Methicillin-Resistant *Staphylococcus aureus*: An Evolving Pathogen

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The horizontal transmission of methicillin resistance to *Staphylococcus aureus* (MRSA) in hospital and community settings, and growing prevalence of these strains, presents a significant clinical challenge to the management of serious infections worldwide. While infection control initiatives have stemmed the rising prevalence, MRSA remains a significant pathogen. More recently, evidence that MRSA is becoming resistant to glycopeptides and newer therapies raises concern about the use of these therapies in clinical practice. Vancomycin resistance has become evident in select clinical settings through rising MICs, growing awareness of heteroresistance, and emergence of intermediate-resistant and fully resistant strains. While resistance to linezolid and daptomycin remains low overall, point mutations leading to resistance have been described for linezolid, and horizontal transmission of cfr-mediated resistance to linezolid has been reported in clinical isolates. These resistance trends for newer therapies highlight the ongoing need for new and more potent antimicrobial therapies.

*Staphylococcus* has plagued man for centuries [1]. Although staphylococci were probably causing diseases such as the “incurable boils” described in the sixth plague of Egypt [2], these organisms were only first described and classified as *Staphylococcus* (from the Greek *staphylos* [“grape”] and *kokkos* [“berry” or “seed”]) in 1882 by the Scottish surgeon Sir Alexander Ogston [3]. Two years later a German physician, Friedrich J. Rosenbach, described 2 pigmented colonies of staphylococci and proposed the nomenclature *Staphylococcus albus* (Latin for “white”) and *Staphylococcus aureus* (from the Latin *aurum* [“gold”]) [4]. Since that time, *S. aureus* has continued to surprise scientists and physicians while infecting and decimating millions of patients.

RESISTANCE TO BETA-LACTAMS AND THE ORIGIN OF MRSA

At the core of the success of *S. aureus* as a human pathogen is its versatility. As part of its adaptation in the antibiotic era, *S. aureus* has been able to evolve, acquiring resistance to nearly all antibiotics used to treat it. Resistance to penicillin was reported in 1942, only 1 year after the miraculous drug was introduced [5]. In the mid-1940s the mechanism of penicillin resistance based on an inducible beta-lactamase was revealed [6]. By the 1950s half or more of *S. aureus* strains in large hospitals were resistant to penicillin [7, 8]. In addition, *S. aureus* was able to develop resistance to the other available antibiotics such as erythromycin, streptomycin, and the tetracyclines [7, 9, 10]. Unfortunately, history soon repeated itself. Methicillin was introduced in 1959 to overcome penicillin resistance. However, methicillin-resistant *S. aureus* (MRSA) was reported only 2 years later [11].

The mechanism leading to methicillin resistance was finally identified in 1981 [12] and involved the expression of a transpeptidase (PBP2a) with reduced affinity.
for all available beta-lactam agents, including penicillin. PBP2a transpeptidase is encoded by chromosomal gene mecA, located in a mobile genomic element known as the staphylococcal cassette chromosome (SCC), in this case SCCmec [13]. SCCmec elements are classified by a hierarchical system into types and subtypes [14, 15]; to date, 11 types of SCCmec have been identified.

Although the origin of MRSA is not fully understood, it is suspected that methicillin-susceptible S. aureus (MSSA) acquired the mecA gene through horizontal transfer from coagulase-negative staphylococci [16, 17]. Subsequent evidence indicates that major MRSA clones repeatedly arose from successful epidemic MSSA strains [18]. The presence of distantly related MRSA lineages indicates that a single ancestral clone was unlikely to have arisen from a common origin [18, 19]. Despite the fact that ancestral clones seem to have successful adaptation characteristics, isolates with ancestral genotypes have not been proven to be more frequently associated with human disease than colonizing isolates [20]. In addition to the ancestral inheritance, S. aureus from certain clonal complexes (eg, CC30 and CC5) seems to be more commonly associated with invasive disease [21]. In addition, other candidate genes (within and outside SCCmec) have been proposed to explain the association between S. aureus and invasive disease [22, 23]. While SCCmec is crucial for antibiotic resistance, there is no direct evidence that SCCmec plays a clear role in MRSA virulence.

