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REVIEW ARTICLE

Clinical implications of vancomycin heteroresistant and intermediately susceptible *Staphylococcus aureus*

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Abstract. *Staphylococcus aureus* has proven to be a major pathogen with the emergence of methicillin-resistant *S. aureus* (MRSA) infections and recently with heteroresistant vancomycin intermediate *S. aureus* (hVISA) and vancomycin intermediate *S. aureus* (VISA) infections. While vancomycin is traditionally a first line and relatively effective antibiotic, its continued use is under question, as reports of heteroresistance in *S. aureus* isolates are increasing. Both hVISA and VISA infections are associated with complicated clinical courses and treatment failures. The prevalence, mechanism of resistance, clinical significance, and laboratory detection of hVISA and VISA infections are not conclusive, making it difficult to apply research findings to clinical situations. We provide an evidence based review of *S. aureus* isolates expressing heterogenic and reduced susceptibility to vancomycin.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most commonly encountered bacteria in hospitals and community settings[1] and is associated with invasive infections ranging in severity from mild to fatal.[2] Vancomycin is considered the standard treatment for empiric and definitive serious MRSA infections.[2] In recent years, infections caused by MRSA with reduced susceptibility to vancomycin have emerged. The formation of intermediate resistant isolates is likely caused by selection pressure from ever-present and longstanding use of vancomycin.[3-5] Poor patient outcomes are attributed to heteroresistant vancomycin intermediate *S. aureus* (hVISA) and vancomycin intermediate *S. aureus* (VISA) infections.[6-8] Herein we review the prevalence, laboratory detection and interpretation, resistance mechanisms, risk factors and outcomes, treatment options, and infection control strategies for hVISA and VISA. Peer-reviewed publications were identified using PubMed, Embase, and Cochrane Central Register of Controlled Trials.

Prevalence of hVISA and VISA

The first clinical strain of *S. aureus* with intermediate resistance to vancomycin, designated Mu50, was reported in 1997 from Japan.[9,10] The first hVISA isolate, designated Mu3, was identified in Japan one year earlier from a patient with MRSA pneumonia unresponsive to vancomycin.[9] Since then, hVISA and VISA cases have been reported in the United States, United Kingdom, China, Australia, Turkey, France, Belgium, Germany, Italy, Brazil, and South Korea.[11] The true prevalence of hVISA is unknown, and estimates vary widely because of non-standardized detection methodologies or absence of routine hVISA screening, variation in interpretation, geographical location, clinical setting, and differing patient populations.[12-19] Reported rates of hVISA throughout the world range from 0 to 73.7%.[18]

One retrospective study evaluated MRSA strains with heterogenic intermediate resistance to vancomycin over a 22-year period in three Detroit hospitals. The prevalence of these organisms increased from 2.2% (1986 – 1993) and 7.6% (1992 – 2002) to 8.3% between 2003 and 2007.[16] Only 14 of the 1,498 (0.93%) MRSA isolates were identified as VISA. There was no apparent pattern of increasing prevalence over the three time periods for VISA isolates. An increase in hVISA was also described in a similar retrospective study from Turkey of 1.6% in 1998 to 36% in 2001.[20] Because clonality was not evaluated in either study, the increase in prevalence may have reflected clonal spread rather than true prevalence. Prevalence may have been underestimated because the isolates were stored for prolonged periods in glycopeptide-free media, which may result in a loss of resistance.[21] Two surveillance studies conducted in 2009 and 2011 in over 40 U.S. medical centers determined rates of antimicrobial resistance among *S. aureus* isolates collected from patients with infections.[22,23] The rates of hVISA among MRSA isolates in 2011 were higher than in 2009 (1.2% vs. 0.4%, P = 0.003).[22] Of note, strains of VISA were not detected.[22,23] While the current prevalence of VISA is low, these organisms may become more common in the future. Data suggests that heteroresistance is a precursor to VISA, therefore the suspected increase in prevalence of hVISA may predict more VISA infections. Increased use of vancomycin provides selection pressure for further emergence of VISA. Based on available data, hVISA appears to be on the rise, yet VISA still remains a rare occurrence. Additional studies are needed to determine appropriate surveillance methods because retrospective studies are complicated by the ability of hVISA to revert back to vancomycin-susceptible *S. aureus* (VSSA) and VISA to revert back to hVISA.

