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Endodontic Infections, Microbiology, Progress, Treatment

Prashanth Kumar Katta

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Abstract

The most evident way for endodontic infection to occur is when the dental pulp is directly exposed to the oral cavity. The most frequent cause of pulp exposure is caries, however trauma or iatrogenic restorative therapies can also expose the pulp directly to microorganisms. The oral bacteria from carious sores, saliva, or plaque that has accumulated on the exposed surface come into direct touch with the exposed pulp tissue. Exposed pulps almost always experience inflammation, necrosis, and infection. Although the amount of time between pulp exposure and canal wide infection is uncertain, the process is typically gradual.

Keywords: Infection routes; Bacteria; Necrosis; Abscess; Granuloma; Intraradicular infection.

INTRODUCTION

Endodontic infection occurs when the pulp becomes necrotic (as a result of caries, trauma, periodontal disease, or invasive surgical treatments) or when the pulp is removed for treatment in root canals that lack host defenses.

Only teeth with radiographic signs of apical periodontitis had bacteria identified in their root canals, proving that the illness is caused by an infectious agent. Almost 90% of the isolates were anaerobic bacteria. The results of Sundqvist's investigation also showed that apical periodontitis lesions cannot be caused by or continue to exist in the presence of necrotic pulp tissue or stagnant tissue fluid in the root canal.[1,2]

Endodontic infections can be classified according to their anatomical location (intra radicular or extra radicular infection) and to how long it took micro-organisms to reach the root canal (primary, secondary, or persistent infection). Usually, primary and secondary/persistent endodontic infections are located intra-radicularly, and may originate in extraradicular infections if left untreated or inadequately treated.

Similar to alterations in other connective tissues, the dental pulp's disease development follows a similar pattern. The following stages are typically experienced by the tissue and disease: normal, inflammation (also known as pulpitis), necrosis, infection, and loss of pulp tissue (i.e., pulpless canals). Chronic illnesses may experience acute exacerbations at any time, and inflammatory

Author's Affiliation: [1]Assistant Professor, Department of Restorative Dental Sciences, College of Dentistry, King Faisal University, Al Hasa, Kingdom of Saudi Arabia.

Corresponding Author: Prashanth Kumar Katta, Assistant Professor, Department of Restorative Dental Sciences, College of Dentistry, King Faisal University, Al Hasa, Kingdom of Saudi Arabia.

E-mail: drprashanthkumar@yahoo.com

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alterations might be brief, chronic, reversible, or irreversible.[3,4]

Root canal infection routes[5,6]

Dentinal Tubules: Whenever dentin is exposed.

Direct Pulp Exposure: Direct exposure of the dental pulp to the oral cavity is the most obvious route of endodontic infection.

Periodontal Disease: In either normal or diseased periodontal tissues, microorganisms in subgingival biofilms could reach the pulp through dentinal tubules or lateral/furcal canals.

Anachoresis: Anachoresis is a process by which microorganisms are transported in the blood or lymph to an area of tissue damage, where they leave the vessel, enter the damaged tissue, and establish an infection.

Primary Intraradicular Infection: Micro organisms that initially invade and colonize the necrotic pulp tissue cause primary intra radicular infection.

Abscess versus Granuloma[7-12]

One of the most common pathological abnormalities with in alveolar bone are peripical abscesses. Recently, we discovered that the most prevalent distinct component in periapical abscesses was 17-octadecynoic acid (17-ODYA). The inflammatory tissue found in apical periodontitis lesions is often composed of lymphocytes, macrophages, and the fibroblasts that make up the periodontium. Granulomas are inflammatory lesions featuring these components. Granulomatous lesions may contain varying numbers of PMNs, mostly close to the apical foramen. It is important to remember that unlike lymphocytes, macrophages, and fibroblasts, PMNs do not have a long lifespan. About 95 percent of the total of periapical exudates contain detectable amounts of IL-8, pointing to IL-8's crucial function in the acute stages of apical illness.[7] Endodontic bacteria can cause by pulp fibroblasts and osteoblasts to produce IL-8.[8] Leukotriene B4 (LTB4) is discovered to be produced when arachidonic acid undergoes lipoxygenase mediated oxidation. This process results in the attachment of polymorphonuclear leukocytes (PMNs) to endothelium walls, which draws macrophages to the area and causes severe damage to the host tissues.

