

IDENTIFYINGTM RESISTANCE

INTERNATIONAL NEWSLETTER

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no 3

STATE-OF-THE-ART

■ **Vancomycin resistance in *Staphylococcus aureus***

THE BIOMERIEUX SOLUTION

■ **VITEK[®] 2 performance**

DID YOU KNOW?

- **Web site**
- **10th ISSSI**
- **NCCLS recommendations**
- **Population analysis**

PRACTICAL ADVICE

- **When to suspect a VISA strain?**
- **How to confirm?**
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The threat from the increase in antibiotic-resistant infections will not disappear overnight. More and more organisms are developing resistance to more and more drugs, while the spread of drug-resistant organisms from hospitals into the community at unprecedented rates is an additional cause for concern.

The emergence of vancomycin-resistant *Staphylococcus aureus* strains is particularly preoccupying since vancomycin is referred to as medicine's last line of defense against staphylococcal infection.

A defense which is narrowing as *Staphylococcus* is becoming increasingly resistant to methicillin.

The work of Professor Keichi Hiramatsu brilliantly demonstrates how important is a thorough understanding of resistance mechanisms to be able to develop the right methodologies and tools to identify vancomycin-resistant *Staphylococcus aureus* strains. More effective surveillance strategies can therefore be developed to better control this major threat of VRSA emergence.

We, at bioMérieux, are fully committed to being on the front-line of the combat against antibiotic-resistance through a close relationship with the academic research to swiftly transform research output into accurate, reliable and state-of-the-art diagnostic tools and services for *Identifying Resistance*.

Dr Xavier Fargetton
Director of Strategy & Marketing

STATE-OF-THE-ART

Vancomycin resistance in *Staphylococcus aureus*



Keichi Hiramatsu MD

Pr Keichi Hiramatsu* is professor of bacteriology (Juntendo University in Tokyo, Japan). He has been working on *S. aureus* since 1988. He found the first VRSA clinical strain in 1996; identified the carrier DNA of methicillin resistance (SCCmec) in 1999, and determined the *S. aureus* whole genome sequence in 2001. In 2002, he found a novel SCCmec (type-IV) distributed community-acquired MRSA

The first *S. aureus* strain with a vancomycin MIC 8 mg/L was isolated in 1996 from a Japanese patient to whom vancomycin therapy was ineffective [1]. Subsequently, dozens of VRSA strains were isolated from USA, France, Korea, South Africa, Scotland and Brazil [2], indicating that the issue is a global one [3]. However, the real essence of the issue lies in the latent dissemination of «hetero-VRSA», which has lower MIC values than 8 mg/L, but is capable of generating VRSA at high frequency upon exposure to vancomycin.

In 1997, we reported 23 Japanese MRSA strains with reduced susceptibility to vancomycin [4]. As is the case for methicillin resistance, we recognized two classes of phenotype in vancomycin resistance. One is vancomycin-resistant *Staphylococcus aureus* (VRSA) that is defined by a vancomycin MIC of 8 mg/l as measured by the broth-dilution susceptibility test. The nomenclature of VRSA is based on the British Society for Chemotherapy. The same strain is called vancomycin-intermediate *S. aureus* (VISA) or glycopeptide-intermediate *S. aureus* (GISA) in the USA, according to American interpretation criteria for

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MIC values [5]. The other class is hetero-VRSA, which though having MICs equal to or less than 4 mg/L, spontaneously generates VRSA within its cell population at a frequency of one in 10⁶ or greater [4].

Hetero-VRSA is regarded as 'susceptible' to vancomycin according to any of the routine susceptibility tests, of which the MIC test is representative [5]. Population analysis (analysis of resistant subpopulations) must be used to discriminate hetero-VRSA from truly vancomycin-susceptible *S. aureus* (VSSA) as illustrated in Figure 1. Population analysis is the gold standard to identify hetero-VRSA [6]. The figure also shows that the vancomycin resistance of *S. aureus* has a characteristic media-dependence, which is closely associated with the mechanism by which VRSA expresses vancomycin resistance [7].

