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Qualitative and quantitative molecular analysis of bacteria in root canals of primary teeth with pulp necrosis

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Abstract: Information about bacterial diversity, such as the number of each species in the root canals of primary teeth, contributes to improving our effective management of infections of endodontic origin in primary teeth. This study made a qualitative and quantitative assessment of the bacteria in the root canals of primary teeth with necrotic pulp, using the fluorescence *in situ* hybridization (FISH) technique. Thirty-one primary teeth with pulp necrosis from 31 children were evaluated using the FISH technique, to detect the presence and density of *Aggregatibacter actinomycetemcomitans, Campylobacter rectus, Enterococcus faecalis, Fusobacterium nucleatum, Porphyromonas gingivalis, Prevotella intermedia, Prevotella nigrescens, Streptococcus, Streptococcus mutans, Streptococcus sobrinus, Tannerella forsythia* and *Treponema denticola*. Descriptive measures explained the data related to density, and Student's *t*-test assessed the differences among the densities of each bacterium, according to signs and symptoms. The bacterial density was paired and correlated. All bacteria tested were detected and identified in all the samples. The average number of bacterial individuals from each species ranged from 1.9 x 10^8 cells/mL (*S. mutans*) to 3.1 x 10^8 cells/mL (*F. nucleatum*) ($p > 0.05$). The sum of the mean counts of each bacterium represented almost 80% of the entire microbial community. Patients with pain had significantly more *T. denticola*, and those with edema showed a greater density of *Streptococcus* and *P. nigrescens* ($p < 0.05$). This study revealed that all 12 bacteria evaluated were found in all primary teeth with pulp necrosis. There was no predominance among the species studied; all species had a similar number of individuals.

Keywords: Tooth; Dental Pulp Necrosis; Bacteria; In Situ Hybridization; Fluorescence.

Introduction

Endodontic infections are caused by microorganisms in the pulp cavity. Pulpal infections in primary teeth are related to microorganism incursion and multiplication in the pulp chamber and root canals. Depending on the virulence and the number of microorganisms in the root canal, acute or chronic inflammation can be established in the periapical region.[1,2,3,4] Pathological microorganisms in endodontic infections can cause abscess,

mobility, severe pain, swelling, cysts, and even tooth loss. Moreover, they can recur after treatment.[3]

Endodontic treatment of primary teeth is designed to keep the teeth in healthy condition until physiological exfoliation. Treatment success is directly related to decreasing the number of microorganisms in the root canals.[5] Recognizing the role played by bacteria in the pathogenesis of pulp and periapical infections makes the elimination of endodontic infection the treatment goal for teeth with necrotic pulp and periapical lesions.[6] Thus, it is important to have in-depth knowledge of microbial diversity, including not only the number of species (species richness), but also the number of individuals of each species (uniformity). From an ecological perspective, a microbial community may change in regard to the number of individuals per species, in response to changing conditions that favor their growth.[7] This characterization of microbial diversity in root canals of primary teeth can enable the development of more effective pulp therapies. However, knowledge of the microbiota in the endodontic infection of primary teeth is far from complete, especially in regard to component uniformity.

Few studies have assessed the microbiota of primary teeth with necrosis.[4,6,8,9,10,11] The techniques for culturing anaerobic bacteria have shown that endodontic infections are polymicrobial. However, the diversity of bacteria in any environment is almost always underestimated when evaluated by culture-based techniques. Molecular methods for detecting microorganisms are being used with greater frequency, because they allow the species unidentified by culture methods to be identified, and also the species to be directly determined from the clinical sample.[12] Molecular methods, such as polymerase chain reaction (PCR), DNA-DNA hybridization, denaturing gradient gel electrophoresis (DGGE) and pyrosequencing, are being used to identify bacterial species that are difficult to cultivate, such as bacteria of the genera *Tannerella*, *Treponema*, *Prevotella,* and *Porphyromonas*.[3,4,8-,9,10,11,13,14] A large interindividual variability was observed in analyses by DGGE, and suggested the existence of polymicrobial communities.[11]

The fluorescence *in situ* hybridization (FISH) technique allows individual microbial cells to be visualized, identified, and quantified, and is considered a quick and objective technique for direct quantification of microorganisms.[15,16] The aim of the present study was to conduct a qualitative and quantitative evaluation of the bacteria in the root canals of primary teeth with necrotic pulp, using the FISH technique.

