Letter to the Editor

Unrecognized Cross-Infection With Vancomycin-Resistant *Enterococcus faecium* and *faecalis* Detected by Molecular Typing of Blood Isolates

To the Editor:

Studying the epidemiology of vancomycin-resistant enterococci (VRE) represents a major challenge.[1-10] Their epidemiology is complex, involving clonal spread, transfer of genetic elements, and the introduction of new strains.[11,12] Additionally, clinically apparent infection represents the tip of the iceberg regarding the pool of colonized subjects.[2] Generally, phenotyping is inadequate.[10] Therefore, typing of the isolates appears to be necessary.[9,10,13,14] Furthermore, traditional surveillance efforts that focus on location and date of identified infection are not sensitive enough to detect outbreaks caused by common bacteria, especially in a nonepidemic setting.[13]

In our institution, infection control professionals assess all patients from whom VRE is isolated for implementation of appropriate barriers and identification of possible linkages between affected patients. Because we had saved all VRE blood isolates during a 3-year period, we elected to characterize their molecular fingerprinting to determine the frequency of clonal cross-infection that was not recognized by routine surveillance.

Our institution is a 603-bed teaching hospital. The results of patients' blood cultures were prospectively monitored from January 1996 through December 1998. All VRE blood isolates were saved and stored at −70°C for typing. The medical records of identified patients were retrospectively reviewed for demographics, underlying illnesses, suspected sources of infection, dates of hospitalization, and geographic location(s).

The identification and susceptibility of organisms was determined using the Vitek gram-positive identification (GPI) card (bioMérieux-Vitek, Hazelwood, MO). Vancomycin resistance was determined by growth on vancomycin screen agar (Becton Dickinson Microbiology Systems, Cockeysville, MD). Molecular analysis was performed by preparing genomic DNA of individual vancomycin-resistant *E. faecium* and *faecalis* isolates in agarose plugs. Sample plugs were digested overnight with 40 U of *Sma*I (Gibco BRL Life Technologies, Inc., Gaithersburg, MD), were loaded in a 1.2% agarose gel in 0.5 × TRIS–boric acid/EDTA buffer, and underwent pulsed-field gel electrophoresis on a CHEF-DR II apparatus (Bio-Rad, Inc., Hercules, CA). The gel was stained with ethidium bromide, photographed, and analyzed by a ChemiImager 4000 (Alpha Innotech Corp., San Leandro, CA). Strains were considered indistinguishable, potentially related (1 to 3 bands different), or unrelated (> 3 bands different).[9,13] Cross-infection was considered possible when indistinguishable or closely related strains were recovered from patients with overlapping dates of hospitalization and locations.

Forty episodes of VRE bacteremia were detected among 35 patients during the 3-year study period (vancomycin-resistant *E. faecium*, 29; vancomycin-resistant *E. faecalis*, 11). All episodes were nosocomial or occurred in patients with prior hospitalization (median time from last discharge, 21.5 days; interquartile range, 135 days). The median age was 60 years (range, 2 months to 91 years; interquartile range, 29 years) and the male-to-female ratio was 1:1.6. All of the patients had one comorbid condition or more. The source of infection was unknown (65%), a vascular access (26%), or an intra-abdominal focus (9%). The median length of hospital stay prior to bacteremia was 10.5 days (interquartile range, 18 days). The overall mortality rate was 48.4%.

Genotyping of the 29 vancomycin-resistant *E. faecium* isolates from 26 patients identified 27 patterns: 25 patterns were detected in 24 patients, 1 other pattern was noted twice in 1 of these 24 patients who had multiple bacteremias, and 1 pattern was shared by 2 patients with overlapping hospital stays on the pediatric unit.

Genotyping of the 11 vancomycin-resistant *E. faecalis* isolates from 9 patients yielded 7 indistinguishable patterns: 5 different patterns in 5 patients, 1 pattern in 2 patients, and 1 pattern in 2 patients each with 2 separate bacteremias. The shared isolates and another isolate from a patient with a unique pattern displayed a 1- to 2-band difference, implying close relatedness. All patients with related or indistinguishable isolates were receiving hemodialysis and had overlapping hospital stays or outpatient dialysis. They emerged as 0 to 2 cases per month during a 7-month period (Figure).

Our findings suggest that nearly all vancomycin-resistant *E. faecium* blood infections represented individual occurrences. Documentation of cross-infection was limited to a set of two pediatric patients. In contrast, apparent cross-infection with vancomycin-resistant *E. faecalis* took place on the hemodialysis unit. Four cases emerged within a 3-month period and one emerged 3 months later, suggesting strain persistence in the unit or earlier acquisition by the patient.[15] The pattern of emergence (0 to 2 cases per month) made it possible for these cases to escape detection by routine surveillance. By the time we became aware of the possible cross-infection, no additional cases were noted to characterize this cluster.

