Reduced susceptibility of *Staphylococcus aureus* to vancomycin - a review of current knowledge

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Abstract

Antibiotic options for patients with methicillin resistant *Staphylococcus aureus* infections are severely limited. Unfortunately, infections with *S. aureus* with reduced susceptibility to vancomycin and teicoplanin have been recently reported for the first time. Commonly used laboratory methods for determining antibiotic susceptibility may be inadequate for detecting reduced susceptibility to vancomycin. Even though no confirmed cases have yet been detected in Australia, a high index of suspicion must be maintained for the occurrence of such organisms. Strategies for prevention of the spread of *S. aureus* with reduced susceptibility to vancomycin should be prepared by Australian hospitals prior to their first cases being identified. This article outlines the background to this developing issue and discusses laboratory methods and findings, with some current recommendations for diagnostic laboratories. *Comm Dis Intell* 1999;24:69-73

Introduction

For many years it has been recognised that *Staphylococcus haemolyticus* (a relatively rare coagulase negative *Staphylococcus*) may exhibit some degree of vancomycin resistance.[1] Vancomycin resistance in *Staphylococcus epidermidis* has also been demonstrated, although rarely.[2,3] However it is the possibility of vancomycin resistance in *Staphylococcus aureus (S. aureus)* which has become a much more significant concern. Unfortunately, hypothetical concern has now developed into practical reality.[4-12]

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History

Known cases of infection with 'vancomycin intermediate *Staphylococcus aureus*'

The first reported case of *S. aureus* infection with reduced susceptibility to vancomycin occurred in Japan in May 1996.[4] Since this report, three cases of *S. aureus* with reduced susceptibility have been reported in the United States of America and one in France. A further clinical specimen has been isolated in Slovakia, although confirmation that it truly has reduced susceptibility to vancomycin has not yet been established.[13]

The Japanese case was a 4 month old infant who underwent heart surgery for pulmonary atresia. Two weeks after surgery the patient developed a sternal wound infection with methicillin resistant *Staphylococcus aureus* (MRSA). The patient was treated with vancomycin (45 mg/kg/day) for 29 days, but fever and discharge of pus continued. The infection eventually resolved with debridement of the infected area and 23 days of therapy with ampicillin/sulbactam and arbekacin (an aminoglycoside available in Japan). The MRSA strain (designated Mu50) obtained both from the original sternal incision site and the debridement sample had a vancomycin minimal inhibitory concentration (MIC) of 8 mg/L by the broth microdilution method. The National Committee for Clinical Laboratory Standards (NCCLS) gives the following guidelines as to the susceptibilities of *S. aureus* to vancomycin; MIC less than or equal to 4 mg/L = susceptible, MIC 8-16 mg/L = intermediate, MIC greater than or equal to 32 mg/L = vancomycin resistant.[14]

The first case from the United States of America was isolated in July 1997 in Detroit from a 59 year old with metastatic lung cancer and end-stage renal disease (on peritoneal dialysis) who had a history of repeated episodes of peritonitis treated with both intravenous and intraperitoneal vancomycin for six months.[5] Peritoneal fluid cultures grew multiple organisms including vancomycin resistant *Enterococcus faecium* and multiple strains of *S. aureus*. All but one of the *S. aureus* strains were susceptible to vancomycin; the exception (designated *S. aureus* 14342) had a vancomycin MIC of 8 mg/L. The organism was methicillin resistant and resistant to teicoplanin (MIC 16 mg/L). The patient improved following treatment with trimethoprim-sulfamethoxazole plus rifampicin.

Another case of infection with MRSA exhibiting a vancomycin MIC of 8 mg/L was isolated in August 1997 from a patient from New Jersey.[10] The patient was known to be colonised with both MRSA and vancomycin resistant Enterococci. From March to August the patient had repeated MRSA bacteremias for which multiple courses of vancomycin had been given (he had received vancomycin for 18/23 weeks from March 1997).[6] In August, a blood culture from the patient grew an MRSA strain with intermediate resistance to vancomycin. The patient stabilised after treatment with a combination of vancomycin, gentamicin and rifampin but died in October 1997 from candidemia.

