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Original Article

Molecular and Epidemiological Study of the First Outbreak of *vanB* Type Vancomycin-Resistant *Enterococcus faecalis* in Japan

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SUMMARY: In July, 1999, an outbreak of vancomycin-resistant *Enterococcus faecalis* (VREF) with the *vanB* genotype occurred for the first time in Japan at Hokushin General Hospital, Nakano City, Nagano Prefecture. Four VREF strains were isolated from the clinical specimens of four inpatients, and 16 VREF strains were isolated by the screening of asymptomatic carriers and by surveillance of the hospital environment. All of the isolates possessed *vanB* genes. In a pulsed-field gel electrophoresis analysis, 19 out of 20 VREF isolates exhibited the indistinguishable restriction endonuclease digestion patterns of the chromosomal DNA. Additional investigation by Southern hybridization using the *vanB* probe implied that the *vanB* gene of these 19 isolates was encoded on a 110-kb plasmid. These findings indicate that the outbreak was principally caused by a single clone. The restriction endonuclease digestion patterns of the remaining single isolate was different from those of the other isolates. The *vanB* gene was encoded on the chromosome.

INTRODUCTION

Particularly in Europe and the United States, vancomycin-resistant enterococci (VRE) have emerged as a significant cause of nosocomial infections and colonizations since their first description in 1988 (1). In Japan, a *vanB* genotype *Enterococcus gallinarum* was first isolated in 1996 (2). Since then, only two sporadic cases of VRE infection, both of which were due to *E. faecium* (3,4), had been reported in Japan. One case was an 81-year-old female inpatient with acute pyelonephritis caused by a *vanA* genotype VRE (3), and the other was a 27-year-old female inpatient with post-operative abdominal lymphocyst infection by a *vanB* genotype VRE (4). However, in July of 1999, an outbreak of nosocomial infections of vancomycin-resistant *E. faecalis* (VREF) occurred at Hokushin General Hospital, Nakano City, Nagano Prefecture (5). In this report, we describe the results of molecular and epidemiological studies of the 20 VREF strains isolated from the outbreak, which was the first VREF outbreak in Japan. The results indicate that a single clone with the *vanB* genotype principally caused the outbreak. Our data also suggested that the *vanB* gene of the clone was encoded on a 110-kb plasmid.

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MATERIALS AND METHODS

Isolation, identification, and screening of VRE: Hokushin General Hospital is a 640-bed hospital with four wards (Wards A-D). Four VREF (designated H1-H4) were isolated from the clinical specimens of four aged inpatients (76 to 87 years old) on Wards A and B during a 3-week period in July 1999. Eight VREF strains were isolated by the screening of asymptomatic carriers (P-numbered isolates), and eight were isolated by the surveillance of the hospital environment on Wards A and B (E-numbered isolates).

Identification of *E. faecalis* was made by Vitek GPI Cards (bioMérieux Vitek systems, Inc., Hazelwood, Mo., USA) and the MicroScan WalkAway system (Dade International, West Sacramento, Calif., USA). To screen the asymptomatic carriers, 819 rectal swabs and/or sputa were collected from 759 inpatients and 57 inpatients' family members. Rectal swabs were collected from the 969 hospital personnel as well. The rectal swabs were collected using sterilized cotton swabs moistened with sterilized saline. Each rectal swab or one loopfull of each sputum was spread onto the Enterococcosel agar plates supplemented with 8 μg/ml of vancomycin (Nippon Becton Dickinson, Tokyo).

For the surveillance of VRE in the hospital environment, specimens were collected from 1,054 hospital environmental sites on Wards A and B by swabbing an area of about 100

cm^2 with sterilized cotton swabs moistened with sterilized saline. Specimens were subjected to the isolation and identification procedures described above. After disinfecting the ward environment, the bacteriological investigation of the 840 sites of Wards A and B was carried out by the same procedure in order to ascertain the elimination of VRE contamination.

Antimicrobial susceptibility testing: Resistance levels of each VRE isolate against various antimicrobial agents except teicoplanin were determined both by Vitek GPS Cards and the MicroScan WalkAway system according to the instructions provided with each system. Both systems yielded the same susceptibility results. Since we only used the standard protocols provided by the manufactures, the exact minimum inhibitory concentrations (MICs) were not determined. In this report, the values obtained from the analyses using the two systems were described as the MICs. Susceptibilities to teicoplanin were determined by the disk diffusion method using Sensidisks (Nippon Becton Dickinson).

