Bacteria recovered from teeth with apical periodontitis after antimicrobial endodontic treatment

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Abstract

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Aim To determine whether there is a pattern for certain bacteria to remain after chemo-mechanical treatment of root canals in teeth with apical periodontitis.

Methodology Consecutive root-canal samples of 200 teeth receiving root-canal treatment, referred from general practitioners and endodontic specialists for analyses of cultivable microbes, were studied prospectively. To be included, samples had to be taken at a treatment session subsequent to the one at which endodontic therapy was initiated. All samples were from teeth that either presented with clinical or radiographic evidence of apical periodontitis or both. Bacteriological findings were linked to clinical and radiographic parameters including status of the root canal prior to treatment, namely, vital pulp, necrotic pulp or root filled.

Results A total of 248 strains were isolated from 107 teeth giving bacterial growth. Gram-positives predominated (85%). *Lactobacillus* spp. (22%), nonmutans streptococci (18%), and *Enterococcus* spp. (12%) were the most common isolates. Gram-negative anaerobes were relatively sporadic. Large radiographic bone lesions, persistent pain and use of intracanal calcium hydroxide dressing correlated with bacterial presence ($P < 0.05$).

Conclusions Once established, nonmutans streptococci, enterococci and lactobacilli appear to survive commonly following root-canal treatment of teeth with clinical and radiographical signs of apical periodontitis.

Keywords: bacterial resistance, microbiology, prospective study, pulpal infection, root-canal therapy.

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Introduction

Complete elimination of the microbes in teeth with an infected dental pulp is a clear objective of root-canal treatment (Trope & Bergenholtz 2002). However, in clinical studies, despite thorough mechanical instrumentation and disinfection of the root-canal system, microorganisms have been recovered both at the end of the treatment procedure and at subsequent treatment sessions (Engström 1964, Olgart 1969, Byström 1986, Gomes *et al.* 1996, Molander *et al.* 1998). Such residual organisms are likely to play a role in treatment failures (Molander *et al.* 1998, Sundqvist *et al.* 1998).

Samples from root filled teeth have frequently shown Gram-positive facultatives including enterococci, streptococci, and lactobacilli (Möller 1966, Molander *et al.* 1998, Sundqvist *et al.* 1998), suggesting that anaerobes normally do not survive in the restricted nutritional environment found in treated root canals. Compared to anaerobes, facultative anaerobes are also likely to be more resistant to antimicrobial and mechanical endodontic procedures; hence, inefficient treatments may select for the most robust segment of the root canal microbiota. This supposition is, however, not well substantiated. For example, *Enterococcus faecalis*, an organism recovered frequently from endodontically

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treated teeth with apical lesions (Molander *et al.* 1998, Sundqvist *et al.* 1998) is rarely isolated in untreated infected pulps (Sundqvist 1994). The frequent presence of facultative anaerobes, including *E. faecalis*, in root canals of failing endodontically treated teeth could be as a consequence of fluid leakage from the oral environment via gaps at the restoration–tooth interface following coronal leakage (Saunders & Saunders 1994, Siqueira 2001).

While clinical follow-up studies have reported that biomechanical procedures reduce microrganisms in the root-canal system (Byström & Sundqvist 1981, Gomes *et al.* 1996, Peters *et al.* 2002), and that this effect is enhanced by the use of intracanal medication between appointments (Byström 1986, Molander *et al.* 1999), there is still insufficient knowledge as to which organisms survive these procedures. Based on the exploration of a large number of cases with diverse pulpal diagnoses at the initiation of therapy, the aim of the present study was to seek a more distinct understanding of the microflora that may endure antimicrobial endodontic treatment in teeth with evidence of apical periodontitis.

Materials and methods

Over a period of 10 months (October 2000–July 2001) consecutive microbial samples from root canals referred to the Department of Oral Microbiology, Göteborg University, were gathered on a prospective basis and analysed for cultivable organisms.

The samples were recovered from a total of 200 teeth (77 anteriors, 38 premolars and 85 molars) from 196 patients (104 females and 92 males, age range 16–85 years). The referrals were from endodontic specialists in the Public Dental Service (59%) and general practitioners in the private sector in the Göteborg area of Sweden (41%) (Table 1).

