/or pneumonia (78–81). Initially the outbreaks were limited to hospital settings; however, gradually infections were also acquired by people outside hospitals (82). This pandemic lasted approximately 10 years, after which a decrease in the phage type 80/81 was observed and methicillin was introduced into the market (83).

Methicillin was put to clinical use in 1959 as the first semisynthetic β-lactamase-resistant penicillin. It was designed for the treatment of β-lactamase-producing staphylococci. Within a year of its introduction, methicillin-resistant *Staphylococcus aureus* (MRSA) emerged (14). These strains were first reported in 1961 in the United Kingdom

and acquired resistance via the incorporation of a *mecA* gene into the chromosome at a specific site (25, 26). *mecA* encodes an alternative penicillin-binding protein (PBP2a or PBP2′) that has low affinity for most semisynthetic penicillins, including methicillin, nafcillin, and oxacillin, as well as most cephem agents (21–23). Unlike penicillinase-mediated resistance, resistance conferred by *mecA* is broad spectrum, conferring resistance to the entire class of β-lactam drugs except for ceftaroline and ceftobiprole (15). *mecA* is extensively dispersed among many staphylococcal species and is carried on a mobile genetic element. This element is transferred among staphylococcal species, conferring broad-spectrum β-lactam resistance (84, 85). Outbreaks of MRSA were hence reported throughout the world during the 1980s and 1990s, including in the United Kingdom, Ireland, Australia, the Far East, and the United States (86–90). MRSA has now globally become a well-known risk of hospital admission in immunocompromised people and a huge problem faced by numerous infection control teams.

Although first identified in the United Kingdom, MRSA infections were rare until the 1980s after which MRSA increased predominantly as a nosocomial pathogen (91). Griffiths and coworkers analyzed the morbidity and mortality due to MRSA in England and Wales over the period of 10 years. Their report showed increases in both the number of deaths and the number of laboratory reports over the researched period. The mortality due to MRSA increased 15-fold and the rate of bacteremia increased 24-fold during the 10-year period from 1993 to 2002 (91). During 10 years after MRSA introduction, i.e., from 1960 to the early 1970s, MRSA infections in Europe were limited to hospital outbreaks caused mainly by *S. aureus* phage type 83A. This strain, also called the archaic clone, gradually became infrequent and was replaced by five prevalent clonal lineages by the 1980s (92–94).

The first Australian case of MRSA infection was reported in Sydney in 1965, after which reports of sporadic nosocomial MRSA infections were reported in Melbourne, Sydney, and other cities (95, 96). Western Australia stayed rather free from MRSA until the appearance of a gentamicin-susceptible non-multidrug (MDR)-resistant MRSA strain in a remote northern region in the late 1980s (97). However, it quickly spread to the rest of the Western Australia.

In the United States, the first hospital epidemic of MRSA was declared in 1968 in Boston, MA (98). It then gradually became rooted as an endemic pathogen in large, urban university hospitals in the United States, mainly their intensive care units (ICUs). The percentage of MRSA isolates from hospitalized patients in the United States rose from 2.4% to 29% from 1975 to 1991 (99). From early 1998 to mid-2003 the annual average percentage increased further to 51.6% for ICU patients and 42% for non-ICU inpatient isolates in the United States (100). Similarly, high rates of MRSA isolates have been observed in health care settings worldwide (101–109).

MRSA infections in Japan have been prevailing in academic hospitals since the late 1980s. Their spread in community hospitals was witnessed in the 1990s (110). The frequency of MRSA patients in Japan increased from 3.8% in 1990 to 9.6% in 1994. Of all patients colonized with MRSA, outpatients accounted for 4.5% in 1990 and 35% in 1994. The authors suggested that this increase in outpatient MRSA colonization could be due to the spread of MRSA into the community. Although there were reports of community-associated MRSA (110–112), the first clinical isolate know to carry the PVL gene in the CA-MRSA era was only observed in 2003 (113, 114).

In Finland, the annual number of MRSA cases has constantly increased since 1995. In addition, the notifications to National Infectious Disease Register (NIDR) have increased up to 10-fold, i.e., from 120 in 1997 to 1,458 in 2004 (115). In contrast, MRSA infections in Norway, Sweden, Denmark, and the Netherlands have remained occasional even in health care settings. This perhaps could be due to the strict surveillance programs in these nations (109, 116–118).

From 2000 to 2006 in Queensland, Eastern Australia, a population-based surveillance study of antibiotic resistance profiles of inpatient MRSA strains revealed an increase from 71 to 315 cases per million for non-MDR strains. These strains were resistant to at least one non-β lactam antibiotic and susceptible to ciprofloxacin (119). During the

same period, a parallel boost was seen in the outpatient department, from 52 to 490 cases per million. The study proposed a speedy propagation of non-MDR MRSA strains.

Very high prevalence rates of MRSA are also detected in East Asia. A multinational surveillance study was performed by Song et al. (120) in 2011 and determined the prevalence of MRSA in different Asian countries. They concluded that HA-MRSA accounted for 86.5% in Sri Lanka, 74.1% in Vietnam, 77.6% in South Korea, 65% in Taiwan, 57% in Thailand, and 56.8% in Hong Kong. However, the prevalence values were much lower in India and the Philippines, i.e., 22.6% and 38.1%, respectively (120).

Intercountry and intercontinental spread of MRSA has occurred via infected patients and staff. New strains of MRSA have continued to emerge and decline for decades for unknown reasons. Although global in its distribution, MRSA was once confined to only hospitals and health care settings. However, it has now invaded the community and emerged in livestock as well. Hence, the ever-increasing burden of MRSA infections has led to the increased use of vancomycin, the last resort for the treatment of MRSA infections, to which MRSA was susceptible. However, this increased use of vancomycin has now resulted in the emergence of vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) strains. These strains are inhibited only at concentrations above 4 to 8 μg/ml, and 16 μg/ml, respectively (68–70), causing a fear of them becoming completely resistant.

mecA, *mecC*, and Other Homologues

***mecA*.** Unlike resistance to penicillin, methicillin resistance in *S. aureus* in not mediated by plasmid-borne β-lactamases (121). In other bacterial pathogens, this kind of resistance was found to be associated with alterations in the amount or affinity of PBPs to β-lactams. Since the methicillin resistance in *S. aureus* was not mediated by plasmid-borne β-lactamase, attention was drawn toward the PBPs (122, 123). Utsui and Yokota (23) in 1985 confirmed the role of an altered PBP in conferring methicillin and cephem resistance in *S. aureus*. They referred to this new PBP as PBP2′. Subsequently, the gene responsible for conferring the methicillin resistance phenotype to *S. aureus* was found to be located on the chromosome, unlike the β-lactamase gene which was found on a plasmid. This region on the chromosome was traced as a "foreign DNA" present only in resistant strains and absent in susceptible ones (124, 125). Cloning and expression of this gene resulted in the heterologous expression of PBP2a (24, 126). Sequencing of the gene by Song et al. (127) later showed that it encodes a PBP, and the gene was later named *mecA*, with confirmation of its role in methicillin resistance coming from the work in the Tomasz laboratory. Tomasz and coworkers conducted transposon mutagenesis experiments to confirm the role of *mecA* in conferring the methicillin resistance phenotype (128–130).

Resistance bestowed by the *mecA* gene product is demonstrated via a reduced rate of β-lactam-mediated enzyme acylation and decreased affinity for β-lactams compared to that of native PBPs. The crystal structure of the *mecA* gene product (i.e., PBP2a) provided the structural basis for this resistance. PBP2a is an elongated protein with a transpeptidase domain, a transmembrane domain, and a non-penicillin-binding domain, which possesses an allosteric site (131). Compared to the active sites of native PBPs, the active site of PBP2a is less accessible to β-lactams, as it is located in a narrow extended cleft. Hence, it does not affect the synthesis of peptidoglycan, given the antibiotic strength reached *in vivo* (132).

Another structurally significant feature of PBP2a is that it is under allosteric control. Binding of the nascent peptidoglycan to the allosteric site, which is located in the non-penicillin-binding domain, initiates a conformational change opening the active site to assist substrate binding (131). This binding of peptidoglycan at the allosteric site, which opens the active site, appears to ensue only in the presence of nascent peptidoglycan substrate.

As mentioned above, the methicillin resistance determinant *mecA* is a part of a mobile genetic element termed SCC*mec*. The genetic determinant of methicillin resistance in MRSA is not a gene native to *S. aureus* but rather is an acquired gene from an

extraspecies source via an unknown mechanism. For decades, the evolutionary origin of *mecA* has remained largely a matter of speculation (127, 133–136). However, Wu et al. (137) in 2001 proposed *Staphylococcus sciuri*, an antibiotic-susceptible animal species, as the probable evolutionary precursor for the *mecA* gene to strains of MRSA. They found a close homologue of the methicillin resistance (*mecA*) gene of *S. aureus* in *S. sciuri*; however, this homologue did not confer any β-lactam resistance in *S. sciuri*. In an attempt to activate the so called silent *mecA* gene, they exposed the susceptible *S. sciuri* parental strain to stepwise-increasing concentrations of methicillin (137). This exposure eventually resulted in a point mutation in the -10 consensus sequence of the promoter, replacing a thymine residue with adenine at nucleotide position 1577. The result was a drastic increase in transcription of the protein that reacted with monoclonal antibodies prepared against the gene product of *mecA* from MRSA, i.e., PBP2a. Transduction of this mutated *mecA* into methicillin-susceptible *S. aureus* (MSSA) conferred resistance to the susceptible strains and led to the production of a protein that reacted with the monoclonal antibodies against PBP2a. These observations led to the conclusion that the *mecA* gene of *S. sciuri* is the probable evolutionary precursor of the *mecA* gene in pathogenic strains of MRSA (137). Fuda et al. (138) in 2007 studied the kinship between the two proteins, i.e., between the *mecA* gene product of *S. sciuri* and PBP2a of MRSA. They compared the biochemical characteristics of the two proteins and found them to be highly similar. These characteristics include not only sequence similarities but also the behavior in inhibition of β-lactam antibiotics, the secluded active site, the need for a conformational change for making the active site available, and the existence of an allosteric site for the binding of the peptidoglycan moiety (138). These observations strongly support the connection between the two proteins and the argument of the possible acquisition of the pathogenic *mecA* gene from the animal commensal species *S. sciuri*. More recent data, however, suggest that *Staphylococcus fleurettii* may be the evolutionary origin of *mecA* in MRSA (139). Tsubakishita and coworkers proposed that SCC*mec* was adopted from the *S. fleurettii mecA* gene and its surrounding chromosomal region. The exact origin and evolution of the *mecA* gene in MRSA, however, is still a matter under debate.

mecA is not unique to *S. aureus* but has been reported in other staphylococcal species of human and other origins. Apart from *S. aureus* and *S. sciuri*, *mecA* has also been reported in methicillin-resistant *S. pseudintermedius*, *S. intermedius*, *S. vitulinus*, *S. epidermidis*, *S. haemolyticus*, and *S. saprophyticus* (139–142). Some of these organisms are now becoming an increasing concern in human as well as veterinary medicine. So far, two *mecA* gene allotypes, named *mecA1* and *mecA2*, have been identified in various species of staphylococci. These allotypes and their naming are discussed later in this review.

***mecC*.** In May 2007, in southwest England, an epidemiological survey of bovine mastitis led to the isolation of *S. aureus* LGA251 from a bulk milk sample in a farm tank (143). It was the first report of *S. aureus* resistant to methicillin detected in a dairy herd in the United Kingdom. The isolate showed resistance to oxacillin and cefoxitin, but tests for the *mecA* gene and PBP2a were repeatedly negative. Later, the Wellcome Trust Sanger Institute revealed the genome sequence of *S. aureus* LGA251, showing the presence of a novel *mecA*-like gene with around 69% sequence identity to the original *mecA*. This gene was initially termed $mecA_{LGA251}$ and showed about 63% homology at the amino acid level to the original PBP2a, explaining the repeated negative tests for *mecA* by PCR and for PBP2a by slide agglutination. This *mecA* homologue, i.e., $mecA_{LGA251}$, was renamed *mecC* in 2012 by the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC) (144). Garcia-Alvarez et al. (143) further isolated and identified 65 isolates positive for *mecC* not only from dairy cattle but also from humans. This included an isolate obtained in 1975 from Danish human blood, suggesting that although this *mecC*-containing MRSA is recently identified, it has probably been causing infections for over 40 years.

Like *mecA*, *mecC* is also located in an SCC*mec* element at the 3′ region of *orfX*. Similar

to *mecA*, *mecC* is also found in other staphylococcal species, including *S. stepanovicii*, *S. xylosus*, and *S. sciuri* (145–147). To date, two *mecC* allotypes have been reported, one in *S. xylosus* isolated from bovine milk in France and the other in *S. saprophyticus* isolated from small wild mammals, including rodents and insectivores (146, 148). These allotypes, *mecC1* and *mecC2*, show approximately 93.5% and 92.9% nucleotide sequence similarity, respectively, to the original *mecC* in *S. aureus* LGA251 (146, 148). It is possible that *mecC* may have originated from coagulase-negative staphylococci as was suggested for *mecA*. However, this issue is still debated, and further investigations including the whole genome sequence of *mecC*-positive staphylococci may offer hints into the origin and evolution of this resistance determinant.

In 2012, Kim et al. (149) demonstrated for the first time the role of *mecC*-encoded PBP2a and its part in determining β-lactam resistance. Their work emphasized major disparities in the properties of the *mecA*- and *mecC*-encoded proteins. The gene was cloned into a plasmid and introduced into *S. aureus* COL-S. The recombinant PBP2a from *mecC* showed higher affinity for oxacillin than for cefoxitin. In contrast, PBP2a from *mecA* showed higher resistance to cefoxitin (MIC = 400 μg/ml) than to oxacillin (MIC = 200 μg/ml). Both proteins exhibited dissimilarities in their thermostability and optimum temperature. *mecC*-encoded PBP2a seemed less stable at 37°C than *mecA*-encoded PBP2a. An interesting discovery was that even though $PBP2a_{mecC}$ does not possess transglycosylase activity, it does not require the presence of native PBP2 to provide high-level oxacillin resistance as does $PBP2a_{mecA}$. This could be due to the collaboration between monofunctional glycotransferases and $PBP2a_{mecC}$. Although the study confirmed the role of *mecC*-encoded PBP2a in methicillin resistance and also its role as a transpeptidase, there are significant variations in the performances of both proteins (149).

Although *mecA*- and *mecC*-encoded proteins possess different biochemical properties, *mecC* nevertheless confers methicillin resistance. Hence, the need for the correct identification of these strains as MRSA is particularly important from a clinical diagnostic perspective. Laboratories using antimicrobial susceptibility testing will likely correctly identify these strains as MRSA; however, a problem exists where molecular methods are used for the identification and confirmation of MRSA. Laboratories need to incorporate universal *mec* gene primers for PCR detection or add *mecC*-specific primers to differentiate between *mecA* and *mecC* MRSA. This would also be beneficial for surveillance studies of *mecC* MRSA and for further isolation of strains for characterization. Several PCR-based methods and commercial PCR assays are now available to detect and differentiate *mecC* MRSA (150–152). However, commercial slide agglutination assays using *mecA*-encoded PBP2a will misidentify *mecC* MRSA as MSSA. Hence, *mecC* MRSA possesses a dodge from the diagnostic point of view. Diagnostic laboratories therefore need to validate their testing methods to identify *mecC* MRSA correctly as MRSA and must modify these tests to detect *mecC* MRSA by using *mecC*-encoded PBP2a.

***mecB*, *mecD*, and other homologues.** A third group of *mec* gene homologues has been reported to occur both chromosomally and on a plasmid of *Macrococcus caseolyticus*, a close genetic relative of *Staphylococcus* classified as *Staphylococcus caseolyticus* until 1998, when it was reclassified as a member of a separate genus, *Macrococcus* (153, 154). Baba et al. (153) in 2009 determined the genome sequence of methicillin-resistant *M. caseolyticus* strain JCSC5402, isolated form the skin of a chicken from Japan. They identified a *mecA* gene homologue carried by these strains. The homologue showed 62% nucleotide sequence similarity to the original *mecA* from *S. aureus* N315 and was later named *mecB* by the IWG-SCC in 2012 (144).

Becker et al. (155), however, in 2018 discovered a plasmid-carried *mecB* gene during routine MRSA screening in an *S. aureus* isolate which tested negative for *mecA* and *mecC* but was found to be resistant to methicillin. The isolate was recovered from a nasal-throat swab of a 67-year-old cardiology patient with no signs of infection. Comparative analysis of *mecB* DNA from *S. aureus* revealed 100% sequence identity with the reported *mecB* gene of *M. caseolyticus*, and therefore it belonged to the same allotype (155). The *mecB* homologue from *S. aureus* shows 60% nucleotide sequence

similarity to the original first-identified *mecA* gene from *S. aureus* N315. Similar to *mecA* and *mecC*, *mecB* in *S. aureus* also confers methicillin resistance and therefore strains carrying it need to be correctly identified as MRSA and not MSSA. Laboratories using antibiotic susceptibility testing can correctly identify *mecB* MRSA as MRSA and not MSSA. However, in the case of PCR, *mecB*-specific primers need to be incorporated to correctly identify these strains as MRSA.

In March 2017, Schwendener et al. (156) reported yet another *mecA* homologue in an *M. caseolyticus* bovine and canine strain and named it *mecD*. The *mecD* gene was found to confer resistance to all classes of β-lactam antibiotics, including anti-MRSA cephalosporins, ceftobiprole, and ceftaroline. The *mecD* gene was found to be located on genomic resistance islands called $McRI_{mecD}$-1 and $McRI_{mecD}$-2. The islands are associated with a putative virulence gene and a site-specific integrase, suggesting potential for propagation. *mecD* shows around 69% sequence identity to *mecB* at the nucleotide level and about 63% at the amino acid level. However, with *mecA* of *S. aureus* strain N315, it shows about 61% and 51% sequence similarity at the nucleotide and amino acid levels, respectively (156).

Classification and naming system for *mecA* gene homologues. In 2012, the IWG-SCC proposed a classification and naming system for *mecA* gene homologues. The schema was based on the similarity of their nucleotide sequences and the dates of publication, i.e., the chronological order of their discovery (144), and was designed to facilitate determining the phylogenetic relationships between different *mec* genes identified in various species of bacteria. According to this system, a *mec* gene would be defined as a genetic determinant encoding a PBP similar to PBP2a or PBP2′, and the product should be composed of three domains, including a characteristic N-terminal domain, a transpeptidase domain, and a non-penicillin binding domain (144).

Various divergent *mec* genes have been identified to date. These include the original *mecA* gene identified in *S. aureus* N315 (24, 157, 158). *mecA* has been identified in various other species of staphylococci, including *S. aureus*, *S. epidermidis*, *S. haemolyticus*, *S. saprophyticus*, and *S. fleurettii* (139–142). The *mecA* genes from the above-mentioned organisms show greater than 98% sequence similarity to that of the original (first identified and fully sequenced) MRSA prototype strain N315 (159). The first divergent *mecA* homologue was discovered in *S. sciuri* and showed about 80% nucleotide similarity to the original *mecA* from *S. aureus* strain N315 (160–162). The second divergent homologue was identified in *S. vitulinus* and showed about 90% sequence similarity to the original *mecA* (163).

The third group of *mecA* genes was found on the genome and/or plasmid of *M. caseolyticus* and showed about 61.6% nucleotide identity to the original *mecA* (153). The fourth homologue was identified in *S. aureus* strain LGA251 isolated from a bulk tank milk sample in 2007. This homologue showed about 69% similarity to the *mecA* from *S. aureus* N315 (143, 164). The next one in the sequence was reported in *S. xylosus* isolated from bovine milk (146). This homologue showed about 69.9% sequence similarity to the original *mecA*. Following in line is the *mecA* gene reported in *S. saprophyticus* isolated from small rats and rodents (148). The last homologue was most recently identified and was reported in March 2017 from an *M. caseolyticus* strain isolated from bovine and canine sources; it shows about 61% nucleotide sequence identity to the original *mecA* gene (156).

According to the proposed system for naming and classification, *mec* genes sharing more than or equal to 70% nucleotide sequence similarity would be placed in a single prototype. These types would be designated *mecA*, *mecB*, *mecC*, and so on, reflecting their chronological order of discovery. *mecA* of *S. aureus* N315, *mecB* of *M. caseolyticus*, *mecC* of *S. aureus* LGA251 and *mecD* of *M. caseolyticus* IMD0819 are suggested to be used as prototype *mec* genes in the definition of new types. Hence, the *mec* nomenclature system is not limited to the genus *Staphylococcus*, as these genes are located on mobile genetic elements and are likely to be found outside the confined boundaries of specific species or genera (144).

The *mec* gene types are further divided into allotypes. *mec* genes of one single type

are divided into allotypes based on the percentage of their sequence identity. If *mec* genes of the same type share ≥70% identity but <95% identity with the prototype *mec* gene, then they would be regarded as a different allotype. For example, the allotypes of *mecA* would be referred to as *mecA1*, *mecA2*, so on, again based on their chronological order of discovery. The same would be true for *mecB*, *mecC*, and so on (144). Figure 1 shows the detail of this classification.

According to this new nomenclature system, the *mecA* gene homologues described before 2012 were renamed by the IWG-SCC (144). As a consequence, *mecA* homologues which were formerly called *mecAm* and $mecA_{LGA251}$ in *M. caseolyticus* and *S. aureus* LGA251, respectively, were renamed *mecB* and *mecC* according to the order of their discovery. *mecA* genes having nucleotide sequence similarity of greater than or equal to 95% were regarded as *mecA*, indicating that they were the members of the same allotype. *mecA* genes that had <95% but ≥70% sequence similarity to the original *mecA* of *S. aureus* N315 were placed in different allotypes. Hence, the *mecA* of *S. sciuri* was renamed *mecA1*, as it had 80% sequence identity to the original *mecA*. Likewise, *mecA* of *S. vitulinus* was renamed *mecA2* based on 90% identity to the original *mecA*. Similarly, based on the same rule, there are two allotypes discovered so far for the *mecC* gene, and they are referred to as *mecC1* and *mecC2*. They show about 93.5% and 92.9% nucleotide sequence similarity, respectively, to the prototype *mecC* gene of *S. aureus* LGA215 (146, 148). Recently, Becker and coworkers (155) identified the *mecB* gene in *S. aureus*, showing 100% nucleotide sequence similarity to original *mecB* from *M. caseolyticus* and 60% to the original *mecA* (155).

More recently, Schwendener et al. (156) reported a novel methicillin resistance gene. Schwendener, et al. (156) named this gene *mecD*, based on the same guidelines as given by the IWG-SCC (144) The gene was discovered in *M. caseolyticus* IMD0819 and showed approximately 61% sequence identity to the original *mecA* and less than 70% to any of the prototype *mec* genes; hence, it was assigned the name *mecD*, signifying a complete new prototype.

Staphylococcal Cassette Chromosome *mec*

The mobile genetic element SCC*mec* and its structural organization. The realization that *mecA* was widely disseminated among staphylococcal species led to the hypothesis that it could be carried on a mobile element having the capacity to easily transfer from organism to organism. It was discovered that the emergence of methicillin-resistant staphylococcal lineages was due to the acquisition and insertion of the staphylococcal cassette chromosome *mec* (SCC*mec*) element into the chromosome of susceptible strains. This mobile 21- to 60-kb genetic element is the defining feature of MRSA strains and is responsible for conferring the broad-spectrum β-lactam resistance (25). SCC*mec* may also contain other genetic structures, such as Tn*554*, pT181, and pUB110, which are responsible for conferring resistance to other non-β-lactam drugs (165). The high diversity in the structural organization and the genetic content of these elements has resulted in their classification into types and subtypes. However, these elements share several common structural characteristics. To summarize, there are three basic structural/genetic elements in SCC*mec*: the *mec* gene complex, containing the *mec* gene (*mecA*, *mecB*, *mecC*, and/or *mecD*) and its regulatory elements that control its expression (*mecR1*, encoding a signal transducer protein, and *mecI*, encoding a repressor protein); the *ccr* gene complex, encoding the site-specific recombinases, i.e., cassette chromosome recombinase (*ccr*) genes (*ccrAB* and/or *ccrC*); and the joining regions (J regions).

(i) *mec* gene complex. The *mec* gene complex is composed of the *mec* gene, its regulatory components (including *mecR1* and *mecI*), and the associated insertion sequences (IS). Based on differences in insertion sequences and regulatory elements upstream and downstream of the *mec* gene, the *mec* gene complex has been classified into 5 different classes (164, 166, 167), classes A to E. Figure 2 is a diagrammatic illustration of the various classes (as well as the variants within those classes) of the *mec* gene complex. Class A is a prototype complex containing *mecA*, the complete *mecR1*,

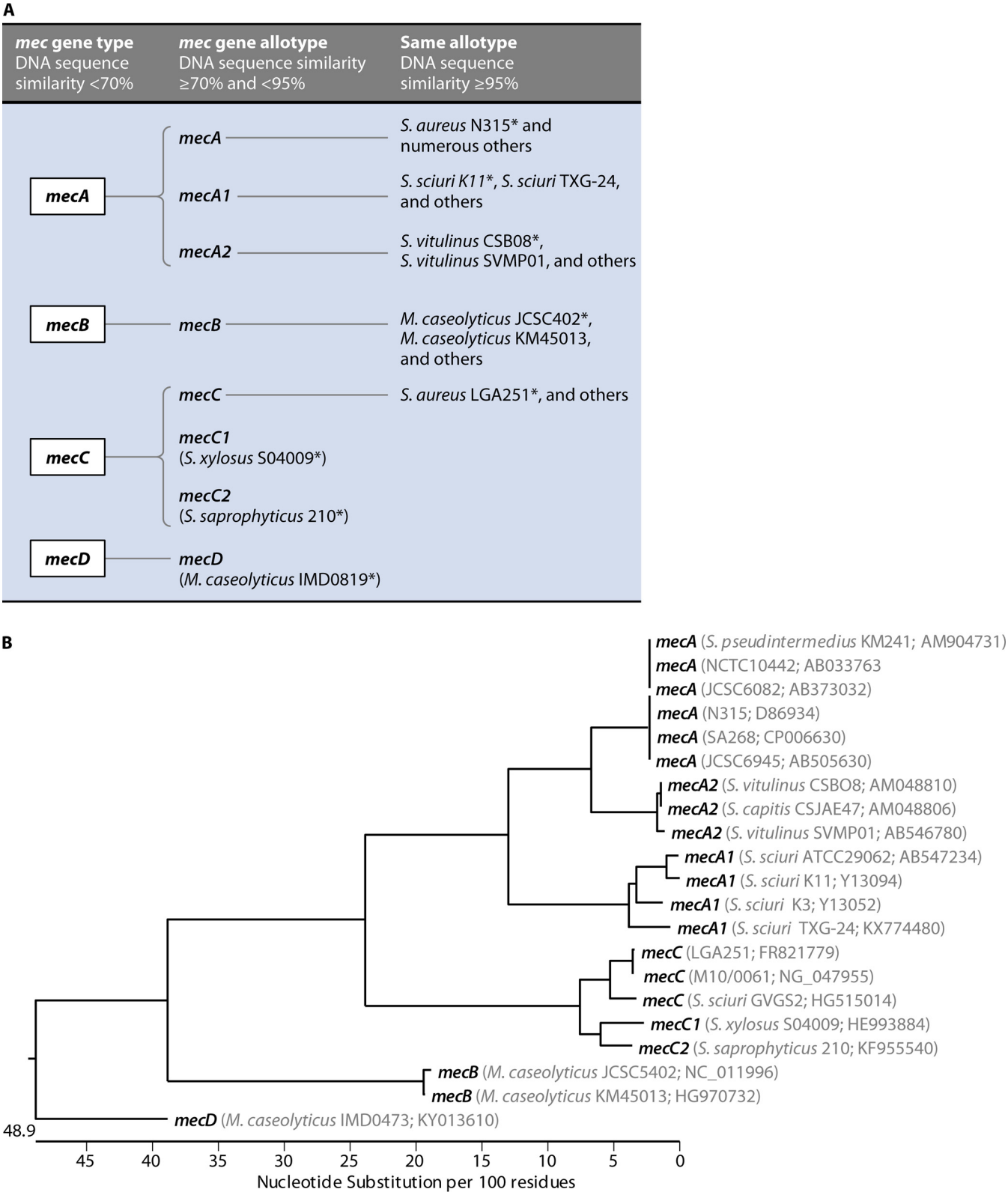

FIG 1 *mec* gene homologue classification and phylogeny. (A) Criteria. *mec* gene homologues are classified based on nucleotide sequence similarity to the prototype *mecA* gene identified in MRSA N315. Strains showing <70% similarity are classified as new *mec* gene types, with 4 (*mecA*, *mecB*, *mecC*, and *mecD*) currently described. The types are further subdivided into allotypes based on ≥70% to <95% sequence homology, while genes sharing ≥95% homology belong to the same allotype (144). Prototypic (asterisks) and representative strains carrying each *mec* gene allotype are indicated. (B) Phylogenetic tree showing the relationships between *mec* gene homologues identified to date. The *mecA*, *mecB*, *mecC*, and *mecD* genes cluster separately and distinct from each other, and within each group the different allotypes can be distinguished. Strain names and accession numbers for organisms in which each gene is present are indicated in parentheses. Species are indicated if they are other than *S. aureus*.

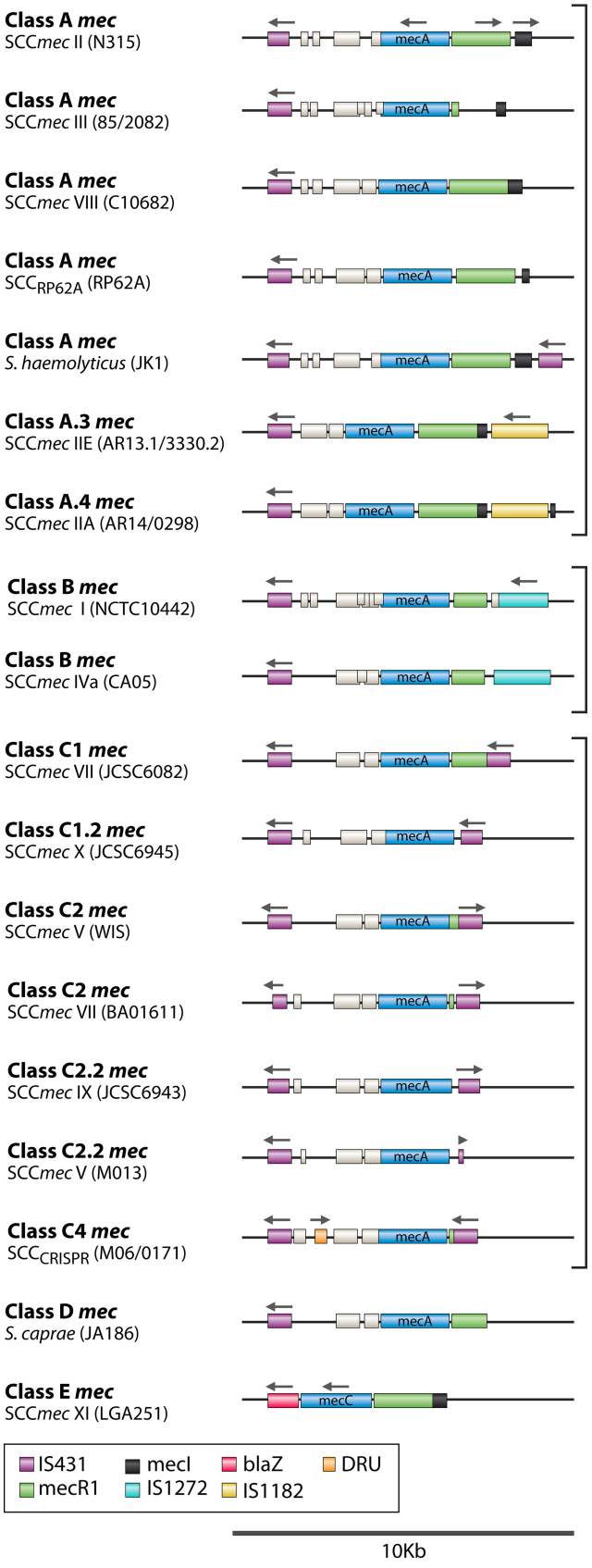

FIG 2 *mec* gene complex variants. Five classes (A to E) of the *mec* gene complex have been reported. The class A complex shows a typical IS*431-mecA-mecR1-mecI* structure. Class B shows the typical IS*431-mecA-*

(Continued on next page)

and *mecI* upstream of *mecA*. Downstream of *mecA* it includes the hypervariable region (HVR) and the insertion sequence IS*431*. The class B *mec* gene complex consist of IS*1272*, *mecA*, truncated *mecR1* (Δ*mecR1*), HVR, and IS*431*. Similarly, the class C *mec* gene complex is composed of IS*431*, *mecA*, Δ*mecR1*, HVR, and IS*431*. There are two distinct versions of the class C *mec* gene complex, termed class C1 and class C2. This distinction is based upon the orientation of IS*431*. In class C1, IS*431* upstream and downstream of *mecA* occurs in same orientation, whereas in class C2 the orientations of IS*431* are reversed and hence are in the opposite direction. The class D *mec* gene complex carries IS*431*, *mecA*, and truncated *mecR1* but no IS downstream of Δ*mecR1*. Finally, the class E *mec* gene complex is composed of *blaZ*, *mecC*, *mecR1*, and *mecI*. Several variants within the major classes of *mec* gene complexes have been described, depending on the truncation of various lengths of *mec* regulatory genes and/or the presence of different insertion sequences or transposons. These variants are specified by a numeral string following the class.

(ii) *ccr* gene complex. *ccr* (cassette chromosome recombinase) genes, as well as the surrounding open reading frames (ORFs), constitute the *ccr* gene complex. Several of these ORFs have unknown functions. Accurate integration and/or excision of SCC*mec* into the chromosome of an *Staphylococcus* strain is catalyzed via *ccrAB* and/or *ccrC*. These recombinases are similar to bacteriophage integrases and are responsible for catalyzing the cleavage of DNA, as well as the exchange of strands and recombination of two attachment sites (168, 169). One of these attachment sites exist on an SCC element, i.e., *attSCC*, and the other attachment site is found on the bacterial chromosome (*attB*). This permits the different *Staphylococcus* strains to trade genetic information among themselves in order to adapt to changing environmental conditions and the pressure of antibiotic selection. The inverted repeats present on either side of the SCC*mec* also play a role in the excision of the element but not integration. The rate and efficacy of the insertion of these elements are determined by these 100- to 200-bp sequences present upstream and downstream of *attB*. The direct repeats (DR) likely also explain why the acquisition of SCC*mec* by *S. aureus* is limited and does not occur in all lineages (170).

