JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1993, p. 653–658
0095-1137/93/030653-06$02.00/0
Copyright © 1993, American Society for Microbiology
Vol. 31, No. 3

Application of DNA Probes for rRNA and *vanA* Genes to Investigation of a Nosocomial Cluster of Vancomycin-Resistant Enterococci

NEIL WOODFORD,* DONALD MORRISON, ALAN P. JOHNSON, VICTORIA BRIANT, ROBERT C. GEORGE, AND BARRY COOKSON

The Laboratory of Hospital Infection, Central Public Health Laboratory, London NW9 5HT, England

Received 23 September 1992/Accepted 14 December 1992

DNA probes specific for genes encoding rRNA and the glycopeptide resistance gene *vanA* were used to investigate a cluster of vancomycin-resistant (MICs, >512 mg/liter) *Enterococcus faecalis* and *Enterococcus faecium* isolated from separate patients in a renal unit in a London hospital. When digested with *Bam*HI, 12 of 13 vancomycin-resistant *E. faecalis* isolates exhibited a common restriction fragment length polymorphism pattern of rRNA genes (ribotype). A *vanA* probe hybridized with chromosomal DNA in these 12 isolates. The other isolate of vancomycin-resistant *E. faecalis* had a different ribotype and the *vanA* gene was located on plasmid DNA. These data suggest that cross-infection with a single strain of vancomycin-resistant *E. faecalis* occurred in most instances. In contrast, 23 vancomycin-resistant *E. faecium* isolates showed greater heterogeneity, comprising 8 ribotypes, suggesting that multiple strains were present in the unit. Twenty-one of these 23 isolates harbored a 24-MDa plasmid which hybridized with the *vanA* probe, implying that interstrain dissemination of a vancomycin resistance plasmid may have occurred among *E. faecium* isolates in the renal unit.

Members of the genus *Enterococcus* are now recognized as important nosocomial pathogens, typically causing between 5 and 20% of nosocomial infections or colonizations in many hospitals (13, 17). Approximately 75 to 80% of such enterococcal episodes are caused by *E. faecalis*, with *E. faecium* responsible for an additional 10 to 15% of such episodes (17). Traditionally, the patient's own endogenous flora has been regarded as the source of enterococci responsible for nosocomial infections or colonizations (10, 17, 33). However, there is increasing evidence which suggests that transfer of enterococci between patients may also occur (17, 33). Carriage of enterococci by attending staff has been implicated as one possible route of transmission (27).

Detailed investigation of the epidemiology of clusters of enterococcal infections or colonizations has been limited by the lack of a suitable typing scheme for these organisms. A number of methods including biotyping (4, 12, 31), bacteriocin typing (14, 15, 25), phage typing (2, 3, 14, 15, 24, 31) and serotyping (29–31) have been applied to enterococci. Antibiotic susceptibility testing, determination of plasmid content, and/or plasmid digestion patterns have also been used (20, 34, 36). However, none provides suitable discrimination of clinical isolates. For this reason, there is still a need for a generally available, reliable typing scheme for both *E. faecalis* and *E. faecium*. Recently, a number of laboratories have begun to assess methods based on analysis of genomic DNA (1, 11, 18, 19).

We have previously described a cluster of infections or colonizations in patients in a renal unit; the infections were caused by enterococci that were highly resistant to the glycopeptide antibiotics vancomycin and teicoplanin (34). The majority of these vancomycin-resistant enterococci (VRE) were identified as *E. faecium* or *E. faecalis*. The resistance was transferable in vitro from both species (34) and was mediated by the *vanA* glycopeptide resistance gene (5). Preliminary evidence from serotyping and plasmid analysis and the unusual nature of the antimicrobial resistance pattern suggested that cross-infection might have occurred in the renal unit for each of the two species (34). However, the transferable nature of the vancomycin resistance determinant raised the possibility of a more complex epidemiological picture. In the present study, we examined VRE from this cluster using DNA probes specific for the genes encoding rRNA (ribotyping) and the *vanA* glycopeptide resistance gene.

