

Vancomycin Resistance in Staphylococci

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INTRODUCTION

The glycopeptide antibiotic vancomycin was introduced clinically in 1958 for the treatment of gram-positive bacteria. Use of this agent has increased dramatically in the last 20 years, in large part because of the increasing prevalence of methicillin resistance in both coagulase-negative staphylococci and *Staphylococcus aureus* (21). Data from the December 2000 report of the National Nosocomial Infection Surveillance (NNIS) System indicated that about 75% of coagulase-negative staphylococci and 47% of *S. aureus* isolates from intensive care units were resistant to methicillin (www.cdc.gov/ncidod/hip/NNIS/DEC2000sar.PDF). Vancomycin remains the drug of choice for these infections.

Vancomycin resistance among staphylococci was developed in laboratories even before the drug was in use clinically (27, 83). However, this resistance was so difficult to induce that many felt it would be unlikely to occur in a clinical setting (42). That no vancomycin-resistant staphylococci were reported in the first 20 years the drug was used only strengthened this assumption. Unfortunately, this confidence was shattered by the first reports of vancomycin resistance in coagulase-negative staphylococci in 1979 and 1983 (61, 74). Though a cause for concern, these reports did not generate a great deal of attention as coagulase-negative staphylococci are generally considered to be relatively avirulent organisms. A very different response greeted the first report of decreased susceptibility to

vancomycin in *S. aureus* in 1997 (13, 35). This report was quickly followed by similar ones from other countries, including the United States (67). Given the well-known virulence of *S. aureus*, the isolation of these organisms generated enormous concern in the medical community and has prompted a flurry of activity aimed at limiting their emergence.

This review will serve as a general overview of vancomycin resistance in staphylococcal species. However, since vancomycin resistance in *S. aureus* is the major problem from both a clinical and public health standpoint, it will serve as the focus of the article.

Definition of Vancomycin Resistance

Unfortunately, confusion over the definitions of vancomycin resistance has been generated by recent literature. The source of this confusion seems to be the different breakpoints in vancomycin susceptibilities used in the various countries where vancomycin-resistant staphylococci have been reported. In the United States, the National Committee for Clinical Laboratory Standards (NCCLS) guidelines should be followed. NCCLS guidelines define staphylococci for which the MIC of vancomycin is ≤ 4 $\mu\text{g/ml}$ to be susceptible, while isolates for which the MIC is 8 to 16 $\mu\text{g/ml}$ are intermediate and those for which the MIC is ≥ 32 $\mu\text{g/ml}$ are resistant (45). Japan, however, considers some isolates for which the MIC is 8 $\mu\text{g/ml}$ to be resistant (68); as a result, some isolates reported as resistant in Japan have been reclassified as intermediate in the United States.

Confusion with respect to vancomycin resistance in staphylococci is also engendered by use of the term “heteroresistant staphylococci.” This phenomenon, which is seen in both coag-

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ulase-negative staphylococci and *S. aureus*, refers to the variability of vancomycin susceptibilities among subpopulations of single isolate. A heteroresistant isolate contains two populations of cells, a majority population that is susceptible to vancomycin and a minority population that is resistant. Heteroresistance is likely more common than pure resistance or diminished susceptibility, as evidenced by the fact that it was found in up to 20% of *S. aureus* isolates in one hospital in Japan (34). A similar study from the United States also found heteroresistant populations to be more common than homogenous populations with reduced susceptibilities; however, the overall incidence was much lower, with only 2 of 630 isolates (0.3%) demonstrating heteroresistance and none showing true reduced susceptibility to vancomycin (36).

The clinical significance of heteroresistance is not fully understood. Although one study (82) did show that patients who were infected with heteroresistant strains did have higher mortality rates than patients infected with sensitive isolates, it is difficult to conclusively determine impact based only on one small, retrospective study. Given the uncertain clinical significance and the difficulty and expense in detecting heteroresistance, there does not appear to be any role for screening outside of research studies (68). If screening is done and heteroresistant isolates are encountered, the MIC for the susceptible, parent strain and not that of the resistant subpopulation should be documented in the patient's record (36, 69).

