Whole Genome Sequencing of Multi-Drug Resistant *Enterococcus faecalis* Isolated from a Camel

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ABSTRACT

The emergence of antimicrobial-resistant, livestock-associated *Enterococcus faecalis* represents a public health concern. Here, we report the isolation, molecular detection of virulence, and antimicrobial resistance and prophage determinants of multi-drug resistant (MDR) *Enterococcus faecalis* isolated from a camel with an oral wound using whole genome sequencing (WGS). Several antimicrobial resistance genes conferring resistance to aminoglycosides (*aac(6')-aph(2"), aph(3')-IIIa*, and *str*), lincosamides (*lnu(B)*, *lsa(A)*, and *lsa(E)*), streptothricin (*sat4*), macrolides (*erm(A)*, *erm(B), msr(A), msr(B)*), trimethoprim (*dfr*, *dfrG*) and tetracyclines (*tet(L)* and *tet(M)*), chloramphenicol (*cat*), linezolid (*optrA*) and florfenicol (*fexA*) were identified. No vancomycin resistance (*van* cluster) genes and no resistance-associated point mutations were detected within the quinolone resistance–determining regions (QRDR) of the parC and gyrA genes in the assembled genome of E. faecalis, suggesting susceptibility to fluoroquinolones at the genetic level. The sequence type (ST) of the isolate was determined as 1116. A total of four different intact prophage were detected in the genome. In addition, 17 putative virulence genes including sex pheromones (*cCF10, cOB1, camE, cad*), adhesins (*efaAfs, ace*), cytolysin toxin-associated genes (*cylA, cylB, cylL, cylM*), endocarditis and biofilm-associated pili genes (*ebpA, ebpB, ebpC*), hyaluronidase (*hylA*), the biofilm- and pilus associated sortase (*SrtA*), and other survival genes (*ElrA, tpx*) were detected. These findings highlight the importance of genomic surveillance to monitor the spread of multidrug-resistant *E. faecalis* in animals. The variety of resistance genes and virulence factors identified in the study indicate the need for effective strategies to control the spread of these resistant bacteria in the food chain.

Keywords: Camel; *Enterococcus faecalis*; Prophage; Resistance; Virulence; Whole Genome Sequencing.

INTRODUCTION

Over the last few decades, enterococci have been recognized as notable nosocomial pathogens worldwide, in particular due to the increasing emergence of antimicrobial resistance phenotypes (1). The vancomycin-resistant enterococci are noteworthy, and their impact in nosocomial settings are of considerable concern (2). Apart from vancomycin, enterococci also exhibit resistance to different classes of antimicrobials such as tetracycline, penicillin, cephalosporin and aminoglycosides (1). Enterococcus faecalis can occasionally be isolated from the oral cavity and has been associated with oral diseases such as caries, endodontic infections, and periodontitis. In addition, it is an opportunistic pathogen responsible for various infections, including urinary tract infections, neonatal infections, wound infections, endocarditis, and meningitis (3). The infections caused by *E. faecalis* has been frequently implicated in failure due to high resistance to antimicrobials, and the ability to form recalcitrant biofilms (1).

Recently, Anderson *et al.* (4) comprehensively evaluated the virulence attributes of *E. faecalis* isolates from the oral cavity, food and clinical specimens, and reported that oral isolates had the highest percentages of virulence genes as well as extracellular enzymes and a capacity to form biofilms. They suggested, therefore, that the oral cavity may constitute a critical reservoir of virulent, antibiotic resistant *E. faecalis* isolates.

E. faecalis is a bacterium that can be found in various animal species and has zoonotic potential. Enterococci of animal origin pose a significant risk to public health with antibiotic resistance. It should also be considered that these bacteria can threaten human health through environmental contamination. Therefore, the spread of *E. faecalis* of animal origin and its impact on public health should be addressed in more detail. Hence, this study was aimed to characterize *E. faecalis* isolated from an oral wound of a camel using whole genome sequencing (WGS).

MATERIALS AND METHODS

Isolation and identification of *Enterococcus faecalis*

The isolate was recovered from an oral wound of a camel brought to the clinics of the Faculty of Veterinary Medicine, Aydın Adnan Menderes University. The isolate was preliminarily identified using phenotypic tests (Gram staining, catalase) and confirmed by MALDI-TOF MS technique (Matrix Assisted Laser Desorption and Ionisation Time of Flight Mass Spectrometry).