A major limitation of our study is that we studied only blood isolates. This approach to the epidemiology of VRE is inadequate because most patients do not have bacteremia. Our intention was to point out the limitation of routine surveillance rather than to examine the spread of VRE. Additionally, the method used cannot detect cross-infections due to transfer of genetic elements or the simultaneous circulation of multiple strains.[11,12] Similar observations were made by Bischoff et al.[3] They performed molecular analysis of all VRE isolates during a 5-year period and demonstrated frequent unrecognized cross-infec-

tions. These findings underscore the difficulties in dealing with VRE. Although limited effectiveness is likely when stringent infection control measures are applied whenever patients with VRE are only identified through routine clinical cultures, the results may be better with active surveillance cultures as demonstrated by Ostrowsky et al.[9]

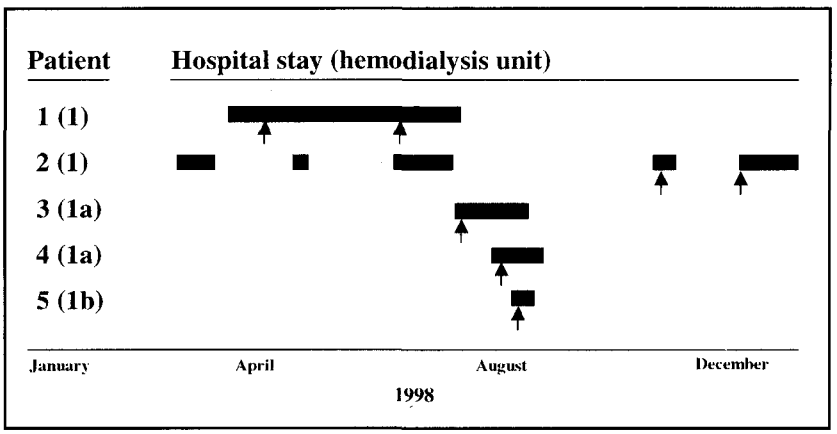

FIGURE. Hospital stay (bars) and sequence of emergence of bacteremia (arrows) of individual patients with indistinguishable or closely related vancomycin-resistant *Enterococcus faecalis* isolates (designated 1, 1a, and 1b), and opportunity for cross-infection.

REFERENCES

1. Dunne WM Jr, Wang W. Clonal dissemination and colony morphotype variation of vancomycin-resistant *Enterococcus faecium* isolates in metropolitan Detroit, Michigan. *J Clin Microbiol* 1997;35:388-392.
2. Moellering RC Jr. Emergence of *Enterococcus* as a significant pathogen. *Clin Infect Dis* 1992;14:1173-1178.
3. Bischoff WE, Reynolds TM, Hall GO, Wenzel RP, Edmond MB. Molecular epidemiology of vancomycin-resistant *Enterococcus faecium* in a large urban hospital over a 5-year period. *J Clin Microbiol* 1999;37:3912-3916.
4. von Gottoberg A, van Nierop W, Duse A, et al. Epidemiology of glycopeptide-resistant enterococci colonizing high-risk patients in hospitals in Johannesburg, Republic of South Africa. *J Clin Microbiol* 2000;38:905-909.
5. Gamabaratto K, Ploy M-C, Turlure P, et al. Prevalence of vancomycin-resistant enterococci in fecal samples from hospitalized patients and nonhospitalized controls in a cattle-rearing area of France. *J Clin Microbiol* 2000;38:620-624.
6. Bertrand X, Thouverez M, Bailly P, Cornette C, Talon D, Members of the Reseau France-Comtois de Lutte contre les infections nosocomiales. Clinical and molecular epidemiology of hospital *Enterococcus faecium* isolates in eastern France. *J Hosp Infect* 2000;45:125-134.
7. McMarthy KM, van Nierop W, Duse A, et al. Control of an outbreak of vancomycin-resistant *Enterococcus faecium* in an oncology ward in South Africa: effective use of limited resources. *J Hosp Infect* 2000;45:294-300.
8. Descheemaeker P, Leven M, Chapelle S, et al. Prevalence and molecular epidemiology of glycopeptide-resistant enterococci in Belgian renal units. *J Infect Dis* 2000;181:235-241.
9. Ostrowsky BE, Trick WE, Sohn AH, et al. Control of vancomycin-resistant *Enterococcus* in health care facilities in a region. *N Engl J Med* 2001;344:1427-1433.
10. D'Agata EMC, Green WK, Schulman G, Li H, Tang Y-W, Schaffner W. Vancomycin-resistant enterococci among chronic hemodialysis patients: a prospective study of acquisition. *Clin Infect Dis* 2001;32:23-29.
11. Kim WJ, Weinstein RA, Hayden MK. The changing epidemiology and the establishment of vancomycin resistance in enterococci at one hospital over a 6-year period. *J Infect Dis* 1999;179:163-171.
12. de Lencastre H, Brown AE, Chung M, Armstrong D, Tomasz A. Role of transposon Tn5482 in the epidemiology of vancomycin-resistant *Enterococcus faecium* in the pediatric oncology unit of a New York city hospital. *Microbial Drug Resistance-Mechanisms Epidemiology & Disease* 1999;5:113-129.
13. Almuneef MA, Baltimore RS, Farrel PA, Reagan-Cirincione P, Dembry LM. Molecular typing demonstrating transmission of gram-negative rods in a neonatal intensive care unit in the absence of a recognized epidemic. *Clin Infect Dis* 2001;32:220-227.
14. Savor C, Pfaller MA, Krusznyski JA, Hollis RJ, Noskin GA, Peterson LR. Comparison of genomic methods for differentiating strains of *Enterococcus faecium*: assessment using clinical epidemiologic data. *J Clin Microbiol* 1998;36:3327-3331.
15. Bonten MJ, Hayden MK, Nathan C, et al. Stability of vancomycin-resistant enterococcal genotypes isolated from long-term-colonized patients. *J Infect Dis* 1998;177:378-382.

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