Staphylococcus aureus with Reduced Susceptibility to Vancomycin

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Infections with *Staphylococcus aureus* with reduced susceptibility to vancomycin continue to be reported, including 2 cases caused by *S. aureus* isolates with full resistance to vancomycin. This review first outlines the definitions of vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) and risk factors for infection. Next, we describe the mechanisms of resistance and methods of laboratory detection of the organisms. Finally, we address infection control and management issues associated with isolation of VISA and VRSA.

The first report of a clinical *Staphylococcus aureus* isolate that demonstrated reduced susceptibility to vancomycin in 1997 has been followed by multiple reports of additional isolates seen in all parts of the world. In the United States, there have now been 9 reported clinical cases of infection with *S. aureus* with intermediate resistance to vancomycin, as well as 2 known clinical cases of infection with *S. aureus* isolates that are fully resistant to vancomycin [1]. This brief review will address the epidemiology, mechanisms of resistance, laboratory identification, containment, and treatment of *S. aureus* isolates with reduced susceptibility to vancomycin.

DEFINITIONS

In the United States, the NCCLS has developed guidelines to define susceptibility for *S. aureus* isolates [2]. Isolates for which the MIC of vancomycin is ≤ 4 μg/mL are susceptible, and isolates for which the MIC of vancomycin is 8–16 μg/mL are intermediate. Resistance is defined as an MIC of vancomycin of ≥ 32 μg/mL. In Japan, *S. aureus* isolates for which the MIC of vancomycin is ≥ 8 μg/mL have been referred to as resistant strains, but these strains would be considered intermediate in the United States [3]. The terms "glycopeptide-resistant *S. aureus*" and "glycopeptide-intermediate *S. aureus*" have been used to refer to resistance to both glycopeptides, vancomycin and teicoplanin; however, because the latter agent is not used in the United States, the convention is to refer to vancomycin-resistant and -intermediate *S. aureus* (VRSA and VISA, respectively).

In addition to *S. aureus* isolates that are identified as VISA or VRSA, there are strains of *S. aureus* that are referred to as "heteroresistant." These strains are susceptible to vancomycin (MIC, ≤ 4 μg/mL); however, they contain subpopulations of organisms for which the MIC of vancomycin is in the intermediate range [4]. These subpopulations become apparent when the original isolate is incubated on a plate containing vancomycin and colonies grow. The clinical significance of heteroresistance is an area of active investigation.

RISK FACTORS

Risk factors associated with isolation of VISA and VRSA determined from case reports of infections with these organisms include prolonged vancomycin use, hemodialysis dependence, and indwelling foreign bodies. A recent study by Fridkin et al. [5] systematically assessed risk factors for infections caused by *S. aureus* with reduced susceptibility to vancomycin in the United States. Nineteen patients with VISA infection ($n = 4$) or *S. aureus* for which the MIC of vancomycin was 4 μg/mL ($n = 15$) were compared with 42 patients infected with methicillin-resistant *S. aureus* (MRSA) strains susceptible to vancomycin (MIC, <4 μg/mL). Patients were similar in underlying illness and exposure to dialysis, and independent predictors of infection with *S. aureus* with reduced susceptibility to vancomycin were receipt of vancomycin in the month before isolation of the organism (OR, 13.1; 95% CI, 1.8–100) and in the 3–6

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months before isolation of the organism (OR, 2.8; 95% CI, 1.1–7.0) and isolation of MRSA from a culture in the 2–3 months before isolation of the organism. Notably, dialysis exposure was not a predictor. These results suggest that patients who are infected or colonized with MRSA and who receive vancomycin frequently over several months are at highest risk for subsequent isolation of *S. aureus* with reduced susceptibility to vancomycin. Also of note, case patients were more likely to die than were control subjects: in-hospital mortality was 63% for case patients, compared with 12% for control subjects.

The epidemiology of VRSA is less well defined because there have been only 2 reported cases of VRSA in the United States (one was in Michigan [reported in July 2002], and the other was in Pennsylvania [reported in September 2002]) [6, 7]. Both patients had recurrent MRSA infection, a prior or concurrent infection or colonization with vancomycin-resistant enterococcus (VRE), and chronic wounds. Of interest, the patient in Pennsylvania had an allergy to vancomycin and thus had not received vancomycin before isolation of the VRSA strain.

