Letters in Applied Microbiology

Letters in Applied Microbiology ISSN 0266-8254

ORIGINAL ARTICLE

Enterococcus faecalis infection in root canals – host-derived or exogenous source?

R. Vidana[1], Å. Sullivan[2], H. Billström[3], M. Ahlquist[1] and B. Lund[4]

1 Division of Cariology and Endodontology, Department of Dental Medicine, Karolinska Institutet, Huddinge, Sweden
2 Medical Products Agency, Uppsala, Sweden
3 Swedish Institute for Infectious Disease Control, Stockholm, Sweden
4 Division of Oral and Maxillofacial Surgery, Department of Dental Medicine, Karolinska Institutet, Huddinge, Sweden

Keywords
Enterococcus faecalis, origin, pulsed-field gel electrophoresis, refractory endodontic infection, root canal.

Correspondence
Bodil Lund, Division of Oral and Maxillofacial Surgery, Department of Dental Medicine, Karolinska Institutet, Alfred Nobels Allé 8, Box 4064, S-141 04 Huddinge, Sweden.
E-mail: bodil.lund@ki.se

2010/1608: received 12 September 2010, revised 2 November 2010 and accepted 3 November 2010

doi:10.1111/j.1472-765X.2010.02972.x

Abstract

Aims: *Enterococcus faecalis* is associated with a significant number of refractory endodontic infections. Previous studies report a prevalence of *Ent. faecalis* ranging from 24% up to 77% in teeth with failed endodontic treatment. The origin of the micro-organism remains unclear, as enterococci do not belong to the normal oral microflora. The aim of this study was to determine whether these enterococci were of endogenous or exogenous origin.
Methods and Results: Fifty consecutive patients with apical periodontitis in need of endodontic orthograde re-treatment were included. Samples were collected from root canals, saliva and faeces and subjected to microbiological culturing. The genetic relationship between *Ent. faecalis* from root canals and isolates from the different host sources was determined using pulsed-field gel electrophoresis. In 16% (8/50) of the patients, enterococci were collected from the root canal samples. The genetic analysis showed that the isolates from the root canals were not related to those from the normal gastrointestinal microflora. None of these patients had enterococci in their saliva samples.
Conclusions: Endodontic infections with *Ent. faecalis* are probably not derived from the patient's own normal microflora, which indicates that these infections ent. faecalis are of exogenous origin.
Significance and Impact of the Study: This is the first study to genetically compare endodontic infectious *Ent. faecalis* isolates with isolates from the hosts' own normal microflora.

Introduction

The eradication of infection from the root canal system by means of mechanical and chemical cleansing presents a major obstacle to combat in endodontic treatment. Alas, because of the complex nature of the root canal anatomy, a complete cleansing and disinfection are almost impossible, leaving residual organic and inorganic matter. Pulpal tissue remnants and exudate from the periapical region serve as substrate for micro-organisms invading the root canal system via either coronal leakage or residing despite treatment. The primary endodontic infection is polymicrobial and consists mainly of obligate anaerobes with a small proportion of facultative anaerobes (Fabricius *et al.* 1982). The obligate anaerobes are fairly easy to eliminate, or at least greatly reduce in number by instrumentation and irrigation, in contrast to the facultative anaerobes which are capable of surviving such chemomechanical treatment and subsequently take over the ecological niche (Chavez de Pas *et al.* 2003). Therefore, the persisting infection in endodontically treated teeth typically consists of facultative anaerobic bacteria.

