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The enterococci have emerged as the third most common cause of nosocomial bacteremia (14, 16, 27). This increase in infections is due in part to an escalation of resistance to antimicrobial therapy, including high-level resistance to aminoglycosides and vancomycin (3, 9, 13, 23, 28). Resistance is most frequently associated with Enterococcus faecium, even though other species conferring resistance are appearing (3, 15, 22, 28). The treatment of infections caused by high-level vancomycin-resistant enterococci is difficult, making it important to recognize these species. Additionally, infections caused by enterococci with intrinsic low-level vancomycin resistance have been shown to cause failure of vancomycin therapy (10, 20). Enterococcus faecalis and E. faecium account for 80 to 90% and 10 to 15% of enterococcal infections, respectively, and other species including E. gallinarum, E. casseliflavus, E. raffinosus, E. durans, E. hirae, and E. avium account for a majority of the remainder (14–17, 19). Identification systems, such as Vitek and MicroScan, have been utilized to identify only a few of the Enterococcus species, including E. faecalis, E. faecium, E. durans, and E. avium (1, 4, 24). Recently, MicroScan revised the gram-positive identification panel by adding Enterococcus species (E. casseliflavus, E. gallinarum, and E. raffinosus and combining E. hirae with E. durans as E. durans/hirae) to the database, reformulating biochemical tests, and including new tests. The purpose of this study was to evaluate the revised Dried Overnight Gram-Positive Identification (CPID2) panel for the ability to accurately identify Enterococcus species.

(This work was presented in part at the 98th General Meeting of the American Society for Microbiology, Atlanta, Ga., 17 to 21 May 1998.)

The Enterococcus species evaluated included 133 stock and 69 fresh isolates (60 E. faecalis, all vancomycin susceptible) isolates, 59 E. gallinarum isolates, 30 E. casseliflavus isolates, 27 E. faecium [7 vancomycin resistant] isolates, 9 E. raffinosus isolates, 8 E. durans isolates, 6 E. hirae isolates, and 3 E. avium isolates). Only one isolate per patient was tested. The stock isolates had been stored frozen at −70°C and prior to testing were passed twice on sheep blood agar.

The revised CPID2 test panels were obtained from the manufacturer (Dade MicroScan, Inc., West Sacramento, Calif.) and inoculated with a turbidity equivalent to that of a 0.5 McFarland standard. The panels were incubated 18 to 20 h at 35°C in ambient air and read manually according to the manufacturer's recommendations. The panels were tested with the following quality control (QC) strains on a weekly basis throughout the study period: Streptococcus aureus, Enterococcus faecalis ATCC 29213, E. faecalis ATCC 29212, Streptococcus bovis ATCC 49147, Micrococcus luteus, Micrococcus luteus ATCC 49732, Staphylococcus saprophyticus, ATCC 49907, Staphylococcus xylosus ATCC 49148, Streptococcus pneumoniae ATCC 49136, and E. avium ATCC 49464. QC testing was within normal limits.

The abbreviated conventional biochemical identification scheme of Facklam and Collins (11) was used as a basis for species identification with modifications as outlined in a previous study (13). The reagent for acidification of 1% methyl-a-d-glucopyranoside (MGP) was prepared and evaluated by the procedure of Devriese et al. (8).

The biotype number was generated by giving a weighted numerical value to all positive reactions. This biotype number was submitted to MicroScan and evaluated by using a BATCHID identification program to generate an identification. Once an identification was determined, it was compared with the results of conventional biochemical testing. No additional testing was done if the CPID2 test panel result was a high-probability species identification (>85%) and it matched the conventional biochemical testing result. If the panel result was a low-probability identification (<85%) and listed multiple possible species, one of which matched the result by conventional testing, additional biochemical tests as indicated on the MicroScan printout were evaluated (e.g., motility test and evaluation for colony pigmentation). Isolates exhibiting a discrepancy between the test panel and conventional biochemical testing were repeat tested with the CPID2 test panel. Biochemical assays were considered the reference standard for all discrepant results.

The initial accuracies of the revised CPID2 test panel for Enterococcus species were 100% for E. faecium (27 of 27), E. casseliflavus (30 of 30), E. durans (8 of 8), E. hirae (6 of 6), and E. avium (8 of 8), but 98.3% for E. faecalis (59 of 60), 88.9% for E. raffinosus (8 of 9), 66.7% for E. avium (2 of 3), and 40.7% for E. gallinarum (24 of 59) (Table 1). The single misidentified E. faecalis isolate was identified as E. avium, the E. avium isolate was identified as E. raffinosus (88.6%), the E.
**TABLE 1. MicroScan Dried Overnight Gram-Positive Identification test panel results compared with conventional biochemicals to identify Enterococcus species**

