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Research article
Ερευνητικό άρθρο

Whole genome sequencing of vancomycin resistant *Enterococcus faecium* isolated from cat and dog

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ABSTRACT: Vancomycin-resistant enterococci (VRE) have emerged a significant public health concern over past few decades due to their association with serious multidrug-resistant (MDR) infections. This study utilized whole genome sequencing (WGS) to perform molecular characterization of three VRE isolates previously recovered from two cats and one dog. The genomic DNA of the isolates was extracted and sequencing was performed using the Illumina Novaseq platform. The genomes of *Enterococcus faecium* HMKU_VREfm_Dog12, *Enterococcus faecium* HMKU_VREfm_Cat95 and *Enterococcus faecium* HMKU_VREfm_Cat103 consisted of 2707111 bp, 2715129 bp, and 2664256 bp, respectively with GC content of 37.95%, 38.03% and 38.01%, respectively. Multidrug antimicrobial resistance genes conferring resistance to high level aminoglycosides (*aac(6')-aph(2')*), lincosamides (*lnu(B)* and *lsa(E)*), macrolides (*erm(A), erm(B), msr(A), msr(C),* and *msr(B)*), trimethoprim (*dfrG*) and tetracyclines (*tet(L)* and *tet(M)*) were identified. The sequence type (ST) of each isolate was determined using the *Enterococcus* PubMLST database. The isolates were found to belong to different STs (ST2248 in VREfm_Dog12, ST43 in VREfm_Cat95, and ST284 in VREfm_Cat103). The isolates carried only *efaAfm* (adhesion-associated protein) as virulence gene. To the best of the authors' knowledge, this study is the first to provide insights into genetic diversity of vancomycin resistant *E. faecium* (VREfm) strains isolated from dogs and cats using whole genome sequencing analysis in Turkey. The findings underscore the importance of genomic surveillance in monitoring the dissemination of MDR VREfm in pet animals.

Keyword: *Enterococcus faecium*; companion animals; vancomycin resistance; whole genome sequencing.

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INTRODUCTION

Antimicrobial resistance is a complex problem involving various bacterial species, resistance mechanisms, transfer mechanisms and reservoirs. Cats and dogs are potential sources of spread of antimicrobial resistance due to the extensive use of antimicrobial agents in these animals and their close contact with humans (Guardabassi et al., 2004). Consequently, antimicrobial resistance in companion animals is a major global concern to public health (Wada et al., 2021).

Vancomycin-resistant *Enterococcus faecium* (VREfm) has emerged as a medically important opportunistic pathogen causing life-threatining infections, and classified by the World Health Organization (WHO) as a high-priority pathogen that urgently requires new antimicrobial strategies.

The success of VREfm in healthcare settings stems from its exceptional adaptability. Hospital-acquired strains have accumulated various traits that enhance their survival and virulence, including antimicrobial resistance genes, specific virulence factors, and specialized metabolic and survival pathways. Moreover, VREfm's ability to survive and persist in abiotic surfaces, contributes to its widespread presence in hospitals. The plasticity of VREfm genome, coupled with its capacity to accumulate multiple plasmids and mobile genetic elements (MGEs), further drives its evolution and adaptation. However, the dominance of certain clonal lineages in hospital settings remains a puzzle that advanced sequencing technologies are only beginning to unravel (Almeida-Santos et al. 2025).

In recent years, high-throughput sequencing technology, such as *whole genome sequencing* (*WGS*) has increasingly been used to investigate in-depth analysis of pathogens including antimicrobial resistant bacteria. Many approaches and bioinformatics tools developed to analyze and extract the relevant genomic data. The aim of the study was to characterize three vancomycin resistant *Enterococcus faecium* (VREfm) isolated from pet animals using whole genome sequencing (WGS).

MATERIALS AND METHODS

Bacterial strains

The study material consisted of three vancomycin-resistant *E. faecium* strains, *Enterococcus faecium* HMKU_VREfm_Dog12, *Enterococcus faecium* HMKU_VREfm_Cat95 and *Enterococcus faecium* HMKU_VREfm_Cat103, previously isolated from rectal swabs of healthy dogs and cats brought to veterinary clinics for either medical checkups or vaccinations in Mersin province, Türkiye (Aslantaş and Tek, 2019).

