Microbiology of Antibiotic Resistance in Staphylococcus aureus

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Methicillin-resistant Staphylococcus aureus (MRSA) isolates came into existence soon after the introduction of methicillin. Historically, MRSA isolates have been associated with nosocomial infections and rapidly developed resistance to multiple drug classes. However, in recent years, different strains with unique phenotypes have emerged in the community, and the reservoir of community-associated MRSA is rapidly expanding. Community-associated pathogens are likely to cause life-threatening systemic infections, especially in children and elderly individuals, and may also cause serious skin and soft-tissue infections in healthy individuals. Compared with nosocomial strains, community-associated MRSA isolates are associated with increased virulence and currently are more likely to be susceptible to a variety of antibiotics. The epidemiological and microbiological differences between community-associated and nosocomial MRSA infections necessitate different strategies to prevent and treat the 2 types of infections. Vancomycin nonsusceptibility in S. aureus is on the increase, further complicating therapy.

Methicillin-resistant Staphylococcus aureus (MRSA) is a major pathogen worldwide; MRSA infections are associated with increased morbidity and mortality, in comparison with other S. aureus infections. Over the past decade, the changing pattern of resistance in S. aureus has underscored the need for new antimicrobial agents [1]. Once confined to health care–associated environments, MRSA has now migrated into the community. Community-associated strains share some characteristics with nosocomial strains but also differ in antimicrobial susceptibility and potential virulence. Of concern is the probable increasing prevalence of heterogeneous vancomycin-intermediate S. aureus (hVISA) and vancomycin-intermediate S. aureus (VISA) MRSA strains in Europe, Asia, and the United States [1]. Although 7 cases of infection with vancomycin-resistant S. aureus (VRSA) strains have been described in the United States, the clinical and epidemiological significance of this resistance phenotype is unclear at the present time [1].

AN OVERVIEW OF THE DEVELOPMENT OF S. AUREUS RESISTANCE

In the 1940s, penicillin was introduced for the treatment of infection; as early as 1942, strains of S. aureus resistant to penicillin had been detected in hospitals. Within 2 decades, ~80% of both hospital- and community-acquired S. aureus isolates were penicillin resistant. The introduction of methicillin in 1961 was rapidly followed by reports of methicillin resistance in S. aureus. Today, MRSA strains are found worldwide, and most are multidrug resistant [1].

Study of early isolates of MRSA showed that a key genetic component responsible for resistance, mecA, is not native to the S. aureus genome. The staphylococcal chromosome cassette mec (SCCmec) has been characterized as a novel, mobile resistance element that differs from both transposons and bacteriophages [2]. MRSA typically spreads through clones; however, it is known that the mec gene has been transmitted between S. aureus strains and, possibly, between other staphylococcal species [2].

Until the 1990s, MRSA was a pathogen associated
with nosocomial infections. However, in the late 1990s, MRSA began to be detected in infections that arose outside of the health care setting. Some cases in the United States have occurred in patients who have never been hospitalized and who have no risk factors for MRSA infection [3]. In 2005, Kazakova et al. [4] reported a community-associated MRSA (CA-MRSA) clone isolated from US football players with skin abscesses. The strain was susceptible to most antimicrobial agents except β-lactams and macrolides. It carried a subtype of the SCCmec unit (SCCmec type IV) that differed from that of health care-associated MRSA (HA-MRSA) and a gene for Panton-Valentine leukocidin (PVL), which may be associated with severe necrotizing infections. Evaluation of the strain involved showed that it was identical to epidemiologically unrelated CA-MRSA isolates from various other regions of the country [4]. Similarities in the genetic backgrounds of early and contemporary epidemic clones of MRSA have also been reported [5]. Recent work has confirmed that these differences between CA-MRSA and HA-MRSA are typical: CA-MRSA strains usually have different subtypes of SCCmec (IV and V), are often resistant to fewer antibiotic classes (frequently only β-lactams and macrolides), and are more virulent, and a high proportion carry the genes encoding PVL [3]. It is noteworthy that the importance of PVL as a determinant of virulence in CA-MRSA has recently been called into question [6]. Although the emergence and virulence of CA-MRSA have been associated with strains USA300 and USA400, both containing PVL, a direct role for PVL in increased virulence had not been systematically evaluated before the study by Voyich et al. [6]. A comparison of the virulence of PVL-positive and PVL-negative strains of CA-MRSA in mouse models has shown them to be equally virulent. Isogenic PVL-negative (knockout) strains of USA300 and USA400 were also found to be as virulent as the wild-type strains [6].

