Journal of Microbiology, Immunology and Infection (2019) 52, 433–442

Available online at www.sciencedirect.com

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Original Article

Molecular characterization of vancomycin-resistant enterococci isolated from a hospital in Beijing, China

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Received 27 November 2018; received in revised form 12 December 2018; accepted 24 December 2018
Available online 11 February 2019

KEYWORDS
Enterococcus;
Antimicrobial susceptibility testing;
Van gene;
Virulence genes;
Molecular typing

Abstract *Objective:* To investigate the occurrence of vancomycin-resistant enterococci (VRE) isolated from patients in Peking Union Medical College Hospital, Beijing, China from 2011 to 2017, and to evaluate their resistance mechanisms and genetic relatedness.
Methods: All isolates were identified using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF). Antibiotic susceptibility testing was performed using the broth microdilution method. Molecular characterization were detected by PCR and sequencing. Genotyping of VRE isolates was performed by multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) analysis. Virulence genes were detected by multiplex PCR.
Results: A total of 87 consecutive VRE were collected, including 84 isolates of vancomycin resistant *Enterococcus faecium* (VREfm) and 3 isolates of *Enterococcus faecalis* (VREfs). Urine (40.2%, 35/87) and blood (17.2%, 15/87) were the most commonly specimens. All VREfm isolates were resistant to ampicillin, and were susceptible to daptomycin, linezolid and tigecycline. The resistant rate of teicoplanin was 47.6%. All of the VREfm isolates carried the *vanA* gene, no isolates carried *vanB*. 11.9% (10/84) VREfm isolates carried both *vanA* and *vanM*. Among them, 76.2% (64/84) and 66.7% (56/84) carried *esp* and *hyl*, respectively. The 3 vancomycin resistant *E. faecalis* (VREfs) isolates were varied, and only one carried *vanB*. A total of 3 and 18 STs were detected among VREfs and VREfm strains, respectively. PFGE results indicated a genetic diversity among VREfm isolates.
Conclusion: This study confirms that VREfm isolates associated with ST78 were the main epidemic lineage responsible for nosocomial infections in China, as were also observed in other nations worldwide.

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https://doi.org/10.1016/j.jmii.2018.12.008

Introduction

Enterococcus is a gram-positive bacterium, widely distributed among the natural and digestive tract of human and animal. It is an important pathogen of urethral infection, soft tissue infection, sepsis and meningitis.

The prevalence of vancomycin-resistant enterococci (VRE) in hospitals has increased recently and has been a serious threat to patients because of the problem of multiple drugs resistance. They can serve as a reservoir of resistance genes and can also transfer to other strains of bacteria.[1] *VanM* was first identified in an *E. faecium* isolate from a hospital in Shanghai in 2006, and then has been predominant in VRE strains since 2011 and showing a greater prevalence than vanA in Shanghai.[2] In our previous research, all *E. faecium* resistent to vancomycin carried the *vanA* gene, but one carried *vanB*.[3] In this study, we detected *vanA*, *vanB* and *vanM* to find the relationship between genotype and phenotype. Moreover, the virulence genes in enterococci isolates are important for colonization and the progress of infections in hospitals, such as *esp* and *hyl*.[4]

There are more and more genetic studies about VRE, and ST78 has been responsible for several outbreaks in hospitals worldwide. Most of them are resistant to ampicillin, aminoglycoside, and glycopeptide, along with virulence genes may likely render the strains from highly adapted to the hospital environment and cause disease. The spread of multidrug-resistant *E. faecium* strains and their resistance genes has serious implications for health care, and control efforts should focus on early detection of *E. faecium* isolates, particularly within those sites with high known dissemination of ST78 strains.[5,6] So we collected *E. aecalis* and *E. faecium* from 2011 to 2017 in Peking Union Medical College Hospital to study the Molecular characterization of them.

Table 1 Antimicrobial susceptibility test results of the VREfm.

Antibiotic name	%R	%I	%S	MIC50	MIC90
Vancomycin	100	0	0	256	≥256
Teicoplanin	47.6	9.5	42.9	16	256
Daptomycin	0	0	100	2	4
Linezolid	0	0	100	2	2
Tigecycline	0	0	100	0.064	0.125
Tetracycline	46.4	1.2	52.4	2	64
Ampicillin	100	0	0	64	64
Gentamicin-High	58.3	0	41.7	≥256	≥256
Streptomycin-High	29.8	0	70.2	≥256	≥256
Chloramphenicol	2.4	16.7	81	8	16
Erythromycin	97.6	2.4	0	32	32
Levofloxacin	98.8	0	1.2	16	16
Nitrofurantoin	28.6	42.9	28.6	64	256

Abbreviations: VREfm, vancomycin resistant *Enterococcus faecium*; R, resistant; I, intermediate; S, susceptible.

Methods

Bacterial isolates and species identification

A study was conducted from January 2011 through December 2017 to evaluate the molecular epidemiology of vancomycin-resistant enterococci in Peking Union Medical College Hospital, Beijing, China, a tertiary teaching hospital with 2000 beds. The present study includes vancomycin-resistant *Enterococcus faecium* (VREfm) and *Enterococcus*

Table 2 The detailed information of the VREfm both carrying *vanA* and *vanM*.

