

# Infection Control in Teeth with Apical Periodontitis Using a Triple Antibiotic Solution or Calcium Hydroxide with Chlorhexidine: A Randomized Clinical Trial

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## Abstract

**Introduction:** This randomized clinical study compared the antibacterial effectiveness of treatment protocols using either a triple antibiotic solution (1 mg/mL) or calcium hydroxide/chlorhexidine paste as interappointment medication in infected canals of teeth with primary apical periodontitis. **Methods:** The root canals of single-rooted teeth with apical periodontitis were prepared by using a reciprocating single-instrument technique with 2.5% sodium hypochlorite irrigation and then medicated for 1 week with either a triple antibiotic solution (minocycline, metronidazole, and ciprofloxacin) at 1 mg/mL ( $n = 24$ ) or a calcium hydroxide paste in 2% chlorhexidine gluconate ( $n = 23$ ). Samples were taken from the canal at the baseline (S1), after chemo-mechanical preparation (S2), and after intracanal medication (S3). DNA extracts from clinical samples were evaluated for total bacterial reduction using a 16S ribosomal RNA gene-based quantitative polymerase chain reaction assay. **Results:** All S1 samples were positive for the presence of bacteria, and counts were substantially reduced after treatment procedures ( $P < .01$ ). Bacterial levels in S2 and S3 samples did not significantly differ between groups ( $P > .05$ ). S2 to S3 reduction was 97% in the antibiotic group and 39% in the calcium hydroxide/chlorhexidine group; only the former reached statistical significance ( $P < .01$ ). There were significantly more quantitative polymerase chain reaction–negative S3 samples in the antibiotic group than in the calcium hydroxide group ( $P < .05$ ). **Conclusions:** Interappointment medication with a triple antibiotic solution at the concentration of 1 mg/mL significantly improved root canal disinfection, and its effects were at least comparable with the calcium hydroxide/chlorhexidine paste. Effec-

tiveness and easy delivery of the antibiotic solution make it an appropriate medicament as part of a disinfecting protocol for conventional nonsurgical endodontic treatment and possibly regenerative endodontic procedures. (*J Endod* 2018; ■:1–6)

## Key Words

Apical periodontitis, calcium hydroxide, endodontic treatment, triple antibiotic mixture

Because bacterial infection is the primary cause of the different forms of apical periodontitis, the successful outcome of endodontic treatment procedures relies on effective infection control (1). However, adequate disinfection of the root canal system of teeth with pulp necrosis

and apical periodontitis remains a crucial challenge to be overcome in endodontics, especially because of the inherent difficulties posed by anatomic variations (2). Intracanal medication has been shown to contribute to canal disinfection by promoting significant additional bacterial elimination after chemomechanical procedures (3–5), possibly by reaching areas that instruments and irrigants could not affect and/or by remaining longer in the canal. However, studies have shown that bacteria can still be detected in some canals even after 1 week or more of calcium hydroxide medication (6–8). Therefore, there is a need for finding medications with better and more predictable disinfection results.

Regenerative endodontic procedures (REPs) have become a treatment alternative for immature teeth with pulp necrosis, showing favorable clinical outcomes including healing of apical periodontitis, continued root development, and, in certain cases, positive responses to vitality tests (9). Disinfection in REPs mostly rely on the chemical debridement promoted by the use of irrigants and intracanal medicaments because most procedures are performed with minimal to no mechanical preparation (10). A

## Significance

Control of endodontic infection is paramount for the successful treatment outcome of teeth with apical periodontitis. In this clinical study, application of an interappointment medication with a triple antibiotic solution (1 mg/mL) significantly improved canal disinfection with effectiveness at least comparable with a calcium hydroxide/chlorhexidine paste.

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## Clinical Research

combination of minocycline, metronidazole, and ciprofloxacin known as triple antibiotic paste (TAP) was used as an intracanal medicament in the first contemporary published case (11), and it has since been the most used form of intracanal medicament in these procedures (10, 12, 13). This antibiotic formulation was introduced as a medication effective against endodontic pathogens from deciduous teeth *in vitro* (14) and *in vivo* (15, 16). In REPs, the drugs are mixed with water, saline, or propylene glycol until a thick creamy mixture is formed. A total concentration of at least 1 g/mL is required for this paste to achieve a physical consistency that aids in its delivery. Thus, TAP concentration has been determined by empirical use and technical convenience.

A substantial concentration of undifferentiated mesenchymal stem cells is transferred from apical tissues into debrided root canals after evoked intracanal bleeding in REPs (17). Different disinfection strategies modify the root canal microenvironment and exert a significant impact on stem cell survival and differentiation potential (12). Concentrations of TAP often used clinically (1 g/mL) resulted in pronounced death when directly in contact with stem cells of the apical papilla, whereas a concentration of 1 mg/mL resulted in moderate toxicity *in vitro* (18). These findings were further confirmed in dental pulp stem cells (19). It is noteworthy that the triple antibiotic combination at a concentration of 1 mg/mL is a solution and not a thick slurry or paste. Although some studies have evaluated whether these lower concentrations of TAP were effective against bacteria *in vitro* (20–22), no study has so far evaluated the antibacterial effectiveness of TAP at a concentration of 1 mg/mL in primary endodontic infections.