Methodology

Thirty-one children (13 females and 18 males), ranging in age from 4 to 9 years (average age 6.29 ± 1.27 years), were recruited from 53 children referred for pulp treatment to the Department of Pediatric Dentistry at the School of Dentistry, Universidade Federal de Juiz de Fora, Brazil. This study was approved by the human research ethics committee of the university (protocol number 226.775). Written informed consent was obtained from the parents of the children enrolled in the study.

One tooth per child was selected as a sample specimen to avert problems of correlation between observations, such as the analysis of more than one selected tooth per individual. Therefore, thirty-one root canals (n = 31) were selected from primary molars (five upper molars and 26 lower molars). A single canal represented the sample used to limit each bacterial evaluation to a single ecological environment.[19] In multiradicular teeth, the canal with the largest caliber was chosen (the palatine canal in the upper molars, and the distal canal in the lower molars), as performed in previous studies.[9,19] The sample size was also based on other Brazilian studies that used 25,[14] 30,[8] 31[17] and 32[9] samples.

The following inclusion criteria were applied: root canals of primary teeth diagnosed with pulp necrosis; with or without periradicular or interradicular inflammation; with intact or non-intact roots, or with resorption less than 2/3; without previous endodontic treatment; enabling absolute isolation of the operative field; and having subsequent restoration. The exclusion criteria were as follows: children having used systemic antimicrobial medication in the three months before the initial exam, using antimicrobial mouthwash, or having a systemic disease.

A clinical chart was filled out for each participant, with identification data and anamnesis. Next, clinical and radiographic exams were performed on the tooth to be treated, in order to record the following signs and symptoms, and classify them as being present or absent: pain; edema; fistula; radiolucency in the periapical or furcation region; and pathological root resorption. These signs and symptoms were adopted in precursory studies.[18,19,20]

Specimen sampling and root canal treatment

First, the children rinsed their mouth for 1 minute with 0.12% chlorhexidinegluconate (Periogard®, Colgate-Palmolive Brasileira, Osasco, Brazil), to ensure antisepsis of the oral cavity. Then, the following procedures were performed to administer local anesthesia: absolute isolation and disinfection of the operative field was ensured using 1% chlorhexidinegluconate (Farmácia Cavalieri®, Juiz de Fora, Brazil), all decayed tissue was removed, and the operative field was again disinfected. After wards, access to the canals was made with sterile diamond burs (KG Sorensen®, Cotia, Brazil), and the canals were irrigated with a small amount of sterile saline. The samples were collected with a type K #15 file (Dentsply/Maillefer Instruments, Ballaigues, Switzerland). This file was inserted up to the working length, delimited by the initial X-ray, and set at 1 mm short of the radiographic apex, or the physiological root resorption limit, and smooth filing movements were made for 1 minute.[19] The file was transferred to a microtube containing 2 mL of 2% paraformaldehyde to preserve the specimen for microbiological analysis. The root canals were cleaned and filled according to a previously described technique.[21] K-files (Dentsply/Maillefer Instruments, Ballaigues, Switzerland) were used to perform the mechanical treatment of the root canals. Initial irrigation was performed with 2.5% sodium hypochlorite (NaClO 0.5%; Farmácia Cavalieri®, Juiz de Fora, Brazil) and Endo-PTC (Fórmula & Ação Farmácia Magistral, São Paulo, Brazil). Final irrigation was made with Tergentol-Furacin, and root canal fillings were completed with Pasta Guedes-Pinto (Fórmula & Ação Farmácia Magistral, São Paulo, Brazil), a paste composed of Rifocort®, iodoform, and camphorated paramonochlorophenol. The pulp chamber was filled with ZOE cement (Pulpo-San; SS White, Rio de Janeiro, Brazil), and the tooth was sealed with self-curing glass ionomer cement (Vidrion-R; SS White, Rio de Janeiro, Brazil) until completely restored. All the steps of the specimen sampling and root canal treatment were performed by an experienced specialist researcher (SSL) in pediatric dentistry and endodontics.