Laboratory Detection of Vancomycin Resistance

Vancomycin resistance can be difficult to detect in the clinical microbiology laboratory. Disk diffusion sensitivity testing using the standard 30- μ g vancomycin disk frequently misclassifies intermediately susceptible isolates as fully susceptible (70). In a recent study, 75% of microbiology laboratories from around the world misreported a glycopeptide-intermediate strain of *Staphylococcus epidermidis* as susceptible based on the results of disk diffusion testing (71).

Automated testing methods like MicroScan rapid panels (Dade Behring) and Vitek (version 7.07; bioMerieux) also have pitfalls. While conventional MicroScan panels performed well in detecting reduced susceptibilities to vancomycin, the rapid panels are less reliable as they do not allow for the recommended 24-h incubation (14, 70). Prior to 1999, Vitek software was not programmed to report vancomycin MICs above 4 μ g/ml and would thus report the MICs of intermediately susceptible or resistant isolates as 4 μ g/ml; a software upgrade in 1999 appears to have corrected this problem (14). At this time, however, nonautomated MIC determinations by broth or agar dilution or by E test are the "gold standard" for determining vancomycin susceptibility (69, 70).

Variation and irreproducibility among quantitative methods can be attributed in large part to difficulties encountered in attempting to detect resistance in a heterogeneous population of susceptible and resistant cells. Since vancomycin resistance has not been a homogenous characteristic of the majority of staphylococci that have been examined, agar-based susceptibility test methods, such as agar dilution and the agar diffusion E test, may be preferred, as they are more sensitive for detecting resistant subpopulations within a strain. However, this detection requires sufficient incubation time for expression of the

resistance determinant and subsequent detectable growth. This usually translates into extended incubation times, i.e., a full 24 to 48 h, and precludes the use of the popular, rapid susceptibility methods (43). Another advantage of the agar methods is the fact that single colonies growing at higher drug concentrations can be visualized on solid media earlier and more readily than in broth-based systems. Whatever method is employed, it should be noted that inconsistencies have been reported even between gold standard testing methods for detecting vancomycin resistance in staphylococci (18, 20).

Further, even the most-sensitive techniques for determining vancomycin susceptibility are still vulnerable to the problems of inoculum size recently reported by Dunne et al. (20). NCCLS standards for susceptibility testing recommend an inoculum density of 5×10^5 CFU/ml for performance of standardized susceptibility testing, while vancomycin-intermediate subpopulations can occur at frequencies ranging from 10^{-6} to 10^{-7} CFU/ml. This raises the possibility that resistant subpopulations may be missed at the time of initial testing due to sampling error.

Though not helpful in MIC determinations, the use of selective agents or growth conditions, such as increased salt concentration or decreased temperature of incubation, to enhance the growth and expression of the resistant subpopulations appears to hold promise as a screening method for detecting vancomycin resistance. Screening isolates for growth on vancomycin-containing media appears to be a sensitive way to detect even low levels of vancomycin resistance. Commercially prepared agar media appear to be more specific, as susceptible isolates will occasionally grow on media prepared in-house, although in-house media appear to be equally sensitive (36, 70). Wong et al. have also described a sensitive system for detecting glycopeptide-intermediate or -resistant subpopulations of staphylococci, which utilizes increased NaCl concentrations (2 to 4%) and the monobactam aztreonam as an inducing agent (82).

The Centers for Disease Control and Prevention (CDC) has published recommendations to guide vancomycin susceptibility testing of *S. aureus* isolates (12, 15). These recommendations state that (i) primary testing of *S. aureus* requires at least 24 h of incubation, (ii) susceptibility determination with disk diffusion is not an acceptable method, and (iii) an MIC testing method should be used to confirm vancomycin susceptibility (15). Any *S. aureus* isolate for which the MIC is ≥ 4 μ g/ml should be sent to the CDC for confirmatory testing.

Unfortunately, there are currently no official recommendations for screening isolates of coagulase-negative staphylococci for reduced susceptibility to vancomycin. At one point, a double zone of growth around an imipenem disk on a disk diffusion test was thought to indicate some degree of vancomycin resistance in coagulase-negative staphylococci (58). However, subsequent studies have revealed this method to be unreliable (33).