To be considered for inclusion in the study, each sample had to come from a tooth that needed to satisfy the following criteria:

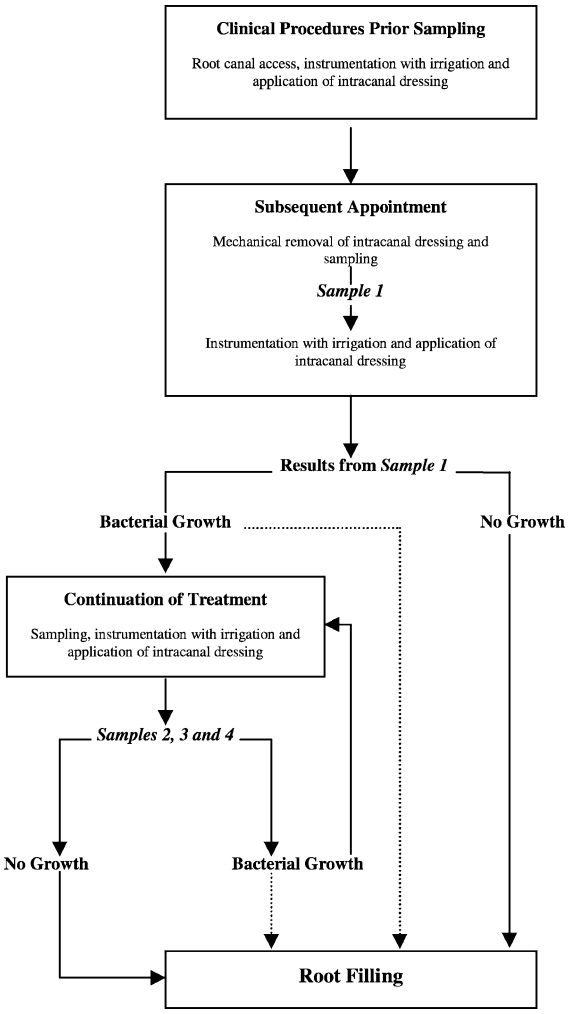

Figure 1 Outline of clinical and laboratory procedures.

- Clinical or radiographic evidence of apical periodontitis or both.
- Root-canal treatment already initiated at one, two or several previous appointments prior to sampling (Fig. 1).
- Root-canal sampling procedures performed according to the method of Möller (1966).

Table 1 Distribution of cases based on bacterial presence, source of referral and intracanal medicament used

	Bacteria present ($n = 107$)			No growth ($n = 93$)		
	$Ca(OH)_2$	IKI	Total	$Ca(OH)_2$	IKI	Total
Endodontists ($n = 118$)	47	25	72	8	38	46
General practitioners ($n = 82$)	27	8	35	44	3	47
Total	74	33	107	52	41	93

On comparing frequencies of bacterial growth between teeth treated with either IKI or $Ca(OH)_2$, more no growth cultures were recorded for IKI than $Ca(OH)_2$ (55% and 41%, respectively, not statistically significant).

• Cases treated only by experienced general practitioners or endodontists, who were regular users of the sampling method.
• Sterility control from the operation field demonstrating no bacterial growth.

Clinical data requested from referring dentists

Referring dentists were requested to complete a form to answer questions regarding the clinical features of each case including the crown status, initial diagnosis, reason for sampling and intracanal medication used. Pre-operative periapical radiographs were also submitted and used to provide confirmation of periapical and clinical crown status. Radiographic images were digitalized and dimensions of radiographic bone lesions were measured using the software Adobe Photoshop v.6 (Adobe Systems Inc., Seattle, WA, USA). The 'size of the lesion' was determined by calculating the mean value between the largest horizontal and vertical measures. Lesions were then classified as small ($\leq$2 mm) or large (>2 mm) (Fig. 2a,b).

Procedures prior to root-canal treatment

Teeth were isolated using rubber dam; then disinfected with 30% hydrogen peroxide and a 10% iodine tincture in accordance with the protocol advocated by Möller (1966). The sterility of the operation field was checked after inactivation of the iodine with 5% sodium thiosulphate solution. Part of the fluid was aspirated with a charcoal-impregnated cotton pellet, which was transferred to transport medium VMGA III (Dahlén & Möller 1992).

Following removal of the interappointment temporary cement, the canal was flushed with sampling fluid (VMG I, Möller 1966) and instrumented to remove

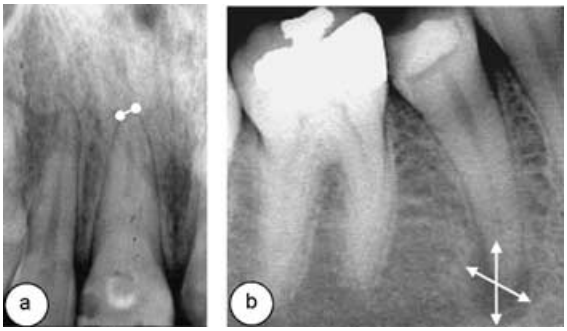

Figure 2 Determination of radiographic bone lesion size (a) small and (b) large.

$Ca(OH)_2$ if present. Then and in cases where IKI had been used for interappointment medication (Table 1), VMGI was added and the canal walls were scaled. The suspension was absorbed into charcoal impregnated paper points (Möller 1966), which were transferred to VMGA III. This sample was designated Sample 1 (Fig. 1). In cases with bacterial growth evident in a previous sample, irrigation and medicament procedures were repeated, and a subsequent sample was taken using the same sampling method in many but not all the cases. These samples were designated as appropriate Samples 2, 3 and 4.