Microbiological analysis

The microbiological analyses were performed by another trained researcher (SWP), who was blinded to the clinical characteristics of the cases under study. Each sample was placed in a vortex, and the contents of the microtube (file + paraformaldehyde) were transferred to a 15 mL Falcon tube. Then, 3 mL of filtered distilled water was added to the sample. The sample was sonicated three times, and filtered using a white polycarbonate filter with a pore size of 0.2 μm. The fluorescence *in situ* hybridization technique was used to identify and quantify the bacteria according to the protocol.[22] Oligonucleotide probes, 16S rRNA (Operon Technologies, USA) were used, marked with Cy3 fluorochrome (indocarbocyanine) to identify bacteria of the *Streptococcus* genus and 11 species of bacteria (Table 1). A probe without any specificity (5'-CCTAGTGACGCCGTCGAC-3') was also used as a negative control to assess hybridization efficiency. Thus, each filter (sample) was divided into 13 parts, a piece for each probe used.

The probes were diluted with hybridization solution to a final concentration of 2.5 ng μL^{-1}. Hybridization solutions had a specific formamide concentration for each probe (Table 1). Aliquots (40 μL) of the diluted probe were placed on a slide coated with parafilm. The slides were incubated in a sealed container (hybridization chamber) at 42°C for at least 4 hours. After hybridization, the samples were transferred to a wash solution with a specific NaCl concentration (Table 1). They were then stained with DAPI (4',6-diamidino-2-phenylindole), dipped three times in 80% alcohol, and placed on absorbent paper to dry. The slide mount was created by placing the filter pieces between the slide and the coverslip, using a glycerol-PBS solution in a proportion of 7:3. The bacterial cells were counted in ten random fields

with an Olympus BX60 epifluorescence microscope equipped with filter sets, a 31000 filter for DAPI, and a 41007 filter for Cy3 at 1000x magnification. The results found for the hybridized samples used a probe without specificity, and were discounted from the densities of the specific probes. The total density of microorganisms was calculated by counting the cells stained with DAPI.

Statistical analysis

All the data were processed by SPSS software (15.0, SPSS, Chicago, USA). Descriptive measures (average, standard deviation, median, minimum and maximum values) were used to explain the continuous variables related to the density (cells/mL x 10^8) of the tested bacteria. The Student's *t*-test was used to detect differences in the density of bacteria in patients with or without the signs and symptoms evaluated (pain, swelling, fistula, radiolucency in the periapical and/or furcation region, and pathological root resorption). The densities of all microorganisms were correlated (paired samples). The significance level for all analyses was set at 5%.

Results

Among the 31 children included in the study, pain was symptomatic for 77.42% of the patients; 38.71% of the teeth presented fistula and radiolucency; 29.03%, edema; and 16.13%, pathological root resorption.

The descriptive measures of the density of the bacteria identified in the 31 samples of primary teeth with pulp necrosis can be seen in Table 2. The twelve bacteria tested were found in all the samples. Only average densities of *F. nucleatum* and *S. mutans* were significantly different among the samples (p = 0.006).

Figure 1 shows the proportion of bacteria identified with the twelve probes. Sample 7 was the sample with the lowest number of identified bacteria (29.72×10^8 cells/mL from a total of 46.92×10^8 cells/mL). On the other hand, sample 28 presented the highest number of identified bacteria (45.26×10^8 cells/mL from a total of 48.11×10^8 cells/mL). The total bacteria-specific mean density (excluding values found for the *Streptococcus* genus) was 27.11×10^8 cells/mL, and corresponded to approximately 74% of the bacterial community of the root canals of primary teeth with pulp necrosis.

Densities of *T. denticola* in children with pain symptoms were significantly higher (p = 0.02) than those of *T. denticola* found in children without pain (Figure 2A). Primary teeth with edema also presented significantly higher densities of the *P. nigrescens* species (p = 0.04) and the *Streptococcus* genus (p = 0.04), compared with teeth without edema (Figure 2B).

