

ER 01

Infections and *van* gene types of vancomycin-resistant *Enterococci* in Korea livestock

HM Kang*, JS Moon, BY Jung,
JM Kim, G Lee¹, CI Chung¹

Bacteriology, National Veterinary Research and Quarantine Service, Anyang, Korea,
Konkuk University, Seoul, Korea¹

Background: It has rarely performed to study on distribution, molecular epidemiology and transfer of vancomycin-resistant *Enterococci* (VRE) in Korea livestock after ban on use and import of avoparcin as a growth promoter since 1997. Therefore, the purpose of this study was to investigate the prevalence of VRE infections and patterns of *Van* genes according to the origins of livestock.

Methods: A total of 885 *Enterococci* was isolated from chicken feces, porcine feces, Korean native cattle (Hanwoo) feces, dairy cow feces and mastitis milk during 1999 to 2001. Antimicrobial susceptibility tests were carried out by disc diffusion methods and MIC, and molecular analysis was performed by PCR-RFLP with *Msp* I.

Results: VRE infections were 0.7%(6/885) in livestock, all of them were shown *VanA* genes as *E. faecium* strains and "a" type in PCR-RFLP. Also, 13 strains of *E. gallinarum* were shown *VanC1* genes (1.5%), and 49 strains of *E. casseliflavus* were found to have *VanC2* genes (5.5%). Total expression rates of *Van* genes were 7.7% (68/885) in domestic animals.

Conclusions: Although VRE infections were not significant, as 0.7%(6/885) at present, future monitoring will be necessary to prevent the potential extension in domestic animals. Distribution of *VanA*, *VanC1* and *VanC2* genes was shown as 0.7%, 1.5%, and 5.5%, respectively. However, none of *VanB* genes were detected in this study.

ER 02

Characterization of *Enterococci* expressing high-level aminoglycoside resistance.

EE Udo*, N Al-Sweih, P John

Department of Microbiology, Faculty of Medicine,
Kuwait University, Kuwait

Background: Recent studies have shown that up to 20% of *Enterococci* isolated in Kuwait hospitals express high-level resistance to aminoglycoside antibiotics. This study investigated the genetic relatedness and the distribution of genes for aminoglycoside modifying enzymes (AME) in high-level aminoglycoside-resistant *Enterococci* isolated in Kuwait hospitals.

Methods: High-level aminoglycoside resistance was confirmed by measuring the MICs of gentamicin, kanamycin tobramycin, amikacin and streptomycin by agar dilution. Genes encoding the AME, AAC (6')-1e-APH (2'')-1a, ANT (4'), APH (3'), APH (2'')-1b, APH (2'')-1c, APH (2'')-1d and ANT(6) were detected by the polymerase chain reaction. Pulsed-field gel electrophoresis (PFGE) was used to study their genetic relatedness.

Results: 118 *Enterococci* consisting of 111 *E. faecalis*, six *E. faecium*, and an *E. casseliflavus* were isolated from urines (56.8%), wounds (14.4%), blood (5.9%), high vaginal swabs (14.4%) and other sources (8.5%). All isolates were resistant to kanamycin (MIC >2000 mg/L), 52 isolates were resistant to gentamicin (MIC >500 mg/L), 71 were resistant to tobramycin (MIC >64 mg/L), 115 were resistant to amikacin (MIC > 64 mg/L) and 97 were resistant to streptomycin (MIC > 1000mg/L). All of the gentamicin-resistant isolates contained genes for AAC (6')-1e-APH (2'') -1a and APH (3''). In addition, genes for AAC (6')-1e-APH (2'')-1a were detected in 15 of 16 isolates with gentamicin MIC, 256mg/L. Genes for ANT (6'') was detected in all the streptomycin-resistant isolates with MIC ≥ 1000mg/L. None of them contained genes for ANT (4'), APH (2'')-1b, APH (2'')-1c and APH (2'')-1d enzymes. PFGE showed no evidence of clonal spread.

Conclusions: AME are common in aminoglycoside resistant *Enterococci* isolated in Kuwait. However, there is no evidence of a dominant clone.

ER 03

Molecular diversity of Tn1546-like elements in *Enterococcus faecium* isolated from Korean hospitals

WG Lee and JY Huh

Department of Laboratory Medicine,
Ajou University School of Medicine,
Suwon, Korea

Background: The *vanA* gene cluster is carried as part of Tn1546-like elements. The molecular epidemiology of Tn1546-like elements has been performed in several papers. Genetic diversity in Tn1546-like elements has been documented previously. The differences described so far have included insertion of IS elements IS1216V, IS1251, IS1476 and IS1542. IS1216V has been widespread among VanA *Enterococci* of diverse geographic areas, whereas IS1542 and IS1476 have only been reported in the UK and Canada. We investigated the distribution of insertion sequences in *vanA*-containing *E. faecium* isolated from patients in Korea.