Bacterial invasion[13-19]

It is very likely that bacteria will get within the ischemic necrotic pulp. Bacterial contamination can come from mechanically injured cervical root surfaces or blood clots that harbor bacteria in a severed PDL along the root surface (bacteria from dental plaque). The primary cause of incomplete revascularization is bacterial colonization of the ischemic pulp. It results in the development of a leukocyte zone that divides infected necrotic tissue from the apical connective tissue that is developing. Both the leukocyte zone and the necrotic pulp contain bacteria, but the neighboring connective tissue rarely does. In developing teeth, pulp necrosis is important because it may cause root growth to stop, leaving weak, brittle roots behind. The pulp will become necrotic and infected when bacteria get access to the coronal fragment in root fractures. This, in turn, causes an accumulation of inflammatory, granulation tissue between the coronal and apical fragments, which prevents the fractured root from healing.

Development of apical lesions[4,5,20-26]

Apical periodontitis, the body's response to the microorganisms that emerge from the apical foramen, begins in the nearby periodontal ligament. This reaction, which is meant to contain and kill the bacteria, also harms the host locally by causing bone resorption. IL-1 and tumor necrosis factor (TNF), two cytokines secreted by the cells of the apical inflammatory response, can stimulate local osteoclastic bone resorption. TNF is a byproduct of activated T-lymphocytes, whereas IL-1 is mostly generated by activated macrophages. The main factors causing localized apical bone resorption are IL-1 and TNF. The signaling protein receptor activator of nuclear factor kappalig and (RANKL) is expressed on the surfaces of bone lining cells when these cytokines are present. This ligand interacts with the RANK receptor, which is located on the surface of nearby preosteoclasts and osteoclasts. As a result, preosteoclasts develop into mature osteoclasts and existing osteoclasts are activated, causing them to show ruffled borders and start aggressively resorbing bone.

The ensuing local bone resorption is initially seen radiographically as a widening of the apical periodontal space; as this space steadily grows, it eventually manifests as an apical lesion, which is a radiolucent lesion in the apical bone.

Thus, apical bone resorption may be viewed as a negative side effect of the host's defensive reaction. The local generation of cytokines causes resorption of the nearby bone as a result of the effective host response being activated to eliminate dangerous

germs.

Periapical (PA) Lesion[6,27-31]

Germs either directly or indirectly cause the PA lesions, which are periapical or periradicular barriers that limit the microorganisms and stop their spread into the surrounding tissues. Resorption of the bone is followed by the replacement of the bone by granulomatous tissue and a thick wall of polymorphonuclear leukocytes (PMN). Less frequently, an epithelial plug is present at the apical foramen to prevent pathogens from entering the extra radicular tissues. Only a few endodontic pathogens are able to pass through these barriers, but microbial byproducts and toxins can do so in order to start and establish periradicular pathosis. The most common clinical manifestations of these lesions are peripapillary radiolucencies. Dental granulomas, periradicular cysts, and radiolucent abscesses make up the majority of periradicular lesions.

The non-vital tooth[5,6,32-37]

It has been established that a non vital pulp facilitates bacterial invasion of dentinal tubules more quickly than a vital pulp. With a healthy pulp, the outward flow of dentinal fluid and the contents of the tubules, such as odontoblast processes, collagen fibrils, and the sheath like lamina limitans that lines the tubules, can potentially slow down bacterial invasion of the tubules. The functional or physiologic diameter of the tubules is only 5% to 10% of the anatomic diameter shown by microscopy due to the presence of tubular contents. Dentin sclerosis beneath a carious lesion, tertiary dentin, the smear layer, and intratubular fibrinogen deposition all restrict dentin permeability and, as a result, limit or even prevent bacterial advance through the dentinal tubules to the pulp.

A root canal is required in cases with pulp necrosis, a clinical diagnostic category that denotes the death of the tooth pulp. The pulp is asymptomatic and unresponsive to pulp tests. Unless the canal is infected, pulp necrosis does not by itself result in apical periodontitis (pain to percussion or radiographic signs of osseous disintegration). Because of calcification, a recent history of trauma, or just because the tooth is not reacting, some teeth may not respond to pulp testing. As previously mentioned, this is the reason why all testing must be comparative in nature (e.g. thermal testing on any teeth may not elicit a response by the patient).

