Effect of endodontic procedures on enterococci, enteric bacteria and yeasts in primary endodontic infections

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Abstract

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Aim To detect enterococci, enteric bacteria and yeast species from the canals of teeth with primary endodontic infections before and after canal preparation and to test the antibiotic susceptibility of enterococcal strains isolated from infected root canals.

Methodology Twenty-five single-rooted teeth with pulp necrosis, intact pulp chambers and periradicular lesions were selected for study. Samples were collected from canals before and after instrumentation. Amongst isolated microorganisms from infected root canals only enterococci, enteric bacteria and yeasts were identified by biochemical tests. The *in vitro* antimicrobial susceptibility of isolated enterococci strains was evaluated by the Etest® system.

Results Microorganisms were isolated from 92% of the samples following intracoronal access, 22% were enterococci, enteric bacteria or yeast species. After biomechanical preparation, these species were no longer detected. After 7 days without intracanal dressing, 100% of the canals contained microorganisms, 52% of which were target species. However, after using paramonochlorophenol [PRP (2.0 g), Rinosoro® and polyethylene glycol (400 equal parts up to 100 mL)] as an intracanal dressing for 7 days, enteric bacteria and yeasts were not detected; only enterococci were still present. All strains of enterococci were susceptible to ampicillin, but exhibited variable susceptibility to rifampin and ciprofloxacin.

Conclusions Enterococci, enteric bacteria and yeasts were present in primary endodontic infections. Enterococci, particularly *Enterococcus faecalis* and *E. faecium* were resistant to removal by root canal preparation followed by intracanal dressing.

Keywords: endodontic therapy, enteric bacteria, enterococci, yeasts.

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Introduction

The close relationship between the development of periapical lesions and the presence of microorganisms inside the root canal system has been studied extensively (Shovelton 1964, Kakehashi *et al.* 1965, Sundqvist 1976, Gomes *et al.* 1996, Sydney 1996). Microorganisms isolated from primary endodontic infections are predominantly strict anaerobes because ecological conditions in the root canal such as nutrients, low oxidation–reduction potential, pH, temperature and bacterial relationships are important ecological determinants to bacterial growth that favour these microorganisms (Sundqvist 1976, 1992, Fabricius *et al.* 1982, Gomes *et al.* 1996, Sydney 1996, Le Goff *et al.* 1997). On the other hand, facultative anaerobic bacteria are usually in the minority and prevail in therapy-resistant cases (Nair *et al.* 1990, Waltimo *et al.* 1997, Molander *et al.*

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1998, Sundqvist *et al.* 1998). Previous studies have demonstrated that obligate anaerobic bacteria are more easily eliminated by endodontic procedures (Gomes *et al.* 1996, Le Goff *et al.* 1997, Lana *et al.* 2001, Chávez de Paz *et al.* 2003). Compared to anaerobes, facultative microorganisms are also likely to be more resistant to antimicrobial and mechanical endodontic procedures (Evans *et al.* 2002) and many of them may be resistant to antibiotics (Wittgow & Sabiston 1975). Antibiotic sensitivity has been performed on endodontic isolates particularly in persistent root canal infections to aid the clinician during treatment (Le Goff *et al.* 1997, Dahlén *et al.* 2000). Systemic antibiotics may support conventional treatment in these persistent cases. The most frequently isolated pathogens are various enteric bacteria (Haapasalo *et al.* 1983, Siren *et al.* 1997), yeasts, especially those belonging to *Candida* spp. (Nair *et al.* 1990, 1999, Waltimo *et al.* 1997, Molander *et al.* 1998) and enterococci, particularly *Enterococcus faecalis* (Gomes *et al.* 1996, Sydney 1996, Siren *et al.* 1997, Sundqvist *et al.* 1998, Dahlén *et al.* 2000).

Enteric bacteria are facultative Gram-negative anaerobe rods frequently associated with hospital infections due to their intrinsic genetic resistance. How they enter the root canal system, however, is still debated. Possibilities such as contamination of irrigation solution, lack of coronal seal, multiple clinical sessions or even the prolonged use of antibiotic therapy have been implicated (Haapasalo *et al.* 1983, Siren *et al.* 1997). Due to their low frequency and to their low prevalence in mixed infections, little attention has been paid to this group of bacteria. The enteric rods do not exceed 5% of the microflora in infected canals but their persistence inside the root canal system can lead to continuation of periapical disease (Haapasalo *et al.* 1983).

