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ORIGINAL ARTICLE

Identification of subclinical transmission of vancomycin-resistant enterococcus within an intensive care unit in Taiwan

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Abstract *Background/Purpose:* Colonization, infection, and clonal dissemination of vancomycin-resistant enterococcus (VRE) have been reported in the literature. We aimed to investigate the incidence rate of VRE acquisition and route of transmission of VRE within the medical intensive care unit (ICU) to prove whether subclinical transmission occurs in medical ICUs.
Methods: Between March 1, 2012 and September 30, 2013, rectal cultures were obtained from all inpatients on admission and after admission to medical ICU. Strain types of VRE were determined by both multilocus sequence typing and pulsed-field gel electrophoresis.
Results: A total of 66 of the 405 rectal swab surveillance cultures obtained from 46 inpatients were positive for VRE, among which 27 inpatients were culture-positive for VRE on admission to medical ICU, and 19 inpatients were initially culture-negative but converted to culture-positive after admission. All isolates carried *van*A gene consisting of 51 *Enterococcus gallinarum*, 13 *Enterococcus faecium*, and two *Eenterococcus casseliflavus*. Of the 51 *E. gallinarum* isolates, 40 were type ST 341, seven were ST 252, two were ST 78, and two were ST 64. The *Enterococcus* spp., MLST and PFGE subtypes were almost similar among these two groups of inpatients. Linezolid and tigecycline were most active against VRE *in vitro*.

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Conclusion: Subclinical VRE cross transmission may occur in ICU. Active surveillance and maximal barrier precautions of VRE are required at ICU with high colonization rate of VRE and shall be beneficial.

Background

Vancomycin-resistant enterococci (VRE) colonization and infection have been reported worldwide, particularly among hemodialysis patients and intensive care unit (ICU) patients[1–5], causing unexpected hospital outbreaks.[6–11] As nosocomial infections due to VRE are usually preceded by colonization and is an international problem, it is necessary to investigate, especially amongst the ICU patients, VRE colonization rate, sequence types, and the route of transmission to prove whether subclinical transmission occurs in ICU.[6–12] Effective measures to control VRE in ICU such as maximal barrier precautions can therefore be established.[8] We performed an active surveillance culture with rectal swabs for every inpatient on admission and after admission to our ICU during the 1 year and 8 months period. Environmental culture for VRE in the ICU was also performed. All VRE isolates from the ICU inpatients and environment were studied with molecular typing to investigate the epidemiology and route of transmission of VRE in the ICU. Genotypes and antibiotic susceptibility of the VRE isolates were also conducted.

Methods

Setting and study design

Chang Gung Memorial Hospital at Keelung in Taiwan is a 1088-bed tertiary-care teaching hospital with ~50,000 admissions per year. Between March 1, 2012 and September 30, 2013, we carried out a surveillance study of VRE amongst patients upon admission to medical ICU (MICU) which cared for both critical cardiac and respiratory failure patients with 20 beds and ~500 admissions per year. All strains of VRE isolated from surveillance rectal culture from MICU patients were collected and stored for further epidemiological and antibiotics susceptibility study. Clinical isolates of VRE causing symptomatic infections in ICU during this period were also collected and stored for further epidemiological study. This study was performed after the research plan was approved by the Committee of Medical Research and the Committee of Human Trials and Medical Ethics of our hospital Chang Gung Memorial Hospital, Keelung (CMRPG2B0261-2 and IRB 100-3609B). Written informed consents were obtained from all participants in the study.

Surveillance rectal swab cultures for VRE from patients of medical ICU

Critical patients in all rooms of the medical ICU from March 1, 2012 and September 30, 2013 excluding those with acute myocardial ischemia, acute myocardial infarctions in whom rectal swabs were contraindicated and those unwilling to accept rectal swab culture were included in this study. Screening with baseline rectal surveillance cultures for VRE were performed within 72 hours of admission to medical ICU after receiving patients' consent and repeated screening at 5–7-day intervals until transfer to general wards or expiry in ICU. All rectal swabs were inoculated on to blood agar for further analysis of VRE.[13]

Infection control policy

All rooms in our medical ICU were single patient rooms with routine cleaning. Every nurse cared for only two patients in two single rooms in medical ICU. Once VRE was isolated from any ICU patient, the head nurse of ICU was notified and barrier precautions including gloves and gowns were performed appropriately. Routine environmental cleaning of these rooms were also intensified.[8–11]

Surveillance culture of environment for VRE to detect contamination

Cultures for VRE were also performed from the environment, including bedrails, ventilator monitor surface, ventilator tubing, electrocardiogram (EKG) monitor surface, emergency button, inside room desk surface, and interroom nurse desk surface for onethird of the rooms rotating every 2–3 months at the medical ICU to detect VRE contamination.