MATERIALS AND METHODS

Bacterial isolates. Thirteen *E. faecalis* and 23 *E. faecium* isolates that were highly resistant to vancomycin (MICs, >512 mg/liter) and that were isolated from separate patients in the renal unit of Dulwich Hospital, London, between November 1986 and May 1988 (34) were recovered from storage at −70°C for inclusion in the present study. Two additional isolates from the renal unit (one *E. faecalis* and one *E. faecium*) which had lost their vancomycin resistance during storage were also included (Table 1). To assess the discrimination afforded by ribotyping for isolates of these two species, 16 other *E. faecalis* isolates and 18 other *E. faecium* isolates were also investigated. These comparison groups included National Collection of Type Cultures type strains of both *E. faecalis* (NCTC 775) and *E. faecium* (NCTC 7171), together with enterococci from diverse geographical areas. The latter group comprised organisms isolated over a 4-year period in 27 hospitals in five countries. The serotypes of *E. faecalis* isolates were determined by immunodiffusion by the scheme of Sharpe and Shattock (29, 30).

Preparation of a cDNA probe for 16S and 23S rRNA genes. The 16S and 23S rRNAs were extracted from the type strain

* Corresponding author.

of *E. faecalis* (NCTC 775), and a biotinylated cDNA probe was prepared by reverse transcription as described previously (22).

Preparation of the *vanA* gene probe. The probe consisted of a 399-bp digoxigenin-labeled intragenic fragment of *vanA*. This was amplified by the polymerase chain reaction (PCR) by using two 22-bp oligonucleotide primers from the published *vanA* sequence (6). The primers were (i) ATGGCAA GTCAGGTGAAGATGG (nucleotides 672 to 693; upstream) and (ii) TCCACCTCGCCAACAACTAACG (nucleotides 1071 to 1050; downstream).

E. faecalis 206, a vancomycin-resistant transconjugant, was used as the source of the *vanA* gene. It is derived from *E. faecium* NCTC 12202 (34), which is known to hybridize with a *vanA* gene probe (5). Strain 206 was grown overnight on Columbia blood agar, and the growth was resuspended in 1 ml of distilled water (tissue culture grade; Sigma, Poole, United Kingdom). After boiling for 15 min, the bacteria were pelleted in a microcentrifuge for 5 min and the supernatant (containing the template DNA) was stored on ice.

The PCR mixture consisted of the nucleotides dATP, dGTP dCTP, and dTTP (each at a final concentration of 250 μM; Perkin-Elmer Cetus purchased through ILS Ltd., London, United Kingdom), 10 μl of 10× *Taq* reaction buffer, 1 μl of template DNA, and 1 μg of each of the two primers. After the addition of 3 U of *AmpliTaq* polymerase (Perkin-Elmer Cetus), the total reaction volume was adjusted to 100 μl with distilled water (tissue culture grade; Sigma) and the sample was transferred on ice to a thermal cycler (Trio-thermoblock; Biometra, Maidstone, United Kingdom). The PCR program consisted of an initial denaturation step at 96°C for 2 min and then 35 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 60 s. After the final cycle, primer extension was allowed to continue at 72°C for 10 min. The sample was then stored at 4°C until required. A 1-μl aliquot of the 399-bp product of this first round of PCR was used as a template in a second PCR round. The conditions were identical to those described above, except that the concentration of dTTP was reduced to 166 μM and digoxigenin-11-dUTP (Boehringer Mannheim UK, Lewes, United Kingdom) was added to a concentration of 83 μM. The ratio of substituted dUTP to unsubstituted dTTP was therefore 1:2, as recommended previously (7).

PCR products were analyzed by electrophoresis through 2% agarose gels at 150 V for 1 h in 0.5× TBE buffer (28). The final 399-bp digoxigenin-labeled fragment to be used as the *vanA* probe was excised from the gel and purified with a Prep-a-Gene kit (Bio-Rad Laboratories, Hemel Hempstead, United Kingdom).

Extraction of genomic and plasmid DNAs. For ribotyping, genomic DNA was extracted from enterococci by using guanidium thiocyanate (23) and 5-μg aliquots were digested with 8 to 10 U of restriction endonucleases (Life Technologies Ltd., Uxbridge, United Kingdom) for 4 h. Plasmid DNA was extracted by a modified alkaline lysis method (34), in order to determine the location of the *vanA* gene. The molecular sizes of the enterococcal plasmids were determined by comparing them with plasmids of known size from *Escherichia coli* V517 and 39R861, which have been used previously (34–36). The extracted DNA was separated on 0.8% agarose gels by electrophoresis at 30 V for 20 h (genomic DNA) or 90 V for 2.5 h (plasmid DNA) in 0.5× TBE buffer. The DNA was then transferred to nylon membranes (Hybond-N; Amersham, Aylesbury, United Kingdom) by using a VacuGene vacuum blotting apparatus (Pharmacia-LKB, Milton Keynes, United Kingdom) and was fixed by baking at 80°C for 2 h.

