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Review Article

VANCOMYCIN INTERMEDIATE AND VANCOMYCIN RESISTANT *STAPHYLOCOCCUS AUREUS* - MECHANISMS, CLINICAL SIGNIFICANCE, AND DETECTION

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ABSTRACT

Vancomycin is used as the antibiotic of choice for severe infection caused by methicillin-resistant *Staphylococcus aureus*. Increased use of vancomycin and the selective pressure has resulted in the emergence of *S. aureus* with reduced susceptibility to vancomycin and vancomycin-resistant *S. aureus*. This review summarizes the definition, mechanism, clinical significance, and epidemiology of *S. aureus* with reduced susceptibility to vancomycin. It also discusses laboratory methods for detection and treatment options available for these pathogens.

Keywords: Vancomycin, Vancomycin intermediate *Staphylococcus aureus*, Heteroresistant vancomycin intermediate *Staphylococcus aureus*, Vancomycin resistant *Staphylococcus aureus*.

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INTRODUCTION

Staphylococcus aureus is a major cause of variety of infections in health-care setting and community [1]. It causes a variety of clinical conditions ranging from asymptomatic colonization to different kinds of infections ranging from superficial skin infection to severe infection such as sepsis [1]. This pathogen has the unique ability to overcome unfavorable conditions such as desiccation, heat, and tolerance to high salt concentrations [2]. Resistance to penicillin among *S. aureus* leads to the introduction of methicillin, a semi-synthetic antibiotic. But in 1960s appearance of methicillin-resistant *S. aureus* (MRSA) was reported, which was also resistant to carbapenems, cephalosporins and all beta-lactam antibiotics [3,4]. MRSA over the time has evolved and become multidrug resistant hence the rate of mortality and morbidity has also increased simultaneously [5]. Due to limited therapeutic options vancomycin, a glycopeptide was considered as the drug of choice for severe infections due to MRSA.

As the prevalence of MRSA increased worldwide so did the use of vancomycin for its treatment, hence it was just a matter of time when appearance of *S. aureus* with reduced susceptibility to vancomycin was observed. Vancomycin was approved in 1958 by the US Food and Drug Administration. Approximately, after 40 years, in 1997 first case of infection by *S. aureus* with reduced susceptibility to vancomycin was documented in Japan [6]. Soon several countries reported similar cases of infection due to these mutated pathogens [7-9]. The first case of vancomycin-resistant *S. aureus* (VRSA) was reported from the USA in 2002 [10]. Several other reports of isolated cases of VRSA infection have also been documented over the years [10]. Isolation of heterogeneous vancomycin – intermediate *S.aureus* (hVISA) created further problem in the existing crisis of vancomycin treatment as the rate of vancomycin treatment failure for these isolates was higher, and also detection of this pathogen was difficult [8].

Due to severity and extent of infections caused by *S. aureus* with lowered susceptibility to vancomycin, its isolation is a matter of great concern in the medical society. Furthermore, treatment of *S. aureus* with reduced susceptibility to vancomycin is difficult as the alternative treatment is expensive and toxic. Hence, rapid identification and proper treatment are required to reduce the morbidity and mortality in patients infected with these pathogens. This article summarizes the information available about *S. aureus* with reduced susceptibility to vancomycin.

DEFINITIONS

The Clinical and Laboratory Standards Institute (CLSI) recommended tests such as broth dilution and agar dilution are used to determine minimum inhibitory concentration (MIC) of vancomycin to *S. aureus*. The results are then interpreted, and *S. aureus* isolates are classified as vancomycin-susceptible *S. aureus* (VSSA), VISA, and VRSA [11].

The definitions of VISA and VRSA are clear as their definitions are based on the value of MICs obtained by standard CLSI procedures. Heterogeneous VISA (hVISA) definition, on the other hand, is not yet clearly defined as a standardized method for determination of its MIC is not yet approved.