Detection of *vanA*, *vanB*, and *vanC* genes by polymerase chain reaction (PCR): Oligonucleotide primers for the amplification of *vanA*, *vanB*, and *vanC1* genes were prepared as previously described (6). Bacterial isolates to be tested were grown at 35°C overnight on sheep blood agar plates (Nippon Becton Dickinson). Colonies were suspended in 300 μl of distilled water and were boiled for 10 min. Boiled samples were chilled quickly on ice and were subjected to centrifugation at 30,000 G for 5 min, and the supernatants were used as templates for PCR. PCR reaction mixtures of 25 μl final volume contained 5 μl of the sample, 1 μl of each primer solution (25 μM), 2.5 μl of dNTP mixture (2 mM each), 0.15 μl of Taq Gold Polymerase (50 units/μl, Roche Molecular Biochemicals, Mannheim, Germany), 2.5 μl of 10 × PCR buffer supplemented with Taq Gold Polymerase, and 12.85 μl of distilled water. PCR was performed on a DNA thermal cycler (Gene Amp 9600; Applied Biosystems, Foster City, Calif., USA) with cycles of 95°C for 12 min for the first cycle, 94°C for 30 sec, 58°C for 30 sec, and 72°C for 1 min for the next 40 cycles, and then 72°C for 7 min for the last cycle. PCR products were analyzed on 2% agarose gel prepared with 0.5 × Tris-borate-EDTA (TBE) buffer (1 × TBE buffer: 89 mM Tris, 89 mM boric acid, and 2.5 mM EDTA [pH8.0]) containing ethidium bromide (0.5 μg/ml). *E. faecalis* strain RV1, which was provided by Dr. Y. Arakawa (National Institute of Infectious Diseases, Tokyo), was used as a *vanB*-positive control strain.

Pulsed-field gel electrophoresis (PFGE): Preparation of intact genomic DNAs and restriction endonuclease-digestion of the DNAs were performed as described previously (7), except that achromopeptidase (Wako Junyaku, Tokyo) was included in the lysis solution at a final concentration of 4 mg/ml. All the restriction endonucleases used were purchased from Takara Shuzo (Shiga). Electrophoresis was performed on 1% agarose gel prepared with 0.5 × TBE buffer using a CHEF MAPPER system (Bio-Rad Laboratories, Hercules, Calif., USA) according to the manufacture's instruction. Running conditions were described in the figure legend of Fig. 2.

Southern hybridization analysis: DNAs were transferred from PFGE gels to nylon membranes (Hybond N^+; Amershamm Pharmacia Biotech, Buckinghamshire, UK) using a VacuGene blotter apparatus (Amershamm Pharmacia Biotech). The *vanB* probe, a 635 bp fragment of the *vanB* coding region, was prepared by PCR, and was purified from an agarose gel using a Gene clean II Kit (Bio 101, Carlsbad, Calif., USA). Probe labeling, hybridization, and signal detection were carried out using an ECL™ Direct Nucleic Acid Labeling and Detection System (Amershamm Pharmacia Biotech).

RESULTS

Isolation of VRE: During a 3-week period in July 1999, four inpatients in Wards A or B yielded VREF (H1-H4 in Table 1). Each isolate was originated from the sputum of an

Table 1. 20 *vanB* genotype VRE isolates and their antimicrobial susceptibilities