**EPIDEMIOLOGY OF MRSA**

Resistance to methicillin was uncommon until the late 1960s, when a multidrug-resistant MRSA (eg, phage type 83A complex) emerged in Europe [10, 24, 25]. For unknown reasons, the incidence of this MRSA in human infections gradually declined [25, 26]. For nearly a decade following this decline, MRSA clones were infrequently encountered and limited primarily to major urban hospitals [27]. However, a successful expansion of MRSA, which began in the late 1970s, turned into a nonstop evolutionary journey. MRSA resistant to gentamicin emerged in Europe and the United States [28, 29], and a multidrug-resistant MRSA became epidemic in Australia [30]. In the late 1980s MRSA rates in teaching hospitals reached 14% in Australia [31], while in the United States they increased from 8% to 22% by the end of the decade [32]. At the same time, an epidemic clone (EMRSA), thought to have been imported from Australia, was propagating in the United Kingdom [33, 34], and soon all of Europe and the United States were seeing dramatic increases in MRSA infections [18]. To illustrate this expansion, MRSA comprised nearly 60% of S. aureus organisms isolated in US intensive care units (ICUs) in 2003 [35]. Similarly in Latin America, rates of MRSA surpassed 50% in over half of the countries [36], and a similar situation was observed in many institutions from the Asia-Pacific region [37].

Names of MRSA clones commonly refer to a specific pulse field gel electrophoresis pattern (eg, USA100), although they are further classified using other complementary techniques such as multilocus sequence typing (of 7 housekeeping genes), SCCmec, and spa type (variants of S. aureus classified according to protein A). For example, the MRSA clone belonging to multilocus sequence type 5, which carries SCCmec II and spa-type 002 (ST5-MRSA-II t-002), is widely known as the New York/Japan clone or USA100.

Remarkably, only a select few MRSA lineages were widely disseminated and responsible for the majority of MRSA infections. Molecular-based epidemiologic studies have shown that 5 major pandemic clones (Iberian, Brazilian, Hungarian, New York/Japan, and Pediatric) accounted for almost 70% of hospital MRSA isolates [38, 39]. In support of this observation, a recent European study determined that MRSA-causing invasive infections are less diverse than MSSA-causing invasive infections and that MRSA spa types have a predominant geographic distribution [40].

Interestingly, recent reports from the United States and the United Kingdom indicate that rates of selected MRSA infections have remained static or have decreased. A combined survey of the National Nosocomial Infections Surveillance system and its successor, the National Healthcare Safety Network, demonstrated that in a large group of US ICUs, the percentage of methicillin resistance in central line–associated bloodstream infections (CLABIs) due to S. aureus increased from 47.9% in 1997 to 64.5% in 2007 [41]. However, the incidence of CLABIs due to MRSA (infections/catheter days) decreased by almost 50% in these same ICUs during the study period. Similar decrements were observed for other CLABIs, including those associated with MSSA [41]. Another study, conducted in more than 9 million US military beneficiaries enrolled in the TRICARE program, indicated that annual rates of hospital-onset MRSA bacteremia decreased (from 0.7 per 100 000 person-years in 2005 to 0.4 per 100 000 person-years in 2010) [42]. Estimates indicate that the number of CLABIs in US ICUs decreased from 43 000 in 2001 to 18 000 in 2009, with reductions in infections due to S. aureus being more marked than those caused by other pathogens [43]. Taken together, these observations suggest that the reduced incidence in CLABIs due to MRSA has probably resulted more from careful, sterile central-line insertion and improved infection control practices than from a change in the organism itself.