hVISA and VISA Laboratory Detection and Interpretation

Further discussion of hVISA and VISA require that clinical and microbiologic definitions are addressed. In 2006, the Clinical and Laboratory Standards Institute (CLSI) lowered vancomycin minimum inhibitory concentration (MIC) breakpoints for *S. aureus*.[24] The CLSI breakpoints by broth microdilution (BMD) currently define vancomycin susceptibility as an MIC ≤ 2 μg/mL, vancomycin-intermediate susceptibility as an MIC of 4 to 8 μg/mL, and vancomycin resistance as an MIC of ≥16 μg/mL (**Table 1**).[25] Vancomycin MIC breakpoints were lowered in an effort to increase detection of potentially heterogeneous-intermediate isolates because of reported associations between vancomycin treatment failure and *S. aureus* isolates with MICs ≥ 4 μg/mL.[7,8,25] Heteroresistance refers to the presence of less susceptible subsets within a larger population of fully antimicrobial-susceptible microorganisms.[5] When tested using routine methods, hVISA isolates are susceptible to vancomycin (MIC ≤2 μg/mL) but contain subpopulations that express reduced vancomycin susceptibility (MIC ≥ 4 μg/mL).[11]

Detection of hVISA is a great challenge in clinical microbiology laboratories because reliable and practical methods are not currently available for routine use. Heteroresistant subpopulations are present in low frequencies (≥1 x 10^6) and can grow in higher vancomycin concentrations than the MIC predicts. Such small populations may not be detected by the inocula (5 x 10^5 CFU/mL) used in standard CLSI microbiology methods. As a result, hVISA isolates are likely undetected in clinical laboratories that use traditional MIC testing methodology.[13] Population analysis profiling with area under the curve (PAP-AUC) is the current reference standard method for confirming hVISA and is the most reliable and reproducible test. However PAP-AUC is labor-intensive, time consuming (3 to 5 days), and costly for use in clinical microbiology laboratories.[17,19,26] Consequently, several screening methods have been developed, such as glycopeptide resistance detection (GRD), marcromethod E-test (MET) and brain heart infusion (BHI) screen agar plates (**Table 2**).[27-29] However, none of these tests have the same degree of sensitivity and specificity as the PAP-AUC test, with issues of reproducibility and variability, in reporting results.[19] Until a suitable hVISA detection method becomes available for use in clinical microbiology laboratories, routine testing is not currently recommended.[2] Currently, clinical screening for hVISA isolates in high-risk patients is favored **(Table 3)**, particularly in patients who do not respond to vancomycin. Further research is warranted to develop a detection method that is practical, cost-effective, and reliable for routine use in clinical settings.

Non-automated MIC methods for the detection of VISA are recommended by the Centers for Disease Prevention and Control (CDC).[30] Acceptable non-automated MIC methods for detecting VISA include BMD per CLSI, agar dilution, and Etest (0.5 McFarland). [30] Though automated methods and vancomycin screen agar plates can be useful in the detection of VISA isolates with a vancomycin MIC of 8 μg/mL, sensitivity levels have not been determined for *S. aureus* with vancomycin MICs of 4 μg/mL.[30] In these situations, a second method, such as BMD per CLSI criteria, should be used to confirm VISA isolates.[30]

