Two Structurally Distinct VanA Resistance Elements at Different Locations in a Glycopeptide-Resistant Strain of Enterococcus faecalis

Glycopeptide-resistant enterococci (GRE) can carry more than one VanA element (3, 5), but we are unaware of previous evidence for separate transfer of such elements. Enterococcus faecalis JS3B was examined as one of 46 GRE isolated from fecal screens of a hematology patient at Addenbrooke’s Hospital, Cambridge, United Kingdom (7). Overlapping PCR (1) showed that this strain contained a group H element (11). VanA resistance appeared to be associated with a 35-MDa plasmid, but Palepou et al. (6) suggested that group H elements reside on the chromosome. Strain JS3B was therefore investigated for carriage of multiple VanA elements.

Transfer of vancomycin resistance was performed by cross-streak conjugation (10). The recipient strains were E. faecalis JH2-2 and E. faecium GE-1, which are resistant to fusidic acid and rifampin and lack pheromone response genes prgA and prgB (see below). After incubation at 37°C for 72 h, the transconjugants were selected on brain heart infusion agar (Oxoid) containing 100 μg of rifampin per ml, 25 μg of fusidic acid per ml, and 10 μg of vancomycin per ml. Plates were incubated at 37°C and examined daily for 5 days. Eight to 12 individual colonies were subcultured onto Columbia horse blood agar. The colonial characteristics of selected transconjugants were noted, and plasmid profiles were examined following alkaline lysis and agarose gel electrophoresis (8).

Five transconjugants, representing each combination of plasmid profile, hemolysis reaction, and colonial morphology, were subjected to overlapping PCR (1, 11). Plasmids were resubjected to alkaline lysis, followed by Southern blotting onto a nylon membrane, and hybridized with a digoxigenin-labeled vanA-specific probe (2). Strain JS3B and the five transconjugants were also examined for the conserved pheromone response genes prgA and prgB (which encodes aggregation substance protein) and prgB (which encodes entry exclusion protein) and prgB (which encodes aggregation substance protein) with previously described PCR primers (4) and the same cycling conditions as for overlapping PCR.

Three transconjugants represented the E. faecalis JH2-2 host and contained group U elements. Two transconjugants represented E. faecium GE-1; one contained a group U element, which yielded multiple amplicons, masked the group U element, which yielded fewer amplicons (11). These results stress the importance of using multiple molecular techniques to identify the structure and location of VanA elements in GRE.

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REFERENCES

<table>
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<tr>
<th>Transconjugant</th>
<th>Recipient strain</th>
<th>Hemolysis reaction on Columbia horse blood agar</th>
<th>VanA element</th>
<th>Band or plasmid that hybridized with vanA probe</th>
<th>Result of PCR specific for:</th>
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<tbody>
<tr>
<td>1</td>
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<td>U</td>
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<td>E. faecium GE-1</td>
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<td>35-MDa plasmid</td>
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</tbody>
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