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A Gene Conferring Resistance to Vancomycin but Not Teicoplanin in Isolates of *Enterococcus faecalis* and *Enterococcus faecium* Demonstrates Homology with *vanB*, *vanA*, and *vanC* Genes of Enterococci

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We report the sequence of a 630-bp fragment of a gene associated with resistance to high levels of vancomycin in a clinical isolate of *Enterococcus faecalis* which retained susceptibility to teicoplanin. This gene was similar to the recently sequenced *vanB* and partially homologous with *vanA*, but it showed less-marked similarity to *vanC*. A DNA probe, derived from this polymerase chain reaction-amplified gene fragment, hybridized specifically with genomic DNA from *Enterococcus faecium* and *E. faecalis* isolates which were vancomycin resistant (MICs ranged from 8 to 512 μg/ml) but susceptible to teicoplanin. Curing of vancomycin resistance was associated with loss of DNA hybridization with the gene probe. Transfer of DNA which hybridized with the probe accompanied transfer of vancomycin resistance to a susceptible recipient strain. Neither curing nor transfer of vancomycin resistance was consistently related to loss or acquisition, respectively, of plasmid DNA.

Reports of glycopeptide-resistant enterococci began to appear in 1988 (21, 36). Since that time, such isolates have become a significant clinical problem in some medical centers (19, 23, 29, 37), and considerable effort to categorize and explore the resistance mechanisms involved has been expended. Isolates with resistance to high levels of vancomycin (MIC ≥ 64 μg/ml) and to teicoplanin (MIC ≥ 16 μg/ml) have been classified as phenotypic class A. Glycopeptide resistance among members of this class is both inducible and transferable (21, 32) and results from interaction of a novel ligase (VanA) (12) and a dehydrogenase (VanH) yielding D-Ala:D-hydroxy acid depsipeptides which, when added to peptidoglycan precursors, bind vancomycin poorly (4, 7, 8). Strains of *Enterococcus faecium* and *E. faecalis* which demonstrate moderate levels of resistance to vancomycin (MIC ≅ 16 to 64 μg/ml) and retain susceptibility to teicoplanin (MIC < 2 μg/ml) (30, 39) are assigned to phenotypic class B. Initial attempts to transfer vancomycin resistance of this phenotype were unsuccessful (11, 39). Phenotypic class C vancomycin resistance is found in *E. gallinarum* and *E. casseliflavus*, which possess constitutive low-level resistance to vancomycin (MIC ≅ 4 to 32 μg/ml) but remain susceptible to teicoplanin (38).

The *vanA* gene, encoding the VanA protein, was localized to a 34-kb plasmid in *E. faecium* BM4147 (6) and subsequently sequenced (12). By using conserved regions in the amino acid sequences of VanA and *Escherichia coli* D-Ala:D-Ala ligases, oligonucleotides which function as universal primers for the polymerase chain reaction (PCR) amplification of homologous segments of this family of genes were synthesized by Dutka-Malen et al. (13). This resulted in the identification and sequencing of the *vanC* gene, which is specific to *E. gallinarum* (13, 22). The genetic basis for vancomycin resistance in *E. casseliflavus* has yet to be elucidated. Recently, Evers and coworkers (16) sequenced a portion of a ligase gene, which they designated *vanB*, derived from a putative class B isolate, *E. faecalis* V583 (vancomycin MIC ≅ 64 μg/ml) (30). Beginning in 1991, a number of reports described isolates of *E. faecium* and *E. faecalis* with high levels of resistance to vancomycin (for which the MIC was as high as 1,024 μg/ml) yet which retained teicoplanin susceptibility (20, 25, 31, 35). Such isolates failed to fit into any of the phenotypic classes described above. Questions arose as to whether these strains, primarily American isolates, had the same genotype as class B organisms or whether they represented a unique genotype, particularly after high-level vancomycin resistance was successfully transferred to a susceptible recipient by cross-streak mating (35).

Utilizing the approach employed in the cloning of *vanC* (13), we sought to identify the gene encoding transferable, high-level vancomycin resistance in a teicoplanin-susceptible American isolate. We report the sequence of a PCR-amplified segment of this gene, its relationship to *vanA*, *vanB*, and *vanC* resistance genes, and its application as a gene probe which demonstrated hybridization specifically with DNA from vancomycin-resistant, teicoplanin-susceptible isolates representing a wide range of vancomycin resistance levels.