The most recent case from the United States of America was from New York.[12] A 79 year old man with a history of renal failure requiring haemodialysis presented in December 1997 with MRSA bacteremia. The source was thought to be an infected dialysis catheter. The catheter was removed and the patient treated with vancomycin for four weeks. In January 1998 the patient had recurrent MRSA bacteremia again treated with vancomycin. Finally, in March 1998 the patient presented with fever, confusion and respiratory distress and died within 12 hours of admission. MRSA was again grown from blood cultures. The vancomycin MIC for this final isolate was 8 mg/L.

Finally, a case has been reported from France of MRSA bacteremia of presumed central venous line origin from a 2 year old girl with leukaemia.[11] The organism was initially susceptible to vancomycin (MIC 2 mg/L), but after 10 days of vancomycin therapy a blood culture isolate with a vancomycin MIC of 8 mg/L and teicoplanin MIC of 16 mg/L was obtained. Her infection was eventually successfully treated with the drainage of pus and administration of quinupristin plus dalfopristin for ten days.

Methods

Laboratory detection of *S. aureus* with reduced susceptibility to vancomycin

In each of the confirmed cases above, the MIC of vancomycin was 8 mg/L. Hence these organisms have been referred to as vancomycin intermediate *S. aureus* (VISA), although the finding of associated teicoplanin resistance has prompted other authorities to refer to the isolates as glycopeptide intermediate *S. aureus* (GISA). (At least 15 patients have been previously reported who had infection with *S. aureus* with decreased susceptibility to teicoplanin (MIC 8-16 mg/L) but whose isolates were susceptible to vancomycin).[15-17]

Of major practical importance is that the use of disc diffusion methods in determining susceptibility of *S. aureus* to vancomycin may be inadequate for the detection of VISA. Using NCCLS recommended methods, inhibitory zone diameters of the VISA strains for vancomycin overlap those produced by susceptible isolates (that is, 17-19 mm). Use of 30 µg teicoplanin discs as a screen for VISA appears more useful. Each of the first three confirmed strains of VISA described above had a zone of 15mm or less around teicoplanin discs (F. Tenover, personal communication).

Contents, ***continued***

Two VISA strains have been tested by the CDS method. One strain was not recognised as VISA by the CDS method, while the other was recognised without difficulty (SM Bell, personal communication). The strain which was not recognised (Mu 50 from Japan) gave an annular radius on Sensitest agar of 2.5 mm (cut-off annular radius of 2mm for resistance to vancomycin). The other strain (HIP 5827 from Detroit) gave an annular radius of less than 2.0 mm. It should be noted that both VISA strains had a fuzzy growth at the edge of the zone of inhibition; this fuzzy edge is not seen in susceptible Staphylococci and corresponds to the light growth that was recorded with these strains on agar dilution plates containing 2.0 or 4.0 mg/L vancomycin (SM Bell, personal communication).

Automated systems are also somewhat unreliable in the detection of VISA. The 'Vitek' test has measured the first three known strains at 4 mg/L consistently (that is, they would have been recorded as susceptible). However, MICs of 4mg/L are extremely rare for vancomycin susceptible strains of *S. aureus* (occurring in only 0.4% of all MRSA strains), so such a finding should prompt testing of the isolate by reference dilution methods.[12] Microscan conventional panels recorded the MICs as 8-16 mg/L in the field, but 4 mg/L when repeated in a reference laboratory. Microscan rapid panels recorded MICs from less than 2 mg/L to 16 mg/L.[12]

Manual MIC methods are therefore necessary to reliably record the MIC as in the intermediate range.[18] When 'E test' was used, for each of the strains, the MIC was 6 mg/L, which would round up to 8 mg/L (since E test results should be reported to the next higher doubling dilution). Broth dilution or agar dilution methods are an alternative reference method.

