

# Extraradicular Infection as the Cause of Persistent Symptoms: A Case Series

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## Abstract

**Introduction:** This article describes 3 cases that presented persistent symptoms after appropriate endodontic treatment. Histopathologic and histobacteriologic investigation were conducted for determination of the cause. **Methods:** Three cases are reported that presented with persistent symptoms after endodontic re-treatment (cases 1 and 2) or treatment (case 3). Periapical surgery was indicated and performed in these cases. The biopsy specimens, consisting of root apices and the apical periodontitis lesions, were subjected to histopathologic and histobacteriologic analyses. **Results:** Case 1 was an apical cyst with necrotic debris, heavily colonized by ramifying bacteria, in the lumen. No bacteria were found in the apical root canal system. Case 2 was a granuloma displaying numerous bacterial aggregations through the inflammatory tissue. Infection was also present in the dentinal tubules at the apical root canal. Case 3 was a cyst with bacterial colonies floating in its lumen; bacterial biofilms were also seen on the external apical root surface, filling a large lateral canal and other apical ramifications, and between layers of cementum detached from the root surface. No bacteria were detected in the main root canal. **Conclusions:** Different forms of extraradicular infection were associated with symptoms in these cases, leading to short-term endodontic failure only solved by periapical surgery. (*J Endod* 2015;41:265–273)

## Key Words

Endodontic re-treatment, extraradicular infection, post-treatment apical periodontitis, treatment outcome

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Post-treatment apical periodontitis is usually caused by persistent or secondary intraradicular infections (1, 2). It has also been suggested that infection located beyond the confines of the root canal system, either in the form of a biofilm attached to the external root surface (3, 4) or as cohesive colonies present within the mass of the inflammatory lesion (5, 6), may be responsible for post-treatment disease in some cases. One of the most debatable issues in the field of endodontic microbiology is whether or not infection can establish itself outside the canal system (except for abscess cases) and as such be the independent cause of post-treatment apical periodontitis.

Culture-dependent (7–11) and culture-independent studies (12–16) have reported the extraradicular occurrence of a complex microbiota associated with apical periodontitis lesions that have not responded favorably to the root canal treatment. One important discussion on this topic refers to whether contamination can be effectively ruled out during surgical sampling of apical periodontitis lesions for microbiological analysis. Bacteria located in the very apical part of the canal may be displaced into the biopsy specimen during surgical procedures and be regarded as "extraradicular" bacteria by culture and molecular studies (17). Most previous studies have not evaluated the bacteriologic conditions of the apical part of the root canal, making it difficult to ascertain whether the extraradicular infection was dependent on or independent of an intraradicular infection (18). As a consequence, there is no sufficient evidence supporting that an extraradicular infection can exist as a self-sustained process independent of the intraradicular infection (19). In a histologic study, Ricucci et al (20) evaluated several root canal-treated teeth with apical periodontitis and found no case of independent extraradicular infection. In the few instances that bacteria were observed outside the root canal system, a concomitant intraradicular infection was present.

Histologic analysis of block specimens composed of the lesion attached to the root apex in their original spatial relationship can circumvent most of the shortcomings of previous studies because it may permit one to distinguish infection from contamination, detect artifactual bacterial displacement into the lesion, and reveal the microbiological conditions of the apical part of the root canal. This article is intended to contribute to the knowledge of the causes of endodontic treatment failure by reporting 3 cases of post-treatment apical periodontitis showing persisting symptoms associated with different types of extraradicular infection.

