Review

Current Knowledge of Enterococcal Endocarditis: A Disease Lurking in Plain Sight to Health Provider

Francesco Nappi *

Posted Date: 5 February 2024

doi: 10.20944/preprints202402.0204.v1

Keywords: Enterococcus faecalis; vegetation; infective endocarditis; biofim; cardiac endovascular infection; gastrointestinal

Review

Current Knowledge of Enterococcal Endocarditis: A Disease Lurking in Plain Sight to Health Provider

Francesco Nappi

Department of Cardiac Surgery, Centre Cardiologique du Nord, 93200 Saint-Denis, France; francesconappi2@gmail.com or f.nappi@ccn.fr; Tel.: +33-149334104; Fax: +33-149334119

Abstract: *Enterococcus faecalis* is a bacterial pathogen that can cause opportunistic infections. Studies indicate that initial biofilm formation plays a crucial regulatory role in these infections, as well as in colonising and maintaining the gastrointestinal tract as a commensal member of the microbiome of most land animals. It has long been thought that vegetation of endocarditis resulting from bacterial attachment to the endocardial endothelium requires some pre-existing tissue damage, and in animal models of experimental endocarditis, mechanical valve damage is typically induced by cardiac catheterisation preceding infection. This section reviews historical and contemporary animal model studies that demonstrate *E. faecalis* ability to colonise the undamaged endovascular endothelial surface directly and produce robust microcolony biofilms encapsulated within a bacterially derived extracellular matrix. This report reviews both previous and current animal model studies demonstrating the resilient capacity of *E. faecalis* to colonise the undamaged endovascular endothelial surface directly and produce robust microcolony biofilms encapsulated in a bacterially derived extracellular matrix. The article also considers the morphological similarities when these biofilms develop on different host sites, for example when *E. faecalis* colonises the gastrointestinal epithelium as a commensal member of the common vertebrate microbiome, lurking in plain sight and transmitting systemic infection. These phenotypes may enable the organism to survive as an unrecognised infection in asymptomatic subjects, providing an infectious resource for subsequent clinical process of endocarditis.

Keywords: *Enterococcus faecalis;* vegetation; infective endocarditis; biofim; cardiac endovascular infection; gastrointestinal

1. Introduction

Enterococci are a unique type of bacteria due to their ability to withstand a broad range of different environmental parameters such as pH, temperature, salinity, bile acids and so on. They are resistant to many antibiotic compounds and have the flexibility to flourish as both common commensal and opportunistic pathogens in a broad range of clinical settings. [1-6] Enterococci commonly live in the body and can cause chronic endocarditis, especially *Enterococcus faecalis*. [5,7-9] They account for approximately 10% of valvular endocarditis cases, with *E. faecalis* being the main causative agent. [5,7-11] **Figure 1**

 doi:10.20944/preprints202402.0204.v1

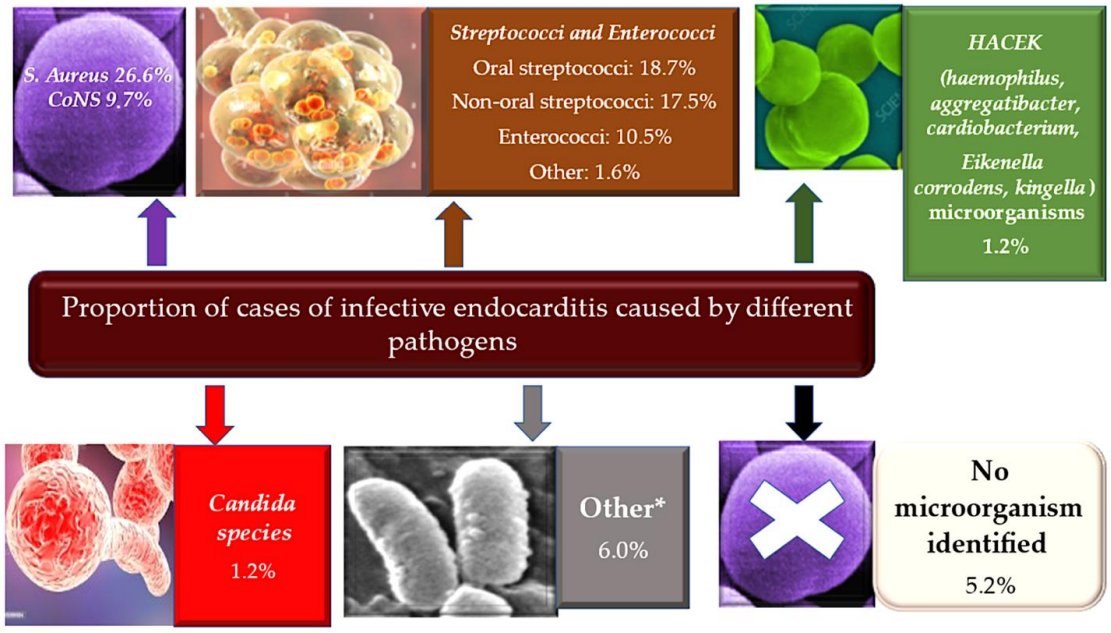

Figure 1. Elderly patients with a history of CIED and younger patients with a history of PWID have a higher incidence of IE. Low incidence of IE in patients with central venous catheters, HIV, CHD and immunosuppression. 26.6% of IE cases are due to Staphilococcus Aureus and 9.7% of these are due to CoNS. Enterococci are involved in more than 10% of cases * Small numbers of *Coxiella burnetii, Bartonella quintana, Pseudomonas aeruginosa, Tropheryma whipplei, Enterobacteriaceae, Acinetobacter ursingii, Listeria monocytogenes, Propionibacterium acnes, Neisseria elongata* and *Veillonella spp.* Abbreviations: CIED, cardiac implantable electronic devices; CHD, congenital heart disease; CoNS, coagulase negative; HIV, human immunodeficiency virus; IE, infective endocarditis; PWID; persons who inject drugs. From Nappi et al Ref [11].

To improve patient outcomes, it is important to accurately diagnose and treat enterococcal infections. During colonization of the murine gastrointestinal (GI) tract, *E. faecalis* has been shown to form and develop bacterial biofilms. These biofilms consist of bacteria attached to a host surface and surrounded by a bacterially-derived extracellular matrix (ECM).[12,13] In animal models of enterococcal catheter-associated urinary tract infection and endocarditis, *E. faecalis* has been identified as a significant pathogenic factor.[14-21] This finding was first reported in 2007.[21] Colonization results in the formation of a defensive bacterial biofilm on the native or engineered tissue: biofilm formation often results in markedly enhanced levels of resilience to antimicrobial agents.[22-24]

Bacteria colonise a prestaged, abacterial collection of host factors according to the classical or canonical model of bacterial endocarditis. The model proposes a two-step process: First, platelets, components of the coagulation chain including fibrinogen, thrombin, etc., and other host factors are deposited in reaction to an initial injury, thereby creating a "sterile vegetation". Bacteria already circulating in the bloodstream then populate this aberrant site, establishing a largely quiescent infection nidus. [10] Likewise, enterococcal infection is a significant cause of dysfunction in allogeneic tissue used as a biological valve replacement for patients who have received an allograft, whether for endocarditis or non-endocarditis of the aortic valve. [11,25]

Barnes et al. [13] have previously reported that *E. faecalis* directly engages and colonises the surface of the intestinal epithelium, producing distinct biofilm microcolonies across the gastrointestinal tract in a germ-free mouse module of infection. In a rabbit model of cardiac endovascular infection, a comparable pattern of colonization of the native host surface is also observed.[12] These observations suggest that the adhesion of enterococci to the cardiac endothelium has a similar role in the development of pathogenic endocarditis as it does in non-pathogenic

 doi:10.20944/preprints202402.0204.v1

intestinal epithelial colonisation. This is supported by the absence of significant systemic host responses to this colonization over several weeks and the ability of *E. faecalis* to adhere to intact endothelium.

This section reviews both the current and past findings for this kind of infection, shows how the conventional model fits, and fails to fit, with recent findings in the area, and considers possible future directions to better understand the pathophysiology of this increasingly important clinical infection. **Table 1.**

Table 1. Pathogen in allograft infection in non-endocarditis and comparison of pathogen in allograft implant and in allograft infection in endocarditis.

Pathogen	Non-IE Allograft infection		IE Pathogen at allograft implant		IE Pathogen at allograft infection	
	n* 22	No.%	nγ 46	No.%	nλ 42	No.%
Staphylococcus aureus	0 (0)		9 (20)		11 (26)	
CoNS	0 (0)		4 (8,7)		3 (7.1)	
Virdans group strep	10 (45)		5 (11)		7 (17)	
Enterococcus	0 (0)		7 (15)		3 (7.1)	
Others	3 (14)		5 (11)		5 (12)	
Pathogen not identified	3 (14)		9 (20)		5 (12)	
Other GPC	3 (14)		4 (8.7)		4 (9.5)	
Fungus	3 (14)		3 (6.5)		4 (9.5)	

Table 1. The infection rate was assessed in patients who received an allograft, both those who underwent surgery for aortic valve endocarditis and those who underwent surgery for reasons unrelated to infection. The causative pathogen type was investigated in previous cardiac surgery and reoperation. Abbreviations; IE, Infective endocarditis; CoNS, coagulase-negative Staphylococci; GPC, gram-positive cocci. *Data available for 22/30 non-IE patients with new allograft infection. γData available for 46 of 49 allograft recipients with IE at index procedure. λData available for 42 of 49 patients with IE with recurrent allograft infection.

2. History

Originally described in the early 20th century and named *Streptococcus faecalis* before being placed in the genus Enterococcus in 1984, *Enterococcus faecalis* has been known to cause endocarditis since the seminal paper published by Andrewes and Horder in 1906. [26]

As previously mentioned, the conventional paradigm for bacterial colonisation of the heart involves an abiotic accumulation of host factors. This is usually accompanied by an endovascular injury. Nevertheless, it is noteworthy that numerous papers in the earlier literature (prior to 1975) reported that enterococcal endocarditis appeared to arise in a substantial proportion of individuals without obvious prior gross endothelial damage or structural cardiac defects. [27,28] As is frequently the case in earlier literature, the exact determination of the particular bacterial strain can be challenging. Several animal model models, notably pigs [29] and rabbits [30] , have also described these clinical findings.

During the 1970s and 1980s, the medical community focused on enterococci because of their high level of intrinsic and transmissible antibiotic resistance in comparison to pathogenic streptococci. It

 doi:10.20944/preprints202402.0204.v1

is worth noting that until the 1980s, enterococci were phylogenetically classified as members of the genus Streptococcus.[31]

The interest of the medical and health care community in enterococci during the 1970s and 1980s was largely driven by the relatively high level of inherent and transmissible antibiotic resistance of these bacteria compared to the pathogenic streptococci routinely found in the population. It is noteworthy to mention that enterococci were classified phylogenetically as belonging to the species Streptococcus right up to the 1980s. [31] During this time, genetic and molecular studies of both plasmids and trans-spliced genetic material provided an important experimental basis for future genomic approaches to enterococcal virulence.[32,33] Yet the global clinical frequency of clinically ascertained enterococcal infections continued to be low throughout much of this time frame, although it is unclear whether this represents a real incidence rate or merely a reflection of a more restricted diagnostic landscape.

In the 1980s, the widespread use of oral prophylaxis with cephalosporins led to the emergence of enterococci (mainly E. faecalis) as the most important hospital pathogens. Certain genotypes were able to achieve epidemic dissemination, both nationally and internationally.[34-40] Starting in the 1990s, systematic attempts to determine crucial genetic factors of virulence in nosocomial and other opportunistic enterococcal infections were intensified as a result of these clinical developments. Pioneering studies in this field aimed to identify enterococcal antigens that triggered an antibody response in patients with infections.[41-49] In early studies, most of the prominent antigens discovered were surface-exposed antigens of the enterococcal cell coat (Ebp, Ace, Epa). Subsequent studies using in vitro assays and animal models, including experimental endocarditis, have identified critical roles for these constituents in host adherence and virulence. [41-49] In addition to the factors mentioned above, which are genetically determined, there is also evidence that plasmid-encoded surface adhesins, such as Aggregation Substance.[50-56]

Enterococci have become increasingly significant in healthcare-associated infections over the last two decades. This trend is likely to be driven by a number of factors. Among them are increased access to diagnostics, an increasingly elderly population, greater invasiveness of medical interventions and the continued emergence of antimicrobial resistance.[57-62] During this time period, the number of studies in the general area of bacterial biofilms increased markedly. [63-71] Additionally, the full genome sequence for *E. faecalis* V583 was published.[72-74] In 2003, Bourgogne et al. [75] identified OG1RF, and since then, several other strains have been extensively studied [76] using enhanced genetic research tools to investigate *E. faecalis* infection. [77-81] Our knowledge of the genetic basis of biofilm development in *E. faecalis*, both during in vitro propagation and infection, has been greatly enhanced as a result.[8,82-85] Barnes et al [12,13] conducted a thorough study on transposon mutagenesis and recombinase-based in vivo expression technology (RIVET) genetic screens. The results were non-overlapping but mutually supportive, identifying several factors involved multiple in vitro biofilm production in the chromosome of strain OG1RF. These findings were previously reported by Kristich et al [79] and Ballering et al [80] When the same RIVET library was tested in a rabbit model of subcutaneously implanted foreign body infection, 28 genes identified in these in vitro tests (2 from the transposon screen, 26 from the RIVET screen) were also found to have promoters. [18] However, only two genes (ahrC and eep) were considered to play a significant role in endocarditis pathogenesis when ten strains with mutations in biofilm-associated genes from these candidate genes were tested for in vivo virulence impairment in a rabbit model of infective endocarditis.[18,86] Leuck et al [87] found that *E. faecalis* clinical strains that were classed as poor biofilm producers in a standard in vitro microtitre dish assay colonised porcine heart valves in an ex vivo assay just as well as strong biofilm-forming clinical strains, supporting the conclusion that in vitro biofilm phenotypes do not closely predict infective endocarditis.

