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Emergence of Methicillin-Resistant *Staphylococcus aureus* with Intermediate Glycopeptide Resistance

Clinical Significance and Treatment Options

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Abstract

Methicillin-resistant *Staphylococcus aureus* is a pathogen that is associated with serious infections that pose a significant risk of morbidity and mortality because of their multidrug resistant nature. Until recently, therapeutic options were limited to vancomycin, making the use of this drug widespread. Unfortunately, the continued application of this drug has led to the emergence of glycopeptide intermediate susceptible *S. aureus* (GISA). By definition, these organisms demonstrated a vancomycin minimum inhibitory concentration (MIC) of >4 mg/L and <32 mg/L. However, although the mechanism of resistance is not fully elucidated at this time, GISA strains have demonstrated thickened or aggregated cell walls, an increase in penicillin binding proteins and greater autolytic activity. At present, the overall number of reported cases of GISA is relatively low. In most cases, thus far, prolonged courses of vancomycin were reported. A few cases reported monitoring serum vancomycin concentrations but because of limited information, no association with outcome can be made. Whether these GISA strains will become more widespread or evolve into fully glycopeptide resistant strains is unknown at this time. Although there are a number of new agents that possess activity against these pathogens, there is no consensus regarding specific recommendations for treatment. Strict infection control practices, routine screening for resistance and controlled use of antibacterial agents, especially vancomycin, are critical steps in preventing the further development of resistance among staphylococci.

1. Background

Staphylococcus aureus is a problematic pathogen responsible for a variety of infections including pneumonia, skin and soft tissue infections, osteomyelitis, endocarditis and meningitis. In hospital settings it is frequently associated with surgical infections and is one of the leading causes of bloodstream infections.[1-5]

This organism has a long history of resistance milestones beginning in the 1940s when soon after the introduction of penicillin, *S. aureus* developed the ability to produce penicillinase, rendering this drug ineffective. Over the next few decades, *S. aureus*

developed resistance to tetracyclines, chloramphenicol and erythromycin. During the 1960s, *S. aureus* altered its penicillin binding proteins and methicillin-resistant *S. aureus* (MRSA) emerged and has since spread worldwide. Multidrug-resistant *S. aureus* strains are now common and have been reported in both hospital and community settings. Vancomycin has been the only reliable agent to treat this pathogen for the last several decades.[1-5]

Coagulase-negative staphylococci with reduced susceptibility to vancomycin have been reported over the years.[6,7] However, it was not until the emergence of glycopeptide intermediate-susceptible *S. aureus* (GISA) strains, with increased minimum inhibitory concentrations (MICs) to vancomycin, that worldwide concern became apparent.[8-15] Much of this fear can be attributed to the lack of alternative agents available to treat this new threat should it become commonplace.

2. Glycopeptide Intermediate-Susceptible *Staphylococcus aureus* (GISA)

2.1 Definition of GISA

GISA is defined as a strain of *S. aureus* that is intermediately susceptible to glycopeptides. Therefore, based on the National Committee for Clinical Laboratory Standards (NCCLS) guidelines, a strain is classified as susceptible with a MIC <4 mg/L, intermediate-resistant with a MIC ≥4 mg/L, and fully resistant with a MIC ≥32 mg/L. The following is a summary of the patient cases in which GISA strains have been documented thus far.

2.2 GISA Case Summaries

Currently, several countries, including Japan and the US, have reported a total of 8 cases of GISA. Several other countries around the world, including the UK, Germany, Italy and Brazil, have evaluated clinical isolates of MRSA and determined that some strains demonstrate the same characteristics of the reported GISA isolates.[16-19] The following are 6 of these GISA cases that have been described in the literature.

In 1996, a MRSA strain (Mu-50) with reduced susceptibility to vancomycin was isolated from a 4-year-old patient in Japan. This was the first reported strain. The patient developed an infected surgical site over the sternum after heart surgery for pulmonary atresia. This nosocomial infection was treated with vancomycin with no resolution of symptoms for 29 days. Effective healing occurred only after arbekacin, an aminoglycoside from Japan, was added for an additional 12 days. Unfortunately, 12 days after discontinuation of therapy recurrence of the infection occurred. At this point ampicillin/sulbactam in combination with arbekacin was employed for 23 days along with surgical debridement resulting in complete resolution of the infection.[20-22]