Laboratory analyses

All samples arrived at the microbiological laboratory within 24 h of sampling. Transport tubes were placed in 37 °C for 15 min and vigorously mixed for 20–30 s using a vortex mixer. Dilution aliquots of 100 μL were distributed onto two Brucella agar plates (BBL Microbiological Systems, Cockeysville, MD, USA); one plate was used for aerobic incubation for 3–5 days at 37 °C and the other plate was used for anaerobic incubation using the hydrogen combustion method for 5–7 days at 37 °C (Möller 1966). Charcoaled paper points, as well as the remaining fluid, were placed in a semi-liquid medium (HCMG-Sula, Möller 1966) and incubated at 37 °C for up to 14 days. In cases of no growth on the agar plates, tubes were checked daily for turbidity during 14 days. Bacteria growing on agar plates or semi-liquid medium were subcultured for identification on the basis of anaerobic or aerobic growth and further identification was performed as outlined by Dahlén & Möller (1992).

Colonies were semi-quantified according to the method described by Dahlén *et al.* (1982). Briefly: 1–10 colony forming units (CFU) were considered as very sparse growth, 11–100 as sparse growth, 101–1000 moderate, 1001–10 000 as heavy and more than 10 000 as very heavy growth.

Strains isolated were Gram-stained and classified by colony morphology, oxygen tolerance and biochemical tests into species, genus or other main groups. Selective media were used for subculturing the following strains: Mitis-Salivarius agar plates (MS agar, Difco Laboratories, Dertroit, MI, USA) on which distinction was made between mutans group and nonmutans group streptococci, MSB agar plates (Mitis-Salivarius with 3.3 mg L^{-1} of Bacitracin (Sigma, St Louis, MO, USA) for identification of *Streptococcus mutans*/*Streptococcus sobrinus*, Staphylococcus 110 medium agar plates (Difco) on which *Staphylococcus aureus* was distinguished from

coagulase-negative *Staphylococcus* spp. by being DNase positive, Enterococcosel agar plates (BBL Microbiology Systems, Cockeysville, MD) for identification of *Enterococcus* spp., Rogosa agar plates (Difco) for identification of *Lactobacillus* spp. and *Bifidobacterium* spp., CFAT agar plates (Zylber & Jordan 1982) for identification of *Actinomyces* spp., Drigalski agar plates for identification of lactose positive enterobacteria and Saboraud dextrose agar plates (Difco) for identification of yeasts (*Candida* spp.).

Gram-positive rods not growing on selective media, e.g. *Propionibacterium* spp., *Clostridium* spp. and *Eubacterium* spp. were identified after subculturing on Brucella blood agar (BBL) enriched with defibrinated horse blood plus 0.5% haemolysed horse blood and 5 μg L^{-1} of menadione, and were analysed by gas-liquid chromatography. Other obligate anaerobic strains, e.g. *Peptostreptococcus* spp., black-pigmented (*Prevotella* spp., *Porphyromonas* spp.) and nonpigmented Gram-negative anaerobes (*Fusobacterium* spp., *Veillonella* spp.) were subcultured on Brucella blood agar plates and further identified by cell and colony morphology.

Plates for anaerobic culturing were incubated in anaerobic jars for 7–10 days while other plates were incubated in air with 10% CO_2 for 3–5 days.

Gas-liquid chromatography

The methodology was performed as outlined by Holdeman *et al.* (1977). Bacteria were grown on RS-30 media for 5 days under anaerobic conditions. Their metabolic products were analysed with a chromatograph equipped with an ionization detector. The glass column of chromatography was packed with 5% AT 1000 (Altech Associates Inc., Deerfield, Ill, USA.) on Chromosorb GHP 100/120 mesh (Johns-Manville, Denver, Co., USA). The carrier gas was nitrogen (30 mL min^{-1}) with an injection port temperature of 120 °C. One microlitre portions of the ether extracted and methylated samples were used, and the results were compared with those obtained with standard solutions of volatile fatty acids.

Statistical analysis

The material from the first samples was distributed into two groups: 'bacteria present' (BP) and 'no growth' (NG). Clinical and radiographic data from BP and NG groups were compared using the Chi-square test (significance level set at $P < 0.05$). Within the BP group, bacterial findings were compared to clinical and radiographic data using Chi-square test (significance level set at: $P < 0.05$).

Results

General findings

Of the 200 cases analysed, 107 presented with bacterial growth in the root-canal sample designated as Sample 1 (BP group), while 93 cases did not yield bacterial growth (NG group).

