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Microorganisms involved in endodontic infection of permanent teeth: A systematic review

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This study investigated the microbial species present in necrotic pulps of permanent teeth needing endodontic treatment. The search for articles was conducted on the Health Science Database and considered all articles published until October 2011, with selected human clinical studies examined, through molecular biology the microorganisms that are present in root canals of permanent teeth requiring root canal treatment with necrosis pulps (exposition). The selected articles were categorized according to the methodological quality and evidence in levels A (high), B (moderate), and C (low). The search strategy was based on PubMed, Bireme, Cochrane and OVID databases. The data extracted from the studies were also tabulated. Eighty-four titles and abstracts were assessed and eight articles met the inclusion criteria, showing that there is a high diversity of microorganisms involved in necrotic permanent teeth. The microorganisms found in these articles were: Porphyromonas gingivalis (27.8%), Porphyromonas endodontalis (42.6%), Prevotella intermedia (5.6%), Prevotella nigrescens (7.4%), Bacteroides forsythus (21%) and Enterococcus faecalis (>50%). This systematic review found high evidence of the polymicrobial nature of primary endodontic infections in necrotic permanent teeth, and also showed that there is a dominance of anaerobic bacteria.

Key words: Microorganisms, endodontic infection, root canal.

INTRODUCTION

The oral cavity is potentially susceptible to a variety of infections due to the presence of numerous proliferative microorganisms that cause fungal, viral and bacterial infections (Riviére et al., 2007; Sakamoto et al., 2007). Dental caries can be characterized as a multifactorial infection directly related to three main factors: Microflora, substrate and host. Some carious processes may develop to the point where dental pulp is affected (Barcelos et al., 2001; Siqueira, 2003). Microbial involvement has been indicated as the main factor associated with the etiology of endodontic diseases (Siqueira et al., 2002, 2005).

Culture procedures have traditionally been used as the reference in the assessment of the microbiota associated with various infectious diseases, including infections of endodontic origin (Siqueira, 2003). Isolation and
identification of infectious agents by phenotypic traits (culture) has some serious pitfalls, most notably the difficulty in simulating the environmental conditions required for most microorganisms to grow. Therefore, non-viable or uncultivable bacteria cannot be isolated using culture methods (Siqueira, 2003; Siqueira et al., 2004; Ruviére et al., 2007).

Microorganism detection methods that identify microbial DNA rather than the microorganisms themselves have been introduced in both research and clinical laboratories and are revolutionizing our knowledge of infectious diseases and allowing effective and rapid diagnosis of many diseases (Siqueira, 2003). Molecular approaches (PCR, Checkerboard, DGGE) have revealed some uncultivable microorganisms involved in endodontic infections, such as Micromonas micros, Fusobacterium nucleatum ss, Tannnerella forsythia, Treponema denticola, Veillonella parvula, Eubacterium nodatum, Porphyromonas gingivalis, Actinomyces odontolyticus, Streptococcus constellatus, Synergistes phylotypes and Enterococcus faecalis (Siqueira et al., 1998, 2002, 2004, 2005, 2007, 2008; Munson et al., 2002; Siqueira and Rôças, 2004a,b, 2005, 2007; Vianna et al., 2005; Seol et al., 2006; Sakamoto et al., 2008).

Knowledge of molecular biology and endodontic infections has increased significantly over recent years, but several questions have yet to be elucidated. For example, in primary infections, not all microorganisms involved, neither the most virulent species have been identified yet (Siqueira, 2003). Despite there are many papers about endodontic microbiology, a systematic review could be more elucidative and this study propose add news aspects in the literature. This paper intends to find evidence of different types of microbial species present in necrotic pulps in permanent teeth needing endodontic treatment.

MATERIALS AND METHODS

A specific protocol and research questions were developed for this study whose research included observational studies that approached the prevalence of microorganisms in root canal. The search for potentially relevant studies was carried out through to October 2011, in the databases PUBMED (1966-2011), BIREME (1967-2011), OVID ALL EMB Reviews (1950-2011), COCHRANE using the MeSH terms: “molecular techniques” AND “microorganisms”, “molecular techniques” AND “oral microorganisms”, “anaerobic bacteria” AND “endodontic”, “molecular techniques” AND “anaerobic bacteria and endodontic” AND “root canal”. Moreover, the reference lists of selected articles and literature review articles were also manually searched.