One of the species of bacteria frequently retrieved from previously root-filled canals is *Enterococcus faecalis*, which is a Gram-positive facultative anaerobe. In previous studies, the prevalence of *Ent. faecalis* in failed endodontic cases ranged between 24 and 70%, when culture-based techniques were used (Engström 1964; Möller 1966;

Molander *et al.* 1998; Sundqvist *et al.* 1998; Peciuliene *et al.* 2000, 2001; Hancock *et al.* 2001; Pinheiro *et al.* 2003a,b). Studies applying polymerase chain reaction (PCR) as the detection method reported a prevalence of 67–77% (Rôças *et al.* 2004; Siqueira and Rôças 2004). As such, *Ent. faecalis* has come to symbolize the refractory endodontic infection. Consequently, enterococci are frequently the target micro-organism when evaluating the antibacterial efficacy of irrigating solutions, intracanal medications and preparation techniques (Portenier *et al.* 2003; Stuart *et al.* 2006; Estrela *et al.* 2008). Numerous studies demonstrate the hardy nature of enterococci, which are able to resist various intracanal medications and adapt to harsh environmental conditions (Gilmore 2002; Figdor *et al.* 2003; Tendolkar *et al.* 2003). Therefore, it is not surprising that *Ent. faecalis* is depicted as a worthy opponent in the effort in re-stabilizing periradicular health, fuelling further research in respective areas of interest.

Enterococci were earlier considered to be assigned to the group of streptococci and to comprise a part of the normal oral microflora. Engström proposed in 1964 that there was 'direct correlation between the occurrence of enterococci in the oral cavity and in the pulp cavity' (Engström 1964). As such, they should easily be sampled in saliva, but studies showed the contrary (Sedgley *et al.* 2004, 2005, 2006a,b). With growing knowledge about the composition of the commensal oral microflora, enterococci are now recognized to be only transient oral bacteria (Aas *et al.* 2005). Their normal habitat is the gastrointestinal and genitourinary tract and not the oral cavity. The source of the enterococci found in the root canal system is thus still unclear, but evidence is gathering pointing towards an exogenous origin (Zehnder and Guggenheim 2009). However, an endogenous origin that is derived from the host's normal gastrointestinal microflora cannot be excluded.

The aim of this study was thus to investigate whether *Ent. faecalis* isolates recovered from previously root-filled teeth with apical periodontitis derived from the patient's own normal microflora. Root canal, saliva and faecal samples were collected from patients and subjected to microbiological culturing. The genetic relationship between the collected *Ent. faecalis* isolates from the different sources was determined using pulsed-field gel electrophoresis (PFGE).

Materials and methods

Patients and study design

The study was conducted during a period of 2 years (March 2007–April 2009). Inclusion criteria were adult patients referred to a private specialist clinic in Stockholm and the Division of Cariology and Endodontology, Department of Dental Medicine at Karolinska Institutet, needing endodontic orthograde treatment for apical periodontitis in root-filled teeth. Exclusion criteria were teeth that rendered an aseptic working field impossible, because of gross tooth loss complicating rubber dam application, or teeth that needed disassembling of postretained crowns. Written consent was obtained from all participants. The study was approved by the regional ethics committee at Karolinska Institutet, Stockholm, Sweden.

Collection of samples

Bacterial samples from the root canals were taken by one and the same operator and in accordance with a modification of the protocol previously proposed (Möller 1966). Initial access preparation, including removal of caries and restorations with defective margins, was made without exposing root-filling material. After rubber dam application, the operating field and tooth crown were carefully disinfected with 30% hydrogen peroxide followed by a 0·5% chlorhexidine–ethanol solution. Access preparation could then be completed with sterile burs without water cooling. Root-filling material was extracted with Profile rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland) and Hedström files (Sendoline, Sweden). The removal of gutta-percha was conducted without the use of chemical solvents as advised by Molander *et al.*(1998). Produced frictional heat was also kept to a minimum to avoid negative effects on the micro-organisms. Sparse irrigation with VMG I (viable transport medium Gothenburg) sampling fluid occurred if lubrication was needed (Möller 1966). Working length was determined using radiographs, and the canals were when possible instrumented to within 0·5–1 mm of the radiographical apex and to an ISO file size apically of 25. Before sampling, VMG I viable transport medium Gothenburg sampling fluid was introduced into the canal to a level just below the canal orifice and agitated with a sterile ISO size 20 Hedström file. The solution inside the canal was then completely absorbed into sterile charcoal impregnated paper points inserted to the full working length. The paper points were immediately transferred into 3 ml of VMGA III (viable transport medium Gothenburg) transport medium and sent for prompt microbiological analysis (Dahlén *et al.* 1993).

