Comparison of Simple and Rapid Methods for Identifying Enterococci Intrinsically Resistant to Vancomycin

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Three different methodologies, reduction of litmus milk (LM) and acidification of arabinose (ARA), acidification of methyl-α-D-glucopyranoside (MGP), and rapid motility (RM), for differentiating isolates of Enterococcus casseliflavus and Enterococcus gallinarum (intrinsically vancomycin-resistant enterococci [IVRE]) from Enterococcus faecalis and Enterococcus faecium were evaluated. All 33 isolates of E. faecalis tested reduced LM within 4 h and were negative in all other tests, while the 53 isolates of E. faecium were ARA positive only. In contrast, 45 of 46 (98%) IVRE isolates examined (26 E. casseliflavus and 20 E. gallinarum isolates) acidified MGP, 41 of 46 (89%) were LM and ARA positive, and 45 of 46 (98%) were RM positive. Acidification of MGP was therefore the single most useful test for differentiating IVRE from vancomycin-resistant E. faecium and E. faecalis; however, a combination of LM-ARA and RM testing enabled the correct designation of organisms without the need for overnight incubation.

The identification of hospitalized patients infected with vancomycin-resistant enterococci (VRE) has become an important component of infection control programs aimed at minimizing patient-to-patient transmission of these organisms (4). A variety of vancomycin-containing media have been shown to provide a simple and cost-effective means for differentiating VRE from non-VRE (1, 12), and use of these media has been adopted in many clinical laboratories. In addition to organisms possessing high-level, transferable vancomycin resistance (predominantly Enterococcus faecalis and Enterococcus faecium), those enterococci that intrinsically express low-level resistance to glycopeptide antibiotics, namely, Enterococcus casseliflavus and Enterococcus gallinarum, will also grow on vancomycin-containing media. In contrast to vancomycin-resistant isolates of E. faecalis and E. faecium, isolates of intrinsically vancomycin-resistant enterococci (IVRE) have not been implicated in outbreaks of VRE infection and appear to be of minimal concern from an infection control standpoint (10, 12, 14). Since occasional isolates of both E. gallinarum and E. casseliflavus that harbor the transmissible vanA gene have been identified (6), however, determination of the level of vancomycin resistance in clinically significant isolates of IVRE may still be warranted. The ability to accurately differentiate IVRE from other VRE, especially E. faecalis and E. faecium, is nonetheless of considerable importance and, unfortunately, has proven to be somewhat problematic for commercial biochemical identification systems (9, 11). A number of tests for rapidly and inexpensively identifying IVRE, including motility (7, 15), pigment production (7), acidification of methyl-α-D-glucopyranoside (MGP) (3, 5), and susceptibility to efomycin (3), have been described in the literature. A previous study conducted in our laboratory demonstrated that determining the ability of organisms to rapidly reduce litmus milk (LM) and acidify arabinose (ARA) was a useful approach for differentiating between the clinically significant enterococcal species (8). In the present study we examined the rapid LM and ARA acidification assay (LM-ARA), MGP acidification, and a modified motility test (readable in 4 h) for their ability to differentiate IVRE from E. faecalis and E. faecium.

A total of 132 enterococcal isolates were analyzed in the study: 49 were obtained from the culture collection of the Microbiology Laboratory, Hennepin County Medical Center; 36 were generously provided by the Bacteriology Laboratory, Minnesota Department of Health, Minneapolis; and 47 were from the Microbiology Service, National Institutes of Health, Bethesda, Md. These isolates included 33 E. faecalis isolates (11 vancomycin-resistant isolates), 53 E. faecium isolates (23 vancomycin-resistant isolates), 26 E. casseliflavus isolates, and 20 E. gallinarum isolates. The identification of isolates was established with a commercial identification system, Vitek GPI (bioMérieux, Hazelwood, Mo.), and conventional biochemical tests (7).

The rapid LM-ARA assay was performed as follows. LM reagent (BBL, Cockeysville, Md.) was formulated according to the manufacturer’s recommendations; ARA test reagent consisted of brain heart infusion broth containing 1% (wt/vol) 1-ARA (Sigma Chemical Co., St. Louis, Mo.) and 0.0016% (wt/vol) bromoresol purple (Baker, Phillipsburg, N.J.). Several drops (5 or 6) of LM and ARA reagent were dispensed into two test tubes (6 by 50 mm), a visible inoculum of test organism was then added (2 to 3 colonies from sheep blood agar), and the tubes were incubated at 42°C for 4 h. A color change of pink-purple to white was indicative of a positive LM result; a positive ARA test was recorded when the broth indicator changed color from purple to yellow. The ability of organisms to acidify MGP was determined essentially as described previously (5), except that the volumes and inoculum used were as in the LM and ARA assays. A change in the indicator color from red to yellow was indicative of a positive result. Motility was determined with a modified motility agar formulation consisting of casitone (1%, wt/vol), yeast extract (0.3%, wt/vol), sodium chloride (0.5%, wt/vol), and a decreased concentration (1.5%, wt/vol) of agar. Inoculated slants were incubated for 4 h at 35°C prior to reading.