VISA

In 2006, due to increase in vancomycin treatment failure CLSI revised the vancomycin breakpoint. According to recent CLSI guidelines MIC of vancomycin was changed from ≤4 μg/ml to ≤2 μg/ml and the isolate having this MIC is considered as VSSA while for VISA the MIC of vancomycin which was initially 8-16 μg/ml was revised to 4-8 μg/ml [12]. Vancomycin MIC results differ based on the methods used, therefore CLSI recommended broth macro or microdilution should be performed before identifying the isolate as VISA [13].

VRSA

The definition for VRSA generates slight confusion because of different cutoff values used in different countries to classify vancomycin susceptibility. CLSI has revised the vancomycin MIC for defining VRSA according to which instead of a MIC value of ≥32 μg/ml, isolates with a MIC of ≥16 μg/ml are considered as VRSA [12]. In the United States and several other countries which uses CLSI guidelines, the above MIC value is used for classifying VRSA. *S. aureus* with MIC of vancomycin ≥6 μg/ml was described in 2002 in Michigan and in New York in 2004 [11].

Heterogeneous vancomycin-intermediate *S. aureus* (hVISA)

The definition of hVISA has not been clearly stated as an approved standardized method for the detection of this pathogen is not yet

available. These isolates have two population of cell; a major part of the population is susceptible to vancomycin (MIC ≤2 µg/ml) while a minor portion is resistant and has a MIC of 8 µg/ml. The resistant population is present in a very low rate of 10^{-5}-10^{-6} hence they escape detection by the routine recommended CLSI method where an inoculum of 5×10^4 CFU/well in case of broth dilution or 1×10^4 CFU/spot in case of agar dilution is used [12,14]. Population analysis profile (PAP) is used for the detection of hVISA even when *S. aureus* strains with a vancomycin MIC as less as 0.5-1 µg/ml is isolated [15].

Table 1 summarizes classification of *S. aureus* based on vancomycin MIC results obtained by CLSI recommended method.

MECHANISM OF VANCOMYCIN RESISTANCE

Vancomycin acts by binding to D-alanyl-D-alanine (D-ala-D-ala) located at C-terminus of late peptidoglycan precursors. It forms a stable, noncovalent complex with the cell wall precursor thus making it unavailable for cell wall synthesis in *S. aureus* [16]. The precursors for cell wall synthesis are located at the tip of division septum making it a major site for cell wall division as the whole cell membrane is not involved in the synthesis of *S. aureus* cell wall [16]. Therefore, it is required for vancomycin to diffuse to the tip of division septum so as to prevent cell wall synthesis in *S. aureus* and any mechanism which prevents either diffusion or binding of vancomycin results in reduced susceptibility to this antibiotic [16].

VISA is believed to arise from hVISA strain after prolonged exposure to glycopeptides [13,17]. Increase in the thickness of cell wall has been attributed to decreased vancomycin susceptibility. Mutation and/or modulation of regulatory systems results in changes in its cellular physiology; the cell wall metabolism is enhanced leading to increased production of D-ala-D-ala residues. More murein monomers and layers of peptidoglycan increase the thickness of cell wall. Thus, vancomycin gets entrapped in the outermost layer of cell wall and the amount of vancomycin reaching the target site is greatly reduced [18]. This mechanism is known as "affinity trapping" [18]. The entrapped vancomycin destroys the outer peptidoglycan layer and blocks the movement of vancomycin to the inner part of cell wall resulting in "clogging phenomenon" [18]. Binding of vancomycin to cell wall results in reduced autolytic activity by blocking the activity of peptidoglycan hydrolase enzyme (an enzyme responsible for shedding the old outer layer of peptidoglycan) [19]. VISA and hVISA strains also show a reduced acetate catabolism which results in alteration in growth pattern, increased production of intercellular adhesion, and change in apoptosis as well as increases antibiotic tolerance [19].

Agr operon has been identified as a significant factor which helps in reducing vancomycin susceptibility [20]. Isogenic mutation in *agr* group II polymorphism leads to increase in heteroresistance to glycopeptides in *S. aureus* [20]. Genetic makeup of VISA includes mutations frequently associated with *walKR*, *vraSR*, *rpoB*, *yvqF/vraSR* genes [11,21]. These genes in most cases are directly or indirectly involved with either synthesis or metabolism of cell wall in *S. aureus* [21]. Fig. 1 shows the difference in the cell wall thickness of *S. aureus* ATCC 25923 and Mu50 as seen under electron microscope [20].