Although the above results are preliminary and are only based on a small number of strains, it is clear that detection of vancomycin resistance may create problems for clinical microbiologists across Australia. Should manual MIC methods be used on all *S. aureus* isolates in our clinical microbiology laboratories? Since all isolates detected so far have been concurrently methicillin-resistant (MRSA) it would be practical to restrict manual methods to isolates known to be MRSA. Although by doing this detection of reduced susceptibility to vancomycin will be delayed by 24 hours, (given that detection of methicillin susceptibility will be determined first), this delay seems justifiable given the current rarity of VISA isolates. Individual laboratories will have to devise their own strategies, depending on resources and current prevalence of MRSA, but restricting MIC testing to MRSA isolates from patients who have had failure of vancomycin therapy may be preferable to blanket MIC testing of all MRSA isolates at the present time. There may also be a place for performing intermittent surveillance of all MRSA isolates in a specified time period or in regular surveillance of isolates from patients in high-risk units (for example, renal or intensive care units). In this circumstance, use of brain heart infusion plates containing 6 mg/L of vancomycin, may be a useful screening method.[12] Appropriate negative controls include *S. aureus* ATCC 29213 and *E. faecalis* ATCC 51299.

Some may argue against a targeted approach and suggest that, given the potential global seriousness of VRSA, all laboratories should screen all MRSA isolates for vancomycin resistance now.

Discussion

Mechanism of Resistance

The mechanism by which the VISA isolates have reduced susceptibility to glycopeptides has not yet been determined. What is known, however, is that the current isolates have not acquired the vancomycin resistance genes of enterococci (van A or van B).[4,5] It is known however, that acquisition of these genes by Staphylococci can occur in the test tube.[19]

VISA isolates have a markedly thick cell wall on electron microscopy.[12] The Japanese isolate of VISA (Mu50) has also been found to have a high level of production of the penicillin-binding protein, PBP-2.[20] Laboratory selected mutants of *S. aureus* with decreased susceptibility to glycopeptides (obtained by incubating previously susceptible clinical isolates in vancomycin or teicoplanin) have also been shown to have elevated PBP-2 production.[20] Although it is possible that the genes encoding PBP-2 may be coregulated along with genes encoding another protein which is responsible for vancomycin resistance, present data suggest hyperproduction of PBP-2 as a possible mechanism of resistance in the current clinical isolates. It has been suggested that increased production of PBP-2 by VISA might increase the concentration of glycopeptide that is needed to interfere with the interaction between the PBP and D-alanyl-D-alanine during peptidoglycan synthesis.[20]

The first clinical strain from Japan (Mu50) exhibited homogeneous insensitivity to vancomycin (all individual bacterial cells in the culture population expressed resistance). However, a strain from the sputum of a patient from the same hospital (strain Mu3) exhibited heterogenous resistance (dubbed 'hetero VISA'. Strain Mu3 had a vancomycin MIC of 3 mg/L but a small fraction (one in a million) of the cell population had subclones with MICs of 8 mg/L.[21,22] Heterogeneously resistant *S. aureus* has now been found in hospitals throughout Japan. A case has of heterogeneously resistant *S. aureus* also been reported from Bristol in the United Kingdom.[23] Heteroresistance to vancomycin in coagulase negative Staphylococci has now also been well-described in New York City.[24] It has been hypothesised that heteroresistant *S. aureus* may swiftly evolve into homogeneous VISA during exposure to glycopeptide antibiotics. Further investigation into the mechanisms of occurrence of heteroresistant VISA is currently in progress.