Strain No.	Glycopeptide-resistance genes			Virulence genes					MLST	MIC(μg/ml)											
	vanA	*vanB*	*vanM*	*asal*	*gele*	*cyla*	*esp*	*hyl*		VAN	TEC	DAP	LNZ	TGC	GEH	STH	NIT	LVX	CHL	ERY	TCY	AMP
128	+	−	+	−	−	−	+	+	78	>256	16	2	1	0.064	>1000	<=1000	64	>8	8	>16	<=1	>32
188	+	−	+	−	−	−	−	+	789	128	32	2	2	0.25	<=500	<=1000	32	8	8	16	32	32
179	+	−	+	−	−	−	+	+	78	256	4	2	1	0.032	>1000	>2000	64	>8	4	>16	<=1	>32
174	+	−	+	−	−	−	+	−	78	256	4	4	2	0.064	>1000	<=1000	64	>8	16	>16	<=1	>32
240	+	−	+	+	−	−	+	+	78	>256	128	2	2	0.125	>1000	>2000	>128	>8	16	>16	2	>32
234	+	−	+	−	−	+	+	−	78	128	32	0.5	1	0.064	>1000	>2000	64	>8	4	>16	4	>32
148	+	−	+	−	−	−	+	−	78	64	4	2	1	0.064	<=500	<=1000	64	>8	4	>16	<=1	>32
196	+	−	+	−	−	−	+	+	192	>256	256	1	2	0.064	>1000	<=1000	128	>8	>32	>16	>32	>32
195	+	−	+	−	−	−	+	−	192	128	32	1	2	0.032	>1000	<=1000	<=16	>8	16	>16	>32	>32
245	+	−	+	+	+	+	+	−	78	>256	128	2	2	0.125	<=500	>2000	128	>8	16	>16	>32	>32

Abbreviations: VAN, vancomycin; TEC, teicoplanin; DAP, daptomycin; LNZ, linezolid; TGC, tigecycline; GEH, gentamicin high-level; STH, streptomycin high-level; NIT, nitrofurantoin; LVX, levofloxacin; CHL, chloramphenicol; ERY, erythromycin; TCY, tetracycline; AMP, ampicillin.

faecalis (VREfs) isolates from the following clinical specimens: blood, urine, secretions from surgical wounds, peritoneal fluid, abdominal abscesses, pleural fluid, cerebrospinal fluid (CSF), bronchoalveolar lavage fluid, sputum and rectal swab. This study collected consecutive enterococcal isolates and only one isolate per patient was included. The microorganisms were identified by using either automated methods (performed with the Vitek or BD Phoenix system) or manual methods, and once the corresponding isolate was included in the study, it was confirmed the identification by molecular methods using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) (MicroflexTM; Bruker Daltonic, Bremen, Germany).

Antimicrobial susceptibility testing

Antimicrobial drug susceptibility was determined by the broth microdilution method with cation-adjusted Mueller-Hinton broth according to the Clinical and Laboratory Standards Institute (CLSI) recommendations.[7,8] Antimicrobial agents tested were: vancomycin, teicoplanin, daptomycin, ampicillin, gentamicin and streptomycin high-level, levofloxacin, nitrofurantoin, tetracycline, erythromycin, chloramphenicol, tigecycline, and linezolid. Validation of the minimum inhibitory concentration (MIC) values was performed by concurrent testing of CLSI-recommended quality control reference strains (*S. aureus* ATCC 29213, *E. faecalis* ATCC 29212 and ATCC 51299). MIC interpretations were based on CLSI (M100-S28) and EUCAST (2018) breakpoint criteria, when available.[8,9]

Detection of van genes and virulence genes

Multiplex PCR assay was performed to detect vancomycin resistance genes: vanA, vanB, vanC1, vanC2/3, and vanM as described previously.[10,11] *E. faecium* ATCC 51559 (vanA) and *E. faecalis* ATCC 51299 (vanB) were used as positive control strains for PCR assays. Five virulence genes *asa1*, *gelE*, *cylA*, *esp*, and *hyl* of enterococci in the VREfm and VREfs isolates from different sources were detected by multiplex PCR, as described previously.[12]

Molecular typing

Pulsed-field gel electrophoresis (PFGE) of the VREfm and VREfs isolates was performed by use of some modifications of a previously described method.[13] The banding patterns were clustered by the unweighted pair group method with arithmetic averages (UPGMA) and interpreted using the guidelines proposed by Tenover et al.[14] The band tolerance was set at 1.2%, and the threshold cutoff value was set at 82%. All the VREfm and VREfs isolates were further characterized by multilocus sequence typing (MLST) using a standard protocol available at the MLST website (http://efaecium.mlst.net/misc/info.asp and http://efaecalis.mlst.net/misc/info.asp). Sequence types (ST) were designated using the MLST website (http://efaecium.mlst.net and http://efaecalis.mlst.net).

Results

Antimicrobial susceptibility results and van-associated genes

A total of 87 consecutive VRE isolates were collected in this study, including 84 VREfm and 3 VREfs. All samples were collected from different sources. The urine (40.2%, 35/87) was the most commonly specimen, followed by blood (17.2%, 15/87) and drain (12.6%, 11/87). All of the VRE isolates collected in this study exhibited multidrug resistance (Table 1). All *E. faecium* isolates were highly resistant to vancomycin (MIC $\geq$256 μg/ml). For teicoplanin, 47.6% isolates were resistant, 9.5% isolates were intermediate and 42.9% isolates were susceptible. All *E. faecium* isolates carried the *vanA* gene, no isolate carried *vanB*. Of 84 *E. faecium* isolates, 11.9% (10) carried both *vanA* and

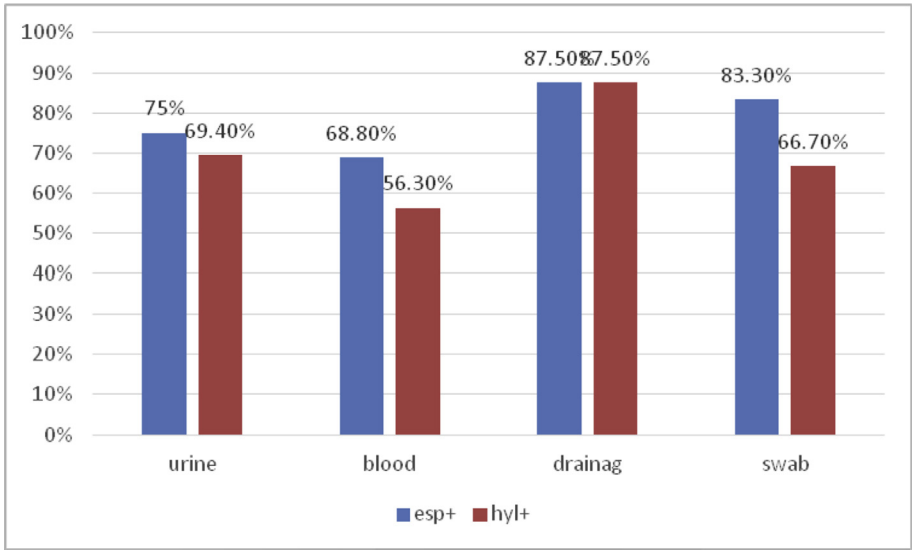


Figure 1. The rates of *esp* and *hyl* virulence genes of VREfm strain isolated from the main sample types.

