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Comparison of Inoculation Methods for Testing Enterococci by Using Vancomycin Screening Agar

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One hundred four recent clinical isolates of *Enterococcus* species were screened for vancomycin resistance by using inocula of 10^5 or 10^6 CFU dispensed by pipet and by use of a cotton swab dipped in a 0.5 McFarland standard organism suspension applied to the surface of brain heart infusion agar containing 6 μg of vancomycin per ml. The three inoculation methods were equivalent in the detection of nonsusceptible isolates. The use of swab inoculation was convenient and less costly than the use of micropipets.

The prevalence of vancomycin-resistant enterococci (VRE) has increased sharply in the United States in the last 5 years (1). The Centers for Disease Control and Prevention has published recommendations for preventing the spread of VRE within health care facilities that include prompt and accurate detection of such strains by clinical microbiology laboratories (2). However, there have been problems with accurate detection of VRE by certain conventional and automated antimicrobial susceptibility testing methods (7). For this reason, the National Committee for Clinical Laboratory Standards has recommended (4) use of the vancomycin agar screening test originally described by Willey et al. (8) for reliable detection of VRE clinical isolates. Studies conducted by the National Committee for Clinical Laboratory Standards VRE working group documented that inocula of either 10^5 or 10^6 CFU provided satisfactory detection of VRE strains with well-characterized resistance genotypes (6). Because it appeared in that study that some latitude existed in the final inoculum density applied to the screening agar and because of the preliminary findings of Willey et al. (8), we have investigated a simplified inoculum delivery method incorporating the use of a cotton swab dipped in a 0.5 McFarland standard organism suspension.

A group of 104 recent enterococcal clinical isolates or previously frozen cultures of clinical origin were used in this study. They included 48 isolates of *Enterococcus faecium*, 26 of *E. faecalis*, 9 of *E. gallinarum*, 3 of *E. casseliflavus*, and 18 of *Enterococcus* spp. not further classified. Included were 63 vancomycin-nonsusceptible isolates that could be further described by phenotype as 39 VanA^r, 12 VanB^r, and 12 VanC^r strains on the basis of vancomycin and teicoplanin MICs (9). The susceptibilities of all of the vancomycin-nonsusceptible strains to these two glycopeptides were determined by use of either the National Committee for Clinical Laboratory Standards broth microdilution method (3) or E-test strips (AB BIODISK, Piscataway, N.J.) placed on unsupplemented Mueller-Hinton agar plates (5). *E. faecalis* ATCC 51299 and ATCC 29212 were used as positive and negative control strains, respectively, for the vancomycin screening agar plates (4).

Vancomycin screening agar plates. Vancomycin screening agar plates were prepared as recommended by the National Committee for Clinical Laboratory Standards (4), by incorporation of 6 μg of vancomycin (Sigma Chemical Company, St. Louis, Mo.) per ml into melted and cooled brain heart infusion agar (Difco Laboratories, Detroit, Mich.) in sterile plastic petri plates (15 by 100 mm). The plates were stored in sealed plastic bags and kept refrigerated until used.

Preparation of test organism inocula. Standardized inoculum suspensions of all isolates were prepared by suspending colonies of *Enterococcus* spp. grown overnight on sheep blood agar plates to the density of a 0.5 McFarland standard in 0.9% NaCl. The back of each vancomycin screening agar plate was marked off into nine sections to test three isolates per plate at three different inoculum densities. Each isolate suspension was placed on the surface of the vancomycin screening agar by dispensing 10^5- and 10^6-CFU inocula with a pipet (0.001 and 0.01 ml, respectively) onto the agar surface and by using a cotton swab dipped in the 0.5 McFarland standard organism suspension and applied to the agar surface as a single spot. Plates were incubated for 24 h at 35°C prior to interpretation of results. A positive (growth) result was defined as a definite spot of growth or greater than one colony present at the site of inoculation. A faint haze on the agar surface or the presence of only a single colony was disregarded.

There was 100% correspondence in the presence or absence of growth among the three inoculum preparation methods used with this collection of isolates (Table 1). However, there was a single isolate of *E. casseliflavus* with a vancomycin MIC of 4 μg/ml that grew fewer than 10 colonies at the 10^5-CFU and swab inoculum concentrations but produced confluent growth with the 10^6-CFU inoculum. All VanA^r and VanB^r strains were accurately detected by the vancomycin screening agar. In addition, 11 *E. gallinarum* and 2 *E. casseliflavus* VanC^r isolates with an intermediate MIC of 8 or 16 μg/ml also grew on the agar at all three inoculum densities. Thus, the cotton swab inoculation method provided results equivalent to those of the more expensive micropipets used in this study. This is consistent with the earlier findings of Willey et al. (8), although those investigators employed Mueller-Hinton agar instead of brain heart infusion agar.

Because vancomycin screening agar represents a very sensitive method for detection of vancomycin-nonsusceptible isolates of *Enterococcus* spp., it is important to determine the species identification and the vancomycin MICs of isolates that grow on the screening plates. Not every isolate that produces growth on the screening agar represents a VanA^r or VanB^r type of VRE. Isolates of species possessing the VanC^r phenotype (i.e., *E. casseliflavus* and *E. gallinarum*) may be expected

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TABLE 1. Growth of 104 *Enterococcus* sp. isolates on vancomycin screening agar plates at three inoculum sizes according to resistance phenotype

Phenotype	No. of isolates	No. of isolates with growth at specified inoculum size		
		10^5 CFU	10^6 CFU	Swab
$VanA^r$	39	39	39	39
$VanB^r$	12	12	12	12
$VanC^r$	12	12	12	12
Van^s	41	1	1	1

to grow routinely on vancomycin screening agar. The findings of the present study should be confirmed by further studies incorporating larger numbers of isolates and additional species.

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