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Whole Genome Sequencing of Vancomycin Resistant *Enterococcus faecium* Isolated from Saudi Arabia

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Enterococcusfaecium **are one of the most prevalent species cultured from humans andthey have become increasingly common cause of infections in the hospital settings globally. The objective of current study was to characterize26** ***E. faecium*** **isolatescollected from different patients attending MaternityHospital in Ha'il, Saudi Arabia.The bacterial isolates were identified by MALDI-TOF-MS and the antibiotic susceptibility was performed by Microscan. Whole genome sequencing of a single vancomycin resistant** ***E. faecium*****(VRE) was performed using MiSeq. The results of antimicrobial susceptibility revealed that, 99%, 90%, 83% and 73%of isolates were resistant to Clindamycin, Gentamicin, Oxacillin and Tetracycline respectively. One(3%) among 26*E. faecium*isolates was found to produce resistance tovancomycin. The WGS analysis of VRE showed that it belonged to ST280 and was found to harbor*vanB* gene cassette.This is the first report of VRE from the Ha'il region of Saudi Arabia. VRE may act as a reservoir for multidrug resistant genes and other important virulence factors that favor the dissemination of antimicrobial resistance. Therefore, the surveillance studies to prevent dissemination of VRE shall be implemented in the healthcare facilities all across the Saudi Arabia.**

Keywords: VRE; multi-drug resistant; *vanB*; whole genome sequencing; virulence.

Enterococci arecommensals of the gastrointestinal tract, prevalent in environmental, human and nosocomial settings.Enterococciare equipped with the capabilities to cause infections in the patients within the healthcare facilities; for example the patients with lowerimmune system and those with serious injuries and trauma, particularly if the patient has previously been treated with antibiotics[1-3]. *Enterococcus faecalis* and *Enterococcus faecium* are the two species involved in human nosocomial infections compared to other members of the genus enterococci[4]. The *E. faecium* has been proved to cause a number of infections such as, bacteremia, UTI, intra-abdominal infections, skin and soft tissue infections and endocarditis[5-9]. In addition of harboring virulence genes, the development of multi-drug resistance among enterococci further complicates thesituation regarding the treatment options and poses a challenge to healthcare professionals. The development of vancomycin-resistance in *Enterococcus faecium* (VRE) in

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1986 followed by the dissemination of this highly resistant and virulent pathogen globally is one of the most challenging aspects of antimicrobial resistance among enterococci[10]. Extensive use of vancomycin within the hospitals worldwide is considered to be the major factor for the spread of nosocomial VRE[10, 11]. Vancomycin,resistance (intrinsic and acquired) among enterococcihas been studied in detail. Eight types of acquired vancomycin resistance (vanA-vanN) have been reported among enterococci so far, with vanA being the most prevalent genotype worldwide followed by vanB[12]. The difference between expression levels of vancomycin resistance vary and are found to below in VanB type strains and high in VanA type strains. Because of the low expression vancomycin resistance levels, VanB type strains are characterized by resistance to vancomycin and susceptibility to other glycopeptides like teicoplanin.VanA strains are found to be resistant to all glycopeptides due to the higher expression of vancomycin resistance type. During recent years, there is a significant increase in vanB genotype *E. faecium* colonization and infections in many European countries[13, 14]. Among these outbreaks, a recent outbreak of VanB strains in Sweden reportedtransferable pRUM-like plasmids harboringvanB2-Tn5382 elements[15]. Furthermore, a mobile genetic element called transposon1546 (Tn1546), which is involved in VanA-type *E. faecium* vancomycin resistance was reported by French researchers in 1986[16]. Subsequently, this transposon was found to be present in enterococci from humans, animals and the environment[16-19].

The molecular characterizations of vancomycin resistance among *E. faecium* have been reportedin several studies from Saudi Arabia. However, none of the study has been reported from Ha'il region, therefore the current study aimed to characterize vancomycin resistant *E. faecium* isolated from patients attending Maternity Hospital, Ha'il Saudi Arabia during 2014.

MATERIALS AND METHODS

Bacterial isolates

In this study, 26-*E. faecium*bacterial strains were cultured from clinical specimens collected from patients attending Maternity hospital, Ha'il, Saudi Arabia. The specimens were collected from, axilla (15.4%), blood (11.5%), fistula (23.2%), groin (30.8%), HVS (3.8%), nose (3.8%) and urine (11.5%).

Identification by MALDI-TOF-MS

MALDI-TOF-MS (Bruker Daltonics, Germany), one of the most advanced automated systems was used for identification of bacterial strains[20]. In this method, a fresh bacterial colony from overnight culture was smeared on target plate draped with 1 ìl of a saturated a-cyano-4-hydroxy-cinnamic acid (HCCA) matrix solution in 50% acetonitrile-2.5% trifluoroacetic acid (Bruker Daltonics) with the help of a sterile toothpick. The target plate was kept at room temperature until dry. The plate was loaded in to the machine and the operation was run. The identification of bacterial strains was performed by using MALDI Biotyper software package (version 3.0).