Whole genome sequencing

DNA extraction was performed using the QIAamp DNA Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions. DNA concentration was evaluated using fluorometric method (Qubit 3.0, ThermoFisher Scientific, Waltham, MA, USA). Sequencing libraries were generated with Nextera XT library preparation kit (Illumina Inc., CA, USA) according to the manufacturer's instructions, whole-genome sequencing was performed with an Illumina Novaseq 6000 platform, which yielded 150-bp paired-end reads.

Bioinformatic analyses

After trimming low-quality reads and removing adapter sequences using Trimmomatic v0.36 (5), the quality of both raw reads and trimmed reads was assessed using FastQC (v 0.11.9). The de novo genome assembly was conducted using the Megahit v1.2.9 by applying the default parameters (6). The quality of assemblies was evaluated using QUAST v4.5 (7), and contigs longer than >200 bp were included in further analysis. The genome annotation was carried out with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (http://www.ncbi.nlm.nih.gov/books/NBK174280/) (8). The assembled genome was deposited at NCBI under accession number JBEUQJ000000000. The acquired antibiotic resistance genes and chromosomal mutations mediating AMR (ResFinder 4.6.0), virulence genes (VirulenceFinder 2.0) and multilocus sequence type (MLST 2.0) were searched using the Center for Genomic Epidemiology (CGE) pipeline (https://cge.food.dtu.dk/services). The PlasmidFinder 2.0 tool available from the CGE was used to identity plasmid incompatibility groups.

Detection of prophage sequences

The integrated prophage determinants within the genomes of the isolate were identified with PHASTEST (9). The criteria for scoring prophage regions (as intact, questionable or incomplete) have been described in PHASTEST. If the region's total score was less than 70, it was marked as incomplete, between 70-90 as questionable, and when greater than 90 defined as intact.

RESULTS

Complete genome sequencing

WGS analysis showed that the strain consists of one chromosome and four plasmids (rep9a, rep9c, repUS43, and repUS11). The genomic sizes of the *Enterococcus faecalis* strain ADU_VET_2023 consisted of 2,984,001 bp with a GC content of 37.8%. The genomic features of the strain included 2 845 coding genes and 70 coding regions for RNAs, of which 59 were transfer RNAs (tRNAs).

In silico identification of antimicrobial resistance and virulence genes

WGS analysis revealed the presence of several antimicrobial resistance genes conferring resistance to aminoglycosides (*aac(6')-aph(2"), aph(3')-IIIa*, and *str*), lincosamides (*lnu(B)*, *lsa(A)*, and *lsa(E)*), streptothricin (*sat4*), macrolides (*erm(A)*, *erm(B), msr(A), msr(B)*), trimethoprim (*dfr*, *dfrG*) and tetracyclines (*tet(L)* and *tet(M)*, chloramphenicol (*cat*), linezolid