Charles et al. [8] performed the largest evaluation to date of the clinical features associated with bacteremia due to *S. aureus* isolates manifesting heteroresistance to vancomycin. These investigators compared data from 48 episodes of MRSA bacteremia with 5 episodes of heteroresistant MRSA bacteremia and found that heteroresistant MRSA bacteremia was significantly associated with infections that have high bacteria loads, such as endocarditis; longer duration of fever, time until clearance of bacteremia, and length of hospitalization; and failure of vancomycin treatment. There was no difference in the mortality rate at 1 month between the 2 groups. Further investigation into the clinical significance of MRSA strains with heteroresistance to vancomycin, as well as improved methods of detection, are essential.

MECHANISMS OF RESISTANCE

A review of the mechanism of action of vancomycin is helpful in understanding the mechanisms of resistance of VISA and VRSA. The *S. aureus* cell wall is made up of peptidoglycan layers that consist of a series of murein monomers, each of which has a D-alanine–D-alanine residue. The monomers are synthesized within the cell, transferred through the cytoplasmic membrane, and assembled into peptidoglycan outside of the cytoplasmic membrane by enzymes located within the membrane. Vancomycin inhibits bacterial cell growth by binding to D-alanine–D-alanine residues of the monomers and preventing the assembly of peptidoglycan outside of the cytoplasmic membrane [3]. The drug can also bind to the D-alanine–D-alanine residues present in the completed peptidoglycan layers; however, this binding does not affect bacterial cell growth.

The proposed mechanisms of resistance described for VISA and VRSA strains are distinctly different from each other. The primary factor that causes reduced susceptibility to vancomycin among VISA isolates is the presence of a thickened cell wall with several more peptidoglycan layers, compared with non-VISA isolates. Vancomycin binds to the many D-alanine–D-alanine residues within the additional peptidoglycan layers and never reaches the surface of the cytoplasmic membrane to exert an effect on the synthesis of peptidoglycan [9]. This phenomenon was demonstrated in the first clinical VISA strain (known as Mu50), which made 7.3 times more murein monomer precursor and had a cell wall that was twice as thick (determined by transmission electron micrography), compared with control isolates [10]. The authors also observed that there was accelerated uptake of N-acetylglucosamine into the cell, accelerated release of cell-wall material into the culture medium, and increased production and autolytic activity, all of which suggest that there is increased synthesis and turnover of the cell wall in the Mu50 isolate. Cui et al. [9] went on to study 16 clinical VISA strains from 7 countries and demonstrated that the mean cell-wall thickness of the VISA strains was significantly greater than that of control strains and that the MIC of vancomycin correlated with cell wall thickness.

The *vanA, vanB,* and *vanC* genes that mediate vancomycin resistance in enterococci were not found in VISA strains, although conjugative transfer of the *vanA* gene from enterococci to *S. aureus* was demonstrated in the laboratory in 1992 [11, 12]. However, the 2 reported clinical isolates of VRSA in the United States have contained the *vanA* gene, which is believed to confer true vancomycin resistance (MIC, $\geq$32 μg/mL) [6, 7, 13–15]. Of note, the Michigan patient was coinfected with vancomycin-resistant *Enterococcus faecalis.* The DNA sequence of the *vanA* gene isolated from the VRSA isolate was identical to that from the *E. faecalis* isolate, suggesting that transfer of this genetic element had occurred. No other vancomycin resistance genes were identified. VRE was not recovered from the Pennsylvania patient; however, he was known to have been colonized with VRE during the preceding year.

LABORATORY DETECTION

All microbiology laboratories should have procedures in place for selection of *S. aureus* strains that should be screened for decreased susceptibility to vancomycin. Microbiologists must be knowledgeable about the most appropriate methods of VISA and VRSA detection. In addition, laboratories should have procedures for confirmation of suspected strains of VISA and for notification of the health care provider, infection-control personnel, and public health officials at the local and national levels (table 1).

