Prevalence and Molecular Epidemiology of Vancomycin-Resistant Enterococci (VRE) Strains Isolated from Animals and Humans in Korea

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Background: To assess the possibility of VRE transmission from animals to humans, we studied the prevalence of vancomycin-resistant enterococci (VRE) in farm animals, raw chicken meat, and healthy people. We then determined the molecular relatedness of VRE isolates between animals and humans in Korea.

Methods: We aimed to isolate VRE from 150 enterococci specimens of farm animals, 15 raw chicken meat samples, and stools from 200 healthy people. Species differentiation was done with conventional biochemical tests. Vancomycin resistance genotyping was done by polymerase chain reaction (PCR). Using the agar dilution method, antimicrobial susceptibility was tested for 8 antimicrobials and pulsed-field gel electrophoresis (PFGE) was done to evaluate the molecular relatedness of VRE isolates.

Results: The prevalence of VRE was 14.7% (22/150) in farm animal specimens, 1% (2/200) in healthy people, and 60% (9/15) in raw chicken meat. Of 22 animal VRE isolates, 1 vanA E. faecium, 15 vanC1 E. gallinarum, and 6 vanC2 E. casseliflavus were identified. All of the 9 VRE from raw chicken meat and all of the 20 clinical VRE strains were vanA E. faecium. However, in healthy people, only 2 vanC2 E. casseliflavus were isolated. These showed low-level resistance to vancomycin and susceptibility to teicoplanin. However, 9 VRE strains from raw chicken meat had high-level resistance to vancomycin (MIC<sub>50/90</sub>: >128 μg/mL), teicoplanin (MIC<sub>50/90</sub>: >128 μg/mL), ampicillin (MIC<sub>50/90</sub>: >128 μg/mL), erythromycin (MIC<sub>50/90</sub>: >128 μg/mL), and tetracycline (MIC<sub>50/90</sub>: >128 μg/mL).

Conclusion: This study demonstrated little evidence of VRE colonization in healthy people despite high recovery of VRE among raw chicken meat. It is suggested that there is little evidence of VRE transmission from animals to healthy people. However, we assumed that there exists the possibility of VRE contamination during the processing of chicken meat.

Key Words: Vancomycin-resistant enterococcus, Domestic animals, Healthy person, PCR, PFGE (electrophoresis, gel, pulsed-field)

INTRODUCTION

Enterococcus spp. is a facultatively anaerobic gram-positive coccus which is part of the normal intestinal flora. Some have also been isolated from the oral cavity and female reproductive tract. Enterococcus faecium colonizes in 10–25% of healthy individuals and Enterococcus faecalis exists in the gastrointestinal tract in almost 100% of people. Enterococcus spp. has a relatively low virulence and does not easily induce disease in healthy individuals, but it causes a variety of opportunistic infections, such as bacteremia, urinary tract infection, endocarditis,
and osteomyelitis. Because the use of antimicrobial agents, such as cephalosporin, in hospitals has rapidly increased since 1980, Enterococcus spp., which shows a relative resistance to these agents, has been highlighted as an important causative bacterium of nosocomial infections. It ranked second to *E. coli* as a nosocomial pathogen in the US in 1995. According to the National Nosocomial Infection Surveillance Study by the Korean Society of Nosocomial Infection Control in 1996, *Enterococcus* spp. accounts for 7.6% among the causative bacteria of nosocomial infections and ranks the fifth most frequently isolated pathogen. With the increasing isolation of *Enterococcus* spp., enterococci resistant to vancomycin, which has very effectively been used in treating *Enterococcus* spp. infection until recently, were reported in France for the first time in 1989. Since then, vancomycin-resistant enterococci (VRE) have continuously been on increasing trends in Europe and North America, and it was isolated domestically for the first time in 1992, showing a rapidly increasing trend centering around large hospitals since 1998. The vancomycin resistance genotypes reported thus far include: vanA, vanB, vanC, vanD, vanE, and vanG, most of which are vanA and vanB types. They have clinical significance because they allow for plasmid-mediated transmission.

In the US, VRE infections are basically hospital-acquired infections and the prevalence of VRE among the general population is only 1%, whereas in Europe the subsisting rates of VRE are reported to be 0.5~14% in healthy individuals, and 12~28% in stock farm residents. The first susceptible route of VRE in healthy individuals is the oral administration of non-vancomycin-resistant *Streptococcus faecium* as a sort of bacterial treatment after being mixed with lactobacillus, which has an intrinsic vancomycin resistance in the intestinal tract, and subsequent resistance acquisition from it. The second susceptible route is contamination from food. In Europe, VRE was isolated when livestock were fed with avoparcin that had cross-resistance to vancomycin as a growth promoter, and since avoparcin was legally banned in 1997, VRE isolation rate has decreased. Although it is also restricted domestically, its use was continued in stockbreeding. In 1998, its usage was banned. However, tylosin, bacitracin, and spiramycin, which can show cross-resistance to vancomycin, are still being used. Domestically, Park et al. first reported the prevalence of VRE within poultry farms in 1999. According to their report, highly resistant VRE (vanA or vanB) were isolated in 2.58% of enterococcal isolates from chicken feces.