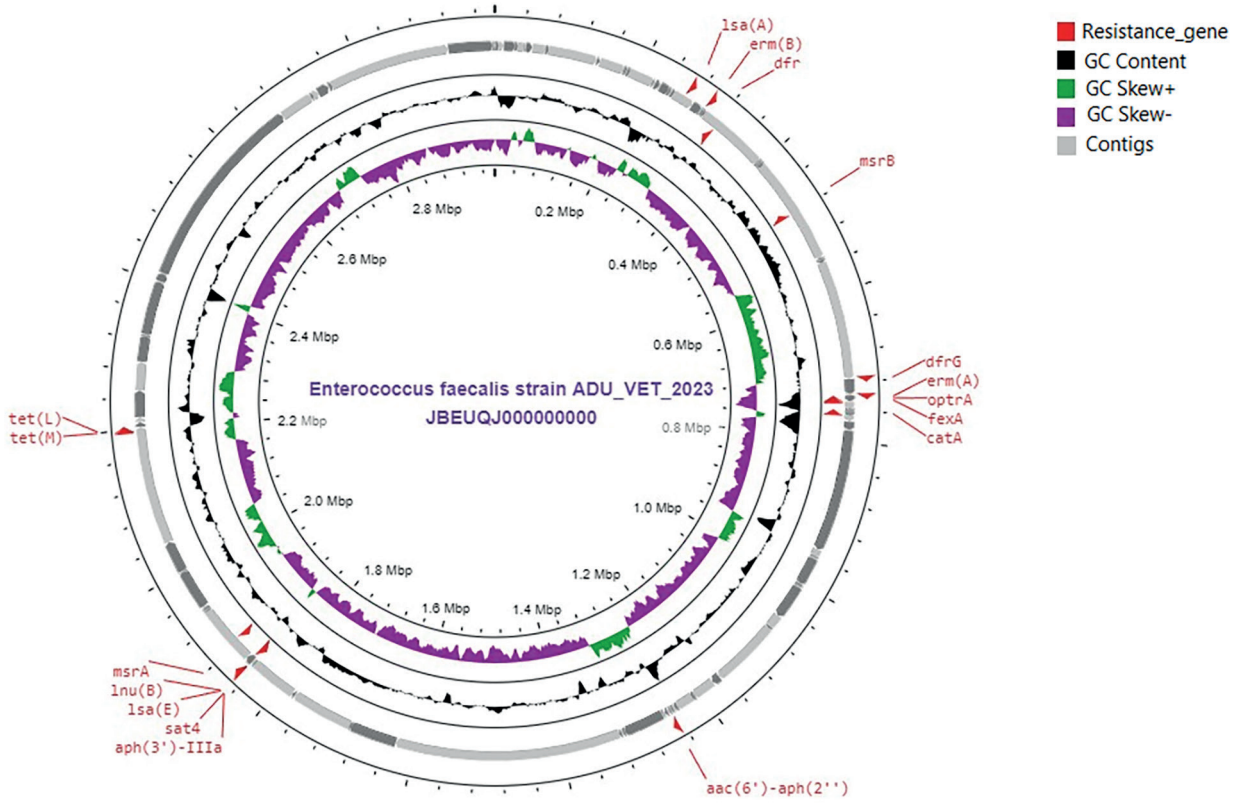

Figure 1. The genome map of *Enterococcus faecalis* strain ADU_VET_2023 was generated using Proksee. Antimicrobial resistance genes are indicated by red arrows, and the contigs are represented by grey arrows. GC skew+ and GC skew- are represented by green and purple peaks, respectively, and GC content is indicated by black peaks.

(*optrA*) and florfenicol (*fexA*) were identified in *E. faecalis* (Figure 1). No vancomycin resistance (*van* cluster) genes were detected in the genome sequences. No known mutations in *parC* and *gyrA* conferring resistance to quinolone was identified in the *E. faecalis* genome.

The isolate harbored 17 putative virulence genes including sex pheromones (cCF10, cOB1, camE, cad), adhesins (*efaAfs*, *ace*), cytolysin toxin-associated genes (*cylA*, *cylB*, *cylL*, *cylM*), endocarditis and biofilm-associated pili genes (*ebpA*, *ebpB*, *ebpC*), hyaluronidase (*hylA*), the biofilm- and pilus associated sortase (*SrtA*), and other survival genes (*ElrA*, *tpx*).

Multilocus sequence typing (MLST)

The multilocus sequence type (ST) of the isolate was determined as ST1116. The MLST alleles were as follow: *aroE* (7), *gdh* (1), *gki* (22), *gyd* (1), *pstS* (104), *xpt* (17), *yqiL* (6).

Identification of phage and plasmid sequences

DNA sequence analysis of the isolate revealed the presence of two intact and two questionable prophage sequences (Table 1, Figure 2). Plasmids detected were: rep9a, rep9c (RepA_N), repUS43 (Rep1), and repUS11 (Inc18).

DISCUSSION

The detection of antibiotic resistance genes in livestock-associated enterococci with zoonotic potential is an escalating public health concern due to the potential risk of transferring their antimicrobial resistance genes to human enterococci or disseminating to humans through the food chain (10). In this study, many antimicrobial resistance genes were identified. Of these, presence of *optrA* gene that conferring resistance to linezolid, is very striking since this antimicrobial is the clinical last resort antibiotic to treat drug-resistant Gram-positive bacteria, including

Table 1. Prophages detected in the isolate.