Identification and Antibiotic susceptibility by Microscan

Microscan walkaway (Siemens Healthcare Diagnostics, Sacramento, CA, USA); an automated system used for bacterial identification and antibiotic susceptibility test was used for confirmation of identification and antimicrobial susceptibility of the bacterial strains. In this method, a small portion of a well isolated colony was taken and added to a Gram-positive Microscan combo panel. The panel was loaded into the Microscan walkaway machine according to the manufacturer's protocol. Results were available after 24- 48 hrs.

Whole Genome Sequencing

The sequencing of the bacterial genome for detection of antibiotic resistant genes, virulence factors, plasmids and MLST types was performed by using Illumina methodology using NextEra kit for library preparation[21]. The fasta files of the sequences were used for the analysis and the ResFinder web server (www.genomicepidemiology.org)and Basespace from Illumina was used to identify acquired antimicrobial resistance genes, MLST types and the presence of different plasmids in the WGS data, using a threshold of 98% identity.

RESULTS

In this study, the characterization of 26-*E. faecium* isolates collected from the patients attending a hospital in Ha'il region of Saudi

Arabia was performed. The antibiotic susceptibility results (Table1) revealed that 99%, 90%, 83% and 73% of the *E. faecium* isolates were resistant to Clindamycin, Gentamicin, Oxacillin and Tetracycline respectively. Additionally, 3.8% (1/26) *E. faecium* were found to be vancomycin resistant. The data revealed that a high percentage of clindamycin, erythromycin, and gentamicin resistance were observed in all *E. faecium* isolates. Additionally, the resistance of VRE to ciprofloxacin, clindamycin, erythromycin, gentamicin, tetracycline and trimethoprim/ sulfamethoxazole was significantly higher in comparison to the vancomycin susceptible *E. faecium* isolates.

The whole genome sequencing results of the sole VRE are presented in Table 2 and Table 3. The data showed that VRE from current study exhibited VanB type of resistance with MLST type 280. Additionally, this VRE revealed the aminoglycosides, macrolides, tetracycline and vancomycin resistant genes. It also exhibited *acm*a virulence factor (collagen binding protein) similar to adhesion-associated protein *EfaA*(*E. faecalis* endocarditis antigen A).

Table 1. Antibiotic susceptibility patterns of 26- *E. faecium*isolates

Antibiotic	Resistance %
Augmentin	3.08
Ampicillin	4.55
Ciprofloxacin	28.79
Clindamycin	98.44
Daptomycin	0
Erythromycin	62.5
Fosfomycin	0
Fucidin	14.06
Gentamicin	89.23
Levofloxacin	25.76
Moxifloxacin	28.79
Nitrofurantoin	3.03
Oxacilin	82.81
Penicillin	7.94
Rifampicin	26.56
Teichoplanin	6.25
Tetracyclinee	72.73
Trimethoprim/Sulfamethoxazole	36.36
Vancomycin	4.69

DISCUSSION

*E. faecium*is a member of normal flora of the gastrointestinal tract of humans and animals. The*E. faecium*are prevalent in human, environmental and healthcare settings, and generally are not virulent[2,3]. Multi-drug resistant *E. faecium* have become increasingly common in the hospital settings and it poses a challenge to clinicians.MDR- *E. faecium* including VRE have been reported worldwide including Saudi Arabia[1, 22-24]. The aim of current research was, molecular characterization including whole genome sequencing of *E. faecium* isolates collected from patients attending a hospital in Ha'il, Saudi Arabia. This study is important because of capability of *E. faecium* specifically VRE to produce outbreaks and severe infections in hospital settings, which put the patients and the staff at a high healthcare risk. The *E. faecium*has been proved to causea number of infections such as, bacteremia, UTI, intra-abdominal infections, skin and soft tissue infections and endocarditis[5-9]. The

Table 2. Sequence Type: ST280 (MLST Scheme: *E. faecium*) of 1- vancomycin resistant *E. faecium*

Gene	% Identity	Alignment Length	DB Allele Length	Gaps	Best Match
adk	100	437	437	0	adk_1
atpa	100	556	556	0	atpa_1
ddl	100	465	465	0	ddl_3
gdh	100	530	530	0	gdh_1
gyd	100	395	395	0	gyd_1
psts	100	583	583	0	psts_1
purk	100	492	492	0	purk_1

Table 3. Different antibiotic resistance genes of vancomycin resistant *E. faecium* using NGS