Mechanism of resistance in VRSA is similar to that of vancomycin-resistant Enterococci (VRE) [22]. Transposon Tn1546, an 11-kb mobile genetic element results in vancomycin resistance [22]. It belongs to Tn3 family of transposons and codes 9 polypeptides ORF1 and ORF2 results in transposition, vanR and vanS responsible for expression of vancomycin resistance, vanH and vanA synthesize modified peptidoglycan precursors which ends in D-lactate (D-lac), vanX and vanY brings about hydrolysis of normal precursors and vanZ, the function of which is yet unknown. vanR/vanS regulate inducible expression of vanA resistance. In the cytoplasmic domain of vanS, histidine residue is present [22]. This histidine residue gets phosphorylated in the presence of glycopeptides and in turn activates the aspartate residue present in vanR by phosphorylating it too [22]. The phosphorylated vanA binds to P_{RES} promoter and activates cotranscription of *vanH*, *vanA*, *vanX*, and *vanY* genes [22]. Binding of vanA to P_{REG} promoter leads to activation of *vanR* and *vanS* [22]. Vancomycin resistance can result by two genetic pathways [22]. Either by plasmid transfer through conjugation from *Enterococcus* species to *S. aureus* or by transposition through insertion of Tn1546 from donor (*Enterococcus* species) to a resident plasmid or chromosome present in the recipient (*S. aureus*) [22]. Some enterococcal plasmid replicate successfully in Staphylococci, but others may be lost during cell division (Fig. 2) [22].

The inserted Tn1546 vanA type resistance element produces D-ala-D-lac in place of D-ala-D-ala which has low affinity for vancomycin thus resulting in vancomycin resistance [23].

Table 1: Interpretation of vancomycin susceptibility in *S. aureus*

MIC (µg/ml) of vancomycin as per CLSI recommended broth microdilution	Interpretation	Classification
≤2	Susceptible	VSSA
4-8	Intermediate	VISA
≥16	Resistant	VRSA

VSSA: Vancomycin susceptible *Staphylococcus aureus*, MIC: Minimum inhibitory concentration, *S. aureus: Staphylococcus aureus,* VISA: Vancomycin intermediate *Staphylococcus aureus,* VRSA: Vancomycin resistant *Staphylococcus aureus*

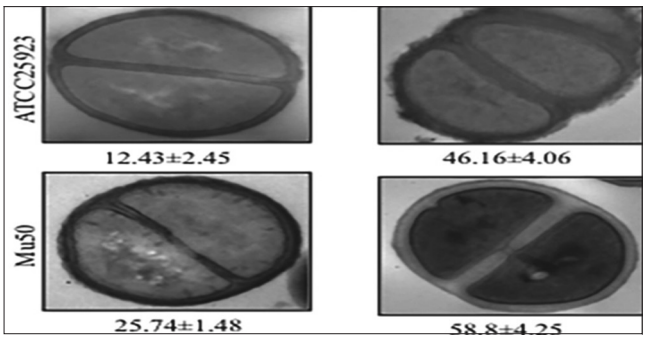

Fig. 1: Difference in cell wall thickness of *Staphylococcus aureus* ATCC 25923 and Mu50 in the presence (right side) and absence of vancomycin (left side) after cultivation in BHI broth. Mean and standard deviation of the cell wall thickness in nanometers is mentioned below each cell (Figure was adapted from reference 20 with approval of author)

