

Fecal Colonization with Vancomycin-Resistant Enterococci in Australia

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To assess the rate of fecal vancomycin-resistant enterococci (VRE) colonization in Australia, we examined specimens from 1,085 healthy volunteers. VRE was cultured from 2 (0.2%) of 1,085 specimens; both were *vanB* *Enterococcus faecium*, identical by pulsed-field gel electrophoresis, but with a pattern rare in Melbourne hospitals.

Colonization and infection with vancomycin-resistant enterococci (VRE) are emerging worldwide. In Europe, agricultural use of the glycopeptide growth promoter avoparcin has been implicated in the emergence of *vanA* VRE in the food chain (1), and fecal colonization rates of 2%-28% have been reported in some communities (2,3). In the United States, where avoparcin is not used, both *vanA* and *vanB* VRE strains appear to be largely nosocomially spread, with fecal VRE colonization rare in nonhospitalized patients (4,5). By contrast, in Australia, where avoparcin has been used widely (10,000 kg/year) in agriculture for many years (6) and the per capita consumption of antibiotics is one of the highest in the world (7), VRE (mostly *vanB* [8]) has only recently emerged as an important nosocomial pathogen. We assessed the rate of fecal VRE colonization in a population of healthy Australians.

The Study

In mid-1997, fecal specimens from 1,085 healthy volunteers were collected and frozen at -70°C as baseline specimens for a water quality study in Melbourne, Australia. These previously described (9) volunteers were from 294 families with young children who lived in a lower- to middle-class suburb. They were specifically

chosen because they were representative of young Australian families in eating habits and medical care. Thus, the elderly, the unmarried, and the very poor were not represented. Study participants had no history of diarrhea or antibiotic use at the time of specimen collection.

For VRE screening, frozen samples were thawed and spread onto enterococcosel agar (BBL Microbiology Systems, Cockeysville, MD) containing 6 µg/mL vancomycin and incubating at 35°C for 48-72 hours. Each sample was also incubated in enterococcosel broth (BBL) without vancomycin for 48 hours, before being spread onto enterococcosel agar containing 6 µg/mL vancomycin. From esculin-positive colonies, three of each morphologic appearance were selected from the direct agar and the subcultured enterococcosel broth cultures of each specimen and provisionally identified as either *Enterococcus faecium* or *E. faecalis* by routine criteria (gram-positive cocci, L-pyrrolidonyl-β-naphthylamide hydrolase-positive, nonmotile, catalase-negative, and pigment-negative) (10,11). All isolates fulfilling these criteria were assessed for susceptibility to vancomycin and teicoplanin by the E-test method (AB Biodisk, Dalvagen, Sweden). Isolates with an MIC to vancomycin ≥2 µg/mL were analyzed for the presence of *vanA*, *B*, *C1*, or *C2/3* genes by polymerase chain reaction (8); if *vanA*- or *vanB*-positive, they were then confirmed as either *E. faecium* or *E. faecalis* by automated biochemical analysis (BioMerieux Vitek Inc., MO) and by polymerase chain

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reaction, and were assessed by pulsed-field gel electrophoresis (PFGE) (8,12,13).

To be certain that this analysis of frozen specimens was a valid assessment of the rate of fecal VRE carriage in this population, two additional studies were conducted. First, to verify that -70°C storage did not affect the overall recovery of enterococci, a randomly selected subset of 112 of the 1,085 specimens were cultured on enterococcosel agar (BBL) without antibiotic and assessed for enterococci by routine identification techniques (10,11). In addition, to assess whether -70°C storage could selectively impair recovery of VRE, fresh fecal specimens were spiked with concentrations (10^4 - 10^8 /mL) of isolates of *E. faecalis* (*vanA*, n=1, *vanB*, n=2) or *E. faecium* (*vanA*, n=2; *vanB*, n=2), then stored at -70°C for 2 weeks before being thawed and cultured.

Results

Enterococci were identified in 106 of the subset of 112 specimens, a rate consistent with previous reports (14). VRE were also consistently recovered from our frozen spiked specimens. These findings suggest that -70°C storage of specimens does not affect the recovery of enterococci in general, or VRE in particular.

In the community study, VRE was isolated from 2 (0.2%) of the 1,085 (95% confidence intervals 0%-0.4%) specimens. Both isolates were *vanB E. faecium* and were detected in both agar and broth cultures. E-test MIC results at 24 hours for both isolates were 12 $\mu\text{g}/\text{mL}$ for vancomycin and 0.125 $\mu\text{g}/\text{mL}$ for teicoplanin. Although they were identical by PFGE, they displayed a PFGE pattern that is uncommon among VRE isolates from Melbourne hospitals. The two participants who were *vanB E. faecium* positive were not related. One was a 34-year-old man and the other a 30-year-old woman; both were married, and each had two children. None of the other family members had detectable fecal VRE colonization. The man had a history of stable ulcerative colitis and was receiving sulfasalazine.

Conclusions

This study suggests that fecal colonization with VRE is present but uncommon in Australia. Despite widespread agricultural use of avoparcin and high community rates of antibiotic use, *vanA*

E. faecium was not identified. *vanB E. faecium*, which is the most common clinical VRE strain in Australia (8) and whose nosocomial transmission remains a concern (15), was identified in healthy Australians.

Acknowledgments

The authors thank the staff at the Microbiological Diagnostic Unit, University of Melbourne, for the PFGE analysis.

Funding for this project was provided by the Department of Infectious Diseases and Clinical Epidemiology, Monash Medical Centre.

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