Carleton et al. [7] evaluated MRSA reservoirs in the community, in an effort to understand the population dynamics. Random sampling (n = 490) from 2154 MRSA isolates collected during a 7-year period from both inpatients and outpatients in San Francisco revealed that a 4-fold increase in the frequency of methicillin resistance between 1998 and 2002 was due to 4 specific genotypes. Contrary to what might have been postulated, these genotypes were associated primarily with community-onset disease and were significantly less likely to be associated with hospital- or health care–associated infections. Thus, the reservoir of CA-MRSA seems to be rapidly increasing. In recent literature, there are increasing reports of the emergence of health care–associated infections caused by MRSA strains with molecular characteristics of CA-MRSA, suggesting that the phenotype of CA-MRSA may become that of HA-MRSA in the future [8], with serious implications for therapy for these infections.

MRSA has been associated with a variety of infections, ranging from superficial to more deep rooted. Life-threatening CA-MRSA infections have been reported, including neonatal sepsis and community-acquired pneumonia [3, 9]. In infants treated since birth in a neonatal intensive-care unit (NICU), 8 of 17 cases of S. aureus bacteremia were found to be due to MRSA.

Six of the MRSA strains had SCCmec genes found in community isolates; all of these were resistant to β-lactam antibiotics and erythromycin, and 1 was also resistant to clindamycin [3]. A majority of infants (88%) presented with septic shock, and, despite rapid treatment with vancomycin, 3 of the infants died, and another 3 had long, difficult hospital courses [3]. The authors indicated that the “introduction of community strains of MRSA into our NICU reflects the high prevalence of these strains in the Houston community” [3, p. 1464]. They also noted that, despite causing superficial infections in healthy adults, CA-MRSA can be highly virulent in some hosts, such as the young children in their study [3]. Francis et al. [9] reported CA-MRSA infection in 4 patients with severe necrotizing MRSA pneumonia who did not have typical risk factors for MRSA infection. All were infected with strains that carried the PVL gene and the SCCmec type IV cassette, which is typical of community strains of MRSA. Two patients had coinfections with influenza A virus, which the authors felt contributed to the severity of disease. One patient died, and the other 3 required prolonged hospitalization as a result of significant complications [9].

**KEY FEATURES OF MRSA**

Methicillin resistance in S. aureus involves an altered target site due to an acquired penicillin-binding protein (PBP 2a) with decreased affinity to β-lactams [2]. The mecA gene encodes this protein and is located on a mobile SCCmec cassette chromosome [10, 11]. This genetic element confers resistance to most currently available β-lactam antibiotics [2]. However, not all mecA clones are resistant to methicillin, and overall resistance levels in a population of MRSA depend on efficient production of PBP 2a, which is modulated by a variety of chromosomal factors. This explains why MRSA resistance levels range from phenotypically susceptible to highly resistant [2].

There are 5 SCCmec subtypes (types I–V), which vary in size from ~20 kb to 68 kb. The smaller subtypes (I, IV, and V) encode only recombinase genes and the structural and regulatory genes for resistance to methicillin; they do not carry transposable elements and genes encoding resistance to non–β-lactam antibiotics [12]. HA-MRSA isolates typically have SCCmec subtypes I–III and rarely carry the gene for PVL. In contrast, types IV and V are associated with CA-MRSA isolates,
and at least type IV frequently carries PVL [12]. Healy et al. [3, p. 1465] note that the SCCmec cassette in community strains is smaller than that in HA-MRSA and speculate that “its genetic composition likely enhances mobility and ability to be transferred between strains.”