A tooth with a necrotic pulp is considered to be nonvital. When a tooth does not respond to heat, electrical, or mechanical stimulation, pulp necrosis is suspected; however, the precise diagnosis is not made until the pulp chamber and the root canal have been examined and probed. As was mentioned above, even in teeth that clinically appear to be healthy, the necrotic pulp tissue and the root canal area are infected virtually always. An apical granuloma or cyst will eventually grow as a result of inflammation caused by microorganisms in the tooth's root canal over time. Therefore, treating a nonvital tooth always entails treating an infectious disease process.

If the pulp is completely necrotic, the predominantly anaerobic microbes have a safe haven because there are no defense cells if there is no vascularity.

Radiographic alterations from a necrotic pulp won't appear until the cortical plate starts to demineralize. (Significant medullary bone degradation may take place prior to the onset of radiographic symptoms).

A polymicrobial mixture with roughly equal amounts of gram-positive and gram-negative species, dominated by obligate anaerobes, causes primary root canal infection in untreated root canals.

Extraradicular infection[7,38-47]

Extraradicular infection typically develops from an intraradicular infection that has traveled through the apical foramen to the periradicular tissues. An acute apical abscess, in which there has been a significant bacterial invasion and a buildup of pus in the periapical region, is a typical instance of an extraradicular infection. Pus is produced as a result of microbial invasion of the periapical tissues, which defines an acute apical abscess. Extraradicular infections and periapical biofilms are thought to be potential causes of endodontic failure since they have mostly been linked to post-treatment apical periodontitis. Using 16S rRNA cloning, sequencing, and pyrosequencing, the microbial communities of root filled canals with post-treatment apical periodontitis have been examined. In untreated teeth, bacterial intraradicular biofilms are the primary cause of apical periodontitis. The majority of such biofilms are eliminated during a primary root canal treatment by the chemomechanical action of tools and irrigants during the expansion and shaping of the main and minor channels.

Management of Endodontic Infection[22,27,48-56]

Adequate debridement of the diseased root canal and drainage for both soft and hard tissue are essential to the effective therapy of infection of endodontic origin. Removing the pathogenic bacteria, their byproducts, and pulpal debris from the infected root canal system that produced the periapical pathosis and creating conditions that will help the lesion heal are the goals of treating infections of endodontic origin. The localized soft tissue swelling of endodontic origin should be incised and drained concurrently, along with sufficient debridement of the root canal system. Studies have shown that when adequate local debridement, medication, and incision for drainage, if necessary, have been accomplished in cases with irreversible pulpitis, symptomatic apical periodontitis, or localized acute apical abscess, adjunctive antibiotics are ineffective in preventing or alleviating signs and symptoms.

Additionally, it is considered best practice to give patients with spreading infections primary or adjunctive antibiotic prescriptions along with local debridement and surgical drainage and to closely monitor their recovery since these prescriptions are made based on clinical judgment and may not be effective or sufficient for adequate treatment.[33,57-64]

A complicated regeneration process including bone, periodontal ligaments, and cementum is used to repair the periradicular tissues. Bone eventually fills in the area of mineral loss, and the radiographic density rises. If the cortical plate is punctured, healing starts with the external cortical plate's regeneration and moves inside the lesion from the outside. Because the maxilla has a larger circulatory network than the mandible, maxillary lesions heal more quickly than mandibular lesions. Due to the proximity of the buccal and lingual plates in the front segments, anterior lesions of the maxilla and mandible heal more quickly than posterior lesions.[65-73]

It was also hypothesized that the infection could enter the periapical tissue through the pulp that had survived and the apical papilla, leading to significant bone resorption. Additionally, it may be feasible for periapical illness to develop even when the pulp is only partially necrotic and infected because the open apex allows for adequate connection between the pulp tissue and the periapical tissue.[74-81]

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Persistent apical periodontitis may be brought on by an endodontic root infection that persisted after therapy. In addition, excessive instrumentation and/or overfilling with the resulting extrusion of debris and material during root canal therapy may potentially cause inflammation to continue.[90-93]

