

Features of the microbiota in chronic apical periodontitis. A systematic review

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Abstract:

Introduction. The article describes the microbiota found in teeth with chronic apical periodontitis.

Materials and methods. Electronic research of English and Russian literature was carried out in January 2022, in the databases of PubMed, Google scholar, Scopus, ResearchGate and ScienceDirect. The search started on the 10th of January 2022 and ended on the 12th of January 2022. A total of 23 articles were selected, 17 of which were selected for detailed review. Chief question in this article was: which microbial communities are present in root canals diagnosed with CAP? And how are they associated with clinical symptomatology?

Results. The inhabitants of the root canals with chronic apical periodontitis are mainly facultative anaerobes.

Conclusions. The microbiota of chronic apical periodontitis is very diverse, some of its main inhabitants are *E. faecalis*, *P. acnes*, *F. nucleatum*, *Streptococcus* spp, and *P. gingivalis*. Some authors have found that *E. faecalis* corresponded to 17% and up to 99.8% of the total bacterial counts present in the root canal with CAP. the presence of *P. gingivalis* was associated with tenderness to percussion, the presence of *E. faecalis*, *F. nucleatum*, and *P. gingivalis* were associated with periapical lesions > 3 mm, and the presence of *P. acnes*, *S. mitis* and *E. faecium* showed persistent exudation.

Keywords: apical periodontitis, apical microbiota, *E. faecalis*, chronic apical periodontitis, symptomatology, microbial flora.

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INTRODUCTION

Despite the continuous advancements in dental materials and techniques, the occurrence of caries and its complications remains at high. Chronic apical periodontitis (CAP) is considered as an infectious disease, in which microorganisms, mainly bacteria, invade and colonize the necrotic root canal system and periradicular tissues, causing inflammation and destruction of periradicular tissues [5,9]. CAP remains a controversial problem as it causes tooth extraction in 48-80% of cases [2, 4]. The main objective of an endodontic treatment is the complete eradication or in some cases, the "reduction" of microorganisms in the root canal system by the means of chemomechanical preparation (CMP) and intracanal medication (ICM) followed by an adequate obturation of the root canal system, which is sometimes difficult to achieve due to the complex anatomical structure of the root canal system and the formation of microbial biofilm outside the apical foramen, out of the reach of CMP and ICM [8, 15, 16, 21]. Studies suggest that due to the strict anatomical coloration between accessory and lateral canals, apical foramen and dentinal tubules, bacteria may migrate from one tissue to another. This pathological communication between these tissues is mainly due to a carious or traumatic lesion that affects the pulp, and secondarily, the periodontium [2, 15, 17]. Due to the deficiency of blood circulation in the necrotic pulpal tissues, the root canal system may serve as a reservoir for microorganisms that cannot be reached by systemic and local immune responses. CAP mainly goes by without any severe symptoms, such as slight discomfort while chewing,

so it is mainly diagnosed based on signs of radiolucency in the periapical area on dental radiographs [4, 10, 13].

The occurrence of oral diseases, such as dental caries, endodontic infection, and periodontal disease is linked to the oral microbiome; Moreover, the oral cavity takes the second place in microbiome complexity right after the colon [15, 20, 23]. The oral cavity has about 700 species present in its microbiome, where about 54% have been cultivated and named, 14% cultivated and unnamed, and about 32% are yet to be uncharted [4, 15, 20]. The oral microbiome is very complex and is dominated by the main phyla of: Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, Spirochetes and Fusobacteria [5, 6, 8, 15]. Recent studies have confirmed the predominance of anaerobic bacteria in infected root canals [3, 4].

Over the last century, it has been thought that *Enterococcus faecalis* is main microorganism responsible for the recurrence of CAP, a Gram-positive (G+) facultative anaerobe that has several mechanisms that improve its survivability, such as the ability to: form biofilms on the root canal walls, to resist ICM during the treatment process; enter an inactive, low metabolic state to survive in fastidious conditions, and is resistant to many antiseptics [2, 4, 8, 13, 15, 19]. But more recently, this theory has been questioned. Some correlate this with errors during the sample collection process, such as: inadequate rubber dam isolation; contamination of instruments; coronal microleakage; invasion of saliva through sinus tracts; the root canal being left open between treatments and treating in multiple visits. [6, 13]. Others in more recent studies suggest that it's connected with the chosen laboratorial method. Traditionally, samples are

taken from root canals using paper points, then cultivated, but unfortunately, there is no single culture medium that is suitable for the growth of the microbiota present in the root canals [8]. Therefore, more recently, the 16S ribosomal RNA (rRNA) gene sequencing approach and polymerase chain reaction (PCR) has been favored by most of the authors in this literature review, as they are more reliable in detecting hard-to-grow microorganisms [7, 8, 16, 23].

