Heteroresistance refers to the presence, within a larger population of fully antimicrobial-susceptible microorganisms, of subpopulations with lesser susceptibility. This phenomenon has been described in a wide range of microorganisms, but much recent attention has been directed toward its expression in *Staphylococcus aureus*. Although it has long been known that resistance to methicillin is characteristic heterogeneously expressed by this organism [1], the current focus revolves around its heteroresistance to vancomycin. Heteroresistant subpopulations of vancomycin-susceptible *S. aureus* (hVISA) were first described in 1997 [2], shortly after the initial description of vancomycin intermediate susceptible strains (VISA) [3]. hVISA have minimum inhibitory concentrations (MICs) in the intermediate susceptible range and likely represent a step on the path to the development of a fully VISA population.

hVISA have been identified among clinical isolates of MRSA at widely varying frequencies, but some recent US reports have been more consistent. During 2003–2007, the proportion of MRSA found to harbor hVISA by the macro Etest in 3 Detroit-area hospitals was 8.3%; this represented an increase from 2.2% in 1986–1993 and occurred without a contemporaneous increase in VISA isolations [4]. This prevalence in the most recent period is similar to that found in a prospective trial of treatment of *S. aureus* bacteremia and endocarditis (mostly in the United States), in which 7 (8%) of 89 MRSA isolates, were vancomycin heteroresistant [5]. Vancomycin heteroresistance is present in both hospital and community strains of *S. aureus*; in the Detroit experience, 56.9% of hVISA belonged to SCC*meC* type II and 39.4% to SCC*meC* type IV [4]. The proportion of methicillin-resistant *S. aureus* (MRSA) isolates demonstrating heteroresistance increases with increasing vancomycin MICs, but heteroresistance is observed in strains with MICs as low as 1.0 μg/mL [6]. The phenomenon is inducible and may be either stable or unstable [7].

hVISA are not detected by standard susceptibility testing methods because they are ordinarily present at frequencies of only 10⁻⁷ to 10⁻⁵ [6]. The gold standard for the identification of hVISA is population analysis, but this is a labor-intensive process not suitable for the clinical microbiology laboratory. An alternative method, the macro Etest, has recently been reported to have, relative to population analysis, sensitivities of 80% and 94% and specificities of 87% and 96% for detection of hVISA/VISA when examined at 24 h and 48 h, respectively [8]. The macro Etest, however, requires use of a non-standard medium and inoculum. A newer method, the Etest GRD strip with incorporation of a nutrient that enhances growth of hVISA/VISA, uses a standard inoculum and medium and has sensitivities of 74% and 94% and specificities of 100% and 95% when read at 24 h and 48 h, respectively [8].

hVISA and VISA generally have a characteristic phenotype, with a thickened cell wall and increased d-ala-d-ala moieties, together with abnormal muropeptide residues and diminished cross-linking of peptidoglycan in association with reduced autolytic activity and slow growth in vitro [9, 10]. The excess dipeptides are believed to “trap” vancomycin, and this, together with the thickened cell wall, may act as a barrier to the diffusion of this large (molecular weight, 1485.7) glycopeptide molecule [11, 12]. It has been speculated that the thickened cell wall may also act as a barrier to daptomycin (molecular weight, 1620.7), thus accounting for vancomycin induction of daptomycin heteroresistance and a reported parallel increase in MIC for this lipopeptide in some
isolates [13, 14]. The development of this phenotype, which can be demonstrated in vitro passage in the presence of vancomycin, has previously been associated with overexpression of genes of the cell wall stimulon [15], a group of genes induced by stress, such as exposure to cell wall–active antibiotics [16]. Mutations resulting in upregulation of one of these operons, VraSR (vancomycin resistance–associated sensor/regulator), which encodes a 2-component histidine kinase response regulator, have been associated with the emergence of VISA in some but not all isolates [17–19]. Mutation of graS, the sensor component of another putative 2-component system, has alternatively been implicated, but full expression of reduced vancomycin susceptibility apparently requires >1 locus mutation [18, 20]. In contrast to these findings, however, transcriptome analysis of a set of strains found no evidence of upregulation of vraSR or the cell wall stimulon but did identify multiple varying mutations, even among isolates of the same multilocus sequence type [19]. This suggests that, instead of single initial signature mutation, there may be multiple potential transcriptional pathways leading to the development of reduced vancomycin susceptibility [19]. Although changes in some global regulators, especially agr, have been associated with reduced vancomycin susceptibility, the association is not consistent [18, 21]. Thus, the genetic basis for hVISA/VISA requires further elucidation.