Yeasts, as well as the enteric bacteria, can be isolated in approximately 7% of the canals (Waltimo *et al.* 2000) and are constituents of the normal oral microflora. Yeasts have been isolated from dental plaque, dental caries and subgingival microflora. They were also observed in the apical end of root canals and in dentine tubules, being considered to be dentinophilic microorganisms (Nair *et al.* 1990). Amongst the yeasts, *C. albicans* is the most common and the most resistant to endodontic procedures, and infections caused by this pathogen can be facilitated by the presence of enteric bacteria with which they favourably coexist (Siren *et al.* 1997).

Undoubtedly, the most frequent microorganisms recovered from cases with post-treatment disease are enterococci. They usually reside in the human intestine but can also be isolated from the oral cavity. *Enterococcus faecalis* has been recovered in 30–70% of canals of root filled teeth with persistent periapical lesions (Molander *et al.* 1998, Sundqvist *et al.* 1998, Dahlén *et al.* 2000, Peciuliene *et al.* 2001, Evans *et al.* 2002, Pinheiro *et al.* 2003a,b). This species usually occurs in a small percentage of the flora in primary root canal infections but is able to survive after root canal treatment. *Enterococcus faecalis* appears to be highly resistant to intracanal dressings and is known to resist the antibacterial effect of calcium hydroxide (Engström 1964, Sydney 1996, Evans *et al.* 2002). Once established in the root canal *E. faecalis* faces several challenges for survival, including antimicrobial agents used during treatment (Evans *et al.* 2002). This microorganism has been isolated from dentine tubules, dental cementum and from extraradicular biofilms (Akpata & Blechman 1982, Ando & Hoshino 1990, Siqueira *et al.* 1996).

Many microbiological investigations have been conducted on teeth with primary endodontic infections and all of them emphasize the role of obligate anaerobe bacteria. However, little attention has been given to facultative anaerobe microorganisms and fungi in these situations (Siqueira *et al.* 2002). When these microorganisms are present in the canal system they could delay the healing process (Heintz *et al.* 1975, Haapasalo *et al.* 1983, Waltimo *et al.* 1997, Molander *et al.* 1998, Trope *et al.* 1999). The purpose of the present study was to detect enterococci, enteric bacteria and yeast species in teeth with primary endodontic infections before and after root canal preparation.

Materials and methods

Patient selection

Twenty-five systemically healthy patients 23–49 years of age, who were referred for endodontic treatment to the São Paulo Dental School, University of São Paulo (USP), São Paulo, Brazil, were involved in this study. The patients were selected at random and included both males and females as well as a variety of races. None of them had received systemic antibiotic therapy in the preceding 3 months. All selected teeth had single roots, intact pulp chambers and showed an asymptomatic apical periodontitis without communication to the mouth through fistula or otherwise. Approval to undertake the study was obtained from the Ethical Committee in Research of the Dental School of São Paulo and informed consent was obtained from all participants/patients.

