Species Identities of Enterococci Isolated from Clinical Specimens

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Conventional tests and commercially available systems were used to determine the species identities of clinical isolates of enterococci. Strict adherence to the conventional test scheme of Facklam and Collins (R. R. Facklam and M. D. Collins, J. Clin. Microbiol. 27:731–734, 1989) resulted in the misidentification of lactose-negative Enterococcus faecalis isolates as Enterococcus solitarius, but this problem was overcome by the application of additional tests. The commercially available systems tested were unable to recognize some of the more recently described enterococcal species. E. faecalis accounted for 87.1% of 302 consecutive isolates, Enterococcus faecium (8.6%), Enterococcus avium (0.7%), Enterococcus durans (0.3%), Enterococcus gallinarum (1.0%), Enterococcus casseliflavus (1.0%), Enterococcus hirae (0.3%), and Enterococcus raffinosus (0.3%) isolates were also identified. None of the isolates produced β-lactamase, but 15.4% of 235 isolates tested, including 1 strain of E. gallinarum, displayed high-level resistance to gentamicin.

Recent studies of the enterococci have advocated their placement in a separate genus, Enterococcus, and have led to the description of new enterococcal species (1–3, 9, 14, 15). An identification scheme utilizing conventional testing media for recognition of all 12 currently described enterococcal species has been proposed by Facklam and Collins (7). They noted that although the majority of clinical enterococcal isolates are Enterococcus faecalis, members of almost all of the recently described species have been found in clinical specimens. Since most studies on the distribution of clinically isolated enterococcal species were done prior to the description of many of the newer species and since studies on newer species have been conducted on selected isolates submitted to a reference laboratory, we decided to assess the distribution of species in 302 consecutive isolates of enterococci from routine cultures at our institution. Identities obtained with the scheme of Facklam and Collins (7) were compared with those provided by two commercially available identification systems. The isolates were also examined for high-level gentamicin resistance and β-lactamase production.

MATERIALS AND METHODS

Bacterial isolates. Sequential, unique isolates of enterococci from a variety of specimens were collected over a period of approximately 3 months. These strains were subcultured onto brucella agar containing 5% horse blood (BBL Microbiology Systems, Cockeysville, Md.) or brain heart infusion agar deeps (Difco Laboratories, Detroit, Mich.) and stored at 2 to 8°C until tested. Before being tested, each isolate was subcultured onto horse blood agar, and a single colony was used to inoculate 5 ml of Todd-Hewitt broth (Difco). After overnight incubation at 35°C, the broth culture was used to inoculate all conventional tests.

The following stock strains, obtained from the American Type Culture Collection, Rockville, Md., were also examined: Enterococcus faecalis ATCC 19433, Enterococcus faecium ATCC 19434, Enterococcus avium ATCC 14025, Enterococcus durans ATCC 19432, Enterococcus gallinarum ATCC 35038, Enterococcus casseliflavus ATCC 25788, Enterococcus hirae ATCC 8043, Enterococcus mundtii ATCC 43186, and Enterococcus malodoratus ATCC 43197.

Conventional tests. Isolates were tested on the following media: heart infusion broths supplemented with mannitol, sorbitol, sorbose, arabinose, raffinose, lactose, and sucrose (6); pyruvate broth (10); bile esculin agar, brain heart infusion broth with 6.5% NaCl, motility medium, and Trypticase soy agar (BBL Microbiology Systems); and Moeller deoxycholate medium with arginine (Northeast Laboratory Services, Waterville, Maine). Ingredients for the carbohydrate fermentation media were obtained from Difco and Sigma Chemical Co., St. Louis, Mo. All tests were incubated and interpreted as described by Facklam and Collins (7).

The first 100 clinical isolates were tested with conventional media and the commercially available systems described below. Since most of these isolates were identified as E. faecalis by all methods, the remaining 202 strains were first subjected to six screening tests to detect non-E. faecalis isolates. The strains were tested for raffinose, lactose, and pyruvate acidification; arginine hydrolysis; motility; and pigment production. The complete battery of conventional tests and the commercially available systems were then used to identify all strains yielding one or more of the following reactions, which are not typical of E. faecalis: raffinose acidification; failure to acidify lactose or pyruvate or hydrolyze arginine; and positive motility reaction or pigment production. Isolates with typical E. faecalis reactions in the screening tests were identified as E. faecalis.

