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Original Article

The prevalence and molecular epidemiology of vancomycin-resistant *Enterococcus* (VRE) carriage in patients admitted to intensive care units in Beijing, China

Meng-Yao Yan [1], Yuan-Hui He [1], Gen-Jie Ruan, Feng Xue, Bo Zheng*, Yuan Lv **

Institute of Clinical Pharmacology, Peking University First Hospital, Beijing 100191, China

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KEYWORDS
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Abstract *Background:* Vancomycin-resistant *Enterococcus* (VRE) can be carried in the gut for a long period and its carriage status is associated with subsequent infections. This study aimed to investigate the frequency of intestinal VRE carriage in intensive care patients in Beijing.
Methods: A multicenter, retrospective cross-sectional study was conducted at six hospitals in Beijing, China. All patients admitted to intensive care units (ICUs) between April 2 and May 1, 2017, were enrolled, and their clinical data were gathered by reviewing electronic medical records. Rectal swabs collected from patients were stored at −80 °C in the Institute of Clinical Pharmacology, Peking University First Hospital, and they were selectively cultured for VRE, then the identified strains were analyzed by polymerase chain reaction (PCR) to detect the glycopeptide resistance gene and were characterized by multilocus sequence typing (MLST).
Results: Of 148 patients recruited, 46 (31.1%) carried VRE, with the majority (n = 42) being *Enterococcus faecium*. In total, 78.3% of the VRE were *vanA* positive and 15.2% *vanM* positive, while 6.5% undetected glycopeptide resistance gene. The predominant ST was ST78 (47.6%) followed by ST192 (14.3%), ST555 (9.5%), and ST789 (9.5%). Multivariate analysis showed that factors associated VRE carriage were patients aged >65 years (odds ratio [OR], 3.786; 95% confidence interval [CI], 1.402–10.222) and recent third-generation cephalosporins use (OR, 6.360; 95% CI, 1.873–21.601).

* Corresponding author.
** Corresponding author.
E-mail addresses: doctorzhengbo@163.com (B. Zheng), lvyuan0901@sina.com (Y. Lv).
[1] Meng-Yao Yan and Yuan-Hui He contributed equally as first co-authors.

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Conclusions: The overall proportion of VRE carriage in patients admitted to ICUs was markedly high in Beijing, China. The *vanM* gene has been spread widely but *vanA* gene was the dominant resistance determinant in VRE in Beijing.

Introduction

Enterococcus is a prominent cause of healthcare-associated infections, and hospital-adapted lineages are increasingly resistant to vancomycin and widely disseminated.[1–3] The gastrointestinal (GI) tract is the primary reservoir for *Enterococcus*, which normally constitutes a small proportion of the gut microbiota.[4,5] However, the exposure of hospitalized patients to antibiotics results in substantial changes in the gut microbiota that facilitate vancomycin-resistant *Enterococcus* (VRE) colonization, especially among patients with critical illness in the intensive care units (ICUs).[6–8] The process seems to be the important first step toward nosocomial enterococcal infections.[4] Because the colonized patients are generally asymptomatic, the GI tract reservoir can easily go unnoticed unless surveillance culture specimens are obtained from patients at risk.

The prevalence of VRE in China is relatively low. The China Antimicrobial Resistance Surveillance System (CARSS) reported that the nationwide incidence of vancomycin-resistant *Enterococcus faecium* (VRE*fm*) was 1.4% in 2017, 1.4% in 2018, 1.1% in 2019 and 1.0% in 2020 (http://www.carss.cn/). Of note, the prevalence of VRE*fm* in Beijing, the capital of China, was markedly higher with an isolation rate of 6.8% in 2017, 7.8% in 2018, 7.7% in 2019, and 8.3% in 2020 (http://www.carss.cn/). The prevalence of VRE in Beijing was serious and its incidence was significantly higher than in all other regions of China. However, a comprehensive epidemiological picture of VRE in Beijing is lacking. In the present study, we conducted a multicenter study to assess the prevalence of VRE carriage in ICUs in Beijing.

Methods

Study design and participants

An active surveillance program for VRE in ICUs was conducted from April, 2 to May 1, 2017, at six tertiary-care hospitals in Beijing, China. In the participating ICUs, a rectal swab was performed on patients every Tuesday morning during this four-week period and submitted to the laboratory for screening of VRE. Rectal swabs were collected by nurses with patients turned onto the left lateral decubitus position and the swab was inserted deeply into the rectal canal and rotated three times. Swabs were transported in liquid Amies media for VRE culture and then flash-frozen at −80 °C in the Institute of Clinical Pharmacology, Peking University First Hospital.