Hybridization with gene probes. Hybridization and development of membranes with either the biotinylated cDNA probe specific for rRNA or the digoxigenin-labeled *vanA* probe were performed under stringent conditions by previously described methods (9, 21, 35, 36).

Analysis of ribotyping results. The banding patterns obtained for *E. faecalis* and *E. faecium* were analyzed separately. Patterns were recorded by visual examination of the blots, and all loci were scored for the presence or absence of a band (21). Patterns were differentiated into ribotypes according to a one-band difference rule. The ribotypes of *E. faecalis* and *E. faecium* were designated by roman numerals prefixed by Fs or Fm, respectively. The number of band differences between ribotypes was determined by using the simple matching coefficient. Intergel comparisons were made possible by the inclusion on each gel of the appropriate type strain and isolates of the predominant ribotypes (FsI and FmI) as standards.

RESULTS

Ribotyping of enterococci. Nine restriction endonucleases (*Bam*HI, *Cla*I, *Eco*RI, *Hin*dIII, *Pst*I, *Sac*I, *Sau*3A, *Xba*I, and *Xho*I) were assessed in order to ascertain which one showed the greatest potential for discrimination of epidemiologically unrelated enterococci. Genomic DNAs from two *E. faecalis* isolates (the type strain NCTC 775 and a vancomycin-resistant isolate from Dulwich Hospital) and two *E. faecium* isolates (the type strain NCTC 7171 and a vancomycin-resistant isolate from Dulwich Hospital) were digested with each of the endonucleases, and the restriction fragment length polymorphisms (RFLPs) of the rRNA genes were compared. For both *E. faecalis* and *E. faecium*, *Bam*HI gave the greatest degree of discrimination between the pairs of strains tested and was used in all subsequent studies. With this enzyme we detected 6 to 12 bands per strain. The bands ranged in size from greater than 23 to 2.9 kb. There were 11 band differences between the *Bam*HI ribotypes of the two *E. faecalis* isolates tested and 12 band differences between the two *E. faecium* isolates (Fig. 1). The ribotypes observed for the two species were distinct.

(i) VRE isolated from the renal unit of Dulwich Hospital. Hybridization of *Bam*HI-digested genomic DNAs with the cDNA probe for rRNA genes showed that 13 of 14 (93%) *E. faecalis* isolates, including one vancomycin-susceptible revertant, had identical ribotypes (designated pattern FsI; Table 1). These 13 isolates were all serotype 9. The other vancomycin-resistant isolate, which was serotype 9/19, had a distinct ribotype (designated FsII). There were six band differences between ribotypes FsI and FsII.

The 24 isolates of *E. faecium* from Dulwich Hospital were more heterogeneous than the *E. faecalis* isolates and were divided into eight ribotypes, designated FmI to FmVIII (Table 1). There were 1 to 18 band differences between these ribotypes. All of these ribotypes were distinct from those of *E. faecalis* isolates. Five ribotypes were represented by single isolates, while organisms belonging to ribotypes FmI, FmII, and FmIII were isolated from 13, 4, and 2 patients, respectively. The largest group, ribotype FmI, included a vancomycin-susceptible revertant (Table 1).

