AMERICAN SOCIETY FOR MICROBIOLOGY | Clinical Microbiology Reviews

Bacteriology | Review

Virulence attributes of successful methicillin-resistant *Staphylococcus aureus* lineages

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SUMMARY Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of severe and often fatal infections. MRSA epidemics have occurred in waves, whereby

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The authors declare no conflict of interest.

See the funding table on p. 33.

Published 20 November 2023

a previously successful lineage has been replaced by a more fit and better adapted lineage. Selection pressures in both hospital and community settings are not uniform across the globe, which has resulted in geographically distinct epidemiology. This review focuses on the mechanisms that trigger the establishment and maintenance of current, dominant MRSA lineages across the globe. While the important role of antibiotic resistance will be mentioned throughout, factors which influence the capacity of *S. aureus* to colonize and cause disease within a host will be the primary focus of this review. We show that while MRSA possesses a diverse arsenal of toxins including alpha-toxin, the success of a lineage involves more than just producing toxins that damage the host. Success is often attributed to the acquisition or loss of genetic elements involved in colonization and niche adaptation such as the arginine catabolic mobile element, as well as the activity of regulatory systems, and shift metabolism accordingly (e.g., the accessory genome regulator, *agr*). Understanding exactly how specific MRSA clones cause prolonged epidemics may reveal targets for therapies, whereby both core (e.g., the alpha toxin) and acquired virulence factors (e.g., the Panton-Valentine leukocidin) may be nullified using anti-virulence strategies.

KEYWORDS methicillin-resistant *Staphylococcus aureus*, virulence, toxins, superantigens, metabolism, gene regulation, mobile genetic elements

INTRODUCTION

Staphylococcus aureus remains a threat to public health despite collective efforts designed to mitigate its impact upon healthcare systems, and the community. *S. aureus* is a commensal of the skin and mucosal surface of about 30% of the human population (1–3). However, when opportunity presents, it is capable of causing a range of infections affecting virtually all of the body's tissues including the skin, soft tissue, blood, bone, heart, and lungs (4–13).

Compounding the problem of *S. aureus* infection is the emergence of antibiotic-resistant strains (14–19). Methicillin-resistant *S. aureus* (MRSA) infections have been a clinical challenge for decades, and compared with infections due to methicillin-susceptible *S. aureus* (MSSA), morbidity and mortality rates are typically higher, which is associated with increased length of hospital stay and associated economic expenses (20–24). The deployment of promising anti-staphylococcal agents has been met with the rapid emergence of resistance, including against last-line agents such as linezolid, daptomycin, and anti-MRSA cephalosporins (18, 25–30). Vancomycin resistance in MRSA remains rare, but the rise of vancomycin-intermediate *S. aureus* strains is concerning (31, 32).

To define the global epidemiology of MRSA, strains are grouped into lineages based on shared molecular characteristics. The success and spread of MRSA lineages are not uniform, with some remaining geographically restricted and others capable of causing global pandemics (33, 34). The regional distribution, frequency, and persistence of dominant MRSA lineages are multifactorial and involve a range of pathogenic factors. In addition to developing resistance to antibiotics, hospital disinfectants, and other toxic compounds, a selection of surface proteins may be present in a given lineage to mediate human infection, including adherence and immune interaction, as well as a range of lytic proteins that contribute to host tissue damage (35–44). Pathogenic factors are often carried on mobile genetic elements that originate in other non-pathogenic species, which serve as a genetic reservoir for the adaptive evolution of MRSA (45, 46). Additionally, subtle genetic changes such as single nucleotide polymorphisms (SNPs), small insertions/deletions (InDels), and genome rearrangements can emerge under selection pressure. This genetic evolution can affect bacterial clone survival and success within a given host through its impact on metabolism and/or virulence gene expression (47–50). The purpose of this review is to summarize current knowledge of the pathogenic mechanisms that contribute to the successes of current epidemic and pandemic MRSA lineages from around the world.

EMERGENCE OF MRSA

Penicillin-resistant *S. aureus*

Mortality rates associated with staphylococcal bloodstream infections in the pre-antibiotic era exceeded 80% (51). In 1941, the outlook for patients with *S. aureus* bacteremia dramatically changed following the introduction of the β-lactam antibiotic, penicillin. β-Lactams bind covalently to penicillin-binding protein transpeptidases (PBPs), inhibiting the final cross-linking reaction in the synthesis of peptidoglycan, which is a critical component of the bacterial cell wall (52). However, penicillin resistance became prevalent soon after the clinical introduction of β-lactams, and by 1948, close to 60% of isolates were penicillin resistant (53). Penicillin resistance was subsequently attributed to the production of β-lactamase, an enzyme that hydrolyzes the β-lactam ring of penicillin resulting in drug deactivation (54–57). The *bla* gene, coding for β-lactamase, is typically carried on plasmids that facilitate horizontal gene transfer (HGT) between bacterial strains and species (54–57).

Methicillin-resistant *S. aureus*

In 1961, the semi-synthetic β-lactam methicillin was introduced, which was resistant to the action of β-lactamases that hydrolyzed penicillin. However, shortly after its clinical introduction, MRSA isolates were reported in the United Kingdom (58), and by the late 1960s, MRSA infections had been described in Australia, the United States (US), Switzerland, Denmark, France, India, and Japan (59–62). MRSA evolved from MSSA via the acquisition of a mobile genetic element known as the staphylococcal cassette chromosome *mec* (SCC*mec*) (63). The *mec* gene complex contains a structural gene (*mecA*, *mecB*, *mecC*, or *mecD*), which encodes a specific penicillin-binding protein (PBP2a, also known as PBP2′), as well as regulatory elements *mecI* and *mecRI* that control *mec* gene expression (34, 63–65). PBP2a has transpeptidase activity and harbors lower affinity for β-lactams when compared to native PBPs (66, 67). In concert with the transglycosylase activity of PBP2, PBP2a can restore peptidoglycan synthesis and generate high-level β-lactam resistance (68). It was thought that MRSA was restricted to healthcare settings until the early 1980s, when cases of community-acquired MRSA (CA-MRSA) infection were first reported (69–71).

Community-acquired *S. aureus*

CA-MRSA and hospital-associated MRSA (HA-MRSA) were traditionally classified by epidemiological definitions. The term "community-acquired" was applied loosely, as the acquisition of infection was often unclear, and many reported CA-MRSA cases that were associated with healthcare risk factors. The term was soon replaced by "community-associated MRSA" and the US Centers for Disease Control and Prevention (CDC) introduced a case definition to distinguish CA-MRSA from healthcare-associated infections (72). An infection was classified as CA-MRSA if it was diagnosed in an outpatient setting or less than 48 hours after hospital admission (72). In addition, the patient would have none of the following healthcare risk factors: history of hospitalization, surgery, dialysis, and residence in a long-term care facility within the previous year of MRSA culture date; permanent indwelling catheter or percutaneous device; or previous isolation of MRSA (72). It is clear that the distinction between CA-MRSA and HA-MRSA has become increasingly blurred as both continue to cause infections and outbreaks in hospital and community settings (72, 73).

Livestock-associated MRSA

MRSA is also an important veterinary and zoonotic pathogen present in a broad range of animal species, including pigs, cattle, horses, rabbits, poultry, dogs, and cats (74–80). The emergence of livestock-associated MRSA (LA-MRSA) in animals is often associated with an increase of LA-MRSA colonization in humans, particularly in farm workers (81,

82). LA-MRSA is capable of host adaptation to animals, and the selection pressure of antibiotics used in animal husbandry has raised concerns of multidrug-resistant LA-MRSA as a reservoir for human MRSA infections (83–85). Control measures for the evolution of antibiotic resistance, virulence, and transmission of LA-MRSA are required to reduce the impact of emerging LA-MRSA lineages on public health.

In addition to *mecA*, *mecC* is another genetic determinant that was first identified in human and bovine MRSA isolates from Denmark and the UK in 2011 (86). The origin of *mecC* remains unknown. Interestingly, Larsen et al. recently reported that particular MRSA lineages carrying *mecC* emerged in European hedgehogs prior to the clinical use of antibiotics (87). One speculative theory of the *mecC* appearance in these MRSA lineages was likely driven by co-evolution, in which *S. aureus* adapted to hedgehog dermatophyte *Trichophyton erinacei* that naturally produces two β-lactams (87).

DEFINING MRSA LINEAGES

Several well-established techniques have been used to genetically categorize MRSA strains in order to describe population structure, and these methods have been discussed in a recent review (34). Here, the focus is on relevant techniques that will provide context when dominant lineages are subsequently described, including pulsed-field gel electrophoresis (PFGE), multi-locus sequence typing (MLST), SCC*mec* typing, and whole-genome sequencing (WGS) (Fig. 1). For other typing methods such as *spa* typing, readers are referred to other reviews (34). Of note, the techniques are not mutually exclusive; often, multiple techniques can be used in combination to define a lineage, and two different techniques can generate definitions that can be used interchangeably.

Pulsed-field gel electrophoresis

Traditionally, PFGE has been a standard method for typing MRSA. This method involves in-gel digestion of chromosomal DNA with a restriction endonuclease, typically SmaI. The DNA fragments are resolved by gel electrophoresis using an instrument that switches current directions based on a predetermined pattern. The relatedness of *S. aureus* isolates can subsequently be determined by comparing the DNA restriction patterns (91, 92) (Fig. 1A). PFGE was formerly the standard method used by the CDC for bacterial strain typing (91). MRSA strains were classified into pulsed-field types (PFTs), for example, PFT USA100 (88). In the early 2000s, PFGE played an important role in identifying USA300 as a dominant CA-MRSA lineage in the US (88, 93). However, PFGE is time consuming and requires costly reagents and specialized equipment, which are its major limitations (94–97). Furthermore, there is insufficient resolution for bands dissimilar in size by <5% and inconsistent interpretation of PFGE bands between different facilities (94–97). Consequently and due to the advent of newer methods for defining MRSA lineages, PFGE is a less commonly used approach nowadays.

Multi-locus sequence typing

MLST is the most widely recognized typing technique for describing MRSA populations. MLST involves sequencing internal fragments of seven housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *ygiL*) that are present in all strains of *S. aureus* (Fig. 1B). The strain of interest is assigned a sequence type (ST) based on the allele profiles of the seven genes via an online database (https://pubmlst.org/saureus/) (98). MLST has the advantage of comparing strains between laboratories and provides the opportunity to explore patterns of evolutionary descent using the algorithm, eBURST, which arranges isolates of similar STs into groups known as clonal complexes (CC) and facilitates the prediction of founder STs (99) (Fig. 1D). STs that differ at only one of the seven MLST loci are grouped together, and the ST with the largest number strains in the group is determined as the founding ST using the principle of parsimony (99). This principle based on the simplest explanation, which is initial diversification of a strain from the founder would differ at

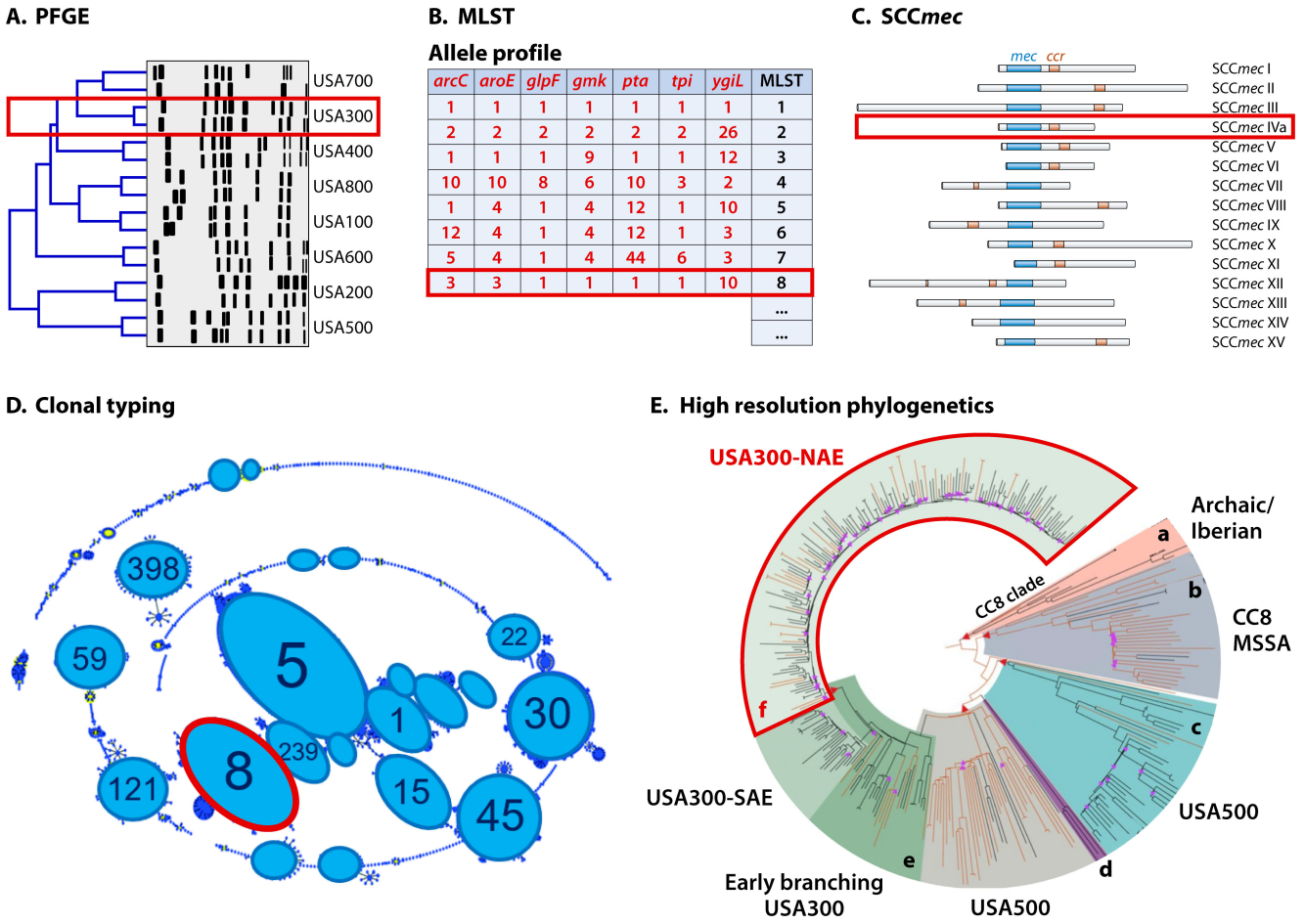

FIG 1 Defining MRSA lineages. (A) Pulsed-field gel electrophoresis (PFGE) was previously the standard method to discriminate *S. aureus* lineages and is based on comparing DNA patterns following restriction enzyme digestion [adapted from reference (88)]. (B) Characterization of the relatedness between *S. aureus* strains was advanced by comparing the sequence of seven housekeeping genes, which is known as multi-locus sequence typing (MLST). (C) As all MRSA strains carry the staphylococcal cassette chromosome *mec* (SCC*mec*), identifying sequence and structural similarities of SCC*mec* between the isolates provides another dimension for lineage definition [based on reference (34)]. (D) Sequence types of MRSA strains defined by MLST can be grouped into clonal complexes (CCs) to infer evolutionary descent across MRSA lineages [adapted from reference (89) with permission of the publisher). (E) Whole-genome sequencing is increasingly being used for phylogenetic analysis to trace the evolution and transmission of successful MRSA clones at high resolution. In the example shown, phylogenetic analysis of 348 genomes illustrates the relationship structure of clinically important CC8 groups. These groups include (A) CC8a Archaic/Iberian, (B) CC8b MSSA clade, (C) CC8c USA500, (D) CC8d CMRSA-9, (E) CC8e (USA500, EB-USA300, USA300-SAE), and (F) CC8f USA300-NAE. Throughout the figure, red boxes indicate how these technologies can be integrated to define a lineage. Here, we have highlighted MRSA-ST8-IVa (USA300-NAE) [adapted from reference (90)].

only one of the seven alleles (99). For example, CC8 harbors genetically similar STs including ST8 and ST247, whereby ST247 most likely originated from ST8. Conversely, ST93 is a singleton and does not cluster with other STs.