Figure 3 is a diagrammatic illustration of the classification of *ccr* gene homologues as well as various species in which these homologues have been identified and their phylogenetic relationships to each other. Three phylogenetically distinct *ccr* genes with DNA sequence similarity of less than 50% have been identified in *S. aureus*: *ccrA*, *ccrB*, and *ccrC* (166). According to the proposed nomenclature system, novel *ccr* genes are defined based on DNA sequence similarities of less than 50%. If their DNA sequences show 50 to 85% identity, they are termed novel allotypes of the *ccr* gene. However, if the *ccr* genes show more than 85% identity at the level of DNA, they are assigned the same allotype. Several allotypes of both *ccrA* and *ccrB* (*ccrA1* to *-7* and *ccrB1* to *-7*) have been identified based on their nucleotide similarity of between 60% and 82%. Until recently, all *ccrC* variants identified showed more than 87% identity and were placed in the same allotype; however, in 2015 Wu et al. (171) reported a novel *ccrC* gene sharing 62.6 to 69.4% similarity to all published *ccrC* sequences. Based on the proposed nomenclature system, it was named *ccrC2* (166, 171).

Different combinations of *ccr* allotypes have given rise to 9 different *ccr* gene

FIG 2 Legend (Continued)
truncated *mecR1*-IS*1272* structure. Class C is defined by its IS*431-mecA*-truncated *mecR1*-IS431 structure, with class C1 having the two IS*431* elements coding in the same direction and C2 having them coding in opposite directions. The class D complex contains IS*431-mecA*-truncated *mecR1* but no downstream IS element. The class E *mec* gene complex is composed of *blaZ-mecC-mecR1-mecI*. Variations within a *mec* gene complex type are seen as a result of differences in the lengths of the regulatory genes, as well as the presence or absence of insertion sequences (IS) and transposons. IS*431* is represented by purple, *mecI* by black, *mecA* or *mecC* by dark blue, *blaZ* by pink, direct-repeat units (DRU) by orange, *mecR1* by green, IS*1272* by light blue, and IS*1182* by yellow. Genes in the hypervariable region (HVR) are represented by white boxes. The included complexes and variants are numbered based on published data rather than being sequential representatives.

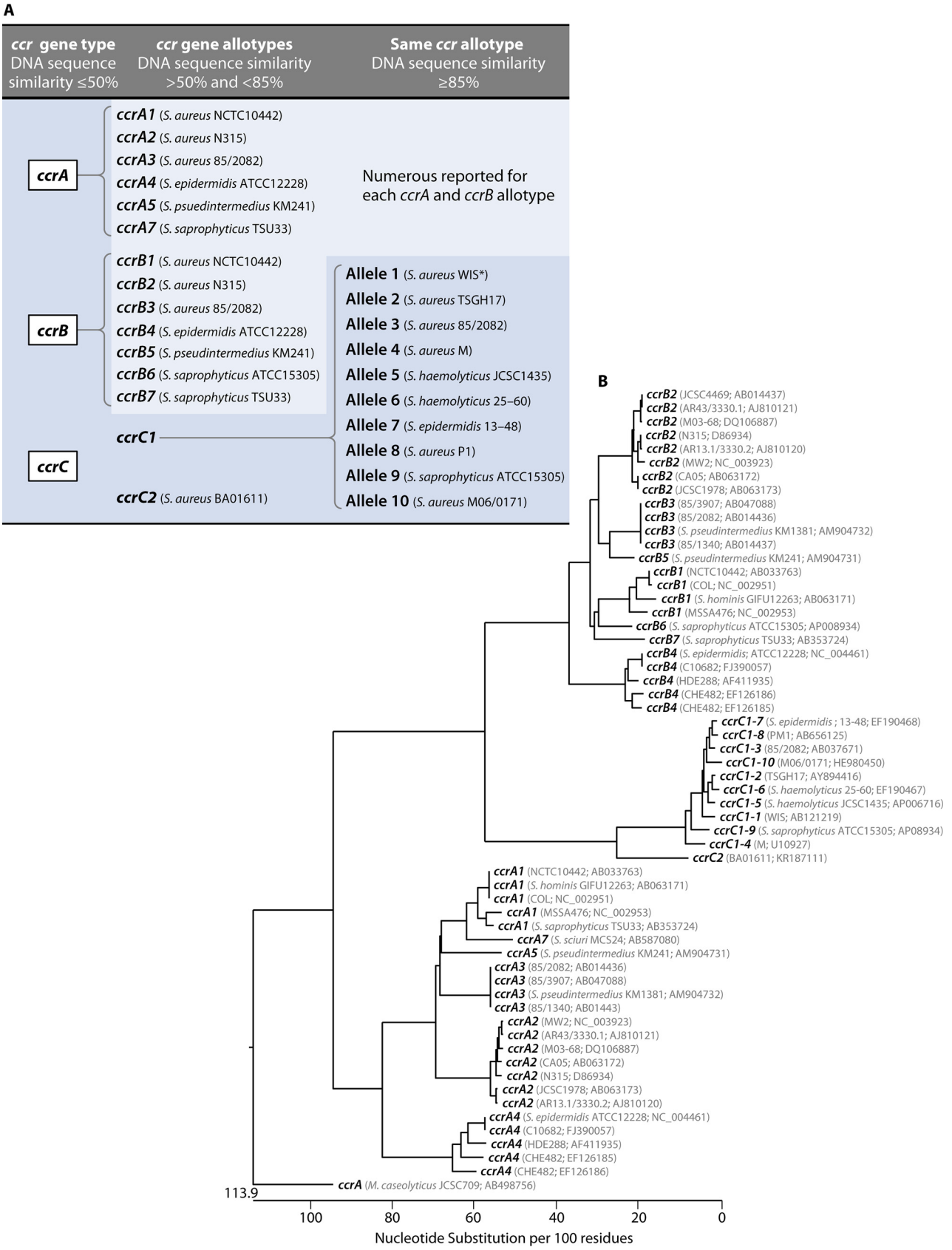

FIG 3 *ccr* gene homologue classification. (A) Novel *ccr* genes are defined based on DNA sequence similarity of ≤50%, and to date 3 types have been described (*ccrA*, *ccrB*, and *ccrC*). The types are further subdivided into allotypes based on a nucleotide sequence similarity between 50% and 85%, while

(Continued on next page)

complex types: 1 (A1B1), 2 (A2B2), 3 (A3B3), 4 (A4B4), 5 (C), 6 (A5B3), 7 (A1B6), 8 (A1B3), and 9 (C2) (171). The type 6 *ccr* gene complex is found exclusively in non-*S. aureus* staphylococci, whereas types 7 and 8 are found only in MRSA (166, 172–175). Two novel *ccr* gene complex combinations have been identified in non-*S. aureus* staphylococci, i.e., *ccrA1B4* in *S. saprophyticus* and *ccrA7B3* in *S. sciuri*, but these have not yet been designated any *ccr* type number (175, 176).

(iii) J regions. Regions other than the *ccr* gene complex and *mec* gene complex are regarded as joining (J) regions. These cassette components are nonessential and may contain determinants for additional antimicrobial resistance (166, 177). Based on their location within the SCC*mec* element, they are classified as J1, J2, and J3. The J1 region (formerly called the L-C region) is the region located between the right chromosomal junction and the *ccr* gene, upstream of the *ccr* gene. The J2 region (formerly called the C-M region) is the region between the *ccr* gene complex and the *mec* gene complex. Similarly, the J3 region (previously known as the I-R region) is located between the *mec* gene complex and the left chromosomal junction, i.e., downstream of the *mec* gene complex. The J1 region often includes several ORFs and regulator genes, whereas J2 contains genetic elements such as the integrase gene or transposon Tn*554*. J3 often includes plasmid-encoded antibiotic resistance, such as for tetracycline, aminoglycosides, etc. Variation in the J-region DNA segment is the basis for defining SCC*mec* subtypes.

Classification and naming regulations for SCC*mec* elements. Due to the highly diverse nature of SCC*mec* elements, variation exists in the structural organization and genetic content of these elements. These variations result in the classification of these elements into types and subtypes. Previously many types, subtypes, and variants of SCC*mec* were reported in the literature without following any standardized rule for nomenclature agreed upon at the international level. Consequently, a committee was set up to rule out these ambiguities and inconsistencies in the published literature. The committee was organized to form an intellectual network for contributions to the study of these elements and to create a unified nomenclature scheme. In addition, the group was responsible for outlining minimum prerequisites for the identification of new SCC elements as well as establishing guidelines for the recognition of these elements for epidemiological studies (166).

As discussed above, SCC*mec* elements share several common characteristics, including the presence of a *mec* gene in the *mec* gene complex, the presence of one or more *ccr* genes in a *ccr* gene complex, integration site sequences (ISS) for SCC on the staphylococcal chromosome acting as a target for recombination mediated by *ccr*, and the presence of direct-repeat sequences flanking ISS. Based on the location of the regulatory genes upstream or downstream of *mec* gene and/or disparities in the insertion sequences, the *mec* gene complex has been categorized into various classes. In addition, various combinations of *ccr* gene allotypes have given rise to different types of *ccr* gene complex. It is the combination of these classes of the *mec* gene complex and the type of *ccr* gene complex that results in the classification of SCC*mec* elements into types. These elements are further divided into subtypes centered on the variations in the J regions within the same combination of *mec-ccr* complex (166). In addition, a number of variants in these major classes of *mec* gene complex exist. These variants are based on, for example, integration of insertion sequences and/or transposon. They are specified by a numeral string following the class (e.g., class B_2) (166). Moreover, there are SCC elements in staphylococci that do not harbor *mecA* but carry genes for resistance to metals and/or different antibiotics. In some cases, two or more amalgam-

FIG 3 Legend (Continued)
sequences with >85% similarity would be classified as the same allotype (166). *ccrA* and *ccrB* each have 7 allotypes (1 to 7), with numerous reported, while *ccrC* has 2 allotypes reported (C1 and C2). The *ccrC1* allotype is further subdivided into 10 alleles (1 to 10). Prototypic strains for each allotype, along with their corresponding accession numbers, are indicated. An asterisk indicates the prototypic strain for the *ccrC1* allotype. (B) *ccr* gene homologues identified to date and their phylogenetic relationships to each other. Strain names and accession numbers for organisms in which each gene is present are indicated in parentheses. Species are indicated if they are other than *S. aureus*.

ated SCC elements have also been observed. These elements and their naming regulations are discussed later in this review.

SCC*mec* types. To date there are a total of 13 types of SCC*mec* identified in MRSA strains. Figure 4 illustrates the variant structure of all 13 SCC*mec* types from each of the prototypic MRSA strains.

(i) SCC*mec* type I. SCC*mec* type I was first described in 2001 in MRSA strain NCTC10442, isolated in the United Kingdom in 1961 (178). This SCC*mec* type was also identified in MRSA strains from Malaysia, South Africa, and Italy collected during the 1980s and has subsequently been found worldwide, predominantly in HA-MRSA strains (179). It carries a characteristic class B *mec* gene complex (*IS1272-ΔmecR1-mecA-IS431*) with a type 1 *ccr* gene complex (*ccrA1* and *ccrB1*) and a *pls* regulator in the J1 region of the element. Plasmid pUB110 is present in the J3 region of subtype IA along with a partial deletion of the hypervariable region (180). SCC*mec* type I in strain NCTC10442 is 34,359 nucleotides long and contains 4 repeat regions, 2 mobile elements, and 41 coding DNA sequences (CDS), of which 36 CDS apparently have no functionally clear ORFs. The whole element is structurally divided into the upstream region of the *ccr* gene complex containing one repeat region and 17 CDS, the *ccr* gene complex having *ccr* genes and 7 CDS, the *mec* gene complex with class B in addition to 10 CDS, and the downstream region of the *mec* gene complex having one repeat region and 3 CDS. The type I element also has a characteristic inverted-repeat sequence that is 22 bp long at both extremities of the element.

(ii) SCC*mec* type II. SCC*mec* type II was first described in 1999 in the characteristic MRSA strain N315 and is found predominantly in HA-MRSA strains. SCC*mec* type II carries a class A *mec* gene complex, a type 2 *ccr* gene complex, an integrated copy of staphylococcal plasmid pUB110 in the J3 region, and a *kdp* regulator in the J1 region (25, 178, 181). SCC*mec* type II in N315 is 53,017 nucleotides long and contains 4 repeat regions, 3 mobile elements, and 51 CDS, of which 33 CDS apparently have no functionally clear ORFs. The whole element is structurally divided into the upstream region of the *ccr* gene complex containing one repeat region and 15 CDS, the *ccr* gene complex having *ccr* genes and 6 CDS, the region between the *ccr* gene complex and the *mec* gene complex with one mobile element and 12 CDS, the *mec* gene complex with class A in addition to 5 CDS, and lastly the downstream region of the *mec* gene complex having 1 mobile element, 2 repeat regions, and 8 CDS. The type II element also has degenerative inverted repeats at the left and right termini of the element.

(iii) SCC*mec* type III. SCC*mec* type III was first discovered in Zelanian isolate 85/2082 in 2001 and, as with types I and II, is found predominantly in HA-MRSA strains. SCC*mec* III carries a class A *mec* gene complex, a type 3 *ccr* gene complex, and, in the J3 region, an integrated copy of plasmid pT181 encoding tetracycline and mercury resistance (178). SCC*mec* type III from 85/2082 is 66,896 nucleotides long and contains 10 repeat regions, 6 mobile elements, and 97 CDS, of which 22 CDS apparently have no functionally clear ORFs. The whole element is structurally divided into the upstream region of the *ccr* gene complex containing one repeat region and 2 CDS, the *ccr* gene complex having *ccr* genes and 11 CDS, the region between the *ccr* gene complex and the *mec* gene complex with one mobile element and 18 CDS, the *mec* gene complex with class A in addition to 9 CDS, 1 mobile element, and 2 repeat regions, and lastly the downstream region of the *mec* gene complex having 4 mobile elements, 7 repeat regions, and 52 CDS. Type III elements also carry the characteristic inverted repeats at both edges. Unlike type I and II, type III bears several antibiotic resistance genes in its J region. These include a transposon encoding cadmium resistance determinants (ΨTn*554*) in the J2 region. A copy of tetracycline and mercury resistance plasmid pT181 and a transposon, Tn*554*, encoding erythromycin and spectinomycin resistance is present in the J3 region.

At 67 kb in size, SCC*mec* type III was considered to be the longest SCC element; however, Chongtrakool and coworkers (182) reported in 2006 that the type III element is actually composed of two smaller SCC elements integrated in tandem, SCC*mercury*

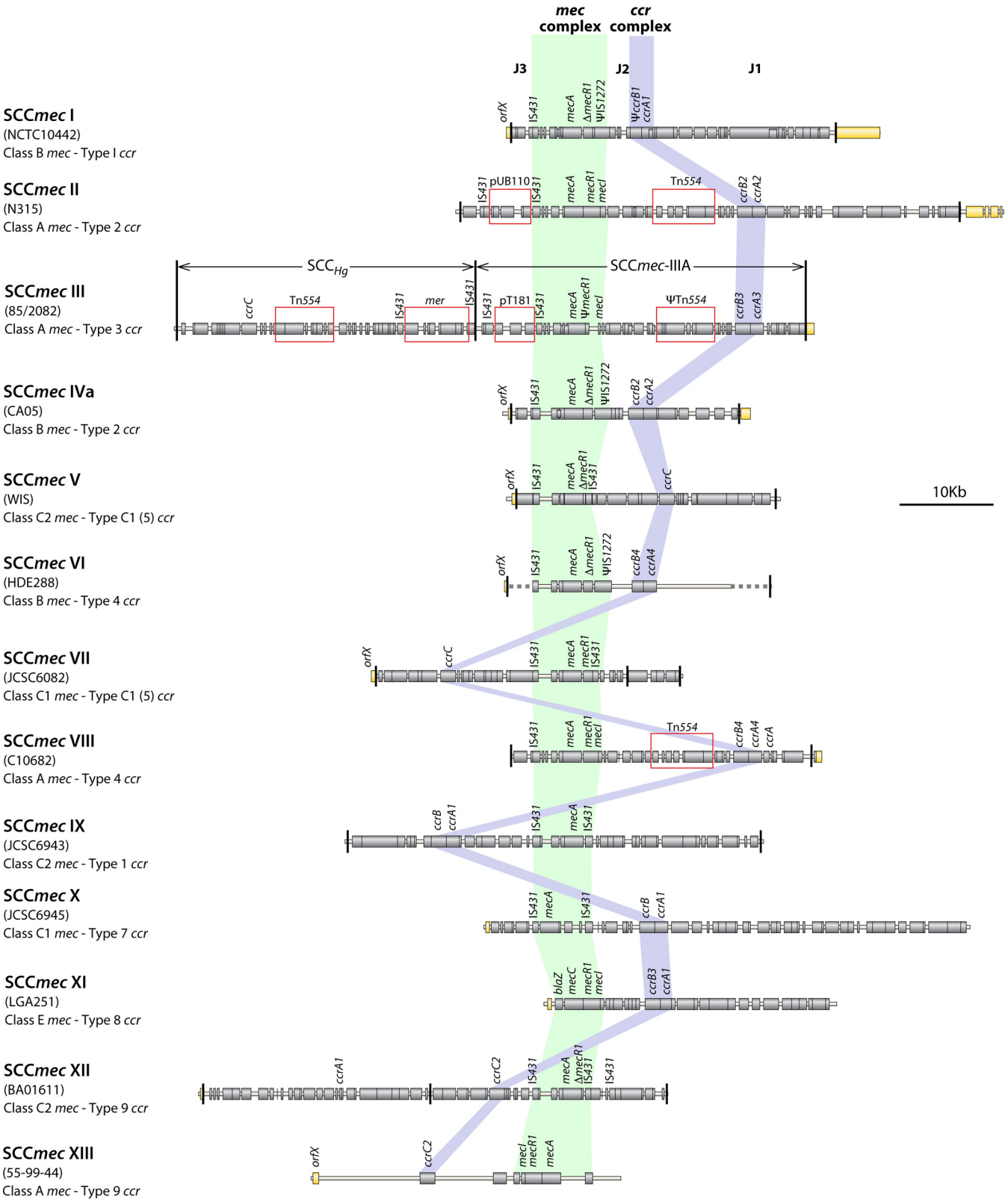

FIG 4 Diagrammatic illustration of the reported SCC*mec* types. To date, 13 SCC*mec* types (I to XIII) have been identified. Scale representations of the 13 SCC*mec* types are shown, with the *mec* gene complex indicated by green shading, the *ccr* gene complex indicated by purple shading, and the J1, J2, and J3 regions surrounding. ORFs within the SCC*mec* elements are represented by red boxes, while chromosomal ORFs are represented by yellow boxes. Important elements in the J regions, such as transposons and plasmids, are also indicated. Different combinations of the *mec* gene complex and *ccr* gene complexes, and occasionally different placements with respect to each other, give rise to the various SCC*mec* types.

and SCC*mec* type III (3A), carrying type 3 *ccr* and class A *mec* gene complexes. Hence to avoid confusion, the name of SCC*mercury* was changed to SCC*Hg*.

(iv) SCC*mec* type IV. SCC*mec* type IV was first discovered in 2002 in two characteristic MRSA strains, CA05 and 8/6-3p, isolated from the joint fluid of patients. While SCC*mec* types I, II, and III were the most frequently encountered types in earlier years, SCC*mec* IV has gone on to become one of the most frequently isolated SCC*mec* types due to its presence in 2 strains of the rapidly expanding CA-MRSA group. SCC*mec* type IV is the smallest one so far and has a unique combination of a class B *mec* gene complex with a type 2 *ccr* gene complex, and it harbors transposon Tn*4001* in the J3 region (36). SCC*mec* type IV from CA05 contains 24,244 nucleotides with 4 repeat regions, 2 mobile elements, and 22 CDS, of which 17 CDS apparently have no functionally clear ORFs. The whole element is structurally divided into the upstream region of the *ccr* gene complex containing one repeat region and 4 CDS, the *ccr* gene complex having *ccr* genes and 6 CDS, the *mec* gene complex with class B in addition to 5 CDS, 2 mobile elements, and 2 repeat regions, and lastly the downstream region of the *mec* gene complex having 1 repeat region and 3 CDS. However, SCC*mec* type IV from 8/6-3p is 20,916 bp long with same features as that from CA05 except for the difference in the J1 region, giving rise to a different subtype. As mentioned above, differences in the J region give rise to SCC*mec* subtypes. Despite the smaller size of SCC*mec* type IV, the genetic makeup of the region varies considerably, with many subtypes (IVa to -n) described. Again, both edges of type IV are characterized by inverted repeats. Due to its smaller size and simpler genetic makeup, no antibiotic resistance genes are found in SCC*mec* type IV, except for *mecA*.

(v) SCC*mec* type V. SCC*mec* type V was first identified in 2004 in the Australian strain WIS (JCSC3624) and is found predominantly in CA-MRSA. After finding several strains with untypeable SCC*mec*, sequencing of the region in strain WIS was done and identified a novel SCC*mec* cassette containing a class C2 *mec* gene complex and a type 5 *ccr* gene complex (183, 184). This element is 27,638 bp long with 6 repeat regions, 2 mobile elements, and 23 CDS, of which 15 CDS apparently have no functionally clear ORFs. The whole element is structurally divided into the upstream region of the *mec* gene complex containing one repeat region and 1 CDS, the *mec* gene complex with class B in addition to 4 CDS, 2 mobile elements, and 4 repeat regions, the region between the *mec* gene complex and the *ccr* gene complex with 2 CDS, the *ccr* gene complex having *ccr* genes and 6 CDS, the *mec* gene complex, and lastly the downstream region of the *ccr* gene complex with 1 repeat region and 7 CDS. The recombination of this type of SCC*mec* element is carried out by neither *ccrA* nor *ccrB* but by a novel *ccrC* gene. Hence, the integration and excision are performed by a single cassette chromosome recombinase C (*ccrC*), unlike the case for *ccrA* and *ccrB*, where integration is performed by one gene and excision by the other. Similar to type IV, type V does not have any identifiable antibiotic resistance genes other than *mecA*.

(vi) SCC*mec* type VI. SCC*mec* type VI was first discovered in 2001 in strain HDE288 isolated from a pediatric hospital in Portugal (180, 185, 186). It harbors a class B *mec* gene complex and a type 4 *ccr* gene complex. The type 4 *ccr* gene complex is identical to the type 3 *ccr* gene complex, and the downstream region of the element is 99% similar to the corresponding part of SCC*mec* type I. It is worth mentioning here that SCC*mec* VI from strain HDE288 was first reported as SCC*mec* type IV based on the order of its discovery (180). However, at the time when SCC*mec* naming regulations were proposed by the IWG-SCC (166), SCC*mec* IV and V from strains CA05 and WIS (JCSC3624) were receiving much attention due to the emergence of CA-MRSA and their association with these elements. Hence, in order to avoid any confusion, SCC*mec* from strain HDE288 was redefined as SCC*mec* type VI (186). In addition, this is the only SCC*mec* element so far for which the complete sequence is not yet available.

(vii) SCC*mec* type VII. First identified in the Swedish CA-MRSA strain JCSC6082, SCC*mec* type VII was reported in 2008 (187). It harbors the class C1 *mec* gene complex and the type 5 *ccr* gene complex. SCC*mec* type VII from JCSC6082 carries 26,753 nucleotides with 2 repeat regions and 29 CDS, of which 16 CDS apparently have no

functionally clear ORFs. The whole element is structurally divided into the upstream region of the *ccr* gene complex containing one repeat region and 3 CDS, the *ccr* gene complex having *ccr* genes and 6 CDS, the region between the *ccr* gene complex and the *mec* gene complex with 8 CDS, the *mec* gene complex with class C1 in addition to 4 CDS, and lastly the downstream region of the *mec* gene complex having 1 repeat region and 5 CDS.

(viii) SCC*mec* type VIII. SCC*mec* type VIII was first reported in a Canadian MRSA isolate, C10628, in 2009. This strain is one of the predominant MRSA strains in Canada (CMRSA9). It carries a novel combination of the class A *mec* gene complex and type 4 *ccr* gene complex (188). SCC*mec* type VIII from C10628 contains 32,184 bp with 6 repeat regions, 1 mobile element, and 36 CDS, of which 14 CDS apparently have no functionally clear ORFs. The whole element is structurally divided into the upstream region of the *mec* gene complex containing 2 repeat regions and 2 CDS, the *mec* gene complex with class A in addition to 6 CDS, 1 mobile element, and 2 repeat regions, the region between the *mec* gene complex and the *ccr* gene complex with 19 CDS, the *ccr* gene complex having *ccr* genes, and lastly the downstream region of the *ccr* gene complex with 2 repeat region and 5 CDS. SCC*mec* type VIII is flanked by a pair of dyad repeats, i.e., DyaR-L and DyaR-R, which are 97 nucleotides away from the direct repeats of this element, i.e., DR_{SCC}-L and DR_{SCC}-R. The structure of the *mec* gene complex of SCC*mec* type VIII is similar to that of the class A *mec* gene complex of SCC*mec* type II from strain N315. Similarly, the *ccr* genes in this element are identical to the *ccr* genes of SCC*mec* type VI from HDE288. The structure of this element is suggestive of recombination between 2 other SCC elements, namely, between *S. epidermidis* strain RP62A and *S. epidermidis* ATCC 12228, whereby the *mec* gene complex originates from RP62A and the *ccr* gene complex originates from ATCC 12228.

(ix) SCC*mec* type IX. SCC*mec* type IX was identified in MRSA strain JCSC6943, recovered from a participant at an international porcine veterinary conference in Denmark. The participant originated in Thailand and carried an isolate belonging to the traditional LA-MRSA lineage, ST398, with a SCC*mec* element that could not be typed. Sequencing of the element revealed that SCC*mec* type IX carries a class C2 *mec* gene complex and a type 1 *ccr* gene complex (189). SCC*mec* type IX from JCSC6943 contain 43,710 nucleotides with 6 repeat regions, 2 mobile elements, and 42 CDS, of which 22 CDS apparently have no functionally clear ORFs. The whole element is structurally divided into the upstream region of the *ccr* gene complex containing one repeat region and 3 CDS, the *ccr* gene complex having *ccr* genes and 6 CDS, the region between the *ccr* gene complex and the *mec* gene complex with 5 CDS, the *mec* gene complex with class C2 in addition to 5 CDS, 2 mobile elements, and 4 repeat regions, and lastly the downstream region of the *mec* gene complex having 1 repeat region and 20 CDS. The element is flanked by two direct repeats at both sides. The structure of the class C2 *mec* gene complex from SCC*mec* type IX is similar to that of SCC*mec* type VII from JCSC6082 and harbors two IS*431* elements integrated into the *mecR1* gene at different positions. Among the J regions, J1 from SCC*mec* type IX is unique and complicated. The J1 ORFs are related to the detoxification of cadmium, copper, and arsenic, containing the *cadDX* operon, the *copA* gene (related to copper detoxification), and two arsenic resistance operons, i.e., *arsRBC* and *arsDARBC*.

(x) SCC*mec* type X. SCC*mec* type X was identified as part of the same study that identified SCC*mec* type IX, from a Canadian participant carrying MRSA of the ST398 lineage. It was identified in MRSA strain JCSC6945 carrying the class C1 *mec* gene complex and a novel type 7 *ccr* gene complex (189). SCC*mec* type X from JCSC6945 contains 50,802 nucleotides with 6 repeat regions, 2 mobile elements, and 54 CDS, of which 33 CDS apparently have no functionally clear ORFs. The whole element is structurally divided into the upstream region of the *mec* gene complex containing 1 repeat region and 6 CDS, the *mec* gene complex with class C1 in addition to 5 CDS, 2 mobile elements, and 4 repeat regions, the region between the *mec* gene complex and the *ccr* gene complex with 4 CDS, the *ccr* gene complex having *ccr* genes and 5 CDS, and lastly the downstream region of the *ccr* gene complex with 1 repeat region and 30

CDS. There are many similarities in the structures of SCC*mec* type X and SCC*mec* type IX. These include the direct repeats at both sides of the element, two IS*431* regions flanked by *tnp* genes, the *ccrA1* gene, and the genes related to heavy metal detoxification, including the *cadDX* operon and *copA* gene. It also incorporates an *arsRBC* operon in the J3 region which is highly similar to the *arsRBC* operon in the J1 region of SCC*mec* type IX. Moreover, it also contains an *arsRBC* operon in the J1 region in addition to an insertion sequence, IS*Sha1*. However, the orientation of the class C1 complex in type X is opposite to that in types I to VII. The combination of the *ccr* gene in type X is classified as novel combination because it includes *ccrA1* and *ccrB6*, and hence it is termed *ccr* type 7.

(xi) SCC*mec* type XI. SCC*mec* type XI was identified in 2011 simultaneously in MRSA strains LGA251 and M10/0061 isolated from southwest England in 2007 and southeast Ireland in 2010, respectively (143, 164). It harbors a novel class E *mec* gene complex (which carries the newly described *mecC* gene, sharing only 69% homology with *mecA*) and a type 8 *ccr* gene complex. The length of SCC*mec* type XI is around 29.4 kb, and it has a wide geographical distribution in Europe with a broad diversity of host species, including companion animals, livestock, and wildlife, which can all serve as potential sources of human infections. SCC*mec* type XI from LGA251 contains 29 CDS. The *mec* gene from SCC*mec* type XI is divergent in comparison with all other *mecA* homologues apart from type III, and the combination of *ccrA1* and *ccrB3* renders SCC*mec* type XI a unique allotype.

(xii) SCC*mec* type XII. With the discovery of a new *ccrC* allotype, *ccrC2*, a novel SCC*mec* type XII was also discovered by Wu and coworkers (171) in *S. aureus* isolate BA01611. The strain was isolated from a bovine mastitis sample collected from northwestern China. SCC*mec* type XII is flanked by a pseudo-SCC (ΨSCC) element, ΨSCCBA01611, carrying a truncated *ccrA1*. Hence, SCC*mec* type XII is a composite consisting of a pseudo-SCC and an intact SCC*mec* designated $SCC mec_{BA01611}$. This composite SCC, however, does not contain any antibiotic resistance gene other than *mecA*. The element is estimated to be 25 kb in size, demarcated via two direct repeats, DR2 and DR3, carrying 31 ORFs. It carries a novel *ccrC* allotype called *ccrC2*. In addition, two type III restriction-modification (RM) system methylation subunits (Mod) were identified in SCC*mec* type XII, which appears to be extremely rare in *S. aureus*. The pseudo-SCC element in SCC*mec* type XII carries 30 ORFs immediately downstream of *orfX*. It is approximately 24.3 kb in length, outlined by DR1 and DR2, carrying the type 1 *ccr* gene complex with a truncated *ccrA1* (171).

(xiii) SCC*mec* type XIII. Recently Baig et al. (190) discovered a novel SCC*mec* type XIII in MRSA ST152, isolated from a 30-year-old Danish male with bacteremia. The element is 32.3 kb in length and harbors a novel *ccrC2* gene. The structure of its *mec* gene complex, however, resembles that of the *mec* class A complex (*mecI-mecR1-mecA*-IS*431*) with an additional IS*431* downstream of *mecI*. Additionally, the order of the genes in the *mec* gene complex of SCC*mec* type XIII is inverted compared to that of the prototype class A *mec* gene complex. Moreover, it contains a gentamicin resistance gene on a transposon, Tn*4001*, found in the J2 region of the element (190).

SCC*mec* subtypes. Polymorphism in J regions within the same *mec* gene complex and the *ccr* gene complex combination gives rise to subtypes of SCC*mec* elements. Many different insertion sequences and transposons have been identified among the major SCC*mec* types. These different IS and transposons result in the classification of SCC*mec* types into subtypes. The reporting of novel SCC*mec* elements should therefore be based upon the nucleotide sequence of the entire element and not just fragments, which can be misleading (166).

Novel SCC*mec* subtypes are therefore defined not just by the mobile genetic elements (plasmids, transposons, and insertion sequences) but also by the noncoding regions, pseudogenes, and other characteristic genes present in the J region of the element (166). There have been different methods used to describe the subtypes. In one, the difference in the J1 region is expressed as lowercase letters, i.e., IIIa, IIIb, IIIc, and so on. In another, the difference due to the presence or absence of mobile genetic

elements is expressed as capital letters. However, considering the ever-increasing diversity of J regions, the number of alphabet letters will be insufficient, and hence a structure based on a binary system with periods, which helps recognize or stipulate these differences, would be helpful. This system would express the differences in each of the three J regions as Arabic numerals in the order of their discovery, e.g., III.1.1.1, III.1.2.1, and III.2.1.1. This system will help assign new SCC*mec* subtype numbers in an informative and definitive way and, importantly, without the artificial limitation of an alphabetic system.

SCC elements harboring genes other than *mecA*. SCC elements carrying genes other than *mecA* have also been identified in staphylococci. These elements carry genes for fusidic acid resistance, a capsule gene cluster, and a mercury resistance operon. They share mutual characteristics with SCC*mec* by carrying *ccr* genes in a *ccr* gene complex, integration at a specific site in staphylococcal chromosome ISS, and the occurrence of flanking DR containing ISS. These elements are defined by adding a suffix defining the gene's name or function after "SCC," such as SCC*fur* for fusidic acid resistance, SCC*cap1* for capsule gene cluster, and SCC*Hg* for mercury resistance operon (166). If the genes in these elements do not have specific functions, they could be described by adding the name of the strain.

Staphylococci are also seen to harbor SCC-like regions. They are similar to SCC in that they are integrated and bracketed by ISS but differ from SCC in that they do not carry a *ccr* gene. They vary in size from 1 kb to 34 kb and should be regarded as pseudo-SCC (ΨSCC) elements. These ΨSCC elements could be designated by adding a suffix describing the name of the gene or its function or by adding the name of the strain harboring it (166).

Composites of two or more SCC elements. SCC elements carrying two or more *ccr* gene complexes have been identified in various *S. aureus* strains and are regarded as composite SCC elements. When identified, the association of *ccr* genes, *mec* gene complexes, and J regions should be compared to that for the previously described SCC*mec* type to determine if it holds any existing SCC*mec* type. Subsequently, the relationship of the element to the earlier *ccr* genes should be determined to see if the occurrence of two *ccr* genes is the consequence of two distinctly combined SCC elements or if the compound was created by the union of the two elements followed by removal of the junction region containing the DR in ISS (166).