Analysis of VRE

All rectal swabs were inoculated onto blood agar. Plates were examined after 48 hours of incubation at 36°C. Colonies were identified as *Enterococcus* spp. by matching with the characteristics of enterococcus, such as Gram-positive cocci, no inhibition by optochin, changing bile-esculin to black color, and growth in 6.5% NaCl. *Enterococcus* spp. were classified to specific species by differential utilization of arginine, sorbitol, arabinose, raffinose, and the rapid 32 Strep kit test (BioMerieux Vitek Inc., Hazelwood, MO, USA). Vancomycin-resistant enterococcus were confirmed by growth in brain heart infusion agar containing 6 ug/mL vancomycin.[13–16] Antibiotic susceptibility was tested by Etest methods.[17]

Genotype analysis

Isolates of VRE were typed with multilocus sequence typing (MLST) using seven primers followed by gene sequence

Table 1 Basic data of VRE surveillance cultures isolates, molecular types and antibiotic susceptibility from patients on and after admission to MICU excluding six duplicate isolates.[a]

Total SC / patients No./+ SC/+ patients No.	VRE spp. (*n*)	*van*A gene	MLST type (*N*)	PFGE(*N*)	MIC ug/mL (mean/range/susceptibility %)							
					Van	Teic	Fusi	Mup	Line	TS[b]	Tige	Dapt
405/248/60/46	*Enterococcus gallinarum* (45)	(+)	341(34)	A4(10)	> 256	316.4/12—>256	1.98/0.25—3	0.43/0.19—1	0.65/0.19—1.5	> 32	0.63/0.125—0.94	4.12/0.38—24
				A3(10)	> 256	322.8/12—>256	2.09/0.125—3	0.43/0.125—0.75	0.93/<0.016—2	57.6/0.032—> 32	0.33/0.064—0.94	2.23/0.75—3
				B1(3)	> 256	> 256	2.67/2—3	0.58/0.5—0.75	0.83/0.75—1	> 32	0.47	2.31/0.94—3
				C3(5)	> 256	208.8/6—>256	1/0.25—3	0.55/0.5—0.75	1.8/1—3	> 32	0.48/0.094—2	3.6/2—6
				C1(1)	> 256	8	2	0.38	2	> 32	0.064	3
				D1(5)	> 256	7.2/6—12	0.5/0.25—0.75	0.48/0.38—0.5	2.1/1.5—3	> 32	0.11/0.094—0.125	3.8/3—4
			252(7)	A1(2)	> 256	268/24—> 256	1.69/0.38—3	0.63/0.5—0.75	1.75/1.5—2	> 32	0.5/0.064—0.94	3.5/3—4
				A2(1)	> 256	16	3	0.38	2	> 32	0.064	0.25
				A3(1)	> 256	> 256	2	32	1	> 32	0.64	4
				D1(1)	> 256	32	0.38	0.75	3	> 32	0.064	3
				D2(1)	> 256	8	0.75	0.5	1.5	> 32	0.125	4
				D3(1)	> 256	32	3	0.5	1.5	> 32	0.094	3
			78(2)	A4(2)	> 256	> 256	3	0.69/0.38—1	256.5/1—> 256	> 32	0.94	1.47/0.94—2
			64(2)	A3(1)	> 256	> 256	0.25	0.75	0.75	> 32	6	2
				D1(1)	> 256	24	0.5	0.5	1.5	0.047	0.064	2
			ALL	> 256/> 256/0	249.2/6—>256/20	1.73/0.125—3/37.8	1.2/0.125—32/97.8	12.6/<0.016—> 256/97.8	61.2/0.032—>32/4.44	0.51/0.064—6/97.8	3.08/0.13—24/93.3	
	Enterococcus faecium (13)	(+)	341(10)	A1(1)	> 256	24	3	0.5	2	> 32	0.094	2
				A4(1)	> 256	12	1.5	0.125	0.19	12	0.125	0.38
				B1(1)	> 256	> 256	3	0.5	0.75	> 32	0.47	3
				C3(2)	> 256	19/6—32	0.44/0.38—0.5	0.44/0.38—0.5	1.75/1.5—2	32.02/0.032—>32	0.16/0.064—0.25	3
				C2(1)	> 256	16	2	0.38	1.5	0.094	0.064	3
				D1(4)	> 256	8.5/6—12	0.85/0.38—2	0.44/0.38—0.5	1.88/1.5—2	> 32	0.12/0.094—0.125	4.5/4—6
			252(2)	A4(1)	> 256	32	0.38	0.5	3	> 32	0.064	4
				D1(1)	> 256	> 256	1.5	0.5	2	0.032	0.064	3
			64(1)	A3(1)	> 256	12	3	0.5	1.5	> 32	0.125	4
			ALL	> 256/	91.7/6—	1.43/	0.43/	1.69/	45.2/0.032—	0.14/	3.34/	

(*continued on next page*)

Table 1 (*continued*)

Total SC / patients No./+ SC/+ patients No.	VRE spp. (*n*)	*van*A gene	MLST type (*N*)	PFGE(*N*)	MIC ug/mL (mean/range/susceptibility %)						
					Van	Teic	Fusi	Mup	Line	TS[b]	Tige	Dapt
				> 256/0	> 256/ 30.8	0.38–3/46.2	0.13–0.5/100	0.19–3/92.3	> 32/23.1	0.064–0.47/ 100	0.38–6/92.3	
	Enterococcus casseliflavus (2)	(+)	341(2)	A4(1)	> 256	> 256	1.5	1	0.5	> 32	0.32	1
				D1(1)	> 256	8	0.25	0.5	1.5	> 32	0.125	4
			ALL	512/ > 256/0	260/8– > 256/50	0.88/ 0.25–1.5/50	0.75/0.5–1 100	1/0.5–1.5/100	64/ > 32/0	0.22/ 0.13–0.32/ 100	2.5/1–4/ 100	

[a] Duplicate isolates: identical VRE spp., MLST, and PFGE subtypes from same inpatients of ICU.
[b] MICs of sulfamethoxazole.
Dapt = daptomycin; Fusi = fusidic acid; Line = linezolid; MIC = Minimal inhibitory concentrations; MICU = medical intensive care unit; MLST = multilocus sequence typing; Mup = mupirocin; PFGE = pulsed-field gel electrophoresis; SC = surveillance culture; Teic = teicoplanin; TS = trimethoprim/sulfamethoxazole; Van = vancomycin; VRE = vancomycin-resistant enterococcus.