Current susceptibility testing methods do not consistently distinguish between MICs of 1 and 2 μg/mL.[2,31] Therefore, laboratory results should indicate the methodology used, because vancomycin MIC results will differ between methods and may alter treatment decisions.[11] In comparison to the CLSI BMD method, automated detection methods, particularly Phoenix system and Vitek, tend to underestimate the MIC, while E-test and MicroScan (prompt method) may overestimate the MIC.[31] Precision of these methods is clinically important as higher vancomycin MICs (> 1.5 μg/mL) are associated with poorer outcomes (e.g., increased mortality, recurrence, delayed response, treatment failure, prolonged hospitalization), particularly in high inoculum infections and with a higher proportion of hVISA presence.[25,32] Alternative therapies should be considered for patients receiving vancomycin therapy who are persistently bacteremic (≥ 7 days) or who have no clinical improvement despite source control with an MIC of ≥ 1.5 μg/mL by Etest.[2,31,32]

Resistance Mechanisms of hVISA and VISA

Evidence suggests that hVISA and VISA arise during continued or sub-optimal exposure to vancomycin.[7,33] The proposed mechanism is selective pressure by vancomycin resulting in the development of rare vancomycin-resistant clones that progress to hVISA and, with continued-exposure, to a uniform population of VISA clones.[5,9] These isolates have significant differences in cell physiology, including morphologic changes and genetic alterations. Strains of hVISA and VISA are characterized by thicker cell walls that correlate with increased vancomycin MICs.[34] Cell wall thickening impairs intracellular penetration of vancomycin rendering it ineffective.[5,34] In addition, hVISA and VISA are associated with slower growth rates than fully susceptible strains, which may contribute to persistent and recurrent infections.[35] Other mechanisms of resistance include alterations in transcriptional and metabolic genes and loss-of-function mutations that disturb critical cell wall biosynthesis.[11] The accessory gene regulator (*agr*) operon directs many critical virulence pathways, particularly the production of exotoxins.[11] In hVISA and VISA strains, *agr* function is reduced, favoring the development of vancomycin resistance and potentially promoting biofilm production that ultimately enhances the survival of hVISA and VISA.[33,36,37]

Risk Factors and Outcomes Associated with hVISA and VISA

Heteroresistance has been reported in MRSA isolates with MICs as low as 0.5 µg/mL and in cases where vancomycin was minimally effective.[6,16] Several studies have noted an increase in vancomycin treatment failures and mortality with vancomycin susceptible MRSA strains, particularly those with MICs of 1.5 or 2 µg/mL.[25,32,38-40] A recent meta-analysis of 20 studies evaluated high versus low vancomycin MICs ($\geq$ 1.5 µg/mL vs $<$ 1.5 µg/mL, respectively) on clinical outcomes in adults with MRSA infections.[40] An increased risk of failure was observed in the high MIC group compared to the low MIC group (relative risk [RR], 1.40; 95% confidence interval [CI], 1.15 – 1.71). There was also a greater risk of overall mortality (RR, 1.45; 95% CI,1.08-1.87) in the high MIC group. Although the investigators attempted to exclude hVISA isolates, hVISA presence was not tested in every study, which may have contributed to vancomycin treatment responses. While most of the isolates were from blood, clinical heterogeneity cannot be excluded. Another study evaluated 559 MRSA isolates and found an increased incidence of hVISA when the vancomycin MIC shifted from 1 to 2 µg/mL.[41] The incidence of hVISA was nearly 40% in isolates with an MIC of 2 µg/mL, supporting the results of other studies that suggest the proportion of hVISA isolates are directly related to increases in vancomycin MIC.[6,15,23,41] Increases in vancomycin MICs are hospital specific and perhaps caused by clonal outbreaks. However, this highlights the trends of vancomycin tolerance, which may be caused by overuse of vancomycin, sub-therapeutic vancomycin concentrations, high bacterial load, or slow vancomycin bactericidal activity.[3,42]