Whole genome sequencing

DNA extraction was performed using the QIAamp DNA Mini Kit (QIAGEN, Hilden, 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. WGS 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 (Bolger et al., 2014), 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 the SPAdes algorithm (version 3.1.14) by applying the default parameters (Bankevich et al., 2012). Assembly metrics were calculated using QUAST v.5.0.0 (Gurevich et al., 2013), 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/) (Tatusova et al., 2016). The assembled genomes were deposited at NCBI under accession number JAPHPU000000000.1, JAPHPT000000000.1 and JAPHPS000000000.1, respectively. 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 identify plasmid incompatibility groups.

Detection of Prophage Sequences

The integrated prophage determinants within the genomes of the isolates were identified using PHASTEST (Wishart et al., 2023). PHASTEST categorizes prophage regions as intact, questionable, or incomplete based on specific scoring criteria. Regions with a total score below 70 were classified as incomplete, those scoring between 70 and 90 were considered

questionable, and regions with scores exceeding 90 were designated as intact.

RESULTS

Genomic Assembly Features

The isolates possessed vanA resistance gene together with several resistance genes and their genome sequences were submitted to NCBI GenBank. Data derived from the assembly and the annotation of the genomes studied are summarized in Table 1.

Genes Involved in Virulence and Antimicrobial Resistance

Whole genome sequencing (WGS) analysis of the isolates' genomes revealed the presence of several antimicrobial resistance genes (ARGs); however, only a single virulence gene, *efaAfm* encoding adhesion-associated protein, was identified. Furthermore, multiple mutations associated with ampicillin resistance were detected in the *pbp5* gene. Antimicrobial resistance genes conferring resistance to aminoglycosides (*aac(6')-aph(2')* and *ant(6)-Ia*), lincosamides (*lnu(B)* and *lsa(E)*), macrolides (*erm(A), erm(B), msr(A), msr(C), msr(B)*), trimethoprim (*dfrG*) and tetracyclines (*tet(L)* and *tet(M)*) were identified in VREfm isolates using the ResFinder database and CGE pipeline (Fig.1-3). Vancomycin resistance (*vanHAX* cluster) gene was detected in the genome sequences of the isolates in the present study (Table 2).

There was no signifant difference in resistance genes profiles between the doh and cat isolates, which might suggest host-specific adaptations. All isolates were resistant to the same classes of antimicrobials and had almost same resistance genes.

Determination of MLST type

The multilocus sequence type (ST) of each *Enterococcus faecium* isolate was determined using the *Enterococcus faecium* PubMLST database (https://pubmlst.org/organisms/enterococcus-faecium, accessed on 20 November 2024). Three STs (ST43, ST284, ST2248) were identified in the isolates (Table 2).

Assessment of Phages and Plasmids

WGS analysis identified two or three distinct prophage sequences, either intact or incomplete, across the three VREfm isolates (Fig. 4-6). Two of the isolates harbored one intact and two incomplete prophage sequences, while the remaining isolate contained only two incomplete prophage sequences (Table 3). Eight different replicon plasmid sequences were identified among the isolates (rep1, rep2, rep14a, rep18b, rep22, rep29, repUS15, and repUS43) (Table 4).

DISCUSSION

The emergence and dissemination of antimicrobial resistance to critically important antimicrobials in zoonotic bacteria pose a significant threat to public health worldwide (Vidovic and Vidovic, 2020). This study highlights genomic features of three VREfm strains isolated from one dog and two cats. WGS data were utilized to gain insights into the resistome and virulence factors of these isolates.

The results of WGS analysis revealed that the isolates had *vanA* gene, which lead high resistance to both vancomycin and teicoplanin. Bakthavatchalam et al. (2022) reported that mobile genetic elements (MGEs), particularly plasmids carrying *vanA* cluster plays a central role in facilitating horizontal transfer

Table 1. Assembly reports of the genomes of the strains

Features	Enterococcus faecium HMKU_VREfm_Dog12	Enterococcus faecium HMKU_VREfm_Cat95	Enterococcus faecium HMKU_VREfm_Cat103
Accession No	JAPHPU000000000.1	JAPHPT000000000.1	JAPHPS000000000.1
Genome size (bp)	2706136	2715129	2663743
GC Content (%)	37.947	38.0358	38.0149
No of contigs	145	152	133
Contig N50	62925	63779	66440
Contig L50	15	14	14
CDS	2777	2796	2711
tRNA	62	61	61
rRNA	2	2	2