Within the BP group, a second sample (Sample 2) was submitted in 99 cases, demonstrating bacterial growth in 56 cases (57%). Sample 3 was obtained from 52 cases, which displayed bacterial growth in 7 cases (13%). Six of these cases were further sampled (Sample 4), with none demonstrating bacterial growth.

Table 1 shows a distribution based on sources of sampling and intracanal medicaments used between appointments. Overall, calcium hydroxide was the most commonly used intracanal dressing (126 cases, 63%). When analysed by source, endodontists used IKI in 53% of the cases in contrast to the general practitioners who more frequently used $Ca(OH)_2$ (87%).

Table 2 Comparisons between bacteria present (BP) and no growth (NG) groups based on clinical and radiographic data

	BP	NG	χ^{2*}
Tooth group			
Upper anterior ($n = 48$)	27	21	NS
Lower anterior ($n = 29$)	15	14	NS
Upper premolar ($n = 18$)	9	9	NS
Lower premolar ($n = 20$)	7	13	NS
Upper molar ($n = 29$)	18	11	NS
Lower molar ($n = 56$)	31	25	NS
Radiographic bone lesion			
No lesion ($n = 6$)	1	5	NS
Small lesion (=2 mm) ($n = 67$)	20	47	0.0001
Large lesion (>2 mm) ($n = 127$)	86	41	0.001
Crown status			
1 surface filling ($n = 42$)	23	19	NS
>1 surface filling ($n = 99$)	51	48	NS
Full coverage crown ($n = 37$)	21	16	NS
Bridge abutment ($n = 22$)	12	10	NS
Initial diagnosis			
Vital pulp ($n = 31$)	15	16	NS
Non-vital pulp ($n = 109$)	63	46	NS
Re-treatment ($n = 60$)	29	31	NS
Reason for sampling			
Persistent pain/tenderness ($n = 72$)	52	20	0.001
Persistent swelling ($n = 9$)	6	3	NS
Persistent fistula ($n = 28$)	12	16	NS
No radiographic healing ($n = 39$)	17	22	NS
Sterility control ($n = 52$)	20	32	NS

*P- value in χ^2-test, NS = not statistically significant.

Clinical parameters

Table 2 shows various clinical and radiographic features associated with the cases investigated namely, tooth category, periapical lesion size, crown status, initial diagnosis and reason given for submitting the sample. Mandibular molar and maxillary anterior teeth were the most regularly sampled tooth type, although there was no association with the presence of bacteria.

Radiographic analyses demonstrated 6 teeth with no lesions, five of them belonging to the NG group. Large bone lesions were the most common (64%), and correlated significantly with presence of bacteria ($P = 0.001$). Occurrence of small lesions was positively correlated with no bacterial growth ($P = 0.0001$).

Almost 50% of the cases had two-surface, or larger, restorations. There was no correlation between size or type of restoration and bacterial growth (Table 2).

More than half of the cases had an initial diagnosis of pulp necrosis, 31 (16%) had a vital pulp diagnosis and 60 (30%) were retreatments. Results of bacterial growth were not correlated to these diagnoses (Table 2).

The most common reason for sampling was persistence of pain and tenderness (36%). These causes significantly correlated with bacterial growth in Sample 1 ($P = 0.001$).

Microbial isolates

Table 3 shows the distribution of the bacterial strains isolated from the root-canal samples. There were a total of 235 strains isolated in Sample 1, with approximately 2 strains per case. Samples 2 and 3 presented an average of 1 isolate per case. *Lactobacillus* spp., nonmutans streptococci and *Enterococcus* spp., were the most prevalent species. In none of the samples were yeasts isolated.

Thirteen strains isolated in Sample 2 had not been recovered from the previous Sample 1. These new strains were: *Lactobacillus* spp. (five strains), nonmutans streptococci (three strains), *Enterococcus* spp. (three strains), *Propionibacterium* spp. (one strain) and lactose-positive enterobacterium (one strain).

In Sample 3 the 7 bacterial strains that prevailed in monoculture had been isolated also in Samples 1 and 2.

Reapplication of intracanal dressing produced a reduction in quantity and growth rate of microbial species isolated from Sample 1 through Sample 3 (Table 3).

