Available online at www.sciencedirect.com
SciVerse ScienceDirect
journal homepage: http://www.elsevier.com/locate/aob

Detection of antibiotic resistance genes in samples from acute and chronic endodontic infections and after treatment

*Isabela N. Rôças, José F. Siqueira Jr.**

Department of Endodontics and Molecular Microbiology Laboratory, Faculty of Dentistry, Estácio de Sá University, Rio de Janeiro, RJ, Brazil

ARTICLE INFO

Article history:
Accepted 20 March 2013

Keywords:
Endodontic treatment
Acute apical abscess
Asymptomatic apical periodontitis
Antibiotic resistance genes

ABSTRACT

Objective: The purpose of this study was twofold: survey samples from acute and chronic endodontic infections for the presence of genes encoding resistance to beta-lactams, tetracycline and erythromycin, and evaluate the ability of treatment to eliminate these genes from root canals.

Design: DNA extracts from samples of abscess aspirates ($n = 25$) and root canals of teeth with asymptomatic apical periodontitis ($n = 24$) were used as template for direct detection of the genes *bla*TEM, *cfx*A, *tet*M, *tet*Q, *tet*W, and *erm*C using real-time polymerase chain reaction (PCR). Bacterial presence was determined using PCR with universal bacterial primers. Root canals of the asymptomatic cases were also sampled and evaluated after chemomechanical procedures using NiTi instruments with 2.5% NaOCl irrigation.

Results: All abscess and initial root canal samples were positive for bacteria. At least one of the target resistance genes was found in 36% of the abscess samples and 67% of the asymptomatic cases. The most prevalent genes in abscesses were *bla*TEM (24%) and *erm*C (24%), while *tet*M (42%) and *tet*W (29%) prevailed in asymptomatic cases. The *bla*TEM gene was significantly associated with acute cases ($p = 0.02$). Conversely, *tet*M was significantly more prevalent in asymptomatic cases ($p = 0.008$). Treatment eliminated resistance genes from most cases.

Conclusions: Acute and chronic endodontic infections harboured resistance genes for 3 classes of widely used antibiotics. In most cases, treatment was effective in eliminating these genes, but there were a few cases in which they persisted. The implications of persistence are unknown. Direct detection of resistance genes in abscesses may be a potential method for rapid diagnosis and establishment of proactive antimicrobial therapy.

1. Introduction

The oral microbiota has been suggested to function as a reservoir for several antibiotic resistance genes, including those encoding resistance to commonly used classes of antibiotics, e.g., beta-lactams, tetracyclines, and macrolides.[1–5] This is a matter of concern since these antibiotics have been widely recommended to treat oral infectious conditions, including those of endodontic origin.[6–8]

Antibiotics have been proposed for some specific indications, either for systemic or topical use. Systemic use of antibiotics in endodontics is usually indicated for acute apical abscesses associated with systemic involvement like fever and malaise, spreading infections, localized infections in medically compromised patients, prophylaxis for medically

* *Corresponding author at*: Faculty of Dentistry, Estácio de Sá University, Rua Alfredo Baltazar da Silveira, 580/cobertura, Recreio, 22790-710 Rio de Janeiro, RJ, Brazil. Tel.: +55 2124978988.
E-mail addresses: jf_siqueira@yahoo.com, jose.siqueira@estacio.br (J.F. Siqueira Jr.).
0003–9969/$ – see front matter
http://dx.doi.org/10.1016/j.archoralbio.2013.03.010

compromised patients during routine endodontic therapy, and replantation of avulsed teeth.[7] Topical use of antibiotics in the root canal has been recently recommended as final irrigants[9] or intracanal medication in the so-called ''revascularization'' procedures.[10] Therefore, selection of the most effective antibiotics to be used for systemic or topical use will depend on a better understanding of the patterns of antibiotic resistance in endodontic bacterial communities and their response to treatment.

Inappropriate prescribing and use of antibiotics have been regarded as one of the major causes of emergence and spread of bacterial resistance.[11,12] Studies analysing the antibiotics prescribing habits of endodontists and oral surgeons have revealed both abuse and misuse.[13,14] For instance, antibiotics have been prescribed for infections that can be usually uneventfully treated without antibiotic therapy (e.g., localized abscesses in uncompromised patients), or in cases with no infection (e.g., irreversible pulpitis). These approaches can contribute to the widespread problem of antibiotic resistance.