Subsequently, two strains of GISA, 963sm (Michigan) and 992 (New Jersey), were reported in the US in 1997. Similarly to the first reported case in Japan, these 2 strains were derived from patients with prolonged exposure to vancomycin. In February, a 59-year-old patient from Michigan with chronic renal failure requiring peritoneal dialysis was admitted with peritonitis.[8,23] After receiving multiple courses of intravenous and intraperitoneal vancomycin for 5 months, eventually GISA was cultured. Finally in September, after 49 days of multi-drug therapy, including rifampicin (rifampin), trimethoprim/sulfamethoxazole and gentamicin, the patient's cultures became negative. Although bacteriological cure was achieved, the patient died only a few months later as a result of complications of his underlying disease. The third case was reported in a 66-year-old New Jersey patient with a history of recurrent MRSA bacteraemia, treated with repeated courses of vancomycin for 18 of 23 possible weeks on both an inpatient and outpatient basis from March until August when the GISA strain was isolated.[8] Once GISA was cultured, gentamicin was added to the drug regimen. However, 15 days later the patient developed pulmonary and pedal oedema at which point rifampin was also added to the regimen. All antimicrobials were discontinued after 4 weeks of treatment for GISA. In September the patient also had multiple fungal cultures from

the peritoneal fluid and positive Gram-negative urine cultures. Irrespective of treatment, 34 days after initialisation of therapy with amphotericin B, doxycycline and ciprofloxacin the patient died.

In 1998, France reported the next GISA case that had occurred in a patient in 1995. This isolate was cultured from a 2-year-old patient with leukaemia with a MRSA bacteraemia (LIM-1).[24] Unlike earlier reported cases, this patient had not had previously received prolonged treatment with vancomycin. The patient received 10 days of vancomycin and amikacin with no improvement in the MRSA bacteraemia and developed a purulent discharge from her central line. Treatment was changed to teicoplanin and amikacin and when blood cultures were rechecked 2 days after this change of therapy, a GISA isolate (LIM-2) was isolated. This isolate was also cross-resistant to teicoplanin. Susceptibility testing revealed that the isolate was only sensitive to pristinamycin (an oral streptogramin used in France) and trimethoprim-sulfamethoxazole. Treatment consisted of drainage and a 10-day regimen of quinupristin/dalfopristin.

The New York strain was similar to the first 3 reported cases. A 79-year-old patient was treated for an MRSA bacteraemia with a prolonged course of vancomycin.[10,25] He was readmitted with an infected jugular catheter about a month later, when the line was removed, and vancomycin therapy restarted and continued after discharge. In March 1998, the patient was admitted for sepsis and subsequently died the next day. The blood culture of *S. aureus* from the last admission was determined to have a vancomycin MIC of 8 mg/L.

Hong Kong has been the fourth and latest country to report the identification of a GISA isolate. Although limited information is available on this strain, it appears that the patient was a middle-aged woman with cancer who was admitted for fever. After 2 weeks of treatment with an unreported agent(s), she died from an uncontrolled bacteraemia.[26]

Unfortunately, vancomycin concentrations were not reported in any of the cases that occurred outside the US. The cases in the US only reported minimal data. The Michigan (963sm) strain reported a median peak serum vancomycin concentration of 33 mg/L (range: 20.6 to 42.3 mg/L) and a median trough concentration of 10.4 mg/L (range: 6.2 to 19.7 mg/L).[8] The New Jersey (992) strain was reported as having 2 peaks measured as 32.5 and 26.4 mg/L, trough and random levels had a median of 16.6 mg/L (range: 4.6 to 26.2 mg/L).[8] In the case from New York there were only 5 random vancomycin levels drawn with the range being 6.3 to 17.3 mg/L.[10] Therefore, because of limited data, it is difficult to determine if vancomycin serum concentrations had any effect on patient outcome.

