Staphylococcus epidermidis with Intermediate Resistance to Vancomycin: Elusive Phenotype or Laboratory Artifact?

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The recent and troubling isolation of Staphylococcus aureus and coagulase-negative staphylococci that have increased resistance to glycopeptide antibiotics has prompted the use of aggressive surveillance measures in the clinical microbiology laboratory to aid in the recognition of these strains. Despite increasing awareness, the confirmation of glycopeptide resistance among staphylococci can be problematic; we present a case of catheter-associated peritonitis caused by Staphylococcus epidermidis to illustrate the dilemma.

Clinical microbiology laboratories worldwide have been on heightened alert to watch for glycopeptide-resistant strains of staphylococci isolated from clinical specimens. This state of heightened alert was prompted by reports of clinically significant strains of Staphylococcus aureus or CONS that has been tested at Henry Ford Hospital during a 7-year period (of ∼28,000 isolates tested) demonstrated a phenotype that showed verifiable intermediate or full resistance to vancomycin when primary susceptibility results (by use of the VITEK system [bioMérieux VITEK] or disk diffusion) were followed with confirmatory testing (by use of E-test; AB Biodisk).

Taken as a whole, these data indicate that rapid and widespread transfer of stable glycopeptide resistance from one strain to another has yet to occur in our region, despite the recent isolation of a glycopeptide-resistant strain of S. aureus in the metropolitan Detroit area [11]. Rather, glycopeptide resistance among the staphylococci appears to be a function of heteroresistant subpopulations [3, 8, 13] and antibiotic selection in vivo [2, 4, 5, 7–11]. For some strains, this phenomenon can be replicated in the laboratory by serial exposure to increasing concentrations of glycopeptide antibiotics [3, 5–8].

Until recently, all instances of falsely elevated MICs of vancomycin among staphylococci at Henry Ford Hospital could be ascribed to mixed inocula or unreproducible results. However, the following case report illustrates a potential dilemma for clinical microbiologists when the vancomycin resistance of a strain of S. epidermidis cannot be substantiated or dismissed.

Case report. A 59-year-old African American woman with end-stage renal disease secondary to diabetes mellitus and uncontrolled hypertension had been receiving continuous ambulatory peritoneal dialysis (CAPD) since October 1996. Her medical history was significant for coronary artery disease, congestive heart failure, peripheral vascular occlusive disease, chronically infected decubitus foot ulcers, and allergy to penicillin. During the 4 years before presentation, the patient had experienced multiple episodes of CAPD-associated peritonitis caused by Enterobacter asburiae (December 1996), Acinetobacter calcoaceticus (November 1997), methicillin-resistant Staphylococcus aureus (MRSA; December 1997), methicillin-resistant CONS (December 1997 and February 1998), Pseudomonas aeruginosa (July 1998), Proteus mirabilis (July 1998), Enteroccus faecalis (September 1998), Propionibacterium acne (December 1998), and viridans group Streptococcus species (January 1999). These episodes had required multiple courses of anti-

fusion agar plate containing 6 μg/mL vancomycin and examining the plates for growth after 24 h of incubation. While primitive by current standards, this method failed to disclose a single strain with intermediate resistance to glycopeptides (unpublished data). Furthermore, not a single isolate of S. aureus or CONS that was tested at Henry Ford Hospital during a 7-year period (of ∼28,000 isolates tested) demonstrated a phenotype that showed verifiable intermediate or full resistance to vancomycin when primary susceptibility results (by use of the VITEK system [bioMérieux VITEK] or disk diffusion) were followed with confirmatory testing (by use of E-test; AB Biodisk).

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microbial therapy, including long-term treatment (3 months total) with vancomycin.

At the time of admission (September 1999), cultures of dialysis fluid specimens yielded Enterococcus faecalis that was susceptible to both ampicillin (MIC <0.5 μg/mL) and vancomycin (MIC <0.5 μg/mL). MRSA was recovered from a chronic de- cubitus ulcer on her right foot at the same time and vancomycin therapy was initiated. Cultures of specimens from the right foot wound remained positive for MRSA and also yielded Enterococcus faecalis and CONS, but the wound eventually improved and the peritonitis resolved. Vancomycin therapy was discontinued and the patient remained free of infection until November 1999, when methicillin-resistant CONS was recovered from the peritoneal dialysate, for which she was again treated with vancomycin. Susceptibility testing of the CONS isolate indicated that it was resistant to oxacillin (MIC >8 μg/mL) but susceptible to vancomycin (MIC, 1.0 μg/mL).

The peritonitis resolved and the patient was free of infection until April 2000, when peritonitis developed secondary to infection with methicillin-susceptible, vancomycin-susceptible CONS. Vancomycin therapy was initiated because of previous experience with methicillin-resistant CONS, but the infection did not resolve, and in June 2000 a sample of peritoneal dialysate was obtained for analysis and culture. The fluid contained 320 WBCs/mm³ (93% neutrophils, 1% lymphocytes, and 6% monocytes). CONS were recovered from a culture of the fluid, and susceptibility testing revealed the following MICs: ampicillin, >8 μg/mL; clindamycin, <0.5 μg/mL; erythromycin, <0.5 μg/mL; gentamicin, >16 μg/mL; oxacillin, 4 μg/mL; rifampin, <1 μg/mL; tetracycline, <1 μg/mL; trimethoprim-sulfamethoxazole, 8 and 152 μg/mL, respectively; vancomycin, 16 μg/mL.