CONCLUSION

Different bacterial species appear to predominate at various stages of the infectious process during root canal infection, which is a dynamic process. Facultative bacteria predominate in the early stages of the pulpal infectious process. After a few days or weeks, the root canal loses oxygen due to pulp necrosis and facultative bacteria eating it. As a result, a low redox potential anaerobic environment forms, which is extremely favorable for the survival and expansion of obligate anaerobic bacteria. As a result, effective infection control is the prerequisite and basis for healing, where root canal preparation is a crucial step. Because different grades of root canal infection require varied disinfection schedules to aid tissue healing and assure the long term success rate of endodontic treatment, an accurate diagnosis of the grades of infected root canals is required before treatment.

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REFERENCES

1. Hernández Vigueras S, Donoso Zúñiga M, Jané-Salas E, Salazar Navarrete L, Segura-Egea JJ, Velasco-Ortega E, *et al.* Viruses in pulp and periapical inflammation: a review. Odontology.
2. Rohlin M, Kullendorff B, Ahlqwist M, Henrikson CO, Hollender L, Stenström B. Comparison between panoramic and periapical radiography in the diagnosis of periapical bone lesions. Dentomaxillofac Radiol. 1989;18:151–155.
3. Persoon IF, Buijs MJ, Özok AR, Crielaard W, Krom BP, Zaura E, *et al.* The mycobiome of root canal infections is correlated to the bacteriome. Clin Oral Investig. 2017;21:1871–1881.
4. Özok AR, Persoon IF, Huse SM, Keijser BJF, Wesselink PR, Crielaard W, Zaura E. Ecology of the microbiome of the infected root canal system: a comparison between apical and coronal root segments. Int Endod J. 2012;45:530–541.
5. Vianna ME, Conrads G, Gomes BP, Horz HP. Identification and quantification of archaea involved in primary endodontic infections. J Clin Microbiol. 2006;44:1274–1282.
6. Strindberg IZ. The dependence of the results of pulp therapy on certain factors. Acta Odontol Scand. 1956;14 (Suppl 21):1–175.
7. Silva TA, Garlet GP, Fukada SY, Silva JS, Cunha FQ. Chemokines in Oral Inflammatory Diseases: Apical Periodontitis and Periodontal Disease. J DentRes (2007) 86(4):306–19.
8. Alaa M. Altaie PhD, Mohammad G. Mohammad PhD, Mohamed I. Madkour PhD, Sarra B. Shakartalla PhD, Manju Nidagodu Jayakumar MS, Aghila Rani K.G. PhD, The Essential Role of 17-Octadecynoic Acid in the Pathogenesis of Periapical Abscesses, Journal of Endodontics, Volume 49, Issue 2, February 2023, Pages 169-177. e3.
9. Yang L-C, Huang F-M, Lin C-S, Liu C-M, Lai C-C, Chang Y-C. Induction of Interleukin-8 Gene Expression by Black-Pigmented Bacteroides in Human Pulp Fibroblasts and Osteoblasts. Int Endod J (2003) 36(11):774–9.
10. Altaie AM, Venkatachalam T, Samaranayake LP, Soliman SSM and Hamoudi R (2021) Comparative Metabolomics Reveals the Microenvironment of Common T-Helper Cells and Differential Immune Cells Linked to Unique Periapical Lesions. Front. Immunol. 12:707267.
11. Paster BJ, Olsen I, Aas JA, Dewhirst FE. The breadth of bacterial diversity in the human periodontal pocket and other oral sites. Periodontol. 2000;2006;42:80–7.
12. Tronstad L, Barnett F, Riso K, Slots J. Extraradicular endodontic infections. Endod Dent Traumatol. 1987;3:86–90.
13. Gatti JJ, Dobeck JM, Smith C, White RR, Socransky SS, Skobe Z. Bacteria of asymptomatic periradicular endodontic lesions identified by DNA – DNA hybridization. Endod Dent Traumatol. 2000;16:197–204.
14. Nair PN. On the causes of persistent apical periodontitis, a review. Int Endod J. 2006;39:249–81.
15. Nair PN, Henry S, Cano V, Vera J. Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after "one-visit" endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod.
16. Costerton JW, Lewandowski Z, DeBeer D, Caldwell D, Korber D, James G. Biofilms, the customized microniche. J Bacteriol. 1994;176:2137–42.
17. Thomas T, Thomas TJ. Polyamines in Cell growth and cell death: Molecular mechanisms and therapeutic applications. Cell Mol Life Sci. 2001;58:244–58.
18. Ramachandran P, Rachuri NK, Martha S, Shakthivel R, Gundala A, Battu TS. Implications of overprescription of antibiotics: A cross-sectional study. J Pharm Bioall Sci. 2019;11 (Suppl S2):434–7.
19. Wasan H, Gupta P, Mathur A, Mutneja E, Mathur VP, Gupta YK. Influence of qualification and practice settings of dental practitioners on antimicrobial prescribing in Delhi and National Capital Region, India. J Nat Sci Biol Med. 2017;8:229–34.
20. Bansal R, Jain A. Overview on the current antibiotic containing agents used in endodontics. N Am J Med Sci. 2014;6:351–8.
21. Carrotte P. Endodontics: Part 1. The modern concept of root canal treatment. Br Dent J. 2004;197:181–183.
22. Holland GR, Davis SB. Ingle's Endodontics. 6th ed. Ontario: BC Decker Inc.; 2008. Pulpal Pathosis; pp. 468–493.
23. Iwu C, MacFarlane TW, MacKenzie D, Stenhouse D. The microbiology of periapical granulomas. Oral Surg Oral Med Oral Pathol. 1990;69:502–505.
24. Johansen JR, Karlsen K. The effect of denervation on trauma from occlusion. J Oral Rehabil. 1979;6:27–34.
25. Haapasalo M., Orstavik D. In vitro infection and disinfection of dentinal tubules. J. Dent. Res. 1987;66:1375–1379.
26. Braz-Silva P.H., Bergamini M.L., Mardegan A.P.,