(ii) Other clinical isolates of enterococci. *Bam*HI differentiated 16 other isolates of *E. faecalis* from diverse geographical areas into 12 ribotypes. The number of band differences between these ribotypes ranged from 4 to 15. Three (19%) of

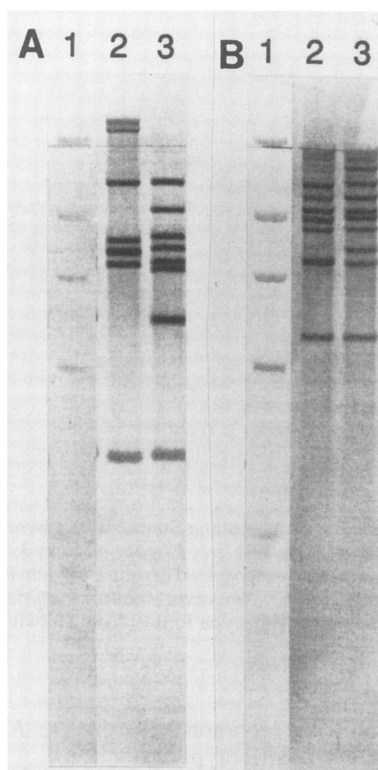

FIG. 1. Southern blots showing the RFLPs of rRNA genes of enterococci after digestion of genomic DNA with *Bam*HI. (A) *E. faecalis* NCTC 775 (lane 2) and a vancomycin-resistant *E. faecalis* isolate of ribotype FsI from Dulwich Hospital (lane 3); (B) *E. faecium* NCTC 7171 (lane 2) and a vancomycin-resistant *E. faecium* isolate of ribotype FmI from Dulwich Hospital (lane 3). Phage lambda DNA digested with *Hin*dIII served as a molecular weight marker (A and B, lanes 1).

these *E. faecalis* isolates belonged to ribotype FsI, which was the most common ribotype among the vancomycin-resistant isolates from Dulwich Hospital and included a VRE isolated from a renal patient at a second hospital in London. Ribotype FsII was seen in 1 of the 16 *E. faecalis* isolates not associated with the cluster of VRE in the renal unit. Ribotyping was able to subdivide isolates of *E. faecalis* which had identical serotypes (data not shown).

Eighteen geographically diverse, vancomycin-susceptible isolates of *E. faecium* could be divided into nine ribotypes. The numbers of band differences among the ribotypes of *E. faecium* ranged from 1 to 20. Ten isolates (56%) belonged to ribotype FmI, which was also the most prevalent ribotype among the isolates from Dulwich Hospital. The remaining isolates each belonged to distinct ribotypes not seen among the vancomycin-resistant isolates.

Hybridization with the *vanA* gene probe. The *vanA* gene probe hybridized with plasmid DNA in only 1 of 13 vancomycin-resistant *E. faecalis* isolates from the renal unit of Dulwich Hospital. In 12 of these isolates, which were all serotype 9 and ribotype FsI, the probe hybridized with only chromosomal DNA (Fig. 2). Although a vancomycin-resistant *E. faecalis* isolate from a second hospital in London had a ribotype identical to that of the 12 isolates, the *vanA* probe hybridized with a plasmid of approximately 60 MDa in this isolate. In the isolate from the renal unit of Dulwich Hospital, which was serotype 9/19 and ribotype FsII, plasmids of approximately 35 and 45 MDa hybridized with the probe.

TABLE 1. Ribotypes of vancomycin-resistant *Enterococcus* spp. isolated from patients in the renal unit of Dulwich Hospital between November 1986 and May 1988

Species	Serotype	No. of isolates	Ribotype
E. faecalis	9	13[a]	FsI
E. faecalis	9/19	1	FsII
E. faecium	—[b]	13[a]	FmI
E. faecium	—	4	FmII
E. faecium	—	2	FmIII
E. faecium	—	1	FmIV
E. faecium	—	1	FmV
E. faecium	—	1	FmVI
E. faecium	—	1	FmVII
E. faecium	—	1	FmVIII

[a] These groups of isolates each included one isolate which had reverted from vancomycin resistance (MIC, >512 mg/liter) to susceptibility (MIC, ≤4 mg/liter) during storage.
[b] —, the serotyping scheme used was not applicable to *E. faecium*.

The probe hybridized with plasmid DNA in all of the 23 vancomycin-resistant *E. faecium* isolates examined. The probe hybridized with a plasmid of approximately 24 MDa in 21 isolates, while in 2 isolates of ribotypes FmI and FmIV, respectively, it hybridized with a plasmid of approximately 40 MDa.

In those isolates of *E. faecalis* and *E. faecium* in which the probe hybridized with plasmid DNA, faint hybridization with the chromosomal DNA band was also observed (Fig. 2). In contrast, isolates of both species which had reverted to vancomycin susceptibility showed no hybridization with the *vanA* probe. The probe also did not react with the type strains *E. faecalis* NCTC 775 (Fig. 2) and *E. faecium* NCTC 7171 or with enterococci of the VanB and VanC glycopeptide resistance phenotypes (data not shown).

