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Potential Protein Receptors in *Enterococcus faecalis* as Antibacterial and Anti-Biofilm Target Therapy in Endodontic treatment: A Review

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ABSTRACT

E. Faecalis is responsible for 80–90% of hospital-acquired enterococcal infections and failed endodontic tretment. The emergence of *E. Faecalis* multidrug resistance including to common endodontic medication and its ability to form biofilms have significantly complicated its clinical management. Thereby the development of novel antibacterial and antibiofilm agents is essential. Identifying and targeting key proteins is critical for drug discovery and development. This review aims to provide a brief information about potential protein receptors in *E. Faecalis* that could serve as targets for antibacterial and antibiofilm therapies.

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INTRODUCTION

Enterococcus faecalis (*E. Faecalis*), an aerobic gram-positive bacterium, frequently responsible for the persistent periradicular lesions and root canal treatment failures due to its ability to from biofilm in harsh environments.(Alghamdi & Shakir, 2020; Boreak et al., 2024a) *E. Faecalis* survived in pH 11.5 and reistance to several antibacterial agent.(Alghamdi & Shakir, 2020) Which is resulting in high prevalence of *E. Faecalis* in 90% of failed endodontic therapy.(Alghamdi & Shakir, 2020) In agreement, previous study by Thammasitboon and collegue found *E. Faecalis* in 88.9% of post endodontic treatment teeth with periradicular lesion using real-time PCR. Moreover, heamollitic strain *E. Faecalis* found resistant to tetracycline and erythromycin. The expression of virulence genes also enhances which may related to its severe manifestations.(Thammasitboon et al., 2024) Furthermore, recent study by Jayavarsha and colleagues, revealed that *E. Faecalis* isolat in the root caries samples resisten to several antibiotic including vancomisin. The present of vanA gene suspected responsible for vancomycin-resistant Enterococci (VRE). VanA gene mediate resistance to vancomycin by alteration the peptidoglycan synthesis.(Jayavarsha et al., 2023)

The resilience of Enterococcus faecalis, its ability to form biofilms, and the rise of multidrug-resistant strains—including vancomycin-resistant enterococci (VRE)—have made eradication from the root canal system increasingly difficult.(Boreak et al., 2024b; Radha et al., 2024)

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Moreover, several study revealed that root canal ,medicament, Calcium hydroxide and sodium hypochlorite, failed to completely eliminate *E. Faecalis* .(Alghamdi & Shakir, 2020) Therefore, the development of novel antibacterial and antibiofilm agents is essential. Elucidating the molecular mechanisms underlying Enterococcus faecalis biofilm formation may offer valuable insights for therapeutic intervention. The identification and targeting of key proteins involved in this process are essential steps in the discovery and development of novel antimicrobial agents.(Basak Erol et al., 2024; Suriyanarayanan et al., 2018) This study aims to provide a brief review of potential protein receptors in *E. Faecalis* that could serve as targets for antibacterial and antibiofilm therapies.

METHODS

The literature search was made in two electronic databases (PubMed and Google Scholar) with the following key words combinations as the following: “Endodontic Infections" OR "Endodontic Pathogens," OR “failed endodontic treatment”, and "Enterococcus Faecalis") from December 2019 and then updated in February 2020. Experimental studies or literature reviews written in English, published during the preceding 10 years (2015to 2025) were used. Article screening by review the abstract abstracts or full articles examination if needed. 26 articles were finally selected for this.

Review

Enterococcus faecalis resistence and its protein receptors therapeutic targets

Enterococcus faecalis is a commensal microorganism commonly found in the oral cavity, gastrointestinal tract, and female genital tract of humans. Despite its benign presence in these sites, it is an opportunistic pathogen implicated in various infections, including urinary tract infections, surgical wound infections, bacteremia, and endocarditis bacterialis.(Thammasitboon et al., 2024) *E. Faecalis* is responsible for 80–90% of hospital-acquired enterococcal infections.(Niaz et al., 2022) The emergence of multidrug resistance and the ability of *E. Faecalis* to form biofilms have significantly complicated its clinical management. its high level of antimicrobial resistance and biofilm-associated persistence limited therapeutic options. Recent study by Niaz and collegue found that *E. Faecalis* isolated from hospital microbilology laboratories showed high levels of resistance against erythromycin (94%), tetracycline (91%), and ciprofloxacin (89%) (Figure 1)

