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ORIGINAL ARTICLE

Can the *Enterococcus faecalis* identified in the root canals of primary teeth be a cause of failure of endodontic treatment?

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ABSTRACT

Objective: This study investigated the presence of *Enterococcus faecalis* in primary teeth with primary root canal infections and related to the possible failure of pulpectomy outcome after 36 months.

Material and methods: Root canal samples were obtained from 25 out of 244 patients using the sterile paper cone method. The identification of *E. faecalis* was done with culture and molecular tests using species-specific 16S rRNA gene-based polymerase chain reaction (PCR). After 36 months, the pulpectomy outcome was evaluated.

Results: *Enterococcus faecalis* was found in five (20%) samples, and dental caries were the cause of primary infection in all of them. Pulpectomy outcome was evaluated only in teeth that completed the entire clinical protocol and were followed up to 36 months ($n = 8$). From these, 75% ($n = 6$) were successful and 25% ($n = 2$) failed. *E. faecalis* was present in 50% of both successful and failed cases.

Conclusions: *Enterococcus faecalis* was not related to the failure of endodontic treatment of primary teeth.

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Introduction

Enterococcus faecalis (*E. faecalis*) is one of the most commonly found bacteria in human gastrointestinal tracts [1], and it is also able to colonise the oral cavity [2]. This microorganism has been associated with oral mucosal lesions in immunocompromised patients [3], periodontitis [4] and root canal infections [1–4]. *E. faecalis* is the most common pathogen in failed root canal treatments of permanent teeth [5,6]. However, there are only a few studies [7–11] that have investigated the microbiota of primary teeth, even though pulp necrosis can lead to periapical disease, affecting the permanent tooth germ of paediatric patients [12,13].

Different methods such as culture [7–11], DNA–DNA hybridisation [14], polymerase chain reaction (PCR) [2,12,13], and denaturing gradient gel electrophoresis fingerprint techniques (DGGE) [15] have been used to detect and identify bacteria in endodontic microbiota. Culture methods have contributed significantly to the elucidation of endodontic diseases [12]. However, molecular approaches, particularly PCR, have shown several advantages in identifying microbial species compared to culture, since they are more specific, more accurate, more sensitive, and faster than culture [4,7,12,13]. In addition, PCR allows the detection of uncultivable and fastidious microorganisms [13]. Although culture and molecular techniques have been widely used to detect bacteria in endodontic infections in permanent teeth [14–16], only a small number of studies have used these methods to investigate the microbiota of primary teeth [7–11]. Also, there have been few investigations about *E. faecalis* as the aetiological agent of canal infections in primary teeth.

The assessment of treatment success of pulpectomy should be made through clinical and radiographic criteria [17]. The clinical success of endodontic treatment in primary teeth is high but often shows a lower proportion of solving the initial pathological radiographic signs [18]. Treatment is considered successful when all clinical signs disappear and a reduction of the periapical radiolucency and bone regeneration occur. This success is dependent on several factors, with the reduction or elimination of bacterial infection being among the most important. Thus, it is crucial to identify the relationship between the microorganisms present in the root canals and the endodontic

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therapy outcome. This study investigated the presence of *Enterococcus faecalis* in primary teeth with primary root canal infections and related to the possible failure of pulpectomy outcome after 36 months.

Materials and methods

Patient selection

This is a longitudinal study to evaluate pulpectomy outcome and its relation to the prior presence of *E. faecalis* in the root canal. Clinical procedures were approved by a local ethics committee (no 46/2010), and written informed consent was obtained from the parents of each patient.

Root canal samples were obtained from 25 out of 244 patients. Eligible participants were recruited during 12 months from among patients of the Paediatric Dentistry Clinic at the Federal University of Rio de Janeiro, Brazil. Healthy patients between 2 and 10 years old who had at least one primary tooth with clinical evidence of pulp necrosis and/or interradicular or periradicular radioluscency were candidates for inclusion if any of the following criteria were fulfilled: (i) presence of sinus tract, abscess, or purulent discharge from the root canal and (ii) no pulp tissue remaining when the pulp chamber was accessed. Patients who had any of the following criteria were excluded from the study: (i) tooth that may not be fully isolated, (ii) obliteration of the root canal, (iii) clinical signs and symptoms of an active spreading infection with systemic involvement requiring antibiotic therapy such as tachycardia, facial swelling, cellulitis, limited mouth opening, high temperature, difficulty swallowing and regional lymphadenitis and (iv) patients who had received antibiotic therapy in the three months prior to the examination or who had a systemic disease.

