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Searching for *Helicobacter pylori* and *Chlamydia pneumoniae* in primary endodontic infections

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ABSTRACT

Objectives: The purpose of this study was to search samples from primary endodontic infections for the presence of two common human bacterial pathogens - *Helicobacter pylori* and *Chlamydia pneumoniae*.

Methods: Genomic DNA isolated from samples taken from 25 root canals of teeth with asymptomatic (chronic) apical periodontitis and 25 aspirates from acute apical abscess was initially amplified by the multiple displacement amplification approach and then used as template in species-specific polymerase chain reaction (PCR) for detection of *H. pylori* and *C. pneumoniae*.

Results: All clinical samples were positive for the presence of bacterial DNA. However, no clinical sample was positive for either *H. pylori* or *C. pneumoniae*.

Conclusions: Neither *H. pylori* nor *C. pneumoniae* were found in samples from primary endodontic infections. These findings suggest that these species are not candidate endodontic pathogens and that the necrotic root canal does not serve as a reservoir for these human pathogens in healthy patients. (Eur J Dent 2012;6:158-162)

Key words: *Helicobacter pylori; Chlamydia pneumoniae;* apical periodontitis; acute apical abscess; polymerase chain reaction

INTRODUCTION

Endodontic infections establish in root canals devoid of vital pulp tissue, which may occur because of pulp necrosis or pulp removal for treatment. The infection may spread locally to cause acute abscesses and the systemic implications of endodontic infections remain to be consistently investigated.[1] Because the root canal devoid of pulp tissue is unable to mount an effective inflammatory defense against infection, the environment becomes conducive to the establishment of bacteria that are resident as well as transient in the oral cavity. Thus, the possibility exists that some important human pathogens may colonize the pulpless tooth.

Helicobacter pylori is a motile non-sporing microaerophilic curved or helical gram-negative rod that has been considered an important grastrointestinal

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pathogen. This species has been associated with gastritis and peptic ulcers, and may represent a risk factor for gastric cancer.[2] It has been shown that approximately 50% of the world's population is infected with *H. pylori*.[2-4] Overt clinical disease is found in about 5-10% of the infected individuals.[5]

Several researchers have suggested that the oral cavity may serve as the primary extragastric reservoir for *H. pylori*.[6-14] Actually, *H. pylori* has been detected in samples from saliva,[7,10,13,15-16] supragingival biofilms,[8,16-17] subgingival biofilms,[6-8,16] and tongue dorsum.[16] At the time of writing, no study had yet investigated the occurrence of *H. pylori* in samples of endodontic origin.

Chlamydia pneumoniae is a spherical or ovoid obligately intracellular bacterial pathogen. This species is gram-negative in architecture and composition, with an outer membrane containing lypopolysaccharides. However, chlamydia apparently lacks peptidoglycan, even though genes for synthesis of this molecule have been identified in their genome. In the extracellular environment, chlamydiae occurs in an infective or dispersal form called elementary body, while within cells they exist in a replicative form called reticulate body.[18] *C. pneumoniae is* one of the leading pathogens of community-acquired pneumonia,[19] and has been associated with pharyngitis, sinusitis, and bronchitis.[20] There is suggestive evidence that infection with this species and dental infections may be associated with atherosclerosis.[21]

C. pneumoniae has been rarely found to infect oral tissues. Tran et al[22] used a species-specific 16S rRNA gene-based polymerase chain reaction (PCR) identification method and did not find *C. pneumoniae* in any samples from subgingival biofilms from 50 adult patients with advanced marginal periodontitis. *C. pneumoniae* has been identified in the oropharynx, possibly involved with about 9% of cases of pharyngitis.[23] Mantyla et al[24] sought *C. pneumoniae* in subgingival biofilm samples from adults with marginal periodontitis and found this species in only one out of 12 patients using a quantitative PCR technique. To the best of our knowledge, only one previous study surveyed endodontic samples for the presence of *C. pneumoniae*. Nandakumar et al[25] evaluated samples from primary and persistent/secondary endodontic infections of 40 patients for the presence of *C. pneumoniae* using single and nested PCR assays. Both methods failed to disclose *C. pneumoniae* in any of the endodontic samples examined.