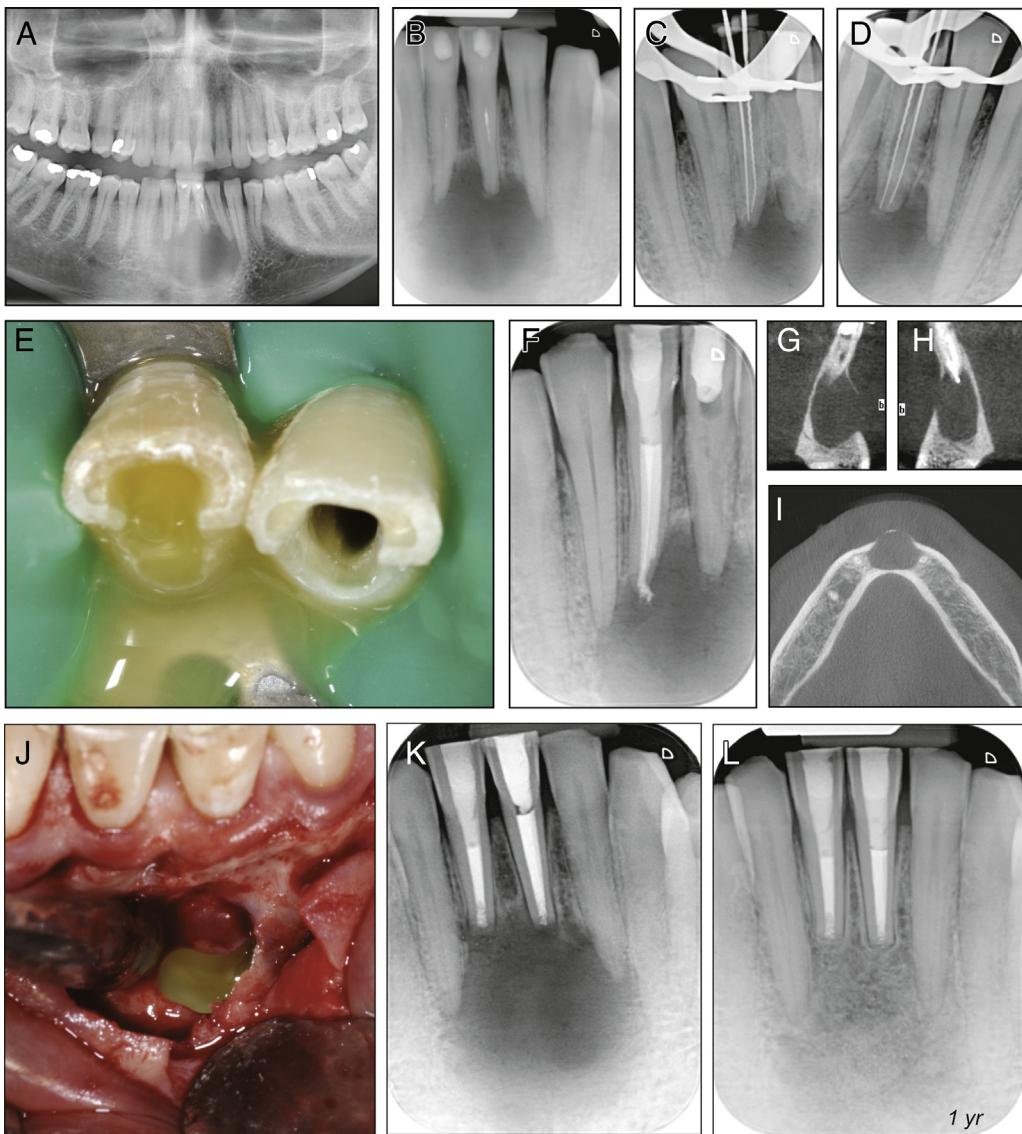
## Case Series

### Case 1

This case relates to a 35-year-old man with a history of repeated abscesses in the anterior mandible with severe pain and swelling. The medical history was noncontributory. His general dentist had initiated treatment of the 2 mandibular central incisors, which had necrotic pulps and were associated with a large periapical radiolucency. The canals (1 per tooth) were instrumented and medicated with an iodoform-based paste. Clinical symptoms did not recede even after 2 sessions of instrumentation and intracanal medication with calcium oxide. At this point (6 months after beginning of the treatment), the lesion had increased in size (Fig. 1A), and the patient was then referred to an endodontist.

At the first visit with the endodontist, signs of severe attrition were noted for all the anterior teeth. The patient denied any acute traumatic event. The access cavities on both

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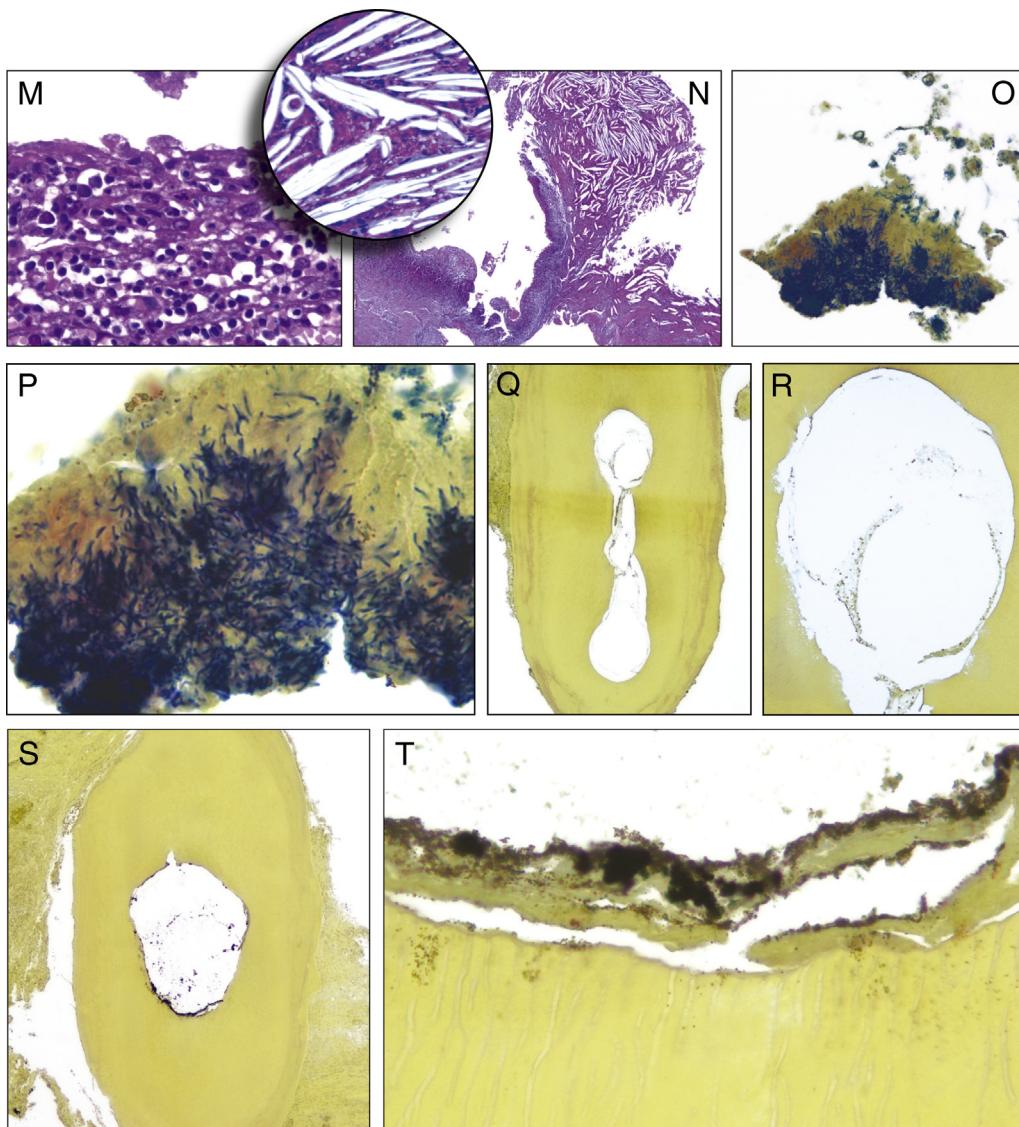
**Figure 1.** Case 1: (A) a panoramic radiograph taken by the general dentist, showing the large radiolucency apparently involving all mandibular incisors. (B) A periapical radiograph taken by the endodontist. (C and D) Lingual canals were found in both teeth and the WL established. (E) Purulent exudate was observed draining from the canals 2 weeks after the first instrumentation session. (F) After 2 more weeks, the canals of tooth #25 were dry; obturation followed. (G and H) CBCT sagittal scans of teeth #24 and #25. (I) A CBCT axial scan. (J) Pus was evident in the pathologic cavity. (K) A postoperative radiograph. (L) A follow-up radiograph taken after 1 year shows complete healing of the lesion. No symptoms were present.

the central incisors were apparently not sufficiently extended lingually and were filled with a temporary material. These 2 teeth were tender to percussion and slightly mobile (grade 1). Fluctuant swelling was present in the vestibule. The 2 lateral incisors and canines responded normally to thermal and electric pulp tests. A periapical radiograph confirmed the presence of the radiolucent lesion, which apparently involved the periapical regions of lateral incisors and canines, and disclosed remnants of a highly radiopaque material (likely iodoform-based paste) in the canals of the central incisors (Fig. 1B).