Correlation of microbial isolates with clinical parameters

Nonmutans group streptococci and enterococci were recovered from cases diagnosed initially with vital pulps

Table 3 Distribution based on growth rate of strains isolated from consecutive root canal samples

	Sample 1 ($n = 107$ cases)						Sample 2 ($n = 56$ cases)				Sample 3 ($n = 7$ cases)		
	+	++	+++	++++	+++++	Total	+	++	+++	Total	+	++	Total
Gram-positive cocci													
Non-mutans group streptococci	5	11	14	9	2	41	8	3	2	13			
Enterococcus spp.	3	8	4	9	2	26	6	7	2	15	1	1	2
Coagulase negative staphylococci	1	5	10	1		17							
Peptostreptococcus spp.		5	2	2		9							
Mutans group streptococci	1	2	1			4							
Gram-positive rods													
Lactobacillus spp.	4	11	16	15	3	49	5	10	3	18	3		3
Bifidobacterium spp.	1	6	8	3		18	2			2			
Propionibacterium spp.	1	4	4	5	1	15	3	1	2	6	2		2
Actinomyces spp.		2	6	4		12	1	2	1	4			
Eubacterium spp.	1	3	1	1		6	1			1			
Clostridium spp.	2	2			4		1		1				
Gram-negative cocci													
Veillonella spp.	2	2	1	1		6							
Gram-negative rods													
Prevotella spp.		9	2			11							
Fusobacterium spp.	1	5	2	1		9							
Enterobacteria (lactose positive)		4	1	2		7	1			1			
Porphyromonas spp.		1				1							
Total isolates	20	80	74	53	8	235	27	24	10	61	6	1	7

Growth rate: + very sparse, ++ sparse; +++ moderate; ++++ heavy, +++++ very heavy growth.

Table 4 Correlation between initial diagnosis and bacteria isolated in Sample 1

	Vital ($n = 15$)	Necrotic ($n = 63$)	Retreatment ($n = 29$)	χ^{2*}
Lactobacillus spp. ($n = 49$)	6	26	17	NS
Non-mutans group streptococci ($n = 41$)	2	19	20	0.0002
Enterococcus spp. ($n = 26$)	0	10	16	0.0001
Bifidobacterium spp. ($n = 18$)	7	10	1	0.001
Coagulase negative staphylococci ($n = 17$)	3	14	0	0.02
Propionibacterium spp. ($n = 15$)	3	6	6	NS
Actinomyces spp. ($n = 12$)	0	10	2	NS
Peptostreptococcus spp. ($n = 9$)	2	6	1	NS
Gram-negative anaerobic rods ($n = 28$)	5	21	2	0.02

*P-value in Chi-square test, NS = not statistically significant.

in $n = 2$ and $n = 0$ cases, respectively. While these two bacterial groups were more commonly recovered from teeth with diagnosis of necrotic pulps ($n = 19$ and $n = 10$, respectively), their presence was significantly more common in the retreatment cases ($P = 0.0002$ and $P = 0.0001$, respectively) (Table 4). *Bifidobacterium* and coagulase negative staphylococci, on the other hand, were uncommon in retreatment cases ($P = 0.001$ and $P = 0.02$, respectively). Gram-negative anaerobic rods (*Prevotella*, *Fusobacterium*, lactose positive enterobacteria and *Porphyromonas*), were mainly present in teeth originally diagnosed with necrotic pulp and were infrequent in re-treatment cases ($P = 0.02$).

Compared to IKI, calcium hydroxide as an interappointment dressing was more often associated with a positive culture (Table 1). $Ca(OH)_2$ was also significantly associated with the presence of nonmutans streptococci ($P = 0.003$) and *Enterococcus* spp. ($P = 0.001$) (Table 5).

Discussion

In the present study, Gram-positive bacteria were recovered from root canals of teeth receiving root-canal treatment for either symptomatic or nonsymptomatic apical periodontitis. In several cases the organisms remained or new ones appeared in subsequent samples, despite chemo-mechanical treatment and antimicrobial intracanal dressings, suggesting that conditions for their survival and growth prevailed in these root canals. Byström & Sundqvist (1981), Gomes *et al.* (1996) and Peters *et al.* (2002) have reported similar findings, although their studies were based on smaller number of cases and with treatment methodologies somewhat different to those applied by the referring dentists of the present study.

Gram-negative anaerobes were conspicuous by their relatively rare occurrence in contrast to primary infections of teeth with necrotic pulps and apical periodontitis

Table 5 Correlation between intracanal medicament used and isolated strains from Sample 1

	$Ca(OH)_2$ ($n = 74$)	IKI ($n = 33$)	χ^{2*}
Lactobacillus spp. ($n = 49$)	40	9	NS
Non-mutans group streptococci ($n = 41$)	37	4	0.003
Enterococcus spp. ($n = 26$)	26	0	0.001
Bifidobacterium spp. ($n = 18$)	9	9	NS
Coagulase negative staphylococci ($n = 17$)	9	8	NS
Propionibacterium spp. ($n = 15$)	13	2	NS
Actinomyces spp. ($n = 12$)	9	3	NS
Prevotella spp. ($n = 11$)	5	6	NS
Fusobacterium spp. ($n = 9$)	7	2	NS
Peptostreptococcus spp. ($n = 9$)	7	2	NS
Enterobacteria (lactose positive) ($n = 7$)	3	4	NS
Veillonella spp. ($n = 6$)	5	1	NS
Eubacterium spp. ($n = 6$)	3	3	NS
Mutans group streptococci ($n = 4$)	1	3	NS
Clostridium spp. ($n = 4$)	2	2	NS
Porphyromonas spp. ($n = 1$)	1	0	NS

*P-value in Chi-square test, NS = not statistically significant.