DISCUSSION

Direct analysis of RFLPs of digested enterococcal genomic DNA by conventional electrophoretic methods has previously been advocated for epidemiological studies (1, 11). However, in the present study nine restriction endonucleases were evaluated, but all produced large numbers of genomic fragments, which made comparison of isolates difficult (data not shown). As an alternative, we used a combination of DNA probes specific for genes encoding rRNA and the glycopeptide resistance protein VANA to investigate a cluster of vancomycin-resistant *E. faecalis* and *E. faecium* isolated from patients in the renal unit of Dulwich Hospital. Extended antimicrobial susceptibility testing, plasmid profiling, and serotyping previously suggested that cross-infection with both species of enterococci occurred in patients from this unit (34).

Twelve of 13 vancomycin-resistant *E. faecalis* isolates and a vancomycin-susceptible revertant from the renal unit had indistinguishable *Bam*HI ribotypes and all were serotype 9. These isolates had very similar plasmid profiles (34) and could not be subdivided by ribotyping with other restriction endonucleases. Furthermore, in Southern blots the *vanA* gene appeared to hybridize with chromosomal DNA in each of these 12 resistant isolates. It is unclear whether the transferable nature of *vanA* in these isolates reflects the presence of a conjugative transposon within the chromo-

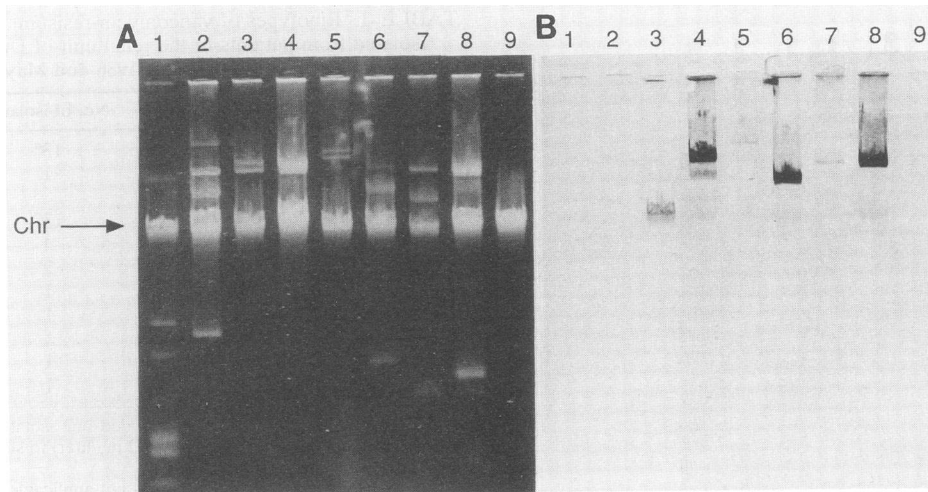

FIG. 2. (A) Agarose gel electrophoresis of plasmid DNA from *E. faecalis* and *E. faecium*; (B) corresponding Southern blot hybridized with the digoxigenin-labeled probe specific for the *vanA* glycopeptide resistance gene. Lanes: 1, *E. coli* V517; 2, *E. coli* 39R861; 3, *E. faecalis* isolate (serotype 9, ribotype FsI) from Dulwich Hospital; 4, *E. faecalis* isolate (serotype 9/19, ribotype FsII) from Dulwich Hospital; 5, vancomycin-resistant *E. faecalis* isolate (serotype 9, ribotype FsI) from a second London hospital; 6, *E. faecium* isolate (ribotype FmI) from Dulwich Hospital; 7, *E. faecium* isolate (ribotype FmI) from Dulwich Hospital; 8, *E. faecium* isolate (ribotype FmIV) from Dulwich Hospital; and 9, *E. faecalis* type strain, NCTC 775.

some, as suggested previously (34), or other possibilities such as the presence of an unusual transferable element which comigrates with chromosomal DNA (26). A distinct strain of vancomycin-resistant *E. faecalis* was isolated from a single patient. This strain was of a different ribotype and the *vanA* gene was present on plasmid DNA. These data suggest that the cluster of infections or colonizations caused by vancomycin-resistant *E. faecalis* isolates in the renal unit was due predominantly to the spread of a single strain between patients.