analysis of the seven housekeeping genes amplified.[18–20] Methods of MLST and primers for polymerase chain reaction (PCR) were based on published literature.[19,20] We also typed all VRE isolates with pulsed-field gel electrophoresis (PFGE).[21–24] Genomic DNA was digested with *Sma*I and subjected to PFGE. We used the criteria for analysis of genomic DNA previously described.[23,24] Isolates were judged to be distinct when their pulsed-field gel electrophoresis profiles differed by more than three bands.[24] For an additional epidemiological marker, we investigated the presence of *van*A, *van*B, *van*C1, or *van*C2 genes by PCR. Primers for PCR were based on published gene sequences for *Enterococcus faecalis*, *Enterococcus faecium*, and *Enterococcus gallinarum*.[15,16]

Antibiotics susceptibility

Antimicrobial susceptibility was evaluated by Etest according to the guidelines of the Clinical and Laboratory Standards Institute.[17] Minimal inhibitory concentrations (MICs) of the eight antimicrobial agents, including daptomycin (Cubist Pharmaceuticals, Lexington, MA, USA), fusidic acid (Leo, London, UK), linezolid (Pfizer, New York City, USA), mupirocin (GlaxoSmithKline, Brentford, London, UK), teicoplanin (Sanofi-Aventis, Gentilly, France), tigecycline (Pfizer) trimethoprim/sulfamethoxazole (Sandoz, Geneva, Switzerland), and vancomycin (Eli Lilly, Indianapolis, Indiana, USA) for the VRE isolates were determined by Etest (AB Biodisk, Solna, Sweden). The tested antibiotics and their concentration ranges were: daptomycin (0.002–32 μg/mL), fusidic acid (0.016–256 μg/mL), linezolid (0.016–256 μg/mL), mupirocin (0.064–1024 μg/mL), teicoplanin (0.016–256 μg/mL), tigecycline (0.016–256 μg/mL), trimethoprim/sulfamethoxazole (0.002–32 μg/mL), and vancomycin (0.016–256 μg/mL). The MIC breakpoints of susceptibility of the five among these eight antimicrobial agents were: daptomycin (≤ 4 μg/mL), linezolid (≤2 μg/mL), teicoplanin (≤ 8 μg/mL), tigecycline (≤ 2 μg/mL, proposed breakpoint from the FDA), and vancomycin (≤ 4 μg/mL) for VRE according to CLSI M100-S22.[17] There were no approved MIC breakpoints of fusidic acid, mupirocin, and trimethoprim/sulfamethoxazole for VRE. In *Staphylococcus aureus*, the MIC breakpoints of susceptibility for fusidic acid, mupirocin, and trimethoprim/sulfamethoxazole were ≤ 0.5 μg/mL, ≤ 2 μg/mL, and ≤2/38 μg/mL respectively.[25,26] *S. aureus* MIC breakpoints of susceptibility for fusidic acid, mupirocin, and trimethoprim/sulfamethoxazole were used for VRE in this study. The following organism with acceptable MICs (μg/mL) limits were included as control strains according to CLSI M100-S22 published in January 2012: *Enterococcus faecalis* ATCC 29212 (1–4 for daptomycin, 1–4 for linezolid, 0.125–0.5 for teicoplanin, 0.03–0.12 for tigecycline, and 1–4 for vancomycin).

Results

Between March 1, 2012 and September 30, 2013, there were 710 critical inpatients admitted to medical ICU and 248 of these 710 inpatients that fit the inclusion criteria were included in this study. A total of 462 critical inpatients were excluded from this study due to acute myocardial

ischemia, acute myocardial infarction, and because they were unwilling to accept rectal swab culture. As shown in Table 1 for surveillance purpose, 405 rectal swabs were collected and cultured from these 248 inpatients on and after admission to our MICU. A total of 66 of these 405 rectal swab cultures were positive for VRE in 46 inpatients on and after admission to ICU. Of these 66 VRE isolates, six were duplicate isolates (identical VRE strain, MLST, and PFGE subtypes from the same inpatient; Table 1). After removing six duplicate clinical isolates, basic data of 60 nonduplicate VRE isolates are shown in Table 1. The epidemiological link of VRE in inpatients on and after admission to ICU are shown in Figure 1. All VRE isolates carried the *van*A gene (45 *E. gallinarum*, 13 *E. faecium*, and 2 *E. casseliflavus* isolates). Of the 45 *E. gallinarum* isolates, 34 were type ST 341, seven were ST 252, two were ST 78, and two were ST 64 (Tables 1 and 3). The PFGE subtypes of these *E. gallinarum* are shown in Table 1 and Figure 2. Of the 13 *E. faecium* isolates, 10 were ST 341, two were ST 252, and one was ST 64. The PFGE subtypes of these *E. faecium* isolates are shown in Table 1 and Figure 2. The two *E. casseliflavus* isolates belonged to ST 341 (Table 1). Seven ICU inpatients acquired more than one VRE strainson admission to ICU and five inpatients acquired more than one VRE strain after admission to ICU. Although identical VRE strains with the same MLST were repeatedly isolated from the same ICU inpatients, their PFGE subtypes were sometimes different.