SCC*mec* typing

The SCC*mec* element, which harbors the *mec* gene facilitating methicillin resistance, can also be used to describe MRSA epidemiology. SCC*mec* is heterogeneous and ranges in size from 21 to 72.5 kb (100, 101). SCC*mec* is comprised of three basic elements: the *mec* gene complex, the *ccr* gene complex, and the joining region (J region) (63). The *ccr* gene complex of SCC*mec* contains different kinds of recombinases (*ccrA*, *ccrB*, or *ccrC*) that are responsible for the excision and integration of the SCC*mec* element into the chromosome at a specific site (*att*SCC) at the 3′ end of the rRNA methyltransferase gene *orfX/rlmH* (63, 65, 102–104). SCC*mec* also harbors open reading frames in addition to *mec* and *ccr* gene complexes, including additional antibiotic and heavy metal resistance

genes (65, 102, 105). It is the combination of these elements (*mec*, *ccr* complex, and additional genes) that allow for the classification of MRSA into various SCC*mec* types and subtypes (34, 106). To date, there are 15 SCC*mec* types (I to XV) that have been discovered, with subtypes (e.g., IVa and IVb) categorized by variations in the linking region between the *mec* and *ccr* elements (34, 100, 107, 108) (Fig. 1C). Although multiplex PCR is a commonly used method for SCC*mec* typing (109, 110), web-based tools such as SCC*mec*Finder have been develop to determine SCC*mec* types using whole-genome sequencing data (111). MLST is often used in combination with SCC*mec* typing to define a lineage. For instance, PFGE type (pulsotype) USA300 belongs to ST8 and harbors a SCC*mec* type IVa element and can be described as ST8-IVa.

Whole-genome sequencing

The application of WGS to strain typing has risen dramatically due to the advances in sequencing technology coupled with significant reductions in cost (112). At the time of writing, 31,252 whole genomes of *S. aureus* had been deposited at the National Center for Biotechnology Information, of which 1,279 were classed as "complete." Based on these data, the *S. aureus* genome is on average 2.84 Mbp in length, has a guanine-cytosine (GC) content of 32.8%, and has 2,728 predicted coding sequences (CDS). Phylogenomics of *S. aureus* is based on divergence of SNPs between genomes (113). Difference between genomes within the same CC is up to 3,000 SNPs (113). In contrast, a difference of more than 15,000 SNPs can be found when comparing genomes from different CCs (113).

Recent studies have demonstrated the promise of routine WGS of *S. aureus* isolates for epidemiological surveillance and identification of high-risk clones based on clonal relatedness, abundance, virulence, and antimicrobial resistance properties inferred from WGS data (114). WGS together with phylogenomic analyses have also been utilized in the clinical setting to define outbreaks, characterize transmission, and exclude unrelated cases (115–118). WGS enables the expansion of traditional MLST (seven genes) to core genome MLST (cgMLST) that includes 1,861 gene loci (119, 120). Leopoid et al. show that cgMLST identified MRSA transmission events that were unsuspected during epidemiological investigation using *spa* typing, showing the precision and discriminatory power of cgMLST (119). An example is the delineation of clonal relationships between previously indistinguishable MRSA ST398 isolates (n = 66) using SeqSphere+ software to process WGS for cgMLST, which differentiated the isolates by between 3 and 78 alleles (121). Whole-genome MLST (wgMLST) is an extension of cgMLST and uses both the core and accessory genomes for analysis, potentially providing higher resolution than cgMLST (120). A consistent naming system for clones or lineages defined using cgMLST and wgMLST will be required for sharing data and reproducibility in the future (120).

The identification of genome-wide SNPs across MRSA isolates is a common method to define the genetic relatedness. Unlike cgMLST and wgMLST that require a reference genome, SNP calling can be performed with or without a reference genome (120). Using high-resolution SNPs analysis, chains of transmission that were originally unsuspected were uncovered during MRSA outbreaks in neonatal intensive care units (115, 122). Defining genetic relatedness using a consensus SNP threshold is crucial for the interpretation of outbreak and infection control management. Recently, Coll et al. have suggested guidelines for determining MRSA transmission based on genetic differences between strains measured as SNPs (123). If the differences are greater than 25 whole-genome SNPs or 15 core-genome SNPs, it suggests that MRSA transmission within the past 6 months is unlikely (123).

The wealth of information garnered from WGS facilitates the *in silico* prediction of antibiotic resistance profiles (114). One approach involves establishing databases of antibiotic resistance determinants based on existing literature (114, 124). This is followed by cross-referencing the genome of an inquiry sequence against these databases to identify the presence of genes or mutations associated with antibiotic resistance (114,

124). Another method employs genome-wide association studies to pinpoint specific genetic variations in antibiotic-resistant strains in comparison to susceptible strains (125). These identified genetic variations can then be utilized to predict antibiotic resistance in genomes of unknown strains (126). However, the potential limitation lies in our incomplete understanding of the genetic basis of antibiotic resistance when using WGS-based prediction method (126). Therefore, it is advisable to incorporate traditional culture-based antimicrobial testing as a quality control measure to validate phenotypic resistance predictions obtained through these approaches (126).

WGS is poised to replace other typing methods and become the new gold standard for epidemiological surveillance (114, 118). It assists us in precisely defining lineages and, through comparative genomics, identifying genes that may be crucial for patho-adaptation (Fig. 1E). Importantly, the current key challenge lies in the need for universal bioinformatic tools that can seamlessly integrate biological and clinical data with WGS data in a timely manner. The development of WGS bioinformatic pipelines, such as EpiSeq and BacPipe, will help overcome the hurdle of data analysis and promote the routine use of WGS in monitoring infection transmissions in hospitals and public health (127, 128).

FACTORS CONTRIBUTING TO MRSA CLONAL EXPANSION

Virulence factors

S. aureus is a versatile pathogen that, when interacting with a host, can be either colonizing, persistent, or disease causing (129). Here, we will define virulence as "the relative capacity of a microbe to cause damage in a host" (130), and we will thus define any microbial component that contributes to virulence, by facilitating colonization, persistence, immune evasion, or damage to the host, as a virulence factor (Fig. 2). The success of a lineage can thus be determined by the acquisition of a virulence factor on a mobile genetic element (e.g., the *sasX* gene facilitating colonization) or the specific regulation of an intrinsic factor (for example, the accessory gene regulator, Agr). When possible, we will highlight instances where the contribution of a virulence factor to pathogenesis was confirmed using the molecular postulates, including gene deletion, complementation, and overexpression studies.

Cell envelope

The *S. aureus* cell envelope is at the forefront of the dynamic interaction between host and pathogen during colonization. The cell envelope is mainly composed of capsular polysaccharides, peptidoglycans, wall teichoic acids (WTAs), lipoteichoic acids (LTAs), and surface proteins. The role of cell envelope components in *S. aureus* pathogenesis is multifaceted. For example, polysaccharide encapsulation of *S. aureus* promotes colonization on mucosal surfaces and interferes with opsonophagocytosis to facilitate bacterial persistence in human blood [for detailed review, see reference (131)]. Thus, the serotype and extent of capsular polysaccharide produced by a lineage are likely to contribute to its success.

Surface-associated proteins are important for adherence to host tissue, a critical factor in colonization and the initiation of MRSA infection. These proteins facilitate binding to host extracellular matrices (ECM), including collagen, fibrinogen, fibronectin, elastin, and bone sialoprotein (132–136). Based on structural and functional analyses, surface proteins are categorized into five distinct groups: microbial surface component recognizing adhesive matrix molecules (MSCRAMMs), near iron transporter motif family, three-helical bundle, G5-E repeat family, and structurally uncharacterized proteins (35). MSCRAMMs are the largest class of surface proteins and have important roles in adhesion to ECM and immune evasion (136). MSCRAMMs include clumping factor A (ClfA) and B (ClfB); collagen adhesin (Cna); fibronectin-binding proteins A (FnBPA) and B (FnBPB); and serine-aspartate repeat proteins C (SdrC), D (SdrD), and E (SdrE) (35). The full repertoire of surface proteins varies among strains, and many surface proteins have

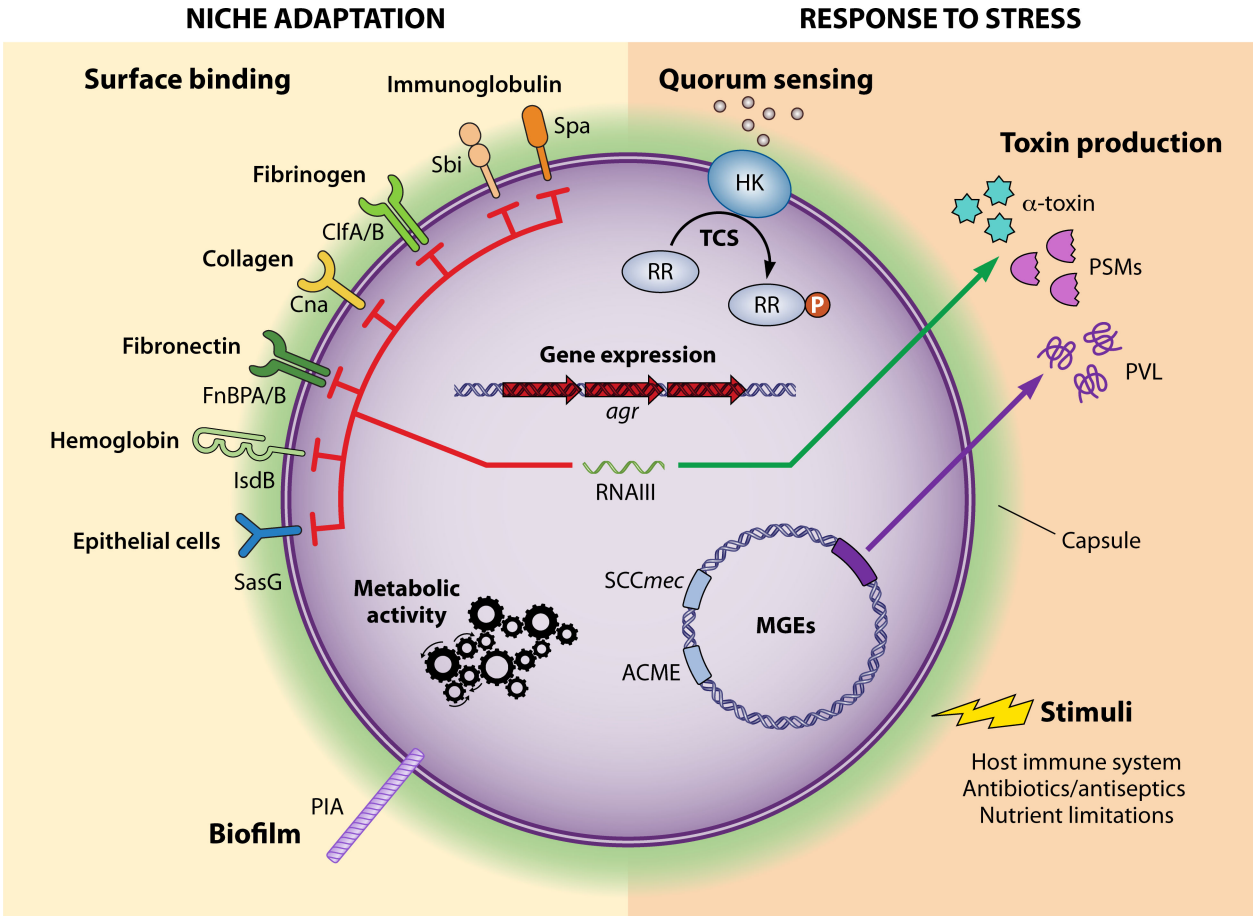

FIG 2 Virulence factors in *Staphylococcus aureus*. The factors are encoded in the genome or acquired via horizontal gene transfer and can be grouped into two categories: establishing niches and responding to stress. Control of these factors is intertwined via two-component regulatory systems (TCS) and the accessory genome regulator (agr). ACME, arginine catabolic mobile element; ClfA/B, clumping factor A/B; Cna, collagen adhesin; FnBPA/B, fibronectin-binding protein A/B; HK, histidine kinase; IsdB, iron-regulated surface determinant B; MGEs, mobile genetic elements; PIA, polysaccharide intercellular adhesin; PSMs, phenol soluble modulins; PVL, Panton-Valentine leucocidin; RR, response regulator; SasG, *S. aureus* surface protein G; SCC*mec*, staphylococcal cassette chromosome *mec*; Sbi, immunoglobulin-binding protein; Spa, staphylococcal protein A.

multiple roles in virulence, with functional redundancy between these surface proteins (35, 137).

Once infection is established, MRSA surface proteins play an integral role in disturbing the host immune system. Protein A (SpA) is a three-helical bundle surface protein that binds human immunoglobulin Fc fragment to inhibit opsonophagocytosis and B-cell receptors to induce B-cell apoptosis (138–142). Inflammatory responses of epithelial cells can also be manipulated by SpA via activating tumor-necrosis factor receptor 1 (TNFR1) and type I interferon (IFN) signaling to promote the pathogenesis of staphylococcal pneumonia (143–145). Sbi is an additional immunoglobulin-binding protein that is released and is capable of inactivating the host complement pathway via interacting with complement component C3 (146, 147).

In addition to facilitating colonization and immune evasion, cell envelope components, peptidoglycan, WTA, and LTA, are major bacterial factors to induce cytokine release and systemic inflammation in the host, which can lead to sepsis, septic shock, and multiple-organ failure if infection is not rapidly controlled (148–151). In MRSA, the alteration of peptidoglycan linkage caused by PBP2a, the product of *mec*A, results in the release of peptidoglycan and the induction of exacerbated inflammation in the host (152). Recent findings also show that specific MRSA strains with the capacity to cause more severe skin abscess in a mouse infection model are closely correlated with higher

content of WTA (153). Taken together, coordination of the cell envelope components is critical for MRSA to establish a niche, to evade the host immune system, and to cause disease. The intricacies of a given lineage's outer surface is likely to play a role in defining its clinical impact.