There is an obvious need for simple and accurate phenotypic screening and confirmatory tests for glycopeptide resistance in staphylococci. Unfortunately, such tests will require the elucidation of the molecular and biochemical mechanisms of resistance for validation. Until this occurs, clinical microbiologists should carefully pursue the evaluation of staphylococci for which the MICs of vancomycin initially appear to be elevated, particularly those from patients who are not responding to

appropriate glycopeptide therapy or who have risk factors for the development of vancomycin-intermediate or -resistant staphylococci.

Infection Control Issues

The epidemiologic importance of vancomycin resistance has brought a great deal of attention to infection control issues regarding the spread of vancomycin-resistant staphylococci. The infection control community is understandably nervous, given the rapid dissemination of vancomycin-resistant enterococci that has occurred in hospitals around the world (11).

It has been established that *S. aureus* can be transmitted between patients by several routes, including environmental surfaces (9), hands of health care workers (44), and nasal shedding (8). Though less is known about the transmission of coagulase-negative staphylococci, there have been well-documented outbreaks of these organisms, and it is assumed that they are transmitted by many of the same routes as *S. aureus* (7, 41, 49). It is also assumed that vancomycin-resistant staphylococci can be transmitted by the same mechanisms as their sensitive counterparts. Indeed, there has been one reported outbreak of vancomycin-intermediate *S. aureus* (VISA) in a French hospital among patients who had not received any glycopeptide antibiotics and whose primary risk for acquiring the organism was environmental exposure (50).

Given the enormous public health concerns regarding the dissemination of these organisms, the Hospital Infection Control Practices Advisory Committee (HICPAC) of the CDC published infection control guidelines for all staphylococci for which the MIC of vancomycin is ≥ 8 $\mu\text{g/ml}$ in 1997 (12). There were those, however, who felt the HICPAC guidelines did not go far enough, and a more elaborate set of infection control guidelines was published by a group from the Medical College of Virginia in 1998 (81). The similarities and differences between these two sets of guidelines are shown in Table 1. In addition to the various precautions listed in the table, both groups emphasize that vancomycin use must be limited to situations where it is deemed appropriate by current CDC guidelines (11).

The results of contact tracing and culturing have been reported for three of the VISA cases in this country (67; L. Conway, T. Ross, M. O'Brien, J. Dick, and T. Perl, Abstr. 11th Annu. Meet. Soc. Healthcare Epidemiol. Am., abstr. 48, 2001). In each of these cases, the patients were already isolated on methicillin-resistant *S. aureus* (MRSA) precautions, and the HICPAC guidelines for VISA isolation were implemented as soon as the reduced vancomycin susceptibility was known. There was no transmission of VISA to any of the 285 contacts that were tested. However, in one case, a family member was colonized with the MRSA strain that later developed reduced vancomycin susceptibility in the patient (Conway et al., 11th Annu. Meet. Soc. Healthcare Epidemiol. Am.).

VANCOMYCIN RESISTANCE IN COAGULASE-NEGATIVE STAPHYLOCOCCI

Epidemiology of Vancomycin Resistance

Resistance to vancomycin among coagulase-negative staphylococci was first reported more than 20 years ago (61). How-

TABLE 1. Infection control guidelines for vancomycin-resistant staphylococci^a

Precaution	MCV ^b	HICPAC
Private room	Yes	Yes
Gloves	Yes	Yes
Gowns to enter room	Yes	No
Gowns for patient contact	Yes	Yes
Antibacterial hand washing agent	Yes	Yes
Record of all health care workers entering room	Yes	No
Mask and/or eye protection from aerosols	Yes	Yes
Mupirocin for nasal colonization	Yes	No
Limit number of health care workers caring for patient	Yes	Yes
Nares cultures for all health care workers who entered room	Yes	Yes
Health care workers at high risk for staphylococcal colonization should not care for patient	Yes	No
Isolation for duration of hospital stay	Yes	No
Environmental cultures after terminal room cleaning	Yes	Optional
Isolation on readmission until nares and previously infected, open sites are staphylococcus negative	Yes	No
Close unit to new admissions if nosocomial transmission occurs	Yes	No
All specimens carried to laboratory (no pneumatic tubes)	Yes	No
When possible, postpone tests that require patient to leave room	Yes	No
Post staff at doorway to ensure compliance with precautions	Yes	No

^a Adapted from reference 81 with permission of the publisher.