According to the use of antibiotics and dietary habits of each country, various research results were shown. Although clinically significant factors such as the restriction on the use of antibiotics, the need for isolation, and the transmission of resistance are significant, domestic studies on the correlation between livestock isolates and clinical isolates, especially in healthy individuals, are insufficient. Therefore, we decided to begin by studying the colonization rate of VRE in livestock, raw chicken, and healthy individuals respectively, and secondly, to study the existence of cross infection between livestock and humans through antimicrobial susceptibility tests and molecular genetic studies.

### MATERIALS AND METHODS

#### Materials and subjects

The presence of VRE was looked for in the following sources: (i) 150 enterococci isolates from fecal samples of farm animals (114 chickens, 25 pigs, and 11 cows); (ii) intestinal secretions of 15 raw chicken meat samples that were purchased at 3 large dairy groceries (5 chickens for each grocery); (iii) fecal samples from 200 healthy people who had visited the Department of Health Examination in the Korea University Guro Hospital from March to May of 2002. VRE strains from each group were compared with 20 clinical strains that were available among 50 VRE isolates from hospitalized patients between January 2000 and December 2001.

#### Culture and Identification Isolation of VRE

Each of the samples were suspended in BHI (brain heart infusion) broth (BBL, Becton Dickinson, USA) supplemented with 6 μg/mL of vancomycin. After 16 hours of incubation, swabs were streaked onto enterococcal agar (BBL, Becton Dickinson, USA) plates containing 6 μg/mL of vancomycin if the broth was turbid. On the following day, black colonies from the enterococcal agar plate were subcultured onto blood agar plates. Growth from the blood agar plate was used to test for and identify VRE. Colonies with the appearance of enterococci were tested for pyrrolidonyl arylamidase activity (PYR: Oxoid, England) to differentiate from lactococcus, pediococcus, and other bacteria. PYR-negative isolates were not investigated further.

#### Vancomycin resistance genotyping and species Identification of VRE by PCR

Determination of glycopeptide resistance genotypes and confirmation of species identification were performed by polymerase chain reaction (PCR). The vancomycin resistance genes (vanA, vanB, vanC7, and vanC2) and *ddl* genes (*E. faecium* and *E. faecalis*) were amplified with the primers listed in Table 1. The PCR amplification mixture consisted of PCR buffer, 200 mM dNTPs, 25 pM primer, 1 U of Taq polymerase (BIOTOOLS, Spain), and DNA template. Reactions were set up.
in a final volume of 20 μL, containing 100 ng of boiled cellular supernatant and primer mixtures. Amplification conditions were 94°C initially for 3 min; 94°C for 1 min, 55°C (50°C for C2) for 30 s, 72°C for 1 min over 30 cycles; and a final 10-min extension period at 72°C. A reagent blank (containing all of the components of the reaction mixture except DNA) and positive control strains for each van genotype (E. faecium 1024 [vanA], E. faecium JB7 [vanB], E. gallinarum 11 [vanC1], and E. casseliflavus ATCC 25788 [vanC2]) were run in every PCR procedure as control. DNA amplification was carried out with a PCR 2600 system (Perkin-Elmer Cetus, USA). Yellow pigmentation test and motility test was done for enterococcal isolates of vanC1 and vanC2 genotypes. For all of the VRE isolates, the β-lactamase stock (Oxoid, England) test was done.

Antibiotic susceptibility testing of VRE

MICs of vancomycin, teicoplanin, ampicillin, erythromycin, tetracycline, chloramphenicol, gentamycin, and streptomycin were determined by the agar dilution method following the National Committee for Clinical Laboratory Standards guidelines. E. faecium JB7 was used as a reference strain. High-level resistance to aminoglycosides was determined by plating onto BHI agar containing 2,000 μg of streptomycin per mL and BHI agar containing 500 μg of gentamycin per mL. We analyzed the difference in the antimicrobial resistance rates of VRE isolates among farm animals, raw chicken meat samples, healthy people, and hospitalized patients.

