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Microorganisms: the reason to perform Endodontics

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That we perform Endodontics because there are microorganisms is now beyond doubt. Nevertheless, not only the microorganisms, but also the host response have a profound effect on the progression of the disease.

Many papers confirmed the polymicrobial nature of pulpal and periapical diseases of endodontic origin and the efficiency of the chemo-mechanical procedures based on physical and chemical elimination of their etiologic factors, whose principles were first presented as far as 1928 by Hall.

Since not only bacterial load may be related to the clinical outcome, but also the bacterial composition of the microbiological canal ecosystem, we aimed at the enumeration of the microorganisms present in the different types of endodontic infections.

Although the emerging picture is clearly a complex one, not allowing clear-cut association of bacteria and clinical situation, only the further pursuit of elucidation of the many factors involved (including geographical variability) will ultimately lead to rational treatment solutions.

Keywords Apical Periodontitis, Microbiology, Endodontics, Review

1. Introduction

In the oral cavity, distinct microenvironments at various soft and hard tissue surfaces influence the composition of the microbiota¹.

For endodontic infection to develop, the root canal must be devoid of vital pulp tissue and its defenses, as a consequence of either pulp necrosis (as a sequel to caries, trauma, periodontal disease, or iatrogenic operative procedures) or pulp removal for treatment. The borderline between the infecting microbiota and the host defenses is often located intraradicularly, i.e., short of or at the apical foramen. In some cases, however, microorganisms may reach the periradicular tissues, and the borderline is then situated extraradicularly, i.e., beyond the boundaries of the apical foramen² creating a clinical situation known as Apical Periodontitis (AP).

AP is a more widespread disease than moderate or severe marginal periodontitis, affecting 50% of the population by age 50, and 62% of individuals over age 60³. That's to say it is a very prevalent health problem.

In addition to bacteria (the most prevalent in diseases of endodontic origin), fungi and archaea have been only occasionally found in intraradicular infections^{4,5,6}, while herpes viruses and HIV have been detected in AP^{7,8}.

The application of Nucleic Acid approaches to endodontic microbiology provided a more complete and holistic picture, compared to the one assessed by Classic Culture.

The goal of this article is to proceed to an international literature review published on this thematic, summarizing the important features related, in order to update the existing information in a pedagogical way.

2. Methods

For the article' elaboration the bases MEDLINE / PUBMED, B-On and library files of Oporto University were accessed, using the terms: "endodontics", "microbiology", "review", "apical periodontitis", "culture" and "molecular biology". The references were selected under inclusion criteria like English language, accessibility, relevance to the theme and scientific rigor.

3. Pathogenesis of Apical Periodontitis

Of the major dental diseases, infection of the root canal is unique for the oral cavity since infection establishes where microorganisms have not previously been present. The most common causes for pulpal inflammation (pulpitis) are bacteria and/or their products entering the pulp through a deep caries lesion or a leaking filling. Loss of the mineralized tooth' structures open an avenue for penetration of bacteria into the pulp space. Leaving behind the nutritionally rich and diverse environment of the oral cavity, microorganisms that establish in the root canal system (RCS) must breach

enamel, invade dentine, overwhelm the immune response of the pulp and settle in the remaining necrotic tissue⁹. Once necrotic, the RCS becomes a “privileged sanctuary” for clusters of bacteria, bacterial by-products, and degradation products of both the microorganisms and the pulpal tissue^{10,11}.

The invading restricted group of species seem to go through some selection mechanism. Low oxygen tension, bacterial by-products, interactions between microbial factors and the availability of nutrients such as polypeptides and amino acids, driven by the route of infection and the ecological pressure in the RCS¹² are factors that determine which microorganisms will predominate.

Actually, studies on the dynamics of root canal infections have shown that the relative proportions of anaerobic microorganisms increase with time and that the facultative and obligate anaerobic bacteria are outnumbered when the canals have been infected for three months or more^{9,13}.