Both hVISA and VISA have been identified in hospital and community strains of MRSA and in MSSA.[16] The findings of studies that evaluated clinical predictors and outcomes of hVISA infections are inconsistent. This may be attributed to the considerable heterogeneity of these studies, including differences in study design, clinical definitions, selection of isolates (initial isolate, final isolate, or random selection), patient populations, and testing methodologies. Commonly reported associations with hVISA infections include vancomycin treatment failure and high-inoculum MRSA infections (e.g., bacteremia, infective endocarditis, osteomyelitis, deep abscesses, and prosthetic device infections).[6,7,14,33,43,44] Other potential predictors of hVISA and VISA infections are prior MRSA infection or colonization (previous 3 months), previous vancomycin exposure (prior 6 months), initial low serum vancomycin trough levels (< 10 µg/mL), persistent bacteremia (≥ 7 days), and presence of indwelling devices **(Table 2).**[7,8,12,14,44,45 46]

Patients with hVISA infections tend to experience prolonged clinical courses, suboptimal response to vancomycin therapy, and prolonged hospital stays.[6-8,14,33,42,44] One retrospective case-control study compared the clinical features and outcomes of hVISA bacteremia (n = 27) and MRSA bacteremia (n = 223).[14] Compared with MRSA bacteremia, patients with hVISA infections had significantly more days of bacteremia (median duration, 12 days vs. 2 days, respectively; P = 0.005) and significantly higher rates of endocarditis (18.5% vs. 3.6%, respectively; P = 0.007) and osteomyelitis (25.9% vs. 7.2%, respectively; P = 0.006).[14] Of note, patients in the hVISA group had significantly more prosthetic/implant devices (e.g., artificial heart valves, pacemakers, or orthopedic implants) and surgical site infections (in the previous month) at baseline, which may have attributed to poorer outcomes. In a small case series, glycopeptide treatment failure, (defined as a positive *S. aureus* blood culture after ≥ 7 days of glycopeptide therapy or a sterile site culture positive for *S. aureus* after ≥ 21 days of glycopeptide therapy) occurred in 19 of 25 (76%) patients with hVISA infections (bacteremia, endocarditis, osteomyelitis, or septic arthritis).[8]

A retrospective, multicenter, matched cohort study compared the outcomes of hVISA versus vancomycin susceptible-MRSA (VS-MRSA) bloodstream infections (BSI) and found similar results.[6] Study investigators concluded that rates of vancomycin treatment failure were 11 times higher for a patient with hVISA BSI (50/61, 82%) than VS-MRSA BSI (20/61, 32.8%; $P < 0.001$). Patients with hVISA BSI were also more likely than patients with VS-MRSA BSI to have persistent bacteremia (59% vs. 21.3%, respectively; $P < 0.001$), infection recurrence at 60 days (25.5% vs. 1.9%, respectively; $P < 0.001$), and longer hospital length of stay (median in days, 24 vs. 16, respectively; $P = 0.022$). While differences in 30-day MRSA infection-related mortality and all-cause 30-day mortality were not observed between the hVISA BSI group and VS-MRSA BSI group (21.3% vs. 9.8%; $P = 0.081$ and 24.6% vs. 11.5%; $P = 0.076$, respectively). Similarly, no other studies have been powered to detect a significant difference in mortality between hVISA and non-hVISA infections. A recent systematic review and meta-analysis evaluated 30-day mortality from eight comparative hVISA studies.[18] After combining the data, 30-day mortality between hVISA and VSSA infections were similar (OR, 1.18; 95% CI, 0.81-1.74).[18] However, these findings may be limited by the variability in definitions used and the predominately retrospective designs of the original studies. While the lack of association between hVISA and mortality can be partly explained by strain characteristics (e.g., decreased virulence) and host immune responses, sufficiently sized studies are needed to accurately determine if such an association exits.[47]

Infections caused by VISA may also lead to recurrent infections, prolonged fevers and bacteremia, vancomycin treatment failure, and increased hospital stay.[7,12,33,44] In a single–center, retrospective study, 6 patients with VISA had a significantly longer duration of bacteremia compared to 22 with hVISA (12.1 ± 13.1 days vs. 3.3 ± 3.9 days, respectively; $P = 0.001$).[43] Significant differences in mortality between VISA and hVISA were not observed. However, rates of attributable mortality between hVISA and VSSA ($n = 215$) were similar (9.1% vs. 8.4%, respectively) while those between VISA and VSSA (33.3% vs. 8.4%) were not.[43] Although this study had several limitations including a small sample size and bias through selective inclusion of isolates, the findings suggest that VISA may have more severe clinical implications and impact on patient outcomes. To date, no other published study has evaluated the outcomes of VISA infections, possibly because of the rarity of VISA infections.