Pulsed Field Gel Electrophoresis (PFGE)

For VRE strains, genotyping was done by PFGE. Overnight cultures grown on 5 mL of BHI broth were centrifuged for 1 min at 13,000 rpm. The pellet was suspended in 0.5 mL of 2X lysis buffer (12 mM Tris, 0.2 M EDTA, 2 M NaCl, 1.0% SLS, 1.0% deoxycholate) containing 1 mg/mL of lysozyme and 50 μg/mL of RNase. An aliquot of this suspension (100 μL) was mixed with 100 μL of 1.6% agarose at 55°C. This mixture was transferred into two 100 mL sample plug molds. The plugs were removed from the molds and were incubated for 18 h at 37°C in 3 mL of 1X lysis buffer (6 mM Tris, 0.1 M EDTA, 1 M NaCl, 0.5% SLS, 0.5% deoxycholate). This lysis solution was replaced by 3 mL of ESP buffer (10% SDS, 50 μg/mL of proteinase K) and the mixture was incubated for 4 h at 55°C. The plug was then washed for 1 h at 55°C in 5 mL of diluted TE buffer (10 mM Tris, 0.1 mM EDTA) and the plug was washed again for 1 h with 20 mL of diluted TE buffer in a petri dish on the shaker. A slice of plug was digested with 20 U of the Small restriction enzyme (MBI Fermentas, Germany) and incubated for 18 h at 30°C. DNA fragments were separated in a 1% agarose gel in 0.5X TBE buffer. Electrophoresis was performed with a CHEF-DR III apparatus (Bio-Rad, USA). Parameters for electrophoresis were 6 V/cm at 14°C for 23 h, with pulse times ramped from 1 s to 30 s. The gels were stained with 0.1 mg of ethidium bromide solution per liter for 30 min and were then placed onto a UV source. The size of the DNA fragments was then determined according to the size of the PFGE marker (Boehringer, Germany).

Analysis of PFGE patterns

To assess the molecular relatedness of PFGE patterns of VRE isolates, the criteria of Tenover et al. were used: (i) if an isolate differed from a main type by only three or fewer bands, it was considered to be a closely related subtype; (ii) if an isolate differed from a main type by 4–6 bands, it was considered to possibly be a related subtype; (iii) if an isolate differed from a main type by seven or more bands, it was considered to be a different type.

Comparison of the PFGE fingerprints was made using computer assisted analysis (Gel compar version 4.1; Kortrijk, Belgium). Comparisons of patterns were made using the unweighted pair group method using the arithmetic averages (UPGMA) clustering method by Dice coefficient. The band-width tolerance was set critically at 4.0%.

Statistics

Data processing and evaluation were carried out in PC SPSS [version 10.0].

RESULTS

Isolation rate of VRE from various sources

In the livestock, only 1 (0.67%) VRE isolate among the 150 enterococcal isolates was identified as vanA E. faecium. On the
Table 1. Primer sequences for PCR of vancomycin resistance genotyping and enterococci species identification among vancomycin-resistant enterococci.

| Genotype | Species       | Primer sequence (5' -> 3') | Product size |
|----------|---------------|----------------------------|--------------|
| vanA (F) | E. faecium    | GATAGCTACTCCGCTTTTGGG       | 380 bp       |
| vanA (R) | E. faecalis   | TATCCGCTCCTGCTCTCTG         |              |
| vanB (F) | E. faecium    | CATCCGCTCCTGGAATTCTCAA      | 667 bp       |
| vanB (R) | E. faecalis   | GATGCCGAGAGATCCGTTGGCT      |              |
| vanC1 (F) | E. faecium   | TGACCCGCTGAA ATATGA AG      | 400 bp       |
| vanC2 (F) | E. faecalis   | TCGGCTGATAAGATCGGG           |              |
| vanC2 (R) | E. faecalis   | CTCCTAGATTCTCTTG            | 439 bp       |
| ddl E. faecalis (F) | | CGAGCAAGACCTTTAAG           |              |
| ddl E. faecalis (R) | | ACGATTCAAAGCTAATCGT       |              |
| ddl E. faecium (F) | | TAGAGACATTGAATATGC         | 550 bp       |
| ddl E. faecium (R) | | TCGAATGTCCTACCAATC         |              |

Table 2. Isolation rates, species, and genotype distribution of VRE strains from various sources.