So far, these composite elements have been identified in *S. aureus* strain ZH47, Taiwanese *S. aureus* strains TSGH17 and PM1, *S. aureus* isolate BA01611, and a single-locus variant of EMRSA-15 strain Lul (171, 191–194). In ZH47, the composite is composed of an SCC with *ccrC* and an SCC*mec* carrying class B2 subclass of *mec* gene complex with an integrated transposon *mec* gene complex and a type 2 *ccr* gene complex. The J1 region of this composite has homology to type IVc SCC*mec*. There were no DR sequences identified at the junction of the composite, but DR sequences were detected at the fringes of the amalgamated element (191). Eventually, this element in ZH47 was classified as a type IV SCC*mec* element.

The elements from the Taiwanese *S. aureus* strains were composed of an SCC with *ccrC1* allele 8 and an SCC*mec* with a class C2 *mec* gene complex and a type 5 *ccr* gene complex harboring *ccrC1* allele 2 (192, 193). The composite is delineated by direct repeats at both ends. The elements from these strains were eventually classified as a type V SCC*mec* element; however, strain PM1 had previously been registered as type VII SCC*mec*. Vignaroli et al. in 2014 reported epidemic MRSA (EMRSA) strain Lul carrying a composite SCC*mec* featuring *ccr* complexes type 2 and 5 (194). The element consists of a combination of a class B *mec* gene complex and a type 2 *ccr* gene complex as well as an additional *ccrC* gene complex, i.e., a type 5 *ccr* gene. The element was been classified as a composite type IV SCC element.

According to the above-mentioned criteria, the type IIIA SCC*mec* carried by *S. aureus* strain HU25 should also be viewed as a composite SCC element carrying a type III SCC*mec* and SCC*Hg*. This is because only two DR are present in the element, one at each extremity. In addition, the characteristic nucleotide sequence at the junction of

the two elements is missing (166). These composite elements should be categorized as SCC*mec* type variants based on the known type of SCC*mec* present in them rather than giving them a roman numeral as a novel type.

MRSA TYPING METHODS AND TECHNIQUES

MRSA infections continue to increase in frequency, and with an awareness of their constantly changing epidemiology comes the need for quick and trustworthy methods for the characterization of isolates to aide in thorough investigations of clonal spreading. This in-depth knowledge related to dissemination and molecular epidemiology is in turn required to implement effective control measures preventing the spread of MRSA, as well as allowing the containment of outbreaks (195). The epidemiology of infectious diseases relies on typing methods as a means for the characterization and discrimination of isolates based on their phenotypic and/or genotypic characteristics. This may then be used to determine clonal relationships among strains and to outline the topographical propagation of the clones (196). Nowadays, most classification schemes are based on molecular methods rather than phenotypic methods, providing more discriminatory power. Numerous typing techniques are currently used and applied by both clinicians and epidemiologists. These methods contribute to the understanding of the epidemiology of infections and are used for the investigation of outbreaks as well as to assist physicians in the clinical treatment of patients by permitting discrimination between successive and/or recurrent infections (197).

There are two general types of criteria for the evaluation of typing techniques: performance/efficacy criteria and convenience/efficiency criteria. Typeability, reproducibility, discriminatory power, and agreement between typing techniques are all included in the performance/efficacy criteria, whereas convenience/efficiency criteria include versatility, rapidity, and ease of execution and interpretation (198–201). A typing technique should ideally possess most if not all of the following characteristics: high portability, unambiguity, and reproducibility of data; inter- and intralaboratory comparability; low-cost methodology with high throughput (ideally less than 3 days); ease of processing, storage, and exchange of data; standardized international nomenclature; quality control of raw typing data (external quality assessment [EQA]), providing information for epidemiological investigations and phylogenetic analysis; and flexibility to type any pathogen with little modification of the protocol.

Several typing techniques have been developed in the last few decades. While they were initially used for research purposes, they are now more commonly employed in clinical labs as well (200).

Historical Typing Methods

Phage typing. The ability of bacteria to be infected by different bacteriophages varies between different strains even if they are very closely related, forming the principle of phage typing. Originally developed in the 1940s, phage typing was the method of choice for characterizing outbreaks (202, 203) and relies on the pattern of bacterial susceptibility in relation to a defined set of phages (204). The clonal nature of MRSA along with the epidemic spread of MRSA in hospitals and intercontinental spread of particular lineages was first recognized and demonstrated using the technique of phage typing (95). A set of 23 internationally accepted phages is used for typing human strains of *S. aureus*, with a window of two local phages (202). *S. aureus* is subjected to attack by a series of phages, and depending on the outcome of the attack (whether the bacteria are killed or not killed by groups of phages), the bacterium is given a number representing the phage type. The technique possesses greater discriminatory power than some other typing techniques, such as capsular typing and/or zymotyping, and has defined several outbreaks in the past.

Phage typing has been the first-line approach in epidemiological studies of MRSA for many years and has also been reported to be valuable in the identification of known epidemic strains among endemic strains (205). There remain some disadvantages associated with phage typing, including a reliance on the outcome of a complex

reliability, often lacking reproducibility. Phage typing is time-consuming and technically challenging, involving phage stocks that can be maintained only at reference laboratories (205). Phage typing can be done more efficiently on larger batches and hence is confined to large laboratories. More importantly, a high proportion of MRSA isolates remain nontypeable (NT) when this technique is used in an outbreak situation (206). This nontypeability is frequently as high as 20 to 30% of tested samples, reaching up to 75% in some cases (202, 207–210). This reduces the value of the information obtained, as there is no way of knowing if the nontypeable bacteria were related. Efforts have been made to reduce the number of untypeable bacteria by this technique by using routine test dilution (RTD) $\times$ 100, by incubating at 48°C prior to the test, and/or by introducing new phages (210–212).

MLEE. Multilocus enzyme electrophoresis (MLEE), also called isoenzyme typing, has long been used as a standard method to study eukaryotic population genetics and systematics (213, 214). Beginning in the early 1980s, medical microbiologists began using this technique to investigate the epidemiology of infectious diseases (213, 215–218). MLEE allows the identification of genetically related types within a species, which can be associated with particular characteristics (219).

Bacterial typing via MLEE involves the extraction of constitutively expressed proteins from the cell and their separation by electrophoresis on gels. The rate of protein migration on the gel depends on the amino acid composition, and the technique equates the variation in the charge of each enzyme/protein with alleles at the underlying genetic locus. Approximately 80% of single substitutions can be detected by a change in electrophoretic properties (213), and on the basis of similarities between enzymes, the bacteria are assigned an electrophoretic type (ET). The degree of similarity between isolates can be assessed by the proportion of loci which show differences (220).

When MLEE is applied to MRSA, isolates are generally typeable with good reproducibility, yet the process remains laborious and somewhat subjective (207, 221). In addition, the results produced are difficult to compare between laboratories. The discriminatory power of MLEE, to a certain extent, also depends on the proteins included in a given study, as some enzymes have been found to be monomorphic even within large collections of MRSA isolates (220). The number of enzymes assessed varies between 12 and 20; however, the individual discriminatory power of each one has not been assessed, and therefore it is not possible to say what optimum combination will work best or how this combination would perform in relation to other techniques (207, 220, 222).

In outbreak investigations, this technique has successfully linked outbreak bacteria and classified them correctly, although on occasion there have been some unrelated bacteria mistakenly included in the same ET (207). On other occasions, the majority of isolates were clustered into just one or two ETs, casting doubt on the validity of the grouping (220, 222). While the patterns produced by MLEE are relatively simple to read and interpret, the comparison can be difficult and is best done with the aid of a computer program (207). As discussed, MLEE reflects the expression of a protein genotype according to its mobility; two bands of the same protein in different positions reflect two different proteins with different conformations and hence two alleles of the same gene, conferring on the sample its type (213). However, there is an obvious drawback to this technique in that two different base sequences could express the same protein, or two different proteins might have the same electrophoretic mobility, and hence be detected as the same band in MLEE. A modified version of this technique which focuses on the actual sequence of the gene, rather than its expression, was developed in 1998 by Maiden et al. (223) for studying the genetic structure of *Neisseria meningitides* (223, 224). While MLEE is a dated technique, the concept of indexing variation at multiple neutral loci remains as valid as ever and forms the principle of the technique called multilocus sequence typing (MLST). MLST takes advantage of the simplicity and speed of automated DNA sequencing and is now employed for charac-

terization, typing, and classification of members of bacterial populations; it is discussed in detail below (223).

PCR-based typing systems. There are a number of typing techniques that involve PCR, including amplified fragment length polymorphism (AFLP), random amplification of polymorphic DNA (RAPD) and arbitrarily primed PCR (AP-PCR), repetitive element PCR (Rep-PCR), and accessory gene regulator (*agr*) typing.

(i) AFLP. Amplified fragment length polymorphism (AFLP) is based on the polymorphisms of amplified fragments of genomic DNA. It involves the digestion of genomic DNA with restriction enzymes, followed by ligation of double-stranded adaptors to the sticky ends of the restriction fragments (225). Subsequently, there is amplification of the fragments using primers complementary to the adaptor sequence, the restriction site sequence, and a few nucleotides inside the restriction site. To ensure the efficient binding of primers to fully complementary nucleotide sequences on the DNA, highly stringent conditions are used for the amplification. The primers are usually fluorescently labeled and typically allow coamplification of 50 to 100 restriction fragments. After separation on the basis of size, the fragments are detected on an automated DNA sequencer, followed by computer-assisted comparison. The genetic relatedness among studied bacterial isolates is determined via high-resolution banding patterns generated during AFLP analysis (226). In addition to a high level of reproducibility, it has considerable discriminatory power (227, 228). The technique can also be automated, and the results are portable. The main drawback to this technique is the fact that it is labor-intensive, time-consuming (with a typical analysis taking up to 3 days), and expensive.

(ii) RAPD and AP-PCR. First described by Williams et al. (229) and Welsh and McClelland (230) in 1990, random amplification of polymorphic DNA (RAPD) and arbitrarily primed PCR (AP-PCR) are based on the principle of rapid parallel amplification of random DNA segments under nonstringent conditions, producing a gel map unique to a particular bacterial strain (229, 230). Short arbitrary sequences up to 10 bases long are used as primers in an amplification reaction under low-, nonstringent-annealing conditions, allowing primer hybridization at multiple mismatched sites. Several amplicons of different size are generated during PCR (231), and the number/size of the fragments forms the basis for typing an isolate. Depending on primer labeling with appropriate dyes, the amplicons are analyzed by either gel electrophoresis or DNA sequencing.

AP-PCR is a variant of RAPD that is also known as RAPD (232). The differences between the two techniques include the amplification, which in AP-PCR is conducted in three different parts, each part with its own stringency and concentration of components. The first part of AP-PCR uses high primer concentrations. These primers are of variable length, and primers often designed for other purposes are also used in AP-PCR.

RAPD is less discriminatory, and the discriminatory power depends on the number and nucleotide sequences of primers. It has, however, been widely used for typing of isolates in outbreaks (233–237). Although it is relatively simple, inexpensive, quick, and easy to use, the main drawback is its low inter- and intralaboratory reproducibility due to low annealing temperatures and sensitivities to subtle differences in reagents, protocols, and/or machines.

(iii) Rep-PCR. Repetitive element sequence-based PCR (Rep-PCR) utilizes primers complementary to specific sequences in the bacterial genome and, hence, is based on genomic fingerprint patterns to classify bacterial isolates (238). The technique utilizes primers based on short sequences of repetitive elements dispersed throughout the prokaryote domain. These primers hybridize to noncoding intergenic repetitive sequences which are known to be repeated throughout the genome, with variable numbers and positions. The repetitive palindromic extragenic elements (Rep) are sequences of 35 to 38 bp, comprised of 6 degenerated positions and variable loops of 5 bp among each conserved side (239). Amplification of the DNA between these binding sites results in products of varied length, producing a genomic fingerprint pattern following electrophoresis. Comparisons of the fingerprint patterns between

strains allow one to determine the genetic relatedness between the analyzed bacterial isolates.

This technique is easy to use and can be applied to large or small numbers of isolates, with a higher discriminatory power than many other typing techniques. As Rep-PCR targets specific sequences, allowing the use of stringent PCR conditions, the reproducibility of this technique is much better than that of RAPD (240). The results obtained via the Rep-PCR technique have good correlation with PFGE, although the discriminatory power is slightly lower. A disadvantage is the need for an extra DNA purification step, increasing the overall time for conducting the procedure.

The target sequences used for typing MRSA isolates include RepMP3, inter-IS*256*, and Tn*916* (240–245). Van der Zee et al. (240) directly compared RepMP3 with primers targeting inter-IS*256* sequences and concluded that although all MRSA isolates included in the study were typeable via both primer sets, RepMP3 had some distinct advantages. The patterns created via RepMP3 were reproducible, easy to compare, and stable after subculture. Amplification using inter-IS*256*, on the other hand, required low annealing temperatures, adversely affecting reproducibility. In addition, a larger number of strains were defined using RepMP3. None of these primers could match the discriminatory power of PFGE, but they operated well compared to other PCR-based typing techniques (240, 242).

Although Rep-PCR can be highly discriminatory for some organisms, the main limitation of this technique lies in its need for PCR combined with electrophoresis using traditional gels, which lack sufficient reproducibility due to variability in reagents and gel electrophoresis systems (246, 247). This limitation can partly be overcome by using the DiversiLab system, which is a semiautomated method using the Rep-PCR approach and is used in infection control settings by a number of hospitals worldwide (248). Although studies have shown that the DiversiLab system is a useful tool for the identification of numerous organisms involved in hospital outbreaks, it is an inadequate and insufficiently discriminative typing method for some organisms, including MRSA (249–251).

(iv) *agr* typing. The accessory gene regulator (*agr*) is a crucial regulatory component in *S. aureus*. Conserved throughout the genus, *agr* is involved in the control of bacterial virulence factor expression. *S. aureus agr* is a 3-kb locus showing highly conserved and hypervariable regions (252). The *agr* locus encodes a two-component signaling pathway which is activated by an *agr*-encoded autoinducing peptide (AIP), whose amino acid sequence and its corresponding receptor divides *S. aureus* strains into groups/types. One-third of the N-terminal region of the *agrB* product and nearly half of the C-terminal region of the *agrC* product are highly conserved. The intervening sequences, which include two-thirds of the C-terminal region of the *agrB* product, the whole of *agrD*, and about half of the N-terminal region of the *agrC* product, are highly divergent and constitute the hypervariable region. It is the variations among these hypervariable regions that divide *S. aureus* into at least 4 *agr* specificity groups (I to IV) (252).

Typing of *agr* uses primers directed against the variable region of the *agr* gene (253). Francois et al. (254) described a method for rapid determination of *agr* type via a novel multiplex real-time quantitative PCR assay for high-throughput epidemiological screening. They selected type-specific oligonucleotides targeting the variable moiety of the *agrC* gene and validated them against reference strains. The assay allowed rapid, specific, and efficient screening of *S. aureus* clinical isolates with moderate turnaround time and reasonable reagent cost for utilization in routine laboratories (254).

There is evidence that specific *agr* biotypes are associated with specific clinical features. For example, most menstrual toxic shock syndrome (TSST-1) strains belong to *agr* group III, whereas most of the VISA strains and leucocidin-induced necrotizing pneumonia strains belong to *agr* group II (255–257). a*gr* type II strains are also isolated from anterior nares or blood and are found harboring a bicomponent toxin gene, *lukD-lukE* (258). Those belonging to *agr* group IV have been found to be associated with the production of exfoliatin and are also found to be involved in bullous impetigo (254,

259, 260). *agr* group I was prevalent in a collection of 192 *S. aureus* strains, most of which were resistant to methicillin (261).

Pulsed-Field Gel Electrophoresis

First described by Schwarz and Cantor in 1984, pulsed-field gel electrophoresis (PFGE) was once considered the gold standard for MRSA typing (262). The technique is based on the digestion of bacterial DNA with restriction endonucleases recognizing a limited number of sites on the bacterial genome, generating fragments ranging from 30 kb to over 1 Mb (263). These fragments cannot be separated via conventional gel electrophoresis, which employs a single and uniform electric field and where DNA travels from cathode to anode through a molecular sieve of gel in one direction only. These larger bands would appear as a single diffuse band due to their size-independent comigration, a phenomenon called reptation (264, 265). In PFGE the orientation of the electrical field is pulsed (i.e., periodically shifted or changed), enabling a change of DNA movement direction and allowing the fragments of megabase pairs to be separated effectively according to their size (200, 202, 231, 241). The pattern generated from chromosomal DNA travel along the pulsed-field gel is much simpler than the pattern generated from digestion with high-frequency-cutting restriction endonucleases (202).

PFGE, however, requires the use of intact genomic DNA, and therefore special care needs to be taken during the extraction of DNA to prevent it from mechanical shearing. For this purpose, bacterial cells are incorporated into low-melting-point agarose plugs, which allow the free flow of solutions necessary for lysing the cell wall and enzymatic digestion of cellular proteins, while at the same time protecting the DNA from breakage (266). While remaining in the agarose, DNA is subjected to treatment with rare-cutting restriction endonucleases, generating high-molecular-weight DNA fragments. The agarose plugs containing digested DNA are then loaded into an agarose gel and subjected to PFGE (200, 202, 231, 241). During electrophoresis, these fragments migrate toward the anode; however, before migration they need to align themselves with the direction of the electric current. The time taken for alignment by DNA fragments depends on the molecular mass of the fragments; the larger fragments need longer to reorient themselves toward the direction of the new field, while the smaller fragments quickly reorient themselves and start migrating. This results in a sharper resolution of larger fragments when the electrical field is pulsed and/or alternated during electrophoresis (262, 267).

Field inversion gel electrophoresis (FIGE) and the contour-clamped homogenous electric field (CHEF) technique are two among the various techniques used for DNA fragment separation using pulsed-field electrophoresis. The former involves alteration of the angle of the electric field in alternating forward and reverse directions, with the forward pulse lasting 3 times longer than the reverse pulse, and seems best for separation of fragments ranging from 0.1 to 200 kb. The CHEF technique, in contrast, is best for the separation of fragments of up to 3 Mb and involves the generation of uniform electric fields at an angle of 120°, with a hexagonal arrangement of electrodes. The fragments move in straight line with little or no distortion (202).

A number of studies have compared the usefulness of PFGE with that of other methods for MRSA isotyping (198, 207, 209, 235, 268–270), and a number of restriction endonucleases have also been tested; SmaI was found to be the most useful for MRSA, allowing nearly all isolates to be typeable and results from standard strains to be reproducible after extensive subculturing (207, 209, 271–275). Although nearly all pathogenic bacteria are typeable via PFGE, the SmaI-based method has been proposed as the gold standard for MRSA typing, mainly due to its performance, including discriminatory power and reproducibility, as well as the ease of execution, data interpretation, and availability. In addition, there is no interference from plasmid DNA, as the fragments generated by plasmid digestion are too small for the profile to be affected (276).

The major limitations to this technique are the long interval before results are obtained, the technically demanding, labor-intensive protocols, the cost of the re-

agents, and the specialized equipment needed (202, 263). It may also lack sufficient resolving power to discriminate bands differing in size by <5%. In addition, though PFGE generates a limited number of gel bands, it can still create problems with interpretation. This is especially true in cases of interlaboratory studies, as small differences in electrophoresis conditions such as temperature, solutions, or equipment can alter results by affecting the distance traveled by each band. This therefore complicates comparisons between isolates run on different gels and in different laboratories, making international comparisons problematic (277, 278).

Despite these limitations, PFGE remains a useful technique for the characterization of outbreaks and has been extensively used for the understanding of the epidemiology of both endemic and epidemic MRSA strains (202). In these situations, the data analysis criteria developed by Tenover et al. (279) have been useful. They proposed a criterion for the interpretation of PFGE patterns generated via standardized schemes. In addition, the BioNumerics software allows PFGE profiles to be normalized and the images to be matched within and between different laboratories. In addition, the experimental protocols have been standardized over the past few years. In this connection, a molecular subtyping standard has been established by the Centers for Disease Control and Prevention (CDC) (279), and all *S. aureus* PFGE profiles have been assembled in a national database for the investigation of MRSA outbreaks and global tracking of MRSA strain types (280, 281).

Multilocus Sequence Typing

As mentioned above, multilocus sequence typing (MLST) is a modification of a phenotypic typing technique, multilocus enzyme electrophoresis (MLEE). MLST analyzes seven constitutively expressed (housekeeping) genes that are essential to cellular functioning of organisms and hence are present in every organism. It assigns alleles to the genes following nucleotide sequencing of 450- to 500-bp internal fragments obtained by PCR for each locus (223, 224). The alleles evolve slowly and are not subjected to direct evolutionary pressure; hence, a point mutation would confer a new allele, as would a recombination change, irrespective of the number of changes in the bases. The extent to which the alleles are different is not relevant and is not considered important. The sequence type (ST) (or allelic profile) is based on the seven assigned numbers, as different sequences for each of the loci are assigned arbitrary allele numbers.

In 2000, Enright et al. (224) applied and validated the MLST scheme for *S. aureus* against PFGE. The seven housekeeping genes were *arcC* (encoding carbamate kinase), *aroE* (shikimate dehydrogenase), *glpF* (glycerol kinase), *gmk* (guanylate kinase), *pta* (phosphate acetyltransferase), *tpi* (triosephosphate isomerase), and *yqiL* (acetyl coenzyme A). They were selected out of 14 genes investigated because they provided the highest number of alleles and ample resolution to characterize the genetic diversity among the population. The authors observed that the strain types grouped via MLST had similar PFGE profiles, whereas those that had different PFGE profiles were also distinct when typed using the MLST technique.

A major strength of MLST is its portability and ease of data comparison between laboratories around the world. Because MLST is defined by its allelic profile, which consists of a string of seven numbers, its unambiguity makes it easily transmittable and communicable around the world via electronic media (282). In addition, the existence of a web-based database containing all allelic profiles and strain types has made this technique an extremely useful global epidemiological tool. The database is located under the URL http://www.mlst.net, uses a universal descriptive formatting language, and provides online software for sequence analysis. MLST has provided a useful tool for studying the origin and evolution of *S. aureus* strains (39, 93, 283–289) and, in conjunction with SCC*mec* typing, offers a universal nomenclature system for *S. aureus* strains.

As mentioned above, based on the allelic profile of the seven loci, MLST assigns a numerical sequence type (ST) to each isolate (for example, ST36 has the allelic profile

2, 2, 2, 2, 3, 3, 2). Although this is useful for typing, the relationship between sequences and isolates is not very clear from these notions. To solve this, Feil and coworkers developed the BURST (based upon related sequence type) algorithm for interpreting and analyzing the data, as well as developing evolutionary relationships among isolates (290–292). Isolates sharing the exact same allelic profile belong to the same ST and, hence, the same genetic lineage. However, isolates differing by a one or two loci (single-locus variants [SLVs] or double-locus variants [DLVs]) are considered to be genetically related and belonging to a cluster of related lineages, termed a clonal cluster (CC). The progenitor of the clonal cluster is the ST which is most prevalent among the population and has the widest geographical dissemination compared to its progenies. This ST should have the largest number of SLVs and must be present among the earliest isolates.

Following the development and validation of the MLST scheme for *S. aureus* in 2000, MRSA lineages have been defined in terms of ST, and for the first time there is an unambiguous, widespread, common, and universal language for MRSA (224). In addition, application of MLST to the study of clonal populations of MRSA has provided important insights into the population structure of MRSA. It validated previous PFGE data that MRSA strains have a very strong clonal population and pandemic MRSA clones belong mainly to few genetic lineages (39, 93, 94). In addition, the value of the MLST scheme for *S. aureus* has been greatly enhanced by the creation and maintenance of the website http://saureus.mlst.net/. This website, hosted at Imperial College and funded by the Wellcome Trust, provides the main hub for the assignment of new alleles and sequence types (282). In addition to collecting MLSTs, other relevant data, such as clinical and drug resistance of strains isolated globally, are also included. The website is maintained manually by a curator who assigns new allele numbers and maintains the allele database. Although MLST is an invaluable tool for creating an evolutionary framework of *S. aureus* strains, the housekeeping genes analyzed in this technique do not have any direct relationship to the virulence of the strains. Some investigators analyze additional genes along with the seven housekeeping genes (289). Verghese and coworkers (293) developed a combined multivirulence-locus sequence typing and SCC*mec* typing scheme in order to demonstrate enhanced discriminatory power with the combined techniques for MRSA strain typing.

The major drawback to MLST is its cost and the sophisticated equipment necessary for execution. This fact makes it unlikely to be used as a technique for studying putative outbreaks in a hospital and limits its use to large centers involved in global epidemiology studies (294). Another major barrier to its widespread use is that it is important to obtain high-quality sequence data on all of the seven alleles. This means that it requires 14 sequence reads to determine each ST, which for many laboratories is an expensive and time-consuming procedure. This drawback is overcome by the introduction of a new generation of parsimonious capillary sequencers which make the process largely automated and reduce material costs (295). Despite some drawbacks, because of its reproducibility, standardization, discriminatory power, and ease of interpretation, MLST is no doubt gaining popularity among researchers.

spa Typing

As mentioned in the section above, MLST involves characterizing genetic variability among seven housekeeping gene targets, which is a labor-intensive, time-consuming, and costly approach for clinical laboratories. In addition, in certain subpopulation of *S. aureus* (as with some MRSA strains), genetic variability is limited and discrimination could be restricted. The ability to use a single-locus target, with adequate discriminatory power, would provide an inexpensive, rapid, and more portable method to type bacteria. However, single-locus sequence typing (SLST) is dependent on finding a target for sequencing that is polymorphic enough to provide a useful strain resolution. Loci with short sequence repeats (SSR) provide appropriate variability to discriminate among outbreaks (296). The *spa* gene, coding for protein A, is conserved among *S. aureus* strains and provides suitable SSR regions to be used as a target for SLST, i.e., *spa*

typing. This is the first DNA sequence-based typing method developed specifically for the characterization of *S. aureus*. The method is based upon PCR amplification and sequencing of protein A specific for *S. aureus* (297). The *spa* gene, coding for protein A, is approximately 2,150 bp in length and encodes three regions: the Fc-binding region, the X region, and the C-terminal region. The X region, also called the repeat region, consist of variable-number tandem repeats (VNTR). These VNTR contain 2 to 15 repetitive sequences consisting of 21 to 27 bp (mainly 24 bp), which are polymorphic and diverse due to deletions and duplications of the repeats and occasionally due to point mutations. The polymorphism is reflected by the number, character, and order of repetitive sequences (298–300).

In *spa* typing, each identified repeat is associated with a numerical or letter code, and the *spa* type is deduced from the order of the specific repeats. Since the main source of variation is the events of duplication and/or deletion of repeat units in the polymorphic X region of the gene, strain lineages cannot be constructed by direct sequence comparisons (299). Therefore, in order to examine strain relatedness, all possible variations are identified, followed by comparing the organization of the repeats in different isolates. Each repeat unit in *spa* typing is given a unique identifier, and each *spa* type denotes a collection of specific repeat units arranged in a precise pattern. Two strains with identical repeat sequences (both content and organization) are considered genetically related and are assigned the same *spa* type. It is important to note that the numerical notion used is not an index of relatedness; the types are named in the order they were analyzed.

Unlike MLST, *spa* typing can be used for the investigation of both molecular evolution and hospital outbreaks (298). As it involves the interrogation of a single polymorphic locus, it is the most suitable typing method for local and short-term epidemiological studies (299, 301). In addition, *spa* type clusters specifically associated with MRSA lineages seem to be stable over time, making this method valuable for long-term global epidemiological studies as well (196, 298, 302–304). Moreover, an algorithm (BURP [based upon repeat pattern]) allows the exploration of *spa* typing for long-term epidemiological studies of MRSA (305, 306), and this has made cluster analysis based upon putting *spa* types into *spa* clonal complexes (CCs) possible (304).

The discriminatory power of *spa* typing falls between those of PFGE and MLST (307); however, compared to both methods it is cost-effective, easy to use, and rapid and has excellent reproducibility. *spa* typing is stable, with a standardized international nomenclature, and is amenable to high throughput using the StaphType software, and the data are fully portable via the Ridom database. These features make it the most useful instrument and method of choice for characterizing *S. aureus* isolates at local, national, and international levels (301, 303, 308–311). Two major nomenclature systems developed by Harmsen et al. (301) and Koreen and coworkers (298), called Ridom and eGenomics, respectively, are widely used for comparison purposes and allow the interchange of *spa* types between the two systems. However, comparison between the two nomenclatures is possible only via computerized tools. For both nomenclatures, the general approach is similar; each repeat is assigned a numerical or alphanumerical code, respectively, and each profile corresponds to a code constituted by the progression of the repeat's codes. The "Ridom Staph type" software and database system enables straightforward semiautomated sequence analysis and type assignment by synchronizing laboratory typing data to a central *spa* server at www.SpaServer.ridom.de. The server is curated by the SeqNet.org initiative (www.seqnet.org), which ensures a universal nomenclature, 100% reproducibility between laboratories, and public access to the typing data (301, 312, 313). This server is the largest known sequence-based typing database for *S. aureus*, and using this server, it is possible to generate a DNA sequence-based electronic early warning system for the automatic detection of MRSA outbreaks in hospitals, institutions, or regions where MRSA is endemic (314). The "eGenomics system," on the other hand, distinguishes among similar pathogens by comparing their DNAs, thereby fingerprinting the bacteria. The resulting DNA sequences are stored in a central database and analyzed using proprietary algorithms

(http://www.egenomics.com, http://tools.egenomics.com). The software tracks the distinct flow of organisms in both time and location so as to understand transmission patterns in order to identify outbreaks before they become endemic. It allows infection control teams to monitor the real-time spread of harmful pathogens and also distinguishes between actual outbreaks and random events, allowing infection control teams to focus their efforts on the most important areas.

Nevertheless, there are drawbacks associated with *spa* typing. The major one is that *spa* typing is based upon single-locus typing, which can easily misclassify particular types due to recombination and/or homoplasy (315). In addition, the difference in the two major nomenclature systems sometimes makes comparison of published *spa* typing data difficult.

SCC*mec* Typing

As discussed earlier in this review, SCC*mec* is a mobile genetic element and a determinant for broad-spectrum β-lactam resistance. So far, 13 different types of SCC*mec* elements have been discovered, which are further divided into subtypes based on the difference in their joining regions. As different types of SCC*mec* elements were discovered, it became quite evident that two MRSA strains carrying different SCC*mec* elements are different, even if they belong to the same MLST type or pulsotype. Hence, it became essential for epidemiological purposes to determine the type of SCC*mec* element carried by an MRSA strain. From a proposal by Enright and colleagues (93), a consensus was reached that MRSA clones should be defined by both the type of SCC*mec* element and the type of chromosome in which this element is integrated (i.e., MLST type). This nomenclature (for example, ST5-II) was accepted in 2002 by a subcommittee of the International Union of Microbiology Societies in Tokyo, Japan. However, under this rationale, quick and simple assays are critical for the detection of SCC*mec* types to properly characterize MRSA clones.

Since SCC*mec* gene variants are discovered regularly, choosing the best SCC*mec* typing scheme with respect to feasibility and discrimination is becoming complicated. Before 2000, SCC*mec* determinants were typed using conventional molecular cloning and sequencing (26, 181). However, a 2001 publication by Ito el. al. (178) introduced a method in which parts of the *mec/ccr* gene complexes were amplified using long-range conventional PCR with several sets of primers. These traditional PCR typing schemes required several primer sets and multiple individual PCR experiments. Since 2002, several improved strategies have been developed for SCC*mec* characterization, including conventional PCR detection of several type-specific loci (316), RFLP analysis of PCR or multiplex PCR products (317, 318), and multiplex real-time PCRs (319, 320). The most common and widely used of these techniques is the multiplex PCR. SCC*mec* typing via multiplex PCR in which *mecA* and different loci on SCC*mec* are detected was initially developed by Oliveira and de Lencastre (321) in 2002. They used this multiplex PCR strategy for quick identification of the structural type of the *mec* element in MRSA strains. However, there results were very difficult to interpret, and the technique was limited in its ability to detect SCC*mec* subtypes and newly described types. Since then, several strategies have been developed based on a variety of approaches for the characterization of SCC*mec* elements. In 2005, Zhang and colleagues (322) developed a novel multiplex PCR assay for the characterization and concomitant subtyping of SCC*mec* types I to V in MRSA strains. They proposed and verified the feasibility, ease, and usefulness of the new sets of primers to type and subtype the elements. At approximately the same time, another PCR using a single pair of primers targeting different types of SCC*mec* elements and the *orfX* region was developed (323). Subsequently, van der Zee et al. (317) proposed a new simplified typing method called multienzyme multiplex PCR-amplified fragment length polymorphism (ME-AFLP). In 2007, Boye and colleagues (324) developed yet another method of multiplex PCR screening for easy typing of MRSA SCC*mec* types I to V using four pairs of primers. For subtyping of SCC*mec* IV, Milheirico and coworkers (325) in 2007 developed a multiplex PCR strategy in which seven primer pairs were designed to simultaneously detect the

ccrB allotype 2 and polymorphic J1 regions described for SCC*mec* type IV and a new J1 region specific for EMRSA-15. At the same time, Milheirico and coworkers (326) updated their multiplex PCR strategy previously described in 2002 to incorporate subtypes of SCC*mec* IV, as well as the newly described SCC*mec* type V. However, the assay still required the use of several primers, as well as two separate PCR experiments. Zhang et al. (327) updated their multiplex PCR assay to make it more reliable and accurate in identifying common and major SCC*mec* types and subtypes.

Unfortunately, to date no single PCR method is available that can identify all SCC*mec* types and subtypes. The multiplex PCRs proposed by Milheirico and coworkers (325) and Zhang et al. (327) are by far the most commonly used method for SCC*mec* typing. Despite the plethora of methodologies available, careful primer design is required, which remains the primary challenge in developing discriminatory typing strategies. Currently, SCC*mec* typing by multiplex PCR is limited to SCC*mec* types I to V. Other methods are therefore needed for typing the increasing number of SCC*mec* types, including types VI to XIII. Moreover, since previously described methods detect specific structural properties of each SCC*mec* element, a single, universal method that can be applied equally to all SCC*mec* types still needs to be developed.

The use of targeted DNA microarrays represents another technique to detect genes associated with SCC*mec*, including *mecA*, its regulatory elements, various allotypes, and J regions, and consequently can be used for the identification of known SCC*mec* types (177, 328). To detect SCC*mec* cassettes of any type, probes for *mecA* and *ugpQ* (encoding glycerophosphoryldiesterase) are used. Hybridization results positive for *ccrA1*, *ccrB1*, Δ*mecR*, *pls*, and *dcs* are used to detect SCC*mec* type I. Similarly, *crrA2*, *crrB2*, *mecI*, *mecR*, the *kdp* operon (*kdpA*–*kdpE*), *xylR*, and *dcs* are used to detect type II, and *crrA3*, *crrB3*, *mecI*, *xylR*, and *mecR* probes are used to detect type III. Probes could be designed based on specific genes for each type; however, only known SCC*mec* types could be identified using this technique. Like previous techniques, it cannot be applied to the detection of novel SCC*mec* types and subtypes, for which whole-genome sequencing (WGS) has been applied (164). Both microarrays and whole-genome sequencing require highly trained personnel and expensive equipment, leaving multiplex PCR for SCC*mec* typing as the best option available today.