Ward	Origin	Isolate Number	Date of isolation	Source	Drug resistance levels (MIC, μg/ml)*						
					VCM	TEIC	PCG	ABPC	GM	AMK	LVFX
B	Inpatient	H1	9, July	Sputum	≧32	S	≧16	2	≧16	≧64	≧8
A	Inpatient	H2	19, July	Urine	≧32	S	≧16	2	≧16	≧64	≧8
A	Inpatient	H3	27, July	Urine	≧32	S	≧16	2	≧16	≧64	≧8
B	Inpatient	H4	31, July	Rectal swab	≧32	S	≧16	2	≧16	≧64	≧8
B	Inpatient	P35	1, Aug.	Rectal swab	≧32	S	≧16	2	≧16	≧64	≧8
B	Inpatient	P38	1, Aug.	Rectal swab	≧32	S	≧16	2	≧16	≧64	≧8
B	Inpatient	P40	1, Aug.	Rectal swab	≧32	S	≧16	2	≧16	≧64	≧8
A	Inpatient	P93	1, Aug.	Rectal swab	16	S	2	0.5	≧16	≧64	≦1
B	Inpatient	P473	1, Aug.	Rectal swab	≧32	S	≧16	2	≧16	≧64	≧8
A	Inpatient	P488	1, Aug.	Rectal swab	≧32	S	≧16	2	≧16	≧64	≧8
A	Inpatient	P1130	1, Aug.	Rectal swab	≧32	S	≧16	2	≧16	≧64	≧8
A	Inpatient	P8065	1, Aug.	Rectal swab	≧32	S	≧16	2	≧16	≧64	≧8
B	Environment	E201	4, Aug.	ward floor	≧32	S	≧16	2	≧16	≧64	≧8
B	Environment	E207	4, Aug.	bed side table	≧32	S	≧16	2	≧16	≧64	≧8
B	Environment	E208	4, Aug.	over table	≧32	S	≧16	2	≧16	≧64	≧8
B	Environment	E213	4, Aug.	ward floor	≧32	S	≧16	2	≧16	≧64	≧8
B	Environment	E222	4, Aug.	bed side table	≧32	S	≧16	2	≧16	≧64	≧8
B	Environment	E225	4, Aug.	bed pan	≧32	S	≧16	2	≧16	≧64	≧8
A	Environment	E398	4, Aug.	ward floor	≧32	S	≧16	2	≧16	≧64	≧8
B	Environment	E518	4, Aug.	slipper	≧32	S	≧16	2	≧16	≧64	≧8

* All the values were the ones determined by the Vitek GPS Cards and the MicroScan WalkAway system using the standard protocols that the manufactures provided. VCM: vancomycin, TEIC: teicoplanin, PCG: benzylpenicillin, ABPC: ampicillin, GM: gentamicin, AMK: amikacin, LVFX: levofloxacin, S: susceptible.

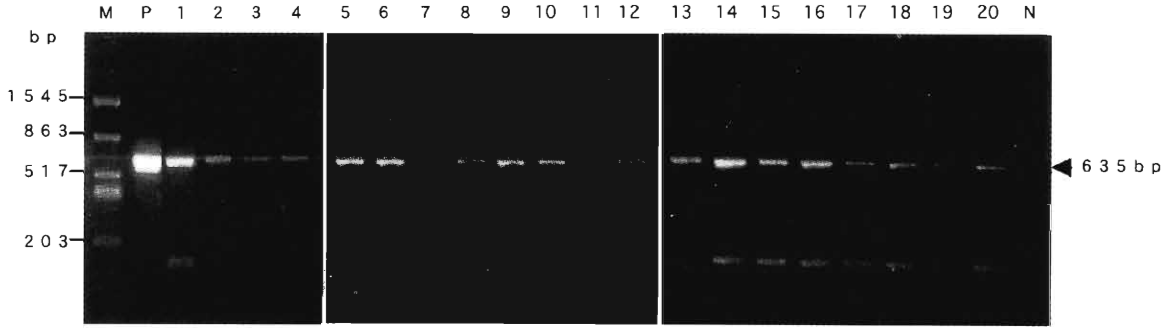

Fig. 1. Detection of *vanB* genes in 20 VRE isolates. PCR products were analyzed on 2% agarose gel. Lanes: M: molecular weight marker, P: *vanB*-positive strain (*E. faecalis* RV1), N: negative control, 1: H1, 2: H2, 3: H3, 4: H4, 5: P35, 6: P38, 7: P40, 8: P93, 9: P473, 10: P488, 11: P1130, 12: P8065, 13: E201, 14: E207, 15: E208, 16: E213, 17: E222, 18: E225, 19: E398, 20: E518.

85-year-old female with bronchitis (H1), from the urine of a 76-year-old female with cerebral infarction (H2), from the urine of an 87-year-old female with cerebral infarction (H3), and from the rectal swab of a 76-year-old female suffering from gallbladder cancer (H4). From the sputum of the 85-year-old female and the urine of the 76-year-old female, methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* were also isolated, respectively. MRSA was also isolated from the stool of the 76-year-old female and the sputum and stool of the 87-year-old female.