Table 1. Oligonucleotide probes of rRNA from microorganisms used in this study. All probes were labeled with Cy3 fluorochrome.

Probe	Specificity	Probe sequence	% FA*	NaCl (mM)**	Formamide concentration	Reference
ACAC	*Aggregatibacter actinomycetemcomitans*	TCCATAAGACAGATTC'	30	112	30	Sunde et al. (2003)[23]
B/TAFO	*Tannerella forsythia*	CGTATCTCATTTTATTCCCCTGTA	20	225	30	Sunde et al. (2003)[23]
CARE	*Campylobacter rectus*	TTAACTTATGTA AAGAAG	35	80	20	Riep et al. (2009)[24]
Efs129	*Enterococcus faecalis*	CCCTCTGATGGG TAGGTT	40	56	35	Behr et al. (2000)[25]
FUS664	*Fusobacterium nucleatum*	CTTGTAGTTCCG C(C/T)TACCTC	30	112	40	Thurneer; Gmür; Guggenheim (2004)[26]
MUT590	*Streptococcus mutans*	ACTCCAGACTTT CCTGAC	40	56	30	Trebesius et al. (2000)[27]
Pint649	*Prevotella intermedia*	GCCGCCRCTGA ASTCAAGCC	40	56	40	Gmür; Thurnheer (2002)[16]
Pnig657	*Prevotella nigrescens*	TCCGCCTGCGCTGCGTGTA	30	112	40	Gmür; Thurnheer (2002)[16]
POGI	*Porphyromonas gingivalis*	CAATACTCGTATCGCCCGTTATTC	30	112	30	Sunde et al. (2003)[23]
TRE II	*Treponema denticola*	GCTCCTTTCCTC ATTTACCTTTAT	40	56	30	Moter et al. (1998)[28]
SOB	*Streptococcus sobrinus*	TTAACTCCTCTT ATGCGG	30	112	40	Trebesius et al. (2000)[27]
STR	*Streptococcus*	TAGCCGTCCCTT TCTGGT	30	112	30	Trebesius et al. (2000)[27]

*Percentage of formamide (FA) in hybridization solution; **NaCl concentration in wash solution.

Table 2. Descriptive measurements (average, standard deviation, median and maximum and minimum numbers) for density of bacteria (cell/mL x 10^8) in primary teeth with pulp canal and chamber necrosis.

Bacteria	Descriptive measurements				
	Average	Standard deviation	Median	Minimum	Maximum
A. actinomycetemcomitans	2.46	2.04	1.82	0.52	9.76
C. rectus	2.74	0.78	2.73	1.08	4.84
E. faecalis	2.05	0.76	2.21	0.35	3.24
*F. nucleatum**	3.12	2.14	2.65	0.56	11.27
P. gingivalis	2.32	0.55	2.45	1.31	3.13
P. intermedia	2.33	0.90	2.18	0.76	5.44
P. nigrescens	2.59	1.55	2.42	1.15	9.70
Streptococcus	2.53	1.45	2.35	0.13	7.86
*S. mutans**	1.90	0.84	1.71	0.49	4.04
S. sobrinus	2.38	0.97	2.20	0.52	4.36
T. forsythia	2.40	1.07	2.16	0.62	6.01
T. denticola	2.82	1.33	2.39	1.24	7.23
Total microorganisms	36.83	8.72	37.76	20.69	56.31

*Significant difference ($p < 0.05$) between *F. nucleatum* and *S. mutans*.