Microarrays

DNA microarray analysis, also known as biochip or DNA chip analysis, is a technique employed for typing studies using a collection of DNA probes attached in an orderly fashion to a solid surface. The probes provide a medium for matching known and unknown DNA samples, thereby automating the process of identifying unknowns (329). The probes could be oligonucleotides (up to 70-mers) or gene segments (PCR amplicons of >200 bp) that can be used to detect the presence of complementary nucleotide sequences in a particular bacterial species. Based on the number of probes on the chip, one can distinguish low-density (hundreds) or high-density (hundred thousands) DNA microarrays. Microarrays represent a tool for spotting genes that aid as markers for specific bacterial strains or to uncover allelic variants of a gene present in all strains of a particular species (315).

Practically speaking, total DNA is extracted from the bacteria and is labeled either chemically or via enzymatic reaction, followed by hybridization to a DNA microarray. After washing off the unbound target DNA, hybridization signals between the labeled DNA and immobilized probe are detected and measured with a scanner (315). Previously reported microarrays could covalently immobilize probes for approximately 180 genes and 300 alleles of *S. aureus* (330–333). These include genes for antimicrobial resistance, toxins, surface components of microorganisms, and typing targets, giving them the potential for simultaneous detection of large number of loci.

Microarrays appear to be well suited for bacterial typing and are widely used for the analysis of genomic mutations such as single-nucleotide polymorphisms (SNPs). They are an efficient tool for the detection of extragenomic elements, including uncommon antibiotic resistance genes, as well as complex patterns of virulence genes, and

therefore have the potential to detect new epidemiological markers for clones (334, 335). Using microarray-based technology, regulons of regulatory systems of *S. aureus* which help us to better understand the molecular mechanisms of pathogenesis have also been elucidated. These regulons of pathogenesis include Agr (336), ArlRS (337), SaeRS (338), YhcSR/AirSR (339, 340), Sar (336), SigB (341), Rot (342), and Mgr (343), among others. Through the technique of microarray-based gene content analysis, pathogens can simultaneously be genotyped and profiled to investigate their resistance and virulence capabilities. Using whole-genome microarrays, comparative genomics has revealed 10 major *S. aureus* lineages responsible for causing the majority of human infections (344). In addition, Sam-62, a recently developed microarray application based on 62 *S. aureus* whole-genome sequences and 153 plasmid sequences, has shown the potential to identify MRSA using microarray profiling, as it is capable of distinguishing extremely similar but nonidentical sequences (334). Typing bacteria using high-density microarrays in routine laboratories is expensive and requires specialized equipment. Alere Technologies has developed the Alere StaphType DNA microarray for *S. aureus*, which covers 334 targets, including 170 distinct genes and their allelic variants (287). It is a rapid and economic microarray assay, in miniaturized microtiter strip format, for the simultaneous detection of 8 to 96 samples. The arrays are read on the scanner, with the assignation of isolates to particular genetic lineages done automatically via software, based on their hybridization profiles.

While DNA microarray analysis is highly accurate, reproducibility within and between different laboratories needs to be established. Another shortfall is that for highly clonal species where SNPs are the target for typing them, DNA microarrays are not the best method to apply. In addition, sequences not included in the array cannot be detected. Moreover, it takes a few days to achieve conclusive results and involves expensive and tedious procedures; hence, is restricted to reference laboratories only (315).

Whole-Genome Sequencing

Whole-genome sequencing (WGS) is the ultimate identification of DNA diversity in any organism. Next-generation sequencing (NGS) provides a cost-effective method of identifying genome-wide variations. NGS is clearly advantageous over the traditional Sanger sequencing, with the ability to generate millions of reads approximating 35 to 700 bp in length. To construct a complete genome, two methods are used: *de novo* assembly and resequencing. In *de novo* assembly, multiple short sequence reads are assembled based on overlapping regions, while in resequencing, reads are assembled against a previously assembled genome sequence. WGS is an extremely powerful and highly attractive tool for epidemiological purposes (345–348). It is highly likely that, in the near future, this technology will take over from routine investigation techniques currently used in clinical practice for the identification and characterization of bacterial isolates.

The postgenomic era for *S. aureus* began in 2001, when the whole genomes of two HA strains of *S. aureus* were published by Hiramatsu and colleagues (159). This was followed by the publication of the whole-genome sequencing of a CA-MRSA strain, MW2, by the same group (349). The genome sequences revealed important information regarding the genomes of the bacteria; these strains contain circular genomes having approximately 2,800,000 bp coding for around 2,600 proteins. With appropriate bioinformatics software, these genome sequences allow one to predict the number of open reading frames, eventually deducing the amino acid sequence of the whole proteome. Several *S. aureus* genomes (including the genomes of methicillin-resistant strains) are now publically available, making the study of its biological systems possible. For example, the whole genome sequences of strains COL (350) and MRSA252 and MRSA476 (351) have allowed researchers to develop strategies to study the genetic backgrounds in detail and extract relevant information regarding virulence and resistance in these clinical isolates.

Whole-genome sequencing of MRSA isolates has revealed that these isolates are significantly similar to their MSSA counterparts from the same lineage, apart from the acquired SCC*mec* gene region (351). In addition, sequencing has revealed that the core genome of each specific lineage is genetically highly variable, especially in the carriage of surface and regulatory genes. Within lineages, up to 20% of the genome consists of mobile genetic elements (MGEs), which vary substantially between isolates, and there is evidence of horizontal transfers of these elements between isolates of the same lineage (344, 352). Sequencing of multiple isolates from geographically diverse locations, but belonging to the same clone, has been done in several studies. Studies with ST239, CC30, and ST225 have revealed that their genomes are stable, with minor variations only in SNPs and/or regular attainment or loss of MGEs (353–355).

With WGS, the key challenge is not the production of sequence data but the rapid analysis of those data to interpret and extract relevant information. This information should, ideally, enable one to directly compare results obtained from traditional typing methods such as PFGE and/or MLST and should be stored in a database that is publically accessible. Depending on the technology used, reads produced via WGS are sometimes short, making *de novo* assembly a challenge. For this reason, the term "whole genome sequence" sometimes refers to around 90% of the genome, as it is represented in contigs (with gaps between assembled regions), which result from the occurrence of dispersed or tandemly arrayed repeats (315). Of note, PFGE profiles cannot be accurately predicted without complete closure of the genome: gaps between the contigs need to close completely in order for an *in silico* restriction digest to simulate PFGE. To improve *de novo* assembly, platforms that can generate much longer reads are needed.

Pacific Biosciences has launched a "third-generation" sequencer, the PacBio, which is able to generate average read lengths of $>$10 kb, with maximum read lengths of 60 kb. Another system, developed by Oxford Nanopore and called nanopore sequencing technologies, is able to generate approximately 100-kb reads (356). The main limitations or drawbacks of these systems, however, are the very high cost, along with low read accuracy (approximately 15% error rate). Pacific Biosciences has promised improvements with longer reads and higher accuracy, and hybrid sequencing methods have also been developed to make use of more accurate short reads in conjunction with PacBio long reads (356–360).

Although costs associated with next-generation sequencing continue to decline and benchtop sequencers are now within the financial reach of many laboratories, the sequencing workflow remains too slow and genome assembly too technically laborious for implementation of routine clinical surveillance. In addition, NGS requires significant computer resources and well-trained bioinformaticians (315). Software such as BioNumerics and Lasergene exist to assist with assembly of the genomes, as well as with querying them against reference genomes or sequences. Therefore, the important prerequisite for use of WGS technology for typing microorganism is the availability of web-developed bioinformatics software pipelines for rapid processing and data analysis. Ideally, these bioinformatics tools should be simple enough to be useful in clinical settings.

In the future, WGS will eventually become the most powerful surveillance tool for outbreak investigations in clinical settings. This, however, requires standard operating procedures to identify variations by examining similarities and difference between genomes over time. In this regard, the approaches used to develop a genome-wide gene-by-gene analysis tool include extended MLST (eMLST) involving ribosomal MLST (rMLST), core genome MLST (cgMLST), whole-genome MLST (wgMLST), and a pan-genome approach (315). eMLST, in addition to utilizing conventional MLST genes, also uses extended genes present in all isolates of a species. The allelic profile produced would therefore be composed of hundreds to thousands of alleles, depending on the species under examination. rMLST uses 7 housekeeping MLST genes as well as 53 ribosomal genes. cgMLST balances the number of loci used with the maximum possible resolution. It does that by including core genome loci (ranging from 95% to 99%)

present in the majority of isolates in a given group of bacteria. These genes ideally imitate the actual genealogy within the species and therefore do not change over time. In this regard, it is important to exclude elements that are not under strict selection pressures, such as, for example, repetitive genes and pseudogenes. wgMLST includes a nonredundant set of genes present across a set of genes in a representing species. It therefore includes a greater number of genes and might also include, if present in a genome, highly variable elements such as pseudogenes and repetitive elements that are not under strict selective pressure. The pan-genome approach, however, uses the full component of genes, including the core genome, the dispensable genome (the pool of genetic material found in a variable number of isolates within the species), and the unique genes that are specific to a single strain of that species. Using this approach, the relatedness of isolates could be measured by the presence or absence of genes across all genomes within a species. Much higher discriminatory power will be endowed, allowing discrimination of very closely related isolates which fall under the same type by traditional MLST. However, for these approaches to be applicable, one must first identify and determine the core, dispensable, and unique genes at the species level within a bacterial genome. This process is assisted by the Bacterial Isolate Genome Sequence Database (BIGSdb) comparator and PubMLST database accessible software (http://pubmlst.org/software/database/bigsdb/). The software is created to store and compare sequence data for bacterial isolates, and it defines large numbers of loci and allelic profiles for each isolate. This method is highly useful for the investigation of real-time outbreaks and will probably take over as a first-line surveillance typing method once the associated costs and analysis time drop to appropriate levels.

For accurate characterization of transmission events and outbreaks, WGS has the potential to compare different genomes with single-nucleotide resolution to determine the genetic relatedness among isolates. This, however, needs extensive investigations before it can be translated into routine practice. In 2010, Harris and colleagues (353) showed that WGS can be used to describe the intercontinental and local transmission of MRSA, and the approach was successfully used to provide a high-resolution view of the epidemiology and microevolution of a dominant strain of MRSA. The global geographic structure within the lineage was revealed, as well as its intercontinental transmission through 4 decades of time. They also revealed the technique's potential to trace person-to-person transmission of MRSA within a hospital (353). WGS also permits detailed targeted analyses of variation within related species via methods based on SNPs. Being capable of illuminating the evolutionary histories of homogenous groups, SNPs can be extremely informative markers. The sequence data from isolates of interest are compared and recorded with a reference genome and nucleotides that vary within the data set. However, quality assurance criteria, such as the distance allowed between SNPs and minimum coverage, must be applied due to inherit imprecisions in single reads of high-throughput sequencing technologies to certify precision, accuracy, and consistency. The discriminatory power of whole-genome SNP analysis to discriminate MRSA outbreak and nonoutbreak strains was put into application by Koser and coworkers (361). They successfully identified MRSA isolates associated with an outbreak and clearly separated them from nonoutbreak isolates. They also created a "resistome" and a "toxome"' to demonstrate concordance between antibiotic resistance genes and the results of phenotypic susceptibility testing and to identify toxin genes, respectively. However, one outbreak isolate had a hypermutator phenotype with higher numbers of SNPs than the remaining outbreak isolates. Further investigation is needed to define a cutoff for differences in identified SNPs and develop a strategy for automated data interpretation of an outbreak in clinical practice (361).

Harris et al. (362) in 2013 used WGS to analyze an outbreak of MRSA on a special-care baby unit (SCBU) at a National Health Service Foundation Trust in the United Kingdom. They validated and expanded the findings of conventionally analyzed epidemiological data and antibiogram profiles of the outbreak strain via WGS. The isolates from colonized patients in the SCBU as well as from patients in the hospital and

community with the same antibiotic susceptibility profiles were sequenced. The authors confirmed the occurrence of transmission between mothers on a postnatal ward and in the community and identified 26 related cases of MRSA carriage. WGS also confirmed the carriage of MRSA by staff members, which allowed the outbreak to persist during periods without known infection and after a deep clean. It was therefore confirmed that WGS is a reliable tool for fast, precise, and comprehensive recognition of MRSA transmission pathway in hospital and community settings (362).

In 2013, Price and coworkers (363) used WGS in order to examine the role of colonized patients as a source of new *S. aureus* acquisition. They also tracked and compared the reliability of conventional methods of *spa* typing and overlapping patient stays to recognize patient-to-patient transmission. The authors concluded that patient-to-patient transmission explained only a minority of *S. aureus* acquisitions. In addition, the WGS contradicted transmission events indicated via conventional methods and revealed unsuspected transmission events. They therefore recommended that WGS should ideally replace conventional methods to detect nosocomial transmission events (363).

Perspectives in MRSA Typing

Over the coming decades, high-speed technological innovation will continue to evolve in the field of microbial typing. This technological advancement will undoubtedly impact the way pathogenic microorganisms can be distinguished and defined in the future. This progression toward automation, resolution, throughput, and design of new bioinformatics tools allows easier comparison, analysis, and long-term epidemiological surveillance of bacterial infections. Currently there is no single typing method available that is ideal, and depending on the setting, each method has its own advantages and limitations. One or more typing methods need to be applied, depending on the objective and the target to be achieved. In cases where speed matters to contain a local outbreak, PCR-based methods are quick and have considerable discriminatory power. Where the outbreak is disseminated in various geographical locations, PFGE seems to be a more robust approach, as it allows a reliable comparison between different laboratories. Some newer methods, such as MLST, SNPs, and microarrays, allow quick typing in urgent cases, and the results obtained are equally comparable to the gold standard PFGE. However, some of these new methods have drawbacks, including the need for highly trained staff and expensive equipment such as automated DNA sequencers. This fact makes it impractical to replace traditional techniques with these newer approaches at the international level. Moreover, the newer method will need to have an unambiguous nomenclature, which needs to be developed during the validation procedure, in such a way that historic information is preserved as well.

Traditional typing methods detect different genomic target sequences. Consequently, it is important to note that one method can detect certain strain variation which may or may not be detected via a second method. As a result, it is sometimes important to use a combination of methods for a more precise discrimination, rather than relying on a single method. A complete, unambiguous typing can be achieved with WGS, which has the potential to resolve even a single-base-pair difference between genomes. WGS and SNP-based methods surpass all methods used initially, such as *spa* typing, PFGE, MLST, etc. *spa* types could be inferred from genome assemblies with 97% and 99% accuracy to ensure backward compatibility with traditional genotyping methods. SCC*mec* typing can also be done using WGS, allowing the detection of new types or subtypes. Using SNP-based approaches during outbreak investigations, studies could rule in or out the direct transmission of closely related isolates. rMLST, cgMLST, wgMLST, or pan-genome MLST all show high discriminatory power and are of great use for standardization and interstudy comparisons, if used often. In the future, these methods promise to deliver the ultimate high-resolution genomic epidemiology. The promise of cost reductions, along with the advancement in bioinformatics software for rapid extraction of information, will nevertheless make it a primary typing tool in the near future, at least in industrialized countries. An added

bonus is that data sets produced via this method would be readily readable in the future, since it is based on the universal genetic code.

MOLECULAR EVOLUTION OF MRSA

As mentioned above, MRSA emerged within a year of the introduction of methicillin (14). After its isolation in the United Kingdom, MRSA was isolated from various other European countries (83, 86, 87, 364, 365), followed by its isolation in the late 1960s and onwards from the other parts of the world, such as Australia (88), Malaysia (89), and the United States (90). Apart from differences in the genetic background, one of the major differences between the MRSA strains and MSSA strains is the acquisition of the SCC*mec* element (25, 128–130). The worldwide dissemination of MRSA is marked by the propagation of a number of clones harboring specific genetic backgrounds (93, 366–375). Several MRSA clones have appeared and dispersed worldwide, and SCC*mec* has been incorporated on at least 20 occasions by different lineages of MSSA (94). Although most MRSA strains are hospital acquired, community-acquired strains are now been increasingly recognized worldwide and are both phenotypically and genotypically different from HA-MRSA strains.

Molecular typing techniques have been widely applied to study the molecular epidemiology of MRSA, with the aim of distinguishing isolates that are related from those that are not epidemiologically related. Numerous and massive typing efforts with large collections of MRSA in the past have produced important insights into the origin, spread, and evolutionary pathways of MRSA clones. The results of these studies suggested that the population of *S. aureus* before the acquisition of the SCC*mec* was quite diverse but contained only a few epidemic lineages (376). Under the selective pressure of antibiotics, SCC*mec* was integrated and sustained in some accommodating lineages. An epidemic MRSA strain, able to spread worldwide in the form of clonal expansion, originates whenever an epidemic lineage of MSSA acquires SCC*mec*. This scenario would explain the observation that the great majority of MRSA infections are caused by only a few epidemic clones and would also explain the relatively limited number of variations seen in the MRSA population structure. It also clarifies the fact that *de novo* SCC*mec* procurement, or horizontal transfer between *S. aureus* strains, is an infrequent event and that resistance propagation is mainly due to the clonal expansion of a limited number of successful lineages (376). However, in order to fully understand the evolutionary relationship between international MRSA strains and the fluctuation of methicillin resistance determinants, combinations of typing techniques have been adopted.

The understanding of MRSA evolution has benefited from the development of molecular tools allowing characterization of strain phylogeny via MLST, as well as of the methicillin resistance determinants via SCC*mec* typing. A combination of these techniques has revealed the fact that over time MRSA has evolved into phylogenetically distinct lineages. Additionally, MRSA has emerged on numerous instances within a given phylogenetic lineage, and most MRSA diseases are caused by only a small number of successful pandemic clones (93).

The Single-Clone Theory versus the Multiclone Theory

An MRSA clone is defined a the group of strains having indistinguishable allelic profiles and SCC*mec* elements and isolated from more than one country. The lineages are therefore classified according to their sequence type (ST), resistance phenotype, and SCC*mec* types. At least two opposing theories, the single-clone theory and the multiclone theory, have been proposed in the past to depict the relationship between current MRSA clones and the first-isolated MRSA strains. According to the single-clone theory, all MRSA clones have a mutual ancestor, and that SCC*mec* was introduced once into *S. aureus*. However, the multiclone theory implies that SCC*mec* was introduced multiple times into various genetic lineages of *S. aureus*. While there is only one study supporting the single-clone theory (377), there are now several studies that support the hypothesis of multiclone theory (93, 94, 220, 378).

To support the single-clone theory, Kreiswirth et al. (377) analyzed the clonality of 472 MRSA isolates. According to their investigation, all the isolates could be divided into 6 temporally ordered *mecA* hybridization patterns, 3 of which were subdivided by Tn*554*, a chromosomal transposon. However, they stated that each Tn*554* pattern occurred in association with only one *mecA* pattern, signifying that *mecA* divergence preceded the acquisition of Tn*554* in all cases and hence leading to the conclusion that *mecA* was attained only once by *S. aureus* (377).

In 2002, using both SCC*mec* typing and MLST, Enright and colleagues (93) studied 553 MSSA clones and 359 MRSA clones collected during 1961 to 1999 from 20 different countries around the world. They recovered five clonal complexes among MRSA populations and found that isolates with the same ST harbored different SCC*mec* elements. They also revealed that the major clones of MRSA, demarcated as groups of isolates from more than one country with the same ST and SCC*mec* element, belonged to one of the five major clonal complexes, i.e., CC5, CC8 (including the CC8-ST239 sub group), CC22, CC30, or CC45, suggesting that the SCC*mec* element was acquired at multiple times by *S. aureus* strains of different genetic backgrounds (93). They also showed that ST8-MSSA, belonging to CC8, is the ancestor of the first-isolated MRSA strain, ST250-MRSA-I, differing from ST8 by a point mutation in the locus *yqiL*. This ST8-MSSA has been outlined as the frequent cause of epidemic MSSA disease and has gained SCC*mec* types I, II, and IV. The Iberian clone, ST247-MRSA-I, is another clone closely related to ST250, differing at the *gmk* locus by a single point mutation. It was once a major clone isolated from European hospitals. The study also showed that apart from CC8, all other CCs (i.e., CC5, CC22, CC30, and CC45) were derived from epidemic MSSA lineages that had acquired SCC*mec*, since they differed from each other and the predicted ancestor of CC8 at 6 or 7 loci used in MLST. In addition, ST59 and ST996 are unique MRSA isolates and were genotypically very different from all other MRSA isolates. Hence, the study concluded that the presence of *mecA* in such widely divergent genotypes is most likely the consequence of horizontal gene transfer into distinctly related *S. aureus* lineages (93).

Another study involving 147 MRSA isolates from diverse geographical locations indicated that MRSA has emerged at least 20 times by acquiring SCC*mec* and that this acquisition by MSSA was four times more common than the replacement of one SCC*mec* with another one, again supporting the multiclone theory (94). In addition, it has also been demonstrated that MRSA clones belonging to the major CCs are much easier to transform with *mecA*-expressing plasmids, suggesting that the genetic background of the strain plays an important role in the stability of SCC*mec* elements. Fitzgerald et al. (378) used DNA microarray technology and concluded that the *mec* gene has been horizontally transferred at least 5 times into distinct genetic backgrounds of *S. aureus*, further supporting the multiclone theory that MRSA strains have evolved multiple independent times.

Mechanisms of Clonal Divergence: Mutation versus Recombination

The remarkable ability of *S. aureus* to adapt to the selective pressure of antibiotics has been discussed earlier in this review. Strains resistant to penicillin and methicillin emerged within a couple of years following clinical antibiotic use (14, 379). This, along with MRSA strains increasingly evolving resistance to vancomycin, is a major public health concern (69, 380). Moreover, the expansion of MRSA from being only a nosocomial only pathogen to being also a community- and livestock-associated pathogen has triggered the interest of researchers in better understanding how natural populations of MRSA have evolved (381, 382). Additionally, the fact that some of these strains have the capacity to grow in frequency and create epidemics highlights the importance of understanding the mechanism behind the emergence of new strains with extraordinary pathogenic potential and new biological characteristics.

Crucial for the survival of bacteria is their ability to generate genetic variations, one mechanism of which could be horizontal gene transfer, enabling them to rapidly adapt to new ecological niches (383). The mechanism of horizontal or lateral gene transfer

includes the introduction of novel sequences in the chromosome, such as SCC*mec* in case of *S. aureus*, and/or homologous recombination resulting in the development of a new haplotypes (383). This introduction of new gene combinations may play an important role in the diversification process and could lead to the adaptation of bacteria. Recombination, for example, could increase the pathogenicity of a bacterium or increase its ability to disseminate, by diffusing genetic material throughout the rest of the bacterial population (384).

In order to understand the creation of genetic diversity in a given species, it is vital to determine the mechanism of the evolutionary process that actually generate this variation, i.e., point mutation and/or recombination. There are several studies highlighting the mechanism of clonal evolution among *S. aureus* and/or MRSA strains (93, 94, 285, 290, 385, 386).

As mentioned above, Enright et al. (93) studied 912 MSSA and MRSA isolates from 20 different countries and concluded that these isolates belong to five major clonal complexes. They pointed out the presence of three major STs within CC8, apart from ST8. This included ST250, which differs from ST8 by a point mutation in the *yqiL* gene, as well as ST247, which is similar to ST250, differing again by a single point mutation in the *gmk* locus. However, within CC8, ST239-MRSA-III, corresponding to a Brazilian clone, emerged as a result of the homologous recombination of a chunk of DNA 557 kb long from the chromosome of ST30 into ST8-MRSA-III (93). In 2003 Feil et al, (290) estimated the rate of recombination within clonal complexes and concluded that the specific alleles and individual nucleotides are more prone to mutation, being at least 15-fold more likely to change by point mutation than by recombination. The study noted that 12 out of 13 single-locus variants appeared to arise due to point mutation, compared to one that possibly arose via recombination. However, the authors pointed out that although there is less evidence of recombination in *S. aureus* and recombination has had a negligible impact on the diversification of the core genome of this species, going further back in the phylogenetic tree and considering longer time scales suggest that at least some recombination has occurred (290).

Studies within the last 15 years, however, seem to favor recombination as a mechanism for evolution. Kuhn and colleagues (385) showed evidence of homologous exchange, showing the passage of an allele from CC5 to CC8. They showed that all alleles of CC8 for *clfB* were acquired from other CCs, and the ancestral allele could not be detected in their collection of isolates. They also showed that recombination in *S. aureus* might occur more frequently between closely related strains than between phylogenetically distant strains, concluding that the phenomenon of recombination might contribute significantly to the evolution of CCs (385). Their hypothesis that recombination within closely related strains is more common than that between phylogenetically distant strains was also supported by Waldron and Lindsay (387), who discovered a restriction-modification system, SauI type I, on the chromosomes of all their sequenced strains. This system functions to block horizontal transfers between *S. aureus* strains of different CCs and provides a mechanism by which gene flow and recombination may be higher within CCs than between CCs (387).

More recently, Basic-Hammer and coworkers (388) proceeded to measure the relative levels of recombination within and between CCs in order to test the hypothesis that recombination rates are higher within CCs than between CCs and to understand how genetic diversity is created and maintained in a highly clonal *S. aureus* population. Although they were not able to show a high level of recombination within CCs, they did indicate it as being an important mechanism generating diversity within CCs, since they did detect a few recombinant genotypes having significant impact on the genetic diversity within CCs. Although they were not able to conclude that recombination occurs at a higher frequency within CCs than between CCs, their study did nevertheless highlight the importance of recombination in the evolution of highly clonal *S. aureus* and suggested that recombination, when combined with demographic mechanisms and selection, might favor the rapid creation of new clonal complexes with increased fitness (388). In addition, a study comparing five genomes of *S. aureus* also revealed

signs of recombination at 45 genes, many of which were likely involved in pathogenesis, confirming the role of recombination in the emergence of epidemic, virulent and resistant strains (389).

Clonal Population Dynamics: Temporal Evolution of MRSA

S. aureus appears to be a very adaptable organism, thriving in unique environments, with the constant emergence of new strains causing new types of disease and showing rapid epidemiological spread and/or response to interventions, resulting in a significant impact on the health care. The emergence of each new strain stimulates the search for new genes or elements in order to increase our understanding of pathogenesis, diagnostics, and targets for therapy. The first-ever example of *S. aureus* adaptability includes acquisition of SCC*mec* conferring methicillin resistance in *S. aureus* (MRSA) and the strain COL (14, 350). Epidemic HA-MRSA is another example, with dramatically rising incidence worldwide over the past 20 years (390). CA-MRSA evolved independently of hospital strains, being associated with severe skin and soft tissue infections and rare cases of fatal pneumonia in young children and healthy adults (27). The emergence of livestock-associated MRSA (LA-MRSA) is another example of the ever-changing nature of these strains (64, 391–393). Vancomycin-resistant *S. aureus* (VRSA) strains have acquired vancomycin resistance genes from enterococci via horizontal transfer (394). Furthermore, although HA-MRSA and CA-MRSA are different entities having their own ecological niches, interchange in these epidemiological alcoves has been observed, with reports of CA-MRSA being isolated from hospitals and HA-MRSA being isolated outside hospitals (395–398). Moreover, the invasive potential of HA-MRSA strains varies considerably, suggesting the dynamic nature and/or temporal evolution of *S. aureus* populations (399).

The reason for the dynamic nature of MRSA populations is not yet clearly understood. MRSA evolved into a number of different lineages by variation and recombination, with selection into a few successful lineages. However, in each geographical location, only one or two of these MRSA lineages predominate (400). Moreover, the successful lineage in a particular geographical location reaches a peak before starting to decline and then disappearing, with the emergence of a new strain. This phenomenon of clonal replacement, where clones that were disseminated widely during a certain time period becomes less dominant and are replaced by other epidemic clones, has been observed worldwide. For example, in Hungary, the Hungarian clone (ST239-III) was the most predominant clone during 1994 to 1998. This clone disappeared and was replaced by the southern German clone (ST228-I) and the New York/Japan epidemic clone (ST5-II), representing about 85% of the isolates in 2001 to 2004 (102). In Portuguese hospitals, the Portuguese clone (ST239-III variant) was the most prevalent clone during the mid-1980s but was later replaced by the Iberian clone (ST247-IA) in 1992 to 1993 and eventually by the Brazilian clone (ST239-III/IIIA) (366). Between 1994 and 2002, the northern German clone (ST247-I) and the Hannover clone (ST254-IV) were the most prevalent clones in German hospitals. However, by the 2000s, they were replaced by the Berlin (ST45-IV), southern German, Barnim (ST22-IV), and Rhine-Hesse (ST5-II) MRSA epidemic clones (401). It is worth mentioning that ST254-IV, ST45-IV, and ST22-IV are community-associated clones. As mentioned above, despite the fact that HA-MRSA and CA-MRSA are genetically different and have their own epidemiological niches, switches in these niches have been observed, with reports of CA-MRSA being isolated from hospitals. In the Czech Republic, the Brazilian (ST239-related) clone and the Iberian (ST247-IA) clone were replaced by the arrival of epidemic EMRSA-15 (ST22) (398).

The epidemiology of major MRSA lineages resembles a wave of expansion followed by population equilibrium and then decline (400). MRSA CC22 (ST22, EMRSA-15) remains the dominant lineage in United Kingdom hospitals. However, this dominant MRSA clone (ST22-IV) belongs to a CA-MRSA lineage, again emphasizing the fact that CA-MRSA clones are being isolated from hospital settings. Nonetheless, MRSA epidemiology has been shifting in United Kingdom health care settings. A fall in prevalence

of EMRSA-16 (CC30, ST36), together with a decline in isolation rates, has been widely reported in the literature (400, 402, 403). Hsu et al. (404) examined the evolutionary dynamics of MRSA within the Singaporean health care system over 3 decades and reported the predominance of MRSA ST239 as the dominant clone in the mid-1980s. However, it lost its dominance to the ST22 CA-MRSA clone around the beginning of the millennium, when this clone rapidly spread through Singaporean hospitals, displacing the endemic ST239 population. Coalescent analysis revealed that although its genetic diversity was masked initially with the appearance of ST22, from 2007 onwards the genetic diversity of ST239 began to increase once more (404).

In Asia, such as in Taiwan and China, ST254-IV, a CA-MRSA clone, was the major prevailing clone during the early 1990s but lost its prevalence to ST239 in the late 1990s and eventually to ST5 and ST59 (405–409). ST59, a CA-MRSA clone, appears to be the most prevalent clone in Asia, and although it has been reported sporadically in North America, it has not become a prevalent clone in that part of the world. USA100 was the typical nosocomial strain in the United States but currently seems to have lost its dominance to USA300, again a CA-MRSA clone, which is increasingly isolated from hospital settings in North America (410, 411). When first recognized, USA400 was the most dominating CA-MRSA clone in the United States (349); however, at the turn of the century it lost its dominance to USA300, which became the most prevalent genetic background in the contiguous 48 states in the United States (412–418). Nevertheless, like USA400, USA300 now seems to be declining as well (419). The reason for this dynamic nature of the clonal MRSA population is an area that needs further study.

Global Clonal Structure

Since the 1940s, MRSA evolution has been analogous to that of penicillin-resistant *S. aureus* (420). MRSA is now pandemic with worldwide broadcasting of strains, including the dissemination of HA-MRSA clones from the 1960s, CA-MRSA clones from the mid-1990s, and LA-MRSA clones from the beginning of this century (15, 421, 422). Although the mentioned years mark the official beginnings of these particular MRSA strains, they existed in nature for quite some time before they were first reported.

Ever since its detection in 1959, MRSA has emerged into different clones. However, of all MRSA clones detected worldwide, the most frequently reported clones belong to one of the five major clonal complexes (CCs), i.e., CC5, CC8 (including the CC8-ST239 subgroup), CC22, CC30, and CC45 (15, 290, 423). Of these major CCs, CC5 and CC8 are the most prevalent clones throughout the world. Both of these CCs contain various STs, differing by point mutations in the genes used for assigning MLST STs, and are distributed in many different countries/regions of the world. Figure 5 shows a population snapshot of MRSA worldwide. While CC30-ST36 is common in the United States and the United Kingdom and CC45 is common in the United States (ST45-II) and Europe (ST45-IV/V), CC22 is a globally widespread CC (15, 287, 370, 423–425). The CC8-ST239 subgroup, CC5 (ST5), and CC22 (ST22) are the most frequently reported CCs in Asian countries (120, 374, 426–430). Evidence suggests that the CC8-ST239 subgroup (ST239-III) lineage from South Korea, Hong Kong, Taiwan, and Vietnam and CC5-ST5-II from South Korea and Sri Lanka have traveled from hospitals into the community (120). ST239-III and ST5-II are both the major HA-MRSA clones. The presence of these isolates in the community again demonstrates the exchange in ecological niches of HA-MRSA and CA-MRSA clones. Predominant clones in Latin America include CC5 (ST5), CC8 (ST239 subgroup), and CC30, while in Africa the CC8 (ST239 subgroup), CC5 (ST5), and CC30 (ST36) lineages predominate. CC8 (ST612) has been described only infrequently in South Africa and Australia (396, 425, 431, 432).