A total of 405 rectal swab surveillance cultures were collected and cultured from 248 inpatients on admission to ICU (Table 2). A group of 27 of these 248 inpatients (10.9%) were culture-positive for VRE on admission to ICU (Table 2). There were 37 VRE isolates obtained from these 27 inpatients on and after admission to ICU (Table 2). All carried the *van*A gene (28 *E. gallinarum* and 6 *E. faecium*). Of these 28 *E. gallinarum* isolates, 22 were type ST 341, five were ST 252 and one was ST 64 (Table 2). The PFGE subtypes of *E. gallinarum* isolates are shown in Table 2. Of the six *E. faecium* isolates obtained on admission, five were ST 341 and one was ST 252 (Table 2). The PFGE subtypes of *E. faecium* isolates are shown in Table 2. Among the 248 inpatients admitted to ICU, 221 patients were culture-negative for VRE on admission to ICU. Of these 221 inpatients that were culture-negative for VRE on admission to ICU, follow-up surveillance rectal swab cultures once every week were performed and 19 converted to culture-positive for VRE at least once after admission to ICU. The shortest interval from VRE culture-negative to VRE culture-positive was 6 days, the longest interval was 22 days, and the mean was 10.6 days. There were 26 VRE isolates obtained from these 19 inpatients after admission to ICU. All carried the *van*A gene (17 *E. gallinarum*, 7 *E. faecium*, and 2 *E. casseliflavus* isolates). Of these 17 *E. gallinarum* isolates, 12 were type ST 341, two ST 252, two ST 78, and one ST 64 (Table 2). The PFGE subtypes of these *E. gallinarum* isolates are shown in Table 2. Of these seven *E. faecium*

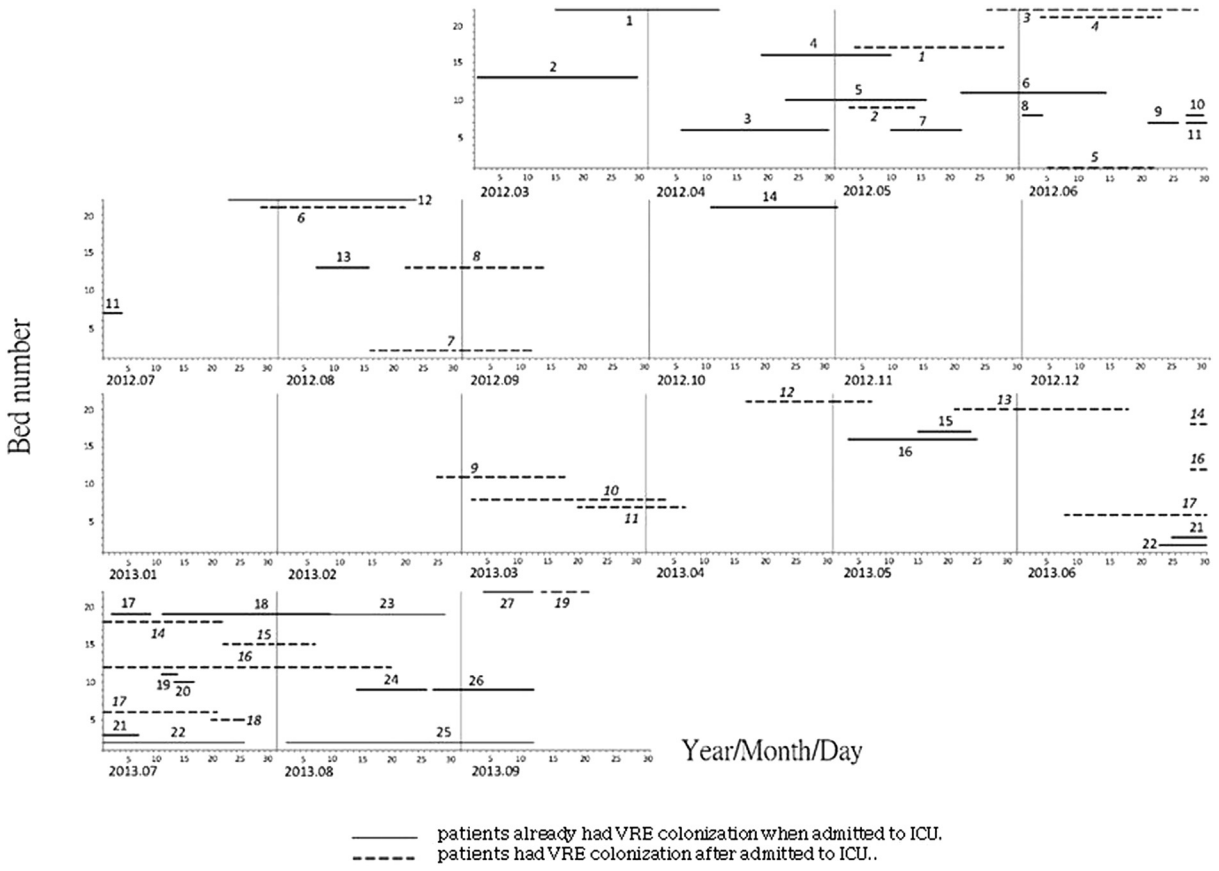

Figure 1. Vancomycin-resistant *Enterococcus* (VRE) colonization in the intensive care unit (ICU) of Keelung Chang Gung Hospital, Taiwan, 2012—2013.