No	Total lenght (Kb)	Completeness	Score	Total proteins	Region Position	Most Common Phage	GC%
1	34.8	Intact	110	44	1-34814	PHAGE_Entero_phiFLA_NC_013646	35.3
2	12.3	Questionable	80	12	169-12474	PHAGE_Strept_phi3396_NC_009018	37.8
3	14.6	Questionable	70	21	149439-164065	PHAGE_Entero_vB_IME197_NC_02867	38.7
4	37.7	Intact	150	62	1812-39529	PHAGE_Entero_phiFLA_NC_013646	35.3

vancomycin-resistant enterococci and methicillin-resistant *Staphylococcus aureus* (MRSA). Linezolid resistance is mediated by two mechanisms in Gram-positive bacteria. The first is associated with a point mutation in the 23S ribosomal ribonucleic acid (rRNA) binding site (11). The second mechanism involves the acquisition of transferable genes, including *cfr*, which encodes methyltransferase, and *optrA* and *poxtA*, which encode the ABC-F ATP-binding cassette (12, 13, 14). The *optrA*, *poxtA*, and *cfr* genes are considered as multiple resistance genes as well. The *optrA* gene identified in the current study, confers resistance to both phenicols (chloramphenicol and florfenicol) and oxazolidinones (linezolid and tedizolid) (11). Additionally, presence of these genes as part of a plasmid or as a transposon composite increases the possibility of transferring these genes to other bacteria (15). Similarly, phenicol-oxazolidinone resistance has been reported to be mediated by the transferable phenicol-oxazolidinone resistance gene (16, 17). Kim *et al.* (18) reported that *optrA* and *fexA* genes were present on the chromosome in *E. faecalis* isolated from pork meat and contained several mobile gene elements and transposase-associated genes belonging *Tn554*, enabling the horizontal transfer of the phenicol-oxazolidinone resistance genes.

Prophages play essential roles in *E. faecalis* genetic exchange, which results in many useful traits such as the acquisition of antimicrobial resistance genes, evolution, and adaptation, conferring an ecological advantage for persistence and survival over time (19).

WGS analysis revealed four different intact prophages in the isolate. The previous studies showed that prophages were highly prevalent in the genomes of *E. faecalis* (20, 21). Therefore, studying multiple strains of *E. faecalis* can provide insight into different traits associated with infection.

A large group of genes conferring virulence (biofilm production, adhesins, sex pheromones, cytolysin toxin-associated genes and other survival genes) were found in the genome of *E. faecalis* isolate. *E. faecalis* conserves many genetic factors that are associated with the production of biofilm and play an essential role in pathogenicity and infection as they promote virulence and antimicrobial resistance (22). Mature biofilms contribute to survival against antimicrobial substances at 10 to1000-fold greater concentrations compared to the required dose to inhibit planktonic bacteria (23). Several genes were identified in the present study, which are involved in biofilm formation, including endocarditis and biofilm associated pili genes (*ebpA*, *ebpB*, and *ebpC*), collagen adhesion precursor (*ace*) and *sortase* (*srtE*). Similarly, these biofilm-conferring genes were isolated from *E. faecalis* from humans, food, and animals in other studies in different parts of the world (24, 25, 26).

The multilocus sequence type (ST) of the isolate was determined as 1116. This ST was previously reported in hospitalized patient in Norway in PubMLST database.

To the best of the authors' knowldege, the study is the first to date to report the whole genome sequencing and sequence type of *Enterococcus* isolated from livestock in Türkiye up until now. The study also demonstrates the importance of food animals as reservoir of MDR pathogenic *E. faecalis*. The shedding of MDR, animal-related *Enterococcus* spp. in the environment via faecal contamination is a serious concern. Therefore, important food pathogens, including *Enterococcus* species with zoonotic potential in livestock farming, should be regularly surveyed and monitored for public health risks.