Actions to reduce healthcare-associated infections need to be emphasized. In the United Kingdom (England and Wales) such actions have included better antibiotic selection, isolation of infected patients and use of gloves to treat them, decontamination with skin and nose treatment prior to surgery if
Resistance to Glycopeptides and New Agents

Vancomycin received US Food and Drug Administration approval in 1958 [45]. Unlike other antibiotics, MRSA took almost 40 years to develop even partial resistance to glycopeptides such as vancomycin. Clinical failures of this ponderous, slowly bactericidal agent in patients with MRSA infections have resulted in a reevaluation of vancomycin minimal inhibitory concentration (MIC) breakpoints. In 2006 the Clinical and Laboratory Standards Institute (CLSI) lowered the vancomycin MIC breakpoints for susceptibility from 4 µg/mL or less to 2 µg/mL or less for MRSA [46]. Despite this realignment, concerns remain about the historical decrement of MRSA susceptibility to glycopeptides. This phenomenon, named “MIC creep,” has only been documented in selected centers [47, 48] (Figure 1).

Glycopeptide intermediate-susceptible S. aureus (GISA or VISA for this article; current MIC breakpoint between 4 and 8 µg/mL) was first described in Japan in 1996 [49] and involved a thicker cell wall with an excess of binding sites able to “trap” glycopeptides [50, 51] (Figure 2). This mechanism, which is not clonal, is commonly related to previous exposure to vancomycin [52]. Importantly, GISA isolates can return in vitro to vancomycin susceptible when the antibiotic pressure is removed [53]. Despite this, outbreaks of GISA already have been reported [54]. A recent study of 33 GISA strains obtained from the Network on Antimicrobial Resistance in S. aureus (NARSA) program indicates that GISA strains frequently carry SCCmec type II and are usually susceptible (>90%) to linezolid, telavancin, tigecycline, and minocycline [55]. Interestingly, not all GISA strains are MRSA, and a minority is susceptible to methicillin [55].

Although extremely uncommon, full vancomycin-resistant S. aureus (VRSA, current MIC breakpoint ≥16 µg/mL) emerged clinically in 2002 [56]. Unlike GISA strains, the mechanism of resistance in VRSA is due to acquisition of a vanA gene transferred from vancomycin-resistant enterococci [57]. To date, only 13 isolates of VRSA (8 from Michigan) are listed on the NARS website [58]. These VRSA isolates were all susceptible to ceftaroline, daptomycin, linezolid, minocycline, and trimethoprim/sulfamethoxazole.

Soon after GISA was described, reports arose of vancomycin-susceptible strains of MRSA containing subpopulations resistant to glycopeptides (typically at a rate of 1 in 10^5 organisms) [59] not detected by conventional laboratory methods. These heteroresistant (hVISA) isolates represent an intermediary stage between fully vancomycin-susceptible S. aureus (VSSA) and GISA isolates. Patients with hVISA were commonly exposed to “low levels” of vancomycin (eg, <10 µg/mL) [60]. As a result, hVISA isolates have emerged from every lineage that has produced pandemic MRSA clones [18]. The reference method for identifying hVISA strains is the population analysis profile—area under the curve (PAP–AUC) calculation, which is labor intensive and not available in most laboratories. Given these difficulties, different screening methods to detect hVISA have been proposed [61]. Using the new screening methods, most hVISA isolates contain vancomycin MICs of 2 µg/mL or higher, but a minority will still have MICs <2 µg/mL [62]. Although the prevalence of hVISA (by reference method) seems to be low in the United States (eg, <1%) [63], there is some evidence that it is increasing in selected locations [64]. However, hVISA is more prevalent than originally thought in patients with invasive and difficult-to-treat MRSA infections. An international study found that 29% of patients with MRSA infective endocarditis had the hVISA phenotype [65]. This finding is in agreement with studies showing that most isolated hVISA came from bloodstream infections [64], although heteroresistance has also been found in patients with other invasive MRSA infections.
infections [66]. Finally, an outbreak of hVISA has been recently described [67].