Saliva samples were collected immediately after the termination of the endodontic treatment. Salivary secretion was stimulated by chewing on a small piece of paraffin for 5 min and secreted saliva was accumulated in a plastic container. A total of 2 ml saliva was stored in 4 ml of VMG II at −70°C (Jordan *et al.* 1968).

A faecal sample was obtained from the patient if the bacterial sample from the root canals positively identified *Ent. faecalis*. The patients were supplied with a sterile

plastic container for collection of faeces and instructed to send the collected sample swiftly to the laboratory via mail. The time between recovery of bacteria from root canals and faeces was approximately 21 days. At the laboratory, the samples were stored at −70°C until analysed.

Microbiological analyses

All collected root canal samples were subjected to culturing. Identification of aerobic and anaerobic bacteria to the genus level was based on Gram staining, colony morphology and biochemical tests, performed according to descriptions in the Manual of Clinical Microbiology (Murray *et al.* 1999).

In case of isolation of *Ent. faecalis* from the root canal, the corresponding saliva and faecal samples from the patient were subjected to microbiological analysis. A total of 2 ml from the saliva samples and 0·5 g faeces were suspended in 4 ml or 4·5 ml phosphate-buffered saline, respectively. A tenfold dilution series up to 10^{-5} for saliva and 10^{-6} for faecal samples were made. From each dilution step, 100 μl was plated on Enterococcosel agar (BBL Microbiological Systems, Cockeysville, MD, USA) and incubated aerobically for 48 h at 37°C for detection of enterococci. Suspected *Ent. faecalis* colonies were picked based on morphology and inoculated on blood agar (LabMKemila, Bury, UK). Coccal isolates, which were Gram-positive, catalase-negative and able to grow in 6·5% (w/v) sodium chloride containing agar, were considered to be enterococci and further tested for the fermentation of sorbitol and arabinose (Teixeira and Facklam 2003). Sorbitol-fermenting isolates were identified as *Ent. faecalis*. Six random colonies of positively identified *Ent. faecalis* were, when available, transferred to a glycerine-containing broth and stored in −70°C. The species identification was confirmed by PCR using species-specific primers (Dutka-Malen *et al.* 1995).

Genotyping by pulsed-field gel electrophoresis

Enterococcal isolates, overnight cultured on agar plates containing Colombia agar base II supplemented with 0·01% tryptophan and 5% citrated horse blood, were embedded in low-melting agarose (SeaPlaque® agarose; FMC BioProducts, Rockland, ME, USA) before lysis, protein degradation and eventually digested by a restriction enzyme, as previously described (Lund *et al.* 2002). The lysis solution contained 6 mmol l^{-1} Tris pH 8·0, 1·0 mol l^{-1} NaCl, 0·1 mol l^{-1} EDTA pH 8·0, 0·2% Na-deoxycholate, 0·5% sarkosyl, 0·5% Brij-58, 500 μg ml^{-1} RNase and 1 mg ml^{-1} lysozyme. All reagents were purchased from Sigma (Saint Louis, MO, USA). The subsequent proteolysis was performed with a buffer consisting of 0·5% EDTA pH 9·0, 1% sarkosyl and 2 mg ml^{-1} proteinase K (Promega Corporation, Madison, WI, USA) in which the discs were incubated overnight at 50°C. The DNA-containing disks were, after repeated washing in 1× TE (10 mmol l^{-1} Tris pH 7·5, 1 mmol l^{-1} EDTA pH 7·5), digested overnight with *SmaI* (Promega Corporation) at 37°C and thereafter loaded in a 1·2% agarose gel (SeaKem® LE agarose; FMC BioProducts). Electrophoresis was run for 20 h at 14°C in a contour-clamped homogenous electric field apparatus (Bio-Rad GenePath™ System; Bio-Rad Laboratories, Hercules, CA, USA). The pulse time was linearly ramped from 5·3 to 34·9 s at 6·0 (V Cm^{-1}). The gels were stained in ethidium bromide prior to the visualization of the DNA bands with ultraviolet illumination. The banding patterns of the gels were visually interpreted according to the following criteria: isolates were considered identical when no band differences between the isolates occurred, a difference of three bands or less rendered the strains as genetically related and at band differences greater than three the strains were regarded as unrelated (Tenover *et al.* 1995).