A number of additional microbial factors adversely affect the antibacterial activity of vancomycin. These include intracellular residence [22], growth as small colony variants [23] and biofilm [24], and tolerance [25]. Vancomycin is inactive against MRSA in biofilm [24], a mode of growth believed to play an important role in many staphylococcal infections. The relationship of hVISA/VISA to biofilm formation appears unsettled. Thus, in contrast to a previous report that used a single S. aureus strain with intermediate vancomycin resistance induced in the laboratory [26], diminished susceptibility to vancomycin was found to be associated with a reduced ability to form biofilm in several clinical isolates associated with persistent bacteremia [27]. This may appear surprising, given the association of hVISA with agr dysfunction [28], a condition generally associated with increased biofilm formation [29]. Tolerance (reduced susceptibility to killing by an antibiotic) has been associated with poor therapeutic responses of patients with MRSA bacteremia to vancomycin [25, 30]. Defined as a minimum bactericidal concentration [MBC]/MIC ratio of ≥32 or an MBC of ≥16 μg/mL, tolerance was detected in one study in all 17 VISA strains tested, 69.3% of 88 hVISA, and only 14.7% of vancomycin-susceptible MRSA [31]. Tolerance is predominantly a consequence of reduced autolytic activity resulting from the impaired access of staphylococcal murein hydrolases to cell wall substrate caused by steric hindrance due to the binding of vancomycin to excess D-alanine-D-alanine [32]. Autolytic activity may also be inhibited by a second mechanism, given that the majority of VISA strains have impaired acetate catabolism (71%, compared with 8% of vancomycin-susceptible S. aureus) [33], which could lead to tolerance because this catabolic activity plays a role in inducing expression of murein hydrolase operons [34]. There is increasing evidence that S. aureus is often an intracellular pathogen [22] and that vancomycin, like many other antibiotics, is poorly active against S. aureus, including VISA, in this location [35, 36]. There appears to be no evidence, however, that hVISA/VISA is more likely than fully susceptible strains to reside intracellularly.

Altered host-pathogen interaction may play a role in the persistence of infection. In an in vitro macrophage model, hVISA/VISA strains causing persistent bacteremia were found to have increased capsule and reduced protein A production, as well as reduced activation of NF-kB, with associated diminished expression of TNF-α and IL-1β [19]. Some hVISA/VISA have also been found to have diminished susceptibility to host-derived, thrombin-induced platelet microbicidal proteins [28]. The reduced TNF-α expression elicited by hVISA/VISA infection of macrophages [19] may result in impaired elimination of S. aureus residing in vascular endothelial cells [37]. These findings suggest that one reason for persistence of bacteremia may be related to a limited host response to the organism.

How strong, however, is the evidence that the presence of hVISA is associated with failure of vancomycin therapy? First, not all investigators have concluded that hVISA infection constitutes a risk for such failure. At a Detroit hospital in 2002, only 3 (13.6%) of 22 of patients with persistent (duration, 10–39 days) or recurrent MRSA bacteremia were infected with hVISA [38]. In Seoul, South Korea, chart review found that all 7 patients with hVISA infections identified over a 9-month period and treated with glycopeptide clinically improved, and 4 had bacteriologic cure [39]. None, however, had bacteremia: 3 had wound infection, 2 had pneumonia, and 1 each had central venous access site infection and urinary tract infection. Glycopeptide treatment was successful in 7 of 9 liver transplant recipients with hVISA infection, but a single infection–related death occurred in one of the 3 bacteremic patients despite a mean trough vancomycin serum concentration of 15.3 μg/mL [40]. Finally, in a prospective trial in which patients with MRSA bacteremia with or without endocarditis received either daptomycin or vancomycin (plus gentamicin for 4 days), there was no association of hVISA infection with persistence of bacteremia [5]. There were, however, only 7 hVISA isolates identified in that trial.

