Enterococcus faecalis (*E. faecalis*): A silent endogenous pathogen from oral through systemic infections

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Abstract

Enterococci are commensal bacteria in animal and human gastrointestinal tracts. However, in term of pathogens, they are responsible for various serious infections. Enterococci have emerged as nosocomial infections with high mortality rate, especially *Enterococcus faecalis* (*E. faecalis*), which is known related to recurrent infection in root canal and failure in endodontic treatment. Infections from oral sources within root canal or periodontal tissues can be disseminated via bloodstream and passed into cardiovascular system. Occurring septicemia can cause following systemic diseases such as infectious endocarditis, particularly, in patients who are compromised or having defects at heart valves. Since *E. faecalis* has been found to highly resist multiple types of antibitotics, studies of this pathogen is currently of interest. Understanding the nature of one of the most infectious agents related to endocarditis and life threatening are therefore important and likely shades light on alternative options for lesser use of antibiotic agents presently problematic due to microbial resistance.

Keywords: Enterococci, *E. faecalis*, Nosocomial infection, Antibiotic resistance, Root canal, Biofilm

Introduction

Enterococci species were thought to be harmless to human in the past (Foulquie et al., 2006). These microorganisms are widely used in food industries and medicine areas because they produce an antibacterial agent, bacteriocin (De Kwaadstenit et al., 2005). Recently, Enterococci become of more interest since several reports demonstrated their relationships with nosocomial infection, causing mortality in patients as high as 61% (De Fa'tima Silva Lopes et al., 2005; Willems et al., 2005; Wisplinghoff et al., 2004). Increasing number of cases reported in Europe and US show incidences of bacteremia caused by these microbes (Butler 2006; de Perio et al., 2006; McDonald et al., 2005). Enterococci are found hard to control due to their ability to resist many antibiotics (Billstrom et al., 2008; Borgmann et al., 2004; Busani et al., 2004; Fisher & Phillips., 2009). Interestingly, percentage of risk to death by vancomycin-resistant enterococci (VRE) is 75 % compared to 45% of those infected with susceptible strains (Bearman & Wenzel, 2005). Over a decade, 20-fold increase in VRE has been associated with nosocomial infection worldwide (Borgmann et al., 2004; Busani et al., 2004; Murray, 1997; Rice, 2007; Shay et al., 1995; Zirakzadeh & Pate, 2006). So far, no case of nosocomial infection caused by oral cavity-originated Enterococci has been reported in Thailand. The increase of Enterococcal antibiotic resistance and the less responsiveness of patients in many medical treatments are still unraveled.

Enterococcus faecalis (*E. faecalis*)

Enterococcus faecalis is Gram-positive facultative anaerobic cocci and typically catalase-negative. It possesses abilities not only to ferment carbohydrate and produce lactic acid, but also to catabolize a wide range of energy sources, such as glycerol, lactate, malate, citrate, diamino acids and many α-keto acids (Van den Berghe, 2006).

This microbe grows at a temperature range of 10 to 45°C, in bile salts, and in environments with broad range of pH (Klein, 2003; Domig et al., 2003; Van den Berghe, 2006). Growth could be enhanced through oxidative phosphorylation using a proton motive force established by electron transport (Klein et al., 1998).

E. faecalis is commonly found in soil, water, and plants (Franz et al., 1999). Survival from harsh environmental niches is due to its capabilities to tolerate stresses ranging from oxidative stress, azide, detergents, ethanol to heavy metals (Klein, 2003). It is also able to live in extreme alkaline pH and high salt concentration (Klein, 2003). These traits require cation transport system to maintain the constant cytosolic ion composition, which is essential for homeostasis (Martinez et al., 2003; Kayaoglu & Orstavik, 2004). Several strains of *E. faecalis* are used in processing lines of food industries but some cause serious infectious problems in human and animals (De Fa′tima Silva Lopes et al., 2005, 2006), especially, opportunistic infection in both hospital and community (Klein, 2003; Klare, 2003; Koch, 2004). Normally this bacterium colonizes in gastrointestinal and genital tracts of human (Klein et al., 1998). Growth under such conditions drives *E. faecalis* to gain flexible metabolisms for their survival. Ability to utilize a variety of sugar sources, enable it to live in a limited nutrient place as intestine (Klein et al., 1998; Koch, 2004). Additionally, capacity to acquire a vast array of antimicrobial factors, allows *E. faecalis* to resist many groups of antibiotic drugs, such as aminoglycosides, penicillin, ampicillin, vancomycin, thus leading to a more complicated treatment in patients who infected (Fisher & Phillips, 2009; Murray, 2004).