If *H. pylori* and *C. pneumonia* are present in the oral cavity, both species might be able to survive in the necrotic root canal and then participate in the pathogenesis of apical periodontitis. Moreover, the necrotic root canal might act as a reservoir for these important human pathogens to exert systemic effects. The present study was undertaken to survey samples of primary endodontic infections from healthy patients for the presence of *H. pylori* and *C. pneumoniae*.

MATERIALS AND METHODS

The study protocol was approved by the Ethics Committee of the Estácio de Sá University, Rio de Janeiro, Brazil. Samples were taken from patients who had been referred for root canal treatment or emergency treatment to the Department of Endodontics, Estácio de Sá University. Only single-rooted teeth from adult patients (ages ranging from 18 to 74 years), all of them having carious lesions, necrotic pulps and radiographic evidence of apical periodontitis, were included in this study. Selected teeth showed an absence of periodontal pockets deeper than 4 mm. In general, 50 samples of primary endodontic infections were obtained: 25 asymptomatic cases diagnosed as chronic apical periodontitis and 25 cases diagnosed as acute apical abscesses, which showed pain and localized or diffuse swellings along with fever, lymphadenopathy, or malaise. No apparent communication from the abscess to the oral cavity or the skin surface was observed. Patients reported no gastric diseases and no other significant systemic condition. Patients who have taken antibiotics over the previous 3 months were excluded from the study.

In cases of chronic apical periodontitis, samples were obtained from the root canals using sterile paper points. After the tooth crown was cleansed with pumice, rubber dam was placed and the tooth and the surrounding field were cleansed with 3% hydrogen peroxide and disinfected with a 2.5% NaOCl solution. Caries was removed and the access cavity was prepared. The operative field, including the pulp chamber, was again swabbed with 2.5% NaOCl, which was then inactivated by sterile 5% sodium thiosulphate. If the root canal was dry, a small amount of sterile saline solution was introduced into the canal. Samples were initially collected by

means of a #15 K-type file with the handle cut off. The file was introduced to a level approximately 1mm short of the root apex, based on diagnostic radiographs, and a gentle filing motion was applied. Afterwards, two sequential paper points were placed to the same level and used to soak up the fluid in the canal. Each paper point was retained in position for 1 min. The cut file and the two paper points were transferred to cryotubes containing 1 ml of Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.6) and immediately frozen at –20° C.

Abscesses were sampled by aspiration of purulent exudate from the swollen mucosa using a sterile syringe. The overlying mucosa was disinfected with 2% chlorhexidine, and a sterile disposable syringe was used to aspirate pus, which was immediately injected into cryotubes containing Tris-EDTA buffer. Pus samples were then immediately frozen.

DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. DNA extracted from *H. pylori* ATCC 43629 and *C. pneumoniae* ATCC VR1310 served as positive controls.

DNA extracts from clinical samples and positive controls were subjected to multiple displacement amplification (MDA) by using the Illustra GenomiPhi V2 DNA Amplification kit (GE Healthcare, Piscataway, NJ, USA) following the manufacturer´s instructions. A negative control with sterile ultrapure water instead of sample was included in every batch of MDA. This MDA step was carried out to achieve a better performance of the subsequent PCR assays, particularly for samples with low number of bacteria.

In order to check if bacterial DNA was available for analysis after extraction, aliquots of the amplified DNA extracts from clinical samples were evaluated by polymerase chain reaction (PCR) using a broad-range 16S rRNA gene primer set.