MATERIALS AND METHODS

Bacterial strains. Vancomycin-resistant enterococci were obtained from a number of sources in the United States, including hospitals in Boston, Mass.; Providence, R.I.; Long Island, N.Y.; and Philadelphia, Pa., and the Centers for Disease Control, as well as England and Spain. *E. faecalis* SF300, a clinical isolate from urine, was referred by Paul Edelstein, Philadelphia, Pa.; *E. faecalis* V583 was a gift of Daniel Sahm (30); and *E. faecium* 228 (17) was provided by Fred Tenover at the Centers for Disease Control.

Media. Isolates were maintained on brucella agar supplemented with 5% horse blood (Northeast Laboratory, Water-

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ville, Maine). Dextrose phosphate broth (Adams Scientific, West Warwick, R.I.) supplemented with 0.1% citrate (DPBC) was used for broth cultures and dilutions, unless otherwise stated. For cloning experiments with *E. coli*, Luria-Bertani broth (24) and Luria-Bertani agar (15 g of agar added to 1 liter of broth) were used. In the preparation of template DNA for sequencing, *E. coli* cells were grown in Terrific broth (34), as recommended by the manufacturer of the automated DNA sequencer.

In vitro susceptibility testing. Antibiotic susceptibility testing was carried out by using broth macrodilution or agar dilution methods. Agar dilution studies utilized Mueller-Hinton II plates (Becton Dickinson, Cockeysville, Md.) inoculated with $\cong 10^4$ CFU per spot. Vancomycin was provided by Eli Lilly & Co., Indianapolis, Ind., while teicoplanin was a gift from Marion Merrell Dow Inc., Cincinnati, Ohio. Some strains were screened for high-level gentamicin resistance with DPBC agar plates containing 500 μg of gentamicin per ml.

DNA isolation. Genomic DNA was extracted with guanidium thiocyanate by the method of Pitcher et al. (27). This method was modified by the use of log-phase cultures in 5 ml of DPBC and incubation in glycine (0.25 g/5 ml) at 37°C for 1 h prior to lysozyme treatment. Plasmid DNA was extracted by a mini alkaline lysis-polyethylene glycol precipitation procedure (1), with the following modifications for enterococci: the use of log-phase cultures in 5 ml of DPBC, glycine incubation (as described above), and lysozyme treatment at 5 mg/ml at 37°C for 30 min. In other experiments, enterococcal plasmid DNA was isolated by the technique described by Birnboim and Doly (5), with minor modifications (15). Chemical reagents were obtained from Sigma Chemical Co., St. Louis, Mo.