A vancomycin-resistant *E. faecalis* isolate from a renal patient at a second hospital in London was also of ribotype FsI. There was a transfer of at least one renal patient between Dulwich Hospital and the other hospital during the period of the cluster of vancomycin-resistant enterococcal infections at Dulwich Hospital (32), which raises the possibility of the interhospital spread of vancomycin-resistant *E. faecalis*. However, ribotype FsI was observed in a relatively high proportion (19%) of *E. faecalis* isolates not associated with the renal unit cluster of infections. This fact, together with the different locations of the *vanA* gene in the *E. faecalis* isolates from the two hospitals, means that the alternative possibility that vancomycin resistance arose independently cannot be excluded.

Results obtained with the combination of two DNA probes suggested that the epidemiology underlying the cluster of infections caused by vancomycin-resistant *E. faecium* differed from that seen with those caused by vancomycin-resistant *E. faecalis*. The 23 isolates of vancomycin-resistant *E. faecium* belonged to eight distinct *Bam*HI ribotypes, indicating that the isolates on the renal unit were heterogeneous. As seen with the ribotypes among vancomycin-resistant *E. faecalis* isolates, it was not possible to subdivide those of the *E. faecium* isolates by using other restriction endonucleases. A similar heterogeneity of ribotypes has also been observed among vancomycin-resistant *E. faecium* isolates in a children's hospital in France (1). In the present study, three ribotypes were each isolated from more than one patient on the renal unit, and although one ribotype predominated, 56% of geographically diverse *E. faecium* isolates were also of this ribotype.

Despite the heterogeneity of the vancomycin-resistant *E. faecium* isolates from patients in the renal unit, the *vanA* gene was present on a 24-MDa transferable plasmid in most isolates, suggesting that transfer of this plasmid between strains had occurred. However, the different location of the *vanA* gene in isolates of *E. faecalis* did not suggest the interspecies transfer of this plasmid. Isolates containing plasmids which hybridized with the *vanA* probe also gave faint hybridization with the chromosomal DNA band. This phenomenon, which we have seen with other probes (36), may be due to integration of a transposable element into the chromosome or degradation of plasmid DNA to its linear form during the extraction procedure. The original source of the *vanA* gene which emerged in both *E. faecalis* and *E. faecium* isolates from patients in this unit is unknown, although French et al. (8) have suggested that the spread of the *vanA* gene during outbreaks may be due to dissemination of a common resistance plasmid between enterococci and other genera in the commensal bowel flora.

Previous workers have found ribotyping of enterococci to be poorly discriminative and more suited to determination of an isolate's species than to epidemiological investigations (1, 11). We feel that our results suggest that ribotyping with *Bam*HI chromosomal digests may be of use for the investigation of clusters of infections and colonizations caused by enterococci. This enzyme discriminated isolates of both *E. faecalis* and *E. faecium*. In addition, it allowed subdivision of some isolates of *E. faecalis* which were indistinguishable by serotyping. This may be useful, because most clinical isolates are either nontypeable or are serotype 9 (16). Despite this, the predominant ribotypes found in both species from patients in the renal unit were also common among enterococci unrelated to this cluster. This limits the usefulness of ribotyping when used alone, particularly when clusters of infections are caused by enterococci of the common ribotypes. For this reason, ribotyping used in combination with other traditional or molecular typing meth-

ods may allow greater discrimination of enterococci. In prospective investigations it is also important to determine the prevalence of particular ribotypes present within a hospital population. However, this was not possible in the retrospective study described here.

In summary, ribotyping used in combination with a *vanA* probe demonstrated differences between the epidemiologies of two species of vancomycin-resistant enterococci from patients in the renal unit of Dulwich Hospital and suggested that dissemination of both strains and resistance determinants occurred concurrently. These findings serve to illustrate the fact that clusters of enterococci with unusual antimicrobial resistances are not necessarily indicative of the spread of single strains.