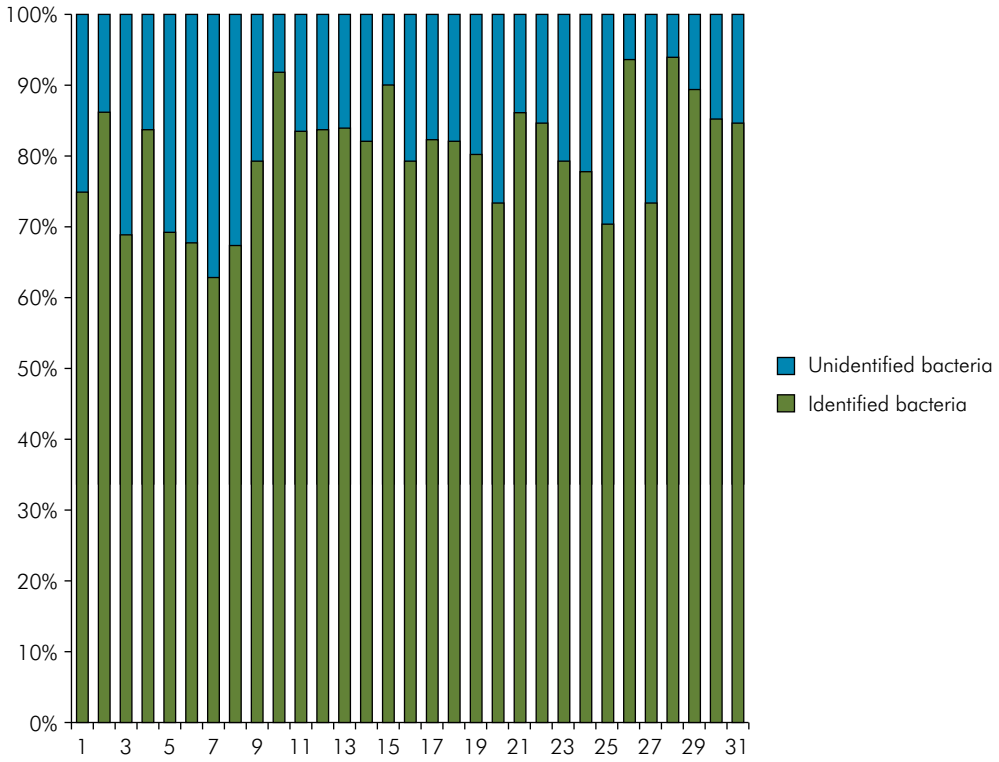

Figure 1. Average proportion of identified bacteria with the microorganisms stained with DAPI in all 31 primary teeth with pulp necrosis.

The density of *A. actinomycetemcomitans* was not significantly correlated with the density of any other bacteria studied. Table 3 shows the correlations between the densities of other bacteria. A high correlation (*r* between 0.8 and 0.6) was found between *F. nucleatum* and *P. nigrescens* ($p< 0.001$), and between *S. mutans* and *S. sobrinus* ($p< 0.001$). The density of *F. nucleatum* was moderately correlated