Results

Subjects

Fifty consecutive adult patients were included in the study. The population consisted of 23 men and 27 women with a mean age of 52 (range from 23 to 76 years). The studied material consisted of one tooth per patient needing treatment, thus 50 teeth. The majority of the investigated teeth were molars. For details regarding included subjects and investigated teeth, see Table 1.

Microbiological analysis

Micro-organisms were recovered from 37 (74%) of the previously endodontically treated teeth. Thirteen root canal samples (26%) showed no growth of bacteria. The most commonly isolated micro-organism was *Ent. faecalis* or viridans streptococci. The micro-organisms found are listed in Table 2. *Ent. faecalis* was recovered from canals in root-filled teeth with apical periodontitis in 8 of 50 patients (16%). In six of these eight cases, the samples yielded *Ent. faecalis* in pure culture. In one tooth, *Ent. faecalis* coexisted with *Enterobacter* spp., and in another with *Actinomyces* spp. *Ent. faecalis* was not identified in any of these eight patient's saliva samples analysed with the utilized culturing method.

Genotyping

Of the eight patients having *Ent. faecalis* in the treated tooth, only six had *Ent. faecalis* in the faecal sample.

Table 1 Characteristics of the included patients

Patient no	*Enterococcus faecalis* in root canal	Age (years)	Gender (M/F)	Tooth type		
				Incisive/canine	Premolar	Molar
1	+	30	M			36
2		66	F			16
3		43	F			16
4		70	M	21		
5		63	F			36
6		46	F			46
7		65	M			46
8		46	M			16
9		65	F		15	
10	+	69	M			46
11		33	M			46
12	+	64	M			46
13		45	M			36
14		49	F			27
15	+	43	F		24	
16		63	F			16
17		36	F		24	
18		70	M		25	
19	+	66	F			46
20		44	F			36
21		69	F			36
22		62	M		15	
23	+	59	F			16
24		38	F			36
25		70	F			26
26		69	F			26
27		40	F		24	
28		61	M			46
29		37	F	31		
30		38	M			27
31		36	M			36
32	+	58	M			46
33		55	M			26
34	+	63	F			26
35		27	F		15	
36		32	F			46
37		31	M			36
38		37	M	21		
39		64	M		45	
40		40	F			27
41		67	F	13		
42		45	F	21		
43		52	M			16
44		75	F		14	
45		76	M		25	
46		59	M	31		
47		68	F	42		
48		40	M			26
49		37	M			36
50		23	F			36
Total	8	Ø 52	23 M/27 F	7	10	33

M/F, Male/Female; Ø, mean age.

Table 2 Micro-organisms isolated from 37 previously root-filled teeth with apical periodontitis

Type of organism	No. of isolates (%)
Facultative anaerobic cocci	
Enterococcus faecalis	8 (16)
Streptococcus spp.*	8 (16)
Staphylococcus spp.†	1 (2)
Facultative anaerobic rods	
Lactobacillus spp.	1 (2)
Actinomyces spp.	3 (6)
Enterobacter spp.	3 (6)
Pseudomonas spp.	1 (2)
Acinetobacter baumanii	1 (2)
Anaerobic cocci and rods‡	17 (34)

*Viridans group.
†Coagulase-negative staphylococci.
‡Not further specified.