Collection of clinical specimens

Microbial samples and endodontic treatment were conducted by one individual with sterile instruments. At each visit, a mouthwash was performed for 60 s with a 0.12% chlorhexidine solution. Teeth under treatment were isolated by a rubber dam. The operating area and the dental surface were disinfected by three 60 s successive applications of 30% hydrogen peroxide, 10% iodine tincture and 5% sodium thiosulphate solution to inactivate the iodine so that remnants of iodine would not influence the bacteriologic sampling (Möller 1966, Le Goff *et al.* 1997). A sterile dry cotton pellet was used to swab the tooth surface and it was transferred to a vial containing sterilized transport medium-VMGA III to check the sterility of the operating field (Dahlén *et al.* 1993). The pulp cavity was opened with sterile round burs of appropriate size under distilled water spray. Briefly, the first collection was made by means of size 15 or size 20 sterile absorbent paper points per root canal introduced to an approximate level of 1 mm short of the tooth apex (as determined by preoperative radiograph) and maintained in place for 30 s (Le Goff *et al.* 1997). Then two sequential paper points were placed to the same level and were retained in position for 30 s. If the root canal was dry, a small amount of sterile saline solution was introduced into the canal to ensure viable sample acquisition and then another collection was taken. The paper points were immediately transferred to transport medium-VMGA III (Dahlén & Möller 1992). The canals were instrumented using a simple stepback technique and conventional files with Endo PTC cream (Tween 80, urea peroxide, carbowax; Formula & Ação, São Paulo, SP, Brazil), 0.5% sodium hypochlorite solution (Paiva & Antoniazzi 1973) and final irrigation with 10 mL of SDS-EDTA solution. Irrigation solutions were delivered by means of a syringe with a sterile thin needle that was introduced to middle portion of the root. The canals were prepared using six instruments in order to complete the apical stop to a size that varied from 50 to 70, depending on the anatomy of the canal. After this, the canal was flushed thoroughly with a final rinse of 20 mL sterile saline solution using a syringe and needle that approached the apical third. At that time, a further sample (sample 2) was taken using the same paper point method. The pulp space was then dried, a sterile cotton pellet placed in the chamber and the access cavity filled with Cimpat® cement (Septodont, Saint Maur, France) of sufficient thickness to ensure a stable seal between appointments (Gomes *et al.* 1996). Sampling at a second visit, after 7 days, was undertaken after isolation and creation of an aseptic operative field. The temporary restoration was removed using round burs in a high-speed handpiece with distilled water spray and three sequential absorbent paper points were immediately inserted inside the root canals for the third sample without the use of any chemical substances. The diameter of the paper points used in this sample was size 30. At that time, the canals were prepared as previously described employing the two last instruments and then the canals were medicated with PRP solution (Formula & Ação) by a syringe with thin needle. The PRP solution was applied into the canal at its entrance, a cotton pellet was placed and the access cavity was sealed with Cimpat temporary cement (Septodont). At the third appointment, 7 days later, the teeth under treatment were isolated under rubber dam and the operating field treated as described previously. The temporary restoration was removed and the fourth sample was collected, as described previously, using size 30 paper points. After this, the canal was rinsed with sterile saline solution and filling of the root canal system was completed. The pulp chamber was sealed with glass–ionomer cement.

Isolation and identification of the microorganisms

The average time between sample collection and laboratory processing was 6 h. Transport tubes were placed in 37 °C for 30 min and vigorously mixed for 20–30 s using a vortex mixer. Each sample was then serially diluted in peptone water and aliquots (25 μL) were plated onto several media, as follows: m-Enterococcus agar (Difco Laboratories, Detroit, MI, USA) for enterococci, MacConkey agar (Difco Laboratories) for enteric bacteria, Sabouraud dextrose agar plates (Difco Laboratories) supplemented with 0.1% chloramphenicol (Medley, Campinas, SP, Brazil) for yeasts and Brucella agar (Difco Laboratories) enriched with 5% defibrinated sheep blood, 10 μg mL^{-1} haemin and 1 μg mL^{-1} menadione, as described by Le Goff *et al.* (1997) for anaerobes. MacConkey agar plates and m-Enterococcus agar plates were incubated aerobically at 37 °C for 48 h and Sabouraud agar plates were kept at room temperature for up to 4 days. Brucella agar plates were incubated at 37 °C in anaerobic jars for 7 days. Experiments were repeated in triplicate.

The different morphologic types of the colonies that grew in MacConkey agar, m-Enterococcus agar and Sabouraud agar plates in all collections were Gram-stained and classified by colony morphology. The

strains were further identified at the species level using biochemical tests: API 20Strep (BioMeriéux SA, Marcy l'Etoile, France) for streptococci and enterococci; API 20E (BioMeriéux SA) for enteric bacteria; ALBICANS ID2 (BioMeriéux SA), API CANDIDA (BioMeriéux SA) and API 20C AUX (BioMeriéux SA) for yeast identification. These kits consist of microtubes containing dehydrated substrates and they are inoculated with a bacterial/yeasts suspension. After a period of incubation and subsequent addition of the reagents, precipitation or colour changes appear in individual cupules. The reactions were read visually and the identification of the strains was obtained by referring to the Analytical Profile Index. Additional tests were performed to ensure the identification of bacteria from the genus *Enterococcus*: motility and yellow pigment production.