Methods: Twenty isolates of *vanA*-containing *E. faecium* isolated from 10 university hospitals in Korea. PCR amplification of internal regions of Tn1546 was performed and primers were used according to the published sequence of Tn1546. To detect the presence of published insertion sequences in the *vanA* gene cluster, specific primer sets for IS1216V, IS1251, IS1476 and IS1542 were used. PCR amplicons were directly sequenced on both DNA strands by the dideoxy termination method.

Results: All isolates contained IS1216V. IS1251 was only detected in 2 isolates from same hospital and found in the *vanSH* intergenic region. IS1542 was detected in the genomes of 16 isolates and IS1476 was not detected in Korean isolates.

Conclusions: IS1542 has appeared to be geographically restricted; it has only been reported in UK. Most of Korean isolates contained IS1542 sequences. Therefore the identification of insertion sequence within *vanA* gene cluster can be a useful tool in epidemiological investigations.

ER 04

Prevalence of streptogramin resistance genes among *Enterococcus faecium* isolates recovered from chickens in Taiwan and Korea

**T-L Lauderdale^{1*}, J-F Lai¹,
LC McDonald¹, Y Lee², M Ho¹**

¹National Health Research Institutes, Taiwan

²Seoul Women's University, Korea

Background: The streptogramin quinupristin/dalfopristin (Q/D) has not been approved for clinical use in Taiwan. Virginiamycin, also a streptogramin, has been used as a feed additive for many years in Taiwan. Q/D resistant *E. faecium* isolates have been recovered from retail chickens and humans in Taiwan. The present study investigated the presence of streptogramin resistance genes in Q/D resistant *E. faecium* isolated from retail chicken carcasses in Taiwan and compared with isolates recovered from chickens in Korea.

Methods: Genes involved in streptogramin A resistance *vat* (D), *vat* (E) and *vga*, and genes involved in streptogramin B resistance *vgb* and *ermB*, were screened for by PCR. The presence of the newly described *msrC* gene was also determined. Sequencing was performed on selected PCR amplicons.

Results: *Vat* (E) gene was found in 9 of the 11 *E. faecium* isolates (1 from Taiwan, 10 from Korea) having high-level Q/D resistance (MIC \geq 32 ug/ml) and 2 of the 47 isolates (30 from Taiwan, 17 from Korea) having low-level Q/D resistance (MIC 3-12 ug/ml); all were erythromycin resistant and *ermB* positive. No *ermB* was detected in the 3 erythromycin susceptible isolates from Korea. *msrC* was detected in 21 of the 31 isolates from Taiwan and 24 of the 30 isolates from Korea. No *vat* (D), *vga*, or *vgb* was found in any of the isolates studied.

Conclusions: The finding of only *vat* (E), which is prevalent in the United States, but not *vat* (D), which is prevalent in Europe, in the present study indicated differences in the distribution of streptogramin A resistance genes in different geographic regions. The newly described *msrC* gene is present in most but not all of the *E. faecium* isolates. Similar to reports from other countries, other undefined resistance mechanisms exist in the low-level Q/D-resistant *E. faecium* isolates in Taiwan and Korea.

ER 05

Antibiotic resistance of Korean isolates *Enterococci* of poultry origin

SJ Kim^{*}, HB Basnet, MC Kim, EK Lee
College of Veterinary Medicine,
Seoul National University, Korea

Background: The practice of using antimicrobials as growth promoters and performance enhancer in poultry industry may facilitate the dissemination of resistance to *Enterococci*. In this study; antibiotic resistance patterns of enterococci isolated from poultry and poultry related samples in Korea were studied.

Methods: *Enterococci* were isolated from chickens and poultry environmental samples and tested for antibiotic sensitivities by the disk diffusion method.

Results: Of 188 Enterococcal isolates made from chickens and their farming environments, 11 different species could be identified. Of the 114 identified *Enterococcus* (*E.*) isolates, *E. faecalis* and *E. faecium* were the highest in isolation frequencies being 36.8% and 36.0%, respectively, followed by *E. gallinarum* (7.0%), *E. durans* and *E. saccharolyticus* (3.5% each).

Most of the 161 isolates tested for sensitivity to 12 different antibiotics were multiply resistant. Vancomycin (11.9%), followed by rifampin (26.7%) and penicillin (37.3%) were the least resistant antibiotics. Resistance frequencies of *E. faecalis* and *E. faecium* isolates were, respectively, 28.2% and 13.0% to vancomycin, 41.0% and 13.0% to rifampin, and 5.1% and 65.2% to penicillin.