Methicillin resistance in S. aureus is expressed in a stepwise fashion [11]. In the pre-MRSA that has the mecA gene together with regulator genes mecI and mecR1, S. aureus strains do not express methicillin resistance. In the hetero-MRSA, the mecI-mediated repression is released by mutation, and the strain becomes resistant to low concentrations of methicillin but remains susceptible to high concentrations. Finally, a homo-MRSA develops that has homogeneously high methicillin resistance.

Naimi et al. [13] conducted a prospective study of 1100 MRSA infections and found 131 (12%) CA-MRSA and 937 (85%) HA-MRSA strains (32 could not be classified). The CA-MRSA isolates were more likely to be more susceptible to ciprofloxacin, clindamycin, erythromycin, and gentamicin than were the HA-MRSA isolates (P ≤ .001). However, most CA-MRSA infections were initially treated with antimicrobials to which the isolate was not susceptible. In a recent analysis by LaPlante et al. [14], CA-MRSA isolates were found to be significantly more susceptible to trimethoprim-sulfamethoxazole, clindamycin, and ciprofloxacin than were HA-MRSA isolates (P ≤ .05).

It is noteworthy, however, that some SCCmec types carry various additional genetic elements (Tn554, which encodes resistance to macrolides, clindamycin, and streptogramin B; and pT181, which encodes resistance to tetracyclines) that can confer resistance to additional antibiotic classes; these genetic elements are especially common in HA-MRSA [12]. In addition, strains that are resistant to erythromycin can quickly become resistant to clindamycin because of the inducibility of resistance. Inducible resistance to clindamycin is not detected by routine susceptibility testing but requires the use of other methods, such as the D-test [15]. The standard disk diffusion (DD) test is performed by placing a 15-µg erythromycin disk and a 2-µg clindamycin disk at a distance of 15–20 mm (closer than the standard dispenser spacing of 26–28 mm). Flattening of the clindamycin zone of inhibition in the area between the disks to resemble the letter “D” indicates inducible resistance [15]. If these tests are not readily available, it is better not to use clindamycin in these cases.

**ADVANCES IN MRSA DETECTION METHODS AND SUSCEPTIBILITY TESTING**

Recently, the British Society for Antimicrobial Chemotherapy, the Hospital Infection Society, and the Infection Control Nurses Association published guidelines for the laboratory diagnosis and susceptibility testing of MRSA [16]. Routine identification of S. aureus should be performed via tube coagulase tests or latex agglutination tests; routine use of molecular tests for identification is not currently practical for most diagnostic laboratories. However, molecular testing may be useful when there is a high index of suspicion for MRSA. Susceptibility testing may be performed through any standard recognized method, and latex methods to detect PBP 2a and/or PCR tests to detect the mecA gene can provide confirmation of equivocal results [16].

DD testing with cefoxitin has been well correlated with the presence of mecA-mediated oxacillin resistance in S. aureus and has excellent sensitivity (98%) and specificity (100%) [17]. Swenson et al. [17] identified cefoxitin DD breakpoints of ≤19 mm (resistant) and ≥20 mm (susceptible) for S. aureus. These authors also reported that the cefoxitin DD test gave results equivalent to oxacillin broth microdilution tests and oxacillin DD tests but was easier to interpret and did not require transmitted light for identification of resistance. On the basis of this study, the Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical Laboratory Standards) now recommends the use of the cefoxitin DD test for detecting methicillin resistance in S. aureus, and the method has been validated in an international collection of staphylococci from the SENTRY antimicrobial surveillance program [18].