Table 2 Comparison of basic data of VRE surveillance cultures isolates, molecular types, and antibiotic susceptibility from MICU patients on and after admission to MICU excluding six duplicate isolates.[a]

On admission to MICU									
Total patients no. /positive patients no.	VRE spp (*n*).	*van*A gene	MLST type (N)	PFGE (N)	MIC ug/mL (mean/range)				
					Fusi	Mup	Line	Tige	Dapt
248/27 [b]	*Enterococcus gallinarum* (28)	(+)	341(22)	A4(8)	1.84/0.25–3	0.41/0.19–1	0.56/0.19–1	0.65/0.125–0.94	1.48/0.38–3
				A3(6)	1.81/0.125–3	0.4/0.125–0.75	0.74/<0.016–2	0.38/0.064–0.94	2.04/0.75–3
				B1(1)	2	0.75	1	0.47	0.94
				C3(4)	0.5/0.25–0.75	0.56/0.5–0.75	2/1.5–3	0.58/0.094–2	4/2–6
				C1(1)	2	0.38	2	0.064	3
				D1(2)	0.5	0.44/0.38–0.5	1.75/1.5–2	0.1/0.094–0.125	3.5/3–4
			252(5)	A1(1)	3	0.5	2	0.064	3
				A3(1)	2	32	1	0.64	4
				D1(1)	0.38	0.75	3	0.064	3
				D2(1)	0.75	0.5	1.5	0.125	4
				D3(1)	3	0.5	1.5	0.094	3
			64(1)	A3(1)	0.25	0.75	0.75	6	2
	Enterococcus faecium (6)	(+)	341(5)	A4(1)	1.5	0.125	0.19	0.125	0.38
				C3(2)	0.44/0.38–0.5	0.44/0.38–0.5	1.75/1.5–2	0.16/0.064–0.25	3
				C2(1)	2	0.38	1.5	0.064	3
				D1(1)	2	0.38	2	0.094	4
			252(1)	D1(1)	1.5	0.5	2	0.064	3
Patients culture-negative on admission									
Total patients No. [c] /+ patients No.[d]	VRE spp (*n*).	*vanA gene*	MLST type (N)	PFGE (N)	MIC ug/mL (mean/range)				
					Fusi	Mup	Line	Tige	Dapt
221/19[e]	*Enterococcus gallinarum* (17)	(+)	341(12)	A3(4)	2.5/1–3	0.47/0.5–0.38	1.22/0.38–2	0.25/0.064–0.64	2.5/1–3
				A4(2)	2.5/2–3	0.5	1/0.5–1.5	0.55/0.47–0.64	15/6–24
				B1(2)	3	0.5	0.75	0.47	3
				C3(1)	3	0.5	1	0.094	2
				D1(3)	0.5/0.25–0.75	0.5	2	0.11/0.094–0.125	4
			78(2)	A4(2)	3	0.69/0.38–1	256.5/1–>256	0.94	1.47/0.94–2

		252(2)	A1(1)	0.38	0.75	1.5	0.94	4	
			A2(1)	3	0.38	2	0.064	0.25	
		64(1)	D1(1)	0.5	0.5	1.5	0.064	2	
Enterococcus faecium (7)	(+)	341(5)	A1(1)	3	0.5	2	0.094	2	
			B1(1)	3	0.5	0.75	0.47	3	
			D1(3)	0.46/0.38 −0.5	0.46/0.38 −0.5	1.83/1.5–2	0.125	4.67/4–6	
		252(1)	A4(1)	0.38	0.5	3	0.064	4	
		64(1)	A3(1)	3	0.5	1.5	0.125	4	
Enterococcus casseliflavus (2)	(+)	341(2)	A4(1)	1.5	1	0.5	0.32	1	
			D1(1)	0.25	0.5	1.5	0.125	4	

[a] Duplicate isolates: identical VRE spp., MLST and PFGE subtypes from same inpatients of ICU.
[b] 19 positive × 1, six positive × 2, two positive × 3.
[c] Total patient no. culture-negative on admission.
[d] Total patient no. culture-negative on admission changed to culture-positive after admission to MICU.
[e] 13 positive × 1 , two positive × 2, four positive × 3.
Dapt = daptomycin; Fusi = fusidic acid; Line = linezolid; MIC = Minimal inhibitory concentrations; MICU = medical intensive care unit; MLST = multilocus sequence typing; Mup = mupirocin; PFGE = pulsed-field gel electrophoresis; Tige = tigecycline; VRE = vancomycin-resistant enterococcus.

isolates, five were type ST 341, one was ST 252, and one was ST 64 (Table 2). The two *E. casseliflavus* isolates belonged to ST 341 (Table 2). Those inpatients that were VRE(−) on admission to ICU and converted to VRE(+) after admission to ICU were closed to those inpatients that were VRE(+) on admission to ICU at the same time and mostly were cared for by the same nurses (Figure 1).

Between March 1, 2012 and September 30, 2013, there were seven clinically overt infections in seven inpatients caused by seven VRE isolates of which two were bacteremia and five were symptomatic urinary tract infections. Of these seven cases with VRE infection, four VRE infections were likely present on admission to ICU and the remaining three VRE infections were more likely acquired in the ICU because these three VRE were isolated > 48 hours after admission to ICU (Table 4). One of the two blood VRE isolates belonged to *E. faecalis* ST 414 and the other, *E. faecium* ST 341 which was catheter-related. Both of the two bacteremia cases due to VRE were treated with daptomycin that were active *in vitro* against VRE but ultimately died of sepsis despite intensive treatment. Of the other five clinical VRE isolates, three were *E. gallinarum* ST 341 and two were *E. faecium* ST 341. These five urinary tract infections caused by VRE were not treated with any VRE-active antibiotic such as daptomycin or linezolid but two cases expired due to other causes and the other three cases remained stable.