Because of skepticism regarding the MIC of vancomycin, an E-test was performed. Results indicated a MIC of 12 μg/mL, which indicates intermediate resistance, and a disk-diffusion zone of inhibition 13 mm in diameter (i.e., no susceptibility). A biochemical profile of the isolate performed with use of the VITEK system allowed us to tentatively identify it as S. epidermidis, and the isolate was forwarded to the Michigan Department of Community Health Bacteriology Laboratory (Lansing, Michigan) for assessment. Analysis of 3 subsequent peritoneal dialysate samples with use of the VITEK system was also positive for vancomycin-intermediate S. epidermidis (VISE). At first isolation, these isolates had the same antibiotic and MIC of vancomycin as did the initial isolate (i.e., 16 μg/mL); a second morphotype of CONS was susceptible to vancomycin (MIC, 2 μg/mL). However, subculture and testing by use of VITEK, E-test, disk diffusion, and microbroth dilution (MicroScan; Dade Behring) revealed that all 4 VISE isolates were susceptible to vancomycin (MIC <4 μg/mL; zone of inhibition, >17 mm diameter); there was no change in interpretation for the other antimicrobial agents. Subsequent testing also showed that each isolate of VISE was susceptible to linezolid, quinupristin/dalfopristin, teicoplanin, chloramphenicol, and doxycycline.

To select for stable vancomycin-intermediate subpopulations, we cultured each isolate on vancomycin screening agar with an aztreonam disk for induction of resistance [8]. We recovered 8 colonies that had MICs of vancomycin that ranged from 6 to 12 μg/mL, as determined by means of the E-test. The frequency of isolation of the vancomycin-intermediate subpopulations ranged from $8 \times 10^{-7}$ to $4 \times 10^{-7}$ cfu/mL; that low frequency could be easily missed using the recommended National Committee for Clinical Laboratory Standards inoculum density of $5 \times 10^{5}$ cfu/mL. The Michigan Department of Community Health Bacteriology Laboratory confirmed the identity of the initial isolate as VISE (S. epidermidis with an MIC of vancomycin of 6 μg/mL). A subculture of the isolate was forwarded to the Centers for Disease Control and Prevention (Atlanta) where the MIC of vancomycin was determined to be 4 μg/mL by use of standard microbroth dilution testing. The patient was subsequently readmitted to the hospital in July 2000 with abdominal pain and cloudy peritoneal fluid. Her catheter was removed following repeated isolation of S. epidermidis that had susceptibility patterns identical to those of the initial isolate. Her abdominal symptoms subsequently resolved.

This case illustrates the potential for staphylococci with reduced susceptibility to vancomycin to be overlooked in the clinical microbiology laboratory. While primary testing of 4 strains of S. epidermidis recovered from this patient indicated increased resistance to vancomycin, subsequent testing of subcultures of these isolates failed to confirm the initial findings. This suggests that either the susceptibility results of the primary cultures were in error or the expression of intermediate-level resistance by these isolates was lost or diluted upon subculture. Although both explanations have merit, a number of findings in this case support the latter conclusion. First, the results of the E-test and disk diffusion testing of the first of this patient’s isolates and the results of subsequent testing of colonies selected on vancomycin screening agar all corroborate the MIC of vancomycin obtained by use of VITEK for all primary isolates. Secondly, the patient’s risk factors (i.e., diabetes, CAPD, chronic infections with MRSA or methicillin-resistant CONS, indwelling catheter, and multiple courses of antimicrobial therapy that included vancomycin) closely parallel those of a number of reported cases of infection caused by strains of S. aureus with intermediate resistance to glycopeptides [8, 9–12, 14] and CONS [2, 4, 5, 7].

The decreased susceptibility of CONS to glycopeptide antibiotics appears to be the function of heterogeneous subpopulations of resistant organisms that occur at variable frequencies [3, 5, 8, 10]. The ability of any susceptibility test to
identify heteroresistant subpopulations requires that the inoculum tested be sufficiently large to compensate for the frequency of expression. It is possible, therefore, that the frequency of subpopulations with intermediate resistance to vancomycin in subcultures was much less than the frequency of such subpopulations that were originally present in the primary cultures; it is also possible that that, in the absence of vancomycin, subcultures effectively deselected for the expression of resistance altogether.

Del’Alamo et al. [1] noted the inconsistency of different susceptibility methods to detect increasing vancomycin resistance in 2 strains of CONS, quite possibly due to the inability of any one method to detect low-frequency heteroresistance. Whatever the explanation, the unsuspecting laboratorian in this circumstance would likely retest the isolate and (if they did not know the pertinent patient history) would conclude that the MIC of vancomycin measured initially was in error and would report the subsequent interpretation that the isolate was susceptible. It seems reasonable, therefore, to approach the interpretation of an elevated MIC of glycopeptide for staphylococci with caution rather than skepticism, especially in light of clinical risk factors such as those that were present in the patient we describe. The use of an induction method such as the one described by Wong et al. [8]—one that has an increased inoculum density to permit the detection of a low-frequency event—might be the only way to confirm whether there is increased glycopeptide resistance among certain strains of staphylococci.

References