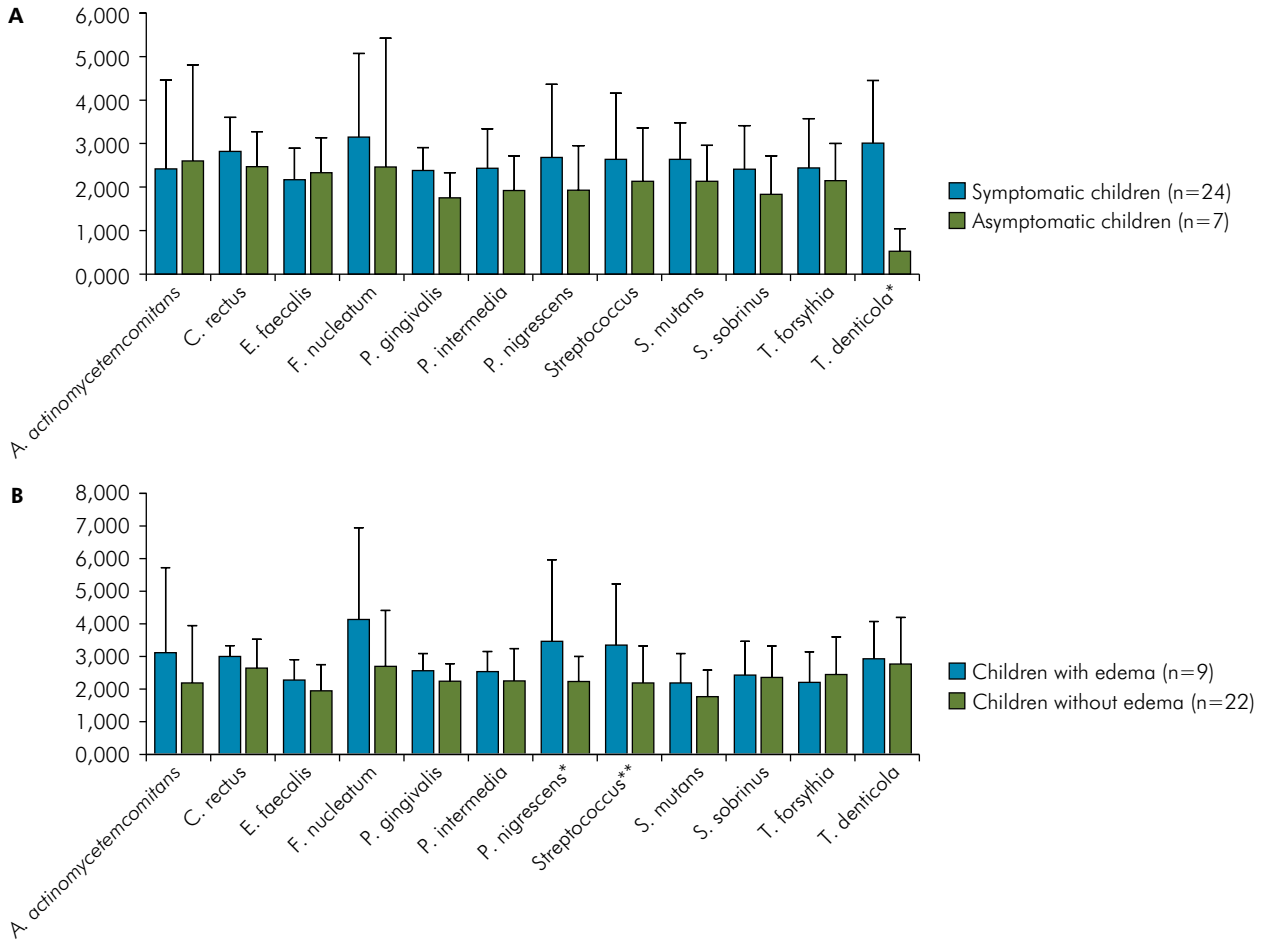

Figure 2. A.Density of bacteria (cells $10^8 mL^{-1}$) in children with and without pain symptoms. Bars = standard deviation, and asterisks = significant difference.B. Density of bacteria (cells $10^8 mL^{-1}$) in children with and without edema. Bars = standard deviation, and asterisks = significant difference.

(*r* between 0.6 and 0.4) with the density of *P. intermedia* (p = 0.022). The density of *S. sobrinus* was significantly correlated with the density of six other bacteria. Additionally, the density of *E. faecalis* was correlated with the densities of seven other analyzed bacteria. *Streptococcus* density was correlated with the two species belonging to that genus (*S. mutans* and *S. sobrinus*). Both *Streptococcus* species were also correlated with the density of *C. rectus*, *E. faecalis*, and *T. forsythia*. The density of *S. sobrinus was also* correlated with *P. gingivalis* and *T. denticola*. The densities of the two evaluated species of *Prevotella* (*P. intermedia* and *P. nigrescens*) were moderately correlated with each other (p = 0.003). The moderate correlation of the density of *P. intermedia* and *T. denticola* was extremely significant (p< 0.001).

Discussion

The data obtained demonstrated the polybacterial nature of endodontic infections of primary teeth with a predominance of mandatory and facultative anaerobic bacteria. The bacteria identified corresponded to approximately 80% of the total bacteria (including values found for the *Streptococcus* genus) and approximately 74% of the bacterial community (excluding values found for the *Streptococcus* genus) of the root canals of primary teeth with pulp necrosis. This result underscores that this species of bacteria was appropriately chosen for our test purposes, despite the diversity of species that can exist in cases of infections in root canals with necrotic pulp. It is also worth noting that the bacteria that were identified are

Table 3. Correlation (*r*) between the densities of the bacteria tested in this study.