Cell Structure

E. faecalis is Gram-positive cocci that typically forms short chains or grows in pairs. In certain growth conditions, elongate form or coccobacillary can be observed (Klein et al., 1998). *E. faecalis* contains 20 to 38% of the dry cell weight (in the exponential and stationary phase cells) (Klein, 2003). Cell wall is composed of peptidoglycan around 40% while rhamnose-containing polysaccharide and a ribitol-containing teichoic acid are the rest (Klein, 2003). Peptidioglycan, like in most Gram-positive cells, has resistance to osmotic bursting (Klein, 2003). Generally, *E. faecalis* is considered as a non-encapsulated organism; however, some strains isolated possess a capsular polysaccharide (Sedgley et al., 2004). *E. faecalis* has ability to exchange genetic material (plasmids) by conjugation process induced by small peptide pheromones (Paulsen et al., 2003). This bacterium also forms biofilm using their pilli appendages as a tool for initial adhesion on surfaces (Nallapareddy et al., 2006; Budzik & Schneewind., 2006). Strains causing endocarditis usually contain large amounts of pili and produce biofilm significantly more often and greater degree than non-endocarditis isolates (Nallapareddy et al., 2006).

Ecology

The Enterococci are members of the bacterial community inhabiting the large bowel in human (Singh et al., 2007). Although, appeared in soil, plants, and water, they are a natural part of the intestinal flora in most mammals and birds (Franz et al., 1999; Peters et al., 2003). *E. faecalis* was found related to failure of endontic-treated root canals (Sundqvist, 1998). This suggests its role in endodontic. A study showed 69% of the isolated bacteria from 100 root-filled teeth with apical periodontitis were facultative anaerobes and 50% of those were enterococci (Dahlen et al., 2000). Human enterococi endocarditis showed evidences of 80-90% endodontic infection caused by *E. faecalis*. Most of them were isolated from medically filled root canal (Love, 2001; Peciuliene et al., 2001).

Virulence and survival factors of *E. faecalis*

E. faecalis possesses vast arrays of virulence factors such as lytic enzymes, aggregation substance (AS), pheromones and lipoteichoic acid, as following described;

a) Aggregation Substance (AS): AS is a 37 kDa surface membrane protein that facilitates *E. faecalis* to interact with host cells (Hällgren et al., 2009). As an adhesin, it helps disseminating plasmid-encoded virulence factors, protecting bacteria from phagocytosis by host immune cells like polymorphonuclear cells and macrophages, and enhancing bacteria virulence by activating quorum-sensing that regulates cytosine, another virulence determinant; hence contributing *E. faecalis* for pathogenesis (Kayaoglu & Orstavik, 2004).

b) Surface protein: A large chromosome-encoded surface protein which has multiple repeat motifs. The presence of this surface protein increases hydrophobicity, biofilm formation and adherence to anbiotic surfaces (Billstrom et al., 2008). Ability of *E. faecalis* to form biofilm is the main factor that initiates progress of endodontic, uninary infections, as well as endocarditis. Gene cluster such as *ebp* (endocarditis-and biofilm-associated pili) is responsible for this manner. The ebp operon contains *ebpA*, *ebpB*, *ebpC* and an associated *srtC* (encoding sortase C) gene (Singh et al., 2007). All are associated with pilli formation. A non-piliated mutant of *E. faecalis* was reported not able to produce biofilm (Budzik & Schneewind, 2006).

c) Collagen-binding protein (adhesion of collagen; ACE): ACE is another cell surface protein of *E. faecalis* and a member of microbial surface components recognizing adhesive matrix molecule (MSCRAMM) family (Kosh, 2004). This collagen-binding protein was found to facilitate *E. faecalis* to bind collagen within dentin (Kayaoglu & Orstavik, 2004). It is suspected to play roles in pathogenesis of endocarditis caused by *E. faecalis* (Budzik & Schneewind, 2006).