Therefore, knowing the diversity of results over the last century, this literature review aimed at understanding the microbiota of CAP, understanding its shifts, and investigating the diversity of microbial communities in regard to clinical symptomatology.

MATERIALS AND METHODS

• Protocol

This review follows the PRISMA (Preferred Reporting Items for Systematic review and Meta-analysis. A detailed protocol following the PICO protocol was designed to answer the following question: which microbial communities are present in root canals diagnosed with CAP? And how are they associated with clinical symptomatology? (P) Patient/problem: Chronic apical periodontitis. (I) intervention: individualized treatment approach. (C) control: control samples from non-infected teeth. (O) outcome: adoption of an effective treatment method in regard to the specific microbial community.

• Selection criteria

Publications that met the following criteria were included:

1. Full-text articles in English or Russian, not older than 10 years.
2. Articles containing relative information about the topic of research.
3. Studies conducted on human teeth.

Publications that had no relative data to the topic of study and literature reviews were dropped out.

• Information sources

Electronic search of English and Russian literature was carried out in January 2022, in the databases of PubMed, Google scholar, Scopus, ResearchGate and ScienceDirect. The search started on the 10th of January 2022 and ended on the 12th of January 2022.

• Search and selection of studies

The combination of these keywords was used in the search: Microbiota, apical periodontitis, microbial flora, asymptomatic apical periodontitis, chronic apical periodontitis, secondary apical periodontitis, microbial complex, microbial communities. As a result, 74 articles from PubMed, Google scholar, Scopus, ResearchGate and ScienceDirect were analyzed.

• Data collection process

Data was extracted from the studies in accordance with the interest of the current review.

• Inclusion and exclusion criteria

The literature search was limited to publications published in the English or Russian languages. The inclusion criteria were: studies containing information about the microbiota present in the root canal with apical periodontitis; studies conducted on human teeth, from randomized, non-randomized clinical studies, and clinical research. Studies involving patients with a history of other chronic diseases or the use of systemic antibiotics in the last 30 days were dropped out.

• Outcome variables

The following outcome variables were defined: A) microbiota present in the root canal with CAP, B) association of clinical symptomatology to the present microbiota.

• Data extraction

All headlines were screened to drop out irrelevant results. Onwards, abstracts were screened to analyze the number of 584 teeth and the main characteristics of the study. The publications that remained after the abstract screening were analyzed according to inclusion/exclusion criteria. At last, 17 articles were included in the present review.

• Statistical analysis

A meta-analysis of the data could not be performed, due to the heterogeneity of the data of the manuscripts included.

• Risk of bias.

Risk of Bias was not conducted.

RESULTS

74 titles were obtained from the electronic search, ranging from 2012 to 2022. The first screening of headlines led to the inclusion of 17 manuscripts.

Regarding the 17 included articles, all of which were clinical research articles. The number of teeth of the diagnosis of CAP included in the selected publications was at least 10 among all articles. In the selected literature, a total number of 584 samples were taken. Authors had different approaches toward their research, most compared the microbial culture of post (failed) treatment of CAP ($n = 6$), and others compared non-treated teeth with CAP with post treatment CAP ($n = 4$), while 4 authors accessed the microbiota before and after CMP. In order to evaluate the microbial cultures, 16S rDNA gene-based broad-range PCR was favored and used by all authors; Except for Horlenko et al. [2], that used material plating cultivation method. Microbial samples were collected from the root canals using sterile paper points [2,8,9,10,12,14,15,19,20,23]; from pulverized root apexes after root-end surgery (apicoectomy) [1, 6, 13, 21, 22]; or from pulverized root apexes after tooth extraction [3, 4, 5].