^b MCV, Medical College of Virginia.

ever, the first report of a clinically significant isolate was in 1987 (59). Since that time, there have been at least five other case reports of clinically relevant coagulase-negative staphylococci that had diminished susceptibility to vancomycin (3, 20, 38, 57, 78).

Since the initial report of reduced susceptibility to vancomycin in coagulase-negative staphylococci, there have been at least 20 studies that have screened large numbers of isolates in an attempt to define the prevalence of this problem. Eleven of these studies did not find any isolates of coagulase-negative staphylococci with reduced vancomycin susceptibility (4, 5, 22, 31, 39, 52, 65, 72, 75, 77, 79), and the nine that did are described in more detail in Table 2. From the studies, it appears that the incidence of these organisms is very low. The notable exception is the study by Froggatt et al., in which 42% of *Staphylococcus haemolyticus* isolates were intermediately resistant (MIC ≥ 6.25 $\mu\text{g/ml}$) to vancomycin. It is unclear from the study why this institution had such a high prevalence of vancomycin resistance. Potential explanations include high vancomycin use in the months preceding the survey, the fact that these isolates were all part of an outbreak, or the possibility that the investigators used a more sensitive technique for detecting vancomycin resistance. Unfortunately, none of these factors was addressed in the study. Nonetheless, the report is a cause for alarm, as it indicates that these organisms can become endemic in a health care setting.

TABLE 2. Summary of in vitro studies that examined reduced susceptibility to vancomycin among coagulase-negative staphylococci

Reference	Location	Study design	No. of isolates tested	% of isolates for which MIC of vancomycin was >4 µg/ml (resistant species [if reported])	Susceptibility testing method(s)
Henwood et al. (32)	England	Multicenter	769	0.5	E test
Santos Sanches et al. (56)	Worldwide	Multicenter	1,351	0.07	
Luh et al. (40)	Taiwan	Single center	405	5.0 (<i>S. epidermidis</i> , <i>S. haemolyticus</i> , <i>S. simulans</i> , and others)	Agar dilution
de Neeling et al. (19)	The Netherlands	Multicenter	7,334	0.4%	Agar dilution
Felmingham et al. (23)	Europe	Multicenter	1,444	0.2 (<i>S. haemolyticus</i>)	Agar dilution
Gruneberg et al. (29)	Europe	Multicenter	1,480	0.03	Agar dilution
Herwaldt et al. (33)	Iowa	Single center	28	0.04 (<i>S. haemolyticus</i>)	Agar and broth dilution
Goldstein et al. (28)	France	Single center	362	0.03	Agar dilution
Froggatt et al. (25)	Virginia	Single center	70	42 (<i>S. haemolyticus</i>)	Agar dilution

Risk Factors for Vancomycin Resistance

Because of the small number of cases, determining risk factors for the development of reduced vancomycin susceptibility among coagulase-negative staphylococci is difficult. Thus far, all resistant strains have been recovered from patients in acute-care hospitals. There was no common underlying illness in all the cases, although two of the five patients were on peritoneal dialysis. Exposure to a glycopeptide antibiotic would certainly appear to play an important role, as four of the five reported patients received at least 30 days of vancomycin before a less-susceptible isolate was recovered. The fifth patient had received a prolonged course of teicoplanin (a glycopeptide antibiotic not available in the United States) before the *S. haemolyticus* developed intermediate resistance to vancomycin. Though not a risk factor for developing vancomycin resistance, it should be noted that all of these isolates were resistant to multiple other antibiotics, including methicillin, quinolones, cephalosporins, and macrolides.

