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Emergence of *vanA* Genotype Vancomycin-Resistant Enterococci with Low or Moderate Levels of Teicoplanin Resistance in Korea

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In Korea, vancomycin-resistant enterococci have become important nosocomial pathogens since the late 1990s, and most vancomycin-resistant enterococcal isolates have been VanA phenotype-*vanA* genotype strains. In 2001, we experienced an outbreak of VanB phenotype-*vanA* genotype vancomycin-resistant enterococci at a university hospital. This is the first report of VanB-*vanA* vancomycin-resistant enterococci from humans in Korea.

Vancomycin-resistant enterococci have spread with unanticipated rapidity and are now encountered in hospitals located in many countries worldwide after being first reported in the United Kingdom (9, 13). In Korea, vancomycin-resistant enterococci have become important pathogens involved in nosocomial infections since the late 1990s (4). The first human vancomycin-resistant enterococcal isolate (*Enterococcus durans*) in Korea was reported in 1992 (12). In 1994, Kim et al. first reported a monoclonal outbreak of *vanB* vancomycin-resistant *Enteroccus faecium* in an intensive care unit, but most of the reported cases in Korea were caused by isolates with *vanA* genotypes, which have the antibiotic susceptibility of the VanA phenotype (2, 3, 4, 8). In 2001, we experienced an outbreak of vancomycin-resistant enterococci with a low or moderate level of teicoplanin resistance among 20 patients in Korea University's Guro Hospital. We initially considered that these isolates were phenotypically VanB and genotypically *vanB*. However, a recent report by Hashimoto et al. suggested that mutations in the *vanS* regulatory gene could result in impaired resistance to teicoplanin among vancomycin-resistant enterococcal isolates possessing the *vanA* gene cluster (7), and there was a report of isolation of VanB-*vanA* vancomycin-resistant enterococci from humans and retail chickens in Taiwan (9). These findings prompted us to suspect that the VanB vancomycin-resistant enterococcal isolates in our hospital may have a VanB phenotype-*vanA* genotype incongruence. We performed clinicoepidemiologic, microbiological, and genetic analyses for this outbreak.

We reviewed patients' medical records to evaluate the clinical characteristics. Vancomycin-resistant enterococci with a low or moderate level of teicoplanin resistance were isolated from 20 inpatients (10 male, 10 female) aged between 13 and 81 years (mean age, 53.9 ± 18.0 years). All of the patients had been in the intensive care unit. The duration of the stay in the intensive care unit before VanB vancomycin-resistant enterococcal isolation ranged from 2 to 207 days (mean, 53.0 ± 56.5 days). The underlying diseases of patients were cerebrovascular accident (eight cases), pneumonia (two cases), pancreatitis (two cases), panperitonitis (two cases), and six other diseases (pancreatic cancer, hemophilia, psoas abscess, pelvic bone fracture, septic knee, and liver cirrhosis). Fourteen strains were isolated from urine, two strains were isolated from abscesses, two strains were isolated from open pus, and two strains were isolated from other sources (one blood and one catheter tip), but only nine strains were evaluable.

All of the vancomycin-resistant enterococcal isolates were identified by the Rapid ID 32 Strep kit (bioMérieux, Lyon, France), and drug susceptibility was tested with an ATB Strep panel (bioMérieux). They were processed by brain heart infusion agar screening in the presence of vancomycin (6 μg/ml) (11). The vancomycin and teicoplanin resistance of nine evaluable vancomycin-resistant enterococci were confirmed by measuring the MIC by agar dilution methods according to recommendations (11). Total DNA was extracted by a boiling method (1).

To confirm the species of vancomycin-resistant enterococci, species-specific PCR was performed with primers specific for *Enteroccus faecalis* and *E. faecium* (5). Control organisms used included *E. faecalis* ATCC 29212 and *E. faecium* BM4147. All PCRs in this study were carried out in a GeneAmp PCR System 2700 (Applied Biosystems). A *vanA-vanB* duplex PCR assay with primers specific for the *vanA* and *vanB* vancomycin resistance genes was performed (5, 6). Genetic mutations in the *vanS* gene of the vancomycin-resistant enterococcal isolates were studied by direct PCR sequencing. To analyze the *vanS* gene of the VanA-type determinants, primers specific for the *vanS* gene were used for PCR (10). The PCR products were purified with QIAquick minicolumns (Qiagen GmbH, Hilden, Germany). The PCR products were sequenced with the Basestation DNA fragment analyzer (MJ Research, Inc.). The clonalities of vancomycin-resistant enterococcal strains were analyzed by pulsed-field gel electrophoresis. Pulsed-field gel electrophoresis was performed with a CHEF-DR III appa-

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ratus (Bio-Rad Laboratories). The gel was stained with ethidium bromide solution and then placed onto a UV source. The sizes of the DNA fragments were determined by comparison to the pulsed-field gel electrophoresis markers (Roche Diagnostics, Basel, Switzerland).