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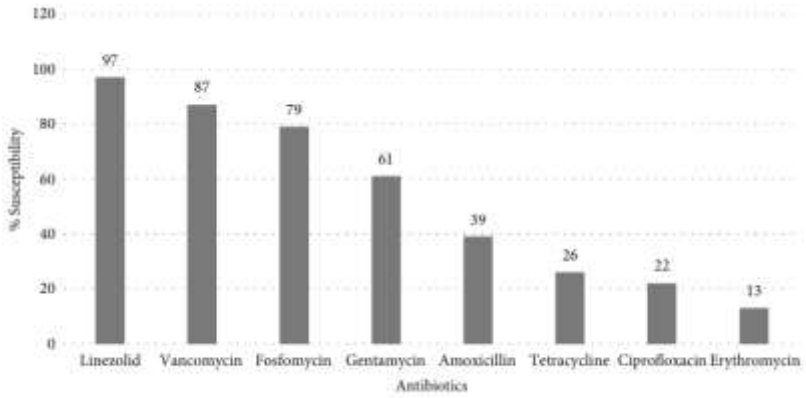

FIGURE 1: Antimicrobial susceptibility pattern of *E. faecalis*.

Figure 1. Antimicrobial susceptibility of *E. Faecalis*

Notably, *E. Faecalis* is frequently associated with persistent intraradicular infections and is considered a leading cause of endodontic treatment failure, mainly due to its capacity for biofilm formation and resistance to antimicrobial agents. The prevalence of *E. Faecalis* in teeth with failed endodontic treatment has been reported to range from approximately 88.9% to 90%.(Thammasitboon et al., 2024) Calcium hydroxide and sodium hypochlorite are commonly intracanal medicaments during root canal treatment. However, several studies have demonstrated that *E. Faecalis* exhibits resistance to these agents.(Alghamdi & Shakir, 2020) Calcium hydroxide exerts its antimicrobial effect by creating an alkaline environment through the release of hydroxyl ions. While *E. Faecalis* cannot survive at pH levels above 11.5, it can adapt to alkaline stress through an active proton pump mechanism. This pump is activated upon cytoplasmic penetration of hydroxyl ions, enabling the bacterium to acidify its cytoplasm and maintain homeostasis. The activation of the proton pump facilitates potassium ion influx, reducing cytoplasmic alkalinity and thereby preserving enzymatic function essential for survival.(Rodríguez-Niklitschek & Oporto V, 2015) Sodium hypochlorite is widely utilized in root canal theatment due to its strong antibiofilm properties.(Basak Erol et al., 2024) Nevertheless, multiple studies have reported the persistence of residual microbial contamination within the root canal system following its use. Additionally, its cytotoxic nature poses a risk of inducing adverse effects, including hypersensitivity reactions.(L. Chen et al., 2018) Therefore, alternative medication with a fewer side effects are urgently required.

Several proteins in *E. Faecalis* are crucial for bacterial metabolism and represent promising targets for therapeutic target. These include proteins involved in cell wall biosynthesis, metabolic processes, biofilm formation, and quorum sensing (QS) pathways. Furthermore, prior genetic screening studies have implicated several genes in the biofilm formation of *Enterococcus faecalis*, including those encoding the enterococcal surface protein (Esp), aggregation substance (AS), gelatinase (GelE), histidine kinase YycFG and components of the Fsr quorum sensing system.(Z. Chen et al., 2021; Suriyanarayanan et al., 2018)

Sortase A (SrtA)

Sortase A is a cysteine transpeptidase that anchors surface proteins to the Gram-positive bacterial cell wall, playing a key role in biofilm formation and virulence.(Parolia et al., 2021) Genomic analyses of *E. Faecalis* have revealed the presence of key sortase enzymes,

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notably the class A sortase (SrtA), which plays a critical role in pilus assembly, biofilm formation, and colonization of host tissues, as well as the class C sortase (SrtC), which is involved in pilus polymerization. Due to its strategic localization on the bacterial cell membrane, SrtA is readily accessible to inhibitory compounds, making it an attractive target for the development of anti-virulence therapeutics. Targeting SrtA by specific inhibitors are considered promising candidates for novel antimicrobial agents.(Boreak et al., 2024a) Inhibiting SrtA disrupts biofilm formation and reduces bacterial virulence.(V et al., 2025)

