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Note

The Reason of High Prevalence of Vancomycin-Resistant (VR) *E. faecium* in Nosocomial Infection

Hyun-Jung Jo[1], Hee-Jeong Kim[1], Hyo-Jin Lee[1], Gyu-Nam Park[1], Min-Ju Kim[1], Dong-Jun An[2] and Kyung-Soo Chang[1,†]

[1]Department of Clinical Laboratory Science, College of Health Sciences, Catholic University of Pusan, Busan 609-757, Korea
[2]National Veterinary Research and Quarantine Service, Anyang, Gyeonggi-do 430-824, Korea

Vancomycin-resistant (VR)-*E. faecium* and VR-*E. faecalis* were isolated simultaneously from a rectal swab of a patient diagnosed with pneumonia in an intensive care unit (ICU). The patient was treated with various antibiotics including vancomycin. Only VR-*E. faecium* was continually isolated from the rectal swab at one and two weeks of the treatment. Identical *vanA*, IS*1216V*, and IS*1542* genes were detected in both VR-*E. faecium* and VR-*E. faecalis* isolates which showed equal resistance against vancomycin and teicoplanin, but IS*1251* was not detected. VR-*E. faecium* showed stronger multi-drug resistance than VE-*E. faecalis*. This result supports the reason why VR-*E. faecium* is one of the major pathogens in nosocomial infections.

Key Words: Vancomycin resistant *Enterococcus* (VRE), *E. faecium*, *E. faecalis*, Multi-drug resistance, *van* gene

Recently, hospitals-acquired infections by *Enterococcus* spp. have dramatically increased. *Enterococcu*s spp. is disseminated from the hands of medical personnels, medical appliances, an environment and other patients. Immuno-compromised patients are infected easily (Murray et al., 1991; Livornese et al., 1992; Murray et al., 1992). Moreover, antibiotics resistant *Enterococcus* are increasing because of overuse of antibiotics. Vancomycin resistant *Enterococcus* (VRE) is a problem with cohabitation in hospitals, and is being treated by antibiotic therapy for multi-drug resistance (Leclercq et al., 1988; Uttley et al., 1988). VRE infection becomes a serious domestic and international problem in hospital-acquired infection. In China, the incidence of VRE was appeared to be increasing. Nosocomial outbreaks by VRE were reported in all hospital and clinics including a tertiary institution (Xu et al., 2011). In Canada, clinical specimens were collected from patients in ICU. VanA phenotype of *E. faecium* made up 88.2% of all VRE. However, the continuous ubiquitous active surveillance programs in Canadian hospitals should bring low level of VRE in Canadian ICU (Zhanel et al., 2008).

Enterococcus has a self-mechanism for antibiotics resistance and an ability to accept plasmids harboring antibiotics resistances. These plasmids can be inserted by conjugation to *Enterococcus* and other bacteria. It induces diffusion of antibiotics resistance. *In vitro*, it was proved that plasmid of VRE can transform to *Staphylococcus aureus* resulting in vancomycin-resistant *Staphylococcus aureus* (VRSA) (Leclercq et al., 1989; Schaberg et al., 1991; Noble et al., 1992).

We report a clinical importance of VR-*E. faecium* and VR-*E. faecalis* isolated from the patient diagnosed with pneumonia at the same time. The isolates were identified on phenotype and genotype, and analyzed on antibiotics resistant patterns by an antimicrobial susceptibility test.

The 62-year-old man who was showing insensibility

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†Corresponding author: Kyung-Soo Chang. Department of Clinical Laboratory Science, College of Health Sciences, Catholic University of Pusan, 9 Bugok 3-dong, Geumjeong-gu, Busan 609-757, Korea.
Tel: 82-051-510-0565, Fax: 82-051-510-0565
e-mail: kschang@cup.ac.kr

Table 1. Comparison of antimicrobial susceptibility between VR-*Enterococcus* spp and Standard *Enterococcus* spp*

Antibiotics (μg)	VR-*Enterococcus* spp		Standard *Enterococcus* spp	
	E. faecium	*E. faecalis*	*E. faecium*	*E. faecalis*
Quinupristin/Dalfopristin (4.5/10.5)	S	R	S	R
Ampicillin/Sulbactam (10/10)	R	S	S	S
Nitrofurantoin (300)	R	S	S	S
Imipenem (10)	R	S	R	S
Teicoplanin (30)	R	R	S	S
Vancomycin (30)	R	R	S	S
Van phenotype	VanA	VanA		
van genotype	vanA	vanA		

*We referred to antibiotic resistant range in CLSI Guidelines, Antibiotics resistant patterns of four VR-*E. faecium* isolates were identical, R, resistant; S, sensitive.

was hospitalized on 13th March in 2010. He had an intracerebral hemorrhage and dyspnea. He was underwent surgery to remove the hematoma. Then he showed symptom of pneumonia and was transferred to the department to internal medicine. ESBL(+) *K. pneumoniae* was isolated from his sputum. He got a treatment in intensive care unit (ICU). VR-*E. faecium* was isolated from rectal swab on 28th April at first, and both VR-*E. faecalis* and VR-*E. faecium* were isolated from rectal swab on 20th May. VR-*E. faecalis* was disappeared from third and fourth isolation by antibiotics but VR-*E. faecium* still remained.