(Baumgartner & Falkler 1991, Hoshino *et al.* 1992, Wasfy *et al.* 1992, Sundqvist 1994). Of the isolates in teeth with an initial diagnosis of pulp necrosis, Gram-negative anaerobes were less than 20% of the total isolates recovered (Table 4). Given this observation, and realizing that a microbiological analysis of the original microbiota in the cases observed was not possible, our findings suggest that the treatment procedures carried out, especially in teeth with necrotic pulps, were more effective against Gram-negative bacteria but less so towards Gram-positive organisms. Hence, these results seem to provide support for the hypothesis that endodontic procedures may select for the more resilient organisms, while the susceptible Gram-negative anaerobes are more easily eliminated. The supposedly higher resistance of Gram-positive bacteria may be related to different factors, as for example cell-wall structure, metabolic products secreted, and resistance towards medicaments. The true nature and implication of such factors need further clarification (Sriskandan & Cohen 1999).

Gram-positive rods were the most frequently isolated group of bacteria. Within this group *Lactobacillus* spp. was by far the most predominant. Screening a variety of cases with diverse pulpal and periapical diagnosis, Sundqvist & Carlsson (1974) reported lactobacilli to be a prevalent bacterium in initial root-canal samples. This organism is not usually recognized as an endodontic pathogen. Sundqvist & Carlsson proposed, on the basis of the simultaneous occurrence of lactobacilli in other sites of the oral cavity, that they are likely to be transient contaminants rather than regular colonizers of necrotic pulps. Indeed they are ubiquitous in the deep portions of caries lesions (Edwardsson 1974, McKay 1976, Hoshino *et al.* 1992) and it is not unreasonable to assume that they are derived from such origins. While their pathogenic potential is not well established, the present data suggest that they are able to survive in restricted nutritional environments. Further, as they have been recovered in root-canal samples from teeth with apical lesions persisting after endodontic treatment (Molander *et al.* 1998, Sundqvist *et al.* 1998), lactobacilli may play a role in pathogenesis.

Gram-positive cocci were the second largest group of bacteria isolated in the samples, of which nonpolysaccharide producing streptococci were the most prevalent. Such organisms have also been recognized in necrotic pulps of untreated root canals (Sundqvist 1994). Gomes *et al.* (1996) isolated diverse streptococcal species after endodontic instrumentation without medication in between treatment sessions, namely, *S. milleri* group, *S. anginosus*, *S. constellatus*, *S. intermedius* and *S. gordonii*. Méjare (1975) observed *S. milleri* in samples obtained from root canals at the time of obturation and that they were most often recovered along with *S. sanguis* and *S. mitior*. In semblance with our results, the origin of these organisms could not be determined and it is quite possible that they were colonizers owing to previous direct exposure of the root canal to the oral environment (Méjare 1975).

Another commonly recovered bacterial group was *Enterococcus* spp. In recent years enterococci have attracted considerable interest owing to their frequent recovery from endodontic re-treatment cases (Molander *et al.* 1998, Sundqvist *et al.* 1998, Peculiene *et al.* 2001). These organisms were also recovered from many of the current samples (12%), especially from the re-treatment cases, supporting previous findings and the view that they are able to survive in environments with sparse nutritional supply. Further, our findings confirm the treatment resilient nature of enterococci as demonstrated *in vitro* (Dahlén *et al.* 2000). Hence, of 26 isolates in Sample 1, 15 isolates were also present in Sample 2. This means that in comparison with all other organisms, *Enterococcus* spp. more than doubled their percentage share of the total number of strains over the treatment period, while other organisms either had a slight increase or decrease or had disappeared totally in Sample 2 (Table 3).

In a similar study, Molander *et al.* (1996) analysed 574 samples submitted in 1986 to the same microbiology laboratory in Göteborg. The results showed that streptococci, enterococci and lactobacilli were also the most common isolates. However, all samplers were general practitioners and sterility checks were not consistently carried out for oral contamination of the sampling site. While oral contamination during sampling may not be ruled out as a factor for the presence of bacteria in the current study, special precautions were undertaken to include only habitual samplers and teeth where dentists were likely to have been able to apply proper rubber dam and aseptic operative procedures.

