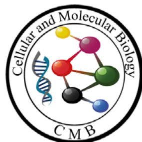
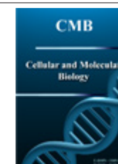
Cell. Mol. Biol. (ISSN: 1165-158X)



Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology



Original Article

Genomic insights into *Enterococcus faecalis* implicated in endodontic infections: resistance, virulence, and genetic variability

Nezar Boreak[1*], Ahlam Abdu Mohammed Mowkly[2], Amnah Sharwani[1], Shroog Ali Almasoudi[1], Ahmed Huraysi[1], Ibrahim Ali Sulily[1], Ghadi Ghamdhan Jali[1], Mohammed Abed Basihi[1], Osama Alfaifi[1], Elham Ali Tohari[1], Rehaf Madkhali[1]

[1] *Department of Restorative Dental Sciences, College of Dentistry, Jazan University, Kingdom of Saudi Arabia.*
[2] *Dentist, Ministry of Health, Kingdom of Saudi Arabia*

Article Info

Article history:

Received: October 14, 2024
Accepted: December 18, 2024
Published: January 31, 2025

Use your device to scan and read the article online

Abstract

Endodontic infections, often involving multispecies bacterial communities, present significant challenges in treatment due to their complex pathogenic mechanisms and resistance to con-ventional therapies. *Enterococcus faecalis* is a facultative anaerobic gram-positive bacterium that has been frequently recovered from secondary or persistent endodontic infections. This study investigates the population structure, resistome, mobilome, and virulome of *E. faecalis* isolated from oral cavity sources, focusing on 22 genomes sequenced from saliva and root canal samples. The genome sequence analysis revealed a diversity of 14 sequence types (STs), high-lighting genetic variability within oral *E. faecalis* populations. Virulence profiling identified 39 genes involved in adherence, biofilm formation, toxin production, stress response, and immune evasion. Antimicrobial resistance (AMR) genes, including lsa(A), efrA, and tetM, were prev-alent across all genomes, indicating potential multidrug resistance. Mobile genetic elements (MGEs), such as insertion sequences, transposons, prophages, and plasmids, were also identi-fied, facilitating genetic exchange within and between species. Network analyses identified central virulence genes (e.g., asa1, gelE) and AMR genes (e.g., ANT (6)-Ia, dfrE) crucial for pathogenicity and resistance, highlighting their pivotal roles in *E. faecalis* infections. This study provides comprehensive insights into the genomic characteristics, AMR genes, virulence fac-tors, and genetic mobile elements associated with *E. faecalis* isolates from the oral cavity, offering implications for dental health and potential strategies for infection control and treat-ment.

Keywords: *Enterococcus faecalis*, Endodontic infection, Antimicrobial resistance, Virulence factors, Genetic diversity.

1. Introduction

Enterococcus faecalis is an anaerobic, gram-positive coccus commonly found in the human oral cavity, gastro-intestinal tract, and vagina due to its ability to adapt well to nutrient-rich, low-oxygen environments with complex ecology [1]. Initially considered non-virulent, enter-ococci are now recognized as major causes of nosocomial infections globally. In particular, *E. faecalis* has been linked to chronic endodontic infections and failed root canal treatments [2, 3].

It is widely assumed that microorganisms in the root canal originate from those colonizing the oral cavity [4]. Various studies support the prevalence of *E. faecalis* in root canals as associated with its presence in saliva [5]. Endodontic infections, which begin in the dental pulp, create a unique microbial environment where *E. faecalis* thrives [6]. This bacterium's resilience in harsh conditions contributes to its persistence and treatment resistance. *E. faecalis* forms biofilms and acquires mobile genetic elements (MGEs) carrying drug resistance, posing significant treatment challenges [7].

Several virulence factors enhance *E. faecalis* pathogenicity by enabling colonization and host tissue invasion, translocation through epithelial cells, and host's immune response evasion [8]. Furthermore, *E. faecalis* is highly proficient at exchanging and transmitting resistance and virulence genes through horizontal gene transfer [9]. Over the past decade, antibiotic-resistant genes have been transferred between different strains [10].

The advent of whole genome sequencing (WGS) has facilitated a more comprehensive exam-ination of enterococcal antimicrobial resistance genes (ARGs), phylogenetics, and virulence [11]. As WGS becomes more accessible and cost-effective, previously archived isolate collec-tions are being re-examined and compared with newer isolates.

Despite the clinical significance of *E. faecalis* in endodontic infections, comprehensive studies focusing on the genetic basis of its resistance and virulence in this context are limited. The current work aims to uncover the prevalence and distribution of population structure, resistome,

* Corresponding author.
E-mail address: nboraak@jazanu.edu.sa (N. Boreak).
Doi: http://dx.doi.org/10.14715/cmb/2025.70.1.11

mobilome, and virulome in *E. faecalis* isolated from the oral cavity. By bioinformatics analyses, and gene interaction network construction, we seek to identify specific genes and determine their potential impact on treatment outcomes. Our findings will contribute to a deeper understanding of *E. faecalis* 's role in persistent endodontic infections and provide insights into developing targeted interventions to mitigate this growing threat.

2. Materials and methods

2.1. Collection of genomic data and sequence analysis

Twenty-two complete genome sequence files of *E. faecalis* isolated from oral cavity including saliva and root canal were downloaded in FASTA format from the NCBI (Accession: PRJNA891504) [11]. The Multi-locus sequence type (MLST) scheme and composition of the sequences were obtained from the BV-BRC database Bacterial and Viral Bioinformatics Re-source Center (BV-BRC) database [12]. The whole genome sequences of the study sequences were aligned using MUSCLE in Molecular Evolutionary Genetics Analysis Version 11 (MEGA11) [13]. The phylogenetic tree is constructed based on the Neighbor-joining method in MEGA11. The generated output file (.tree) was visualized and annotated on the Interactive Tree of Life (iTOL) interface v5.

2.2. Antimicrobial resistance and virulence genes, their plasmid, and genomic context in *E. faecalis*

The presence of resistance genes was identified using ABRicate 1.0.1 [14], ResFinder [15], AMRFinderPlus version 3.11.11 [16], and Comprehensive Antibiotic Resistance Database (CARD) [17]. Intrinsic virulence factors were identified using ABRicate 1.0.1 with the Viru-lence Factor Database (VFDB) and Victors knowledgebase from BV-BRC. Plasmids associ-ated with *E. faecalis* were identified using ABRicate 1.0.1 with PlasmidFinder. The assembled genomes were further analysed for mobile genetic elements (MGEs), including insertion se-quences, using ISFinder [18] and MGEFinder [19] and intact prophages using PHASTEST [20], respectively. Integrative Conjugative Elements (ICE) and integrative and mobilizable elements (IME) were identified using MGEFinder and ISFinder. Virulence factors, resistance genes, and MGEs were considered significant if the identity and coverage percentage were greater than 90%.