Several studies have reported on the antibiotic susceptibilities of isolates from endodontic infections.[15–18] These studies have been based on bacteriological culture and antibiotic susceptibility testing of the isolated strains through phenotype-based approaches. While highly reliable and considered the gold-standard, these tests for anaerobic bacteria are usually time-consuming and expensive, in addition to not detecting resistance in difficult-to-grow or uncultivable bacteria. Detection of antibiotic resistance genes in clinical samples by molecular methods has the potential to be an efficient and rapid method of predicting resistance to specific antibiotics. A study surveyed clinical samples directly for the presence of *cfx*A genes in clinical samples (pus and root canal exudates) from dentoalveolar infections and found this gene in 45% of the samples.[19] Moreover, because root canal bacteria may serve as a reservoir for antibiotic resistance genes,[20] it seems important to determine the efficacy of endodontic treatment procedures in eliminating bacteria carrying antibiotic resistance genes.

The present study surveyed acute apical abscess aspirates and root canal samples from teeth with asymptomatic apical periodontitis for the presence of genes encoding resistance to beta-lactams (*bla*TEM and *cfx*A), tetracycline (*tet*M, *tet*Q and *tet*W) and erythromycin (*ermC*). Moreover, elimination of bacteria carrying these genes was evaluated after chemomechanical procedures. The choice for the 6 antibiotic resistance genes targeted in this study was based on a previous study showing that these genes have already been detected in bacterial isolates from primary endodontic infections.[21]

2. Materials and methods

2.1. Subjects, sample taking and treatment procedures

Samples were taken from 50 patients who were seeking treatment in the Department of Endodontics, Estácio de Sá University, Rio de Janeiro. Only single-rooted teeth from adult patients (ages ranging from 19 to 64 years), all of them having carious lesions, necrotic pulps and radiographic evidence of periradicular bone loss were included in this study. In general, samples of primary endodontic infections were distributed as follows: 25 cases diagnosed as asymptomatic apical periodontitis and 25 cases diagnosed as acute apical abscesses. Diagnosis of acute apical abscess was based on the presence of spontaneous pain, exacerbated by mastication, and localized or diffuse swelling, along with fever, lymphadenopathy, or malaise. No fistula connecting the abscess to the oral cavity or skin surface was observed. Patients included in the study have not made use of antibiotics within the previous 3 months. All teeth showed no periodontal pockets deeper than 4 mm. The study protocol was approved by the Ethics Committee of the Estácio de Sá University.

All patients were asked to rinse the oral cavity for 1 min with 0.12% chlorhexidine before sampling procedures. Abscesses were sampled by aspiration of the purulent exudate from the swollen mucosa over each abscess. The overlying mucosa was disinfected with 2% chlorhexidine solution, and a sterile disposable syringe was used to aspirate the purulent exudate, which was immediately injected into cryotubes containing Tris–EDTA (TE) buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.6) and frozen at −20 °C. In cases of asymptomatic apical periodontitis, samples were obtained from the root canals under strict aseptic conditions, which included rubber dam isolation and a two-step disinfection protocol of the operative field with 2.5% NaOCl, as previously described.[22] Paper points used for sampling the root canals were transferred to cryotubes containing TE buffer and immediately frozen at −20 °C. Sterility control samples taken from the tooth crown were tested by using polymerase chain reaction (PCR) with universal primers for the bacterial 16S rRNA gene. Accordingly, one case was excluded because of a positive result.

Root canal samples from the teeth with asymptomatic apical periodontitis were also taken after chemomechanical procedures in order to evaluate the effects of treatment on endodontic bacterial communities that were positive for antibiotic resistance genes. Root canals were instrumented with NiTi hand or rotary instruments at a working length (WL) established 1 mm short of the apical foramen with the aid of an electronic apex locator (Novapex, Forum Technologies, Rishon le-Zion, Israel) and confirmed by radiographs. Patency of the apical foramen was confirmed with a small file throughout the procedures and under control with the apex locator. The size of apical preparation ranged from #40 to #55. For irrigation, 2.5% NaOCl was used in all canals, 2 ml after each file size, and delivered by disposable syringes and NaviTip needles (Ultradent, South Jordan, UT) inserted up to 4 mm short of the WL.

After preparation, smear layer was removed by rinsing the canal with 17% EDTA and 2.5% NaOCl. The canal was dried using sterile paper points and then flushed with 5 ml of 5% sodium thiosulfate to inactivate NaOCl. Next, a postpreparation (S2) sample was taken from the canals as for the initial sample.

2.2. Real-time PCR for antibiotic resistance genes

DNA was extracted from all samples using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA) following the protocol recommended by the manufacturer. The presence of bacteria

Please cite this article in press as: Rôças IN, Siqueira Jr JF, Detection of antibiotic resistance genes in samples from acute and chronic endodontic infections and after treatment. *Archives of Oral Biology* (2013), http://dx.doi.org/10.1016/j.archoralbio.2013.03.010