Root canal sample procedures

All samples were collected by one of the authors at the clinic. At the first appointment, supragingival biofilm was removed by scaling and cleaning with pumice. After local anaesthesia and rubber dam isolation, the tooth and the surrounding field were cleansed with 3% hydrogen peroxide and disinfected with 2.5% sodium hypochlorite (NaOCl). Then, carious tissue was removed and access to the pulp chamber was achieved with sterile high-speed spherical diamond burs (KG Sorensen Indústria e Comércio, São Paulo, SP, Brazil). After accessing the pulp chamber, the tooth, clamp, and adjacent rubber dam were once again disinfected with 2.5% NaOCl.

As a sterility control, two paper points were rubbed on the disinfected tooth crown (one in lingual face and the other one buccal face) and transferred to a tube containing enterococcosel broth (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA), a selective medium with bile-esculin and sodium azide, and then incubated for 72 h at 35 °C. Using a sterile K-type file (no. 15), the material from the apical region was retrieved. A small amount of sterile 5% sodium thiosulfate solution was introduced into the root canal, a sterile file was inserted to a level 1 mm radiographically short of the root apex, and a gentle filing motion was applied. The root canal contents were absorbed onto four paper points. Each paper point was retained in position for one minute. The first and fourth points were transferred to enterococcosel broth and incubated for 72 h at 35 °C. The second and third paper points were transferred to 1 ml of 0.5% dimethyl sulfoxide in trypticase-soy broth and immediately frozen at −20 °C [2].

After the root canal sample procedures, each tooth received treatment – pulpectomy or exodontia – according to its clinical and radiographic characteristics. Pulpectomy was indicated according Barcelos et al. [17] criteria. In cases indicated to pulpectomy, following the chemomechanical preparation with K-files and 2.5% sodium hypochlorite (NaOCl), teeth were irrigated with 6% citric acid and 0.9% physiologic solution for smear layer removal and camphorated paramonochlorophenol was used as intracanal medication. At a second appointment, one week after, root canals were filled with iodoform based paste and received coronal restoration with composite. Patients were evaluated in six-month intervals up to 36 months. During the follow-up appointments, patients received clinical and radiographic examination to determine pulpectomy outcome. Treatment was judged to be successful when both clinical and radiographic criteria were fulfilled. Clinical success was achieved when there were no signs or symptoms of infection, including the absence of swelling, fistula, or sensitivity to percussion [17]. Radiographic success was achieved when there was no newly formed radiolucency in the cases without radiolucency at the beginning of the treatment or when there was no evidence of reduction in the size of the radiolucent area that was previously observed in initial radiography [17].

Culture procedures

The samples with growth in the enterococcosel medium were plated onto a blood agar plate (CQA-Brazil) and incubated for 72 h at 35 °C. Resulting colonies were submitted to Gram staining, analysis of colony morphology, catalase test, growth in NaCl broth, hydrolysis of esculin in the presence of bile salts, hydrolysis of pyrrolidonyl-α-naphthylamide (PYR) and leucine aminopeptidase (LAP), hydrolysis of arginine, pyruvate utilisation, motility, pigmentation production, and carbohydrate fermentation tests (arabinose, mannitol, metal-α-D-glucopyranoside, raffinose, sucrose, sorbitol and sorbose) [7,19].

PCR procedures

One-millilitre aliquots from samples, as well as from positive cultures in selective enterococcosel broth, were transferred to microtubes, and the DNA was extracted by boiling. *E. faecalis* was identified using PCR amplification of the signature sequences of the 16S rRNA gene [2]. The oligonucleotide species-specific primers for *E. faecalis* produced a PCR amplicon of 310 bp [2]. The PCR was done following the protocol established by Zoletti et al. [20].