d) Gelatinase, Serine protease and Hyalurodinase: Gelatinase is a non plasmid-encoded metalloendopeptidase responsible for resorption of bone and degradation of dentin organic matrix, leading to pathogenesis of periapical inflammation (Johnson et al., 2006). Serine protease can cleave peptide bond and contribute to *E. faecalis*-dentin bond. Its production is auto-regulated in a growth-phase-dependent mode by the quorum-sensing system encoded by the *fsr* (faecal streptococci regulator) (Sifri, 2002). Hyaluronidase is considered as a spreading factor since its degradation activity involved in the presence of *E. faecalis* and other bacteria at periapical region (Peciuliene et al., 2001). All of these hydrolytic enzymes are known to degrade extracellular components in connective tissues, thus facilitating spreading of organisms from oral to other systemic tissues as well as their toxins (Mohamed & Huang, 2007).

e) Cytolysin (Secreted virulence factor): Cytolysin is a pheromones-responsive plasmid-encoded toxin produced by beta-hemolytic *E. faecalis* isolates (Fisher & Phillips, 2009). It is regulated by a quorum-sensing mechanism and is used to kill other bacteria. Cytolysin can lyse red blood cells, polymorphonuclear cells and macrophages, thus lessening phagocytosis by host immune cells (Semedo et al., 2003).

f) Lipoteichoic acid (LTA): Lipoteichoic acid is a virulence factor of *E. faecalis* involved in aggregate formation and plasmid transfer which contributes to pathogenesis (Kayaoglu & Orstavik, 2004). Viable *E. faecalis* produced two folds of LTA molecules but nothing changed in non cultivable state. This suggests a role for LTA during this stage (Kayaoglu & Orstavik, 2004).

g) Extracellular superoxide: This survival determinant is associated with invasive trait of virulence isolates rather than that of the commensal isolates (Kayaoglu & Orstavik, 2004).

h) Pheromones: *E. faecalis* produces pheromones which induce chemotaxis of polymorphonuclaer cells and trigger superoxide production (Kayaoglu & Orstavik, 2004). Pheromones help transfer antibiotic resistance and other virulence traits such as cytolysin production through sex pheromones system (Kosh, 2004).

i) Quorum sensing: Quorum sensing is a two-component bacterial communication system established by production of signals via an autoinducing peptide (AIP) (Fisher & Phillips, 2009). Lack of corresponding genes in the locus, *E. faecalis* cannot form biofilm (Sifri et al., 2002). All *E. faecalis* strains related to endocarditis contain *fsr* system whereas only 10 from 19 commensal stool isolates equip with this regulator (Sifri et al., 2002). The *fsr* quorum sensing system thus exhibits its role in virulence (Podbielski & Kreikemeyer et al., 2004).

E. faecalis is a persister regarded to its capability of surviving within such a harsh environmental conditions (Klein, 2003). It can grow in an environment of high level of salt, wide temperature range, broad pH range and in adequate nutrition (Ivanov et al., 1999). *E. faecalis* may require serum from periapical tissues which sustains other microbial flora for growth (Rams et al., 1992). With serum, it is able to be alive but not to be cultivated. A survival mechanism adopted by a group of bacteria can reverse harsh to favorable condition (Kosh, 2004). Some population can invade and live within the dentinal tubules (Love, 2001). They can bind dentin using a collagen binding protein (Love, 2001). *E. faecalis* can infect medicated root canals in form of mono-infections. Acquiring, accumulating and sharing extrachromosomal elements encoding virulence traits are abilities that facilitate this microorganism to succeed in colonization, competition with other bacteria, escaping from host defense mechanisms and production of the pathological factors in initiation and progression of diseases (Sedgley et al., 2004).

E. faecalis is a competent biofilm former (Estrela et al., 2009). It comprises a variety of biofilm-required factors which help the group to protect homeostasis of their biofilm (Nallapareddy et al., 2006). Biofilm is known to protect bacteria from host defenses and antibiotics (Mohamed & Huang, 2007). Bacteria residing within biofilm can communicate and exchange genetic materials to acquire new traits which may enhance their ability to resist antibiotics. This communication is established by quorum sensing (Sifri et al., 2002). Like other biofilm bacteria, *E. faecalis* gains higher resistance against many types of antibiotics and also calcium hydroxide as intra-canal medicaments by maintaining pH homeostasis (Estrela et al., 2009).