DNA cloning and sequencing. PCR primers were designed on the basis of the published sequences of *vanA* (12), *vanC* (13), the *Salmonella typhimurium* D-Ala:D-Ala ligase gene (10), and *E. coli* ligase genes *ddlA* (40) and *ddlB* (28) to produce universal primers for this family of genes by a method based on the approach used by Dutka-Malen et al. (13). The primers, synthesized by Oligos Etc. Inc., Williamsville, Oreg., were 5′-GA(AG) GAT GGI T(CG)C AT(AC) CA(AG) GG(AT)-3′ (primer 1) and 5′-(AC)GT (AG)AA ICC IGG CA(GT) (AG)GT (AG)TT-3′ (primer 2). PCR reagents were obtained from Perkin-Elmer Cetus, Norwalk, Conn. The PCR protocol was as recommended by the manufacturer, and specimens were processed with the DNA Thermal Cycler 480 (Perkin-Elmer Cetus). PCR conditions consisted of 30 cycles of denaturation (94°C for 30 s), annealing (55°C for 30 s), and extension (72°C for 2 min). PCR products were examined by electrophoresis in 0.8% agarose mini gels (GTG agarose; SeaKem, Rockland, Maine) run at 100 V and stained with ethidium bromide. The 0.6-kb product of PCR amplification of DNA from *E. faecalis* SF300 (MIC of vancomycin = 512 μg/ml; MIC of teicoplanin = 0.5 μg/ml) was cut from the gel, and the DNA was extracted from the agarose with a Geneclean II kit (Bio 101 Inc., La Jolla, Calif.). For cloning purposes, the PCR primers were modified by the addition of GAGAATTC to the 5′ end of primer 1 and of ACGAATTC to the 5′ end of primer 2. This resulted in a PCR product with restriction sites for *Eco*RI at each end. Insert DNA was derived from the combination of five individual PCR vessels. The *Eco*RI-digested PCR product was ligated into pBluescript II phagemid (Stratagene, La Jolla, Calif.), which had been cut with *Eco*RI and treated with calf intestinal alkaline phosphatase. *E. coli* XL1-Blue (Stratagene), made competent with calcium chloride, was used as the cloning host. Screening for transformants was done on Luria-Bertani agar plates containing ampicillin (50 μg/ml), tetracycline (12.5 μg/ml), X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) (80 μg/ml), and IPTG (isopropyl-β-D-thiogalactopyranoside) (20 mM). Plasmid mini preps were performed on apparent transformants, and then the gels were examined for the electrophoretic appearance of the plasmids before and after endonuclease digestion with *Eco*RI. The identity of the transforming DNA was confirmed by a PCR using the primers described above. Sequencing of the DNA insert was performed with three transformed clones at the Molecular Biology Core Facility at the Dana-Farber Cancer Institute (Boston, Mass.) with a model 373A *Taq* cycle sequencer (Applied Biosystems, Foster City, Calif.). Protocols for ligation and selection of transformants were from Stratagene. Enzymes, including *Eco*RI, calf intestinal alkaline phosphatase, and T4 DNA ligase, were obtained from Boehringer Mannheim Biochemicals (Indianapolis, Ind.) and used under conditions recommended by the manufacturer. X-Gal and IPTG were purchased from the same source.

Probe generation. After the PCR product from SF300 was cloned into pBluescript, the transformed plasmid was used as a template for production of probe DNA. The 0.6-kb fragment was purified by agarose gel electrophoresis as described above and then labeled with digoxigenin-dUTP with a kit purchased from Boehringer Mannheim and used according to the accompanying protocol.

DNA hybridization. A 10-μl volume of heat-denatured genomic or plasmid DNA was spotted onto Hybond-N 0.45-μm-pore-size nylon hybridization membranes (Amersham Corporation, Arlington Heights, Ill.). The DNA was bound to filters by baking at 85°C for 2 h under a vacuum. Hybridization reagents and protocol were obtained from Boehringer Mannheim (DIG nucleic acid detection kit), and stringent conditions were observed. Hybridization was carried out at 68°C overnight in a buffer containing 5× SSC (20× SSC is 3 M NaCl plus 0.3 M sodium citrate) (pH 7.0), 0.5% blocking reagent, 0.1% *N*-lauroylsarcosine, and 0.02% sodium dodecyl sulfate. The filters were washed with 2× SSC at room temperature and then with 0.1× SSC at 68°C. Filters were photographed after the color reaction had been allowed to proceed for 2 to 3 h.

Curing and transfer of vancomycin resistance. The MIC of novobiocin (Sigma) against *E. faecalis* SF300 was determined by broth macrodilution. The contents of the tubes with the MIC and the first dilution below the MIC were plated onto blood agar. Individual colonies were streaked simultaneously onto blood agar plates and DPBC agar plates containing 10 μg of vancomycin per ml. Cured strains were selected for their inability to grow on vancomycin-containing plates. The vancomycin MICs for the cured strains were determined by macrodilution in DPBC.

