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Prevalence of Vancomycin-Resistant *Enterococcus* in Prenatal Screening Cultures

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Recommendations for the perinatal treatment of women colonized with *Streptococcus agalactiae* include vancomycin prophylaxis for those with severe penicillin allergies and antibiotic-resistant organisms. Because of potential postpartum infections due to vancomycin-resistant enterococci (VRE) and the possible spread of vancomycin resistance, the prevalence of VRE in prenatal screening cultures was determined.

Enterococcus spp. are a component of the human intestinal flora and may be found naturally in the birth canals of women. These organisms are also a common cause of hospital-acquired urinary tract, wound, and bloodstream infections. Although *Enterococcus* is rarely reported to cause perinatally acquired neonatal infections, it is commonly isolated from women with postcesarean endometritis and wound infections (3, 18, 24, 29). *Streptococcus agalactiae* can also colonize the vaginas and/or recta of women and is a well-described neonatal pathogen that is transmitted perinatally. Vertical transmission of *S. agalactiae* is associated with increased morbidity and mortality in neonates due to pneumonia, sepsis, and meningitis (11, 17). Antimicrobial resistance is an increasing problem for both of these organisms. Vancomycin-resistant enterococci (VRE) are more refractory to treatment than their sensitive counterparts, and *S. agalactiae* is becoming increasingly resistant to erythromycin and clindamycin (1, 14–16, 23).

In 2002, the Centers for Disease Control and Prevention recommended that all pregnant women be screened for the presence of *S. agalactiae* (group B *Streptococcus*) and published guidelines for the treatment of colonized pregnant women (5). Penicillin is the drug of choice for the prophylactic treatment of *S. agalactiae*. For severely penicillin-allergic mothers, the alternative treatment is either erythromycin or clindamycin. However, antibiotic resistance among *S. agalactiae* isolates is increasing. At the University of North Carolina Hospitals, 31% of *S. agalactiae* isolates were found to be resistant to erythromycin and two-thirds of these isolates were also resistant to clindamycin (M. B. Miller, M. C. Jones, E. Rogers, V. Lang, and P. H. Gilligan, Abstr. 103rd Gen. Meet. Am. Soc. Microbiol., abstr. C-124, 2003). Further, in some cases, in vitro susceptibility data may not be available for prenatal isolates. In these instances, vancomycin has been recommended for prophylaxis of perinatal *S. agalactiae* infection.

The use of vancomycin in penicillin-allergic women may precipitate an increase in the presence of VRE in this population, which raises at least two concerns. First, if postpartum infections develop in either the mother or the neonate, empirical therapy with vancomycin will not be effective. Second, since vancomycin resistance can be transferred between bacteria, the presence of VRE might promote an increase in vancomycin resistance in other vaginal or rectal gram-positive organisms (6, 12, 19). Therefore, to assess the potential for VRE-associated postpartum infections and the possible bacterial transfer of vancomycin resistance in this patient population, the prevalence of VRE in routine prenatal screening cultures was determined.

(A preliminary report of this work has been presented previously [S. L. Allen, M. E. Mangum, M. B. Miller, A. Doutova, and P. H. Gilligan, Abstr. 103rd Gen. Meet. Am. Soc. Microbiol., abstr. C-413, 2003].)

Specimens (n = 447) received for prenatal *S. agalactiae* screening were inoculated into LIM enrichment broth and subsequently plated on sheep blood agar and Thayer-Martin (improved) medium (Remel, Lenexa, Kans.). Cultured sources included vaginal and rectal (197 specimens), vaginal (185 specimens), cervical (54 specimens), endocervical (5 specimens), rectal (5 specimens), and unspecified (1 specimen) sites. However, it should be noted that a chart review revealed that many of the swabs labeled as vaginal were, in fact, vaginal and rectal swabs. Growth on Thayer-Martin (improved) medium (3 μg of vancomycin/ml) was used as a first screen for the presence of *Enterococcus* with reduced susceptibility to vancomycin. Colonies consistent with *Enterococcus* were identified with Gram stain, catalase, bile esculin, and 6.5% NaCl media and by the pyrrolidonyl aminopeptidase test. Vancomycin MICs were determined by the Etest (Remel). All other susceptibility results were determined by disk diffusion (Becton Dickinson, Cockeysville, Md.) by using NCCLS interpretation guidelines. Isolates exhibiting resistance to vancomycin were further identified to species level on the basis of arabinose utilization, arginine decarboxylase activity, methyl-α-D-glucopyranoside acidification, motility, and pigmentation (4, 7, 9, 22, 30). Multiplex PCR for the *van* genes was performed with *Enterococcus* isolates as previously described (8, 10, 20). Genomic DNA for pulsed-field gel electrophoresis (PFGE) was prepared, di-

