J Microbiol Immunol Infect.
2009;42:63-68

Original Article

Vancomycin-resistant enterococci in a tertiary teaching hospital in Taiwan

Yu-Chia Hsieh[1], Tsong-Yih Ou[1], Sing-On Teng[1], Wuan-Chan Lee[2], Yi-Chun Lin[2], Jann-Tay Wang[3], Shan-Chwen Chang[3], Wen-Sen Lee[1]

[1]Section of Infectious Disease, Department of Internal Medicine, Taipei Medical University, Wan Fang Hospital; [2]Taipei Medical University Hospital; and [3]Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan

Received: January 27, 2008 Revised: May 23, 2008 Accepted: July 22, 2008

Background and purpose: In 2007, an outbreak of vancomycin-resistant enterococci (VRE) occurred at Taipei Medical University, Wan Fang Hospital, Taipei, Taiwan. The aim of this study was to characterize the mechanism of glycopeptide resistance and to investigate the genetic relatedness among isolates of VRE.

Methods: Between May and October 2007, bacterial isolates from 16 patients identified as colonized or infected with VRE were collected. Polymerase chain reaction and pulsed-field gel electrophoresis (PFGE) were used to determine resistant genes and molecular typing.

Results: All 16 isolates of VRE presented with the VanA phenotype with the *vanA* gene except for 1 isolate of *Enterococcus faecalis*, which had the VanB phenotype with the *vanA* gene. PFGE analysis revealed a major clone containing 12 isolates, and 4 other distinct clones containing 1 to 2 isolates each. Five patients had VRE colonized in their gastrointestinal tract, the genotype of which was the same as the clinical isolates. Fourteen isolates (87.5%) had the *esp* gene.

Conclusions: An outbreak of VRE was caused by the simultaneous existence of monoclonal and polyclonal spread. Rigorous infection control, active surveillance, and decreasing pressure of antibiotic use are important for controlling the emergence of VRE.

Key words: Drug resistance, bacterial; Electrophoresis, gel, pulsed-field; *Enterococcus*; Genes; Vancomycin resistance

Introduction

Enterococci, which are normal inhabitants of the gut flora, have adapted to several antibiotics by acquiring resistance to high concentrations of aminoglycosides, β-lactams, and glycopeptides [1-3]. Since vancomycin-resistant enterococci (VRE) were first described in 1987 [1,3], this pathogen has emerged as an important nosocomial pathogen worldwide [1,3,4]. The use of vancomycin, cephalosporin, and antibiotics with anti-anaerobic activity have been reported to be associated with VRE colonization or infection [5,6]. In Europe, the use of avoparcin in the agricultural industry has contributed to the selection of VRE [7,8]. VRE colonization or infection frequently occurs in critically ill and immunocompromised patients in wards in which the use of antibiotics is high. With enhanced infection-control strategies, VRE outbreaks have been successfully controlled in some hospitals [9-11], although VRE has become endemic in some institutions [12]. Studies have found that VRE has an impact on the mortality, morbidity, and adverse outcomes of patients, and causes substantial medical care costs [13-15].

Taipei Medical University, Wan Fang Hospital, has been established for 11 years and has been a medical center for 3 years. VRE has been isolated only once, in a patient referred from another hospital 10 years ago. In May 2007, a VRE isolate was recovered

Corresponding author: Dr. Wen-Sen Lee, Section of Infectious Disease, Department of Internal Medicine, Taipei Medical University, Wan Fang Hospital, Hsing-Long Rd, Section 3, No111, Taipei116, Taiwan. E-mail: 89425@wanfang.gov.tw

from the blood of a patient admitted to the hematology ward. In the following 6 months, another 15 patients were newly identified as colonized or infected with VRE. This study was performed to investigate the clinical characteristics, and microbiological and molecular epidemiology of the VRE outbreak in the hospital.

Methods

Bacteria, antimicrobial agents, and minimal inhibitory concentration determination

From May to October 2007, 16 isolates of VRE were recovered from 16 adult patients (median age, 78.5 years) admitted to Taipei Medical University, Wan Fang Hospital, Taipei, Taiwan. All the VRE isolates were cultured within 72 h of the patients being admitted to hospital.