The mechanism of MRSA resistance to different antibacterials was elegantly reviewed by Lowy [68]. Table 1 displays the most common mechanisms of antibiotic resistance for S. aureus. Although uncommon, it is important to mention that MRSA resistance to new antibiotics such as linezolid or daptomycin has been described in clinical settings. Linezolid resistance associated with ribosomal point mutations in the 23S rRNA gene, or ribosomal proteins L3 and L4, have been associated with outbreaks of healthcare-associated linezolid-resistant infections in several countries [70]. Since pathogens susceptible to linezolid contain multiple rRNA genes, a cumulative threshold needs to be achieved before clinical resistance can be observed. More recently a plasmid-borne methyltransferase-mediated resistance mechanism cfr (for chloramphenicol-florfenicol resistance gene) has been identified; it conveys resistance to a range of antibiotics, including linezolid [70] (Figure 3). A recent outbreak of linezolid-resistant MRSA was reported in a Spanish ICU, mediated by the acquisition of cfr and associated with the extensive use of this antibiotic [73]. Resistance to daptomycin has also been described in a landmark bacteremia study [74]. Interestingly, several of these patients had incompletely drained infections. In addition, a US study showed a correlation between reduced susceptibility to vancomycin and daptomycin resistance, particularly in patients infected with MRSA demonstrating MICs to vancomycin of 4 µg/mL or greater [74]. From these data, it is clear that although new antibacterial agents are essential to treat this dynamic pathogen, it is equally important to understand and use these agents appropriately.

**COMMUNITY-ASSOCIATED MRSA**

Since the beginning of the MRSA expansion, infections due to this organism were primarily limited to major hospital centers and their healthcare systems. Community-acquired MRSA was rarely reported [75]. However, during the 1990s, a new epidemic of MRSA began. A unique clone of MRSA acquired in the
community was first described in Western Australia [76]. A few years later, other community-acquired MRSA clones were recognized in Europe [77], the United States [78], Latin America [79], and Asia [80]. These clones often affected young people without healthcare contact, producing purulent skin infections [81] or pneumonia [82]. All these community-acquired MRSA strains differed from hospital strains of MRSA (eg, major MRSA pandemic clones). They were usually susceptible to multiple non–beta-lactam antibiotics and commonly carried SCCmec type IV (less commonly, type V) as well as genes for Panton–Valentine leukocidin (PVL).

Some of these new MRSA clones were extremely successful in displacing both emergent and endemic clones and spread geographically, infecting thousands. The clone USA400 (ST1-MRSA-IV, the first community-acquired MRSA clone in the United States), for example, was rapidly displaced by USA300 (ST8-MRSA-IV), which became the primary cause of purulent skin lesions in adults [83, 84]. Similarly, in France an emerging community-acquired MRSA clone, ST5 Geraldine, is now more prevalent than the previous clone, ST80 [85].

It was not long before community-acquired MRSA entered hospitals [86] and healthcare systems in the United States [87].

### Table 1. Representative Mechanisms of *Staphylococcus aureus* Resistance to Selected Antimicrobials [68, 69]

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Resistance Gene(s)</th>
<th>Gene Product(s)</th>
<th>Mechanism(s) of Resistance</th>
<th>Location(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Lactams</td>
<td>blaZ, mecA</td>
<td>β-Lactamase, PBP2a</td>
<td>Enzymatic hydrolysis of β-lactam nucleus, Reduced affinity for β-lactam</td>
<td>Plasmid: Transposon, Chromosome: SCCmec</td>
</tr>
<tr>
<td>Glycopeptides</td>
<td>GISA: unknown, VRSA: vanA</td>
<td>Altered peptidoglycan, D-Ala-D-Lac</td>
<td>Trapping of vancomycin in the cell wall, Synthesis of dipeptide with reduced affinity for vancomycin</td>
<td>Chromosome</td>
</tr>
<tr>
<td>Quinolones</td>
<td>parC, gyrA or gyrB</td>
<td>ParC (or GrlA) component of topoisomerase IV, GyrA or GyrB components of gyrase</td>
<td>Mutations in the QRDR region, reducing affinity of enzyme-DNA complex for quinolones</td>
<td>Chromosome</td>
</tr>
<tr>
<td>Aminoglycosides (eg, gentamycin)</td>
<td>Aminoglycoside-modifying enzymes (eg, aac, aph)</td>
<td>Acetyltransferase, phosphotransferase</td>
<td>Acetylation and/or phosphorylation of aminoglycosides</td>
<td>Plasmid, Plasmid: Transposon</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole (TMP-SMZ)</td>
<td>Sulphonamide: sulA, TMP: dfrB</td>
<td>Dihydropteroate synthase, DHFR</td>
<td>Overproduction of p-aminobenzoic acid by enzyme, Reduced affinity for DHFR</td>
<td>Chromosome</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>Tetracycline, doxycycline, and minocycline: tetM</td>
<td>Ribosome protection protein</td>
<td>Binding to the ribosome and clearing the drug from its binding site</td>
<td>Plasmid: Transposon</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>erm (A, C)</td>
<td>Ribosomal methylase (constitutive or inducible)</td>
<td>Alteration of 23S rRNA</td>
<td>Plasmid: Transposons</td>
</tr>
<tr>
<td>Linezolid*</td>
<td>cfr</td>
<td>Ribosomal methyltransferase</td>
<td>Methylation of the 23S rRNA that interferes with ribosomal binding</td>
<td>Plasmid</td>
</tr>
<tr>
<td>Daptomycin*</td>
<td>mprF</td>
<td>Lysylphosphatidylglycerol synthetase (LPG) synthetase</td>
<td>Increasing synthesis of total LPG, outer LPG translocation and positive net charges on cell membrane</td>
<td>Chromosomal</td>
</tr>
</tbody>
</table>