Species Variation in Vancomycin Susceptibility

There may be species differences in the coagulase-negative staphylococci with respect to vancomycin susceptibility. Despite a few case reports of intermediate susceptibility (26, 38, 57), almost all *S. epidermidis* isolates, which represent 60 to 90% of clinical isolates (47), remain sensitive to vancomycin. This relatively uniform susceptibility has been demonstrated in the in vitro prevalence studies in Table 2, in which only one group reported *S. epidermidis* isolates with reduced susceptibility to vancomycin (40). This finding seems to be supported in the laboratory, where some investigators have reported that vancomycin resistance in *S. epidermidis* has been difficult to induce (1, 59).

Although still unusual, reduced susceptibility to vancomycin may be more common in *S. haemolyticus*, as indicated in the prevalence studies in Table 2. Initially, this higher incidence of reduced susceptibility in *S. haemolyticus* was supported by in vitro studies in which researchers attempted to induce vancomycin resistance in several coagulase-negative staphylococcal species but were successful only with isolates of *S. haemolyticus* (58, 59). However, in another study, vancomycin resistance was induced with similar frequencies in both *S. haemolyticus* and *S. epidermidis* (33). Given the mixed results of these in vitro studies and the small numbers of resistant isolates detected in

the prevalence studies, it is impossible to say if the increased frequency of reduced susceptibility in *S. haemolyticus* is truly a species phenomenon. Only one prevalence study (40) has reported reduced vancomycin susceptibility among species of coagulase-negative staphylococci other than *S. epidermidis* and *S. haemolyticus*. Thus, commenting on other potential species differences is simply not possible at this time.

Mechanisms of Vancomycin Resistance

The true mechanism of vancomycin resistance in these species, though currently unknown, is a topic of active investigation. Vancomycin acts by binding irreversibly to the terminal D-alanyl-D-alanine of cell wall disaccharide-pentapeptide precursors, thereby inhibiting production of the bacterial cell wall. Enterococci have developed vancomycin resistance by replacing the terminal alanine with a lactate, which has a greatly reduced affinity for vancomycin (10). Studies of vancomycin-resistant, coagulase-negative staphylococci have shown that these altered cell wall precursors are produced, but in amounts that are likely too small to account for the degree of resistance observed (6). Analysis of the cell wall peptidoglycans from highly resistant coagulase-negative staphylococci has demonstrated the presence of altered cross-links compared to susceptible strains (6). It has been suggested that these altered cross-links may inhibit vancomycin binding to target peptides, but this hypothesis has yet to be proven. Vancomycin resistance in coagulase-negative staphylococci is likely multifactorial, but the exact mechanisms await elucidation.

Treatment Options for Coagulase-Negative Staphylococci with Reduced Susceptibility to Vancomycin

Given the paucity of cases, there are no formal recommendations regarding the treatment of infections with coagulase-negative staphylococci with reduced susceptibility to vancomycin. In the cases reported to date, the infections were successfully treated by the addition of rifampin to vancomycin in one case (59) and by changes in therapy to rifampin and fusidic acid in one case (3) and to erythromycin in another (57).

Recently, two new agents to treat gram-positive infections have been approved for use in this country, the streptogramin quinupristin-dalfopristin and the oxazolidinone linezolid. Unfortunately, there are few in vitro and no in vivo data on the

TABLE 3. VISA cases in the United States

State and yr (reference)	Source	Underlying illness(es)	Vancomycin exposure (wk)
Michigan, 1997 (67)	Peritoneal fluid	Renal failure, MRSA peritonitis, cancer	18
New Jersey, 1997 (67)	Blood	Acute renal failure, MRSA bacteremia	18
New York, 1998 (53)	Blood	Renal failure, MRSA bacteremia	6
Illinois, 1999 (15)	Blood	Renal failure, MRSA endocarditis	3.5
Minnesota, 2000 (24)	Blood	Renal failure, MRSA osteomyelitis	18
Nevada, 2000 (24)	Abscess fluid	Complicated cholecystectomy with polymicrobial intrahepatic abscess (including MRSA)	10
Maryland, 2000	Blood	MRSA endocarditis, psoriasis, sleep apnea	14