The identities of the organisms tested and the results of the
LM-ARA, MGP, and rapid motility (RM) assays are shown in Table 1. All 33 isolates of *E. faecalis* were LM positive and ARA negative, MGP negative, and RM negative. All 53 isolates of *E. faecium* were LM negative and ARA positive, MGP negative, and RM negative. Of the 26 isolates of *E. casseliflavus* tested, 22 were LM and ARA positive, MGP positive, and RM positive; 1 was LM negative and ARA positive, MGP positive, and RM positive; 2 were LM positive and ARA negative, MGP positive, and RM positive; and 1 was LM and ARA positive, MGP positive, and RM negative. Of the 20 isolates of *E. gallinarum* tested, 18 were LM and ARA positive, MGP positive, and RM positive, and 2 were LM positive, ARA negative, MGP positive, and RM positive. The sensitivity of all three test methodologies for differentiating *E. faecalis* or *E. faecium* from IVRE was 100% (59 of 59). The specificity of the MGP and RM assays was 98% (45 of 46), and the LM-ARA combination was 89% (41 of 46) specific.

These results confirm previous observations concerning the utility of MGP acidification for differentiating IVRE from other enterococcal species (3, 5). Unfortunately, an overnight incubation step is necessary to obtain adequate sensitivity with the MGP assay. Attempts to accelerate the test by increasing the concentration of MGP (up to 7.5%) were unsuccessful, resulting in false-positive results with *E. faecium* (data not shown). A combination of LM and ARA testing resulted in ready differentiation of *E. faecalis* or *E. faecium* from the majority of isolates of IVRE within a 4-h time period. All four ARA-negative isolates of IVRE were also ARA negative after overnight incubation, indicating that the rapid assay reliably detects ARA positivity and that the ability to acidify ARA is not found in all isolates of *E. casseliflavus* and *E. gallinarum*. Following our initial evaluation of the LM and ARA assays (8), we have used this test combination routinely in our laboratory to identify *E. faecalis*, since this is the only enterococcal species commonly isolated from human clinical specimens that is LM positive and ARA negative. Both *E. faecium* and *E. avium* are LM negative and ARA positive, and consequently, further testing is required for the definitive identification of *E. faecium*. Since *E. faecium* is MGP negative and *E. avium* is MGP positive (3), however, a combination of LM-ARA and MGP testing permits differentiation of these organisms, and thus use of these three simple tests enables us to identify the vast majority of clinical isolates of *Enterococcus* spp.

Motility and yellow-pigment production have been the most widely used rapid tests for differentiating IVRE from *E. faecalis* or *E. faecium*, and *E. gallinarum* from *E. casseliflavus*, and are typically used as supplemental tests when commercial biochemical test systems are employed for enterococcal identification (9, 11). The accuracy of these methods has, however, varied considerably in published studies (2, 3, 9, 11, 15), hence the interest in alternative approaches to differentiating these organisms. We developed and evaluated an RM assay that, by decreasing the agar concentration by 50%, enables the detection of motility in only 4 h. Only one isolate of the motile enterococcal species examined did not demonstrate motility in our study (in either the RM or conventional motility tests), giving this test the same specificity as MGP acidification.

In addition, 17 of 18 isolates of *E. casseliflavus* were visibly pigmented; no isolates of any of the other species tested produced pigment. These results support the use of motility and pigment production as adjunctive tests for identification of enterococci, a conclusion reached in a previous study comparing PCR with conventional methods for identifying these organisms (2).

Since the RM assay enables motility to be detected in only 4 h, this test can be used in conjunction with rapid LM and ARA tests for differentiating IVRE from other VRE. By using a combination of the LM-ARA and RM assays, identification of IVRE can be made with a high degree of accuracy, in a single working day, for approximately $0.07 in reagent acquisition cost. In addition, use of the LM-ARA combination enables same-day identification of *E. faecalis*, obviating the need to use commercial biochemical test systems for the identification of most enterococcal isolates. In conclusion, the results of this study demonstrate that the use of a small number of simple, inexpensive, biochemical and morphologic tests can determine the identity of vancomycin-resistant isolates of *Enterococcus* spp. Wider utilization of these tests should enable laboratories to provide prompt, accurate, and cost-effective test results on these organisms of increasing clinical and epidemiologic importance.

**REFERENCES**


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<th>LM (no.) (%)</th>
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Volume 37, no. 3, p. 815–817, 1999. Page 815, column 2, line 3 from bottom: “1.5%” should read “0.15%.”