Table 2. Antimicrobial resistance pattern, AMR genes, and STs identified in VREfm isolates in the present study

Isolate	Host	MLST	AMR Genes	Phenotypic antimicrobial resistance pattern	Mutations in pbp5 gene
Enterococcus faecium HMKU_VREfm_Dog12	Dog	ST2248	vanA, aac(6')-aph(2'), ant(6')-Ia, ermB, msrA, msrC, lnu(B), lsa(E), tetL, tetM, dfrG	VA, AMP, TE, CN, E	V24A, S27G, R34Q, G66E, A68T, E85D, E100Q, K144Q, T172A, L177I, D204G, A216S, T324A, M485A, N496K, A499T
Enterococcus faecium HMKU_VREfm_Cat95	Cat	ST43	vanA, aac(6')-aph(2'), ermB, msrA, msrB, msrC, lnu(B), lsa(E), tetL, dfrG	VA, AMP, TE, CN, E, CIP	V24A, S27G, R34Q, G66E, A68T, E85D, E100Q, K144Q, T172A, L177I, D204G, A216S, T324A, M485A, N496K, A499T
Enterococcus faecium HMKU_VREfm_Cat103	Cat	ST284	vanA, aac(6')-aph(2'), ant(6')-Ia, ermA, ermB, msrA, msrB, lnu(B), lsa(E), tetM, dfrG	VA, AMP, TE, CN, E	V24A, S27G, R34Q, G66E, A68T, E85D, E100Q, K144Q, T172A, L177I, D204G, A216S, T324A, M485A, N496K, A499T

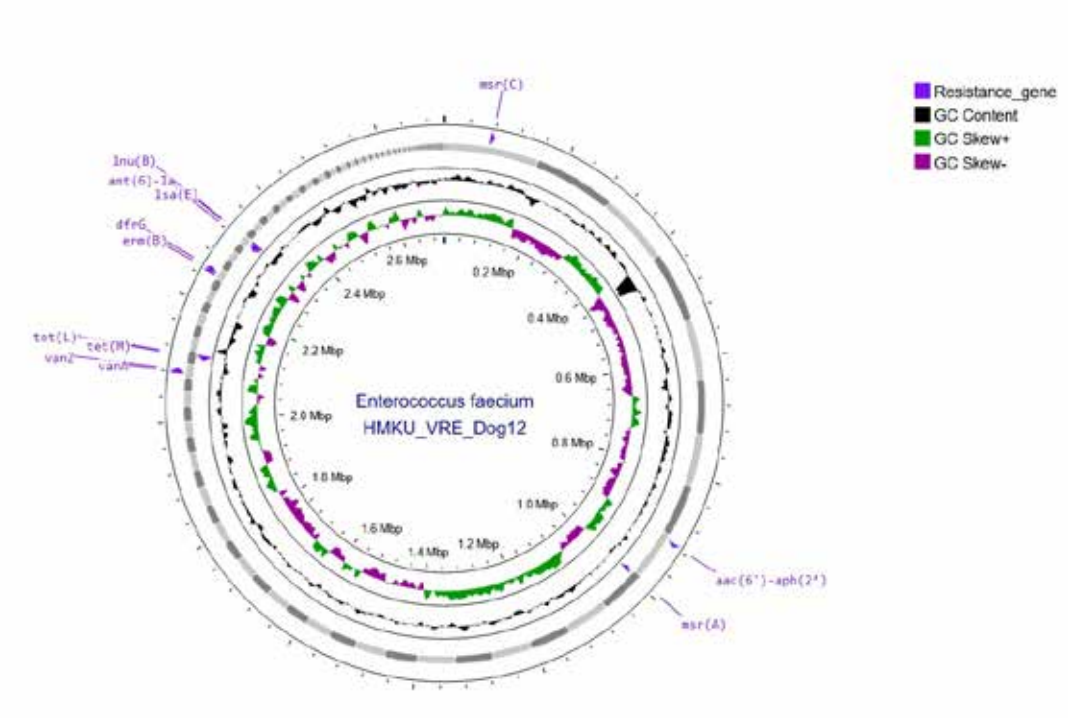

Figure 1. Circular draft genomic maps of *E. faecium* HMKU_VRE_Dog12 constructed using Proksee. The resistance genes denoted as purple arrows