2.3. Gene interaction network analysis

The functional partners of the genes mediating antibiotic resistance and virulence were identi-fied using the STRING database [21]. STRING includes physical and functional association partners obtained from high-throughput experimental data, co-expressed genes, databases, and literature. The identified antibiotic resistance and virulence protein sequences were input into the STRING database, using the *E. faecalis* V583 strain as a reference. Sequences with more than 90% similarity were considered for further data retrieval. Interacting partners with a confidence score greater than 0.4 were considered for network construction. Various topo-logical parameters, and centrality measures such as degree, betweenness, eccentricity, closeness and topological coefficient were calculated using the Network analysis option in Cytoscape.3.10.2 [22]. The antibiotic resistance and virulence gene interaction networks were con-structed and visualized using Cytoscape 3.10.2.

2.4. Statistical analysis

The statistical analysis section of this study focuses on the genomic characteristics and anti-microbial resistance (AMR) profiles of *E. faecalis* isolates obtained from oral cavity sources. A total of 22 complete genome sequences were analyzed, revealing significant genetic diversity with 14 distinct sequence types (STs). The presence of virulence factors was assessed through bioinformatics tools, identifying 39 genes associated with pathogenicity, including those in-volved in biofilm formation and immune evasion. Statistical methods, such as Multi-locus Sequence Typing (MLST) and phylogenetic analysis using the Neighbor-Joining method, were employed to investigate the relationships among isolates. Additionally, network analysis was performed to identify interactions between AMR and virulence genes, providing insights into their functional associations and potential implications for treatment strategies. These analyses underscore the complexity of *E. faecalis* infections and highlight the urgent need for targeted therapeutic approaches to combat resistance and virulence in clinical settings.

3. Results

3.1. Genomic features and population structure of *E. faecalis* from oral cavity isolates

The *E. faecalis* genome size of the study isolates ranged between 3.18 Mbp to 2.74 Mbp. The genomic map of the study isolates is shown in Fig. 1 which shows that the majority of the study isolates are sequentially similar. The MLST analysis assigned 14 different STs to the 22 isolates of our study. The analysis of bacterial strains from saliva (S), and root canal (RC) revealed significant patterns. Strains from the same patients often shared iden-

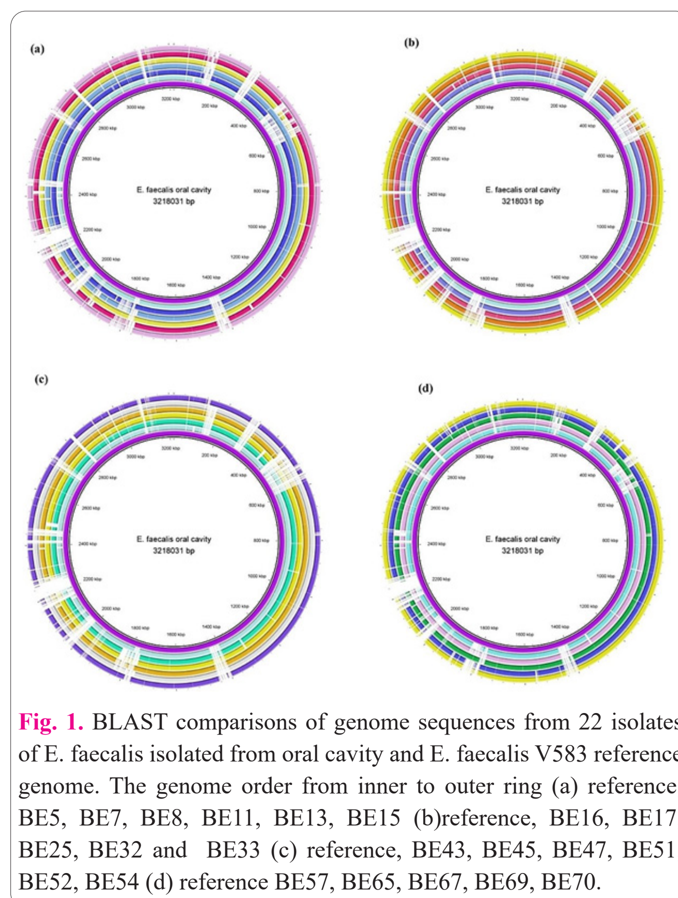


Fig. 1. BLAST comparisons of genome sequences from 22 isolates of E. faecalis isolated from oral cavity and E. faecalis V583 reference genome. The genome order from inner to outer ring (a) reference, BE5, BE7, BE8, BE11, BE13, BE15 (b)reference, BE16, BE17, BE25, BE32 and BE33 (c) reference, BE43, BE45, BE47, BE51, BE52, BE54 (d) reference BE57, BE65, BE67, BE69, BE70.

tical sequence types (STs), such as BE15/BE43 (ST25), BE16/BE17 (ST260), BE7/BE8 (ST16), and BE32/BE33 (ST55), indicating persistence in the individuals. Notably, BE11 and BE32, both ST55, were isolated from the same patient's saliva four months apart, suggesting long-term persistence (Table 1). The diversity of Sequence Types (STs) identified in our study reflects varied genetic profiles within the oral cavity. ST300, ST40, ST55, and ST16, commonly reported in clinical and environ-mental contexts, highlight adaptability and potential transmission routes. Conversely, ST179, and ST380, and others less frequently observed in oral isolates underscore unique microbial ecologies. The phylogenetic tree reveals significant clustering of strains from the same patients, such as BE15/BE43, BE16/BE17, BE7/BE8, BE32/BE33, and BE51/BE52, indicating high genetic similarity and persistence within hosts irrespective of the isolation site. Strains BE11 and BE32, isolated from the same patient's saliva four months apart, also cluster closely, demonstrating temporal consistency (Fig. 2).