Antimicrobial Susceptibility of VISA

One of the major concerns regarding vancomycin resistance in *S. aureus* is that this common bacteria would therefore become resistant to all antibiotics which are currently available. Fortunately the clinical isolates of VISA so far obtained have retained susceptibility to other antibiotics. For example, the isolate from Detroit was susceptible to low concentrations of trimethoprim-sulfamethoxazole, rifampin, chloramphenicol, mupirocin and tetracycline.[5] It was also susceptible to investigational agents such as quinupristin-dalfopristin (MIC 0.5 mg/L), arbekacin (MIC < 0.12 mg/L), clinafloxacin (MIC 1 mg/L), LY 333328 (MIC 2 mg/L) and the oxazolidinones, eperezolid and linezolid (MIC for both 1 mg/L).[5] The isolate from the patient in New Jersey was susceptible to chloramphenicol, gentamicin, tetracycline and

Table 1. Suggestions for the microbiology laboratory in the identification and control of vancomycin-resistant *S. aureus* or Vancomycin intermediate *Staphylococcus aureus* (VISA)

1. Recognise that failure to correctly identify *S. aureus* strains as VISA has occurred with NCCLS disc diffusion methods, the CDS method and automated susceptibility tests, such as Vitek. Clues to the presence of VISA include a teicoplanin zone size of 15mm or less (NCCLS disc diffusion), a 'fuzzy edge' (CDS method) and MIC of 4 mg/L (Vitek).
2. Strains exhibiting any of these characteristics or strains of *S. aureus* from patients with persistent infection despite use of vancomycin, should be considered for testing by a manual MIC test.
3. Strains with an MIC of 8mg/L or more should have the MIC reconfirmed by a reference laboratory.
4. The Director of Microbiology and the Director of Infection Control should be contacted upon suspicion that an isolate is VISA or VRSA.
5. The organism should be stored for future studies at -70^{o}C in a freezer located in an area of the laboratory to which there is limited access. Consideration should be given to sending the isolate to the Nosocomial Pathogens Laboratory Branch, Centers for Disease Control and Prevention, Atlanta, United States of America, which has studied all of the known strains of VISA

trimethoprim-sulfamethoxazole, and was susceptible to quinupristin-dalfopristin (MIC 0.5 mg/L) and linezolid (MIC 1 mg/L).[6]

In the absence of extensive clinical experience, optimal treatment of VISA is unknown. Teicoplanin is unlikely to offer any advantage over vancomycin since those VISA isolates which have been assessed also have reduced susceptibility to teicoplanin. Synergy has been observed in vitro when vancomycin and anti-staphylococcal beta-lactams have been used in combination.[25]

Infection Control Issues

Since other resistant microbes are well known to spread from patient to patient (often via the hands of healthcare workers) concern existed that patients in addition to those reported may have become infected or colonised with VISA. In Juntendo University Hospital (Tokyo, Japan) where the first clinical isolate was discovered, 20% of MRSA isolates now have heteroresistant VISA.[22] By pulsed field gel electrophoresis these isolates were found to be identical to, or similar to, Mu50 (the originally described strain). Four other Japanese hospitals have isolated heteroresistant VISA. In contrast, no contacts of the first three VISA isolates in the United States of America have been found to be positive for VISA (although 16% of nares and 25% of hand cultures of healthcare providers and hospital roommates were positive for *S. aureus).*[6]

Guidelines on the control of spread of vancomycin resistant *S. aureus* have been published.[26-29] In general they are similar to guidelines already in place across Australia for control of MRSA infection. It should be noted that many of the recommendations have failed to control the spread of MRSA, so may not control spread of VISA. However the guidelines also draw attention to nasal colonisation with *S. aureus*, and possible ways to prevent nasal colonisation of healthcare workers.

In the absence of known cases of VISA or vancomycin-resistant *S. aureus* in Australia, present attention should be concentrated upon restriction of vancomycin usage in the hospital. Guidelines on the prevention of vancomycin resistant enterococci have recently been developed by the Australasian Society for Infectious Diseases and include guidance as to situations where vancomycin use is appropriate. For example, vancomycin use is inappropriate as first line treatment of *C. difficile* colitis, as prophylaxis in the absence of penicillin allergy or a significant risk of MRSA infection, and as treatment of methicillin susceptible gram-positive infections in the absence of penicillin allergy. Table 1 presents some suggested guidelines for laboratories.