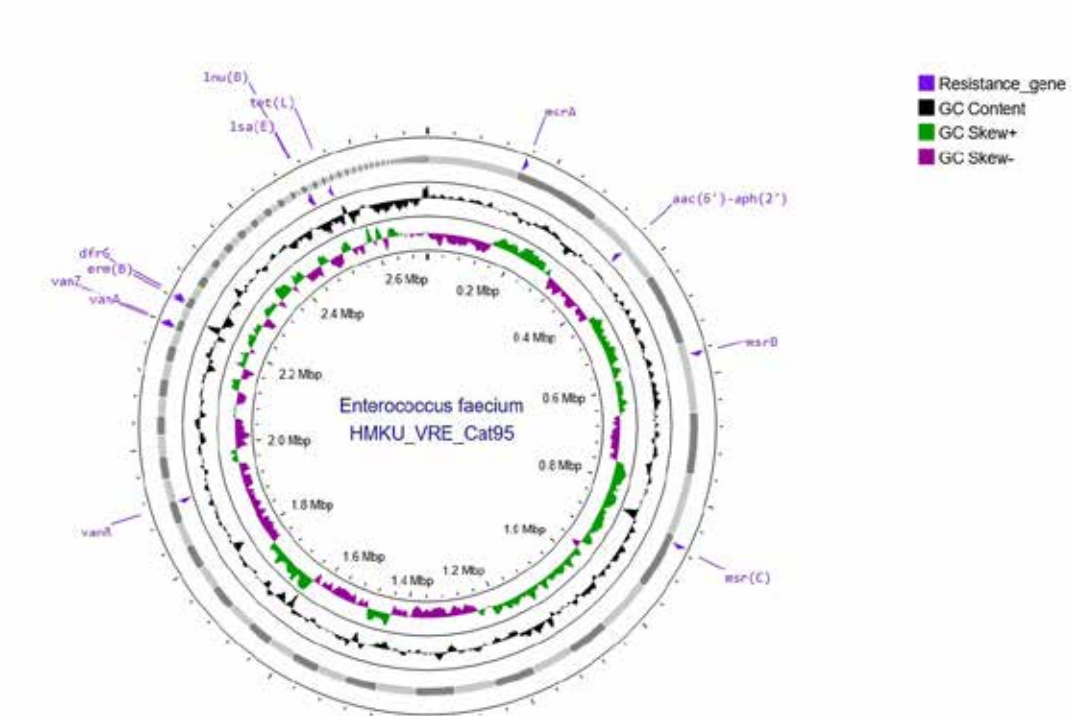

Figure 2. Circular draft genomic maps of *E. faecium* HMKU_VRE_Cat95 constructed using Proksee. The resistance genes denoted as purple arrows

