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Virulence determinants and pathogenicity in *Enterococcus faecalis* and *E. faecium*

Samantha Estephania Valencia-Estrada [1], Jackeline Lizzeta Arvizu-Gómez [2], Angélica Nallelhy Rodríguez-Ocampo [1] and Christian González-Reyes [1,*]

[1] *Academic Unit of Biological and Pharmaceutical Chemical Sciences, Autonomous University of Nayarit, Tepic 63000, Mexico.*

[2] *Research and Postgraduate Secretary, Center for Innovation and Technology Transfer (CENITT), Autonomous University of Nayarit, Tepic 63000, Mexico.*

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Abstract

Enterococci are Gram-positive bacteria that inhabit the gastrointestinal tract of many organisms, including humans. These bacteria can easily adapt to their surrounding environment, even under stress conditions that can be lethal to other microorganisms. Among the species described in this genus, *faecalis* and *faecium* stand out, considered one of the main causes of nosocomial infections due to their ability to adapt and resist in clinical environments. They are recognized as opportunistic pathogens that can cause a variety of serious infections, such as endocarditis, urinary tract infections, and bacteremia, especially in immunocompromised patients or those with invasive medical devices. Recently, it has been observed that *Enterococcus* presents an increase in resistance to antibiotics, heavy metals, as well as external environmental conditions such as heat and desiccation. This resistance has been directly related to the capacity to form biofilm, an organized microbial ecosystem that makes it almost impermeable and serves as a shield for the bacteria. In addition, biofilm has been linked to bacterial virulence due to the presence of virulence factors. Therefore, researchers are interested in conducting research on the correlation between biofilm formation, virulence factors and antimicrobial resistance, which is essential to understanding the pathogenesis of these infections and improving treatment strategies.

Keywords: *Enterococcus faecalis*; *E. faecium*; Biofilms; Virulence factor; Antibiotic resistance

1. Introduction

Enterococcus are Gram-positive bacteria belonging to the Enterococcaceae family. Morphologically they are spherical or ovoid, are usually found grouped in pairs or short chains and are non-motile cells. The environment in which they live is the gastrointestinal tract of many organisms, including humans [1]. They are facultative anaerobes, generally catalase negative and commonly non-hemolytic, but on certain occasions they can cause α-hemolysis and very little β-hemolysis. They hydrolyze bile-esculin and L-pyrrolidonyl-B-naphthylamide (PYR), produce leucine-aminopeptidase (LAP). They differ from other cocci due to a teichoic acid present in their cell wall, called antigen D [1, 2]. They can adapt to their environment, so they can grow at temperatures ranging from 10°-60 °C, with 37 °C being the optimal growth temperature. This genus consists of 58 described species, among *E. faecalis* and *E. faecium* stand out, which are the most frequent species in clinical isolates, causing 90% of enterococcal infections [3, 4].

Enterococci are part of the intestinal microbiota of humans, mammals and birds, however, *E. faecium* is considered one of the main causes of enterococcal diseases in humans, representing up to 40% of these, in hosts with compromised immune systems, it can cause death [5, 6]. It presents high resistance to a wide variety of antibiotics, including

* Corresponding author: Christian González-Reyes

glycopeptides, due to the gene transfer mechanisms that have allowed them to evolve to evade or interrupt antimicrobial action [5, 7]. On the other hand, *E. faecalis* is one of the main causes of diseases in humans, being this species one of the most common nosocomial pathogens. Specifically, *E. faecalis* can adapt to stress conditions that can be lethal to other microorganisms, since it can grow in environments where nutrients are scarce, very resistant to anaerobic conditions compared to other bacteria and tolerates environments rich in NaCl [8, 9]. In addition, it uses a survival mechanism which makes its clinical identification very complicated since under stress conditions it does not grow, but if subjected to adequate conditions it proliferates again and fulfills its function as a pathogen [8].