A retrospective, multicenter, cross-sectional study was conducted to investigate the prevalence and molecular epidemiology of VRE carriage. Adult patients (≥18 years) were considered for the study if they were admitted to the ICUs during the active surveillance period. More specifically, patients were eligible if they were either directly admitted to the ICUs or transferred to the ICUs from a hospital ward. Only the first eligible screening of VRE for each patient was considered for analysis if there were multiple clinical samples from the same patient. The present study was approved by the Institutional Review Board of Peking University First Hospital (2020 Research 202). Due to the retrospective nature of the study, written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Epidemiologic and clinical data

The patients' epidemiologic and clinical data collected included sociodemographics (age, gender, and ethnicity), comorbidity (cardiovascular disease, liver disease, renal disease, neoplasm, central nervous system disease, chronic pulmonary disease, and diabetes mellitus), prior exposure to the medical device (central venous catheterization, endotracheal tube, and indwelling urinary catheter), antibiotics exposure in the preceding 7 days, a history of surgical procedures (including abdominal, trauma, neuro, vascular and thoracic surgery) and prior anti-cancer chemotherapies.

Data were obtained electronically from medical and pharmacy records maintained by the hospitals and then manually summarized in a paper-based questionnaire by the coordinating physicians.

VRE screening and identification

The screening culture was performed by streaking a rectal swab in trilinear method onto an esculin agar medium containing vancomycin (6 μg/mL) followed by incubation at 37 °C for 24 or 48 h. Suspicious single colonies were transferred to 5% sheep blood-enriched Columbia agar plates containing vancomycin (6 μg/mL) for 24—48 h for purification culture. Then the bacterial isolates were identified by the Analytical Profile Index system (API Rapid ID 32 strip [bioMérieux]) and 16S ribosomal RNA (rRNA) analysis. Total DNA as the template for polymerase chain reaction (PCR) was extracted from the bacterial isolates using a DNA extraction Kit (Bacterial Genome DNA Extraction Kit; TIANGEN, Catalog no. DP 302) according to the manufacturer's guidelines (Bacterial Genome DNA Extraction Handbook DP190814) and stored at −20 °C. The product was then subjected to PCR to amplify the nearly complete 16S rRNA gene with universal primers 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTAC

GACTT-3′), followed by Sanger sequencing. The sequence similarity was determined using the BLAST program from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST). PCR and sequencing were also used to determine the presence of glycopeptide resistance genes *vanA*, *B*, and *M* using a previously published protocol.[9]

Antimicrobial susceptibility testing

The minimal inhibitory concentrations (MICs) were determined by the agar dilution method or the broth microdilution method (tigecycline and daptomycin), and interpretative breakpoint criteria were in accordance with those recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines,[10,11] with the exception of tigecycline, which was in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST).[12] A total of 14 antimicrobial agents were tested: ampicillin, vancomycin, teicoplanin, erythromycin, tetracycline, minocycline, tigecycline, ciprofloxacin, linezolid, levofloxacin, nitrofurantoin, rifampin, chloramphenicol, and daptomycin. Bacterial suspensions ($>10^4$ CFU of each bacterium) were obtained by a multipoint inoculator. *Enterococcus faecalis* ATCC 29212 was used as quality control reference strains.

Molecular epidemiology

E. faecium multilocus sequence typing (MLST) was conducted using previously published methods.[13] Sequence types (STs) were determined using the MLST database (http://efaecium.mlst.net) and genetic relatedness was explored using the BURST algorithm.

Statistical approach

We used frequencies and percentages for categorical variables and medians and interquartile ranges (IQR) for continuous variables for descriptive analyses. The differences in characteristics between VRE carrier patients and VRE non-carrier patients were compared using the chi-square test or Fisher's exact test where appropriate. Difference in age was compared using the Mann–Whitney U test, as the data did not follow a normal distribution. Factors that had significance at a $p < 0.05$ level in the univariate analysis were considered candidates for the building of logistic regression multivariable model. All reported p values were two-tailed with an α level of 0.05. All data were analyzed using Statistical Package for the Social Sciences 26.0 software (SPSS, Inc., Chicago, IL, USA). Minimum spanning tree (MST) analysis was carried out using the BURST algorithm for related STs between different backgrounds by BioNumerics 7.5 software (Applied Maths, Belgium).