There is evidence that some HA-MRSA clones have dispersed internationally and have evolved multiple times. For example, SNP analysis of CC5 (ST5) validates that this clone evolved numerous times in several countries via the attainment of the SCC*mec* cassette in a local CC5 (ST5) MSSA strain (286). Similarly, phylogenetic analysis revealed the intercontinental dissemination and hospital transmission of CC8-ST239 subgroup isolates through North America, Europe, South America, and Asia (353). In addition, in

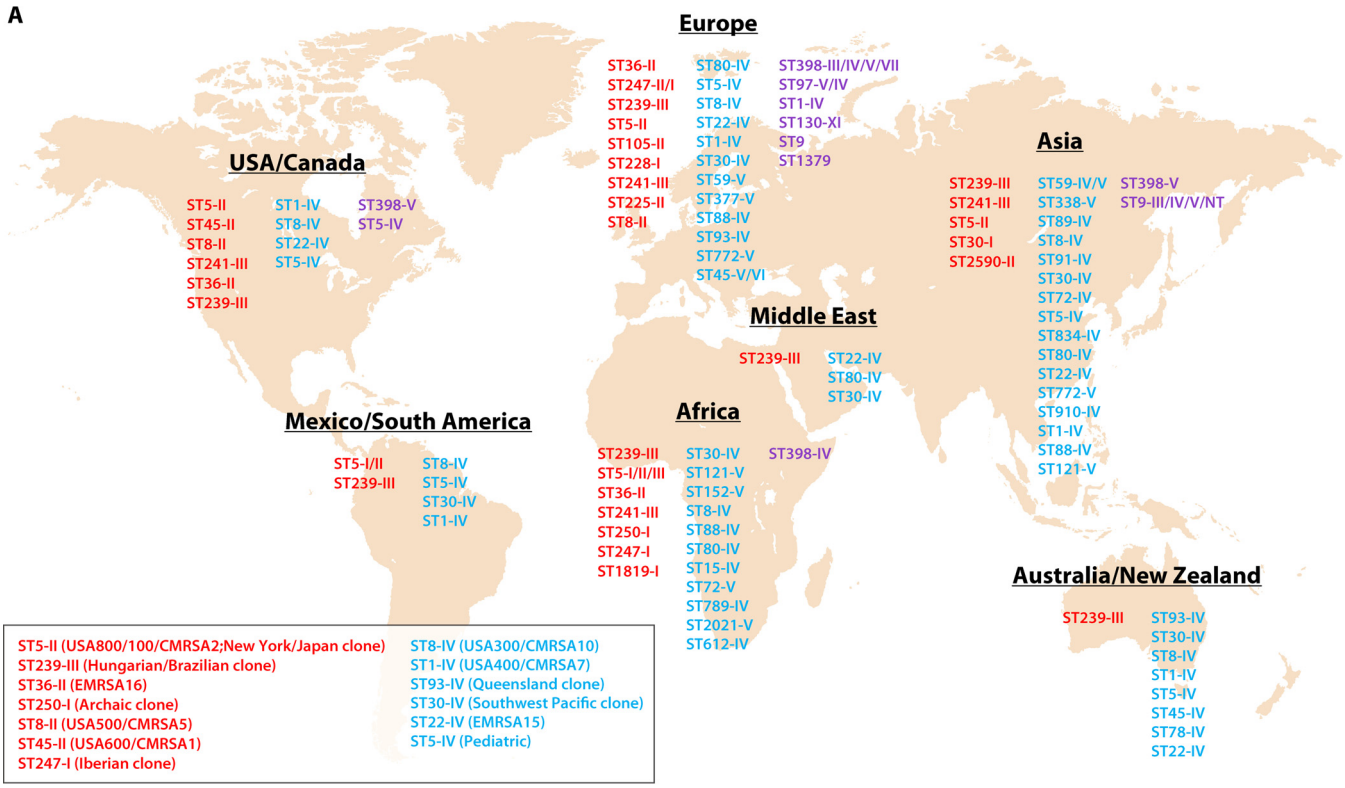

FIG 5 Global MRSA population snapshot. (A) MRSA population structure, showing the major clones reported in each continent or region along with the commonly associated SCC*mec* types. While there is overlap in terms of STs between the continents, there are many STs that show marked region specificity. Red represents STs belonging to HA-MRSA, blue represents those belonging to CA-MRSA, and purple represents belonging to LA-MRSA. Alternate or traditional names for the mainly predominant epidemic strains are at the bottom. (B) Evolutionary relationships between the predominant MRSA STs listed in panel A are represented by eBURST analysis (compared with the international MLST database, updated 31 January 2018). Individual STs, as well as the clonal complexes to which they belong, are indicated. As in panel A, red (HA-MRSA), blue (CA-MRSA), and purple (LA-MRSA) are used to distinguish the types. Peach color is used to denote STs present in more than one source group.

the 1990s, the CC8-ST239 subgroup dispersed from South America to Europe and from Thailand to China (433). This dispersal pattern, however, may not be true for all HA-MRSA strains. Analysis of a large collection of MRSA isolates from southern Europe, the United States, and South America revealed that nearly 70% of the isolates which dominated internationally up to the early 2000s belonged to five major pandemic clones, including Iberian or UK-EMRSA5 (ST247-IA), Brazilian (ST239-IIIA), Hungarian (ST239-III), New York/Japan (ST5-II), and the pediatric (ST5-IV) clone (93). Again it is worth mentioning here that although ST5-IV is a CA-MRSA clone, it was also isolated from hospital settings.

CA-MRSA has evolved, and the number of cases has subsequently increased rapidly. Ever since its emergence and isolation, CA-MRSA strains have evolved differently in separate geographical areas. The earliest reported cases of CA-MRSA in the United States were caused by USA400 (ST1-IV) (434). This clone was then replaced by USA300 (ST8-IV), which is currently the most common cause of CA-MRSA infections in North America (435). In the United States, the spread of CA-MRSA was almost exclusively due to the spread of these two major clones, USA300 and USA400; however, USA300 rapidly took over and became endemic (412–418). There is evidence for the international spread of USA300 and USA400 (45, 436–438), which has sporadically also been reported in Europe as well as in travelers visiting these countries (439).

Compared to those in the United States, CA-MRSA infections in Europe have remained infrequent, with ST80 (CC80) being the most predominant circulating clone. Unlike the pattern seen in the United States, different CA-MRSA clones belonging to diverse STs have been reported in Europe, including ST1, ST5, ST8, ST22, ST30, ST59, ST80, ST88, ST93, and ST772 (330, 440, 441). To date, USA300 has not been widely reported from western Europe and ST80 has not been reported from North America, although the precise reason for this remains unknown (442). In the United Kingdom, most infections within the community are caused by EMRSA-15 (ST22) and EMRSA-16 (ST36), which are also the major clones in hospitals (443). ST80 is also present in the United Kingdom but accounts for only a small proportion of isolates (444). A new clone of swine origin, ST398, has been reported in Europe and described as being transmitted to humans (392, 445). In East Asia, ST59 is the most dominant CA-MRSA clone; however, ST30, ST239, and ST5 also prevail in the community (120). In addition, there are reports of ST59-IV, ST30-IV, and ST72-IV (CA-MRSA clones) being spread from the community to hospitals and of ST239 and ST5 (HA-MRSA clones) moving in the opposite direction, again pointing toward the interchange in the epidemiological niches of these MRSA clones (120). In eastern Australia, ST30 seems to be the most frequently circulating clone (437).

Based on Swedish data, the greatest risk of travelers importing CA-MRSA was from, in decreasing order, North Africa and the Middle East, sub-Saharan Africa, Oceania, East Asia, South America, the northeast Mediterranean and North America (446). Intercontinental exchange of ST8, ST30, ST59, and ST80 has been demonstrated, and the data indicate that the main areas of spread have been between North and South America, between individual European countries, and between North African and East Asian/Australian regions. Trends include the movement of ST8 from the United States to Europe and of ST80 from the Middle East to Europe and then to Asia (447).

Figure 5 summarizes the global population structure of MRSA. HA-MRSA worldwide predominant clones are likely clustering, primarily in 2 clonal complexes (CC5 and CC8) which are also closely associated with each other genetically. On the other hand, CA-MRSA clones are more diverse, dispersing in many CCs with only a few being closely associated genetically with each other, while many are not interrelated. It is also evident from Fig. 5 that some CA-MRSA and HA-MRSA clones do overlap each other, showing a very close genetic association. Conversely, the LA-MRSA main clone ST398 lies far apart, demonstrating that it has little correlation with either CA-MRSA or HA-MRSA clones.

HOSPITAL-ASSOCIATED MRSA

Hospital-associated MRSA (HA-MRSA) remains one of the most common causes of multidrug-resistant hospital-associated nosocomial infection (390). While patients with compromised immune systems or other comorbid conditions are at increased risk, any patients with increased infection risk profiles are at higher risk of HA-MRSA infections. Since the emergence of MRSA in 1960, it has been recognized as a nosocomial pathogen with very high incidence rates in many parts of the world, and even today it is a significant cause of illness among the population (448). Infections with HA-MRSA are often clinically difficult to treat due to their resistance to multiple classes of antibiotics, creating an enormous economic and logistical problem, with significant mortality and morbidity (390). Meta-analyses show that MRSA bacteremia is twice as likely to prove fatal as MSSA bacteremia, resulting in longer hospital stays and increased utilization of hospital resources (including pharmaceuticals and staffing), resulting in a 3-fold increase in treatment costs (17–20). Infection control strategies, including handwashing vigilance programs, antibiotic stewardship policies, mandatory infection rate reporting laws, and screening and decolonization programs, have helped decrease the incidence of MRSA infections (449).

HA-MRSA describes the epidemiological behavior of particular MRSA strains that are specifically successful in hospital settings. They may also be endemic in certain health care institutes, causing epidemic levels of infections that are typically above the threshold *S. aureus* infection rate. They are the dominant MRSA clones causing the majority of this type of infections, with only a small number of clones that are actually successful (448, 450). Epidemics due to the spread of successful clones have been registered in practically every geographic region (15). Many factors contribute to the success of this pathogen; however, its capacity to persist as a commensal, its ability to combat multiple antimicrobial agents, and its multitude of virulence determinants, often with redundant functions, are among the most important contributors (105, 451, 452). HA-MRSA strains are among the most common cause of intravenous-catheter-associated infections, ventilator-associated pneumonias, nosocomial infective endocarditis, and surgical wound infections, even in industrialized countries such as the United States (453–455).

As HA-MRSA accounts for a high proportion of infection in hospitalized patients, in order to control the infection rate, it is important to understand the associated risk factors (448). One of the major MRSA infection-associated risk factors is nasal colonization with *S. aureus*. The relationship between MRSA colonization and the development of infection is complex and not clearly understood; nonetheless, it appears to be the major risk factor for MRSA infections, especially during hospitalization (2, 456–459). It has been estimated that nasal MRSA carriers are 3.9 times more likely to develop nosocomial bacteremia than the carriers of susceptible strains (457). Moreover, in most cases the isolated bloodstream strain belongs to the same genotype as the nasal strain (458, 460, 461). MRSA colonization in hospitalized patients is associated with old age, prolonged hospital stay, enteral feeding, catheters and intravenous medical devices, the presence of skin lesions, wounds, ulcers, and receipt of antibiotics (462–464). Other factors associated with the development of HA-MRSA infection include hemodialysis, diabetes mellitus, cancer, history of MRSA colonization, previous or prolonged hospitalization and/or surgery, ICU admission, and prior antimicrobial therapy (464–469). Furthermore, admission into a hospital room previously occupied by an MRSA-infected patient has also been found to be associated with increased risk of MRSA acquisition. This risk, however, accounted for less than 5% of all incident MRSA cases (470).

Major Continental MRSA Strains/Clones

Not all MRSA clones are able to establish a successful foothold in the health care environment. When first recognized in 1961, the incidence of MRSA infection was very low and the isolates did not become endemic in hospital settings, nor did they spread significantly between hospitals. However, in the 1980s and 1990s, epidemic MRSA emerged in most developed countries due to the emergence of these new clones (15,

TABLE 1 Molecular characteristics of HA-MRSA clones around the world[a]

Location	Standard name	Common name	Molecular characteristics					
			PFGE	MLST	*spa* type	SCC*mec* type	*agr* type	PVL
USA/Canada	ST5-MRSA II	USA100	USA100/CMRSA2	ST5	t002/t242/t311	II	2	–
	ST45-MRSA II	USA600	USA600/CMRSA1	ST45		II	1/4	–
	ST36-MRSA II	USA200	USA200/CMRSA4,8,9	ST36	t018	II	3	–
	ST8-MRSA II			ST8	t064/t068	II	1	
	ST241-MRSA II			ST241	t037/t138	II	1	–
	ST239-MRSA III		PFGE type B	ST239	t037/t138	III	1	
Mexico/South America	ST5-MRSA I	Cordobes/Chilean clone	PFGE type C	ST5	t002/t003	I	2	–
	ST239-MRSA III	Brazilian clone	PFGE type B	ST239	t037	III	1	–
	ST5-MRSA II	New York/Japan clone	USA100/CMRSA2	ST5	t002/t003/t311	II	2	
Europe	ST36-MRSA II	EMRSA-16	USA200/CMRSA4,8,9	ST36	t018	II	3	
	ST5-MRSA I	EMRSA-3	PFGE type C	ST5	t002/t003	I	2	–
	ST239-MRSA III	EMRSA-1,4,11, Vienna/ Brazilian/Hungarian clone	PFGE type B	ST239	t037/t138	III	1	
	ST247-MRSA I/II	EMRSA-5,7 Iberian clone	PFGE type A	ST247	t051/t052	I/II	1	–
	ST241-MRSA III	UK-Finland clone		ST241	t037/t138	III	1	–
	ST8-MRSA II			ST8	t064/t068	II	1	
	ST105-MRSA II			ST105		II	2	
	ST228-MRSA I			ST228		I	2	
	ST5-MRSA II	New York/Japan clone	USA100/CMRSA2	ST5	t002/t003/t311	II	2	
	ST239-MRSA III variant	Portuguese clone	PFGE type B	ST239		III variant	1	
	ST225-MRSA II			ST225		II		
Africa	ST239-MRSA III	Brazilian/Hungarian clone	PFGE type B	ST239	t037	III/III mercury	1	
	ST241-MRSA III			ST241		III	1	
	ST250-MRSA I			ST250	t194/t292	I	1/4	
	ST5-MRSA I		PFGE type C	ST5	t002/t003	I	2	–
	ST36-MRSA II		USA200/CMRSA4,8,9	ST36	t018	II	3	
	ST5-MRSA III		USA100/CMRSA2	ST5		III	2	
	ST247-MRSA I			ST247	t051/t052	I	1	–
	ST1819-MRSA I			ST1819		I	1	–
Middle East	ST239-MRSA III		PFGE type B	ST239	t037/t030	III	1	–
Asia	ST239-MRSA III		PFGE type B	ST239	t030/t037	III/IIIA	1	–
	ST241-MRSA III			ST241		III/IIIA	1	–
	ST5-MRSA II	New York/Japan clone	USA100	ST5	t002/t003/t242	II	2	
	ST254-MRSA I			ST254		I	1	
	ST30-MRSA I			ST30	t019/t318	I	3	
	ST2590-MRSA II			ST2590	t002	II	2	–
Australia/New Zealand	ST239-MRSA III		PFGE type B	ST239	t030/t037	III	1	–

[a]–, absence of PVL. Blank entries indicate a lack of available information.

471). The new clones were able to rapidly disseminate within and between hospitals, causing infection well above the baseline infection rate for *S. aureus* (472, 473). A study conducted in 2012 by Hetem and coworkers (474) suggested that successful HA-MRSA clones have a tendency to transfer at higher rates between hospitalized patients. The distribution of successful HA-MRSA clones varies with geography (448, 450); hence, the dominant clone in North America might not be dominant, or even present, in Europe, for example. Figure 5 shows a snapshot of HA-MRSA populations geographically. The incidence of HA-MRSA in each country is reported as the proportion of *S. aureus* infections in hospitals, and therefore baseline incidences cannot be easily compared among different health care settings. Eastern Asia has a high incidence of HA-MRSA (greater than 70%), while the Netherlands, Denmark, Norway, Sweden, Finland, and Iceland are included in the countries with the lowest prevalence rates, at less than 5% (448). As mentioned above, it is not clear why certain MRSA clones are successful in specific regions of the world while remaining completely nonexistent in other parts of the world.

A summary of the molecular characteristics of some common HA-MRSA clones around the world is given in Table 1.

United States and Canada. *S. aureus* is one of the most common etiological agents of bloodstream infection, as well as a frequent cause of infective endocarditis, in the developed world (475–477). Among these *S. aureus* infections, MRSA accounts for more than 60% of isolates in ICU settings (478). According to CDC's estimate, MRSA septicemia was responsible for more than 30,000 hospitalizations in 2000 in the United States (479). From 1999 to 2005, it has been estimated that hospitalizations due to MRSA have increased more than 80% (480).

The epidemiology of MRSA is continuously evolving. Tsuji et al. (481) characterized 50 HA-MRSA isolates obtained from infected patients at the Detroit Medical Center, Detroit, MI. They concluded that among all HA-MRSA isolates included in their study, 75% of them belonged to the USA100 PFGE profile carrying SCC*mec* II (481). The USA100 strain is typically associated with nosocomial acquisition in the United States (410, 411). Chua and colleagues (395) described the molecular epidemiology of MRSA bloodstream isolates collected from Henry Ford Hospital (Detroit, MI) during July 2005 to February 2007. They evaluated 210 isolates, and the predominant strains again included USA100. Recently, another MRSA strain, USA600, predominantly carrying SCC*mec* II and accounting for less than 5% of MRSA bacteremia, has also been reported in Detroit, MI (482). USA600 is clonally related to Berlin MRSA strain ST45, first reported as an epidemic strain in Germany (483) and the Netherlands (484) as well as in Ontario, Canada (485).

During the Canadian Ward Surveillance (CANWARD) study from 2007 to 2009, 3,589 *S. aureus* isolates were collected from all geographic regions of Canada (486). Among those isolates, 889 were identified as MRSA. HA-MRSA comprised 72.4% (644) isolates, of which the predominating genotype belonged to CMRSA2 (USA100/800), accounting for 83.5% of these HA-MRSA isolates. Other HA-MRSA isolates identified included CAMRSA3/6 (11.8%), CMRSA1 (USA600, 2.0%), and CMRSA8, CMRSA9, and CMRSA4, accounting for less than 1%. Among these HA-MRSA strains, the majority (81.2%) carried SCC*mec* II (486). Bush and coworkers (487) recently (April 2011 to March 2013) conducted a complete provincial surveillance of all acute-care facilities in Alberta, Canada, and reported the predominance of CMRSA2/USA100 (65.1% of all HA-MRSA strains) as the most predominant HA-MRSA strain in Alberta.

Mexico and South America. MRSA is an increasing problem in Latin America, with the frequency of nosocomial MRSA infections exceeding 50% in greater than half of South America. Surveillance programs, however, have just been initiated and are still undergoing refinement, meaning many limitations remain. Data are unobtainable from several countries, while data from countries participating in regional surveillance programs may not reflect the true state and may not be comprehensive. Most communities and populations within the continent are served by small community centers where resources and microbiological facilities may not be ideal. The available data reflect a few larger hospitals and/or reference laboratories. Nonetheless, most HA-MRSA clones detected in South America belong to two major lineages, CC8 and CC5, and the identified genotypes within these lineages are characterized as ST5-I and the CC8-ST239-III subgroup.

The earliest study from Brazil indicates the spread of a single MRSA clone in 8 of 9 hospitals in Sao Paulo between 1990 and 1992 (488). The following year, MRSA isolates collected from 5 different teaching hospitals from various parts of the country also indicated the spread of a single epidemic clone, the Brazilian clone (ST239-III) (489). Since then, variants of this clone, collectively termed the "Brazilian epidemic clonal complex" (BECC), have been widespread and highly prevalent in the country's hospitals (490). Some studies, however, suggest that the frequency of these clones among hospital MRSA isolates has decreased to approximately 50% of all *S. aureus* isolates (491, 492). In Argentina, earlier studies confirmed the presence and dominance of the Brazilian clone (ST239-III) (493–495). However, in 2002, Sola et al. (496) reported codominance of the Brazilian clone along with the Chilean clone (ST5-I) in one of the largest cities in Argentina. Isolates belonging to the Chilean clone had a multiresistant

phenotype. The clone increased in prevalence over time, causing nosocomial outbreaks in Cordoba and Buenos Aires (494, 496–500).

Aires De Sousa and coworkers (494) reported the presence of a variant of the Brazilian clone, along with the Chilean clone, in Chile in 2001. Later studies, however, indicated that the Chilean/Cordobes clone predominates in the country (431). In 2013 Medina and colleagues (501) demonstrated the stability of the Cordobes/Chilean clone over time as the major HA-MRSA clone in southern Chile.

In Colombia, studies have noted the presence and dissemination of the Cordobes/Chilean clone (502, 503), while in Paraguay, the Brazilian clone coexisted with the dominant Cordobes/Chilean clone (504). In Uruguayan hospitals, the presence of the Brazilian clone was demonstrated between 1996 and 1998 (494), and this clone was isolated from 4 of the 5 hospitals included in a study by Senna and coworkers (505), though it predominated in only one of the hospitals. In another study, all 244 MRSA isolates from three major hospitals in Trinidad and Tobago (isolated from Jan 2000 to December 2001) belonged to a single major PFGE genotype. These clones had a PFGE banding pattern similar to that of the Canadian strain CMRSA6 (506). Finally, a study from Mexico found that in 2001, the New York/Japan clone (ST5-II) was introduced into hospitals, completely replacing the previously dominant clone in that country within a year (507).

Europe. Within the health care setting, MRSA infections are predicted to affect more than 150,000 patients within the European Union (EU) annually, adding €380 million in costs to the EU health care systems. Among EU member states, marked variability exists in the proportion of *S. aureus* strains that are resistant to methicillin, ranging from 1% to more than 50%, as shown by Pan-European surveillance data on bloodstream infections (390). However, in the past 10 years, countries with higher rates of endemic MRSA infection have shown significantly decreased MRSA bacteremia rates. In 2008, over 380,000 hospital-acquired infections due to antibiotic-resistant bacteria were estimated to be acquired in EU member states, Iceland, and Norway annually. Of these bacteria, MRSA accounted for 44% of infections, 22% of excess deaths, and 41% of extra hospitalization days (390).

The majority of HA-MRSA strains isolated from Europe have emerged from five *S. aureus* clonal complexes, i.e., CC5, CC8 (CC8-ST239 subgroup), CC22, CC30, and CC45, as defined by MLST (508). Following the initial isolation of MRSA from a United Kingdom hospital in 1961 (14), MRSA remained uncommon for several years. This situation, however, changed in 1967, when a strain of multiresistant MRSA, belonging to phage type 83A, was isolated from various parts of the world, including England, Denmark, France, Switzerland, etc. (83, 95, 364, 365, 509). Although widely disseminated, this strain began to decline in the 1970s to 1980s (510). The cause of this decline remains unclear. However, this decline was followed by the emergence of gentamicin-resistant MRSA strains reported from several countries, including the United Kingdom and Ireland (511–513). After 1982, an epidemic multidrug-resistant MRSA strain caused outbreaks in London hospitals and was believed to be imported by an Australian health care worker (HCWs) (514, 515). A numerical suffix for epidemic MRSA was introduced by the staphylococcal reference laboratory of the United Kingdom public health laboratory service after the second epidemic MRSA strain became prevalent in United Kingdom hospitals (516). Sixteen epidemic MRSA strains were identified until 1995 throughout England and Wales; however, only three of these strains, EMRSA-3, EMRSA-15, and EMRSA-16, were reported throughout the 1990s. Among these strains, EMRSA-15 (ST22) and EMRSA-16 (ST36) showed the most dynamic behavior and have been endemic in United Kingdom hospitals for over 20 years, in addition to in other European countries (517). Although ST22-IV is a major CA-MRSA clone, its predominance in hospitals around the world once again indicated a change in the ecological niches of the MRSA strains. The rapid spread of MRSA infections, along with their high prevalence in United Kingdom hospitals, led the United Kingdom government to introduce stringent infection control legislation. This legislation resulted in a decrease in the rates of nosocomial

MRSA infections in the United Kingdom, with EMRSA-16 rates declining more rapidly than EMRSA-15 rates, likely due to strain-dependent factors (402, 403).

The MRSA prevalence in European countries varies from less than 3% in the Nordic countries and the Netherlands to more than 50% in southern European countries and the United Kingdom (286, 368, 518, 519). A 2011 EARSS-Net report showed stabilization or a decreasing MRSA trend in several European countries. Mlynarczyk et al. (520) conducted a molecular characterization of HA-MRSA strains isolated from hospitalized patients in transplant wards in a hospital in Warsaw, Poland, over a 17-year period from 1991 to 2007. Seventy-eight percent of the isolated strains belonged to CC8, including ST239-III (EMRSA-1, -4, -11, Brazilian, Hungarian clone), which occurred with a frequency of 35.9%, ST247-I (EMRSA-5, -7, Iberian clone; 20.5%), and ST241-III (UK-Finland; 5.15%). Although ST239-III was found to be the most dominant clone in Warsaw hospitals, the predominance of clones fluctuated over the whole study period (520). In 1994, 50% of the isolates comprised the Brazilian-Hungarian clone. In 1996, the Iberian clone (53.9%) was most frequently isolated, whereas from 2005 to 2007, the Brazilian-Hungarian ST239-III clone (41.3%) was again the most predominant one (520). In 2010 to 2011, Szymanek-Majchrzak and coworkers (521) studied the molecular epidemiology of HA-MRSA in the surgical and transplantation wards of the same hospital mentioned above and reported the predominance of another clone, ST36/CC30, for the first time in Poland. A total of 65.4% of the isolates belonged to ST36/CC8, followed by CC8/ST8 (15.4%). Although this was the first report of ST36 HA-MRSA in Poland, the strain has been previously reported in other countries of the world and included the epidemic UK EMRSA-16 and USA200 clones carrying SCC*mec* II (521).

In 2007, Blanc et al., (522) reported on the changing molecular epidemiology of MRSA at multiple sites in western Switzerland over an 8-year period from 1997 to 2004. The major clone, accounting for 32% of patients, belonged to ST105-II. It first appeared in 1998 and was responsible for a large outbreak in a tertiary-care hospital in 1999 (522). The clone then declined, likely due to strict infection control policies, but it subsequently increased in frequency again from 2001 to 2004, where it accounted for large outbreaks in several health care institutions in western Switzerland. The second major clone in this study, accounting for 23% of the patients, belonged to ST228-I. First recovered in 1999, in 2001 it was involved in the largest outbreak recorded in a tertiary-care hospital. ST228-I has also been reported in a Geneva tertiary-care hospital, being responsible for causing 80% of MRSA bacteremia (522).

The nosocomial prevalence rate of MRSA in Portugal continues to increase, reaching approximately 54.3% and representing one of the highest rates in Europe (523). The highly disseminated clones responsible for the majority of HA-MRSA infections in Portugal include the New York/Japan clone (ST5-II), the Iberian clone (ST247-I), and the Portuguese clone (ST239-III variant) (397, 518, 524–526). Of these, the Iberian clone and the Portuguese clone were the most prominent HA-MRSA clones in the early 1990s (397, 526). Currently, the New York/Japan clone is the most prevalent HA-MRSA clone in the country (397, 518, 524, 525). Moreover, in 2010, clones belonging to CC5 (ST105-II) accounted for approximately 20% of the MRSA isolates in the country's hospitals (397).

In Turkey, the prevalence of HA-MRSA varies considerably, from 12 to 75% in various Turkish hospitals (527). More than 80% of the HA-MRSA strains belong to SCC*mec* type III and, less frequently, SCC*mec* IIIb (527–529). Rare isolates have been reported to carry SCC*mec* type I and II. Alp et al. (527) performed a multicenter study isolating MRSA from patients with invasive *S. aureus* from 8 different university hospitals geographically distributed over the six main regions of Turkey. They concluded that ST239-III is the major clone in Turkish hospitals and showed its persistence and predominance over the last 10 years (527, 530). In 2013, Oksuz and colleagues (531) characterized MRSA clones collected from the Istanbul Medical Faculty Hospital during a 5-year period and reported the prevalence of the Vienna/Hungarian/Brazilian clone (ST239-III), which accounted for 53.9% of isolates. These isolates were multidrug resistant and showed

resistance to penicillin, tetracycline, rifampin, kanamycin, tobramycin, gentamicin, levofloxacin, erythromycin, lincomycin, and fosfomycin.

In 2011, a European-level survey was conducted, including 350 laboratories serving 453 hospitals in 25 different European countries (532). Isolates were collected from *S. aureus* bloodstream infections and characterized using molecular typing techniques. The most frequently isolated MRSA clone belonged to ST22, accounting for 24.5% of total MRSA isolates, again showing the presence of a CA-MRSA clone in hospital settings. ST22 was followed by three major clones, i.e., ST8, ST5, and ST225, accounting for 9.6% of total MRSA isolates. This study was a repeat of a previous survey conducted in 2006 for the investigation of temporal and spatial changes in *S. aureus* clones in Europe (310). In 2006, the most common MRSA strains also belonged to ST22, accounting for 16.9% of total MRSA isolates from 26 different European countries, making ST22 the major European CA-MRSA clone predominant in the hospital environment.

Africa. Since 2000, the prevalence of MRSA in Africa appears to be increasing in many countries, posing an evident threat to the continent (533). ST239-III, the Brazilian/Hungarian clone, is the major clone associated with hospital-acquired infections and has been reported from various parts of Africa, including Algeria (534, 535), Ghana (536), Kenya (537), Morocco (396), Niger (396), Nigeria (538), Senegal (396), South Africa (425), and Tunisia (539). Information regarding the molecular epidemiology of MRSA from North African countries, including Egypt, Tunisia, and Algeria, is scarce but shows an increase in the proportion of MRSA (540). Basset and colleagues (541) collected 84 MRSA isolates between January 2006 and July 2011 from Bologhine Ibn Ziri University Hospital in Algeria and characterized them using double-locus sequence typing (DLST). Their analysis identified DLST type 28-30 in 8.3% of the isolates, corresponding to Brazilian clone ST239 carrying SCC*mec* III$_{mercury}$ (541). Mariem et al. (539) characterized HA-MRSA clones isolated from two Tunisian hospitals from 2004 to 2008 (539). They identified several HA-MRSA clones, including ST247-I, ST1819-I, ST239-III, and ST241-III (539).

Breurec et al., (396) studied the epidemiology of MRSA isolates from 5 African cities, including one each from the countries of Cameroon, Madagascar, Morocco, Niger, and Senegal. They isolated 86 MRSA strains from 5 African towns during January 2007 to March 2008 and concluded that 40% of the HA-MRSA clones belonged to ST239/241-III (396). Egyir et al. (536) conducted molecular epidemiological studies and antimicrobial susceptibility testing of clinical *S. aureus* isolates from 6 health care institutions across northern, central, and southern Ghana and reported that HA-MRSA in this region belongs to clones ST250-I and ST239-III.

In 2010, Moodley and coworkers (425) described the population structure of MRSA by isolating 320 clinical MRSA isolates from nine different provinces of South Africa. They identified a few major HA-MRSA lineages circulating in South Africa, including ST5-I (CC5), ST239-III (CC8), and ST36-II (CC30) (425). A study conducted in the KwaZulu-Natal (KZN) province of South Africa also identified the presence of ST239-III as the major clone in 3 of 4 health care institution in Durban and in health care facilities in Pietermaritzburg and Empangeni (542). Another major clone detected in their study was ST5, similar to the New York/Japan clone (ST5-II), except that the ST5 from South Africa carried SCC*mec* III. It was observed in 2 hospitals in Durban and Pietermaritzburg and in health care facilities in Eshowe and Scottburg (542). This finding is consistent with findings by Nubel et al. (286) that the ST5 clone is associated with at least six SCC*mec* types, suggesting that ST5 MRSA clones have emerged multiple independent times by acquiring SCC*mec* in methicillin-susceptible ST5. In addition, their findings also revealed that ST5 MRSA strains from South Africa and Kenya form a unique sublineage not closely related to MRSA strains from other continents sharing identical *spa* types.

In Kenya, a cross-sectional study was conducted to determine the carriage of *S. aureus* in a midsized government hospital. Only 7% of all *S. aureus* isolates were MRSA; they were isolated from burn patients with prolonged admission, belonged to ST239-III, and were resistant to multiple antibiotics (537). As mentioned, this strain is typically associated with hospital acquisition and has been reported from sub-Saharan Africa in

Senegal and Niger (396) and South Africa (425), and it is the most common one among hospitalize burns patients in Nigeria (538). The multiple-antibiotic-resistance pattern found in the Kenyan hospital is typical of this strain in African settings (396). In conclusion, available data on the prevalence of predominant MRSA clones from Africa is limited, and this points toward the need for increased surveillance and molecular epidemiology studies in order to better understand the origin of newly emerging clones.

Middle East. Few data are available from the Middle East, and the majority of them point toward the dominance of CA-MRSA in both the community and hospitals. Senok et al. (543) conducted a study looking at the molecular characterization of MRSA isolates causing nosocomial infections in a tertiary-care hospital from 2009 to 2015 in Saudi Arabia. Their study determined that ST239-III was the prevalent HA-MRSA isolate in the tertiary-care facility in Riyadh (543). Apart from this single study pointing toward the presence of ST239-III, not many data regarding the prevalence of HA-MRSA in the Middle East are available.

Asia. MRSA is widely disseminated in nearly all health care facilities and constitutes an enormous infectious disease burden in Asia; however, the incidence differs considerably between countries and has changed significantly over time (544-548). Most molecular epidemiology reports are from high-income countries such as Japan, Taiwan, South Korea, Hong Kong, and Singapore. Reports from the resource-limited countries of South Asia and Southeast Asia are substantially limited, limiting our understanding of MRSA epidemiology (549).

During the early 1990s, the majority of Taiwanese MRSA isolates in hospitals belonged to ST254, but in the late 1990s they lost their predominance to the CC8-ST239 subgroup (mainly ST239 or ST241) with SCC*mec* III (405). As reported by Huang et al. (406) in 2004, approximately 73% of HA-MRSA isolates collected from six major hospitals in Taiwan belonged to ST239. Another study conducted in 2003 in China and Taiwan reported that 95% of clinical MRSA strains in an institute in northern Taiwan belonged to ST239 or ST241 with SCC*mec* III or IIIA (407). The early 2000s saw a change in the molecular epidemiology of MRSA, with the appearance of another pandemic clone, ST5-II (408). A recent study conducted in 6 major Taiwanese hospitals confirmed the decreasing prevalence of ST239 and increasing prevalence of ST5 in this region (409).

Since 1996, two predominant clones, ST5 and ST239, have been circulating in South Korean hospitals (550–553). The majority of ST5 strains carried SCC*mec* II, whereas ST239 carried either SCC*mec* III or IIIA (550–552). ST5-SCC*mec* II belonged to the pandemic New York/Japan MRSA clone that is widely prevalent in North America and Europe (366). Prevailing in South Korean and Japanese hospitals in 1990s, in the 2000s this clone gradually disseminated to other Asian countries, including Taiwan, China, and Hong Kong (408, 554–556). ST239 has extremely high levels of antibiotic resistance and is resistant to trimethoprim-sulfamethoxazole (SXT), ciprofloxacin, tobramycin, gentamicin, erythromycin, and tetracycline. In contrast, ST5 shows comparatively low levels of resistance and is susceptible to trimethoprim-sulfamethoxazole (552).