2. Review content

2.1. Pathologies

Although *Enterococcus* are part of the intestinal microbiota, they are considered one of the main causes of nosocomial infections and, unlike other bacteria that cause this type of infections, *Enterococcus* are multidrug resistant, making the treatment of infections caused by these organisms a challenge [10, 11]. Among the infections produced by *Enterococcus* in humans, urinary tract infections are common, causing up to 16% of nosocomial diseases. Among other diseases that they cause, there are wound infections, bacteremia and endocarditis. Currently, they represent approximately 20% of native valve bacterial endocarditis and less than 10% of prosthetic valve endocarditis. Enterococci have become a serious public health problem due to the limitations of antimicrobial treatments, and the resistance they acquire through gene transfer, as is the case of vancomycin-resistant enterococci (VRE). It should be noted that enterococcal infections have now been associated with endodontics, implants and medical devices [12].

2.1.1. Endocarditis

It is an infection caused mainly by *E. faecalis*; it consists of the formation of bacterial clusters inside the heart, specifically in the heart valves, followed by the formation of clots that can spread throughout the body, making it a serious disease. Enterococci cause subacute chronic endocarditis, native valve endocarditis and prosthetic valve endocarditis. Several bacterial species produce extracellular proteases that contribute to the pathogenesis of endocarditis through the manipulation of the host's immune response. These proteases are directed at several components of the innate immune system, specifically, the complement system, antimicrobial peptides, cytokines and cytokine receptors [13].

2.1.2. Urinary tract infections (UTIs)

UTIs caused by enterococci are one of the most common human bacterial infections, both in and out of the hospital setting. They are a challenge for public health, responsible for significant levels of morbidity and loss of productivity worldwide, with an estimated 150 million cases per year of this disease, affecting mostly women [14]. They are frequently acquired in the hospital and consist of complicated UTIs such as pyelonephritis, prostatitis, perinephric abscess, related to urinary tract malformations, urinary catheters, and prolonged antibiotic treatments. The enterococcal polysaccharide antigen has been implicated in the pathogenicity of these infections, responsible for the binding of enterococci to epithelial cells, biofilm formation, and evasion of the phagocytic response by neutrophils. Other factors associated with pathogenicity and biofilm formation in urinary catheters are endocarditis and biofilm-associated pili (Ebp) and enterococcal surface protein (Esp), of wide clinical interest because it promotes persistence in the bladder and subsequent dissemination to the kidneys, which could trigger bacteremia [15].

2.1.3. Bacteremia

Bacteremia is the presence of bacteria in the bloodstream. It is characterized by bacterial dissemination to various tissues. It is an urgent public health problem that can trigger devastating diseases, with a global economic cost that reaches billions of dollars each year. Clinical bacteremia can cause sepsis, defined as a potentially fatal organ dysfunction caused by a dysregulated host response to infection [16]. Gene expression plays an important role in enterococcal bacteremia, because virulence factors facilitate colonization, transmission and invasion, specifically gelatinase, an extracellular metalloendopeptidase that can hydrolyze bioactive proteins such as gelatin, hemoglobin and endothelin-1. This enzyme promotes the degradation of polymerized fibrin and facilitates bacterial spread [17].

2.1.4. Intra-abdominal infections (IAI)

IAIs comprise a large group of intra- and retroperitoneal processes ranging from uncomplicated pathologies, which affect a single organ and can be treated with antibiotics or surgical resection, such as appendicitis, to complicated ones, such as peritonitis, which can cause damage to more than one organ [18, 19]. They are considered an important cause of morbidity and mortality. Various disorders such as perforation of hollow viscera, inflammatory bowel disease, and post-surgical complications can produce intra-abdominal and pelvic abscesses [20]. It should be mentioned

Enterococcus is not the only bacterial agent involved in IAI, since more than one bacterial genus can commonly be found in this type of pathology, such as *Escherichia coli*, *Streptococcus*, and *Staphylococcus*. On the other hand, factors such as advanced age, ventilation and long stays in the intensive care unit could be associated with a high mortality rate [21].

2.1.5. Endodontics

After endodontic treatments, there is the possibility of generating infections, either due to poor hygiene or poor sterilization of surgical instruments to eliminate microorganisms. *E. faecalis* is one of the main microorganisms causing these infections, found in 23-70% of cultures of filled root canals. Studies that have reported the presence of *E. faecalis* in root canal treated teeth with periradicular lesions confirm that resistance to antibiotics used to treat these infections and the ability to form biofilms increase the survival of these bacteria, allowing them to resist phagocytosis, antibodies and antimicrobials. The antimicrobial resistance of bacteria has been attributed to the protective barrier provided by the extracellular polymeric matrix (biofilms) [22].

