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SHORT REPORT

Genomic Characterization of a Vancomycin-Resistant Strain of *Enterococcus faecium* Harboring a *rep2* Plasmid

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Purpose: In China, vancomycin-resistant enterococci (VRE) was not a common occurrence, and research on the genetic context and transmission mechanism of *vanA*-plasmid was scarce. The aim of this study was to molecularly characterise a vancomycin-resistant *Enterococcus faecium* isolate from a bloodstream infection and determine the genetic environment and delivery pattern of the plasmid carrying vancomycin-resistant gene.

Materials and Methods: In May 2022, a vancomycin-resistant strain of Enterococci was identified during routine screening for VRE bacteria at the First Affiliated Hospital, Zhejiang University School of Medicine. Utilizing matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), the isolate was accurately identified. Antimicrobial susceptibility and whole-genome sequencing (WGS) were employed to perform phenotypic and genomic analysis, respectively. Further bioinformatics analyses was carried out to characterize the *vanA*-bearing plasmid.

Results: The antimicrobial susceptibility test showed that SJ2 strain was resistant to multiple antimicrobials, including ampicillin, benzylpenicillin, ciprofloxacin, erythromycin, levofloxacin, streptomycin, and vancomycin. Whole-genome analysis revealed that SJ2 strain carries several antimicrobial resistance genes and virulence determinants. MLST analysis found that SJ2 strain belongs to an unknown ST type. Plasmid analysis confirmed that the *vanA* gene was located on a variant of ~50 kb *rep2* plasmid.

Conclusion: Our study found that *vanA*-bearing *rep2* plasmid is a potential source of dissemination and outbreak, and continuous surveillance is necessary to control its spread in Hangzhou, China.

Keywords: vancomycin-resistant *Enterococcus faecium*, *vanA*, whole genome sequencing, *rep2*

Introduction

Enterococcus is a genus of Gram-positive, facultative anaerobe and oxidase-negative cocci classified as lactic acid bacteria and belonging to the Enterococcaceae family.[1] Enterococci is a type of bacteria that is often found in the gastrointestinal tract of humans and other mammals.[2,3] It comprises a ubiquitous group of bacteria that are commonly found in the environment and are known to cause infections in humans, especially in healthcare settings.[4] Enterococci emerged as a leading cause of a variety of life-threatening infections in the 1970s.[1] *Enterococcus faecalis* and *Enterococcus faecium* are the primary causative agents of opportunistic infection in humans among the enterococci.[5]

Over the past decades, the increasing antibiotic resistance of *Enterococcus* has been a major concern worldwide.[6,7] The growing problem of vancomycin-resistant enterococcus (VRE) is worrying because of its increasing resistance to antibiotics,

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longer hospital stays, and increased death rates.[8] The effectiveness of some VRE infection control practices is disputed, as newer developments in infection prevention strategies are continually being discovered.[9,10]

Several studies have been conducted to investigate the prevalence of vancomycin-resistant *E. faecium* (VREfm) in clinical settings in China, with considerable variation among the VREfm strains; ST78 and ST192 being the most frequently identified.[11–15] So far, the *vanA* and *vanM* determinants are the primary determinants of resistance to vancomycin. Understanding the dissemination of VREfm strains is important to comprehending the dynamics of how VRE emerges and spreads in hospital environments. The ability of antibiotic resistance plasmids to be horizontally transferred is crucial to the spread of VRE strains.[16–18] However, the genetic context and transmission mechanism of *vanA*-plasmids from *Enterococcus* spp. remains largely uncharacterized. In this study, we report a vancomycin-resistant *E. faecium* SJ2 strain carrying *vanA* from an intensive care medicine (ICU) patient. Whole-genome sequencing (WGS) was employed to unveil the genetic identity of the isolate and the *vanA*-bearing plasmid.

Methods and Materials

Bacterial Isolate

Our routine screening of vancomycin-resistant bacteria in the First Affiliated Hospital, Zhejiang University School of Medicine, uncovered a VRE isolate from an ICU patient (designated as SJ2 strain) in May, 2022. Identification of SJ2 was based on the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker, Bremen, Germany) as described previously.[19,20]

Antimicrobial Susceptibility Testing (AST)

The minimum inhibitory concentration (MIC) was determined using both the agar dilution and broth microdilution methods. Results were interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI) standards, with the exception of colistin and tigecycline, which were interpreted according to the EUCAST clinical breakpoints. *E. faecalis* ATCC strains ATCC 51299 and ATCC 29212 were used as resistant and susceptible controls, respectively.