| Genotype | Species       | Number of strains from: | Farm animals (N=150) | Raw chicken meats (N=15) | Healthy people (N=200) | Hospitalized Patients* (N=20) |
|----------|---------------|-------------------------|----------------------|--------------------------|------------------------|-------------------------------|
| vanA     | E. faecium    | 1                       | 1                    | 0                        | 0                      | 20                            |
| vanB     | E. faecalis   | 0                       | 0                    | 0                        | 0                      | 0                             |
| vanC1    | E. gallinarum | 15                      | 0                    | 0                        | 0                      | 0                             |
| vanC2    | E. casseliflavus | 6                 | 0                    | 2                        | 0                      | 0                             |
| Total    | vanA or vanB  | 21                      | 9 (60%)              | 0 (10%)                  | 2 (1%)                 | 20                            |
| vanC     |               | 21 (14%)                | 0                    | 2 (1%)                   |                        |                               |

*150 enterococcal isolates from 114 chickens, 25 pigs, and 11 cows.
* 20 clinical strains that were available among 50 VRE isolates from hospitalized patients between January 2000 and December 2001.

On the other hand, 21 (14%) isolates were identified to be vanC VRE, while 15 of them were E. gallinarum and 6 were E. casseliflavus. VRE was cultivated in 18 out of 114 chicken fecal samples (16%): 1 isolate of E. faecium, 15 isolates of E. gallinarum, and 2 isolates of E. casseliflavus, in 1 out of 25 pig fecal samples (4%: 3 isolates of E. casseliflavus), and 3 out of 11 cow fecal samples (27%: 3 isolates of E. casseliflavus).

In the samples obtained from the raw chicken meat, 11 out of 15 showed black colonies when cultured in the enterococcal agar, but 9 (60%) of them were PYR-positive and the 9 isolates were all identified to be E. faecium.

Among the 200 fecal samples of healthy individuals, 82 of them showed black colonies when cultivated in the enterococcal agar. However, only 21 (14%) of them were PYR-positive and all were identified as E. casseliflavus. Clinically isolated VRE was vanA E. faecium in all 20 isolates, and E. faecalis was not isolated (Table 2).

Vancomycin resistance genotypes of VRE

According to vancomycin resistance genotyping using PCR, the 22 livestock isolates included 1 vanA isolate, 15 vanC1 isolates, and 6 vanC2 isolates, and the 9 isolates from the raw chicken meat were all vanA type. The 2 E. casseliflavus isolates from healthy individuals were all vanC2 genotype, but the 20 clinical VRE isolates were all of the vanA genotype. The ddl PCR results for vanA VRE strains showed E. faecium in 1 livestock (chicken), 9 raw chicken meat samples, and 20 clinical isolates (Table 2).

According to the β-lactamase test, only 2 isolates (3.8%) out of the entire 53 VRE isolates were β-lactamase positive and all of them were E. faecium isolates from raw chicken meat.

Results of antibiotic-susceptibility of VRE

The 22 VRE isolates from the livestock showed a low degree of resistance (MIC<sub>50</sub>: 8 μg/mL) to vancomycin, susceptibility to teicoplanin (MIC<sub>90</sub>: 0.25/1 μg/mL), and
Table 3. Comparison of antibiotic susceptibility of VRE strains from various sources.

|                         | Farm animals (N=22) | Raw chicken meats (N=9) | Healthy people (N=2) | Hospitalized patients' (N=20) |
|-------------------------|---------------------|-------------------------|----------------------|-------------------------------|
|                         | MIC<sub>50</sub>   | MIC<sub>50</sub>   | MIC<sub>50</sub>   | MIC<sub>50</sub>   | MIC<sub>50</sub>   | MIC<sub>50</sub>   | MIC<sub>50</sub>   | MIC<sub>50</sub>   |
|                         | (μg/mL)            | (μg/mL)            | (μg/mL)            | (μg/mL)            | (μg/mL)            | (μg/mL)            | (μg/mL)            | (μg/mL)            |
| Vancomycin              | 8                  | 8                  | > 128              | > 128              | 8                  | 8                  | > 128              | > 128              |
| Teicoplanin             | 0.25               | 1                  | > 128              | > 128              | 0.25               | 1                  | 16                 | 128               |
| Tetracycline            | 64                 | 128                | 128                | > 128              | 0.25               | 0.5                | 0.5                | 16                |
| Erythromycin            | 0.5                | > 128              | > 128              | > 128              | 1                  | 1                  | > 128              | > 128              |
| Chloramphenicol         | 2                  | 4                  | 8                  | 64                 | 2                  | 2                  | 4                  | 16                |
| Ampicillin              | 16                 | 16                 | > 128              | > 128              | 4                  | 8                  | > 128              | > 128              |
| Gentamicin              | 8                  | > 128              | 32                 | > 128              | 4                  | 8                  | > 128              | > 128              |
| Streptomycin            | > 128              | > 128              | > 128              | > 128              | 32                 | 32                 | 64                 | > 128              |

20 clinical strains that were available among 50 VRE isolates from hospitalized patients between January 2000 and December 2001.