PFGE utilized to investigate the relationship between *Ent. faecalis* strains from root canals and faeces was consequently used for these six cases. The PFGE-derived banding pattern clearly showed no genetic relationship between the recovered root canal and faecal strains for each and every individual as the macrorestriction profiles obtained differed with more than three bands between isolates from these two sources (Fig. 1). The root canal strains within each patient were identical or genetically related, as opposed to the faecal strains that could show a greater genotypic polymorphism on the intraindividual level (Table 3). There was no genetic relationship between

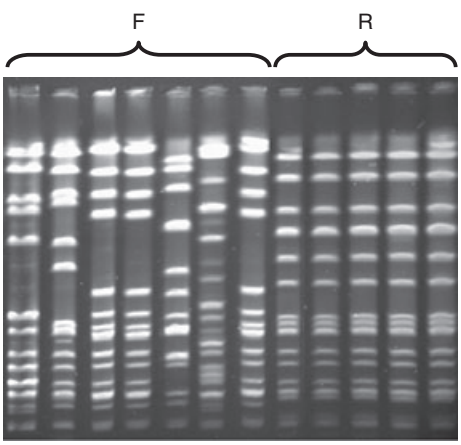

Figure 1 Photograph of gel presenting pulsed-field gel electrophoresis macrorestriction profiles for *Enterococcus faecalis* isolated from root canals (R) and faeces (F) in patient 1. Strains from the different sites differ with more than three bands and are thus unrelated suggesting that *Ent. faecalis* recovered from root canals is not a part of the endogenous microflora and probably has an exogenous origin.

Table 3 *Enterococcus faecalis* strains in root canal and faecal samples compared with pulsed-field gel electrophoresis according to criteria by Tenover *et al.* 1995

Sample	No. of analysed colonies	No. of strains	Genetic relationship between the isolates
Root canal			
Patient 1	6	1	Identical
Patient 10	12	3	Related
Patient 12	6	2	Related
Patient 15	6	1	Identical
Patient 19	6	2	Related
Patient 23	6	1	Identical
Patient 32	6	1	Identical
Patient 34	6	1	Identical
Faecal			
Patient 1	8	5	Not related
Patient 10	12	6	Not related
Patient 12	6	1	Identical
Patient 19	6	1	Identical
Patient 23	1	1	Identical
Patient 34	6	1	Identical

the different root canal-derived *Ent. faecalis* isolates from the eight patients.

Discussion

To our knowledge, this is the first study that attempts to elucidate the origin of *Ent. faecalis* strains present in root-filled canals by comparing them with strains from the intestinal tract. If the source was the endogenous normal microflora, the strains from the different locations should be identical or genetically related, taking into account the changes in genetical material that occurs spontaneously or via exchange. The results, on the contrary, indicate that *Ent. faecalis* strains recovered from root canals do not originate from the patient. The strains were genetically unrelated to the strains found in the faecal samples, if any were found at all. In the faecal samples from two patients (patients 15 and 32), *Ent. faecalis* could not be recovered, and in another, only one strain was detected first after enrichment culturing (patient 23). Common for those three patients was that the predominant enterococcal species were *Enterococcus faecium* or *Enterococcus durans*. This is in accordance with observations that *Ent. faecium*, in some individuals and some countries, is present in a higher proportion in the intestine than *Ent. faecalis* (Devriese and Pot 1995). Interestingly, one of the patients (patient 23) had been subjected to a colostomy, which in turn could have altered the gastrointestinal microflora. Another possibility for the absence of *Ent. faecalis* could be that the faecal sample does not accurately mirror the true composition of the intestinal microflora in terms of quality and quantity (Zoetendal *et al.* 2002). Nonetheless, if *Ent. faecalis* in the root canals had an endogenous origin, it seems unlikely that strains exhibiting clonal relationship with strains in the root canal would be found in the faecal sample when no longer present in saliva. Consequently, *Ent. faecalis* in previously root-filled canals is probably of exogenous origin. The opportunity for enterococci to invade or, if already present as a small part of the primary infection, overtake the root canal system presumably arises during endodontic treatment, between appointments or after the treatment has been completed (Rôças *et al.* 2004). An iatrogenic transmission during treatment via contaminated endodontic instruments is in that perspective plausible. Studies on hospital outbreaks of *Ent. faecium* strains harbouring antibiotic resistance have identified cross-contamination between patients by the hands of personnel as a cause (Boyce *et al.* 1994). Another possible source of enterococci is contamination of the root canal by food-borne *Ent. faecalis*, as it is commonly utilized in the fermentation of cheese or present in certain fermented food products such as sausages and olives (Foulquié Moreno *et al.* 2006).