Pore-forming toxins

MRSA expresses multiple extracellular toxins and enzymes that facilitate tissue dissemination and host cell lysis during the course of infection (154). One major group is the β-barrel pore-forming toxins, including α-toxin (Hla) and the bicomponent leukocidins (Luk) (155, 156). Hla inserts into the plasma membrane of host cells to form a monomeric heptamer complex, leading to the uncontrolled flux of ions and water followed by membrane damage and cell lysis (157–159). The binding affinity of Hla to cells contributes to the sensitivity of different cell types or host species to Hla (160). Hla reduces dendritic cell accumulation in skin during infection, leading to the suppression of antigen-specific T cell responses (161). A disintegrin and metalloprotease 10 (ADAM10) has been identified as a high-affinity binding receptor for Hla on cells to cleave the adherence junction protein E-cadherin, contributing to lethal infection and skin infection in mice (162–164). The important role of Hla in virulence has been confirmed in mouse lung and skin infection models (165–169). Deletion of *hla* resulted in smaller skin lesion in the model while complementation of *hla* in the mutant restored the abscess size at the same level as wild type (165). Vaccination with a non-cytolytic mutant form of Hla (Hla_{H35L}) or targeting of ADAM10 was validated as an effective strategy to reduce the severity of *S. aureus* infections (165–169). Interestingly, a recent study indicates that Hla induces specialized pro-resolving mediators in human M2-like macrophages to resolve infectious inflammation (170), highlighting the multifaceted functions of Hla during host-pathogen interactions.

The bicomponent leukocidins are comprised of the paired fast-eluting F-subunit and the slow-eluting S-subunit, which are so-called based on the rate of migration in liquid chromatography (171, 172). So far, five leukocidins related to human infections have been characterized: LukSF-PV [originally known as Panton-Valentine leukocidin (PVL)], LukED, γ-hemolysins AB and CB (HlgAB and HlgCB), and LukAB (also known as LukGH) (44, 155, 156). The S-subunit of the leukocidins recognizes and binds with high affinity to a target protein receptor on the host cell membrane, which causes the recruitment of the F-subunit to the cell surface. Dimerization of F-S subunits leads to oligomer formation and the assembly of an octameric β-barrel pore that spans the host cell membrane bilayer, resulting in cell lysis (44, 155, 156). Each of these leukocidins target different cell receptors and have host species specificity for toxin binding. Further details of these bicomponent leukocidins are provided in other reviews (44, 155, 156, 173).

Phenol-soluble modulins

Phenol-soluble modulins (PSMs) are a family of secreted short peptides with α-helical and amphipathic physicochemical properties (42, 174). PSMs are grouped into the smaller α-type (PSMα1 to PSMα4, δ-toxin, and PSM-*mec*) and the larger β-type (PSMβ1 and PSMβ2) (174, 175). The *psmα* and *psmβ* operons encode four PSMα peptides (PSMα1 to PSMα4) and two PSMβ peptides (PSMβ1 and PSMβ2), respectively (42, 174). The δ-toxin is encoded by *hld* within the *agr* effector, RNAIII, while PSM-*mec* is found in SCC*mec* types II, III, and VIII (174, 176, 177). All *S. aureus* lineages contain a single highly conserved allele of the *psmα* operon, while *psmβ2* is absent in some lineages (178).

PSMs are able to attract, activate, and lyse human neutrophils and have demonstrated significant contribution to the virulence of CA-MRSA strains in murine bacteremia and skin infection models using PSMα deletion strains (42). Complementation of PSMα in the deletion strain completely restored lytic activities against human neutrophils (42). PSMα3 causes the most profound effects on human neutrophils and inflammatory response compared with other PSMs (42). The cytotoxicity of PSMα3 to human cells is highly related to self-associating amyloid-like PSMα3 fibrils, which are formed by

stacking amphipathic α helices perpendicular to the fibril axis (179). The δ-toxin is a strong inducer of mast cell degranulation to release immunoglobulin-E, which mediates allergic skin diseases such as atopic dermatitis (AD) (180). Replication within the host cell cytoplasm as an intracellular pathogen is another strategy for MRSA to evade the host immune system, and PSMα is important for the escape of MRSA from the phagosome to grow intracellularly (181). All PSMs mediate structural biofilm formation and detachment processes, which was demonstrated by the dissemination of *S. aureus* cells in a murine catheter infection model (182). PSMs are critical to form fibrils that have amyloid-like properties to stabilize *S. aureus* biofilms, and PSMα1 and PSMα4 assemble these fibrils through joining steric zipper interfaces of β-sheets (183, 184).

Superantigens

S. aureus superantigens are secreted virulence factors that disrupt the host adaptive immunity by stimulating T cell hyper-activation and, therefore, contribute to toxic shock syndrome, pneumonia, and sepsis (38, 40, 185, 186). The family of *S. aureus* superantigens contain toxic shock syndrome toxin-1 (TSST-1) and staphylococcal enterotoxins (SEs) and enterotoxin-like (SE*l*s) proteins (186). SEs have demonstrated emetic activity in monkeys; however, TSST-1 and SE*l*s have no proven emetic activity in the non-human primate model despite these toxins being structurally similar to SEs (187). Genes encoding enterotoxins are often found as a cluster of genes in an operon present on a variety of different mobile genetic elements (188). For example, the enterotoxin gene clusters (*egc*) containing *seg*, *sei*, *sem*, *sen*, and *seo* are found in a genomic island νSAβ (189). Enterotoxin genes *sea*, *sep*, *sek,* and *seq* form an immune evasion gene cluster (IEC), which is present in a prophage φSa3int (190, 191). Staphylococcal enterotoxins B (SEB) and C (SEC) promote pathogenic production of interferon γ (IFN-γ) to facilitate bacterial colonization in the liver, contributing to MRSA bloodstream infections in mice expressing human MHC class II (192). The findings of SEB and SEC in liver colonization were validated by successfully complementing the genes *in trans* to restore their functions (192). This excessive IFN-γ from $CD4^+$ T cells induced by SEB and SEC allows MRSA to replicate efficiently within macrophages (192). In addition to superantigen activity, recent data indicate that SEC has antiangiogenic effects to inhibit branching microvessel formation and the expression of angiogenesis mediator, contributing to MRSA endocarditis (193). The critical roles of SEC and staphylococcal enterotoxin-like X (SE*l*X) for MRSA are shown in rabbit infection models and were associated with infective endocarditis, sepsis, acute kidney injury, and necrotizing pneumonia (194, 195). Loss of SEC or SE*l*X in MRSA led to attenuated virulence, while complementation or mutation repair restored disease production (194, 195).

Other secreted enzymes

In addition to exotoxins, MRSA produces other excreted enzymes including lipases, phospholipases, proteases, esterases, and hyaluronidases that contribute to host tissue invasion, immune evasion, and pathogenesis (196, 197). Secreted factors including formyl peptide receptor-like 1 inhibitor, chemotaxis inhibitory protein of *S. aureus* (CHIPS), and staphylococcal complement inhibitor (SCIN) contribute to immune evasion by inhibiting neutrophil chemotaxis and complement activation (154, 198–200). CHIPS is encoded by the gene *chp*, and CHIPS specifically binds to human neutrophils to inhibit calcium mobilization induced by formylated peptides and complement activation C5a (199). A secreted lipase of MRSA, glycerol ester hydrolase (Geh), hydrolyzes host-derived lipoprotein particles to utilize liberated free fatty acids for the bacterial membrane phospholipid biosynthesis as an adaptive strategy (201). Geh is also capable of inhibiting activation of the innate immune system via ester hydrolysis of MRSA lipoproteins, which is a major pathogen-associated molecular pattern recognized by Toll-like receptor 2 of host immune cells (197). Phosphatidylinositol (PI)-specific phospholipase C releases glycosyl-PI-linked proteins from the host cell membrane and contributes to the survival of MRSA in human blood and neutrophils (202). IEC also contains genes responsible for

evasion of the human immune response, including the staphylokinase *sak*, *chp*, and *scn* encoding SCIN (190, 191).

Shaping virulence in MRSA

Regulation

The response to stress both inside and outside of a mammalian host has a fundamental role in the success of MRSA (203). These responses are coordinated by a complex and finely tuned regulatory network controlling virulence and metabolism to adapt to different environments. A bioinformatic analysis predicted MRSA to code for 135 transcription factors; a large number of which are yet to be experimentally characterized (204). Three well-studied systems pertaining to virulence are the two-component regulatory systems (TCRSs), the staphylococcal accessory regulator (Sar) nucleic acid-binding protein family, and alternative sigma factors (205–207). Of these, the most well-characterized and central virulence regulator is Agr, which will be a key focus of this discussion. The functioning of these regulatory systems is further influenced by central metabolism and is dependent on numerous environmental factors.

Two-component regulatory systems

TCRSs are critical mediators of signal transduction in prokaryotes. In their simplest form, they consist of a sensor histidine kinase that responds to an environmental signal by autophosphorylating a response regulator (208, 209). The activated response regulator then binds specifically to target DNA sequences resulting in a transcriptional response (208). Most strains of *S. aureus* have 16 TCRSs, while MRSA strains harbor an additional TCRS within SCC*mec* that mediates methicillin resistance (210, 211). AgrCA is one of the best characterized staphylococcal TCRSs. The *agr* locus encodes two transcripts, RNAII and RNAIII, which are controlled by two divergent promoters, P2 and P3, respectively (205). RNAII contains *agrC* and *agrA*, which encode the histidine kinase and response regulator, respectively, as well as *agrB* and *agrD* (205, 212, 213). AgrD is the precursor of the *agr* autoinducing peptide (AIP), and AgrB catalyzes the formation of an AIP biosynthesis intermediate, the AgrD (1–32) thiolactone (214). Maturation of AIP is mediated by the protease regulator MroQ in *agr* specificity groups I and II (215, 216). AgrC binds AIP resulting in phosphorylation of AgrA, which drives the transcription of RNAIII (217). In addition to coding for the δ-toxin, RNAIII is the effector of the *agr* system, regulating the expression of numerous extracellular toxins and enzymes including α-toxin, PSMs, PVL, enterotoxins, TSST-1, exfoliative toxin and serine proteases, as well as surface-associated virulence factors including SpA, FnBPA, FnBPB, and the capsule (42, 217–221). In addition to AgrCA, numerous TCRSs are confirmed regulators of virulence as determined by gene deletion followed by complementation *in trans*, including SaeRS, LytRS, GraRS, VraRS, SsrAB, ArlRS, and WalKR (222–228).

The association between dysfunctional *agr* and reduced vancomycin susceptibility in MRSA is of interest and has been reported in several studies (229–231). However, other researchers found no significant difference of *agr* dysfunction between high- (≥2 μg/mL) and low-vancomycin minimum inhibitory concentration (MIC) (≤1.0 μg/mL) groups (232). Furthermore, Butterfield et al. showed that there was no association between *agr* dysfunction and vancomycin-intermediate *S. aureus* (MIC ≥4 μg/mL) (233). In line with this discrepancy, *agr* dysfunction did not correlate with vancomycin treatment failure of MRSA in a rabbit infective endocarditis (IE) model (234). The relationship between *agr* and reduced vancomycin susceptibility is further complicated by mutations in *walKR* during vancomycin exposure (235, 236). Rao et al. recently found that the WalK(S221P) mutation that is responsible for a vancomycin-intermediate phenotype failed to activate WalR to bind the promoter of the *agr* system, leading to silenced Agr gene expression and attenuated virulence (235). This study showcases how gene expression is moderated by this type of regulatory crosstalk and can influence colonization, persistence, and disease-causing behaviors (235).

Sar transcriptional regulators

Sar transcriptional regulators are classed based on their homology to the SarA prototype. The *sar* locus consists of three overlapping transcripts driven from three promoters (P1, P2, and P3), each of which contain the *sarA* gene (237). SarA binds to promoter regions termed Sar boxes (238), which directly enhances the expression of hemolysins and surface proteins, including SpA, FnBPA, FnBPB, and Cna (239–242). In addition, SarA binds to both P2 and P3 of the *agr* locus, regulating the production of RNAII and RNAIII (243). As such, SarA regulates virulence factor expression in both *agr*-dependent and *agr*-independent manners (240, 243), further highlighting the complex and intertwined nature of *S. aureus* virulence regulation. The role of SarA-like homologs and repressor of toxins (Rot) in the regulation of virulence factor expression has also been described (244–250).

Alternative sigma factors

Sigma factors bind to RNA polymerase providing gene target specificity during the process of transcription initiation. The primary sigma factor, σ^{70}, is responsible for the expression of housekeeping genes typical of exponential growth, while alternative sigma factors take over in response to adverse conditions. Sigma factor B (σ^{B}) influences the expression of 200–250 genes with various functions, many of which relate to virulence (251, 252). SigB activity has been shown to influence virulence gene expression independently and in concert with other regulatory systems including SarA and Agr (253). The *S. aureus* genome codes for an additional alternative sigma factor, σ^{H}; however, its contribution to pathogenesis is currently unclear (254).

Influence of metabolism

S. aureus virulence is influenced by nutrient composition in the environment, as well as the activity of seemingly unrelated metabolic pathways (255). The contribution of carbohydrate and amino acid metabolism has been appreciated for many years, whereas the role of nucleotide metabolism and lipid biosynthesis is only beginning to be delineated.

S. aureus responds to changes in carbohydrate (glucose, fructose, and glycerol) accessibility via carbon catabolite repression regulators, CcpA and CcpE, each of which have been shown to influence virulence gene expression (256, 257). In modeled diabetic infections, MRSA acquires excess glucose via two glucose transporters to significantly enhance the production of Hla, leading to worse skin infection outcomes (258). Deletion of CcpA results in reduced Hla production, attenuated virulence in the murine diabetic model, and decreased level of bacterial cellular ATP (259). Additionally, pH shifts resulting from glucose catabolism have been shown to inhibit Agr function and downstream virulence gene transcription (260). More recently, the central metabolite, pyruvate, which is a key nutrient in the human host, has been shown to induce the expression of leukocidins and increase the virulence of CA-MRSA by inactivating key TCRSs (AgrCA, SaeRS, and ArlRS) (261). Fatty acid metabolism regulated by NADH-dependent respiration is sensed by SaeRS in *S. aureus* (262). Deficiency of NADH dehydrogenase NdhC impairs *S. aureus* biofilm formation, Hla production, and bacterial colonization in a murine model of systemic infection (262). Complementation of *ndhC* in the *ndhC* mutant restored biofilm formation and Hla production (262).