For studies of resistance transfer, *E. faecalis* SF298 (MIC of vancomycin = 64 μg/ml; MIC of teicoplanin = 0.25 μg/ml) was plated onto blood agar (see "Media" above) in a broad swath. This was cross-streaked with vancomycin-susceptible, rifampin- and fusidic acid-resistant strains, specifically *E. faecalis* JH2-2 (18) and *E. faecium* GE-1 (14). Confluent growth, present at the intersections after overnight culture at 37°C, was transferred to DPBC agar plates containing fusidic acid (25 μg/ml), rifampin (100 μg/ml), and vancomycin (20 μg/ml). These plates were incubated for 4 days. Colonies which appeared were replated on the same selective medium to confirm the presence of both donor and recipient resistance determinants. The plasmid and genomic DNAs of the

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1    GAGGATGGGTGCATCCAGGGACTGTTTGAATTGTCTGGTATCCCCTATGTGGGCTGTGATATTCAAAGCTCCGCAGCTTGCATGGACAAATCACTGGCCT  100
101  ACATTCTTACAAAAAATGCGGGCATCGCCGTTCCCGAATTTCAAATGATTGATAAAGGTGACAAGCCGGAGGCGGGTGCGCTTACCTACCCTGTCTTTGT  200
201  GAAGCCGGCACGGTCAGGTTCGTCCTTTGGCGTAACCAAAGTAAACGGTACGGAAGAACTTAACGCTGCGATAGAAGCGGCAGGACAATATGATGGAAAA  300
301  ATCTTAATTGAGCAAGCGATTTCGGGCTGTGAGGTCGGGTGTGCGGTCATGGGGAACGAGGATGATTTGATTGTCGGCGAAGTGGATCAAATCCGGCTGA  400
401  GCCACGGTATCTTCCGCATCCATCAGGAAAACGAGCCGGAAAAAGGCTCAGAAAATGCGATGATTACAGTTCCCGCAGACATTCCGGTCGAGGAACGAAA  500
501  TCGGGTGCAGGAAACGGCAAAGAAAGTATATCGGGTGCTTGGATGCAGAGGGCTTGCCCGTGTTGATCTTTTTTTGCAGGAGGATGGCGGCATCGTTCTA  600
601  AATGAGGTCAACACCCTGCCCGGCTTCACG  630
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FIG. 1. Sequence of the 630-bp PCR-amplified segment of the vancomycin resistance gene in *E. faecalis* SF300. Nucleotides which are complementary to the PCR primers are underlined.

resulting strains were examined by agarose gel electrophoresis and DNA hybridization.

Nucleotide sequence accession number. The nucleotide sequence of the 630-bp PCR-amplified segment of the vancomycin resistance gene in *E. faecalis* SF300 has been lodged in the GenBank data base under accession number L15304.

RESULTS

Glycopeptide susceptibility of study isolates. Clinical isolates possessing various phenotypes of glycopeptide resistance were studied. For 19 isolates of teicoplanin-susceptible *E. faecium* and *E. faecalis*, the MICs of vancomycin ranged from 8 to 512 μg/ml (teicoplanin MICs were ≤1 μg/ml for all of these strains). MICs of vancomycin and teicoplanin for four class A isolates ranged from 256 to 512 and 64 to 128 μg/ml, respectively. Phenotypic class C was represented by three isolates of *E. gallinarum* for which the MIC of vancomycin was 8 μg/ml and the MIC of teicoplanin was 0.5 μg/ml and by one *E. casseliflavus* isolate for which the MICs of vancomycin and teicoplanin were 4 and <0.125 μg/ml, respectively. Glycopeptide-susceptible strains included *E. faecalis* JH2-2 and EBC22.

DNA sequence of the 0.6-kb PCR product from *E. faecalis* SF300. PCR amplification of DNA from a highly vancomycin-resistant strain, *E. faecalis* SF300, consistently produced a fragment of approximately 0.6 kb with the degenerate primers we selected. This fragment was cloned into *E. coli*, and three individual transformants were sequenced. Excluding the primers, only two bases showed less than complete agreement, and at these two loci, two of the three sequences were in agreement, allowing the creation of a consensus sequence. This 630-bp sequence (Fig. 1) showed significant homology to *vanB* and, to a lesser extent, to *vanA* and *vanC*. Specifically, there was 96.4% base pair identity between the gene fragment and the published sequence of *vanB* (16). The deduced amino acid sequence of the protein encoded by the novel gene was 97.5% homologous with the deduced amino acid sequence of VanB, 77% homologous with VanA, and 36% homologous with VanC as determined by comparison with previously published aligned, gapped sequences of the last two proteins (13) (Fig. 2).