This randomized clinical study sought to compare the clinical antibacterial effectiveness of endodontic treatment protocols using either a triple antibiotic solution (1 mg/mL) or calcium hydroxide/chlorhexidine paste as interappointment medication in infected canals of teeth with primary apical periodontitis. The reduction of the total bacterial levels was evaluated by means of a culture-independent molecular microbiology assay (quantitative real-time polymerase chain reaction [qPCR]).

## Material and Methods

### Patients and Case Selection

The study population consisted of 48 subjects (23 females and 25 males; mean age = 41 years; range, 13–71 years) who attended the endodontic clinic at the School of Dentistry, Estácio de Sá University, Rio de Janeiro, Rio de Janeiro, Brazil, for the treatment of apical periodontitis associated with mature teeth from January 2016 to October 2017. The inclusion criteria consisted of teeth with a single root and a single canal presenting with a carious lesion and necrotic pulp as clinically confirmed by pulp sensibility tests and clinical/radiographic evidence of asymptomatic apical periodontitis. The exclusion criteria were teeth with extensive crown destruction by caries that could not be restored or isolated with a rubber dam, root or crown fracture, previous endodontic treatment, symptoms, periodontal pockets measuring >4 mm deep, and patients subjected to systemic antibiotic therapy over the previous 3 months. Patients had no significant systemic conditions. Approval for the study protocol was obtained from the Ethics Committee of Estácio de Sá University, and informed consent was obtained from all individuals or their parents.

### Sample Size Calculation and Randomization

A reduction in intracanal bacterial counts after using treatment protocols with either an antibiotic mixture or a calcium hydroxide paste as interappointment medication was the primary outcome evaluated. Calculation of the sample size using STATISTICA v8.0 software (StatSoft,

Tulsa, OK) revealed that a minimum of 21 teeth per group would suffice to show a 5% difference in bacterial counts with a power of 90%. Therefore, teeth were randomly distributed into 2 groups of 24 each according to the medication used. Randomization with equal proportion allocation was obtained by drawing slips of paper with the group name from a bowl; the drawing for group assignment was conducted after chemomechanical procedures and immediately before application of the medication.

### Sample Collection

Samples were collected from the root canals under strict asepsis. The tooth was scaled and cleaned with pumice, a rubber dam was applied, and caries and/or previous coronal restorations were removed under saline irrigation. Before and after preparation of the access cavity, the operative field (tooth, clamp, and dam) was disinfected using a protocol with the sequential use of 3% H<sub>2</sub>O<sub>2</sub> and 2.5% sodium hypochlorite (NaOCl). The second approach (conducted after access preparation) also included the pulp chamber. Next, 10% sodium thiosulfate was used to inactivate NaOCl, and sterility control samples were taken from the internal surfaces of the cavosurface angle of the access cavity by using sterile paper points as described elsewhere (23, 24). The area sampled to serve as the sterility control included the access cavity walls where the paper points used later for taking root canal samples might accidentally touch. Paper points were transferred aseptically to a tube containing Tris-EDTA buffer (10 mmol/L Tris hydrochloride and 1 mmol/L EDTA, pH = 7.6) and immediately frozen at –20°C. For inclusion of the tooth in the study, the sterility control samples taken at the first and second visit had to be negative for bacterial presence in the same qPCR assay as used for root canal sample analysis (see later).

Before chemomechanical procedures, an initial sample (S1) was taken from the root canal and served as the baseline. Sterile 10% sodium thiosulfate solution was applied to the pulp chamber without over-flowing, and a small K-type hand file (Dentsply Maillefer, Ballaigues, Switzerland) was placed 1 mm short of the root apex as radiographically determined. This instrument was gently used in a circumferential filing motion. In sequence, the canal was sampled by using sterile paper points up to that level. Care was taken to avoid touching the access cavity walls with the paper points. Each paper point was left in the canal for about 1 minute to soak up the intracanal fluid. Paper points were sequentially used until moisture was no longer evident on their surfaces. All points were placed in cryotubes containing RNA *later* (Ambion, Austin, TX), stored at –4°C for 12 hours, and then frozen at –20°C.

### Root Canal Treatment Procedures

After irrigation with 2 mL 2.5% NaOCl, the working length (WL) was established 1 mm short of the apical foramen based on an electronic apex locator (Novapex; Forum Technologies, Rishon Le-Zion, Israel) and radiographs. A size 20 K-type hand file (Dentsply Maillefer) was used to initially enlarge the canal and establish the apical foramen patency.

Chemomechanical preparation was completed in a single visit using Reciproc instruments (VDW, Munich, Germany) as follows. The R40 (for mandibular incisors and maxillary lateral incisors) or R50 (for canines and maxillary central incisors) instrument was used in reciprocating motion powered by a torque-limited electric motor (VDW Silver, VDW) using the preset adjustments. The instrument was placed in the canal until resistance was felt and then activated and moved in the apical direction using in-and-out pecking motions of approximately a 3-mm amplitude. After 3 pecking motions, the instrument was removed and cleaned, and the canal was irrigated with 2.5% NaOCl. Patency of the apical canal was assessed using a size 15 K-type hand file (Dentsply

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