Primers and PCR cycling conditions used for broad-range analysis or specific detection of *H. pylori* and *C. pneumoniae* were as described previously.[11,22,26] PCR amplifications were performed in a 50 µl of reaction mixture containing 5 µl of DNA extract, 0.8 µM of each primer, 5 µl of 10× PCR buffer (Fermentas, Burlington, Canada), 2 mM $MgCl_2$, 1.3U of Taq DNA polymerase (Fermentas) and 0.2 mM of each deoxyribonucleoside triphosphate (dNTPs) (Biotools, Madrid, Spain). Positive control comprising DNA extracted from *H. pylori* and *C. pneumoniae* and negative controls consisting of sterile ultrapure water instead of sample were included with each batch of samples analyzed. DNA was amplified using a DNA thermocycler (Mastercycler personal, Eppendorff, Hamburg, Germany).

PCR products were subjected to electrophoresis in a 1.5% agarose gel–Tris-borate-EDTA buffer. The gel was stained with GelRed (Biotium, Hayward, CA, USA) and visualized under UV illumination. A 100-bp DNA ladder (New England Biolabs, Beverly, MA) was used as a molecular size standard.

RESULTS

All genomic extracts from clinical samples exhibited the expected amplicon after PCR amplification with broad-range 16S rRNA gene primers. This indicates that the DNA extraction and amplification procedures were adequate, bacteria were present in all examined samples, and significant PCR inhibitors were not present. No PCR products were observed in negative controls using sterile ultrapure water instead of sample. Positive controls yielded the amplicons of predicted sizes for each tested species. No clinical sample was positive for either *H. pylori* or *C. pneumoniae*.

DISCUSSION

To increase the sensitivity of the method, the present study made use of nonspecific whole genomic amplification by means of MDA so as to generate large quantity of assay-ready DNA. MDA is of special value in experiments involving samples with low amount of DNA and has been used as a pre-PCR strategy for the detection of low copy number sequences.[27-28] This can be the case for endodontic infections, because of the recognized limitations of root canal sampling techniques.[29-30]

In the present study, endodontic samples were taken from healthy patients with no informed gastric infection and all samples were negative for *H. pylori*. Although the oral cavity has been suggested to be a reservoir for *H. pylori*, there appears to be no consensus in this regard. Prevalence of *H. pylori* in the oral cavity has been shown to range from 0% [31] to 97%.[10] Specifically, detection of *H. pylori* in subgingival biofilms has also been demonstrated to vary, with studies showing absence[32] and others finding this species in about 25-40% of the samples.[7-9,11] A study concluded that the oral cavity may not be a reservoir for *H. pylori* in patients with

epigastric pain syndrome, with the bacterium being detected exclusively in the stomach.[33] Even so, it remains to be evaluated if this bacterial species occurs in necrotic root canals from patients with established gastric infection.

C. pneumoniae was not detected in any endodontic samples either. As for its occurrence in the oral cavity, there are fewer reports as compared to *H. pylori* and data, while somewhat inconclusive, suggest that this species is not a common oral inhabitant. Tran et al[22] did not detect *C. pneumoniae* in the subgingival dental plaque, whereas Huovinen et al[23] identified it in oropharynx. Mantyla et al[24] detected *C. pneumoniae* RNA in the subgingival plaque samples from only 1 of 12 patients tested. Similar to the present study, Nandakumar et al[25] did not find this species in samples from primary and persistent/secondary endodontic infections using two sensitive molecular methods.

Bacteria colonizing the necrotic root canal are usually members of the oral microbiota.[34] Absence of both target species in endodontic samples may indicate that either they are not commonly present in the oral cavity or that the root canal environmental conditions are not conducive to their establishment. The former is in agreement with some studies mentioned above that reported absence or low prevalence of these species in the oral cavity. The latter may be related, for instance, to ecological conditions established by bacterial interactions in the endodontic bacterial community. Future studies investigating the concomitant presence of these species in saliva or dental plaque and root canal infections may help elucidate these questions.

In any circumstance, our findings make it reasonable to conclude that *H. pylori* and *C. pneumoniae* are not candidate endodontic pathogens and that the necrotic root canal does not serve as a reservoir for these two important human pathogens.

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