



## Epidemiology and Clinical Implications of Glycopeptide-resistant *Staphylococcus aureus*

Michael Jacobs

Department of Pathology  
Case Western Reserve University, USA

Development of resistance in *Staphylococcus aureus* to antimicrobial agents is a major problem, starting with resistance to penicillin G due to  $\beta$ -lactamase production, followed by development of methicillin resistant *S. aureus* (MRSA) in the 1960s through an altered penicillin binding protein 2a encoded by the *mecA* gene.<sup>1,3</sup> Until recently, MRSA was predominantly a problem of institutions such as acute and chronic care hospitals, but it is now also a problem in other institutions such as jails and child care centers, and is increasingly community-acquired, such as in competitive contact sports participants.<sup>4</sup> *S. aureus* had, until recently, retained its susceptibility to glycopeptides such as vancomycin, with MICs of vancomycin ranging from  $\leq 0.25$  to  $2 \mu\text{g}/\text{mL}$ , and with MIC<sub>50</sub> and MIC<sub>90</sub> values of  $1 \mu\text{g}/\text{mL}$ .<sup>5,6</sup> Current susceptibility breakpoints for vancomycin are as follows: MICs of  $\leq 4 \mu\text{g}/\text{mL}$ , susceptible;  $8\text{-}16 \mu\text{g}/\text{mL}$ , intermediate; and  $\geq 32 \mu\text{g}/\text{mL}$ , resistant.<sup>7</sup> However, strains with vancomycin MICs of  $2 \mu\text{g}/\text{mL}$  may not always be susceptible and should be retested especially if the patients do not respond to vancomycin.

### Development of vancomycin resistance in enterococci

High-level vancomycin resistance in enterococci (VRE) (VanA or VanB type) developed in the mid-1980s associated with the acquisition of around 10 kb of DNA encoding polypeptides resulting in synthesis of abnormal pentapeptide peptidoglycan precursors to which neither vancomycin nor teicoplanin bind with high affinity, and hydrolysis of precursors of normal peptidoglycan.<sup>8,9</sup> Unlike the normal peptidoglycan precursors, which have D-alanyl D-alanine (D-Ala D-Ala) dipeptide termini, those of VRE end with the depsipeptide D-alanyl D-lactate (D-Ala D-Lac). The affinity of vancomycin for these altered molecules is 1,000 times lower than its affinity for the native PG precursor. VanA enterococci are resistant to high levels of vancomycin (MIC,  $\geq 64 \mu\text{g}/\text{mL}$ ) and teicoplanin (MIC,  $\geq 8 \mu\text{g}/\text{mL}$ ), and resistance is induced by the presence of either drug. VanB organisms are resistant to a range of vancomycin concentrations, from 4 to  $>1024 \mu\text{g}/\text{mL}$ , but typically retain susceptibility to teicoplanin, which does not induce resistance.

### Experimental transfer of vancomycin resistance from *Enterococcus* to *S. aureus*

Transfer of resistance genes from a vancomycin resistant *Enterococcus faecalis* to *S. aureus* was reported by Noble, *et al.* in 1992.<sup>10</sup> Resistance transfer was shown *in vitro* on filters ( $<1$  in  $10^6$  recipients), and *in*

*in vivo* on the occluded skin of mice ( $10^0$  to  $10^3$  per  $10^6$  recipients), and the authors predicted that as high vancomycin resistance can be transferred from an *Enterococcus* to a *Staphylococcus* under conditions similar to those that exist in nature, wild-type *S. aureus* will acquire vancomycin resistance in time.

