Vancomycin-Resistant Enterococcus

by Peter C. Iwen, MS

The enterococci have emerged as major causes of nosocomial infections, recognized as the 3rd most common cause of bacteremia. This increase in infection is due in part to resistance to standard therapies, such as high level aminoglycosides and the beta-lactam antimicrobial agents--and more recently, to the glycopeptides, including vancomycin and the non-FDA approved agent, teicoplanin . Currently, there are no known effective antimicrobial Vancomycin-Resistant Enterococcus agents to treat infections caused by the vancomycin-resistant entorococci (VRE), with prevention and early detection the best approaches to control.

VRE can remain viable in the environment for an extended time period, and therefore poise a problem for infection control in hospitals and nursing homes. In addition, these enterococci have been detected as part of the enteric flora in non-symptomatic patients. These colonized patients serve as potential sources for transfer of this organism to other patients and medical personnel.

Classification

Currently, 14 species of enterooocci have been recovered from humans. *Enterococcus faecalis* accounts for 80 to 90% of enterococcal infections from all sources, with *E. faecium* responsible for a majority of the rest. The number of other species is generally less than 5%, although this may be higher, since methods to identify enterococci other than *E. faecalis* and *E. faecium*, are not widely used by clinical laboratories. In a study conducted at University Hospital evaluating enterococcal isolates recovered from blood cultures over eight years, *E. faecalis* was responsible for 68.5%, *E. faecium* for 26.2%, and the other enterococci for 5.3% **(Table 1)**. In this study, resistance was most evident with *E.faecium*, which was also responsible for all cases of vancomycin resistance. Nationally, resistance to vancomycin also occurs most frequently with *E.faecium*, even though other species of enterococci have become resistant. Intrinsic low-level vancomycin resistamce occurs with *E. casseliflavus* and *E. gallinarum*, generally the most common *nonf a ecal i s / fae c ium* enterococcal species detected. These VRE species are found as normal stool flora and are not usually considered clinically significant even though sporadic blood stream infections have been det e c t ed in se v e r e l y immunocompromised patients.

Recently, a phenotypic classification system was devised to categorize the VRE into three groups: *van*A strains, which show high-level vancomycin resistance (minimum inhibitory concentrations [MIC] of >32 mcg per ml) and resistance to teicoplanin; *van*B strains, which have variable resistance to vancomycin (MICs of 4 to >128 mcg per ml) and susceptibility to teicoplanin; and *van*C strains, which show intrinsic resistance to low-levels of vancomycin (MICs of 2 to l6 mcg per ml) and susceptibility to teicoplanin. These *van*C enterococci which include *E. casseliflavus* and *E. gallinarum* can be differentiated from other enterococci since they are usually positive for motility. It is important for the laboratorian to distinguish these motile species from the other enterococci which show high-level vancomycin resistance, since the former are not considered an epidemiological threat for nosocomial transfer and are usually susceptible susceptible to standard therapies.

Laboratory identification

VRE at the Nebraska Public Health Laboratory (NPHL) are generally detected by routine "Aerobic Culture" of a normally sterile body site, by a “VRE Culture Screen", or as an incidental finding during the culture of stool for "Enteric Pathogens". Gram positive cocci with atypical macroscopic appearance, which are catalase-negative and spot pyrrolidonyl arylamidase- (PYR) positive are suspected *Enterococcus* species. Also, growth of an isolate with these characteristics on CVA medium, which is a selective medium used in an enteric

pathogen screen culture to detect *Campylobacter* in stool, should be considered suspicious for the presence of VRE. This medium supports the growth of enterococci and contains vancomycin in a concentration adequate to screen for resistance.

Suspected enterococcal isolates considered clinically significant or isolates which grow on CVA medium are subsequently tested by biochemicals for identification and by tests for susceptibility to, high-levels of gentamicin, high-levels of streptomycin, and ampicillin. Additionally, an agar dilution test - containing vancomycin is also inoculated as an initial screen for vancomycin resistance. Isolates identified as *Enterococcus* species which grow in the presence of vancomycin on the agar dilution plate are subsequently confirmation tested. This includes vancomycin and teicoplanin disk diffusion and motility tests to screen for the low-level vancomycin resistant motile enterococci (*van*C strains). Isolates confirmed as nonmotile and by the disk diffusion as resistant to vancomycin, are identified as VRE. New patients with VRE detected, whether colonized or infected, are subsequently reported to Infection Control to initiate isolation procedures. Teicoplanin results are used only for epidemiological purposes to classify isolates as a *van*A or *van*B phenotype.

Conclusion

The first vancomycin resistant Enterococci was detected in August 1993 at NHS-University (formally University Hospital). Since that time, numerous additiorral isolates have been reported. At present, *E. faecium* has been the only Enterococcus species associated with highlevel resistance to vancomycin, with an approximate 70 to 30 ratio between *van*A and *van*B phenotypes, respectively. A majority of patients have been identified as colonized by a “VRE Culture Screen", or as an incidental finding from slool. Blood and peritoneal fluid have been the most common source of VRE-caused infection. Personnel at the NPHL are interested in conducting a a statewide surveillance for VRE to evaluate clonality among isolates and they would welcome submission of these isolates from through out the State of Nebraska for banking and additional testing. Additionally, the NPHL offers verification testing for the identification of VRE. To submit isolates for verification or for banking , complete a "Special Microbiology Requisition Form", and submit this along with the isolate to the NPHL. For additional information or to receive a copyof the requisition form by FAX, call Peter Iwen at (402) 559-7774.

Table 1.

Antimicrobial susceptibility of *Enterococcus* species isolated from blood cultures (1988 through 1995). [a,b,c]

Species and time period	Total no. identified	% Resistant			
		AM	GM	ST	VA
E.faecalis					
1988-91	142	0	4.9	7.7	0
1992-95	148	0.7	32.4	17.7	0
E.faecium					
1988-91	35	17.1	0	20.0	0
1992-95	75	60.0	32.0	42.7	22.7
E.gallinarum[d]	6	0	0	0	0
E.casseliflavus[d]	5	0	0	0	0
E.raffinosus	5	60.0	0	0	0
E.durans	3	0	0	0	0
E.hirae	2	0	0	0	0
E.avium	1	0	0	0	0

Abbreviations: AM = ampicillin, GM = high-level gentamicin, ST = high-level streptomycin, VA = vancomycin

[a]Condensed from; Iwen, et al. 1997. Change in prevalence and antibiotic resistance of *Enterococcus* species isolated from blood cultures over an 8-year period. **Antimicrob. Agents Chemother.**, 41:494.

[b]One isolate per patient only.

[c]Species were identified using conventional macrotube biochemical tests.

[d]These isolates have intrinsic low-level resistance to vancomycin.