VRE isolation from clinical specimens was carried out using 5% sheep blood and bile esculin agar plates (bioMérieux, Marcy l'Étoile, France). Routine antibiograms were determined on Mueller-Hinton agar by the disc diffusion method. The minimal inhibitory concentrations (MICs) of enterococci to ampicillin, vancomycin, and teicoplanin were determined by the agar dilution methods, which were performed and interpreted according to the guidelines established by the Clinical and Laboratory Standards Institute [16]. *Staphylococcus aureus* American Type Culture Collection (ATCC) 29213, and *Enterococcus faecalis* ATCC 29212 were used as control strains.

Detection of the *vanA*, *vanB*, and *esp* genes

The DNA of the isolates was purified by using the DNase Tissue kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's instructions. *vanA* was amplified by primers 5'-GGGAAAACGACAATT GC-3' and 5'-GTACAATGCGGCCGTTA-3'. *vanB* was amplified by primers 5'-ATGGGAAGCCGATAGTC-3' and 5'-GATTT CGTTCCTCGACC-3'. Detection of the N-terminal region of the *esp* gene by primers 5'-TT GCTAATGCTA GTCCACGACC-3' and 5'-GCGT CAACACTTGCATTG CCGAA-3' was used to screen for the presence of the *esp* gene [17]. Polymerase chain reaction to detect the *vanA*, *vanB*, and *esp* genes was done as previously described [17,18].

Pulsed-field gel electrophoresis typing

Pulsed-field gel electrophoresis (PFGE) was performed according to the methodology described elsewhere [17]. The DNA was digested with *Sma*I. Bands were stained with ethidium bromide and visualized with ultraviolet light. PFGE patterns differing by ≤3 bands were defined as 1 pulsotype [19].

Results

Among the 16 patients, 12 had true VRE infection. The most common underlying diseases were diabetes mellitus, congestive heart failure, and chronic renal failure (Table 1). Seven patients were in the intensive care unit (ICU) — 5 in the medical ICU and 2 in the surgical ICU — and 9 patients were in medical wards — 4 in a nephrology ward, 3 in a respiratory ward, 1 in a hematology ward, and 1 in a cardiology ward (Table 1). Twelve of the 16 VRE isolates (75.0%) were from pus or urine, and 3 (18.8%) were from blood (Table 1).

Among the 16 VRE isolates, 15 were *Enterococcus faecium*, and one was *E. faecalis* (Table 1). The *vanA* gene was detected in all 16 isolates (Table 2). Fifteen isolates of *E. faecium* had high MICs for vancomycin (>128 μg/mL) and teicoplanin (32-64 μg/mL) [Table 2]. These isolates were also resistant to ampicillin (MIC, >128 μg/mL), erythromycin, gentamicin, and levofloxacin. One isolate of *E. faecalis* was resistant to vancomycin (MIC, >128 μg/mL), but was susceptible to teicoplanin (MIC, 8 μg/mL). This *E. faecalis* isolate was a VanB phenotype with a *vanA* genotype. One isolate of *E. faecalis* and 1 isolate of *E. faecium* were resistant to chloramphenicol.

The PFGE analysis of the 16 isolates showed 4 different pulsotypes (Table 2, Fig. 1). Pulsotype A accounted for 12 isolates (75.00%), pulsotype B accounted for 2 isolates (12.50%), and pulsotypes C and D accounted for 1 isolate (6.25%) each. Five isolates belonging to pulsotype A were from patients in the medical ICU and 3 were from the nephrology ward; 2 isolates belonging to pulsotype B were from the respiratory ward. There was no other clonal dissemination among the wards. The *esp* gene was present in 15 isolates of E. faecium. All isolates in pulsotype A had the *esp* gene. Isolates in pulsotypes B, and D also had the *esp* gene.