ACKNOWLEDGMENTS

We are grateful to Jon Clewley (Virus Reference Laboratory, Central Public Health Laboratory) and Androulla Efstratiou (Streptococcus Reference Laboratory, Central Public Health Laboratory) for advice in preparing the *vanA* probe. Mark Broughton (Virus Reference Laboratory, Central Public Health Laboratory) kindly synthesized the oligonucleotide primers.

V.B. was supported in part by a grant from Eli Lilly.

REFERENCES

1. **Bingen, E. H., E. Denamur, N. Y. Lambert-Zechovsky, and J. Elion.** 1991. Evidence for the genetic unrelatedness of nosocomial vancomycin-resistant *Enterococcus faecium* strains in a pediatric hospital. J. Clin. Microbiol. **29:**1888–1892.
2. **Brandis, H., P. Pleceas, and L. Andries.** 1985. Typing of *Streptococcus faecalis* and *Streptococcus faecium* strains by means of bacteriophages. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I Orig. Reihe A **260:**206–215.
3. **Caprioli, T., F. Zaccour, and S. S. Kasatiya.** 1975. Phage typing scheme for group D streptococci isolated from human urogenital tract. J. Clin. Microbiol. **2:**311–317.
4. **Coudron, P. E., C. G. Mayhall, R. R. Facklam, A. C. Spadora, V. A. Lamb, M. R. Lybrand, and H. P. Dalton.** 1984. *Streptococcus faecium* outbreak in a neonatal intensive care unit. J. Clin. Microbiol. **20:**1044–1048.
5. **Dutka-Malen, S., R. Leclercq, V. Coutant, J. Duval, and P. Courvalin.** 1990. Phenotypic and genotypic heterogeneity of glycopeptide resistance determinants in gram-positive bacteria. Antimicrob. Agents Chemother. **34:**1875–1879.
6. **Dutka-Malen, S., C. Molinas, M. Arthur, and P. Courvalin.** 1990. The VANA glycopeptide resistance protein is related to D-alanyl-D-alanine ligase cell wall biosynthesis enzymes. Mol. Gen. Genet. **224:**364–372.
7. **Emanuel, J. R.** 1991. Simple and efficient system for synthesis of nonradioactive nucleic acid hybridization probes using PCR. Nucleic Acids Res. **19:**2790.
8. **French, G., Y. Abdulla, R. Heathcock, S. Poston, and J. Cameron.** 1992. Vancomycin resistance in south London. Lancet **339:**818–819. (Letter.)
9. **Garaizar, J., M. E. Kaufmann, and T. L. Pitt.** 1991. Comparison of ribotyping with conventional methods for the type identification of *Enterobacter cloacae*. J. Clin. Microbiol. **29:**1303–1307.
10. **Gross, P. A., L. M. Harkavy, G. E. Barden, and M. F. Flower.** 1976. The epidemiology of nosocomial enterococcal urinary tract infection. Am. J. Med. Sci. **272:**75–81.
11. **Hall, L. M. C., B. Duke, M. Guiney, and R. Williams.** 1992. Typing of *Enterococcus* species by DNA restriction fragment analysis. J. Clin. Microbiol. **30:**915–919.
12. **Hussain, Z., M. Kuhn, R. Lannigan, and T. W. Austin.** 1988. Microbiological investigation of an outbreak of bacteraemia due to *Streptococcus faecalis* in an intensive care unit. J. Hosp. Infect. **12:**263–271.
13. **Jarvis, W. R., and W. J. Martone.** 1992. Predominant pathogens in hospital infections. J. Antimicrob. Chemother. **29**(Suppl. A): 19–24.
14. **Kuhnen, E., F. Richter, K. Richter, and L. Andries.** 1988. Establishment of a typing system for group D streptococci. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I. Orig. Reihe A **267:**322–330.
15. **Kuhnen, E., K. Rommelsheim, and L. Andries.** 1987. Combined use of phage typing, enterococcinotyping and species differentiation of group D streptococci as an effective epidemiological tool. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I. Orig. Reihe A **266:**586–595.
16. **Morrison, D., and B. D. Cookson.** Unpublished data.
17. **Murray, B. E.** 1990. The life and times of the enterococcus. Clin. Microbiol. Rev. **3:**46–65.