Detection of VISA isolates can be difficult. In our experience and that reported by others, VISA are slow growing and may not appear on the primary culture plate until $\geq$2 days of incubation [16]. Consequently, the Centers for Disease Control

and Prevention (CDC; Atlanta, GA) recommends that primary testing of *S. aureus* requires ⩾24 h of incubation. In addition, colonies may initially appear pinpoint and may have variable—even atypical—morphologies. Loss of typical phenotypic characteristics, such as β-hemolysis and thermostable nuclease activity, has been observed as isolates develop increasing MICs to vancomycin [17].

To accurately detect staphylococci with reduced susceptibility to vancomycin, antimicrobial susceptibility optimally should be determined with a quantitative method. These include broth dilution, agar dilution, and agar gradient diffusion (Etest; AB-Biodisk). The NCCLS currently recommends broth dilution testing in cation-adjusted Mueller-Hinton broth using a 0.5 McFarland standard as the inoculum, with incubation at 35°C for a full 24 h [2].

Strains of staphylococci for which the MIC of vancomycin is 4–8 μg/mL are not detected reliably using disk-diffusion procedures and some automated systems [11]. In a study by Tenover et al. [11], the MicroScan Rapid panels (Dade Behring-MicroScan) failed to successfully recognize any of the VISA strains evaluated, calling them either susceptible (MIC, ⩽2 μg/mL) or completely resistant (MIC, ⩾16 μg/mL). In this evaluation, the Vitek system (bioMérieux) reported MICs that were consistently 4 μg/mL; higher MICs were not reported. A subsequent evaluation of later versions of Vitek software (version 7.01 and above) reported acceptable results [18]. However, in an evaluation of the Pennsylvania VRSA strain, Microscan, Vitek, and Vitek 2 all failed to report MICs in the resistant range [15]. Commercial systems that produce results that most closely approximate those of the reference methods include the agar gradient diffusion method (Etest) and nonautomated broth-based MIC tests, such as Sensititre panels (Trek Diagnostics) and PASCO panels (PASCO Laboratories) [11, 18].

Laboratories that routinely use disk diffusion or an automated system for staphylococcal susceptibility testing should consider using a commercially prepared brain-heart infusion agar plate with 6 μg/mL of vancomycin to screen *S. aureus* isolates for reduced susceptibility to vancomycin [15]. Lot-to-lot variation has been observed when agar screen plates are prepared in-house [11]. An inoculum of 10^6 colony-forming units (CFU)/mL and both a negative control (*S. aureus* ATCC 25923) and a positive control (*E. faecalis* ATCC 51299) are recommended when using the agar screening method [11]. Isolates growing on the agar screen plates should have MICs of vancomycin determined by an acceptable method.

Any *S. aureus* isolate that has an MIC of ⩾4 μg/mL should be confirmed with retesting using an MIC method. If the isolate again has an MIC of ⩾4 μg/mL, it should be reported to health care providers, infection control, the health department, and the CDC as presumptive VISA (or as presumptive VRSA if the MIC is ⩾32 μg/mL). All presumptive VISA or VRSA isolates should be sent to the CDC for confirmatory testing. The CDC offers expedited confirmation (within 72 h after receipt) of suspected VISA or VRSA isolates to all laboratories. Laboratories may e-mail SEARCH@cdc.gov to facilitate testing.

Routine screening of clinical *S. aureus* isolates for vancomycin heteroresistance is not recommended by the NCCLS or the CDC, is difficult to perform, and is not done in the majority of microbiology laboratories. Isolates shown to contain heteroresistant subpopulations should not be reported as VISA isolates. The optimal method of detection of heteroresistant strains and their clinical significance are areas of active research.

Table 1. Recommendations for detecting *Staphylococcus aureus* with decreased susceptibility to vancomycin.

Strategies for selecting which strains require additional testing
Select isolates with MICs of vancomycin of >4 μg/mL, or
Select isolates with MICs of vancomycin of >8 μg/mL, or
Select all MRSA isolates, or
Select all *S. aureus* isolates
Laboratory testing and confirmation
Ensure that culture is not mixed—i.e., pure isolate of *S. aureus*
Use a quantitative method to determine the MIC in accordance with NCCLS guidelines
Broth microdilution
Agar dilution
Agar gradient diffusion
Acceptable commercial system (see Laboratory Detection)
Retest isolates that have MICs of >4 μg/mL
Retest isolates recovered from patients who do not improve with vancomycin treatment
Notify infection-control personnel, the health care provider, the local public health department, and the CDC (http://www.SEARCH@cdc.gov) when an *S. aureus* isolate with an MIC of ⩾4 μg/mL has been recovered