The mechanism of vancomycin resistance in *S. aureus*

Vancomycin binds to D-alanyl-D-alanine residues of murein monomer (Figure A). There are two classes of binding targets in the *S. aureus* cell: firstly D-alanyl-D-alanine residues in the completed peptidoglycan layers; secondly those of the murein monomer substrates for glycosyltransferase, which are located in the cytoplasmic membrane. Binding of vancomycin to the former targets does not inhibit peptidoglycan synthesis, though it interferes with cross-bridge formation by PBPs. If bound to murein monomers, on the other hand, vancomycin inhibits peptidoglycan synthesis, presumably because glycosyltransferase cannot use the murein monomer bound to vancomycin as a substrate. The latter is the 'real' target for vancomycin to exert desirable antimicrobial activity [8]. In order for vancomycin molecules to bind to the second targets, they have to pass through about 20 peptidoglycan layers (only five layers are drawn in Figure B) without being trapped by the first targets. Since there are many D-alanyl-D-alanine targets in the peptidoglycan layers, many vancomycin molecules are lost in the peptidoglycan layers [8] [9]. For this reason, accurate susceptibility tests of vancomycin (and also teicoplanin) are much more difficult to perform than with other antibiotics, because the inoculated cells consume vancomycin in the medium and causes a drop in the effective concentration of the drug. Therefore, even a subtle difference in the inoculum size, that is negligible for the susceptibility test using other antibiotics, may cause substantial variance in the MIC values [8].

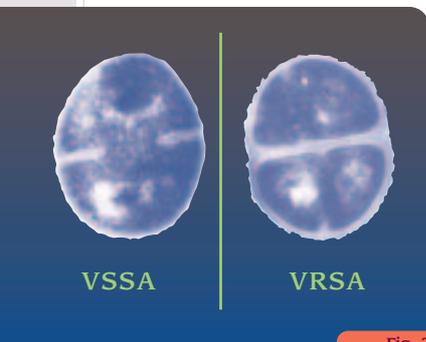


Fig. 2*

Transmission electron microscopy examination shows the difference in thickness of bacterial wall between VSSA and VRSA, due to increased amount of peptidoglycan.

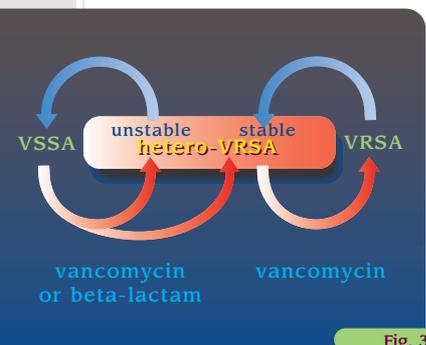


Fig. 3*

Hetero-VRSA: Vancomycin resistance is not easily detectable by routine susceptibility tests and thus latent in its distribution and prevalence. It generates VRSA on exposure to vancomycin.