Since the frequent isolation of VRE within a short period strongly suggested an outbreak due to VRE, 819 rectal swabs and/or sputa from all of the 759 inpatients and 57 inpatients' family members who visited the hospital were examined for the colonization of VRE. Rectal swabs from all 969 hospital personnel were also examined. By this screening, VRE were isolated from the rectal swabs of 8 inpatients (P-numbered isolates in Table 1): 4 from inpatients in Ward A and 4 from inpatients in Ward B. No VRE was isolated from the hospital personnel and inpatients' family members. In addition, we examined 1,054 environment sites in Wards A and B for VRE contamination. VRE were isolated from 8 sites in Wards A and B (E-numbered isolates in Table 1). No VRE was recovered from the hospital environment after disinfection.

Characteristics of the 20 VRE isolates: With one exception (an isolate designated P93), susceptibilities to various antibiotics were the same for all the VRE isolates. As shown in Table 1, 19 isolates exhibited a high degree of resistance to vancomycin (MICs were 32 μg/ml or higher) as well as to benzylpenicillin, gentamicin, amikacin, and levofloxacin, but were susceptible to aminobenzylpenicillin. In contrast, isolate P93 exhibited a lower MIC value to vancomycin (16 μg/ml), and was susceptible to benzylpenicillin and levofloxacin. The susceptibility to aminobenzylpenicillin was also different from that of the other 19 isolates (MIC = 0.5 μg/ml).

All of the VRE isolates, including P93, revealed the typical VanB phenotype, i.e., resistant to vancomycin but susceptible to teicoplanin (8). These isolates possessed the *vanB* gene, as demonstrated by PCR using *vanB*-specific primers (Fig. 1). No PCR products were detected when either the *vanA*- or *vanC*-specific primers were used.

In the PFGE analysis of the restriction endonuclease digestion patterns of the genomic DNAs, all of the isolates except P93 showed almost the same *Not*I or *Sma*I-digestion pattern (Fig. 2A and 2B, respectively). *Apa*I-digestion patterns of the 19 isolates were also indistinguishable (data not shown), indicating that they were derived from a single clone. In contrast, P93 exhibited completely different PFGE patterns in the case of the *Not*I, *Sma*I, and *Apa*I-digestion (Fig. 2A, 2B, and data not shown, respectively).

Southern hybridization analysis using the *vanB* probe: In the Southern hybridization analysis of the *Not*I-digests of genomic DNAs, the 19 isolates exhibited the same hybridization pattern as that of the *vanB* probe, namely, a weak signal of approximately 110 kb in size was observed (Fig. 2A). Their *Sma*I- and *Apa*I-digests also gave 110-kb signals (Fig. 2B, and data not shown, respectively). In contrast, P93 exhibited completely different hybridization patterns: a >400-kb signal in the *Not*I-digest and a 200-kb signal in the *Sma*I-digest (Fig. 2A and 2B, respectively). Interestingly, *vanB*-hybridization signals of the 19 isolates were the same size for all three restriction enzymes, and the strengths of the signals were significantly weaker than both those of the control *vanB*-positive strain and the P93. In addition, the *Not*I-digests of the 19 isolates exhibited no visible DNA band at the positions of the 110-kb signals. These data suggest the possibility that the *vanB* genes of the 19 isolates were located on 110-kb plasmids which had no restriction sites for the three enzymes. Furthermore, the data suggest that only a small portion of plasmid DNAs which were linearized by mechanical or non-specific cleavage gave weak 110-kb signals. Strong signals were actually observed at the positions of plugs, which were most likely from the intact, circular plasmid DNAs that did not migrate on the PFGE gels (Fig. 2A and 2B). Furthermore, Southern hybridization analysis of undigested genomic DNAs of the 19 isolates also gave weak 110-kb signals, whereas the undigested DNAs from the control strain and isolate P93 gave no signal (Fig. 2C). These results also suggest that *vanB* genes of the 19 isolates are located on 110-kb plasmids. Although our repeated efforts to isolate plasmid DNA from these 19 VRE were for unknown reasons unsuccessful, the results of the Southern hybridization analyses using other restriction enzymes were consistent with the following notion. That is, *Sal*I- and *Eco*RI-digested genomic DNAs from the 19 isolates gave 76-kb and 7.8-kb signals, respectively, and the signals at the positions of plugs disappeared (Fig. 2D and 2E).