Treatment Options for hVISA/VISA Infections

Although reports of vancomycin failure have emerged, no data demonstrate superior outcomes with alternative antimicrobials. Alternative antimicrobial agents with activity against hVISA/VISA include daptomycin, linezolid, ceftaroline, trimethoprim/sulfamethoxazole, tigecycline, quinupristin/dalfopristin, and the combination of vancomycin or daptomycin with a beta-lactam.[12]

Daptomycin

Daptomycin is a potential treatment option for hVISA and VISA infections and, although it does have activity against MRSA, previous vancomycin exposure can result in some degree of cross-resistance to daptomycin.[48,49] Several studies have noted an *in vitro* association between increasing vancomycin MICs and increasing daptomycin non-susceptibility.[48-50] The highest rate of daptomycin non-susceptibility was reported in a study evaluating 47 Australian hVISA and VISA isolates never exposed to daptomycin.[50] The investigators noted daptomycin non-susceptibly in 15% of hVISA and 38% of VISA strains.[50] Because bactericidal activity with daptomycin is concentration dependent, higher doses may be necessary to treat hVISA and VISA infections with elevated daptomycin MICs, high inoculum infections (e.g., endocarditis), and infection sites characterized by poor antimicrobial penetration.[51] High-dose daptomycin may prevent the selection or development of isolates with reduced susceptibility to daptomycin and subsequent treatment failure.[51]

An *in vitro* study observed more rapid reduction of bacterial burden of hVISA and VISA in simulated endocardial vegetations with high-dose daptomycin (10 mg/kg/day for 8 days) and dose de-escalation (10 mg/kg/day for 4 days followed by 6 mg/kg/day for 4 days) regimens compared to that of the standard (6 mg/kg/day for 8 days) and dose escalation (6 mg/kg/day for 4 days followed by 10 mg/kg/day for 4 days) regimens.[51] With respect to hVISA, the dose de-escalation regimen had a significantly increased killing effect on the hVISA strain compared to the dose escalation regimen ($P < 0.024$).[51] The investigators concluded that these daptomycin dosing approaches may lead to a faster cure of bacteremia *in vivo* and prevent the emergence of daptomycin non-susceptibility.[51] However, no *in vivo* studies evaluating de-escalation dosing and the appropriate duration of high-dose daptomycin have been published. The role of high-dose daptomycin alone in patients with hVISA or VISA infections is unclear. Until more evidence is available, caution is required when considering daptomycin in patients who may be at risk for hVISA or VISA infections (e.g. high-bacterial load infections, vancomycin failure). The determination of daptomycin susceptibility in these patients may also guide therapeutic decision making.

<u>Linezolid</u>

The role of linezolid for the treatment of invasive hVISA and VISA infections is also in question. Successful use of linezolid alone or in combination with other antimicrobial agents has been described in several case reports of vancomycin heteroresistant and intermediate MRSA endocarditis and bacteremias after vancomycin failure and in some cases after daptomycin failure.[8,52-55] In one case report, a 60 year old male with an automatic implantable cardioverter-defibrillator (AICD) presented with bacteremia and endocarditis initially caused by MRSA which later developed into hVISA, then daptomycin non-susceptible VISA after exposure to vancomycin and daptomycin.[55] The patient initially received 6 weeks of vancomycin (trough concentrations between ≥ 15 μg/mL and ≤ 21 μg/mL), followed by approximately 25 days of daptomycin (6 mg/kg every 48 hours, renal dose adjusted). During therapy with daptomycin the defibrillator generator and leads were removed however, the patient was persistently bacteremic and febrile. Blood cultures cleared after therapy was switched to linezolid and trimethoprim/sulfamethoxazole.