3.2. Virulence factors associated with the isolates

The virulence genes were identified by comparing the *E. faecalis* genomes against VFDB, using ABRicate and Victors supported by BV-BRC. A total of thirty-nine different virulence genes were identified in the study isolates (Table 2). These virulence factors were classified based on their functions into several categories: adherence factors, biofilm formation factors, capsule formation factors, toxins, secretion systems, immune evasion factors, enzymes, and regulatory factors. Most of the virulence genes were associated with immunomodulation (cpsA, cpsB, cpsC, cpsD, cpsE, cpsF, cpsG, cpsH, cpsI, and cpsK), adherence (ebpA, ebpB, ebpC, srtC, and Ef0485), biofilm formation (ebpA, ebpB, ebpC, and efaA), and biofilm modulation (bopD, fsrA, fsrB, and fsrC).

3.3. Anti-microbial resistance genes associated with the isolates

The presence of multiple antimicrobial resistance (AMR) genes in *E. faecalis* isolates were identified by comparing the results obtained from different tools (Table 3). A total of eighteen AMR genes were identified in the study isolates. All isolates harbored the lsa(A) gene. The widespread occurrence of the lsa(A) gene among all isolates is particularly concerning as it confers resistance to clindamycin, quinupristin-dalfopristin, and dalfopristin, limiting treatment options for severe infections. Most isolates also displayed biocide resistance (efrA) and trimethoprim resistance (tetM), except a few isolates, further complicates therapeutic strategies, potentially reducing the efficacy of commonly used antibiotics and disinfectants. The diversity of additional AMR genes observed highlights the adaptability of *E. faecalis* in acquiring resistance mechanisms against various antimicrobial agents. The presence of genes such as tetS, ErmB, ANT (6)-Ia, IsaE, dfrG, lnuB, catA, SAT-4, ANT (9)-Ia, aph(3')-III, Str, and bcrA in different isolates indicates a broad spectrum of resistance capabilities, encompassing antibiot-ics, biocides, and disinfectants. Overall, these findings indicate that the isolates may contain diverse antimicrobial resistance determinants, potentially indicative of a multidrug-resistant (MDR) phenotype irrespective of their location in the oral cavity. However, it's important to interpret these results cautiously, as the actual expression was not assessed phenotypically in this study.

3.4. Characterization and association of mobile genetic elements

The study utilized Plasmid finder as a reference database and employed the ABRicate program to identify plasmids within the isolates. This approach successfully detected plasmids in fourteen (BE67, BE65, BE54, BE52, BE51, BE43, BE33, BE32, BE25, BE15, BE13, BE11, BE8, BE7) of the isolates, highlighting the presence of the plasmid replicon DOp1 across these samples. The detection of this replicon suggests a potential commonality or shared mechanism of plasmid carriage among these isolates, which may have implications for understanding plasmid-mediated gene transfer in this microbial community. Additionally, the analysis re-vealed that nearly all isolates contained intact prophages, with a variety of specific prophages being identified. The most prevalent prophage, Entero_phiFL3A_NC_013648, was found in fifteen isolates, followed by Entero_phiFL1A_NC_013646 in nine isolates and several others in fewer numbers (Table S1 1). The presence of these prophages could indicate a significant role in the genetic and phenotypic diversity of the isolates, potentially affecting their virulence, adaptability, and resistance to environmental stresses. The study also identified six distinct insertion sequence (IS) families within the isolates: IS3, IS1380, IS256, IS6, ISL3, and IS30. The specific IS elements, such as ISLmo19 and ISS1N of IS6, and others, suggest a complex landscape of mobile genetic elements that contribute to genomic variability. These IS elements can facilitate gene rearrangements, horizontal gene transfer, and the spread of antibiotic re-sistance genes, which are crucial factors in microbial evolution and pathogenicity. The detection of simple transposons, including Tn6009, Tn6000, and Tn5405, further underscores the dynamic nature of the genomes in these isolates (Table S2). Transposons are known to play a critical role in the mobility of genetic elements, potentially leading to the dis-

Fig. 2. Phylogenetic relationships among E. faecalis isolates. Orange and green labels represent samples from intracanal and saliva, respectively. Samples from the same patient are indicated by the same node color. The genome of the reference laboratory strain E. faecalis V583 was used as an outgroup.

Table 1. Genomic composition and population structure of the study isolates.

GenBank accession	Strain	MLST	Chromosomes	Contigs	Genome Length	GC Content	tRNA	rRNA	CDS	Partial CDS
CP110019.1	BE70	300	1	1	2872849	37.25588	65	12	2858	9
CP110020.1	BE69	300	1	1	2874917	37.26393	65	12	2828	6
CP110022.1	BE67	179	1	4	2980122	37.40216	60	12	3029	11
CP110030.1	BE65	1272	1	7	3182451	37.32652	68	12	4286	18
CP110032.1	BE57	40	1	2	2978663	37.38123	61	12	2977	9
CP110035.1	BE54	179	1	2	3013236	37.38874	61	12	3101	13
CP110036.1	BE52	74	1	1	2845082	37.64155	60	12	2777	8
CP110038.1	BE51	239	1	2	2846585	37.59322	61	12	2888	9
CP110039.1	BE47	380	1	1	2757740	37.59658	60	12	2687	2
CP110040.1	BE45	380	1	1	2745951	37.59514	60	12	2682	2
CP110041.1	BE43	25	1	6	3025526	37.39115	60	12	2995	10
CP110047.1	BE33	55	1	3	2986815	37.3524	61	12	2945	10
CP110050.1	BE32	55	1	3	2986731	37.35439	61	12	2924	7
CP110053.1	BE25	173	1	1	2761658	37.68048	60	12	2634	4
CP110054.1	BE17	260	1	5	2977527	37.38895	59	12	2930	5
CP110059.1	BE16	260	1	4	2978319	37.39485	62	12	2925	4
CP110063.1	BE15	25	1	5	3022182	37.39583	60	12	3001	11
CP110068.1	BE13	72	1	1	2995083	37.55722	63	12	2921	10
CP110069.1	BE11	55	1	2	2957563	37.40228	61	12	2866	7
CP110071.1	BE8	16	1	1	2863584	37.44629	62	12	2746	13
CP110072.1	BE7	16	1	2	2977930	37.29819	66	12	2919	13
CP110074.1	BE5	326	1	5	3034464	37.44253	61	12	3027	13

Table 2. List of virulent genes identified in the study isolates.