In Japan, a molecular study conducted in 2006, covering a 20-year period from 1979 to 1999, showed a high prevalence of ST30 strains before 1985, with the strains carrying SCC*mec* I (554). After the 1990s, however, the dominance of ST30 waned and ST5-SCC*mec* II took its place as the major MRSA clone, accounting for more than 95% of clinical MRSA strains in Japanese hospitals (554). In Hong Kong, a study conducted in 2003 and 2004 on isolates collected from Prince of Wales Hospital, spanning a 12-year period from 1988 to 2000, found that the isolates clustered into two major phage types and five pulsotypes. The two dominant pulsotypes, A and B, contained strains belonging to the ST239-SCC*mec* III lineage (557, 558). Another multicenter study conducted in four hospitals in Hong Kong during 2000 to 2001 showed ST239-SCC*mec* III as a major clone, with ST5-SCC*mec* II representing a minor clone, among MRSA bloodstream isolates (555). Since the late 1990s, ST239-SCC*mec* III/IIIA has been the most dominating nosocomial strain in China (370, 407). A nationwide study held in 2005 to 2006,

conducted in 18 hospitals in 14 different cities, concluded that 77.1% of the MRSA nosocomial isolates belonged to the ST239-SCC*mec* III lineage, whereas only 15.5% belonged to the ST5-SCC*mec* II lineage (556). ST239 showed resistance to tetracycline, erythromycin, clindamycin, gentamicin, tobramycin, and ciprofloxacin (555, 556), while ST5 strains were resistant to the above-mentioned antibiotics except for clindamycin. In 2011, a laboratory-based multicenter surveillance study involving 27 provinces in China was conducted, and again, ST239-III-t030, ST239-III-t037, and ST5-II-t002 were noted as the predominant strains (559) The study also pointed out the existence of a newly emerging ST, designated ST2590, which is a single-locus variant of ST5-II-t002, identified in 3 hospitals in 2 different cities and accounting for a total of 17 isolates, suggesting a changing epidemiology of HA-MRSA in China (559).

Molecular epidemiology studies conducted in the Philippines, Singapore, Thailand, and Vietnam on isolates collected during 1998 to 2003 indicated that the majority of the strains belonged to ST239 or ST241, with SCC*mec* III/IIIA (370). In addition, during 2006 to 2010, ST239-SCC*mec* III was again the dominant clone in Singapore. Few studies have examined the MRSA distribution in South Asia. A study conducted by Zafar and colleagues (560) on MRSA isolates collected during 2006 to 2007 showed the prevalence of ST239-SCC*mec* III among HA-MRSA strains in Pakistan. Another study, conducted by Shabir and coworkers (561), also reported the dominance of ST239 throughout Pakistan and India. A single-institute study conducted in southern India in 2011 also reported the dominance of ST239-SCC*mec* III strains (560). Moreover, the isolates were multidrug resistant, showing resistance to mupirocin, amikacin, cotrimoxazole, erythromycin, rifampin, and tetracycline (560).

Australia and New Zealand. The epidemiology of *S. aureus* in Oceania has distinct socio-demographic features, mainly because of the uneven burden of *S. aureus* disease in the aboriginal population.

The variety of HA-MRSA clones circulating in Australia is more limited, with ST239-III being endemic in the region since the 1970s (562). ST239-III was the dominant HA-MRSA clone in the country until recently, when it was replaced by the British epidemic strain EMRSA-15 (ST22-IV) again showing evidence of CA-MRSA clones replacing HA-MRSA in hospitals (563). Limited molecular epidemiology data are available from New Zealand; the most common HA-MRSA in hospital setting belongs to ST239-III, with the addition of clonal complexes such as CC1, CC30, CC59, and CC101 (564).

EMERGENCE OF COMMUNITY-ASSOCIATED MRSA

MRSA was considered a nosocomial pathogen, and absent from the community, when it was first reported in 1961. This notion has changed significantly in the past 2 decades. The epidemiology of MRSA is changing worldwide, with growing anxiety concerning strains of MRSA that are emerging in the community. Community-associated MRSA (CA-MRSA) has appeared lately as a clinically significant and potentially virulent pathogen associated with serious skin and soft tissue infections, particularly in young, healthy individuals (27). Less frequently, it has been recognized as the cause of rapidly lethal and severe infections such as necrotizing pneumonia and fasciitis. Although the first few reported cases of CA-MRSA were from Detroit, MI, and Dublin, Ireland, in the 1980s, these patients either were intravenous drug users or had a previous hospitalization history and hence had inclining risk factors for MRSA infection (28, 29, 565). The first definite case of CA-MRSA was reported in 1993 from an aboriginal population in Western Australia (112, 566). These patients were from Kimberly, a remote and sparsely populated area in Western Australia, and therefore did not have any interaction with individuals who had access to large medical centers. Additionally, these MRSA isolates, known as WA-MRSA-1 or WA-1, were not multidrug resistant and were distinct from the usual MRSA strains identified at that time.

The second report of CA-MRSA came from Minnesota and North Dakota, United States, describing four otherwise healthy children who died from sepsis or necrotizing pneumonia between 1997 and 1999 (434). These pediatric fatalities were caused by CA-MRSA, as the children were apparently healthy and had no identifiable risk factor for

MRSA infection. Following this, CA-MRSA was recognized as a distinct clinical entity, and the strain responsible for this small outbreak became known as MW2, a close relative of WA-1 (349, 442, 566, 567). These small early outbreaks were the beginning of what is now known as an epidemic of CA-MRSA. Both these strains were classified as MLST clonal complex 1 (CC1).

MW2 and its close relatives were collectively known as pulsed-field type USA400 and MLST 1 and were the most common CA-MRSA strains in the United States before 2001 (224, 280, 434, 568). Beginning in 2000, further outbreaks of CA-MRSA were reported in apparently healthy and diverse populations, including athletes, military personnel, children in day care centers, Native Americans, prisoners, Pacific Islanders, and other apparently healthy individuals (568–574). These outbreaks were subsequently associated with a different MRSA lineage (CC8), a strain unrelated to the MW2/ST1 lineage, which was dubbed USA300. This new strain rapidly replaced USA400 and is now the leading cause of CA-MRSA infections in the United States and Canada. Distinct CA-MRSA lineages are now reported from various parts of the world, with some exhibiting more restricted geographical ranges than others (575).

The U.S. Centers for Disease Control and Prevention (CDC) Active Bacterial Core (ABC) surveillance sites created a standard definition of CA-MRSA in 2005 (576). They defined CA-MRSA infections as infections caused by MRSA which result in a positive culture from any part of the body in an outpatient individual or in a person within 48 h of hospital admission. In addition, the individual should have no history of hospitalization, surgery, or residency in a long-term-care facility in the past year, no indwelling catheters at the time of isolation, and no previous positive MRSA cultures. This CDC definition is important, but it underestimates the actual burden of the disease, as it does not account for many CA-MRSA infections arising in the community among people who have one or more health care-associated risk factors (577). In addition, it may be difficult to determine the origin of MRSA infection arising in the community, because MRSA colonization persists for months or years and may go unobserved, leading to infection months and/or years after hospital discharge (578). CA-MRSA has now spread into hospitals, appearing as a nosocomial pathogen, meaning that based on above definition, the true prevalence of this community-dwelling pathogen could be underestimated or overestimated (30–33). As a consequence, people now prefer a strain-based definition of CA-MRSA, as it has a unique genetic profile, antibiotic resistance pattern, presentation, epidemiology, and treatment (442, 577, 579, 580). Having said that, the definition of CA-MRSA needs to be further investigated in detail, as although the antibiotic resistance pattern of CA-MRSA isolates is limited to β-lactam antibiotics, multidrug resistance has been reported among them (581–583). At present, molecular epidemiological definitions centered on SCC*mec* typing and phylogenetic analysis are thought to be the most trustworthy (584).

Various hypotheses have been proposed for the rapid global emergence of CA-MRSA clones within the last 2 decades; however, none of these theories completely explain the epidemiological data. Some connect the increased use of fluoroquinolones (FQs) with the rapid appearance of CA-MRSA, since the emergence of these isolates occurred in tandem with the augmented use of FQs. Increased use of FQs has been associated with abolition of sensitive strains from colonizing the nasal mucosa, predisposing it to be colonized by resistant strains (585). Alternatively, CA-MRSA in the United States may have exploited the colonization niche created following global pneumococcal immunization efforts. Recommendations for use of the Prevnar vaccine in Canada in 2002 may have correlated with the rise of CA-MRSA in Canada (586, 587). Some authors have therefore suggested that decreases in *Streptococcus pneumoniae* after Prevnar vaccination may have created new ecological niches for CA-MRSA colonization. Epidemiological evidence against this hypothesis includes the fact that CA-MRSA was already commonly reported in Australia at the time Prevnar was introduced in 2005.

The theory that gained significant public attention was one proposed by Vandenesch and coworkers (575). Those authors studied virulence factors and SCC*mec* types in

117 CA-MRSA isolates from different countries around the world. They found that virtually all newly evolving CA-MRSA strains, irrespective of their genetic background and antibiogram, carried the gene for Panton-Valentine leucocidin (PVL) toxin and a novel small SCC*mec* IV element (575). PVL is a leucocidin that was first reported by Panton and Valentine in 1932 (588). It is a bicomponent phage-encoded toxin that targets leukocytes. Encoded by two genes, *lukS-PV* and *lukF-PV*, it is readily incorporated into the chromosome of *S. aureus* by a bacteriophage. It causes tissue necrosis and leukocyte destruction by forming pores in cellular membranes. The authors concluded that there is a strong association between CA-MRSA strains and the PVL genes and suggested that PVL could be the marker for CA-MRSA (575). Others, however, argue that the emergence of SCC*mec* type IV during late 1980s drove the concurrent emergence of multiple CA-MRSA lineages (15, 447).To date, no single explanation can provide the conclusion, and perhaps MRSA represents an unceasingly evolving wonder compelled by multiple factors, including host, pathogen, and environment.

Controversial Role of Panton-Valentine Leucocidin

The report by Vandenesch et al. (575) generated a debate regarding the role of Panton-Valentine leucocidin (PVL) as a possible culprit in the origin and distribution of CA-MRSA. At the 45th Interscience Conference on Antimicrobial Agents and Chemotherapy in 2005 in Washington, DC, Zhang and colleagues (589) were the first to challenge this notion. They determined the genetic diversity among PVL-positive *S. aureus* isolates from 1989 to 2004, over a period of 16 years, in a large Canadian health care region. Isolates of MSSA, MRSA, and coagulase-negative staphylococci (CoNS) from frozen collection were randomly selected and tested for PVL genes via multiplex PCR assay. The PVL-positive isolates were further characterized via PFGE, SCC*mec* typing, MLST, *spa* typing, and *agr* typing, and clonal complexes were determined using eBURST analysis. The results revealed no significant difference in mean PVL-positive rates between MRSA and MSSA. With slight yearly variation, the mean PVL-positive rates were 1.4% and 1.6% for MSSA and MRSA, respectively; however, no CoNS were found to be positive for PVL. Irrespective of the antibiotic resistance profile and MLST types, PVL genes were found in CA-MRSA clones as well as MSSA strains. Interestingly, the PVL genes were also detected in clones that had genetic backgrounds similar to that of typical epidemic HA-MRSA, associated with SCC*mec* types II and III and MLST types ST5, ST30, ST22, ST45, and ST105. Figure 6 shows the eBURST analysis and molecular, genomic, and antibiotic susceptibility profiles. The eBURST analysis did not reveal any clear descent or evolution of these PVL-positive strains. In addition, the antibiotic resistance profiles of the isolates closely matched their SCC*mec* type rather than their PVL genes. These results therefore provided the first indirect evidence from a population-based study that the genomic background, rather than PVL genes, may play a greater role in the emergence of CA-MRSA infections and that PVL genes are not the key elements of the newly emerging CA-MRSA clones.

Voyich and coworkers (590), however, were the first to directly challenge the notion of PVL being the major virulence determinant associated with CA-MRSA disease. They conducted studies in mouse sepsis and abscess models with PVL-knockout isogenic strains of USA300 (LAC) and USA400 (MW2). They concluded that PVL-negative and -positive strains caused similar skin disease and were as virulent and toxic as their wild-type strains (590). Consequently, they concluded that PVL is not a major determinant of virulence among CA-MRSA strains. However, another study conducted using lab MRSA strains expressing phage- and/or plasmid-encoded PVL in BALB/c mice concluded that PVL promoted staphylococcal lung infection and was sufficient to cause necrotizing pneumonia (591). With this model, death was apparent only with plasmid-encoded PVL, likely due to high levels of PVL expression. This is in contrast to another study conducted in a different murine model, which concluded that PVL does not have any impact on staphylococcal pneumonia and anti-PVL serum does not protect mice from staphylococcal pneumonia (592). These conflicting reports could be due to the

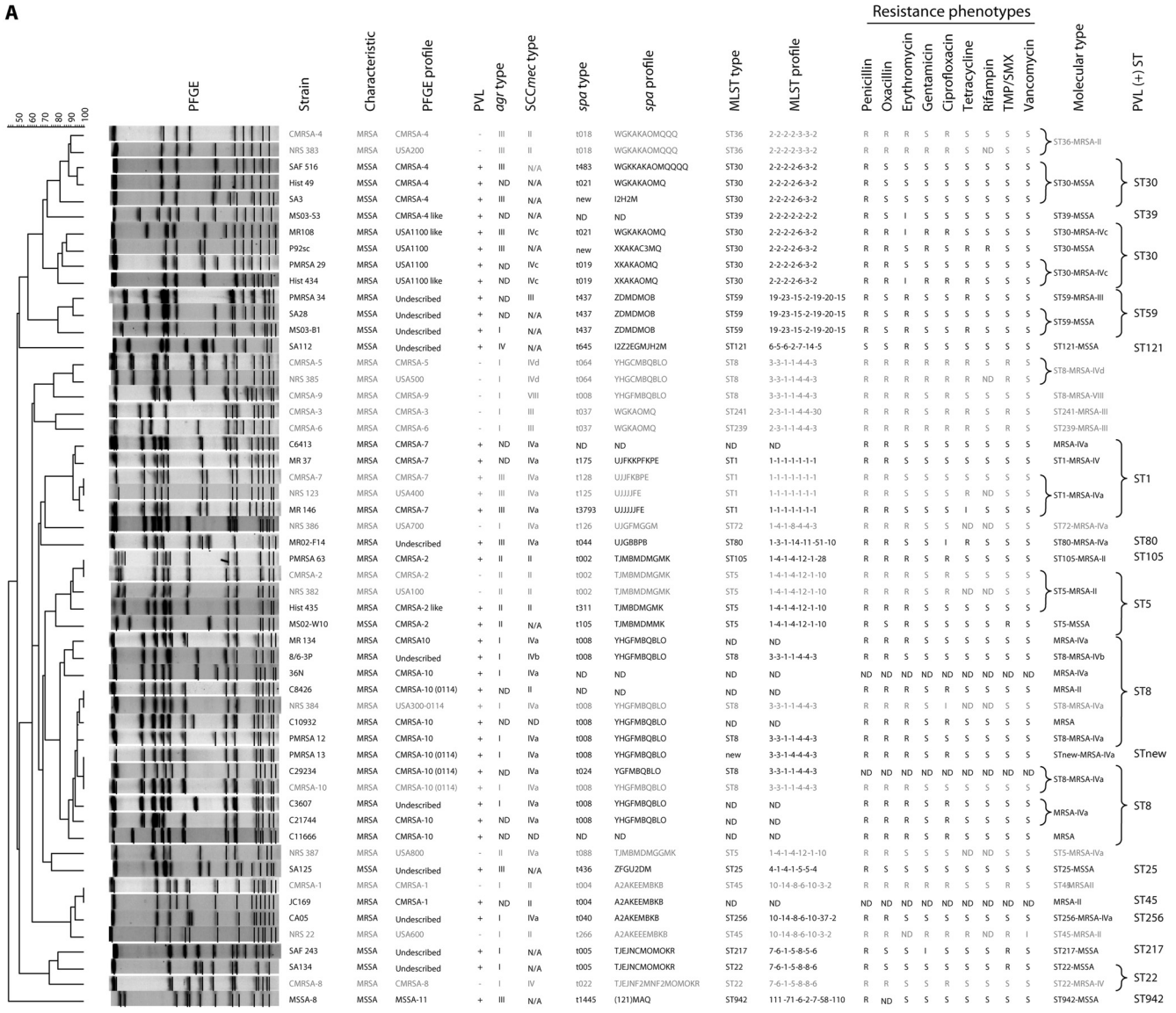

FIG 6 Genetic diversity among PVL-positive *Staphylococcus aureus* clinical isolates (589). (A) Molecular characterization of representative PVL-positive strains, isolated over 16 years (1989 to 2004) in a large Canadian health care region, via pulsed-field gel electrophoresis (PFGE), SCC*mec* typing, MLST, *spa* typing, and

(Continued on next page)

different genetic makeups of the host species, and hence, to exclude this possibility, Bubeck and coworkers (593) extended their work using the same mouse model as used by Labandeira-Rey et al. (591). Their results still contradicted the report of Labandeira-Rey et al., and they concluded that PVL does not contribute to the pathogenesis of staphylococcal infection in BALB/c mice (593). An important point, however, is the fact that these studies were conducted in a murine model, the granulocytes of which are not susceptible to the action of PVL (594).

Diep and coworkers (595) subsequently developed a rabbit model of CA-MRSA bacteremia to better study the link, as rabbit granulocytes are sensitive to the effects of PVL. Their data indicated a modest and transiently positive role of PVL in the acute phases of infection, offering signs to support the function of PVL in the pathogenesis of CA-MRSA infections (595). However, none of these studies provided direct data or evidence from human populations, and this indirect evidence is not sufficient to establish the contribution of PVL in the dissemination and emergence of CA-MRSA.

The first direct evidence that PVL genes are not the key factor associated with the increased dissemination of CA-MRSA strains came from the work of Zhang and colleagues (596) in 2008. They conducted a retrospective study on a natural population of MRSA clinical isolates recovered from patients during 2000 to 2005 in a large Canadian health care region. The study identified coexisting PVL-positive and -negative sibling USA400 strains, with the PVL-negative strain as the dominant one. USA400 was the early CA-MRSA clone described in the United States and Canada, and it was implicated in community-onset outbreaks of CA-MRSA with serious consequences (434, 597, 598). Molecular and sequencing studies indicated the presence of PVL phage in PVL-positive strains, whereas this phage was found to be absent from PVL-negative strains. However, both of these strains were phenotypically identical in every other aspect, sharing MLST, SCC*mec*, *spa*, and *agr* profiles and possessing the same toxins, adhesions, and coenzymes. In addition, the strains showed similar resistance profiles and clinical characteristics (596). The study therefore concluded that rather than PVL genes, the genomic milieu and the repertoire of virulence and resistance genes may play a greater role in increased distribution and pathogenesis of CA-MRSA strains (596).

The work by Zhang and colleagues (596) remains the strongest evidence regarding the role of PVL in CA-MRSA emergence in humans. To further support their conclusion and to better understand the role of PVL in virulence and pathogenesis, PVL-positive and -negative sister strains of USA400 were used in various *in vitro* cell culture and animal models (599). The results from these various studies showed no significant difference in the virulence and the cytotoxicity between PVL-positive and -negative USA400 strains (599). Hep-2 (human laryngeal carcinoma) cell lines were used to test bacterial invasion and survival ability, as well as to test their cytotoxic effects on cell lines. The results showed no difference in the invasion and survival ability or in epithelial cell cytotoxicity between PVL-positive and -negative strains (Fig. 7 and 8) (599). The nematode *Caenorhabditis elegans* (600) and fruit fly *Drosophila melanogaster* (601) models were also developed to study the virulence and pathogenesis of *S. aureus*. Three clinical isolates with detailed characterization, including PVL-positive and -negative USA400 sibling strains as well as USA400 MW2 PVL-positive and -negative isogenic (PVL knockout) strains, were selected to test in these models. Both the positive and the negative USA400 strains showed no significant difference in the rate of killing. All these strains had high nematocidal and fly-killing ability, regardless of the presence or absence of PVL genes, indicating no significant difference between the killing rates in PVL-positive and PVL-negative strains (Fig. 9 and 10) (599). A murine model capable of demonstrating dermatopathological differences between various CA-MRSA strains, developed by Zhang et al. (602), was also used to test these PVL-positive and -negative

FIG 6 Legend (Continued)
accessory gene regulator (*agr*) typing, as well as antimicrobial susceptibility profiles of the strains. (B) Determination of clonal complexes via eBURST analysis comparing our MLST data (16 STs) with the international MLST database (updated 9 December 2008). (Adapted from reference 909 with permission of the publisher.)

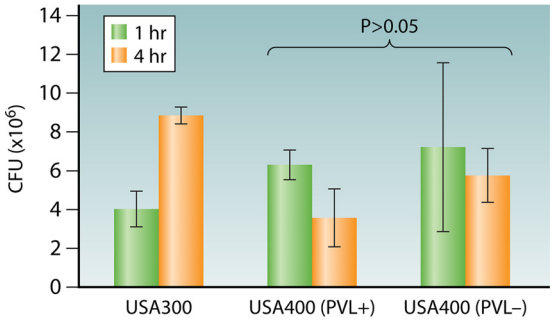

FIG 7 PVL-positive (PVL+) and PVL-negative (PVL−) USA400 sibling strains show no significant differences in invasion and survival abilities in human laryngeal carcinoma (HEp-2) cells (599). Sixteen-hour-old Hep-2 cells were infected with 10^8 CFU of *S. aureus*. After an hour of incubation, extracellular bacteria were removed, and intracellular bacteria were enumerated at 1 h and 4 h by serial dilution and plating. No significant differences in invasion or survival ability was noted between PVL(+) and PVL(−) isolates. (Adapted from reference 909 with permission of the publisher.)

USA400 sibling strains. Both of these strains presented with identical localized skin infections associated with a focal inflammatory response without skin ulceration, and hence, no dermatopathological difference was observed (Fig. 11) (599). All these studies therefore demonstrated that the PVL genes are not the major virulence determinant and that other factors play a greater role in the cytotoxicity and pathogenesis of CA-MRSA strains (599).

There have been several other reported studies focusing on the role of PVL in the life of CA-MRSA (603–605); however, the exact function of PVL in the emergence, worldwide dissemination, and pathogenesis remains contentious. Virulence and pathogenesis of CA-MRSA appear to be complex, and it is likely that multiple factors are implicated in the transmission and establishment of disease by CA-MRSA. Nonetheless, the issue is still disputed and has remained unsettled even after a decade.

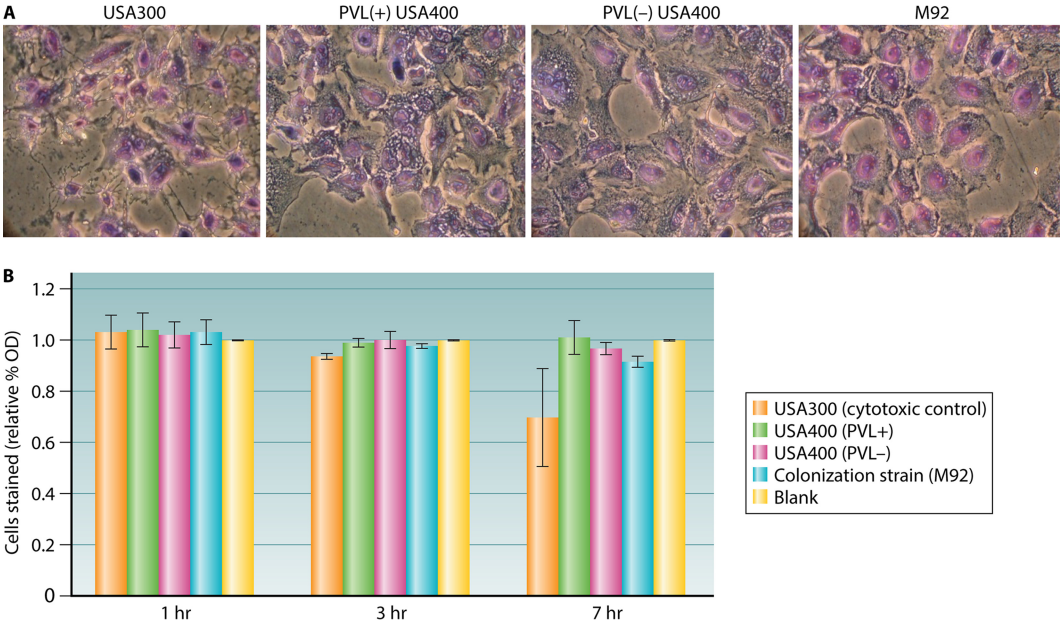

FIG 8 PVL(+) and PVL(−) USA400 sibling strains show no significant differences in cytotoxicity toward human lung epithelial (A549) cells (599). Sixteen-hour-old A549 cells were infected with 10^8 CFU of *S. aureus*, and bacteria were removed after an hour of incubation. (A) The cells were fixed and stained, using methanol and Giemsa stain, at 1, 3, and 7 h. (B) Bacterial cytotoxicity on A549 cells was determined by reading the optical density (OD) of Giemsa stain. The results are expressed as the relative percent optical density against a blank control with no bacterial infection. No significant difference in the cytotoxicity was noted between PVL(+) and PVL(−) strains. (Adapted from reference 909 with permission of the publisher.)

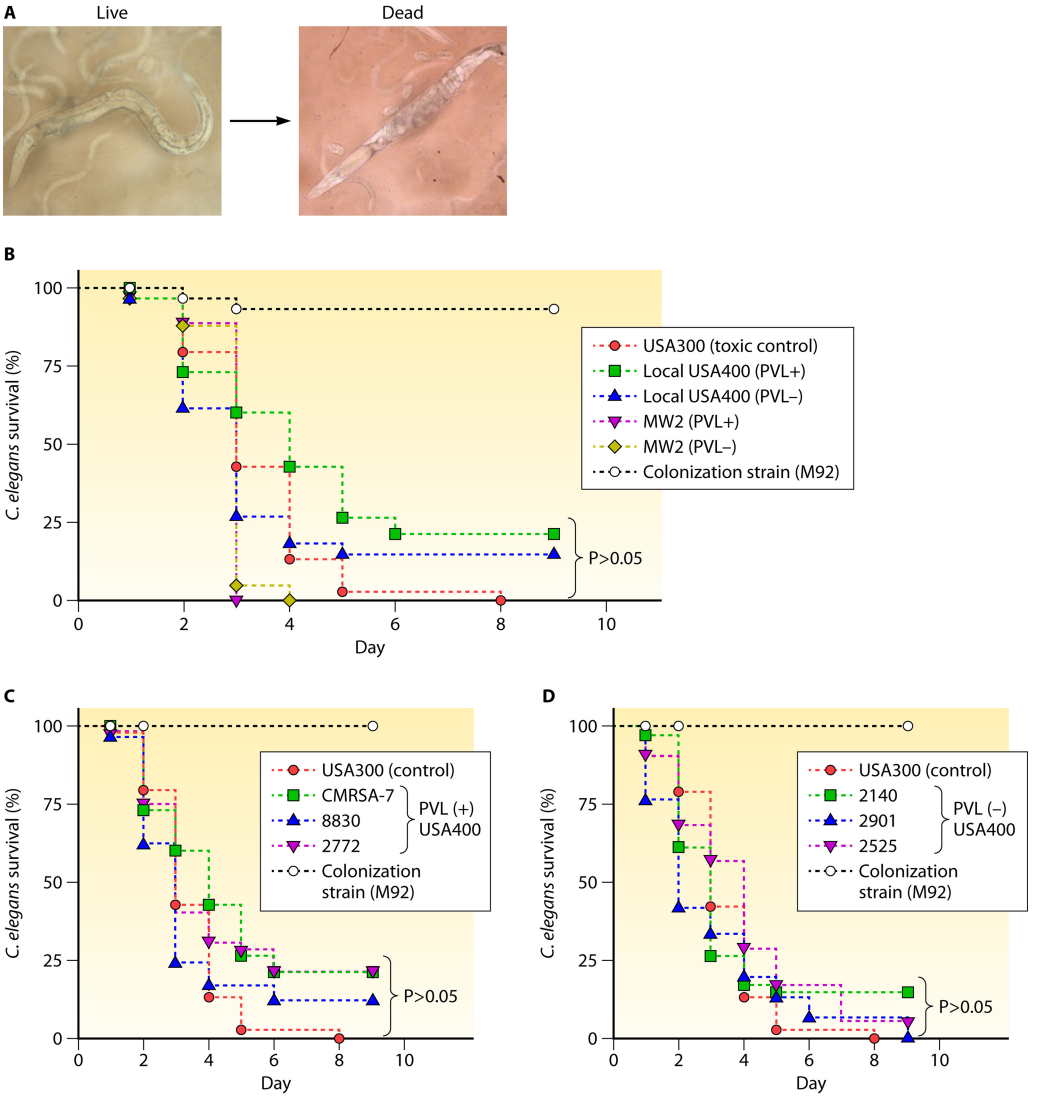

FIG 9 PVL(+) and PVL(−) sibling strains and USA400(MW2) and USA400(MW2 PVL-knockout) control strains are both highly lethal in the *C. elegans* model (599). Tryptic soy agar supplemented with 7 μg/ml nalidixic acid was inoculated with bacteria 3 h prior to the addition of 30 L4-stage nematodes. Plates were incubated at 25°C, and scoring for live and dead worms was performed every 24 h. The experiment was performed thrice in triplicate, and Kaplan-Meier and log rank tests were used to analyze nematode survival data. (A) Pictorial representation of live and dead *C. elegans* feeding on the PVL-positive USA400 strain. (B) Kaplan-Meier survival plots of nematodes fed with PVL(+) and PVL(−) USA400 and control strains indicate that no significant difference was seen in killing activities between these two groups. (C and D) Three representative clinical isolates each from PVL(+)/(−) USA400 sibling strains demonstrate similar nematocidal activity, with no significant difference between the groups. (Adapted from reference 909 with permission of the publisher.)

HA-MRSA versus CA-MRSA

CA-MRSA strains differ significantly from their health care-associated counterparts, including in the configuration of the gene cassette coding for methicillin resistance, in the resistance to other classes of antibiotics carried on plasmids, and in their growth rates They also differ in their associated toxins and/or virulence factors and enzymes, as well as the route and site of infection, thereby affecting different risk groups.

The large SCC*mec* types (i.e., SCC*mec* types I to III) are present in HA-MRSA strains and were likely conveyed from commensal *Staphylococcus* species on a few occasions only (34, 35). However, smaller SCC*mec* types (i.e., IV and V) are assumed to be transferred from methicillin-susceptible backgrounds frequently, resulting in the emergence of unique-fit MRSA clones (36, 37). Unlike SCC*mec* types I to III, which bear genes for multiple antibiotic resistance, SCC*mec* types IV and V carry only the *mecA* gene for

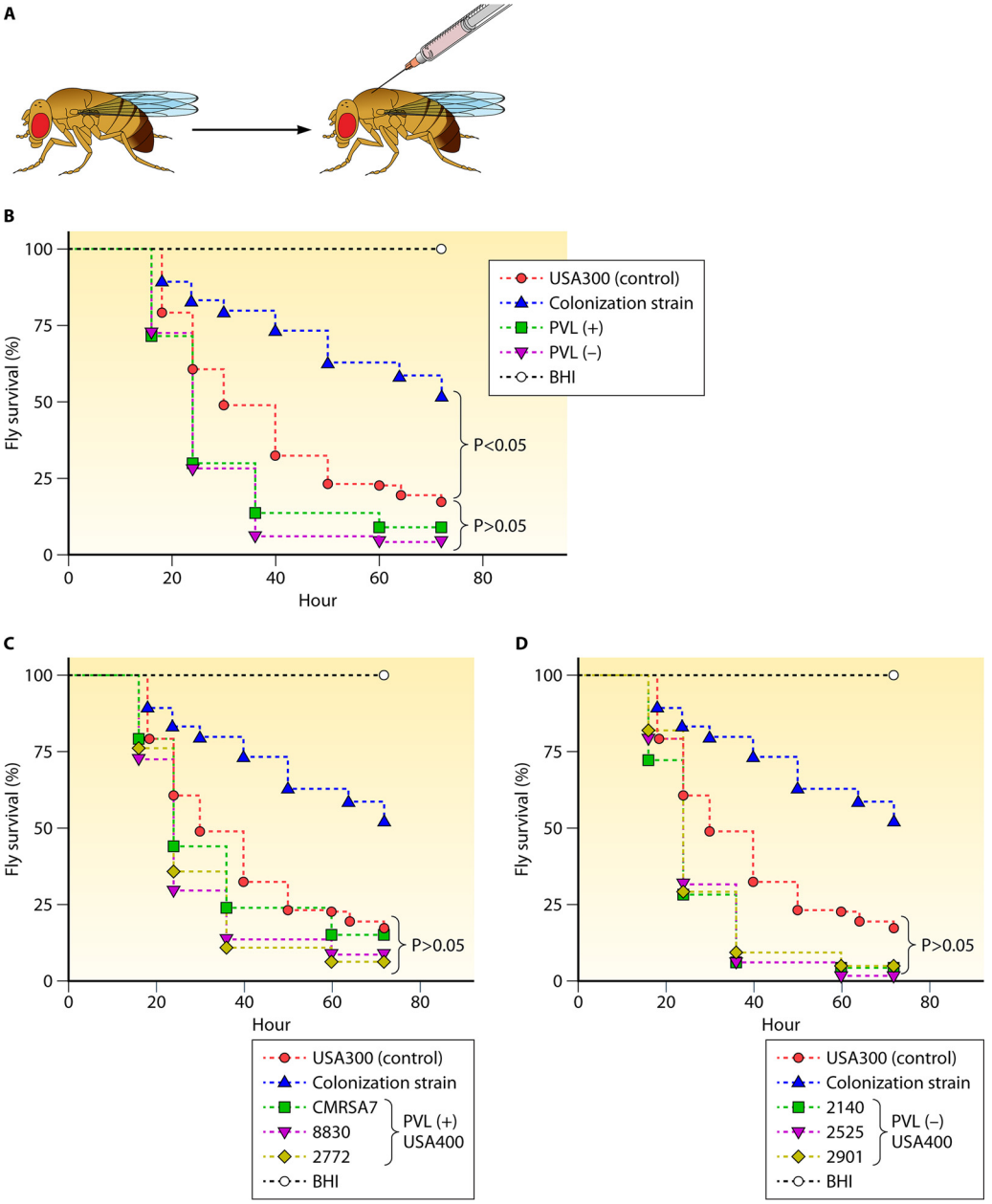

FIG 10 PVL(+) and PVL(−) USA400 sibling strains both have high fly-killing activities in the *Drosophila melanogaster* model (599). (A) For the determination of fly-killing activity, 2- to 5-day-old female *Drosophila* flies were pricked in the dorsal thorax with a 27.5-gauge needle dipped in bacterial suspension (8×10^8 CFU/ml of *S. aureus*). The flies were kept at room temperature, fed with sucrose, and monitored daily to be scored as live or dead. The experiment was performed thrice in triplicate, and Kaplan-Meier and log rank tests were used to analyze fly survival data. (B) Kaplan-Meier survival plots of flies injected with PVL(+) and PVL(−) USA400 and control strains indicate that no significant differences were seen between the two groups. (C and D) Three representative clinical isolates each from PVL(+)/(−) USA400 sibling strains show similar fly-killing activities. (Adapted from reference 909 with permission of the publisher.)

resistance to β-lactam antibiotics (36, 38–40). This lack of genes conferring resistance to non-β-lactam antibiotics partly accounts for their non-multidrug-resistant phenotype. The antibiogram and gene composition, therefore, support the hypothesis that they are newly emerged MRSA clones arising from the integration of smaller SCC*mec* types into MSSA strains. The analysis of a large number of MRSA clones identified twice as many clones with smaller SCC*mec* types, indicating their successful endurance and better promiscuity (39). This could be due to a greater efficiency of transfer to recipient clones, due to their smaller size and the lower fitness costs associated with carrying fewer

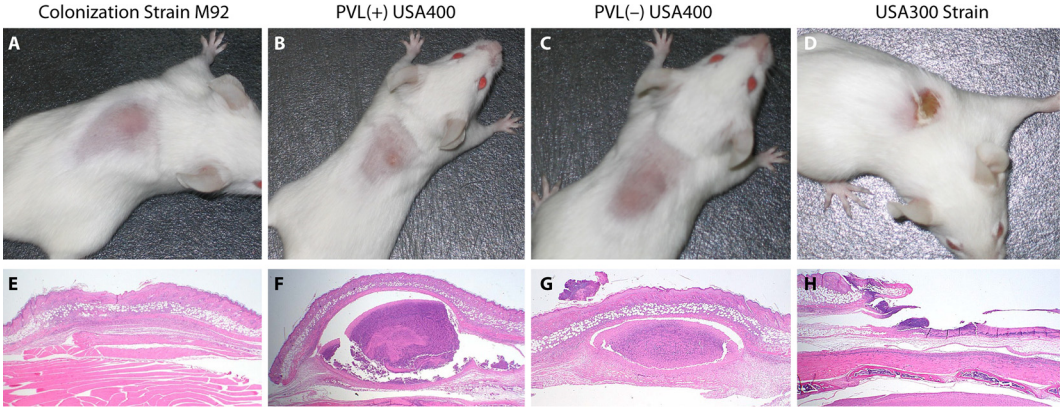

FIG 11 No dermatopathological differences are seen between PVL-positive and -negative sibling strains in a murine intradermal infection model (599). No dermatopathological difference was observed on day 4 following infection with PVL(+) and PVL(−) USA400 sibling strains. Skin lesions (A to D) and histopathological sections (E to H) of mouse skin from intradermally infected BABL/c mice are shown. Similar pustules/abscesses with confined dermal abscess damage without ulceration were observed with both PVL(+) (B and F) and PVL(−) (C and G) USA400 sibling strains. In contrast, a large cutaneous ulcer with a predominantly neutrophilic inflammation was observed with the USA300 strain (D and H), and the colonizing strain M92 (A and E) caused only localized edema with a localized inflammatory reaction without ulceration. (Adapted from reference 909 with permission of the publisher.)

genes (316, 368, 606, 607). It has been reported that CA-MRSA strains harboring SCC*mec* types IV and V replicate more rapidly than HA-MRSA strains with larger SCC*mec* types. The decreased efficiency of transfer for larger SCC*mec* types would also account for the lower number of HA-MRSA clones seen worldwide.