Root canal retreatment of the 2 central incisors was initiated. Access cavities were improved, and lingual canal orifices were detected in both teeth. The working length (WL) was established (Fig. 1C and D) and the canals enlarged with Gates-Glidden burs in the coronal two thirds and hand files (up to the size #35) in the apical third. Irrigation was performed with 1% sodium hypochlorite (NaOCl). The canals were medicated with calcium hydroxide mixed with sterile saline to

a creamy consistency, and the access sealed with IRM (Dentsply International, Milford, DE). Symptoms disappeared over the postoperative period.

The canals were then reopened 2 weeks later, and after removal of the calcium hydroxide paste, a purulent exudate was observed draining from the canals (Fig. 1E). Palpation of the mucosa over the periapical area provoked massive drainage through the canals. Canals were instrumented and medicated once again. After 2 more weeks (4 weeks from the beginning of retreatment), the canals of tooth #25 were dry. They were then obturated with gutta-percha and sealer using cold lateral compaction (Fig. 1F). Purulent fluid was still present in the canals of tooth #24, which was remedicated. Periapical surgery was scheduled at this point and a cone-beam computed tomographic (CBCT) scan was requested to ascertain the relationship of the involved teeth with the adjacent structures and the extent of periapical bone loss (Fig. 1G–I).



**Figure 1.** (continued). (M) A high-power view from the epithelial lining showing an ulcerated appearance (hematoxylin-eosin, original magnification  $\times 400$ ). (N) The area of the cyst wall with a mass of cholesterol crystals projecting into the lumen (original magnification  $\times 16$ ; inset  $\times 100$ ). (O and P) Debris from the cyst lumen with ramifying bacteria in a biofilm structure (Taylor-modified Brown and Brenn, original magnification  $\times 400$  and  $\times 1000$ ). (Q and R) A crosscut section of the apex of tooth #24 taken approximately 2 mm coronally to the root tip. No bacteria could be seen (original magnification  $\times 25$  and  $\times 100$ ). (S and T) A crosscut section of the apex of tooth #25 taken approximately 1.5 mm coronally to the root tip. The absence of bacteria (original magnification  $\times 25$  and  $\times 400$ ).

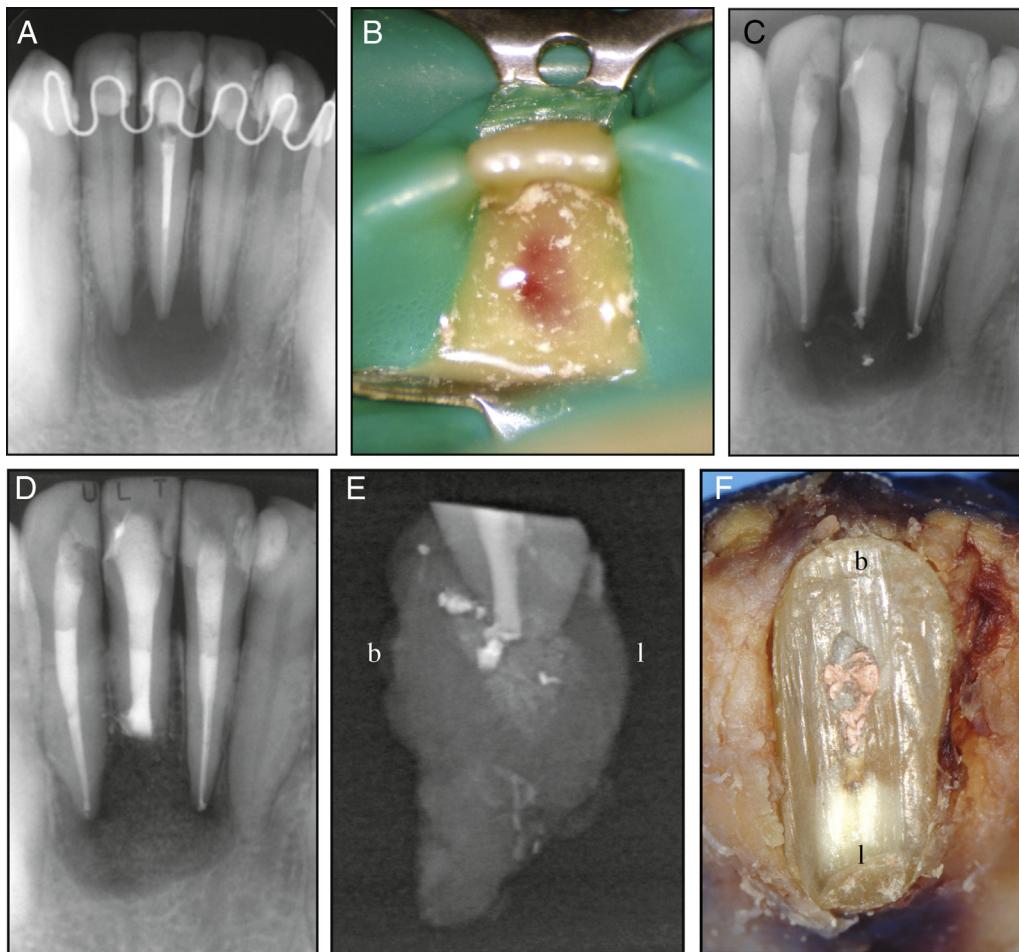
Surgical intervention was performed 10 weeks after the beginning of retreatment. Because exudation still persisted, root canals of tooth #24 were obturated during surgery. The purulent content of the pathologic cavity could be clearly seen after breakage of the wall (Fig. 1J). An attempt was made to detach the tissue from the bone crypt in 1 piece, but this was not possible because of the thin wall, and the lesion was removed in 1 major portion and several minor fragments. Root-end resection was accomplished for both teeth. The canals of tooth #24 were then obturated. Retrocavities were prepared with ultrasonic tips and filled with mineral trioxide aggregate (Tech Bio Sealer Root End; Isasan, Rovello Porro [CO], Italy) (Fig. 1K).

After the bone cavity was filled with blood, the flap was sutured. No complications occurred after surgical treatment. The pathologic soft tissue and the 2 root apices were submitted to histologic and histobacteriologic analysis. A follow-up radiograph taken after

12 months showed that the lesion healed (Fig. 1L). The tooth was asymptomatic.