Adapted with permission of the American Society for Clinical Investigation, from: Lowy FD. Antimicrobial resistance: the example of *Staphylococcus aureus*. J Clin Invest 2003; 111: 1265–1273.

Abbreviations: DHFR, dihydrofolate reductase; GISA, glycopeptide-intermediate susceptible *Staphylococcus aureus*; LPG, lysylphosphatidylglycerol; QRDR, quinolone resistance–determining region; VRSA, vancomycin-resistant *S. aureus*.

* Other mechanisms for linezolid resistance involve mutations to the central loop of domain V of 23S rRNA or in the ribosomal proteins L3 and/or L4 of the peptide translocation center [70].

* Other mechanisms were also proposed, such as increased cell wall thickening, decreased membrane fluidity [71], and increased expression of vraSR [72].
causing invasive infections and making the “community-acquired” or “-associated” label less appropriate. A study of invasive MRSA infections in the United States determined that 16% of MRSA clones classified as hospital acquired were actually the USA300 clone, which originated in the community. In addition, a multidrug-resistant community-associated MRSA (USA300) has already been described in men who have sex with men [88]. VISA phenotype and resistance to daptomycin have also been reported in patients with infective endocarditis due to community-acquired MRSA (USA300) [89, 90]. Clearly, the epidemiology of community-associated MRSA reflects continuous changes in accord with the evolutionary nature of this pathogen [91, 92].

ANTIBIOTIC RESISTANCE AND VIRULENCE

Whether MRSA is more virulent than MSSA is still a matter of debate. Two metaanalyses have shown that patients with MRSA died more often than those with bloodstream infections due to MSSA [93, 94]. However, such differences might be explained by the fact that patients with MRSA infections were usually older, had more severe underlying disease, and often were receiving more inappropriate and/or suboptimal therapy than patients infected with MSSA. When carefully adjusted for other factors, MRSA was not associated with higher mortality in patients with VAP [95, 96]. To further confuse the issue, several investigators found that decreasing susceptibility to vancomycin within susceptible ranges was associated with worse clinical outcomes, particularly in patients with bloodstream infections [97–100] or pneumonia [66].

The link between reduced susceptibility and increased virulence remains unclear. Outcomes in patients with GISA infections have been poor [54], although this may be due to inappropriate treatment in otherwise sick individuals. Similarly, patients with bloodstream infections due to hVISA (bacteremia or endocarditis) had longer durations of bacteremia than patients with MRSA and without the hVISA phenotype [60, 65]. However, different studies did not find increased mortality in patients with bacteremia [101] or infective endocarditis [65] due to MRSA strains with the hVISA phenotype. In addition, 1 study reported higher survival in patients with bloodstream infections due to MRSA (mostly ST239) with the hVISA phenotype [102]. Thus the theories linking antibiotic resistance with either reduced fitness or increased virulence, although still attractive for hVISA, are unproven, and their clinical significance remains to be determined.
Other factors, such as the dysfunction of the accessory gene regulator (agr) may also play a role in MRSA virulence. The agr locus regulates expression of several virulence and housekeeping genes in a growth phase-dependent manner (quorum-sensing mechanism). Conceptually, increased expression of agr augments production of toxin and diminishes expression of surface cell adhesins [103]. Dysfunction in the agr locus was associated with reduced susceptibility to vancomycin [104], persistent MRSA bacteremia [105, 106], and, in 1 study, increased mortality [107].

