Intrahospital Spread of a Single Gentamicin-Resistant, β-Lactamase-Producing Strain of Enterococcus faecalis in Argentina

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β-Lactamase-producing (Bla+) isolates of Enterococcus faecalis are being increasingly reported, particularly from hospitals in the United States (4, 5, 10, 11). Since hybridization studies have, to date, only been performed with isolates from the United States, this study was undertaken to investigate the type of Bla and gentamicin resistance genes in enterococcal isolates from Argentina, where β-lactamase has recently been reported (4). We also investigated the relatedness of the Argentinian isolates by comparing their chromosomal restriction endonuclease digestion patterns (REDPs).

Beginning in January of 1989, all enterococci isolated in the microbiology laboratory of the Hospital Nacional de Pediatria Juan Garrahán in Buenos Aires, Argentina, were tested for Bla with nitrocefin. Six Bla+ isolates were identified, three of which have been previously reported (4).

All Bla+ isolates were identified as E. faecalis by standard methods (2), OG1 (1) was used in conjugation studies. Plasmids pJM13 and pSF815A were the sources of the β-lactamase and gentamicin resistance (Gm+) gene probes (3, 13). Previously described methods were used for bacterial matings (6), plasmid DNA preparation (12), restriction endonuclease digestion (12), and hybridizations (3, 13). Pulsed-field gel electrophoresis of genomic DNA was performed as described by Murray et al. (7, 8), using clamped homogeneous electric fields (CHEF-DRII from Bio-Rad). Escherichia coli MG1655 digested with NotI was the molecular size standard (7).

Clinical data. The first Bla+ enterococcus isolated at Juan Garrahán Hospital was recovered from a patient the day after transfer from another hospital. A total of 5 of 134 enterococci were Bla+ in 1989, and 1 of 150 were Bla+ in 1990. Most of the patients had been given multiple antibiotics prior to isolation of Bla+ enterococci, and all the children were on different wards on the same floor of the hospital. A summary of the available clinical information is given in Table 1.

Resistance transfer and hybridization. All six Bla+ Argentinian enterococcal isolates transferred Bla and gentamicin resistance to isolate OG1. Plasmid DNAs from four of the clinical isolates digested with EcoRI are shown in Fig. 1. Plasmid DNA from two of the isolates (HG6049 [not shown] and HG4354) had identical REDPs; these two had all the bands seen for HG9829 plus additional bands, suggesting the acquisition of new DNA. HG6280 also had all the bands seen for HG9829 plus additional bands different from those in HG6049 and HG4354. The staphylococcal Bla gene probe hybridized to a 2.4-kb HaeIII fragment from all of the isolates (data not shown), and the Gm+ gene probe hybridized to a 3.9-kb HaeIII fragment (Fig. 2). Pulsed-field gel electrophoresis of genomic DNA. Smal-digested genomic DNA of five of the Bla+ isolates is shown in Fig. 3. Isolates HG6280, HG9829, and HG09521 (not shown) had identical REDPs; HG4354 and HG6049 were also identical to each other (as previously described (8)) and differed from HG6280, HG9829, and HG09521 by a change in a single fragment (the second largest fragment). HG10528 was most similar to HG6280 and HG9829. These chromosomal REDPs were different from those of six Gm+ Bla-negative E. faecalis isolates from the same hospital (Fig. 3); five of these isolates were different from each other, while two (lanes g and h) were identical. It has been previously shown that the REDPs of HG6280, HG6049, and HG4354

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differed from Bla" E. faecalis isolated in the United States and in Lebanon (8).