### Case 2

This case relates to a 42-year-old man who was referred to an endodontist by his orthodontist because of the presence of an extensive apical periodontitis lesion in the region of mandibular incisors as detected radiographically. The patient reported a previous trauma in the area caused by a car accident. Tooth #25 had developed crown discoloration since then. The patient reported no significant medical history, and pulp sensibility tests revealed positive results for all mandibular incisors, except for tooth #25, which had been endodontically treated 2 years previously. Percussion and palpation tests yielded negative results, but induration was evident over the root apex of tooth #25. The patient could recover only 1 radiograph from the time of



**Figure 2.** Case 2: (A) A diagnostic radiograph. (B) Pus is seen draining from the canal after removal of the root canal filling material. (C) A radiograph taken after obturation of the canals of teeth #25 (necrotic) and #24 and #26 (both previously vital). (D) A postoperative radiograph. (E) A radiograph of the apical biopsy taken in a mesiodistal projection (b, buccal; l, lingual). (F) A photograph of the resected surface of the apex (b, buccal; l, lingual).

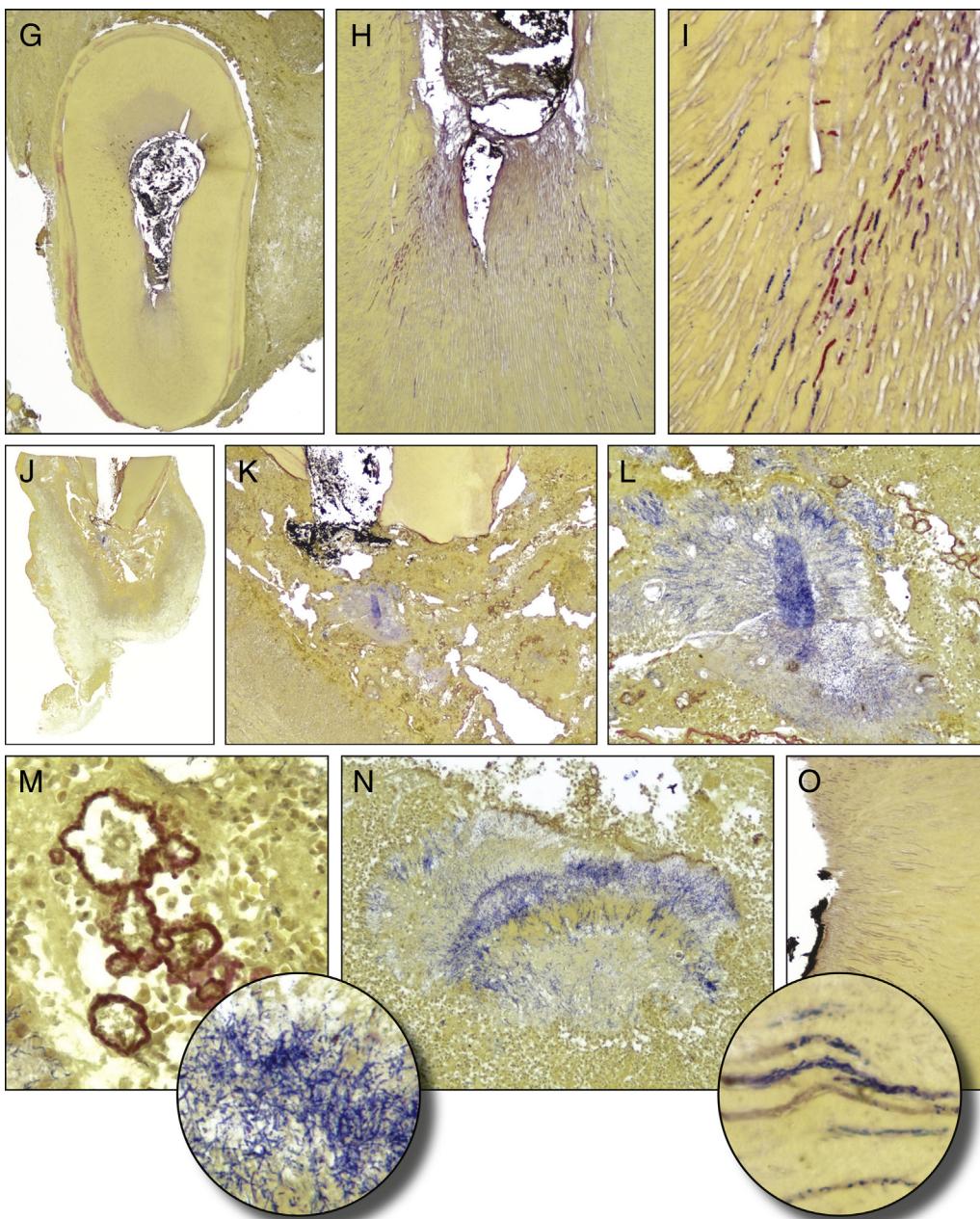
previous treatment, which had been taken for WL determination apparently without rubber dam placement. The apical periodontitis lesion was present at the time of the previous treatment, apparently with a smaller diameter compared with the most recent radiograph taken by the endodontist (Fig. 2A). Endodontic retreatment was scheduled.

Under rubber dam isolation and after disinfection of the operative field, the previous filling material was removed using Gates Glidden burs, hand K-type files, and solvent (chloroform). The WL was established using an electronic apex locator (Apex Finder 7005; Analytic/Endo, Orange, CA) and confirmed by periapical radiographs. A K-type file size 20 was used as the patency instrument throughout the preparation procedures. After removal of the previous filling and establishment of apical patency, a heavy purulent discharge was observed (Fig. 2B). A bacteriologic sample was taken from the canal using sterile paper points as described elsewhere (21). After a few minutes, the canal was irrigated with NaOCl and instrumented up to the apical foramen using the Reciproc instrument R40 (VDW, Munich, Germany) and abundant irrigation with 5.25% NaOCl. After preparation, a small aspiration cannula was taken up to 2 mm short of the WL. A large volume of pus still drained via the canal. After drainage stopped, the canal was irrigated with NaOCl, and the access cavity was temporarily sealed with IRM. Because of the persistent exudation, the patient was instructed to take 750 mg amoxicillin with 125 mg potassium clavulanate every 12 hours for 7 days.