Results

VRE prevalence in ICUs

During the study period, from April 2 to May 1, 2017, a total of 162 patients were admitted to ICUs and they were all screened for rectal carriage with VRE. However, 14 patients were not incorporated into the study, as the related data of the 14 patients' medical records could not be retrieved in time. In the end, 148 patients' information was obtained for analysis.

Participants were predominantly male (n = 90; 60.8%), and the median age was 78 years (IQR, 62–83 years). Of the 148 participants, 46 (31.1%) carried VRE, the majority of which were identified as *E. faecium* (n = 42). PCR analysis revealed that the predominant gene cluster conferring vancomycin resistance among the tested VRE strains was the *vanA* cluster, present in 78.3% of strains (n = 36), while 15.2% of strains (n = 7) harbored the *vanM* cluster and three strains (including one *E. faecalis*, one *Enterococcus casseliflavus* and one *Enterococcus gallinarum*) undetected genotypes. Table 1 summarizes the patients' characteristics.

The prevalence of VRE carriage in six hospitals was 15.4% (6/39), 21.1% (8/38), 14.7% (5/34), 80.0% (12/15), 85.7% (12/14) and 37.5% (3/8), respectively. Apart from hospital D, *vanM*-type VRE was detected in all centers over the study period. There was no VRE outbreak in these six hospitals during the study period.

Antimicrobial susceptibility

A total of 42 VRE*fm* strains were available for antimicrobial susceptibility testing. All 42 strains displayed high levels of resistance to vancomycin, with MICs in the range of 64 to

Table 1 Characteristics of patients and their carried vancomycin-resistant enterococci[a].

Characteristics	Values for patients (n = 148)
Age (yr)	
Median (IQR)	78 (62–83)
Range	25–95
No. (%) of patients >65 yr of age	101 (68.2)
No. (%) of patients by gender	
Male	90 (60.8)
Female	58 (39.2)
No. (%) of patients screened for VRE in the following hospital:	
Hospital A	39 (26.4)
Hospital B	38 (25.7)
Hospital C	34 (23.0)
Hospital D	15 (10.1)
Hospital E	14 (9.5)
Hospital F	8 (5.4)
No. (%) of patients carried VRE	46 (31.1)
No. (%) of patients with VRE of the following type:	
Enterococcus faecium	42 (91.3)
Other	4 (8.7)
No. (%) of patients with VRE of the following genotype:	
vanA gene	36 (78.3)
vanM gene	7 (15.2)

[a] Abbreviations: IQR, interquartile range; VRE, vancomycin-resistant enterococci.

>512 μg/mL. Resistance was found for teicoplanin (50%), ampicillin (100%), erythromycin (83.3%), tetracycline (57.1%), minocycline (31.0%), ciprofloxacin (100%), levofloxacin (100%), nitrofurantoin (59.5%), rifampin (95.2%), chloramphenicol (2.4%), linezolid (0%), daptomycin (0%) and tigecycline (0%). Detailed susceptibilities of VRE strain results are shown in Table 2.

Genetic distribution of isolates

Using seven-locus MLST, all VRE*fm* strains were grouped into 10 STs. The distribution of STs over the study period is depicted in Fig. 1. The predominant ST among the 42 strains was ST78 (n = 20, 47.6%). ST192 (n = 6, 14.3%) was the second most predominant ST. In addition, ST555 and ST789 accounted for four strains (9.5%) each. ST547 and ST922 accounted for two strains (4.8%) each. Finally, four strains were singletons belonging to ST17, ST80, ST343, and ST389, respectively.

Patient factors associated with VRE carriage

Characteristics were compared between the group of VRE carrier patients and VRE non-carrier patients (Table 3). In comparison with VRE non-carrier patients, patients with VRE carriage had a greater proportion of age of >65 years (84.8% vs. 60.8%, $p < 0.05$), more pulmonary infections (76.1% vs. 54.9%, $p < 0.05$), and significant increases in endotracheal tubes (45.7% vs. 23.5%, $p < 0.05$). In comparison with VRE non-carrier patients, patients with VRE carriage had a greater proportion of exposure to third-generation cephalosporins (23.9% vs. 5.9%, $p < 0.05$) and glycopeptides (15.2% vs. 4.9%, $p < 0.05$) in the preceding 7 days. To identify the factors associated with VRE carriage in patients admitted to ICUs, we carried out a multivariate analysis. As shown in Table 4, multivariate logistic regression model analysis showed that the factors associated with VRE carriage were patients >65 years of age (odds ratio [OR], 3.786; 95% confidence interval [CI], 1.402—10.222), and exposure to third-generation cephalosporins in the preceding 7 days (OR, 6.360; 95% CI, 1.873—21.601).