Studies have identified several phytocompounds as potential SrtA inhibitor, showing high binding affinity via molecular docking simulation, performed using Autodock Vina, as shown in the table 1.(Boreak et al., 2024a)

Table 1. phytocompounds as potential SrtA inhibitor

No	Phytocompounds	Binding Energy (kcal/mol)
1	Pinocembrin	-8.0
2	Glabridin	-7.9
3	Ursolic acid	-7.9
4	7-hydroxyflavanone	-7.9
5	Epigallocatechin3-gallate	-7.8
6	Curcumin	-7.7
7	Eriodictyol	-7.7
8	Myricitrin	-7.7
9	6-hydroxyflavanone	-7.7
10	Naringenin	-7.5
11	6,2' -dihydroxyflavanone	-7.5
12	Asiatic acid	-7.5
13	Oleanolic acid	-7.4
14	Quercetin	-7.3
15	2-hydroxyflavanone	-7.2

MurA (UDP-N-acetylenolpyruvoylglutamate reductase)

The cell wall of *Enterococcus* species, like that of other Gram-positive bacteria, is predominantly composed of peptidoglycan (PG), wall teichoic acid (WTA), and lipoteichoic acid (LTA). The peptidoglycan forms a mesh-like lattice overlying the cytoplasmic phospholipid bilayer, providing structural stability, integrity and protection against osmotic stress and mechanical pressure to the cell.(Km Rakhi et al., 2024; Windaryanti et al., 2022) MurA is an essential enzyme in peptidoglycan biosynthesis. It's a critical component of the bacterial cell wall by catalyzes enol pyruvate transfer from phosphoenol-pyruvate (PEP) to UNDP-N_acetylglucosamine. Inhibiting MurA leading to loss of cell integrity and disrupts cell wall synthesis.(Apriyanti et al., 2020; Kurnia et al., 2020) MurA 3D structure could be seen in figure 2.

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Natural compounds like 24-propylcholesterol, allypyrocatechol derivates have shown potential in binding to MurA, with a binding affinity of -7.6 kcal/mol, thereby inhibiting cell wall metabolism and biofilm formation.

cyclic-di-AMP (c-di-AMP)

Cyclic-di-AMP, a second messenger that play important role in bacterial growth, biofilm formation, virulence, and host immune response.(L. Chen et al., 2018) c-di AMP binding to and modulating nucleic acid or protein at a translational or transcriptional level. Enhance c-di-amp resulting in promotion of biofilm formation. Recent in vitro studies by biofilm quantification assay confirmed that, targeting the c-di-AMP syntetase DisA in *E. Faecalis* inhibit bacterial growth and biofilm formation.(L. Chen et al., 2018)

Quorum Sensing Regulator

the quorum sensing (QS) system is a regulatory system of unicellular microorganism that responsible for cell-cell communication and controls the gene expression that can be interrupted at one or more stages. Bacteria communicate through molecules signal called autoinducers.(Windaryanti et al., 2022) Gelatinase biosynthesis-activating pheromone (GBAP) is a cyclic peptide which acts as an autoinducing peptide. It stimulates fsr signal transduction cascade resulting in gelatinase biosinthesis, a virulence factors that contributing in biofilm formation. Targeting GBAP can disrupt bacterial communication and reduce pathogenicity.(Apriyanti et al., 2020; Satari et al., 2021)

Enterococcal Surface Protein (Esp)

Esp is a high-molecular-weight surface protein consisting of 1873 amino acids as well as N-terminal, central nucleus, and C-terminal domains. It is a cell wall protein contributing to bacterial adhesion, colonization of surfaces.(Ghameshlouei et al., 2021) It is play important role in for biofilm formation. E. faecallis isolat without Esp are able to produce biofilm after obtaining the plasmid carrying the Esp gene.(Ghameshlouei et al., 2021) It may be responsible in the colonization and persistence of *E. faecalis* during infections. Inhibiting Esp expression can significantly reduce biofilm formation. Oxadiazole derivatives(such as naphthalene and methoxyphenyl compounds), tymoquinone, ledene oxid have been identified as Esp inhibitors. (Niaz et al., 2022; Radha et al., 2024; Windaryanti et al., 2022) In agreement, Ghameshlouei and collegues revealed Esp gene expression downregulation can significantly reduce biofilm formation.(Ghameshlouei et al., 2021)