The MICs of vancomycin for all nine strains were above 256 μg/ml, and the teicoplanin MIC ranged from 4 to 16 μg/ml (six susceptible, two intermediate), and so all nine strains were of the VanB phenotype. By *vanA-vanB* duplex and species-specific PCR, they were identified as *vanA* genotype *E. faecium*. This confirmed the emergence of VanB-*vanA* vancomycin-resistant enterococci in our hospital. Direct sequencing of the *vanS* gene demonstrated three identical point mutations in the *vanS* gene at positions 148 (T→G), 160 (G→C), and 207 (A→T), resulting in the substitutions Leu148Val, Glu160Gln, and Gln207His, which are identical to those previously reported from Japan and Taiwan (7, 9). These reports did not provide any explanation for the VanB phenotype-*van*A genotype incongruence. Further evaluation is needed to identify the mechanisms and effects of point mutations in the *vanS* gene in the development of VanB-*vanA* vancomycin-resistant enterococci.

Pulsed-field gel electrophoresis of the nine vancomycin-resistant enterococcal strains showed four different types of clonality. We originally speculated that the outbreak of VanB-*vanA* vancomycin-resistant enterococci occurred in the intensive care unit and that it was a monoclonal outbreak or horizontal spread of VanB phenotype resistance; however, it was suspected that transmission of the *vanA* gene cluster containing point mutations in the *vanS* gene was the probable cause of VanB-*vanA* enterococcal outbreak rather than clonal spread.

To our knowledge, this is not only the first reported emergence of VanB-*vanA* enterococci in Korea but also the first clinical outbreak of its type in the world.

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REFERENCES

1. **Bell, J., J. Paton, and J. Turnidge.** 1998. Emergence of vancomycin-resistant enterococci in Australia: phenotypic and genotypic characteristics of isolates. J. Clin. Microbiol. **36:**2187–2190.
2. **Cheong, H. J., J. Y. Song, J. S. Eom, W. J. Kim, S., J. Choi, J. H. Choi, H. Lee, M. H. Chung, K. M. Lee, D. H. Shin, B. R. Sohn, J. S. Choi, H. J. Woo, J. A. Kwon, K. W. Lee, K. W. Choe, and S. C. Park.** 2002. Colonization rate, risk factor for acquisition and genetic diversity of vancomycin-resistant enterococci (VRE) isolated from rectal culture of patients in intensive care units from ten large hospitals in Korea. Kor. J. Infect. Dis. **34:**276–284.
3. **Cheong, H. J., C. W. Park, J. S. Eom, K. I. Ha, J. H. Shun, C. M. Kim, H. S. Lee, Y. H. Park, W. J. Kim, and S. C. Park.** 2002. Clinical features of VRE infection, microbiological characteristics and genetic relatedness of VRE isolates from six university hospitals. Kor. J. Infect. Dis. **34:**285–292.
4. **Cheong, H. J., and W. J. Kim.** 1998. Current status, prevention and control strategy of vancomycin-resistant enterococci in Korea. Kor. J. Nosocom. Infect. Control **3:**57–64.
5. **Dutka-Malen, S., S. Evers, and P. Courvalin.** 1995. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. J. Clin. Microbiol. **33:**24–27.
6. **Free, L., and D. F. Sahm.** 1996. Detection of enterococcal vancomycin resistance by multiplex PCR, p. 150–155. *In* D. H. Persing (ed.), PCR protocols for emerging infectious diseases. American Society for Microbiology, Washington, D.C.
7. **Hashimoto, Y., K. Tanimoto, Y. Ozawa, T. Murata, and Y. Ike.** 2000. Amino acid substitutions in the VanS sensor of the VanA-type vancomycin-resistant Enterococcus strains result in high-level vancomycin resistance and low-level teicoplanin resistance. FEMS Microbiol. Lett. **185:**247–254.
8. **Hong, W. P., M. Kim, J. W. Song, S. H. Kim, J. H. Shin, S. P. Seo, and D. W. Yang.** 1998. Plasmid DNA and random amplified polymorphic DNA (RAPD) analysis of vancomycin-resistant *Enterococcus faecium* isolates. Kor. J. Clin. Pathol. **18:**379–385.
9. **Lauderdale, T. L., L. C. McDonald, Y. R. Shiau, P. C. Chen, H. Y. Wang, J. F. Lai, and M. Ho.** 2002. Vancomycin-resistant enterococci from humans and retail chickens in Taiwan with unique VanB phenotype-*vanA* genotype incongruence. Antimicrob. Agents Chemother. **46:**525–527.
10. **Miele, A., M. Bandera, and B. P. Goldstein.** 1995. Use of primers selective for vancomycin resistance genes to determine *van* genotype in enterococci and to study gene organization in VanA isolates. Antimicrob. Agents Chemother. **39:**1772–1778.
11. **National Committee for Clinical Laboratory Standards.** 1998. Methods of dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 2nd ed. National Committee for Clinical Laboratory Standards, Wayne, Pa.
12. **Park, J. W., Y. R. Kim, W. S. Shin, M. W. Kang, K. J. Han, and S. I. Shim.** 1992. Susceptibi1ity tests of vancomycin-resistant enterococci. Kor. J. Infect. Dis. **24:**133–137.
13. **Uttley, A. H., C. H. Collins, J. Naidoo, and R. C. George.** 1988. Vancomycin-resistant enterococci. Lancet **i:**57–58.