This review selected human (participants) clinical studies that examine, through molecular biology (PCR) the microorganisms (outcomes) that are present in root canals of permanent teeth requiring root canal treatment with necrosis pulps (exposition). Additional articles of potential relevance were identified by manual searches. Observational controlled study designs composed of microorganism matched for root canal were included without language restrictions. The exclusion criteria were human clinical studies in which patients had used antibiotics 6 months prior to material collection, studies in patients with any systemic or periodontal disease, studies with permanent teeth that have undergone re-treatment or in teeth with a periodontal pocket > 3 mm, and studies with radiographic periapical bone loss.

Textbooks, book chapters dissertations, case reports, case series, review articles, and abstracts were excluded. Two examiners (V.A.C.P. and R.S.P.) independently screened each paper by examining the title, abstract, and keywords. If examiners had diverging opinions, the papers were reexamined until a consensus was reached. If relevant data were missing, the authors of the papers in question were contacted for additional information.

Quality assessment and risk of bias

We performed a quality assessment of the remaining studies to control for influence bias, to gain insight into potential comparisons, and to guide interpretation of findings (Meerpohl et al., 2009). The quality of the studies was evaluated using a checklist presented in Table 1. The selected articles were assessed in accordance with some modifications in the criteria of high quality observational studies proposed by STROBE (Meerpohl et al., 2009). Researchers classified the studies into three categories with scores “A” to “C” according to predetermined criteria for method and performance. For each item, there were 2 options of answer (Yes, No), only one option could be mark for each question. None answer were unclear, when this happened the author was contacted.

The articles were categorized as category A (9-10 points): high methodological quality, when the study included least 9 “yes” of the assessed criteria; Category B (6-8 points): moderate methodological quality, when the study included 8 "yes" of the assessed criteria; Category C (0-5 points): low methodological quality, when the study included 5 "yes" or less assessed criteria. Studies classified as B or C was excluded from this research. The prevalence of microorganisms was recorded.

All data were analyzed by using SPSS (SPSS Inc., Chicago, IL, USA) 16.0 software program for windows. Chi-square test was used to compare the data. Significance was established at 5% (p < 0.05).

RESULTS

The electronic search results are presented in Figure 1. Eighty-four titles and abstracts were assessed and eight articles met the inclusion criteria and their full-text was retrieved. All selected studies were cross-sectional (Siqueira et al., 2001; Siqueira and Rôças, 2004, 2006, 2007; Rôças and Siqueira, 2005; Tomazinho and Avila-Campos, 2007). Checkerboard test was used only in two articles (Siqueira et al., 2002, 2008). The main microorganisms found in included studies were: P. endodontalis, P. gingivalis, P. intermedia and P. nigrescens in all selected articles for quality assessment. P. endodontalis was the most prevalent microorganism, with a strong evidence. The difference between the techniques used to identify microorganisms were not statistic significant (p>0.005). The percentages of the microorganisms most frequently found in the papers are shown in Figure 2. Selected articles were categorized according to the quality of evidence, two articles were considered B and the others C and the data extracted from the studies were tabulated (Table 2).
Table 1. Criteria for quality assessment of the studies included.

<table>
<thead>
<tr>
<th>Item</th>
<th>Quality assessment</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Representative sample size</td>
<td>Yes</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2. Samples collected using strict asepsis methods</td>
<td>Yes</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>3. Same operator for the samples collected</td>
<td>Yes</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>4. Description of data collection</td>
<td>Yes</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>5. Cross-sectional study—if applicable, describe analytical methods taking sampling strategy into account</td>
<td>Yes</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>6. Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable</td>
<td>No</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>7. Potential sources of bias addressed</td>
<td>Yes</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>8. Description of all statistical methods, including those used to control confounding factors</td>
<td>Yes</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>9. Discuss limitations of the study</td>
<td>Yes</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>10. Cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence</td>
<td>Yes</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Positive response (Yes) was equal 1 point and negative response (No) equal 0 point.

Figure 1. Flow diagram of literature search.

Figure 2. Microorganisms most frequently found in the articles.