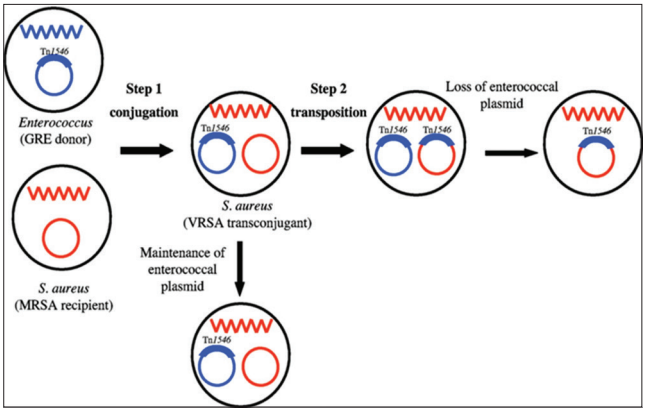

Fig. 2: Genetic pathways for Tn1564 transfer from *Enterococcus* species to *Staphylococcus aureus* (Glycopeptide resistant *Enterococcus*) (Adapted from reference 22 with due permission from the author)

EPIDEMIOLOGY OF *S. AUREUS* WITH REDUCED SUSCEPTIBILITY TO VANCOMYCIN

A 4 months infant from Japan underwent heart surgery in 1996 [6]. 2 weeks after surgery the surgical site was found to produce purulent discharge. Mu50 was isolated from pus culture with vancomycin MIC of 8 mg/l thereby giving world the first published case of VISA in 1997 [6]. hVISA was reported from sputum of a 64 years male patient suffering with pneumonia in Japan, 1997 [24]. Soon after isolation of hVISA and VISA from Japan, cases of infection due to *S. aureus* with reduced susceptibility to vancomycin was reported from several parts of the world [25]. Japan, United States, Australia, France, Brazil, Scotland, South Korea, Hong Kong, South Africa, Thailand, and Israel are some of the many which have reported infection due to hVISA and VISA [16,26]. The rate of VISA varies from 0.04% to 44.9% in Asian countries, in America a rate of 0-28.6% has been recorded while a rate of 0.07-31.7% has been seen in European countries [25,27,28]. A systematic review was conducted by Zhang *et al*. which included data from Asia, Europe, Australia, and America from studies published from 1997 to 2014; these studies revealed that the rate of hVISA has gradually increased from 4.68% to 7.01% over the years. Similarly, the rate of VISA has also increased and the rate of VISA which was initially 2.05% has increased to 7.93% at present [27]. According to this study, the rate of VISA was 3.42% in Asia and 2.75% in Europe/America while hVISA had a rate of 6.81% in Asia and 5.60% in Europe/America. The first case of hVISA from Australia was reported in 2001 and since then increased number of hVISA and VISA has been reported from this country. A study conducted in Australia showed the rate of VISA isolates to be 1.7% [29]. 33 vanA positive VRSA cases have been documented worldwide till date [30]. In India, VISA has been reported from Hyderabad, Pondicherry, Chandigarh, Mangaluru, and Varanasi [31-35]. Investigators have reported 7 VRSA isolates from Hyderabad health-care settings [31]. A study conducted in Bhubaneswar reported 28.86% of VRSA and 45.11% VISA from nosocomial sources while in ICU and NICU the rate of VRSA and VISA was 16.80% and 45.17% respectively [28].

LABORATORY DIAGNOSIS

Vancomycin resistance may go undetected in routine antibiotic susceptibility testing [25]. Disk diffusion test which used 30 µg vancomycin disk was not sensitive for the detection of VISA strains and often misclassified VISA as VSSA; it also failed to detect hVISA and hence was considered as an inappropriate method for determining vancomycin susceptibility [25]. Genetic determinants for detection of hVISA and VISA have not yet been defined. Phenotypic methods are unable to provide accurate detection of hVISA and in some cases also for VISA isolates [25]. However, several methods for screening and confirming hVISA and VISA in the clinical specimen are acknowledged.

Colony morphology of hVISA and VISA on conventional agar plate may provide subtle information about its presence. Growth kinetics of hVISA is supposed to be different from that of standard *S. aureus* culture [16]. hVISA isolates produce small-sized colony or mixed colony variants [16]. Different size, pigmentation, hemolysis, and slow growth rate of colonies in the same pure culture obtained from the same clinical specimen may indicate a possibility of the presence of hVISA or VISA variants [16,35]. However, these changes are not diagnostic and each different monotype should be tested for vancomycin susceptibility with a confirmatory test.