Rectal swabs from 5 of 13 patients had positive VRE cultures (Table 2). The PFGE types of these 5 carrier isolates shared the same pattern as the clinical VRE isolates (data not shown). All of the isolates belonged to pulsotype A. Three patients acquired the VRE in the medical ICU. The presence of the *esp* gene was not consistent with the genotype between clinical isolates and carrier isolates in 1 patient.

Table 1. Demographic data of patients with vancomycin-resistant *Enterococcus* spp. infection.

Patient No.	Age (years)	Sex	Date	Source	Ward	*Enterococcus* spp.	Underlying disease
1	81	Male	May 19, 2007	Blood	Hematology	*E. faecium*	Diabetes mellitus
2	91	Male	June 04, 2007	Urine	Respiratory	*E. faecium*	Chronic rènal failure
3	78	Female	June 25, 2007	Urine	Nephrology	*E. faecium*	Liver cirrhosis
4	69	Male	June 27, 2007	Pus	Cardiology	*E. faecium*	Diabetes mellitus
5	90	Male	July 24, 2007	Urine	Medical ICU	*E. faecium*	Congestive heart failure
6	90	Female	July 24, 2007	Urine	Nephrology	*E. faecium*	Diabetes mellitus
7	85	Male	August 06, 2007	Catheter tip	Medical ICU	*E. faecium*	Chronic renal failure
8	65	Female	August 29, 2007	Pus	Medical ICU	*E. faecium*	Congestive heart failure
9	59	Female	September 12, 2007	Urine	Nephrology	*E. faecium*	Diabetes mellitus
10	79	Male	September 15, 2007	Blood	Medical ICU	*E. faecium*	Congestive heart failure
11	90	Female	September 15, 2007	Urine	Medical ICU	*E. faecium*	Congestive heart failure
12	83	Female	September 18, 2007	Urine	Respiratory	*E. faecium*	Chronic renal failure
13	71	Male	September 19, 2007	Pus	Nephrology	*E. faecalis*	Congestive heart failure
14	46	Male	September 19, 2007	Pus	Surgical ICU	*E. faecium*	Coronary artery disease
15	77	Male	October 01, 2007	Blood	Surgical ICU	*E. faecium*	Coronary artery disease
16	54	Male	September 27, 2007	Urine	Respiratory	*E. faecium*	Old stroke

Abbreviation: ICU = intensive care unit.

Table 2. Minimal inhibitory concentrations (MICs) of vancomycin-resistant *Enterococcus* spp.

Patients/ isolates	MICs			Agar disc diffusion test					*vanA/ vanB*	Pulso- type	*esp* gene
	Ampicillin	Vancomycin	Teicoplanin	Gentamicin	Chloramphenicol	Erythromycin	Penicillin	Levofloxacin			
1	>128	>128	64	I	S	R	R	R	*vanA*	A	+
1R[a]	>128	>128	32						*vanA*	A	+
2	>128	>128	64	R	S	R	R	R	*vanA*	A	+
3	>128	>128	32	R	S	R	R	R	*vanA*	A	+
4	>128	>128	64	R	S	R	R	R	*vanA*	A	+
5	>128	>128	64	R	S	R	R	R	*vanA*	A	+
5R[a]	>128	>128	32						*vanA*	A	+
6	>128	>128	64	R	S	R	R	R	*vanA*	A	+
6R[a]	>128	>128	64						*vanA*	A	–
7	>128	>128	32	R	S	R	R	R	*vanA*	A	+
7R[a]	>128	>128	32						*vanA*	A	+
8	>128	>128	64	R	S	R	R	R	*vanA*	A	+
8R[a]	>128	>128	32						*vanA*	A	+
9	>128	>128	32	R	S	R	R	R	*vanA*	A	+
10	>128	>128	32	R	S	R	R	R	*vanA*	A	+
11	>128	>128	32	R	S	R	R	R	*vanA*	A	+
12	>128	>128	32	R	S	R	R	R	*vanA*	B	+
13	2	>128	8	R	R	R	R	R	*vanA*	C	–
14	>128	>128	64	R	S	R	R	R	*vanA*	D	+
15	>128	>128	32	R	R	R	R	R	*vanA*	A	+
16	>128	>128	64	R	S	R	R	R	*vanA*	B	+

[a]Vancomycin-resistant *Enterococcus* spp. cultured from rectal swabs.
Abbreviations: I = intermediate resistance; R = resistant; S = susceptible.