De Rosa C.S., Hasseus B., Jonasson P. Inflammatory profile of chronic apical periodontitis: A literature review. Acta Odontol. Scand. 2019;77:173–180.

27. Verma D., Garg P.K., Dubey A.K. Insights into the human oral microbiome. Arch. Microbiol. 2018;200:525–540.
28. Kwang S., Abbott P. The presence and distribution of bacteria in dentinal tubules of root filled teeth. Int. Endod. J. 2014;47:600–610.
29. Love R.M., Jenkinson H.F. Invasion of dentinal tubules by oral bacteria. Crit. Rev. Oral Biol. Med. 2002;13:171–183.doi:10.1177/154411130201300207.
30. Narayanan L.L., Vaishnavi C. Endodontic microbiology. J. Conserv. Dent. 2010;13:233–239.
31. Siqueira J.F., Jr., Rocas I.N., Souto R., de Uzeda M., Colombo A.P. Actinomyces species, streptococci, and Enterococcus faecalis in primary root canal infections. J. Endod. 2002;28:168–172.
32. Adib V., Spratt D., Ng Y.L., Gulabivala K. Cultivable microbial flora associated with persistent periapical disease and coronal leakage after root canal treatment: A preliminary study. Int. Endod. J. 2004;37:542–551.
33. Ashley M, Harris I. The assessment of the endodontically treated tooth. Dent Update. 2001;28(5):247–52.
34. Ng YL, Mann V, Gulabivala K. A prospective study of the factors affecting outcomes of non-surgical root canal treatment:part 2:tooth survival. Int Endod J. 2011 Jul;44(7):610–25.
35. Vire DE. Failure of endodontically treated teeth:classification and evaluation. J Endod. 1991;17(7):338–42.
36. Siqueira JF, Jr, de Uzeda M. Disinfection by calcium hydroxide pastes of dentinal tubules infected with two obligate and one facultative anaerobic bacteria. J Endod. 1996;22(12):674–6.
37. Iqbal MK, Johansson AA, Akeel RF, Bergenholtz A, Omar R. A retrospective analysis of factors associated with the periapical status of restored, endodontically treated teeth. Int J Prosthodont. 2003;16(1):31–8.
38. Metzger Z, Huber R, Tobis I, Better H. Enhancement of healing kinetics of periapical lesions in dogs by the Apexum procedure. J Endod. 2009;35(1):40–5.
39. Kvist T, Reit C. Postoperative discomfort associated with surgical and nonsurgical endodontic retreatment. Endod Dent Traumatol. 2000;16(2):71–4.
40. Torabinejad M, Corr R, Handysides R, Shabahang S. Outcomes of nonsurgical retreatment and endodontic surgery:a systematic review. J Endod. 2009;35(7):930–7.
41. Love RM, Firth N. Histopathological profile of surgically removed persistent periapical radiolucent lesions of endodontic origin. Int Endod J. 2009;42(3):198–202.
42. Strindberg LZ. The dependence of the results of pulp therapy on certain factors-an analytical study based on radiographic and clinical follow-up examination. Acta Odontol Scand. 1956;14:1–175.
43. Lalonde ER, Luebke RG. The frequency and distribution of periapical cysts and granulomas. An evaluation of 800 specimens. Oral Surg Oral Med Oral Pathol. 1968;25(6):861–8.
44. Wigler R, Kaufman AY, Lin S, Steinbock N, Hazan-Molina H, Torneck CD. Revascularization:a treatment for permanent teeth with necrotic pulp and incomplete root development. J Endod. 2013;13(39) 3:319–26.
45. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, *et al.* Platelet-rich fibrin (PRF): a second-generation platelet concentrate Part I:technological concepts and evolution. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;101(3):e37–44.
46. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, *et al.* Platelet-rich fibrin (PRF):a second-generation platelet concentrate Part II:platelet-related biologic features. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;101(3):e45–50.
47. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, *et al.* Platelet-rich fibrin (PRF):a second-generation platelet concentrate Part III:leucocyte activation:a new feature for platelet concentrates ? Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;101:e51–5.
48. Kim JH, Woo SM, Choi NK, Kim WJ, Kim SM, Jung JY. Effect of Platelet-rich Fibrin on Odontoblastic Differentiation in Human Dental Pulp Cells Exposed to Lipopolysaccharide. J Endod. 2017;43(3):433–8.
49. He L, Lin Y, Hu X, Zhang Y, Wu H. A comparative study of platelet-rich fibrin (PRF) and platelet-rich plasma (PRP) on the effect of proliferation and differentiation of rat osteoblasts in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009;108(5):707–13.
50. Satish Kumar K, Subbiya A, Vivekanandhan P, Prakash V, Tamilselvi R. Management of an Endodontic Infection with an Extra Oral Sinus Tract in a Single Visit:A Case Report. J Clin Diagn Res. 2013;7(6):1247–9.
51. Carr G. B., Schwartz R. S., Schaudinn C., Gorur A., Costerton J. W. (2009). Ultrastructural Examination of Failed Molar Retreatment With Secondary Apical Periodontitis: An Examination of Endodontic Biofilms in an Endodontic Retreatment Failure. J. Endod. 35 (9), 1303–1309.
52. Di Bella J. M., Bao Y., Gloor G. B., Burton J. P., Reid G. (2013). High Throughput Sequencing Methods and Analysis for Microbiome Research. J. Microbiol. Methods 95 (3), 401–414.
53. Henriques L. C. F., de Brito L. C. N., Tavares W. L.

F., Teles R. P., Vieira L. Q., Teles F. R., *et al.* (2016). Microbial Ecosystem Analysis in Root Canal Infections Refractory to Endodontic Treatment. J. Endod. 42 (8), 1239–1245.