One week later, the patient returned with no symptoms, and the canals were irrigated with NaOCl and reinstrumented at the WL with the R40 instrument. Final irrigation with 5 mL NaOCl was performed followed by passive ultrasonic agitation (PUI) for 1 minute using the E1-Irrisodic tip (Helse, SP, Brazil). Similar irrigation/agitation procedures were performed in sequence using 17% EDTA, saline solution, and finally 2% chlorhexidine. After using several paper points, the canal was still slightly damp. A medication with calcium hydroxide paste in camphorated paramonochlorophenol (Calen; SS White, Rio de Janeiro, RJ, Brazil) was applied to the full extent of the prepared canal. The tooth was temporized with Cavit (3M ESPE, Seefeld, Germany). Systemic antibiotic was continued for 7 more days.

Afterward, the patient returned, the canal was reopened, the calcium hydroxide paste was removed, and PUI was performed once again as described for both NaOCl and EDTA. Attempts to dry the canal completely were without success, and the intracanal medication was placed once again. After 2 weeks, persistent purulent exudation was still present in the canal, and surgery was scheduled. Intracanal medication was repeated.

One day before surgery, the tooth was reopened, and the canal revised with the R40 instrument, another session of PUI, and attempts to dry. To prevent excessive moisture during the filling procedure, an apical plug with calcium hydroxide powder was placed at the last apical

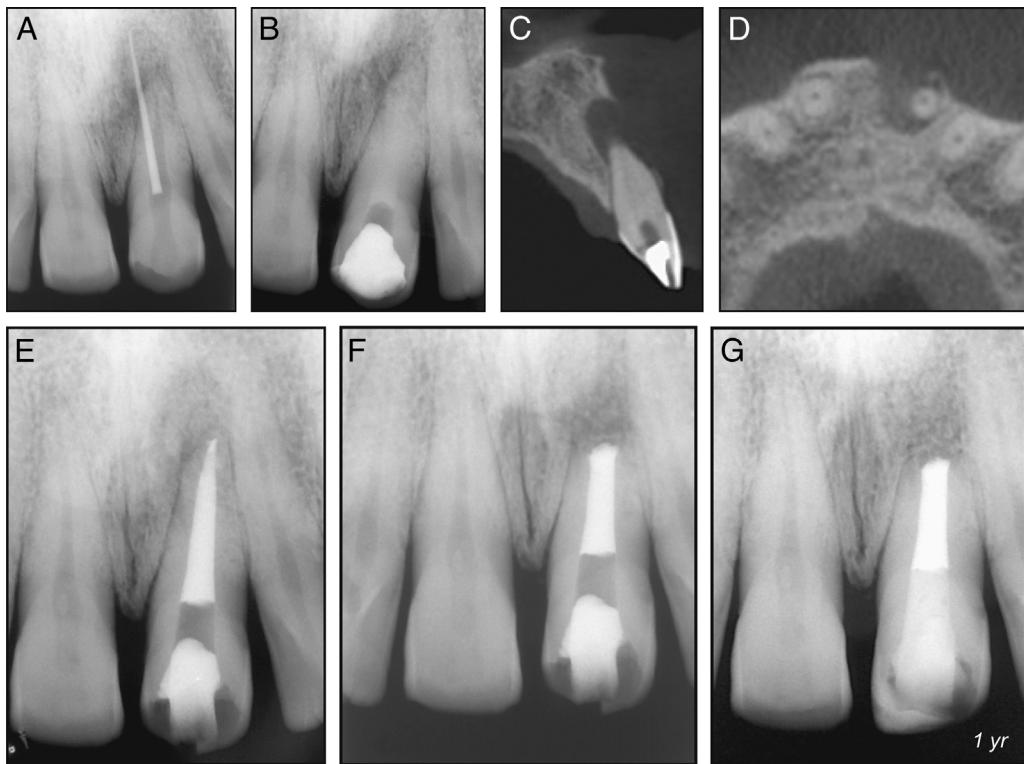


**Figure 2.** (continued). (G) A crosscut section taken at about 2 mm from the root tip. The lingual extension of the canal (bottom) is untouched by the instruments (Taylor-modified Brown and Brenn, original magnification  $\times 16$ ). (H and I) Dentin surrounding the lingual portion of the canal is heavily colonized by bacteria (original magnification  $\times 50$  and  $\times 400$ ). (J) A longitudinal section taken on a buccolingual plane (overview [original magnification  $\times 6$ ]). (K) Detail of the foraminal and periforaminal area from J. Filling material (black) is present beyond the foramen. A large bacterial colony can be seen more apically in the inflammatory tissue (original magnification  $\times 25$ ). (L) Detail of the colony in K (original magnification  $\times 100$ ). (M) Another area of the lesion. Fuchsin-stained bodies (original magnification  $\times 400$ ). (N) Another section. Bacterial colony surrounded by inflammatory cells (original magnification  $\times 100$ ). (Inset) A high-power view showing ramifying bacteria (original magnification  $\times 400$ ). (O) The lingual root canal wall with dentinal tubules colonized by bacteria (original magnification  $\times 100$ , inset  $\times 400$ ).

millimeter. Then, the canal was filled with gutta-percha and sealer using the vertical compaction technique. Nonsurgical root canal treatment was completed on the adjacent incisors because devitalization of these teeth was expected during the surgical procedure (Fig. 2C). These teeth had vital pulps and were treated in a single visit.

Swelling was present on the gingiva buccally to tooth #25 before surgery. After flap elevation, care was taken to enucleate the lesion still attached to the resected root end. Surgery was completed by root-end

preparation with ultrasonic tips and root-end filling with white mineral trioxide aggregate (Angelus, Londrina, PR, Brazil) (Fig. 2D). All of the surgical procedures were performed under magnification with an operating microscope. Radiographs and photographs were taken of the biopsy specimen (Fig. 2E and F), which was submitted to histologic and histobacteriologic analysis. DNA was extracted from the bacteriologic sample taken at the first visit of retreatment and used as a template in a real-time polymerase chain reaction (PCR) procedure for the



**Figure 3.** Case 3: (A) the sinus tract buccally to tooth #9 traced with a gutta-percha cone. The cone pointed to the periapical area of tooth #9 showing a large radiolucent area. (B) A radiograph taken after filling the canal with a calcium hydroxide paste. (C) A CBCT sagittal slice. (D) A CBCT axial slice. (E) A postobturbation radiograph. (F) A radiograph taken after surgery. (G) A 1-year follow-up radiograph. Normal periradicular conditions.

detection of 9 putative endodontic pathogens using primers and conditions described elsewhere (21–24).