Table 3 Characterization of VREfm isolated from 2011 to 2017 in Peking Union Medical College Hospital.

WARD	Strain No	Date	Source	Virulence		MIC(μg/ml)		Typing	
				esp	*hyl*	VAN	TEC	ST	PFGE
der	109	2011/1/7	wd	+	+	256	4	78	P035
eme	155	2013/8/19	ur	+	+	>256	128	17	P007
	135	2012/7/2	ur	+	+	128	8	78	P015
	179	2014/4/10	ur	+	+	256	4	78	P024
	199	2015/1/29	bl	+	+	>256	>256	389	P027
	176	2014/1/22	bl	+	+	>256	32	78	P034
	232	2016/2/15	ur	+	+	256	64	78	P036
	151	2013/4/22	ur	+	+	128	<=0.12	341	P044
	217a	2015/3/26	ur	+	+	>256	16	78	P046
	206	2015/4/2	ur	+	+	256	16	78	P047
	157	2013/8/13	bl	−	+	64	8	78	P052
	156	2013/8/7	bl	+	+	128	4	78	P052
	159	2013/9/29	bl	−	+	128	8	78	P053
	153	2013/8/10	ur	+	+	128	8	78	P055
	231	2016/8/31	ur	+	+	128	16	78	P070
	128	2012/8/27	bl	+	+	>256	16	78	P071
	238	2017/1/29	ur	+	+	>256	128	78	P072
	239a	2017/2/3	dr	+	+	>256	32	789	P073
	132	2012/4/14	ur	−	+	>256	32	18	P075
	188	2014/11/3	ur	−	+	128	32	789	P076
	244	2017/8/22	ur	+	+	>256	64	230	P077
icu	171	2014/1/3	ur	−	+	128	8	78	P001
	183	2014/6/26	ur	+	+	128	0.25	343	P002
	216a	2014/12/2	ur	−	+	256	8	78	P004
	211a	2015/7/15	bl	+	+	256	8	564	P011
	150	2013/3/22	sb	−	+	256	8	389	P017
	193a	2014/11/24	ba	+	+	>256	32	78	P018
	130	2012/9/26	bl	−	+	128	1	new	P020
	137b	2012/9/20	dr	+	+	64	1	78	P032
	243	2017/4/7	dr	+	+	>256	>256	78	P038
	200	2015/2/26	ur	+	+	128	8	17	P039
	174	2014/1/10	wd	+	+	256	4	78	P043
	240	2017/2/11	sb	+	+	>256	128	78	P048
	154a	2013/8/17	dr	+	+	256	64	78	P050
	175	2014/1/21	ba	+	+	256	16	78	P051
	192a	2014/9/10	bl	+	+	256	8	359	P056
	112	2011/11/14	bl	+	+	128	2	343	P059
	233b	2016/5/3	bl	+	+	128	8	343	P061
	212a	2015/9/21	ti	−	+	>256	256	80	P062
	205	2015/3/28	ur	−	+	>256	256	17	P066
	236a	2016/7/16	bl	−	+	>256	128	17	P067
	139a	2012/12/5	dr	−	+	32	4	578	P069
	237	2017/1/7	bl	+	+	>256	>256	343	P074
med	245	2017/10/17	sb	+	+	>256	128	78	P003
	186	2014/9/22	ur	−	+	256	16	78	P005
	223	2014/1/3	ur	+	+	128	8	17	P008
	113	2010/11/15	bl	−	+	128	8	new	P009
	182	2014/5/9	sp	+	+	256	32	17	P010
	227a	2016/1/10	ur	−	+	>256	128	78	P012
	209	2015/12/26	ur	+	+	>256	>256	new	P013
	160	2013/12/19	fl	−	+	128	2	389	P016
	134	2012/6/25	ur	+	+	32	0.25	78	P021
	147	2013/1/11	se	+	+	128	4	78	P022
	158	2013/8/24	sf	−	+	128	8	78	P023
	191	2014/12/29	ur	+	+	>256	128	341	P025
	177	2014/2/18	bl	+	+	>256	8	78	P028

Table 3 (*continued*)

WARD	Strain No	Date	Source	Virulence		MIC(μg/ml)		Typing	
				esp	*hyl*	VAN	TEC	ST	PFGE
	184	2014/7/22	ur	+	+	256	2	78	P033
	204	2015/3/12	bi	+	+	>256	256	389	P041
	234	2016/2/17	sp	+	+	128	32	78	P049
	201	2015/3/4	ur	+	+	16	16	78	P054
	136	2012/9/14	ur	−	+	32	4	17	P057
	207	2015/6/29	sb	+	+	128	8	78	P058
	148	2013/3/4	ur	+	+	64	4	78	P078
	185	2014/7/29	sb	+	+	128	4	78	P079
	187	2014/10/12	dr	+	+	>256	128	389	P080
	229	2016/1/5	sk	+	+	>256	64	64	P081
	196	2015/1/22	ur	+	+	>256	256	192	P082
obg	242a	2017/3/18	dr	+	+	256	16	78	P006
	146	2013/1/4	ur	+	+	32	<=0.12	414	P026
	131a	2012/6/14	ca	+	+	256	32	363	P064
oth	178	2014/3/5	sp	+	+	64	2	78	P014
	215a	2014/12/26	ur	+	+	256	32	78	P019
	138a	2012/11/12	ur	+	+	32	32	78	P040
	202	2015/3/7	ur	+	+	>256	256	389	P042
	208	2015/8/10	ur	+	+	256	128	412	P060
	210a	2014/4/18	ur	−	+	256	8	18	P063
	214a	2015/2/5	ur	+	+	>256	64	192	P068
sur	133	2012/10/31	sb	+	+	128	0.5	78	P020
	197	2015/1/23	dr	+	+	128	2	78	P029
	213a	2015/6/20	mo	+	+	256	4	78	P030
	198	2015/1/27	wd	+	+	256	2	78	P031
	140	2012/11/27	bl	+	+	>256	8	78	P037
	110	2011/5/10	ab	−	+	128	4	78	P045
	195	2015/1/22	sf	+	+	128	32	192	P065