2.3 Description of GISA Strains

All the GISA strains isolated thus far were MRSA in origin. These strains have all met the susceptibility definition displaying a minimal inhibitory concentration of 8 mg/L.[20,23] The strains display multidrug resistant patterns, although several strains are susceptible to tetracycline, chloramphenicol, ampicillin/sulbactam, trimethoprim/sulfamethoxazole and gentamicin.[8,21,22,24,27,28] Some isolates are susceptible to recently approved agents such as quinupristin/dalfopristin and linezolid, as well as a few of the newer generation fluoroquinolones (e.g. trovafloxacin and clinafloxacin) and some investigational agents such as daptomycin, a lipopeptide, and oritavancin (LY-333328), a glyocopeptide (table I).[8,28-30]

3. Mechanism of Resistance

The mechanism of resistance has not yet been elucidated. However, extensive study of laboratory-derived strains and several of the clinical isolates has revealed a great deal of information. Thus far all clinical strains examined have been negative for the presence of plasmid mediated vancomycin resistance *van*A, B and C.[8,31]

Transmission electron microscopy of GISA isolates has demonstrated cell walls that are thicker than typical MRSA isolates. The thickened layer of extracellular material of laboratory derived strains has been shown to sequester vancomycin and decrease the susceptibility of *S. aureus* to vancomycin *in vitro*.[8,32] However, no thickened cell wall was

Table I. Antibacterial susceptibilities of various glycopeptide intermediate-susceptible *Staphylococcus aureus* (GISA) strains. Reproduced from Hershberger et al.,[28] with permission.

Antibacterial agent	MIC/MBC (μg/ml) for *S. aureus*		
	Mu-50 (Japan)	963sm (Michigan)	992 (New Jersey)
Vancomycin	8/12	8/8	6/6
Oritavancin (LY-333328)	2/8	1/2	1/1
Teicoplanin	16/32	8/16	2/4
Daptomycin	0.5/1	1/1	0.5/1
Ampicillin/sulbactam	64/64	32/64	8/8
Gentamicin	128/128	64/>256	0.25/0.5
Rifampin	2048/>2048	1024/>2048	2048/>4096
Quinupristin/dalfopristin	0.5/1	0.25/1	0.25/0.25
Linezolid	2/16	1/4	0.5/2
Trovafloxacin	2/2	0.5/1	1/1
Clinafloxacin	1/1	1/2	0.5/0.5
Tetracycline	128/128	2/16	0.5/0.5
Levofloxacin	8/8	>16/>16	8/16
TMP-SMX	0.06/0.125	4/>64	0.06/0.06

MIC/MBC = minimum inhibitory concentration/minimum bacteriocidal concentration; **TMP-SMX** = trimethoprim/sulfamethoxazole.

observed upon the evaluation of the New York strain, instead multicellular aggregates were noted to occur during bacterial growth with vancomycin present.[10] Interestingly, the concentration of vancomycin in these bacteria during growth actually decreased to a point where no remaining vancomycin was found in the growth medium but could ultimately be retrieved from the bacterial cell walls.[10] Examination of penicillin-binding proteins (PBPs) has revealed that GISA strains contain as much as 3 to 5 times the amount of PBP 2 when compared with vancomycin-susceptible strains of *S. aureus*.[8,27,31,33,34] It has also been demonstrated that these strains have accelerated uptake of N-acetylglucosamine into the cell, increased amounts of cytoplasmic murein monomer precursors, and greater autolytic activities and autolysin production compared with control strains of *S. aureus*.[32,33] The relationship between the increased cell wall turn over and subsequent modifications and vancomycin resistance is currently not known, however, it is likely that these strains were naturally selected mutants which evolved from prolonged exposure to vancomycin.

Data from Japan and the US indicate that most of the GISA strains isolated thus far are heteroresistant to vancomycin.[14,35] Hetero-resistance has been described as an organism which has multiple subpopulations of varying MICs (i.e. varying resistance levels) within a particular strain, with the highest MIC utilised as the representative MIC for that strain. Hiramatsu et al.[14] observed multiple MICs within the subpopulations of several Japanese strains of *S. aureus* with reduced susceptibility to vancomycin, including Mu-50.[14] This study found that about 70% of all MRSA strains tested had clonotype II-A, which was shown to make these strains easily acquire vancomycin resistance. Heterogeneous vancomycin resistance may account for the early emergence of GISA in Japan despite less than a decade of exposure to intravenous vancomycin. Another susceptibility study examining the first 3 GISA strains demonstrated that 963sm (Michigan) was the most heterogeneous strain followed by Mu-50, with the vancomycin MIC decreasing to 2 and 4 mg/L, respectively, after the first serial passage.[35] The New Jersey strain (992) appears to be the most homogeneous strain of GISA as demonstrated by only a 1-fold decrease in vancomycin MIC (4 mg/L), even after 10 serial passages. This study and others have demonstrated that a varying degree of resistance among the subpop-

ulations can be induced (increased MICs) or reduced (decreased MICs) based on the exposure to or removal of vancomycin.[35,36] However, other studies have suggested that even after multiple serial passages that MIC values have remained stable.[8] The differences in these studies could be related to the difficulty in detecting resistant subpopulations and to the methodology for determination of MICs.