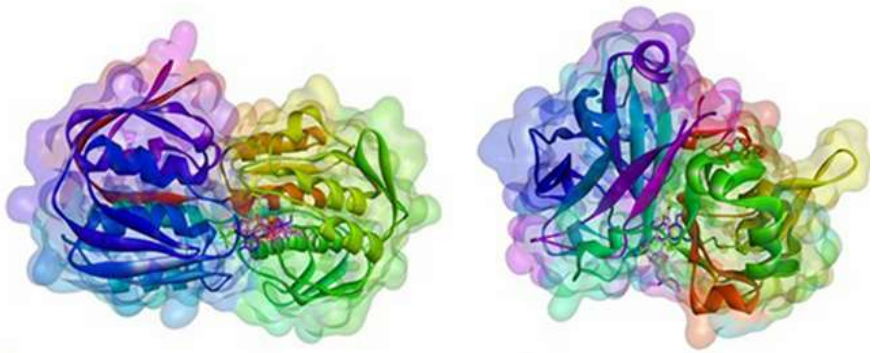

Figure 2. MurA (PDB_ID 1UAE) and Esp (PDB_ID 6ORI)

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Histidine kinase YycG

The YycFG plays a critical role in the regulation of biofilm formation in low-G + C Gram-positive bacteria. YycG, a sensor histidine kinase, is involved in modulating biofilm development, while the activated response regulator YycF can directly bind to the promoter regions of key genes such as the ica operon and codY, thereby enhancing extracellular polysaccharide (EPS) production and facilitating biofilm matrix formation.(Wu et al., 2022) YycG inhibitors may be valuable as a potential target of antibacterial and antibiofilmagainst *E. Faecalis*.

Due to its essential function in biofilm regulation, YycG represents a promising target for the development of novel antibacterial and antibiofilm therapies against Enterococcus faecalis. Previous research shows that YycG inhibitors based on autophosphorylation assays and binding kinetics analyses exhibited potent inhibitory activity against planktonic E. faecalis cells, including strains resistant to vancomycin and linezolid.(Z. Chen et al., 2021)

In silico approaches are efficient, fast, and reliable drugs screening. Moreover, it's capable to provide molecular interaction and binding mechanism.(Boreak et al., 2024a) In silico study, mainly structure based drugs design (SBDD), require 3D protein structure for molecular docking analysis. Following are several protein receptor ID has been used for in silico study in *E. Faecalis* (Table 2).

Table 2. Specific protein receptor for in silico study in *E. Faecalis*

No	Protein target	Organism	PDB ID	Validation
1	Sortase A	B. anthracis (Boreak et al., 2024a; Daood et al., 2021)	2KW8	Modeller, Ramachandran plot, ERRAT, and ProSA
		S. mutans(Parolia et al., 2021)	4TQX	
2	c-di-AMP synthetase DisA	Thermotoga maritima(L. Chen et al., 2018)	3C1Z (apoDisA), 3C21 (apoDisA ATP), 3C23 (apoDisA-Cordycepin-Triphosphate), and 3C1Y (apo DisA-c-di-AMP)	
3	MurA`	Escherichia coli(Windaryanti et al., 2022)	1UAE	
4	Esp	Enterococcus faecalis(Ghameshlouei et al., 2021; Niaz et al., 2022; Radha et al., 2024; Windaryanti et al., 2022)	6ORI	

5	GBAP	Enterococcus faecalis(Apriyanti et al., 2020; Windaryanti et al., 2022)	-
6	Gelatinase	Bacillus thermoproteolyticus(Windaryanti et al., 2022)	5A3Y

CONCLUSION

Several proteins in *E. Faecalis* are crucial for bacterial metabolism and represent promising targets as therapeutic target for novel antimicrobial development, including SrtA, c-d-AMP syntase DisA, MurA, Esp, GBAP and Gelatinasse. Further experimental are required to validate the specificity of this protein.

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