Pathology

Enterococci have been reported as a cause of nosocomial infections. Within the group *E. faecalis* is a main human pathogen involved in both systemic and local infection including urinary tract and abdominal infections, wound infections, bacteremia, and endocarditis (McDonald et al., 2005). *E. faecalis* is presently considered as a major problem due to high resistance to multiple antibiotics (Billstrom et al., 2008). The resistance to vancomycin, a currently effective antibiotics, has been reported (Zirakzadeh & Pate, 2006). This leads to serious health problems owing to lack of available antibiotic therapy for the infections. Most VRE strains harbor resistance to multiple antibiotics (e.g. aminoglyscoides, ampicillin). The transfer of vanocmycin resistant gene from VRE to other Gram-positive pathogens raises a critical public health concern. In hospital, *E. faecalis* transmitted between patients usually occurs in long term care facilities (Wisplinghoff et al., 2004). It is causative pathogens in lower urinary tract infections (UTI), such as cystitis, prostatitis, epididymitis (Hällgren et al., 2009). *E. faecalis* are also observed in intra-abdominal, pelvic, and soft tissue infections (de Perio et al., 2006). Endocarditis is the most serious enterococcal infection because it could leads to inflammation of heart valves. Antibiotic used in infective endocarditis mostly failed and the surgical removal is required. Other human infections caused by *E. faecalis* include meningitis, hematogenous, osteomyelitis, septic arthritis, and sometimes pneumonia (Shay et al., 1995). Treatment of these bacterial diseases is more fastidious due to the resistance of *E. faecalis* to multiple drugs. These abilities are acquired by the distribution of resistance genes amongst bacterial strains (Singh et al., 2007). Drug-challenging is therefore needed for bacteria in this group. As it is known that antibiotics commonly used in the treatment of a bacterial infection have an impact on the intestinal flora (Klein, 2003). Resistant bacteria can be naturally selected during treatment and could become potentially pathogenic (Kristich et al., 2007; Murray, 1997). Searching for novel agents to treat β-lactamase producing and vancomycin-resistant *E. faecalis* infections in human is ongoing (Rice, 2007; Willems et al., 2005).

The consequence of its inherent resistance to certain antibiotics and its abilities to survive and proliferate in the intestinal tract are crucial. The genetic basis of the antibiotics resistant mechanisms in *E. faecalis* is not well known. Kristich et al. (2007) attempted to identify causes of the resistance. They showed that PrkC, a one-component signaling protein containing a eukaryotic-type Ser/Thr kinase domain, allows *E. faecalis* for inherent antimicrobial resistance and intestinal persistence. An *E. faecalis* mutant lacking PrkC grew at a wild-type rate in the absence of antimicrobial stress but showed enhanced sensitivity to cell-envelope-active compounds including bile detergents and antibiotics targeted on cell-wall biogenesis (Kristich et al., 2007). PrkC regulates physiological processes in *E. faecalis* and is a key for its success as a hospital-acquired pathogen (Podbielski & Kreikemeyer, 2004). PrkC is predicted as a cytoplasmic kinase (Kristich et al., 2007). Its domain is separated by a transmembrane segment thought to bind uncross-linked peptidoglycan (Kristich et al., 2007). This suggests that PrkC is a transmembrane receptor that monitors the integrity of the *E. faecalis* cell wall and mediates adaptive responses to maintain integrity. PrkC is essential for *E. faecalis* to cause nosocomial infections, suggesting that the signaling protein is a target for the development of therapies in order to prevent infections by antibiotic-resistant Enterococci (Kristich et al., 2007).

E. faecalis and its importance in dental field

Enterococci are one of top three nosocomial bacterial pathogens highly resisting to presently used antibiotics (Kayaoglu & Orstavik, 2004; Budzik & Schneewind, 2006). Some of them were detected in samples taken from multiple oral sites; 60% of school children with high caries activity and 75% of patients with endodontic infection (Gold et al., 1975). *E. faecalis* was found in 29% of oral-rinsed samples, 55% of tongue dorsum, and 22% of gingival sulcus samples, from 41 endodontic subjects (Sedgley et al., 2006). It occasionally predominates in periodontal tissue (Rams et al., 1992; Souto & Colombo, 2008; Sun et al., 2009) or oral mucosa (Dahlen, 2009), but barely found in peri-implantitits (Charalampakis et al., 2011; Leonhardt et al., 1999,). *E. faecalis* is mainly related to infectious root canals or failed treatment of endodontic treated root canal (Kayaoglu & Orstavik, 2004; Love, 2001). The formation of biofilm facilitates *E. faecalis* as in biofilm group to increase resistance to antibiotics and provides more chance to survive within root canal (Rocas et al., 2004; Sundqvist et al., 1998). This includes, additionally, more resistance against $Ca(OH)_2$ (Estrela et al., 2009; Kayaoglu & Orstavik., 2004).