Abbreviations: der, dermatology department; eme, emergency department; icu, intensive care unit; med, internal medicine department; obg, gynecology and obstetrics; sur, surgery department; oth, other; wd, wound; ur, urine; bl, blood; sb, swab; dr, drainage; ca, catheter; sp, sputum; mo, mouth; ab, Abdominal fluid; sf, cerebrospinal fluid; VAN, vancomycin; TEC, teicoplanin.

vanM (Table 2), and was highly resistant vancomycin (MIC ≥256 μg/ml). But 40% (4 isolates) were susceptible to teicoplanin, *i.e.*, the VanB phenotype. The detailed information was showed in Table 2. All *E. faecium* strains were resistant to ampicillin, erythromycin, levofloxacin and all of them were susceptible to daptomycin, linezolid and tigecycline. Erythromycin and levofloxacin reaching high levels resistant rates (97.6% and 98.8%). For the 3 *E. faecalis*, 2 isolates carried the *vanA* gene were resistant to teicoplanin, and one of isolate carried *vanB* was susceptible to teicoplanin (MIC = 0.25 μg/ml).

Virulence genes

Among the *E. faecium* strains, 76.2% (64/84) and 66.7% (56/84) carried *esp* and *hyl*, respectively. The *asa1*, *gelE* and *cylA* genes were detected only in 15.5% (13/84), 16.7% (14/84) and 10.7% (9/84) of these isolates, respectively. There were 41 *E. faecium* isolates harbored both *esp* and *hyl* gene including 14 types of ST (17、192、230、341、343、359、363、389、412、564、78、789 and a new type), while a part of ST18、78 and a new ST didn't carry these genes. In addition, the STs only positive for *esp* gene were ST64, ST414 and a part of ST78, ST343, ST431,ST192. The STs only positive for *hyl* gene were ST80, ST578 and a part of ST17, ST18, ST389,ST78,789 and a new ST. 75% (27/36) of the VREfm isolated from urine was positive for *esp* gene, meanwhile, 69.4% (25/36) was positive for *hyl*. For the isolates from blood, 68.8% (11/16) was positive for *esp* gene, and 56.3% (9/16) was positive for *hyl* (Fig. 1). The virulence genes of all the 3 *E. faecalis* varied.

Molecular typing

A total of 18 MLST results were obtained among 84 *E. faecium* strains and ST78 (57.1%, 48/84) predominated followed by ST17 (9.5%, 8/84), ST389 (7.1%, 6/84) and ST343 (4.8%, 4/84). 82 PFGE types were detected, with great genetic diversity (Table 3). 7 types were collected in internal medicine department, 54.2% (13/24) was ST78, and others were ST17, ST64, ST192, ST341 and ST389. There was no breakout among 84 VREfm isolates, as shown in Fig. 2. All 3 *E. faecalis* isolates were in different types of ST. Unweighted pair group method with arithmetic mean (UPGMA) dendrogram draw by PFGE typing results of VREfm and VREfs were shown in the Figs. 3a, b and 4.

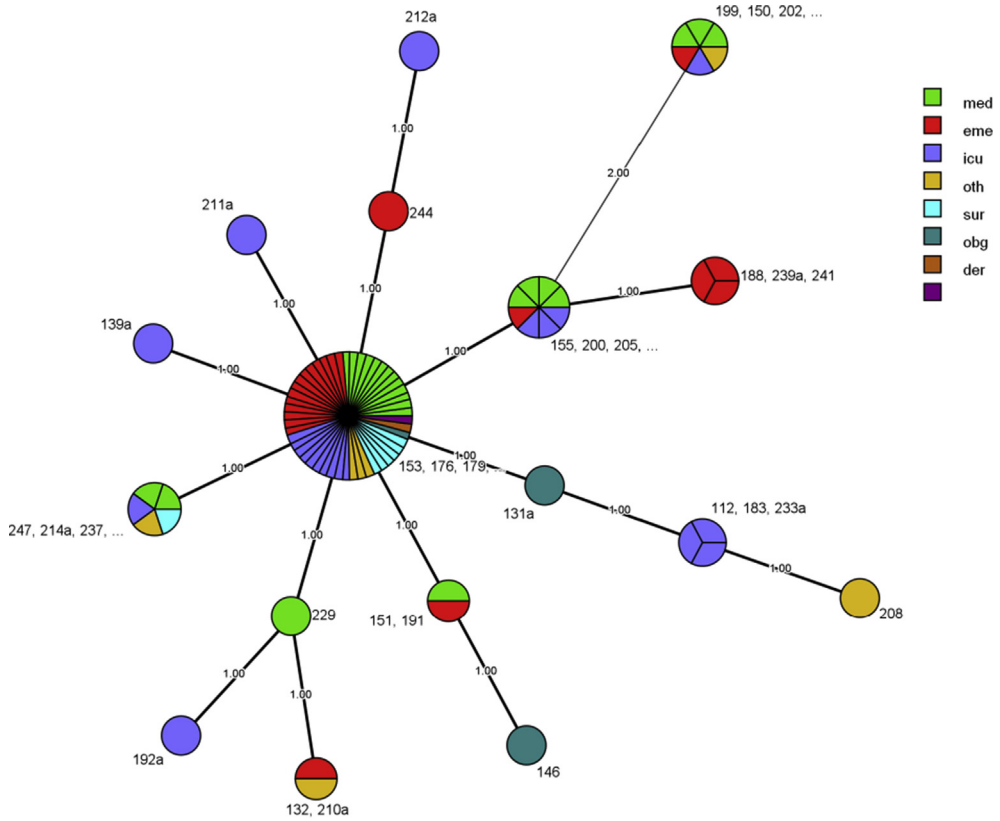


Figure 2. Minimum spanning tree analysis based on MLST and department of VREfm. der: dermatology department, eme: emergency department, icu: intensive care unit, med: internal medicine department,obg: gynecology and obstetrics, sur: surgery department, oth: other.