Bacteria	*C. rectus*	*E. faecalis*	*F. nucleatum*	*P. gingivalis*	*P. intermedia*	*P. nigrescens*	*Streptococcus*	*S. mutans*	*S. sobrinus*	*T. forsythia*
E. faecalis	0.394*									
F. nucleatum	-	-								
P. gingivalis	-	0.390*	-							
P. intermedia	-	0.557**	0.411**	-						
P. nigrescens	-	0.387*	0.724***	-	0.509**					
Streptococcus	0.380*	-	-	0.413*	-	-				
S. mutans	0.525**	0.577**	-	-	-	-	0.403**			
S. sobrinus	0.490**	0.401**	-	0.535**	-	-	0.391*	0.697***		
T. forsythia	-	-	-	0.372*	0.535**	-	-	0.426*	0.562**	
T. denticola	-	0.371*	-	-	0.567***	-	-	-	0.469**	0.432**

Asterisks represent significance levels: *low correlation (*r* between 0.4 and 0.1); **moderate correlation (*r* between 0.6 and 0.4); ***high correlation (*r* between 0.8 and 0.6).

included among the bacterial genera most frequently detected in primary endodontic infections.[29]

Knowledge of this polymicrobial nature of bacteria is of utmost importance, because their behavior can change, and become more virulent due to stresses arising from adverse environmental conditions, such as an increase in population density, lack of nutrients, and changes in pH and temperature. Under these conditions, the microorganisms develop an adaptation or defense mechanism, whereby they communicate intracellularly through molecular signaling, aligned with concepts of the biofilm and microbial community. Thus, the intra- or interspecies relations increase the chance of the microorganisms to survive in the environment, and the infection to become more resistant.[30]

The heterogeneity of the oral microbiota was greater than previously believed. The microbiota of the root canals of primary teeth were previously investigated by culture and molecular methods.[9,10,17,18,19,31] The cultivation technique can be fastidious. Additionally, some microorganisms are uncultured and cannot be identified by this methodology. Endodontic microbiota has been clearly redefined by molecular methods. Thus, variations in prevalence data are changing, and the species richness and uniformity values are increasing. However, in some cases, the number of bacteria present in the environment is below the level of detection by the technique chosen, there by masking its prevalence. Furthermore, dead and inactive bacteria are included in the quantification using molecular techniques.[9]

FISH has a 10^3cell detection limit,[32] but may concentrate larger sample volumes to increase the number of cells. This technique is a rapid and objective method that provides direct quantitative results, without a prior culture or amplification of nucleic acids. The bacterial individuals can be visualized and quantified by microscopy or flow cytometry. Viewing through a microscope also allows analysis of morphological characteristic of microbial cells and spatial distribution patterns (aggregates, adhered and isolated cells).[15,33] Microscopic study shows that dense aggregate-forming bacteria in the root canal wall are formed by various different morphological types, suggesting their co-aggregation.[29]

However, errors may occur, mainly in cases whose target microorganisms are autofluorescent. FISH requires a targeted investigation, because the specificity of the probes used can lead to false positive results. The use of positive and negative control samples, and the choice of probes labeled with Cy3 offset possible limitations.[15,34] As previously described in the methods section, we used a negative control here in, and chose to use probes labeled with Cy3 specifically to offset limitations of the FISH technique.

Fusobacterium nucleatum was, on average, the most abundant bacterium in the root canal system of the examined teeth, and was significantly different only from *S. mutans* regarding average density. *F. nucleatum* is considered an intermediate colonizing species, and can be found in both symptomatic and

asymptomatic infections. It may facilitate invasion by other bacteria, triggered by physical and chemical changes. High prevalence and ability to co-aggregate with other species may be related to its low proteolytic capability.[33,35] Although also present in all the samples, *S. sobrinus* had the lowest average bacterial density. The difference in prevalence can be explained by possible exposure of only a few canals to the oral cavity, an outcome which would increase the presence of *S. mutans*.[17] In this study, none of the teeth selected and treated had root canals that communicated with the oral environment.