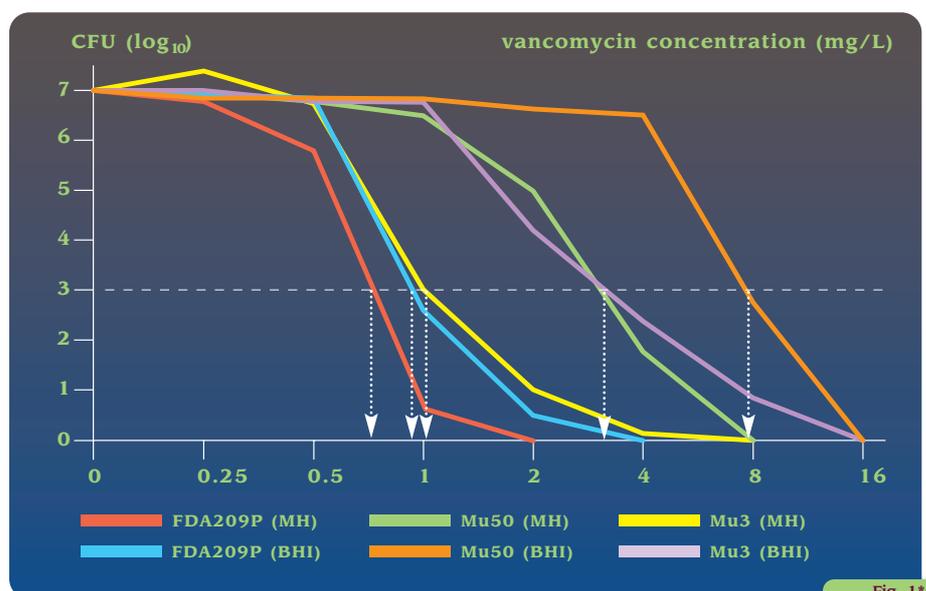


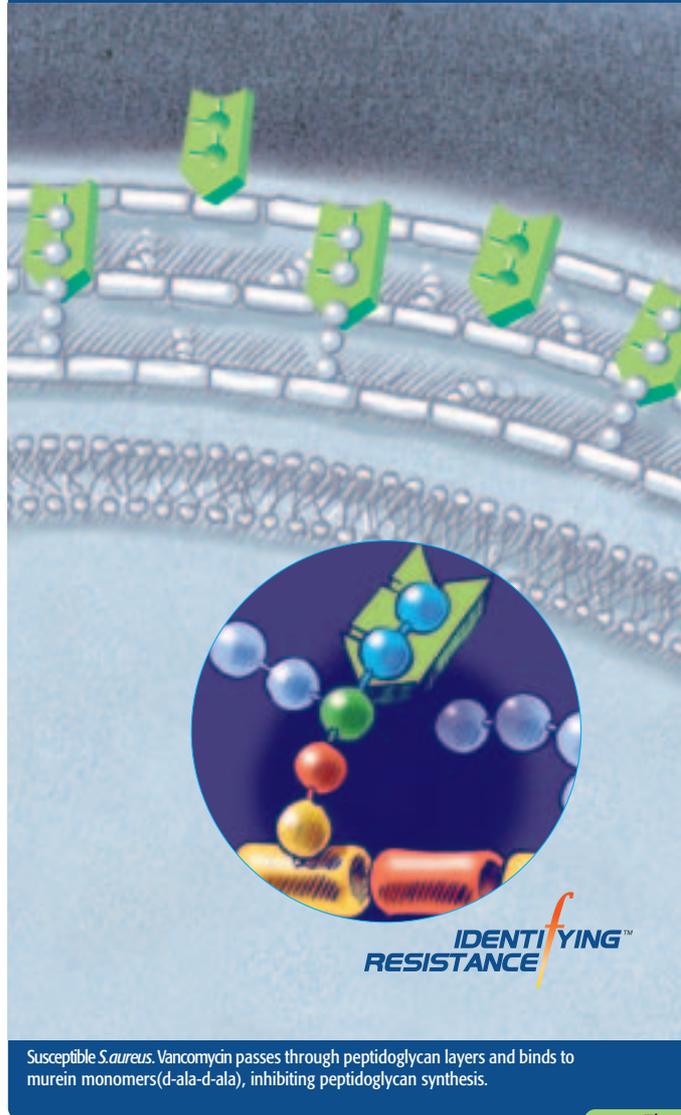
Fig. 1*

Population analysis: influence of media composition on vancomycin resistance

- ◆ FDA209P, Mu50 and Mu3 are respectively VSSA, VRSA and hetero-VRSA strains.
- ◆ Vancomycin resistance is more pronounced in Brain-Heart Infusion (BHI) agar than in Mueller-Hinton (MH) agar.
- ◆ Vancomycin resistance is higher with broth-dilution than with agar-dilution. However, BHI agar gives a MIC = 8 mg/L when used for agar-dilution susceptibility test of Mu50.

in *Staphylococcus aureus*

Susceptible *S. aureus*

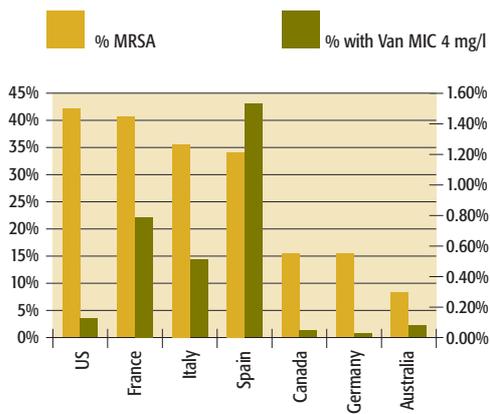


Susceptible *S. aureus*. Vancomycin passes through peptidoglycan layers and binds to murein monomers (d-ala-d-ala), inhibiting peptidoglycan synthesis.