There were 22 single rooms at the MICU of this study. We performed the environmental cultures of these rooms seven times, including surface of bedrails, ventilator monitor, ventilator tubing, EKG monitor, emergency button, inside room desk surface, and interroom nurse desk surface. Environmental cultures for VRE were positive on two surfaces. One VRE isolate, *E. gallinarum*, belonging to ST 341 was isolated from the surface of a nurse desk situated between two rooms. One methicillin-resistant *S. aureus* isolate (MLST 239, SCC*mec* II) was also obtained from this site. Another VRE isolate belonging to ST 341 was isolated from the desk surface inside a single room in the MICU.

Of the 45 *E. gallinarum*, 13 *E. faecium*, and two *E. casseliflavus* isolates, all the MICs of vancomycin were over > 256 ug/mL. The range/mean of MICs and susceptibility rate of teicoplanin, fusidic acid, mupirocin, linezolid, trimethoprim/sulfamethoxazole, tigecycline, and daptomycin of these 60 VRE isolates are described in Tables 1 and 2.

Discussion

From our prior study, there was a close relationship between VRE colonization and VRE symptomatic infections; similar ST types (414, 78, 18, and 341) and PFGE types (A, C, D, and E) were identified in patients both asymptomatic for and clinically manifested VRE.[6] This prior study indicated that infection control policies for VRE would not be successful when it included clinically manifested VRE infections while excluding asymptomatic VRE colonization. Moreover, if the infection control policy could include asymptomatic VRE colonization, an active VRE surveillance would then be required.

This current study evaluated the VRE colonization rate, sequence types, and the route of transmission of VRE to

Table 3 Multilocus sequence types of 66 vancomycin-resistant *Enterococcus* isolates.

Serial No.	Strain	ST [a]	Assigned No. for allele						
			AtpA	Ddl	Gdh	PurK	Gyd	PstS	Adk
1	1051	341	15	5	1	1	1	1	1
2	1053	341	15	5	1	1	1	1	1
3	1055	341	15	5	1	1	1	1	1
4	1057	341	15	5	1	1	1	1	1
5	1059	341	15	5	1	1	1	1	1
6	1060	341	15	5	1	1	1	1	1
7	1062	341	15	5	1	1	1	1	1
8	1063	341	15	5	1	1	1	1	1
9	1068	78	15	1	1	1	1	1	1
10	1069	78	15	1	1	1	1	1	1
11	1071	341	15	5	1	1	1	1	1
12	1073	341	15	5	1	1	1	1	1
13	1075	341	15	5	1	1	1	1	1
14	1077	341	15	5	1	1	1	1	1
15	1082	341	15	5	1	1	1	1	1
16	1083	341	15	5	1	1	1	1	1
17	1084	341	15	5	1	1	1	1	1
18	1086	341	15	5	1	1	1	1	1
19	1089	341	15	5	1	1	1	1	1
20	1090	252	1	5	1	1	1	1	1
21	1092	341	15	5	1	1	1	1	1
22	1093	341	15	5	1	1	1	1	1
23	1095	341	15	5	1	1	1	1	1
24	1096	341	15	5	1	1	1	1	1
25	1097	341	15	5	1	1	1	1	1
26	1103	341	15	5	1	1	1	1	1
27	1117	252	1	5	1	1	1	1	1
28	1118	64	7	1	1	1	1	1	1
29	1121	341	15	5	1	1	1	1	1
30	1122	341	15	5	1	1	1	1	1
31	1124	341	15	5	1	1	1	1	1
32	1130	341	15	5	1	1	1	1	1
33	2004	341	15	5	1	1	1	1	1
34	2005	341	15	5	1	1	1	1	1
35	2011	252	1	5	1	1	1	1	1
36	2015	341	15	5	1	1	1	1	1
37	2017	252	1	5	1	1	1	1	1
38	2019	252	1	5	1	1	1	1	1
39	2020	341	15	5	1	1	1	1	1
40	2021	341	15	5	1	1	1	1	1
41	2022	341	15	5	1	1	1	1	1
42	2023	341	15	5	1	1	1	1	1
43	2024	341	15	5	1	1	1	1	1
44	2025	341	15	5	1	1	1	1	1
45	2026	341	15	5	1	1	1	1	1
46	2027	341	15	5	1	1	1	1	1
47	2028	341	15	5	1	1	1	1	1
48	2029	341	15	5	1	1	1	1	1
39	2031	341	15	5	1	1	1	1	1
50	2032	252	1	5	1	1	1	1	1
51	2034	341	15	5	1	1	1	1	1
52	2035	341	15	5	1	1	1	1	1
53	2036	341	15	5	1	1	1	1	1
54	2037	341	15	5	1	1	1	1	1
55	2038	64	7	1	1	1	1	1	1
56	2040	341	15	5	1	1	1	1	1
57	2041	252	1	5	1	1	1	1	1
58	2042	64	7	1	1	1	1	1	1
59	2045	341	15	5	1	1	1	1	1
60	2046	252	1	5	1	1	1	1	1
61	2047	341	15	5	1	1	1	1	1
62	2050	341	15	5	1	1	1	1	1
63	2051	252	1	5	1	1	1	1	1
64	2052	341	15	5	1	1	1	1	1
65	2053	341	15	5	1	1	1	1	1
66	2054	341	15	5	1	1	1	1	1

[a] Web site for VRE MLST analysis: http://efaecium.mlst.net/sql/singlelocus.asp.