The relationship between metabolism and virulence is finely tuned by moonlighting or multitasking regulators. Branched-chain amino acids (BCAAs; isoleucine, leucine and valine) are vital nutrients for the growth of *S. aureus*, as they are essential to the biosynthesis of proteins and membrane branched-chain fatty acids (263–265). Depletion of BCAAs is sensed by the transcriptional regulator CodY to lift its repression of the operon for BCAA biosynthesis (266, 267). In addition to amino acid metabolism, CodY is also a global repressor of virulence and mediates the expression of *hla*, the *agr* system, and *saeRS* (266, 268–270). Deletion of *codY* in MRSA strains was shown to impact

virulence in mouse models of necrotizing pneumonia, skin infection, and bacteremia (270, 271). However, deletion of *codY* in a MSSA strain had no effect on host survival and bacterial burden in the same murine bacteremia model (272), suggesting that more studies are required to investigate the role of *codY* in virulence.

The intricate relationship between metabolism and virulence is further illustrated by the link between the nucleic acid biosynthesis pathway and virulence control in MRSA (273, 274). PurR is the master negative regulator of *de novo* purine biosynthesis in bacteria (273–276). Inactivation of PurR in murine infection models leads to a greater amount of secreted leukocidins and Hla and up-regulation of FnBPs, resulting in hypervirulence independent of enhanced purine production (273, 274). The role of PurR in virulence is further confirmed by complementation of PurR, which reverses toxin secretion and hypervirulence phenotypes (273). Interestingly, in a comparative study using clinical MRSA bacteremia isolates from the same clonal complexes, the expression of purine synthesis genes is higher in isolates that were persistent in patients longer than 6 days, compared to the isolates that were resolved within 4 days after therapy (277). These studies suggest that purine biosynthesis may have an important role in MRSA persistence *in vivo*.

Lipid metabolism is important as *S. aureus* cell membranes are involved in crucial cellular processes including stress response, antimicrobial resistance, and virulence (278). Host antimicrobial peptides target *S. aureus* cell membranes composed by phospholipids, mainly anionic phosphatidylglycerol (PG) (278). Aminoacylation of PG with an L-lysine group to form lysyl-phosphatidylglycerol (L-PG) is a defense strategy mediated by multiple peptide resistance factor (MprF) in *S. aureus* (279). Depletion of MprF leads to hyper susceptibility to neutrophil killing and attenuated virulence in animal models (279, 280). Zheng et al. recently showed that secretion of virulence factors LukAB on cell surface and to extracellular milieu depends on L-PG and LTA biosynthesis, which are controlled by MprF and YpfP, respectively (281). Stimulation of host fatty acids induces the expression of the *S. aureus* type VII secretion system genes to export virulence factors during infection (282). This process is mediated by *S. aureus* fatty acid kinase (Fak) pathway to incorporate extracellular fatty acids into bacterial membranes (282, 283). Depletion of the kinase FakA compromises α-hemolysin production, enhances proteases SspAB and aureolysin secretions, and increases resistance to host antimicrobial peptides (284–286). The role of FakA in virulence appears to be tissue specific, as deletion of *fakA* enhances *S. aureus* pathogenesis in a murine skin infection model but reduces the virulence in a mouse model of *S. aureus* bacteremia (282, 286).

Core genome diversity and mutations

Genes that are present in all MRSA strains not only constitute a core component of approximately 75% of the genome and are principally responsible for essential cellular functions but also contribute to virulence (287). Natural variations within the core genome can have remarkable effects upon gene expression and protein function and contribute to the evolution of successful clones under various selection pressures (195, 288). These variations include SNPs, InDels, repeat variations, and operon arrangements (287).

SNPs can result in nonsense mutations that introduce premature stop codons, producing pseudogenes without function. A classic example is the *hla* pseudogene of CC30, which eliminates α-toxin production for this lineage (48). SNPs can result in non-synonymous amino acid substitutions that alter protein function (289). An SNP causing a non-synonymous amino acid substitution within the regulator of the pyrimidine biosynthetic operon (PyrR K126I) resulted in upregulation of the operon and promoted colonization and transmission for a dominant subclone of USA300 (47). SNPs outside of coding sequences can also influence virulence gene expression. Polymorphism in the promoter region upstream of *hla* is a genetic marker for hyper α-toxin production for strains of *S. aureus* isolated from bovine mastitis (289, 290).

Recent evidence supports adaptive shaping of MRSA pathogenicity via phase variation. The function of *agr* can be influenced by a multitude of mechanisms including nonsense mutations, non-synonymous mutations, frameshift mutations, poly(A) tract alterations, and inversion duplication mutations (291–296). Reactivation of *agr* function can be triggered by host-mediated stress such as phagocytosis, resulting in functional reversion of the shutdown mutations (291). Ramond et al. recently showed that loss of *agr* function is associated with a proinflammatory response in the lung, contributing to the long-term colonization of *S. aureus* in young cystic fibrosis patients (297). In a Japanese study, *S. aureus* isolates from infants who did not develop AD had increased frequency of *agr* mutations, compared with the isolates from infants who later developed AD (298). More studies are needed to elucidate the contribution of *agr* in the evolution of *S. aureus* during colonization in different host tissues.

Diversity in operon arrangements can extend variation within core genomes and impact upon bacterial competition and host immune responses (299–301). Again, using *agr* as an example, the operon contains an internal variable region ranging from the C terminus of AgrB to the N terminus of AgrC and spanning AgrD (302, 303). This variation results in four distinct *agr* groups with divergent capacity of AIP to activate or inhibit quorum sensing between strains carrying a different *agr* system (300, 302, 304).

A second example of operon arrangement/composition influencing pathogenicity is the *cap* operon, coding for capsular polysaccharide. Most clinical MRSA isolates express capsular polysaccharides serotype 5 (CP5) or 8 (CP8), which consist of the same repeating element of trisaccharide with a difference only in the linkages between the sugars and O acetylation positions (131, 305–307). The gene clusters responsible for CP5 and CP8 biosynthesis contain 12 essentially identical genes and four type-specific genes (*cap5HIJK* and *cap8HIJK*), which display low sequence similarity (308). The capsular serotype is highly associated with strain lineage as most of CC5 and CC8 strains are CP5 while CC30 strains are CP8 (306, 309, 310). In a murine infection model, CP5 was associated with better bacterial survival compared with CP8 in a bacteremia model, indicating that the difference between CP5 and CP8 likely contributes to the relative virulence of serotype 5 and 8 MRSA *in vivo* (311). CP5-specific monoclonal antibodies were shown to protect mice from bacteremia caused by serotype 5 strains (312–314); however, CP8-specific monoclonal antibodies failed to protect against serotype 8 staphylococcal infections in mice and was associated with a high amount of CP8 release from serotype 8 strains, which hindered the development of CP8 vaccines or antibodies for passive immunotherapy (314).

The accessory genome

Much of the genetic material that exists outside of the core genome is present on various distinct elements that are either mobile [termed mobile genetic elements (MGE)] or were once likely mobile but have since become fixed within the genome [termed genomic islands (GI)]. The presence and arrangement of these elements play a crucial role in shaping *S. aureus* lineages.

Genomic islands

Staphylococcal GIs are stably maintained within the chromosome; however, they present evidence of historic mobility including incomplete integration machinery (315). *S. aureus* GIs do not contain core/essential genes, but they typically harbor genes that contribute to virulence and/or niche adaptation. Often, multiple virulence genes with highly similar sequences appear in series of variable length and composition (316–318). The complement of these genes differs between lineages but is highly conserved within them (316, 318), suggesting a key role for GIs in lineage-specific successes.

S. aureus genomes typically contain two major GIs: vSAα and vSAβ (315, 319). Multiple staphylococcal superantigen-like genes (*ssl*, also referred to as staphylococcal enterotoxin-like, *set*) and lipoprotein genes (*lpl*) are located on vSAα, each of which appear in extended series (320). vSAβ typically carries a serine protease-like (*spl*) gene cluster

and an *egc* and often harbors a lantibiotic/bacteriocin biosynthesis operon (*bsa*), a hyaluronate lyase precursor gene (*hysA*), and genes coding for a bicomponent leucocidin (*lukED*) (316, 320).

Mobile genetic elements

S. aureus contains many MGEs that can move between and across species, including bacteriophages, pathogenicity islands, staphylococcal cassette chromosomes (SCC), insertion sequences, transposons, and plasmids (321). MGEs can provide genes that contribute to both virulence and antibiotic resistance. We will focus our discussion on the specific contribution of MGEs to virulence. MGEs related to antibiotic resistance are discussed in another review (322).

Bacteriophages

Temperate bacteriophages of the Siphoviridae family are frequently integrated in *S. aureus* genomes. Siphoviridae have highly organized genomes that are approximately 40 kb and arranged in functional modules that facilitate lysogeny/integration, DNA replication, transcriptional regulation, packaging, head proteins, tail proteins, and lysis (323). A useful classification scheme is centered upon the sequence of the integrase (*int*). Here, the majority of prophages cluster within seven major groups (φSa1int–φSa7int) (191).

Many important virulence genes are carried by temperate phages. The most common prophage is φSa3int, which is present in approximately 75% of *S. aureus* genomes (191). φSa3int harbors the IEC that contains various combinations of *sek*, *seq*, *sea*, *sak*, *scn*, and *chp* (190). Interestingly, φSa3int further modulates *S. aureus* virulence as its typical site of insertion results in inactivation of *hlb* (324). φSa3int integration is strongly associated with human nasal colonization isolates and is less frequent in isolates from acute infection, suggesting that it is an important mediator of the switch from commensal to pathogen (191). Other factors that can affect nasal colonization include nasal microbiome, the specific composition of which can either promote or inhibit *S. aureus* persistence (325). φSa2int is the second most common prophage and is the major carrier of *lukFS-PV*, which codes for Panton-Valentine leucocidin (191, 326). Thus, like PVL, the presence of φSa2int is strongly associated with necrotizing pneumonia and skin and soft tissue infections (SSTIs) in humans (327, 328). Less frequently detected phage groups φSa7int and φSa1int have been shown to harbor the virulence genes coding for staphylokinase (*sak*) and exfoliative toxin A (*eta*), respectively (191). The recently identified virulence gene *sasX*, which codes for the cell wall-anchored virulence determinant SasX, is present on an atypically large (127 kb) staphylococcal φSPβ-like prophage (329). The prophage was a marker for an epidemic lineage of MRSA (ST239) that spreads through Chinese hospitals in the 2000s (329), reaffirming the important contribution of MGEs to *S. aureus* clonal expansion (330).

S. aureus pathogenicity islands (SaPIs)

SaPIs share similarities with bacteriophages, including a modular genetic architecture with conserved regions for integration, regulation, and replication (331). However, they do not encode the machinery that facilitates HGT but instead can be mobilized by hijacking the capsid of so-called helper phages (332). Toxin genes are commonly present as accessory genes in SaPIs, including TSST and a host of superantigens (i.e., *seb*, *sec*, *sek*, *sel*, *sep*, and *seq*) (332, 333).

Additional elements

Plasmids are more commonly involved in the horizontal transfer of antibiotic resistance genes; however, some code for virulence factors (334). For example, plasmids can contain various combinations of enterotoxin genes (including *sea*, *seb*, *sed*, *seg*, *sej*, *sep*, *ser*, *ses*, and *set*) (334–338), exfoliative toxin B (339, 340), and an *ica*-like locus

that may contribute to biofilm formation (341). As is the case for plasmids, the SCC*mec* element is more commonly associated with resistance to antibiotics and heavy metals; however, certain SCC*mec* variants contain a PSM (termed PSM*mec*) (342), and non-*mec* SCC elements can code for capsule genes (SCC*cap*) (343).

Animal host adaptation

Comparative analyses of *S. aureus* isolates from various sources, including humans and animals, have revealed an additional role for MGEs in host-specific adaptation (344). As mentioned above, the *hlb* converting phage φSa3int carries a set of human innate immunomodulatory genes (the IEC), and this element is infrequently related to animal-associated lineages (345, 346). For some avian-adapted MRSA, φSa3int is replaced by an alternative *hlb* converting phage, φAvβ, which is not associated with human isolates and harbors genes predicted to be involved in avian niche-specific adaptation including an ornithine cyclodeaminase and a putative protease (80, 345). SaPIs have also been shown to harbor host-specific adaptive genes including *SaPIbov2*, which codes for the Bap adhesion protein that was shown to contribute to persistence in a bovine intramammary gland infection model (347). Additionally, differential coagulation capacities of ruminant associated *S. aureus* (348) have been attributed to the SaPI encoded von Willebrand factor binding homologs that have livestock blood clotting specificities (349, 350).

Gene reservoirs

S. aureus is a frequent colonizer of the human skin and shares this niche with a multitude of commensals, including the clinically important coagulase-negative staphylococci (CoNS). Due to their close physical proximity and genetic relatedness, genetic material can be exchanged within and between staphylococci via HGT. Multiple MGEs that have contributed to the success of specific *S. aureus* lineages appear to have originated in the CoNS, suggesting that these species may act as a reservoir for pathoadaptive genes (45).

The archetypal *S. aureus* MGE, SCC*mec*, appears to have originated in the commensal species *Staphylococcus sciuri*. Some taxonomists have suggested the reclassification of *S. sciuri* into a novel genus known as *Mammaliicoccus* (351). The *mecA* gene homolog of *M. sciuri* shares high sequence identity (80%–99%) with *mecA* of contemporary MRSA (352, 353). Importantly, *mecA* from *M. sciuri* confers resistance to β-lactams upon introduction into *S. aureus* (354, 355). Additional genes present on prototypical MRSA SCC*mec* elements have been identified in *Staphylococcus vitulinus*, *Staphylococcus fleurettii*, and *M. sciuri*, suggesting that these three early branching species each contributed to the modular assembly of SCC*mec* (356). Homologs of *psm-mec*, which codes for a toxin that contributes to sepsis for *Staphylococcus epidermidis* and acts at the interface between virulence and antibiotic resistance in MRSA (342, 357), also appear to have its origins in the *M. sciuri* group (356).

Multiple lines of evidence support that *S. epidermidis* is a source for the introduction of SCC*mec* into *S. aureus* (45). SCC*mec* elements are widespread in *S. epidermidis*, and they share high sequence identity with those found in MRSA lineages. Of these, SCC*mec* type IV particularly seems to have appeared in *S. epidermidis* earlier than in *S. aureus* (45, 358). *In vivo*, the conversion of an MSSA to MRSA in a patient undergoing antibiotic therapy was attributed to the horizontal acquisition of SCC*mec* from a co-colonizing *S. epidermidis* (359, 360). However, this particular genetic exchange could not be recapitulated in the laboratory (360).

In addition to the acquisition of SCC*mec* from CoNS, select lineages have benefited from additional elements that have provided an adaptive advantage. These include the arginine catabolic mobile element (ACME), which was assembled in *S. epidermidis* and transferred to MRSA USA300 (361), and the φSPβ-like prophage that harbors the *sesI* homolog *sasX*, which contributed to the spread of ST239 in Asia (362). Each of these elements will be discussed in more detail in the section describing USA300.