Screening tests for hVISA

hVISA infection has a low proportion of vancomycin intermediate population (10^{-5}-10^{-6}). Hence, the standard inoculum (McFarland 0.5 standard) used for CLSI broth or agar dilution for MIC determination fails to detect this subpopulation. Therefore, detection of hVISA requires higher inoculum size, longer incubation period or more nutritious media to facilitate its growth.

Macro method Etest uses bacterial inoculum equal to 2 McFarland on brain heart infusion agar (BHIA) and an incubation period of 48 hrs. Both teicoplanin and vancomycin E strips are used on separate plates. The test is considered positive if teicoplanin MIC is ≥12 µg/ml or if both teicoplanin and vancomycin MIC is ≥8 µg/ml. The result of this test is just a cutoff value, and hence the actual MIC value cannot be reported [16]. Another screening test is the glycopeptides resistance detection Etest (GRD Etest). Here, vancomycin and teicoplanin are present on the same E strip and concentration of both ranges from 0.5 to 32 µg/ml. Standard inoculum (McFarland 0.5 standard) and Mueller-Hinton Agar supplemented with 5% blood is used. The initial result can be read after 24 hrs and final result after 48 hrs. GRD Etest is considered positive if either vancomycin or teicoplanin has a MIC of ≥8 µg/ml [16].

Simplified population analysis described by Hiramatsu *et al*. involves the use of BHIA with 4µg of vancomycin per ml (BHIA-V4) [24]. The plate is inoculated with 10 µl of 10^8 CFU/ml of bacterial suspension. Isolates growing at 24 hrs and 48 hrs were considered as potential VISA and hVISA, respectively. If the strains produced subcolonies which had vancomycin MIC 8 µg/ml and remained resistant for >9 days on antibiotic-free medium then such isolates were considered as confirmed hVISA [24].

Confirmatory test for hVISA

The reproducibility of simplified population analysis was poor and hence Wootton *et al*. described a modified PAP method [36]. Accordingly, BHIA plates are prepared with different concentrations of vancomycin (0.5 µg/ml, 1 µg/ml, 2 µg/ml, 2.5 µg/ml and 4 µg/ml). Bacterial suspension is prepared using isolates grown in trypticase soya broth for 24 hrs. This suspension is then diluted to 10^{-3} and 10^{-6} using saline and used for inoculation of BHIA gradient plates. The plates are incubated for 48 hrs at 37°C after which colonies grown on the plates are counted. PAP/area under the curve (AUC) is calculated by dividing AUC of test organism (MRSA) by corresponding AUC for Mu3. If PAP/AUC ratio is <0.9, 0.9-1.3 and >1.3 then the isolate is considered as VSSA, hVISA and VISA [37]. Using GraphPad Prism software viable count is plotted against vancomycin concentration and AUC is then calculated (Fig. 3) [25].

Detection of VISA and VRSA

VISA and VRSA are relatively easy to identify due to the presence of recommended and standardized CLSI testing methods [12]. Broth macrodilution or agar dilution method can be used for determination of vancomycin MIC among test isolates. *S. aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 are used as the quality control in these tests [12]. Other methods such as E test and automated tests such

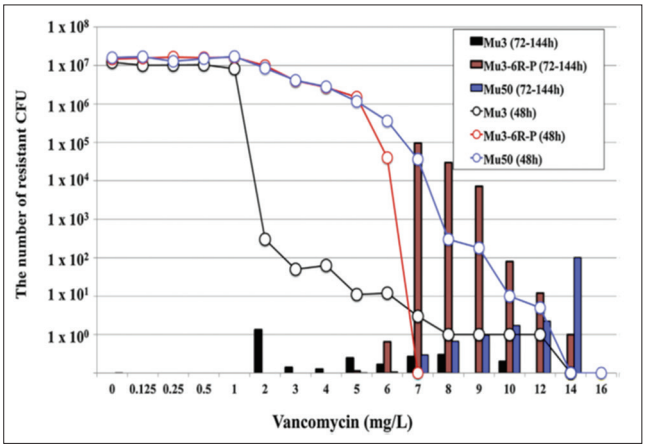

Fig. 3: Population analysis curve of vancomycin-intermediate *Staphylococcus aureus* (vancomycin intermediate *S. aureus* [VISA]; Mu50), hetero-VISA (hVISA; Mu3) and "slow VISA" (sVISA, a laboratory strain derived from Mu3; Mu3-6R-P). Bars show the number of colonies which appeared on each agar plate after 72 hrs up to 144 hrs of incubation (adapted from reference 37 with permission from the author)