MLST = multilocus sequence typing; ST = sequence type; VRE = vancomycin-resistant enterococcus.

prove whether subclinical transmission of VRE occurs in the medical ICU by active surveillance. Of the 248 critical inpatients admitted to the ICU, 27 patients (10.9%) were culture-positive for VRE on admission to ICU (Table 2). A total 10.9% (27/248) VRE colonization rate among inpatients on admission to ICU was unexpectedly high. These VRE isolates from inpatients on admission to ICU might be acquired during stay in the Internal Medicine general ward or already existing in the patient's intestinal tract on admission to the hospital. VRE positive rates among patients were different between different general wards and between different hospitals.[6,10,18,19] Although all the rooms in the ICU were single patient rooms, with one nurse taking care of only two inpatients in the two rooms, cross transmission of VRE via contaminated environment may still occur if maximal barrier precautions were not performed in the ICU with a high VRE colonization rate. Of the 221 critical inpatients culture-negative for VRE on admission to ICU in this study, 19 inpatients converted to culture-positive for VRE after admission to ICU. As the VRE spp., MLST and PFGE subtypes were almost identical among these two groups of inpatients except one ST 64 *E. faecium* and two ST 34 *E. casseliflavus* identified after admission to ICU, it was highly possible that these 19 inpatients culture-negative for VRE on admission to ICU acquired the VRE via cross transmission from those inpatients already culture-positive for VRE on admission to ICU (Table 2). As inpatients VRE culture-negative on admission to ICU become VRE culture-positive after admission to ICU were closed to inpatients VRE culture-positive on admission to ICU at the same time as shown in Figure 1, we could presume that subclinical transmission of VRE occurred in the ICU. This hypothesis was further supported by the discovery of two VRE isolates, one from an inside-room desk surface and the other from an interroom nurse desk surface. Both environment VRE isolates were *E. gallinarum* belonging to ST 341, identical to those of the inpatients at the ICU (Table 3). The reason why one ST 64 *E. faecium* and two ST 34 *E. casseliflavus* were not discovered on admission to ICU was not clear. It is possible that those ICU inpatients that did not meet the

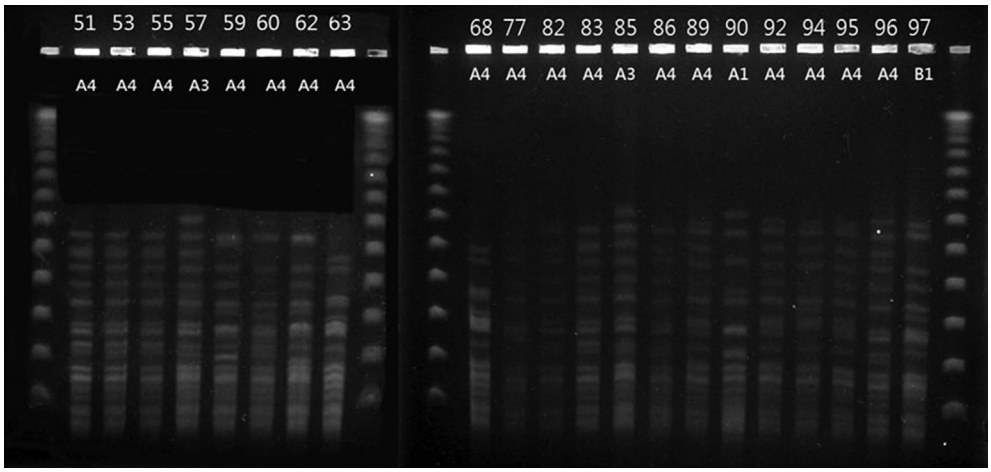

Figure 2. Pulsed-field gel electrophoresis types of 21 isolates from 66 vancomycin resistant *Enterococcus* isolates.

Table 4 Basic data of seven clinically overt VRE infections in ICU.

No.	Age (y)	Sex M/F	Specimen/ isolation day[a]	VRE spp.	Underlying diseases	APACHE III score[b]	Antibiotics active to VRE[c]	Outcome C/E/DOD/ COD
1	56	M	B/1	*Enterococcus faecium*	Traumatic ICH, hemothorax	25	Daptomycin × 20 d	E/22/septic shock, DIC
2	83	F	B/19	*Enterococcus faecalis*	CHF, IHD, CKD, DM	17	Daptomycin × 7 d	E/28/nosocomial pneumonia, septic shock
3	77	F	U/2	*Enterococcus gallinarum*	CVD, CKD, bed-ridden	24	Nil	C/24
4	99	F	U/1	*E. gallinarum*	Cervical cancer with liver, adrenal gland, & bone metastasis, after CPR	50	Nil	E/2/cardiac arrest
5	87	F	U/32	*E. gallinarum*	CHF, pneumoconiosis	14	Nil	E/28/nosocomial pneumonia, septic shock
6	86	F	U/19	*E. faecium*	CVD, CKD, bed-ridden	21	Nil	C/21
7	70	F	U/1	*E. faecium*	CVD	21	Nil	C/6