Resistance gene	% Identity	Allele/ Alignment length	Contig ID	Position in Contig	Phenotype	Accession number
Aph(3')-III	100	795/795	NODE_339_length_2181_COV_140.196243	356..1150	Aminoglycoside resistance	M26832
aadE	100	864/509	NODE_331_length_1301_COV_195.631058	823..1331	Aminoglycoside resistance	KF421157
lnu(B)	99.88	804/804	NODE_171_length_6626_COV_45.661785	1348..2151	Lincosamide resistance	AJ238249
msr(C)	98.99	1479/1479	NODE_363_length_5831_COV_19.333733	1825..3303	Macrolide,Lincosamide and Streptogramin B resistance	AY004350
Erm(B)	100	738/738	NODE_37_length_1642_COV_111.984169	272..1009	Macrolide resistance	AF299292
Tet(M)	96.46	1920/1920	NODE_127_length_20046_COV_45.256859	11316..13235	Tetracycline resistance	EU182585
Tet(L)	100	1377/1377	NODE_127_length_20046_COV_45.256859	13429..14805	Tetracycline resistance	M29725
vanH-B	99.49	972/972	NODE_41_length_30848_COV_24.928553	2001..2972	Vancomycin resistance	AF192329
vanW-B	97.58	828/828	NODE_41_length_30848_COV_24.928553	2969..3796	Vancomycin resistance	AF192329
vanX-B	96.72	609/609	NODE_41_length_30848_COV_24.928553	366..974	Vancomycin resistance	AF192329
vanY-B	100	807/807	NODE_41_length_30848_COV_24.928553	3814..4620	Vancomycin resistance	AF192329
vanS-B	99.85	1344/1344	NODE_41_length_30848_COV_24.928553	4791..6134	Vancomycin resistance	AF192329
vanR-B	99.25	663/663	NODE_41_length_30848_COV_24.928553	6134..6796	Vancomycin resistance	AF192329
vanB	99.13	1029/1029	NODE_41_length_30848_COV_24.928553	980..2008	Vancomycin resistance	AF192329

development of multidrug resistance among these bacterial isolates further complicate the treatment options and one of the most important clinically significant antimicrobial resistance is development of vancomycin-resistant *E. faecium*(VRE).VRE is highly resistant to large number of available antibiotics and this potentially difficult-to-treat pathogen is now being reported all across the world[10]. In our study, molecular characterization of 26-*E. faecium* isolates (including a single VRE) was performed. The antibiotic susceptibility revealed that 99%, 90%, 83% and 73% of the *E. faecium* isolates were resistant to Clindamycin, Gentamicin, and Tetracycline respectively. Additionally, 3.8% (1/26) *E. faecium* were found to be vancomycin resistant. The data revealed that a high percentage of clindamycin, erythromycin, and gentamicin resistance were observed in all *E. faecium* isolates. Additionally, the resistance of VRE to ciprofloxacin, clindamycin, erythromycin, gentamicin, tetracycline and trimethoprim/ sulfamethoxazole was significantly higher in comparison to the vancomycin susceptible *E. faecium* isolates.Although, there are several studies about the molecular characterization and prevalence of VRE conducted in Saudi Arabia, there is no report of prevalence of VRE from Ha'il region. The studies conducted in this aspect used PCR, MLST and PFGEapproaches[22-24], with a recent paper from Makkah region using the whole genome sequencing[1]. As per our knowledge we report the first VRE from Ha'il region. We performed the characterization of the only VRE in our study by whole genome sequencing using MiSeq platform. The whole genome sequencing result of *E. faecium* revealed that a genome size of 2861776 bp with 874 contigs was successfully sequenced. The MLST data revealed that the VRE from our study belong to ST type 280. MLST 280 is part of cluster of *E. faecium* MLST genotype called clonal complex CC17, which has been found to be associated with global nosocomial infections[22-25]. Additionally, the res finder showed that our VRE contained the genes which exhibited the resistance to aminoglycosides, macrolides, tetracycline and vancomycin. Furthermore, the result of current study showed that the VRE harbored *vanH-B, vanW-B, vanX-B, vanY-B, vanS-B vanR-B and van-B genes*, which are known to confer vancomycin resistance.VanB type of VRE has been previously reported from Saudi Arabia[24]. The vanB-type genes have been found to confer resistance to vancomycin and not to teicoplanin in comparison to vanA-type genes which confer resistance to both teicoplanin and vancomycin. Thus, the prevalence and dissemination of resistant enterococci, specifically VRE is a challenging health care problem faced by health care professionals in all geographical areas.

CONCLUSION

This is the first report of VRE from the Ha'il region of Saudi Arabia. VRE may act as a reservoir for multidrug resistant genes and other important virulence factors that favor the dissemination of antimicrobial resistance. Therefore, the surveillance studies to prevent the dissemination of VRE shall be implemented in the healthcare facilities all across the Saudi Arabia.

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