Discussion

The spread of vancomycin resistance in enterococcal species has been reported to be via horizontal transfer of transposon [20], or clonal dissemination of an epidemic strain [21,22]. There are 6 mechanisms involved in vancomycin resistance, involving VanA to VanE and VanG [23,24]; VanA and VanB are the most common mechanisms. High-level resistance to vancomycin and teicoplanin is due to the *vanA* gene, which

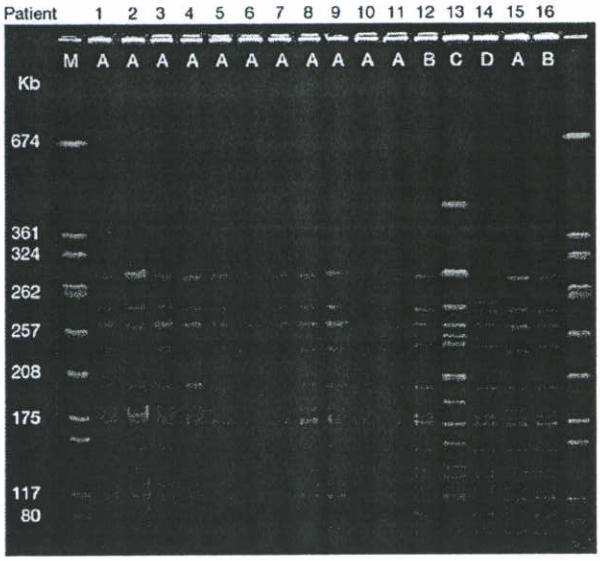

Fig. 1. Pulsed-field gel electrophoresis analysis revealed 4 types among 16 isolates of vancomycin-resistant *Enterococcus* spp.: pulsotypes A (12/75.00%), B (2/12.50%), C (1/6.25%), and D (1/6.25%).
Lane M = marker lane.

is usually located on transposon Tn1546 [25]. Resistance to vancomycin with susceptibility to teicoplanin is due to the *vanB* gene, which is usually located on transposon Tn1547 [26]. The mechanism of the *vanC* gene conferring low-level resistance to vancomycin is chromosome-located in less virulent enterococci such as *Enterococcus gallinarum*, *Enterococcus casseliflavus*, and *Enterococcus flavescens* [27]. Some VRE strains of the VanB phenotype with the *vanA* genotype have resulted from mutations in the *vanS* regulatory gene [28].

The first VRE isolated from a person in Taiwan was discovered in 1996 [29], after which VRE became endemic in some medical centers [18,30,31]. However, VRE with the VanB phenotype with the *vanA* genotype has been documented in both chickens and humans in Taiwan [32]. Hence, acquired VRE in people in Taiwan was not only hospital acquired, but also acquired in the community [30]. In 2007, the first nosocomial outbreak of VRE with the *vanA* gene occurred in Taipei Medical University, Wan Fan Hospital, due to the simultaneous existence of a clonal strain of *E. faecium*, combined with other multiple clones. Clonal spread of VRE occurred in the medical ICU, and the nephrology and respiratory wards, and polyclonal spread of VRE occurred in the surgical ICU, and the nephrology and respiratory wards. All hospital environments were screened for VRE colonization, but there were no positive VRE culture results. Meanwhile, infection control measures were implemented according to the recommendations of the Hospital Infection Control Practice Advisory Committee (HICPAC).

It is known that certain genotypes of VRE are prone to cause hospital outbreaks [30]. The ecologic success of these epidemic strains was due to the presence of the *esp* gene, encoding an enterococcal surface protein. Esp is important for biofilm formation and cell adherence on enterococcal pathogenesis [33]. This study found that all of the isolates belonged to the epidemic pulsotype A clone harboring the *esp* gene, which accounted for its clonal dissemination in the hospital. However, further study is required to elucidate whether horizontal transfer of transposon is responsible for the polyclonal VRE outbreak.