The patient received at least 28 days of the combination and 6 weeks of linezolid monotherapy in total since the last positive blood culture. One year post-treatment the patient had no infection recurrence. After failing vancomycin and daptomycin therapy, this patient's VISA infection was successfully treated with linezolid. While other case reports have shown similar outcomes with the use of linezolid, *in vitro* studies have not shown the same efficacy.[56] Evidence to recommend the use of linezolid for hVISA and VISA is insufficient. Further study is needed to evaluate linezolid alone or in combination for hVISA and VISA infections.

Ceftaroline

Ceftaroline has potent *in vitro* bactericidal activity against MRSA including hVISA, VISA, and daptomycin non-susceptible (DNS) MRSA strains.[57] The use of ceftaroline in the treatment of invasive infections (e.g., endocarditis, bacteremia, osteomyelitis) caused by hVISA, VISA, and DNS MRSA is supported by data from *in vivo* animal studies and human case reports.[58-61] In a recent case series report, a patient with DNS VISA bacteremia and endocarditis was successfully treated with 6 weeks of ceftaroline. The patient initially received and failed vancomycin therapy.[62] Blood cultures cleared within 48 hours of switching to daptomycin (6 mg/kg/day). However, subsequent blood cultures were positive and revealed DNS VISA. Daptomycin was discontinued, and ceftaroline (600 mg IV every 8 hours) was initiated. While on ceftaroline, blood cultures cleared within 48 hours and remained sterile. *In vitro* pharmacokinetic/pharmacodynamic studies reported enhanced ceftaroline activity against hVISA, VISA, and DNS MRSA as vancomycin and daptomycin susceptibilities decreased, which have been referred to as the "seesaw effect".[58-60] While further study is needed, ceftaroline appears to be a safe and effective alternative in the treatment of invasive hVISA, VISA, and DNS MRSA infections given its bactericidal activity, favorable safety profile, and emerging data.

Combination therapy

The combination of vancomycin or daptomycin and a beta-lactam antimicrobial has also been studied for treatment of hVISA and VISA infections. Beta-lactams that have been evaluated for synergistic activity with vancomycin or daptomycin include ceftaroline, cefazolin, and piperacillin-tazobactam.[63-66] *In vitro* and clinical case report data evaluating the combination of high-dose daptomycin (10 mg/kg/day) and trimethoprim/sulfamethoxazole also appear promising for the treatment of hVISA, VISA, and DNS MRSA infections.[67,68] *In vitro* studies have demonstrated improved kill rates with these antimicrobial combinations.[63-65] Investigators hypothesize that beta-lactam exposure may influence vancomycin-cell wall interactions to improve vancomycin activity, although further investigation is warranted.[63] In summary, preliminary experimental studies show possible prospects for the treatment of hVISA and VISA infections. However, it is not yet clear which treatment options correlate with optimal clinical outcomes for patients with confirmed hVISA or VISA infections.

Infection Control: Preventing the Dissemination of hVISA/VISA

As with MRSA, hVISA and VISA can colonize humans and the environment despite eradication efforts. The CDC has made several recommendations in an attempt to prevent the emergence of vancomycin non-susceptible infections.[42] Infections with confirmed VISA should be reported to infection-control personnel, the patient's primary caregiver, medical ward staff, local and state departments of health, and the CDC. Patients and their caregivers should be educated regarding wound care, physical hygiene, and signs of infection.[69] Contact isolation in both the inpatient and outpatient setting may also limit further emergence. Adherence to recommended infection prevention and control guidelines, appropriate antibiotic prescribing through antimicrobial stewardship programs, and active surveillance in a cohesive health care system are essential to prevent further emergence of hVISA and VISA colonization and infection.