Two novel molecular detection methods are worthy of mention, although they are not ready for routine clinical use. The 3-dimensional microarray system described by Nagaoka et al. [19] has a sensitivity 10-fold greater than that of standard PCR methods and provides a high level of data reproducibility. It is a rapid, specific, and easy test for the simultaneous detection of resistance to levofloxacin and the presence of the mecA gene in S. aureus. Zhang et al. [20] reported the development of a novel multiplex PCR assay that both characterizes and subtypes SCCmec in MRSA (e.g., types I–V and subtypes IVa–IVd) using sets of SCCmec type- and subtype-unique and specific primers. This method has demonstrated excellent (100%) sensitivity and specificity in characterizing 54 known strains of MRSA with various SCCmec types and subtypes. This tool may be useful in understanding the epidemiology and clonal relatedness of various MRSA isolates.

As is shown in figure 1, beneficial outcomes are associated with rapid detection of MRSA. An interventional cohort study of a new molecular MRSA screening test used at time of admission to the intensive care unit showed that 55 of 71 previously unknown MRSA carriers would have gone undetected in the intensive care unit without screening. In addition, the median time from intensive care unit admission to reporting of test results was reduced from 4 days to 1 day (P<.001).
resulting in preemptive isolation and cohorting, thus reducing MRSA cross-infections [21].

VANCOMYCIN NONSUSCEPTIBILITY IN MRSA

As is stated above, multidrug resistance is common in HA-MRSA. It is worrisome that, in recent years, VISA have been isolated with an increased incidence. Since 2002, 7 VRSA strains have been isolated in the United States; these strains have not, as far as we know, been reported elsewhere ([1] and M. Rybak, personal communication, 2006). Multidrug-resistant strains of both VISA and VRSA have been detected, suggesting that the efficacy of antimicrobial agents for systemic infections such as bacteremia, endocarditis, and osteomyelitis may soon be significantly compromised [1]. The true incidence of hVISA (strains ostensibly vancomycin susceptible as assessed by use of current CLSI breakpoints but with a subpopulation able to grow in the presence of vancomycin) is currently unknown because of detection problems, which are detailed later in this article.

An hVISA strain (Mu3) was first identified in 1996 in Japan in a 64-year-old patient with MRSA pneumonia whose condition responded poorly to vancomycin treatment [22]. This strain had a vancomycin MIC of 4 μg/mL, which was initially identified as an hVISA but would now be classified as a true VISA according to current CLSI criteria. The first reported strain of VISA (Mu50) was described in a 4-month-old Japanese infant who was unsuccessfully treated with vancomycin: Mu50 was found to be an MRSA strain, with a vancomycin MIC of 8 μg/mL as assessed by broth microdilution [22]. In subsequent years, close to 100 cases of S. aureus with reduced susceptibility to vancomycin have been reported [1, 23, 24], with some strains responsible for life-threatening systemic infections [23, 24]. Risk factors for VISA infection include recurrent MRSA infections treated with vancomycin and chronic renal failure. There have been fewer cases of VRSA infection (7 cases reported), all of which occurred in elderly US patients with multiple health problems ([1] and M. Rybak, personal communication, 2006).

Susceptibility breakpoints established by CLSI for vancomycin are currently as follows: susceptible, ≤2 μg/mL; intermediate (VISA), 4–8 μg/mL; and resistant (VRSA), ≥16 μg/mL [25]. When effective, vancomycin inhibits cell wall synthesis. Resistant staphylococci are able to maintain the cellular wall, and glycopeptides are unable to access cell wall synthesis sites.