EVOLUTION OF SUCCESSFUL MRSA LINEAGES IN THE CONTEXT OF VIRULENCE

The emergence and spread of MRSA across the globe have resulted in distinct clones circulating in different settings and regions. While some clonal types are disseminated, others are restricted to specific geographical locations (Fig. 3). In this section, we will discuss the virulence attributes of current dominant lineages typically associated with HA-MRSA, CA-MRSA, and LA-MRSA from across the globe. The details of the representative strains used to characterize the pathogenicity of dominant lineages are also summarized in Table 1. For each lineage, we will discuss its origins and definitions, epidemiology in humans and/or animals, virulence in animal models, and the current known molecular mechanisms contributing to its success.

CC5

CC5 is a widespread clonal complex, which comprises a large number of different pandemic HA-MRSA clones worldwide (Fig. 3). Although ST5 is the dominant and presumed ancestor of CC5, this lineage comprises many other epidemic clonal types mainly spread within Europe (33), including ST225-II in Central Europe (381), ST125-IV/VI mainly in Spain (421), and ST228-I (South German/Italian clone) mostly in Germany,

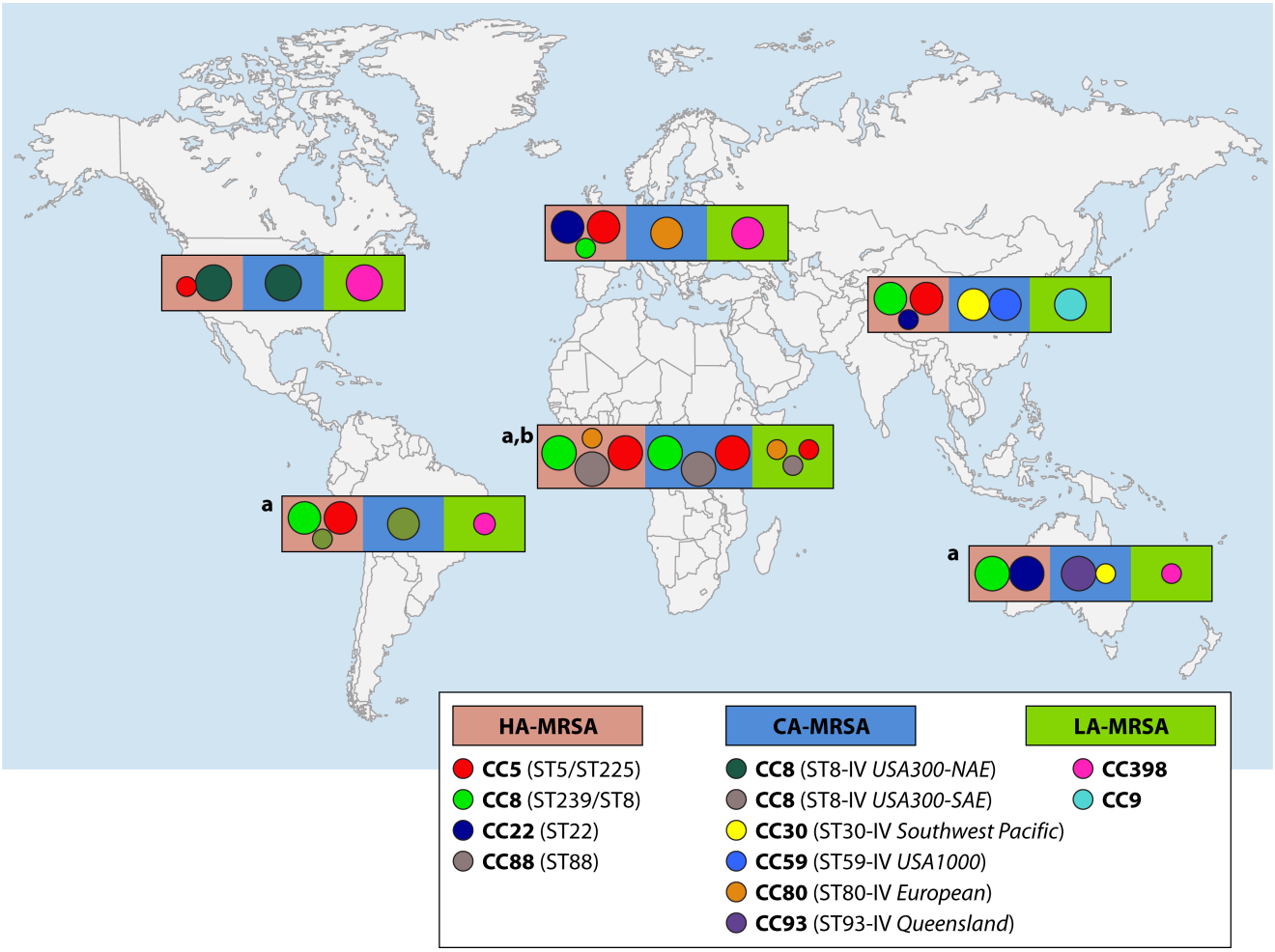

FIG 3 Distribution of current dominant MRSA lineages. The major lineages of HA-MRSA, CA-MRSA, and LA-MRSA reported in each continent or region are shown. (a) Data on LA-MRSA from Africa, Latina America, and Australia were retrieved from single reports due the paucity of available data and, therefore, should not be considered as predominant LA-MRSA lineages in these regions. (b) Although there is no clear distinction between HA- and CA-MRSA clones reported from the African continent, the results should be interpreted with caution since they may reflect the lack of epidemiological data.

TABLE 1 Representative *Staphylococcus aureus* strains of common clonal complexes used in virulence studies

Clone name	Synonyms[a]	Representative	NARSA[b]	ATCC[c]	Region	Accession (reference)	Virulence studies
CC1[d]							
ST1-IV	USA400; Canada-7[e]	MW2	NRS123	BAA-1707	USA	NC_003923 (315)	(167, 301, 363–378)
CC5							
ST5-II/ ST225-II	New York Japan; Rhine Hesse; USA100; UK-3[f]; Canadian-2	N315	NRS70		Japan	NC_002745 (210)	(301, 315, 377, 379, 380)
		04-02981			Germany	CP001844 (381)	
ST5-IV	Pediatric clone; USA800	1045	NRS387		USA	SRR1014708	
ST105		JH1			USA	NC_009632 (382)	
ST228-I	South German Epidemic; Italian clone	16035			Switzerland	NC_020533 (383)	
CC8							
ST8-IV	USA500	2395			USA	CP007499 (384)	(384, 385)
		BD02-25					(364, 386)
		95938	NRS385				
ST8-IVa	USA300; USA300-NAE; Canadian-10; WA-12[g]	FPR3757		BAA-1556	USA	CP000255 (387)	(369, 377)
		LAC			USA		(47, 167, 363, 365–368, 371, 373, 374, 376, 378, 388–393)
		SF8300			USA		(364, 378, 386)
	USA300-BKV				USA	PRJNA497094	(47)
ST8-IVc	USA300-SAE	CA12			South America	CP007672.1 (394)	
ST72-IVa	USA700	CN1			Korea	NC_022226 (395)	(364)
ST239-III	Hungarian, Portuguese, Brazilian, Czech of Vienna clone	TW2			UK	NC_017331 (396)	(372)
	UK-1, UK-4, UK-7, or UK-11; Canadian-3 or Canadian-6.	JKD6008			New Zealand	NC_017341 (397)	(398)
		T0131			China	CP002643.1 (399)	
		GV69			Brazil	CP009681.1 (400)	
ST247-I	Iberian; North German Epidemic; UK-5, UK-8, UK-17	HPV107		BAA-44	Portugal	SAMN06320797 (90)	(386)
ST250-I	Early; ancestral	COL	NRS100		UK	CP000046 (320)	(202, 363, 366, 374, 386)
CC9							
ST9-XII		ZY462471			China	GCA_015070865 (401)	(401)
CC22							
ST22-IV	UK-15; Canadian-8; Barnim Epidemic	H-EMRSA-15			Denmark	CP007659.1 (402)	(372)
CC30							

(*Continued on next page*)

TABLE 1 Representative *Staphylococcus aureus* strains of common clonal complexes used in virulence studies (*Continued*)

Clone name	Synonyms[a]	Representative	NARSA[b]	ATCC[c]	Region	Accession (reference)	Virulence studies
ST36-II	UK-16; USA200; Canadian-4	MRSA252		BAA-1720	UK	BX571856 (403)	(367, 370, 372, 374, 404)
ST30-IV	Southwest Pacific; USA1100	TCH60	NR-10129		USA	CP002110.1	(202)
CC45							
ST45-II	USA600; Canadian-1	CA-347	NRS648		USA	NC_021554 (405)	(406)
ST45-IV	Berlin Epidemic; WA-75						
CC59							
ST59-IV	USA1000	AIS2006061	NRS483		USA		
ST59-VT	Taiwan clone	SA957			Taiwan	CP003603.1 (407)	(371, 407–409)
CC80							
ST80-IV	European CA-MRSA clone	11819-97			Denmark	CP003194.1 (410)	
ST93							
ST93-IV	Queensland clone	JKD6159			Australia	NC_017338 (411)	(372, 391, 412–414)
CC398							
ST398-V/VT		S0385	NR-28983		Netherlands	AM990992.1 (415)	(416–420)

[a]Based on guidelines in reference (33).
[b]NRSA, Network on Antimicrobial Resistance in *Staphylococcus aureus*.
[c]ATCC, American Type Culture Collection.
[d]CC, clonal complex.
[e]Canadian-, Canadian-MRSA-.
[f]UK-, UK-EMRSA-.
[g]WA-, WA-MRSA-.

Hungary, Austria, and Italy (33). ST5-II (also called USA100) was reported as the main clone in New York hospitals in the late 1990s (422). Subsequently, it was also reported in Japanese hospitals and designated as the "New York/Japan clone" (423). ST5-II remained the predominant HA-MRSA clone all over the US during the following 15 years (88) and is still the major HA-MRSA clone in Japan and in other countries in Eastern Asia (424). On the other hand, ST5-IV (also called USA800), which was initially detected among pediatric isolates and referred to as the "Pediatric clone" (425), has also achieved pandemic spread and clinical relevance, including in the African continent (426).

Our current understanding of the virulence mechanisms of CC5 is relatively limited compared with the knowledge of CC8 lineages. Clinical MRSA CC5 isolates were able to cause mortality at similar levels as other major lineages in rabbit endocarditis, murine sepsis, and *Galleria mellonella* infection models (363, 427, 428). *G. mellonella* is a non-mammalian model for studying the pathogenesis of *S. aureus* infections (429). However, contributions of specific virulence factors during CC5 infections have yet to be fully investigated *in vivo*. Gerlach et al. recently showed that CC5 MRSA clones altered cell glycosylation to evade host immunity by abrogating IgG response *in vivo*, which compromised neutrophil phagocytosis of CC5 strains (301). This immune evasion is mediated by TarP encoded in φSa*int*3, which is an alternative WTA glycosyltransferase transferring N-acetylglucosamine to a different hydroxyl group of the WTA ribitol-phosphate than the standard enzyme TarS (301). In addition to immune evasion, CC5 MRSA isolates from patients with bacteremia formed stronger bonds with fibronectin compared with CC45 counterparts, which likely promote binding to target tissues (430).

Phylogenomic analyses and phenotypic studies of clinical isolates provide hints of molecular mechanisms behind the success of MRSA CC5. Clinical CC5 isolates from various infection sites exhibit strong hemolysis of rabbit erythrocytes and strong biofilm formation compared with CC30 (USA200), CC8 (USA300), CC1 (USA400), and CC45

(USA600) isolates in the US, showing the toxicity and virulence of this lineage (363). This is corroborated by a high prevalence of virulence factors in CC5, including IEC in phage φSa*int*3, *egc*, and *lukED* on genomic island υSaβ (363, 431, 432). Among these virulence factors, the TSST-1 gene was strongly associated with lethal infections in a Chinese hospital (433), and staphylococcal enterotoxin P (Sep) was a significant predictor of bacteremia in hospitalized patients colonized with MRSA (434). Sep has been shown to disrupt the immune response by inducing proliferation of human lymphocytes and cytokine production of human T cells (435). Interestingly, clonal expansion of CC5 across the Americas was preceded by convergent loss of *sep* and gains of resistance to fluoroquinolone, macrolide, and lincosamide antibiotics, suggesting that more antibiotic-resistant and less virulent MRSA CC5 clones are more likely to spread geographically (431). However, a new local variant within CC5, ST764, emerged and disseminated endemically via acquiring new virulence determinants, ACME and SaPInn54 (436, 437).

CC8

CC8 is common in the community and in hospital settings, particularly in the US. CC8 encompasses numerous lineages of historic and contemporary importance including the notorious USA300 (ST8-IVa), which will be of particular focus in this review, as well as the closely related USA500 (also mainly ST8-IV), Archaic (ST250-I), and Iberian (ST247-I) clones. ST239 shares sequence similarity with CC8 lineages and will be discussed in the following section.

USA300 (ST8-IVa)

The clonal lineage USA300 emerged in the 1990s and rapidly became the dominant CA-MRSA strain in the US (438–440) (Fig. 3). USA300 was originally defined by its PFGE profile (88). The emergence and spread of USA300 coincided with the increased use of WGS as a diagnostic and epidemiological tool. Comparative genomic analyses revealed several prototypical molecular markers that were then used to define the lineage including MLST ST8, SCC*mec*IVa, genes coding for PVL, as well as either an ACME or copper and mercury resistance (COMER) element, which differentiates the North American epidemic (USA300-NAE) and South American epidemic (USA-300-SAE or USA300-LV) sublineages, respectively (288, 387, 394).

Given the success of USA300, a number of studies compared the virulence of representative strains with isolates from non-USA300 lineages using animal infection models (364, 386). In a landmark study, Li et al. showed that USA300 and the closely related lineage USA500 had enhanced virulence when compared to others related to CC8, including the archaic clone (ST250-I), the Iberian clone (ST247-I), and the Brazilian/Portuguese clone (ST239-III), based on mortality and abscess size in murine bacteremia and skin models, respectively (386). In a subsequent study, the same group showed that CC8 representatives USA300 and USA500, as well as ST80, were more virulent compared with isolates from diverse clonal complexes including CC5 (USA100), CC30 (USA200, USA1100), CC1 (USA400), CC59 (USA1000), and ST72. USA300 produced larger abscesses in a rabbit skin infection model, and this correlated with enhanced host immune markers of infection including leukocyte infiltration and cytokine levels (IL-8 and TNF-α) (364). In a rodent pneumonia model, USA300 produced more severe disease based on mortality and lung tissue pathology compared to an alternative CA-MRSA lineage, USA400 (365). Despite a limited capacity to form robust cardiac vegetations, USA300 isolates were highly lethal in rabbit models of infective endocarditis (363, 366). Taken together, USA300 represents a highly virulent and transmissible clonal lineage, and the molecular characteristics driving these traits are under close scrutiny.