The results presented here indicate that all six Bla" E. faecalis isolates from Buenos Aires recovered over a 17-month period represent a single clonal group. They also illustrate the stability of chromosomal REDPs over this period. The interpretation of clonality is based on the identity or near identity of the chromosomal REDPs. The marked differences between the REDPs of these isolates and other Gm^2 Bla" isolates in Buenos Aires, as well as enterococci from other geographic areas, also support the interpretation that almost identical REDPs indicate a single strain and its derivatives. The differences in the plasmid REDPs of four isolates suggest the presence of at least one common plasmid and the presence of additional nonshared DNA in some of the isolates. We previously reported marked differences in two Bla" Gm^2 plasmids isolates from the United States which were later considered, on the basis of their chromosomal REDPs, to be a single strain (8, 12).

plasmids isolated from the United States showed extensive cross hybridization, indicating that they were in fact extensively related (9). The plasmids isolated in the United States had been obtained more than 5 years apart, while the plasmids isolated in Argentina had been obtained much closer temporally, illustrating the rapidity with which enterococcal plasmid content can be altered.

In conclusion, β-lactamase and gentamicin resistance genes in enterococci from Argentina, like these genes in enterococci from the United States, are highly homologous to those in staphylococci. These resistances were transferable by conjugation, which suggests that they should be able to spread into different enterococcal strains. However, all six Bla" isolates recovered over a 17-month period in this Argentinian pediatric hospital belong to a single clone, having identical or almost identical chromosomal REDPs. Because the first isolate was recovered from a patient the day after transfer from another hospital, this enterococcal strain may have been introduced by this patient and then spread to different patients. The presence of a common strain in different patients emphasizes the need to recognize and contain multiresistant organisms, particularly in the hospital setting. Because we have little control over what resistances bacteria may spread to one another, efforts to

<table>
<thead>
<tr>
<th>Enterococcal isolate</th>
<th>Date (mo-day-yr) of admission to hospital</th>
<th>Date (mo-day-yr) of isolation of Bla&quot; enterococci</th>
<th>Source of isolate</th>
<th>Prior antibiotics</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HG4354</td>
<td>April 26, 1989</td>
<td>April 27, 1989</td>
<td>Decubitus ulcer</td>
<td>Chloramphenicol, cefotaxime, amikacin, norfloxacin</td>
<td>4</td>
</tr>
<tr>
<td>HG6049</td>
<td>April 9, 1989</td>
<td>June 17, 1989</td>
<td>Blood</td>
<td>TMP-SMX^a, metronidazole, cefotaxime</td>
<td>4</td>
</tr>
<tr>
<td>HG6280 HG9829</td>
<td>June 24, 1989 August 2, 1989</td>
<td>July 7, 1989; October 12, 1989</td>
<td>Blood; Gastric fluid</td>
<td>Cefotaxime, TMP-SMX; Cefoxitin, gentamicin, cefoperazone, amikacin, imipenem</td>
<td>4; This study</td>
</tr>
<tr>
<td>HG10528</td>
<td>September 13, 1989</td>
<td>October 31, 1989</td>
<td>Subdiaphragmatic abscess</td>
<td>Penicillin, gentamicin, chloramphenicol, cefoxitin, amikacin, metronidazole</td>
<td>This study</td>
</tr>
<tr>
<td>HG09521</td>
<td>September 4, 1990</td>
<td>September 24, 1990</td>
<td>Cerebrospinal fluid</td>
<td>Amoxicillin</td>
<td>This study</td>
</tr>
</tbody>
</table>

^a The patient admitted on this date had been transferred from another hospital after several weeks of hospitalization.

^b TMP-SMX, trimethoprim-sulfamethoxazole.

FIG. 2. (A) Conventional agarose gel of HaeIII-digested plasmid DNA from HG4354 (lane a) and its transconjugant (lane e), HG6049 (lane b) and its transconjugant (lane f [DNA very faint]), and HG6280 (lane c). The 1-kb ladder is shown in lane d. (B) Autoradiogram after hybridization to the gentamicin resistance gene probe showing hybridization to a 3.9-kb fragment.

FIG. 3. Pulsed-field gel electrophoresis of Smal-digested E. faecalis from Argentina. Bla" isolates are seen in lanes b (HG6280), c (HG9829), d (HG4354), and e (HG6049). Lanes a, f, g, h, and i contain highly gentamicin-resistant Bla" enterococci. Lane j contains E. coli MG1655 digested with NotI.
control spread of bacteria between patients are particularly important.

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REFERENCES