HA-MRSA is often multidrug resistant, while CA-MRSA strains are usually limited to β-lactam resistance. This is consistent with the fact that SCC*mec* types IV and V lack antibiotic resistance genes other than *mecA*, whereas other SCC*mec* types accumulate multiple additional antibiotic resistance genes (39). Most CA-MRSA isolates are therefore susceptible to fluoroquinolones, aminoglycosides, erythromycin, and clindamycin (41). This, however, does not preclude resistance encoded by chromosomal genes or genes carried by plasmids. For example, in Western Australia some CA-MRSA strains with plasmid-borne resistance to tetracycline, trimethoprim, mupirocin, and cadmium have been isolated (581). Fluoroquinolone resistance in CA-MRSA has been reported in homeless youth in San Francisco, CA (582). In other studies, CA-MRSA strains resistant to trimethoprim-sulfamethoxazole, clindamycin, vancomycin, gentamicin, fluoroquinolones, and macrolides have been reported as well (608).

CA-MRSA strains test positive for hemolysins, leucocidin, and exfoliative toxins, whereas HA-MRSA usually does not contain these toxins. CA-MRSA strains also encode β-lactamase and hyaluronidase (609). Among these virulence factors, the most notable is PVL, which is common among CA-MRSA strains from diverse genetic backgrounds (although absent from certain CA-MRSA lineages such as WA-1) but is seldom located in HA-MRSA strains (<5%) worldwide (15, 42–46). As discussed above, while PVL is universally linked epidemiologically to CA-MRSA strains, it is not known how it contributes to the fitness, virulence, and pathogenicity of these strains or if it is merely a marker for other fitness or virulence determinants (610). As new PVL-negative CA-MRSA clones are described, this leucocidin is losing its utility as a marker for CA-MRSA, and the reason behind the global emergence of PVL-positive CA-MRSA lineages during the past decade remains an open question (440, 611).

CA-MRSA is commonly associated with serious skin and soft tissue infections (SSTIs), causing folliculitis, pustular lesions, and abscesses, particularly in young, healthy individuals with no risk factor for acquisition of HA-MRSA. HA-MRSA, on the other hand, causes more invasive infections. In some instances, CA-MRSA has been found to be associated with severe and rapidly fatal necrotizing pneumonia and necrotizing fasciitis (41, 609). CA-MRSA usually enters the bloodstream through the lungs, via a surgical or

implant site, and may also inflame a portion of the skin, causing a pimple or boil which might look like a spider bite. As mentioned, HA-MRSA usually affects immunocompromised patients, people with a recent history of hospitalization, dialysis patients, people in long-term-care facilities, or postsurgical patients.

From the Community Back to the Hospital

Because of the evolving epidemiology of CA-MRSA, these strains have now emerged as a cause of hospital outbreaks as well. Nosocomial outbreaks of CA-MRSA have been reported in various parts of the world since 2003, often affecting special areas of hospitals such as pediatrics or obstetrics, where the prevalence of HA-MRSA is low (47–55). In 2010, Carey et al. (612) studied the changes in the molecular epidemiology of MRSA in a neonatal intensive care unit (NICU) in New York. They showed that HA-MRSA strains have been replaced by CA-MRSA strains as a predominant cause of MRSA infections in NICUs. Similarly, outbreaks of CA-MRSA have been reported in patients, health care workers, patients' relatives, and hospital staff, including security guards, on several occasions (54, 55, 613, 614). In one of these outbreak reported from West Midlands, United Kingdom, a previously healthy Filipino health care worker (HCW) died after she went underwent a cesarean section and acquired a fatal PVL-MRSA infection. The PVL-MRSA isolate belonged to the ST30-IV lineage and appeared to be circulating in the households and contacts of Filipino nurses (613). In a study conducted by Alvarez et al. (615), 10% of CA-MRSA nosocomial infections were caused by USA300, isolated primarily from bacteremia and surgical site infections in Colombia (615). In another study from southeastern Germany, two health care-associated outbreaks with PVL-MRSA occurred during a 9-month period, affecting 83 patients, personnel, and contacts of personnel. The PVL-MRSA from outbreaks I and II belonged to ST22 and ST80, respectively (616).

There have been reports of CA-MRSA emerging as a cause of hospital infections in South Korea (617, 618). The prevalence of community strains causing hospital infections has increased from $<$10% to 25% in recent years, where ST72-IVa accounted for 19 of the 76 MRSA bloodstream isolates in 3 community hospitals (617). Other studies have shown the ST72-IV PVL-negative genotype accounting for a significant fraction of infections in hospitals (619–621).

A surveillance study conducted more than 2 decades ago, in 1996, identified USA100 (ST5-II lineage, also known as the New York/Japan clone) as a major cause of hospital-acquired infection in 12 New York City hospitals (622). Eighteen years later, in 2013 to 2014, an updated epidemiological study in 7 of the same hospitals showed USA300 as the major isolate, replacing USA100 as the dominant clone in these hospitals. The USA300 clone was associated with 84.5% of SSTIs, compared to 5% for the USA100 clone. That study concluded that the USA300 clone replaced the New York/Japan clone as the most dominant type causing nosocomial infections in the New York metropolitan area (622). A more recent study from 20 hospitals across the United States also showed a continued expansion of USA300-like MRSA among hospitalized patients (623), with an increase in the prevalence of t008/SCC*mec* IV isolates. The study characterized 516 MRSA isolates collected between 2011 and 2014 from nares and blood cultures of U.S. patients and found 29.9% t002/SCC*mec* II in nares isolates compared to 30.9% t008/SCC*mec* IV and 24.4% t002/SCC*mec* II in blood isolates compared to 39.9% t008/SCC*mec* IV. Their data concluded that there was a significant change in the overall antimicrobial resistance pattern and displacement of t002/SCC*mec* II by t008/SCC*mec* IV strains in U.S. hospitals (623).

Recent reports suggest that CA-MRSA has nearly taken over from HA-MRSA clones as a cause of endemic hospital-acquired infections (622–625). USA300, which was previously identified as an endemic community-associated pathogen in the United States, now has an equivalent tendency to cause nosocomial infections after every hospital entry. It has been associated with outbreaks in newborns in hospital settings, as well as being a cause of postoperative joint infections and health care-associated bacteremias (48, 626–629). In view of the changing epidemiology of CA-MRSA, national

data from the CDC ABC surveillance system in 2004 to 2005 identified USA300 as the cause of 16% of hospital-onset and 22% of health care-associated community-onset infections (630).

Clinical Significance of CA-MRSA

The global emergence of genetically diverse CA-MRSA strains rivals the former dissemination of HA-MRSA lineages, emphasizing the evolutionary adaptability of MRSA as a pathogen no longer confined to the health care boundaries. Recent mathematical models predict the eventual displacement of conventional HA-MRSA strains by CA-MRSA in hospitals, with substantial clinical and public health consequences (631, 632). Since its emergence in the 1990s, the number of cases of CA-MRSA has rapidly escalated. Studies surveying 11 U.S. hospitals show that 97% of PVL-positive CA-MRSA isolates were USA300 bearing SCC*mec* type IV and were susceptible to clindamycin, rifampin, tetracycline, and trimethoprim-sulfamethoxazole (SXT) (633, 634). Similarly, a study conducted in San Francisco demonstrated a 10-fold increase in the incidence of CA-MRSA compared to HA-MRSA in 2004 to 2005. USA300 was the dominant isolate in both CA and HA infections (416). In addition to USA300, there is also evidence which implies that other CA-MRSA lineages are now becoming responsible for health care-associated infections (33).

The prevalence of CA-MRSA varies worldwide, ranging from less than 1% in some countries to more than 50% in others, with the prevalence been higher in children than in adults (635–638). In the United States, Taiwan, Canada, and Australia, reported outbreaks have been more extensive, with infection becoming endemic in certain populations in each of these countries (437, 442, 447, 639). In other parts of the world, only small outbreaks or cases have been reported. In Europe, for example, the prevalence is low but increasing, while for the developing world only limited data are available. There is fear, however, that if CA-MRSA becomes endemic in resource-poor nations, it would result in devastating consequences.

As stated, CA-MRSA has now emerged as a significant cause of nosocomial infections; however, the reason behind this emergence is largely unknown. However, this influx of CA-MRSA into the hospital presents several challenges. First, it puts a wider group of people at risk, such as hospitalized patients with no risk factor for MRSA infection, health care workers, and their community contacts, which substantially adds to costs in health care facilities (640). Patients with MRSA infections reside longer in the hospital, and the preventive measures taken to isolate patients all add substantial costs. Second, the increased prevalence of PVL-MRSA in health care settings might increase the virulence of nosocomial MRSA infections. In a study conducted in Detroit, MI, it was shown that USA300 was more likely to cause invasive infections when contracted in health care settings than the uncomplicated STIs typically caused in a community setting (641). Also of concern is that exposure of CA-MRSA to antibiotic pressure in the hospital environment might result in the emergence of CA-MRSA strains resistant to multiple antibiotics. As shown by Davis et al., CA-MRSA behave more like HA-MRSA in hospital settings, and the disease profiles of groups of study patients infected with an CA-MRSA strain and an HA-MRSA strain were similar (629). This suggests that these community strains might one day come to resemble hospital strains in their multidrug resistance phenotype. One study found that USA300 strains associated with health care infection were significantly more resistant to ciprofloxacin then their community counterparts. In addition, a clinical isolate of USA300 with intermediate susceptibility to vancomycin and reduced susceptibility to daptomycin has already been detected (642, 643).

Because CA-MRSA can now cause nosocomial infections as well as community-associated infections, the definitions of CA-MRSA and HA-MRSA are becoming confusing, as they were originally based on the epidemiology and emergence of MRSA infection in patients without prior risk (639, 644–646). As a consequence, it is now necessary to analyze these strains based on microbiological and molecular features, which will then define an MRSA strain as community or hospital associated. The

tendency of CA-MRSA to cause hospital-acquired infections forces us to rethink what CA- and HA-MRSA are: should they be considered separate entities, or are CA-MRSA and HA-MRSA merely strains of MRSA with different virulence and genetic backgrounds?

Major Extant CA-MRSA Strains and Clones

Several *S. aureus* genetic backgrounds are responsible for causing CA-MRSA epidemics in various parts of the world. Figure 5 shows a population snapshot of CA-MRSA around the world, and a summary of the molecular characteristics of some common CA-MRSA clones around the world is given in Table 2. CA-MRSA is currently associated with more than 20 distinct genetic lineages (611). CA-MRSA is generally less prevalent in Europe than it is in North America and is characterized by genetic heterogeneity, in contrast to the predominant spread of USA300 (ST8-IV) in North America. The other globally predominant clones include ST1-IV (WA-1, USA400), ST30-IV (southwest Pacific clone), ST59-V (Taiwan clone), and ST80-IV (European clone) (442, 447, 647). Among them, ST8-IV and ST30-IV may be considered pandemic due to their repeated isolation from every continent (287, 447).

United States and Canada. When it was first recognized in the late 1990s, USA400 was the most prominent CA-MRSA clone in the United States (349). It was also identified in the community in Saskatchewan and Manitoba, Canada, in the years 1999 to 2002 and 1995 to 2000, respectively (597, 648). USA400 is an ST1 strain carrying SCC*mec* type IV, is usually susceptible to most non-β-lactam antibiotics, and is commonly found associated with SSTIs (649). Although several other genetic backgrounds were responsible for CA-MRSA infections at that time, by the start of the 21st century one well-characterized genetic background, USA300, became most prevalent in the contiguous 48 states in the United States (412–418). It is the most prevalent CA-MRSA strain in North America and the primary strain in the Native Alaskan population (650). USA300 is probably the only strain which poses a global epidemic threat (651, 652). It has been isolated from all continents in the world except Antarctica; however, its role as the dominant CA-MRSA strain has not yet been duplicated anywhere other than in North America. The first few reports of USA300 were tied to sports teams in Pennsylvania, followed by numerous outbreaks among prisoners in Mississippi and Los Angeles, CA (653). Although associated with particular risk groups, such as military personnel, prisoners, intravenous drug users, athletes, homeless and urban populations, and men who have sex with men, it has nonetheless become the root cause of SSTIs among the general population (417, 653).

The defining characteristic of USA300 include the carriage of SCC*mec* type IV along with the PVL genes and the arginine catabolic mobile element (ACME) (651, 654, 655). This element was previously considered to be the unique marker associated with this lineage and was believed to be connected to its heightened transmissibility; however, this is no longer the case. Unlike traditional HA-MRSA, USA300 prefers colonizing extranasal sites (656, 657). It has now been documented as a cause of serious invasive community-acquired infections, including pneumonia, endocarditis, and necrotizing fasciitis, and is now becoming endemic in nosocomial settings, even displacing ST5-II as the primary cause of bloodstream infection in the United States (651, 652, 654, 658).

USA300 became the most dominant CA-MRSA strain in the United States in a relatively short period of time. Its prevalence has been documented in a number of settings, including a population-based study conducted in San Francisco in 2004 to 2005, which showed that the primary cause of 78.5% of community-onset MRSA infections was the USA300 clone (416). Another study, conducted in Baltimore, MD, reported that USA300 caused no MRSA SSTIs in 2000 but was responsible for 84% of MRSA SSTIs by 2005 (659). USA300 was reported as the cause of 29% of invasive MRSA infection in 2005. This prevalence increased to 31% by 2006 (630, 660). Similarly there are several other studies reporting USA300 as the cause of MRSA infection in the United States, thereby emphasizing the prevalence of USA300 in this part of the world (395, 577, 634, 661). Additionally, USA300 has increasingly become an asymptomatic colonizer among the general population in the United States. Tenover et al. reported an

TABLE 2 Molecular characteristics of CA-MRSA clones around the world[a]

Location	Standard name	Common name	Molecular characteristics					
			PFGE	MLST	*spa* type	SCC*mec* type	*agr* type	PVL
USA/Canada	ST8-MRSA IV	USA300	USA300/CMRSA10	ST8	t622/t008/t121/t451/t024/t064/t068	IV	1	+
	ST1-MRSA IV	USA400	USA400	ST1	t128/t127/t125/t1178/t273	IV	3	+
	ST5-MRSA IV	Pediatric clone	USA800	ST5	t002/t311/t003	IV/IVa	2	
	ST22-MRSA IV	EMRSA-15	PFGE type B	ST22	t022/t032/t223	IV	1/2	−/+
Mexico/South America	ST8-MRSA IV	USA 300 LA variant	PFGE type B	ST8	t622/t008/t451/t121/t024	IV	1	+
	ST5-MRSA IV	Pediatric clone	USA800	ST5	t002/t311/t003	IV/IVa	2	
	ST30-MRSA IVc	Southwest Pacific clone	PFGE type N	ST30	t019/t318	IVc	3	
	ST1-MRSA IV			ST1	t128/t127/t125/t1178/t273	IV	3	+
Europe	ST80-MRSA IV	European clone	PFGE type G2	ST80	t044/t359	IV	2/3	+
	ST22-MRSA IV	EMRSA-15	PFGE type B	ST22	t022/t032/t223	IV	1/2	−/+
	ST1-MRSA IV	USA400	USA400	ST1	t128/t127/t125/t1178/t273	IV	3	+
	ST30-MRSA IV	Southwest Pacific clone	PFGE type N	ST30	t019/t318	IV	3	
	ST59-MRSA V		PFGE type A	ST59	t437	V	1	
	ST5-MRSA IV	Pediatric clone	USA800	ST5	t002/t311/t003	IV	2	+
	ST8-MRSA IV	USA300	USA300	ST8	t622/t008/t121/t451/t024/t064/t068	IV	1	
	ST377-MRSA V			ST377	t355	V	1	+
	ST88-MRSA IV	African clone	PFGE type J	ST88	t168/t186/t729	IV	3	+
	ST93-MRSA IV	Queensland clone	PFGE type E	ST93	t202	IV	3	+
	ST772-MRSA V			ST772	t345	V	2	+
	ST45-MRSA IV/V		PFGE type E	ST45	t004/t026/t040	IV/V	1	
Africa	ST88-MRSA IV	African clone	PFGE type J	ST88	t168/t186/t729	IV	3	+
	ST80-MRSA IV	European clone	PFGE type G2	ST80	t044/t359	IV	2/3	+
	ST30-MRSA IV	Southwest Pacific clone	PFGE type N	ST30	t019/t318	IV	3	+
	ST121-MRSA V			ST121	t314/t159	V	4	+
	ST152-MRSA V			ST152	t355	V	1	+
	ST15-MRSA IV			ST15	t084/t085	IV	2	+
	ST8-MRSA IV	USA300	USA300	ST8	t622/t008/t121/t024/t451/t064/t068	IV	1	+
	ST72-MRSA V			ST72	t537/t324/t664	V	1	+
	ST789-MRSA IV		PFGE type 1B	ST789		IV		+
	ST2021-MRSA V			ST2021		V		+
	ST612-MRSA IV			ST612		IV	1	+
Middle East	ST80-MRSA IV	European clone	PFGE type G2	ST80	t044/t359	IV	2/3	+
	ST22-MRSA IVa		PFGE type B	ST22	t022/t032/t223	IVa/V	1/2	−
	ST30-MRSA IV	Southwest Pacific clone	PFGE type N	ST30	t019/t318	IV	3	+
Asia	ST59-MRSA IV/V		HKU 200	ST59	t437	IV/V/V_T	1	+/−
	ST338-MRSA V_T			ST338		V_T	1	+
	ST910-MRSA IVa			ST910	t318	IVa		+
	ST1-MRSA IV		PFGE type 1I	ST1		IV	3	+/−
	ST89-MRSA IV		PFGE type 1B	ST89		IV	3	−
	ST8-MRSA IV	CA-MRSA/J	USA300	ST8	t622/t008/t121/t024/t451	IV/IVa	1	+/−
	ST91-MRSA IV		PFGE type 3B	ST91	t416/t604	IV	3	−
	ST30-MRSA IV	Southwest Pacific clone	HKU 100	ST30	t019/t318	IV/IVa/IVc	3	+
	ST5-MRSA IV	New York/Japan clone	PFGE type I	ST5	t002/t003/t311	IV	2	−
	ST72-MRSA IVa		USA700	ST72	t324/t664/t537	IVa	1	−
	ST88-MRSA IV		PFGE type J	ST88	t690	IV	3	
	ST80-MRSA IV		PFGE type G2	ST80	t044/t359	IV	2/3	−
	ST834-MRSA IV			ST834		IV	1	−
	ST121-MRSA V			ST121	t314/t159	V	4	+
	ST22-MRSA IV		PFGE type B	ST22	t022, t032/t223	IV	1/2	+
	ST772-MRSA V			ST772	t345	V	2	+
Australia/New Zealand	ST93-MRSA IV	Queensland clone	PFGE type E	ST93	t202	IV	3	+
	ST30-MRSA IV	Southwest Pacific clone	PFGE type N	ST30	t019/t318	IV	3	+
	ST8-MRSA IV	USA300	USA300	ST8	t622/t008	IV	1	+
	ST1-MRSA IV	WA-MRSA-1	PFGE type 1I	ST1	t128/t127/t125/t1178/t273	IV	3	−
	ST5-MRSA IV		PFGE type I	ST5	t002/t003	IV	2	−
	ST45-MRSA IV			ST45	t004/t026/t040	IV	1	
	ST78-MRSA IV			ST78	t186/t690/t786/t1598/t3205	IV	3	−
	ST22-MRSA IV		PFGE type B	ST22	t022, t032/t223	IV	1/2	+

[a]−, absence of PVL; +, presence of PVL. Blank entries indicate a lack of available information.

increase in nasal colonization by USA300 from 8% in 2001 to 2002 to 17% by 2003 to 2004 (413).

In Canada, USA300 is known as CMRSA10, and it emerged later than in the United States. First reported in Canada in 2004 after an outbreak of SSTIs in Calgary, Alberta,

it is currently the primary CA-MRSA strain in Canada as well (662). In the Calgary outbreak, 39 cases of SSTIs and one fatal case of necrotizing pneumonia were reported in individuals with a history of illicit drug use, incarceration, or homelessness. Further investigations documented a 5.5% colonization or infection rate in marginalized populations, with increased risk associated with shared drug use and casual sex partners (663). Between 2003 and 2005, it was found to be the most common cause of skin infections in an emergency department in Vancouver (664). A prevalence survey conducted at the national level from 2007 to 2009 reported that CMRSA10 was responsible for 73.7% of CA-MRSA cases in Canada (486). Another study conducted in Alberta from July 2005 to June 2008 showed that CMRSA10 was responsible for 53% of cases, and the population-based prevalence of CMRSA10 was found to be 16 cases per 100,000 in 2008 (665). Although initially more prevalent in western Canada, it is now becoming more and more prevalent in eastern communities and has been isolated from patients with SSTIs in Toronto and from the nares of an Ontario school teacher (666, 667). Increased incidences of CMRSA10 have been reported from hospital settings, including 21% of hospital-onset cases in Alberta and 32% in Vancouver (668). A hospital outbreak of CMRSA10 involving mothers and newborns has also been reported, mirroring the presentation of USA300 in the United States (52).

Mexico and South America. While the epidemiology of CA-MRSA infections has been well documented in the United States and Canada, the burden of disease and the molecular characterization of MRSA isolates in Latin America remain largely unknown. Little information is available from the few sporadic reports regarding dominating clones in this region; however, the presence of 3 pandemic clones, i.e., ST5, ST8, and ST30, has been witnessed in multiple regions (500, 501, 669–672).

In Ecuador, the prevalence of the USA300 Latin American variant (USA300-LV, ST8-IV) is well known (669). This clone has also been documented in Cuba, Columbia, Peru, and Venezuela (670, 671). The majority of CA-MRSA infections in Argentina and Uruguay have been due to ST5-IVa and ST30-IVc, respectively (500, 672). Medina et al. (501) reported the presence of clones related to the pediatric clone (ST5-IV) and USA300-like clone (ST8-IV) in southern Chile in 2013. Jimenez-Truque and coworkers (673) studied the molecular epidemiology of MRSA in Costa Rican children and reported that 94.5% of the MRSA isolates carried SCC*mec* IV with a USA700-like PFGE type. The second most common pulsotype, however, belonged to USA400. Velazquez-Meza and colleagues (674) were the first to report the presence of ST8-IV in Mexico in 2011. Apart from this, to our knowledge, there are no data available regarding the molecular epidemiology of CA-MRSA in Mexico.

Europe. The prevalence of CA-MRSA in Europe in general is low, but it is rising and has been reported in most European countries, including in the Netherlands and Nordic countries, where HA-MRSA has very low rates due to strict surveillance. CA-MRSA in Europe is characterized by clonal diversity (675). Although USA300 and its variants have been reported in minute numbers in certain parts of Europe, such as Italy (676), Germany (677, 678), Spain (107, 679), Austria (680), and Denmark (681), some other international clones, such as ST1-IV, ST30-IV, and ST59-V, have also been reported in this continent (45). However, the most important and prevalent CA-MRSA isolate in Europe is ST80-IV, the so-called European clone (45, 575). It has been reported in France (575), Switzerland (575), Austria (682), Bulgaria (683), Belgium (684), Germany (677, 678), Greece (685, 686), the Netherlands (436, 441), Denmark (687), Finland (688), Norway (373), and Sweden (689) and shows a characteristic pattern of resistance to fusidic acid, tetracycline, and kanamycin, with variable resistance to ciprofloxacin (45, 575). The first few infected patients had a history of travel to the Mediterranean, Middle East, and/or Africa; therefore, it is presumed that this clone may have its origin in one of those areas. Irrespective of the origin, it is the most commonly reported clone in Europe (438, 690).

Reports of CA-MRSA infection are rare in the United Kingdom, but several studies now suggest that its prevalence might be increasing. A study reported on an increase in presumptive CA-MRSA cases of 46% from 2000 to 2004 (691). Another study, conducted in London during 2000 to 2006, identified an increase in the proportion of

MRSA isolates susceptible to ciprofloxacin, with another proportion being PVL positive (692). Ireland is associated with few reports of CA-MRSA, accounting for <2% of the isolates and being connected to international travelers (42, 693).

In the Netherlands and Nordic countries, the prevalence of HA-MRSA has always been very low, but CA-MRSA has emerged as a significant entity despite strict national antimicrobial restriction and infection control policies (436, 694). The European clone is the most commonly isolated type in both community and hospitalized patients; however, USA300 has also been reported at times.

The European clone is the most frequent isolate in Belgium, and CA-MRSA in that country is characterized by genetic heterogeneity (684). The first report of the European clone came from Germany in 2002, and reports from German reference laboratories show an increasing prevalence of this clone, with an emergence of a USA300-like clone in this region (677, 678, 695). Nosocomial transmission of CA-MRSA is becoming an issue in Germany, with several reports of outbreaks associated with the European clone and an ST22-IV PVL-positive clone in health care facilities (616, 624, 696). In Austria, 10% of MRSA isolates are community associated, with USA300 being the prominent one but with ST80-IV now commonly isolated from some parts of the country (680, 682, 697).

The first few European cases of MRSA infection in patients without any recognized risk factors for HA-MRSA were reported in Geneva and France in 2002 and seemed to have been caused by the same CA-MRSA strain (698, 699). Geneva and Zurich have very low rates of MRSA prevalence, although a study in 2006 reported an outbreak of an ST5-IV strain of PVL-positive CA-MRSA in a Swiss ICU, demonstrating the ability of CA-MRSA to cause hospital outbreaks in that region (608, 700).

Despite high rates of HA-MRSA in Spain and Portugal, low albeit growing rates of CA-MRSA has been observed in the Iberian Peninsula. In two Spanish studies, USA300 was found to be the most prevalent clone, but it was associated with immigrants from South America (107, 679). Reports of CA-MRSA from Italy are scanty, although a recent laboratory-based study showed isolation of 12 MRSA strains from 188 *S. aureus* patients, 9 of which belonged to the USA300 type (676). Limited information is available regarding CA-MRSA prevalence in Greece, although ST80-IV seems to be the most commonly encountered strain, with ST377-V also being reported from certain parts, especially southwest and central Greece (685, 701).

The data from other European countries are scanty, with the exception of a few reported cases from Russia (702), Poland (703, 704), and Croatia (705), making the common types and prevalence in these countries hard to determine.

Africa. Despite the cultural and geographical diversity in Africa, which could significantly affect the epidemiology of MRSA, research on this pathogen has been largely neglected in this part of the world. The epidemiology of MRSA in Africa is unique and fascinating due to the divergence between established urban societies and the remote rural populations in close association with animals, including livestock, domestic animals, and wildlife, along with an uneven allotment of wealth, the poor governance structure of some countries, and high birth rates with low economic growth. For example, high colonization rates have been observed immediately after birth and in teenagers not only in urban but also in rural populations. However, this colonization rate seems to decrease with higher parental education levels (706, 707). The picture of CA-MRSA spread in Africa is largely unclear compared to that in the rest of the world (447).

Detailed molecular characterization of clinically important *S. aureus* isolates is largely neglected in Africa, and the only data available are from travelers returning from Africa. These limited data hint at the different genetic backgrounds of African *S. aureus* strains and also point to the fact that these strains may be more virulent than those isolated from Europe. Fatal *S. aureus* pneumonia and complicated SSTIs have been reported in travelers returning from Africa. In addition, these strains were most often PVL positive, to the extent that Africa is now considered a region where PVL is endemic, with rates of PVL-positive isolates ranging from 17 to 74% (684, 708–711).

ST80-IV is the most prevalent clone in Maghreb, with 91 to 100% of isolates carrying

the PVL genes (712, 713). The antibiogram of ST80-IV from Egypt showed susceptibility to tetracycline and fusidic acid, while isolates from Algeria were resistant to tetracycline and those from Tunisia were resistant to tetracycline and intermediately resistant to fusidic acid (713, 714). ST80-IV is the major CA-MRSA clone in Europe and shows the same resistance pattern as isolates from Tunisia (45). These reports, as well as the close geographical proximity to Europe, suggest the spread of this clone between these two regions.

ST30, ST121, and ST152 are the main isolates from West and Central Africa, with major clones being PVL positive. The prevalence of PVL-positive isolates is between 9 and 100% among the ST30 lineage, between 50 and 93% for ST121, and between 97 and 100% for ST152 (711, 715–717). A study conducted by Egyir et al. (536) determined the antimicrobial susceptibility patterns and clonal diversity of clinical *S. aureus* isolates from Ghana. A total of 308 isolates from 6 health care institutions were characterized. Of 91 *spa* types detected, t355 (ST152), t084 (ST15), and t314 (ST121) were the most frequent ones. About 60% of the isolates were PVL positive and 3% of the isolates were methicillin resistant, belonging to ST88-IV, ST8-IV, ST72-V, ST789-IV, and ST2021-V, suggesting a high frequency of PVL-positive *S. aureus* with significant MRSA lineage diversity in Ghana (536). A recent study from Gabon noted the prevalence of clone related to USA300, indicating the spread of this clone in Central Africa (715). There is, however, only one reported case of severe invasive bacteremia, pneumonia and pericarditis caused by this USA300-related clone in an otherwise healthy Gabonese person (718). Another study by Oosthuysen and coworkers revealed a high prevalence of the PVL genes, as well as a unique local MRSA clone, in the South African Tygerberg Academic Hospital, Western Cape province. Of 367 *S. aureus* isolates collected over a period of 1 year, ST612-IV was the most dominant MRSA clone (719).

In West, Central, and East Africa, ST88-IV is the most prevalent clone; this clone is only sporadically seen around the world, except for in Far East Asia (45, 720–722). In Africa this clone accounts for 24 to 83% of all MRSA isolates, whereas in China and Japan it accounts for fewer than 10 and 12.5% of all MRSA isolates, respectively. As a consequence, it has been referred to as the "African clone" (720, 723, 724).

Middle East. Epidemiological data on CA-MRSA infection and prevalence in the Middle East are limited, with little attention paid to the molecular epidemiology of this pathogen in countries of the Mediterranean region. Biber and coworkers (725) conducted a cross-sectional survey of nasal *S. aureus* carriage in healthy children and their parents and found MRSA in 45% of the isolates, belonging predominantly to ST22-IVa. These ST22 isolates were PVL negative, while the majority of PVL-positive isolates belonged to ST80-IV (725). Another study to determine the prevalence of MRSA nasal carriage among healthy university students, as well as to determine SCC*mec type*, was conducted by Adwan et al. (726). The study revealed that almost half of the MRSA isolates carried SCC*mec* type IVa or V.

Similarly, Khalil and coworkers (727) conducted a molecular characterization of 103 *S. aureus* isolates from nasal and fecal samples of children admitted to the Jordan University hospital; the majority of the isolates belonged to the ST80-IV lineage. More recently Aqel et al. (728) studied the molecular epidemiology of nasal MRSA isolates from HCWs and other healthy individuals from Jordan. All MRSA isolates (7.8%) in their study carried *mecA*, and none carried the *mecC* gene, with the majority of the isolates bearing SCC*mec* types IV and V. The most dominant MRSA lineage among nasal carriers in the community belonged to ST22-IVa (728).