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gested with *Sma*I or *Not*I, and then subjected to PFGE (CHEF-DR III system; Bio-Rad, Hercules, Calif.) as described previously (2, 8, 21). The resulting banding patterns were interpreted according to published recommendations (26).

Sixty-eight percent of the prenatal screening cultures grew *Enterococcus* (305 isolates). For 5.6% of the *Enterococcus* isolates, intermediate vancomycin MICs were 4 to 8 μg/ml (17 isolates). The 17 *Enterococcus* species identified included *E. gallinarum* (14 isolates) and *E. casseliflavus* (3 isolates), which are known to have low-level intrinsic resistance to vancomycin due to nontransferable *van* genes (*vanC-1* and *vanC-2*, respectively). PCR was performed to confirm the *van* resistance determinants of these isolates. A multiplex PCR with *vanA* and *vanB* primers did not produce any PCR products for the 17 isolates in question, whereas the results of *vanC-1* and *vanC-2* multiplex PCR confirmed that all isolates identified as *E. gallinarum* harbored *vanC-1* but that the isolates identified as *E. casseliflavus* contained *vanC-2*.

Susceptibilities to seven commonly tested drugs were determined for the 17 *Enterococcus* isolates that were recovered from prenatal screening cultures. All of the isolates were sensitive to ampicillin, high-concentration gentamicin, chloramphenicol, and linezolid. Two isolates were resistant to high-concentration streptomycin, and resistance to tetracycline was observed for four isolates. Interestingly, all of the isolates that exhibited reduced susceptibility to vancomycin also showed reduced susceptibility to quinupristin-dalfopristin (10 isolates were intermediate, and 7 were resistant). While intrinsic resistance to streptogramins is known for *Enterococcus faecalis*, previous reports have not indicated increased levels of resistance among *E. casseliflavus* and *E. gallinarum* isolates (13, 25). In addition, only two of the 17 enterococci isolated from prenatal cultures appeared to be genetically related as determined by PFGE.

The data presented suggest that there is little resistance among *Enterococcus* strains recovered from screened pregnant women. Of the prenatal cultures, 3.8% contained *Enterococcus* isolates with intermediate resistance to vancomycin. Previous reports have demonstrated rectal colonization rates of *vanC*-containing enterococci ranging from 5 to 12% in various populations (27, 28). The colonization rate observed for prenatal cultures is lower likely due to the screening of specimens that did not include a rectal component. However, there are no published data regarding the prevalence of these organisms in nonrectal sites. Although this study suggests a 2.7% vaginal colonization rate for *vanC*-containing enterococci, this rate may be a misrepresentation due to clerical errors with regard to site identification.

Since none of the *Enterococcus* strains isolated contained *vanA* or *vanB*, there does not presently seem to be a threat of vancomycin resistance genes being transferred to other gram-positive species found in the vaginal and rectal sites of the women involved in this study. However, as the number of *S. agalactiae* isolates that are resistant to erythromycin and clindamycin increases, the use of vancomycin to treat penicillin-allergic pregnant women who harbor *S. agalactiae* will also increase. Thus, the incidence of VRE in this patient population may also increase, leading to the failure of empirical treatment with vancomycin for postpartum infections and the possible transfer of vancomycin resistance genes to other gram-positive species.

This study was approved by the Institutional Review Board of the University of North Carolina School of Medicine.

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