Discussion

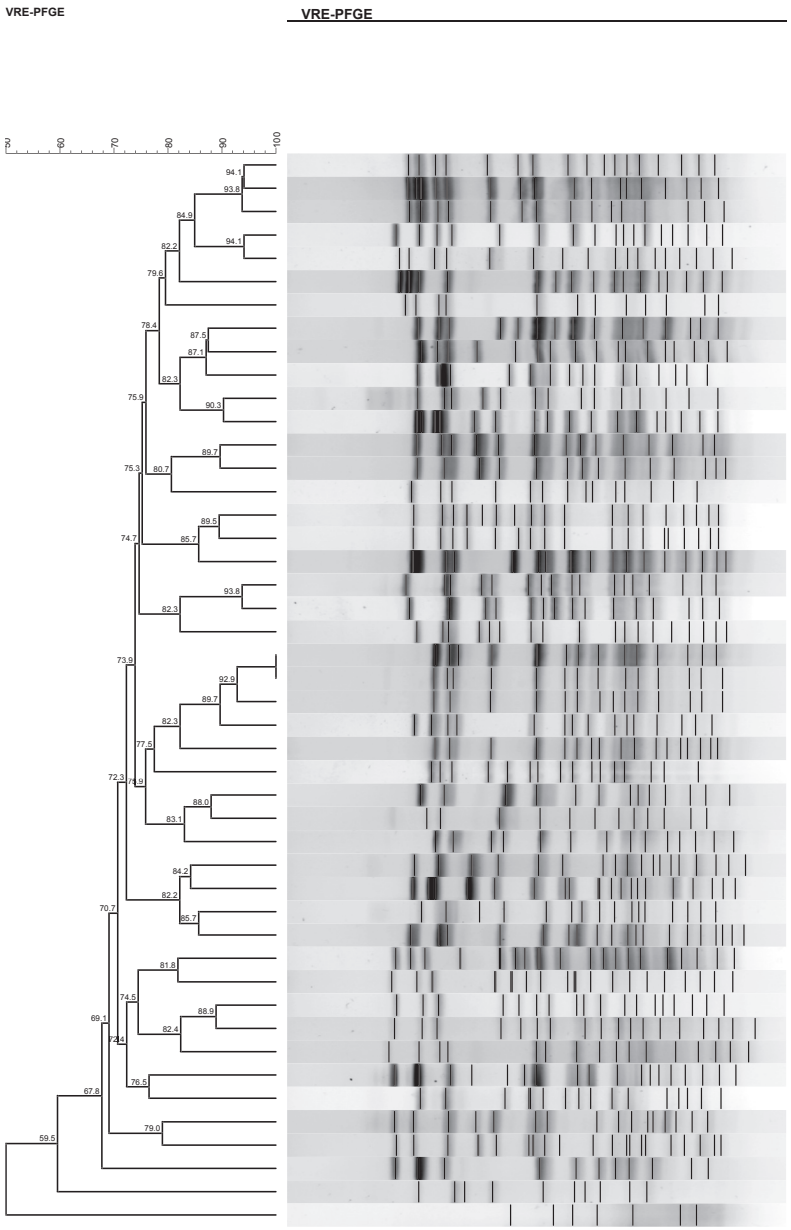
The prevalence of VRE in various healthcare settings has increased dramatically in the last decade in China,[15] Irish,[16] Italian,[17] Iran[18] and other countries. Moreover, *Enterococcus* could persist in the ecosystem for a long time, especially in hospital wards.[4]

This investigation collected a total of 87 clinical isolates of VRE recovered from Peking Union Medical College Hospital. Overall, daptomycin, linezolid, and tigecycline demonstrated complete in vitro activity against this collection. All VREfm isolates were resistant to ampiciline, and carried the *vanA* gene, no isolate carried *vanB*. In this study, we had collected 10 (11.9%) isolates VREfm, which carried both *vanA* and *vanM*. Vancomycin was highly resistant, and 40% (4 isolates) were susceptible to teicoplanin, i.e., the *vanB* phenotype.

VanM was first identified in an *E. faecium* isolate from a hospital in Shanghai in 2006, and then has been predominant in VRE strains since 2011 and showing a greater prevalence than *vanA* in Shanghai.[2,19] Generally, *vanA* genotype is characterized by an acquired high-level of resistance to both vancomycin and teicoplanin, called VanA phenotype.[20] The *vanB* genotype is characterized by variable acquired levels of resistance to vancomycin, but not to teicoplanin, called VanB phenotype.[20] Many studies have reported the emergence of VanB phenotype-*vanA* genotype VRE, however, maybe it is *vanM* that makes it more difficult to determine genotypes by drug susceptibility.

Other antimicrobial agents tested demonstrated more limited or suboptimal coverage. One of VREfs carried *vanB*. The MIC value of vancomycin was 32 μg/ml, and it was susceptible to teicoplanin and ampiciline. Overall, *vanA* is dominant in the prevalence of VRE as other studies described,[21] and *vanB* was limited to one VREfs isolate. In our previous research,[21] all *E. faecium* resistent to vancomycin carried the *vanA* gene, but one carried *vanB*, and ampicillin was susceptible when tested against *E. faecalis* (only 3 strains). A total of 3 and 18 STs were detected among VREfs and VREfm strains in this study, while these strains were clustered within 3 and 82 PFGE types, respectively. PFGE results indicated a genetic diversity among *E. faecium*.