An important result of this study was the observation of the *E. faecalis* species in all samples. Few studies conducted with molecular methods have previously observed *E. faecalis* in deciduous teeth,[9,14,19,31] but none of them with the frequency which we observed. *E. faecalis* is an opportunistic pathogen that can withstand chemical and mechanical treatment, and also high pH levels, owing to its biofilm formation potential, and to the operation of a proton pump that reduces intracytoplasmic pH.[14,36,37] Although *E. faecalis* is the most common pathogen associated with secondary infection in permanent teeth,[14,38,39] the authors did not associate this species with the failure of endodontic treatment of primary teeth. Even so, *E. faecalis* can acquire resistance to the antimicrobials commonly used in endodontic treatments. It should be highlighted that the ability to detect how and why this bacterium favors or rejects different endodontic infections is a matter of particular interest.[36,37,39]

Infections of the root canal space with Gram-negative, facultative, and obligate anaerobic bacteria have been associated with different clinical signs and symptoms. Sensitive and accurate molecular techniques are needed to characterize the root canal bacterial irritants, in order to determine their association with clinical symptoms and treatment prognosis.[19] There was no correlation between the number of bacteria and the presence of fistula, injury or pathological root resorption in our samples.

Teeth with edema had more bacteria of *Streptococcus* genus and *P. nigrescens* species than teeth without edema. These bacteria are frequently associated with acute apical abscesses, as well as other Gram-negative bacilli, including *Fusobacterium*, *Porphyromonas*, *Dialister* and *Treponema*.[40]

A meta-analysis showed the heterogeneity in the prevalence rates of the *Treponema* species in primary and secondary infections, whether symptomatic or not.[41] The authors of a previous study emphasized the importance of these species, particularly in cases of acute primary infection. The participation of *T. denticola* in the pathogenesis of acute apical abscess was evaluated using real-time PCR.[13] The authors of that study found an association between the bacteria of the species and symptomatic infections.[18,19] They proved in their study that *T. denticola* and *E. faecalis* bacteria were highly associated with periapical radiolucency and previous pain, whereas *P. gingivalis* was associated with tenderness to percussion in both primary and permanent teeth. In our study, bacteria of the *T. denticola* species were found in all children's teeth evaluated (100% prevalence). Using the FISH technique, we were able to quantify children with pain symptoms as having more *T. denticola*. In a bacterial community, *T. denticola* does not commonly become more virulent, or increase the virulence of other community species.[18]

Bacterial correlations such as *Porphyromonas* spp./*Prevotella* spp. and *P. gingivalis*/*Enterococcus* spp. were detected, suggesting that these bacteria are able to survive in necrotic tissues, whether alone or in association. The availability of nutrients, low oxygen tension and bacterial interactions are important ecological determinants for these bacteria in root canals with necrotic pulp.[6] *P. gingivalis* has been described as a species with proteolytic enzymes and more pathogenic microorganisms, identified from its black pigment. The association of different bacterial species in infected root canals of primary teeth seems to activate the immune response of the host. However, these same bacteria may compete for the same receptor, or may activate different receptors interfering with the transcription of inflammatory proteins.[1]

Again, the quantification of specific bacteria can contribute to the understanding of this process, since a larger number of bacterial individuals of a given species may proportionally make these individuals better competitors. Likewise, the

concentration of endotoxin is related not only to the microorganisms that produce the bacteria, but also to the metabolic activity of the bacteria and the number of individuals. Whereas all are active, the greater the number of individuals, the greater the production. The degree of severity of an endodontic infection is not related to the mere presence of pathogens, but to the number of these microorganisms at the infected site.[42]

Conclusion

In conclusion, this study showed that all 12 bacteria evaluated were found in all primary teeth with pulp necrosis. There was no predominance among the species studied; all species had a similar number of bacterial individuals. Not only the prevalence, but also the component uniformity of the diversity confirmed the nature of the polymicrobial infection.

Our results provide information on bacterial diversity, such the number of species, and the number of individuals of each species of bacteria present in the root canals of primary teeth, detected using the FISH technique. This method has proven effective in detecting, identifying and enumerating the microbiota of primary teeth with pulpal necrosis. This information improves our effective management of infections of endodontic origin in primary teeth.

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