DISCUSSION

Knowledge of endodontic infections has increased significantly over decades, but several questions still await elucidation. This article has shown that the microflora associated with endodontic infections is diverse. Various microorganisms are associated with primary endodontic infection (Siqueira and Rôças, 2009, 2009). In this paper only studies where patients had a primary infection were analyzed. Black-pigmented anaerobic microorganisms were the most found in the papers (Siqueira et al., 2000, 2001, 2002, 2005, 2007, 2008; Munson et al., 2002; Rôças and Siqueira, 2006; Seol et al., 2006; Sassone et al., 2008; Saito et al., 2009; Siqueira and Rôças, 2009a, b, c). The presence of each...
Table 2. Characteristics of studies included.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Category (Points)</th>
<th>Country</th>
<th>Methods Type of study</th>
<th>Participants</th>
<th>Disinfection</th>
<th>Sample collected by</th>
<th>Cryotubes</th>
<th>Molecular methods</th>
<th>Microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Siqueira et al.</td>
<td>2001</td>
<td>A(9)</td>
<td>Brazil</td>
<td>Cross-sectional</td>
<td>54 18-60 yrs</td>
<td>3% hydrogen peroxide, 2.5% sodium hypochlorite</td>
<td>Sterile paper point</td>
<td>1 ml of TSB</td>
<td>PCR</td>
<td>P. endodontalis (42.6%) P. gingivalis (27.8%) P. intermedia (5.8%) P. nigrescens (7.4%)</td>
</tr>
<tr>
<td>Siqueira et al.</td>
<td>2002</td>
<td>A(9)</td>
<td>Brazil</td>
<td>Cross-sectional</td>
<td>50 Adults⁴</td>
<td>3% hydrogen peroxide, 2.5% sodium hypochlorite solution, 5% sodium thiosulfate</td>
<td># 15 K-type file</td>
<td>1 ml of TSB</td>
<td>PCR and checkerboard</td>
<td>A. actinomycetemcomitans (0%) P. gingivalis (21%) T. denticola (8%) P. endodontalis (16%) B. forsythus (21%) P. micros (0%)</td>
</tr>
<tr>
<td>Siqueira and Rôças</td>
<td>2004</td>
<td>A(9)</td>
<td>Brazil</td>
<td>Cross-sectional</td>
<td>50 18-60 yrs</td>
<td>3% hydrogen peroxide, 2.5% sodium hypochlorite solution, 5% sodium thiosulfate</td>
<td># 15 K-type file</td>
<td>1 ml of TSB</td>
<td>PCR</td>
<td>C. periodontii (14%)</td>
</tr>
<tr>
<td>Rôças and Siqueira</td>
<td>2005</td>
<td>A(9)</td>
<td>Brazil</td>
<td>Cross-sectional</td>
<td>50 Adults⁴</td>
<td>3% hydrogen peroxide, 2.5% sodium hypochlorite solution, 5% sodium thiosulfate</td>
<td># 15 K-type file</td>
<td>1 ml of TSB</td>
<td>PCR</td>
<td>T. parvum (52%) T. putidum (2%)</td>
</tr>
</tbody>
</table>

Species in the included articles is shown in Table 2. None of the articles elucidated the relationships in primary endodontic disease (Siqueira et al., 2002; Sakamoto et al., 2008). P. endodontalis, P. gingivalis, P. nigrescens, P. intermedia appeared in most of the articles found in this systematic review (Siqueira et al., 2001, 2002, 2004; Munson et al., 2002; Seol et al., 2006; Tomazinho and Avila-Campos, 2007; Sakamoto et al., 2008).

Differences between studies may occur for several reasons, including differences in the selection of cases and the methods used for
<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Country</th>
<th>Design</th>
<th>Sample Size</th>
<th>Age Range</th>
<th>Main Procedures</th>
<th>PCR Assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Siqueira and Rôças</td>
<td>2006</td>
<td>Brazil</td>
<td>Cross-sectional</td>
<td>50</td>
<td>18-60 yrs</td>
<td>3% hydrogen peroxide, 2.5% sodium hypochlorite solution, 5% sodium thiosulfate</td>
<td>1 ml TSB, C. morbid (26%), G. adiacens (14%)</td>
</tr>
<tr>
<td>Tomazino and Avila-Campos</td>
<td>2007</td>
<td>Brazil</td>
<td>Cross-sectional</td>
<td>100</td>
<td>Adults</td>
<td>3% hydrogen peroxide, 2.5% sodium hypochlorite solution, 5% sodium thiosulfate</td>
<td>3 mL Möller’s viability medium, PCR</td>
</tr>
<tr>
<td>Siqueira and Rôças</td>
<td>2007</td>
<td>Brazil</td>
<td>Cross-sectional</td>
<td>50</td>
<td>Adults</td>
<td>3% hydrogen peroxide, 2.5% sodium hypochlorite solution, 5% sodium thiosulfate</td>
<td>1 ml TSB, PCR</td>
</tr>
<tr>
<td>Sassone et al.</td>
<td>2008</td>
<td>Brazil</td>
<td>Cross-sectional</td>
<td>60</td>
<td>Adults</td>
<td>3% hydrogen peroxide, 2.5% sodium hypochlorite solution, 5% sodium thiosulfate</td>
<td>Eppendorf tube containing 150 µl TE, Checkerboard</td>
</tr>
</tbody>
</table>