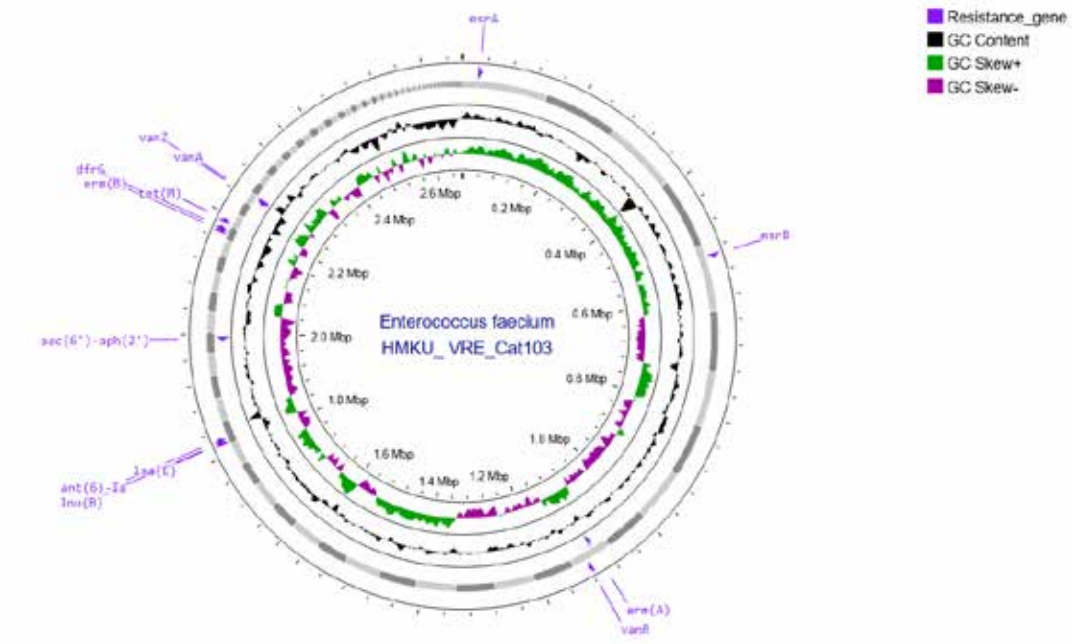

Figure 3. Circular draft genomic maps of *E. faecium* HMKU_VRE_Cat103 constructed using Proksee. The resistance genes denoted as purple arrows

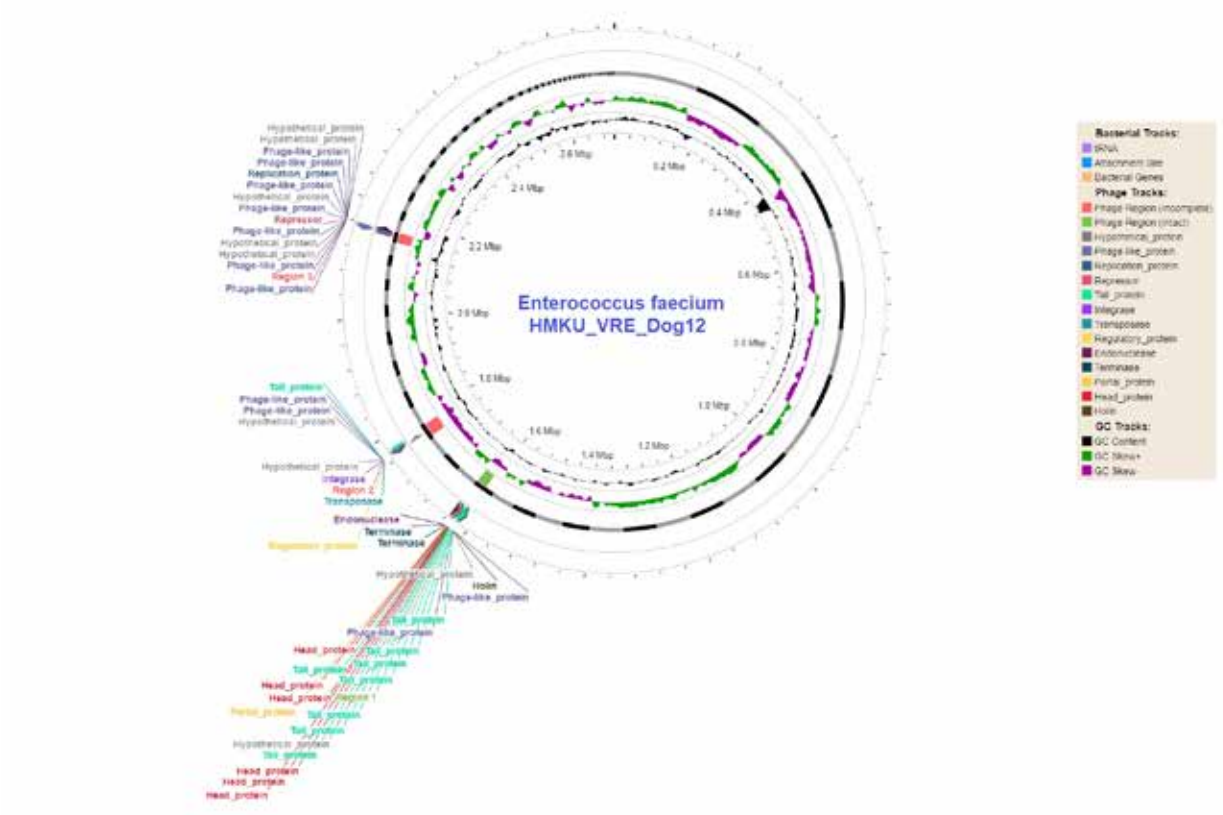

Figure 4. Circular genomic maps of showing prophage sequences in *E. faecium* HMKU_VRE_Dog12 generated using PHASTEST

