

Prevalence of microorganisms in root canals of human deciduous teeth with necrotic pulp and chronic periapical lesions

Prevalência de microrganismos em canais radiculares de dentes decíduos de humanos com necrose pulpar e lesão periapical crônica

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ABSTRACT: The objective of this study was to evaluate bacterial prevalence in 31 root canals of human deciduous teeth with necrotic pulp and periapical lesions using bacterial culture. After crown access, the material was collected using absorbent paper points for microbiological evaluation and determination of colony forming units (CFU). Anaerobic microorganisms were found in 96.7% of the samples, black-pigmented bacilli in 35.5%, aerobic microorganisms in 93.5%, streptococci in 96.7%, and *S. mutans* in 48.4%. We concluded that in human deciduous teeth root canals with necrotic pulp and periapical lesions the infection is polymicrobial, with a large number of microorganisms and a predominance of streptococci and anaerobic microorganisms.

DESCRIPTORS: Tooth, deciduous; Bacteriological techniques; Periapical abscess.

RESUMO: O objetivo deste estudo foi avaliar, por meio de cultura bacteriológica, a prevalência de microrganismos em 31 canais radiculares de dentes decíduos humanos com necrose pulpar e lesão periapical. O material, colhido dos canais radiculares após a realização da cirurgia de acesso, foi submetido ao processamento microbiológico para a determinação das unidades formadoras de colônia de microrganismos. Os resultados mostram que os microrganismos anaeróbios foram quantificados em 96,7% dos casos, os bacilos pigmentados de negro (BPB) em 35,5%, os aeróbios em 93,5%, os estreptococos em 96,7% e os *S. mutans* em 48,4%. Assim, pôde-se concluir que a infecção em canais radiculares de dentes decíduos humanos portadores de necrose pulpar e lesão periapical é polimicrobiana, com grande quantidade de microrganismos e maior prevalência de estreptococos e microrganismos anaeróbios.

DESCRIPTORIOS: Dente decíduo; Técnicas bacteriológicas; Abscesso periapical.

INTRODUCTION

Although oral health education and prevention are priorities in contemporary dentistry, pulp changes in deciduous teeth are very frequent. Thus, curative dentistry should simultaneously evolve and improve concepts and therapeutic procedures.

Despite the controversy, many authors report the need to treat root canals of deciduous teeth with pulp necrosis^{5,6,12} due to the propagation of microorganisms throughout the entire root canal system, including the lumen, lateral, accessory

and secondary canals, dentinal tubules, ramifications of the apical delta, apical foramen, areas of apical cementum resorption and periapical biofilm^{7,11}.

The success of endodontic treatment depends on many factors and the reduction or elimination of bacterial infection^{9,21} is the most important one. However, for this to occur, it is important to identify which microorganisms are present. Little research has been done to identify which bacterial species are present^{3,4,6,10,13,20,21} in deciduous teeth with pulp necrosis and periapical infection.

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Therefore, the aim of this study was to evaluate the bacterial profile in root canals of human deciduous teeth with necrotic pulp and periapical lesions.

MATERIAL AND METHODS

Clinical procedures

The research protocol was approved by the Institution's Ethics Committee (#2000.1.488.58.7).

Fifteen 3- to 7-year-old children, of both sexes, seen at the Pediatric Dental Clinic, School of Dentistry of Ribeirão Preto (University of São Paulo) were selected. None of the children had been treated with antibiotic for at least 3 months. A total of 31 root canals from 18 deciduous teeth (maxillary incisors and canines, and maxillary and mandibular molars) with necrotic pulp and radiographically visible radiolucent areas in the region of the bone furcation and/or the periapical region suggesting chronic periapical lesion were used. The teeth had carious lesions, some with the pulp chamber exposed to the oral cavity. However, it was possible to isolate the surgical field with a rubber dam and restore the tooth. They also had intact roots or less than 2/3 of physiological root resorption, no periodontal pocket and no previous root canal intervention. Among the teeth treated, 19 root canals from 13 teeth had fistulae.