The reduced vancomycin susceptibility of MRSA has been attributed to, among other factors, functional loss of the accessory gene regulator (agr) operon under vancomycin selection pressure, especially with the agr group II phenotype. In vitro studies have shown a greater propensity of agr group II knockout strains to develop vancomycin heteroresistance, compared with other agr groups [26]. In a study by Sakoulas et al. [27], VISA and hVISA clinical isolates from diverse geographic origins were found to be enriched in the agr group II polymorphism, in which several point mutations have been noted. In another study, of 122 MRSA isolates from 87 patients treated with vancomycin, Moise-Broder et al. [28] reported that the conditions of 86% (31/36) of the patients infected with MRSA that had the agr group II polymorphism did not respond to vancomycin. This polymorphism was found to be an independent predictor of vancomycin failure in patients with MRSA infection (P = .005). This genetic marker may provide a survival advantage to the MRSA strains, toward development of reduced vancomycin susceptibility. However, this may not be the only factor that plays a role.

Currently, hVISA and VISA strains are, in all likelihood, being underreported because of nonstandard detection techniques [23]. Typically, DD is the only method used in the clinical laboratory; this may be acceptable for detection of VRSA but is not satisfactory for detection of hVISA and VISA strains [1]. Available automated methods, such as Vitek (bioMerieux) and Microscan (Dade Behring), do not identify all known strains of VRSA adequately and should not be used for the routine detection of hVISA and VISA [1]. A vancomycin agar screening plate should be used. Existing commercially available screening plates still contain a vancomycin concentration of 6 μg/mL, on the basis of the old CLSI breakpoint of ≥4 μg/mL. In view of the new lowered vancomycin susceptibility breakpoint mentioned above, screening plates with a lower vancomycin concentration would appear to be in order and urgently require evaluation. In addition, routine screening for glycopeptide resistance must be done when MRSA strains are isolated, to detect such strains early and to prevent outbreaks [24]. In all cases, at the very least, a vancomycin agar screening test with a more appropriate vancomycin concentration (to be determined, but <6 μg/mL) should be used. A better
approach, in countries with the financial capability, is routine testing of all MRSA strains with an overnight vancomycin Etest strip (AB BIODISK), followed, if necessary, by a macro Etest for all isolates with vancomycin MICs of 1–2 μg/mL isolated from patients with *S. aureus* infections not responding to vancomycin therapy [29]. As is depicted in figure 2, a macro Etest comparing vancomycin and teicoplanin can easily differentiate between VISA and hVISA: vancomycin susceptible when both MICs are low, hVISA when the vancomycin MIC is low but the teicoplanin MIC is high, and VISA when both MICs are high [30]. It has been reported that teicoplanin susceptibility in MRSA is lost before vancomycin susceptibility. The reason for this is not known, but the lower baseline activity of teicoplanin for MRSA, compared with that of vancomycin, may result in easier emergence of resistance to teicoplanin [31]. To improve the detection of vancomycin or glycopeptide resistance in MRSA, it is very important to consider the testing method. The Etest may offer the best alternative, particularly because not all of these strains will be detected by CLSI microbroth dilution methodology [23].

**CONCLUSIONS**

The epidemiological and microbiological characteristics of pathogenic organisms have been rapidly shifting because of selection pressure. MRSA strains are now common both in the health care setting and more widely in the community. Multidrug-resistant strains of MRSA are rapidly evolving, including the more serious glycopeptide-nonsusceptible strains. Compendial advances in delineating the genetic characteristics of MRSA have recently been made, although this aspect is far from being completely understood, and new questions are being raised—for example, the importance of PVL as a virulence factor in CA-MRSA. Options for treating multidrug-resistant *S. aureus* infection are few, and, as new strains emerge, the options are becoming even more limited. Further study of MRSA and evaluation of optimal practices to detect MRSA infections clearly are needed. Active screening and detection of glycopeptide nonsusceptibility in MRSA will almost certainly result in more accurate representation of prevalences of hVISA and VISA strains and, possibly, VRSA strains. A rise in systemic infections caused by non–glycopeptide-susceptible *S. aureus* strains will be a very serious development indeed, leaving the clinician with very few therapeutic options.

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