GenBank accession	Virulent Genes
CP110019.1	bopD, cpsA, cpsB, cpsC, cpsD, cpsE, cpsG, cpsH, cpsI, cpsJ, cpsK, cylI, ebpA, ebpB, ebpC, EF0485, EF0818, efaA, fsrA, fsrB, fsrC, fss1, fss2, gelE, sprE, srtC
CP110020.1	bopD, cpsA, cpsB, cpsC, cpsD, cpsE, cpsG, cpsH, cpsI, cpsJ, cpsK, cylI, ebpA, ebpB, ebpC, EF0485, EF0818, efaA, fsrA, fsrB, fsrC, fss1, fss2, gelE, sprE, srtC
CP110022.1	bopD, cpsA, cpsB, cylA, cylB, cylI, cylL, cylM, cylR1, cylR2, cylS, ebpA, ebpB, ebpC, EF0485, EF3023, efaA, esp, fsrC, fss1, fss2, fss3, gelE, sprE, srtC
CP110030.1	ace, bopD, cpsA, cpsB, ebpA, ebpB, ebpC, EF0818, efaA, fsrC, fss1, gelE, sprE, srtC
CP110032.1	ace, bopD, cpsA, cpsB, ebpA, ebpB, ebpC, EF0818, EF3023, efaA, fsrA, fsrB, fsrC, fss1, fss2, gelE, sprE, srtC
CP110035.1	bopD, cpsA, cpsB, cylA, cylB, cylI, cylL, cylM, cylR1, cylR2, cylS, ebpA, ebpB, ebpC, EF0149, EF0485, EF3023, efaA, esp, fsrC, fss1, fss2, fss3, gelE, sprE, srtC
CP110036.1	ace, bopD, cpsA, cpsB, ebpA, ebpB, ebpC, EF0818, EF3023, efaA, fss1, fss3, srtC
CP110038.1	ace, bopD, cpsA, cpsB, ebpA, ebpB, ebpC, EF0818, EF3023, efaA, fsrC, fss1, gelE, sprE, srtC
CP110039.1	ace, bopD, cpsA, cpsB, ebpA, ebpB, ebpC, EF0818, efaA, fss1, fss3, srtC
CP110040.1	ace, bopD, cpsA, cpsB, ebpA, ebpB, ebpC, EF0818, efaA, fss1, fss3, srtC
CP110041.1	bopD, cpsA, cpsB, ebpA, ebpB, ebpC, EF0818, efaA, fsrC, fss3, gelE, sprE, srtC
CP110047.1	ace, bopD, cpsA, cpsB, ebpA, ebpB, ebpC, EF0818, EF3023, efaA, fss1, srtC
CP110050.1	ace, bopD, cpsA, cpsB, ebpA, ebpB, ebpC, EF0818, EF3023, efaA, fss1, srtC
CP110053.1	ace, bopD, cpsA, cpsB, ebpA, ebpB, ebpC, EF0818, EF3023, efaA, fss1, gelE, sprE, srtC
CP110054.1	bopD, cpsA, cpsB, ebpA, ebpB, ebpC, EF0818, EF3023, efaA, fsrA, fsrB, fsrC, fss1, gelE, sprE, srtC
CP110059.1	bopD, cpsA, cpsB, ebpA, ebpB, ebpC, EF0818, EF3023, efaA, fsrA, fsrB, fsrC, fss1, gelE, sprE, srtC
CP110063.1	bopD, cpsA, cpsB, ebpA, ebpB, ebpC, EF0818, efaA, fsrC, fss3, gelE, sprE, srtC
CP110068.1	bopD, cpsA, cpsB, cpsC, cpsD, cpsE, cpsG, cpsI, cpsJ, cpsK, ebpA, ebpB, ebpC, EF0818, EF3023, efaA, fsrA, fsrB, fsrC, fss1, fss2, gelE, sprE, srtC
CP110069.1	ace, bopD, cpsA, cpsB, ebpA, ebpB, ebpC, EF0818, EF3023, efaA, fss1, fss3, srtC
CP110071.1	bopD, cpsA, cpsB, cpsC, cpsD, cpsE, cpsF, cpsG, cpsH, cpsI, cpsJ, cpsK, cylA, cylB, cylI, cylI, cylL, cylM, cylR1, cylR2, cylS, ebpA, ebpB, ebpC, EF0485, EF3023, efaA, esp, fss1, fss2, srtC
CP110072.1	bopD, cpsA, cpsB, cpsC, cpsD, cpsE, cpsF, cpsG, cpsH, cpsI, cpsJ, cpsK, cylA, cylB, cylI, cylI, cylL, cylM, cylR1, cylR2, cylS, ebpA, ebpB, ebpC, EF0485, EF3023, efaA, esp, fss1, fss2, srtC
CP110074.1	bopD, cpsA, cpsB, ebpA, ebpB, ebpC, EF3023, efaA, fsrC, fss1, fss3, gelE, sprE, srtC

Table 3. List of AMR genes associated with the study isolates.