DISCUSSION

An outbreak of VREF occurred at Hokushin General Hospital, Nakano City, Nagano Prefecture, in July, 1999 (5). All of the 20 VRE isolates possessed *vanB* genes. Antimicrobial susceptibility testing and PFGE analyses revealed that 19 out of the 20 isolates were clonal, indicating that this outbreak

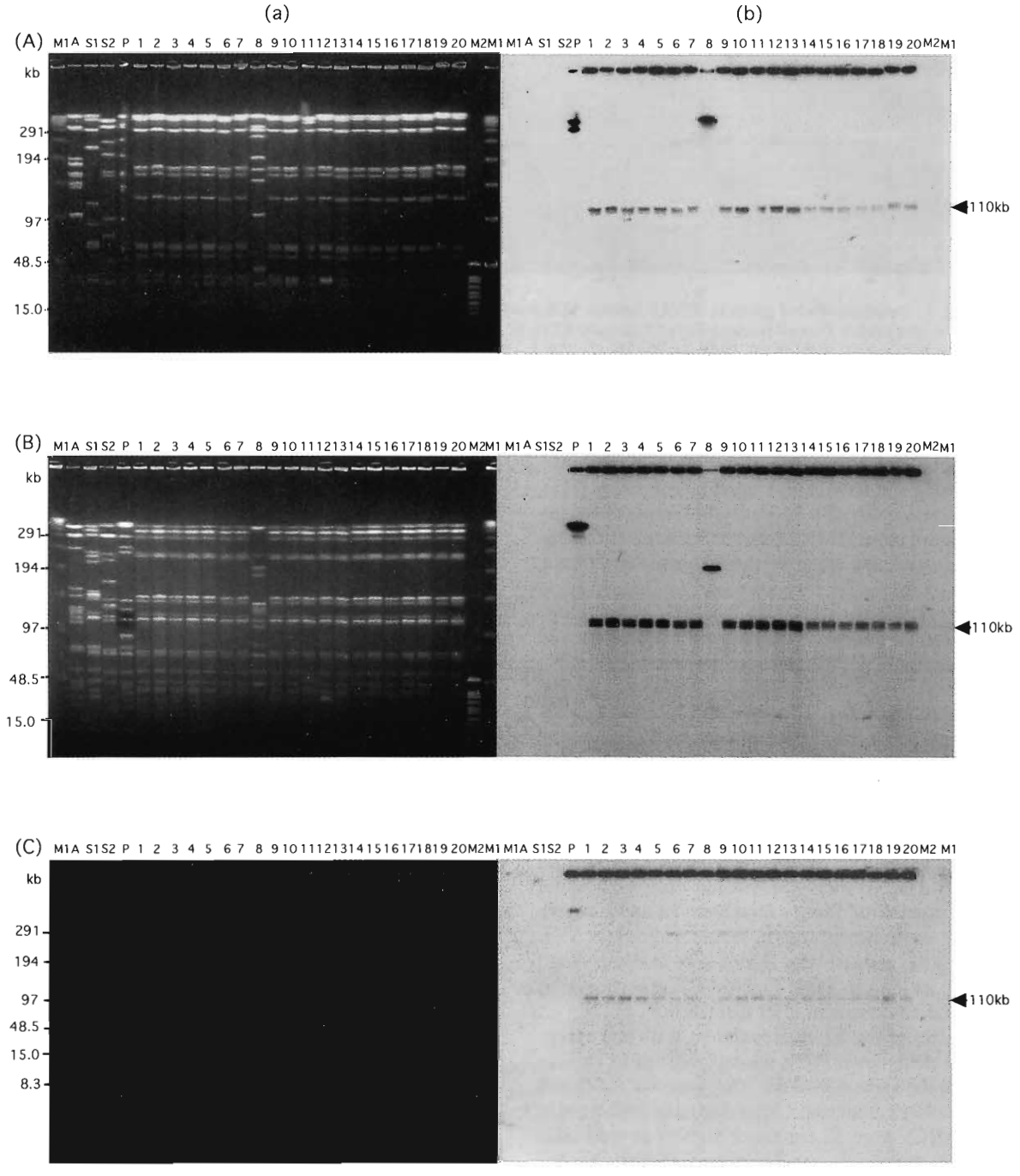

Fig. 2. PFGE analyses and Southern hybridization analyses using a *vanB* probe. Genomic DNAs were digested with *Not*I (A), *Sma*I (B), none (C), *Sal*I (D), or *Eco*RI (E), and were subjected to PFGE analyses (a); then, Southern hybridization analyses were conducted using the *vanB* probe (b). Lanes: M1: lambda phage DNA ladder standard (Bio-Rad Laboratories), M2: low-molecular-weight ladder standard (Bio-Rad Laboratories), M3: lambda *Sty*I digest (Nippon Gene), P: a *vanB*-positive control *E. faecalis* strain (RV1), A: a vancomycin-susceptible *E. faecalis* strain (ATCC 19433), S1 and S2: vancomycin-susceptible *E. faecalis* strains (clinical isolates in Shinshu University Hospital), 1: H1, 2: H2, 3: H3, 4: H4, 5: P35, 6: P38, 7: P40, 8: P93, 9: P473, 10: P488, 11: P1130, 12: P8065, 13: E201, 14: E207, 15: E208, 16: E213, 17: E222, 18: E225, 19: E398, 20: E518. Running conditions for PFGE (pulse time and running time) were as follows: (A) and (B): 0.47 sec-17.33 sec for 20 h 18 min (10-200 kb range for the CHEF MAPPER apparatus), (C): 0.22 sec-26.29 sec for 15 h 16 min (5-300 kb), (D): 2.98 sec-8.53 sec for 26 h 56 min (20-100 kb), (E) 0.11 sec-0.36 sec for 20 h 30 min (5-25 kb).

was principally caused by a single clone. The route through which this clone was brought in the hospital remains unknown, but transmission of the clone within the hospital may have occurred due to the contaminated environment, since the clone was isolated from various sites in the hospital.

The results of Southern hybridization analyses implied that the *vanB* gene is encoded on a 110-kb plasmid, though we could not isolate the plasmid DNA by the conventional procedure. The *vanB* genes are usually encoded on the chromosome (9-11) and occasionally on plasmids (12-17). Plasmids encoding the *vanB* genes were reported to be 60-kb in size by Boyce et al. (12) and Rice et al. (17), and were larger than 50 kb or 60 MDa