<table>
<thead>
<tr>
<th>Enterococcus species</th>
<th>Total no. of isolates</th>
<th>No. of isolates with initial results*</th>
<th>Correct HP</th>
<th>Incorrect HP</th>
<th>Correct LP</th>
<th>Incorrect LP</th>
<th>Correct VRB</th>
<th>Incorrect VRB</th>
<th>Overall % correct following resolution testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. faecalis</td>
<td>60</td>
<td></td>
<td>59</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>98.3</td>
</tr>
<tr>
<td>E. gallinarum</td>
<td>59</td>
<td>15</td>
<td>14</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>59</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>E. casseliflavus</td>
<td>30</td>
<td>14</td>
<td>9</td>
<td>15</td>
<td>16</td>
<td>4</td>
<td>59</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>E. faecium</td>
<td>27</td>
<td>27</td>
<td>27</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. raffinosus</td>
<td>9</td>
<td>8</td>
<td>8</td>
<td>1</td>
<td>8</td>
<td></td>
<td>88.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. durans*</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>8</td>
<td></td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. hirae*</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td></td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. avium</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td></td>
<td>66.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>202</td>
<td>137</td>
<td>27</td>
<td>34</td>
<td>1</td>
<td>3</td>
<td>199</td>
<td>98.5</td>
<td></td>
</tr>
</tbody>
</table>

* High-probability (HP) results were reported as ≥85%, with low-probability (LP) results reported as ≤85%.

Resolution testing was performed only with incorrectly identified E. gallinarum isolates, which were tested for motility and the ability to acidify MGP.

Low-probability results for E. casseliflavus and E. gallinarum required supplemental testing with a motility test and an analysis of colony pigmentation.

All incorrect high- and low-probability results were identified by the MicroScan panel as E. faecium.

The MicroScan database combines E. durans and E. hirae as E. durans/hirae.

A raffinosus isolate was identified as a very rare biotype (VRB), and 33 of the E. gallinarum isolates were identified as E. faecium with 2 VRBs. Both of these VRBs were retested with the revised MicroScan panel, and both were subsequently identified as E. faecium.

Supplemental tests for motility and the ability to acidify MGP were performed with all isolates identified as E. gallinarum and E. faecium. All E. gallinarum isolates, including those incorrectly identified as E. faecium, were positive by the MGP test, and 54 of 59 (91.5%) were positive for motility. All known E. faecium isolates were negative by both tests. Following this resolution testing, the CPID2 test panel correctly identified 98.5% of the Enterococcus isolates.

In the present study, the overall accuracy of the CPID2 test panel was initially 81.2% (164 of 202). One isolate each of E. faecalis, E. raffinosus, and E. avium was misidentified, and 35 of the 59 isolates of E. gallinarum were incorrect. All misidentified E. gallinarum isolates were recognized as E. faecium (33 from initial testing and 2 upon repeat testing). This inability to distinguish these closely related species by using automated tests has been reported by others (5, 7, 8, 11, 17, 25). Since supplemental testing with motility and MGP tests has shown reliability to distinguish these species, these tests were applied to all isolates identified by the CPID2 panel as E. gallinarum and E. faecium (2, 7, 8, 12, 25). All of the E. gallinarum isolates were positive for acidification of MGP, and 91.5% were positive for motility, while none of the E. faecium isolates were positive by either test. Unfortunately, this approach would require supplemental testing of all isolates identified by the panel as E. faecium. An alternative approach would be to only test those E. faecium isolates recognized as ampicillin susceptible. Studies have shown that ampicillin resistance is common for E. faecium (>80%), but uncommon for E. gallinarum (<10%) (14, 21). None of the E. gallinarum isolates in this study showed resistance to ampicillin, while 33 of the misidentified E. gallinarum isolates showed intermediate susceptibility (31 isolates) or resistance (2 isolates) to vancomycin. These findings suggest that isolates identified by the CPID2 test panel as E. faecium, which are susceptible to ampicillin and/or intermediate or resistant to vancomycin, should be screened by motility and MGP tests to rule out or identify E. gallinarum.

Finally, a potential shortcoming of the revised MicroScan panel includes the identification of phenotypically atypical enterococcal isolates, especially the nonmotile E. gallinarum and nonmotile and nonpigmented E. casseliflavus isolates. These isolates are rarely encountered in clinical samples, although extensive screens to detect these isolates have not been done (2, 7, 8, 12, 25). Inulin fermentation may be an alternative way to distinguish these species, since in our study, 29 of 30 E. casseliflavus isolates were positive, while only 1 of 59 E. gallinarum isolates was positive. The inulin-negative E. casseliflavus isolate was motile and had an intense yellow colony, while the inulin-positive E. gallinarum isolate was motile and nonpigmented.

In conclusion, the results of this study showed that the CPID2 test panel was a reliable method for the overnight identification of Enterococcus species; however, supplemental testing is frequently needed to differentiate E. gallinarum from E. faecium.

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**REFERENCES**