AP can be described as a dynamic encounter between microbial factors and host defences at the interface between infected radicular pulp and periodontal ligament that results in local inflammation, resorptions of hard tissues, destruction of other periapical tissues, and eventual formation of various histopathological categories of AP, commonly referred to as periapical lesions¹⁴. Infectious microbes and host defences destroy much of the periapical tissue, resulting in the formation of various categories of AP lesions. In spite of the formidable defence, the body is unable to destroy the microbes well entrenched in the sanctuary of the necrotic root canal, which is beyond the reaches of body defences. Therefore, AP is not self-healing¹⁵.

4. Overview of the classic studies

The establishment of the foundation of Endodontics as the discipline primarily involved with the treatment of an infectious disease started many years ago.

Antony van Leewenhoek was the first to observe oral flora. His description of the “animalcules” observed with his microscopes included those from dental plaque and from an exposed pulp cavity¹¹.

More than a century ago (1890) Miller, based on his finding of numerous types of bacteria in the necrotic dental pulp, raised the hypothesis that bacteria were the causative factors of AP. By means of bacterioscopy of the canal samples, he found bacterial cells in the three basic morphologies known, i.e. cocci (round cells), bacilli (cylindrical cells) and spirilla (helical cells). Morphologically, the endodontic microbiota was clearly different in the coronal, middle and apical parts of the root canal.

However, the essential role of micro-organisms in the etiology of AP remained doubtful for several years. In 1965, Kakehashi et al.¹⁶ resolved the issue: no AP developed in germ-free rats when their molar-pulps were kept exposed to the oral cavity, as compared with control rats with a conventional oral microflora in which massive periapical radiolucencies occurred. Thus, the presence or absence of microbial flora was the major determinant for the destruction or healing of exposed rodent pulps.

The great significance of obligate anaerobes in endodontic infections was soon established¹⁷ with the advent of anaerobic culture techniques. These findings were confirmed by other researchers using anaerobic techniques^{18,19}. Bacteria were found only in the root canals of teeth exhibiting radiographic evidence of AP, confirming the infectious etiology of this disease.

Later, another relevant observation was that bacteria in a root canal infection do not occur *in vivo* as separate colonies, but grow within an extracellular matrix in interconnected communities as a bacterial biofilm. By the application of the precise technique of correlative light and transmission electron microscopy, these biofilms were first reported, describing them as co-aggregating communities with a palisade structure²⁰. The clinical significance of a biofilm growth pattern is that bacteria are relatively protected compared with planktonic forms and are known to be more resistant to antimicrobial treatment²¹. Thus, there is a current trend to include AP in the category of biofilm-induced diseases which is a major step forward in understanding of root canal infection²².

Community profile analyses of the endodontic microbiota have disclosed some interesting findings²³:

a) endodontic infections harbor mixed bacterial communities²⁴, including persistent/secondary infections associated with treated teeth²⁵;

b) some underrepresented uncultivated bacteria may be commonly found in infected root canals^{24,26};

c) bacterial communities may follow a specific pattern according to the clinical condition (chronic AP, acute apical abscesses, and treated teeth)²³;

d) there is a great interindividual variability in endodontic communities associated with the same clinical disease^{23,27}, i.e., each individual harbors a unique endodontic microbiota in terms of species richness and abundance. Thus AP has a heterogeneous etiology, with no single prevalent species²⁸;

e) this interindividual variability is still more pronounced when individuals from different geographical locations are analyzed^{24,29,30,31}.

The fact that the composition of the endodontic microbiota differs consistently between individuals suffering from the same disease^{24,25} denotes a heterogeneous etiology for AP, where multiple species combinations can lead to similar disease outcomes.

The comprehension of species abundance and distribution is essential for understanding the behavior of a microbial community (Table I)³².