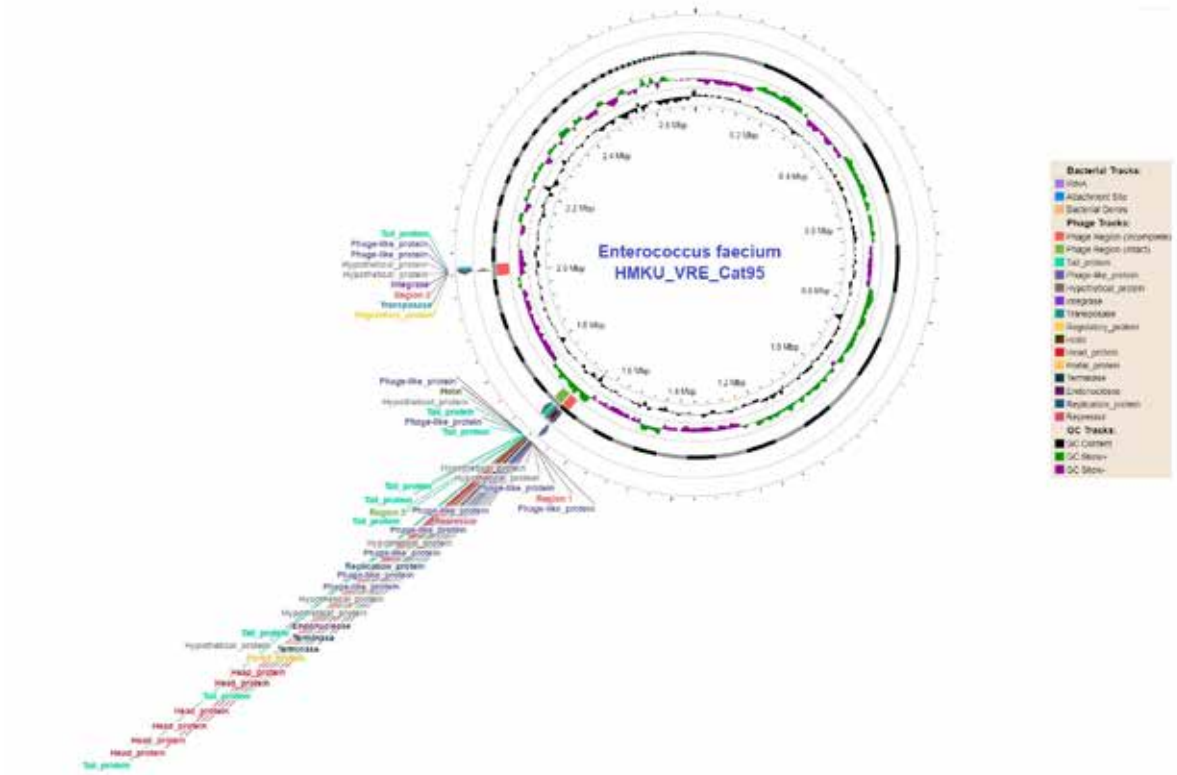

Figure 5. Circular genomic maps of showing prophage sequences in *E. faecium* HMKU_VRE_Cat95 generated using PHASTEST