Whole-Genome Sequencing (WGS)

The total DNA was extracted and purified using the OMEGA Bacterial DNA kit (Omega Bio-tek, Norcross, USA). The DNA was sequenced using both the Illumina NovaSeq 6000 (Illumina, San Diego, CA, USA) and Oxford Nanopore (Oxford Nanopore Technologies, Oxford, UK) platforms as described previously.[21] And the assembly of the complete genome was using Unicycler v0.4.2.[22] The genome was annotated using Prokka v1.14.5.[23]

Data Analysis

The acquired antibiotic resistance genes (ARGs) and plasmid incompatibility types were determined using ResFinder (software version: 4.1; database version: 2022)[24] and PlasmidFinder (software version: 2.0.1; database version: 2021),[25] and the multilocus sequence typing (MLST) was determined by MLST website (https://pubmlst.org/organisms/enterococcus-faecium). The detection of virulence factors was performed with VFDB 2022.[26] The circular map of the plasmid pSJ2_VanA was finally plotted under the BLAST Ring Image Generator (BRIG) 0.95.[27]

Results and Discussion

In January 2022, a bloodstream isolate, SJ2, was recovered from a 76-year-old ICU patient and identified as faecium by MALDI-TOF-MS. This patient had been hospitalized from October 25, 2022 to February 7, 2022, for a total of 4 months. It has been confirmed by earlier studies that *Enterococci* are a major factor in infections among critically ill patients.[28,29] Certain experts contend that the utilization of broad-spectrum antibiotics, coupled with the disruption of the gut microbiota in critically ill patients, can lead to a decrease in the body's natural defenses against infection, thereby increasing the possibility of intestinal colonization by VRE and the development of bloodstream infection.[30] It is possible that the patient in this study contracted VRE infection during his four-month hospitalization. The MIC results indicated that the SJ2 isolate was resistant to vancomycin, with an MIC of greater than 32 mg/L (Table S1).

This resulted in the classification of the isolate as VREfm. Results showed that SJ2 was resistant to various antimicrobials, including but not limited to ampicillin, benzylpenicillin, ciprofloxacin, erythromycin, levofloxacin, and streptomycin. This strain exhibited susceptibility to five antibiotics, including gentamicin, linezolid, quinupristin/dalfopristin, tetracycline, and tigecycline. The patient reacted positively to the linezolid medication, as it proved to be an effective antibiotic.

After de novo assembly of the raw reads, by searching against the pubMLST database, VREfm SJ2 was classified as ST2237, a never reported type with alleles *adk* (3), *atpA* (5), *ddl* (5), *gdh* (1), *gyd* (3), *pstG* (3), and *purK* (3), which belongs to the CC17 of *E. faecium*. Previous studies found the high frequency of intestinal VRE carriage in intensive care patients in Beijing, China, which revealed the predominant ST was ST78, and other STs were also sporadically identified.[12,31] An epidemiological study conducted from 2013 to 2018 revealed that ST78, ST192 and ST570 were the predominant strains of VREfm in Nanjing, China, and their prevalence had been decreasing annually.[14] Molecular analysis revealed that most VRE isolates carried the *vanA* gene, followed by *vanM*. Vancomycin resistance is mainly attributed to these genes.[12,14,32]

SJ2 encompasses the entire sequence of the chromosome alongside five plasmids. Among them, the chromosome of 2839384 bp in length, a plasmid carrying *vanA* of 49,961 bp in length and other four plasmids. The antimicrobial resistance genes and virulence genes are distributed as shown in Table S2. Resfinder's results indicated that strain SJ2 possessed multiple antimicrobial resistance genes, including *msr, vanA, vanX, vanH, erm, ant (6)-Ia*, and *aph (3')-III*, which is in line with the resistance profiles (Table S1). SJ2 was found to contain four virulence genes that encoded multiple functions, such as collagen-binding adhesin (*acm*), cell wall adhesin (*efaAfm*), *enterococcal* surface protein (*espfm*), and hyaluronidase (*hylEfm*). Instances of nosocomial infections caused by VREfm are on the rise, leading to a scarcity of treatments. This is likely a result of the escalating antibiotic resistance and biofilm-related infections. These infections are associated with adhesion, biofilm formation, and collagen binding in *E. faecium*.[33,34] The virulent factors found in this work increased the likelihood of strain SJ2 being virulent.