Table 1 Main distinctive features of endodontic microbiota in different clinical conditions

	Primary infections		Persistent infections	Persistent/secondary infections
	Chronic Apical Periodontitis	Acute apical abscess	Filling stage	Treated teeth
Community	mixed	mixed	Mixed, sometimes single	Mixed, sometimes single
Number of taxa/case	10-20	10-20	1-5	Adequate treatment: 1-5 Inadequate treatment: 2-30
Uncultivated bacteria	40-55%	40%	42%	55%
Most prevalent groups	Gram-negative / Gram-positive anaerobes	Gram-negative anaerobes	Gram-positive facultative /anaerobes	Gram-positive facultative
Most frequent taxa	<i>Treponema spp.</i> <i>Tannerella forsythia</i> <i>Porphyromonas spp.</i> <i>Dialister spp.</i> <i>Filifactor alocis</i> <i>Pseudoramibacter alactolyticus</i> <i>Fusobacterium nucleatum</i> <i>Synergistes spp.</i> <i>Eikenella corrodens</i> <i>Prevotella spp.</i> <i>Olsenella spp.</i> <i>Parvimonas micra</i> <i>Peptostreptococcus spp.</i> <i>Campylobacter spp.</i>	<i>Treponema spp.</i> <i>Tannerella forsythia</i> <i>Porphyromonas spp.</i> <i>Dialister spp.</i> <i>Fusobacterium nucleatum</i> <i>Eikenella corrodens</i> <i>Synergistes spp.</i> <i>Prevotella spp.</i> <i>Olsenella spp.</i> <i>Parvimonas micra</i>	<i>Streptococcus mitis</i> <i>Other streptococci</i> <i>Propionibacterium spp.</i> <i>Fusobacterium nucleatum</i> <i>Prevotella spp.</i> <i>Pseudoramibacter alactolyticus</i> <i>Parvimonas micra</i> <i>Lactobacilli</i> <i>Olsenella spp.</i> <i>Actinomyces spp.</i> <i>Pseudomonas aeruginosa</i> <i>Enteric rods</i>	<i>Enterococcus faecalis</i> <i>Candida albicans (yeast)</i> <i>Streptococcus spp.</i> <i>Pseudoramibacter alactolyticus</i> <i>Propionibacterium propionicum</i> <i>Filifactor alocis</i> <i>Dialister spp.</i> <i>Actinomyces spp.</i> <i>Pseudomonas aeruginosa</i> <i>Enteric rods</i>

5. Composition and localization of flora in endodontic infections

Since the root canal environment and nutritional supply govern the dynamics of the microbial flora, it means that the bacteria present in the root canal will depend on the stage of the infection.

Furthermore, knowledge of the patterns of microbial colonization allows the establishment of antimicrobial therapeutic strategies to reach and eliminate microorganisms located not only in the main canal, but also in other areas of the RCS in which they can propagate²³ like dentine tubules, lateral and accessory canals, isthmus, fins, “loops”, etc..

5.1. Primary endodontic infection

Primary Endodontic infections can be regarded as the initial or ‘wild’ infection, in the sense that there has not been any professional intervention yet. Participating microorganisms may have been involved in the earlier stages of pulp invasion or they can be latecomers that took advantage of the environmental conditions in the root canal after pulp necrosis.

They are the cause of primary AP, which can manifest itself as a chronic or acute disease. Some acute conditions may evolve to an abscess, which in some cases can spread to head and neck spaces to establish a life-threatening condition²³.

The number of bacterial species in an infected root canal may vary from one to more than twelve, and the number of colony-forming units (CFUs) in an infected root canal varies from 10^3 to $10^{8, 27, 33, 34}$.

As for species richness, a mean of 10-20 species have been found per canal of teeth with chronic AP as revealed by molecular biology studies³⁵. Root canals of teeth with apical radiolucency associated with a draining sinus tract (chronic apical abscess or suppurative AP) have been reported to harbor a mean number of 17 species²⁸.

A correlation seems to exist between the size of the periapical lesion and the number of bacterial species and cells in the root canal. Teeth with long-standing infections and large lesions usually harbor more bacterial species and have a higher density and a more complex association of bacteria than teeth with small lesions^{9,23,34,36} (Figure 1).