Antimicrobial susceptibility

The antimicrobial susceptibility of enterococci was determined using the Etest® System (AB Biodisk, Solna, Sweden), an agar diffusion susceptibility test. The isolated strains of enterococci [*E. casseliflavus* ($n = 2$), *E. faecalis* ($n = 6$), *E. faecium* ($n = 4$)], facultative anaerobic Gram-positive cocci were tested for their susceptibility/resistance to ciprofloxacin, ampicillin and rifampin. The Etest® was evaluated using Mueller–Hinton agar (Difco Laboratories). Sterile swabs were used to inoculate plates, to which Etest® strips were then applied. Plates were incubated for 24 h at 37 °C. After incubation, an elliptical zone of growth inhibition was observed around the strip. The minimum inhibitory concentration (MIC), in μg mL^{-1}, of each antibiotic was read from the scale on the strip at the intersection of the growth with the E-strip. The sensitivity values were interpreted according to the guidelines of National Committee for Clinical Laboratory Standards data (NCCLS 1999). All tests were completed in duplicate.

Results

After disinfection of the teeth using the protocol proposed by Möller (1966), none of the 25 teeth had bacterial growth.

During the first sample at the time of intracoronal access, microorganisms were isolated from 23/25 (92%) root canals and 22% of them were infected by enterococci, enteric bacteria or yeasts (Fig. 1). Three (13%) of the 23 infected canals harboured enterococci, one (4%) was infected by enteric bacteria associated with enterococci and another (4%) by yeasts (Fig. 2).

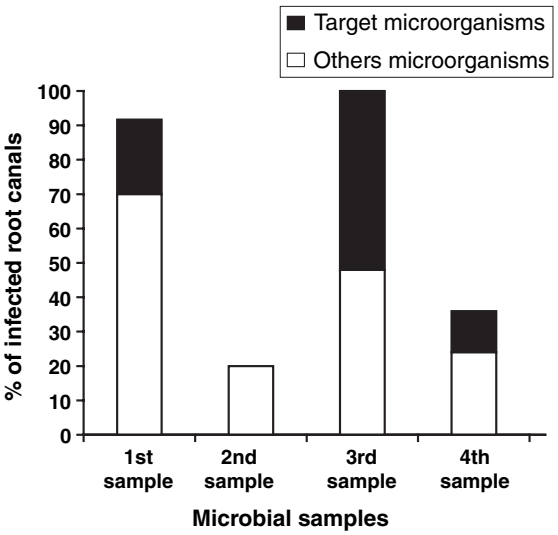


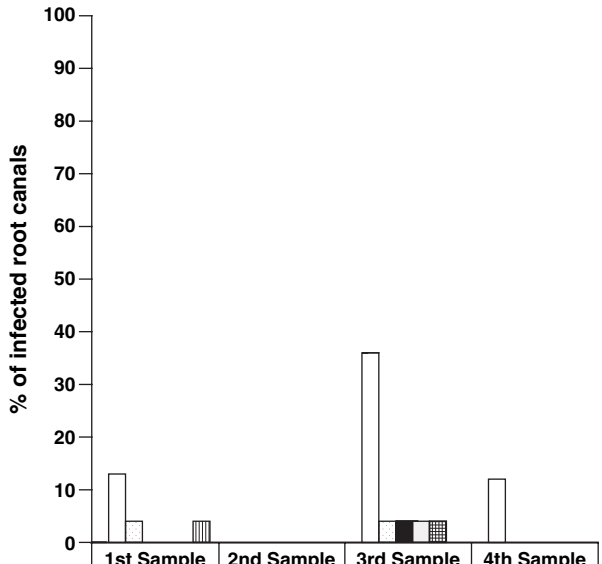
Figure 1 Percentage of infected root canals by the time the samplings were performed.

Two root canals had no cultivable bacteria. After biomechanical instrumentation (sample 2), enterococci, yeasts and enteric bacteria were no longer detected but other microorganisms were still present in five root canals (20%) (Fig. 1).

After 7 days without intracanal dressing, microorganisms were isolated from 25/25 (100%) root canals (sample 3). Nine (36%) of these were infected by enterococci only, one (4%) harboured enteric bacteria, the other (4%) with enteric bacteria associated with enterococci and the other (4%) with enterococci associated with yeasts or (4%) enterococci coexisted with enteric bacteria and yeasts (Figs 1 and 2).

After 7 days of intracanal dressing, microorganisms were recovered (sample 4) from nine (36%) root canals (Fig. 1). Enteric bacteria and yeasts were no longer isolated, whilst three root canals were infected with enterococci (Fig. 2). These strains were identified as *E. faecalis* and *E. faecium*.

