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

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Six β-lactamase-producing (Bla$^+$) isolates of *Enterococcus faecalis* recovered over a 17-month period from an Argentinian pediatric hospital were found to have identical or almost identical chromosomal restriction patterns by pulsed-field gel electrophoresis, although the plasmid patterns were different. These isolates, like Bla$^+$ enterococci in the United States, hybridized to a staphylococcal Bla gene probe. The presence of a single strain was somewhat surprising, since all isolates transferred Bla by conjugation.

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 Pediatría Juan Garrahan 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^r) 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 *Not*I was the molecular size standard (7).

Clinical data. The first Bla$^+$ enterococcus isolated at Juan Garrahan 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 *Eco*RI 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 *Hae*III fragment from all of the isolates (data not shown), and the Gm^r gene probe hybridized to a 3.9-kb *Hae*III fragment (Fig. 2).

Pulsed-field gel electrophoresis of genomic DNA. *Sma*I-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^r 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

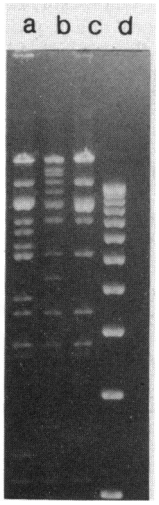

FIG. 1. Conventional agarose gel electrophoresis of plasmids from HG4354 (lane a), HG6280 (lane b), and HG9829 (lane C) after digestion with *Eco*RI. The 1-kb ladder is shown in lane d.

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TABLE 1. Isolation of Bla$^+$ enterococci at Hospital Nacional de Pediatría Juan Garrahan

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

[a] The patient admitted on this date had been transferred from another hospital after several weeks of hospitalization.
[b] TMP-SMX, trimethoprim-sulfamethoxazole.

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^r 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^r plasmids isolates from the United States which were later considered, on the basis of their chromosomal REDPs, to be a single strain (8, 12). The 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 transferrable 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

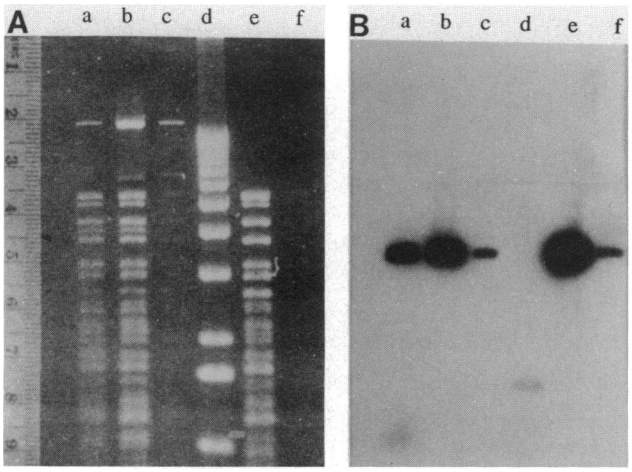

FIG. 2. (A) Conventional agarose gel of *Hae*III-digested plasmid DNA from HG4354 (lane a) and its transconjugant (lane e), HG6049 (lane b) and its transconjugant (lane f [DNA very faint]), and HG6280 (lane c). The 1-kb ladder is shown in lane d. (B) Autoradiogram after hybridization to the gentamicin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

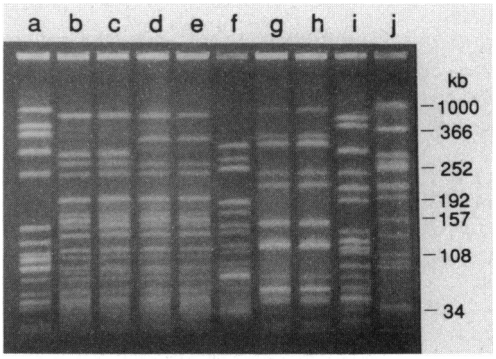

FIG. 3. Pulsed-field gel electrophoresis of *Sma*I-digested *E. faecalis* from Argentina. Bla$^+$ isolates are seen in lanes b (HG6280), c (HG9829), d (HG4354), and e (HG6049). Lanes a, f, g, h, and i contain highly gentamicin-resistant Bla$^-$ enterococci. Lane j contains *E. coli* MG1655 digested with *Not*I.

control spread of bacteria between patients are particularly important.