54. Murad C. F., Sassone L. M., Faveri M., Hirata R., Jr., Figueiredo L., Feres M. (2014). Microbial Siversity in Persistent Root Canal Infections Investigated by Checkerboard DNA-DNA Hybridization. J. Endod. 40 (7), 899–906.
55. Qi Z., Cao H., Jiang H., Zhao J., Tang Z. (2016). Combinations of Bacterial Species Associated With Symptomatic Endodontic Infections in a Chinese Population. Int. Endod. J. 49 (1), 17–25.
56. Siqueira J. F., Rôças I. N. (2009). Diversity of Endodontic Microbiota Revisited. J. Dent. Res. 88 (11), 969–981.
57. Takahama A., Jr., Rocas I. N., Faustino S. P., Alves F. R. F., Azevedo R. S., Gomes C. C., *et al.*. (2018). Association Between Bacteria Occurring in the Apical Canal System and Expression of Bone-Resorbing Mediators and Matrix Metalloproteinases in Apical Periodontitis. Int. Endod. J. 51 (7), 738–746.
58. Zhang C., Yang Z., Hou B. (2021). Diverse Bacterial Profile in Extraradicular Biofilms and Periradicular Lesions Associated With Persistent Apical Periodontitis. Int. Endod. J. 54 (9), 1425–1433.
59. Tzanetakis G. N., Azcarate-Peril M. A., Zachaki S., Panopoulos P., Kontakiotis E. G., Madianos P. N., *et al.*. (2015). Comparison of Bacterial Community Composition of Primary and Persistent Endodontic Infections Using Pyrosequencing. J. Endod. 41 (8), 1226–1233.
60. Siqueira J. F., Alves F. R. F., Rocas I. N. (2011). Pyrosequencing Analysis of the Apical Root Canal Microbiota. J. Endod. 37 (11), 1499–1503.
61. Saber M. H., Schwarzberg K., Alonaizan F. A., Kelley S. T., Sedghizadeh P. P., Furlan M., et al.. (2012). Bacterial Flora of Dental Periradicular Lesions Analyzed by the 454-Pyrosequencing Technology. J. Endod. 38 (11), 1484–1488.
62. Noguchi N., Noiri Y., Narimatsu M., Ebisu S. (2005). Identification and Localization of Extraradicular Biofilm-Forming Bacteria Associated With Refractory Endodontic Pathogens. Appl. Environ. 71 (12), 8738–8743.
63. Keijser B. J. F., Zaura E., Huse S. M., van der, Vossen J. M. B. M., Schuren F. H. J., Montijn R. C., *et al.*. (2008). Pyrosequencing Analysis of the Oral Microflora of Healthy Adults. J. Dent. Res. 87 (11), 1016–1020.
64. Farias B. C., Souza P. R. E., Ferreira B., Melo R. S. A., Machado F. B., Gusmao E. S., *et al.*. (2012). Occurrence of Periodontal Pathogens Among Patients With Chronic Periodontitis. Braz. J. Microbiol. 43 (3), 909–916.