as Vitek, Phoenix, and MicroScan rapid panels are used in diagnostic laboratories for determination of vancomycin susceptibility [38-40]. These methods though are easy, less labor intensive and quick compared to the recommended CLSI methods; they have their own drawbacks and produces a variable result which is not 100% reproducible [38,39]. Hence, vancomycin MIC results determined by any other MIC method should always be confirmed by CLSI reference method [12,41]. According to CLSI, VISA and VRSA are reported when vancomycin MIC is 4-8 μg/ml and ≥16 μg/ml, respectively [12,41]. The laboratory methods for detection of hVISA, VISA and VRSA is summarized in Table 2.

CLINICAL SIGNIFICANCE OF HVISA, VISA, AND VRSA

Significance of hVISA and VISA in the clinical setting remains unclear. Treatment failure in case of infection by these strains is whether due to its virulence or level of resistance has yet to be investigated.

β-lactam antibiotics are considered superior to vancomycin by several clinicians for treating bacteremia and endocarditis caused by *S. aureus*. Patients with such infection fail vancomycin therapy even if the isolate causing the infection is susceptible to the antibiotic when tested [42].

Animal studies have been conducted to ascertain the efficacy of vancomycin when used against hVISA and VISA. A rabbit endocarditis model harboring Mu50 showed vancomycin treatment failure [16]. The presence of high inoculum of hVISA and VISA *in vitro* models has shown decreased activity of vancomycin [43]. A rabbit endocarditis model was used which has been infected with clinical strain of MRSA derived from an endocarditis patient with vancomycin treatment failure. PAP identified the isolate as hVISA which persisted even after vancomycin therapy [44].

Several clinical studies around the world suggest treatment failure and existence of infection even after vancomycin therapy when this antibiotic is used for the treatment of infection caused by hVISA/VISA [16]. The first case of VISA reported from Japan showed vancomycin to be ineffective in the treatment of infection when used alone and relapse of infection occurred. The infant was cured after the therapy had been changed from vancomycin to arbekacin and ampicillin sulbactam [8]. From the United States, cases of vancomycin treatment failure have been documented starting from 1999. Blood which was collected from a patient undergoing renal dialysis gave a positive culture of MRSA having vancomycin MIC of 2 μg/ml [45]. Later just before patient's death MRSA which was isolated had vancomycin MIC of 8 μg/ml [45]. In this incidence, it was not clear if treatment failure was primarily due to increasing vancomycin MIC level in the isolate or due to secondary adaptation to the antibiotic as a deep-seated MRSA focus present in the patient went undetected and was not removed [45]. Two other cases were reported from the United States in which patients were infected with VISA (MIC 8-16 μg/ml) [46]. These patients had invasive MRSA infection which was either persistent or recurrent. In Spain 19 cases of MRSA having vancomycin MIC <4 μg/ml were noted [47]. Metallic implant was present in 14 of these patients and vancomycin treatment failed in 12 of these patients. In the remaining five, only one underwent vancomycin treatment failure [47].

About 30 days of vancomycin treatment failed to eradicate MRSA from four burns and one osteomyelitis case reported from Brazil [48]. Another case of vancomycin treatment failure was recorded in an endocarditis patient where MRSA had a vancomycin MIC of 4-8 μg/ml [49]. The patient failed to respond to 43 μg/ml trough level of vancomycin but responded immediately to linezolid. Long vancomycin exposure of about 6-18 weeks, 3-6 month before VISA infection has been suggested as an important contributing factor for emergence of VISA [47,49]. Studies have shown that this phenotype arises from pre-existing MRSA strain which had caused infection months before in the patient [48,50].