E. faecalis was also found within subgingival plaque collected from chronic refractory periodontitis patients (Balaei-Gajan et al., 2010). It caused recurrent periodontal disease as high as 51.8% (Balaei-Gajan et al., 2010). Since periodontal destructive parameters such as clinical attachment loss, pocket depth, are associated with the presence of *E. faecalis*, its role in the severity and/or progression of periodontitis is suggested (Souto & Colombo., 2008). The virulence factors may be involved in pathogenesis of periodontal disease including aggregation substance, surface adhesin, lipoteichoic acid, extracellular superoxide production, cytolysin, haemolysin, and lytic enzymes as gelatinase, hyaluronidase and elastase (Fisher & Phillips, 2009; Sedgley et al., 2005). Clinical isolates of *E. faecalis* from deep oral infections and oral lesions showed bacteriocin production and abundant plasmid presence. They were found to express virulence factors via genes e.g. *efa* and *esp* as high as 93% (Dahlen et al., 2012). This agrees with previous studies in which similar virulence genes were reported in medical isolates (Eaton & Gasson, 2001; Sedgley et al., 2009). Interestingly, clindamycin, an antibiotics commonly used in dental treatment, was failed to inhibit growth of *E. faecalis* (Dahlen et al., 2012). Although the amount of *E. faecalis* is not much in dental biofilm, their presence as a reservoir of antibiotics-resistant for other oral bacteria could cause complicated problems in dental and medical treatment (Sedgley et al., 2005, 2009).

Growth of *E. faecalis* amongst other periodontal pathogens in subgingival region may increase pathogenicity so that finally leads to apical periodontitis (Socransky et al., 1998). Periodontal inflammation results in bleeding of gingival tissue (Rams et al., 1992; Socransky et al., 1998). Dental procedures during periodontal or endodontic treatments and vasodilatation of capillar allow the passage of oral bacteria including *E. faecalis* into blood stream and later the body system, causing consequently hospital-acquired endocarditis in compromised patients (Johnson et al., 2006; Rams et al., 1992). The signature of enteroccocci in medical and dental fields and a massive increase in antibiotics resistance worldwide light up the curiosity for their nature. Further studies are needed in order to provide proper management for this silent endogenous pathogen.

Conclusion

Enterococci were previously thought as endogenous nonpathogenic microflora in human and considered not medically important (Franz et al., 1999). Nowadays, they become one of the most common nosocomial pathogens that raise a great concern in hospitals around the world (Rice, 2007; McDonald et al., 2005; William et al., 2005; Zirakzadeh & Pate, 2006). Abilities of oral-originated *E. faecalis* to survive a broad range of adverse environments, to cause systematic infection, and to resist a vast group of antibiotics are well known and their mechanisms in molecular level are waiting to be elucidated (Sedgley et al., 2009; Van den Berghe et al., 2006). Understanding virulence traits, antibiotic resistance, how to cope with stresses and survival within oral cavity environment, becomes more important as the emergence of *E. faecalis* in nosocomial infection is increasing. Oral cavity is a main source of the cross-contamination that this pathogen can easily disseminate through body, therefore mechanisms and alternative management of this species is required.

References

Balaei-Gajan, E., Shirmohammadi A., Abashov R., Agazadeh M. & Faramarzie M. (2010). Detection of *Enterococcus faecalis* in subgingival biofilm of patients with chronic refractory periodontitis. *Med Oral Patol Oral Cir Bucal, 15*, 667-670.

Bearman, G. M. L. & Wenzel, R. P. (2005). Bacteraemias: a leading cause of death. *Arch Med Res, 36*, 646659.

Billstrom, H., Lund, B., Sullivan, A. & Nord, C. E. (2008). Virulence and antimicrobial resistance in clinical *Enterococcus faecium*. *Int J Antimicrob Agents, 32*, 374377.

Borgmann, S., Niklas, D. M., Klare, I., Zabel, L. T., Buchenau, P., Autenrieth, I. B. & Heeg, P. (2004). Two episodes of vancomycin resistant *Enterococcus faecium* outbreaks caused by two genetically different clones in a newborn intensive care unit. *Int J Hyg Environ Health, 207*, 386389.