Table 2 Susceptibilities of vancomycin-resistant *Enterococcus faecium* strains to antimicrobial agents[a].

Antibacterial agents	MIC (μg/ml)			
	Range	MIC_{50}	MIC_{90}	R (%)
Vancomycin	64 to > 512	256	512	100
Teicoplanin	0.25 to 128	16	64	50
Linezolid	1 to 4	1	2	0
Daptomycin	0.06 to 2	1	2	0
Tigecycline	0.01 to 0.12	0.06	0.12	0[b]
Ampicillin	128 to > 512	512	>512	100
Erythromycin	<0.06 to >512	512	512	83.3
Tetracycline	0.25 to 256	32	128	57.1
Minocycline	0.03 to 32	8	16	31.0
Ciprofloxacin	16 to > 512	128	512	100
Levofloxacin	32 to 128	64	128	100
Nitrofurantoin	32 to 512	128	256	59.5
Rifampin	<0.06 to 64	16	32	95.2
Chloramphenicol	4 to 64	8	16	2.4

[a] CLSI 2020 breakpoints were applied except tigecycline.
[b] ECUST 2020 breakpoints were applied.

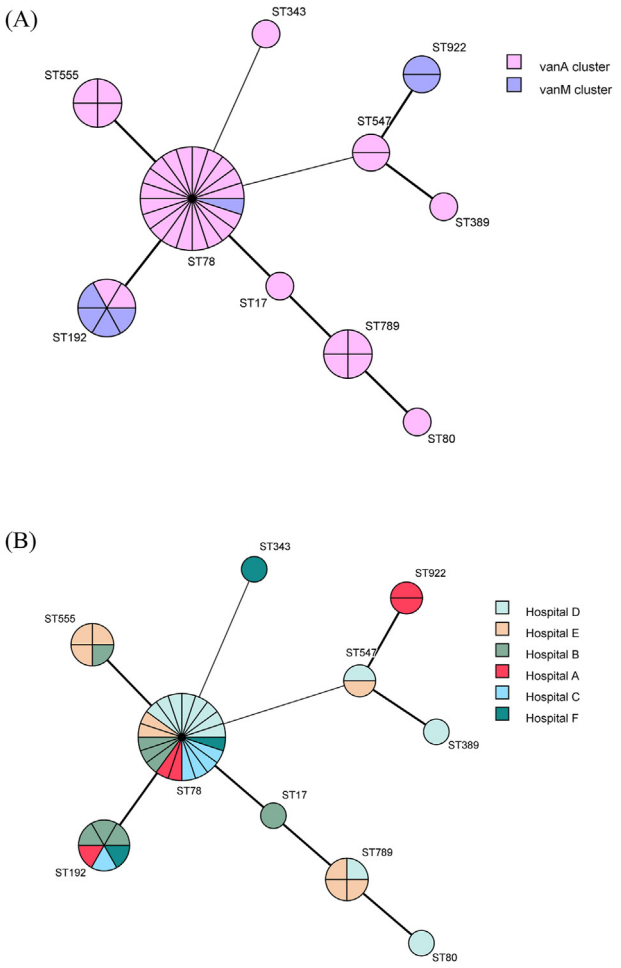

Figure 1. MST analysis of the 42 vancomycin-resistant *Enterococcus faecium* strains generated from MLST data. Each ST is displayed as a circle and the size of the circle denotes the number of strains belonging to that particular ST. Lengths of lines between each circle/ST proportionally demonstrate the number of different alleles. Each circle is labeled with the corresponding ST. Different groups of strains are identified by different colors. Strains were divided into two groups according to *van* genotype (A), and six groups according to the hospital of isolation (B).

Discussion

Enterococcus, a globally important opportunistic pathogen, can be carried in the GI tract for a long period without any symptoms of infection and likewise persist in the hospital environment.[14] *Enterococcus* is inherently resistant to several antimicrobial classes, and over recent decades there has been a significant increase in the rates of acquired antimicrobial resistance (AMR) in *E. faecalis* and *E. faecium*, including VRE.[15,16] Previous studies have demonstrated that enterococcus status at ICU admission was

Table 3 Comparison of the characteristics between groups of VRE carrier patients and VRE non-carrier patients[a].