Conclusions: A total of 188 isolations of *Enterococci* with eleven different species identifications were made from chickens and related samples. *E. faecalis* and *E. faecium* were the most frequently isolated species. Most of the enterococcal isolates were multiply resistant to 12 different antibiotics tested. Resistance frequencies of the isolates of *E. faecalis* and *E. faecium* were, respectively, 28.2% and 13.0% to vancomycin, 41.0% and 13.0% to rifampin, and 5.1% and 65.2% to penicillin.

ER 06

First report of vanB-vanA incongruent vancomycin-resistant enterococcus in Korea

EJ Shin^{1*}, Y Park², WJ Kim³, JH Lee⁴,
TL Lauderdale⁵, Y Lee⁶

¹Seoul Women's University, Seoul, Korea

²College of Veterinary Sciences,

Seoul National University, Seoul, Korea

³Department of Infectious Diseases, Kuro Hospital,
Korea University, Korea

⁴Chunbuk National University, Chunju, Korea

⁵National Health Research Institutes,

Taipei, Taiwan

Background: Since avoparcin is one of glycopeptide antibiotics and avoparcin-resistant bacteria are resistant to vancomycin, avoparcin has been prohibited in agricultural industry by law in Korea since 1999. In this work, the occurrence of VRE after avoparcin prohibition has been monitored in 2001.

Methods: During one-year period, total five hundred ninety four isolates of *Enterococcus* were obtained from chicken intestines in slaughterhouses. Among these, vancomycin-resistant *Enterococcus* were selected and identified with biochemical and physiological tests and 16S rRNA sequencing. Genes responsible for vancomycin-resistance were detected with multiplex-PCR.

Results: Sixty-nine *Enterococcus* isolates (11.6%) were vancomycin-resistant. These VRE were composed of fifty-two isolates (75.4%) of *E. faecium*, sixteen isolates (23.2%) of *E. gallinarum*, and one isolate (1.4%) of *E. caesselifalvus* while *E. faecalis* was not found. Among *E. faecium*, twenty-eight isolates were VanA and twenty-four isolates were VanB. Every *E. gallinarum* was VanC1 and *E. caesselifalvus* was VanC2. When multiplex PCR was performed, *vanA* gene not *vanB* gene was found in every *E. faecium* with VanB phenotype. With pulse field gel electrophoresis, these VanB-*vanA* incongruent VRE showed no similarity to each other and with those isolates in Taiwan.

Conclusions: This is the first report of VanB-*vanA* incongruent VRE in Korea. These VRE were not similar to each other and isolates in Taiwan. Results suggested that there are special mechanisms responsible for incongruence by inhibiting *vanA* expression.

ER 07

Emergence of vancomycin-resistant *Enterococci* (VRE) with vanB-phenotype, vanA-genotype in a Korean Hospital

JS Eom^{*}, IS Hwang, YJ Lee, DW Park,
HJ Cheong, WJ Kim, SC Park
Department of Internal Medicine, Korea Univ.
Coll. of Med., Seoul, Korea

Background: In Korea, VRE has become an important nosocomial pathogen since 1995 and most of VRE were *vanA*-genotype with antibiotic susceptibility of VanA-phenotype. During 2001, we experienced the outbreak of VRE with VanB-phenotype, *vanA*-genotype in one university hospital. We analyzed the clinical findings of patients and analyzed the molecular genetics of VRE isolates.

Methods: In 2001, 20 VRE were isolated from 20 patients in Korea University Guro Hospital. We reviewed medical records of those patients. 9 VRE isolates were studied by agar dilution antimicrobial susceptibility test, *vanA-vanB* duplex PCR and pulsed-field gel electrophoresis (PFGE). Genetic mutations in *vanS* gene of VRE isolates was studied by direct PCR sequencing.

Results: All of 20 VRE patients (10 males, mean age 53.9 ± 18.0 years) had underlying diseases; the most common disease was cerebrovascular accident in 8(40%). All of patients had been in intensive care unit and mean durations of hospital stay were 178.9 ± 165.0 days. Vancomycin were used in 7 patients (35%), and teicoplanin in 3 patients (15%). Site of isolations included urine in 14(70%), abscess in 2, wound in 2, blood in 1, and catheter tip in 1. All of 9 VRE strains studied showed VanB phenotype (vancomycin MICs $> 256 \mu\text{g/ml}$, teicoplanin MICs 4-16 $\mu\text{g/ml}$) and *vanA* genotype by *vanA-vanB* duplex PCR. PFGE showed 4 different clones among 9 VRE strains. Nucleotide sequencing of *vanS* gene demonstrated 3 identical mutations described by Hashimoto *et al* (2000).