Budzik, J. M. & Schneewind, O. (2006). Pili prove pertinent to enterococcal endocarditis. *J Clin Invest, 116*, 25822584.

Busani, L., Del Grosso, M., Paladini, C., Graziani, C., Pantosti, A., Biavasco, F. & Caprioli, A. (2004). Antimicrobial susceptibility of vancomycin-susceptible and -resistant enterococci isolated in Italy from raw meat products, farm animals, and human infections. *Int J Food Microbiol, 97*, 1722.

Butler, K. M. (2006). Enterococcal infection in children. *Semin Pediatr Infect Dis, 17*, 128-139.

Charalampakis, G., Leonhardt, A., Rabe, P. & Dahlen G. (2011). Clinical and microbiological characteristics of peri-implantitis cases; a retrospective multi-center study. *Clin Oral Implant Res, 38*, 864-871.

Dahlen G. (2009). Bacterial infections of the oral mucosa. *Periodontology 2000, 49*, 13-38.

Dahlen, G., Samuelsson, W. & Molander, A. (2000). Identification and antimicrobial susceptibility of enterococci isolated from root canal. *Oral Microbiol Immunol, 15*, 309312.

Dahlen, G., Suanne B., Annica, A. & Anette C. (2012). Virulence factors and antibiotic susceptibility in enterococci isolated from oral mucosa and deep infections. *J.OralMicrobiol, 4*, 10855.

De Fa´ tima Silva Lopes, M., Ribeiro, T., Abrantes, M., Figueiredo Marques, J. J., Tenreiro, R. & Crespo, M. T. B. (2005). Antimicrobial resistance profiles of dairy and clinical isolates and type strains of enterococci. *Int J Food Microbiol 103*, 191198.

De Fa´ tima Silva Lopes, M., Simo es, A. P., Tenreiro, R., Figueiredo Marques, J. J. & Barreto Crespo, M. T. B. (2006). Activity and expression of a virulence factor, gelatinase, in dairy enterococci. *Int J Food Microbiol, 112*, 208214.

De Kwaadsteniet, M., Todorov, S. D., Knoetze, H. & Dicks, L. M. T. (2005). Characterization of a 3944 Da bacteriocin, produced by *Enterococcus mundtii* ST15, with activity against Gram-positive and Gram-negative bacteria. *Int J Food Microbiol, 105*, 433444.

De Perio, M. A., Yarnold, P. R., Warren, J. et al. (2006). Risk factors and outcomes associated with non-*Enterococcus faecalis*, non-*Enterococcus faecium* enterococcal bacteremia. *Infect Control Hosp Epidemiol, 27*, 28-33.

Domig, K. J., Mayer, H. K. & Kneifel, W. (2003). Methods used for the isolation, enumeration, characterisation and identification of Enterococcus spp. 2. Pheno- and genotypic criteria. *Int J Food Microbiol, 88*, 165188.

Eaton, T. J. & Gasson, M. J. (2001). Molecular screening of Enterococcus virulence determinants and potential for genetic exchange between food and medical isolates. *Appl Environ Microbiol, 67*, 1628-1635.

Estrela, C., Sydney, G. B., Figueiredo, J. A. & Estrela., S. R. (2009). Antibacterial efficacy of intracanal medicaments on bacterial biofilm: a critical review. *J Appl Oral Sci, 17*, 1-7.

Fisher, K. & Phillips, C. (2009). The ecology, epidemiology and virulence of Enterococcus. *Microbiol, 155*, 174957.

Foulquie Moreno, M. R., Sarantinopoulos, P., Tsakalidou, E. & De Vuyst, L. (2006). The role and application of enterococci in food and health. *Int J Food Microbiol, 106*, 124.

Franz, C. M. A. P., Holzapfel, W. H. & Stiles, M. E. (1999). Enterococci at the crossroads of food safety? *Int J Food Microbiol*, *47*, 124.

Gold, O. G., Jordan, H. V. & van Houte, J. (1975). The prevalence of enterococci in the human mouth and their pathogenicity in animal models. *Arch Oral Biol, 20*, 473-477.