Figure 2. Comparison of antibiotic resistance rates of vanA E. faecium isolates between people and animals. (TE: tetracycline, EM: erythromycin, AM: ampicillin, CM: chloramphenicol, GM: gentamicin, SM: streptomycin)

Figure 3. PFGE patterns of vanA E. faecium isolates (A1—A11: human, J1—K2: raw chicken meat, L1: animal and raw chicken meat).

susceptibility or a low degree of resistance to ampicillin, erythromycin, and chloramphenicol but not tetracycline (MIC<sub>50</sub>: 6/128 μg/mL), and streptomycin (MIC<sub>50</sub>: >128/128 μg/mL). All of the 9 isolates from raw chicken meat were vanA E. faecium, and also showed resistance to most of the antibiotics, including vancomycin (MIC<sub>50</sub>: >128/128 μg/mL) and teicoplanin (MIC<sub>50</sub>: >128/128 μg/mL) (Table 3).

The 2 vanC2 E. casseliflavus isolates from healthy individuals showed a low degree of resistance (MIC<sub>50</sub>: 8 μg/mL) to vancomycin and susceptibility to teicoplanin (MIC<sub>50</sub>: 0.25/1 μg/mL), while showing susceptibility or a low degree of resistance to ampicillin (MIC<sub>50</sub>: 4/8 μg/mL) and tetracycline (MIC<sub>50</sub>: 0.25/0.5 μg/mL), erythromycin (MIC<sub>50</sub>: 1/1 μg/mL), chloramphenicol (MIC<sub>50</sub>: 2.2 μg/mL), gentamicin (MIC<sub>50</sub>: 4/8 μg/mL), and streptomycin (MIC<sub>50</sub>: 32 μg/mL). All of the 20 clinical isolates were vanA E. faecium and showed resistance to most of the antibiotics including vancomycin (MIC<sub>50</sub>: >128/ >128 μg/mL) and teicoplanin (MIC<sub>50</sub>: 16/128 μg/mL).

Interestingly, VRE isolates from farm animals or raw chicken meat showed a high resistance to tetracycline, but the isolates from healthy individuals and clinical isolates all showed susceptibility to this antimicrobial. Also, the raw chicken meat, livestock, healthy individuals, and clinical isolates all showed susceptibility to chloramphenicol, except the 2 raw chicken meat isolates.

The vanA E. faecium isolates from the humans (20 clinical isolates) and the ones from the animals (9 raw chicken isolates and 1 livestock isolate) were compared in terms of the differences in their susceptibility to antibiotics. Erythromycin and ampicillin showed a 100% resistance in both humans and animals, but chloramphenicol showed a very low resistance rate, and all showed susceptibility to humans (humans/animals, 0/20% p<0.038). Tetracycline and streptomycin each showed a 100% resistance in the animals, but the resistance was relatively low in humans (tetracycline 15%, p<0.001, streptomycin 25% p<0.001), and gentamicin showed a rather higher resistance rate in humans (p<0.001) (Figure 2).

Analysis of PFGE patterns of VRE

When the patterns of PFGE of vanA isolates were analyzed by the criteria of Tenover et al., they were isolated in 12 types (A–L). For the clinical isolates, the PFGE patterns of the 20
isolates were manifested in 9 different types (A-1), and one type did not appear noticeably frequently. For the raw chicken meat isolates, 5 J-type isolates, 3 K-type or its subtype isolates, and 1 L-type were shown. The livestock vanA isolates did not show molecular types or the subtypes as the clinical isolates did, but showed molecular types such as the L-type of the raw chicken meat isolates (Figure 3).

On dendrogram analysis, clinical isolates were diverse, but the raw chicken meat isolates showed relatively high similarity (Figure 4). There were no characteristic band patterns that differentiated the clinical isolates from the raw chicken meat isolates.

As a result of PFGE of vanC1 and vanC2 isolates, there were no identical bacterial strains observed and they appeared variously.