Although the current study does not provide evidence as to the origin of the organisms found in the treated cases, our findings do raise concerns about their pathogenic capacity, once they are established in the root-canal environment. This is regardless of whether they were present from the very beginning, or became selected in the process of root-canal instrumentation and medication. Over the past 20 years, the field of endodontics has been very much preoccupied with the role of the anaerobic segment of the root-canal microbiota especially the Gram-negatives owing to their dominance

in untreated cases of necrotic pulp and the fact that they exert distinct pathogenic properties allowing them to escape various host defence mechanisms (Haapasalo *et al.* 1986, Sundqvist *et al.* 1979). The risk of a flora being dominated by Gram-positives after treatments, of which several are facultatives, necessitates efforts to elucidate whether or not treatment outcome depends on their complete elimination. Further to the acquisition of such knowledge there is a great clinical need to understand which medication assists in their eradication. The observation that the use of calcium hydroxide, the most commonly employed intracanal dressing in the current study, was associated with the continual presence of some of these organisms confirms its relative inefficiency as an antimicrobial agent in root-canal treatment (Stevens & Grossman 1983, Haapasalo & Ørstavik 1987, Reit & Dahlén 1988). The present findings support the contention that it is difficult to kill by this medicament not only enterococci but also other Gram-positive organisms. Most likely the continued recoveries of certain Gram-positives is related to an inability of clinicians to properly instrument and disperse this intracanal dressing to all areas of the root-canal system where bacteria may remain, i.e. in isthmuses, dentinal tubules and ramifications (Siqueira & Lopes 1999).

Although our findings revealed associations of bacterial presence to lesion size, intracanal medication and reason for sampling, we did not find it meaningful to further explore links to genus type. In studies of this nature, a variety of other confounders may be of significant influence some of which are more or less uncontrollable. To these belong variations in root-canal anatomy and the efficiency which operators are able to carry out the instrumentation procedures.

Bacteriological sampling procedures and culture processing may not provide a complete outlook of the root-canal microbiota, because many types of organisms fail to survive for identification under regular laboratory conditions. In the present study, spirochetes and yeasts, previously reported in root-canals samples (Waltimo *et al.* 1997, Baumgartner *et al.* 2000, Siqueira *et al.* 2000), were not detected. As an alternative method for the identification of the endodontic microbiota, further molecular analyses of 16S-RNA genes have shown a far more diverse bacterial flora (Munson *et al.* 2002), however, the true implication of these 'uncultivable' species, in clinical endodontics has yet to be demonstrated. So far, there is no evidence suggesting that cultivable bacteria should not be regarded relevant in endodontic infections and their elimination from root canals a critical treatment goal.

Conclusion

The findings of the current study justify the view that nonmutans group streptococci, enterococci and lactobacilli commonly survive antimicrobial endodontic treatment.

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References

Baumgartner JC, Falkler WA (1991) Bacteria in the apical 5 mm of infected root canals. *Journal of Endodontics* **17**, 380–3.

Baumgartner JC, Watts CM, Xia T (2000) Occurrence of *Candida albicans* in infections of endodontic origin. *Journal of Endodontics* **26**, 695–8.

Byström A (1986) Evaluation of Endodontic Treatment of Teeth with Apical Periodontitis. Odontological Dissertation. University of Umeå (Umeå, Sweden).

Byström A, Sundqvist G (1981) Bacteriological evaluation of the efficacy of mechanical root-canal instrumentation in endodontic therapy. *Scandinavian Journal of Dental Research* **89**, 321–8.

Dahlén G, Linde A, Möller ÅJR, Öhman A (1982) A retrospective study of microbiological samples from oral mucosal lesions. *Oral Surgery Oral Medicine Oral Pathology* **53**, 250–5.

Dahlén G, Möller ÅJR (1992) Microbiology of endodontic infections. In: Slots, J, Taubman, M, eds. *Contemporary Oral Microbiology and Immunology*, 1st edn, pp. 444–75. St. Louis, Missouri, USA: Mosby-Year Book.

Dahlén G, Samuelsson W, Molander A, Reit C (2000) Identification and antimicrobial susceptibility of enterococci isolated from the root canal. *Oral Microbiology and Immunology* **15**, 309–12.

Edwardsson S (1974) Bacteriological studies of deep areas of carious dentine. *Odontologisk Revy.* **32** (Suppl.), 1–143.

Engström B (1964) Bacteriological Cultures in Root-Canal Therapy. PhD Thesis. University of Umeå (Umeå, Sweden).

Gomes BPFA, Lilley JD, Drucker DB (1996) Variations in the susceptibilities of components of the endodontic microflora to biomechanical procedures. *International Endodontic Journal* **29**, 235–41.

Haapasalo M, Ørstavik D (1987) *In vitro* infection and disinfection of dentinal tubules. *Journal of Dental Research* **66**, 1375–9.

Haapasalo M, Ranta H, Ranta K, Shah H (1986) Black-pigmented *Bacteroides* spp. in human apical periodontitis. *Infection and Immunity* **53**, 149–53.