The prevalence of *Ent. faecalis* (16%) was low in comparison with previous studies, and all the isolated micro-organisms were not entirely categorized on a genus and species level. The intention with this study was not to elaborate on the precise composition of the intracanal microbiota or the prevalence of *Ent. faecalis* in teeth with refractory apical periodontitis. In that case, perhaps a more sensitive method, such as 16S rRNA-based endpoint PCR or real-time quantitative PCR combined with a more cautious sampling would have been more suitable (Sedgley *et al.* 2006a,b; Zoletti *et al.* 2006). A sterility control of the operation field after disinfection of the coronal portion of the tooth prior to sampling, as recommended by Möller, was not undertaken (Möller 1966). This measure was considered unnecessary for the reason previously stated, and with the assumption that if enterococci were present in the root canal sample as a result of contamination from the oral cavity, they would also be present in the saliva sample. The objective was to retrieve cultivable *Ent. faecalis* strains from root canals/saliva and faeces for comparison by PFGE and future studies on differences in characteristics between them, such as virulence traits and antibiotic resistance. Such analyses are not possible without preceding isolation and amplification of bacteria, which was the reason for choosing culturing despite its disadvantages.

Thirteen of the root canal samples showed no growth when cultured, which could be attributed to the sampling procedure. A negative impact on the micro-organisms when removing the root-filling material cannot be disregarded, although the gutta-percha was removed carefully without the use of solvents while keeping frictional heat produced by the rotating files as low as possible. It is also likely that remnants of root-filling material adhering to the dentinal walls hindered the retrieval of micro-organisms resulting in false-negative samples (Molander *et al.* 1998). Another explanation could be sought after in the culturing process, as it is known that only a fraction of the total microflora is cultivable, despite the use of controlled culture conditions (Siqueira and Rôças 2005). One can also speculate that the bacteria were present in numbers below the detection limit of bacterial culturing. There are reports indicating that some bacteria, including *Ent. faecalis*, are able to enter a viable but noncultivable state (Oliver 2005). It is plausible that certain micro-organisms in the root-filled canal enter a dormancy state, in which metabolic activity is almost completely arrested, as a way to cope with the extreme milieu a filled canal space with sparse nutrition poses. This phenomenon, however, does not explain why *Ent. faecalis* was not recovered from the saliva samples. A more likely explanation is that the normal microflora in a healthy individual prevents the opportunistic enterococci to colonize the oral cavity by competition for substrate and attachment sites, as well as production of bacteriocins and hydrogen peroxide. Enterococci transient in the oral microflora are therefore eliminated by oral clearance as long as the colonization barrier is intact. Razavi *et al.* administered food-associated *Ent. faecalis* to healthy individuals and could show that bacterial counts in oral rinse samples were significantly reduced after nearly 2 h and below the detection limit after 1 week (Razavi *et al.* 2007). The composition of the normal microflora within the same individual is in that way kept fairly stable. It is therefore not surprising that the endodontically treated root canal with an absent or reduced colonization resistance offers an unchallenged target for enterococcal invasion, whereas the rest of the oral cavity often merely serves as a transit zone.

In conclusion, the results of this study indicate that *Ent. faecalis* isolated from refractory endodontic infections probably does not derive from the patients' own normal microflora. An exogenous route of infection can therefore not be ruled out as a source of these infections.

Acknowledgements

This study was supported by grants from Ollie and Elof Ericssons foundation, the Scandinavian Society of Antimicrobial Agents and Chemotherapy and the Swedish Dental Association.

References

Aas, J.A., Paster, B.J., Stokes, L.N., Olsen, I. and Dewhirst, F.E. (2005) Defining the normal bacterial flora of the oral cavity. *J Clin Microbiol* **43**, 5721–5732.

Boyce, J.M., Opal, S.M., Chow, J.W., Zervos, M.J., Potter-Bynoe, G., Sherman, C.B., Romulo, R.L., Fortna, S. *et al.* (1994) Outbreak of multidrug-resistant *Enterococcus faecium* with transferable vanB class vancomycin resistance. *J Clin Microbiol* **32**, 1148–1153.