Characteristic	VRE positive (n = 46; 31.1%)	VRE negative (n = 102; 68.9%)	*p*-value
Demographics			
Median (IQR) age (years)	83 (76–86)	72 (61–83)	0.001[b]
No. (%) of patients >65 years of age	39 (84.8)	62 (60.8)	0.004
No. (%) of male patients	31 (67.4)	59 (57.8)	0.271
Comorbidities			
Median (IQR) CCI	5 (4–5)	4 (3–5)	0.252[b]
No. (%) of patients with CCI of >5	11 (23.9)	23 (22.5)	0.855
No. (%) of patients with the following:			
Cardiovascular disease	12 (26.1)	18 (17.6)	0.237
Chronic pulmonary disease	9 (19.6)	24 (23.5)	0.592
Hepatic dysfunction	3 (6.5)	1 (0.9)	0.089[c]
Renal dysfunction	7 (15.2)	7 (6.7)	0.132[c]
Diabetes	4 (8.7)	12 (11.8)	0.776[c]
No. (%) of patients with the following infection diagnosis:			
Urinary tract infection	4 (8.7)	3 (2.9)	0.204[c]
Intra-abdominal infections	1 (2.2)	3 (2.9)	1.000[c]
Pulmonary infection	35 (76.1)	56 (54.9)	0.014
No. (%) of patients with the following medical devices:			
Central venous catheterization	21 (45.7)	36 (35.3)	0.231
Endotracheal tube	21 (45.7)	24 (23.5)	0.007
Indwelling urinary catheter	35 (76.1)	66 (64.7)	0.169
No. (%) patients with the following type of antibiotic therapy in the past 7 days:			
Second-generation cephalosporins	0 (0)	11 (10.8)	0.018[c]
Third-generation cephalosporins	11 (23.9)	6 (5.9)	0.002
β-Lactam combination agents	15 (32.6)	31 (30.4)	0.787
Carbapenems	14 (30.4)	39 (38.2)	0.360
Glycopeptides	7 (15.2)	5 (4.9)	0.049[c]
Fluoroquinolones	5 (10.9)	6 (5.9)	0.318[c]
Linezolid	4 (8.7)	9 (8.8)	1.000[c]
Nitroimidazoles	2 (4.3)	8 (7.8)	0.725[c]
Other healthcare-associated factors			
Chemotherapy	0 (0)	2 (2.0)	1.000[c]
Surgery	7 (15.2)	40 (39.2)	0.004

[a] Abbreviations: IQR, interquartile range; CCI, Charlson's Comorbidity Index.
[b] Mann–Whitney U test.
[c] Fisher's exact test.

associated with risk for death or all-cause infection, and the gastrointestinal microbiome may have a role in risk stratification and early diagnosis of ICU infections.[8] There is a need for active surveillance to better prevent the emergence and dissemination of VRE.

Table 4 Multivariable logistic regression of factors associated with VRE carriage.

Characteristic	OR (95% CI)	*p*-value
Patients >65 years of age	3.786 (1.402–10.222)	0.009
Pulmonary infection	1.590 (0.671–3.768)	0.292
Endotracheal tube	2.287 (1.001–5.222)	0.050
Third-generation cephalosporins use in the preceding 7 days	6.360 (1.873–21.601)	0.003
Glycopeptides use in the preceding 7 days	3.192 (0.819–12.431)	0.094

Abbreviations: OR, odds ratio; CI, confidence interval.

We performed a multicenter study investigating the prevalence of gastrointestinal VRE carriage in hospital patients in Beijing and analyzing the molecular epidemiology of VRE. Although this study was performed on a selection of hospital patients, i.e., patients admitted to ICUs, the results are important since these patients are especially prone to colonization and (subsequent) infection. We reported a mean proportion of 31.1% (46 of 148) for culture-positive intestinal carriage of VRE in ICU, and the result of the high prevalence of VRE carriage was surprising. To our knowledge, this is the largest study investigating the prevalence of VRE carriage in ICUs in mainland China, and the results are further strengthened by the multicenter design.