GenBank accession	CARD	ResFinder	AMRfinder	ABRicate
CP110019.1	efrA, dfrE, lsa(A)	lsa(A)	lsa(A)	lsa(A), dfrE
CP110020.1	efrA, dfrE, lsa(A)	lsa(A)	lsa(A)	lsa(A), dfrE
CP110022.1	dfrE, tet(M), lsa(A)	lsa(A), tet(M)	lsa(A), tet(M)	lsa(A), tet(M), dfrE
CP110030.1	dfrE, tet(M), lsa(A)	lsa(A), tet(M)	lsa(A), tet(M)	lsa(A), tet(M), dfrE
CP110032.1	efrA, dfrE, lsa(A)	lsa(A)	lsa(A)	lsa(A), dfrE
CP110035.1	efrA, tet(M), dfrE, ErmB, lsa(A)	lsa(A), tet(M), ErmB	lsa(A), tet(M), ErmB, ant(6)-Ia	lsa(A), erm(B), dfrE, tet(M)
CP110036.1	lsa(A), efrA, tet(M), dfrE	lsa(A), tet(M)	lsa(A), tet(M), bcrA	lsa(A), tet(M), dfrE
CP110038.1	efrA, tet(M), dfrE, lsa(A)	lsa(A), tet(M)	lsa(A), tet(M)	lsa(A), tet(M), dfrE
CP110039.1	efrA, dfrE, lsa(A)	lsa(A)	lsa(A)	lsa(A), dfrE
CP110040.1	efrA, dfrE, lsa(A)	lsa(A)	lsa(A)	lsa(A), dfrE
CP110041.1	efrA, tet(M), dfrE, lsa(A)	lsa(A), tet(M)	lsa(A), tet(M)	lsa(A), tet(M), dfrE
CP110047.1	tet(S), lsa(A), efrA, tet(M), dfrE	lsa(A), tet(M), tet(S)	lsa(A), tet(M), tet(S)	lsa(A), tet(M), tet(S), dfrE
CP110050.1	tet(S), lsaA, efrA, tet(M), dfrE, lsa(A)	lsa(A), tet(M), tet(S)	lsa(A), tet(M), tet(S)	lsa(A), tet(M), tet(S), dfrE
CP110053.1	efrA, tet(M), dfrE, lsa(A)	lsa(A), tet(M)	lsa(A), tet(M)	lsa(A), tet(M), dfrE
CP110054.1	efrA, dfrE, lsa(A)	lsa(A)	lsa(A)	lsa(A), dfrE
CP110059.1	efrA, dfrE, lsa(A)	lsa(A)	lsa(A)	lsa(A), dfrE
CP110063.1	efrA, tet(M), dfrE, lsa(A)	lsa(A), tet(M)	lsa(A), tet(M)	lsa(A), tet(M), dfrE
CP110068.1	efrA, dfrE, lsa(A)	lsa(A)	lsa(A)	dfrE, lsa(A)
CP110069.1	tet(S), lsa(A), efrA, tet(M), dfrE	lsa(A), tet(M), tet(S)	lsa(A), tet(M), tet(S)	lsa(A), tet(M), tet(S), dfrE
CP110071.1	dfrG, APH(3')-IIIa, lsaE, ANT(6)-Ia, lnuB, catA8, SAT-4, efrA, ErmB, dfrE, tet(M), lsa(A)	ant(6)-Ia , str, aph(3')-III, dfrG, lsa(E), lsa(A), lnu(B), erm(B), tet(M), cat	dfrG, lsa(A), str, catA, sat4, aph(3')-IIIa, ant(6)-Ia, spw, lsa(E), lnu(B), erm(B), tet(M)	dfrG, lsa(A), str, catA8, sat4, aph(3')-IIIa, spw, lsa(E), lnu(B), ant(6)-Ia, erm(B), dfrE, tet(M)
CP110072.1	dfrG, APH(3')-IIIa, lsaE, ANT(6)-Ia, lnuB, catA8, SAT-4, efrA, ErmB, dfrE, tet(M), lsa(A)	ant(6)-Ia, str, aph(3')-III, dfrG, lsa(E), lsa(A), lnu(B), erm(B), tet(M), cat	dfrG, lsa(A), str, catA, sat4, aph(3')-IIIa, ant(6)-Ia, spw, lsa(E), lnu(B), ant(6)-Ia, erm(B), tet(M)	dfrG, lsa(A), str, catA8, sat4, aph(3')-IIIa, spw, lsa(E), lnu(B), ant(6)-Ia, erm(B), dfrE, tet(M)
CP110074.1	efrA, dfrE, lsa(A)	lsa(A)	lsa(A)	lsa(A), dfrE

semination of resistance genes and other functional genes that confer adaptive advantages. The identification of approximately 120 different composite transposons across 14 isolates highlights the exten-sive genetic diversity and the potential for horizontal gene transfer events within this microbial population. We also observed that Tetracycline resistance gene, tet(M) of BE43 strain and linezolid resistance genes lsa(E) gene of BE16 strain and fsrA gene of BE54 strain were pre-dicted to be within prophage sequences.

3.5. AMR Gene interaction network analysis

The mining of interaction partners for the AMR based on sequence similarity with *E. faecalis* V583 genome identified functional partners for five AMR genes. The network analysis of gene interactions of AMR genes revealed intricate relationships among key genes (Fig. 3). ANT (6)-Ia emerged as central due to its interactions with metabolic enzymes like mprF and transport proteins such as ecfA1, highlighting its role in antibiotic resistance and cellular transport. The bcrA gene, associated with bacitracin resistance, interacted with ecfA1, ecfA2, and other transport-related genes, indicating its involvement in resistance and membrane transport func-tions. The dfrE (folA) gene, crucial for folate biosynthesis, showed interactions with genes involved in nucleotide (thyA, purN, purH) and amino acid metabolism (glyA), suggesting a key role in these metabolic pathways. Lastly, efrA and lsa(A) were linked to efflux mechanisms and ribosomal protection, un-

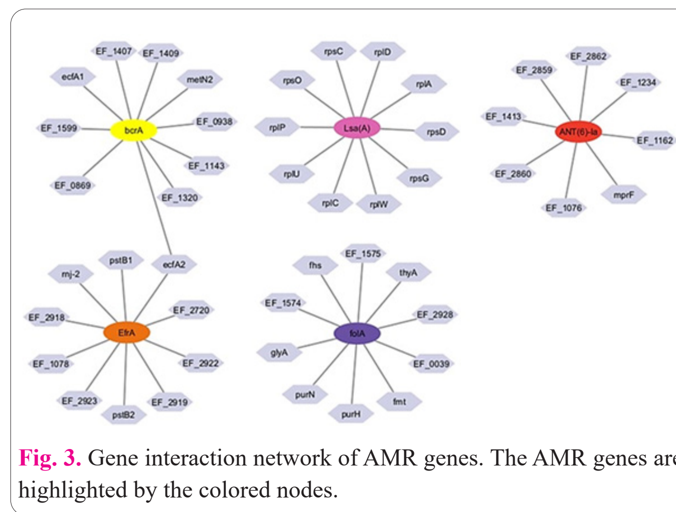


Fig. 3. Gene interaction network of AMR genes. The AMR genes are highlighted by the colored nodes.

derscoring their contributions to antibiotic resistance and the regulation of protein synthesis.

The network properties of antimicrobial resistance (AMR) genes in *E. faecalis* were analyzed, revealing key insights into their connectivity and potential roles within the bacterial genome. The genes included in the analysis are ANT (6)-Ia, bcrA, dfrE (folA), efrA, and lsa(A). Their respective network properties such as Betweenness Centrality, Closeness Centrality, Degree, Eccentricity, and Topological Coefficient were calculated and summarized in Table 4. Network topological metrics of the AMR gene networks.

ANT (6)-Ia, dfrE (folA), and lsa(A) have the highest Betweenness and Closeness Centrality values of 1, highlighting their crucial roles as central hubs in the network. These genes also show the lowest Eccentricity, underscoring their central position. The Degree analysis shows that bcrA, folA, EfrA, and lsa(A) are highly connected, with bcrA and EfrA having the highest Topological Coefficient at 0.1, indicating shared interaction partners. Overall, ANT (6)-Ia, dfrE (folA), and lsa(A) are pivotal in maintaining network connectivity, while bcrA and efrA, de-spite high connectivity, are more peripheral, suggesting roles in specific resistance mechanisms.