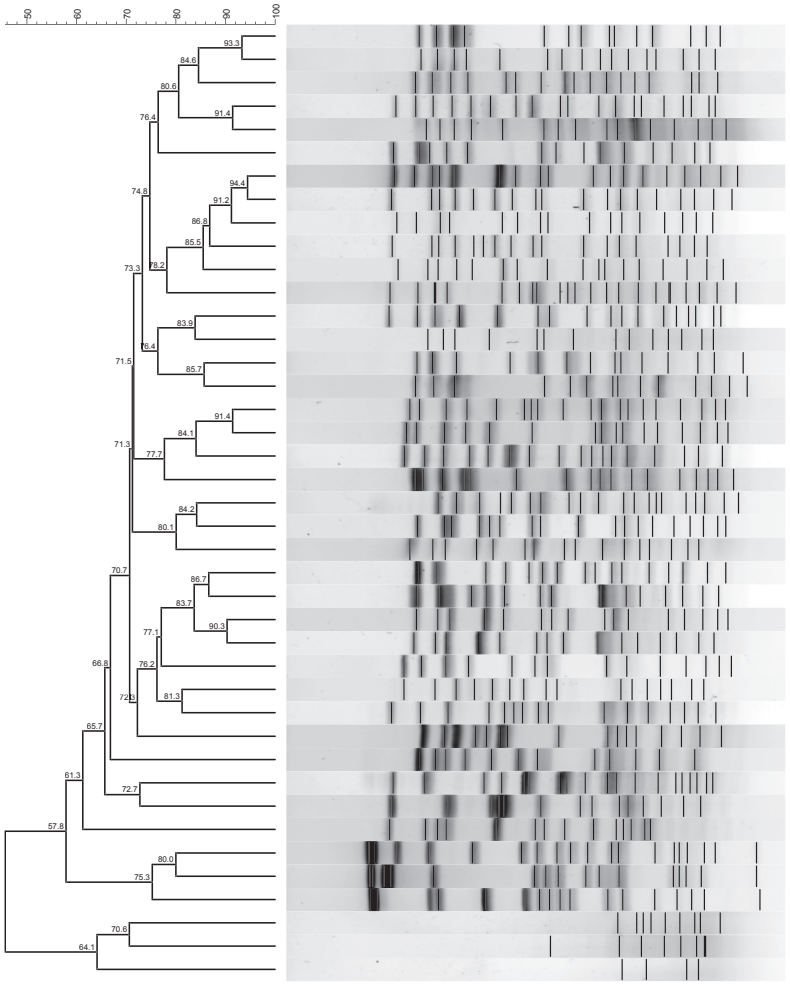
Esp (*Enterococcus* surface protein) plays an important role in initial attachment of bacteria in the urinary tract, and the formation of biofilm.[12,22] The *hyl* gene in the *E. faecium* encodes hyaluronidase, the presence of this gene is believed to facilitate intestinal colonization and invasion by bacteria.[12,22] In this study, all *E. faecalis* and 76.2% of *E. faecium* included in this study carried *esp*, respectively. The rate observed among *E. faecium* corroborates those previously reported.[15,23] Studies have shown that *E. faecium* strains causing human infections are more likely to harbor *esp*.[22] However, the collection of *E. faecalis*



Key	Location type	Date	Source	esp	hyl	VAN	TEC	ST	PFGE type
197	in	2015-01-23	dr	+	+	128	2	78	P029
213a	in	2015-06-20	mo	+	+	256	4	78	P030
198	in	2015-01-27	wd	+	+	256	2	78	P031
137b	in	2012-09-20	dr	+	+	64	1	78	P032
184	in	2014-07-22	ur	+	+	256	2	78	P033
176	out	2014-01-22	bl	+	+	>256	32	78	P034
109	in	2011-01-07	wd	+	+	256	4	78	P035
232	in	2016-02-15	ur	+	+	256	64	78	P036
140	in	2012-11-27	bl	+	+	>256	8	78	P037
243	in	2017-04-07	dr	+	+	>256	>256	78	P038
174	in	2014-01-10	wd	+	+	256	4	78	P043
138a	in	2012-11-12	ur	+	+	32	32	78	P040
217a	out	2015-03-26	ur	+	+	>256	16	78	P046
206	in	2015-04-02	ur	+	+	256	16	78	P047
110	in	2011-05-10	ab	-	+	128	4	78	P045
147	in	2013-01-11	se	+	+	128	4	78	P022
158	in	2013-08-24	sf	-	+	128	8	78	P023
179	out	2014-04-10	ur	+	+	256	4	78	P024
193a	in	2014-11-24	ba	+	+	>256	32	78	P018
215a	in	2014-12-26	ur	+	+	256	32	78	P019
133	in	2012-10-31	sb	+	+	128	0.5	78	P020
157	out	2013-08-13	bl	-	+	64	8	78	P052
156	in	2013-08-07	bl	+	+	128	4	78	P052
159	in	2013-09-29	bl	-	+	128	8	78	P053
201	in	2015-03-04	ur	+	+	16	16	78	P054
153	out	2013-08-10	ur	+	+	128	8	78	P055
207	in	2015-06-29	sb	+	+	128	8	78	P058
231	in	2016-08-31	ur	+	+	128	16	78	P070
128	out	2012-08-27	bl	+	+	>256	16	78	P071
177	in	2014-02-18	bl	+	+	>256	8	78	P028
240	in	2017-02-11	sb	+	+	>256	128	78	P048
234	in	2016-02-17	sp	+	+	128	32	78	P049
154a	in	2013-08-17	dr	+	+	256	64	78	P050
175	in	2014-01-21	ba	+	+	256	16	78	P051
178	in	2014-03-05	sp	+	+	64	2	78	P014
227a	in	2016-01-10	ur	-	+	>256	128	78	P012
216a	in	2014-12-02	ur	-	+	256	8	78	P004
186	in	2014-09-22	ur	-	+	256	16	78	P005
242a	in	2017-03-18	dr	+	+	256	16	78	P006
171	in	2014-01-03	ur	-	+	128	8	78	P001
245	in	2017-10-17	sb	+	+	>256	128	78	P003
135	out	2012-07-02	ur	+	+	128	8	78	P015
134	out	2012-06-25	ur	+	+	32	0.25	78	P021
238	in	2017-01-29	ur	+	+	>256	128	78	P072
148	in	2013-03-04	ur	+	+	64	4	78	P078
185	in	2014-07-29	sb	+	+	128	4	78	P079