The findings of this study highlight the potential role of food-producing animals, particularly camels, as reservoirs of multidrug-resistant E. faecalis strains carrying diverse resistance and virulence determinants. The detection of plasmid- and prophage-associated resistance factors emphasizes the importance of continuous genomic surveillance to monitor potential routes of transmission to humans through

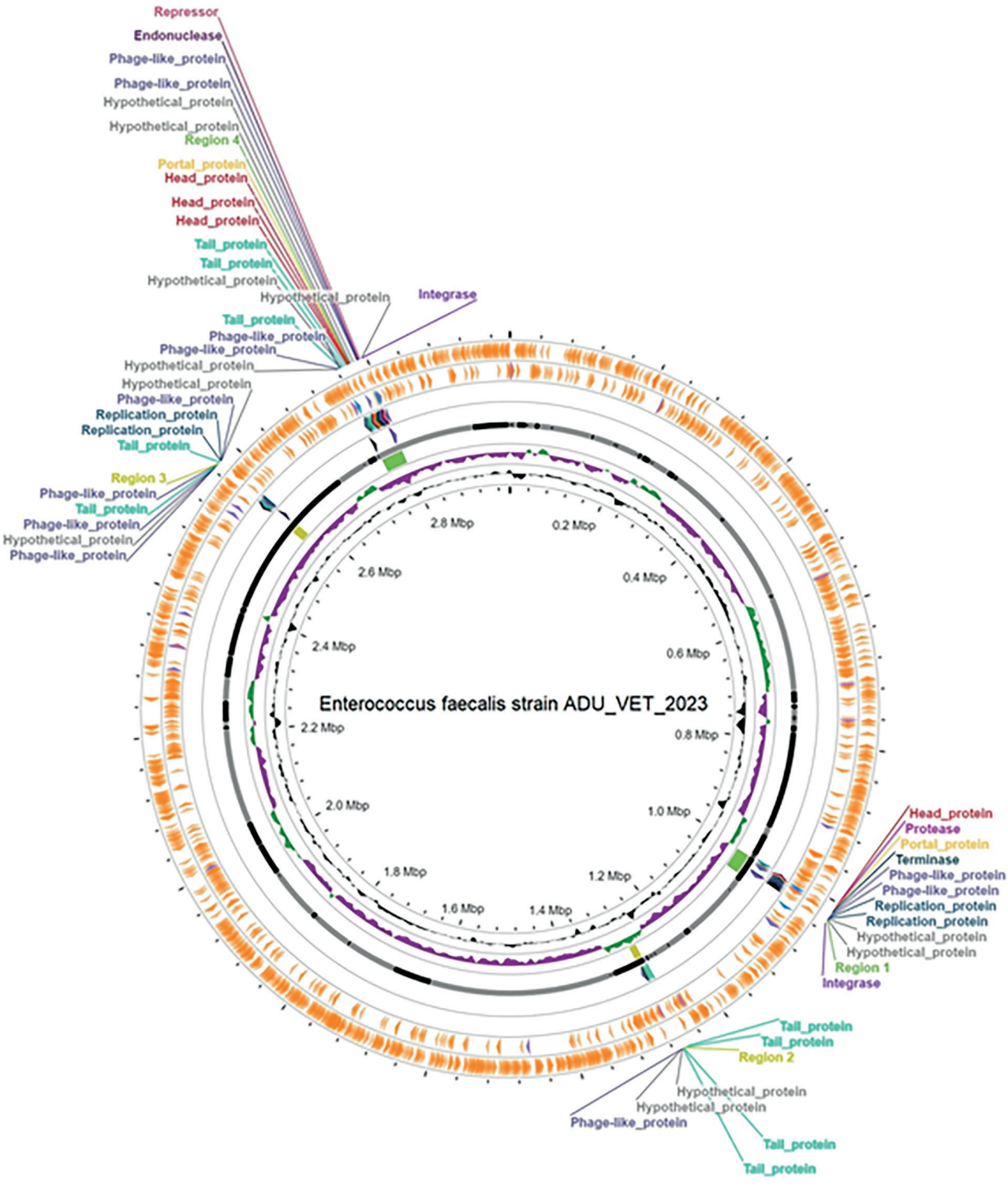

Figure 2. Prophage sequences determined in *E. faecalis* strain ADU_VET_2023 genome by PHASTEST.

both direct contact and the food chain. Given the isolate's profile, which includes resistance to critical antimicrobials such as linezolid and the presence of mobile genetic elements, this study underscores the need for integrated approaches in veterinary and public health sectors to mitigate the spread of these pathogens. Future research focusing on the molecular epidemiology and ecological dynamics of such strains in different livestock populations will provide a clearer understanding of their role in the global dissemination of antimicrobial resistance.

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Ethical Statement

The study doesn't require ethical approval.

Conflict of Interest

The authors declared that there is no conflict of interest.

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