Madsen et al conducted a systematic literature review that summarised nine virulence factors of *E. faecalis* infective endocarditis.[16] This information is highly useful for readers. The virulence factors listed therein comprise the aggregation substance, cell wall glycolipids, the Ebp pili proteins, haemolysin, the stress protein gls24, the secreted protease GelE, the membrane metalloprotease Eep, and the adhesins Ace and EfbA. [16,88] The transcriptional regulator AhrC is the tenth virulence

 doi:10.20944/preprints202402.0204.v1

factor of E. faecalis endocarditis. It affects the expression of the ace and ebp genes, as reported by Frank et al [18] and Manias and Dunny. [53,84,89] **Figure 2** [90-93]

The genetic drivers involved in E. faecalis biofilm formation are shown in **Table 2.** [82]

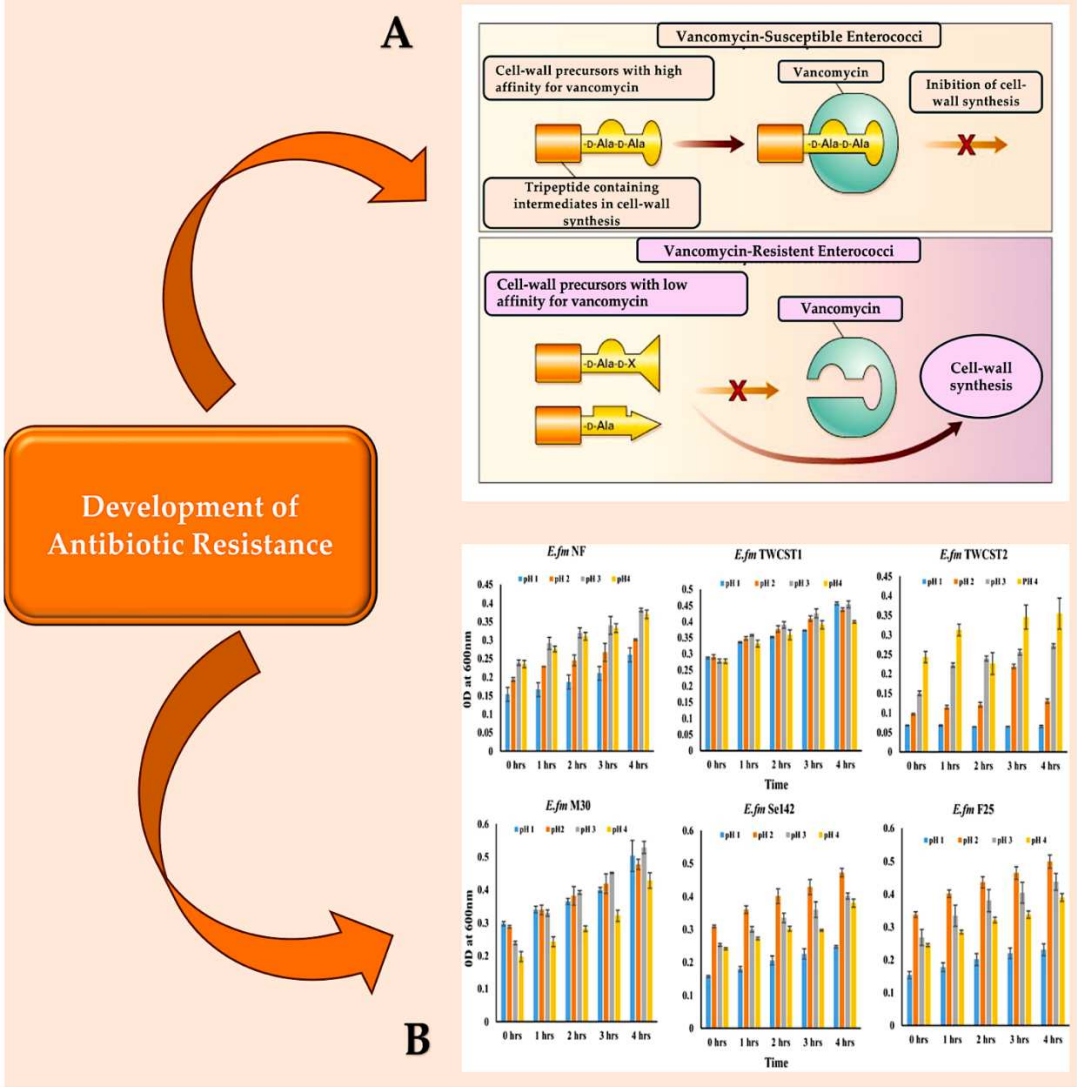

Figure 2. A: Vancomycin-susceptible enterococci synthesize cell wall precursors that bind vancomycin with high affinity. These precursors end in D-Ala-D-Ala and are translocated from the cytoplasm to the cell surface where, once bound, they cannot participate in cell wall synthesis. In the presence of an inducer like vancomycin, vancomycin-resistant enterococci produce intermediates with different end groups (D-Ala-D-Lac, D-Ala or D-Ala-D-Ser), which have a low affinity for vancomycin33-36 and can therefore be used for cell wall synthesis. **B:** The resistance of the chosen enterococcal strains to artificial gastric juice, containing pepsin and acidified to pH 1.0 to pH 4.0, was tested by incubating them for 4 hours at 37°C. This mimics the transient time of food in the stomach. Abbreviations: 'LA' denotes either alanyl or alanine, while 'X lactate' is used for VanA, VanB, and VanD types of resistance, and 'serine' is used for VanC and VanE types. Ref [90-93].

3. Causes of E. Faecalis bacteremia

Bacteremia is evidently required for endothelial bacterial colonisation of the endothelium and the development of IE. In cases of acute bacterial, the initial source of infection is often identifiable. This is due to the short period of time between the spread of bacteremia and the onset of IE. Chronic endocarditis, which is similar to the classic enterococcal endocarditis, is often much more ambiguous.[94,95] A variety of causes have been proposed, varying from colonisation of the oral cavity in endodontic disease to translocation of commensal enterococci in the gastrointestinal tract.

 doi:10.20944/preprints202402.0204.v1

[96,97] Enterococcus is the second leading cause of hospital-acquired bacteremia, due in part to its ability to thrive in challenging environments. Contamination of environmental surfaces in healthcare settings can cause exogenous infection, leading to direct seeding of the vasculature through catheterization or contamination of implantable medical devices. Indirect infection can also occur through colonization of the urinary or gastrointestinal. Endogenous infections can also result from translocation through the epithelium of the GI tract. [96-101] **Figure 3**

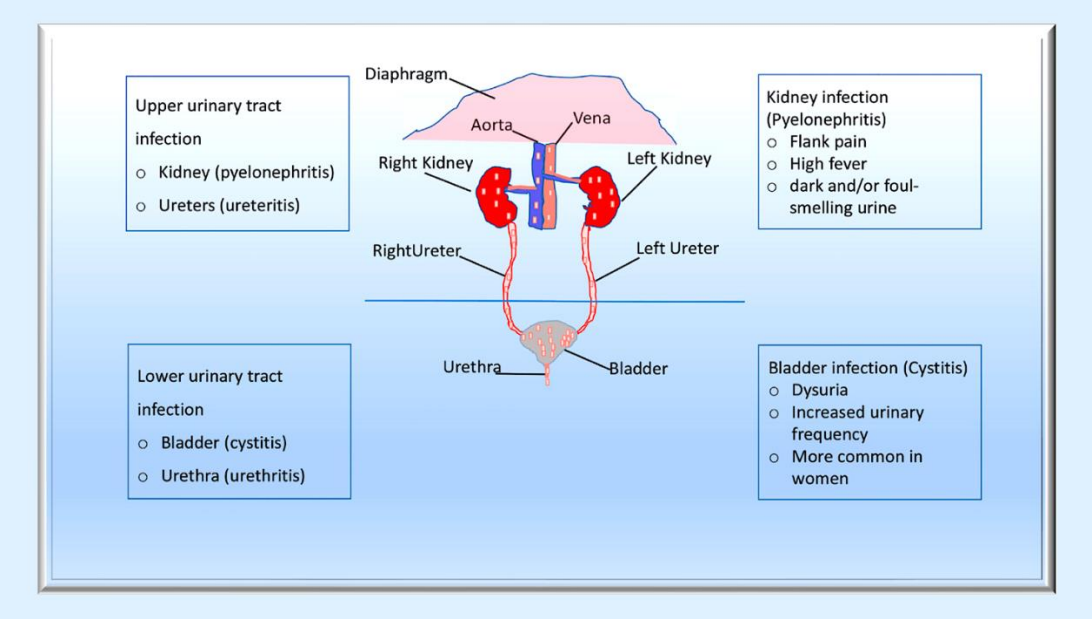

Figure 3. The pathogenesis of urinary tract infections (UTIs) begins with the colonization of uropathogens in the urethra, followed by the bladder, facilitated by specific adhesins. Causative bacteria proliferate and form biofilms if they are not eliminated by the immune system. Pathogen can ascend from the lower urinary tract to the kidney, leading to bacteremia. Uropathogens can bind to the catheter and multiply within the biofilm in the case of complicated UTIs. The infection may progress to pyelonephritis and bacteraemia if left uncontreated. From Mancuso et al. Ref [98].

This process is facilitated by conventional antibiotic regimens, which can drastically increase the amount of enterococci in the intestinal flora. [102,103] More than 3 decades ago, Wells et al [104] experimentally demonstrated translocation of *E. faecalis* across the epithelial barrier of the GI system and subsequent penetration into the circulation in a mouse model. More advanced work has followed, including detection of invasion-defective *E. faecalis* mutant strains in a T84 cell culture model [105-107] and high-resolution imaging of the process with complementary findings on intracellular migration. [108] Despite the long-standing belief that oral enterococci are a likely source for endocarditis, cohort evidence has shown that oral infections are not a common factor in IE, despite the fact that enterococci are also commonly found in the oral cavity and are a leading etiology of endodontic disease. [108] For instance, only 1.6% of enterococcal cases could be attributed to oral routes of transmission versus 6.7% of non-enterococcal cases in a recent large Spanish cohort study comparing enterococcal IE (516 patients) and non-enterococcal IE cases (3,308 patients). [58]

Severe physiological challenge, in combination with the possibility of organism-specific translocation, may result in enough GI barrier breakdown to permit bacterial penetration via systemic host immunosuppression.[109-112] It is unclear whether enterococcal translocation is a result of host immunosuppression or if enterococci themselves are immunomodulatory and can initiate the suppressive response. [110] In a mouse model, common antibiotics at clinically relevant doses can cause GI barrier dysfunction and bacterial translocation, in some cases after a single dose. Again, *E. faecalis* is a key player. [113-115]

Brown et al [116] have recently reported the discovery of cardiac microlesions during severe bacteremia caused by *E. faecalis* infection in mice. These microinjuries are similar to those caused by

 doi:10.20944/preprints202402.0204.v1

Streptococcus pneumoniae during invasive pneumococcal disease. However, *E. faecalis* does not encode the virulence determinants involved in pneumococcal microinjury formation. The study discovered that the protein DsbA, which forms disulfide bonds, is essential for E. faecalis virulence in a C. elegans model and for efficient formation of cardiac microlesions. Additionally, *E. faecalis* facilitated necroptotic cell death of cardiomyocytes at sites of microlesion formation. Unlike the wild-type strain, which suppressed the immune response, loss of DsbA resulted in an increase in pro-inflammatory cytokines. Furthermore, E. faecalis was able to induce microlesions in the heart. This study has identified the features of both the bacterium and the host response that are involved in this process.

Although there is only a paucity of clinical evidence to date, there is also some emerging data of an association between enterococcal endocarditis events and cryptic colorectal cancers. [117-119] It is uncertain whether there is a significant association between these clinical conditions, as seen in most cases of Streptococcus gallolyticus subsp. gallolyticus endocarditis, previously associated with Streptococcus bovis biotype I. [120-124] Stanley et al [125] found that a murine model of ischemic-reperfusion stroke showed bacteremia caused by a specific group of commensal bacterial strains, with enterococci being the most prevalent.

3.1. Induced enterococcal colonisation involves cell surface mechanisms. Ultra-large von Willebrand factor and sortase are key players in this process.

The accepted developmental pathway for bacterial endocarditis includes the primary production of a host-derived thrombus, with subsequent processes promoting colonisation of the thrombus by bloodstream bacteria. However, there are multiple instances where direct colonisation of host epithelial surfaces has been reported, and in practise, this mode of adhesion may be more prevalent than is currently recognised. *S. aureus* is one of the most studied of those bacterial pathogens that have been demonstrated to directly adhere to the endothelium, at least under some circumstances. *S. aureus* expresses three fundamental molecules on its surface: fibronectin-binding protein A (FnBPA) and B (FnBPB), as well as clumping factor A (ClfA). These molecules promote bacterial adherence and identify the cultured human endothelial cells (ECs) that interact with gram-positive cocci. Three recent reports have investigated the adherence of gram positive cocci to endothelial cells (ECs) and have highlighted the fundamental importance of these molecules in IE. [126–128]

Pappelbaum et al [129] showed that *Staphylococcus aureus* adhesion to healthy endothelial cells is associated with elevated levels of ultra-large von Willebrand factor, a host cofactor that deserves in-depth analysis due to its peculiarities of action. Bacterial proteins, such as ClfA and FnBPA, help *S. aureus* stick to EC surface molecules. This is also done by subendothelial matrix proteins, like fibrinogen, fibrin, fibronectin, and von Willebrand factor (vWF). In the setting of undamaged endothelium, evidence suggests that ultra-large von Willebrand factors (ULVWF) significantly facilitate the initial pathogenic phase of *S. aureus*-induced endocarditis. When activated human endothelial cells were perfused with fluorescent bacteria under high-shear-rate conditions, 95% of the *S aureus* attached to ultralarge von Willebrand factor (ULVWF). [129] Flow experiments using VWF deletion mutants and heparin indicated that the A-type domains of VWF contribute to bacterial binding. The role of wall teichoic acid, but not staphylococcal protein A, was suggested by analysis of several bacterial deletion mutants. ULVWF-mediated bacterial adherence significantly increased with the presence of inactivated platelets and serum. ADAMTS13, a thrombospondin 13 disintegrin and metalloproteinase, reduced bacterial binding and shortened the length of ULVWF in a dose-dependent manner, but even at physiological levels of ADAMTS13, individual cocci remained bound by ULVWF. To further demonstrate the role of VWF in vivo, wild-type mice were compared with VWF knockout mice. Using the dorsal skinfold chamber model and intravital microscopy, fluorescent bacteria binding was observed in tumour necrosis factor-α-stimulated tissue. VWF knockout mice had fewer bacteria in their postcapillary and collecting venules compared to wild-type mice. Using heparin and ADAMTS13 can reduce ULVWF formation and may provide a novel therapeutic option to prevent IE. [129]

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 February 2024 doi:10.20944/preprints202402.0204.v1