After antisepsis of the oral cavity by rinsing for 1 min with 5.0 ml of 0.12% chlorhexidine digluconate (Periogard, Colgate-Palmolive Ind. Brasileira, Osasco, SP, Brazil), local anesthesia was applied with 2.0% mepivacaine (DFL Ind. e Comércio Ltda., Rio de Janeiro, RJ, Brazil). A rubber dam was placed and 1.0% chlorhexidine digluconate was used for antisepsis.

All carious tissue was removed with curettes and low-speed spherical burs, and antisepsis was performed again. High-speed spherical diamond burs, cooled with air and water, were used for surgical access of the root canals. Compensatory wearing was carried out with a high-speed Endo-Z stainless steel bur (Les Fils d'August, Maillefer, Ballaigues, Switzerland), cooled with air and water, and followed by irrigation/suction with sterile saline (School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, SP, Brazil).

Absorbent paper points (Tanari Industrial Ltda., Manaus, AM, Brazil) were used for collection of root canal samples. At the largest end of the paper points, "wings" made out of a stainless steel matrix for amalgam (0.5 mm) were adapted in order to prevent them from floating when placed in test tubes containing solution (reduced transport

fluid - RTF). The different caliber paper points were placed individually in 12 x 75 mm tubes, which were then labeled and closed with cotton and sterilized in an autoclave at 120°C, for 20 min.

Bacteriological samples were collected immediately after crown access by introducing 3-4 sequential sterile absorbent paper points, of a size visually compatible with the root canal diameter, 2-3 mm before the radiographic apex or the limit of the physiological root resorption, as determined by diagnostic radiographs.

After approximately 1 min, the paper points were removed from each root canal and placed in a test tube (13 x 100 mm) containing 2.0 ml of reduced transport fluid (RTF), prepared according to Syed and Loesche¹⁹, that is recommended for the maintenance of viable anaerobic microorganisms. The tube was closed hermetically and processed for microbiological analysis.

After samples collection, the root canals were treated for the immediate and progressive neutralization of septic/toxic content using K-files and copious irrigation/suction with 2.5% sodium hypochlorite followed by odontometry 1 mm before the radiographic apex or the limit of the physiological root resorption. Biomechanical preparation was carried out with sequential K-files and irrigation with 2.5% sodium hypochlorite. The canals were subsequently dried with sterile absorbent paper points and filled with EDTA (Odashcam Herpo Produtos Dentários Ltda., Rio de Janeiro, RJ, Brazil), and mixed for 3 min with a K-file to remove smear layer. The canals were irrigated and dried, and then filled with calcium hydroxide paste (Calen PMCC, S.S. White Artigos Dentários Ltda., Rio de Janeiro, RJ, Brazil) with a special syringe (ML, S.S. White Artigos Dentários Ltda., Brazil). The pulp chamber was sealed with zinc oxide/eugenol cement (IRM, Dentsply Indústria e Comércio Ltda., Petrópolis, RJ, Brazil). After 14-30 days, the intracanal dressing was removed and the canals were filled with Calen thickened with zinc oxide, as recommended by Silva and Leonardo¹⁵, and restored.

Microbiological processing

The tubes containing the samples were mixed with 4-6 sterile glass beads in a mixer (Mixtron-Toptronix, SP, Brazil) for 2 min at maximum speed. Subsequently, serial decimal dilutions up to 10⁻⁵ were made in Sorensen Phosphate Buffer (PBS) under laminar airflow. A volume of 0.05 ml of the pure samples and of each dilution was sown, with a sterile calibrated pipette, onto plates containing As (Blood agar, Difco, Detroit, USA), Ms (Mitis

Salivarius agar, Difco, Detroit, USA) and Ask media (Blood agar supplemented with hemin and menadione, Sigma Chemical Co., St. Louis, USA). Plates containing SB₂₀ agar (Bacitracin sucrose agar, Difco, Detroit, USA) received dilutions only up to 10⁻¹. The samples were then distributed uniformly from the most diluted to the least diluted using a sterile glass L-shaped rod.