Certain isolates (see Results) were also tested for the ability to grow and reduce tellurite (indicated by blackening of the medium) in Todd-Hewitt broth supplemented with 0.05% potassium tellurite (Fisher Scientific Co., Fair Lawn, N.J.). These strains were also tested for the ability to ferment ribose (Sigma) in brain heart infusion broth containing this sugar (6). All isolates were tested for pyrrolidonyl arylamidase activity with Identicult AE (Scott Laboratories, Inc., Fiskeville, R.I.).

Commercially available identification systems. Todd-
plates. After overnight incubation of the blood GPI card was used in accordance with manufacturer instructions.

**Antimicrobial susceptibility testing.** All clinical isolates were screened for β-lactamase production with a large inoculum and Cefinase disks (BBL Microbiology Systems) in accordance with manufacturer instructions. Resistance to high levels of gentamicin was tested in the first 234 consecutive isolates and 1 additional isolate of E. gallinarum by lightly inoculating (one or two colonies) glucose phosphate agar (GIBCO Laboratories, Lawrence, Mass.) containing 2,000 μg of gentamicin per ml with the organism to be tested. Growth after overnight incubation at 35°C was interpreted as high-level gentamicin resistance. All clinical isolates were tested by the disk diffusion method (12) for susceptibility to the antimicrobial agents methicillin, clindamycin, and vancomycin.

**RESULTS**

Strains were identified as enterococci on the basis of a positive bile esculin reaction, growth in heart infusion broth containing 6.5% NaCl, and the presence of pyrrolidonyl arylamidase activity. All strains were also resistant to methicillin and clindamycin when tested by the disk diffusion method.Resistance to these two antibiotics is used along with other characteristics as a criterion for identification of isolates as enterococci in our laboratory.

Table 1 displays the sources and species identities according to a slightly modified version (see below) of the scheme of Facklam and Collins (7) for the 302 clinical isolates. The majority of strains (87.7%) were E. faecalis, while E. faecium accounted for 8.6%. Only 3.6% of the isolates were identified as members of other species. E. faecalis accounted for a greater percentage of isolates from urine and wound cultures than from fluid and blood cultures, although the smaller samples of isolates from the latter two culture types make it difficult to draw firm conclusions on E. faecalis distribution. Six of the 10 non-E. faecalis, non-E. faecium species were represented among the isolates. No strains of Enterococcus solitarius, Enterococcus pseudoavium, E. malodoratus, or E. mundii were identified.

Strict adherence to the identification scheme of Facklam and Collins (7), with only the key tests recommended for enterococcal identification, resulted in the misidentification of 22 isolates (15 from urine, 2 from wounds, 3 from fluids, and 2 from other specimen types) as E. solitarius. The API Rapid Strep system identified these isolates as E. faecalis, as did the GPI card for 20 of the 22 strains. These isolates all fermented ribose, reduced tellurite, and failed to ferment lactose, suggesting that they were really lactose-negative strains of E. faecalis (1, 14). Therefore, isolates identified as E. solitarius by the abbreviated scheme of Facklam and Collins (7) should be subjected to additional tests designed to differentiate E. solitarius from lactose-negative strains of E. faecalis. Tellurite reduction, noted as a useful additional test by these authors, seems to be essential for the differentiation of E. faecalis and E. solitarius in their scheme (7).

All three identification systems tested agreed in their identifications of all of the 89 lactose-positive E. faecalis strains tested. Agreement was noted among the three systems for 26 isolates identified as E. faecium, 2 E. avium isolates, and 1 E. durans isolate. Strains producing discrepant identifications are displayed in Table 2; differences in identifications by the scheme of Facklam and Collins (7) with and without added tests are also shown.

None of the clinical isolates was positive for β-lactamase, but high-level gentamicin resistance was detected in 36 (15.4%) of the 235 strains tested. Twenty-six (12.6%) of 206 E. faecalis isolates tested and 9 (50%) of 18 E. faecium isolates tested were resistant to high levels of gentamicin. This characteristic was also found in one of three E. gallinarum strains tested.