Research has been conducted on the cell biology of NETosis in the context of infection. [130] The enzyme PAD4, which stands for protein arginine deiminase 4, plays a crucial role in this process. PAD4 is the only member of the PAD family that possesses a nuclear localization signal. [131–134] Furthermore, it is believed that PAD4 has particular targets within the cytoplasm that affect the cell biology of NETosis and the composition of the neutrophil inflammasome. During an infection, functional cytoplasts (enucleated cells) capable of supporting phagocytosis can be identified. In blood vessels, NETs act as a platform for platelet adhesion and initiation of coagulation, similar to VWF. [132,133,135,136] Active PAD4, which is released in conjunction with NETs, also facilitates the citrullination of ADAMTS13. This impedes VWF scission and allows platelet aggregates to remain close to the vessel wall in the presence of PAD4. [137,138] Recent studies have linked NETosis and the increase in NET-associated tissue factor (TF) to systemic inflammation and IL-1β levels, indicating a common regulatory pathway. [139] Additionally, TF secretion from activated macrophages and monocytes is stimulated by the activation of both canonical and non-canonical inflammasomes, as demonstrated by recent research.[140,141] **Figure 4**

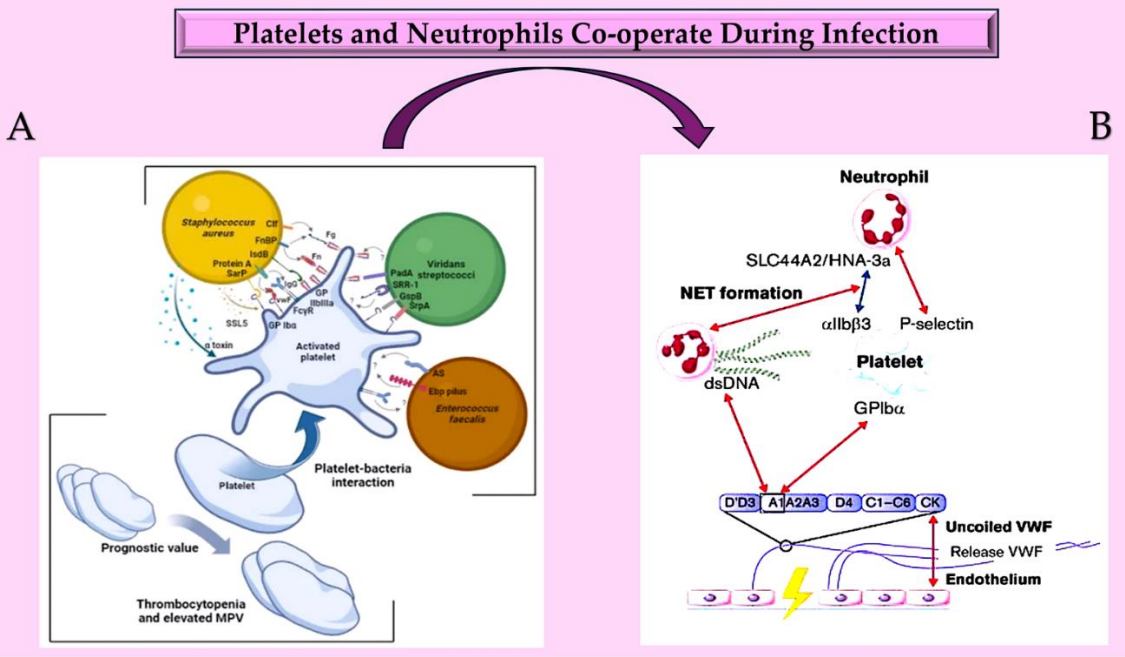

Figure 4. A*: Platelets play a crucial role in infective endocarditis. The interactions between platelets and the bacterial species involved in IE are complex and involve numerous ligand-receptor pairs. Changes in platelet parameters have predictive value. Additionally, von Willebrand factor is also involved. **B:** The diagram illustrates the interactions between von Willebrand factor (VWF) and neutrophils at an infection site. It provides insights into the relationships between the A1 domain of VWF multimers, platelets, neutrophils, and NETs under conditions of high and low shear flow (indicated by red and blue arrows, respectively). Abbreviations; AS: aggregation substance; Clf: clumping factor; Ebp: endocarditis- and biofilm-associated pili; FcγR: crystallizable fragment gamma receptor; Fg: ds, double strand; fibrinogen; Fn: fibronectin; FnBP: fibronectin-binding protein; GP, glycoprotei; GspB: Streptococcus gordonii surface platelet B; IgG: immunoglobulin G; IsdB: iron-responsive surface determinant B; PadA: platelet adherence protein A; Sar P: staphylococcal accessory regulator protein; SrpA: serine-rich glycoprotein A; SRR-1: serine-rich repeat glycoprotein 1; SSL5: staphylococcal superantigen-like 5; VWF: von Willebrand factor. Ref [126–141] *From Brai et al [142].

The role of vWbp and sortase-assembled pilus family emerged during the analysis of adhesion mechanisms in gram-positive cocci infections. Claes et al [126] discovered that the interaction between vWbp and surface proteins of *S. aureus* reduces bacterial adhesion to VWF and vascular endothelium under shear stress. Mutants deficient in Sortase A (SrtA) and SrtA-surface proteins, as well as *Lactococcus lactis* transmitting single staphylo-surface proteins, have been employed. S. aureus

 doi:10.20944/preprints202402.0204.v1

attaches to the endothelium via vWF. The VWF-binding protein (vWbp) facilitates adhesion under shear stress. The vWbp interacts with vWF to complete the adhesion process. It is suggested that the synergistic action of Sortase, a ClfA-dependent surface protein, plays a role in this process.

Similarly, *Enterococcus faecalis* is an opportunistic bacterium that causes various hospital-acquired infections, including catheter-associated urinary tract infections. It may contribute to virulence and the development of infective endocarditis. In a mouse model of *E. faecalis* ascending urinary tract infection, the role of the endocarditis- and biofilm-associated pilus (Ebp), a member of the sortase-assembled pilus family, was demonstrated. The Ebp pilus consists of the major EbpC shaft subunit and the minor subunits EbpA and EbpB. In experimental catheter-associated urinary tract infections, the EbpABC (-) strain, a non-piliated pilus knockout mutant, was significantly less virulent than its isogenic parent OG1RF. In contrast, the EbpC (-) strain, which is a mutant with a deleted nonpiliated ebpC gene, exhibited similar behaviour to OG1RF in vivo because it expressed EbpA and EbpB. Deletion of either the minor pilin gene ebpA or ebpB disrupted pilus biogenesis and resulted in defects in experimental catheter-associated urinary tract infection. The Ebp pilus has been identified as a virulence factor in *E. faecalis* catheter-associated urinary tract infections. Its in vivo function depends on a metal ion-dependent adhesion site motif that is predicted in EbpA's von Willebrand factor A domain. Understanding the molecular basis of this common protein domain among the tip subunits of sortase-assembled pili is important in preventing and treating catheter-associated urinary tract infections caused by *Enterococcus faecalis*. The Ebp pilus of E. faecalis and its subunits are crucial in the virulence of enterococcal infections in a mouse model of catheter-associated urinary tract infections. The metal ion-dependent adhesion site motif in EbpA is crucial for Ebp function in vivo. This discovery has implications for the molecular basis of virulence in *E. faecalis* catheter-associated urinary tract infection, as well as other infections caused by enterococci and other Gram-positive pathogens. The metal ion-dependent adhesion sitemotif is also present in other sortase-assembled pili. [126]

3.2. The role of endocardium and enterococcal pathoadaptation

The endothelium is a specialized type of epithelium. This concept offers an intriguing explanation. Several studies have confirmed that the endocardium is indeed a modified endothelium, [143–147] although there has been some uncertainty about the specifics of endocardial development. *E. faecalis* can directly colonise different host epithelial surfaces in a variety of animal experimental models. In a germ-free mouse model, Barnes et al [13] demonstrated that *E. faecalis* can successfully colonise the surface of the intact, normal intestinal epithelium directly. Barnes et al [12] have recently suggested that enterococcal coverage of endocardial and endovascular surfaces is possible without the need for host tissue destruction or even restricted surgical intervention, using a rabbit model of endocarditis.

Endocarditis caused by *E. faecalis* is a serious clinical manifestation, commonly acquired in a community setting. Understanding the extrinsic pathogenesis at the valve level is a priority. Infective endocarditis is a complex disease with many host and microbial components contributing to the formation of bacterial biofilm-like vegetations on the aortic valve and adjacent areas of the heart. Thurlow et al [20] reported further evidence supporting a non-valvular role in early endocardial colonization. In their model, even after the inflamed valve was harvested, cardiac tissue homogenates still showed greatly elevated bacterial loads.

In a rabbit model of enterococcal endocarditis, the pathogenic capacity of vancomycin-resistant *E. faecalis* V583 and three isogenic protease mutants (ΔgelE, ΔsprE and ΔgelE ΔsprE mutants) were compared. [148] Compared to V583 or the SprE(-) mutant, the bacterial load in the heart of the

GelE(-) mutants (ΔgelE and ΔgelE ΔsprE mutants) was considerably reduced. A marked deposition of the fibrinous matrix layer and increased chemotaxis of inflammatory cells was also observed on aortic valves infected with GelE(-) mutants (ΔgelE and ΔgelE ΔsprE mutants). This suggests a role for proteolytic modulation of the immune response to *E. faecalis*. Furthermore, it was observed that GelE can degrade the anaphylatoxin complement C5a and that this proteolysis leads to reduced neutrophil recruitment in vitro, supporting a role for proteolytic modulation of the

 doi:10.20944/preprints202402.0204.v1

immune response to *E. faecalis*. In vivo, GelE-producing strains were observed to cause a decrease in heterophil migration at infected tissue sites, while SprE-producing strains did not show this effect. These results indicate that of the two enterococcal proteases, GelE is the most important in mediating the pathogenesis of endocarditis. Perez et al published an important study in which the gene encoding gelatinase (gelE) was found to be under the control of the Fsr quorum sensing system, whose encoding genes (fsrA, fsrB, fsrC and fsrD) are situated immediately upstream of gelE. Biofilm formation was prevented and gelatinase activity was suppressed in a derived mutant of *E. faecalis* V583 when a DNA fragment was integrated into the fsr locus. Sequence analysis revealed the presence of IS256 integrated into the fsrC gene at nucleotide position 321. It is worth noting that IS256 is also linked to biofilm formation in *Staphylococcus epidermidis* and *Staphylococcus aureus*. [148] **Figure 5** [149]

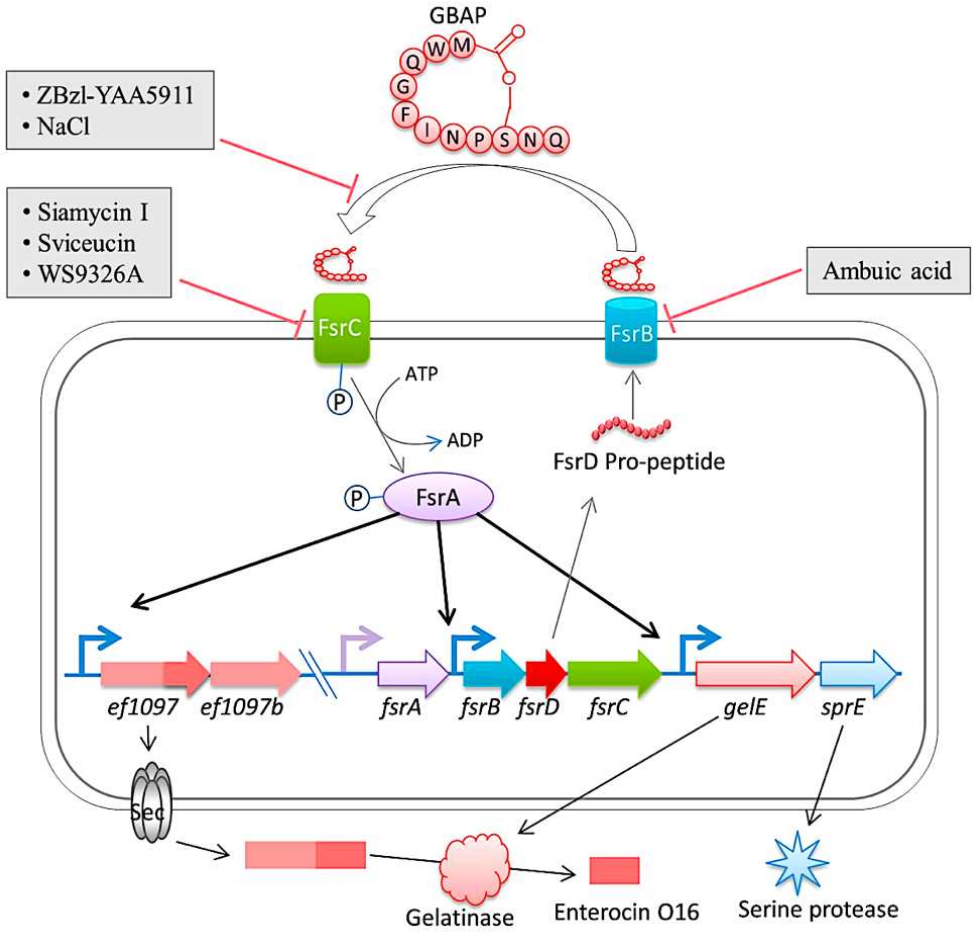

Figure 5. The diagram is a representation of the Fsr quorum sensing system and its regulation in *E. faecalis*. The system involves the export and processing of the FsrD propeptide (encoded by fsrD) to produce the small lactone gelatinase biosynthesis activating pheromone (GBAP) via FsrB. FsrC is part of a two-component regulatory system that reacts to extracellular GBAP by phosphorylating the intracellular response regulator FsrA, which in turn induces the expression of ef1097, ef1097b, the fsr locus, gelE (encoding a gelatinase) and sprE (encoding a serine protease). The pre-protein encoded by ef1097 is cleaved and transported via the Sec-dependent pathway and the precursor is further truncated by the gelatinase to form enterocin O16. The interaction of GBAP with FsrC is inhibited by ZBzl-YAA5911 in a competitive manner, whereas NaCl inhibits it in a concentration-dependent manner. The inhibition of FsrB activity is due to the presence of ambuic acid. Siamycin I, sviceucin and WS9326A inhibited the phosphorylation of FsrC. From Ali et al Ref [149].

Enterococcal pathoadaptation to the endocardium is believed to be facilitated by the IS256 element, which causes gene inactivation and recombination. However, the regulation and activation mechanisms of IS256 remain poorly understood. To describe how chronic lytic phage infection leads

 doi:10.20944/preprints202402.0204.v1

to extensive amplification of IS256 in *E. faecalis* and how antibiotic exposure is associated with amplification of IS256 in *E. faecium* during clinical human infection, Kirsch et al [150] recently applied an IS256-specific deep sequencing approach. Comparative genomics assessment revealed that IS256 is predominantly expressed in hospital-acquired enterococcal isolates. IS256 mobility in *E. faecalis* is transcriptionally regulated by multiple mechanisms, indicating tight control of IS256 activation in the absence of selective pressure. The results show that rapid genome-scale transposition in enterococci is driven by stressors such as phages and antibiotic load. IS256 diversification may thereby illustrate how evolutionary selection mediates enterococcal genome evolution, ultimately leading to the development of dominant nosocomial lineages threatening human health.