The sample was collected every two weeks, and cultured in ChromID VRE agar (Biomerieux) and blood agar plate (BAP). VR-*E. faecium* and VR-*E. faecalis* were violet and bluish-green colony on ChromID VRE agar, respectively. They were gram positive cocci and catalase negative. VR-*E. faecalis* and VR-*E. faecium* isolates were confirmed by using Vitek-II gram-positive (GP) identification card (Biomerieux) and by genotyping using 16S rRNA sequences. *Enterococcus faeciem* (ATCC 19434) and *Enteorococcus faecalis* (ATCC 19433) were used as reference bacteria.

Antimicrobial susceptibility of VR-*E. faecium* and VR-*E. faecalis* isolates were tested by GPS-600kit (Biomerieux) and antibiotics disk diffusion test. Mueller Hinton agar (MH agar) and antibiotics disks such as vancomycin, teicoplanin, quinupristin/dalfopristin, imipenem, ampicillin/sulbactam and nitrofuantoin (Gibco BRL) were used. Antimicrobial susceptibility of the isolates was determined by CLSI standard. VR-*E. faecium* and VR-*E. faecalis* were resistant

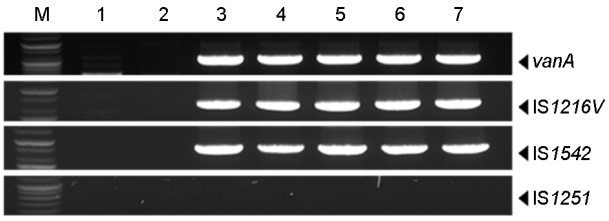

Fig. 1. Analysis of *van* and IS genes of VRE isolates from patient by isolation time
M: marker, 1: *Enterococcus faecium* (ATCC 19434), 2: *Enteoroccus faecalis* (ATCC 19433), 3: First isolated VR-*E. faecium* from the patient, 4: Second isolated VR-*E. faecalis* from the patient, 5: Second isolated VR-*E. faecium* from the patient, 6: Third isolated VR-*E. faecium* from the patient, 7: Forth isolated VR-*E. faecium* from the patient.

to vancomycin and teicoplanin. Van phenotypes of them were VanA. Antibiotics resistant patterns of four VR-*E. faecium* isolates were identical. VR-*E. faecium* isolates showed multi-drug resistance compared to VR-*E. faecalis*. In addition, only VR-*E. faecium* showing multi-drug resistance has survived in the patient with various antibiotics treatment and still remained in the intestine of the patient (Table 1).

For analysis of *van* gene and IS genes typing, PCR using primer sets was conducted as described previously (Kim et al., 2010). *vanA* gene were detected in all isolates, but *vanB* and *vanC* genes were not. IS*1216V* and IS*1542* were detected in all isolates, but IS*1251* not. *van* genotype and IS genotype of VRE isolates from patient were identical (Fig. 1). This result suggests that origin of *van* gene and IS gene from VRE isolates were the same. Four VR-*E. faecium*

isolated from the patient periodically were same bacteria.

We suggest that *vanA* of VR-*E. faecium* was transferred to *E. faecalis* being normal flora. The *E. faecalis* was transformed to VR-*E. faecalis* by *vanA* from VR-*E. faecium*. VR-*E. faecium* was more multi-drug resistant than VR-*E. faecalis*. Therefore, VR-*E. faecium* might survived for a long time in intestine compared to VR-*E. faecalis* in presence of other antibiotics. Finally, VR-*E. faecium* might be only isolated.

Otherwise *E. faecalis* is mostly isolated from patients, VR-*E. faecium* is more frequently found than VR-*E. faecalis* in hospital-acquired infection. We anticipate that *E. faecium* has various systems for acquisition of antibiotics resistance or natural antibiotics resistance to survive in presence of general antibiotics. The mechanism on natural resistance of *E. faecium* against antibiotics has not been clearly elucidated. To overcome *E. faecium* showing multi-drug resistance depending on *van* gene as well as other resistance systems, we should study natural antibiotics resistance of *E. faecium* such as imipenem.

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