Few data exist regarding the molecular characterization of CA-MRSA isolates from Saudi Arabia; however, studies that exist have shown an increase in the prevalence of CA-MRSA (729, 730). Abou Shady et al. (731) determined the prevalence and molecular epidemiology of MRSA nasal carriage among outpatients attending a primary health care center in Saudi Arabia. They collected 103 swab samples and concluded that the majority of the MRSA isolates belonged to SCC*mec* types V and IVa and were included in four clonal complexes, CC5, CC8, CC22, and CC80 (731). Similarly, in Lebanon limited data are available on the molecular epidemiology of CA-MRSA isolates; however, two

studies have shown that ST80-IV is the prevalent CA-MRSA clone in Lebanon (732, 733). CA-MRSA in Kuwait was first isolated in hospitals in 2001, accounting for up to 1.8% of MRSA isolated from patients in seven hospitals (734). Among the isolates, the two most common clones detected included 38.5% ST30-IV PVL-positive and 30.8% ST80-IV PVL-positive isolates. This prevalence increased to 17% during the next 4 years (735) with additional CA-MRSA clones identified, including 2 dominant clones (ST80 [51%] and ST30 [22%]), and was attributed to patients who returned after seeking medical care abroad or to the immigrant staff (734, 736). HA-MRSA is 40 to 60% prevalent in Iranian hospitals; however, not much information is available about the molecular epidemiology of CA-MRSA in Iran (737).

Asia. Because of a scarcity of diagnostic facilities and limited resources, data about the surveillance and epidemiology of CA-MRSA in Asia come predominantly from developed countries rather than from the resource-poor nations (738). In Asian countries, the rate of CA-MRSA infections varies substantially between countries, ranging from as low as 2.5% to as high as 39%, and is characterized by clonal heterogeneity, similar to the case in Europe (120). Many epidemic clones circulate in Asia, with limited data available regarding their surveillance. There is a fear that CA-MRSA could have devastating effects if it becomes epidemic in developing nations with limited resources.

In 2004 Wang and coworkers (739) were the first to report on the molecular characterization of CA-MRSA in Taiwan. They found that 16 of the 17 CA-MRSA isolates belonged to PVL-positive ST59, while 13 of these isolates carried a specific subtype of SCC*mec* V called SCC*mec*V_T (192). More than 80% of the isolates in Taiwan now belong to either one of the two major clones, called ST59 (or its single-locus variant ST338)-V_T PVL positive or ST59-IV PVL negative (740). The PVL-positive isolate was the most common one isolated, whereas the PVL-negative clone dominates among colonizing isolates (741–745). The major lineage in China, accounting for up to two-thirds of isolates, belongs to ST59 and its single-locus variant ST338. ST59-IV-t437 was found to be the most common clone between 2006 and 2008, followed by ST910-IVa-t318 and ST1 (427, 746, 747). The majority of isolates from China carry the PVL genes and show a multidrug resistance phenotype. A study conducted by Du et al. (748) reported 3% nasal MRSA carriage, with isolates showing much molecular heterogeneity and ST59 and ST338 accounting for only 14.3% and 3.6%, respectively.

A nationwide survey conducted in Japan from 2008 to 2009 showed an increase in the prevalence of CA-MRSA since 2000 (749). Most of the CA-MRSA isolates in the survey were PVL-negative SCC*mec* type IV and belonged to ST89, ST8, or ST91-IV (114, 750–754). The PVL-positive CA-MRSA cases reported from 2000 to 2008 were caused by ST30-IVa-19 (113). A study in 2009 by Ozaki and coworkers reported MRSA nasal colonization rates from 0.7% to 3.7% in children of various age groups (755), with isolates belonging to diverse genetic backgrounds. The first nosocomial outbreak of USA300 was reported in Japan in 2010 in a general hospital ward, infecting 6 HCWs and 4 patients (756), although there were a few sporadic cases of USA300 reported in Japan before that (757, 758). The first outbreak was followed by another outbreak in the following year, in which an immunocompromised patient and 3 healthy staff members at a dermatology hospital were affected (759). Taiwanese clone ST59-V-PVL-positive has also been identified in Japan since 2010 (760, 761). A clone highly similar to USA300, but carrying SCC*mec* IV (ST8 CA-MRSA/J), was identified in Japan in 2003 (762). Since its emergence, it has become a highly successful native clone associated with SSTIs and invasive infections, similar to USA300. Through a Japanese family, this strain spread to Hong Kong in 2005. Although highly similar to USA300, this strain lacked the PVL phage and arginine catabolic mobile element and possessed cytolytic peptide genes of CA-MRSA. Other, less commonly isolated strains in different studies include ST5, ST8, ST88, ST89, ST8 CA-MRSA/J, and the ST5 New York/Japan clone.

The first report of CA-MRSA infection in South Korea came from the province of Kyungnam in 2004 to 2005, when 23 CA-MRSA isolates were identified. The isolates belonged to ST72-IVa-t324 or -t664 and ST5-IV, and all lacked the PVL genes (763). Around the same time, a hospital laboratory-based survey was conducted in seven

major hospitals in South Korea, which identified CA-MRSA rates of 5.9%, with strains belonging to diverse genotypes. The most common clone belonged to ST72-IVa PVL negative, and only one strain from ST72 was found to be multidrug resistant (44, 764, 765). Studies of nasal CA-MRSA carriage among healthy children in 2008 identified predominantly ST72 and its single-locus variant SCC*mec* IVa PVL negative (766, 767). The first report of USA300 in South Korea came in 2008 when a case of perianal abscess was reported and suggested to be imported from Hawaii, USA (768). However, in 2012 a case of pneumonia caused by USA300 followed by pandemic influenza was reported in a patient who had never traveled (neither had family members) to any part of the United States, indicating the gradual spread of this clone in South Korea (769).

The prevalence of CA-MRSA has risen rapidly in Hong Kong since the first report in 2004 (770). In a 2-year study conducted in Hong Kong from January 2004 to December 2005, 24 episodes of SSTIs and a single episode of meningitis due to CA-MRSA were identified (771). A total of 29 isolates were analyzed, and it was shown that CA-MRSA infections might be more common in families originating from outside Hong Kong. The isolates analyzed belonged to ST30-IV (identical to the southwest Pacific clone), ST59-V multidrug resistant carrying an *ermB* gene responsible for macrolide resistance (a characteristic shared with the predominant CA-MRSA clone in Taiwan), and ST8-IVa PVL negative (typical of the CA-MRSA clone from Japan). In addition, CA-MRSA carriage was found in 13% of household contacts, indicating that intrafamilial spread might be common. In another study, the molecular typing of CA-MRSA isolates from 6 regional hospitals providing services to half of the Hong Kong population showed two major clones, ST30-HKU100-IV-t019 PVL positive and ST59-HKU200-IV-t437 PVL positive (772).

In Singapore, there have been reports of the spread of ST30 MRSA isolates among various ethnic groups (773), with most MRSA isolates since 2004 belonging to the PVL-positive ST30-IVc genotype (774, 775). No data about MRSA molecular epidemiology are available from Indonesia. In Malaysia, a study conducted between 2006 and 2008 detected MRSA rates of 3.2% from 9 different hospitals, with isolates carrying SCC*mec* IV and the predominant clone being ST30. Nine of the 20 strains isolated were CA-MRSA, of which 8 belong to ST30 and one was ST80 PVL negative (776). Few data are available regarding the molecular genotyping of CA-MRSA from Thailand. In Cambodia, the diagnostic microbiology facilities at Angkor Children's Hospital identified CA-MRSA from 3 different northwest provinces in 2006 to 2007 (777). Fifteen of these isolates were ST834-IV PVL negative, belonging to CC9 (similar to the Western Australia isolate known as WA MRSA-41), and 2 of the isolates were ST121-V PVL positive. A carriage study in Cambodia showed that 91% of the MRSA isolates belonged to ST834 (778). Reported in 2007, an MRSA outbreak in Vietnam associated with vaccinations showed PVL genes among all isolates, which carried SCC*mec* V and belonged to the ST59 lineage, similar to the endemic CA-MRSA clone in Taiwan (779).

In India, during 2006 to 2009, 412 MRSA isolates were identified from patients in Mumbai. Among them, 34% isolates carried SCC*mec* IV and 41% carried SCC*mec* V (430), with 90% of SCC*mec* IV isolates and 62% of SCC*mec* V isolates being PVL positive. ST22-IV and ST772-V were identified as the major clones; however, ST772 was first reported in Bangladesh and has also been reported in Malaysia (780, 781). In addition, ST22-IV, a variant of the United Kingdom epidemic clone EMRSA-15, was identified (782). Nadig et al. (783) also reported finding variants of EMRSA-15 clones among carriers and patients with SSTIs and neurological diseases in Bengaluru (783). In Pakistan, a study conducted during 2006 to 2007 showed that 15% of MRSA isolates were community onset, with ST8-IV as a predominant strain (560). Apart from this, not many data are available from these countries.

Australia and New Zealand. The molecular epidemiology of CA-MRSA in Australia and New Zealand has changed considerably over the past decades. Some of the first global reports of CA-MRSA were from remote aboriginal communities in Western Australia, and the epidemiology of CA-MRSA in this region has several unique features. This is mostly due to the geographical isolation and exclusively indigenous communi-

ties dwelling in this region. Hence, there are a number of distinct CA-MRSA clones circulate in this region.

The Queensland clone (ST93-IV) has been the predominant clone in Australia since 2008 (784). First identified in 2000 (785), this clone harbors PVL genes and is typically susceptible to non-β-lactam antibiotics. The clone has successfully managed to spread throughout Australia and has been found to be associated with both SSTIs and severe invasive infections (786). Facilitated by international travel, this clone has also been reported in other parts of the world as well (787, 788). Other CA-MRSA clones known to circulate in Australia, in decreasing order of prevalence, include ST30-IV, ST1-IV, ST45-IV, ST78-IV, and ST5-IV (789).

ST30-IV, the major cause of CA-MRSA infections throughout New Zealand, was first identified in Auckland in 1992 in individuals who had contact with Western Samoa (790, 791). The clone then spread throughout the mid-1990s and early 2000s, when it became a major etiology of CA-MRSA infections in the country. In contrast, in 2007 the three most common CA-MRSA clones detected in Samoa were ST8-IV, ST93-IV, and ST1-IV, while ST30-IV accounted for only 12% of MRSA isolates (792). ST30-IV isolates are PVL positive and predominantly associated with SSTIs in otherwise healthy individuals. Since 2005, ST30-IV in New Zealand has largely been replaced by ST5-IV, which is becoming a predominant cause of CA-MRSA infections in that country (789); however, the fundamental cause of this rapid and sustained emergence is not clear.

LIVESTOCK-ASSOCIATED MRSA (LA-MRSA)

When it first appeared in hospitals in 1961, MRSA was considered purely a nosocomial pathogen (14, 91). Years following its first report, it emerged in the community as community-associated MRSA (27). However, an ever-changing epidemiology of MRSA has now raised concerns about its presence in livestock, as livestock-associated MRSA (LA-MRSA). Although it was first isolated in 1972 from a Belgian cow (56), LA-MRSA gained significant attention over a decade ago, with an alarming report about infections and high rates of MRSA colonization among Dutch pig farmers in 2005 (64, 391, 392). LA-MRSA isolates are genetically distinct from human isolates, comprised mostly of MLST type ST398 from CC398 and representing the largest reservoir of MRSA outside hospitals (393). Since ST398 strains are the major MRSA type reported in pigs at the international level, it is conceivable that this strain originated in pigs and then subsequently was dispersed to other species (57). CC398 is reported from various parts of the world, where it is associated mainly with food animal species such as pigs and veal calves but has the capacity to colonize a wide range of hosts, including dogs, cats, sheep, cows, goat, poultry, rabbits, and horses (57–61). Figure 5 shows a population snapshot of LA-MRSA worldwide.

LA-MRSA strains are important from a monetary prospective as they cause infections in economically important livestock animals. For example, intramammary infections in dairy cows leading to mastitis result in a major financial burden on the dairy industry worldwide (793). Similarly, small ruminants affected by the disease are a particular issue in regions producing sheep and goat cheeses (794). It also causes skeletal infections in commercial broiler chickens, initiating lameness in the poultry (795). *S. aureus* epidemics causing skin abscesses, mastitis, and septicemia in rabbits are frequent in continental Europe, where rabbit farming is an expanding industry (796).

The origin and molecular evolution of LA-MRSA seem to be associated primarily with pigs. Since MSSA strain ST398 has been described only in humans and pigs, it is suggested that one of these species is the original host of MRSA ST398. Data supporting MSSA ST398's association with pigs come from an early French study where ST398 was found in certain MSSA clones in healthy pig farmers but not in nonfarmer controls (57, 797). Nearly all MRSA strains found in Dutch pigs were nontypeable using PFGE, and therefore a closer look was taken at all nontypeable human strains to see if there was any link with pig farming. It was found that nontypeable MRSA carriers or infected humans were 12 times more likely to be pig farmers and 18 to 20 times more likely to be cattle farmers. The authors hence concluded that there is an association of non-

typeable MRSA that causes serious diseases with pigs and cattle and that it is also transmitted between humans (57, 797). However, more data are required to reliably conclude that there is relationship between MSSA ST398 and commercially raised pigs.

The main force driving the current pandemic of antimicrobial resistance in pathogens appears to be the excessive use of antimicrobials, along with insufficient infection control measures. This, in addition to the use of antibiotics in animal food (often as a growth stimulant), contributes to resistant microorganisms and genetic spread from animals to humans, either via direct contact or through the food chain by contact with or ingestion of animal food products. The continued use of antibiotics in animal husbandry and agricultural activities has contributed to a significant increase in their resistance and transmission (798–801).

With the emergence of LA-MRSA, surveys have directly demonstrated the presence of MRSA in meat and meat products. Slaughtering of MRSA-carrying animals may result in contamination of the carcass. Various meat samples were collected from the retail trade by the Dutch Food and Safety Agency to test for the presence of MRSA in meat samples. Of 2,217 samples analyzed, 264 (11.9%) were contaminated with MRSA. The various kinds of contaminated meat included beef, veal, lamb and mutton, pork, chicken, turkey, fowl, and game, with ST398 as the major strain responsible for 85% of the contamination (802). Another Dutch survey found that 46% of retail meat samples were contaminated with *S. aureus*. Of them 2% were MRSA belonging to ST398 and USA300 (803). In a U.S. survey, 39.2% of retail meat samples had *S. aureus* contamination, 5% of which were MRSA (804). On the other hand, 7.7% of Canadian retail meat samples harbored MRSA belonging to ST398 (30%), ST8 (40%), and ST5 (30%). Interestingly, ST5 is a common strain found in humans in the United States and Canada (805). The surveys clearly indicate the presence of MRSA in food, posing an imminent risk for human health.

There are limited data available regarding the rates of colonization of humans with LA-MRSA. Within a cohort study among 1,878 volunteers from the general population in a "pig-dense" region of Germany, only 0.8% of individuals were found to be colonized with MRSA. In contrast, LA-MRSA colonization in people with livestock contact was significantly higher, with about 40% of MRSA strains belonging to *spa* types indicative of the CC398 lineage (806).

Since the emergence of this new MRSA clone associated with livestock, molecular typing methods have confirmed the relationship of this strain with food production, animals, and humans in contact with these animals. From these reservoirs, MRSA can be introduced into hospitals, causing serious infections and outbreaks, which have been incidentally reported. However, how critical this new development is to human health and the possibilities of infection control are currently subjects under exploration. Bearing in mind the amount of LA-MRSA dissemination among production animals, it is unlikely that attempted eradication efforts would be successful. While MRSA is frequently found in retail meat, with the potential for widespread propagation in the population, there are signs that ST398 does not easily spread among humans and is potentially less virulent than other MRSA strains (65). This assumption, however, needs extensive investigation before coming to a conclusion. Regardless, the potential implications for MRSA reservoirs in food animals and meat demand careful monitoring of the epidemiology of this strain to design appropriate control measures before a catastrophe occurs.

A summary of the molecular characteristics of some common LA-MRSA clones around the world is given in Table 3.

Transmission of LA-MRSA between Livestock and Humans

The potential for animals to act a source and/or reservoir of *S. aureus* zoonotic infections has been exemplified by a few recent reports of human infections caused by pig-associated strains of MRSA. Persons in direct contact with livestock colonized by MRSA, such as farmers, personnel at slaughterhouses, transporters of livestock, and veterinarians, are at increased risk for colonization with LA-MRSA. They in turn may

TABLE 3 Molecular characteristics of LA-MRSA clones around the world[b]

Location	Standard name	Molecular characteristics					
		PFGE	MLST	*spa* type	SCC*mec* type	*agr* type	PVL
USA/Canada	ST398-MRSA V	NT[a]	ST398	t571/t011/t034/t1197/t1250/t1451/t1456/t2510	V	1	−
	ST5-MRSA IV	PFGE type I	ST5	t002/t003/t311	IV	2	−
Europe	ST398-MRSA V	NT	ST398	t571/t011/t034/t1197/t1250/t1451/t1456/t2510	III/IV/V/VII	1	
	ST9		ST9	t100/t411/t899/t4358		2	
	ST97-MRSA V		ST97	t1234	V/IV	1	−
	ST1379-MRSA V		ST1379	t3992	V	1	
	ST1-MRSA IV	USA400	ST1	t128/t127/t125/t1178	IV	3	−
	ST130-MRSA XI		ST130	t373	XI		−
Africa	ST398-MRSA IV	NT	ST398	t571/t011/t034/t1197/t1250/t1451/t1456/t2510	IV	1	
Asia	ST9-MRSA		ST9	t100/t411/t899/t4358	III/IV/V/NT	2	
	ST398-MRSA V	NT	ST398	t571/t011/t034/t1197/t1250/t1451/t1456/t2510	IV	1	

[a]NT, nontypeable.
[b]−, absence of PVL. Blank entries indicate a lack of available information.

become a source of MRSA transmission to other animals and humans. In addition, contact with household members may also result in the subsequent transfer of the bacteria. Animal-to-human transmission could therefore occur via three routes: direct contact, environmental contamination, or handling of an infected animal's products.

As with HA-MRSA and CA-MRSA, the most obvious route of transmission for LA-MRSA is via direct contact with colonized sources (i.e., animals), meaning that those who have direct contact with animals are at highest risk of acquiring LA-MRSA (797). A study conducted with Dutch pig farms revealed that 50% of humans living there were carriers of MRSA, some of whom also developed serious infections (807). Similarly, compared to the general Dutch population, a 760-fold-higher risk of colonization with LA-MRSA was reported for Dutch pig farmers in a study conducted by Voss and coworkers in 2005 (64). MRSA transmission could also occur between cattle and their farmers and between chickens and farmers. Similarly, veterinarians handling infected animals are also at risk of colonization and becoming infected (808). In an international study conducted by Wulf and colleagues (809), 12.5% of veterinarians worldwide were found to be contaminated with MRSA. In 2009, the MRSA prevalence among veterinarians in Switzerland was found to be 3% (810). These studies suggest that people in direct contact with livestock are at potential risk of becoming colonized with and suffering from infection caused by LA-MRSA.

Infected/colonized animals are not the only source of transmission. The first LA-MRSA ST398 outbreak in a Dutch hospital was reported in patients with no apparent contact with pigs or veal calves, suggesting possible human-to-human transmission (811). Van Cleef et al. reported on the prevalence of low proportions (1.17%) of ST398 among humans in 8 of the 15 European countries with a high pig density (422). In high-density pig farming areas in Germany, it was reported that 25% of hospitalized patients had LA-MRSA colonization, and 7% had infections with the same clonal type (812). A comparative longitudinal study performed in three European countries (the Netherlands, Denmark, and Belgium) demonstrated that contact with pigs was the most important determinant for carriage of MRSA among household members of pig farmers (813). Moreover, a study performed in the Netherlands also concluded that working with sows and living with an MRSA-positive pig farmer increased MRSA carriage among household members (814). A study conducted in Taiwan demonstrated that pig nasal carriage of LA-MRSA ST9 was higher in larger farms than in smaller farms (34% versus 7%, respectively), which was echoed by the carriage rates in human contacts (36.8% versus 9.1%, respectively) (815). Studies from organic farms in Germany and the Netherlands indicate that LA-MRSA CC398 is less prevalent in pigs at these farms (816, 817). On the other hand, LA-MRSA dissemination among humans beyond

farms seems to be rare, even in an area with a high density of pig farming (818). Although human-to-human transmission of LA-MRSA seems to be rare, recent reports from Spain and Germany on LA-MRSA infections in humans who had no contact with animals contradict this assumption (819, 820). In addition, a report from the Netherlands revealed that 15% of all LA-MRSA CC398 human cases were connected to people having no direct contact with pigs or veal calves (821).

An alternative hypothesis suggests that the origin of LA-MRSA CC398 is from a human MSSA strain that acquired tetracycline and methicillin resistance (822). Those authors characterized a diverse collection of MRSA and MSSA CC398 isolates from animals and humans from 19 different countries in 4 continents via WGS typing. They discovered that MSSA from humans formed the most ancestral clade upon phylogenetic analysis, while the LA-MRSA was composed of the most derived lineages, with three different SCC*mec* types, IV, V, and VII-like. In addition, the LA-MRSA isolates were largely missing the phages encoding human innate immune modulators that were present in human-associated isolates from the basal clades. These results strongly suggested that the origin of LA-MRSA CC398 was from humans as MSSA and that the jump from humans to livestock was accompanied by a loss of human virulence genes carried by the phage. The lineages subsequently acquired tetracycline and methicillin resistance, which is strongly suggestive of the diverse antimicrobial use associated with food animal production (822).

In addition to direct physical contact, transmission of LA-MRSA has also been shown via the environment, when resistant bacteria from farm animals escape into the environment through manure and/or by being carried in the air. As farm animal manure becomes disseminated on land and contaminates the water supply and crops, there is a danger of MRSA being spread with it. An American study indicated that people living near MRSA-positive pig farms may also be exposed to high MRSA concentrations in the air (797). Dust in stables with MRSA-colonized pigs is also highly contaminated (823), and hence the inhalation of MRSA-contaminated air by humans working in these areas could result in nasal colonization by MRSA (824). This has also been supported by studies where 77 to 86% of humans working in such areas were found to be colonized (818, 825). This extensive colonization, however, seems to be dependent upon the duration of exposure, as well as the intensity of contact with contaminated animals (58). LA-MRSA emitted from air exhausted from pig stables has been found in air up to 350 m downwind from stables and up to 500 m distant on the soil surfaces (826). It has also been detected in manure in chicken farms and in the soil where this manure has been used as a fertilizer (827). Interestingly, in a study from Pennsylvania, USA, skin and soft tissue infections with MRSA were more prevalent in people living in close proximity to fields where manure from conventional farms was used as a fertilizer (828). LA-MRSA has also been reported in dogs and other companion animals, which in turn could also serve as a point of transmission to humans, which serves as a reminder of the importance of basic hygiene in households (62).

As discussed above, besides human-to-human, animal-to-human, and environment-to-human transmission, LA-MRSA could also be transmitted via contaminated meat and meat products (829). To date there are not signs that this has significantly contributed to the dissemination of LA-MRSA to humans; however, handling raw meat with bare hands could allow MRSA to bypass the cooking process and, combined with *S. aureus*'s ability to live easily on skin and soft tissues, could potentially contribute to its dissemination by direct contact with other humans.

LA-MRSA CC398

CC398 is the most common LA-MRSA strain worldwide. However, its prevalence varies geographically, and in certain regions of the world other clonal complexes are more established. In Europe and North America, CC398 is the most dominant LA-MRSA strain, although it has also been detected sporadically in Asia and Africa (64, 830–833). Mainly associated with the colonization of pigs and veal calves, it has been detected in poultry and horses (834–842). The genotypic and phenotypic characteristics associated

with MRSA CC398 include its nontypeability with PFGE using the SmaI restriction enzyme (71). Additionally, most of the CC398 strains belong to the sequence type ST398 or related STs associated with CC398 (843). The SCC*mec* cassettes carried by them are different from those carried by other MRSA genotypes belonging to health care and community settings and mainly include SCC*mec* type IVa or V (189). Variants of SCC*mec* IV, such as 2B and 5, as well as III and nontypeable types have also been reported in pigs and bovines (836, 844). Moreover, coresistance to many non-β-lactam antibiotics is their typical feature. Characteristic resistance genes detected in CC398 LA-MRSA include resistance against trimethoprim, tetracycline, macrolides, lincosamides, gentamicin, ciprofloxacin, and trimethoprim-sulfamethoxazole, as well as those found in animal feed (845). In addition, there is evidence that CC398 can acquire rare genes such as the multiresistance gene *cfr*, encoding resistance to "PHLOPSA" antibiotics. PHLOPSA antibiotics include phenicols, lincosamides, oxazolidines, pleuromutilins, and streptogramins (846). The majority of CC398 strains lack toxins, including Panton-Valentine leucocidin and other enterotoxins (847). However, there are reports of its ability to acquire foreign DNA, which could be one of its most dangerous features. The capability of acquiring virulence genes and the PVL gene (*pvl*) has been demonstrated already (848–850). Other virulence factors, such as staphylococcal enterotoxins, have also been reported occasionally in LA-MRSA CC398 in pigs and turkeys (845, 851–853). Genes encoding adhesion factors such as proteases, hemolysins, leukocidins, and superantigen-like proteins have been detected often in CC398 strains from pigs, poultry, and bovines (836, 839, 851).

In animals, LA-MRSA has been identified as the causative agent in bovine mastitis (842, 854–857) and infected foot joints in turkeys (851) and has been isolated from lesions in pigs (858). The spectrum of infections with MRSA CC398 in humans ranges from minor localized infections such as abscesses (849) and SSTIs (65) to urinary tract infections (UTIs) (849), wound infections (849, 859), mastitis (392), and conjunctivitis (860), as well as more severe invasive infections, including bacteremia (849), necrotizing pneumonia (861), osteomyelitis (65), pyomyositis (862), and postoperative infections (863). Although these cases were reported in the last 10 years, a survey of invasive *S. aureus* infections conducted during a 6-month period among 26 European countries concluded that CC398 strains were isolated in only 0.4% of the samples, and they were all MSSA (310).

Human-origin CC398 contains immune evasion cluster (IEC) genes, which are usually absent in animal isolates (864). These genes, however, have been identified in isolates from nosocomial infections in horses, as well as in veterinarians (864). CC398 also shows little host specificity compared to other *S. aureus* isolates and can move easily between hosts. Considering its ability to acquire genes, there is a real threat that it may become more virulent and dangerous for various species, including humans. An increasing number of severe infections, including necrotizing pneumonia and invasive bloodstream infections, in young healthy people caused by ST398 MSSA strains have been observed in China, France, and the Netherlands. Concerns that these strains represent a more virulent ST398 subtype were raised by a Dutch surveillance study. The study reported that only 0.2% of healthy individuals were colonized by this strain; however, the frequency of its recovery from bloodstream infections is 10 times higher, at 2.1%. In addition, most of these MSSA ST398 infections were acquired in the absence of livestock exposure, indicating that variants of ST398 may be present in humans with no animal contact. Due to the limited surveillance of MSSA, an accurate assessment of its global spread and its clinical relevance could not be done. However, recently, Uhlemann et al. (865) reported ST398 MSSA as a common cause of infections in Manhattan, NY, and documented its frequent transmission between household members. Their analysis noted fewer mobile genetic elements (MGEs) in the chromosome of human-associated MSSA ST398 than in LA-MRSA ST398. While the core genome was conserved between the two strains, the HA-MSSA harbored prophage and IEC genes, giving it increased adhesion to human skin keratinocytes and keratin. It was proposed that, using an augmented inventory of MGEs and adhesion molecules, HA-MSSA ST398 can

spread independent of animal contact, highlighting the importance of molecular surveillance of MSSA ST398.

MRSA CC398 is rapidly evolving. When it was first discovered, CC398 only had a few sequence types and *spa* types, but they seem to be increasing with time. Currently, CC398 harbors 43 sequence types (866); however, the main sequence type colonizing pigs still remains ST398. Other STs in pigs include ST541, ST1965, ST1966, ST1967, and ST1968 (867, 868). The present situation indicates livestock as a major reservoir of continuously evolving MRSA CC398. The impact of this clone appears to be low at the moment; however, with its ability to procure genetic elements, such as genes for virulence factors and antibiotic resistance genes, it may pose a considerable threat to human health in the future. Vigilantly monitoring the evolution and epidemiology of CC398 is crucial from a public health perspective.

Other LA-MRSA Lineages

Over the last 2 decades, the epidemiology of MRSA has changed significantly. While the emergence of LA-MRSA was initially restricted to a single clonal complex, CC398, it has now expanded into several clonal complexes, along with an increased diversity of subtypes within the clonal complexes. It is now clear that the diversity of LA-MRSA is greater and is rapidly changing with time.

CC9. As mentioned above, CC398 is the major LA-MRSA lineage in Europe and North America (64, 830). In Asia, CC9 is the dominant strain, with a prevalence that varies substantially among Asian countries (832). CC9 has also been detected in Europe, first in Italy in 2008 (869), followed by LA-MRSA ST9 isolates found in pigs (870) and poultry (871) from Germany and retail meat in the United Kingdom (872). Recent evidence suggests that ST9 was present in Europe before the emergence of CC398 (873). In a study conducted in the United States, ST9 was one of the most frequent MSSA STs in pigs (873, 874). Due to the differences seen between Asia and the rest of the continents, it is imaginable that the SCC*mec* was acquired only by Asian strains and not by the European ones. ST9 is less studied than CC398 and has typically been found only associated with swine, although it has sporadically been found infecting humans as well (556, 875, 876). Several SCC*mec* types have been noted, including III, IV, V, and nontypeable (NT) types (832). Similar to the case for ST398, ST9 has also been found to be multiresistant and carries rare resistance genes such as those for resistance to lincosamides, pleuromutilin-lincosamide-streptogramin A, and PHLOPSA antibiotics, in addition to the typical resistance genes (846, 877). In addition to carrying at least one enterotoxin gene, some strains are PVL positive, as well as the cause of toxic shock syndrome (832, 875, 878). Asian as well as European CC9 isolates show a great variety of *spa* types (832, 873).

CC97. The leading cause of bovine mastitis worldwide, CC97, is occasionally recovered in small ruminants, pigs, and humans (857, 879–881). The MSSA CC97 strain circulating in humans is believed to have jumped from livestock to the human host approximately 40 years ago, followed by acquisition of SCC*mec* and methicillin resistance (882). This clonal complex is present not only in current collections but also in historic collections dating back to the 1970s and has been found associated with pigs for some time (873). It was discovered in pigs in Italy and in Spain during 2008 to 2009 as a new single-locus variant of ST97, namely, ST1379 (869, 883). However, only about 10% of isolates belonged to ST1379, while the remaining LA-MRSA isolates belonged to CC398 (883).This strain has also been detected in humans and pigs in Spain (884–886).

CC1. A very successful human CA-MRSA lineage, CC1, has only recently been reported in pigs and dairy cows in Italy and now seems to be spreading to other countries (857, 887, 888), such as Denmark and Belgium (844, 889). To date, no PVL-positive CC1 isolates have been detected in livestock; however, their human counterparts are positive for the PVL toxin. Livestock associated-CC1 isolates were found to carry IEC genes, such as *sak* and *scn*, enterotoxin genes *sea*, and genes for β-hemolysins, γ-hemolysin, enterotoxin H, and superantigen X (887, 890). These strains may carry different SCC*mec* types, such as IV or type 5 (5C2), and the strain typically found in animals belongs to *spa* type t127 (857,

887–889). Because of the multidrug resistance capability of ST1 MRSA from animals along with the high virulence capacity and immunomodulatory genes, combined with its ability to readily colonize humans, serious measures need to be implemented at the farm level in order to prevent spillover (887).

Other lineages. Several human-associated strains of MRSA, such as ST239 and ST5, are also found in livestock. In Belgium, ST239 has been detected at low prevalence in pigs, bovines, and poultry (836, 839, 891); however, Belgium is the only location where ST239 has been detected in livestock so far. Another human-associated CA-MRSA strain, the ST80-IV European clone, has also been detected in pigs in Belgium (891). ST5 has been detected at high frequencies in pigs and pork in the United States and Canada (892–895) and, interestingly, has also jumped to poultry, where it is frequently implicated in diseases. The significance of ST5 in pork and pigs remains unclear (822, 851, 896), but it could be a newly emerging LA-MRSA clone in the United States, as it is one of the three most commonly detected MSSA clones in pigs in Minnesota (874). Some other clonal complexes detected in livestock include CC30, CC8, CC20, CC45, CC479, CC522, and CC705 (836, 844, 889).

LA-MRSA XI (*mecC*). As mentioned above, SCC*mec* XI, containing a new methicillin resistance gene, *mecC*, was first identified in MRSA strains that originated from mastitis in cows, as well as from humans in the United Kingdom and Denmark (143). This SCC*mec* type is associated mainly with CC130 but has also been detected in many other CCs, including CC1943, CC425, CC599, and CC59 (897–899). These strains appear to be widespread in Europe and are found in a variety of animal species, as well as in humans and the environment (897, 900). Livestock animals involved are mainly dairy cattle, although other animals involved include sea mammals, pets, wildlife, and zoo animals, while pigs and poultry remain free from it so far (897–899, 901–906). ST130 strains carrying SCC*mec* type XI appear to have a diverse array of virulence factors, such as hemolysins, enterotoxins, immune evasion factors, and toxic shock syndrome toxin, and are therefore likely to be zoonotic, causing infections in animals (898, 907). *mecC*-containing MRSA strains have so far not been detected outside Europe, and a study conducted in France indicates that these strains might have a limited geographic spread (907).

CONCLUDING REMARKS

MRSA, a virulent and difficult-to-treat "superbug," can optimize its gene content and expression to create new strains with augmented virulence and colonization capabilities. Being an extraordinarily adaptable pathogen with the proven ability to develop resistance, MRSA is now considered an urgent threat to public health by the CDC. It is regarded as one of the top-priority antimicrobial-resistant pathogens by the Public Health Agency of Canada (PHAC) (908). It can be considered a continuously evolving wonder with constant emergence of new strains, often resulting in sustained epidemics. Initially only a nosocomial pathogen found in hospitals, it has now created its home in the community and has found a new ecological niche in animals. It is, therefore, an important task for current MRSA research to delineate factors defining the virulence of the entire range of infectious MRSA strains. However, as the phrase states, "prevention is better than cure." Clinicians and investigators have always believed that preventing MRSA infections would be better and simpler then treating them. Much effort has been made toward finding the perfect decolonizing agent to serve as a "magic bullet" to solve this problem, but despite all the hard work, this goal has not been achieved yet. For the years to come, MRSA will remain an important area of research and development. Staying ahead of the problem is best achieved through research to overcome this unceasingly advancing marvel.

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