Brown et al. have recently reported in an experimental mouse model setting that peritoneal inoculation of *E. faecalis* can result in sub-endothelial microlesions in the heart. [116,151] The study also showed a strong immune response to the infection, indicating that different inoculation routes may result in varying outcomes for both the host and the bacteria. *E. faecalis* invades the vascular endothelium to enter myocardial tissue and induce cell death.[116] Notably, *E. faecalis* lacks homologs of pneumococcal surface adhesin CbpA, pneumolysin (ply), and pyruvate oxidase (spxB), suggesting the involvement of other factors. However, it can produce reactive oxygen species (ROS). [152] ROS release by *E. faecalis* may therefore also be involved in cell death and microlesion development. One protein that has been found to affect *E. faecalis* cardiac microlesion formation is a disulfide bond forming (Dsb) protein called DsbA. Thioredoxins, such as DsbA, play a crucial role in various bacterial fitness and pathogenicity factors, including biofilm formation, cell division, virulence, motility, cell wall synthesis, and growth. Proteins with a highly expressed CXXC active site motif interact with the free thiol groups of substrate cysteines, catalysing a disulfide linkage. Gram-positive bacteria have a lesser understanding of oxidative protein folding than gram-negative bacteria. [153]

4. Point and Counterpoint

From a clinical point of view, the pathophysiology of IE is centred on the functional changes caused by bacterial damage to the cardiac valves. This process is generally believed to follow a foreseeable course: deployment of host factors at a site of endocardial surface injury or impairment, development of vegetations, valvular insufficiency and decline in cardiac function. Staphylococci or streptococci are the most common causes of acute infective endocarditis in clinical practice, usually with a fast-moving, febrile course. [11] Chronic (subacute) IE, on the other hand, is more often related to a slowly developing, more insidious course with prodromal malaise and non-specific findings: oral streptococci and enterococci are the most likely pathogens in these instances. [154] For complex reasons previously discussed, [59,155] although the incidence of bacterial endocarditis is generally steady or decreasing in modern health care systems, the proportion of cases due to enterococci has been on the rise. [59,155]

From the 1970 onwards, a substantial proportion of both fundamental and clinical investigations in the endocarditis literature have suggested that physical injury to the vascular endothelium is a prerequisite for the active pathogenesis of IE. Most current frameworks assume an initial host immune reaction involving platelets, soluble components of the coagulation cascade, etc., with subsequent bacterial invasion of the emerging thrombus. [156,157] Upon close scrutiny of the historical references prior to 1975, however, IE has been described in a wide variety of animal experimental settings in the absence of such damage. [158–163] The researchers found that removing the endothelium prior to infection increased the rate of vegetation formation and reduced the number of animals required for the experiments. But this is simply an issue of convenience and efficiency, not biological need. [164–172]

Therefore, while it is possible that pre-existing cardiac structural abnormalities or disorders of the cardiac endothelium in humans may increase the risk of bacterial colonisation and endocarditis, there is little evidence to suggest that overt endothelial surface disruption is necessary for bacterial colonisation, as previously reported. [171,173] However, even in previously published experimental studies in which pre-inoculation endothelial injury was not included, the process of bacterial invasion is still considered to rely on an existing host-derived thrombus as a precondition. [154] It is worth

 doi:10.20944/preprints202402.0204.v1

noting that certain pathogens can directly colonize the endothelial surface in certain circumstances. [10] In a recent study by Barnes et al, [12] it was reported that *E. faecalis* directly colonized the undamaged endothelial surface in a rabbit model system of endocarditis, without any obvious participation of host factors. Specifically, Barnes et al. discussed endothelial colonization, which refers to the assembly of non-valvular microcolonies and biofilm formation as a bacterial mechanism for persistent infection, rather than classic frank valvular endocarditis. Further investigation of this aetiology is relevant, although there is no evidence to suggest that the attachment of enterococci to the valve surfaces is markedly distinct. Importantly, endothelial coverage and establishment of biofilm on valvular surfaces may be temporally distinct. This suggests that a suspected gastrointestinal source of enterococcal bacteremia may progress through multiple steps before presenting with clinical signs of endocarditis. [12,15]

The conventional endocarditis research and development studies show platelets and fibrin as the bare subendothelial components. The main question is how enterococci interact with the surface of normal cells. Jamet et al [174] found that in the vasculature, enterococci may bind to circulating von Willebrand factor (vWF), similar to Staphylococcus aureus and Streptococcus pneumoniae. [126,127,175,176] Moreover, vWF is a crucial constituent of vertebrate haemostatic signaling pathways, [177–179] and *E. faecalis* strain OG1RF contains virulence factors (ElrA) that seem to be involved in dealing with vWF domains.[174] This mechanism involves circulating von Willebrand factor (vWF) binding to free-floating bacteria. The bacteria then attach to surface-bound vWF on endothelial cells, which allows them to adhere to the cell surface. This process is believed to inhibit platelet recruitment and other responses of the host coagulation cascade by shrouding the bacteria in host vWF. Or conversely, a rprevious paper report by Gaytán et al [180] showed that a new adhesin that binds to sialic acid was crucial for infective endocarditis in several bacterial species. However, it is unclear how this relates to enterococcal endocarditis. Although host-factor interactions cannot be excluded in enterococcal IE, Barnes et al [12,13,15] have shown that *E. faecalis* microcolonies form in a similar way in the vasculature and other non-circulatory disease settings, such as the murine gastrointestinal tract and in vitro polymer surfaces. This suggests the existence of another, perhaps more common, mechanism of adhesion. This suggests the existence of another, perhaps more common, mechanism of adhesion.

The potential for patients with enterococcal endocarditis to infect themselves through GI translocation would resolve several clinical problems in identifying the source of infection in many instances. Antibiotic and systemic stress can cause increased gut permeability to enterococci, which is a common occurrence in both outpatient and inpatient settings. Furthermore, in some endovascular infection models, there is no clear systemic, cell-mediated immune response observed, indicating that *E. faecalis* may evade the host immune system for extended periods. This complicates the establishment of definitive links between the onset of (potentially temporary) bacteremia and endovascular colonization. Further investigation is required to understand the potential and actual routes of patient self-infection in this area of research. [181–184]

A multifaceted process is involved in the induction of enterococcal biofilm. It includes adherence to the surface, attachment, maturation of the microcolony and the subsequent development of chronic disease. Despite extensive in vitro studies on the mechanisms of surface attachment, enterococcal virulence factors, plasmid exchange, and antibiotic resistance, their role in causing disease in vivo is still a matter of considerable debate. Furthermore, numerous laboratory-scale in vitro systems for studying biofilm formation have proven to be inconsistent with in vivo studies, numerous laboratory-based in vitro systems used to study biofilm formation are inconsistent, indicating the need for further improvements. Additionally, the general mechanisms of biofilm formation in clinical disease states, including endocarditis, have been understudied. [181–190]

Over the past ten years, basic in vitro research has revealed that the genetic and physiological drivers of biofilm formation are likely to be highly variable between bacterial species: a universal biofilm inhibitor probably does not exist. Although some species may share similarities, it is also important to study the outliers, which include enterococci that have played a significant role for

 doi:10.20944/preprints202402.0204.v1

years. The genetic drivers involved in E. faecalis biofilm formation are shown in **Table 2.** [46,82,191-200]

Table 2. Genetic determinants that are involved in the formation of E. faecalis biofilm.

Gene/locus	Protein/function	Reference
srtC	Sortase C/an enzyme that anchors surface proteins to the cell wall	Nallapareddy et al. (2006); Ref [46]
atn	Autolysin	Mohamed et al. (2004); Ref [191]
salB	Secretory antigen-like B/cell-shape determinant	Mohamed et al. (2006); Ref [192]
bee	Biofilm enhancer in Enterococcus/a putative cell wall-anchored protein	Tendolkar et al. (2006); Ref [193]
salA	Secretory antigen-like A	Mohamed et al. (2006); Ref [192]
bop	Biofilm on plastic surface/a putative sugar-binding transcriptional regulator	Hufnagel et al. (2004); Ref [194]
gelE	Secretory metalloprotease gelatinase E	Mohamed et al. (2004); Kristich et al. (2004); Hancock & Perego (2004); Ref [191,195,196]
dltA	D-alanine lipoteichoic acid/D-alanine-D-alanyl carrier protein ligase	Fabretti et al. (2006); Ref [197]
ebpA, ebpB, ebpC	Endocarditis and biofilm-associated pili	Nallapareddy et al. (2006); Ref [46]
ebpR	Transcriptional regulator of ebpABC	Bourgogne et al. (2007); Ref [198]
epa (orfde4)	Enterococcal polysaccharide antigen/a putative glycosyltransferase involved in polysaccharide synthesis	Mohamed et al. (2004) ; Ref [191]
esp	Enterococcal surface protein	Toledo-Arana et al. (2001); Tendolkar et al. (2004, 2006); Ref [193,199,200]
etaR	Enterococcal two-component system regulator	Mohamed et al. (2004); Ref [191]
fsrA, fsrB, fsrC	E. faecalis regulator/two-component quorum-sensing signal transduction system, regulates the expression of gelatinase and serine protease	Mohamed et al. (2004, 2006) ; Pillai et al. (2004); Hancock & Perego (2004)

Table 2. Shows the genetic determinants that are involved in the formation of E. faecalis biofilm. Ref [46,82191-200]

In clinical settings, approximately half of enterococcal IE cases fail to identify a definitive source. This new framework suggests that prolonged persistence of enterococcal microcolonies on the cardiac endothelium may be consistent with a cloaked mechanism of enterococcal infection. [182,201–204]

In-vitro mechanistic studies provide evidence that platelets play a crucial role in the initial phase of infective endocarditis by constituting the first line of the immune response. This disease's first phase is supported by the interaction of pathogens with platelets, making it a priority to counteract

platelet antimicrobial activity. Experimental in vitro and animal models have suggested that aspirin can limit bacterial-platelet interactions, preventing vegetation development. These findings are promising. Clinical trial data on the outcome of patients with infective endocarditis treated with aspirin remain controversial. Contradictory findings cast a cloud of uncertainty over the benefit of antiplatelet agents in the prevention of infective endocarditis. In addition to aspirin, ticagrelor, an antagonist of the platelet receptor P2Y12, has been attributed with a therapeutic effect. This is due to its powerful antiplatelet activity and well-known antibacterial activity. In addition, a more recent study using a mouse model reported a significant capacity of ticagrelor to eradicate Staphylococcus aureus bacteraemia. [205–207]

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The author declares no conflict of interest.

References

1. Gaca AO, Lemos JA. Adaptation to Adversity: the Intermingling of Stress Tolerance and Pathogenesis in Enterococci. Microbiol Mol Biol Rev. 2019 Jul 17;83(3):e00008-19. doi: 10.1128/MMBR.00008-19.
2. Fiore E, Van Tyne D, Gilmore MS. Pathogenicity of Enterococci.Microbiol Spectr. 2019Jul;7(4): 10.1128/microbiolspec.GPP3-0053-2018. doi: 10.1128/microbiolspec.GPP3-0053-2
3. Goh HMS, Yong MHA, Chong KKL, Kline KA. Model systems for the study of Enterococcal colonization and infection.Virulence. 2017 Nov 17;8(8):1525-1562. doi: 10.1080/21505594.2017.1279766.
4. Ramsey M, Hartke A, Huycke M. The Physiology and Metabolism of Enterococci. 2014 Feb 15. In: Gilmore MS, Clewell DB, Ike Y, Shankar N, editors. Enterococci: From Commensals to Leading Causes of Drug Resistant Infection [Internet]. Boston: Massachusetts Eye and Ear Infirmary; 2014
5. Lebreton F, Willems RJL, Gilmore MS.2014 Feb 2. *Enterococcus* Diversity, Origins in Nature, and Gut Colonization. In: Gilmore MS, Clewell DB, Ike Y, Shankar N, editors Enterococci: From Commensals to Leading Causes of Drug Resistant Infection [Internet]. Boston: Massachusetts Eye and Ear Infirmary; 2014
6. Boehm AB, Sassoubre LM.2014 Feb 5. Enterococci as Indicators of Environmental Fecal Contamination. In: Gilmore MS, Clewell DB, Ike Y, Shankar N, editors. Enterococci: From Commensals to Leading Causes of Drug Resistant Infection [Internet]. Boston: Massachusetts Eye and Ear Infirmary; 2014
7. Nappi F, Avtaar Singh SS, Jitendra V, Fiore A. Bridging Molecular and Clinical Sciences to Achieve the Best Treatment of *Enterococcus faecalis* Endocarditis.Microorganisms. 2023 Oct 21;11(10):2604. doi: 10.3390/microorganisms11102604.
8. Ch'ng JH, Chong KKL, Lam LN, Wong JJ, Kline KA. Biofilm-associated infection by enterococci. Nat Rev Microbiol. 2019 Jan;17(2):82-94. doi: 10.1038/s41579-018-0107-z.
9. Ramos S, Silva V, Dapkevicius MLE, Igrejas G, Poeta P. Enterococci, from Harmless Bacteria to a Pathogen. Microorganisms. 2020 Jul 25;8(8):1118. doi: 10.3390/microorganisms8081118.
10. Holland TL, Baddour LM, Bayer AS, Hoen B, Miro JM, Fowler VG Jr. Infective endocarditis. Nat Rev Dis Primers. 2016 Sep 1;2:16059. doi: 10.1038/nrdp.2016.59.
11. Nappi F, Martuscelli G, Bellomo F, Avtaar Singh SS, Moon MR. Infective Endocarditis in High-Income Countries. Metabolites. 2022 Jul 25;12(8):682. doi: 10.3390/metabo12080682.
12. Barnes AMT, Frank KL, Dale JL, Manias DA, Powers JL, Dunny GM. *Enterococcus faecalis* colonizes and forms persistent biofilm microcolonies on undamaged endothelial surfaces in a rabbit endovascular infection model.FEMS Microbes. 2021 Sep 25;2:xtab014. doi: 10.1093/femsmc/xtab014. eCollection 2021.
13. Barnes AMT, Dale JL, Chen Y, Manias DA, Greenwood Quaintance KE, Karau MK, Kashyap PC, Patel R, Wells CL, Dunny GM. Enterococcus faecalis readily colonizes the entire gastrointestinal tract and forms biofilms in a germ-free mouse model. Virulence. 2017 Apr 3;8(3):282-296. doi: 10.1080/21505594.2016.1208890.
14. Mazzantini D, Calvigioni M, Celandroni F, Lupetti A, Ghelardi E. Spotlight on the Compositional Quality of Probiotic Formulations Marketed Worldwide. Front Microbiol. 2021 Jul 20; 12:693973. doi: 10.3389/fmicb.2021.693973. eCollection 2021.
15. Barnes AMT, Frank KL, Dunny GM. Enterococcal Endocarditis: Hiding in Plain Sight. Front Cell Infect Microbiol. 2021 Aug 30; 11:722482. doi: 10.3389/fcimb.2021.722482. eCollection 2021.