In June 2002, the first case of VRSA was reported in a 40 years diabetic female patient with chronic renal failure from Michigan, United States. The patient had received several courses of antibiotic treatment for chronic foot ulcer in the past 15 years with vancomycin being included in some of these treatments. In April 2002, before isolation of VRSA patient was treated with vancomycin for MRSA bacteremia. VRSA (MIC >128 μg/ml) was isolated from a catheter site infection and a swab culture from infected foot ulcer. VRSA isolate contained *vanA* gene and *mecA* gene. The patient was treated with trimethoprim/sulfamethoxazole and wound care [46]. Second VRSA case was reported in September 2002 from a 70 years male patient undergoing treatment for chronic plantar ulcer. The patient had multiple lower extremities ulcer and osteomyelitis [51]. Before developing VRSA infection, MRSA and VRE were frequently isolated from the same site. Main difference in this case compared to the first VRSA infection was that the patient had no prior exposure to vancomycin except in 1997 when he received vancomycin-

Table 2: Methods for detection of hVISA, VISA and VRSA in laboratory

Media used	Inoculum	Reference
Detection of hVISA		
Screening tests		
BHIA+vancomycin 6 μg/ml	10 μl of McFarland 0.5 standard suspension	[16]
MHA+vancomycin 5 μg/ml	10 μl of McFarland 0.5 standard suspension	[16,59]
MHA+teicoplanin 5 μg/ml	10 μl of McFarland 2 standard suspension	[16,59]
Simplified PAP: BHIA+4 μg/ml	10 μl of McFarland 0.5 standard suspension	[16,59]
MET: BHIA	McFarland 2 standard suspension	[16,59]
GRD Etest: MHA with 5% blood	McFarland 0.5 standard suspension	[16]
Confirmatory test		
Modified PAP: BHIA+vancomycin (0.5 μg/ml, 1 μg/ml, 2 μg/ml, 2.5 μg/ml, 4 μg/ml)	Culture incubated in TSB for 24 hrs, then diluted to 10^{-3} and 10^{-6} and used for plating	[37]
Detection of VISA and VRSA		
Screening test		
Etest	McFarland 0.5 standard suspension	[16,39,40]
Vancomycin screen agar: BHIA+vancomycin 6 μg/ml	10 μl of McFarland 0.5 standard suspension	[12]
Confirmatory test		
CLSI recommended broth microdilution	McFarland 0.5 standard suspension diluted to obtain 5×10^4 CFU/well	[12]
CLSI recommended agar dilution method	McFarland 0.5 standard suspension adjusted to obtain 10^4 CFU/spot of 5-8 mm diameter	[12]

BHIA: Brain heart infusion agar, MHA: Mueller hinton agar, MET: Macromethod Etest, GRD Etest: Glycopeptides resistance detection Etest, PAP: Population analysis profile, TSB: Trypticase soya broth, VISA: Vancomycin intermediate *Staphylococcus aureus,* VRSA: Vancomycin resistant *Staphylococcus aureus*

impregnated beads for 5 days. This case thus demonstrated that prior prolonged vancomycin exposure need not necessarily resulted in emergence of VRSA; even frequent use of other antibiotics may create selective pressure resulting in a favorable growing site for both MRSA and VRE together [51]. This facilitates horizontal *vanA* gene transfer from VRE to MRSA resulting in emergence of VRSA. Till date, 13 confirmed VRSA cases had been documented from the United States. All the patients had a history of prior infection with *Enterococcus* and *S. aureus* at the same time and at the same site, also most of VRSA infected patients had received prior vancomycin treatment [16]. Although vancomycin is routinely used in the treatment of MRSA, only a few cases of vancomycin resistance has been reported from another part of the world [30].

A previous study has shown that the chances of vancomycin treatment failure were eleven times more in patients with hVISA bloodstream infection than a patient with VSSA bloodstream infection [52]. Patients with hVISA and VISA infections have a longer duration of hospital stay, recurrent infections, longer treatment regime and while in hVISA the response to vancomycin is suboptimal, vancomycin therapy fails in cases of VISA infections [53].