65. Peciuliene V, Reynaud AH, Balciuniene I, Haapasalo M. Isolation of yeasts and enteric bacteria in root-filled teeth with chronic apical periodontitis. Int Endod J. 2001. Sep;34(6):429–34.
66. Sundqvist G, Figdor D. Life as an endodontic pathogen: ecological differences between the untreated and root-filled root canals. Endod Topics. 2003;6(1):3–28.
67. Sunde PT, Olsen I, Debelian GJ, Tronstad L. Microbiota of periapical lesions refractory to endodontic therapy. J Endod. 2002. Apr;28(4):304–10.
68. Ricucci D, Siqueira JF., Jr Biofilms and apical periodontitis: study of prevalence and association with clinical and histopathologic findings. J Endod. 2010. Aug;36(8):1277–88.
69. Gomes BP, Pinheiro ET, Jacinto RC, Zaia AA, Ferraz CC, Souza-Filho FJ. Microbial analysis of canals of root-filled teeth with periapical lesions using polymerase chain reaction. J Endod. 2008. May;34(5):537–40.
70. Ricucci D, Candeiro GT, Bugea C, Siqueira JF., Jr Complex Apical Intraradicular Infection and Extraradicular Mineralized Biofilms as the Cause of Wet Canals and Treatment Failure: Report of 2 Cases. J Endod. 2016. Mar;42(3):509–15.
71. Munson, M. A., T. Pitt-Ford, B. Chong, A. Weightman, and W. G. Wade. 2002. Molecular and cultural analysis of the microflora associated with endodontic infections. J. Dent. Res. 81:761-766.
72. Noiri, Y., L. Li, and S. Ebisu. 2001. The localization of periodontal-disease-associated bacteria in human periodontal pockets. J. Dent. Res. 80:1930-1934.
73. Ramachandran Nair, P. N. 1987. Light and electron microscopic studies of root canal flora and periapical lesions. J. Endodont. 13:29-39.
74. Sunde, P. T., I. Olsen, G. J. Debelian, and L. Tronstad. 2002. Microbiota of periapical lesions refractory to endodontic therapy. J. Endodont. 28:304-310.
75. Takemura, N., Y. Noiri, A. Ehara, T. Kawahara, N. Noguchi, and S. Ebisu. 2004. Single species biofilm-forming ability of root canal isolates on gutta-percha points. Eur. J. Oral Sci. 112:523-529.
76. Tronstad, L., F. Barnett, and F. Cervone. 1990. Periapical bacterial plaque in teeth refractory to endodontic treatment. Endodont. Dent. Traumatol. 6:73-77.
77. Tronstad, L., F. Barnett, K. Riso, and J. Slots. 1987. Extraradicular endodontic infections. Endodont. Dent. Traumatol. 3:86-90.
78. Sundqvist, G. 1992. Associations between microbial species in dental root canal infections. Oral Microbiol. Immunol. 7:257-262.
79. Sundqvist, G. 1994. Taxonomy, ecology, and pathogenicity of the root canal flora. Oral Surg. Oral Med. Oral Pathol. 78:522-530.