### Case 3

This case pertains to a 42-year-old man who presented for treatment because of a sinus tract near the apex of tooth #9. The patient reported trauma to the face caused by a car accident 10 years previously, and the sinus tract was present for 6 months. Increased tooth mobility was noticed after the accident, but it reverted to normal a few days later. Clinically, the tooth presented with significant color change and a simple crown fracture involving the mesial and incisal regions. Tooth #9 responded negatively to pulp sensibility tests. Percussion and palpation tests resulted in slight sensitivity. A gutta-percha cone was inserted into the sinus tract, and a radiograph was taken (Fig. 3A), which indicated that tooth #9 was the origin of the sinus tract. A radiolucent lesion was detected in association with the apical region of this tooth (Fig. 3A).

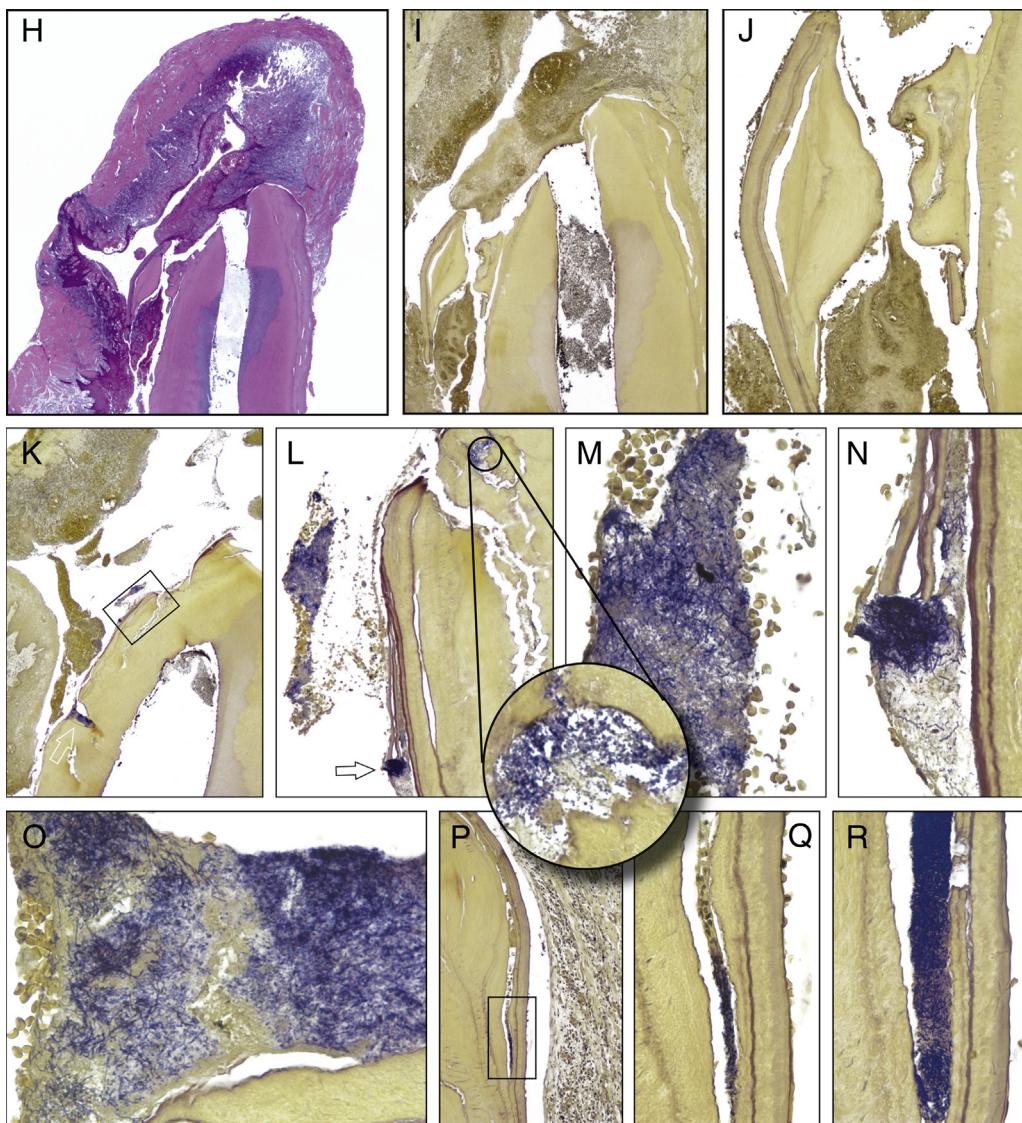
Endodontic treatment was performed in multiple sessions. The incisal fracture was firstly restored with composite, and an endodontic access cavity was prepared. The root canal was instrumented using Gates Glidden burs and K-type files in a crown-down approach. The WL was established with the aid of an electronic apex locator (Bingo-1020; Forum Engineering Technologies, Rishon, LeZion, Israel). The apical foramen, whose initial diameter was already large as a consequence of the periapical pathologic process, was cleaned with a #50 K-type file. Preparation was completed with an instrument ProTaper F5 (Dentsply Maillefer, Ballaigues, Switzerland) working up to the apical foramen under irrigation with 5.25% NaOCl. PUI was performed first using EDTA and then NaOCl. The canal was medicated with a calcium hydroxide

paste (Calen) and coronally sealed with IRM. A radiograph taken at the end of this session showed that the canal was uniformly filled by the medicament with no voids (Fig. 3B). After removal of the rubber dam, calcium hydroxide paste was seen at the exit of the sinus tract.

In the second appointment 10 days later, no clinical improvement was observed, the sinus tract was still present, and purulent discharge was evident after pressing the apical region of the tooth. The canal was reopened, calcium hydroxide paste was removed, and the apical foramen was cleaned once again with a K-type file #50. After additional sessions of PUI with EDTA and then NaOCl, the calcium hydroxide paste was placed once again.

In the third session after 15 days, no improvement in clinical conditions was observed. Instrumentation was revised, and another calcium hydroxide dressing was placed. Thirty days later, the clinical picture remained without significant improvement even after an overall 60 days of calcium hydroxide treatment. Procedures for cleaning and dressing were repeated, and the patient was scheduled for periapical surgery. CBCT examination was requested to ascertain the relationship of the apical periodontitis lesion with the surrounding anatomic structures (Fig. 3C and D). Root canal filling was placed 5 days after the last appointment using the vertical compaction technique (Fig. 3E).