16. Madsen KT, Skov MN, Gill S, Kemp M. Virulence Factors Associated with *Enterococcus Faecalis* Infective Endocarditis: A Mini Review.Open Microbiol J. 2017 Mar 31;11:1-11. doi: 10.2174/1874285801711010001. eCollection 2017.
17. Kafil HS, Mobarez AM. Spread of Enterococcal Surface Protein in Antibiotic Resistant Entero-coccus faecium and Enterococcus faecalis isolates from Urinary Tract Infections. Open Microbiol J. 2015 Jun 26;9:14-7. doi: 10.2174/1874285801509010014. eCollection 2015
18. Frank KL, Guiton PS, Barnes AM, Manias DA, Chuang-Smith ON, Kohler PL, Spaulding AR, Hultgren SJ, Schlievert PM, Dunny GM. AhrC and Eep are biofilm infection-associated virulence factors in Enterococcus faecalis. Infect Immun. 2013 May;81(5):1696-708. doi: 10.1128/IAI.01210-12.
19. Sillanpää J, Chang C, Singh KV, Montealegre MC, Nallapareddy SR, Harvey BR, Ton-That H, Murray BE. Contribution of individual Ebp Pilus subunits of Enterococcus faecalis OG1RF to pilus biogenesis, biofilm formation and urinary tract infection.PLoS One. 2013 Jul 11;8(7): e68813. doi: 10.1371/journal.pone.0068813.
20. Thurlow LR, Thomas VC, Narayanan S, Olson S, Fleming SD, Hancock LE. Gelatinase contributes to the pathogenesis of endocarditis caused by Enterococcus faecalis. Infect Immun. 2010 Nov;78(11):4936-43. doi: 10.1128/IAI.01118-09.
21. Singh KV, Nallapareddy SR, Murray BE. Importance of the ebp (endocarditis- and biofilm-associated pilus) locus in the pathogenesis of Enterococcus faecalis ascending urinary tract infection. J Infect Dis. 2007 Jun 1;195(11):1671-7. doi: 10.1086/517524.
22. Rouchon CN, Harris J, Zubair-Nizami Z, Weinstein AJ, Roky M, Frank KL. The Cationic Antimicrobial Peptide Activity of Lysozyme Reduces Viable *Enterococcus faecalis* Cells in Biofilms. Antimicrob Agents Chemother. 2022 May 17;66(5):e0233921. doi: 10.1128/aac.02339-21.
23. Qu Q, Chen T, He P, Geng H, Zeng P, Luan G. Isolation and characterization of a novel lytic bacteriophage vB_Efm_LG62 infecting Enterococcus faecium. Virus Genes. 2023 Oct;59(5):763-774. doi: 10.1007/s11262-023-02016-9.
24. Holmberg A, Rasmussen M. Mature biofilms of Enterococcus faecalis and Enterococcus faecium are highly resistant to antibiotics. Diagn Microbiol Infect Dis. 2016 Jan;84(1):19-21. Doi 10.1016/j.diagmicrobio.2015.09.012.
25. Nappi F, Schoell T, Spadaccio C, Acar C, da Costa FDA. A Literature Review on the Use of Aortic Allografts in Modern Cardiac Surgery for the Treatment of Infective Endocarditis: Is There Clear Evidence or Is It Merely a Perception?Life (Basel). 2023 Sep 28;13(10):1980. doi: 10.3390/life13101980.
26. Andrewes, F. W., and Horder, T. J. A Study of the Streptococci Pathogenic for Man. Lancet 1906. 2, 708–713. doi: 10.1016/S0140-6736(01)31538-6
27. Geraci JE, Martin WJ. Antibiotic therapy of bacterial endocarditis. VI. Subacute enterococcal endocarditis; clinical, pathologic and therapeutic consideration of 33 cases. Circulation. 1954 Aug;10(2):173-94. doi: 10.1161/01.cir.10.2.173.
28. Toh, C. C., Ball, K. Natural History of Streptococcus faecalis Endocarditis. Br Med J 1960 Aug 27;2(5199):640-4. doi: 10.1136/bmj.2.5199.640.
29. Jones, J. E. The Experimental Production of Streptococcal Endocarditis in the Pig. J Pathol 1969 Dec;99(4):307-18. doi: 10.1002/path.1710990406
30. Durack DT, Beeson PB, Petersdorf RG. Experimental bacterial endocarditis. 3. Production and progress of the disease in rabbits. Br J Exp Pathol. 1973 Apr;54(2):142-51
31. Schleifer KH, Kilpper-Bälz R, Kraus J, Gehring F. Relatedness and classification of Streptococcus mutans and "mutans-like" streptococci. J Dent Res. 1984 Aug;63(8):1047-50. doi: 10.1177/00220345840630080701.
32. Clewell DB. Movable genetic elements and antibiotic resistance in enterococci. Eur J Clin Microbiol Infect Dis. 1990 Feb;9(2):90-102. doi: 10.1007/BF01963632.
33. Murray BE. The life and times of the Enterococcus. Clin Microbiol Rev. 1990 Jan;3(1):46-65. doi: 10.1128/CMR.3.1.46.
34. Donati L, Scamazzo F, Gervasoni M, Magliano A, Stankov B, Fraschini F. Infection and antibiotic therapy in 4000 burned patients treated in Milan, Italy, between 1976 and 1988. Burns. 1993 Aug;19(4):345-8. doi: 10.1016/0305-4179(93)90125-r
35. Peng MY, Young TG, Yang CH, Chou MY. Enterococcal bacteremia in a medical center. Zhonghua Yi Xue Za Zhi (Taipei). 1994 Nov;54(5):306-11.
36. Nicoletti G, Stefani S. Enterococci: susceptibility patterns and therapeutic options. Eur J Clin Microbiol Infect Dis. 1995;14 Suppl 1: S33-7.
37. de Vera ME, Simmons RL. Antibiotic-resistant enterococci and the changing face of surgical infections. Arch Surg. 1996 Mar;131(3):338-42. doi: 10.1001/archsurg.1996.01430150116021.
38. Gin AS, Zhanel GG. Vancomycin-resistant enterococci. Ann Pharmacother. 1996 Jun;30(6):615-24. doi: 10.1177/106002809603000610.
39. Evers S, Quintiliani R Jr, Courvalin P. Genetics of glycopeptide resistance in enterococci. Microb Drug Resist. 1996 Summer;2(2):219-23. doi: 10.1089/mdr.1996.2.219.

40. Biavasco F, Miele A, Vignaroli C, Manso E, Lupidi R, Varaldo PE. Genotypic characterization of a nosocomial outbreak of VanA Enterococcus faecalis. Microb Drug Resist. 1996 Summer;2(2):231-7. doi: 10.1089/mdr.1996.2.231.
41. Shorrock PJ, Lambert PA, Aitchison EJ, Smith EG, Farrell ID, Gutschik E. Serological response in Enterococcus faecalis endocarditis determined by enzyme-linked immunosorbent assay. J Clin Microbiol. 1990 Feb;28(2):195-200. doi: 10.1128/jcm.28.2.195-200.1990.
42. Xu Y, Jiang L, Murray BE, Weinstock GM. Enterococcus faecalis antigens in human infections. Infect Immun. 1997 Oct;65(10):4207-15. doi: 10.1128/iai.65.10.4207-4215.1997.
43. Rich RL, Kreikemeyer B, Owens RT, LaBrenz S, Narayana SV, Weinstock GM, Murray BE, Höök M. Ace is a collagen-binding MSCRAMM from Enterococcus faecalis. J Biol Chem. 1999 Sep 17;274(38):26939-45. doi: 10.1074/jbc.274.38.26939.
44. Teng F, Jacques-Palaz KD, Weinstock GM, Murray BE. Evidence that the enterococcal polysaccharide antigen gene (epa) cluster is widespread in Enterococcus faecalis and influences resistance to phagocytic killing of E. faecalis.Infect Immun. 2002 Apr;70(4):2010-5. doi: 10.1128/IAI.70.4.2010-2015.2002.
45. Ton-That H, Schneewind O. Assembly of pili in Gram-positive bacteria. Trends Microbiol. 2004 May;12(5):228-34. doi: 10.1016/j.tim.2004.03.004.
46. Nallapareddy SR, Singh KV, Sillanpää J, Garsin DA, Höök M, Erlandsen SL, Murray BE. Endocarditis and biofilm-associated pili of Enterococcus faecalis. J Clin Invest. 2006 Oct;116(10):2799-807. doi: 10.1172/JCI29021.
47. Budzik JM, Schneewind O. Pili prove pertinent to enterococcal endocarditis. J Clin Invest. 2006 Oct;116(10):2582-4. doi: 10.1172/JCI30088.
48. Kemp KD, Singh KV, Nallapareddy SR, Murray BE. Relative contributions of Enterococcus faecalis OG1RF sortase-encoding genes, srtA and bps (srtC), to biofilm formation and a murine model of urinary tract infection. Infect Immun. 2007 Nov;75(11):5399-404. doi: 10.1128/IAI.00663-07.
49. Scott JR, Zähner D. Pili with strong attachments: Gram-positive bacteria do it differently. Mol Microbiol. 2006 Oct;62(2):320-30. doi: 10.1111/j.1365-2958.2006.05279.x.
50. Galli D, Wirth R, Wanner G. Identification of aggregation substances of Enterococcus faecalis cells after induction by sex pheromones. An immunological and ultrastructural investigation. Arch Microbiol. 1989;151(6):486-90. doi: 10.1007/BF00454863.
51. Olmsted SB, Kao SM, van Putte LJ, Gallo JC, Dunny GMRole of the pheromone-inducible surface protein Asc10 in mating aggregate formation and conjugal transfer of the Enterococcus faecalis plasmid pCF10. J Bacteriol. 1991 Dec;173(23):7665-72. doi: 10.1128/jb.173.23.7665-7672.1991.
52. Hirt H, Wanner G, Galli D, Wirth R. Biochemical, immunological and ultrastructural characterization of aggregation substances encoded by Enterococcus faecalis sex-pheromone plasmids. Eur J Biochem. 1993 Feb 1;211(3):711-6. doi: 10.1111/j.1432-1033. 1993.tb17600. x.
53. Dunny GM, Leonard BA, Hedberg PJ. Pheromone-inducible conjugation in Enterococcus faecalis: interbacterial and host-parasite chemical communication. J Bacteriol. 1995 Feb;177(4):871-6. doi: 10.1128/jb.177.4.871-876.1995.
54. Leonard BA, Bensing BA, Hedberg PJ, Ruhfel RE, Chung JW, Dunny GM. Pheromone-inducible gene regulation and signalling for the control of aggregation substance expression in the conjugative plasmid pCF10. Dev Biol Stand. 1995; 85:27-34.
55. Nakayama J, Clewell DB, Suzuki A. Targeted disruption of the PD78 gene (traF) reduces pheromone-inducible conjugal transfer of the bacteriocin plasmid pPD1 in Enterococcus faecalis. FEMS Microbiol Lett. 1995 May 15;128(3):283-8. doi: 10.1111/j.1574-6968. 1995.tb07537. x.
56. Bae T, Kozlowicz B, Dunny GM. Two targets in pCF10 DNA for PrgX binding: their role in production of Qa and prgX mRNA and in regulation of pheromone-inducible conjugation. J Mol Biol. 2002 Feb 1;315(5):995-1007. doi: 10.1006/jmbi.2001.5294.
57. Fernández-Hidalgo N, Escolà-Vergé L, Pericàs JM. *Enterococcus faecalis* endocarditis: what's next? Future Microbiol. 2020 Mar; 15:349-364. doi: 10.2217/fmb-2019-0247.
58. Pericàs JM, Llopis J, Muñoz P, Gálvez-Acebal J, Kestler M, Valerio M, Hernández-Meneses M, Goenaga MÁ, Cobo-Belaustegui M, Montejo M, Ojeda-Burgos G, Sousa-Regueiro MD, de Alarcón A, Ramos-Martínez A, Miró JM; GAMES Investigators. A Contemporary Picture of Enterococcal Endocarditis. J Am Coll Cardiol. 2020 Feb 11;75(5):482-494. doi: 10.1016/j.jacc.2019.11.047.
59. Escolà-Vergé L, Fernández-Hidalgo N, Larrosa MN, Fernandez-Galera R, Almirante B. Secular trends in the epidemiology and clinical characteristics of Enterococcus faecalis infective endocarditis at a referral center (2007-2018). Eur J Clin Microbiol Infect Dis. 2021 Jun;40(6):1137-1148. doi: 10.1007/s10096-020-04117-x.
60. Bashore TM, Turner NA. Addressing the Menace of Enterococcal Endocarditis. J Am Coll Cardiol. 2020 Feb 11;75(5):495-497. doi: 10.1016/j.jacc.2019.12.009.
61. Ramos-Martínez A, Domínguez F, Muñoz P, Marín M, Pedraz Á, Fariñas MC, Tascón V, de Alarcón A, Rodríguez-García R, Miró JM, Goikoetxea J, Ojeda-Burgos G, Escrihuela-Vidal F, Calderón-Parra J; GAMES

investigators. Clinical presentation, microbiology, and prognostic factors of prosthetic valve endocarditis. Lessons learned from a large prospective registry. PLoS One. 2023 Sep 8;18(9):e0290998. doi: 10.1371/journal.pone.0290998. eCollection 2023.