80. Tawakoli PN, Ragnarsson KT, Rechenberg DK, Mohn D, Zehnder M. Effect of endodontic irrigants on biofilm matrix polysaccharides. Int Endod J. (2017) 50:153–60.
81. Neelakantan P, Romero M, Vera J, Daood U, Khan AU, Yan A, *et al*.. Biofilms in endodontics-current status and future directions. Int J Mol Sci. (2017) 18:1748.
82. Shen Y, Stojicic S, Haapasalo M. Antimicrobial efficacy of chlorhexidine against bacteria in biofilms at different stages of development. J Endod. (2011) 37:657–61.
83. Thilo BE, Baehni P, Holz J. Dark-field observation of the bacterial distribution in root canals following pulp necrosis. J Endod 1986; 12:202-5.
84. Baumgartner JC, Falkler WAJ. Bacteria in the apical 5 mm of infected root canals. J Endod 1991; 17:380-3.
85. Munson MA, Pitt-Ford T, Chong B, Weightman A, Wade WG. Molecular and cultural analysis of the microflora associated with endodontic infections. J Dent Res 2002; 81:761-6.
86. Vianna ME, Conrads G, Gomes BP, Horz HP. Quantification and characterization of Synergistes in endodontic infections. Oral Microbiol Immunol 2007; 22:260-5.
87. Zehnder M, Guggenheim B. The mysterious appearance of enterococci in filled root canals. Int Endod J 2009; 42:277-87.
88. Hahn CL, Liewehr FR. Relationships between caries bacteria, host responses, and clinical signs and symptoms of pulpitis. J Endod 2007; 33:213-9.
89. Whitworth JM. Apparent periapical repair without operative intervention: a case report and discussion. Int Endod J 2000; 33:286-9.
90. Marending M, Peters OA, Zehnder M. Factors affecting the outcome of orthograde root canal therapy in a general dentistry hospital practice. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005; 99:119-24.
91. Ricucci D, Siqueira JFJ. Biofilms and apical periodontitis: study of prevalence and association with clinical and histopathologic findings. J Endod 2010; 36:1277-88.
92. Hajishengallis G. The inflammophilic character of the periodontitis-associated microbiota. Mol Oral Microbiol 2014; 29(6):248-57.
93. Whitworth JM. Apparent periapical repair without operative intervention: a case report and discussion. Int Endod J 2000; 33:286-9.
94. Slots J, Sabeti M, Simon JH. Herpesviruses in periapical pathosis: an etiopathogenic relationship? Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003; 96:327-31.
95. Baumgartner JC, Falkler WAJ, Beckerman T. Experimentally induced infection by oral anaerobic microorganisms in a mouse model. Oral Microbiol Immunol 1992; 7:253-6.
96. Rocas IN, Siqueira JFJ. Detection of novel oral species and phylotypes in symptomatic endodontic infections including abscesses. FEMS Microbiol Lett 2005; 250:279-85.
97. Fredericks DN, Relman DA. Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. Clin Microbiol Rev 1996; 9:18-33.