### Table 2: Strains yielding discrepant identifications when tested by conventional and commercially available methods

<table>
<thead>
<tr>
<th>Scheme of Facklam and Collins</th>
<th>GPI card</th>
<th>Rapid Strep system</th>
</tr>
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<tbody>
<tr>
<td>Modified*</td>
<td>Unmodified*</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Strain</th>
<th>Identification (no. of strains) by:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>E. faecalis, lactose negative (22)</td>
<td>E. faecalis (20), Streptococcus uberis (1), unidentified (1)</td>
<td>E. faecalis (22)</td>
</tr>
<tr>
<td>E. casseliflavus (3)</td>
<td>E. casseliflavus (3)</td>
<td>E. faecium (3)</td>
</tr>
<tr>
<td>E. gallinarum (3)</td>
<td>E. gallinarum (3)</td>
<td>E. faecium (3)</td>
</tr>
<tr>
<td>E. hirae (1)</td>
<td>E. hirae (1)</td>
<td>E. durans (1)</td>
</tr>
<tr>
<td>E. raffinosus (1)</td>
<td>E. raffinosus (1)</td>
<td>E. avium (1)</td>
</tr>
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</table>

* Positive reactions in additional tests (ribose fermentation and tellurite reduction) are needed to accurately identify lactose-negative E. faecalis strains.

* Identification scheme described in reference 7, without added tests.
DISCUSSION

Our observations on the incidence of various enterococcal species are similar to those of other authors (4, 6, 11, 13) who noted, prior to the recognition of new species, that the majority (63 to 81%) of clinical isolates were _E. faecalis_, followed by _E. faecium_ (13 to 23%). Other species (_E. avium_, _E. durans_, _E. casseliflavus_, _E. gallinarum_, _E. hirae_, and _Enterococcus raffinosus_) accounted for only 3.6% of our isolates. While these and other enterococcal species are rare among clinical isolates (7), they may be encountered in clinical laboratories. Despite the recognition of β-lactamase-producing enterococci in a number of U.S. cities (R. C. Moellering, Jr., Clin. Microbiol. News. 10:129–132, 1988), including our own (E. Rhinehart, C. Wennersten, E. Goss, G. Eliopoulos, R. Moellering, N. Smith, and D. Goldmann, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1073, 1988), none was detected among the 302 isolates that we screened. To our knowledge, however, this is the first time that _E. gallinarum_ displaying high-level resistance to gentamicin has been reported. More information on possible correlations between a given species and pathogenic potential or susceptibility patterns would be desirable.

The commercially available systems examined in this study provided accurate identifications for the majority of isolates examined. Previous studies have demonstrated good performance of these products for the identification of _E. faecalis_, _E. faecium_, and _E. avium_ (5, 8). Both the GPI card and the Rapid Strep system correctly identified lactose-negative variants of _E. faecalis_. These isolates would have been misidentified as _E. solitarius_ by the scheme of Facklam and Collins (7) if additional tests had not been done. Alternatively, tellurite reduction or ribose fermentation could be substituted for lactose fermentation to distinguish between _E. faecalis_ and _E. solitarius_ in their scheme. In general, it seems that the commercially available systems are highly accurate in their identification of _E. faecalis_ isolates. Identifications of isolates as _E. faecium_ and other species may not be as reliable, because of phenotypic similarities among _E. faecium_, _E. avium_, _E. durans_, and the more recently described species (1–3, 9). Database and nomenclature updates may allow these systems to accurately recognize the newer species of enterococci.

Because we recovered only a small number of isolates belonging to species other than _E. faecalis_ or _E. faecium_ and because we could see no obvious differences between disk diffusion susceptibility patterns of _E. faecium_ and the non- _E. faecalis_, non- _E. faecalis_ strains, we decided to continue using commercially available systems for enterococcal species identification. At this point, we feel that more information on the importance of accurately identifying these isolates to the species level is needed. It should be noted that a study done with a limited number of isolates suggested that _E. gallinarum_ may be more resistant to vancomycin than are other enterococci (16). The collection of additional information of this kind may justify more accurate identification of enterococcal isolates.

LITERATURE CITED