62. Herrera-Hidalgo L, Fernández-Rubio B, Luque-Márquez R, López-Cortés LE, Gil-Navarro MV, de Alarcón A. Treatment of *Enterococcus faecalis* Infective Endocarditis: A Continuing Challenge. Antibiotics (Basel). 2023 Apr 4;12(4):704. doi: 10.3390/antibiotics12040704.
63. Parsek MR, Fuqua C. Biofilms 2003: emerging themes and challenges in studies of surface-associated microbial life. J Bacteriol. 2004 Jul;186(14):4427-40. doi: 10.1128/JB.186.14.4427-4440.2004.
64. Häussler S, Parsek MR. Biofilms 2009: new perspectives at the heart of surface-associated microbial communities. J Bacteriol. 2010 Jun;192(12):2941-9. doi: 10.1128/JB.00332-10.
65. Bjarnsholt T. The role of bacterial biofilms in chronic infections. APMIS Suppl. 2013 May;(136):1-51. doi: 10.1111/apm.12099.
66. Haussler S, Fuqua C. Biofilms 2012: new discoveries and significant wrinkles in a dynamic field. J Bacteriol. 2013 Jul;195(13):2947-58. doi: 10.1128/JB.00239-13.
67. Visick KL, Schembri MA, Yildiz F, Ghigo JM. Biofilms 2015: Multidisciplinary Approaches Shed Light into Microbial Life on Surfaces. J Bacteriol. 2016 Sep 9;198(19):2553-63. doi: 10.1128/JB.00156-16. Print 2016 Oct 1.
68. Høiby N. A short history of microbial biofilms and biofilm infections. APMIS. 2017 Apr;125(4):272-275. doi: 10.1111/apm.12686.
69. Fuqua C, Filloux A, Ghigo JM, Visick KL. Biofilms 2018: A diversity of microbes and mechanisms. J Bacteriol. 2019 Feb 19;201(18):e00118-19. doi: 10.1128/JB.00118-19
70. Wang T, Zhang R, Chen Z, Cao P, Zhou Q, Wu Q. A global bibliometric and visualized analysis of bacterial biofilm eradication from 2012 to 2022. Front Microbiol. 2023 Nov 22; 14:1287964. doi: 10.3389/fmicb.2023.1287964. eCollection 2023.
71. Săndulescu O, Săndulescu M. Oral biofilms - pivotal role in understanding microbes and their relevance to the human host. Germs. 2023 Mar 31;13(1):7-9. doi: 10.18683/germs.2023.1361. eCollection 2023 Mar.
72. Hegstad K, Mikalsen T, Coque TM, Werner G, Sundsfjord A. Mobile genetic elements and their contribution to the emergence of antimicrobial resistant Enterococcus faecalis and Enterococcus faecium. Clin Microbiol Infect. 2010 Jun;16(6):541-54. doi: 10.1111/j.1469-0691.2010.03226. x.
73. Paulsen IT, Banerjei L, Myers GS, Nelson KE, Seshadri R, Read TD, Fouts DE, Eisen JA, Gill SR, Heidelberg JF, Tettelin H, Dodson RJ, Umayam L, Brinkac L, Beanan M, Daugherty S, DeBoy RT, Durkin S, Kolonay J, Madupu R, Nelson W, Vamathevan J, Tran B, Upton J, Hansen T, Shetty J, Khouri H, Utterback T, Radune D, Ketchum KA, Dougherty BA, Fraser CM. Role of mobile DNA in the evolution of vancomycin-resistant Enterococcus faecalis. Science. 2003 Mar 28;299(5615):2071-4. doi: 10.1126/science.1080613.
74. Weigel LM, Clewell DB, Gill SR, Clark NC, McDougal LK, Flannagan SE, Kolonay JF, Shetty J, Killgore GE, Tenover FC. Genetic analysis of a high-level vancomycin-resistant isolate of Staphylococcus aureus. Science. 2003 Nov 28;302(5650):1569-71. doi: 10.1126/science.1090956.
75. Bourgogne A, Garsin DA, Qin X, Singh KV, Sillanpaa J, Yerrapragada S, Ding Y, Dugan-Rocha S, Buhay C, Shen H, Chen G, Williams G, Muzny D, Maadani A, Fox KA, Gioia J, Chen L, Shang Y, Arias CA, Nallapareddy SR, Zhao M, Prakash VP, Chowdhury S, Jiang H, Gibbs RA, Murray BE, Highlander SK, Weinstock GM. Large scale variation in Enterococcus faecalis illustrated by the genome analysis of strain OG1RF. Genome Biol. 2008;9(7): R110. doi: 10.1186/gb-2008-9-7-r110.
76. Palmer KL, Carniol K, Manson JM, Heiman D, Shea T, Young S, Zeng Q, Gevers D, Feldgarden M, Birren B, Gilmore MS. High-quality draft genome sequences of 28 Enterococcus sp. isolates. J Bacteriol. 2010 May;192(9):2469-70. doi: 10.1128/JB.00153-10.
77. Kristich CJ, Chandler JR, Dunny GM. Development of a host-genotype-independent counterselectable marker and a high-frequency conjugative delivery system and their use in genetic analysis of Enterococcus faecalis. Plasmid. 2007 Mar;57(2):131-44. doi: 10.1016/j.plasmid.2006.08.003
78. Kristich CJ, Manias DA, Dunny GM. Development of a method for markerless genetic exchange in Enterococcus faecalis and its use in construction of a srtA mutant. Appl Environ Microbiol. 2005 Oct;71(10):5837-49. doi: 10.1128/AEM.71.10.5837-5849.2005.
79. Kristich CJ, Nguyen VT, Le T, Barnes AM, Grindle S, Dunny GM. Development and use of an efficient system for random mariner transposon mutagenesis to identify novel genetic determinants of biofilm formation in the core Enterococcus faecalis genome. Appl Environ Microbiol. 2008 Jun ;74(11):3377-86. doi: 10.1128/AEM.02665-07
80. Ballering KS, Kristich CJ, Grindle SM, Oromendia A, Beattie DT, Dunny GM. Functional genomics of Enterococcus faecalis: multiple novel genetic determinants for biofilm formation in the core genome. J Bacteriol. 2009 Apr;191(8):2806-14. doi: 10.1128/JB.01688-08
81. Frank KL, Barnes AM, Grindle SM, Manias DA, Schlievert PM, Dunny GM. Use of recombinase-based in vivo expression technology to characterize Enterococcus faecalis gene expression during infection

identifies in vivo-expressed antisense RNAs and implicates the protease Eep in pathogenesis. Infect Immun. 2012 Feb;80(2):539-49. doi: 10.1128/IAI.05964-11.
82. Mohamed JA, Huang DB. Biofilm formation by enterococci. J Med Microbiol. 2007 Dec;56(Pt 12):1581-1588. doi: 10.1099/jmm.0.47331-0.
83. Paganelli FL, Willems RJ, Leavis HL. Optimizing future treatment of enterococcal infections: attacking the biofilm? Trends Microbiol. 2012 Jan;20(1):40-9. doi: 10.1016/j.tim.2011.11.001.
84. Dunny, G. M., Hancock, L. E., and Shankar, N. (2014). "Enterococcal Biofilm Structure and Role in Colonization and Disease. 2014 Feb 14," in Enterococci: From Commensals to Leading Causes of Drug Resistant Infection. Eds. M. S. Gilmore, D. B. Clewell, Y. Ike and N. Shankar (Boston: Massachusetts Eye and Ear Infirmary). Available at: https://www.ncbi.nlm.nih.gov/books/ NBK190433.
85. Tan CAZ, Antypas H, Kline KA. Overcoming the challenge of establishing biofilms in vivo: a roadmap for Enterococci. Curr Opin Microbiol. 2020 Feb; 53:9-18. doi: 10.1016/j.mib.2020.01.013
86. Frank KL, Vergidis P, Brinkman CL, Greenwood Quaintance KE, Barnes AM, Mandrekar JN, Schlievert PM, Dunny GM, Patel R. Evaluation of the Enterococcus faecalis Biofilm-Associated Virulence Factors AhrC and Eep in Rat Foreign Body Osteomyelitis and In Vitro Biofilm-Associated Antimicrobial Resistance. PLoS One. 2015 Jun 15;10(6):e0130187. doi: 10.1371/journal.pone.0130187. eCollection 2015.
87. Leuck AM, Johnson JR, Dunny GM. A widely used in vitro biofilm assay has questionable clinical significance for enterococcal endocarditis.PLoS One. 2014 Sep 25;9(9):e107282. doi: 10.1371/journal.pone.0107282. eCollection 2014.
88. Colomer-Winter C, Gaca AO, Chuang-Smith ON, Lemos JA, Frank KL. Basal levels of (p)ppGpp differentially affect the pathogenesis of infective endocarditis in Enterococcus faecalis. Microbiology (Reading). 2018 Oct;164(10):1254-1265. doi: 10.1099/mic.0.000703.
89. Manias DA, Dunny GM. Expression of Adhesive Pili and the Collagen-Binding Adhesin Ace Is Activated by ArgR Family Transcription Factors in Enterococcus faecalis. J Bacteriol. 2018 Aug 24;200(18): e00269-18. doi: 10.1128/JB.00269-18.
90. Evers S, Quintiliani R Jr, Courvalin P. Genetics of glycopeptide resist- ance in enterococci. Microb Drug Resist 1996; 2:219-23.
91. Arthur M, Reynolds PE, Depardieu F, et al. Mechanisms of glycopep- tide resistance in enterococci. J Infect 1996; 32:11-6.
92. Arthur M, Depardieu F, Gerbaud G, Galimand M, Leclercq R, Cour- valin P. The VanS sensor negatively controls VanR-mediated transcriptional activation of glycopeptide resistance genes of Tn *1546* and related elements in the absence of induction. J Bacteriol 1997; 179:97-106.
93. Bugg TDH, Wright GD, Dutka-Malen S, Arthur M, Courvalin P, Walsh CT. Molecular basis for vancomycin resistance in *Enterococcus faecium* BM4147: biosynthesis of a depsipeptide peptidoglycan precursor by vancomycin resistance proteins VanH and VanA. Biochemistry 1991;30: 10408-15.
94. Milbrandt, E. A novel source of enterococcal endocarditis. Clin Cardiol 1998 Feb;21(2):123-6. doi: 10.1002/clc.4960210211
95. Khan Z, Siddiqui N, Saif MW. *Enterococcus Faecalis* Infective Endocarditis and Colorectal Carcinoma: Case of New Association Gaining Ground. Gastroenterology Res. 2018 Jun;11(3):238-240. doi: 10.14740/gr996w.
96. Manoil D, Cerit EE, Fang H, Durual S, Brundin M, Belibasakis GN. Profiling Antibiotic Susceptibility among Distinct *Enterococcus faecalis* Isolates from Dental Root Canals. Antibiotics (Basel). 2023 Dec 24;13(1):18. doi: 10.3390/antibiotics13010018
97. Pandova M, Kizheva Y, Tsenova M, Rusinova M, Borisova T, Hristova P. Pathogenic Potential and Antibiotic Susceptibility: A Comprehensive Study of Enterococci from Different Ecological Settings. Pathogens. 2023 Dec 29;13(1):36. doi: 10.3390/pathogens13010036
98. Mancuso G, Midiri A, Gerace E, Marra M, Zummo S, Biondo C. Urinary Tract Infections: The Current Scenario and Future Prospects. Pathogens. 2023 Apr 20;12(4):623. doi: 10.3390/pathogens12040623.
99. Arias CA, Murray BE. The rise of the Enterococcus: beyond vancomycin resistance. Nat Rev Microbiol. 2012 Mar 16 ;10(4):266-78. doi: 10.1038/nrmicro2761.
100. Jahansepas A, Aghazadeh M, Rezaee MA, Hasani A, Sharifi Y, Aghazadeh T, Mardaneh J. Occurrence of Enterococcus faecalis and Enterococcus faecium in Various Clinical Infections: Detection of Their Drug Resistance and Virulence Determinants. Microb Drug Resist. 2018 Jan/Feb;24(1):76-82. doi: 10.1089/mdr.2017.0049
101. Coccitto SN, Cinthi M, Simoni S, Pocognoli A, Zeni G, Mazzariol A, Morroni G, Mingoia M, Giovanetti E, Brenciani A, Vignaroli C. Genetic analysis of vancomycin-variable Enterococcus faecium clinical isolates in Italy. Eur J Clin Microbiol Infect Dis. 2024 Jan 31. doi: 10.1007/s10096-024-04768-0.
102. Dubin K, Pamer EG. Enterococci and Their Interactions with the Intestinal Microbiome. Microbiol Spectr. 2014 Nov;5(6): 10.1128/microbiolspec.BAD-0014-2016. doi: 10.1128/microbiolspec.BAD-0014-2016.
103. Hendrickx AP, Top J, Bayjanov JR, Kemperman H, Rogers MR, Paganelli FL, Bonten MJ, Willems RJ. Antibiotic-Driven Dysbiosis Mediates Intraluminal Agglutination and Alternative Segregation of