Several previous studies have reported a prevalence of VRE carriage on ICU admission ranging between 2.5% and 40%.[17–19] In this study, we found the overall prevalence of VRE carriage at ICU was markedly high. Moreover, there was a huge difference in the isolation rates of VRE among the six hospitals, and the lowest isolation rate was 14.7% (5/34) but the highest rate up to 85.7% (12/14). Hospital variations are

not unusual for drug-resistant bacteria, as different compliance with isolation practices, infection control, and antibiotic stewardship programs that affect behavior among the health care personnel, account for this difference. In addition, the variations may be related to the patients' characteristics prior to ICU admission, as well as, the limited sample size in part of the hospitals.[20,21] Altogether, the incidence of VRE carriage was comparatively high in Beijing and might be neglected before. The previous study has indicated that pathogens can be cultured from stool or swabs that predict specific infections, and VRE colonization has been established as a risk factor for subsequent infection.[4]

E. faecium (42/46) predominated among the isolated VRE in this study. Among the tested VRE, 36 strains harbored *vanA* resistance gene and 7 strains carried *vanM* gene, and there was no *vanB* gene detected. Glycopeptide resistance in enterococci is mediated by *van* gene clusters, among which *vanA* and *vanB* are the most commonly reported worldwide.[22–25] In 2006, *vanM* was first reported as a new and prevalent resistance determinant in clinical enterococci in China.[29] Subsequently, *vanM*-type VRE has spread rapidly around the country, especially in the cities of Shanghai and Hangzhou.[26,27] Epidemiology data for strains with *vanM* gene remain rare in Beijing, and previous results were limited to single-center investigations. In this study, *vanM*-type VRE was detected in almost every participating hospital, thus suggesting that *vanM* gene plays an important role in vancomycin resistance and *van* gene dissemination in Beijing. Despite this, our study showed that the *vanA* gene still was the dominant resistance determinant in enterococcus in Beijing. Generally, *vanA* genotype is characterized by an acquired high-level of resistance to both vancomycin and teicoplanin, called VanA phenotype. The *vanB* genotype is characterized by variable acquired levels of resistance to vancomycin, but not to teicoplanin, called VanB phenotype.[1,28] Many studies have reported the emergence of VanB phenotype-*vanA* genotype VRE.[28] In the present study, 15 isolates were VanB phenotype-*vanA* genotype VRE*fm*. Most of the isolated VRE were resistant to several kinds of antimicrobial agents, and they belong to multi-drug resistant (MDR) (resistant to three or more antimicrobial classes). Fortunately, linezolid, daptomycin, and tigecycline demonstrated complete *in vitro* activity against these strains.

MLST typing displayed two dominant STs, including ST78, and ST192, which were frequently identified in VRE*fm* strains in China, and the most common ST78 occurred in each participating hospital.[13,29,30] All but one *E. faecium* strain (ST922) in this study belonged to clonal complexes (CC) 17, which represents a lineage of a virulent VRE hospital clone that has been observed worldwide.[2,31–36] The results described here clearly suggest a clonal spread of the highly adapted and resistant lineage CC17 of *E. faecium* strains among hospitals. In addition, two *E. faecium* strains both carrying *vanM* resistance gene belonged to ST922, and they were detected in the same hospital. The fact that ST922 was not included in CC17, and no previous study has found ST922 strains carrying *vanM* gene in China, goes some way to suggest a horizontal transfer of *van* cluster among *E. faecium* strains. Overall, these data indicate that clonal expansion and horizontal transfer of resistance genes have contributed to VRE increased prevalence in hospitals.

We found that clinical and demographic were different between groups of VRE carrier patients and VRE non-carrier patients. This suggests that some factors might be associated with VRE carriage. In multivariate analysis, patients aged >65 years were associated with VRE carriage. This is easily explainable by the fact that older adults have lower immunity than younger people which is in favor of VRE acquisition. Our study also showed the recent third-generation cephalosporins use was associated with VRE carriage. This phenomenon may be attributed to the third-generation cephalosporins disrupting the normal gut flora, thus the likelihood of VRE carriage may have increased.

The study has some limitations. First, the study period was relatively short, and thus the population was not large enough, which may influence the determination of factors for VRE carriage. Second, the rectal surveillance swab was not applied to patients at the time of admission but every Tuesday morning during the study period, which lead to the VRE status of some patients being unclear while ICU admission. Another limitation is the problem of the screening process. We evaluated the VRE colonization status by way of phenotype identification, and thus some patients carrying VRE might be neglected and the prevalence of *van* gene might be underestimated.

Conclusion

In conclusion, the overall proportion of VRE carriage in patients admitted to ICUs was markedly high in Beijing. The *vanM* gene has been spread widely but *vanA* gene was the dominant resistance determinant in VRE in Beijing. Clonal expansion and horizontal transmission were both found in VRE strains.

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Ethics approval and consent to participate

The study was approved by the Institutional Review Board of Peking University First Hospital (2020 Research 202). Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements. We make sure to keep patient data confidential and in compliance with the Declaration of Helsinki.

Declaration of competing interest

The authors declare that they have no competing interests.

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