NOTE. CDC, Centers for Disease Control and Prevention; MRSA, methicillin-resistant *S. aureus.*

The CDC uses an inoculum of 10^6 CFU/mL on brain-heart infusion agar containing 6 μg/mL of vancomycin to detect vancomycin heteroresistance [18]; however, other investigators have used other methods, including varying the concentrations of vancomycin in agar media in combination with a higher inoculum (2.0 McFarland vs. 0.5 McFarland) and prolonged incubation (48 vs. 24 h) [19–21].

INFECTION CONTROL

Given the rapid spread of MRSA and VRE within hospitals shortly after their emergence as pathogens, the specter of a similar pattern of spread with VISA and VRSA has elicited great concern in the infection-control and public health arenas. It is prudent to assume that spread from patient to patient can occur via the same mechanisms as described for less resistant *S. aureus* strains (on the hands of health care workers, on contaminated equipment, and from nasal shedding). Indeed, transmission of a clonal VISA strain has been reported among a group of patients in France who were residents of the same long-term care facility and had never received glycopeptide antibiotics [22].

Contact investigations of 3 VISA cases and 2 VRSA cases in the United States have not demonstrated transmission of VISA or VRSA to health care workers [14, 23, 24]. A total of 830 contacts of the VRSA-infected index patients were identified (including hospital, podiatry clinic, and dialysis workers; household members; and employees of a nail salon frequented by the Michigan index case), 635 cultures were obtained, and none grew VRSA. However, close personal contacts of both index cases were found to be colonized with vancomycin-susceptible MRSA strains that were found to be identical by PFGE to the VISA strains seen in the index cases. This finding underscores the potential risk of transmission to close hospital contacts but also suggests that prolonged vancomycin use may drive transformation of MRSA isolates toward reduced susceptibility to vancomycin. Clearly, limiting prolonged or inappropriate use of vancomycin is important in reducing the risk of VISA and VRSA.

The lack of documented transmission of the US VISA and VRSA strains within the health care setting may be related to the use of contact precautions for MRSA before the isolation

Table 2. Recommendations for infection control for patients infected with *Staphylococcus aureus* with decreased susceptibility to vancomycin.

CDC recommendations for prevention of spread
Isolate patient in a private room
Don gowns and gloves to enter the room
Don mask and eye protection if aerosolization is possible[a]
Practice hand hygiene with an antibacterial agent
Use dedicated equipment that is not shared among patients
Continue isolation until results of tests of nares and infected sites are negative 3 times over 3 weeks[b]
Minimize number of staff caring for patient
Educate staff about appropriate precautions and assess compliance
CDC recommendations for evaluation for spread
Perform baseline cultures of specimens from hands and nares of persons with extensive patient contact[c]
Perform baseline cultures for other contacts if results of cultures for those with extensive contacts are positive
Perform weekly cultures of specimens from the nares of persons with extensive patient contact
Decolonize index patient and HCWs with VISA, VRSA, and MRSA with mupirocin[d]
Other recommendations beyond CDC guidelines[f]
Use sign-in sheets to monitor who enters patient's room
Limit nonessential tests that require patient to leave room
HCWs at risk for staphylococcal colonization should not care for patient[e]
Specimens should not be sent to the lab via pneumatic tubes
Close unit if nosocomial transmission is documented
Perform environmental cultures after terminal room cleaning

NOTE. CDC, Centers for Disease Control and Prevention; HCW, health care worker; MRSA, methicillin-resistant *S. aureus;* VISA, vancomycin-intermediate *S. aureus;* VRSA, vancomycin-resistant *S. aureus.*

[a] Aerosolization would be expected during manipulation of wounds or suctioning or in patients with pneumonia.

[b] Including readmission to facility.

[c] The CDC recommends that priority be given to contacts with extensive interaction with patient (i.e., hospital roommates, nursing staff and physicians responsible for direct care of patient [bathing, turning, dressing changes, suctioning, and performing extensive physical examination], ancillary staff with prolonged patient contact [rehabilitation, dialysis, wound care, and respiratory therapy], and household contacts who provide care or share rooms with the patient).