4. Screening Methods

Screening strains for vancomycin reduced susceptibility is not easily accomplished. Disk diffusion methods are not reliable and therefore, not recommended. E-test strips, agar or broth dilution methods should be employed for the detection of these strains. The Centers for Disease Control and Prevention (CDC) has made a number of recommendations to help curtail the spread of vancomycin-resistant strains of Gram-positive organisms, including GISA. Strict infection control practices and a reduction in the routine use of vancomycin are the primary suggestions from these guidelines.[8,15]

5. Patient Characteristics

To date, all patient isolates of GISA, except the French case, have emerged after prolonged use of vancomycin. Susceptibility studies and the majority of cases demonstrate that exposure to vancomycin over time could lend itself to creating the environment for the potential development of resistance. Therefore, routine or prolonged use of vancomycin in patients, especially patients receiving peritoneal or haemodialysis, should be avoided. Medical histories have been somewhat similar in all the cases reported, with several patients having chronic renal failure (requiring dialysis), oncological disorders or diabetes mellitus. Therefore, any patient with a condition that could impair the immune system or require long term catheter placement could possibly be at increased risk for acquiring a resistant organism, including GISA.

6. Treatment Options

To date, there are no recommended treatment strategies and a standard of therapy has yet to be determined. However, at present time, potential treatment should be patient specific, based on susceptibility profiles of the current cases and the limited *in vitro* data available.

Kill curve experiments and *in vitro* infection models utilising the standard dosage regimen of vancomycin (i.e. 1g every 12 hours) have been ineffective. However, in an *in vitro* study, higher dosage regimens (i.e. high dose continuous infusion or 1.5g every 12 hours, etc.) have been shown to have more consistent effects and added activity against the GISA strains, although this is specific-strain-dependent.[37] In a neutropenic mouse model, lower pharmacodynamic parameters resulted in similar bacterial kill against 2 GISA strains compared with 3 sensitive strains.[38] Therefore, although increased dosage regimens may be able to achieve higher area under the plasma concentration-time curve (AUC)/MIC ratios, the anticipated benefit may not outweigh the increased potential resistance and/or toxicity.

Currently, there are two newly approved antimicrobial agents, quinupristin/dalfopristin and linezolid, that offer clinicians an alternative to vancomycin as well as a potential treatment for GISA.

Unfortunately, it may be necessary to use investigational agents or unproven drug combinations in treating patients with GISA. Preliminary results with the investigational lipopeptide agent daptomycin alone or in combination with arbekacin has shown significant activity against some GISA strains.[29] Another possible treatment option is the investigational glycopeptide oritavancin, which has also shown activity against GISA.[39] Studies have shown that β-lactam antibacterials (i.e. methicillin, oxacillin) have decreased resistance against some GISA strains, leading to a possible treatment of combination therapy with vancomycin plus multiple β-lactams including methicillin, oxacillin, nafcillin or cefotaxime.[10,40,41] Ampicillin/sulbactam ± arbekacin has also demonstrated activity against

these strains, which may be due to the increased production of PBPs of the isolates.[20-22,42]

7. Current Opinions

At this point, clinicians are faced with the dilemma of what is the best treatment for these patients. Based on current evidence, there are numerous components that need to be considered. First, identify which patients are at risk of developing GISA and specifically look at their comorbid disease states. Once a patient has been identified and is receiving vancomycin, prudent use of this agent is required. Serum monitoring in this patient population will be of more importance, especially trough levels which will need to be higher than in most patients (i.e. 10 to 20 mg/L). If a GISA isolate is identified in a patient, susceptibilities need to be performed as well as initial aggressive empirical therapy utilising currently available antibacterial agents. Upon susceptibility results or little to no patient improvement, adjustment of the antibacterials used will be necessary. Ultimately, investigational agents or combination therapy may be warranted.

8. Conclusions

As of now there are few alternative treatment options, which are limited to new investigational agents or agents that remain susceptible upon sensitivity testing against each particular isolate. These organisms will continue to present difficult situations for clinicians until more established treatment alternatives can be determined. Therefore, it is imperative that we as healthcare providers be aware of the increasing isolation of resistant organisms, practice infection control guidelines and curtail the current misuse of antimicrobials.

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