Periapical surgery was performed 4 days after obturation of the root canal. Care was taken to enucleate the lesion while adhered to the root apex. The root end was resected, root-end preparation was performed with an ultrasonic tip, and the cavity was filled with white mineral trioxide aggregate (Angelus) (Fig. 3F). The biopsy specimen was submitted to histologic and histobacteriologic analysis. Twelve months postoperatively, the tooth was asymptomatic, and a radiograph showed healing of the radiolucency (Fig. 3G).



**Figure 3.** (continued). (H) A section encompassing the root canal, foramen, and the cyst cavity. Overview (hematoxylin-eosin, original magnification  $\times 8$ ). (I) The section proximal to that in H (Taylor modified Brown & Brenn, original magnification  $\times 16$ ). (J) Detail from the left radicular surface in I. Cementum spicules detached from the root (original magnification  $\times 50$ ). (K) The section passing through the canal, not encompassing the main foramen. Several ramifications can be seen in the thickness of the left canal wall (original magnification  $\times 16$ ). (L) Magnification of the area demarcated by the rectangle in K. Bacterial colonies free and adhered to the root canal surface (original magnification  $\times 100$ ). (Inset) Magnification of the upper ramification in L. Coccoid prevail in this biofilm (original magnification  $\times 400$ ). (M) A high-power view of the elongated free colony in L (original magnification  $\times 400$ ). (N) A high-power view of the area indicated by the arrow in L. Filaments prevail in a biofilm structure adhering to the radicular surface and showing varying bacterial concentrations. Note that the biofilm is also formed in the space created by the detachment of layers of cementum (original magnification  $\times 400$ ). (O) A high-power view of the exit of the lateral canal indicated by the arrow in K (original magnification  $\times 400$ ). (P) Another section. Right radicular surface. A layer of cementum appears detached from the root surface (original magnification  $\times 100$ ). (Q) A high-power view of the rectangular area in P. The space is occupied by a bacterial biofilm and inflammatory cells on top (original magnification  $\times 400$ ). (R) Another section. Dense bacterial biofilm filling the space between cementum layers (original magnification  $\times 400$ ).

### Tissue Processing

After fixation in a 10% neutral buffered formalin solution, the biopsy specimens were demineralized in an aqueous solution consisting of a mixture of 22.5% (vol/vol) formic acid and 10% (wt/vol) sodium citrate for 3–4 weeks with the end point being determined radiographically. All specimens were washed in running water for 48 hours, dehydrated in ascending grades of ethanol, cleared in xylene, infiltrated, and embedded in paraffin (melting point 56°C) according to standard procedures. With the microtome set at 4–5  $\mu\text{m}$ , meticulous longitudinal or transversal serial sections were taken until each specimen was exhausted. Sections were stained with a modified Brown and Brenn technique

for staining bacteria (25, 26). Selected slides were stained with hematoxylin-eosin. Slides were examined under a light microscope.

### Results

#### Case 1

Analysis of the soft tissue revealed that the lesion was a cyst with the epithelial wall composed of stratified squamous epithelium laying on a connective tissue severely infiltrated by inflammatory cells. In most areas, the epithelium was also infiltrated to the extent that the cyst epithelial lining appeared ulcerated (Fig. 1M). In different areas of

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the cyst wall, empty spaces with pointed appearance in the form of “broken glass,” typical of cholesterol crystals, could be observed. The crystals were surrounded by a dense accumulation of both acute and chronic inflammatory cells, and in some areas they formed masses projecting into the cyst lumen (Fig. 1N).

Histobacteriologic staining did not reveal bacterial colonization in the cyst wall, but several masses of necrotic debris were present, apparently floating in the cyst lumen and heavily colonized by ramifying bacteria arranged in a typical bacterial biofilm structure with abundant extracellular matrix (Fig. 1O and P). Crosscut serial sections of the 2 apices did not reveal bacteria in the root canal system (Fig. 1O–T).

### Case 2

The biopsy specimen was embedded in the paraffin block to obtain crosscut sections in an apical direction. Approximately 200 consecutive sections were taken. These showed that the lingual portion of the single canal was underinstrumented and contained abundant tissue debris packed by the obturation material (Fig. 2G). This debris did not show bacterial colonization. Instead, the surrounding dentin was colonized by bacteria to a considerable depth (Fig. 2H and I).

The specimen was then removed from the paraffin block and reembedded to obtain longitudinal sections on a buccolingual plane. Sections passing through the foramen confirmed the overinstrumentation and overfilling, with masses of filling material spread throughout the periapical inflammatory tissue (Fig. 2J and K). The pathologic tissue appeared firmly attached to the root tip through a sleeve of connective tissue that extended to form the outer part of the apical periodontitis lesion, the so-called pseudocapsule (Fig. 2J). This connective tissue showed an abundance of collagen bundles and few or no inflammatory cells. The central portions of the lesion showed a severely inflamed connective tissue, with an abundance of both acute and chronic inflammatory cells. No epithelium was observed in this lesion.

Histobacteriologic analysis disclosed several bacterial aggregations of varying sizes and densities spread through the inflammatory tissue. These colonies exhibited the typical structure of “ray fungus” or actinomycotic rosettes, with intertwining and branching filaments held together by an extracellular matrix with peripheral filaments in the form of clubs or rods. The colonies were surrounded by several layers of polymorphonuclear neutrophils (Fig. 2K, L, and N). The inflammatory tissue also displayed numerous unusual fuchsin-stained bodies showing elongated, round, or polycyclic forms. These appeared to encircle masses of necrotic tissue and were surrounded by inflammatory cells (Fig. 2M). Analysis of the dentin walls in the apical third disclosed bacterial colonization of the dentin tubules to a considerable depth (Fig. 2O).

The pus sample taken at the first visit of retreatment was analyzed by 16S ribosomal RNA gene-based real-time PCR using universal bacterial primers and primers targeting bacterial groups and species commonly associated with endodontic infections. PCR analysis yielded positive results for the universal bacterial primers and revealed the presence of *Actinobacteria* species, *Streptococcus* species, *Porphyromonas endodontalis*, and *Fusobacterium nucleatum*. Negative results were found for *Enterococcus faecalis*, *Dialister* species, *Treponema denticola*, *Tannerella forsythia*, and *Porphyromonas gingivalis*.