**DISCUSSION**

*Enterococcus* spp. infection including VRE has been known mainly as a hospital-acquired infection occurring in groups through sporadic infection or the transmission of clonal bacterial strains. Different from the US, there have been reports in many European countries, such as England, Belgium, and the Netherlands, that although VRE isolation rate is low, VRE is being colonized in the intestinal tract through livestock or pets in the community. Based on the fact that most of the nosocomial VRE bacterial strains have different genetic heterogeneities from one another, it was first thought in Europe that VRE might have entered from the community, and it has been verified by many reports that genetically associated VRE bacterial strains have been isolated from livestock on farms, raw meats, and healthy individuals. As stated in the introduction, avoparcin, a glycopeptide antibiotic, has been added in the feeds of livestock to be used as growth promoter in Europe. Thus, VRE is speculated to have spread through the livestock. In the US, on the other hand, many studies are reporting that there is a very small possibility that VRE had spread from healthy individuals in the community, and that avoparcin has never been used as a growth promoter for the livestock. Also, the amount of vancomycin used in the US is 5 times as much as that of the European countries, and since oral vancomycin use is not being restricted, the glycopeptide resistance rate is considered high in the clinical field. Actually, there has been a report of a clinical study by Auwerda et al. that administered oral vancomycin into a subject, and as a result, vanA VRE was selectively colonized in the feces. Domestically, VRE has been considered to cause nosocomial infections as it had in America since vancomycin-resistant *E. durans* was first reported in a patient with acute leukemia in 1992. However, avoparcin had been used in Korea from 1983 to 1998, and since Park et al. have reported 2.58% of VRE colonization rate (10 vanA, 1 vanB) in the study with 32 chickens and 70 pigeons, spreading of VRE among healthy individuals in the community through the livestock cannot be excluded. VRE can easily spread, as it can survive for about 7 days in the environment, and its carriers, while carrying VRE for 2 years, can spread it through the feces. Therefore, dissemination of VRE in the community is said to have a very important clinical significance. As reported in this study and many others, VRE was cultivated in the suspension culture of BHI broth and it was then cultivated again in the selective medium (enterococcosel agar) in order to increase its detection rate. In Europe, however, the subsisting rate of VRE in the healthy individuals in the community is being reported from up to 5% to 12-28%, whereas this study showed as low as 1%, compared to 14% in the livestock and 60% in the raw chicken meat.

Although the identification of bacterial strains was not mentioned in the results, the phenotype identification method that used API 20 STREP (bioMerieux, Marcyl' Etoile, France) and the genotype identification method showed differences in the results of the livestock. In the identification that used API 20 STREP, most of the cases that were identified as *E. faecium* were verified as *E. gallinarum* or *E. casseliflavus* as a result of PCR. This seems to be due to the differences caused by the glucose fermentation between the human body isolates and livestock isolates, as presented in the study by Beatriz et al. As a result of the bacterial strain identification, as known from before, *E. gallinarum* and *E. casseliflavus* have rated high compared to *E. faecium* or *E. faecalis* in the livestock, but all were vanA type *E. faecium* in the clinical isolates.

The most important characteristics of *Enterococcus* spp. are not only that it essentially shows its intrinsic resistance to
various kinds of antibiotics, but also that it easily acquires resistance. *Enterococcus* spp. produces PBPs (penicillin-binding proteins), which have low binding affinity to the β-lactam antibacterial agent, and shows resistance because of the tolerance (the trait that the bacteriophage effects are not shown even if the growth has been suppressed once) one exposure to the β-lactam antibacterial agent, and therefore, use of the β-lactam antibacterial agent alone is difficult to expect. Additionally, in this study, it was shown that resistance to the β-lactam antibacterial agent was not due to β-lactamase production by showing a low β-lactamase production rate. Also, aminoglycoside mostly shows a low degree of resistance due to the inactivating enzyme (6-acetyltransferase) produced by *Enterococcus* spp. and the low permeability to *Enterococcus* spp. Therefore, only gentamycin and streptomycin can be used therapeutically in many cases, and it shows a high degree of resistance in some cases, due to the plasmid-mediated gene transfer and ribosomal mutation. Vancomycin shows disinfecting effects by blocking the transglycosylation and transpeptidation steps that are needed for the multimerization of peptidoglycan by binding with D-alanyl-D-alanine, the peptidoglycan precursor in the process of cell wall synthesis of gram-positive cocci, and resistance is induced by the ligase activity which shows modified matrix-specificity. As such, VRE mostly shows resistance to conventional antibiotics. Thus, there is no effective therapeutic agent, and it is perceived to be a very serious pathogenic bacterium because vancomycin-resistant *Staphylococcus aureus* (VRSA) can emerge through the transferring of vancomycin-resistant genes to *S. aureus*. In Japan, VRSA with higher than 8 μg/mL of MIC to vancomycin was actually isolated from a patient for the first time in 1996. Afterwards, it was also found in the US, France, and domestically, and this issue is being raised as a serious problem. In this study, according to the resistant genotype test, all of the nosocomial isolates and raw chicken meat isolates showed resistance to most of the antibiotics with vanA genotype, but healthy individual isolates and the clinical isolates obtained from patients mostly showed susceptibility to tetracycline. Almost all of the isolates showed susceptibility to chloramphenicol. Such findings are identical to the report by Choi et al. The causes of these differences are thought to be that tetracycline-type antibiotics are still frequently being used as growth promoters for livestock, but its use has been decreased in humans. It is also considered to be due to side effects, such as aplastic anemia in the case of chloramphenicol, although it is rare, and its use has been restricted. Therefore, tetracycline and chloramphenicol can be considered as treating agents. According to the result of the testing of the high resistance of vanA-type VRE to aminoglycoside antibiotics, the livestock and raw chicken isolates showed high resistance rates to streptomycin when compared to the human isolates, while showing low resistance rates to gentamicin. It is believed that this is because streptomycin is being used for treatment purposes in livestock, but gentamicin is not being used. The results from the phenotyping of vanA, vanB, and vanC types using MIC and the results from the genotyping of vanA, vanB, and vanC types using PCR were identical. In this study, mostly vanC-type VRE was isolated in the cases that used the fecal samples of the livestock, while vanA-type *E. faecium* was isolated in the cases of the raw chicken meat samples that used intestinal secretion as the specimen. Based on this, the possibility of the selective spread and isolation of highly resistant vanA VRE in the manufacturing process of raw chicken meat or differences in the sample-collecting process can be considered.

