Vancomycin-resistant enterococci (VRE) were first reported from Europe in the late 1980s (1). The proposed origin of VRE was nosocomial, but these bacteria have also been isolated from several animal sources. The growth promoter avoparcin in animal husbandry has probably played a role in the development of resistance (2).

The rapid dissemination of VRE among humans has urged clinical microbiology laboratories to detect these organisms in clinical samples with more efficient and reliable methods. PCR-based techniques are increasingly used to verify vancomycin resistance and to determine the Van-type, but they are still not used in many routine laboratories. We report of a urine sample from which an Enterococcus faecium strain co-isolated with lactobacilli mimicked vancomycin resistance. It demonstrates some of the difficulties in identifying VRE and the need for molecular diagnostics.

The isolate was catalase negative, exhibited pyrrolidinyl arylamidase (PYR) activity, and had a Gram stain and colony morphology consistent with enterococci on blood and cephalaxin-aztreonam-arabinose agar (CAA) (3). It was identified as Enterococcus faecium due to its arabinose utilization. Antimicrobial susceptibility profile was established by disk diffusion (Oxoid) by methods recommended by Swedish Reference Group for Antibiotics (www.srga.org). The isolate was resistant to ampicillin, and the zone around the vancomycin disk (5 μg) was 19 mm with a sharp edge after 18 h, but a few colonies appeared in the inhibition zone, suggesting vancomycin resistance. The resistant colonies were analyzed further by a PCR method described by Dutka-Malen and coworkers (4). A single vancomycin-resistant colony was also subcultured on CAA, and the minimal inhibitory concentration (MIC) of vancomycin was determined by repeated Etests (AB Biodisk, Solna, Sweden) (5). The PCR detected gene sequences specific for Enterococcus faecium but not for vanA or vanB, and the CAA agar showed growth of Enterococcus faecium, as earlier, with a vancomycin MIC of >256 mg/l. However, the colonies that grew close to the Etest strip yielded no PCR products. Due to the suspicion of a mixed culture, subcultured colonies from the CAA-plate were inoculated onto chocolate agar. After 18–24 h, two different colony variants were distinguishable, one corresponding to Enterococcus faecium and the other to a PYR-negative bacterium, morphologically and phenotypically consistent with a Lactobacillus species from the acidophilus group. The vancomycin MIC of the pure enterococcal culture was 0.5 mg/l by Etest.

The resistance to glycopeptides in Lactobacillus spp. is principally the same as in VRE, but the enzymes involved are not closely related to vanA or vanB, and the resistance is not considered to be transferable (6). However, Lactobacilli may act as a reservoir of van genes (7).

Several lactobacilli have features in common with VRE, including natural habitat, microscopic appearance, growth on the CAA medium, traditional tests for phenotyping, and resistance patterns. Colonies of lactobacilli are usually quite small on most common media after an overnight incubation, and the activity of enterococcal bacteriocins may suppress the cogrowth of intrinsically vancomycin-resistant bacteria (8). Furthermore, lactobacilli in milk products have been used to improve clearance of VRE in humans (9), and mixtures of lactobacilli and enterococci have been used to improve broiler performance (10). This increases the risk of misidentification. In human medicine, unnecessary infection control measures might also be taken and inappropriate antibiotics prescribed.

How often enterococci and lactobacilli coexist in clinical samples is not known, but coisolation of these
bacteria on CAA medium has occurred more than once at our laboratories. Whenever a suspected VRE is isolated from a clinical sample, the purity of the culture should be carefully checked, or PCR techniques applied, to avoid expensive errors.

Conflict of interest and funding
The authors have not received any funding or benefits from industry or elsewhere to conduct this study.

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*Åsa Melhus
Department of Medical Sciences/Clinical Bacteriology
Uppsala University
SE-751 85 Uppsala, Sweden
Email: asa.melhus@akademiska.se