### Case 3

Longitudinal sections passing through the main foramen showed a dense connective tissue at the periphery of the lesion and a central cavity layered by stratified squamous epithelium (Fig. 3H). The epithelium seemed to seal the main apical foramen in all sections so that no communication was apparent between the cyst cavity and the main foramen (Fig. 3H). The connective tissue subjacent to the epithelium was heavily

inflamed, with an abundance of acute and chronic inflammatory cells. Accumulations of cholesterol crystals could be observed in some areas of the cyst wall. The cyst cavity, containing some tissue debris, was open to the oral environment through a sinus tract whose lumen appeared completely lined by nonkeratinized stratified squamous epithelium. Hypercementosis was present, which in some areas presented with a thick multilayered band of normal cementum, whereas in other places it manifested as an irregular deposition of calcified tissue on the external radicular surface with holes entrapping necrotic debris (Fig. 3H–J). In some sections, cementum spicules appeared to have detached from the root surface, being apparently free in the cyst lumen (Fig. 3I and J).

Histobacteriologic analysis did not reveal stainable bacteria in the main root canal (Fig. 3I). However, large actinomycoticlike colonies could be observed in the debris apparently free in the cyst lumen. A bacterial biofilm was present on the external root surface (Fig. 3L–N) and filling a large lateral canal (Fig. 3K and O). Biofilms were also noted in other irregular apical ramifications (inset in Fig. 3). The major lateral canal and the apical ramifications were in direct communication with the cyst lumen, and this allowed the lesion to be categorized as a “pocket cyst.” A peculiar observation was that a bacterial biofilm was also observed in a quite unusual location (ie, between the layers of cementum that were detached from the radicular surface) (Fig. 3N and P–R).

## Discussion

Extraradicular infections have been regarded as one of the possible causes of post-treatment apical periodontitis (3, 8, 10, 15). Although it is conceivably difficult for bacteria to leave the canal and establish an infection outside the boundaries of the root canal in direct contact with the host defenses, there may be certain conditions in which this can occur and result in persistent disease (2). This article describes 3 cases of persistent symptoms associated with extraradicular infection that resulted in short-term endodontic treatment/retreatment failure. The first 2 cases consisted of previously inadequately treated canals subjected to retreatment, whereas the third case consisted of a previously untreated tooth.

In the first case, even though the purulent exudate filling in the cyst had been aspirated during surgery, analysis of the debris that escaped aspiration showed a heavy infection of the cyst cavity by bacterial colonies exhibiting typical actinomycotic morphology. No infection was evident in the apical root canal system, suggesting that this extraradicular infection was independent of the intraradicular infection. Actually, it has been suggested that pocket cysts may be prone to becoming infected by bacteria leaving the root canal directly into the cyst lumen (27). This case indicates that an extraradicular infection independent of the intraradicular infection may be the cause of persistent symptoms in some specific and rare occasions.

In the second case, an extraradicular infection composed of several bacterial colonies also resembling actinomycotic arrangements was observed near the apical foramen in a lesion diagnosed as granuloma. Differently from case 1, bacteria were also observed in the apical root canal heavily infecting the dentinal tubules. In this specific case, molecular microbiologic analysis confirmed the presence of *Actinobacteria*, which is coherent with the finding of actinomycotic colonies. Moreover, some other common endodontic pathogens were also found, indicating a mixed infection. The large amount of bacterial colonies outside the canal and the morphology of the apical foramen suggested that overinstrumentation of the infected root canal was the cause of the persistent symptoms by projecting infected tissue and dentin debris into the periradicular tissues.

In the third case, a large cyst was diagnosed, with no apparent contact of the cyst lumen with the apical foramen because of the presence of an epithelial plug on the latter. However, a lateral canal and several

apical ramifications clogged with bacterial biofilms were in direct contact with the cyst lumen. Adjacent to the lateral canal and attached to the outer root surface, bacterial biofilms were also observed. In addition, large actinomycoticlike colonies were observed in the cyst lumen. It is noteworthy bacterial biofilms were observed between the layers of cementum detached from the radicular surface. The high complexity of infection in this case, including biofilms present in a lateral canal, on the outer root surface, and between cementum layers, was the cause of persistent disease regardless of the extensive antimicrobial intracanal efforts using PUI and calcium hydroxide medication. To the best of our knowledge, this is the first report of bacterial biofilms of endodontic origin formed between layers of apical cementum.

A recent concept regarding the issue of extraradicular infections as the cause of treatment failure refers to the infection being dependent on or independent of the intraradicular infection (2). Independent extraradicular infections are those no longer fostered by the intraradicular infection and can persist even after successful eradication of the latter. Of the 3 cases reported herein, only case 1 had no infection detected in the root canal system, which suggests an independent extraradicular infection. The presence of bacterial colonies in the cyst lumen may be derived from the direct advancement of the root canal infection into the cyst lumen or extrusion during previous root canal instrumentation. Whatever the cause, even after successful eradication of the intraradicular infection, the extraradicular bacteria succeeded in sustaining inflammation and disease. Colonies resembled those formed by *Actinomyces* species and *Propionibacterium propionicum* in apical actinomycosis (5, 28–30). However, a study has shown that bacteria other than these typical species may be present in actinomycoticlike colonies (8). Bacteria in the cyst lumen are conceivably difficult to eliminate by neutrophils infiltrating in the cyst lumen because of their organization as floating biofilms (flocs) and the difficulties of polymorphonuclear neutrophils entrapping large colonies in a fluid or semisolid environment. In the other 2 cases, an intraradicular component of infection was also detected. However, in case 2, despite the presence of bacteria within dentinal tubules in the apical part of the canal, the large amount of extraradicular bacteria present in the tissue close to the apical foramen was certainly more likely to have been the cause of purulent exudation and persistent symptomatology.

It is important to emphasize that most cases of post-treatment apical periodontitis are caused by persistent or secondary intraradicular infection. However, the involvement of an extraradicular infection in some cases cannot be discarded. The 3 cases reported herein presented with persistent symptoms/exudation that did not respond well to treatment or retreatment, which is in agreement with the finding that extraradicular bacteria, if present, are more frequent in symptomatic teeth (20, 31). Actually, even though post-treatment disease associated with adequately treated canals is more often caused by intraradicular infections, when symptoms persist after adequate treatment, one may suspect an extraradicular component of infection. The 3 cases were only successfully managed by periradicular surgery.

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