Enterococcus faecium from the Intestinal Epithelium. mBio. 2015 Nov 10;6(6):e01346-15. doi: 10.1128/mBio.01346-15.
104. Wells CL, Jechorek RP, Erlandsen SL. Evidence for the translocation of Enterococcus faecalis across the mouse intestinal tract. J Infect Dis. 1990 Jul;162(1):82-90. doi: 10.1093/infdis/162.1.82
105. Qin X, Singh KV, Weinstock GM, Murray BE. Effects of Enterococcus faecalis fsr genes on production of gelatinase and a serine protease and virulence. Infect Immun. 2000 May;68(5):2579-86. doi: 10.1128/IAI.68.5.2579-2586.200
106. Zeng J, Teng F, Weinstock GM, Murray BE. Translocation of Enterococcus faecalis strains across a monolayer of polarized human enterocyte-like T84 cells. J Clin Microbiol. 2004 Mar;42(3):1149-54. doi: 10.1128/JCM.42.3.1149-1154.2004.
107. Zeng J, Teng F, Murray BE. Gelatinase is important for translocation of Enterococcus faecalis across polarized human enterocyte-like T84 cells. Infect Immun. 2005 Mar;73(3):1606-12. doi: 10.1128/IAI.73.3.1606-1612.2005.
108. Archambaud C, Derré-Bobillot A, Lapaque N, Rigottier-Gois L, Serror P. Intestinal translocation of enterococci requires a threshold level of enterococcal overgrowth in the lumen. Sci Rep. 2019 Jun 20;9(1):8926. doi: 10.1038/s41598-019-45441-3.
109. Manfredo Vieira S, Hiltensperger M, Kumar V, Zegarra-Ruiz D, Dehner C, Khan N, Costa FRC, Tiniakou E, Greiling T, Ruff W, Barbieri A, Kriegel C, Mehta SS, Knight JR, Jain D, Goodman AL, Kriegel MA. Translocation of a gut pathobiont drives autoimmunity in mice and humans. Science. 2018 Mar 9;359(6380):1156-1161. doi: 10.1126/science. aar7201.
110. Fine RL, Manfredo Vieira S, Gilmore MS, Kriegel MA. Mechanisms and consequences of gut commensal translocation in chronic diseases. Gut Microbes. 2020;11(2):217-230. doi: 10.1080/19490976.2019.1629236.
111. Little R, Wine E, Kamath BM, Griffiths AM, Ricciuto A. Gut microbiome in primary sclerosing cholangitis: A review. World J Gastroenterol. 2020 Jun 7;26(21):2768-2780. doi: 10.3748/wjg.v26.i21.2768.
112. Tie Y, Huang Y, Chen R, Li L, Chen M, Zhang S. Current insights on the roles of gut microbiota in inflammatory bowel disease-associated extra-intestinal manifestations : pathophysiology and therapeutic targets. Gut Microbes. 2023 Dec;15(2):2265028. doi: 10.1080/19490976.2023.2265028.
113. Knoop KA, McDonald KG, Kulkarni DH, Newberry RD. Antibiotics promote inflammation through the translocation of native commensal colonic bacteria. Gut. 2016 Jul;65(7):1100-9. doi: 10.1136/gutjnl-2014-309059.
114. Kulkarni DH, Rusconi B, Floyd AN, Joyce EL, Talati KB, Kousik H, Alleyne D, Harris DL, Garnica L, McDonough R, Bidani SS, Kulkarni HS, Newberry EP, McDonald KG, Newberry RD. Gut microbiota induces weight gain and inflammation in the gut and adipose tissue independent of manipulations in diet, genetics, and immune development. Gut Microbes. 2023 Dec;15(2):2284240. doi: 10.1080/19490976.2023.2284240
115. Hill CA, Casterline BW, Valguarnera E, Hecht AL, Shepherd ES, Sonnenburg JL, Bubeck Wardenburg J. Bacteroides fragilis toxin expression enables lamina propria niche acquisition in the developing mouse gut. Nat Microbiol. 2024 Jan;9(1):85-94. doi: 10.1038/s41564-023-01559-9
116. Brown AO, Singh KV, Cruz MR, Kaval KG, Francisco LE, Murray BE, Garsin DA. Cardiac Microlesions Form During Severe Bacteremic Enterococcus faecalis Infection. J Infect Dis. 2021 Feb 13;223(3):508-516. doi: 10.1093/infdis/jiaa371
117. Khan Z, Siddiqui N, Saif MW. *Enterococcus Faecalis* Infective Endocarditis and Colorectal Carcinoma: Case of New Association Gaining Ground. Gastroenterology Res. 2018 Jun;11(3):238-240. doi: 10.14740/gr996w
118. Cabiltes I, Coghill S, Bowe SJ, Athan E. Enterococcal bacteraemia 'silent but deadly': a population-based cohort study. Intern Med J. 2020 Apr;50(4):434-440. doi: 10.1111/imj.14396
119. Pericàs JM, Ambrosioni J, Muñoz P, de Alarcón A, Kestler M, Mari-Hualde A, Moreno A, Goenaga MÁ, Fariñas MC, Rodríguez-Álvarez R, Ojeda-Burgos G, Gálvez-Acebal J, Hidalgo-Tenorio C, Noureddine M, Miró JM; GAMES Investigators. Prevalence of Colorectal Neoplasms Among Patients With Enterococcus faecalis Endocarditis in the GAMES Cohort (2008-2017).Mayo Clin Proc. 2021 Jan;96(1):132-146. doi: 10.1016/j.mayocp.2020.06.056.
120. Pasquereau-Kotula E, Martins M, Aymeric L, Dramsi S. Significance of Streptococcus gallolyticus subsp. gallolyticus Association With Colorectal Cancer. Front Microbiol. 2018 Apr 3;9:614. doi: 10.3389/fmicb.2018.00614. eCollection 2018.
121. Jans C, Boleij A. The Road to Infection: Host-Microbe Interactions Defining the Pathogenicity of *Streptococcus bovis/Streptococcus equinus* Complex Members. Front Microbiol. 2018 Apr 10;9:603. doi: 10.3389/fmicb.2018.00603. eCollection 2018.
122. Aymeric L, Donnadieu F, Mulet C, du Merle L, Nigro G, Saffarian A, Bérard M, Poyart C, Robine S, Regnault B, Trieu-Cuot P, Sansonetti PJ, Dramsi S. Colorectal cancer specific conditions promote *Streptococcus gallolyticus* gut colonization.Proc Natl Acad Sci U S A. 2018 Jan 9;115(2):E283-E291. doi: 10.1073/pnas.1715112115.

123. Taylor JC, Gao X, Xu J, Holder M, Petrosino J, Kumar R, Liu W, Höök M, Mackenzie C, Hillhouse A, Brashear W, Nunez MP, Xu Y. A type VII secretion system of Streptococcus gallolyticus subsp. gallolyticus contributes to gut colonization and the development of colon tumors. PLoS Pathog. 2021 Jan 6;17(1):e1009182. doi: 10.1371/journal.ppat.1009182. eCollection 2021 Jan
124. Taylor JC, Kumar R, Xu J, Xu Y. A pathogenicity locus of Streptococcus gallolyticus subspecies gallolyticus. Sci Rep. 2023 Apr 18;13(1):6291. doi: 10.1038/s41598-023-33178-z.
125. Stanley D, Mason LJ, Mackin KE, Srikhanta YN, Lyras D, Prakash MD, Nurgali K, Venegas A, Hill MD, Moore RJ, Wong CH. Translocation and dissemination of commensal bacteria in post-stroke infection. Nat Med. 2016 Nov;22(11):1277-1284. doi: 10.1038/nm.4194.
126. Claes,J.;Liesenborghs,L.;Peetermans,M.;Veloso,T.R.;Missiakas,D.;Schneewind,O.;Mancini,S.;Entenza,J.M.; Hoylaerts,M.F.; Heying, R.; et al. Clumping factor A, von Willebrand factor-binding protein and von Willebrand factor anchor *Staphylococcus aureus* to the vessel wall. *J. Thromb. Haemost.* **2017**, *15*, 1009–1019.
127. Claes,J.;Ditkowski,B.;Liesenborghs,L.;Veloso,T.R.;Entenza,J.M.;Moreillon,P.;Vanassche,T.;Verhamme,P.;H oylaerts,M.F.; Heying, R. Assessment of the Dual Role of Clumping Factor A in *S. Aureus* Adhesion to Endothelium in Absence and Presence of Plasma. *Thromb. Haemost.* **2018**, *118*, 1230–1241.
128. Ko,Y.P.;Kang,M.;Ganesh,V.K.;Ravirajan,D.;Li,B.;Höök,M. Coagulase and Efb of *Staphylococcus aureus* Have a Common Fibrinogen Binding Motif. *mBio* **2016**, *7*, e01885-15
129. Pappelbaum, K.I.; Gorzelanny, C.; Gräßle, S.; Suckau, J.; Laschke, M.W.; Bischoff, M.; Bauer, C.; Schorpp-Kistner, M.; Weidenmaier, C.; Schneppenheim, R.; et al. Ultralarge von Willebrand factor fibers mediate luminal *Staphylococcus aureus* adhesion to an intact endothelial cell layer under shear stress. *Circulation* 2013,*128*, 50–59.
130. Fuchs TA, Abed U, Goosmann C, Hurwiu R, Schulze I, Wahn V, Weinrauch Y, Brinkmann V, Zychlinsky A. Novel cell death program leads to neutro- phil extracellular traps. *J Cell Biol*. 2007; 176:231–241. doi: 10.1083/jcb.200606027
131. Thiam HR, Wong SL, Wagner DD, Waterman CM. Cellular mechanisms of NETosis. *Annu Rev Cell Dev Biol*. 2020; 36:191–218. doi: 10.1146/ annurev-cellbio-020520-111016
132. Nappi F, Bellomo F, Avtaar Singh SS. Insights into the Role of Neutrophils and Neutrophil Extracellular Traps in Causing Cardiovascular Complications in Patients with COVID-19: A Systematic Review. J Clin Med. 2022 Apr 27;11(9):2460. doi: 10.3390/jcm11092460.
133. Nappi F, Iervolino A, Avtaar Singh SS. Thromboembolic Complications of SARS-CoV-2 and Metabolic Derangements: Suggestions from Clinical Practice Evidence to Causative Agents. Metabolites. 2021 May 25;11(6):341. doi: 10.3390/metabo11060341
134. Wong SL, Demers M, Martinod K, Gallant M, Wang Y, Goldfine AB, Kahn CR, Wagner DD. Diabetes primes neutrophils to undergo NETosis, which impairs wound healing. *Nat Med*. 2015; 21:815–819. doi: 10.1038/nm.3887
135. Nappi F, Bellomo F, Avtaar Singh SS. Worsening Thrombotic Complication of Atherosclerotic Plaques Due to Neutrophils Extracellular Traps: A Systematic Review.Biomedicines. 2023 Jan 2;11(1):113. doi: 10.3390/biomedicines11010113
136. Nappi F, Nappi P, Gambardella I, Avtaar Singh SS. Thromboembolic Disease and Cardiac Thrombotic Complication in COVID-19: A Systematic Review. Metabolites. 2022 Sep 22;12(10):889. doi: 10.3390/metabo12100889.
137. Morrell CN, Hilt ZT, Pariser DN, Maurya P. PAD4 and von Willebrand Factor Link Inflammation and Thrombosis. Circ Res. 2019 Aug 16;125(5):520-522. doi: 10.1161/CIRCRESAHA.119.315601.
138. Sorvillo N, Mizurini DM, Coxon C, Martinod K, Tilvawala R, Cherpokova D, Salinger AJ, Seward RJ, Staudinger C, Weerapana E, et al. Plasma peptidy- larginine deiminase IV promotes VWF-platelet string formation and accel- erates thrombosis after vessel injury. *Circ Res*. 2019; 125:507–519. doi: 10.1161/CIRCRESAHA.118.314571
139. Liberale L, Holy EW, Akhmedov A, Bonedi NR, Nietlispach F, Mader CM, Mach F, Montecucco F, Beer JH, Paneni F, et al. Interleukin-1β mediates arterial thrombus formation via NET-associated tissue factor. *J ClinMed*. 2019;8:E2072. doi: 10.3390/jcm8122072
140. Wu R, Wang N, Comish PB, Tang D, Kang R. Inflammasome-dependent coagulation activation in sepsis. *Front Immunol*. 2021; 12:641750. doi: 10.3389/fimmu.2021.641750
141. Franklin BS, Bossaller L, De Nardo D, Rader JM, Stuu A, Engels G, Brenker C, Nordhoff M, Mirandola SR, Al- Amoudi A, et al. The adaptor ASC has extracellular and 'prionoid' activities that propagate inflammation. *Nat Immunol*. 2014; 15:727–737. doi: 10.1038/ni.2913
142. Braï MA, Hannachi N, El Gueddari N, Baudoin JP, Dahmani A, Lepidi H, Habib G, Camoin-Jau L. The Role of Platelets in Infective Endocarditis. Int J Mol Sci. 2023 Apr 19;24(8):7540. doi: 10.3390/ijms24087540.
143. Misfeldt AM, Boyle SC, Tompkins KL, Bautch VL, Labosky PA, Baldwin HS. Endocardial cells are a distinct endothelial lineage derived from Flk1+ multipotent cardiovascular progenitors. Dev Biol. 2009 Sep 1;333(1):78-89. doi: 10.1016/j.ydbio.2009.06.033.

144. Dyer LA, Patterson C. Development of the endothelium: an emphasis on heterogeneity. Semin Thromb Hemost. 2010 Apr;36(3):227-35. doi: 10.1055/s-0030-1253446
145. Harris IS, Black BL. Development of the endocardium.Pediatr Cardiol. 2010 Apr;31(3):391-9. doi: 10.1007/s00246-010-9642-8.
146. Milgrom-Hoffman M, Harrelson Z, Ferrara N, Zelzer E, Evans SM, Tzahor E. The heart endocardium is derived from vascular endothelial progenitors. Development. 2011 Nov;138(21):4777-87. doi: 10.1242/dev.061192
147. Borasch K, Richardson K, Plendl J. Cardiogenesis with a focus on vasculogenesis and angiogenesis.Anat Histol Embryol. 2020 Sep;49(5):643-655. doi: 10.1111/ahe.12549.
148. Perez M, Calles-Enríquez M, del Rio B, Ladero V, Martín MC, Fernández M, Alvarez MA. IS256 abolishes gelatinase activity and biofilm formation in a mutant of the nosocomial pathogen Enterococcus faecalis V583. Can J Microbiol. 2015 Jul;61(7):517-9. doi: 10.1139/cjm-2015-0090.
149. Ali L, Goraya MU, Arafat Y, Ajmal M, Chen JL, Yu D.Molecular Mechanism of Quorum-Sensing in Enterococcus faecalis: Its Role in Virulence and Therapeutic Approaches. Int J Mol Sci. 2017 May 3;18(5):960. doi: 10.3390/ijms18050960.
150. Kirsch JM, Ely S, Stellfox ME, Hullahalli K, Luong P, Palmer KL, Van Tyne D, Duerkop BA.Targeted IS-element sequencing uncovers transposition dynamics during selective pressure in enterococci. PLoS Pathog. 2023 Jun 2;19(6):e1011424. doi: 10.1371/journal.ppat.1011424. eCollection 2023 Jun
151. Brown AO, Garsin DA. The pathogenesis of cardiac microlesion formation during severe bacteremic infection. PLoS Pathog. 2020 Nov 19;16(11):e1009021. doi: 10.1371/journal.ppat.1009021. eCollection 2020
152. Wang X, Huycke MM. Extracellular superoxide production by Enterococcus faecalis promotes chromosomal instability in mammalian cells. Gastroenterology. 2007; 132(2):551–61. Epub 2007 Jan 30. https://doi.org/10.1053/j.gastro.2006.11.040
153. Reardon-Robinson ME, Ton-That H. Disulfide-Bond-Forming Pathways in Gram-Positive Bacteria. J Bacteriol. 2015; 198(5):746–54. https://doi.org/10.1128/JB.00769-15
154. McDonald, J. Acute Infective Endocarditis. Infect. 2009; Dis. Clin. North Am. 23 (4), 643–664. doi: 10.1016/j.idc.2009.04.013
155. Dahl A, Iversen K, Tonder N, Hoest N, Arpi M, Dalsgaard M, Chehri M, Soerensen LL, Fanoe S, Junge S, Hoest U, Valeur N, Lauridsen TK, Fosbol E, Hoi-Hansen T, Bruun NE. Prevalence of Infective Endocarditis in Enterococcus faecalis Bacteremia. J Am Coll Cardiol. 2019 Jul 16;74(2):193-201. doi: 10.1016/j.jacc.2019.04.05
156. Keynan Y, Rubinstein E. Pathophysiology of infective endocarditis. Curr Infect Dis Rep. 2013 Aug;15(4):342-6. doi: 10.1007/s11908-013-0346-0.PMID: 23737237
157. Liesenborghs L, Meyers S, Vanassche T, Verhamme P. Coagulation: At the heart of infective endocarditis. J Thromb Haemost. 2020 May;18(5):995-1008. doi: 10.1111/jth.14736. Epub 2020 Feb 3.
158. Bizzini A, Beggah-Möller S, Moreillon P, Entenza JM. Lack of in vitro biofilm formation does not attenuate the virulence of Streptococcus gordonii in experimental endocarditis. FEMS Immunol Med Microbiol. 2006 Dec;48(3):419-23. doi: 10.1111/j.1574-695X.2006.00168. x.
159. Rowlands DT Jr, Vakilzadeh J, Sherwood BF, LeMay JC. Experimental bacterial endocarditis in the opossum (Didelphis virginiana). I. Valvular changes following a single injection of bacteria in unmodified adult opossums. Am J Pathol. 1970 Feb;58(2):295-304.
160. Vakilzadeh J, Rowlands DT Jr, Sherwood BF, LeMay JC. Experimental bacterial endocarditis in the opossum (Didelphis virginiana). II. Induction of endocarditis with a single injection of Streptococcus viridans. J Infect Dis. 1970 Jul-Aug ;122(1):89-92. doi: 10.1093/infdis/122.1-2.89.
161. Sherwood BF, Rowlands DT Jr, Vakilzadeh J, LeMay JC. Experimental bacterial endocarditis in the opossum (Didelphis virginiana). 3. Comparison of spontaneously occurring endocarditis with that induced experimentally by pyogenic bacteria and fungi. Am J Pathol. 1971 Sep;64(3):513-20.
162. Jones JE. Experimental bacterial endocarditis in the pig. Proc R Soc Med. 1972 Nov;65(11):990-4.
163. La Regina MC, Lonigro J, Woods L, Williams GA, Vogler GA. Valvular endocarditis associated with experimental Erysipelothrix rhusiopathiae infection in the opossum (Didelphis virginiana). Lab Anim Sci. 1988 Apr;38(2):159-61.
164. Garrison PK, Freedman LR. Experimental endocarditis I. Staphylococcal endocarditis in rabbits resulting from placement of a polyethylene catheter in the right side of the heart. Yale J Biol Med. 1970 Jun;42(6):394-410.
165. Perlman BB, Freedman LR.Yale Experimental endocarditis. II. Staphylococcal infection of the aortic valve following placement of a polyethylene catheter in the left side of the heart. J Biol Med. 1971 Oct;44(2):206-13.
166. Perlman BB, Freedman LR. Experimental endocarditis. 3. Natural history of catheter induced staphylococcal endocarditis following catheter removal. Yale J Biol Med. 1971 Oct;44(2):214-24.
167. Durack DT, Beeson PB. Experimental bacterial endocarditis. I. Colonization of a sterile vegetation. Br J Exp Pathol. 1972 Feb;53(1):44-9.