A) microbiota present in the root canal with CAP: the microorganisms found in the root canals by the authors were very diverse; mainly facultative anaerobes. Historically, it was entitled that *E. faecalis* was linked with the persistence of CAP, Vengerfeldt et al.'s research concluded that *E. faecalis* was only found in teeth with CAP that have been previously endodontically treated [19]. Moreover, Barbosa-Ribeiro et al. research showed that *E. faecalis* was present in the root canal alongside with *Staphylococcus epidermidis* and *Propionibacterium acnes* after CMP, and *E. faecalis* was the most prevalent species present in the root canal after ICM [8]. Additionally, Bouillaguet et al. and Antunes et al. studies showed similar results, stating that *E. faecalis* corresponded to 17% and up to 99.8% of the total bacterial counts present in the root canal with CAP [4, 22]. Other than that, *fusobacterium nucleatum*, *porphyromonas gingivalis* and *propionibacterium acnes* have shown a high relativity of the microorganisms found in the root canals with persistent CAP [2, 4, 8, 9, 10, 12, 13, 14, 15]. *Streptococcus* spp., a species feeble to endodontic irrigants, were commonly identified in previously endodontically treated teeth [1, 2, 5, 8, 15, 22], which may have occurred due to contamination during sample collection or coronal microleakage during and/or after root canal treatment [6, 8, 9].

B) association of clinical symptomatology to the present microbiota: further analysis of symptoms in correlation to microorganisms revealed that the presence of *P. gingivalis* was associated with tenderness to percussion

[8,10,13]; the presence of *E. faecalis*, *F. nucleatum*, and *P. gingivalis* were associated with periapical lesions > 3mm [8,15,14]; the presence of *P. acnes*, *S. mitis* and *E. faecium* showed persistent exudation [3, 10, 15]. Buonavoglia et al. concluded that the exacerbation of CAP (also known as phoenix abscess) is believed to be provoked by the presence of Socransky red complex (*P. gingivalis*, *Treponema denticola*, and *tannerella forsythia*) as they were found in 12/19 (63.2%) of the teeth diagnosed with phoenix abscess [10].

DISCUSSION

From the analysis of the literature, few studies were published regarding the microbiota of chronic apical periodontitis in the last 10 years. No systematic reviews or meta-analysis were found in the literature. Thus, the purpose of this literature review was to evaluate the reported data in the literature analysing two main aspects: A) microbiota present in the root canal with CAP, B) association of clinical symptomatology to the present microbiota.

The topic was focused on evaluating the microbiota present in teeth with chronic apical periodontitis. According to the literature, most studies showed that *E. faecalis* was found in root canals with CAP, except for Barbosa-Ribeiro et al.'s research, that stated that *E. faecalis* was only found 2/10 samples, which were later undetectable after CMP [9]. Such results may have been related to the paper point sampling method that was used by the author, which have been reported that it might overlook the microorganisms in lateral canals, apical ramifications, isthmuses, root canal irregularities of the root canal, and dentinal tubules; or due to technical issues, such as the used primers [4, 6, 8, 22]. Previous studies have shown the presence of actinomyces genus (mainly actinomyces israelii and actinomyces gerencseriae) and Propionibacterium propionicum have been found in asymptomatic CAP that are refractory to endodontic treatment. Studies revealed that these microorganisms have to ability to: survive in granulation tissue outside the root

canal; adhere to dentinal debris forced out through the apical foramen as they advance in the periapical tissues; adhere to the root apex surface; coaggregate with other bacteria; and survive in the extraradicular area [1, 5, 18, 21]. Furthermore, it has been reported that *P. acnes* could induce the differentiation of T-lymphocytes into CD25 regulatory bright cells that might have an inhibitory response of the immune system [18]. Interestingly noted, microorganism families involved in phototransferase systems and the metabolism of galactose, fructose, glycerolipids and nucleotide sugars were persistently found in persistent/secondary CAP, while the ones responsible with lipopolysaccharides biosynthesis were associated with primary apical periodontitis [4,8,14]. authors reported that the microorganism at different positions, 1/3 apex, 1/3 middle, and 1/3 crown, were different for infected root canals and after all endodontic procedures, the total bacterial reduction was 86.2% [8, 21]. Finally, variations in the results may be explained by distinct clinical conditions, and the fact that all-mentioned above studies have been conducted in different countries, therefore different bacterial profiles were found, due to the use of antibiotics in general practice, which differ from a country to another, geographical related factors, including environmental contaminants from food and other extraoral resources and socioeconomic status that are hard to track [4, 8, 15].

CONCLUSIONS

The microbiota of chronic apical periodontitis is very diverse, some of its main inhabitants are *E. faecalis*, *P. acnes*, *F. nucleatum*, streptococcus spp, and *P. gingivalis*. Some authors have found that *E. faecalis* corresponded to 17% and up to 99.8% of the total bacterial counts present in the root canal with CAP. the presence of *P. gingivalis* was associated with tenderness to percussion, the presence of *E. faecalis*, *F. nucleatum*, and *P. gingivalis* were associated with periapical lesions > 3mm, and the presence of *P. acnes*, *S. mitis* and *E. faecium* showed persistent exudation.

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