effectiveness of these new agents in treating infections caused by coagulase-negative staphylococci with reduced susceptibility to vancomycin. A study from Taiwan (40) found high rates of resistance (16%) to quinupristin-dalfopristin among coagulase-negative staphylococci, suggesting that it would not be a good option for vancomycin-resistant isolates in that country. However, the long-standing use of the streptogramin virginiamycin in animal husbandry in Taiwan is likely to have contributed significantly to the quinupristin-dalfopristin resistance seen in this study. Indeed, studies in other countries (2, 79) have reported excellent activity of quinupristin-dalfopristin against coagulase-negative staphylococci, although no vancomycin-resistant strains were tested. Likewise, linezolid has also shown excellent activity against coagulase-negative staphylococci, with no resistance reported to date (32, 37, 54). One of these studies (32) did test a few isolates that had reduced susceptibility to vancomycin.

VANCOMYCIN RESISTANCE IN *S. AUREUS*

Introduction

Since first being reported in 1997, the threat of vancomycin resistance in *S. aureus* has been the topic of intense research and discussion. Although vancomycin resistance in *S. aureus* remains extremely rare and is less common than vancomycin resistance in enterococci or even coagulase-negative staphylococci, there is widespread concern that vancomycin-resistant *S. aureus* (VRSA) poses, by far, the greatest risk to patients, given the virulence of the organism. Though there have been only a few reports of *S. aureus* isolates with reduced susceptibility to vancomycin, the high prevalence of MRSA and vancomycin use, both thought to be risk factors for VRSA, make the widespread dissemination of these organisms an alarmingly realistic possibility (48). Furthermore, there is the equally alarming threat of the risk of transmission of these organisms between patients.

Epidemiology of Vancomycin Resistance in *S. aureus*

To date, there have been no verified clinical isolates of *S. aureus* that were truly resistant to vancomycin by the NCCLS standards. Instead, the organisms have had intermediate susceptibility, which has led to the term "vancomycin intermediate *S. aureus*" or "VISA." The term "glycopeptide intermediate *S. aureus*" or "GISA" is synonymous, but because vancomycin is the only glycopeptide used in this country, most American physicians are more familiar with the acronym VISA.

VISA isolates were first found in nature more than 15 years ago while investigators were screening isolates for vancomycin susceptibility (80). However, it was not until 1995 that the first clinical isolate was reported, which was from a French child who had been receiving vancomycin for an MRSA line infection (51). In 1996, a wound infection caused by VISA was reported in Japan in a child receiving vancomycin for an MRSA wound infection (35). The following year, the first VISA isolate was reported in the United States from Michigan. Since then, there have been at least seven confirmed cases of VISA from around the country (Table 3).

Interestingly, unlike the case for coagulase-negative staphylococci, it does not appear that there are undetected isolates of VISA in hospital microbiology laboratories. Several studies that have screened *S. aureus* isolates for reduced susceptibility to vancomycin have found none (19, 22, 23, 29, 31, 35, 39, 72, 75).

Risk Factors for Vancomycin Resistance

As is the case with coagulase-negative staphylococci, the relative rarity of decreased vancomycin susceptibility in *S. aureus* makes risk factors difficult to ascertain. Exposure to vancomycin (or other glycopeptide antibiotics) again stands out as a strong risk as every patient in this country who developed a VISA isolate had been on vancomycin therapy for some period of time, though the duration did vary widely, from just a few weeks to several months. Prior infection caused by MRSA would also appear to be a strong risk, as no known cases of VISA have developed from methicillin-susceptible strains. The relative risk posed individually by vancomycin exposure and MRSA infection is difficult to determine, as they tend to go hand-in-hand in most cases. Renal failure appears to be a significant risk factor, as it was present in five of the seven cases from the United States. Again, whether renal failure itself is a risk or merely serves to increase the risk of MRSA infection and vancomycin exposure is unknown.