Virulence of USA300 is commonly attributed to the virulence regulator Agr, as evidenced by deletion of the *agr* locus resulting in reduced abscess size and less dermonecrosis for USA300 in a murine subcutaneous infection model (367). USA300 displays striking *agr*-dependent expression of α-toxin, PSMs and PVL (367). Additionally,

virulence was attenuated in *agr* mutants, as well as *hla* and *psm* deletion mutants, in rabbit infection models, thus supporting the role of these genes in USA300 pathogenesis (388). In contrast, despite the strong epidemiological association between the enigmatic PVL and *S. aureus* SSTIs (328), deletion of PVL had no impact on pathogenicity in a murine skin infection model (388) or murine models of pneumonia and bacteremia (167, 368, 389, 441). The lack of a pathogenic contribution for PVL in animal models has been attributed to host specificity of the toxin, whereby PVL is lytic toward human neutrophils, but its effect against murine neutrophils is benign (369). Interestingly, the lytic effects of PVL were most pronounced in the presence of an additional virulence factor, PSMα3 (369). Additionally, the lytic effects of USA300 culture supernatants toward human cells were neutralized by an anti-PVL monoclonal antibody. In a separate study using an *ex vivo* human skin model, PVL was toxic, albeit to a lesser extent when compared to α-toxin (442). More recently, virulence attenuation was observed for PVL deletion mutants in SSTI and pneumonia models when using humanized mice expressing PVL-sensitive receptors (390, 443). Deletion of PVL reduces bacterial burden in lung tissues in the humanized mice and improves clearance of PVL-deficient cells, while complementation of PVL restores these phenotypes (390). Together, there may be a role for PVL in human disease; however, it is likely to be less pronounced compared to other toxins and may be dependent on the activity of other virulence factors (444).

The exact reason for the distinct *agr* regulation profile of USA300 is not completely defined. One possible explanation is the presence of SCC*mec*IV. Unlike SCC*mec*II, SCC*mec*III, and SCC*mec*VIII, which are associated with HA-MRSA lineages, SCC*mec*IV does not code for the *psm-mec* locus. The *psm-mec* transcription product binds to *agrA* mRNA, which inhibits its translation (370). Deletion of *psm-mec* from select HA-MRSA strain backgrounds increased AgrA production and enhanced virulence in murine models of skin infection and sepsis (370). Conversely, introduction of *psm-mec* into USA300 reduced the expression of AgrA (370), suggesting that its absence may facilitate high *agr* activity for this lineage. In a similar vein, the *mecA* gene itself has been shown to reduce the virulence of MRSA lineages harboring SCC*mec* types II, III, and VIII, which may represent a general explanation for the reduced toxicity of HA-MRSA when compared to MSSA (404). In contrast, strains harboring SCC*mec*IV had lower levels of oxacillin resistance and expressed less PBP2a, which correlated with high toxicity similar to that of MSSA (404). In addition, SCC*mec*IV is not associated with the *in vitro* and *in vivo* fitness costs described for other SCC*mec* types (445, 446). Together, SCC*mec* type and its relationship with *agr* functionality may explain a selective advantage for SCC*mec*IV harboring lineages; however, given the distribution of SCC*mec*IV among additional CA-MRSA lineages, it does not specifically explain the success of USA300.

Unlike SCC*mec*IVa, the prototypical ACME is rarely detected in non-USA300 isolates making it an attractive potential explanation for the success of USA300-NAE (447, 448). In addition, despite the close proximity of the elements, when SCC*mec* is infrequently lost from USA300, ACME is retained, suggesting that it provides an appreciable selective advantage (90). However, ACME's contribution to acute virulence in *S. aureus* is unclear. While reduced fitness was attributed to ACME deletion in a rabbit model of bacteremia (445), a subsequent study using murine infection models found that deletion of ACME had no appreciable impact upon virulence endpoints (449). Here, the absence of ACME did not impact upon mortality, organ bacterial density, or lung pathology in a necrotizing pneumonia model or skin dermonecrosis in an SSTI model (449). Taken together, there is some evidence to suggest that ACME may improve *in vivo* fitness, but it does not enhance the severity of invasive *S. aureus* disease in animals.

USA300 acquired ACME from *S. epidermidis*, a predominant member of the human skin microflora (387, 450). This horizontal gene transfer event coincided with the rapid emergence of SSTIs caused by USA300 and displacement of other dominant clonal types causing SSTIs, suggesting that ACME likely contributed to improved colonization and/or transmission as opposed to enhanced acute virulence. Indeed, the skin provides an inhospitable environment for bacterial pathogens and in order to colonize it, *S.*

aureus has to overcome low pH as well as innate and adaptive immune responses (451). ACME harbors several genetic systems that support this hypothesis: an auxiliary arginine deiminase pathway coded for by the arc_{ACME} operon, a spermidine (Spd)/spermine (Spm) acetyltransferase (*speG*), and the copper resistance locus *copXL*. Each system mediates subtle metabolic adaptations that improve survival in conditions relevant to human skin.

The Arc_{ACME} system facilitates acid tolerance for USA300 at pH levels associated with the skin (pH ~5.0) (452). Arc converts arginine to ornithine and concomitantly generates ammonia and ATP. The core *S. aureus* genome codes for an intrinsic Arc; however, this system functions in anoxic conditions not typical of the skin (453). In contrast, arc_{ACME} is constitutively active, and Arc_{ACME}-mediated ammonification effectively neutralizes physiologically relevant acid levels (452). However, circumventing skin pH via arginine deamination presents an additional obstacle; excessive ornithine is converted by the host to polyamines such as spermine and spermidine (452). Polyamines are present at high levels during inflammation and wound healing; they synergize with antibiotics and are toxic toward non-USA300 *S. aureus* (454–456). For USA300, polyamines can be mitigated by the function of ACME encoded SpeG (457). The ΔACME and Δ*speG* mutants are susceptible to polyamines, while introduction of *speG* in *trans* in these mutants recovers the resistance to polyamines (457). However, the exact mechanisms underscoring this detoxification remain unclear. In addition to facilitating polyamide resistance, *speG* has recently been shown to provide additional benefits, including improved adherence, biofilm formation, and resistance to keratinocyte-mediated killing (361). Together, there appears to be a strong selective advantage for ACME elements harboring *speG*, whereby Arc and SpeG are both physically and functionally linked within the ACME, and SpeG works to detoxify a byproduct of Arc activity as well as provide tolerance toward naturally occurring polyamines present in human tissues.

ACME is not present in USA300-SAE. In its place, USA300-SAE has acquired the distinct COMER element. The COMER and ACME regions share two genes, *copX* and *copL*. Phylogenetic analysis of the orthologs revealed that the two major USA300 subtypes acquired the genes from other staphylococcal species independently (394, 458), further highlighting the important role of skin commensals as reservoirs for genes involved in USA300 adaptation. The *copXL* locus is involved in copper resistance (458). While copper is an important cofactor, elevated levels are toxic for bacteria, and it is exploited by the innate immune system for its antibacterial properties, particularly by macrophages patrolling the skin and respiratory tract (458–460). The *S. aureus* core genome codes for an intrinsic copper efflux system mediated by the P_{1B-1}-type ATPase copper efflux transporter CopA and the copper chaperone protein CopZ (461). However, acquisition of *copXL* has been shown to confer copper hyper-resistance for USA300. CopX is a P_{1B-3}-type ATPase efflux transporter which extrudes copper with high efficiency, and it is postulated that CopL may sequester copper and interact with the CopX and CopA transporters (458). Copper hyper-resistance for *copXL* harboring strains has been shown to promote survival in macrophages, suggesting that it may enhance USA300 fitness by circumventing innate immunity (458).

Despite its rapid spread over the past 25 years, there is some evidence that USA300 may be in decline in some regions of the US (462). This is thought to be due in part to improved clinical practices focused on hand hygiene, environmental disinfection, and decolonization strategies. Alarmingly, Copin et al. have recently reported the clonal expansion of a USA300 sublineage, Brooklyn variant (USA300-BKV), which was driven by the acquisition of resistance genes for topical antimicrobials chlorhexidine and mupirocin, leading the authors to postulate that the spread was attributable to excessive clinical intervention (47). In addition to resistance, the USA300-BKV sublineage has adaptive genome alterations that has enhanced its pathogenicity. First, USA300-BKV has a mutation in the regulator of pyrimidine biosynthesis, which resulted in a subtle metabolic shift that improved the fitness of the lineage in a murine colonization and transmission model (47). Second, the lineage harbors a mosaic phage that contributed

to abscess formation in a murine SSTI model (47). This continued adaptation toward enhanced virulence and antibiotic resistance suggests that we are a long way from seeing the end of USA300.

USA500 (ST8-IV)

USA500 is an additional pulsotype describing a group of MRSA isolates from CC8 that are closely related to USA300 and commonly cause invasive infections in North America (88, 463). It was originally thought that USA500 was the direct progenitor of USA300 (386); however, multiple comprehensive phylogenetic studies have suggested that the two lineages most likely arose independently from a common ancestor (384, 463–465). Although USA500 and USA300 emerged in clinics at similar times, the prevalence of USA500 decreased as USA300 proliferated to become the most dominant MRSA clonal type in the US (464–467).

Phylogenetic analysis reveals unique features of USA500 compared with USA300 (463). Insertions of the mobile element IS256 are prevalent in USA500 strains, but no USA300 strains have IS256 (463). Unlike USA300 strains, ACME (or COMER) elements, *speG*, and PVL toxins are uncommon in the USA500 lineage (463). USA500 contains a frameshift mutation in *adsA* encoding adenosine synthase, resulting in a truncated protein of 131 amino acids instead of full-length 773 amino acids (463). The cell wall-anchored protein AdsA generates deoxyadenosine and deoxyguanosine extracellularly to modulate host immune responses (468, 469). AdsA is important for *S. aureus* to escape phagocytic clearance in blood, induce cell death of macrophages, and contribute to bacterial survival in organs in a murine infection model (468, 469). However, it is unclear how this *adsA* mutation in USA500 affects virulence and transmission. A recent study has found that deletion of *adsA* in MRSA induces potent inflammatory cytokines release in mice and promotes protective T cell responses against re-infection (470). More studies are required to examine how USA300 outcompetes USA500, including if USA500 elicits enhanced inflammatory and T cell responses as compared to USA300.

USA500 can cause severe invasive infections associated with high morbidity and mortality and has caused outbreaks in both community and hospital settings (463, 466). The severity of disease caused by USA500 in humans has also been modeled in laboratory animals whereby USA500 showed a similarly high virulence potential when compared to that of USA300 (364, 386). In agreement with these findings, USA500 expresses high levels of toxic exoproducts including α-toxin (384, 386). At least for a subset of USA500 isolates, hypervirulence could be attributed to the acquisition and insertion of the mobile element IS256 in the promoter of *rot*, which codes for the Rot (384). This event resulted in derepression of toxin production for USA500 and subsequently increased virulence in a murine infection model. Additionally, IS256 has also occasionally integrated in the *agr* locus suggesting that the movement of this element can result in both increased or decreased toxin production (384). However, this study was limited to strains from one area (New York). By analyzing a larger, more diverse collection of USA500 isolates, Frisch et al. found that 76 of the included 539 isolates harbored at least one copy of IS256 and that these isolates typically clustered in one of three clades within USA500 (463). This suggests that IS256-mediated toxin regulation cannot entirely explain the hypervirulence of USA500.

Taken together, USA500 and USA300 are very closely related, and each have high virulence potential. Of note, USA500 harbors the same SCC*mec* element as USA300, suggesting that it may also have virulence benefits when compared to HA-MRSA. Nevertheless, it is clear that virulence *per se* is not the sole determining factor for an MRSA lineage and that more subtle changes in physiology that result in improved colonization and transmission likely contribute to the comparative success of USA300.

CC9

MRSA CC9 clones were first isolated from pig farms in China in 2008 and have become the predominant LA-MRSA clonal lineage in Asia (471, 472) (Fig. 3). In the US, it is concerning that colonization of the CC9 lineage has emerged among pigs raised in industrial hog operations and persons who work or live close to hog operations (473, 474). Recent genomic analyses of 191 ST9 strains collected from 12 countries suggest that ST9 emerged as a human MSSA approximately two centuries ago (475). The loss of the immune evasion cluster genes (*scn*, *chp*, and *sak*) for human infections and the acquisition of animal-specific virulence factor SaPIbov4-like element-encoding *vwb* in ST9 support the evolution of host shift from human to animals (475).

Phylogenetic analysis of *S. aureus* isolates from different sources has indicated that pigs are an important reservoir for CC9 transmission to human and bovine hosts (476). Close contact is a likely risk factor for transmission given that the carriage rate of MRSA CC9 for pig farm workers was significantly higher than that of the general population (477–480). However, the pathogenicity of CC9 in humans remains largely unknown. Chen et al. recently identified eight MRSA CC9 isolates after screening 3,328 clinical MRSA isolates from a national database (481). The majority of these CC9 isolates carried SCC*mec*XII and were associated with lethal bacteremia and osteomyelitis, indicating that CC9 strains can be pathogenic to humans (481). Jin et al. recently reported a highly virulent ST-SCC*mec*XII isolate with IEC-carrying βC-φ, and phylogenetic analysis indicated that this strain is likely evolved from an MSSA predecessor rather than LA-MRSA ST9 (401).

Virulence factors of MRSA CC9 have not been fully investigated in animal models; only vertical perinatal transmission from sow to newborn piglets was shown in a swine model (482). Nevertheless, WGS analysis has shown that MRSA CC9 has acquired many MGEs harboring functional antimicrobial resistance and virulence genes, possibly due to the use of antimicrobials in industrial animal food productions (476, 479, 483, 484). Taken together, MRSA CC9 presents a potential risk as a zoonotic pathogen, and multidrug resistance is of particular concern.

CC22

CC22 clones cause the majority of HA-MRSA infections worldwide, particularly ST22-IV, which is a highly epidemic MRSA (EMRSA) clone designated as EMRSA-15 (Fig. 3) (33). It is considered the most rapidly expanding and persistent HA-MRSA clone in Europe (485). First isolated in the United Kingdom in the mid-1980s, EMRSA-15 quickly became endemic in UK hospitals and subsequently disseminated throughout Europe and beyond, namely, to Australia, Asia, and the Middle East (33, 486). Currently, EMRSA-15 is the main HA-MRSA clone in Australia (56%) and Singapore (424, 487). Using phylogenomic analyses, Holden et al. (488) showed that the current pandemic ST22-IV clone evolved from a healthcare-associated EMRSA-15 subclone spread in England during the mid-1980s (488).