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REFERENCES

1. **Christie, P. J., R. Z. Korman, S. A. Zahler, J. C. Adsit, and G. M. Dunny.** 1987. Two conjugative systems associated with *Streptococcus faecalis* plasmid pCF10: identification of a conjugative transposon that transfers between *S. faecalis* and *Bacillus subtilis*. J. Bacteriol. **169**:2529–2536.
2. **Facklam, R. R., and M. D. Collins.** 1989. Identification of *Enterococcus* species isolated from human infections by a conventional test scheme. J. Clin. Microbiol. **27**:731–734.
3. **Hodel-Christian, S. L., and B. E. Murray.** 1990. Mobilization of the gentamicin resistance gene in *Enterococcus faecalis*. Antimicrob. Agents Chemother. **34**:1278–1280.
4. **Lopardo, H., L. Casimir, C. Hernandez, and E. A. Rubeglio.** 1990. Isolation of three strains of beta-lactamase-producing *Enterococcus faecalis* in Argentina. Eur. J. Clin. Microbiol. Infect. Dis. **9**:402–405.
5. **Murray, B. E.** 1990. The life and times of the enterococcus. Clin. Microbiol. Rev. **3**:46–65.
6. **Murray, B. E., F. Y. An, and D. B. Clewell.** 1988. Plasmids and pheromone response of the β-lactamase producer *Streptococcus* (*Enterococcus*) *faecalis* HH22. Antimicrob. Agents Chemother. **32**:547–551.
7. **Murray, B. E., K. V. Singh, J. D. Heath, B. R. Sharma, and G. M. Weinstock.** 1990. Comparison of genomic DNA of different enterococcal isolates using restriction endonucleases with infrequent recognition sites. J. Clin. Microbiol. **28**:2059–2063.
8. **Murray, B. E., K. V. Singh, S. M. Markowitz, H. A. Lopardo, J. E. Patterson, M. J. Zervos, E. Rubeglio, G. M. Eliopoulos, L. B. Rice, F. W. Goldstein, S. G. Jenkins, G. M. Caputo, R. Nasnas, L. S. Moore, E. S. Wong, and G. Weinstock.** 1991. Evidence for clonal spread of a single strain of β-lactamase-producing *Enterococcus faecalis* to six hospitals in five states. J. Infect. Dis. **163**:780–785.
9. **Patterson, J. E., A. Wanger, K. K. Zscheck, M. J. Zervos, and B. E. Murray.** 1990. Molecular epidemiology of β-lactamase-producing enterococci. Antimicrob. Agents Chemother. **34**:302–305.
10. **Patterson, J. E., and M. J. Zervos.** 1989. Susceptibility and bactericidal activity studies of four β-lactamase-producing enterococci. Antimicrob. Agents Chemother. **33**:251–253.
11. **Rhinehart, E., N. E. Smith, C. Wennersten, E. Gorss, J. Freeman, G. M. Eliopoulos, R. C. Moellering, and D. A. Goldmann.** 1990. Rapid dissemination of β-lactamase producing aminoglycoside-resistant *Enterococcus faecalis* among patients and staff on an infant toddler surgical ward. N. Engl. J. Med. **323**:1814–1818.
12. **Wanger, A. R., and B. E. Murray.** 1990. Comparison of enterococcal and staphylococcal β-lactamase plasmids. J. Infect. Dis. **161**:54–58.
13. **Zscheck, K. K., R. Hull, and B. E. Murray.** 1988. Restriction mapping and hybridization studies of a β-lactamase-encoding fragment from *Streptococcus* (*Enterococcus*) *faecalis*. Antimicrob. Agents Chemother. **32**:768–769.