It is noteworthy that the most prevalent species in primary infections may vary from study to study, which can be explained by several factors: sensitivity and specificity of the identification method, sampling technique, geographic location, and accuracy or divergence in clinical diagnosis, and disease classification. Even so, one can select 10-20 species that are virtually always among the most frequently detected species in most well conducted studies about the endodontic microbiota²³.

5.1.1. Bacteria found in Primary endodontic Infections

Genera of bacteria frequently detected in primary infections are^{33,37,38,39,40}:

- Gram-negative bacteria (*Fusobacterium*, *Dialister*, *Porphyromonas*, *Prevotella*, *Tannerella*, *Treponema*, *Campylobacter*, and *Veillonella*);
- Gram-positive bacteria (*Parvimonas*, *Filifactor*, *Pseudoramibacter*, *Olsenella*, *Actinomyces*, *Peptostreptococcus*, *Streptococcus*, *Propionibacterium*, and *Eubacterium*);
- Facultative or microaerophilic Streptococci^{28,41,42}.

The species list is much larger and can be summarized as:

- 1) Black pigmented Gram-negative anaerobic rods such as *Prevotella intermedia*, *Prevotella nigrescens*, *Prevotella tanneriae*, *Prevotella multissacharivorax*, *Prevotella baroniae* and *Prevotella denticola*, *Porphyromonas endodontalis* and *Porphyromonas gingivalis*.
- 2) *Tannerella forsythia* (previously called *Bacteroides forsythus* or *Tannerella forsythenis*) was the first periodontal pathogen to be detected in endodontic infection⁴³.
- 3) *Dialister* species are asaccharolytic obligately anaerobic Gram negative coccobacilli which have been consistently detected in endodontic infections, namely *Dialister pneumosintes* and *Dialister invisus*.
- 4) *Fusobacterium nucleatum* and *Fusobacterium periodonticum* is also a common member of endodontic microbiota.
- 5) Spirochetes are highly motile, spiral-shaped, Gram negative bacteria with periplasmic flagella. All oral spirochetes fall into the genus *Treponema*⁴⁴. Prevalent species are: *Treponema denticola*, *Treponema socranskii*, *Treponema parvum*, *Treponema maltophilum* and *Treponema lecithinolyticum*.
- 6) Gram positive anaerobic rods have also been found in endodontic microbiota like: *Pseudoramibacter alactolyticus*, *Filifactor alocis*, *Actinomyces spp.*, *Propionibacterium propionicum*, *Olsenella spp.*, *Slackia exigua*, *Mogibacterium timidum* and *Eubacterium spp.*
- 7) Gram positive cocci, specifically Peptostreptococci and Streptococci are frequently present in primary endodontic infections. Examples are *Parvimonas micra* (previously called *Peptostreptococcus micros* or *Micromonas micros*), *Streptococcus spp.* which include *Streptococcus anginosus*, *Streptococcus mitis*, *Streptococcus sanguinis* and *Enterococcus faecalis*.
- 8) Other bacterial detected more sporadically include: *Campylobacter rectus*, *Campylobacter gracilis*, *Veillonella parvula*, *Eikenella corrodens*, *Neisseria mucosa*, *Centipeda periodontii*, *Gemella morbillorum*, *Capnocytophaga gingivalis* and *Corynebacterium matruchotii*.

5.1.2. Other Pathogens found in Primary endodontic infections

Fungi and most recently archaea and viruses and as yet uncultivable bacteria have been found in association with endodontic infections:

- 1) Fungi – *Candida spp.*, particularly *Candida albicans*^{45,46}.
- 2) Archaea – These are a diverse group of prokaryotes which are distinct from bacteria and that make up the third domain of cellular life of the members of the human oral microflora. They are strikingly less diverse than oral bacteria and appear to be relatively rare with respect to their numerical abundance⁴⁷. Since they have been exclusively found in association with oral infections such as periodontitis and AP⁵ and given their unique physiology and energy metabolism, it is highly plausible that they are more than just secondary colonizers of infected areas, but instead are actively involved in the overall poly-microbial infection process. Conversely, it is a highly challenging task to clearly demonstrate their possible active participation. In fact, since it is extremely difficult to grow them in routine microbiology laboratories⁴⁷, evidence of their existence in this human ecosystem has primarily been provided by PCR-based techniques targeting either the 16S rRNA gene and/or the functional gene *mcrA* encoding for the methyl-coenzyme M reductase (a key enzyme in methanogenesis)⁴⁷. In fact, Culture has only revealed the presence of methanogenic Archaea, namely *Methanobrevibacter oralis*⁴⁸.
- 3) Viruses – Viruses are particles structurally composed of a nucleic acid molecule (DNA or RNA) and a protein coat with or without a membrane envelope. They require viable host cells to infect and use the cell's machinery to replicate

its genome. Hence, they cannot survive in a necrotic root canal. Its presence in the root canal has been reported only for non-inflamed vital pulps of patients infected with human Herpes virus⁴⁹. Among the Herpes spp., the human *Cytomegalovirus* and *Epstein-Barr-virus* may be implicated in the pathogenesis of AP.

4) As Yet-Uncultivated Bacteria

Only after the advent of molecular techniques for bacterial identification, several culture-difficult species have been consistently included in the set of candidate endodontic pathogens. The main examples are *Tannerella forsythia*, *Dialister* species (*Dialister invisus* and *Dialister pneumosintes*), *Filifactor alocis*, *Prevotella baroniae*, *Olsenella uli*, and *Treponema* species^{41,43,50,51,52}. Strengthening of the association between AP and some species previously recognized as candidate pathogens has also become evident by molecular findings. Examples include *Fusobacterium nucleatum*, *Parvimonas micra* (formerly *Peptostreptococcus micros*), *Porphyromonas* species (*Porphyromonas endodontalis* and *Porphyromonas gingivalis*), *Prevotella* species (*Prevotella intermedia* and *Prevotella nigrescens*), and *Pseudoramibacter alactolyticus*, all of which have been detected in higher prevalence values than previously reported by culturing studies^{39,53,54}.

Clone library analyses of primary endodontic infections reveal that a significant proportion of the detected taxa consist of phylotypes that remain to be cultivated and phenotypically characterized^{30,41} i.e. species that are known only by a 16S rRNA gene sequence and that have yet to be cultivated and fully characterized²⁶. These as-yet-uncultivated phylotypes account for approximately 55% of the taxa found in root canals of teeth with chronic AP and in terms of abundance represent more than 38% of the clones sequenced²⁷ and have emerged as candidate pathogens of endodontic infections². Therefore, there is no reason to believe that they are less important than the cultivable proportion of the microbiota when it comes to disease causation²⁸.

Several uncultivated phylotypes from the genera *Synergistes*, *Dialister*, *Megasphaera*, *Solobacterium*, *Eubacterium* and *Selenomonas*, as well as phylotypes related to the family *Lachnospiraceae* or the phylum Bacteroidetes have been identified in primary endodontic infection²⁸.

5.2. Acute apical periodontitis and abscesses

Acute AP and acute apical abscesses are typical examples of symptomatic endodontic infection. Whereas microbial causation of AP is well established, there is no strong evidence disclosing specific involvement of a single species with any particular sign or symptom of AP²⁸. While an acute abscess is usually preceded by acute AP, the latter does not necessarily evolve to the former. Therefore, the acute abscess can be regarded as an advanced stage of the acute disease. In later stages of the disease process, the diagnosis of acute abscesses usually does not represent a difficult task, mostly because of swelling (Figure 2). In symptomatic cases, the infection is located in the root canal but it may also have reached the periradicular tissues and, in abscessed cases, it has the potential to spread to other anatomical spaces of head and neck to form a cellulitis²³.

The acute infection is characterized by an increased bacterial metabolism and uncontrolled cell division. At this stage, the body has one main goal and that is to prevent the infection from spreading. A fibrotic capsule can be formed in order to build a bacteria penetration barrier; however, at the cost of a total destruction of the tissues within the barrier. This results in an abscess with pus⁴⁴.