18. **Murray, B. E., K. V. Singh, J. D. Heath, B. R. Sharma, and G. M. Weinstock.** 1990. Comparison of genomic DNAs of different enterococcal isolates using restriction endonucleases with infrequent recognition sites. J. Clin. Microbiol. **28:**2059–2063.
19. **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. Wienstock.** 1991. Evidence for clonal spread of a single strain of beta-lactamase-producing *Enterococcus* (*Streptococcus*) *faecalis* to six hospitals in five states. J. Infect. Dis. **163:**780–785.
20. **Patterson, J. E., B. L. Masecar, C. A. Kauffman, D. R. Schaberg, W. J. Hierholzer, Jr., and M. J. Zervos.** 1988. Gentamicin resistance plasmids of enterococci from diverse geographic areas are heterogeneous. J. Infect. Dis. **158:**212–216.
21. **Pitcher, D., A. Johnson, F. Allerberger, N. Woodford, and R. George.** 1990. An investigation of nosocomial infection with *Corynebacterium jeikeium* in surgical patients using a ribosomal RNA gene probe. Eur. J. Clin. Microbiol. Infect. Dis. **9:**643–648.
22. **Pitcher, D. G., R. J. Owen, P. Dyal, and A. Beck.** 1987. Synthesis of a biotinylated DNA probe to detect ribosomal RNA cistrons in *Providencia stuartii*. FEMS Microbiol. Lett. **48:**283–287.
23. **Pitcher, D. G., N. A. Saunders, and R. J. Owen.** 1989. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. Lett. Appl. Microbiol. **8:**151–156.
24. **Pleceas, P.** 1979. A phage-typing system for *Streptococcus faecalis* and *Streptococcus faecium*, p. 264–265. *In* M. T. Parker (ed.), Pathogenic streptococci—Proceedings of the VIIth International Symposium on Streptococci and Streptococcal Diseases. Reedbooks, Chertsey, England.
25. **Pleceas, P., C. Bogdan, and A. Vereanu.** 1972. Enterocine-typing of group D streptococci. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I Orig. Reihe A **221:**173–181.
26. **Rahman, M., W. C. Noble, and B. Cookson.** 1989. Transmissible mupirocin resistance in *Staphylococcus aureus*. Epidemiol. Infect. **102:**261–270.
27. **Rhinehart, E., N. E. Smith, C. Wennersten, E. Gorss, J. Freeman, G. M. Eliopoulos, R. C. Moellering, Jr., and D. A. Goldmann.** 1990. Rapid dissemination of beta-lactamase-producing, aminoglycoside-resistant *Enterococcus faecalis* among patients and staff on an infant-toddler surgical ward. N. Engl. J. Med. **323:**1814–1818.
28. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
29. **Sharpe, M. E.** 1964. Serological types of *Streptococcus faecalis* and its varieties and their cell wall type antigen. J. Gen. Microbiol. **36:**151–160.
30. **Sharpe, M. E., and P. M. F. Shattock.** 1952. The serological typing of group D streptococci associated with outbreaks of neonatal diarrhoea. J. Gen. Microbiol. **6:**150–165.
31. **Smyth, C. J., H. Matthews, M. K. Halpenny, H. Brandis, and G. Colman.** 1987. Biotyping, serotyping and phage typing of *Streptococcus faecalis* isolated from dental plaque in the human mouth. J. Med. Microbiol. **23:**45–54.
32. **Uttley, A. H. C. (Dulwich Public Health Laboratory).** 1991. Personal communication.
33. **Uttley, A. H. C., and R. C. George.** 1991. Nosocomial entero-

coccal infection. Curr. Opin. Infect. Dis. **4**:525–529.

34. **Uttley, A. H. C., R. C. George, J. Naidoo, N. Woodford, A. P. Johnson, C. H. Collins, D. Morrison, A. J. Gilfillan, L. E. Fitch, and J. Heptonstall.** 1989. High-level vancomycin-resistant enterococci causing hospital infections. Epidemiol. Infect. **103**: 173–181.
35. **Woodford, N., and R. C. George.** 1992. Trimethoprim resistance linked to high-level gentamicin resistance in *Enterococcus faecium*. Med. Microbiol. Lett. **1**:11–16.
36. **Woodford, N., E. McNamara, E. Smyth, and R. C. George.** 1992. High-level resistance to gentamicin in *Enterococcus faecium*. J. Antimicrob. Chemother. **29**:395–403.