In contrast to these studies, 13 (68.4%) of 19 patients at a Barcelona, Spain, hospital who had surgical hVISA infections after undergoing orthopedic procedures had persistence or recurrence of clinical evidence of infection [41]. Twelve of 13 patients who did not respond to therapy had orthopedic implants, as did 1 of 6 who had a successful response. No information on vancomycin dosing or blood
levels was reported, and, although the device was removed in all patients who did not respond to therapy, the timing of the removal is not explicitly stated. Separately, Charles et al. [42] reported that 5 (9.4%) of 53 of patients with MRSA bacteremia were infected with hVISA strains at a single Australian hospital from July 2001 to June 2002. All 5 had prosthetic devices that could not be promptly removed. Patients with hVISA were more likely to have “high bacterial load infections” (defined as either undrained MRSA collections, infected prosthetic material, or endocarditis) and clinical failure (i.e., persistent fever and bacteremia >7 days after vancomycin initiation). The median duration of bacteremia was 26 days in the patients with hVISA infection and only 3.5 days in the others. All 5, however, had a serum vancomycin trough concentration of <10 μg/mL in the first 7 days of therapy, compared with only 31% in the comparator group.

Analysis of 25 patients with hVISA infection, 17 of whom had bacteremia (8 with endocarditis), found that 19 (76%) did not respond to therapy with vancomycin [43]. Four patients without bacteremia had positive results of sterile-site cultures for >21 days. It should be noted, however, that an overlap in institutions, years of study, and investigators raises concern about the possibility that patients from this study also appeared in the study by Charles et al. [42]. In other reports of patients with bacteremia, the colonization of hVISA has been found to be an independent risk factor for failure and for recurrence of bloodstream infection [44, 45].

Maor et al. [46] previously reported a prevalence of hVISA of 6% among 264 patients with MRSA bacteremia at the Sheba Medical Center in Tel Hashomer, Israel, during 2003 and 2004 and suggested that hVISA may have been associated with persistently positive results of blood cultures. In this issue of the Journal [47], they have extended their observations by prospectively identifying 27 cases of bacteremia due to hVISA, as determined by the Etest method, and retrospectively compared them with 223 cases of MRSA bacteremia absent vancomycin heteroresistance. Patients with hVISA infection were more likely to have an orthopedic or intravascular device (i.e., prosthetic valve or pacemaker) but were not more likely to have received vancomycin in the previous 6 months. Although not associated with greater mortality, hVISA infection was significantly associated with a longer median duration of bacteremia (12 vs. 2 days), a greater likelihood of the presence of endocarditis and of osteomyelitis, and an increased risk of the emergence of rifampin resistance (44% vs. 5.9%) regardless of rifampin exposure. The last finding is of interest, suggesting that an inability of vancomycin to rapidly eradicate hVISA results in a failure to protect rifampin from emergence of resistant clones. The development of rifampin resistance in the absence of rifampin therapy is more difficult to explain but could be related to activation of the SOS system, as has been demonstrated with β-lactam antibiotics, with a consequent increased mutation rate [48].

This study by Maori and colleagues suffers from a number of limitations, including its retrospective nature, the lack of clinician blinding with respect to the presence of hVISA, and the fact that surveillance blood cultures were only obtained a mean of every 2.5–2.9 days during the first 2 weeks. The observed frequency of device infections with hVISA infection and treatment failure, which was also observed in other studies, is an obvious potential confounder. The lack of information about precise vancomycin MIC determinations is also of importance, given the relationship of the presence of hVISA with elevated MICs in the susceptible range [6], because it raises the issue of which of these factors might account for reported treatment failures. Despite these shortcomings, this report adds further evidence that vancomycin is an ineffective therapeutic choice in many patients with serious hVISA infection. Thus, the authors’ conclusion that laboratories should routinely screen for hVISA if vancomycin is to be used in the treatment of MRSA bacteremia must be taken under careful consideration.

References


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