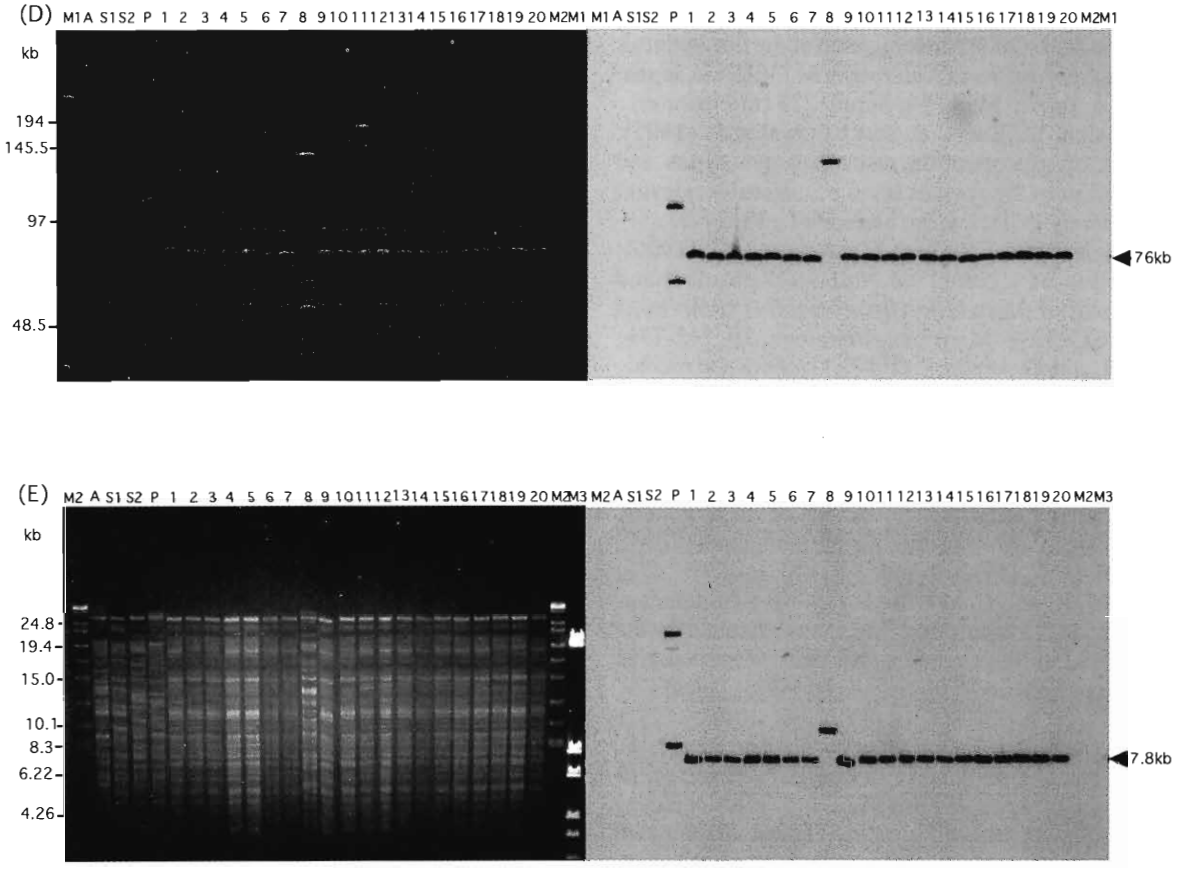

Fig. 2. –Continued.

(approximately 90 kb), as reported by Woodfond et al. (14,16). Molecular analysis of the plasmid will provide important information to aid an understanding of the molecular mechanism of *vanB* gene transmission among enterococcal strains.

Another important result was the isolation of VRE, which was designated as P93, from an asymptomatic carrier. This isolate also possessed a *vanB* gene, but showed PFGE patterns different from the other 19 isolates, indicating that the isolate was of different origin. Susceptibilities of this isolate to various antibiotics were indeed different from those of the other isolates. It is unlikely that the *vanB* gene of P93 was transferred from the predominant clones because the *vanB* gene of P93 is not located on a plasmid but on the chromosome. It is instead more likely that the P93 was isolated by chance during the systematic screening we performed. This finding implies that VRE may already have been distributed widely throughout the community and hospital facilities in Japan. In this regard, it is important to mention our recent finding that VRE with a VanA or VanB phenotype had colonized in the gastrointestinal tracts of a small but significant portion of healthy schoolchildren in the community (18). Healthy individuals whose gastrointestinal tracts have been colonized by VRE could be reservoirs of VRE in the community. Thus, all communities and hospital facilities in Japan appear to be presented with certain risks of encountering VRE infection. Fortunately, VRE infection has thus far been rare in Japan. More attention should be paid to the emergence of VRE, and the appropriate use of glycopeptides should be emphasized. It is also important to establish rapid and accurate methods of epidemiological investigation of VRE. In this regard, the PFGE analysis of *Not*I, *Sma*I, or *Apa*I-digested genomic DNAs, which we employed in this study, is the most effective and accurate method at present. In order to determine the level of contamination of VRE in hospital facilities and to organize the most appropriate cooperation system to the VRE infection, the systematic screening of asymptomatic carriers and the surveillance of high-risk environments is recommended.

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REFERENCES

1. Woodford, N. (1998): Glycopeptide-resistant enterococci: a decade of experience. J. Med. Microbiol., 47, 849-862.
2. Ishii, Y., Ohno, A., Kashitani, S., Iwata, M. and Yamaguchi, K. (1996): Identification of VanB-type vancomycin resistance in *Enterococcus gallinarum* from Japan. J. Infect. Chemother., 2, 102-105.
3. Fujita, N., Tanimoto, K. and Ike, Y. (1998): First report of the isolation of high-level vancomycin resistant *Enterococcus faecium* from a patient in Japan. Antimicrob. Agents Chemother., 42, 2150.
4. Imafuku, Y., Yoshida, H., Sato, T., Yamada, H., Sato, A. and Hiramatsu, K. (1999): A case of lymphocyst infection caused by *vanB* type VRE. J. Jpn. Assoc. Infect. Dis., 73, 473-476 (in Japanese).