Hällgren, A., Claesson, C., Saeedi, B., Monstein, H. J., Hanberger, H. & Nilsson, L. E. (2009). Molecular detection of aggregation substance, enterococcal surface protein, and cytolysin genes and in vitro adhesion to urinary catheters of *Enterococcus faecalis* and *E. faecium* of clinical origin. *Int J Med Microbiol, 299*, 323-332.

Ivanov, I. T., Boytcheva, S. & Mihailova, G. (1999). Parallel study of thermal resistance and permeability barrier stability of *Enterococuc faecalis* as affected by salt composition, growth temperature and preincubation temperature. *J Therm Biol, 24*, 217227.

Johnson, E., M., Flannagan, S., E. & Sedgley C. M. (2006). Coaggregation interactions between oral and endodontic *Enterococcus faecalis* and bacterial species isolated from persistent apical periodontitis. *J Endod., 32*, 946-950.

Kayaoglu, G. & Orstavik, D. (2004). Virulence factors of *Enterococcus faecalis*: relationship to endodontic disease. *Crit Rev Oral Biol Med, 15*, 308320.

Klare, I., Konstabel, C., Badstubner, D., Werner, G. & Witte, W. (2003). Occurrence and spread of antibiotic resistances in *Enterococcus faecium*. *Int J Food Microbiol*, *88*, 269290.

Klein, G. (2003). Taxonomy, ecology and antibiotic resistance of enterococci from food and the gastro-intestinal tract. *Int J Food Microbiol, 88*, 123131.

Klein, G., Pack, A., Bonaparte, C. & Reuter, G. (1998). Taxonomy and physiology of probiotic lactic acid bacteria. *Int J Food Microbiol, 41*, 103125.

Koch, S., Hufnagel, M., Theilacker, C. & Huebner, J. (2004). Enterococcal infections: host response, therapeutic, and prophylactic possibilities. *Vaccine 22*, 822830.

Kristich, C. J., Wells, C. L. & Dunny, G. M. (2007). A eukaryotic-type Ser/Thr kinase in *Enterococcus faecalis* mediates antimicrobial resistance and intestinal persistence. *PNAS, 27*, 3508-3513.

Leonhardt, A., Renvert, S. & Dahlen, G. (1999). Microbial findings at failing implants. *Clin Oral Implant Res, 10*, 339-345.

Love, R. M. (2001). *Enterococcus faecalis* a mechanism for its role in endodontic failure. *Int J Endod, 34*, 399405.

Martinez, S., Lopez, M. & Bernardo, A. (2003). Thermal inactivation of *Enterococcus faecium*: effect of growth temperature and physiological state of microbial cells. *Lett Appl Microbiol, 37*, 475481.

McDonald, J. R., Olaison, L., Anderson, D. J. et al. (2005). Enterococcal endocarditis: 107 cases from the international collaboration on endocarditis merged database. *Am J Med, 118*, 759-66.

Mohamed, J. A. & Huang, D. B. (2007). Biofilm formation by enterococci. *J Med Microbiol, 56*, 15811588.

Murray, B. E. (1997). Vancomycin-resistant enterococci. *Am J Med, 102*, 284-289.

Nallapareddy, S. R., Singh, K. V., Silanpaa, J., Garsin, D. A., Hook, M., Erlandsen, S. L. & Murray, B. A. (2006). Endocarditis and biofilm associated pili of *Enterococcus faecalis*. *J Clin Invest, 116*, 2799807.

Paulsen, I. T., Banerjei, L. G., Myers, S. A., Nelson, K. E., Seshadri, R. T., Read, D., Fouts, D. E. & et al. (2003). Role of Mobile DNA in the Evolution of Vancomycin-Resistant *Enterococcus faecalis*. *Science, 299*, 2071-2074.

Peciuliene, V., Reynaud, A. H., Balciuniene, L. & Haapasalo, M. (2001). Isolation of yeasts and enteric bacteria in root-filled teeth with chronic apical periodontitis. *Int J Endod, 34*, 429434.

Peters, J., Mac, K., Wichmann-Schauer, H., Klein, G. & Ellerbroek, L. (2003). Species distribution and antibiotic resistance patterns of enterococci isolated from food of animal origin in Germany. *Int J Food Microbiol, 88*, 311314.

Podbielski, A. & Kreikemeyer, B. (2004). Cell density-dependent regulation: basic principles and effects on the virulence of Gram positive cocci. *Int J Infect Dis, 8*, 8195.