The Ask plates were incubated anaerobically using the Gas-Pak system, in hermetically sealed jars (Permutation, Equipamentos e produtos químicos Ltda., Curitiba, Brazil) for 7-10 days. The Ask plates were incubated aerobically for 24-48 h, at 37°C, and the Ms and SB₂₀ plates were incubated for 3 days in microaerophilic conditions (candle jar system) in a hermetically sealed jar. After incubation, colonies were counted with a stereomicroscope (Nikon, Tokyo, Japan) under reflect light and the colony forming units (CFU/ml) were calculated.

From the SB₂₀ agar plates, 3-4 colonies suspect of being *mutans* streptococci were isolated and identified according to Ito *et al.*⁸: fermentation of mannitol, sorbitol, raffinose and melibiose; hydrolysis of arginine and sculin; and sensitivity to bacitracin.

RESULTS

The CFU (colony forming units) of the samples from 31 root canals from deciduous teeth with necrotic pulp and radiographically visible periapical lesion are presented in Table 1.

The prevalence of anaerobic microorganisms was 96.8% (30 root canals), and black-pigmented bacillus (BPB) were found in 11 cases (35.5%). Aerobic microorganisms were present in 29 root canals (93.5%) with streptococci present in 30 canals (96.8%). *Streptococcus mutans* was quantified in 15 canals (48.4%) whereas *Streptococcus sobrinus* was not detected.

DISCUSSION

During the 1950s and 1960s, researchers isolated mainly aerobic and facultative bacterial species from root canals with necrotic pulp and periapical lesions due to the limitation of isolation techniques and microbial culture¹⁴. With scientific and technological evolution, anaerobic techniques have been developed and, in the 1980s, this concept was modified showing that in root canals of permanent teeth with necrotic pulp and periapical lesions there is a polymicrobial infection with predominance of strict anaerobic species^{1,17,22}.

The microbiota is constituted of only a few species when compared to the total bacteria of the oral cavity. There are many factors that can influence the growth and development of these microorganisms in root canals, i.e., nutrient availability, low oxygen tension, bacteria interaction, as well as disintegrated pulp tissue and tissue fluids that are essential nutrient sources¹⁷.

In the present study, anaerobic microorganisms were detected in 96.8% of the samples. These results are in agreement with those of Toyoshima *et al.*²¹, Sato *et al.*¹³ and Faria⁶, who reported that in root canals of primary teeth with periapical lesions there is a polymicrobial infection with predominance of anaerobic microorganisms, similar to those of the microbiota of permanent teeth.

Black-pigmented bacilli (BPB) have frequently been isolated from root canals of permanent teeth with necrotic pulp. Sundqvist *et al.*¹⁸ reported their presence in 30% of the cases while Assed *et al.*¹ verified by immunofluorescence that these microorganisms were found in 60% of the samples. In the present study in deciduous teeth, BPB were found in 11 cases (35.5%), a figure which is similar to those found in reports by Tomic-Karovic, Jelinek²⁰ and Faria⁶ who detected these microorganisms in 36% and 30%, respectively, of the root canals of deciduous teeth with necrotic pulp. However, Toyoshima *et al.*²¹ quantified BPB in 44.4% of deciduous root canals in retreatment cases.

Studies of permanent teeth associate the presence of BPB with the development of abscesses¹⁸. We agree with Faria⁶ who did not observe this correlation when considering deciduous teeth. In the present study, 8 of the 19 root canals with fistulae had BPB. However, this microorganism was also detected in 3 cases in which there were no fistulae.

In this study, aerobic microorganisms were quantified in deciduous teeth with necrotic pulp and chronic periapical lesions in 29 root canals (93.5%). Although Sato *et al.*¹³ and Faria⁶ observed a higher prevalence of anaerobic microorganisms over aerobic microorganisms, in our study prevalence rates were similar (93.5% aerobic and 96.8% anaerobic). These results show that endodontic infections in deciduous teeth, similarly to those in permanent teeth, are polymicrobial with the development of microbial interactions.