TREATMENT OPTIONS AVAILABLE FOR *S. AUREUS* WITH REDUCED SUSCEPTIBILITY TO VANCOMYCIN

Emergence of MRSA itself had narrowed the treatment choices available for this pathogen. Emergence of hVISA, VISA and VRSA further created a problem in antibiotics selection. Combination therapy with antibiotics which have synergistic action should be considered for the effective treatment of hVISA, VISA, and VRSA.

hVISA, VISA, and VRSA have been usually isolated from invasive infections such as endocarditis, bacteremia, deep-seated abscess, osteomyelitis, and prosthetic device related infections. About 31% of these cases were treated by the use of antibiotic alone, whereas 69% of cases required surgical debridement along with antibiotic usage for effective therapy [50].

ANTIBIOTICS AVAILABLE FOR TREATMENT OF HVISA, VISA AND VRSA INFECTIONS

Ampicillin when used in presence of sulbactam for the treatment of first case of VISA in Japan in combination with arbekacin was found to be a treatment worth consideration for treatment of VISA infection [8]. Rifampicin and fusidic acid combination have been used for the treatment of complicated MRSA infection. This combination has been successfully used in the treatment of MRSA infection where vancomycin therapy has failed [50].

Linezolid, a synthetic oxazolidinone inhibits protein synthesis at 50S ribosome. It is effectively used in treatment of skin and soft tissue infection and also for healthcare-associated pneumonia. Although linezolid is bacteriostatic *in vitro* against *S. aureus*, it has effectively cured several serious infections due to MRSA, hVISA, VISA and VRSA [50].

Daptomycin, a lipopeptide class of antibiotic has bactericidal activity which is dependent on its concentration [52]. It is effective in the treatment of bacteremia, endocarditis and skin and soft tissue infections [52]. Mutations in mprF and yycG which leads to reduced vancomycin susceptibility in some *S. aureus* strains has also been linked with reduced susceptibility to daptomycin [50]. Hence, an association between hVISA and VISA and increased MIC of daptomycin has been seen. This association is strain specific and not stable [54].

Quinupristin/dalfopristin, a streptogramin is used in the treatment of invasive infection where vancomycin treatment has failed as an intravenous preparation [50,55].

Tigecycline, a member of tetracycline group of antibiotic shows good *in vitro* activity for some of the VISA strains tested [54].

PROMISING ANTIBIOTICS UNDER DEVELOPMENT

Dalbavancin has good activity against MRSA, hVISA, VISA and VRSA. It is also effective for *S. aureus* resistant tolinezolid and quinupristin/dalfopristin. Half life of this antibiotic is long and hence 1 dose/week is sufficient to maintain serum level [56]. Oritavancin with structure almost similar to vancomycin is effective against VISA and VRSA [16]. Telavancin has low MIC for MSSA, MRSA and VISA strains but higher MIC for VRSA [16].

New cephalosporins are also being tested which shows promising results for effective treatment of hVISA and VISA. Ceftaroline in animal studies has been useful for the treatment of MRSA infection and was found to be equal or superior to vancomycin, linezolid, teicoplanin and arbekacin [57]. Doripenem, ranbozolid, telavancin, and iclaprim are some of the other promising antibiotics which can be considered in the treatment of infection where vancomycin therapy fails [58].

CONCLUSION

The worldwide increase in the rate of multidrug resistance MRSA infections, especially in health-care settings, during the past several decades has resulted in the frequent use of vancomycin to treat such infections. This increased selective pressure has resulted in the emergence of MRSA strains with reduced susceptibility to vancomycin (VISA) in 1997 and then MRSA strains with high-level resistance to vancomycin (VRSA) in 2002. Mutations in determinants that control biosynthesis of cell wall and/or mutation in ribosomal gene rpoB results in VISA. In case of VRSA, high-level resistance to vancomycin is due to the acquisition of copies of transposon Tn1546 from VRE through plasmid. VISA strains may develop *in vivo* during treatment with vancomycin VISA and VRSA strains may be detected using CLSI recommended broth dilution, agar dilution or Etest. However, detection of hVISA is normally difficult. PAP can be used for this purpose. Although VISA strains have been isolated from health-care settings, VRSA still is rare.

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