Mechanisms of Vancomycin Resistance

The true mechanism of vancomycin resistance in *S. aureus* is not known. It was initially feared that *S. aureus* would acquire the *van* genes that code for vancomycin resistance in *Enterococcus* species, especially after this transfer was successfully accomplished in the laboratory (46). Further, vancomycin-resistant *Enterococcus faecalis* emits a sex pheromone that promotes plasmid transfer, and it has been recently demonstrated

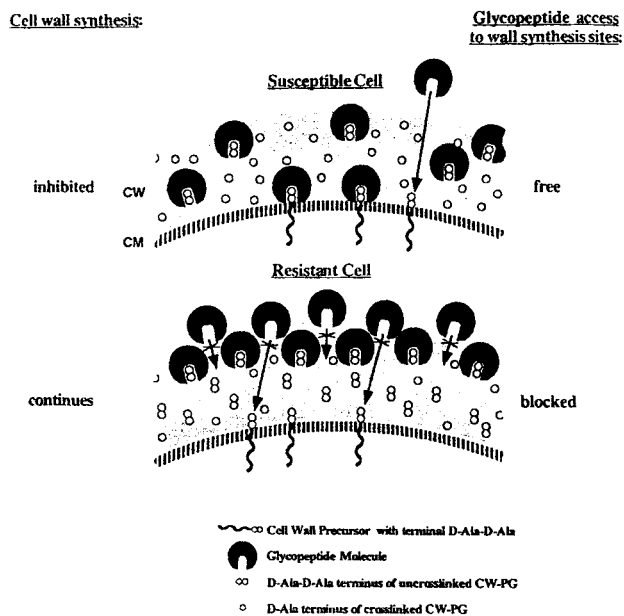


FIG. 1. Proposed model for the capture of vancomycin in the cell wall of VISA organisms. Reprinted from reference 62 with permission of the publisher.

that this same pheromone is produced by *S. aureus*. Emission of this pheromone by *S. aureus* organisms that are in proximity to vancomycin-resistant enterococci that contain plasmids encoding *van* genes could result in transfer of these resistance genes (60). However, thus far, neither the *van* genes nor their altered peptidoglycan products have been recovered in vancomycin-intermediate or resistant *S. aureus* isolates. Instead, it appears that vancomycin resistance in *S. aureus* is conferred by other alterations in the bacterial cell wall.

Several years prior to the first clinical VISA isolate being reported, Daum et al. produced laboratory strains of VISA and VRSA that had much thicker cell walls than the sensitive parent strains (17). Subsequent investigators have demonstrated that cell wall synthesis and turnover are upregulated in VRSA isolates, leading to thicker and more-disorganized cell walls (30). Further, it appears that resistant isolates have significantly less cross-linking in the peptidoglycan component of the cell wall (64). In order to exert an effect, vancomycin must reach the cytoplasmic membrane and bind with nascent cell wall precursors, thereby inhibiting their incorporation into the growing cell wall. It has been proposed that the thicker, disorganized cell walls can actually trap vancomycin at the periphery of the cell, thereby blocking its action (Fig. 1) (62). In fact, it has been shown that vancomycin can be recovered intact from the cell walls of VISA and VRSA isolates (63), indicating that the antibiotic is not being inactivated but merely sequestered by the bacteria. Furthermore, the altered cell walls appear to have a reduced affinity for vancomycin as soluble targets are able to bind more antibiotic in the presence of vancomycin-resistant isolates (6).

The role of penicillin-binding proteins (PBPs) in vancomycin resistance remains unclear. PBPs are a group of enzymes that catalyze various steps in cell wall synthesis and are the targets

of beta-lactam antibiotics. It is a mutation in one of these enzymes, PBP2A, that confers methicillin resistance in MRSA. While some studies have shown an increase in the production of PBPs in VRSA (30, 43), others have shown that these enzymes are down regulated (66). That the MIC of oxacillin for some of the VISA isolates has decreased as the vancomycin MIC goes up (64; Conway et al., 11th Annu. Meet. Soc. Healthcare Epidemiol. Am.) supports the in vitro finding that expression of the mutated PBP2A is down regulated in these isolates (66). What role, if any, this altered expression plays in vancomycin resistance is unknown.

Clearly, more research is needed to further elucidate the exact mechanism of vancomycin resistance. However, given what is currently known, it seems safe to assume that it will be multifactorial.