Probe DNA hybridization with enterococci. Hybridization experiments, using the gene probe described above, were performed with DNA extracted from 30 strains of enterococci. The probe hybridized with genomic DNA from 19 of 20 isolates of vancomycin-resistant, teicoplanin-susceptible *E. faecium* and *E. faecalis* (vancomycin MICs, 8 to 512 μg/ml), including *E. faecalis* SF300 and V583. When plasmid DNA from several of these isolates was examined, the probe failed to hybridize (data not shown). The probe was specific for this phenotype, as it did not hybridize with genomic DNA from class A vancomycin-resistant isolates (including a prototypic class A strain, *E. faecium* 228 [17]) or class C vancomycin-resistant isolates under stringent conditions. In addition, there was no hybridization with DNA from vancomycin-susceptible strains of *E. faecalis* (JH2-2 and EBC22).

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VanB2  EDGCIQGLFELSGIPYVGCDIQSSAACMDKSLAYILTKNAGIAVPEFQMIDKGDKP------EAGALTYPVFVKPARSGSSFGVTKVNGTEELNAAIEAA  100
VanB   xxxxxxxLFELSGIPYVGCDIQSSAACMDKSLAYILTKNAGIAVPEFQMIEKGDKP------EARTLTYPVFVKPARSGSSFGVTKVNSTEELNAAIEAA
VanA   EDGSIQGLFELSGIPFVGCDIQSSAICMDKSLTYIVAKNAGIATPAFWVINKDDRP------VAATFTYPVFVKPARSGSSFGVKKVNSADELDYAIESA
VanC   EDGCIQGLLELMNLPYVGCHVAASALCMNKWLLHQLADTMGIASAPTLLLSRYENDPATIDRFIQDHGFPIFIKPNEAGSSKGITKVTDKTALQSALTTA

VanB2  GQYDGKILIEQAISGCEVGCAVMGNEDDLIVGEVDQIRLSHGIFRIHQENEPEKGSENAMITVPADIPVEERNRVQETAKKVYRVLGCRGLARVDLFLQE  200
VanB   GQYDGKILIEQAISGCEVGCAVMGNEDDLIVGEVDQIRLSHGIFRIHQENEPEKGSENAMIIVPADIPVEERNRVQETAKKVYRVLGCRGLARVDLFLQE
VanA   RQYDSKILIEQAVSGCEVGCAVLGNSAALVVGEVDQIRLQYGIFRIHQEVEPEKGSENAVITVPADLSAEERGRIQETAKKIYKALGCRGLARVDMFLQD
VanC   FAYGSTVLIQKAIAGIEIGCGILGNE-QLTIGACDAISLVDGFFDFEEKYQLIS----ATITVPAPLPLALESQIKEQAQLLYRNLGLTGLARIDFFVTN

VanB2  DGGIVLNEVNTLPGFT
VanB   DGGIVLNEVxxxxxxx
VanA   NGRIVLNEVNTLPGFT
VanC   QGAIYLNEINTMPGFT
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FIG. 2. Aligned deduced amino acid sequences of VanB2, VanB, VanA, and VanC. Gaps have been inserted to produce maximum homology. Amino acids which differ between the sequences of VanB2 and VanB are underlined.