Fig. A*

Statistics of *S. aureus* clinical isolates

Percentage of MRSA among *S. aureus* strains isolated from clinical specimens and percentage of *S. aureus* strains with Van MIC = 4mg/l



Data was derived from The Surveillance Network® (TSN®) Databases in North America and Europe for the year 2000. Kindly provided by Focus Technologies, Inc., Herndon, Virginia, USA.

Biochemical and transmission electron microscopy (TEM) examination of Mu50, a representative VRSA strain, revealed that it produces an increased amount of peptidoglycan [7] [10]. More murein monomers are produced and 30-40 layers of peptidoglycan are present in the cell wall (Figure A ; only three layers are drawn). As a result, more vancomycin molecules are trapped in the peptidoglycan layers before reaching the cytoplasmic membrane where peptidoglycan synthesis is ongoing. Moreover, a higher concentration of vancomycin would be required to saturate all the murein monomers that are supplied at an increased rate in Mu50. Besides the vancomycin-trapping mechanism, designated 'affinity trapping' [9] [3], our recent experiments suggest that dense accumulation of vancomycin molecules within the thickened cell-wall significantly delays the timing of complete inhibition of cell-wall synthesis by not allowing efficient penetration of vancomycin molecules through the thickened cell-wall layers [7] [11]. This apparent «mesh clogging» phenomenon may be due to either physical or electrostatic mechanisms, since vancomycin is a cationic compound with a considerably large molecular size (MW,1449.27).

Hetero-VRSA is the essence of the problem

Unlike vancomycin resistance in enterococci, *S. aureus* cells have to maintain vancomycin resistance by producing a thick cell wall. This requires either accelerated cell-wall synthesis or prolonged generation time. The former mechanism, represented by Mu50, requires many nutrients from the environment and consumes a lot of energy [7] [10].

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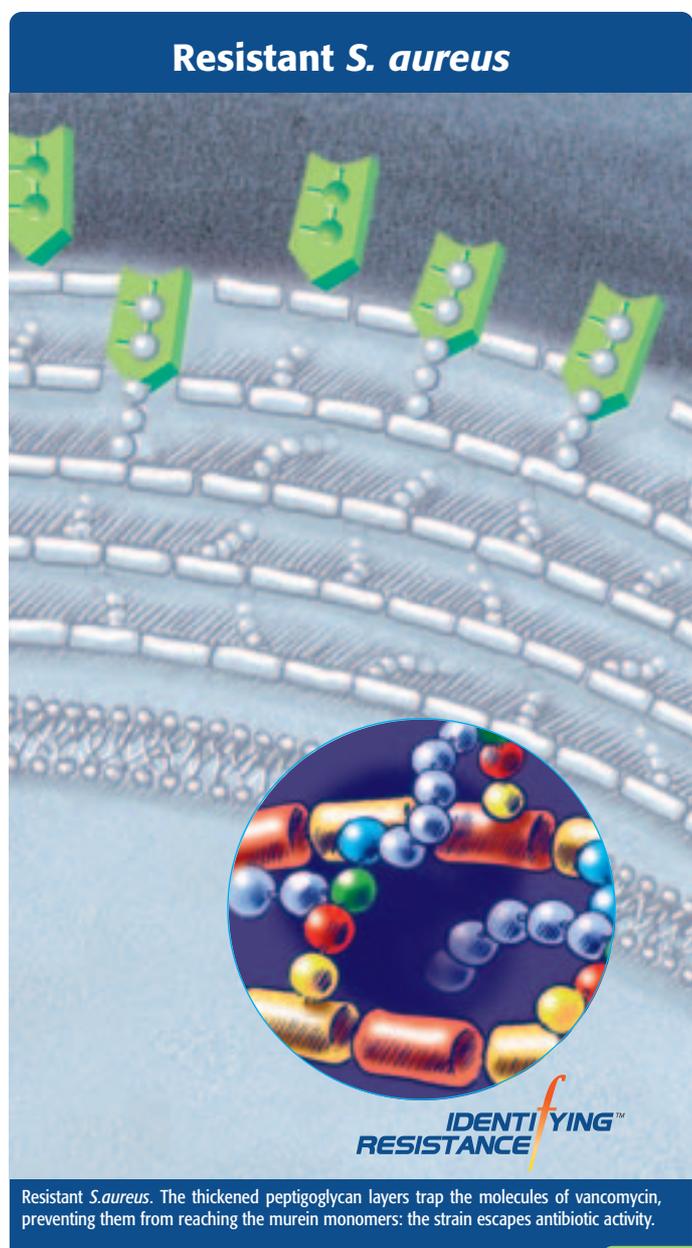


Fig. B*

* Figures are modified from reference 3.