Conclusions

The evolution of *S. aureus* to MRSA and now to hVISA and VISA is an important and ongoing public health concern. Vancomycin is the drug of choice for invasive MRSA infections, however, its use is under question. Over-use, suboptimal concentrations, or inappropriate use of vancomycin is speculated to be a major contributor in the emergence of hVISA and VISA. Most alarming are the poor outcomes that have been associated with hVISA and VISA infections and the limited antimicrobials available to treat these infections. Proper detection methods are necessary for accurate surveillance, guidance on therapeutic decision-making, and a full understanding of the implications of hVISA/VISA infections. Until then, patients who are at risk for hVISA/VISA infections and failing vancomycin therapy may warrant further confirmatory testing for hVISA/VISA. Based on currently available data, clinicians should, with vigilance, continue to use vancomycin per the Infectious Diseases Society of America guidelines.[2,3] Alternative therapies should be considered in patients with risk factors for hVISA/VISA who are not responding clinically to vancomycin despite source control and a vancomycin MIC ≤ 2 μg/mL. In patients infected with VISA (vancomycin MIC 4 – 8 μg/mL), an alternative antimicrobial should be considered. Caution is advised when deciding to use daptomycin in patients with hVISA/VISA infections because of the potential for cross-resistance. To prevent further resistance, appropriate use of antimicrobials and implementation of infection-control guidelines are imperative.

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Table 1. CLSI susceptibility definitions for vancomycin[24,25]

	2006 CLSI Update MIC	Previous CLSI Breakpoints MIC
VSSA	≤ 2 μg/mL[a]	≤ 4 μg/mL
VISA	4 – 8 μg/mL	8 – 16 μg/mL
VRSA	≥ 16 μg/mL	≥ 32 μg/mL

CLSI = Clinical and Laboratory Standards Institute; MIC = minimum inhibitory concentration;
VISA = vancomycin intermediate *S. aureus*; VRSA = vancomycin resistant *S. aureus*; VSSA = vancomycin susceptible *S. aureus;*
[a] May contain heteroresistant intermediate susceptible subpopulations with MIC > 4 μg/mL. Heteroresistant vancomycin intermediate *S. aureus* (hVISA) isolates are not identified by CLSI and can occur at vancomycin MICs as low as 0.5 μg/mL.

Table 2. Advantages and disadvantages of laboratory detection methods for hVISA

Confirmatory Methods		
Method	**Advantages**	**Disadvantages**
PAP[4,11,13,26,70]	• Considered the "gold standard" • High reproducibility and accurate detection • Definitive confirmation: Modified PAP	• No data to show superiority to other techniques • High labor intensity • High-cost • Long turn-around time
Screening Methods		
Method	**Advantages**	**Disadvantages**
GRD E-test (AB Biodisk)[17,19,27]	• Results ready to read following 24 hours of incubation • Uses standard bacterial inoculum	• Unreliable specificity and sensitivity
MET or High inoculum method[11,29]	• 100% reproducibility • Easily performed	• Testing performed on nonstandard media while utilizing a standard McFarland suspension • Results of MET are cut-off points, not true MICs
BHI screen agar plates[7,17,28]	• Easily performed	• Poor reproducibility • Many variations; some studies screened with a different agar, inoculum size, or used suspensions with higher bacterial concentration

BHI = Brain Heart Infusion; GRD = Glycopeptide Resistance Detection; MET = Macromethod E-Test; MIC = minimum inhibitory concentration; PAP = Population Analysis Profiling

Table 3. Predictors and outcomes of hVISA and VISA

Predictors	Outcomes
• Previous vancomycin use • Prior MRSA infection or colonization • High bacterial load infections[a] • Persistent bacteremia • Initally low serum vancomycin levels (<10 μg /mL) • Presence of indwelling devices	• Long duration of bacteremia, days • Persistent fever • Recurrent infections • Vancomycin treatment failure • Prolonged hospitalization

hVISA = heteroresistant vancomycin intermediate *S. aureus*; MRSA = methicillin resistant *S. aureus*; VISA = vancomycin intermediate *S. aureus*

[a] E.g.bacteremia, endocarditis, osteomyelitis, deep abscess, or prosthetic joint infection