The microbiota involved with abscesses is mixed and dominated by anaerobic bacteria^{27,54,55,56}. Bacterial counts per abscess case have been reported to range from 10⁴ to 10⁹ CFUs⁵⁵. The mean number of species is comparatively higher in abscesses than in canals of teeth with chronic AP, with molecular studies revealing an average of 12-18 species/case in abscesses as compared to 7-12 species present in chronic cases^{23,27}. It has been shown that as-yet-uncultivated phylotypes encompass approximately 40% of the species found in abscesses and collectively represent more than 30% of the clones sequenced²⁷. Some Gram-negative anaerobic bacteria have been suggested to be involved with symptomatic lesions^{27,57,58}, but the same species may also be present in somewhat similar frequencies in asymptomatic cases^{27,53}.

Therefore, factors other than the mere presence of a given putative pathogenic species may play a role in the etiology of symptomatic endodontic infections⁵⁹. These factors possibly include: (a) differences in virulence ability among strains of the same species; (b) bacterial interactions resulting in additive or synergistic effects among species in mixed communities; (c) bacterial population density; (d) environment-regulated expression of virulence factors; and (e) host resistance, which may be modulated by diverse aspects including systemic diseases, concomitant virus infection, environmental factors (stress, smoking), and genetic patterns⁵⁹. The diversity of the bacterial communities has been found to differ significantly when the microbiota of chronic AP and acute apical abscesses are compared⁶⁰. Differences are essentially represented by the dominant and the large number of species in abscesses. A shift in the community structure is then suspected to precede the emergence of symptoms. Differences in species richness and abundance, and the resulting interactions among community members may affect virulence of the whole consortium²³.

5.3. Secondary / Persistent endodontic infections

Persistent and secondary infections are responsible for several problems in endodontic practice, including persistent exudation, persistent symptoms, flare-ups, and treatment failure.

There are some reports that show that non-oral bacteria may be involved with secondary infections to cause persistent exudation and/or symptoms^{61,62}. As for flare-ups, the evidence of specificity is even weaker, although there are some reports of involvement of Gram-negative anaerobes, such as black-pigmented rods and *Fusobacterium nucleatum*^{23,63}.

Conversely, the microbiota involved with treatment failure has been extensively studied and is supported by two strong evidence-based arguments. First, there seems to be an increased risk of adverse treatment outcome when bacteria that survived the effects of intracanal disinfection procedures are present in the canal at the time of filling (persistent intraradicular infection)^{64,65,66}. Second, most (if not all) root canal-treated teeth evincing persistent AP lesions have been demonstrated to harbor an intraradicular infection, ie. microorganisms have infected the canal after filling as a result of coronal leakage^{30,67,68} or apical percolation.

Based on these arguments, studies have attempted to identify the microorganisms found at the root canal-filling stage, which are 'short-term survivors' that may put the treatment outcome at risk, and the microorganisms in root canal-treated teeth with AP, which are 'long-standing survivors' that are arguably the cause of post-treatment disease²³.

Even after diligent chemomechanical preparation followed or not by intracanal medication, some canals may harbor detectable levels of cultivable bacteria²³. In these cases, 1-5 species can be detected per canal, with counts reaching 10^2 - 10^5 CFUs per sample^{34,64,69}. No single species has been significantly found to persist after treatment procedures. But, generally, Gram-negative species and fastidious anaerobes have a limited capacity to endure treatment and are readily eliminated by instrumentation and antimicrobial irrigation⁷⁰.

Gram-positive species and facultative anaerobes have a higher rate of recovery in post-instrumentation samples like *Streptococcus* species, *Parvomonas micra*, *Actinomyces* spp., *Propionibacterium* spp., *Pseudoramibacter alactolyticus*, *Lactobacillus* spp., *Enterococcus faecalis*, and *Olsenella uli*^{64,71,72}.