Polymerase chain reaction products from samples that grew in enterococcosel and that were not identified as *E.*

faecalis were sequenced. For this, amplicons were purified using a PCR purification system (Wizard PCR Preps, Promega, Madison, WI, USA) and sequenced with the forward primers on the ABI 377 automated DNA sequencer using dye terminator chemistry (Amersham Biosciences; Little Chalfont, Buckinghamshire, UK). The sequences generated were compared to those of GenBank using the BLAST algorithm [21,22]. Database sequences with the highest similarities to our sequences were chosen for identification.

All data were analysed using the SPSS (SPSS Inc., Chicago, IL, USA) 16.0 software programme for windows. The analysis was carried out through descriptive (distributions of absolute and relative frequencies for categorical variables; mean and standard deviation for continuous variables) and analytical (Exact Fisher Test and Chi-square test) approaches, with a significance level of 5% ($p < .05$). The sequences were deposited in GenBank under accession numbers KY612205–KY612208.

Results

The final sample consisted of 25 teeth in 18 patients aged from 2 to 10 years old (mean age: 5.28 (±2.26) years; 48% male). These patients also participated in a previous study carried out by the group [23]. Of the 25 teeth included in this study, 10 cases were positive in the enterococcosel broth (Table 1). *E. faecalis* was detected by species-specific 16S rRNA gene-based PCR in five of the positive cases. No statistical significance was found between clinical data and number of *E. faecalis* isolates from the patients evaluated (Table 2).

Among the 10 cases that showed growth in the enterococcosel medium, five isolates were not identified as being *E. faecalis* in the biochemical and/or PCR tests, and they looked like coccobacilli with the Gram method. Partial 16S rRNA gene sequences revealed that isolates were affiliated with *Lactobacillus* spp.: two isolates were affiliated with *Lactobacillus plantarum*, one with *L. pentosus* and one with *L. vaccinostercus*. The sequencing of the isolates revealed high identity with these microorganisms (Table 3). One isolate, from P17, showed low quality of sequence, and it was classified as undefined.

The clinical and microbiological data and the pulpectomy outcome of each tooth are summarised in Table 1. Of 25 patients, 13 were indicated to exodontia and 12 to pulpectomy. Among the pulpectomies initiated, 25% ($n = 3$) were not completed, as root canal filling was unfeasible because signs of a persistent infection were found even after two appointments for chemomechanical preparation and intracanal medication. Another tooth was not evaluated because it was avulsioned after a trauma. Thus, pulpectomy outcome was evaluated only in teeth that completed the entire clinical protocol and were followed up to 36 months ($n = 8$). Among these, 75% ($n = 6$) were successful and 25% ($n = 2$) failed. *E. faecalis* was present in 50.0% of both success and failure cases.

Discussion

Enterococcus faecalis is a pathogen normally found in permanent teeth in cases of failed endodontic therapy [6]. In the present study, we found this pathogen in 20% of the samples isolated from primary teeth with primary root canal infections. Although these teeth presented primary infections without a retreatment evaluation, it is important to highlight that the presence of *E. faecalis* in primary teeth of children will add knowledge concerning the early presence of this pathogen in the oral cavity.

Only a few authors have investigated the microorganisms present in root canals of primary teeth, and the available records are from studies using microbial culture [7,8] and molecular methods [9–11]. da Silva et al. [9] and Ruviére et al. [11] evaluated primary teeth using culture and checkerboard DNA–DNA hybridisation, respectively, and did not find *E. faecalis* in their studies. In the first study, black-pigmented bacilli, streptococci, mutans streptococci, and gram-negative aerobic rods were found. In the study by Ruviére et al. [11], *Campylobacter rectus* was the most prevalent species found. In both studies, polymicrobial infections were found in the root canals of primary teeth with necrotic pulp.