In silico analysis confirmed the existence of ~50 kb *rep2* pSJ2_VanA (Figure 1). According to the annotation, the pSJ2_VanA plasmid carried the *vanA* gene cluster, which was carried on the Tn*1546*-like family. Fifteen insertion sequences were identified in this plasmid, including IS*1216E*, IS*1542*, IS*1062*, IS*Efm2*, IS*Efa11*, and IS*1485*. IS*1216* was the most frequently occurring element among the others. The genome alignment of the complete sequence of pSJ2_VanA with the NCBI GenBank database found that pSJ2_VanA is most similar to the *E. faecium* ~40 kb VRE3370 plasmid (CP103318.1), sharing 100% identity and over 67% coverage. Additionally, pSJ2_VanA shares 100% identity and over 56% coverage with the ~82 kb p27 plasmid (CP041258.1), and 100% identity and over 73% coverage with the ~64 kb E6020 plasmid: 3 (LR536642.1). These findings suggest that pSJ2_VanA is a variant of *rep2* plasmid. The *vanA* gene was found to be genetically and environmentally conserved in structure, with *vanY*-IS*1216*-*vanX*-*vanA*-*vanH* (Figure 2). The fact that these three *rep2* plasmids encoding VanA were identified from Australia, the USA, and the Netherlands. Previous research found that *rep2*-type *vanA*-carrying plasmids are present in both Germany[35] and Hangzhou, China.[15] This study's findings, in combination with data that demonstrate the plasmid's wide prevalence, particularly in Hangzhou.

In general, a vancomycin-resistant *E. faecium* strain (SJ2) carrying the vanA-encoding plasmid (rep2) was isolated from a blood infection in Hangzhou, China. The phenotypic and genetic characteristics of isolate SJ2 were further elucidated. The scope of our study was restricted to a single strain that was isolated and characterized. It is essential that additional studies are conducted with VanA-producing isolates to identify reservoirs and track the transmission dynamics of *vanA* genes in Hangzhou, China. This work highlighted the necessity of monitoring vanA-bearing plasmids in the clinic on an ongoing basis to prevent their potential spread in this area.

Nucleotide Sequence Accession Numbers

The whole-genome sequence of the was submitted to GenBank under the following BioProject number: PRJNA896556.