The success of CC22 is likely related to antibiotic resistance, virulence, and stress tolerance. The presence of a fluoroquinolone resistance trait in CC22 provided a selective advantage, while there was extensive use of this class of antibiotic in the early 1990s (488). CC22 MRSA strains show greater fitness, strong biofilm formation, and superior capacity to survive desiccation, antiseptics, oxidative stress, heat, and pH changes compared with other competing clonal types, including CC5 (ST228-I), CC30 (ST36-II), and CC8 (ST239-III) (489–492). Animal models of infection have also been used to assess the virulence of CC22 (489, 493, 494). In a mouse model of acute lung infection, CC22 exhibited higher virulence, resulting in lethal infections and causing a higher bacterial load in the lung, which in turn led to severe inflammation, as compared to CC5 (ST228-I) (489). ST228-I was the predominant clone replaced by CC22 (489). Further comparisons with CC5 (ST228-I) revealed stronger α-hemolysin activity and β-hemolysin production, as well as an active *agr*, which partly explained the enhanced virulence of CC22 (491).

Naturally occurring variations in the *agr* system in CC22 strains can directly impact on virulence, suggesting that it is a virulence switch for this lineage (494). For example, amino acid substitution Y223C in AgrC, caused by an SNP in *argC*, led to the destabilization of the AgrA-AgrC interactions. This caused differential regulation of virulence genes, leading to a switch from a cytotoxic to a colonizing phenotype and less severe skin tissue damage in a murine skin infection model (494). This phenotypic switch likely promotes the emergence of new variants for the ongoing persistence of CC22. However, given that this observation is based on a limited number of isolates, further comprehensive studies are required to investigate the role of the *agr* system in CC22 virulence.

Despite our current understanding of CC22 virulence factors informed by experimental animal studies, it remains to be fully elucidated if these virulence factors are important in human infections. A recent study extensively investigated the role of bacterial factors in determining disease outcome of *S. aureus* bacteremia in humans (495). Recker et al. quantitatively phenotyped cytolytic activity and biofilm formation of a collection of 135 sequenced clinical CC22 isolates from patients with bacteremia. The researchers utilized a machine-learning framework to analyze the pooled data of bacterial phenotype and genotype together with clinical metadata (495). Elevated cytolytic toxicity in combination with low levels of biofilm formation was predictive of an increased risk of mortality in infections caused by CC22 strains (495). A virulence factor for *S. aureus*, CapA, was identified to be predictive of mortality within the CC22 collections in their model (495). The *capA* gene encodes a dual-function phosphodiesterase/kinase activator and forms a protein complex with CapB to positively control multiple enzymatic checkpoints of capsule biosynthesis (496). Expression of capsular polysaccharide is important for protection from host immune responses and is a determinant of virulence in a mouse bacteremia model (497, 498). A naturally occurring SNP in *capA* was identified in CC22 isolates from patients who survived their bacteremia compared with isolates from patients who died. This SNP led to amino acid substitution P146S in CapA and was associated with reduced capsule production, a lack of reactivity to the antisera and susceptibility to human neutrophil killing compared with wild-type CapA (495).

Taken together, antibiotic resistance and high tolerance to environmental stress contribute to the success of CC22. Further genomic and phenotypic analysis of clinical CC22 isolates combined with clinical records will improve our understanding of factors contributing to bacterial virulence and pathogenesis in humans. This knowledge will potentially improve management of infectious diseases caused by this lineage.

CC30

S. aureus isolates belonging to CC30 have been causing bacterial epidemics for close to 70 years in both hospital and community settings (48, 499). The first lineage of CC30 to become prominent was the penicillin-resistant, methicillin-sensitive clone known as phage-type 80/81, which emerged in Australia, Europe, and North America in the early 1950s (500–502). The decline of phage-type 80/81 coincided with the clinical introduction of penicillinase-resistant β-lactams (i.e., methicillin) in 1961. Two CC30 MRSA lineages largely replaced phage-type 80/81: the hospital-acquired ST36-II clone known as EMRSA-16 in the UK (503), which is closely related to USA200 in the US (88), and the community-acquired ST30-IV PVL+ lineage known as the Southwest Pacific Clone (SWP) (499).

EMRSA-16/USA200 (ST36-II)

For the purpose of this review, we will refer to EMRSA-16, USA200, and closely related CC30 MSSA isolates as contemporary CC30 hospital isolates (cCC30), as previously described (48). cCC30 were a predominant source of hospital-acquired infections, particularly in the UK throughout the 1990s (503, 504), but they have more recently been displaced in hospital settings by CC22 (505). Nevertheless, in humans, cCC30 MRSA is associated with persistent bacteremia and hematogenous complications such as IE (506–

511). In a rabbit infection model, cCC30 has a propensity to cause IE rather than lethality (366), which correlates with what has been observed clinically. When compared with the other closely related CC30 clones, phage-type 80/81 and SWP, cCC30 infections were less lethal in both bacteremia and pneumonia models (48). cCC30 isolates taken from patients with persistent bacteremia were more resistant to host innate immune defenses and displayed enhanced adherence to host cells *in vitro*, compared with isolates causing resolving bacteremia (from CC8). However, this did not correlate with any differences in acute virulence endpoints in a rabbit IE model. Nevertheless, in the same IE model, the cCC30 strain was more resistant to vancomycin therapy evidenced by greater cardiac vegetations after treatment, highlighting the potential for enhanced persistence of this lineage (511).

WGS has been crucial for the molecular differentiation of CC30 and has revealed a number of genetic contributors to explain the pathogenicity profile of each sublineage (512). Numerous mechanisms have been identified that appear to direct cCC30 away from acute bloodstream infection and toward persistence and niche adaptation, including gene mutations that directly alter toxicity and virulence gene expression, acquisition of MGEs, amplification of insertion sequence (IS) elements, and altered metabolism (513). cCC30 harbors a mutation that generates a premature stop codon in *hla*, which abolishes the production of functional α-toxin and has been shown to directly contribute to reduced virulence in bacteremia models (48). cCC30 also possesses an SNP mutation in *agrC*, which results in a non-synonymous amino acid change (G55R) that reduces RNAIII transcription. However, the impact of this specific mutation to virulence in animal models was less pronounced when compared to that of *hla* (48, 514). Additionally, CC30 codes for a unique variant of PSMα3, which is typically the most proinflammatory example of these cytolytic peptides (42). The non-synonymous mutation to *psmα3* in CC30 isolates (PSMα3N22Y) reduces the potential for the peptide to stimulate neutrophil chemotaxis and reduces its contribution to cytotoxicity when compared to classical non-CC30 PSMα3 (514). In a murine bacteremia model, the CC30-specific PSMα3N22Y contributed to hematogenous seeding, as determined by kidney abscess formation. No enhanced virulence profile was observed using an SSTI model, suggesting that the contribution of PSMα3N22Y to virulence is infection setting specific, which may explain the association between CC30 and hematogenous infections in humans. Of note, PSMα3N22Y is present in both historic and contemporary CC30 strains, so it cannot explain the particular virulence profile of cCC30 (514). However, it is possible that reduced toxicity mediated by the combination of *hla* and *agr* mutations and PSMα3N22Y may synergistically contribute to persistence in humans by circumventing the host immune response.

In addition to causing persistent bacteremia and infections associated with hematogenous spread, CC30 is largely responsible for cases of toxic shock syndrome (TSS) (515, 516). In CC30, the gene coding for TSST-1 (*tst*) is carried on SaPI2, which is commonly harbored by both MSSA and MRSA strains (516, 517). However, TSS is more commonly caused by CC30 MSSA, and this correlates with increased production of TSST-1 compared to *tst*-positive CC30 MRSA, as demonstrated *in vitro* (515). Reduced production of TSST-1 for *tst*-positive MRSA has been associated with a non-synonymous SNP in *ccpA* that resulted in an amino acid change (T87I) in the catabolite control protein CcpA, which has been shown to directly influence the expression of *tst* (518). While the association was strong [33/39 *tst*-positive MRSA compared with 0/23 *tst*-positive MSSA (518)], the direct contribution for CcpA T87I to TSST-1 production or TSS is yet to be confirmed using allelic replacement. This possibly represents another example of the evolution of cCC30 MRSA away from acute toxicity; however, the benefits of reduced TSST-1 production remain to be elucidated.

SWP clone/USA1100 (ST30-IV)

The SWP clone is a pandemic lineage of CA-MRSA that has been identified from numerous locations across the globe (519). It was originally thought that SWP was a

direct descendant of phage-type 80/81 (499); however, multiple reports using high-resolution molecular differentiation facilitated by genome sequencing revealed that the clone, along with cCC30, arose from a common ancestor (48, 517). Nevertheless, SWP and phage-type 80/81 share high toxin production profiles and are highly virulent in murine sepsis and pneumonia (520). Compared with cCC30 and CA-MRSA clones such as USA300, the lineage-specific molecular contributors to SWP virulence are not well characterized. One study assessed the contribution of PVL to cytotoxicity toward human osteoblasts and found it to be negligible (521). Given its known contribution to severe infection and its global distribution, more work is warranted to address the pathogenicity of the SWP clone.

ST239

One of the more successful global clones of HA-MRSA is ST239 (Fig. 3). The lineage emerged as the result of a major recombination event involving ST8 and ST30 (522). Recent genomic analyses indicate that ST239 originated between 1920 and 1945, predating the clinical use of methicillin in 1959 (523). ST239 has evolved toward antibiotic resistance and virulence, with the cost of lower competitive fitness compared with ST8 and ST30 (523). Comparing with ST8, ST22, and ST93, ST239 and ST36 isolates were less cytotoxic toward monocyte-macrophage THP-1 cells (524). Epidemiological studies have revealed that ST239 MRSA isolates cluster into regional clades, which indicates local expansion and is associated with only limited and sporadic intercontinental spread of evolved representatives (525, 526). In line with this, regional ST239 MRSA sublineages often have distinct pathogenicity profiles.

A contributing factor for the success of ST239-III in many Asian hospitals is the presence of *sasX*, which codes for an LPXTG motif surface-anchored protein (329, 396). The gene is typically found on ϕSPβ-like prophages and shares sequence similarity with *sesI* from *S. epidermidis* (396, 527). A comparative analysis of global ST239 revealed that the majority of isolates from the "Asian clade" harbored *sasX* and, conversely, few non-Asian representatives possessed the gene (528). The rate of *sasX*-positive MRSA in Chinese hospitals increased between 2003 and 2011, and the same increases were not observed in the community, therefore suggesting that *sasX* is specifically linked to hospital-acquired infections (329). However, the adaptive benefit of *sasX* is likely dependent on regional selection pressures, as imported *sasX*-positive ST239-III strains were unable to persist in Japanese hospitals (529).

Cell surface-bound SasX promotes adhesion to human nasal epithelial cells, which has epidemiological significance as nasal carriage is linked with infection (530). Deletion of *sasX* reduces biofilm formation, bacterial survival in human blood, and lysis of human neutrophils, whereas complementation of *sasX* restores these phenotypes (329). SasX also contributes to immune evasion and virulence in animal skin and lung infection models (329), suggesting that it may represent an attractive anti-virulence target. Indeed, passive and active immunization strategies using the SasX protein effectively reduced nasal colonization in mice and reduced the severity of disease in animal infection models (531).

When compared to MSSA ST398, ST239 MRSA has an enhanced capacity for nasal colonization in mice and is less virulent in a septic murine infection model (532). At the proteomic level, the production of AgrCA was lower in ST239 MRSA, and this correlated with enhanced production of surface-related proteins under its repressional control, including SpA, FnbpA, ClfA, IsaA, IsaB, LtaS, SsaA, and Cna (533). Most notably, expression of SpA contributed to the impressive nasal colonization of ST239 MRSA. Additionally, SpA contributed to long-term tissue damage in a persistent murine renal abscess model (532). Taken together with *sasX* acquisition, ST239-III strains from China have a characteristic cell surface decoration, which facilitates efficient colonization and persistence in hospital environments.

In Australian hospitals, ST239-III has persisted for many decades. Two distinct clades have been identified, each of which have undergone convergent adaptation toward

the hospital environment, manifested by temporal increases in antibiotic resistance and virulence attenuation (525). Reduced susceptibility of MRSA ST239-III to antibiotics, including vancomycin, teicoplanin, and daptomycin, has been observed over time in Australia (525). SNPs in the *walKR* locus are significantly associated with increased vancomycin MICs (525). Reduced virulence for late isolates was also observed, and this correlated with *agr* dysfunction. Despite the loss of acute virulence traits in a murine model of septicemia, the adapted isolates maintained the capacity to persist in the kidney, suggesting that the adapted Australian ST239-III clades favor persistence as opposed to acute pathogenicity (525).

CC59

CC59 is an epidemic lineage of CA-MRSA in the Asia-Pacific region (Fig. 3) with carriage and infection commonly observed in children (534–537). CC59 is also becoming part of HA-MRSA in the Asia-Pacific region (538, 539). Phylogenomic analysis of global CC59 strains shows that two distinct major clades emerged in the US and in East Asia/Taiwan between 1960 and 1970, followed by dissemination to Europe and Australia separately (540). Of note, CC59 strains from East Asia/Taiwan contain a greater number of antibiotic resistance genes compared with the USA CC59 strains (540).

Recent population studies of *S. aureus* infections in China show that CC59 is replacing CC8 (ST239-III) and CC5 (ST5-II), indicating the success of this lineage in the region (541, 542). In murine infection models, ST59 CA-MRSA isolates caused significantly larger skin abscesses and more pronounced lung damage compared with HA-MRSA ST5 and ST239 isolates from the same Chinese hospital (543). ST59 also displays higher growth rate and better competitive advantage compared to ST239 but increased susceptibility to rifampicin and fluoroquinolones (541). Regarding virulence, PVL appears not to be essential for the success of CC59 given that PVL is not the common feature of CC59 USA and East Asia/Taiwan clones (543, 544). Genetic association studies identified a possible link between *chp* and enhanced virulence potential of MRSA ST59 when compared with MRSA ST239 isolates (545). This association was validated in the lysis assay of human erythrocytes using *chp* knockout MRSA ST59 mutants (545).

The virulence of ST59 MRSA is correlated with increased secretion of PSMα and δ-toxin compared with ST5 and ST239 MRSA isolates (543). Consistently, deletion of *hla*, *psma*, and *agr* significantly compromised the virulence and pathogenesis of CC59 CA-MRSA in skin, lung, and blood infections, suggesting that these virulence factors play an important role in CC59 MRSA infections (543). Analysis of toxin genes also indicated that CC59 MRSA isolates from Chinese pediatric patients with bloodstream infections contain a specific toxin gene profile, that is, *seb-sek-seq* (546, 547). Recently, Bae et al. identified that Seb has a significant role in the virulence of ST59 MRSA isolates, as deletion of *seb* reduced the cytokine storm and increased host survival in a murine systemic infection model (548). The contribution of *sek* and *seq* in the pathogenesis of CC59 MRSA remains to be determined.

