Rapid Identification of Clinically Relevant Enterococcus Species by Fluorescence In Situ Hybridization

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A fluorescence in situ hybridization assay for the rapid identification of clinically relevant enterococci (Enterococcus faecalis, E. faecium, E. gallinarum, the VanC-type resistance group) was developed and evaluated with 33 reference strains, 68 clinical isolates, and 58 positive blood cultures. All probes showed excellent sensitivities and specificities.

Enterococci are frequent causes of nosocomial infections, like urinary tract infections and sepsis (13). The rapid identification of enterococci to the species level is important for treatment decisions, because different species show different resistance patterns. While most strains of Enterococcus faecalis are susceptible to penicillins, first-line therapy for infections caused by most strains of Enterococcus faecium requires the use of a glycopeptide antibiotic. In turn, members of the VanC-type resistance group, such as Enterococcus gallinarum and Enterococcus casseliflavus, are intrinsically resistant to vancomycin but are mainly susceptible to penicillins. Species identification of enterococci by phenotypic methods is time-consuming (5–7, 14, 20, 21), however, and the misidentification of E. faecium as E. gallinarum or E. casseliflavus and vice versa is a frequent problem (4, 7, 12).

Fluorescence in situ hybridization (FISH) with fluorescently labeled oligonucleotide probes targeting the rRNA is a rapid and easy-to-perform method that has already been used for the detection of E. faecalis and E. faecium in blood cultures (10, 11, 16, 17, 22). The published Enterococcus-specific probes have not been thoroughly evaluated, however (10, 11, 16, 17, 22), and are partly not specific, according to a current GenBank database analysis (1, 10, 11). Others are implemented under unusual conditions, such as with formamide concentrations of 5–7%, 14, 20, 21), however, and the misidentification of E. faecium as E. gallinarum or E. casseliflavus and vice versa is a frequent problem (4, 7, 12).

In the first step, the probes were evaluated with 14 Enterococcus spp. and 19 nonenterococcal reference strains: Enterococcus avium (ATCC 14025), E. casseliflavus (ATCC 25788, ATCC 12755), Enterococcus durans (ATCC 20633), E. faecalis (ATCC 29212, ATCC 51299, ATCC 19433), E. faecium (ATCC 19434), Enterococcus flavescens (ATCC 7370), E. gallinarum (ATCC 700425, ATCC 35038), Enterococcus hirae (ATCC 8043), Enterococcus munditii (ATCC 43186), Enterococcus raffinosus (ATCC 49427), Streptococcus agalactiae (ATCC 13813), Streptococcus anginosus (ATCC 12395), Streptococcus bovis (ATCC 33317), Streptococcus constellatus (ATCC 27823), Streptococcus mitis (ATCC 49456), Streptococcus mutans (ATCC 25175), Streptococcus oralis (ATCC 35037), Streptococcus parasanguis (ATCC 15912), Streptococcus pneumoniae (ATCC 6303, ATCC 49619, ATCC 20566, ATCC 11865), Streptococcus pyogenes (ATCC 12344), Streptococcus salivarius (ATCC 7073), Streptococcus sanguinis (ATCC 10556), Staphylococcus aureus (ATCC 29213, ATCC 43300), Staphylococcus epidermidis (ATCC 12228), and Staphylococcus saprophyticus (ATCC 15305). All probes correctly detected the respective target organisms. Apart from probes ENC 176 and ENU 1470, which cross-reacted with Staphylococcus saprophyticus and one isolate of Enterococcus durans, respectively, all probes had an analytical specificity of 100%. Because of the observed cross-reaction, five well-characterized isolates of E. durans (15) were additionally included and did not show any cross-reaction.