3.6. Virulence gene interaction network analysis

The twenty-two virulent genes were found to have interacting partners in the STRING data-base. The virulence gene network analysis of *E. faecalis* highlights several highly connected genes, which play pivotal roles in pathogenicity (Fig. 4). The gene asa1, associated with adhe-sion and biofilm formation, exhibits extensive interactions with TDC, cylM, vanB, and gelE, indicating its central role in coordinating various virulence mechanisms. The Asa1 gene medi-ates bacterial aggregation and enables plasmid transfer, also promoting biofilm formation. The cytolysin operon is a two-component system consisting of lysin (L) encoded by cylL1, cylL2, cylM, and cylB, and an activator (A) encoded by cylA.

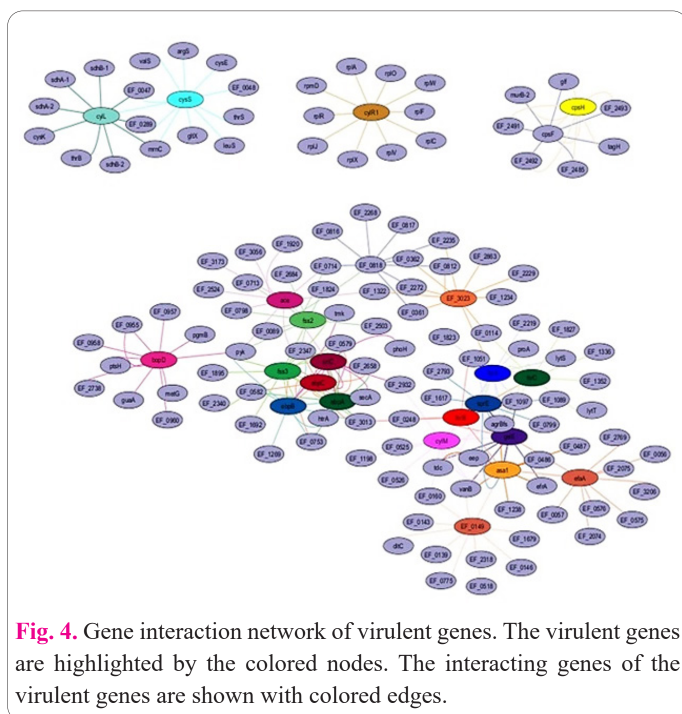


Fig. 4. Gene interaction network of virulent genes. The virulent genes are highlighted by the colored nodes. The interacting genes of the virulent genes are shown with colored edges.

The network analysis of virulent genes in *E. faecalis* reveals a complex interplay of gene in-teractions essential for pathogenicity. Key network properties highlight central genes crucial for coordinating virulence mechanisms. cpsH, cylR1, and cylL emerge as pivotal genes with high Betweenness Centrality, indicating their role as bridges between different parts of the network. They also exhibit high Closeness Centrality, suggesting their efficiency in influencing other genes. Genes like asa1, gelE, sprE, and fss3 show high Degree, indicating their extensive connections within the network. cpsH, cylR1, and cylL have low Eccentricity, implying their central positioning and efficient interaction capabilities.

The Topological Coefficient highlights cpsH, cylL, and cysS as genes likely to share interaction partners, facilitating coordinated virulence functions. This comprehensive analysis sheds light on the structural framework of virulence in *E. faecalis*, identifying key genes pivotal for pathogenicity (Table 5).

4. Discussion

The identification of multiple adherence factors suggests that *E. faecalis* has robust mechanisms for attaching to host tissues, which is critical for colonization and infection[23]. Biofilms pro-vide a protective niche for bacteria, enhancing their resistance to host immune responses and antimicrobial agents. The overlap of biofilm formation and adherence factors indicates that *E. faecalis* employs a coordinated strategy to establish and maintain persistent infections. Capsule formation is another crucial virulence mechanism, providing protection against phagocytosis and contributing to the bacterium's ability to evade the host immune system. The comprehen-sive array of capsule formation cps operon encoding the polysaccharide capsule and 11 open reading frames (cpsA through cpsK) identified in these isolates underscores the importance of this virulence strategy in oral infections [24].

Toxins such as cylA and gelE are directly involved in damaging host tissues and modulating immune responses [24]. The presence of multiple toxin genes points to the aggressive nature of *E. faecalis* in inflicting host damage to facilitate infection and dissemination. Secretion systems and regulatory factors, such as the fsr operon (fsrA, fsrB, fsrC), play critical roles in the ex-pression and secretion

Table 4. Topological metrics of the AMR gene networks.

Gene	Betweenness Centrality	Closeness Centrality	Degree	Eccentricity	Topological Coefficient
ANT (6)-Ia	1.000	1.000	8	1	0.0
bcrA	0.711	0.513	10	3	0.1
dfrE (folA)	1.000	1.000	10	1	0.0
EfrA	0.711	0.513	10	3	0.1
Lsa(A)	1.000	1.000	10	1	0.0

Table 5. Network topological metrics of the virulent gene networks.

Gene	Betweenness Centrality	Closeness Centrality	Degree	Eccentricity	Topological Coefficient
ace	0.143653	0.28133	11	6	0.13913
asa1	0.243	0.306407	16	6	0.144465
bopD	0.157631	0.22449	11	7	0.1
cpsH	0.375	1	9	1	0.34375
cylL	0.606618	0.708333	10	2	0.154545
cylM	0.143818	0.302198	14	6	0.210909
cylR1	1	1	10	1	0
cysS	0.680147	0.73913	11	2	0.145455
ebpA	0.026707	0.289474	14	5	0.265217
ebpB	0.097303	0.297297	14	5	0.26087
ebpC	0.026707	0.289474	14	5	0.265217
EF_0149	0.141729	0.244444	11	7	0.108333
efaA	0.140784	0.259434	10	7	0.152941
fsrA	0.193458	0.308989	14	6	0.171123
fsrB	0.298461	0.340557	10	5	0.192683
fsrC	0.085668	0.292553	13	6	0.196364
fss2	0.153322	0.277778	13	6	0.123563
fss3	0.170564	0.306407	14	5	0.229167
gelE	0.153535	0.319767	17	6	0.155518
sprE	0.092775	0.310734	15	6	0.194805
srtC	0.139091	0.290237	14	5	0.204762

of virulence determinants. The Fsr proteins are essential for producing two secreted proteases: gelatinase (GelE), serine protease (SprE) and enterocin O16 [25]. The fsr quorum-sensing system regulates biofilm development by promoting gelatinase production [26].