Genomic comparison further indicates that the East Asia/Taiwan CC59 clade is composed of a "Taiwan clone" [PVL positive, SCC*mec* V(5C2&5)] and an "Asian-Pacific clone" (PVL negative, SCC*mec* IV) (106, 407, 535). The Taiwan clone is frequently isolated from patients with severe disease, while the Asian-Pacific clone is a common colonizer of healthy children (407). The Taiwan clone induced more severe infections with a higher mortality rate in comparison with an Asia-Pacific clone in a murine bacteremia model (407). Loss of *sak* in prophage φSa*int*3 contributes to virulence in the Taiwan clone, as complementation of *sak* expression was shown to reduce the level of virulence in murine skin infections and bacteremia models (407, 549). The G10S variant of δ-toxin is also a characteristic of CC59, which leads to reduced chemotaxis and lysis of human neutrophils (371).

CC80

CC80 (ST80-IV) emerges as an important CA-MRSA lineage in Europe in late 1990s (550–552). MRSA ST80 was first reported in a Greek hospital and was the dominant CA-MRSA strain to cause SSTIs in Denmark (550, 551). Since then, MRSA CC80 has been prevalent in Europe, the Middle East, and North Africa (553–555). It is likely that this lineage was imported into Europe as many infections caused by MRSA ST80 in Scandinavia were related to travels to the Middle East and Africa (556, 557). Phylogenetic analyses of genomes from global MSSA and MRSA CC80 isolates indicate that a PVL-positive MSSA from sub-Saharan Africa is most likely to be the ancestor of the European epidemic ST80-IV (558). During this evolution, ST80-IV also acquired a plasmid conferring resistance to fusidic acid, which is a commonly used antibiotic to treat skin infections (558).

The success of MRSA ST80 is potentially attributed to specific properties of this lineage (558, 559). In addition to antibiotic resistance, MRSA ST80 harbors a specific non-synonymous SNP in *agrC* that distinguishes this strain from its MSSA ST80 ancestor (558). This SNP results in L184I amino acid substitution within the sensor domain of the AgrC receptor, where the AIP binds (558, 560, 561). Moreover, ST80-IV induces lower cytokine production (TNF-α, IL-1b, IL-6, IL-8, IL-10, IFN-γ, and IL-2) by monocytes compared with the response induced by ST30-IV, ST225-II, ST239-III, and ST5-IV (559). These properties possibly promote persistent colonization of MRSA ST80 by evading host immune responses and increasing its fitness.

PVL, epidermal differentiation inhibitor B (EdinB), and the exfoliative toxin D (EtD) have frequently been associated with MRSA-ST80 (562). EdinB targets host Rho GTPase (563), and deletion of *edinB* in MRSA-ST80 reduced the occurrence of bacteremia in mice with pneumonia, suggesting that EdinB facilitates bacterial dissemination in tissues (564). Furthermore, EtD induced skin exfoliation with the destruction of cell-to-cell adhesion in mice (565). These virulence factors might play a pathogenic role during MRSA ST80 infections.

ST93

In Australia, the dominant CA-MRSA clone is ST93 and is colloquially termed the "Queensland or QLD clone" (566) (Fig. 3). ST93 MRSA is typically SCC*mec* type IVa (2B) and PVL positive and is an MLST singleton as determined by eBURST, suggesting distant molecular relationships with other global lineages (567, 568). Recent studies indicate that ST93-MRSA-IV originated from MSSA strains in remote indigenous communities of northwestern Australia, emerged as MRSA and spread along the Australian East Coast in the early 2000s, followed by spreading overseas to New Zealand, the UK, and Papua New Guinea (569, 570).

ST93 commonly causes SSTIs in humans, as well as additional severe clinical manifestations such as necrotizing pneumonia (571, 572). Accordingly, the common MRSA ST93 reference strain (JKD6159) has shown high virulence potential in animal models, producing larger lesions even when compared to other notorious CA-MRSA lineages such as USA300 in mice skin infection models (391, 412). In support of these findings, enhanced virulence was associated with hyperproduction of α-toxin, whereby deletion of *hla* from JKD6159 reduced the severity of disease (413). In contrast, deletion of the *psm*α locus or PVL had little impact on virulence endpoints, further highlighting the key role of α-toxin for the pathogenicity of MRSA ST93 in this model (413). While high expression of α-toxin is a hallmark feature of ST93, some naturally occurring clinical isolates were shown to produce low levels of exotoxins as a result of convergent mutations affecting the *agr* locus, and this resulted in virulence attenuation (414). Toxin production for MRSA ST93 was also shown to be potentiated by the activity of an AraC/XylS family regulator, AryK (413).

Recently, MRSA ST93 isolates with human origin have been detected in pigs and farm workers in Australia. Transmission was followed by livestock adaptation in many

cases including the loss of φSa*int*3 and its characteristic human invasion gene cluster (~70%), absence of PVL (~30%), and increased antibiotic resistance (573). Thus, ST93 is a well-established CA-MRSA threat and an emerging occupational risk for piggery workers in Australia (574).

ST398

LA-ST398

ST398, originally identified as PFGE non-typeable MRSA, is the major clone of CC398 which dominates LA-MRSA strains isolated from animals in North America, Europe, and Asia (Fig. 3) (34, 575). In particular, strains belonging to CC398 frequently colonize pigs and other livestock hosts as well as people exposed to pigs and pig farmer's households (575–577). The origin of ST398 in farmed animals is not completely clear. Phylogenetic analyses of MRSA and MSSA from animals and humans across 19 countries and four continents indicate that it is likely that ST398 LA-MRSA originated in humans as MSSA and then jumped to livestock and companion animals followed by host adaptive evolution (74, 345, 578). Of note, a recent study has also shown that human-adapted ST398 MSSA can acquire an SCC*mec*-V class D to become CA-MRSA ST398, which is different from SCC*mec*-V class C present in most of MRSA ST398 (579). This lineage is capable of adapting to hosts and environments via obtaining or losing MGEs, raising concerns of MRSA ST398 as a potential zoonotic pathogen (84, 345, 580).

In light of the threat of MRSA ST398, animal models have been developed to examine the colonization, transmission, and virulence factors of this lineage in the hope to develop control strategies (482, 579, 581–586). MRSA has often been detected in the air and housing environments of pig barns, suggesting that transmission of LA-MRSA can occur via the environment (587–589). Colonization of MRSA ST398 in pigs via inhalation was demonstrated in a swine model when piglets were exposed to MRSA in an aerosol chamber (581). Additionally, MRSA ST398 was able to co-colonize piglet skin or the nose in the presence of coagulase-negative staphylococci or MSSA, suggesting that this lineage is able to adapt to the microbial ecology of farmed animals (584, 585). This may provide a reservoir for genes that can be important for adaptation in different animals, as has been seen for human MRSA USA300.

The spread of LA-MRSA by vertical perinatal transmission was observed when vaginal inoculation of a sow with LA-MRSA led to persistent colonization in all newborn piglets (482). In a study carried out in two intensive pig production systems in the US, *S. aureus* was prevalent among vaginal swabs (39.6%) from sows, and 91.1% of pigs had *S. aureus* in at least one site of nares, tonsils, skin, or rectum (590). Transmission of MRSA ST398 between pigs was also evident as naïve pigs were colonized after exposure to pigs orally inoculated with MRSA ST398 (586). Together, these studies provide insights underlying the success of colonizing MRSA ST398 in farmed animals.

Comparative genomic analyses have shown the characteristics that differentiate human- and animal-adapted ST398 strains. Human ST398 MSSA isolate harbors φSa*int*3, which contains IEC and is associated with enhanced adhesion ability to human skin keratinocytes and keratin (416). Host adaptive evolution of ST398 from human to livestock involves loss of φSa*int*3 and IEC and acquisition of resistance to antibiotics, including tetracycline and methicillin (84, 345). However, it is likely that LA ST398 still maintains the capacity to at least temperately colonize humans. In a study involving healthy human volunteers inoculated with a mixture of bovine MSSA ST398 and human MSSA ST931 into the nose, ST398 was able to compete with the human strain and survived in the nose for 21 days (591). Acquisition of prophages was also suggested to increase adhesion expression of LA-MRSA ST398 and virulence in a rat IE model (592). It remains rare but possible that human-to-human transmission of LA-MRSA ST398 can occur, given that dissemination between caretaker and patient in a hospital setting has been reported (593). LA-MRSA ST398 is constantly evolving and is able to re-acquire IEC for the readaptation to human host (580).

LA-MRSA ST398 strains display the lowest content of virulence genes compared with ST5, ST15, ST22, CC30, CC97, CC130, and CC151 (594). The lack of accessory virulence genes and no specific virulence markers in LA-MRSA ST398 suggest that the genetic background of this lineage is unique compared with other major MRSA lineages from humans (594). Recent analysis of superantigens gene distribution reveals that CC398 isolates have only *selw* but no other superantigens (595). SELW is required for a CC398 clinical isolate to induce Vβ-specific T cell proliferation, and SELW contributes to bacterial load in liver by a CC398 isolate in a murine bloodstream infection model (595). Complementation of *selw* in Δ*selw* mutant *in trans* leads to more severe disease outcomes in the model compared with wild type (595). Despite the absence of many perceived virulence factors, LA-MRSA CC398 has retained its capacity to cause infections in humans who have close contact with livestock and companion animals (593, 596, 597).

The pathogenicity of LA-MRSA CC398 strains from industrial hog operation workers was assessed in a mouse SSTI model, which showed that larger lesion sizes and reduced IL-1β expression could be induced by LA-MRSA CC398 compared with an MRSA USA300 strain, SF8300 (598). Comparing ST398 MRSA isolates from pigs with those from humans, Schmidt et al. showed that human ST398 MRSA isolates have better cell adhesion and stronger lysis activity toward human neutrophils (599). This stronger cytolytic activity of human MRSA ST398 is likely due to the regulation of toxin expression and exportation as no pivotal difference in virulence factors was detected between LA-MRSA ST398 and human MRSA ST398 (599).

Human-adapted ST398

In addition to transmission from animals to humans, ST398 MRSA strains can also evolve from human-adapted ST398 MSSA and cause severe infections (579). A recent study shows that severe and fatal human infections in a Chinese community were caused by human-adapted ST398 MRSA, which acquired a low level of methicillin resistance while maintaining high virulence as ST398 MSSA (579). In both murine lung and skin infection models, the ST398 CA-MRSA isolates showed more severe disease than HA-MRSA clones (ST5 and ST239) and LA-MRSA ST398 strain S0385. These human-adapted ST398 MRSA clones harbor a class D SCC*mec*-V element compared with class C SCC*mec*-V from LA-MRSA ST398 clones and have a lower oxacillin MIC (4 μg/mL) compared with HA-MRSA clones' MIC (128 μg/mL) (579). Oxacillin MIC 4 μg/mL is the susceptibility breakpoint to define MRSA. This study shows that distinct ST398 MRSA lineages emerged by multiple recent SCC*mec* uptake events from ST398 MSSA ancestors, indicating that the development of novel and highly virulent MRSA lineages may be a frequently occurring scenario.

At the molecular level, community-acquired ST398 MRSA isolates from China exhibited higher expression levels of ESAT-6 secretion system (ESS) genes compared with ST239, which is the predominant hospital-associated lineage MRSA in China (582). ESS is important for the virulence of the ST398 isolates collected from humans in China (582). The disruption of ESS by deletion of the structural component, *essB*, was shown to significantly reduce resistance to neutrophil killing and decrease virulence in murine skin and blood infection models (582). Neutrophil killing was restored after complementation of *essB* in the Δ*essB* mutant (582). The ESS-secreted protein, EsxX, is highly conserved only in the ST398 MRSA isolates from humans in China (600). EsxX promotes neutrophil lysis, evades neutrophil killing, and contributes to virulence in murine infection models (600). Comparative proteomic analysis of ST398 and ST239 MRSA lineages illustrates that ST398 has a higher expression of the Agr system (AgrA and AgrC), which enhances ESS and Agr interactive factors (PhoP, SrrB, WalK, SarX, SigB, and ClpP) (533, 582). High production of α-toxin and a highly functional *agr* system has also been associated with fatal pneumonia caused by ST398 MRSA isolates in Brazil (417).

Characterization of 35 prophages found in the collection of 76 ST398 MRSA bacteremia isolates showed that these prophages harbor many genes that are associated with virulence or immune evasion (601). An increasing prevalence of poly-lysogeny in

ST398 bloodstream infection isolates over time was identified, therefore highlighting that this lineage may increase its capacity to spread in humans by continuously acquiring virulence and/or antibiotic resistance genes via HGT (601). ST398 MRSA expresses *S. aureus* Toll/IL-1 receptor (TIR)-like protein 1 (SaTlp1) and *S. aureus* TIR-like protein 2 (SaTlp2), which contain domains similar to TIR (583). SaTlp1 and SaTlp2 enhance activation of the transcription factor NF-κb and downstream pro-inflammatory cytokines and immune effectors (583).

Taken together, ST398 represents a successful lineage for its ability to constantly evolve, adapt, and readapt to changing host environments. Surveillance of antimicrobial resistance and factors contributing to transmission and virulence remains a priority for monitoring this lineage. Future studies are required to develop control measures to mitigate the threat of MRSA ST398 as a zoonotic pathogen important to public health.

CONCLUDING REMARKS

Although the rate of MRSA infections has stabilized or even decreased in many countries, it is still a significant concern for society and a threat to global economies. MRSA infections remain severe and difficult to treat, especially with the emergence of strains resistant to last-line antibiotics. As *S. aureus* continues to evolve and to surprise, now is not the time for complacency.

In the current review, we have summarized a large body of work from the last 25 years that has defined the specific character of successful MRSA lineages. WGS and comparative genomics have played a major role in our understanding of MRSA pathobiology. Here, numerous core and lineage-specific virulence factors have been identified and discussed. Factors that contributed to MRSA infections, including toxins, immune evasion proteins, and *agr*, have been investigated to characterize their contribution in the success of a lineage. Recent studies have also shed light on how MRSA pathogenicity and metabolism are closely regulated. The success of USA300 lineage is attributed to virulence regulation and metabolic adaptation, whereas it is less clear how ST80-IV is persistent among European countries. Further investigations on host factors for MRSA colonization and pathogenesis are likely to improve our understanding of the success of a specific MRSA lineage. Together, these findings will identify interesting potential targets for future intervention. Neutralization of toxins, inactivation of the central virulence regulator Agr, inhibition of the transfer of MGEs, and microbiome-mediated exclusion of MRSA colonization are likely to be important strategies to prevent continued global MRSA pandemics.

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FUNDING

Funder	Grant(s)	Author(s)
DHAC | National Health and Medical Research Council (NHMRC)	APP2002921	Jhih-Hang Jiang Anton Y. Peleg

AUTHOR CONTRIBUTIONS

Jhih-Hang Jiang, Conceptualization, Writing – original draft, Writing – review and editing | David R. Cameron, Conceptualization, Writing – original draft, Writing – review and editing | Cara Nethercott, Writing – original draft, Writing – review and editing | Marta Aires-de-Sousa, Writing – review and editing | Anton Y. Peleg, Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review and editing

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