In this study, *E. faecalis* was investigated in primary teeth with primary infection because the role of this pathogen is unclear. Only two studies have been carried out to evaluate the presence of *E. faecalis* in paediatric patients with necrotic pulp. Cogulu et al. [7], using culture and PCR methods, reported that *E. faecalis* was present in 18% of primary (from 45 samples) and 26% of permanent teeth (from 38 samples). The authors showed that both culture and PCR methods are sensitive to detect *E. faecalis* in root canals. In 2008, using PCR, these authors found that this pathogen was the most prevalent species (20%) in 79 primary teeth with primary infection. Our results are in accordance with these findings, even though Cogulu et al. [7], did not use enterococcosel selective medium. Tavares et al. [10] investigated the microbiota involved in endodontic infections in primary teeth from 40 samples using checkerboard DNA–DNA hybridisation. *Prevotella intermedia, Neisseria mucosa, Prevotella tannerae, Prevotella nigrescens* and *Tannerella forsythia* were the most prevalent species, whereas *E. faecalis* was present in only 3.2% of the samples. This disagreement in relation to our results could be explained due to the molecular technique chosen for the analysis. Moreover, it could also be a result of differences in the composition of the oral microbiota. Sensitive and accurate molecular techniques are necessary to characterize the root canal microbial irritants in order to determine their association with clinical symptoms and the prognosis of treatment. Although DNA sequence analysis is the gold standard in microbial identification in this field; PCR assay also represents a new and a sensitive method applied to the study of endodontic bacteria [7].

Enterococcus faecalis is associated with failed root treatment in permanent teeth, as demonstrated by some authors. Siqueira and Rôças [2] detected *E. faecalis* in 77% of root-filled samples from Brazilian patients, whereas Rôças et al. [4] found this species in 64% of samples from South Korean patients. However, there is no retreatment for primary teeth, and consequently it is not possible to study resistant infections in primary teeth. Thus, differences in the microbiota could be associated with primary infections in primary teeth and primary and secondary infections in permanent teeth.

Table 1. General characteristics and pathogens identified after culture from samples of root canal teeth with necrosis of the 25 paediatric patients evaluated.

Case	Sex	Age (years)	Tooth	Cause	Mobility	Root resorption	Swelling	Fistula	Positive by culture in enterococcosel	*E. faecalis* by PCR	*Lactobacillus* by sequencing	Treatment	Pulpectomy completed	Pulpectomy outcome
P1	M	8	75	C	N	N	N	Y	N	N	N	Exodontia	–	–
P2	F	3	51	T	N	Y	N	N	N	N	N	Pulpectomy	Yes	Loss
P3	F	5	85	C	N	N	N	Y	N	N	N	Pulpectomy	Yes	Failure
P4	F	4	61	C	Y	N	N	N	N	N	N	Exodontia	–	–
P5	F	4	51	C	N	N	N	Y	N	N	N	Exodontia	–	–
P6	F	4	84	C	N	N	N	N	N	N	N	Exodontia	–	–
P7	M	8	74	C	N	Y	N	N	N	N	N	Pulpectomy	No	–
P8	M	7	85	C	N	N	N	N	N	N	N	Pulpectomy	Yes	Success
P9	M	7	54	C	N	N	N	N	N	N	N	Pulpectomy	Yes	Success
P10	F	2	61	C	N	N	N	N	Y	Y	N	Pulpectomy	Yes	Failure
P11	F	4	74	C	N	N	N	N	N	N	N	Exodontia	–	–
P12	M	6	65	C	N	N	N	N	N	N	N	Exodontia	–	–
P13	M	5	75	C	N	N	N	Y	Y	Y	N	Pulpectomy	Yes	Success
P14	M	5	74	C	N	N	N	N	Y	Y	N	Pulpectomy	Yes	Success
P15	F	10	65	C	N	N	N	Y	Y	Y	N	Pulpectomy	Yes	Success
P16	F	2	51	T	Y	Y	N	N	Y	N	Y	Pulpectomy	Yes	Success
P17	F	2	61	T	N	N	N	N	Y	N	U	Exodontia	–	–
P18	F	6	85	C	N	N	N	N	Y	N	Y	Exodontia	–	–
P19	M	4	54	C	Y	N	N	Y	Y	N	Y	Pulpectomy	No	–
P20	F	8	54	C	N	Y	N	Y	N	N	N	Exodontia	–	–
P21	M	4	51	C	N	N	N	Y	Y	N	Y	Pulpectomy	No	–
P22	M	6	85	C	Y	N	Y	N	Y	Y	N	Exodontia	–	–
P23	M	8	84	C	N	N	N	N	N	N	N	Exodontia	–	–
P24	M	3	51	T	N	Y	N	Y	N	N	N	Exodontia	–	–
P25	F	7	55	C	N	Y	N	Y	N	N	N	Exodontia	–	–

Patient: P; Male: M; Female: F; Caries: C; Trauma: T; No: N; Yes: Y; Undefined: U.