[d] The CDC recommends that this decision be made in conjunction with infection control and the health department.

[e] Insulin-requiring diabetes and exfoliative dermatitis.

[f] In use at Johns Hopkins Hospital (Baltimore, MD).

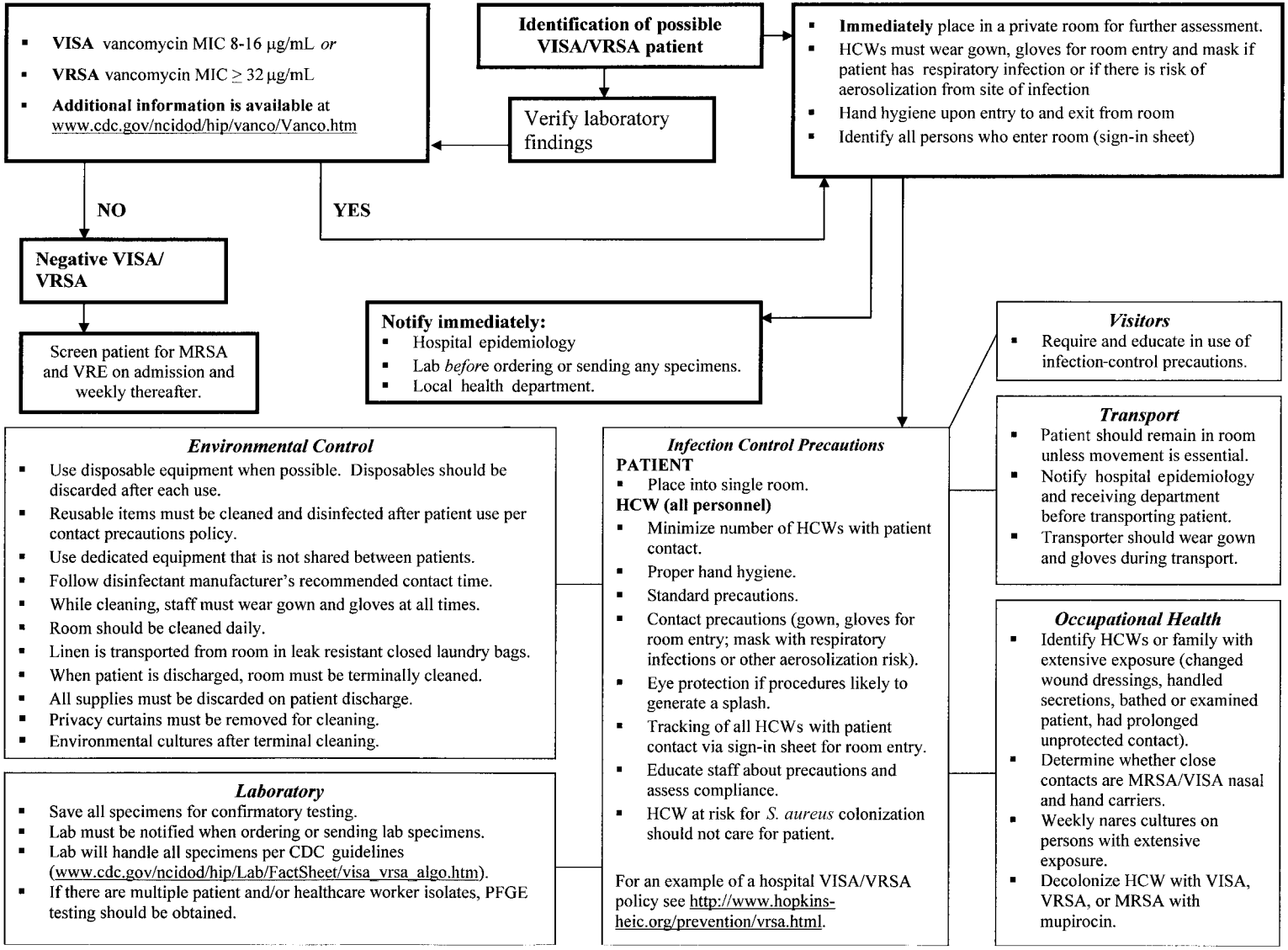

Figure 1. Flow diagram for infection control in vancomycin-intermediate *Staphylococcus aureus* (VISA)/vancomycin-resistant *S. aureus* (VRSA) management. CDC, Centers for Disease Control and Prevention; HCW, health care worker; MRSA, methicillin-resistant *S. aureus*.

