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Research Article

Vancomycin-Variable *Enterococcus faecium* ST1424: Containing the *vanA* gene with the *vanS*, *vanR* and *vanH* gene without *vanX*, *vanY* and *vanZ* Gene in a Clinical Isolate in Australia

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Abstract

In this article we describe a new vancomycin variable *Enterococcus faecium* clone (VVE) isolated from a blood cultured specimen in central Sydney, Australia. The *E. faecium* isolated contained the *vanA* gene, and was susceptible to vancomycin and teicoplanin in vitro. Whole genomic sequencing (WGS) revealed that this isolate contained *vanR, vanS, vanH* and *vanA* genes but did not contain, *vanX, vanY,* or *vanZ* genes. Multi-locus sequence (MLTS) revealed the isolate to be E. faecium ST1424. The *E. faecium* was susceptible to daptomycin. Newly emerging VVE sequence types of *E. faecium* such as the VVE ST1424 described poses challenges to the control of health associated infections and infection control practices in hospitals.

Keywords: Vancomycin Variable *Enterococcus faecium; vanR; vanS; vanA; vanX; vanY; vanZ*; ST1424; ST1421; VVE; VRE

Introduction

Enterococci infections, particularly those caused by antibiotic-resistant strains, are associated with significant mortality and have been designated as a high priority pathogen by the World Health Organization [1]. Glycopepetide-susceptible *vanA*-bearing *E. faecium* have been described previously, first isolated in Canada in 2011 [2]. Clinical cases of isolation have been reported in the literature, their predominant mechanism of resistance is encoded on transposon Tn1546 which is carried on a plasmid and includes the two component regulator gene (*vanRS*) and a gene cluster (*vanHAXYZ*) encoding the resistant mechanisms [3]. Biochemically, the *vanA* gene encodes a ligase that catalyzes the linkage of D-alanine and D-lactate, which replaces the typical D-alanine D-alanine precursor for peptidoglycan, therefore decreasing the affinity of glycopeptide antibiotics for their target sites [3,4]. VVE previously examined did not contain the *vanR* or *vanS* genes, which are believed to be essential to the expression of the *vanH*AX gene cluster. The *vanS* gene is a membrane-bound histidine kinase that senses the presence of vancomycin, resulting in ATP-dependent autophosphorylation. The phosphor-*vanS* then transfers phosphate to the response regulator *vanR* in the cytoplasm. Phospho-*vanR* binds to intergenic region upstream of the *vanH*AX operon and facilitates transcription, resulting in drug resistances [3-5]. While *vanH*, *vanA*, *vanX, vanY* are associated with vancomycin resistance, *vanZ* is associated with teicoplanin resistance. Thaker., *et al*. reported the underlaying molecular mechanism for the variable resistance phenotype in VVE strains and showed that various changes to the region upstream of *vanH*AX can result in the constitutive expression of gene cassette, conferring resistance in the absence of *vanR*S genes [5]. Current studies have shown that a complete *vanH*/*vanA*/*vanX* operon is required for the development of a vancomycin-resistant phenotype [6,7].

This mechanism of resistance in enterococci is a concern since VVE are vancomycin-susceptible enterococci containing a silenced *vanA* gene cluster that has been shown to revert to a resistant phenotype through genetic rearrangements occurring at low frequencies [6]. The presence of such VVEs in hospitals has clinical implications, the resistant subpopulation may be selected for during antibiotic exposure, causing therapeutic failure [5,8,9]. This article describes a newly emerging VVE sequence type of *E. faecium vanA* ST1424 which may pose challenges to the control of health associated infections and infection control practices.

Materials and Methods

Bacterial strain

The *Enterococcus faecium* clone (VVE) described here (MB20075945) was isolated at Concord Hospital from a blood cultured specimen in central Sydney, Australia.

Bacterial identification

Identification was performed by Matrix-Assisted Laser Desorption-Ionization Time of Flight mass spectrometry (MALDI-TOF MS Biotyper sirius one) (Bruker, Germany) in accordance with the manufacture instructions.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of *the E. faecium* isolates was determined by the automated VITEK 2 XL microbiology analyzer (BioMérieux Inc.) using antimicrobial susceptibility testing (AST) AST-P643 card (BioMérieux, Australia). Results were interpreted as susceptible or resistant based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [10]. The isolate was also examined using the 5µg Vancomycin disc as described by EUCAST. The isolate was categorized as resistant if the zone diameter was less than 12 mm. Resistance was suspected when the vancomycin zone edge was fuzzy or colonies were growing within the inhibition zone. *Enterococcus faecalis* ATCC 29212 vancomycin susceptible and *Enterococcus faecalis* ATCC 51299 *Van*B vancomycin resistant were used as controls.

Multiplex PCR

DNA extraction was performed with single colonies into 200µL sterile saline and boiled for 10 minutes at 95°C. After boiling, the liquid contained bacteria was centrifuged for 30 seconds with 12000r/sec. The supernatant was used as the template for real-time PCR as previously described by Merlino., *et al*. [11,12]. *Enterococcus faecalis* ATCC 29212 vancomycin susceptible and *Enterococcus faecalis* ATCC 51299 *Van*B vancomycin resistant were used as controls.