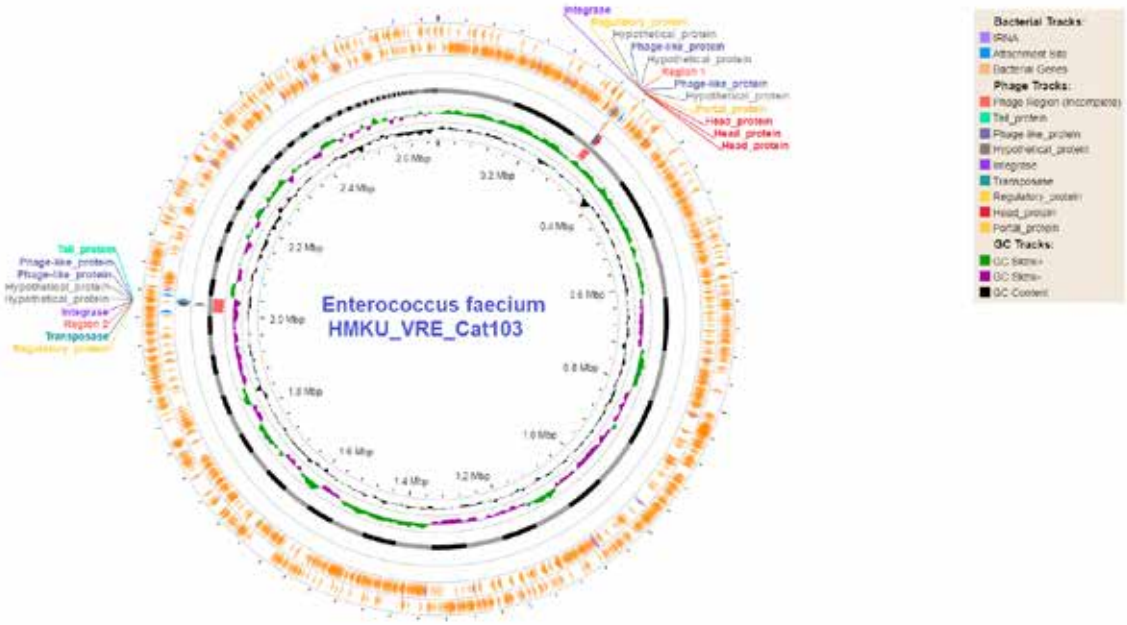

Figure 6. Circular genomic maps of showing prophage sequences in *E. faecium* HMKU_VRE_Cat103 generated using PHASTEST.

Table 3. Prophages detected in VREfm strains

Strain	Region length (Kb)	Completeness	Score	Total Proteins	Node	Length (bp)	Coverage	Region Position	GC %	Most Common Phage
VREfm_ Dog12	20.1	Intact	150	27	20	41783	191.9944	18506-38658	39.15	PHAGE_Entero_IME_ EFm5_NC_028826
	25.7	Incomplete	40	19	24	35166	268.810311	5501-31202	36.72	PHAGE_Thermu_OH2_ NC_021784
	19.7	Incomplete	20	21	38	35166	190.256831	1972-21770	35.63	PHAGE_Entero_phiFL2A_ NC_013643
VREfm_ Cat95	20.1	Intact	150	27	20	42979	157.694583	3126-23278	39.15	PHAGE_Entero_IME_ EFm5_NC028826(9)
	25.7	Incomplete	40	19	21	41783	165.800460	23096-42859	35.66	PHAGE_Entero_phiFL2A_ NC_013643(3)
	19.7	Incomplete	20	21	29	35166	212.168628	5501-31202	36.71	PHAGE_Thermu_OH2_ NC_021784(2)
VREfm_ Cat103	25.7	Incomplete	40	19	29	35164	291.289992	5500-31201	36.72	PHAGE_Thermu_OH2_ NC_021784(2)
	13.7	Incomplete	60	17	3	123831	240.785752	30507-44021	36.24	PHAGE_Entero_EFAP_1_ NC_012419(3)

of vancomycin resistance and virulence genes in *E. faecium* isolates. Indeed, plasmid analysis revealed the presence of plasmid replicon types commonly seen in human clinical VREfm isolates. Chopjjit et al (2025) reported rep17 as the most common plasmid type associated with *vanA* genes in the VREfm isolated from bloodstream infections. On the other hand, Freitas et al. (2011) and Tedim et al. (2021) reported the strong association between carriage of rep2 plasmid replicons and resistance to glycopeptides in enterococci isolated from both humans and food-producing animals. Similar observation also reported by Messele et al. (2023), who found that *vanA* carriage (only in human VREfm isolates) was mostly linked to rep2 plasmid replicons. The presence of *vanA*-carrying plasmids across multiple

Table 4. Plasmid sequences detected among VREfm isolates

Isolate	Plasmid replicon type							
	rep1	rep2	rep14a	rep18b	rep29	rep22	repUS15	repUS43
Enterococcus faecium HMKU_VREfm_Dog12	✓	✓	✓	✓	✓	✓	✓	✓
Enterococcus faecium HMKU_VREfm_Cat95	✓	✓	✓		✓		✓	✓
Enterococcus faecium HMKU_VREfm_Cat103	✓	✓	✓		✓	✓	✓	✓

replicon types highlights the potential for horizontal gene transfer of vancomycin resistance among enterococcal isolates within hospital environments and patient populations. Although the presence of plasmids with different rep origins, including rep2, was detected in this study, it is not possible to ascertain that whether vanA gene cluster is a part of a plasmid or not. Therefore, further studies are needed to elucidate the possible role of plasmids in the transmission of the *vanA* cluster and other resistance genes in VREfm isolates.