As mentioned previously, community-associated MRSA usually carries marker genes (luk-S and luk-F) encoding for PVL. Different studies have suggested that PVL could play a major role in the virulence of community MRSA in both animal models [108] and humans [82, 108, 109]. However, these observations are not supported by large clinical studies. Bae and coworkers analyzed isolates obtained from 522 patients with complicated skin infections caused by MRSA who were enrolled in 2 clinical trials. Patients infected with MRSA strains carrying PVL-positive genes were significantly more likely to be cured than those infected with PVL-negative strains (91.6% vs 80.7%; P = .015) [110]. Similarly, PVL-positive USA300 was not associated with worse clinical outcomes in patients with bloodstream infections enrolled in a multinational trial [111]. Another study involving more than 100 patients demonstrated that HAP/VAP caused by PVL-positive strains of MRSA resulted in mortality equal to that caused by PVL-negative strains [112]. Similar findings were reported by Sharma-Kuinkel and colleagues in 287 patients with HAP/VAP (173 with MRSA) [113]. Thus, although PVL may have a role in virulence, an increasing body of evidence indicates that PVL is not a primary determinant of clinical outcomes in patients with community-acquired MRSA.

THE BURDEN OF DISEASE

Since first being identified, *S. aureus* infections have been associated with significant morbidity and mortality. In the preantibiotic era, bloodstream infections due to *S. aureus* yielded more than 80% mortality [114]. Although the prognosis has since improved, the impact of the disease remains dramatically high. Contemporary studies have shown that overall in-hospital mortality rates for patients with bloodstream infections due to MRSA are in the range of 30% [94, 115] but can be as high as 65% in some centers [115]. A study by the Centers for Disease Control and Prevention from 1999 to 2000 estimated that 125,969 hospitalizations for a diagnosis of MRSA infection occurred annually in the United States, including 31,440 for bloodstream infections and 29,823 for pneumonia [116]. More recent US estimates indicate MRSA causes approximately 95,000 invasive infections and 19,000 deaths per year [117]. This mortality number is higher than the rates of death produced by human immunodeficiency virus, viral hepatitis, tuberculosis, and influenza combined [118].

CONCLUSIONS

MRSA is a versatile, well-equipped pathogen with the potential to evolve and adapt to its host as well as to the treatments developed to control its invasive damage. Clearly, new therapies are needed in the ongoing struggle. In addition, prevention and rapid identification are essential. Determining the optimal methods of treating this evolving organism will require that both clinicians and researchers understand the organism, the patients, and the antibacterials being employed more clearly.

Notes

*Supplement sponsorship.* This article was published as part of a supplement titled “Tedizolid: A Novel Oxazolidinone for Gram-positive Infections,” funded by Cubist Pharmaceuticals.

*Potential conflicts of interest.* M. E. S. has received payment for writing or reviewing this manuscript from Cubist Pharmaceuticals and for manuscript preparation from Cempra and has served as a consultant to Cerexa, Furiex, The Medicines Company, PRA International, Theravance, Trius Therapeutics, Nabriva, and Cempra. G. R. C. has served as a consultant to Merck, AstraZeneca, Astellas, Cempra, Cubist Pharmaceuticals, Cerexa, Forest Pharmaceuticals, Furiex, PRA International, Inmex, Pfizer, GlaxoSmithKline, Polymedix, Achaogen, Trius Therapeutics, Rib-X, Nabriva, Seachaid, BioCryst, Durata, and Gilead. His institution has received grant funding from Theravance, The Medicines Company, and Innocoll.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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2. Exodus 9:8–12 (ESV).