Conclusions: We reported the first nosocomial outbreak caused by VRE with VanB-phenotype, *vanA*-genotype in Korea.

ER 08

Streptogramin resistance among vancomycin resistant *Enterococcus faecium* from poultry and clinical isolates in South Korea

YS Lee^{*}, YH Jung, YH Choi, JK Lee,
JI Yoo, BS Kim
Lab. of antimicrobial resistant pathogens,
Department of bacteriology, NIH, Seoul Korea

Background: The semisynthetic streptogramin B/A combination quinupristin/dalfopristin is one of the few currently approved antimicrobial agents with activity against vancomycin resistant *Enterococcus faecium* (VREF). Its clinical use was approved in the South Korea in July 2000. Due to a wide use of streptogramin (virginiamycin) in commercial animal farming since 1974, the possibility of occurrence of streptogramin-resistant *E. faecium* isolates is very high. We investigated the prevalence of streptogramin resistance among poultry and clinical isolates of VREF in South Korea.

Methods: 98 clinical and 78 poultry isolates of vancomycin resistant VanA *E. faecium* isolated between 2000 and 2002 were examined. The minimal inhibitory concentrations (MICs) of quinupristin-dalfopristin were determined by E-test method. Streptogramin resistance genes, *vatE*, *vatD*, *ermB*, and *vgb*, were detected by multiplex PCR. Variation in the *vatE* allele was examined by PCR and sequencing.

Results: 51 among 78 VREF poultry isolates exhibited resistance to quinupristin/dalfopristin. MICs of quinupristin-dalfopristin of poultry isolates were $\geq 32 \mu\text{g/ml}$ (36 isolates), 24 $\mu\text{g/ml}$ (2 isolates), 12 $\mu\text{g/ml}$ (2 isolates), and 4 $\mu\text{g/ml}$ (11 isolates). The *vatE* and *ermB* gene were detected in 33 isolates and only *ermB* gene in 15 isolates, only *vatE* in 3 isolates. Sequences of *vatE* gene were identical to *vatE-1*. MICs of 4 isolates resistance to quinupristin/dalfopristin among 98 clinical isolates were 4~8 $\mu\text{g/ml}$ and were detected *ermB* gene.

Conclusions: This study suggest that there is a prevalence of high-level streptogramin resistance among VREF in poultry, Korea. It has been speculated that the extensive use of virginiamycin in animal husbandary might contribute to the emergence of quinupristin/dalfopristin resistance among clinical isolates of *E. faecium*.

**Correlation of penicillin and ampicillin
NCCLS susceptibility breakpoints for
E. faecium with imipenem BSAC
susceptibility breakpoints
false susceptibility to imipenem by
vitek GPS card in *Enterococcus faecium***

HK Lee^{*}, YJ Park, EJ Lee, BK Kim, CS Kang

Department of Clinical pathology, College of Medicine,
Catholic University, Seoul, Korea

Background: In Korea, about 40-50% of *Enterococci* isolated from clinical specimens are *E. faecium*. Although imipenem has high activity against a wide variety of bacteria including *Enterococci*, strains of *E. faecium* are known to be less susceptible than *E. faecalis* strains to imipenem. However, there are no NCCLS guidelines for susceptibility testing of imipenem versus *Enterococci*. Also there are no statements that in vitro susceptibility results for other antimicrobial agents can predict the in vitro activity of imipenem against *Enterococci*.

Methods: Fifty-two isolates of *E. faecium* were tested. All isolates were identified in Kangnam St. Mary's Hospital Clinical Microbiology Laboratory using Vitek GPI card and was confirmed by conventional microbiological testing. Each isolate was tested versus penicillin, ampicillin and imipenem. Solutions of all antimicrobials were prepared from standard powders of known potencies obtained either from the manufacturer of the compound or from a commercial source (Sigma, St. Louis, Mo.). MICs were determined by agar dilution method according to NCCLS guidelines (NCCLS, 2000). Imipenem MIC determinations were also repeated by E-test according to manufacturers specification.

Results: The penicillin and ampicillin MICs by agar dilution method coincided with the results from the Vitek GPS card for all the 52 isolates. But for imipenem, the MICs by the agar dilution method did not correspond with the Vitek results. The penicillin and ampicillin results by agar dilution method correctly predicted the results for imipenem for all 52 strains.

Conclusions: Imipenem MICs for *E. faecium* by Vitek system is not reliable but MICs of ampicillin and penicillin are reliable and they can accurately predict imipenem susceptibility. Also imipenem MICs for *Enterococci* by Vitek system should be rechecked by agar dilution method.