* = Not reported.
sampling, sample transport and identification. The higher sensitivity of the PCR method compared to culture is probably the reason for the higher prevalence of Porphyromonas species (Siqueira et al., 2001, 2002; Munson et al., 2002; Rôças and Siqueira, 2008; Siqueira and Rôças, 2009). In addition the PCR methods allow detection of uncultivable strains of these species (Zoleti et al., 2006). P. endodontalis and P. gingivalis were found by the authors of three articles, as shown in Table 2. The “Red complex” (Bacteroides forsythus, Porphyromonas gingivalis, and Treponema denticola) has been associated with primary endodontic infections (Rôças et al., 2001; Siqueira and Rôças, 2009), and was also reported in this systematic review. Tomazinho and Avila-Campos (2007), using PCR, analyzed 60 samples and the DNA of the following organisms was detected: P. gingivalis (43.3%), P. nigrescens (43.3%), P. intermedia (31.7%) and P. endodontalis (23.3%). Saito et al. (2009) detected P. gingivalis in 28% of the subjects analyzed and T. forsythia was detected in 18% to 24% of the primary endodontic infections by conventional PCR.

The PCR is used to investigate the prevalence of bacterial species in infected root canals (Siqueira and Rôças, 2003). The major reagents to be used in PCR are the target DNA to be amplified, single-strand oligonucleotides (primers) complementary to known sequences of the target DNA, the four deoxyribonucleoside triphosphates (dNTPs), and a heat-stable DNA polymerase. Amplification reactions are carried out using special DNA thermocycles (Siqueira and Rôças, 2003, 2004). Nested PCR has increased sensitivity and possibly also improved specificity when compared with PCR (Siqueira and Rôças, 2004). Increased sensitivity is a function of the larger total number of cycles used in nested PCR assays. In addition, target DNA is amplified in the first round of amplification, with subsequent dilution of other DNA and inhibitors present in the sample (Vianna et al., 2005).

The Denaturing Gradient Gel Electrophoresis (DGGE) technique can also be used for the identification of microorganisms. The application of the PCR-DGGE method in endodontic research has revealed that there are significant differences in the predominant bacterial composition between asymptomatic and symptomatic cases (Siqueira et al., 2005). In spite of the fact that PCR-DGGE has been applied to analyze microbial communities from diverse environments this technique has only recently been introduced in endodontic microbiology to fingerprint bacterial communities associated with different types of infection and to identify some dominant members of communities (Machado de Oliveira et al., 2007).

The Checkerboard DNA-DNA hybridization method was introduced for hybridizing larger numbers of DNA samples against large numbers of DNA probes on a single support membrane. This method permits the simultaneous determination of a multitude of bacterial species in single or multiple clinical samples and is particularly applicable in epidemiological research (Siqueira et al., 2002; Sassone et al., 2008). Studies with this molecular method have provided substantial new information for a better understanding of the microbiology of endodontic diseases (Siqueira et al., 2002).

This review revealed that in the literature numerous microorganisms are involved in permanent teeth infections, such as: Porvimonas micra, Fusobacterium nucleatum ssp, Tannerella forsythia, Treponema denticola, Veillonella parvula, Eubacterium nodatum, Porphyromonas gingivalis, Actinomyces odontolyticus, T. parvum, T. putidum, Firmicutes, Actinobacteria, Bacteroidetes, Proteobacteria, Fusobacteria, Synergistes, Flavobacteriaceae, Saprospiraceae, P. alactolyticus, C. durum, P. aeruginosa, P. nigrescens, E. faecalis, and Treptococcus constellatus (Munson et al., 2002; Siqueira et al., 2002, 2004, 2005, 2007; Rôças et al., 2003, 2004; Siqueira and Rôças, 2004, 2007; Rôças and Siqueira, 2005; Vianna et al., 2005; Seol et al., 2006; Sakamoto et al., 2008; Rôças and Siqueira, 2009). Siqueira et al. (2002) in his study using two different molecular methods (Checkerboard and PCR) confirmed that B. forsythus and T. denticola, (two particularly fastidious periodontal pathogens that had not been cultured previously from endodontic infections), are components of the endodontic microbiota and may play a role in the pathogenesis of periapical diseases. The same author (Siqueira and Rôças, 2004) found Dialister pneumosintes and Filifactor alocis in endodontic infections using the PCR multiplex method. And, in another paper, (Rôças and Siqueira, 2005) were the first authors to report the occurrence of T. parvum and T. putidum in samples from primary endodontic infections. The findings of these newly named Treponema species have expanded the list of bacteria involved with infections of endodontic origin.