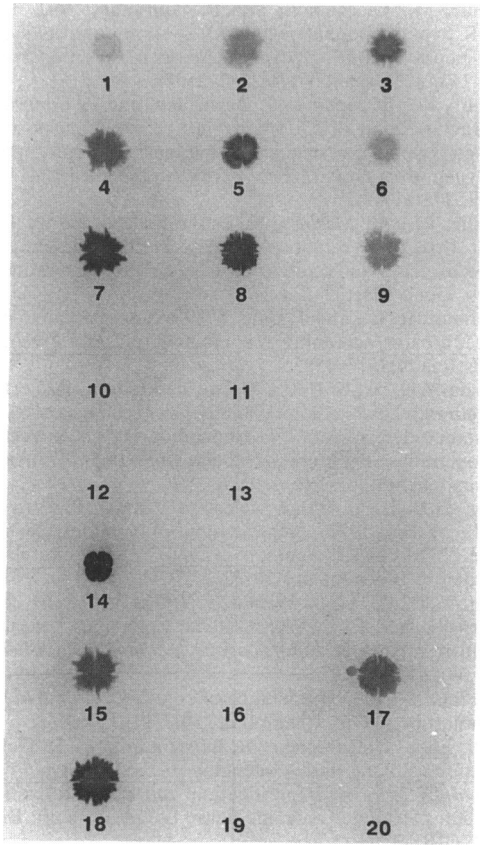

FIG. 3. Representative dot blot of genomic DNA extracted from the following enterococci with and without resistance to glycopeptides, after hybridization with a digoxigenin-labeled probe derived from the vancomycin resistance gene of *E. faecalis* SF300: dots 1 through 9, *E. faecium* and *E. faecalis* isolates susceptible to teicoplanin and for which vancomycin MICs ranged from 8 μg/ml (dot 1) to 512 μg/ml (dot 9); dot 10, *E. faecium* 228 (class A [15]); dot 11, *E. gallinarum* (class C); dots 12 and 13, vancomycin-susceptible strains *E. faecalis* EBC22 and JH2-2, respectively; dot 14, unlabeled probe DNA, used as a positive control; dots 15, 16, and 17, a vancomycin-resistant donor (*E. faecalis* SF298), a vancomycin-susceptible recipient (*E. faecalis* JH2-2), and a vancomycin-resistant transconjugant (*E. faecalis* SF298T), respectively; dot 18, a vancomycin-resistant clinical isolate (*E. faecalis* SF300); and dots 19 and 20, two vancomycin-susceptible laboratory-derived strains (*E. faecalis* SF300CE and SF300C1, respectively).

A representative dot blot, showing positive hybridization signals from nine vancomycin-resistant, teicoplanin-susceptible clinical isolates from the United States, is shown in Fig. 3. Also shown are negative signals from one class A strain, one class C strain, and vancomycin-susceptible strains JH2-2 and EBC22.

Strains cured of high-level vancomycin resistance. Several vancomycin-susceptible clones, including SF300CE and SF300C1 (MICs of vancomycin = 2 to 4 μg/ml), were derived from SF300 (vancomycin MIC = 512 μg/ml) by curing with novobiocin. The cured strains retained resistance to high levels of gentamicin, erythromycin, clindamycin, and tetracycline. Examination of plasmid DNA from five cured strains did not reveal any change in the electrophoretic mobility of the single, high-molecular-weight plasmid seen in SF300 (data not shown). Genomic DNA extracted from SF300CE and SF300C1 did not hybridize with the gene probe derived from the parent strain (Fig. 3).

A transconjugant of SF298 and JH2-2. Cross-streak mating yielded a transconjugant of *E. faecalis* SF298 and JH2-2. The MICs of vancomycin against the two parent strains were 64 and 1 μg/ml, respectively. The vancomycin MIC for the transconjugant, SF298T, was 128 μg/ml. All three strains were susceptible to teicoplanin. High-level resistance to gentamicin, present in the donor, was not transferred. SF298 had at least two plasmids; however, repeated attempts failed to detect plasmid DNA in the transconjugant (data not shown). DNA hybridization with the digoxigenin-labeled probe revealed a strongly positive signal from genomic DNA extracted from SF298 and SF298T, with no evidence of hybridization with JH2-2 (Fig. 3). Attempts to transfer vancomycin resistance from isolate SF300 were hampered by the presence of bacteriocin activity, i.e., growth of recipient strains was inhibited by SF300.

DISCUSSION

Three major phenotypes of glycopeptide resistance in enterococci have been previously well described, with groupings based on the degree of vancomycin resistance, susceptibility to teicoplanin, inducibility, and transferability (9, 33). At this time, much is known about the molecular basis for glycopeptide resistance in enterococci of the class A phenotype. On the basis of early reports, it appears that in most cases the resistance determinants are carried on plasmids (12). Inducible resistance is associated with the synthesis of a 39-kDa cytoplasmic membrane protein (26, 32) which is structurally related to the D-Ala:D-Ala ligases of *E. coli* and *S. typhimurium* (12). This enzyme, identified as VanA, exhibits ligase activity producing altered peptidoglycan precursors which bind vancomycin poorly (7). The *vanA* gene, which encodes this protein, was found to be part of a cluster of structural and regulatory genes, including *vanH*, encoding a dehydrogenase which produces substrate for *vanA* (4, 8), and a two-component regulatory system (*vanR* and *vanS*) (2). Recent work indicates that the *vanA* gene cluster is carried on a transposon (3).