According to previous experience in Taiwan, VRE is difficult to eradicate [18,30]. Active surveillance and implementation of contact and cohort isolation could effectively control the VRE outbreak [30]. After adherence to the recommendations of HICPAC, VRE was eradicated from the medical ICU during a 3-month follow-up period, but was still encountered in other wards. Strict adherence to infection control strategies, active surveillance of health care personnel, and decreasing pressure of antibiotic use are important strategies for control of VRE outbreaks in the future.

References

1. Uttley AH, Collins CH, Naidoo J, George RC. Vancomycin-resistant enterococci. Lancet. 1988;1:57-8.
2. Grayson ML, Eliopoulos GM, Wennersten CB, Ruoff KL, De Girolami PC, Ferraro MJ, et al. Increasing resistance to beta-lactam antibiotics among clinical isolates of *Enterococcus faecium*: a 22-year review at one institution. Antimicrob Agents Chemother. 1991;35:2180-4.
3. Leclercq R, Derlot E, Duval J, Courvalin P. Plasmid-mediated resistance to vancomycin and teicoplanin in *Enterococcus faecium*. N Engl J Med. 1988;319:157-61.
4. National Nosocomial Infections Surveillance (NNIS) system report, data summary from January 1992-April 2000, issued June 2000. Am J Infect Control. 2000;28:429-48.
5. Richards C, Emori TG, Edwards J, Fridkin S, Tolson J, Gaynes R. Characteristics of hospitals and infection control professionals participating in the National Nosocomial Infections Surveillance System 1999. Am J Infect Control. 2001;29:400-3.
6. Edmond MB, Ober JF, Weinbaum DL, Pfaller MA, Hwang T, Sanford MD, et al. Vancomycin-resistant *Enterococcus*

faecium bacteremia: risk factors for infection. Clin Infect Dis. 1995;20:1126-33.