2.1.6. Meningitis

Meningitis is an inflammatory disease that affects the membranes surrounding the brain (meninges) and can be caused by viral or bacterial infections [23]. Enterococcal meningitis is rare and accounts for 0.3-4% of cases of bacterial meningitis. Predisposing factors include neurosurgical conditions such as head trauma, shunt placement, cerebrospinal fluid leak, and enterococcal bacteremia. The concern about enterococcal meningitis lies in the high mortality rate which ranges from 21% to 25% of cases [24].

2.1.7. Catheter-associated infections

Catheter-associated urinary tract infections (CAUTIs) are the most common nosocomial infections, accounting for approximately 80%. One of the main factors associated with the severity of these infections is the formation of microbial biofilms. *Enterococcus* species have been associated with urinary tract infections and account for 15-30% of CAUTIs. *E. faecalis* isolates can produce biofilms on urinary catheters and grow despite an intense inflammatory response [25].

2.2. Biofilms

Biofilms are bacterial accumulations that adhere to each other and to surfaces, either monomicrobial (a single species of bacteria) or polymicrobial (more than one bacterial species), to form resistant and molecule-exchanging microenvironments to proliferate and invade hosts. The removal of these biofilms is complicated, due to the formation of a matrix composed of extracellular polymeric substances (EPS) [26, 27].

2.2.1. Biofilm formation

The development of a microbial biofilm can be described as a dynamic process involving four successive steps; the first step consists of cell adhesion to a biotic or abiotic surface through weak and reversible interactions (Van de Waals forces), which allow cell-surface interaction and allow stronger bonds to be formed through adhesion receptors; the second step consists of the formation of microcolonies, from the division and growth of the first adhered cells; the microcolonies grow and merge, forming a layer of bacteria that covers the surface, this step occurs progressively until multiple bacterial layers are formed; the third step initiates the maturation of the biofilm through bacterial signaling and communication "quorum sensing", this step is characterized by the presence of macrocolonies surrounded by water channels that create an optimal environment for cell survival; Eventually, the biofilm matures and grows to such an extent that nutrients become limited, so bacterial aggregates or individual bacteria break off and migrate to other sites or tissues. In general, biofilm dispersal occurs in response to environmental changes and depends on growth conditions (Figure 1) [27].

2.2.2. Biofilm matrix

The integrity of the biofilm is regulated by a matrix composed of EPS, mainly polysaccharides, proteins and DNA, which varies in physical and chemical properties depending on the bacterial species present in the biofilm [27]. The exopolymer matrix is extracellular material, produced almost entirely by bacteria adhering to the biofilm. Its function is to provide structure, adhesion and cohesion. In addition, EPS keep the biofilm hydrated and act as a diffusion barrier, preventing antibiotics or disinfectants from filtering through it [28-29].

Biofilms are mostly made up of matrix (80-85%) and, to a lesser extent, cells (15-20%). The matrix completely envelops these bacterial aggregates and allows the exchange of genetic material and bacterial communication, the latter through signaling molecules (autoinducers) produced by the bacteria themselves, a process known as “quorum sensing”, which

regulates bacterial gene expression. In certain species, quorum sensing has been implicated as an inducer of biofilm formation and in others, it is also involved in the phenotypic heterogeneity of the same [27].