Microbiology of VISA and VRSA

The cell wall alterations in VRSA can cause difficulties with laboratory identification. Morphologically, colonies of VISA and VRSA isolates often look smaller than their susceptible counterparts, which can lead some to confuse them with coagulase-negative staphylococci (64). Furthermore, vancomycin-resistant strains may require more incubation time for coagulase detection. If the coagulase reactions are incubated for less than 4 h, the result may be falsely negative and the isolate may be misclassified as coagulase-negative staphylococci (43). Thus, for any staphylococcus with suspected vancomycin resistance, the coagulase test should be incubated for more than 4 h before being interpreted (43).

Treatment of Infections Caused by VISA and VRSA

The susceptibilities and treatments of the VISA isolates that have been reported in this country are summarized in Table 4. It is interesting that all isolates have been sensitive to trimethoprim-sulfamethoxazole and tetracycline. Investigators and clinicians have also attempted to exploit the decreased resistance to oxacillin of some of the VISA isolates. In the laboratory, the combination of nafcillin and vancomycin was synergistic in the treatment of VISA endocarditis in rabbits (16). Beta-lactam antibiotics have been used clinically in the treatment of two of the VISA cases, once in combination with an aminoglycoside (35) and once in combination with an aminoglycoside and vancomycin (24). In both cases, the infection was cleared, although only one of the patients survived.

Given the rarity of these infections, it is impossible to say what role the recently approved antibiotics quinupristin-dalfopristin and linezolid will play in their management. One study did show that both agents had good activity against three separate VISA strains (55); however, at least one of the clinical isolates was resistant to quinupristin-dalfopristin (Conway et al., 11th Annu. Meet. Soc. Healthcare Epidemiol. Am.). Only linezolid has been used in reported clinical cases, being used once in conjunction with trimethoprim-sulfamethoxazole and doxycycline (24) and once as a single agent (Conway et al., 11th Annu. Meet. Soc. Healthcare Epidemiol. Am.). Again, though there was a microbiologic cure in both cases, only one of the patients survived. Though VISA isolates thus far have all been susceptible to linezolid, the recent report of linezolid resis-

TABLE 4. Susceptibilities and treatments of U.S. VISA isolates

State and yr (reference)	Drugs to which isolate was susceptible	Treatment
Michigan, 1997 (67)	Trimethoprim-sulfamethoxazole, chloramphenicol, rifampin, tetracycline	Vancomycin, tobramycin then trimethoprim-sulfamethoxazole, rifampin
New Jersey, 1997 (76, 77)	Trimethoprim-sulfamethoxazole, chloramphenicol, tetracycline, gentamicin	Gentamicin, rifampin
New York, 1998 (53)	Trimethoprim-sulfamethoxazole, chloramphenicol, tetracycline, gentamicin, clindamycin	Vancomycin, tobramycin
Illinois, 1999 (15)	Trimethoprim-sulfamethoxazole, tetracycline, gentamicin	Vancomycin, tobramycin, rifampin
Minnesota, 2000 (24)	Not available	Vancomycin, nafcillin, gentamicin
Nevada, 2000 (24)	Trimethoprim-sulfamethoxazole, tetracycline, gentamicin	Linezolid, doxycycline, trimethoprim-sulfamethoxazole
Maryland, 2000	Trimethoprim-sulfamethoxazole, chloramphenicol, tetracycline, linezolid	Linezolid

tance in an isolate of MRSA, combined with growing use of this agent, raises real concern over how long this uniform susceptibility will hold (73).

Thus, there do appear to be at least a few treatment options for VISA and VRSA infections. However, until more experience exists, the best treatment strategy would appear to be to tailor therapy to the susceptibilities of the isolate in each case.

CONCLUSION

Vancomycin resistance in staphylococcal species is only beginning to emerge as a clinical issue, yet the attention it has already received serves to underscore the seriousness of the problem. Although much work has been and is being done on these organisms, much is also yet to be done, especially with respect to determining the true risk factors for infection and the mechanisms of vancomycin resistance. A better understanding of these issues will be key to helping prevent and treat these infections in the future. If past experience with multidrug resistant organisms is any indicator, the problem of vancomycin-resistant staphylococci will only grow in the future. However, heightened awareness of the issues and strict adherence to current guidelines for vancomycin use and infection control practices may help limit the impact of these organisms.

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