Table 2. Correlation between clinical data and number of *Enterococcus faecalis* isolates in root canals with necrosis of the 25 paediatric patients evaluated.

Clinical data	*E. faecalis* + (*N* = 5)	*E. faecalis* − (*N* = 20)	*p value*
Tooth region			
Anterior teeth	1	7	.642
Posterior teeth	4	13	
Age			
2–5 years old	3	11	1.000
6–10 years old	2	9	
Mobility			
Presence	1	3	.642
Absence	4	17	
Pain			
Presence	2	8	1.000
Absence	3	12	
Cause of pulpal involvement			
Trauma	0	4	.549
Caries	5	16	

N: number of cases; +: positive; −: negative.

Table 3. *Lactobacillus* spp. isolates description based on BLAST (GenBank).

Patient isolates	Closest described relative	Percentage similarity (%)	GenBank accession no
P16	*L. plantarum*	100	KX388383.1
P18	*L. pentosus*	100	KX057554.1
P19	*L. plantarum*	99	LC213631.1
P21	*L. vaccinostercus*	100	LN907853.1

Biochemical tests were performed on all 10 samples that showed growth in enterococcosel broth. However, five isolates tested were incompatible with *E. faecalis*, and 16S rRNA gene sequences revealed that these isolates were affiliated with *L. plantarum, L. pentosus* and *L. vaccinostercus. Lactobacillus* spp. are common in the oral cavity, especially in patients with carious lesions [24]. Svec et al. [25] revealed the phenotypes *Lactobacillus fermentum*, *Lactobacillus casei/paracasei*, *Lactobacillus rhamnosus*, *Lactobacillus salivarius*, *Lactobacillus plantarum*, and *Lactobacillus acidophilus* in samples from carious lesions in primary teeth, according to our results. In deep carious lesions, *Lactobacillus* spp. can be present and infect necrotic pulp as well. We hypothesised that *Lactobacillus* can grow in enterococcosel broth, since this pathogen was capable of esculin hydrolysis and resisting bile.

Another important factor in this study is the low age of the patients and their behaviour, which together make such research more difficult; the mean age was 5.68 years old. A lot of time was spent on isolating the tooth with a rubber dam and on collecting the root canal samples.

The success of endodontic treatment depends on several factors. One of the most important is the reduction or elimination of bacterial infections [7]. Therefore, it is important for the clinician to be aware of such bacteria and their ability to grow in an endodontic microenvironment. The presence of *E. faecalis* in primary root canals with primary infection of young individuals adds knowledge to the fact that this microorganism is present in the oral cavity from an early age [7]. This is important for the treatment plan and choice of therapy to achieve success in each case. The endodontic treatment in primary teeth is most often successful when performed in asymptomatic teeth without root resorption or periapical infection. Barcelos et al. [17] emphasise that the pulpectomy outcome for teeth with pulpal necrosis, preoperatory symptoms, or periapical/interradicular radiolucency was significantly improved by the removal of the smear layer with 6% citric acid. In our study, all treated cases were necrotic pulp and the success rate was 75% after a 36-month follow-up, that is a high frequency rate of success considering the long-time of accompaniment during 36-month follow-up.

Previous studies confirm that certain species of microorganisms are associated with clinical signs and symptoms of endodontic infections in primary and permanent teeth [2,10,20,25]. However, in this study *E. faecalis* wasn't associated to any clinical data confirming its role in necrotic process. Also, no study, in the reviewed literature, with a 36-month follow-up related treatment outcome to root canal primary infection microbiota, emphasising that more studies are necessary to better understand this relationship.

Conclusions

Within the study limitations, the *E. faecalis* in primary tooth root canals was not related to the failure of pulpectomy after 36 months, since only two teeth had failed the treatment, and the microorganism was identified in only one. Further studies are needed to investigate the role of this pathogen in primary root canal infections and its possible relation to the failure outcome.

Disclosure statement

No potential conflict of interest was reported by the authors.

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