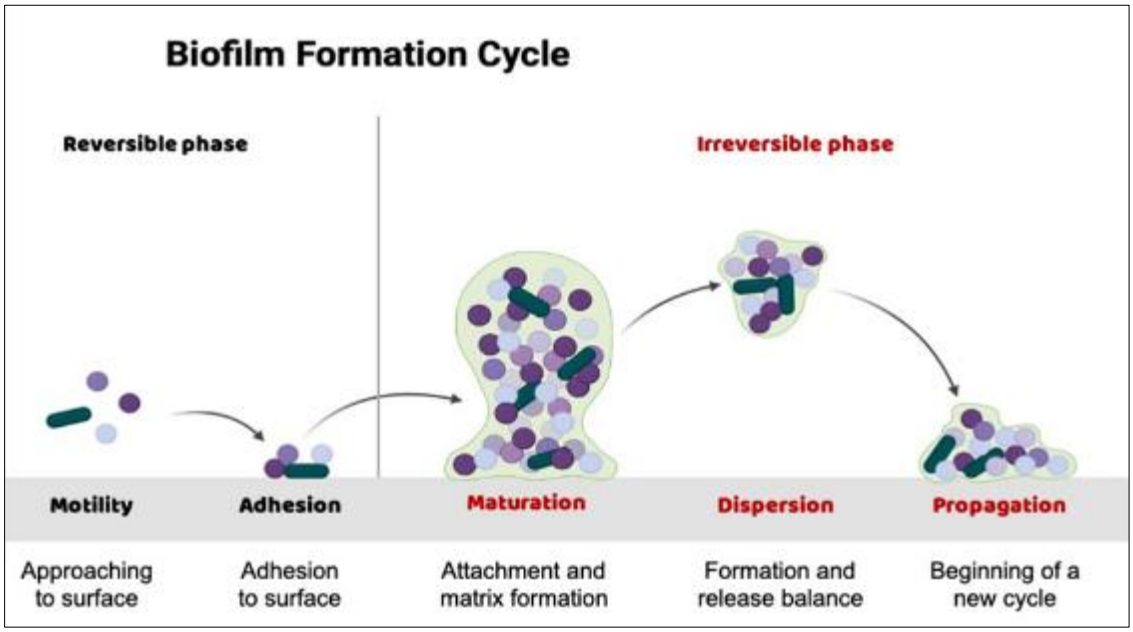

Figure 1 Stages of microbial biofilm biosynthesis. Drawing based on Ortega-Peña [28]

2.3. Quorum sensing (QS)

Biofilm formation is associated with QS. The QS system is the population density-dependent regulatory mechanism by which bacteria communicate through signaling molecules, called autoinducers (AI) or bacterial pheromones, these act by activating specific receptors found in the cytoplasm or on the cell surface (depending on the type of bacteria), once the receptors are activated, the transcription of quorum sensing genes is also activated. QS can regulate several activities such as bioluminescence, expression of virulence factors, sporulation, biofilm formation, and mating [30]. AI in Gram-negative bacteria are modified acyl-homoserine lactones, while peptide autoinducers are commonly used by Gram-positive bacteria. In contrast, autoinducer 2 molecules (AI-2) are used for intra- and interspecies communication in Gram-positive and negative bacteria. In the case of Gram-positive bacteria, autoinducers are exported outside the cell and are modified oligopeptides that act in the cytoplasm, activating specific transcription factors and regulating gene expression. When high concentrations of autoinducers are present outside the cell, they bind to histidine kinase receptors, which is autophosphorylated, activating the response regulator, which in turn activates the quorum sensing regulon [31].

2.3.1. Quorum sensing system by fsr

The Fsr Quorum sensing system of *E. faecalis* consists of a two-component system that senses cell density and regulates virulence. This system participates in the positive regulation of the expression of *gelE* and *sprE*, which encode gelatinase and serine protease, respectively, important for biofilm formation. The *fsr* locus consists of four genes: *fsrA*, *fsrB*, *fsrC* and *fsrD*. Upstream of this operon are the *gelE* and *sprE* genes. The *fsrA* gene encodes the FsrA protein that belongs to the LytTR family, which are characterized by possessing DNA binding domains. The *fsrB* gene gives rise to the FsrB protein, which processes the FsrD propeptide to generate GBAP (Gelatinase Biosynthesis Activating Pheromone), which is exported to the exterior of the cell to be subsequently sensed by FsrC. The *fsrC* gene encodes the transmembrane protein FsrC, which acts as the sensor histidine kinase of the two-component system, Fsr. Fsr has been identified as the only quorum sensing system involved in biofilm formation in *E. faecalis* under various environmental conditions [31-33].

2.4. Virulence factors

The virulence of an organism has been described to be regulated by genes encoding virulence factors, specifically regions called pathogenicity islands. Virulence factors are secreted molecules or surface proteins present in microorganisms that are necessary to cause disease or enhance their ability to do so [34].