Conclusion

There is no doubt that *S. aureus* with reduced susceptibility to vancomycin has arrived. Although there is no evidence the organism exists in Australia (yet), strenuous efforts to prevent dissemination of these organisms in Australian hospitals needs to be instigated now in order to avoid a public health disaster.

Note

Since this article was written, two major papers pertaining to VISA have been published.[25, 30]

References

1. Veach LA, Pfaller MA, Barrett M, Koontz FP, Wenzel RP. Vancomycin resistance in *Staphylococcus heamolyticus* causing colonization and bloodstream infection. *J Clin Microbiol* 1990;28:2064-2068.
2. Sanyal D, Johnson AP, George RC, Cookson BD, Williams AJ. Peritonitis due to vancomycin-resistant *Staphylococcus epidermidis. Lancet* 1991;337:54.
3. Schwalbe RS, Stapleton JT, Gilligan PH. Emergence of vancomycin resistance in coagulase-negative staphylococci. *N Engl J Med* 1987;316:927-931.
4. Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC. Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. *J Antimicrob Chemother* 1997;40:135-136.
5. CDC. Reduced susceptibility of *Staphylococcus aureus* to vancomycin - Japan, 1996. *MMWR* 1997;46:624-626.
6. Robinson-Dunn B, Jennings G, Mitchell J et al. Characterization of a unique isolate of vancomycin-intermediate *Staphylococcus aureus* (VISA). Abstract LB-14 In: Program addendum of the 37th Interscience Conference on Antimicrobial Agents and Chemotherapy. Washington DC: American Society for Microbiology, 1997:11.
7. CDC. *Staphylococcus aureus* with reduced susceptibility to vancomycin - United States, 1997. *MMWR* 1997;46:765-766.