[a] Days after admission to ICU when isolation of VRE was performed.
[b] APACHE (Acute Physiology, Age, Chronic Health Evaluation) III score[28] on VRE culture (+) day.
[c] Antibiotics active to VRE indicate daptomycin or linezolid.
C = cured; CHF = congestive heart failure; CKD = chronic kidney disease; COD = cause of death; CPR = cardiopulmonary resuscitation; CVD = cerebrovascular disease; DIC = disseminated intravascular coagulation; DM = diabetes mellitus; DOD = Days after admission to ICU of discharge or expiry; E = expired; F = female; ICH = intracerebral hemorrhage; ICU = intensive care unit; IHD = ischemic heart disease; M = male; U = urine; VRE = vancomycin-resistant enterococcus.

inclusion criteria might carry these VRE ST strains on admission to ICU, causing a leak in active surveillance of inpatients on admission to ICU. As the VRE spp. and MLST were similar among inpatients with clinical overt infections due to VRE and inpatients being colonized by VRE without overt infection, it is highly possible that the eight inpatients with clinical overt VRE infections acquired the VRE via cross transmission from those inpatients already culture-positive for VRE on admission to ICU (Table 2).

Cross transmission via the contaminated environment and medical personnel are noted as the most possible route of transmission of VRE at the ICU in this study. According to our experience, it is very difficult to isolate VRE from the hands of medical personnel because VRE only stay on the hands of medical personnel transiently, unlike the environment surface on which the VRE can survive for a long time.[13] It is known, VRE are easily washed off the hands of medical personnel during hand washing especially under investigation. Moreover, some medical personnel may refuse to cooperate with hand VRE culture for research purposes when there is no established outbreak of healthcare-associated infections. Based on these two reasons, hand culture of medical personnel was not performed in this study. However, we can still infer from the results of this study that VRE were transmitted via the contaminated environment and the hands of medical personnel because

VR-*E. gallinarum* MLST 341 that were identical to those VRE isolated from culture (+) inpatients on and after admission to ICU were isolated only from the desk surface where the nurses touched frequently during daily regular work. VR-*Enterococcus faecium* might also exist the ICU environment and was not discovered because surveillance cultures of environments for VRE to detect contamination were performed only on certain high risk sites every 2—3 months. After notification of positive desk surface environment cultures, these surfaces were disinfected more frequently every day and subsequent environment cultures of these desk surfaces became negative. No VRE was isolated from the surface of medical devices, indicating that it was unlikely that VRE were transmitted via contaminated medical devices. In the MICU, medical devices were disinfected regularly according to approved methods. Although the culture positive rate of the environment was low, two among seven kinds of surfaces that were most possibly contaminated by the hands of patients or medical personnel were positive.

Cho et al[18] in Korea reported VR-*E. faecium* ST 192, ST 78, ST17, and ST 414 (highest to lowest frequency) in 2011. Lee et al[6] in Taiwan discovered VR-*E. faecium* ST 18 and ST 414 were the most predominant ST types in bacteremia caused by VRE between 2009 and 2010.[18] Our prior study in 2013 also discovered VR-*E. faecium* ST 414 and ST 18 were the two most predominant ST types in our nephrology ward.[6] VR-*E. faecium* ST 341 and ST 78 were also present in this prior study but comprised only a minority. However, in this study, the predominant VRE strain and type are VR-*E. gallinarum* ST 341 and ST 252 that had not to our knowledge been reported in Taiwan. VR-*E. gallinarum* and VR-*E. casseliflavus* were not found in the prior study in our hospital. These data indicated that multiple strains of VRE exist in our hospital and any strain of VRE can cause an outbreak if strict infection control policies are not followed and practiced.

From our antimicrobial susceptibility study of VRE, we found that all VRE isolates were resistant to teicoplanin compatible with *van*A genotype. There were high resistance rates of fusidic acid and trimethoprim/sulfamethoxazole in VRE isolates when MICs breakpoint for *S. aureus* were used for VRE (Table 1). All VRE isolates were susceptible to mupirocin and linezolid which can be used clinically to treat infections caused by VRE. Although there was resistance to daptomycin in some VRE isolates, the resistant rate was not high, indicating that daptomycin may be considered to treat infections caused by VRE. Although there was no resistance to tigecycline in VRE isolates, tigecycline is not appropriate for treatment of clinically overt VRE infections because tigecycline urine concentration is very low according to prior reports and most VRE systemic infection involves the urinary tract.[27] In this study, we found some ICU inpatients acquired more than one VRE strain. Although identical VRE strains with the same MLST were repeatedly isolated from the same ICU inpatients, their PFGE subtypes were sometimes different. This phenomenon might be caused by amplification of endogenous strains after hospitalization and antibiotics usage.

There were some limitations in this study. Inpatients in medical ICU were very critical and many inpatients' family refused to participate in this study. Many inpatients, especially cardiological patients in medical ICU, had myocardial ischemia; in these patients rectal swabs were contraindicated, resulting in exclusion from study. These reasons decreased the number of our patients that fit the criteria for study. Moreover, medical personnel in the medical ICU refused to accept hand culture because there was no proved VRE outbreak in the medical ICU and no regulation enforcing hand culture without an outbreak.

After this study, active surveillance of every critical inpatient on admission to ICU were regularly performed after discussion and decision in our Infection Control Committee. Strict infection control policy including isolation will be performed immediately whenever any inpatient is known to be colonized by VRE. We conclude that subclinical transmission of VRE occurs in the ICU. If maximal barrier precautions can prevent cross-transmission of VRE in the ICU, active surveillance and maximal barrier precautions for VRE carriers shall be cost-effective in units of hospitals with high colonization rates of VRE.

Conflicts of interest

All authors have no conflicts of interest to declare.

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