7. Aarestrup FM, Seyfarth AM, Emborg HD, Pedersen K, Hendriksen RS, Bager F. Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. Antimicrob Agents Chemother. 2001;45:2054-9.
8. van den Bogaard AE, Bruinsma N, Stobberingh EE. The effect of banning avoparcin on VRE carriage in The Netherlands. J Antimicrob Chemother. 2000;46:146-8.
9. Biavasco F, Miele A, Vignaroli C, Manso E, Lupidi R, Varaldo PE. Genotypic characterization of a nosocomial outbreak of VanA *Enterococcus faecalis*. Microb Drug Resist. 1996;2:231-7.
10. Torell E, Fredlund H, Törnquist E, Myhre EB, Sjöberg L, Sundsfjord A. Intrahospital spread of vancomycin-resistant *Enterococcus faecium* in Sweden. Scand J Infect Dis. 1997; 29:259-63.
11. Brown AR, Amyes SG, Paton R, Plant WD, Stevenson GM, Winney RJ, et al. Epidemiology and control of vancomycin-resistant enterococci (VRE) in a renal unit. J Hosp Infect. 1998;40:115-24.
12. Morris JG Jr, Shay DK, Hebden JN, McCarter RJ Jr, Perdue BE, Jarvis W, et al. Enterococci resistant to multiple antimicrobial agents, including vancomycin. Establishment of endemicity in a university medical center. Ann Intern Med. 1995;123:250-9.
13. Edmond MB, Ober JF, Dawson JD, Weinbaum DL, Wenzel RP. Vancomycin-resistant enterococcal bacteremia: natural history and attributable mortality. Clin Infect Dis. 1996;23:1234-9.
14. Stosor V, Peterson LR, Postelnick M, Noskin GA. *Enterococcus faecium* bacteremia: does vancomycin resistance make a difference? Arch Intern Med. 1998;158:522-7.
15. Carmeli Y, Eliopoulos G, Mozaffari E, Samore M. Health and economic outcomes of vancomycin-resistant enterococci. Arch Intern Med. 2002;162:2223-8.
16. Performance standards for antimicrobial susceptibility testing. Seventh informational supplement. CLSI document M100-S17. Wayne: Clinical and Laboratory Standards Institute; 2007.
17. Dutka-Malen S, Evers S, Courvalin P. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. J Clin Microbiol. 1995;33:1434.
18. Shankar V, Baghdayan AS, Huycke MM, Lindahl G, Gilmore MS. Infection-derived *Enterococcus faecalis* strains are enriched in *esp*, a gene encoding a novel surface protein. Infect Immun. 1999;67:193-200.
19. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol. 1995;33:2233-9.
20. Kawalec M, Gniadkowski M, Hryniewicz W. Outbreak of vancomycin-resistant enterococci in a hospital in Gdansk, Poland, due to horizontal transfer of different Tn1546-like transposon variants and clonal spread of several strains. J Clin Microbiol. 2000;38:3317-22.
21. Morrison D, Woodford N, Cookson BD. Epidemic vancomycin-resistant *Enterococcus faecium* in the UK. Clin Microbiol Infect. 1995;1:146-7.
22. Thal L, Donabedian S, Robinson-Dunn B, Chow JW, Dembry L, Clewell DB, et al. Molecular analysis of glycopeptide-resistant *Enterococcus faecium* isolates collected from Michigan hospitals over a 6-year period. J Clin Microbiol. 1998;36:3303-8.
23. Murray BE. Vancomycin-resistant enterococcal infections. N Engl J Med. 2000;342:710-21.
24. McKessar SJ, Berry AM, Bell JM, Turnidge JD, Paton JC. Genetic characterization of *vanG*, a novel vancomycin resistance locus of *Enterococcus faecalis*. Antimicrob Agents Chemother. 2000;44:3224-8.
25. Arthur M, Molinas C, Depardieu F, Courvalin P. Characterization of Tn1546, a Tn3-related transposon conferring glycopeptide resistance by synthesis of depsipeptide peptidoglycan precursors in *Enterococcus faecium* BM4147. J Bacteriol. 1993;175:117-27.
26. Quintiliani R Jr, Courvalin P. Characterization of Tn1547, a composite transposon flanked by the IS16 and IS256-like elements, that confers vancomycin resistance in *Enterococcus faecalis* BM4281. Gene. 1996;172:1-8.
27. Navarro F, Courvalin P. Analysis of genes encoding D-alanine-D-alanine ligase-related enzymes in *Enterococcus casseliflavus* and *Enterococcus flavescens*. Antimicrob Agents Chemother. 1994;38:1788-93.
28. Hashimoto Y, Tanimoto K, Ozawa Y, Murata T, Ike Y. 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. 2000;185:247-54.
29. Ben RJ, Lu JJ, Young TG, Chi WM, Wang CC, Chu ML, et al. Clinical isolation of vancomycin-resistant *Enterococcus faecalis* in Taiwan. J Formos Med Assoc. 1996;95: 946-9.
30. Wang JT, Chen YC, Chang SC, Chen ML, Pan HJ, Chang YY, et al. Control of vancomycin-resistant enterococci in a hospital: a five-year experience in a Taiwanese teaching hospital. J Hosp Infect. 2004;58:97-103.

31. Chang SC, Chen CH, Lu DC, Tai HM, Hsu KC, Lo SS. Vancomycin-resistant enterococci in north-eastern Taiwan. J Microbiol Immunol Infect. 1999;32:63-7. [Article in Chinese, English abstract.]
32. Lauderdale TL, McDonald LC, Shiau YR, Chen PC, Wang HY, Lai JF, et al. Vancomycin-resistant enterococci from humans and retail chickens in Taiwan with unique VanB phenotype-*vanA* genotype incongruence. Antimicrob Agents Chemother. 2002;46:525-7.
33. Heikens E, Bonten MJ, Willems RJ. Enterococcal surface protein Esp is important for biofilm formation of *Enterococcus faecium* E1162. J Bacteriol. 2007;189:8233-40.