8. Smith TL, Pearson M, Wilcox K et al. *Staphylococcus aureus* with reduced susceptibility to vancomycin, United States, 1997. (Abstract LB-16). In: Program addendum of the 37th Interscience Conference on Antimicrobial Agents and Chemotherapy. Washington DC: American Society for Microbiology, 1997:11.
9. CDC. Update: *Staphylococcus aureus* with reduced susceptibility to vancomycin - United States, 1997. *MMWR* 1997;46:813-815.
10. Turco TF, Melko GP, Williams JR. Vancomycin intermediate-resistant *Staphylococcus aureus. Ann Pharmacother* 1998;32:758-760.
11. Ploy MC, Grelaud C, Martin C, de Lumley L, Denis F. First clinical isolate of vancomycin-intermediate in a French hospital. *Lancet* 1998;351:1212.
12. Tenover FC. Staphylococcal susceptibility testing: new challenges. [Abstract S-13]. In: Program of the 38th Interscience Conference on Antimicrobial Agents and Chemotherapy. Washington DC: American Society for Microbiology, 1998:3.
13. Tabaqchali S. Vancomycin-resistant *Staphylococcus aureus*: apocalypse now? *Lancet* 1997;350:1644-1645.
14. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically - fourth edition: approved standard, M7-A4. Villanova, Pennsylvania: National Committee for Clinical Laboratory Standrads, 1997.
15. Brunet G, Vedel G, Dreyfus F et al. Failure of teicoplanin therapy in two neutropenic patients with staphylococcal septicemia who recovered after administration of vancomycin. *Eur J Clin Microb Infect Dis* 1990;9:145-147.
16. Kaatz GW, Seo SM, Dorman NJ, Lerner SA. Emergence of teicoplanin resistance during therapy of *Staphylococcus aureus* endocarditis. *J Infect Dis* 1990;162:103-108.
17. Mainardi J-L, Shlaes DM, Goering RV, Shlaes JH, Acar JF, Goldstein FW. Decreased teicoplanin susceptibility of methicillin-resistant strains of *Staphylococcus aureus. J Infect Dis* 1995;171:1646-1650.
18. Tenover FC, Lancaster MV, Hill BC et al. Characterization of Staphylococci with reduced susceptibilities to vancomycin and other glycopeptides*. J Clin Microbiol* 1998;36:1020-1027.
19. Noble WC, Virani Z, Cree RG. Co-transfer of vancomycin and other resistance genes from *Enterococcus faecalis* NCTC 12201 to *Staphylococcus aureus. FEMS Microbiol Lett* 1992;72:195-198.
20. Moreira B, Boyle-Vavra S, deJonge BLM, Daum RS. Increased production of penicillin-binding protein 2, increased detection of other penicillin-binding proteins, and decreased coagulase activity associated with glycopeptide resistance in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1997;41:1788-1793.
21. Boyce JM, Medeiros AA, Hiramatsu K. Clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) from the United States with subpopulations of cells with reduced susceptibility to vancomycin. (Abstract LB-15). In: Program addendum of the 37th Interscience Conference on Antimicrobial Agents and Chemotherapy. Washington DC: American Society for Microbiology, 1997:11.
22. Hiramatsu K, Aritaka N, Hanaki H et al. Dissemination in Japanese hospitals of strains of *Staphylococcus aureus* heterogeneously resistant to vancomycin. *Lancet* 1997;350:1670-1673.
23. Howe RA, Bowker KE, Walsh TR, Feest TG, MacGowan AP. Vancomycin-resistant *Staphylococcus aureus. Lancet* 1998;351:602.
24. Sieradzki K, Villari P, Tomasz A. Low-level teicoplanin resistance and heteroresistance to vancomycin. *Ann Intern Med* 1998;128:245.
25. Sieradzki K, Roberts RB, Haber SW, Tomasz A. The development of Vancomycin resistance in a patient with methicillin-resistant *Staphylococcus aureus* infection. *N Engl J Med* 1999;340:517-523.
26. Wenzel RP, Edmond MB. Vancomycin-resistant *Staphylococcus aureus*: Infection Control Considerations. *Clin Infect Dis* 1998;27:245-251.
27. Edmond MB, Wenzel RP, Pasculle AW. Vancomycin-resistant *Staphylococcus aureus*: perspectives on measures needed for control. *Ann Intern Med* 1996;124:329-334.
28. Hospital Infection Control Practices Advisory Committee. Recommendations for preventing the spread of vancomycin resistance. *Infection Control and Hospital Epidemiology* 1995;16:105-113.
29. CDC. Interim guidelines for prevention and control of Staphylococcal infection associated with reduced susceptibility to vancomycin. *MMWR* 1997;46:626-8, 635.
30. Smith TL, Pearson ML, Wilcox KR et al. Emergence of Vancomycin resistance in *Staphylococcus aureus*. *N Engl J Med* 1999;340:493-501.

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Salmonellosis outbreak, South Australia

In late February the Communicable Disease Control Branch, South Australia was notified of an unusual number of cases of gastroenteritis. This was later determined to be caused by *Salmonella* Typhimurium phage type 135a. A case control study conducted on 6 and 7 March implicated a brand of commercially packaged fresh unpasteurised orange juice. On 8 March a bacteria presumptively identified as a *Salmonella* was isolated from a sample of the suspect brand purchased unopened from a retailer. A product recall was issued that day. On 10 March the presumptive *Salmonella* isolated from the juice 2 days previous was definitively identified as *S.* Typhimirium PT135a. As at 23 March, 405 cases of infection with this *Salmonella* had been laboratory confirmed and investigations are continuing into the source of the contamination of the orange juice. Although this product may be distributed to States other than South Australia there are, as yet, no reports of this unusual phage type causing recent infections in humans elsewhere in Australia.