Low-level vancomycin resistance characteristic of *E. gallinarum* (class C) is mediated by the *vanC* gene, which displays 29 to 38% sequence homology with *vanA* and the *E. coli* D-Ala:D-Ala ligase genes (13). A DNA probe derived from a 690-bp restriction fragment of the gene hybridized specifically with *E. gallinarum* but not with the phenotypically similar species *E. casseliflavus* (22). The molecular basis for vancomycin resistance in the latter species is as yet undetermined.

An internal fragment of *vanB* derived from an *E. faecalis* isolate characteristic of the class B phenotype was recently sequenced (16). Following the initial description of the class B phenotype, several reports noted high-level vancomycin resistance among *E. faecium* and *E. faecalis* isolates from hospitals in the United States (vancomycin MICs ≥ 64 μg/ml), a level of resistance comparable to that of the class A phenotype. However, these isolates resembled class B strains, as they were teicoplanin susceptible (20, 25, 31, 35). This novel pattern of resistance was noted to be transferable to a vancomycin-susceptible enterococcal recipient and was referred to as "VanAmerican" (35).

We have demonstrated that the DNA sequence of a 630-bp segment amplified from one of these isolates (*E. faecalis* SF300) differed by 3.6% from *vanB*. In light of this degree of homology, we propose to call this gene *vanB2*. Our sequence

was significantly related to the *vanA* gene and had lesser homology with *vanC*. Although the proposed *vanB2* had partial homology with *vanA* and *vanC*, however, a digoxigenin-labeled probe prepared from cloned DNA of *E. faecalis* SF300 was specific in showing no detectable hybridization with DNA from either class A or class C glycopeptide-resistant enterococci under stringent conditions. Loss of vancomycin resistance in a laboratory derivative of SF300 selected following exposure of the parent to novobiocin was associated with failure to hybridize with our vancomycin resistance gene probe, providing further evidence of the specificity of this reaction.

This probe reacted not only with DNA of strains resistant to high concentrations of vancomycin (several of which had previously been shown not to hybridize with a *vanA* gene probe [35]) but also with strains with low and moderate levels of resistance characteristic of originally described class B isolates (30, 39), including *E. faecalis* V583. This is strong evidence that the 630-bp sequence derived from *E. faecalis* SF300, or a sequence that is nearly identical (i.e., *vanB* or closely related genes), is found in *E. faecium* and *E. faecalis* isolates for which vancomycin MICs range from 8 to 512 μg/ml yet which retain teicoplanin susceptibility. It is conceivable that differences in levels of vancomycin resistance among the strains we studied arise from minor alterations in the sequence of this ligase gene which alter phenotypic expression without preventing hybridization with the DNA probe under stringent conditions. An alternative explanation is that differences in factors regulating gene expression produce this wide range of vancomycin resistance.

The experiments we have described above present indirect evidence for a chromosomal location of this transferable vancomycin resistance determinant in some isolates. First, the probe hybridized with genomic but not plasmid DNA of several strains. Also, loss of vancomycin resistance from one isolate occurred without alteration in the electrophoretic mobility of a single high-molecular-weight plasmid, loss of erythromycin, clindamycin, or tetracycline resistance, or loss of high-level gentamicin resistance; transfer of vancomycin resistance from a second isolate occurred without acquisition of plasmid DNA or of high-level gentamicin resistance. These data support a hypothesis of conjugative transposon-mediated vancomycin resistance (as has been recently described for *vanA* [3]), a possibility that merits further investigation.

In summary, the gene conferring resistance to high levels of vancomycin in the absence of resistance to teicoplanin in *E. faecalis* SF300 was closely related by sequence homology to *vanB* and to a lesser degree to *vanA* and *vanC*. Strategies which have been successfully employed to investigate resistance mechanisms in class A strains should be applicable to the further study of enterococcal isolates which have susceptibility to teicoplanin despite having various levels of resistance to vancomycin.

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