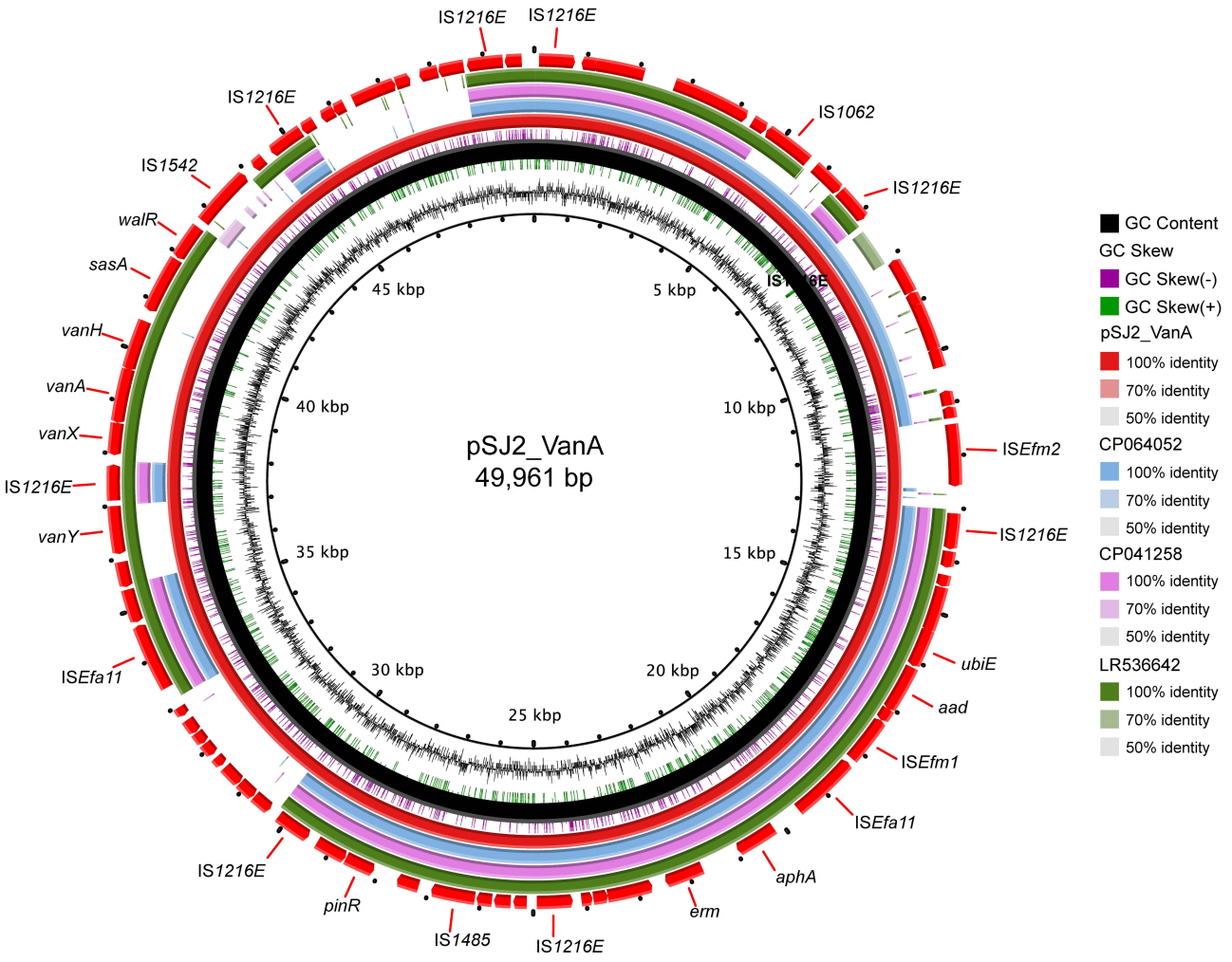

Figure 1 Circular genome alignment of the *vanA*-encoding *rep2* pSJ2_VanA plasmid with three closely related plasmids (CP103318.1, CP041258.1, and LR536642.1). ORFs are portrayed by arrows. The alignment of the plasmids was performed and visualized by BLAST ring image generator (BRIG) software.

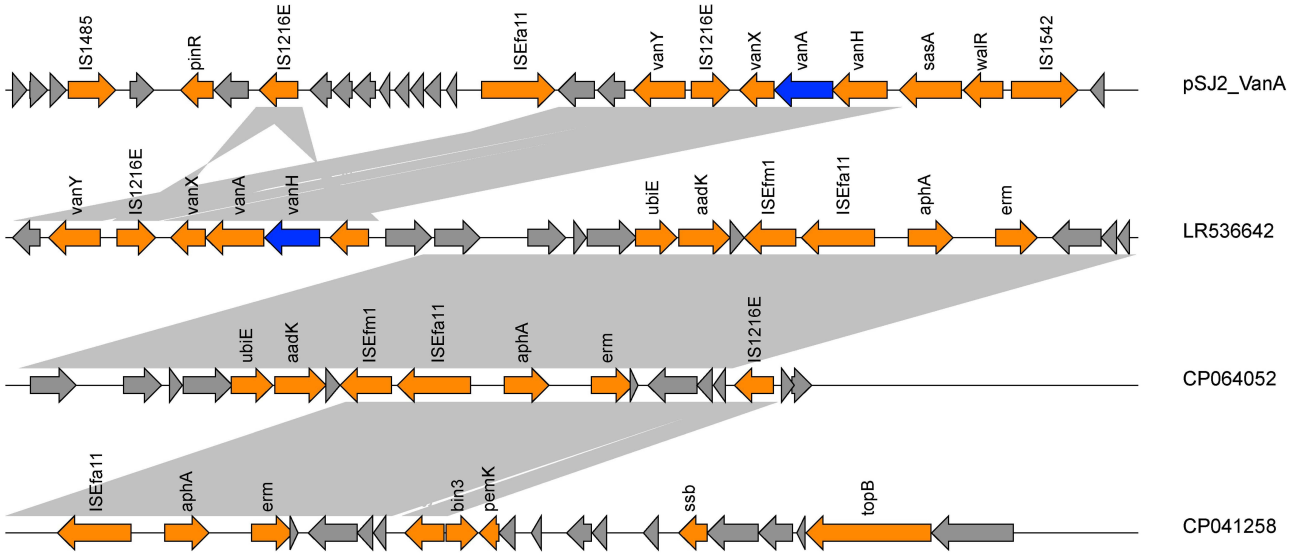

Figure 2 Genetic environment of the *vanA* gene in isolate *Enterococcus faecium* SJ2. Colored arrows represent ORFs, and the shaded region indicates sequence similarity. The arrows in the figure indicate the direction of transcription for the different genes shown in different colors. The ARGs are indicated in blue. The Easyfig program was applied in comparative genomics.

Abbreviation: IS, insertion sequence.

Ethics Approval and Consent to Participate

This study was conducted following the Declaration of Helsinki and obtained approval from the clinical research ethics committee of the First Affiliated Hospital, Zhejiang University School of Medicine [number 2020-IIT-660]. The patient provided written informed consent to allow the case details to be published.

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Disclosure

The authors report no conflicts of interest in this work.

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