Seeking Vancomycin Resistant \textit{Staphylococcus aureus} among Patients with Vancomycin-Resistant Enterococci

Diane Franchi,\textsuperscript{1,2} Michael W. Climo,\textsuperscript{2} Alice H. M. Wong,\textsuperscript{1,2} Michael B. Edmond,\textsuperscript{1,2} and Richard P. Wenzel\textsuperscript{1,2}

From the \textsuperscript{1}Division of Quality Health Care, \textsuperscript{2}Department of Internal Medicine, Medical College of Virginia/Virginia Commonwealth University, Richmond, Virginia

Clinical isolates of \textit{Staphylococcus aureus} displaying intermediate resistance to vancomycin (VISA) have been identified. The objective of our study was to identify VISA colonization among patients known to be colonized or infected with vancomycin-resistant enterococci (VRE). Eight weekly point prevalence screening surveys for VRE and \textit{S. aureus} were conducted on 5 hospital units. Of the 243 patients screened, 31 (12.8\%) were colonized with VRE. In addition, 18 inpatients were already known to be VRE-positive. Fourteen (28.6\%) of the 49 VRE-positive patients were co-colonized with \textit{S. aureus}. All 30 \textit{S. aureus} isolates from these 14 patients were methicillin-resistant (MRSA) but remained vancomycin-susceptible (minimal inhibitory concentration [MIC] range, 0.75–2 $\mu$g/mL). Population analysis profiling demonstrated no evidence of heteroresistant subpopulations that could grow on agar containing 3 $\mu$g/mL vancomycin for any of the MRSA isolates. Although 23 (77\%) of 30 staphylococcal isolates had vancomycin MICs of 1.5 or 2 $\mu$g/mL, no VISA strains (MICs, 8–16 $\mu$g/mL) were recovered.

The emergence of vancomycin-resistant enterococci (VRE) in 1988 heightened concern that vancomycin resistance would spread to \textit{Staphylococcus aureus} \cite{1}. In 1992, vancomycin resistance was transferred from \textit{Enterococcus faecalis} to \textit{S. aureus} in the laboratory by cell-to-cell mating \cite{2}. The transfer of resistance was thought to be transposon-mediated, but this was not established definitively. More recently, the selection of \textit{S. aureus} mutants highly resistant to vancomycin was achieved in the laboratory by gradually exposing these mutants to increasing concentrations of vancomycin during growth \cite{3}.

The first clinical isolate of \textit{S. aureus} with reduced susceptibility to vancomycin (MIC of 4 $\mu$g/mL) was reported from Japan in 1997 \cite{4}. Soon afterward, similar isolates were found in Michigan and New Jersey \cite{5, 6}. Recently a patient from New York was described \cite{7, 8}. All strains were also resistant to methicillin and were isolated from patients who had received multiple courses of vancomycin \cite{9}. In France, however, the first clinical isolate of \textit{S. aureus} with intermediate susceptibility to vancomycin (VISA) was cultured from the blood of a 2-year-old girl with leukemia who had had only one 10-day course of vancomycin \cite{10}.

Even though VISA are rare causes of clinical infections, we sought VISA colonization by surveying a high-risk hospitalized population. The population included patients who were infected and/or colonized with VRE, those likely to have been exposed to vancomycin.

Methods

Hospital Survey

This study was conducted at the Medical College of Virginia Hospital from March to May of 1997. Perianal swabs were used to detect VRE colonization, and swabs of the nares were used to detect \textit{S. aureus} colonization. There were 2 parts to the hospital survey. First, patients known to be colonized with VRE had their nares swabbed weekly to screen for co-colonization with \textit{S. aureus}. Second, perianal swabs and swabs of the nares were obtained from patients who occupied 5 targeted units and were not known to be colonized with VRE. The targeted units included 3 intensive care units, the hematology/oncology unit, and the bone marrow–transplant unit.

Microbiology

\textit{Enterococci}. Perianal swabs were screened on bile-esculin-azide agar containing 6 $\mu$g/mL vancomycin. Colonies representative of enterococcus were subcultured onto brain-heart infusion agar, and gram staining and catalase testing were done. Identification was carried out with API Strep Strips (bio-Mérieux, Marcy l’Etoile, France). Etest (AB BIODISK, Solna, Sweden) strips were used to determine vancomycin MICs in
accordance with National Committee for Clinical Laboratory Standards guidelines [11]. Enterococci with MICs ≥4 μg/mL were considered resistant to vancomycin.

**Staphylococci.** Material obtained by swabbing the nares was initially screened on mannitol-salt agar. Colonies fermenting mannitol were subcultured onto trypticase soy agar. Gram staining and catalase and coagulase tests were conducted to confirm the identification of *S. aureus*. Vancomycin susceptibilities were determined by use of Etest strips in accordance with National Committee for Clinical Laboratory Standards guidelines [11]. Tenover et al. [12] have shown that vancomycin susceptibility testing with Etest strips can identify most strains of *S. aureus* with reduced susceptibility to that drug.

*S. aureus* strains were also screened for oxacillin susceptibility by use of Mueller-Hinton agar containing 6 μg/mL oxacillin. Methicillin MICs were then determined by use of Etest strips. Staphylococci were considered resistant to methicillin (MRSA) if the MIC was ≥12 μg/mL.

The staphylococcal isolates showing a vancomycin MIC ≥1.5 μg/mL by the Etest method were tested for the presence of heteroresistant subpopulations with higher levels of vancomycin resistance by population analysis profiles generated on Mueller-Hinton agar containing 1.0 μg/mL, 2.0 μg/mL, 2.5 μg/mL, and 3.0 μg/mL vancomycin, as described elsewhere [13].

**Vancomycin Use**

The total amount of vancomycin received by each patient colonized with MRSA was tallied for the 6 months prior to the MRSA isolate collection dates. The total dose of vancomycin received by each patient ranged from 0 to 81.5 g (mean, 24.6 g; median, 5 g). Five patients were receiving vancomycin at the time the MRSA isolates were collected.

**Discussion**

In the United States, clinical isolates of VISA have been recovered from patients in the Midwest (Michigan) and the Northeast (New Jersey and New York). None, however, have been identified in the Southeast. We performed prospective surveys for clinical VISA strains among patients colonized with VRE, knowing that these patients are often exposed to vancomycin. However, no VISA isolates were identified in our small study of 261 patients.

There are 3 other important points to emphasize. First, not surprisingly, patients who are identified as colonized or infected with VRE after culturing for clinical reasons account for only a small fraction of patients who are colonized with VRE on a ward at one time, only 16% in our series. Second, the patients who were co-colonized with VRE and *S. aureus* in our study were all co-colonized with MRSA. Third, the vast majority of patients co-colonized with VRE (23 [77%] of 30) had staphylococcal isolates with vancomycin MICs of 1.5 or 2 μg/mL. Future work will examine the sequence in which patients become colonized with VRE and MRSA and the possibility of stepwise increases in vancomycin MICs among patients with VRE.

**References**