As a result of analyzing the patterns of PFGE of vanA VRE, clinical isolates were variously manifested, and there was no particular type that appeared more frequently. However, 9 isolates from the raw chicken meat samples showed findings that were identical in 3 PFGE patterns, and 1 bacterial strain showed identical PFGE patterns with the livestock isolates. Therefore, in addition to the vertical transmission of the same clone, the possibility of both vertical transmission of resistant bacterial strains and horizontal transmission of resistant genes can be also considered. Actually, in the beginning, vertical transmission was considered the main mechanism for vancomycin-resistant transmission. However, although epidemiologically related, due to the many cases that show various PFGE patterns, resistance by the horizontal transmission of vanA genes is now also known to be a main mechanism. To investigate the epidemiology of VRE, further study is needed. These studies must include vanA gene cluster analysis (overlapping PCR), southern hybridization analysis, and conjugation study of gene transfer.

Considering the remarkably low VRE isolation rate in healthy individuals compared to the high isolation rate of vanA resistant genotype in the raw chicken meat and the differences in the results of susceptibility of the animal and human body isolates, the possibility for the vertical transmission of resistant bacteria or the horizontal transmission of resistant genes from animals to humans is very low. Considering the similar PFGE patterns of the raw chicken isolates, VRE contamination during the manufacturing process of raw chicken meat can be considered. It is possible that VRE cross-contamination may occur through poor handwashing or improper cleansing of butchers’ kitchen-boards and knives. The facial specimen was taken by swabbing of stool samples, but intestinal secretion of chicken meat was taken by rinsing after managing in the microwave oven for 1 minute. The differences in the isolation rates might be influenced by the specimen-collection method. Domestically, however,
there is a mixed epidemiological trait between the European
trait, where antibiotics such as avoparcin and tylosin were used
growth promoters for livestock, and the American trait,
where the antibiotics are being widely used in hospitals. Also,
the isolation rate might have been low because the bacterial
cultivation was done with fecal sample cultures instead of
intestinal secretions. Therefore, larger scale studies should
be conducted in the future.