### Development of vancomycin intermediate *S. aureus*

The first report of *S. aureus* with reduced susceptibility to vancomycin (MIC of 8  $\mu\text{g}/\text{mL}$ ) was from Japan.<sup>11</sup> Subsequently, 8 isolates of vancomycin-intermediate *S. aureus* (VISA) have been documented in the US from patients with clinical infections.<sup>12-17</sup> Two mechanisms of resistance have been described in VISA strains : 1) an increased proportion of glutamine nonamidated mucopeptides, and 2) decreased cross-linkage of the peptidoglycan, with drastically reduced levels of PBP4, and change in the structure and/or metabolism of teichoic acids. The effect of reduced vancomycin susceptibility with both mechanisms is abnormal, thickened cell walls.<sup>18,19</sup>

### Development of vancomycin resistant *S. aureus*

Four isolates of VRSA have been reported in the USA, with isolates found in June 2002 and February 2005 in Michigan,<sup>20-22</sup> in September 2002 in Pennsylvania,<sup>23-25</sup> and in March 2004 in New York.<sup>26</sup> Common features of these four patients were a history of chronic underlying diseases (diabetes, morbid obesity, residence in chronic care facility, peripheral vascular insufficiency with skin ulceration) and isolation of VRSA from skin ulcers or urine. These isolates have been highly resistant to vancomycin (MICs  $\geq 32 \mu\text{g}/\text{mL}$ ), although resistance requires induction in some isolates and may be missed by some automated susceptibility test systems. The first two isolates have been shown to have plasmid-mediated vancomycin resistance due to *vanA*, which was probably acquired from VRE present in the lame lesion in the first Michigan case.<sup>25,27</sup> VRSA strains are resistant to glycopeptides, but are susceptible to lipopeptides such as daptomycin.<sup>28</sup> The first Michigan VRSA was highly resistant to vancomycin, with MICs of 1024  $\mu\text{g}/\text{mL}$  by broth microdilution and  $>256 \mu\text{g}/\text{mL}$  by E-test.<sup>21</sup> The isolate was susceptible to chloramphenicol, linezolid, minocycline, quinupristin-dalfopristin, and trimethoprim-sulfamethoxazole. The Pennsylvania VRSA isolate showed inducible vancomycin resistance, with a small area of clearing noted within a zone of reduced growth around vancomycin disks and E-test,<sup>25</sup> growth was observed on agar screen plates containing 6  $\mu\text{g}/\text{mL}$  of vancomycin, and the vancomycin MIC was 64  $\mu\text{g}/\text{mL}$  using the E-test method and 32  $\mu\text{g}/\text{mL}$  by the NCCLS broth microdilution method, while the teicoplanin MIC was 8  $\mu\text{g}/\text{mL}$  by broth microdilution. This isolate was resistant to aminoglycosides, macrolides, oxacillin, rifampin, and tetracycline, but susceptible to chloramphenicol, linezolid, minocycline, quinupristin-dalfopristin, rifampin, and trimethoprim-sulfamethoxazole. Vancomycin susceptibility was tested by three automated methods, i.e., MicroScan conventional MIC plates, Vitek cards, and Vitek 2 cards, and these methods showed the strain to be vancomycin susceptible or intermediate. Based on these limitations, staphylococci should be tested on agar plates containing 6  $\mu\text{g}$  of vancomycin per mL.<sup>28</sup>

### Conclusions

Vancomycin resistance in enterococci developed in the latter half of the 1980s, and was associated

with a complex mechanism encoded by a series of genes carried on a transposon. These changes resulted in remodeling of the cell glycopeptide, with replacement of the terminal D-alanine molecule in the pentapeptide component with D-lactose. This was followed by a report in 1992 of the successful experimental transfer of vancomycin resistance from an enterococcus to *S. aureus* both *in vitro* and *in vivo*, with the frequency of transfer being considerably higher in the *in vivo* model. A number of vancomycin intermediate strains have been described, and these strains have abnormal, thickened cells walls in the presence of vancomycin. The long anticipated development of vancomycin resistance in *S. aureus* has now occurred in four instances, and a number of vancomycin intermediate strains have been described. In two instances, the patients with VRSA had skin lesions coinfecting with vancomycin resistant, *vanA* genotype, *E. faecium*, and the *vanA* resistance genes had been transferred to the *S. aureus* strains. Expression of resistance has been constitutive in some VRSA strains and inducible others, making detection more challenging in the latter instance, and staphylococci should be tested on agar plates containing 6 µg of vancomycin per mL. These developments are of great concern, and every effort should be made to prevent further development and spread of vancomycin resistance in staphylococci.

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