of VISA and VRSA, emphasizing the importance of the use of contact precautions in patients colonized with MRSA. Rapid isolation of infected patients and education of health care workers, the patient, and the patient's family are essential. The importance of hand hygiene and environmental cleaning cannot be over-emphasized. Table 2 summarizes the current CDC recommendations for infection control to reduce the risk of transmission of VISA and VRSA (items 1 and 2), as well as additional recommendations proposed by a group from the Medical College of Virginia and in use at our institution (item 3) [25, 26]. Figure 1 outlines the steps that are taken at our institution when a presumptive VISA or VRSA isolate is identified.

TREATMENT

There is no standardized therapy for VISA and VRSA infections. Removal of infected indwelling hardware and debridement of infected sites is of utmost importance and must be considered for every patient. Vancomycin monotherapy has been associated with treatment failure in VISA infections [27]. Investigators have assessed the utility of combination therapy with β-lactam agents and vancomycin in a rabbit model of endocarditis due to 2 strains of VISA (MU50 and HIP5827) and found that, although monotherapy with either nafcillin or vancomycin was ineffective, the combination resulted in a significant decrease in organism count as well as survival in the majority of animals (15 of 16 animals) [28]. Whether this finding is generalizable to all VISA strains is unknown; synergy testing of these agents could be considered in the clinical management of patients infected with VISA, although many microbiology laboratories do not routinely perform such testing.

All of the VISA isolates identified in the United States have been susceptible to trimethoprim-sulfamethoxazole (TMP-SMX) and tetracycline, and these agents have been used in various combinations with other agents for the treatment of some VISA infections. The 2 reported VRSA isolates were resistant to tetracycline but were both susceptible to TMP-SMX, and this agent was used in combination with aggressive local debridement and contact casting to treat the patient from Michigan, resulting in a clinical cure. TMP-SMX use in combination with linezolid in the patient from Pennsylvania led to a microbiologic cure, although the patient subsequently died of cardiopulmonary disease. TMP-SMX has been used successfully in the treatment of MRSA infections; however, its role in the management of infections caused by VISA and VRSA relative to newer antistaphylococcal agents requires further elucidation [29].

As noted above, linezolid has been used in combination therapy for VRSA infection, as well as for the management of 2 VISA infections (as monotherapy in 1 case). Both linezolid and quinupristin-dalfopristin have in vitro activity against 3 of the VISA strains and the Michigan and Pennsylvania VRSA strains, although both linezolid and quinupristin-dalfopristin were bacteriostatic against the Pennsylvania strain [13, 15, 30, 31]. Daptomycin is a recently approved lipopeptide antibiotic with a unique mechanism of action and bactericidal activity against many gram-positive organisms, including *S. aureus.* Although daptomycin has not been used to treat clinical infections due to VISA or VRSA, it also has been shown in vitro to have bactericidal activity against the aforementioned strains [13, 15, 30, 31]. The addition of rifampin to therapy can also be considered if the isolate is drug susceptible, but rifampin should not be used as monotherapy.

Given the likely colonization of VISA or VRSA or the MRSA precursor in the anterior nares of patients, we recommend that the anterior nares of the patient be cultured once the infection is cleared. If the patient is colonized, decolonization should be attempted with mupirocin applied to the nares. In addition, chlorhexidine washes can be considered in select cases.

CONCLUSIONS

VISA and VRSA are emerging pathogens that have the potential to become more prevalent given the acuity of patients in the health care setting today. Increased use of dialysis and indwelling hardware, more complicated surgical and medical procedures, and the associated increase in vancomycin use create an environment in which VISA and VRSA isolates have emerged. Given the history of rapid spread of MRSA and VRE in hospitals and other arenas, such as dialysis units, clinicians must be aware of issues surrounding the microbiological diagnosis and management of patients who are colonized or infected with VISA and VRSA to prevent further emergence and spread. Research priorities should include enhancement of early detection of and surveillance for VISA and VRSA isolates and further elucidation of the use of both old and new antimicrobial agents in the management of VISA and VRSA infections.

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