Growth and detection of Glycopeptide intermediate *Staphylococcus aureus* in VITEK and VITEK® 2 Systems

D.J. LEAHART*, S.R. FITZSIMMONS, and M. PEYRET
 bioMérieux Vitek, Inc., Hazelwood, MO.
 ASM, Atlanta, May, 1998

The glycopeptide antibiotic vancomycin has been the mainstay of antimicrobial therapy in the treatment of serious infections caused by methicillin-resistant staphylococci (MRSA). The recent appearance of strains of *Staphylococcus aureus* with reduced susceptibility to vancomycin has serious consequences, as limited alternative antimicrobial agents are available for treating serious MRSA infections. Therefore, it is essential that susceptibility methods be able to accurately detect this emerging resistance. In this study two strains of *Staphylococcus aureus*, CDC HIP-5827 and CDC HIP-5836, previously isolated in the United States, and exhibiting reduced susceptibility to vancomycin (MIC = 8 µg/ml) were tested on Vitek and VITEK 2 Systems with 3 different lots of Gram positive susceptibility cards containing vancomycin.

Staphylococcus aureus ATCC 29213 served as a control. MICs for CDC HIP-5827 and CDC HIP-5836 were reported as 4, 4, 4 µg/ml and 4, 8, 4 µg/ml by Vitek and 8, 16, 8 µg/ml and 16, 16, 8 µg/ml respectively by VITEK 2. Current CDC guidelines state that all staphylococci with an MIC of 4 µg/ml be considered as candidates for reduced vancomycin susceptibility. Growth characteristics for HIP-5827 and HIP-5836, in increasing concentrations of vancomycin (4 µg/ml), serve to differentiate these strains from the susceptible ATCC control strain. Reproducibility testing showed these patterns to be consistent, enabling these strains to be used as a predictive model for the growth of strains with reduced susceptibility to vancomycin when tested with the Vitek and VITEK 2 Systems. Reduced susceptibility was also seen with teicoplanin, with the modal MICs for Vitek and VITEK 2 being 4 µg/ml and 4-8 µg/ml respectively. In this study the Vitek and VITEK 2 were shown to be capable of accurately and reproducibly detecting strains of *Staphylococcus aureus* with reduced susceptibility to vancomycin.

VITEK® 2 in Japan



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 Toho University Hospital,
 Tokyo, Japan

CDC Web site: <http://www.cdc.gov/>

Several pages are dealing with GISA/VISA issue.

<http://www.cdc.gov/ncidod/hip/lab/FactSheet/gisa.htm>

Fact sheet on lab aspects.

<http://www.cdc.gov/ncidod/hip/AREST/SEARCH>

Definitions and recommendations
 (how to report and submit isolates).

<http://www.cdc.gov/ncidod/hip/arest/visa.htm>

Fact Sheet focusing on medical aspects

10th ISSSI

10th International Symposium on Staphylococci and Staphylococcal Infections

October 16 - 19, 2002
 Tsukuba Japan (next to Tokyo)

Chairman

Keiichi Hiramatsu,
 Juntendo University Department of Bacteriology

Congress Secretariat

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<http://www.staphylococcus.net/>

The symposium is being hosted by Japanese Symposium on Staphylococci and Staphylococcal Infections (ISSSI). ISSSI has a long history (the first gathering was about 40 years ago), and has greatly promoted Staphylococcal research in Japan. At present, ISSSI is composed of about 300 dedicated basic and clinical researchers in the field of staphylococci.

Venue: Epochal Tsukuba International Congress Center

Organized by: Juntendo University Dept. of Bacteriology



DID YOU KNOW?