The probes were further evaluated with 68 clinical isolates, including 49 enterococcal strains and 1 Abiotrophia adiacens strain from blood cultures and 18 strains of vancomycin-resistant enterococci (VRE). The VRE were grown on VRE screening agar (BD, Heidelberg, Germany) and included 3 E. faecium vanA strains, 1 E. faecium vanB strain, and 14 E. gallinarum strains. All isolates were investigated by FISH. The clinical isolates were identified by growth on bile-esculin agar with esculin hydrolysis, use of the API 20 Strep system (BioMérieux, Nuertingen, Germany), formation of methyl-a-D-glucopyranoside (according to Devriese et al. [3]), and pigmentation. Sequencing of the complete 16S rRNA gene was performed as described previously for isolates with ambiguous phenotypic identification results (n = 5) (1, 9). All FISH probes consistently reacted in accordance with the identification results (Table 2).

It was further assessed whether FISH may also be used for the rapid identification of enterococci directly from positive blood cultures. Samples from 58 positive blood cultures micro-

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Table 1. Sequences of oligonucleotide probes used in this study

<table>
<thead>
<tr>
<th>Probe</th>
<th>Target organism</th>
<th>Probe target</th>
<th>Sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUB 338</td>
<td>All bacteria</td>
<td>16S rRNA</td>
<td>GCT GCC TCC CGT AGG AGT</td>
<td>2</td>
</tr>
<tr>
<td>ENC 176</td>
<td>Enterococcus spp.a</td>
<td>23S rRNA</td>
<td>CA GTT CTC TGC GTC TAC TCC</td>
<td>This study</td>
</tr>
<tr>
<td>ENC 221</td>
<td>Enterococcus spp.b</td>
<td>16S rRNA</td>
<td>CAC CGC GGG TCC ATC CAT CA</td>
<td>This study</td>
</tr>
<tr>
<td>EGAC 183</td>
<td>VanC group of enterococci</td>
<td>16S rRNA</td>
<td>CAA CTT TCT TCC ATG CCG AAD AT</td>
<td>This study</td>
</tr>
<tr>
<td>ENF 191</td>
<td>Enterococcus faecalis</td>
<td>16S rRNA</td>
<td>GAA AGC GCC TTT CAC TCT TAT GC</td>
<td>This study</td>
</tr>
<tr>
<td>ENU 140</td>
<td>Enterococcus faecium</td>
<td>23S rRNA</td>
<td>GAC TCC TTC AGA CTT ACT GCT TGG</td>
<td>This study</td>
</tr>
<tr>
<td>EGA 141</td>
<td>Enterococcus gallinarum</td>
<td>23S rRNA</td>
<td>TTTCACAAGCTGTAACATCTGTTAT</td>
<td>This study</td>
</tr>
</tbody>
</table>

a Also Carnobacterium spp.
b Also Granulicatella spp. and Abiotrophia spp.
c Comprising Enterococcus gallinarum, E. flavescens, and E. casseliflavus.

Table 2. Identification of clinical isolates by FISH

<table>
<thead>
<tr>
<th>Isolated species</th>
<th>No. of isolates identified/total no. tested (%) by:</th>
<th>Direct identification by FISH of enterococci in microscopically positive blood cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Identification by FISH of clinical isolates from subculture</td>
<td></td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>26/26 (100)</td>
<td>18/18 (100)</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>3/3 (100)</td>
<td>12/12 (100)</td>
</tr>
<tr>
<td>Enterococcus gallinarum</td>
<td>15/15 (100)</td>
<td>2/2 (100)</td>
</tr>
<tr>
<td>Streptococcus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abiotrophia adiacens</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>Total</td>
<td>68/68 (100)</td>
<td>60/60 (100)</td>
</tr>
</tbody>
</table>

a Correct identification as Enterococcus spp. other than E. faecalis, E. faecium, E. gallinarum, E. casseliflavus, and E. flavescens.
b Correct identification as non-Enterococcus spp.

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REFERENCES

9. Hiraishi, A. 1992. Direct automated sequencing of 16S rDNA amplified by...


19. Reference deleted.