The dual role of these factors in regulation and secretion highlights their importance in the pathogenicity of *E. faecalis*. Immune evasion factors, including perR and phrB, enable *E. faecalis* to persist within the host by neutralizing host defenses [27]. The identification of such factors emphasizes the adaptive capabilities of *E. faecalis* in hostile environments like the oral cavity. The identification of these virulence factors, especially those with overlapping roles, reveals a sophisticated pathogenic strategy that complicates effective management. Recent studies have clarified the role of phages in the horizontal gene transfer (HGT) of antimicrobial resistance (AMR) genes, as these genes are frequently found in prophage sequences and are stably inherited within the host genome, carrying antibiotic resistance determinants [28].

Gelatinase damages host tissue, facilitating bacterial migration and spread [29]. The fsr quorum-sensing system (fsrA, fsrB, fsrC) emerges as another crucial hub, regulating genes like gelE and cylM, which are involved in extracellular matrix production and stress responses. The ebp operon (ebpA, ebpB, ebpC), linked to srtC, enhances bacterial adhesion and biofilm for-mation, further demonstrating its significance in host colonization [30]. GelE, a gelatinase, interacts with regulatory genes and agrBfs, highlighting its dual role in matrix degradation and antibiotic resistance, tightly regulated by quorum sensing. The production of cytolysin has been demonstrated to contribute to the severity of enterococcal disease [31]. The cyl operon, re-sponsible for cytolysin production, connects with ribosomal and metabolic genes, suggesting a link between virulence expression and the bacterial metabolic state.

5. Conclusions

In conclusion, our study provides a detailed analysis of *Enterococcus faecalis* isolates from the oral cavity, revealing diverse genomic characteristics, including 14 sequence types and a range of virulence and antimicrobial resistance genes. The findings underscore the pathogenic po-tential of *E. faecalis* in oral infections, characterized by robust adherence mechanisms, biofilm formation capabilities, and a spectrum of antimicrobial resistance profiles. Detection of mobile genetic elements such as plasmids and prophages highlight their role in genetic diversity and horizontal gene transfer. Network analyses elucidate central genes like cpsH, cylR1, cylL in virulence, and ANT (6)-Ia, dfrE (folA), lsa(A) in resistance, emphasizing their pivotal roles. The genetic similarity observed between *E. faecalis* genomes in saliva and root canal samples sug-gests that the pathogen's presence in saliva may contribute to root canal contamination, thereby increasing the likelihood of periapical lesion development. This study enhances understanding of *E. faecalis* epidemiology and underscores its implications for oral health management and infection control strategies.

Supplementary materials

The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: List of prophages identified in the study isolates, Table S2: List of Mobile Genetic Elements identified in the study isolates.

Acknowledgments

The authors gratefully acknowledge the funding of the

Deanship of Graduate Studies and Scientific Research, Jazan University, Saudi Arabia, through Project Number: GSSRD-24.

Consent for publication

The author agreed with the final manuscript for publication.

Ethics approval and consent to participate

The present research does not use human or animals.

Conflicts of interest

The authors declare no conflicts of interest.

Author's contribution

N.B: Concept, supervision and writing original draft. A.A.M.M; Formal analysis, data curation and data validation, Visualization and analysis. A.S.A: Editing manuscript and data curation. A.H and I.A.S: Project administration and Investigation. G.G.J and M.A.B: Writing contribution. O.A: Funding. E.A.T and R.M: Writing contribution