Chavez de Pas, L.E., Dahlén, G., Molander, A., Möller, Å. and Bergenholtz, G. (2003) Bacteria recovered from teeth with apical periodontitis after antimicrobial endodontic treatment. *Int Endod J* **36**, 500–508.

Dahlén, G., Pipattanogovit, P., Rosling, B. and Möller, Å.J.R. (1993) A comparison of two transport media for saliva and subgingival samples. *Oral Microbiol Immunol* **8**, 375–382.

Devriese, L.A. and Pot, B. (1995) The genus *Enterococcus*. In *The Genera of Lactic Acid Bacteria, vol. 2; The Lactic Acid Bacteria* ed. Wood, B.J. and Holzapfel, W.H., pp. 327–367. London: Blackie Academic & Professional.

Dutka-Malen, S., Evers, S. and Courvalin, P. (1995) Detection of glycopeptides resistance genotypes and identification to species level of clinically relevant enterococci by PCR. *J Clin Microbiol* **33**, 24–27.

Engström, B. (1964) The significance of enterococci in root canal treatment. *Odontol Revy* **15**, 87–106.

Estrela, C., Silva, J.A., de Alencar, A.H., Leles, C.R. and Decurcio, D.A. (2008) Efficacy of sodium hypochlorite and chlorhexidine against *Enterococcus faecalis* – a systematic review. *J Appl Oral Sci* **16**, 364–368.

Fabricius, L., Dahlén, G., Holm, S.E. and Möller, Å.J.R. (1982) Influence of combinations of oral bacteria on periapical tissues of monkeys. *Scand J Dent Res* **90**, 200–206.

Figdor, D., Davies, J.K. and Sundqvist, G. (2003) Starvation survival, growth and recovery of *Entercoccus faecalis* in human serum. *Oral Microbiol Immunol* **18**, 234–239.

Foulquié Moreno, M.R., Sarantinopoulos, P., Tsakalidou, E. and De Vuyst, L. (2006) The role and application of enterococci in food and health. *Int J Food Microbiol* **106**, 1–24.

Gilmore, M.S. (2002) *The Enterococci: Pathogenesis, Molecular Biology, and Antibiotic Resistance*. Washington: ASM Press.

Hancock, H.H. III, Sigurdsson, A., Trope, M. and Moiseiwitsch, J. (2001) Bacteria isolated after unsuccessful endodontic treatment in a North Am population. *Oral Sur Oral Med Oral Pathol Oral Radiol Endod* **91**, 579–586.

Jordan, H.V., Krasse, B. and Moller, A. (1968) A method of sampling human dental plaque for certain ''caries-inducing'' streptococci. *Arch Oral Biol* **13**, 919–927.

Lund, B., Agvald-Öhman, C., Hultberg, A. and Edlund, C. (2002) Frequent transmission of enterococcal strains between mechanically ventilated patients treated at an intensive care unit. *J Clin Microbiol* **40**, 2084–2088.

Molander, A., Reit, C., Dahlén, G. and Kvist, T. (1998) Microbiological status of root-filled teeth with apical periodontitis. *Int Endod J* **31**, 1–7.

Möller, Å.J.R. (1966) Microbiological examination of root canals and periapical tissues of human teeth. *Odontol Tidskr* **74**, 1–380.

Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C. and Yolken, R.H., ed. (1999) *Manual of Clinical Microbiology*, 7th edn. Washington DC: ASM Press.

Oliver, J.D. (2005) The viable but nonculturable state in bacteria. *J Microbiol* **43**, 93–100.

Peciuliene, V., Balciuniene, I., Eriksen, H.M. and Haapasalo, M. (2000) Isolation of *Enterococcus faecalis* in previously root-filled canals in a Lithuanian population. *J Endod* **26**, 593–595.

Peciuliene, V., Reynaud, A.H., Balciuniene, I. and Haapasalo, M. (2001) Isolation of yeasts and enteric bacteria in root-filled teeth with chronic apical periodontitis. *Int Endod J* **34**, 429–434.