Holdeman LV, Cato EP, Moore WEC (1977) Anaerdee Laboratory Manual, 4th edn, Blacksburg, VI, USA; Virginia Polytechnic Institute and State University.

Hoshino E, Ando N, Sato M, Kota K (1992) Bacterial invasion of nonexposed dental pulp. *International Endodontic Journal* **25**, 2–5.

McKay GS (1976) The histology and microbiology of acute occlusal dentine lesions in human permanent molar teeth. *Archives of Oral Biology* **21**, 51–8.

Méjare B (1975) On the facultative anaerobic streptococci in infected dental root canals in man. *Odontologysk Revy.* **33** (Suppl.), 1–27.

Molander A, Reit C, Dahlén G (1996) Bacteriologic root-canal sampling: diffusion of a technology. *International Endodontic Journal* **29**, 163–7.

Molander A, Reit C, Dahlén G (1999) The antimicrobial effect of calcium hydroxide in root canals pretreated with 5% iodine potassium iodide. *Endodontics and Dental Traumatology* **15**, 205–9.

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

Möller ÅJR (1966) Microbiological Examination of Root Canals and Periapical Tissues of Human Teeth. PhD Thesis. University of Göteborg (Göteborg, Sweden).

Munson MA, Pitt-Ford T, Chong B, Weightman A, Wade WG (2002) Molecular and cultural analysis of the microflora associated with endodontic infections. *Journal of Dental Research* **81**, 761–6.

Olgart LG (1969) Bacteriological sampling from root canals directly after chemomechanical treatment. A clinical and bacteriological study. *Acta Odontologica Scandinavica* **27**, 91–103.

Peculiene V, Reynaud A, Balciuniene I, Haapasalo M (2001) Isolation of yeasts and enteric bacteria in root-filled teeth with chronic apical periodontitis. *International Endodontic Journal* **34**, 429–34.

Peters LB, van Winkelhoff A-J, Buijs JF, Wesselink PR (2002) Effects of instrumentation, irrigation and dressing with calcium hydroxide on infection in pulpless teeth with periapical bone lesions. *International Endodontic Journal* **35**, 13–21.

Reit C, Dahlén G (1988) Decision making analysis of endodontic treatment strategies in teeth with apical periodontitis. *International Endodontic Journal* **21**, 291–9.

Saunders WP, Saunders EM (1994) Coronal leakage as a cause of failure in root-canal therapy: a review. *Endodontics and Dental Traumatology* **10**, 105–8.

Siqueira JF Jr (2001) Aetiology of root-canal treatment failure: why well-treated teeth can fail. *International Endodontic Journal* **34**, 1–10.

Siqueira JF, Jr, Lopes HP (1999) Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. *International Endodontic Journal* **32**, 361–9.

Siqueira JF, Jr, Rocas IN, Favieri A, Santos KR (2000) Detection of Treponema denticola in endodontic infections by 16s r-RNA gene directed polymerase chain reaction. *Oral Microbiology and Immunology* **15**, 335–7.

Sriskandan S, Cohen J (1999) Gram-positive sepsis. Mechanisms and differences from gram-negative sepsis. *Infectious Disease Clinics of North America* **13**, 397–412.

Stevens RH, Grossman L (1983) Evaluation of the antimicrobial potential of calcium hydroxide as an intracanal medicament. *Journal of Endodontics* **9**, 372–4.

Sundqvist G (1994) Taxonomy, ecology and pathogenicity of the root-canal flora. *Oral Surgery Oral Medicine Oral Pathology* **78**, 522–30.

Sundqvist G, Carlsson J (1974) Lactobacilli of infected dental root canals. *Odontologysk Revy* **25**, 233–8.

Sundqvist G, Eckerbom M, Larsson P, Sjögren U (1979) Capacity of anaerobic bacteria from necrotic dental pulps to induce purulent infections. *Infection and Immunity* **25**, 685–93.

Sundqvist G, Figdor D, Persson S, Sjögren U (1998) Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. *Oral Surgery Oral Medicine Oral Pathology* **85**, 86–93.

Trope M, Bergenholtz G (2002) Microbiological basis for endodontic treatment: can a maximal outcome be achieved in one visit?. *Endodontic Topics* **1**, 40–53.

Waltimo TMT, Sirén EK, Torkoo HL, Olsen MP, Haapasalo M (1997) Fungi in therapy resistant apical periodontitis. *International Endodontic Journal* **30**, 96–101.

Wasfy MO, McMahon KT, Minah GE, Falkler WA Jr (1992) Microbiological evaluation of periapical infections in Egypt. *Oral Microbiology and Immunology* **7**, 100–5.

Zylber LJ, Jordan HV (1982) Development of a selective medium for detection and enumeration of *Actinomyces viscosus* and *Actinomyces naeslundii* in dental plaque. *Journal of Clinical Microbiology* **15**, 253–9.