In addition to vancomycin resistance, VREfm commonly exhibits resistance to multiple antimicrobial classes, including macrolides, lincosamides, aminoglycosides, beta-lactams, and folate pathway inhibitors (Coccitto et al., 2024). Recently, acquired resistance to last-line agents such as linezolid and daptomycin has been also reported in VREfm by Wardal et al. (2023).

In this study, it is not feasible to establish an epidemiological link regarding the potential zoonotic transmission of the isolates. Since there was no access to samples from the pet owners, it is not possible to assume that *vanA* gene exchange occured between humans and companion animals.

In previous studies conducted in Türkiye, however, in human clinical VREfm isolates, ST280, ST203, ST117 were reported as the most common sequence types, followed by ST78, ST17, ST18, and ST733 (Arslan et al. 2013; Erdem et al. 2020). Reports of VREfm in companion animals are very scarce, and there is no information regarding STs of VREfm isolated from companion animal. STs detected in VREfm strains in this study have not been reported before in clinical VREfm isolates from human or companion animals. However, it should not be ignored that these VREfm strains could evolve into more virulent strains and become dominant in healthcare settings and cause VRE outbreaks (O'Toole et al. 2023).

In this study, VREfm isolates carried several resistance genes and *pbp5* gene mutations conferring resistance to aminoglycosides, tetracycline, trimethoprim, macrolides, lincosamides and ampicillin. High-level ampicillin resistance in clinical isolates of E. faecium has been primarily associated either increased production of PBP5 or point mutations are located close to the active site of the enzyme. In particular, mutations at positions Met-485-Thr/Ala and Asp466Ser have been responsible for a reduced affinity for penicillin and increased beta-lactam MICs. In addition, other amino acid substitutions at position Ala-499-Ile/Thr, at position Glu-629-Val, and at position Pro-667-Ser have been implicated in resistance to beta-lactams (Montealegre et al. 2017). The detection of the above-mentioned mutations in pbp5 in this study indicates the cause of ampicillin resistance. This means that there would be great challenges in treating infections caused by MDR VREfm isolates with the available antibiotics. Therefore, MDR *Enterococcus* spp. in animals that have zoonotic potential and pose a public health risk should be regularly surveyed and monitored.

In this study, a total of eight prophage genome sequences were detected in isolates. The presence of prophage genome sequences frequently identified in the genome of clinical *E. faecium* isolates is well documented (Lisotto et al., 2021; Mikalsen et al., 2015). Previous studies suggested that prophages, along with other MGSs, are the major contributors to the plasticity of enterococcal genomes (Werner et al., 2013; Hegstad et al., 2010), and clinical *E. faecium* strains have twice as many genes associated with MGEs compared to non-clinical strains (Kim et al., 2013). The impact of prophages on enterococcal diversity is less understood, but whole genome sequencing of *E. faecium* strains identified prophages as a prominent source of genome diversity (Mikalsen et al., 2015).

The pathogenicity of the clinical enterococcal isolates is primarily associated with their virulence factors (Fisher and Phillips, 2009). In the current study, detection of only efaAfm (cell wall adhesin) as the virulence gene could be explained by VREfm being isolated from feacal material. Indeed, clinical VREfm isolates harbor several virulence factors contributing the adaptation to healthcare settings (Almeida-Santos et al. 2025). In a recent study, Coccioto et al. (2024) found *efaAfm* gene together with *acm*, *hylEfm*, *espfm* genes in clinical VREfm isolates. Sacramento et al (2024) isolated a the human-associated vanA-positive VREfm (ST 612) from a dog with an infected wound, found that carried the virulence genes *acm*, *efaAfm*, *hylEfm* and *sgrA*. However, it is more likely that these isolates can acquire virulence via horizontal gene transfer and evolved towards being more virulent.

In summary, we report the first emergence of MDR *vanA* positive VREfm ST43, ST248 and ST2248 in companion animals in Türkiye. Therefore, microbiologists and veterinarians should be aware of these agents. Considering that importance of VRE, continuous surveillance should be performed in small animal practice, and characterized with advanced molecular techniques (e.g. hybrid sequencing).

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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