Pinheiro, E.T., Gomes, B.P.F.A., Ferraz, C.C.R., Sousa, E.L.R., Teixeira, F.B. and Souza-Filho, F.J. (2003a) Microorganisms from canals of root-filled teeth with periapical lesions. *Int Endod J* **36**, 1–11.

Pinheiro, E.T., Gomes, B.P.F.A., Ferraz, C.C.R., Teixeira, F.B., Zaia, A.A. and Souza-Filho, F.J. (2003b) Evaluation of root canal microorganisms isolated from teeth with endodontic failure and their antimicrobial susceptibility. *Oral Microbiol Immunol* **18**, 100–103.

Portenier, I., Waltimo, T.M.T. and Haapasalo, M. (2003) *Enterococcus faecalis* – the root canal survivor and ''star'' in posttreatment disease. *Endod Top* **6**, 135–160.

Razavi, A., Gmür, R., Imfeld, T. and Zehnder, M. (2007) Recovery of *Enterococcus faecalis* from cheese in the oral cavity of health subjects. *Oral Microbiol Immunol* **22**, 248–251.

Rôças, I.N., Siqueira, J.F. and Santos, K.R.N. (2004) Association of *Enterococcus faecalis* with different forms of periradicular diseases. *J Endod* **30**, 315–320.

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

Sedgley, C.M., Nagel, A.C., Shelburne, C.E., Clewell, D.B., Appelbe, O. and Molander, A. (2005) Quantitative real-time PCR detection of oral *Enterococcus faecalis* in humans. *Arch Oral Biol* **50**, 575–583.

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

Sedgley, C., Nagel, A., Dahlén, G., Reit, C. and Molander, A. (2006b) Real-time quantitative polymerase chain reaction and culture analyses of *Enterococcus faecalis* in root canals. *J Endod* **32**, 173–177.

Siqueira, J.F. Jr and Rôças, I.N. (2004) Polymerase chain reaction-based analysis of microorganisms associated with failed endodontic treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **97**, 85–94.

Siqueira, J.D. Jr and Rôças, I.N. (2005) Exploiting molecular methods to explore endodontic infections: part 1 – current molecular technologies for microbiological diagnosis. *J Endod* **31**, 411–423.

Stuart, C.H., Schwartz, S.A., Beeson, T.J. and Owatz, C.B. (2006) *Enterococcus faecalis*: its role in root canal treatment failure and current concepts in retreatment. *J Endod* **32**, 93–98.

Sundqvist, G., Figdor, D., Persson, S. and Sjögren, 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.

Teixeira, L.M. and Facklam, R.R. (2003) *Enterococcus*. In *Manual of Clinical Microbiology*, 8th edn ed. Murray, P.R., Baron, E.J., Jorgensen, J.H., Pfaller, M.A. and Yolken, R.H., pp. 422–433. Washington: ASM Press.

Tendolkar, P.M., Baghdayan, A.S. and Shankar, N. (2003) Pathogenic *Enterococci*: new developments in the 21st century. *Cell Mol Life Sci* **60**, 2622–2636.

Tenover, F.C., Arbeit, R.D., Goering, R.V., Mickelsen, P.A., Murray, B.E., Persing, D.H. and Swaminathan, B. (1995) Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* **33**, 2233–2239.

Zehnder, M. and Guggenheim, B. (2009) The mysterious appearance of enterococci in filled root canals. *Int Endod J* **42**, 277–287.

Zoetendal, E.G., von Wright, A., Vilpponin-Salmela, T., Ben-Amor, K., Akkermans, A.D. and de Vos, W.M. (2002) Mucosa-associated bacteria in the human gastrointestinal tract are uniformly distributed along the colon and differ from the community recovered from feces. *Appl Environ Microbiol* **68**, 3401–3407.

Zoletti, G.O., Siqueira, J.F. Jr and Santos, K.R. (2006) Identification of *Enterococcus faecalis* in root-filled teeth with or without periradicular lesions by culturedependent and – independent approaches. *J Endod* **32**, 722–726.