Amongst the total target microorganisms isolated in this study, three yeast strains were identified as *Candida albicans* (one), *Candida glabrata* (one) and *Candida magnoliae* (one). Three enteric bacteria strains were identified, one being *Klebsiella pneumoniae* and two *Enterobacter sakazaki*. Of the total isolated enterococci, two strains were *E. casseliflavus*, four *E. faecium* and six were *E. faecalis*. It is important to emphasize that all samples of *Enterococcus* tested were positive to the pyruvate, hippurate, esculin, pyrrolidonil 2 naphtylamide, l-leucine-2-naphtylamide, arginine tests and acidificated in the presence of mannitol, lactose,



	1st Sample	2nd Sample	3rd Sample	4th Sample
□ Enterococci	13%		36%	12%
□ Enterococci and enteric bacteria	4%		4%	
■ Enterococci and yeasts			4%	
□ Enterococci and enteric bacteria and yeasts			4%	
▦ Enteric bacteria			4%	
▥ Yeasts	4%			

Figure 2 Percentage of root canals infected by enterococci, enteric bacteria and yeasts isolated by the time the samplings were performed.

trehalose and starch. All the species did not produce β-glucuronidase and alkaline phosphatase, neither acidificated in the presence of inulin and glycogen. Only the *E. casseliflavus* species acidificated in the presence of raffinose, which did not occur with the *E. faecalis* and *E. faecium* species. Additional tests were performed due to the rare isolation of these species in the endodontic literature. Two biochemical tests have been reported to have the greatest utility in differentiating these three microorganisms. They are tests for motility (*E. casseliflavus* was positive, *E. faecium* and *E. faecalis* were negative) and yellow pigment production (*E. casseliflavus* was positive, *E. faecium* and *E. faecalis* were negative) (Facklam & Collins 1989).

The results of the Etest® are showed in Table 1. All strains of enterococci were susceptible to ampicillin, but

Table 1 Microbial sensitivity test of enterococci strains isolated from root canals

Species of Enterococci	Rifampin MIC (μg mL^{-1})	Sensitivity	Ciprofloxacin MIC (μg mL^{-1})	Sensitivity	Ampicillin MIC (μg mL^{-1})	Sensitivity
E. casseliflavus	0.75	S	0.25	S	3.0	S
E. casseliflavus	0.016	S	0.75	S	1.0	S
E. faecalis	8.0	R	1.5	I	1.5	S
E. faecalis	2.0	I	0.50	S	1.5	S
E. faecalis	6.0	R	1.0	S	1.0	S
E. faecalis	32	R	1.5	I	1.5	S
E. faecalis	8.0	R	1.0	S	1.0	S
E. faecalis	12	R	1.0	S	1.5	S
E. faecium	4.0	R	1.0	S	0.032	S
E. faecium	64	R	1.5	I	0.38	S
E. faecium	12	R	1.0	S	1.5	S
E. faecium	0.094	S	3.0	I	0.094	S

S, sensitive; I, intermediate sensitivity; R, resistant. Ampicillin: S ≤8.0 μg mL^{-1}. Rifampin and Ciprofloxacin: S ≤1.0 μg mL^{-1}; R ≥4.0 μg mL^{-1}.

exhibited variable susceptibility to rifampin and ciprofloxacin.

Discussion

Several studies on root canal infections have focused on strict anaerobic bacteria due to their predominance in samples taken from untreated teeth with necrotic pulps (Sundqvist 1976, 1992, Le Goff *et al.* 1997, Dahlén *et al.* 2000). However, the presence of enterococci, enteric bacteria and yeasts in the root canal system can delay or even prevent the healing process (Heintz *et al.* 1975, Haapasalo *et al.* 1983, Waltimo *et al.* 1997, Molander *et al.* 1998, Trope *et al.* 1999).