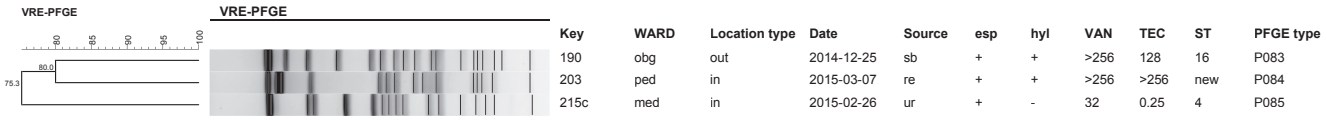
Figure 3. a. The UPGMA dendrogram representing PFGE patterns of ST78 VREfm isolates obtained from different sources. b. The UPGMA dendrogram representing PFGE patterns of non-ST78 VREfm isolates obtained from different sources.

VRE-PFGE



Key	Location type	Date	Source	esp	hyl	VAN	TEC	ST	PFGE type
112	in	2011-11-14	bl	+	+	128	2	343	P059
208	in	2015-08-10	ur	+	+	256	128	412	P060
233b	in	2016-05-03	bl	+	+	128	8	343	P061
191	in	2014-12-29	ur	+	+	>256	128	341	P025
146	in	2013-01-04	ur	+	+	32	<=.12	414	P026
210a	in	2014-04-18	ur	-	+	256	8	18	P063
155	out	2013-08-19	ur	+	+	>256	128	17	P007
223	in	2014-01-03	ur	+	+	128	8	17	P008
113	in	2010-11-15	bl	-	+	128	8	new	P009
182	in	2014-05-09	sp	+	+	256	32	17	P010
211a	in	2015-07-15	bl	+	+	256	8	564	P011
209	out	2015-12-26	ur	+	+	>256	>256	new	P013
136	in	2012-09-14	ur	-	+	32	4	17	P057
192a	in	2014-09-10	bl	+	+	256	8	359	P056
195	in	2015-01-22	sf	+	+	128	32	192	P065
131a	in	2012-06-14	ca	+	+	256	32	363	P064
205	in	2015-03-28	ur	-	+	>256	256	17	P066
236a	in	2016-07-16	bl	-	+	>256	128	17	P067
214a	in	2015-02-05	ur	+	+	>256	64	192	P068
212a	in	2015-09-21	ti	-	+	>256	256	80	P062
183	in	2014-06-26	ur	+	+	128	0.25	343	P002
130	in	2012-09-26	bl	-	+	128	1	new	P020
151	out	2013-04-22	ur	+	+	128	<=.12	341	P044
200	in	2015-02-26	ur	+	+	128	8	17	P039
202	in	2015-03-07	ur	+	+	>256	256	389	P042
199	out	2015-01-29	bl	+	+	>256	>256	389	P027
204	in	2015-03-12	bi	+	+	>256	256	389	P041
139a	in	2012-12-05	dr	-	+	32	4	578	P069
150	in	2013-03-22	sb	-	+	256	8	389	P017
160	in	2013-12-19	fl	-	+	128	2	389	P016
188	in	2014-11-03	ur	-	+	128	32	789	P076
132	in	2012-04-14	ur	-	+	>256	32	18	P075
237	in	2017-01-07	bl	+	+	>256	>256	343	P074
239a	in	2017-02-03	dr	+	+	>256	32	789	P073
244	in	2017-08-22	ur	+	+	>256	64	230	P077
190	out	2014-12-25	sb	+	+	>256	128	16	P083
203	in	2015-03-07	re	+	+	>256	>256	new	P084
215c	in	2015-02-26	ur	+	-	32	0.25	4	P085
187	in	2014-10-12	dr	+	+	>256	128	389	P080
229	in	2016-01-05	sk	+	+	>256	64	64	P081
196	in	2015-01-22	ur	+	+	>256	256	192	P082

(Continued).



Key	WARD	Location type	Date	Source	esp	hyl	VAN	TEC	ST	PFGE type
190	obg	out	2014-12-25	sb	+	+	>256	128	16	P083
203	ped	in	2015-03-07	re	+	+	>256	>256	new	P084
215c	med	in	2015-02-26	ur	+	-	32	0.25	4	P085

Figure 4. The UPGMA dendrogram representing PFGE patterns of 3 VREfs isolates obtained from different sources.

included in this study was limited, and so it was difficult to come to a conclusion. The *asa1*, *gelE*, and *cylA* genes were detected in only 15.5%—16.7% of *E. faecium* isolates, which is in agreement with the results reported by other investigators.[22] In this study, we didn't find a obvious correlation between vriulence genes and STs.

Overall, this study shows most of VREfm isolates with different PFGE pattern belonged to the same ST. ST78 (the CC17 lineage) VREfm was the main epidemic lineage responsible for nosocomial infections in Peking Union Medical College Hospital, Beijing, as also observed in other nations worldwide.[1,24] Cross transmission via the medical personnel and environment is the common way of transmission of VRE in hospital, so we should pay attention and take measures to prevent the transmission of VRE.[25,26]

Acknowledgements

Thanks to all of the excellent technical contributions to this study in our laboratory.