References

1. Alcock BP, Huynh W, Chalil R, Smith KW, Raphenya AR, Wlodarski MA, Edalatmand A, Petkau A, Syed SA, Tsang KK (2023) CARD 2023: expanded curation, support for machine learning, and resistome prediction at the Comprehensive Antibiotic Resistance Database. Nucleic Acids Res 51 (D1): D690-D699. doi: https://doi.org/10.1093/nar/gkac920
2. Borgio JF, AlJindan R, Alghourab LH, Alquwaie R, Aldahhan R, Alhur NF, AlEraky DM, Mahmoud N, Almandil NB, AbdulAzeez S (2023) Genomic Landscape of Multidrug Resistance and Virulence in *Enterococcus faecalis* IRMC827A from a Long-Term Patient. Biol 12 (10): 1296. doi: https://doi.org/10.3390/biology12101296
3. Javadian E, Biabangard A, Ghafari M, Saeeidi S, Fazeli-Nasab B (2024) Green Synthesis of Silver Nanoparticles and Antibacterial Properties of Extracts of Capparis spinosa Leaves. Int J Med Plants By-Prod 13 (2): 329-343. doi: https://doi.org/10.22034/jmpb.2023.361363.1528
4. Alavi M, Hamblin MR (2023) Antibacterial silver nanoparticles: effects on bacterial nucleic acids. Cell Mol Biomed Rep 3 (1): 35-41. doi: Https://doi.org/10.55705/cmbr.2022.361677.1065
5. Evans M, Davies JK, Sundqvist G, Figdor D (2002) Mechanisms involved in the resistance of *Enterococcus faecalis* to calcium hydroxide. Int Endod J 35 (3): 221-228. doi: https://doi.org/10.1046/j.1365-2591.2002.00504.x
6. Feldgarden M, Brover V, Gonzalez-Escalona N, Frye JG, Haendiges J, Haft DH, Hoffmann M, Pettengill JB, Prasad AB, Tillman GE (2021) AMRFinderPlus and the Reference Gene Catalog facilitate examination of the genomic links among antimicrobial resistance, stress response, and virulence. Sci rep 11 (1): 12728. doi: https://doi.org/10.1038/s41598-021-91456-0
7. Gaeta C, Marruganti C, Ali IA, Fabbro A, Pinzauti D, Santoro F, Neelakantan P, Pozzi G, Grandini S (2023) The presence of *Enterococcus faecalis* in saliva as a risk factor for endodontic infection. Front Cell Infect Microbiol 13: 1061645. doi: https://doi.org/10.3389/fcimb.2023.1061645
8. Ghazvinian M, Asgharzadeh Marghmalek S, Gholami M, Amir Gholami S, Amiri E, Goli HR (2024) Antimicrobial resistance patterns, virulence genes, and biofilm formation in enterococci strains collected from different sources. BMC Infect Dis 24 (1): 274. doi: https://doi.org/10.1186/s12879-024-09117-2
9. Hahn C-L, Hanford K (2021) An In Vitro Model to Study the Colonization and Tubular Invasion of *Enterococcus faecalis*. J Endod 47 (3): 451-457. doi: https://doi.org/10.1016/j.joen.2020.12.004
10. Hancock LE, Perego M (2004) The *Enterococcus faecalis* fsr two-component system controls biofilm development through production of gelatinase. J Bacteriol 186 (17): 5629-5639. doi: https://doi.org/10.1128/jb.186.17.5629-5639.2004
11. Komiyama EY, Lepesqueur LSS, Yassuda CG, Samaranayake LP, Parahitiyawa NB, Balducci I, Koga-Ito CY (2016) Enterococcus species in the oral cavity: prevalence, virulence factors and antimicrobial susceptibility. PloS one 11 (9): e0163001. doi: https://doi.org/10.1371/journal.pone.0163001
12. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13 (11): 2498-2504. doi: https://doi.org/10.1101/gr.1239303
13. Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, Doncheva NT, Legeay M, Fang T, Bork P (2021) The STRING database in 2021: customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic acids res 49 (D1): D605-D612. doi: https://doi.org/10.1093/nar/gkaa1074
14. Siguier P, Pérochon J, Lestrade L, Mahillon J, Chandler M (2006) ISfinder: the reference centre for bacterial insertion sequences. Nucleic acids res 34 (suppl_1): D32-D36. doi: https://doi.org/10.1093/nar/gkj014
15. Sillanpää J, Chang C, Singh KV, Montealegre MC, Nallapareddy SR, Harvey BR, Ton-That H, Murray BE (2013) Contribution of individual Ebp Pilus subunits of *Enterococcus faecalis* OG1RF to pilus biogenesis, biofilm formation and urinary tract infection. PloS one 8 (7): e68813. doi: https://doi.org/10.1371/journal.pone.0068813
16. Tamura K, Stecher G, Kumar S (2021) MEGA11: molecular evolutionary genetics analysis version 11. Mol Biol Evol 38 (7): 3022-3027. doi: https://doi.org/10.1093/molbev/msab120
17. Verneuil N, Rincé A, Sanguinetti M, Posteraro B, Fadda G, Auffray Y, Hartke A, Giard J-C (2005) Contribution of a PerR-like regulator to the oxidative-stress response and virulence of *Enterococcus faecalis*. Microbiol 151 (12): 3997-4004. doi: https://doi.org/10.1099/mic.0.28325-0
18. Wang Q-Q, Zhang C-F, Chu C-H, Zhu X-F (2012) Prevalence of *Enterococcus faecalis* in saliva and filled root canals of teeth associated with apical periodontitis. Int J Oral Sci 4 (1): 19-23. doi: https://doi.org/10.1038/ijos.2012.17
19. Wishart DS, Han S, Saha S, Oler E, Peters H, Grant JR, Stothard P, Gautam V (2023) PHASTEST: faster than PHASTER, better than PHAST. Nucleic Acids Res 51 (W1): W443-W450. doi: https://doi.org/10.1093/nar/gkad382
20. Zhu X, Wang Q, Zhang C, Cheung GSP, Shen Y (2010) Prevalence, Phenotype, and Genotype of *Enterococcus faecalis* Isolated from Saliva and Root Canals in Patients with Persistent Apical Periodontitis. J Endod 36 (12): 1950-1955. doi: https://doi.org/10.1016/j.joen.2010.08.053
21. Zhang C, Du J, Peng Z (2015) Correlation between *Enterococcus faecalis* and Persistent Intraradicular Infection Compared with Primary Intraradicular Infection: A Systematic Review. J Endod 41 (8): 1207-1213. doi: https://doi.org/10.1016/j.joen.2015.04.008
22. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB (2006) *Enterococcus faecalis*: its role in root canal treatment failure and current concepts in retreatment. J Endod 32 (2): 93-98. doi: https://doi.org/10.1016/j.joen.2005.10.049
23. Köser CU, Ellington MJ, Peacock SJ (2014) Whole-genome sequencing to control antimicrobial resistance. Trends Genet 30

(9): 401-407. doi: https://doi.org/10.1016/j.tig.2014.07.003

24. Kondo K, Kawano M, Sugai M (2021) Distribution of antimicrobial resistance and virulence genes within the prophage-associated regions in nosocomial pathogens. Msphere 6 (4): 10.1128/msphere. 00452-00421. doi: https://doi.org/10.1128/msphere.00452-21
25. Kiruthiga A, Padmavathy K, Shabana P, Naveenkumar V, Gnanadesikan S, Malaiyan J (2020) Improved detection of esp, hyl, asa1, gelE, cylA virulence genes among clinical isolates of Enterococci. BMC Res Notes 13: 1-7. doi: https://doi.org/10.1186/s13104-020-05018-0
26. Letunic I, Bork P (2021) Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. Nucleic Acids Res 49 (W1): W293-W296. doi: https://doi.org/10.1093/nar/gkab301
27. Manson JM, Hancock LE, Gilmore MS (2010) Mechanism of chromosomal transfer of *Enterococcus faecalis* pathogenicity island, capsule, antimicrobial resistance, and other traits. PNAS 107 (27): 12269-12274. doi: https://doi.org/10.1073/pnas.1000139107
28. Olson RD, Assaf R, Brettin T, Conrad N, Cucinell C, Davis JJ, Dempsey DM, Dickerman A, Dietrich EM, Kenyon RW (2023) Introducing the bacterial and viral bioinformatics resource center (BV-BRC): a resource combining PATRIC, IRD and ViPR. Nucleic Acids Res 51 (D1): D678-D689. doi: https://doi.org/10.1093/nar/gkac1003
29. Pinheiro ET, Penas PP, Endo M, Gomes BPFA, Mayer MPA (2012) Capsule Locus Polymorphism among Distinct Lineages of *Enterococcus faecalis* Isolated from Canals of Root-filled Teeth with Periapical Lesions. J Endod 38 (1): 58-61. doi: https://doi.org/10.1016/j.joen.2011.08.002
30. Rostamzadeh S, Mirnejad R, Masjedian Jazi F, Masoumi Zavaryani S, Saeidi S, Zahedi bialvaei A (2020) Molecular characterization of the virulence genes gelE and cylA in the Enterococcus species isolated from clinical samples. Meta Gene 24: 100695. doi: https://doi.org/10.1016/j.mgene.2020.100695
31. Sengupta M, Sarkar S, SenGupta M, Ghosh S, Sarkar R, Banerjee P (2021) Biofilm producing Enterococcus isolates from vaginal microbiota. Antibiotics 10 (9): 1082. doi: https://doi.org/10.3390/antibiotics10091082