168. Durack DT, Beeson PB. Experimental bacterial endocarditis. II. Survival of a bacteria in endocardial vegetations. Br J Exp Pathol. 1972 Feb;53(1):50-3.
169. Durack DT, Petersdorf RG, Beeson PB. Penicillin prophylaxis of experimental S. viridans endocarditis. Trans Assoc Am Physicians. 1972; 85:222-30.
170. Durack DT, Beeson PB, Petersdorf RG. Experimental bacterial endocarditis. 3. Production and progress of the disease in rabbits. Br J Exp Pathol. 1973 Apr;54(2):142-51
171. Freedman LR, Arnold S, Valone J. Experimental endocarditis. Ann N Y Acad Sci. 1974 Jul 31;236(0):456-65. doi: 10.1111/j.1749-6632. 1974.tb41510. x.
172. Durack DT, Beeson PB. Protective role of complement in experimental Escherichia coli endocarditis. Infect Immun. 1977 Apr ;16(1):213-7. doi: 10.1128/iai.16.1.213-217.1977.
173. Tunkel, A., Scheld, W. "Experimental Models of Endocarditis," in Infective Endocarditis. Ed. D. Kaye (New York: Raven Press), 1992; 37–56.
174. Jamet A, Dervyn R, Lapaque N, Bugli F, Perez-Cortez NG, Blottière HM, Twizere JC, Sanguinetti M, Posteraro B, Serror P, Maguin E. The Enterococcus faecalis virulence factor ElrA interacts with the human Four-and-a-Half LIM Domains Protein 2. Sci Rep. 2017 Jul 4;7(1):4581. doi: 10.1038/s41598-017-04875-3.
175. Huck V, Schneider MF, Gorzelanny C, Schneider SW. The various states of von Willebrand factor and their function in physiology and pathophysiology. Thromb Haemost. 2014 Apr 1;111(4):598-609. doi: 10.1160/TH13-09-0800
176. Steinert M, Ramming I, Bergmann S. Front Med (Lausanne). Impact of Von Willebrand Factor on Bacterial Pathogenesis. 2020 Sep 3;7:543. doi: 10.3389/fmed.2020.00543. eCollection 2020
177. Wagner DD. Cell biology of von Willebrand factor. Annu Rev Cell Biol. 1990;6:217-46. doi: 10.1146/annurev.cb.06.110190.001245
178. Journet AM, Saffaripour S, Cramer EM, Tenza D, Wagner DD. von Willebrand factor storage requires intact prosequence cleavage site. Eur J Cell Biol. 1993 Feb;60(1):31-41.
179. Bowman M, Casey L, Selvam SN, Lima PDA, Rawley O, Hinds M, Tuttle A, Grabell J, Iorio A, Walker I, Lillicrap D, James P. von Willebrand factor propeptide variants lead to impaired storage and ER retention in patient-derived endothelial colony-forming cells. J Thromb Haemost. 2022 Jul;20(7):1599-1609. doi: 10.1111/jth.15740. Epub 2022 May 3.
180. Gaytán MO, Singh AK, Woodiga SA, Patel SA, An SS, Vera-Ponce de León A, McGrath S, Miller AR, Bush JM, van der Linden M, Magrini V, Wilson RK, Kitten T, King SJ. A novel sialic acid-binding adhesin present in multiple species contributes to the pathogenesis of Infective endocarditis. PLoS Pathog. 2021 Jan 19;17(1):e1009222. doi: 10.1371/journal.ppat.1009222. eCollection 2021 Jan
181. Kim KS, Lee G-H, Bak HR, Park YM, Lee SH, Hong S-J, Lee D-W. Complete genome assembly of *Enterococcus faecalis* strain HL1, isolated from an infant fecal sample. Microbiol Resour Announc. 2023 Nov 16;12(11): e0055823. doi: 10.1128/MRA.00558-23.
182. Koch, S., Hufnagel, M., Theilacker, C., Huebner, J. Enterococcal infections: host response, therapeutic, and prophylactic possibilities. Vaccine. 2004 Feb 17;22(7):822-30. doi: 10.1016/j.vaccine.2003.11.027.
183. Rich RL, Kreikemeyer B, Owens RT, LaBrenz S, Narayana SV, Weinstock GM, Murray BE, Höök M 1999. Ace is a collagen binding MSCRAMM from *Enterococcus faecalis*. J Biol Chem. 1999 Sep 17;274(38):26939-45. doi: 10.1074/jbc.274.38.26939.
184. Giuliano S, Angelini J, D'Elia D, Geminiani M, Barison RD, Giacinta A, Sartor A, Campanile F, Curcio F, Cotta MO, Roberts JA, Baraldo M, Tascini C. Ampicillin and Ceftobiprole Combination for the Treatment of *Enterococcus faecalis* Invasive Infections: "The Times They Are A-Changin". Antibiotics (Basel). 2023 May 9;12(5):879. doi: 10.3390/antibiotics12050879.
185. Shankar V, Baghdayan AS, Huycke MM, Lindahl G, Gilmore MS. Infection-derived *Enterococcus faecalis* strains are enriched in *esp*, a gene encoding a novel surface protein. Infect Immun. 1999 Jan;67(1):193-200. doi: 10.1128/IAI.67.1.193-200.1999.
186. El-Telbany M, Lin CY, Abdelaziz MN, Maung AT, El-Shibiny A, Mohammadi TN, Zayda M, Wang C, Zar Chi Lwin S, Zhao J, Masuda Y, Honjoh KI, Miyamoto T, El M. Potential application of phage vB_EfKS5 to control Enterococcus faecalis and its biofilm in food. AMB Express. 2023 Nov 20;13(1):130. doi: 10.1186/s13568-023-01628-6.
187. Galli, D., Wirth, R. Comparative analysis of Enterococcus faecalis sex pheromone plasmids identifies a single homologous DNA region which codes for aggregation substance. J Bacteriol. 1991 May;173(9):3029-33. doi: 10.1128/jb.173.9.3029-3033. 1991.
188. Vlková B, Szemes T, Minárik G, Tóthová L, Drahovská H, Turňa J, Celec P. Food-borne enterococci and their resistance to oxidative stress. J Microbiol. 2011 Aug;49(4):657-62. doi: 10.1007/s12275-011-0296-x. Epub 2011 Sep 2.PMID: 21887651
189. Carniol K, Gilmore MS. Signal transduction, quorum-sensing, and extracellular protease activity in Enterococcus faecalis biofilm formation. J Bacteriol. 2004 Dec;186(24):8161-3. doi: 10.1128/JB.186.24.8161-8163.2004.

190. Khalil MA, Alorabi JA, Al-Otaibi LM, Ali SS, Elsilk SE. Antibiotic Resistance and Biofilm Formation in Enterococcus spp. Isolated from Urinary Tract Infections. Pathogens. 2022 Dec 25;12(1):34. doi: 10.3390/pathogens12010034.
191. Mohamed, J. A., Huang, W., Nallapareddy, S. R., Teng, F. & Murray, B. E. (2004). Influence of origin of isolates, especially endocarditis isolates, and various genes on biofilm formation by Enterococcus faecalis. Infect Immun 72, 3658–3663.
192. Mohamed, J. A., Teng, F., Nallapareddy, S. R. & Murray, B. E. (2006). Pleiotrophic effects of 2 Enterococcus faecalis sagA-like genes, salA and salB, which encode proteins that are antigenic during human infection, on biofilm formation and binding to collagen type I and fibronectin. J Infect Dis 193, 231–240.
193. Tendolkar, P. M., Baghdayan, A. S. & Shankar, N. (2006). Putative surface proteins encoded within a novel transferable locus confer a high-biofilm phenotype to Enterococcus faecalis. J Bacteriol 188, 2063–2072.
194. Hufnagel, M., Koch, S., Creti, R., Baldassarri, L. & Huebner, J. (2004). A putative sugar-binding transcriptional regulator in a novel gene locus in Enterococcus faecalis contributes to production of biofilm and prolonged bacteremia in mice. J Infect Dis 189, 420–430.
195. Kristich, C. J., Li, Y. H., Cvitkovitch, D. G. & Dunny, G. M. (2004). Esp- independent biofilm formation by Enterococcus faecalis. J Bacteriol 186, 154–163.
196. Hancock, L. E. & Perego, M. (2004). The Enterococcus faecalis fsr two- component system controls biofilm development through production of gelatinase. J Bacteriol 186, 5629–5639.
197. Fabretti, F., Theilacker, C., Baldassarri, L., Kaczynski, Z., Kropec, A., Holst, O. & Huebner, J. (2006). Alanine esters of enterococcal lipoteichoic acid play a role in biofilm formation and resistance to antimicrobial peptides. Infect Immun 74, 4164–4171.
198. Bourgogne, A., Singh, K. V., Fox, K. A., Plughoeft, K. J., Murray, B. E. & Garsin, D. A. (2007). EbpR is important for biofilm formation by activating expression of the endocarditis and biofilm-associated pilus operon (ebpABC) of Enterococcus faecalis OG1RF. J Bacteriol 189, 6490–6493.
199. Toledo-Arana, A., Valle, J., Solano, C., Arrizubieta, M. J., Cucarella, C., Lamata, M., Amorena, B., Leiva, J., Penades, J. R. & Lasa, I. (2001). The enterococcal surface protein, Esp, is involved in Enterococcus faecalis biofilm formation. Appl Environ Microbiol 67, 4538–4545.
200. Tendolkar, P. M., Baghdayan, A. S., Gilmore, M. S. & Shankar, N. (2004). Enterococcal surface protein, Esp, enhances biofilm formation by Enterococcus faecalis. Infect Immun 72, 6032–6039.
201. Flemming, H.-C.; Wingender,J.Thebiofilmmatrix.*Nat.Rev.Microbiol.*2010,*8*,623–633.
202. Schwartz,F.A.;Christophersen,L.;Laulund,A.S.;Lundquist,R.;Lerche,C.;Nielsen,P.R.;Bundgaard,H.;Høiby, N.;Moser,C. Novel human in vitro vegetation simulation model for infective endocarditis. *APMIS* 2021, *129*, 653–662.
203. Di Domenico, E.G.; Rimoldi, S.G.; Cavallo, I.; D'Agosto, G.; Trento, E.; Cagnoni, G.; Palazzin, A.; Pagani, C.; Romeri, F.; De Vecchi, E.; et al. Microbial biofilm correlates with an increased antibiotic tolerance and poor therapeutic outcome in infective endocarditis. *BMC Microbiol.* 2019, *19*, 228.
204. Schwartz,F.A.;Nielsen,L.;StruveAndersen,J.;Bock,M.;Christophersen,L.;Sunnerhagen,T.;Lerche,C.J.;Bay,L.; Bundgaard,H.; Høiby, N.; et al. Dynamics of a Staphylococcus aureus infective endocarditis simulation model. *APMIS* 2022, *130*, 515–523.
205. Leeten,K.;Jacques,N.;Lancellotti,P.;Oury,C. Aspirin or Ticagrelor in Staphylococcus aureus Infective Endocarditis :Where Do We Stand? *Front. Cell. Dev. Biol.* 2021, *9*, 716302
206. Ditkowski,B.;BezulskaDitkowska,M.;Jashari,R.;Baatsen,P.;Moreillon,P.;Rega,F.;Veloso,T.R.;Hoylaerts,M.F. ;Heying,R.; Congenital Cardiology and Cardiac Surgery Group. Antiplatelet therapy abrogates platelet-assisted Staphylococcus aureus infectivity of biological heart valve conduits. J. Thorac. Cardiovasc. Surg. 2021, 161, e457-e472.
207. Hannachi, N.; Habib, G.; Camoin-Jau, L. Aspirin Effect on Staphylococcus aureus-Platelet Interactions During Infectious Endocarditis. *Front. Med.* 2019, *6*, 217