The literature shows the presence of streptococci in 70%⁴, in 82%¹⁰, in 76%²⁰ and in 85%⁶ of the root canals of deciduous teeth with pulp necrosis. In our study, streptococci were detected in 30 cases (96.8%). Streptococci and anaerobic microorganisms were the most prevalent bacteria in the

TABLE 1 - Prevalence of microorganisms (CFU) in root canals of human deciduous teeth with pulp necrosis and chronic periapical lesion.

Case	Anaerobic		Aerobic total	Streptococci	
	Total	BPB		Total	<i>S. mutans</i>
1*	500,000	5,400	49,600	51,060	160
2	17,000	120	2,320	2,640	0
3	120	40	240	0	0
4*	8,120	0	0	9,306	0
5*	212,000	0	632,000	222,600	76,900
6*	1,400	40	580	280	0
7*	0	0	760	40	0
8*	214,000	0	21,000	40,200	4,820
9*	1,640	0	80	240	0
10	480	0	80	1,600	0
11	2,000	40	560	620	0
12	7,200	0	1,080	Unc	40
13*	35,600,000	3,240,000	278,000	736,000	144,000
14*	44,440,000	124,000	344,000	2,560,000	196,000
15	22,200	0	5,200	11,600	10,800
16*	2,120,000	0	192,000	680,000	0
17*	921,300	1,420	21,000	94,260	0
18*	3,060,000	280	1,180	400	0
19	53,066	0	16,200	27,400	2,020
20	2,700	0	1,060	720	40
21*	120	0	40	120	120
22*	118,400	0	3,980	58,000	5,680
23*	2,480,000	21,000	234,000	4,920,000	1,220
24*	40	0	0	340	0
25*	340	0	280	680	0
26	609,000	0	86,200	68,200	120
27	768,000	0	9,400	65,200	0
28	506,600	0	76,000	242,000	680
29	541,300	0	244,000	734,660	120
30*	35,800,000	336,000	35,600	4,540	0
31*	736,000	0	52,400	12,200	0
Total of positive cases	30 (96.8%)	11 (35.5%)	29 (93.5%)	30 (96.8%)	15 (48.4%)

*root canals with fistulae; BPB - black-pigmented bacilli; Unc - uncountable.

deciduous teeth with necrotic pulp and periapical lesions.

Mutans streptococci were found in 15 root canals (48.4%); however, only *S. mutans* was found. *S. sobrinus* was not found in any case. Regarding the quantification of *mutans* streptococci in root

canals of permanent teeth, many studies have detected this microorganism, which was found by Stobbering, Eggink¹⁶ in 3.05% of the cases, by Baumgartner, Falkler Jr.² in 33.4%, and by Assed *et al.*¹ in 52.0% of the cases. However, in deciduous teeth root canals only Faria⁶ reported the presence

of *mutans* streptococci (30.0%) in which *S. mutans* was found in 25.0% and *S. sobrinus* in 5.0% of the cases. The variations in the prevalence of these microorganisms in different studies can be explained by the fact that in the cases in which the numbers were higher, some root canals could have been exposed directly to the oral cavity, enhancing the prevalence of *S. mutans*, as observed in our study.

According to the results of this study, anaerobic microorganisms, black-pigmented bacilli, aerobic bacteria, streptococci and *mutans* streptococci are components of root canals of deciduous teeth with periapical lesions. Because of the importance of microorganisms in the etiology of pulp and periapical changes, the lack of *in vitro* and *in vivo* studies evaluating the root canal microbiota of deciduous teeth with different degrees of pulp and periapical

pathoses is not understandable. Thus, there is no consensus on which technique and material is better for the reduction and/or elimination of these microorganisms, so that the deciduous teeth can remain in function in the oral cavity until exfoliation. Therefore, we suggest further research on this subject.

CONCLUSIONS

According to the obtained results, we can conclude that, before biomechanical preparation, the root canals of deciduous teeth with necrotic pulp and chronic periapical lesion presented a high number of microorganisms and polymicrobial infection with the prevalence of anaerobic bacteria and streptococci.

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