NCCLS recommendations

Table 2C M7-MIC

NCCLS Guidelines provide MIC interpretive standards for Antibiotic Susceptibility Testings. Staphylococci are treated in **Table 2C**, along with comment 14.

| test/report Group | | 2 | 4 | 8 | 16 | 32 | 64 |
|-------------------|-------------|---|---|---|----|----|----|
| B | vancomycin | 2 | 4 | 8 | 16 | 32 | 64 |
| Inv | teicoplanin | 2 | 4 | 8 | 16 | 32 | 64 |
| | | S | | I | | R | |

Comment 14

No vancomycin-resistant staphylococci have been isolated. Send any staphylococci determined to have an elevated MIC for vancomycin (≥ 4 mg/l) to a reference laboratory

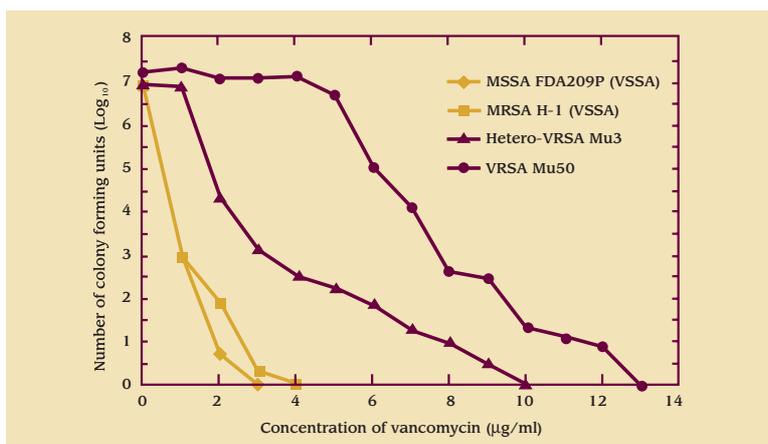
Population Analysis

Ref.: Hanaki H, Hiramatsu K. Detection methods of glycopeptide-resistant *Staphylococcus aureus* I: Susceptibility testing. In: Gillespie SH, ed. Antibiotic Resistance Methods and Protocols. Totowa, New Jersey: Humana Press, 2001: 85-92.

"Population analysis is one of the most important methods employed in the study of glycopeptide resistance in *S.aureus*. It allows heterogeneous resistance to be detected simply."

- ◆ Culture test and control bacteria overnight in TSB, then adjust the optical density to OD 0.3 at 578 nm.
- ◆ Make 10-fold serial dilutions from a portion (μ L) of the cell suspension and spread with sterilized spreader onto the BHIA plates containing varied concentrations of vancomycin: 0, 1, 2... 7, 8, 9, 10 mg/ml.
- ◆ Count the number of colonies growing on each plate after incubation at 37°C for 48 h.
- ◆ Plot the colony counts on a semi-logarithmic graph with colony counts on the vertical axis and vancomycin concentration on the horizontal axis.

Analysis of resistant subpopulations of VRSA Mu50, Hetero-VRSA Mu3, and vancomycin-susceptible strains



Glycopeptide antibiotics

This is a large family of agents with only few used as antibiotics. The classification shows four groups, named after their main molecules: vancomycin, avoparcin, ristocetin, teicoplanin types.

| | | | |
|-------------|---|-----|--------------------------|
| vancomycin | Vancocin [®] , Lyphocin [®] | VAN | Eli Lilly, Shionogi, etc |
| teicoplanin | Targocid [®] | TEC | Aventis |

Others are under development such as daptomycin, ramoplanin, etc...

Phenotype

- wild S
- Tec R
- GISA

PRACTICAL ADVICE

How important is the problem?

- 1 ■ it is very rare
- 2 ■ it is very serious as patient outcome may be fatal

When to suspect a VISA strain?

Any strain with a Van MIC = 4 can be suspected as being a VISA strain.

How to confirm?

MIC determination can be performed according to NCCLS methodology. The best solution is to perform a population analysis test. The strain can be sent to a lab with experience regarding this test.

What therapy is possible?

Main alternative antibiotics are Linezolid and Quinupristine-Dalfopristin.

LATEST NEWS

Sivert et al. just published in MMWR dated July 5, 2002 "Staphylococcus aureus Resistant to Vancomycin".

The strain has been studied by the CDC and MIC results for vancomycin, teicoplanin and oxacillin were >128, 32, >16 mg/l. The isolate contained the *vanA* vancomycin resistance gene from enterococci.

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