Multilocus sequence typing and whole genome sequencing

MLST and WGS was performed by a reference laboratory at Royal Prince Alfred Hospital. Seven housekeeping genes （adk, atpA, ddl, gyd, gdh, purK, and pstS) were selected for the MLST analysis of the *E. faecium* isolate (Table 1). PCR conditions used for MLST and WGS (illumina MiSeq) were performed as previously described [13].

Table 1: Resistome of the VVE *E. faecium* isolate from the whole genome sequencing and the results of the multi-locus sequencing results of the VVE *E. faecium* isolate MB20075945.

Antibiotic class: AG: aminoglycosides, MA: macrolides, OTH: various antibiotics, TE: tetracyclines, TMP: trimethoprim, VAN: vancomycin. Gene present at >90% identity (red) or >60% (orange) coverage.

Resistome	aacA-ENT1	ant(9)-Ia	erm(A)	erm(B)	msr(C)	aph(3')-IIIa	eat(A)	sat4	tet(M)	dfrG	vanA	vanH-A	vanR-A	vanS-A
Antibiotic Class	AG	AG	MA	MA	MA	OTH	OTH	OTH	TE	TMP	VAN	VAN	VAN	VAN
MB20075945	100	100	100	100	100	100	100	47	100	100	100	100	100	100

MLST	Scheme	ST	atpA	ddl	gdh	purK	gyd	pstS	adk
MB20075945	E. faecium	1424	9	1	1	1	12	0	1

Results

Enterococcus faecium was isolated from a blood cultured specimen on horse blood agar and identified by MALDI-TOF with a score of 2.53. Molecular investigation of the *Enterococcus faecium* was found to contain the *vanA* gene, however the isolate was susceptible to vancomycin and teicoplanin *in vitro* on the Vitek 2 XL [6]. The isolate was further examined using the 5 µg Vancomycin disc as described by EUCAST. The isolate was categorized as susceptible with a zone diameter of greater than 12mm. No resistance was suspected since the vancomycin zone edge was not fuzzy and no colonies were growing within the inhibition zone. Further genomic investigations of the *Enterococcus faecium* containing the *vanA* gene by whole genomic sequencing (illumina MiSeq) revealed that this isolate contained *vanR*, *vanS*, *vanH* and *vanA* genes but did not contain, *vanX*, *vanY*, or *vanZ* genes. The resistome of the VVE *E. faecium* isolate from whole genome sequencing is shown in table 1. The isolate was multi-locus sequenced and revealed to be *E. faecium* ST1424. The *Enterococcus faecium* was susceptible to daptomycin. The *Enterococcus faecium* was not isolated again from blood cultures or any rectal swabs once daptomycin was used in therapy.

Discussion and Conclusion

Herein, we report a VVE ST1424 containing *vanR*, *vanS*, *vanH* and *vanA* genes and the deletion of *vanX*, *vanY*, and *vanZ* genes. Therefore, confirming that expression of vancomycin resistance in some VVE may not be completely dependent on the absence of the *vanS* and *vanR* genes. Further genome analysis of our clinical isolate also revealed partial deletion of the *vanA* gene (83bp deletion; 92% coverage). The *vanA* gene present in this isolate is truncated by insertion of IS1216E element together with a complete copy of the *vanH* gene of the assembled genome sequence (figure 1).

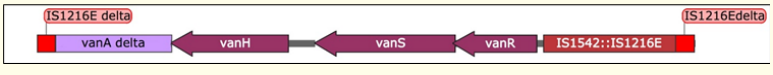

Figure 1: The *vanA* gene present in the isolate MB20075945 truncated by insertion of IS1216E element together with a complete copy of the *vanH, vanS* and *vanR* gene.

While in Australia VVE ST1421 has been extensively studied, very little is known about VVE ST1424 [6]. Further research is required involving both in *vitro* and in *vivo* studies to determine whether vancomycin exposure induces resistances to vancomycin in VVE strains with both *vanS* and *vanR* and partial deletion of the *vanX*, *vanY*, and *vanZ*. Studies in Australia with VVE ST1421 has shown that *vanX* a D-Ala-DAla dipeptidase is required for vancomycin resistance, and the deletion in *vanX* results the VVE phenotypically susceptible to vancomycin [6,8]. VVE ST1424 unlike VVE ST1421 has an additional deletion of both *vanY* and *vanZ* and the presence of both *vanS* and *vanR*. Both *vanS* and *vanR* genes have been found in *vanA* VRE but seldom in VVE susceptible isolates such as ST1421. The potential for vancomycin resistance to rise following vancomycin exposure creates a risk of treatment failure in such isolates. Our initial attempt failed to grow the organism in different concentrations of vancomycin of commercial chromogenic culture media containing 4 µg/ml (Brilliance VRE agar, Thermofisher-Australia) and 8 µg/ml (CHROMID VRE agar, Edwards – Australia) of vancomycin, concentrations used by many commercial companies to detect VRE in hospital infection control screening. Transmission is a major issue with such VVE isolates, the ability of the partial described *vanA* gene being transmitted in enterococci from one patient to another in a hospital setting without infection control detection.

Newly emerging VVE sequence types of *E. faecium* such as VVE ST1424 pose challenges to the control of health associated infections in hospitals an area that requires further investigation in clinical settings and infection control [8].

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Conflict of Interest

The authors state no conflicts of interest exist.

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