The microbiota in root canal-treated teeth with post-treatment AP also exhibit a decreased diversity (both richness and abundance) in comparison to primary infections. Root canals with apparently adequate treatment usually contain 1-5 species, while the number of species in canals with inadequate treatment can reach up to 30 species, which is very similar to primary infections (Siqueira 2008). In terms of bacterial density, a treated canal associated with post-treatment disease can harbor 10^3 - 10^7 CFUs^{71,73,74}.

All these findings indicate that the microbiota of root canal-treated teeth with AP is more complex than previously anticipated by Culture studies. However, it is proportionally less complex than primary infections²³.

A number of microbial properties favor the selection and survive of some species over others. For example, an ability to endure starvation is a beneficial survival characteristic which helps ensure that some species outlast others until they may access nutrients from the local milieu, e.g. serum-type fluid that may seep into the canal space over time¹. Starvation survival characteristics have been demonstrated in selected species known to be involved in persistent infection such as *Enterococcus faecalis*⁷⁵ and *Candida albicans*⁷⁶ whereas others like *Fusobacterium nucleatum*, *Peptostreptococcus anaerobius*, *Prevotella intermedia*, and *Pseudoramibacter alactolyticus* seldom identified in persistent infection have poor starvation survival ability⁷⁷.

Regardless of the identification method, *Enterococcus faecalis* is the most frequently detected species in root canal-treated teeth, with prevalence values reaching up to 90% of the cases^{29,67,73,78}. Root canal-treated teeth are about nine times more likely to harbor *Enterococcus faecalis* than cases of primary infections⁷⁹. This species has been considered as transient in the oral cavity and its source might be food⁸⁰. The fact that *Enterococcus faecalis* has been commonly recovered from cases treated in multiple visits and/or in teeth left open for drainage^{64,79} suggests that it may cause a secondary infection that then becomes persistent.

Candida species, particularly *Candida albicans*, have been detected in root canal-treated teeth in up to 18% of the cases^{71,81,82}.

Other bacteria found in root canal-treated teeth with AP include *Streptococcus* species and some fastidious anaerobic species such as *Pseudoramibacter alactolyticus*, *Propionibacterium propionicum*, *Filifactor alocis*, *Dialister pneumosintes*, and *Dialister invisus*^{26,29,67}.

As-yet-uncultivated phylotypes correspond to 55% of the species detected in treated canals (Siqueira & Rôças 2005a), which confirms their importance in disease etiology.

6. Concluding remarks

Any species found in the pulpal space has the potential to be an endodontic pathogen or at least to play a role in the ecology of the oral microbial community.

Without doubt, bacteria are the most common microorganisms occurring in endodontic infections.

Study of root canal infections is not an easy task. It relies on the clinical significance of the material collected from an anatomical location that is difficult to manipulate under sterile conditions. In fact, if the healthy root canal is expected to be sterile, neither the diseased one nor the mouth environment is sterile in nature. Thus, sample material has to be collected, preserved, transported to the lab and processed in the lab under strict conditions.

Datasets from Culture and Molecular studies showed that more than 400 different microbial species belonging to 100 genera and 9 phyla have been identified in different types of endodontic infections. The phyla with the highest species

richness were Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria. Diversity varies significantly according to the type of infection.

Because AP is a disease of infectious aetiology, the logical goals of the endodontic treatment are to eliminate or substantially reduce the microbial populations within the RCS (through antiseptic means) and to prevent introduction of new microorganisms in the canal (through aseptic means). Prevention of reinfection is also achieved by a tight coronal seal of the root canal provided by both the root canal filling and the permanent coronal restoration. The success rate of the endodontic treatment will depend on how effective the clinician is in accomplishing these goals.

The question now is no longer whether the microbes are involved but specificity of microbial species. New endodontic pathogens are added to the list because of improved technologies like molecular methods which help the endodontist to be more accurate in the treatment planning. Studying the flora will streamline the treatment planning.

Acknowledgments Author M. C. Manso acknowledges Fundação para a Ciência e a Tecnologia through grant n.º PEst-C/EQB/LA0006/2011.

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