Enterococcus virulence factors can be classified into two groups, the first group consists of cell surface-associated or adhesion factors, and the second group consists of secreted factors.

Adhesion factors that have been described in *Enterococcus* are aggregation substance (AsaI), collagen binding protein (Ace), antigen A (EfaA) and enterococcal surface protein (Esp). The secreted factors are secreted substances and enzymes, such as cytolysin (Cyl), gelatinase (GelE) and hyaluronidase (Hyl) (Table 1) [35]. Surface-associated virulence factors are involved in resistance to phagocytosis, promotion of biofilm formation and adhesion to the host or surfaces; on the other hand, secreted virulence factors are associated with tissue damage and binding, inhibition of the immune response, activation of stress and could be involved in cell wall metabolism [7, 35].

Among the virulence factors that have been described as participating in biofilm formation in *E. faecalis in vitro*, are found Ebp pili and Ace, which are surface adhesins and promote attachment to host tissue [36]. In other study, it was shown that the *cylA* gene is involved in cytolysin activation and biofilm formation in urinary tract infections [37]. On the other hand, other investigations associate Esp and secreted antigen A (SagA) with biofilm formation [7, 38].

Table 1 Virulence factors described in *Enterococcus spp*

Virulence factor	Gene	Biological function
Extracellular surface protein	*esp*	Involved in tissue adhesion and colonization, evasion of the immune system; participates in biofilm formation [39].
Cytolysin (hemolysin)	*cylA*	Bactericidal against Gram+ and hemolytic capacity in humans [40].
Gelatinase	*gelE*	Degrades host tissue, which provides nutrients to the bacteria; possibly associated with biofilm formation and facilitates bacterial dissemination [41].
Antigen A	*efaA*	Facilitates adhesion to biotic and abiotic surfaces and is involved in certain stages of biofilm formation [42].
Adhesion of collagen from *E. faecalis*	*ace*	Adhesin that can bind to collagen types I and IV and plays an important role in the pathogenesis of endocarditis and urinary tract infections [43].
Aggregation substance	*asal or agg*	Promotes binding to tissue surfaces, increases internalization and cellular life of the bacteria [44].
Endocarditis and biofilm-associated pili	*ebp*	Group of genes related to pill formation, involved in adhesion to multiple types of human cells and biofilm formation [45].
Secreted antigen A	*sagA*	Protein that contributes to biofilm formation [46].

2.5. Antibiotic resistance

Antibiotic resistance is the ability of a microorganism to resist the effects of an antibiotic. Resistance occurs by natural selection through random mutations, or by genetic transfer from other resistant organisms [47]. The *Enterococcus* genus has intrinsic resistance to a wide variety of antibiotics, including beta-lactams, aminoglycosides, glycopeptides, fluoroquinolones, oxazolidinones, among others, thus being a major problem for public health due to the presence of multi-resistant strains and their alarming resistance to vancomycin, giving it a place on the list of priority pathogens of the World Health Organization (WHO) with a priority 2: high [48-50]. Vancomycin resistance genes have been described to be encoded by extrachromosomal mobile genetic elements called plasmids, which are acquired by conjugative transfer [48, 49]. The mechanism of action of vancomycin consists of inhibiting cell wall formation by strongly interacting with the D-alanine-D-alanine end of the cell wall-forming units. In this sense, vancomycin-resistant species modify the end of the wall-forming units by changing it to D-alanine-D-lactate, which prevents vancomycin from binding to this end and favors cell wall formation [49].

3. Conclusion

Enterococci belonging to human, and animal gastrointestinal flora are widely distributed in the environment. They have clearly emerged as a medically important organism, causing outbreaks of many nosocomial infections. These bacteria have developed a high resistance to antimicrobial agents, due to its multiple virulence factors that promote colonization, infection and contribute to its ability to survive. Moreover, their developed ability to acquire novel determinants for both resistance and virulence has kept them ahead of the many attempts to control the damage that they cause to

patients in health care systems. The recent evolution of *Enterococcus* strains that are hypervirulent and multidrug-resistant highlights the need for a better understanding of the biology of these pathogens and applying the preventive and control measures are required.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest.

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