Rams, T. E., Feik, D., Young, V., Hammond, B. F. & Slots, J. (1992). Enterococci in human periodontitis. *Oral Microbiol Immunol, 7*, 249-252.

Rice, L. B. (2007). Emergence of vancomycin-resistant enterococci. *Emerg Infect Dis, 7*, 183-187.

Rocas, I. N., Siqueira J. F. & Santos K. R. (2004). Association of *Enterococcus faecalis* with different forms of periradicular diseases. *J Endod, 30*, 315-20.

Sedgley, C. M, Buck G. & Appelbe O. (2006). Prevalence of *Enterococcus faecalis* at multiple oral sites in endodontic patients using culture and PCR. *J Endod, 32*, 104-109.

Sedgley, C. M., Clewell, D. B. & Flannagan, S., E. (2009). Plasmid pAMS1- encoded, bacteriocin-related 'siblicide' in *Enterococcus faecalis*. *J Bacteriol, 191*, 3183-3188.

Sedgley, C. M., Lennan, S. L. & Clewell, D. B. (2004). Prevalence, phenotype and genotype of oral enterococci. *Oral Microbiol Immunol, 19*, 95-101.

Sedgley, C. M., Molander, A., Flannagan, S. E., Nagel, A. C., Appelbe, O. K., Clewell, D. B. & et al. (2005). Virulence, phenotype and genotype characteristics of endodontic Enterococcus spp. *Oral Microbiol Immunol, 20*, 10-19.

Semedo, T., Santos, M. A., Lopes, M. F., Marques, J. J. F., Crespo, M. T. & Tenreiro, R. (2003). Virulence factors in food, clinical and reference enterococci: a common trait in the genus? *Syst Appl Microbiol, 26*, 1322.

Shay, D. K, Maloney, S. A., Montecalvo, M. & et al. (1995). Epidemiology and mortality risk of vancomycin-resistant enterococcal bloodstream infections. *J Infect Dis, 172*, 993-1000.

Sifri, C. D., Mylonakis, E., Singh, K. V., Qin, X., Garsin, D. A., Myurray,B. E., Ausubel, F. M. & Calderwood, S. B. (2002). Virulence effect of *Enterococcus faecalis* protease genes and the quorum-sensing locus *fsr* in *Caenorhabditis elegans* and mice. *Infect Immun, 70*, 56475650.

Singh, K. V., Nallapareddy, S. R. & Murray, B. A. (2007). Importance of the *ebp* (endocarditis-and biofilm-associated pilus) locus in the pathogenesis of *Enterococcus faecalis* ascending urinary tract infection. *J Infect Dis, 195*, 16711677.

Socransky, S. S., Haffajee A. D., Cugini M. A., Smith C. & Kent R. L. (1998). Microbial complexes in subgingival plaque. *J Clin Periodontol. 25*, 134-144.

Souto, R. & Colombo, A. P. (2008). Prevalence of *Enterococcus faecalis* in subgingival biofilm and saliva of subjects with chronic periodontal infection. *Arch Oral Biol, 53*, 155-160.

Sun, J., Song, X., Kristiansen, B., Kjaereng, A., Willems, R. J. L., Eriksen, H. M. & et al. (2009). Occurrence, population structure and antimicrobial resistance of enterococci in marginal and apical periodontitis. *J Clin Microbiol, 47*, 2218-2225.

Sundqvist, G., Figdor, D., Persson, S. & Sjogren, U. (1998). Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 85*, 86-93.

Van den Berghe, E., De Winter, T. & De Vuyst, L. (2006). Enterocin A production by *Enterococcus faecium* FAIR-E 406 is characterised by a temperature-and pH-dependent switch-off mechanism when growth is limited due to nutrient depletion. *Int J Food Microbiol, 107*, 159170.

Willems, R. J., Top, J., van Santen, M., Robinson, D. A., Coque, T. M., Baquero, F. & et al. (2005). Global spread of vancomycin-resistant *Enterococcus faecium* from distinct nosocomial genetic complex. *Emerg Infect Dis, 11*, 821-8.

Wisplinghoff, H., Bischoff, T., Tallent, S. M., Seifer, H., Wenzel, R. P. & Edmond, M. B. (2004). Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin Infect Dis, 39*, 309-317.

Zirakzadeh, A. & Pate, R. (2006). Vancomycin-resistant enterococci: colonization, infection, detection, and treatment. *Mayo Clin Proc, 81*, 529-536.