Microorganisms were recovered from the first sample in 23 (92%) root canals in agreement with previous studies that showed the relationship between microorganisms and the development of apical periodontitis (Kakehashi *et al.* 1965, Sundqvist 1976, Fabricius *et al.* 1982, Le Goff *et al.* 1997, Dahlén *et al.* 2000, Lana *et al.* 2001). It is important to emphasize that the samples were processed in the laboratory within 6 h to preserve the reproductive capacity of bacterial cells and to prevent the growth of microorganisms in the sample. In two cases microorganisms were not isolated from root canal due to the limitations of the sampling technique and especially because of the small sample size (Le Goff *et al.* 1997). Bacteriological sampling procedures and culture processing may not provide an accurate reflection of the root canal microbiota because many types of organisms fail to survive for identification under regular laboratory conditions.

In this study, 22% (5:23) of the initial population consisted of facultatives belonging to the enterococci genera, enteric bacteria and yeasts. These microorganisms were found in a large proportion of cases but it is important to emphasize that the selection of patients was random. These findings were similar to those reported by Le Goff *et al.* (1997) who found facultative anaerobes in 19% of canals. In the present study enterococci were the most frequent bacteria recovered from the canals before treatment. Although the role of yeast microorganisms in endodontic infection has not been established, yeasts of the genera *Candida* were recovered from root canals presenting pulp necrosis with intact pulp chamber before the treatment procedures (Sundqvist *et al.* 1998, Lana *et al.* 2001). The reasons for the occurrence of enteric bacteria in root canal infections are poorly understood. Enterococci are seldom found in primary infections or are present in very low numbers in untreated root canals (Molander *et al.* 1998, Dahlén *et al.* 2000, Lana *et al.* 2001) but these microorganisms have been suggested to be important agents in post-treatment disease. This study confirms that enterococci, enteric bacteria and yeasts are involved in root canal infections.

Despite mechanical instrumentation and disinfection of the root canal system, microorganisms were recovered in five (20%) canals (sample 2), clearly showing that root canal preparation is unable to eliminate all bacteria from the root canal system (Gomes *et al.* 1996, Sydney 1996, Sjögren *et al.* 1997, Chávez de Paz *et al.* 2003). However, preparation did reduce the original range of infective microbiota (Lana *et al.* 2001, Peters *et al.* 2002). Enterococci, enteric bacteria or yeasts were not detected in the second sample (immediately after biomechanical instrumentation). The root canals were left empty to allow surviving bacteria in the root canal to multiply to a level that would be detectable at a subsequent appointment (Gomes *et al.* 1996, Sundqvist *et al.* 1998).

At the second appointment, 7 days later, microorganisms were detected in all of the canals (Fig. 1). After 1 week without intracanal dressing, without any chemical or physical barrier, the target species were isolated from 13 (52%) root canals. It is interesting to note that enterococci were recovered from 12 root canals, three of which were infected with enterococci from the outset (first sample), in nine canals the enterococci were isolated only from the third sample. Amongst the enterococci, six strains were identified as *E. faecalis*, four strains as *E. faecium* and two as *E. casseliflavus* by biochemical tests. These microorganisms were probably present from the outset in low numbers that could not be isolated. It is believed that environmental change due the biomechanical preparation, which eliminated the most sensitive microorganisms, provided better growth conditions for enterococci, in addition to the fact that enterococci have the ability to penetrate dentinal tubules (Akpata & Blechman 1982, Ando & Hoshino 1990, Sundqvist 1992, Sydney 1996). Another possibility could be as a consequence of fluid leakage from the oral environment via gaps at the filling-tooth interface following coronal leakage (Kalfas *et al.* 2001, Chávez de Paz *et al.* 2003). Siren *et al.* (1997) reported that dental visits and lack of an adequate seal increased significantly the probability of finding enteric bacteria in the root canal, indicating that these bacteria enter the root canal between treatment visits. The precautions taken in this study, such as sterile instruments, sterility controls before opening the pulp cavity, minimization of the

time taken to culture the samples and having a single practitioner performing the procedure, reduced the risk of contamination to a minimum but the probability of contamination cannot be dismissed (Le Goff *et al.* 1997, Molander *et al.* 1998).