These papers cited above reveal the diversity of microorganisms involved in endodontic infections. The clinical implications of the presence of these microorganisms in endodontic infections is that for a diseases with multiple microbiological causes there is not as yet any kind of irrigants that can be used during the root canal treatment to eliminate all the microorganisms involved in this pathosis. For the endodontists, the success of endodontic treatment depends on several factors and one of the most important is the reduction or elimination of bacterial infections. Therefore, it is important for the clinician to be aware of such bacteria and their ability to grow in an endodontic microenvironment. The clinician has to be prepared for possible treatment fail, because some microorganism(s) might help to maintain the disease. This research is important to disclose the species that are putative pathogens implicated in the pathogenesis of different types of endodontic infections and periradicular diseases. In addition to being of clear academic interest, this knowledge has an unquestionable
clinical importance as it provides clues for the search for effective antimicrobial strategies to reach and eliminate the components of the microbiota located in all irregularities of the root canal system.

Given the small number of studies that were included in this review, the meta-analyses on the effects of several prognostic factors could be considered to be compromised by lack of statistical power to demonstrate a significant influence. Also it is important to highlight that this diversity is not only due to the methodology used since all studies included were “A”, but this is, probably, also related to the kind of infection (primary or not) and the symptoms involved (abcess, pocket >3mm, pain, teeth mobility) (Zoletti et al., 2006, 2010; Sassone et al., 2008; Siqueira and Roças, 2008). Some articles lost points in quality assessment because they did not have a representative sample (Saito et al., 2009), did not describe if the samples were collected using strict asepsis methods (Saito et al., 2009), whether the samples were collected by the same operator (Tomazinho and Avila-Campos, 2007), did not have a description of the data collection (Munson et al., 2002), and other points analyzed based on quality (Munson et al., 2002; Saito et al., 2009).

Black-pigmented anaerobic bacteria may be commonly associated with symptomatic endodontic infections (Sakamoto et al., 2008). That is why A. actinomycetemcomitans and E. faecalis, high virulent microorganisms, were not found in a greater number of papers (Siqueira et al., 2001, 2002; Siqueira and Roças, 2004; Roças and Siqueira, 2005). E. faecalis was much more likely to be found in cases of failed endodontic therapy than in primary infections in the Brazilian population (Seol et al., 2006; Zoletti et al., 2006, 2010; Sakamoto et al., 2008; Siqueira and Roças, 2008). Therefore, the negative results regarding the occurrence of these bacteria in root-canal infections when assessed by culture, or another method, does not necessarily imply that they were absent, but it reflects the inherent limitations of the phenotype-based identification approaches used.

Molecular methods may be successfully used to overcome such limitations and have added further important information with regard to the composition of the endodontic microbiota (Siqueira, 2003). However, much more studies are necessary to identify which microorganisms are involved, their virulence, the association with clinical symptoms and the refractory infections. These aspects are essential to the understanding of the disease process and to the development of more effective and predictable therapeutic measures to deal with endodontic diseases.

Little is known about the virulence traits and mechanisms of the pathogenicity of these species involved in endodontic infections. Further studies are thereby warranted in order to confirm involvement with endodontic disease causation and to look for specific virulence factors. This is an important step in the understanding of the disease process and for the development of more effective and predictable therapeutic measures to deal with endodontic diseases.

**Conclusion**

This review found high evidence to confirm the polymicrobial nature of primary endodontic infections and the dominance of anaerobic bacteria (*Porphyromonas gingivalis, Porphyromonas endodontalis, Bacteroides forsythus, Prevotella intermedia* and *Prevotella nigrescens*). Future clinical studies will be important to confirm the results observed here in permanent and deciduous teeth.

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Sassone, LM; Fidel, RA; Faveri, M; Guerra, R; Figueiredo, L; Fidel, SR, et al. (2008). A microbiological profile of symptomatic teeth with primary endodontic infections. J. Endod. 34(5):541-545.