5. Atarashi, K., Baba, K., Fujiki, K., Takamoto, T., Nishimura, H. and Honda, T. (2000): Investigation of the first outbreak of vancomycin-resistant *Enterococcus* (VRE) in Japan. J. Jpn. Soc. Intern. Med., 89, Suppl. 272 (in Japanese).
6. Dutka-Malen, S., Evers, S. and Courvalin, P. (1995): Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. J. Clin. Microbiol., 33, 24-27.
7. Furihata, K., Sato, K. and Matsumoto, H. (1995): Construction of a combined *Not*I/*Sma*I physical and genetic map of *Moraxella* (*Branhamella*) *catarrhalis* strain ATCC25238. Microbiol. Immunol., 39, 745-751.
8. Arthur, M. and Courvalin, P. (1993): Genetics and mechanisms of glycopeptide resistance in enterococci. Antimicrob. Agents Chemother., 37, 1563-1571.
9. Quintiliani, R. and Courvalin, P. (1994): Conjugal transfer of the vancomycin resistance determinant *vanB* between enterococci involves the movement of large genetic elements from chromosome to chromosome. FEMS Microbiol. Lett., 119, 359-364.
10. Quintiliani, R. and Courvalin, P. (1996): Characterization of Tn*1547*, a composite transposon flanked by the IS*16* and IS*256*-like elements, that confers vancomycin resistance in *Enterococcus faecalis* BM4281. Gene, 172, 1-8.
11. Carias, L. L., Rudin, S. D., Donskey, C. J. and Rice, L. B. (1998): Genetic linkage and cotransfer of a novel, *vanB*-containing transposon (Tn*5382*) and a low-affinity penicillin-binding protein 5 gene in a clinical vancomycin-resistant *Enterococcus faecium* isolate. J. Bacteriol., 180, 4426-4434.
12. Boyce, J. M., Opal, S. M., Chow, J. W., Zervos, M. J., Potter-Bynoe, G., Sherman, C. B., Romulo, R. L. C., Fortna, S. and Medeiros, A. A. (1994): Outbreak of multidrug-resistant *Enterococcus faecium* with transferable *vanB* class vancomycin resistance. J. Clin. Microbiol., 32, 1148-1153.
13. Woodford, N., Jones, B. L., Baccus, Z., Ludlam, H. A. and Brown, D. F. J. (1995): Linkage of vancomycin and high-level gentamicin resistance genes on the same plasmid in a clinical isolate of *Enterococcus faecalis*. J. Antimicrob. Chemother., 35, 179-184.
14. Woodford, N., Morrison, D., Johnson, A. P., Bateman, A. C., Hastings, J. G. M., Elliott, T. S. J. and Cookson, B. (1995): Plasmid-mediated *vanB* glycopeptide resistance in enterococci. Microb. Drug Resist., 1, 235-240.
15. Chadwick, P. R., Oppenheim, B. A., Fox, A., Woodford, N., Morgenstern, G. R. and Scarffe, J. H. (1996): Epidemiology of an outbreak due to glycopeptide-resistant *Enterococcus faecium* on a leukaemia unit. J. Hosp. Infect., 34, 171-182.
16. Woodford, N., Chadwick, P. R., Morrison, D. and Cookson, B. D. (1997): Strains of glycopeptide-resistant *Enterococcus faecium* can alter their *van* genotypes during an outbreak. J. Clin. Microbiol., 35, 2966-2968.
17. Rice, L. B., Carias, L. L., Donskey, C. L. and Rudin, S. D. (1998): Transferable, plasmid-mediated VanB-type glycopeptide resistance in *Enterococcus faecium*. Antimicrob. Agents Chemother., 42, 963-964.
18. Kawakami, Y., Yamaguchi, M., Oana, K., Hama, N., Takahashi, Y., Akahane, T., Okimura, Y., Yanagisawa, E. and Katsuyama, T. (1999): Incidence of vancomycin-resistant enterococci in stool specimens from healthy Japanese schoolchildren. Med. Sci. Res., 27, 61-62.