Such residual microorganisms are likely to play a role in treatment failures (Molander *et al.* 1998, Sundqvist *et al.* 1998). In one case enterococci coexisted with enteric bacteria, in one case with yeasts, in one case with enteric bacteria and yeasts, and in nine cases they did not coexist with other target microorganisms, suggesting a pathogenic role for this organism (Fig. 2). These results support the hypothesis that enterococci do not need any other interactions to survive (Engström 1964, Sydney 1996). Enterococci are often reported to be present in low numbers in untreated infected root canals with necrotic pulps (Sundqvist 1992). However, when the ecological prerequisites are altered, enterococci may thrive and multiply. They are able to survive in environments with sparse nutritional supply (Chávez de Paz *et al.* 2003). These microorganisms appear to have the ability to utilize opportunities created by the removal of other microbes and also to have the capacity to grow in a low-nutrient environment (Trope *et al.* 1999). The results from the second sample reflect the importance of an antimicrobial dressing. To leave the canal empty is an opportunity for endodontic infection or reinfection (Lage-Marques & Antoniazzi 2000).

As mechanical instrumentation and irrigation may not eliminate all microorganisms, it has been emphasized that antimicrobial agents should be used between appointments (Ando & Hoshino 1990, Oguntebi 1994, Siqueira *et al.* 1996, Chávez de Paz *et al.* 2003). Bacteria within dentine tubules can represent an important microbial reservoir with the capacity to promote infection or reinfection of the root canal before and after endodontic therapy (Oguntebi 1994). Thus, in the present study the root canals were instrumented in order to reduce microorganisms and dressed with PRP. This medication has been employed as intracanal dressing in cases of necrosis pulp in School of Dentistry, University of São Paulo, São Paulo, Brazil. It is based on paramochlorophenol which has demonstrated effectiveness on *Candida albicans* (Valera *et al.* 2001). Scelza *et al.* (1999) evaluated the biocompatibility of PRP in the mandible of rats. The results demonstrated similarities between the control group and PRP group regarding the repair process of the surgical site and resorption of implanted material. In this way, studies in culture cells have shown that 2% paramonochlorophenol allowed cellular growth (Bortolotto *et al.* 2003). This is the first report that evaluated the microbiological effectiveness of this intracanal dressing *in vivo*.

At the third visit, 7 days later, following medication with PRP, the fourth sample was performed after removal of the temporary filling. As shown in Fig. 2, the target microorganisms were observed in only three of the 13 cases detected in the previous collection. Two cases were infected by *E. faecalis* and one case by *E. faecium*. In two cases, enterococci were present from the outset and in one case enterococci were isolated from the third sample (7 days without intracanal dressing) and survived into the canal after intracanal dressing. These findings suggest that the treatment procedures were less effective against Gram-positive organisms. The supposedly higher resistance of Gram-positive bacteria may be related to various factors, for example, cell-wall structure, metabolic products secreted and resistance towards medicaments (Chávez de Paz *et al.* 2003). Microorganisms of the genera *Candida* or enteric Gram-negative rods were not found in the fourth sample.

The *in vitro* susceptibility test was performed on enterococcal strains because these organisms are considered to be difficult to eliminate. All strains of *E. casseliflavus* were shown to be sensitive to the antibiotics used. Strains of *E. faecium* and *E. faecalis* were sensitive to ampicillin. These results were similar to those obtained by Heintz *et al.* (1975), who verified the sensitivity of 50 strains of *E. faecalis* to ampicillin. Engström (1964) observed resistance of enterococci to penicillin in only 6% of the studied strains and Dahlén *et al.* (2000) showed that *E. faecalis* and *E. faecium* were resistant to penicillin and ampicillin. It is important to emphasize that two strains of *E. faecalis* showed intermediate sensitivity to ciprofloxacin, whilst the other four were highly susceptible to this antibiotic. All strains showed low susceptibility to rifampin. Antibiotics should not normally be required during endodontic treatment but the microbial sensitivity test can be considered as an important auxiliary resource in cases resistant to endodontic therapy (Dahlén *et al.* 2000).

There is still insufficient knowledge with respect to enterococci, enteric bacteria and yeasts survival after endodontic procedures. *Enterococcus faecalis* can be present in primary endodontic infections and its persistence can lead to post-treatment disease. These bacteria over long periods of time may induce or maintain a periapical lesion (Dahlén *et al.* 2000). The persistence of microorganisms inside the root canals may not lead to treatment failure, but certainly their

absence will favour healing (Lana *et al.* 2001). Further study is required to determine the possible sources of these bacteria.

Conclusion

Enterococci, enteric bacteria and yeasts were present in primary endodontic infections; enterococci were the most frequently isolated. Enterococci, particularly *E. faecalis* and *E. faecium*, were more resistant to root canal preparation and intracanal dressing.

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