References

1. Freitas AR, Tedim AP, Francia MV, Jensen LB, Novais C, Peixe L, et al. Multilevel population genetic analysis of *vanA* and *vanB* *Enterococcus faecium* causing nosocomial outbreaks in 27 countries (1986—2012). *J Antimicrob Chemother* 2016;**71**:3351—66.
2. Zhang G, Lin X, Cai J, Zhang R, Xiu H, Tian B, et al. Characteristics of clinical and environmental *vanM*-carrying vancomycin-resistant *enterococci* isolates from an infected patient. *Int J Antimicrob Agents* 2018;**51**:932—5.
3. Sun H, Wang H, Xu Y, Jones RN, Costello AJ, Liu Y, et al. Molecular characterization of vancomycin-resistant *Enterococcus spp*. clinical isolates recovered from hospitalized patients among several medical institutions in China. *Diagn Microbiol Infect Dis* 2012;**74**:399—403.
4. Arshadi M, Mahmoudi M, Motahar MS, Soltani S, Pourmand MR. Virulence determinants and antimicrobial resistance patterns of vancomycin-resistant *Enterococcus faecium* isolated from different sources in southwest Iran. *Iran J Public Health* 2018;**47**:264—72.
5. Yang J, Jiang Y, Guo L, Ye L, Ma Y, Luo Y. Prevalence of diverse clones of vancomycin-resistant *Enterococcus faecium* ST78 in a Chinese hospital. *Microb Drug Resist* 2016;**22**:294—300.
6. Hsieh YC, Lee WS, Ou TY, Hsueh PR. Clonal spread of CC17 vancomycin-resistant *Enterococcus faecium* with multilocus sequence type 78 (ST78) and a novel ST444 in Taiwan. European journal of clinical microbiology & infectious diseases. *Eur J Clin Microbiol Infect Dis* 2010;**29**:25—30.
7. Clinical and Laboratory Standards Institute (CLSI). *Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically; Approved Standard: Nineth Edition. M07-A9*. Wayne, PA: CLSI; 2012.
8. Clinical and Laboratory Standards Institute (CLSI). *Performance standards for antimicrobial susceptibility testing: 28th Informational Supplement. M100-S28*. Wayne, PA: CLSI; 2018.
9. EUCAST. *European committee on antimicrobial susceptibility testing breakpoint tables for interpretation of MICs and zone diameters*. 2018. Version 8.1 May 2018. Available at: http://www.eucast.org/clinical_breakpoints/.
10. 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**:24—7.
11. Xu X, Lin D, Yan G, Ye X, Wu S, Guo Y, et al. vanM, a new glycopeptide resistance gene cluster found in *Enterococcus faecium*. *Antimicrob Agents Chemother* 2010;**54**:4643—7.
12. Vankerckhoven V, Van Autgaerden Tim, Vael C, Lammens C, Chapelle S, Rossi R, et al. Development of a multiplex PCR for the detection of *asa1*, *gelE*, *cylA*, *esp*, and *hyl* genes in *enterococci* and survey for virulence determinants among European hospital isolates of *Enterococcus faecium*. *J Clin Microbiol* 2004;**42**:4473—9.
13. Murray BE, Singh KV, Heath JD, Sharma BR, Weinstock GM. Comparison of genomic DNAs of different *enterococcal* isolates using restriction endonucleases with infrequent recognition sites. *J Clin Microbiol* 1990;**28**:2059—63.
14. 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.
15. Yang JX, Li T, Ning YZ, Shao DH, Liu J, Wang SQ, et al. Molecular characterization of resistance, virulence and clonality in vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis*: a hospital-based study in Beijing, China. *Infect Genet Evol* 2015;**33**:253—60.
16. Ryan L, O'Mahony E, Wrenn C, FitzGerald S, Fox U, Boyle B, et al. Epidemiology and molecular typing of VRE bloodstream isolates in an Irish tertiary care hospital. *J Antimicrob Chemother* 2015;**70**:2718—24.
17. Fallico L, Boldrin C, Grossato A, Franchin E, De Canale E, Tommasini T, et al. Molecular epidemiology of *Enterococcus faecium* isolates from an Italian hospital. *Infection* 2011;**39**:127—33.
18. Arshadi M, Douraghi M, Shokoohizadeh L, Moosavian SM, Pourmand MR. High prevalence of diverse vancomycin resistance *Enterococcus faecium* isolates in clinical and environmental sources in ICU wards in southwest of Iran. *Microb Pathog* 2017;**111**:212—7.
19. Chen C, Sun J, Guo Y, Lin D, Guo Q, Hu F, et al. High prevalence of *vanM* in vancomycin-resistant *Enterococcus faecium* isolates from Shanghai, China. *Antimicrob Agents Chemother* 2015;**59**:7795—8.
20. Shen H, Liu Y, Qu J, Cao B. Comparison of *vanA* gene mRNA levels between vancomycin-resistant *Enterococci* presenting the VanA or VanB phenotype with identical Tn1546-like elements. *J Microbiol Immunol Infect* 2016;**49**:866—71.
21. Hammerum AM, Baig S, Kamel Y, Roer L, Pinholt M, Gumpert H, et al. Emergence of *vanA Enterococcus faecium* in Denmark, 2005—15. *J Antimicrob Chemother* 2017;**72**:2184—90.

22. Strateva T, Atanasova D, Savov E, Petrova G, Mitov I. Incidence of virulence determinants in clinical *Enterococcus faecalis* and *Enterococcus faecium* isolates collected in Bulgaria. *Braz J Infect Dis* 2016;**20**:127—33.
23. Kang M, Xie Y, He C, Chen ZX, Guo L, Yang Q, et al. Molecular characteristics of vancomycin-resistant *Enterococcus faecium* from a tertiary care hospital in Chengdu, China: molecular characteristics of VRE in China. *Eur J Clin Microbiol Infect Dis* 2014;**33**:933—9.
24. Sacramento AG, Zanella RC, Esposito F, Costa EAS, de Almeida LM, Pires C, et al. Changed epidemiology during intra and inter hospital spread of high-risk clones of *vanA*-containing *Enterococcus* in Brazilian hospitals. *Diagn Microbiol Infect Dis* 2017;**88**:348—51.
25. Lochan H, Moodley C, Rip D, Bamford C, Hendricks M, Davidson A, et al. Emergence of vancomycin-resistant *Enterococcus* at a tertiary paediatric hospital in South Africa. *S Afr Med J* 2016;**106**:39—43.
26. Lee SC, Lee CW, Shih TC, See LC, Chu CM, Liu YC. Identification of subclinical transmission of vancomycin-resistant *enterococcus* within an intensive care unit in Taiwan. *J Microbiol Immunol Infect* 2016;**49**:749—59.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2018.12.008.