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REFERENCES

1) Murray BE. The life and times of the enterococcus. Curr Microbiol Rev 3:46-55, 1999
2) Schaberg DR, Culver DH, Gaynes RP. Major trends in the microbial
etiology of nosocomial infection. Am J Med 91(Suppl 3B):72S-75S, 1991
3) Leclercq R, Derlot E, Duval J, Courvalin P. Plasmid-mediated resistance to vancomycin and teicoplanin in enterococci faecium. N Engl J Med 315:157-161, 1988
4) Goossens H. The epidemiology of vancomycin-resistant enterococcus. Curr Opin Infect Dis 12:537-541, 1998
5) Kim BS. Vancomycin-resistant enterococci in the regional society. CDMR 1:37-41, 2002
6) Park YH, Seo KS, Yoo HS. Development of multiplex PCR for detection of vancomycin-resistant enterococci and epidemiological application in Korea. J Korean Soc Chemother 17:369-374, 1999
7) NCCLS. Performance standards for antimicrobial susceptibility testing: 9th international supplement NCCLS document M100-S9, 1999
8) Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 33:2223-2230, 1995
9) Kim WJ. Global epidemiology of glycopeptide-resistant enterococci: an update. Proceedings of 3rd International Symposium on Antimicrobial Agents and Resistance, pp.129-131, Seoul, 2001
10) Sundsfjord A, Simonsen GS, Courvalin P. Human infections caused by glycopeptide-resistant enterococci spp.: are they a zoonosis? Curr Microbiol 7:16-33, 2001
11) van der Auwera P, Pensaert M, Korten V, Murray BE, Leclercq R. Influence of oral glycopeptides on the fecal flora of human volunteers: selection of highly glycopeptide-resistant enterococci. J Infect Dis 173:119-136, 1996
12) Shin DH, Park JH, Shin JH, Suh SP, Ryang DW, Kim SJ. A molecular epidemiologic study on vancomycin-resistant enterococci isolated from clinical specimens over a 5-year period. Korean J Infect Dis 32:203-211, 2000
13) leven M, Vercauteren E, Descheemaeker P, van Laer F, Goossens H. Comparison of direct plating and broth enrichment culture for the detection of intestinal colonization by glycopeptide-resistant enterococci among hospitalized patients. J Clin Microbiol 37:1436-1440, 1999
14) Robredo B, Singh KV, Baquero F, Murray BE, Torres C. Vancomycin-resistant enterococci isolated from animals and food. Int J Food Microbiol 54:197-204, 2000
15) D'Agata EM, Jitjis J, Goudin C, Tang YW. Community dissemination of vancomycin-resistant Enterococcus faecium. Am J Infect Control 29:216-230, 2001
16) Kanchana MV, Dencer H, Blondeau J. Cost-effective algorithm for detection and identification of vancomycin-resistant enterococci in surveillance cultures. Eur J Clin Microbiol Infect Dis 19:366-369, 2000
17) Wootton M, Howe RA, Hillman R, Walsh TR, Bennett PM, MacGowan AP. A modified population analysis profile (PAP) method to detect hetero-resistance to vancomycin in Staphylococcus aureus in a UK hospital. J Antimicrob Chemother 47:399-403, 2001
18) Choi YH, Lee YS, Lee J, Yoo J, Kim CK, Kim BA. Molecular relationship of vanA glycopeptide resistance gene in enterococci from hospitalized patients in poultry in Korea. Korean J Infect Dis 33:283-301, 2001
19) van den Bogaard AE, Jensen LB, Stoibergh EE. Vancomycin-resistant enterococci in turkey and fowls. N Engl J Med 337:1553-1558, 1997
20) Choeng HJ, Kim WJ, Woo HJ, Kim MJ, Park SC. Study on the infection due to VanA type vancomycin-resistant enterococci. Korean J Infect Dis 30:10-18, 1998
21) Kim DR, Yu CW, Choeng HJ, Woo HJ, Kim WJ, Kim MJ, Park SC, Choi SJ. Rectal surveillance culture of vancomycin-resistant enterococci colonization among patients hospitalized in the intensive care unit. Korean J Infect Dis 31:203-209, 1999
22) Choeng HJ, Kim WJ, Choi SJ, Lee KW, Choi KW, Park SC. Nationwide questionnaire survey for the prevalence, detection methods, and infection control program of VRE among hospitals. Korean J Infect Dis 32:78-87, 2001
23) Arthur M, Courvalin P. Genetics and mechanisms of glycopeptide resistance in enterococci. Antimicrob Agents Chemother 37:1563-1571, 1993
24) van den Bogaard AE, Williams R, London N, Top J, Stoibergh EE. Antibiotic resistance of faecal enterococci in poultry, poultry farmers and poultry slaughtermen. J Antimicrob Chemother 46:479-487, 2000
25) van den Bogaard AE. Antimicrobial resistance: relation to human and animal exposure to antibiotics. J Antimicrob Chemother 40:453-458, 1997
26) Murray BE. Vancomycin-resistant enterococci infection. N Engl J Med 342:710-721, 2000
27) Park JW, Kim YR, Shin WS, Kang MW, Han KJ, Shim SI. Susceptibility tests of vancomycin-resistant enterococci. Korean J Infect Dis 24:133-137, 1992
28) Edmond MB, Ofer DF, Dawson JD, Weinaum DL, Wenzel RP. Vancomycin-resistant enterococcal bacteremia: natural history and attributable mortality. Clin Infect Dis 23:1234-1239, 1996
29) Kim WJ, Weinstein RA, Hayden MK. The changing molecular epidemiology and establishment of endemicity of vancomycin-resistant enterococci at one hospital over a 6-year period. J Infect Dis 179:162-171, 1999
30) Clark NC, Cookeve RC, Hill BC, Swenson JM, Tenover FC. Characterization of glycopeptide-resistant enterococci from U.S. hospitals. Antimicrob Agents Chemother 37:2311-2317, 1993