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ORIGINAL ARTICLE

Bacteria in the apical root canals of teeth with apical periodontitis

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antibiotic sensitivity test;
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Background/Purpose: Bacteria in the tooth root canal may cause apical periodontitis. This study examined the bacterial species present in the apical root canal of teeth with apical periodontitis. Antibiotic sensitivity tests were performed to evaluate whether these identified bacterial species were susceptible to specific kinds of antibiotics.
Methods: Selective media plating and biochemical tests were used first to detect the bacterial species in samples taken from the apical portion of root canals of 62 teeth with apical periodontitis. The isolated bacterial species were further confirmed by matrix-assisted laser desorption ionization-time of flight mass spectrometry.
Results: We found concomitant presence of two (32 teeth) or three species (18 teeth) of bacteria in 50 (80.6%) out of 62 tested teeth. However, only 34 bacterial species were identified. Of a total of 118 bacterial isolates (83 anaerobes and 35 aerobes), *Prophyromonas endodontalis* was detected in 10; *Bacteroides*, *Dialister invisus* or *Fusobacterium nucleatum* in 9; *Treponema denticola* or *Enterococcus faecalis* in 8; *Peptostreptococcus* or *Olsenella uli* in 6; and *Veillonella* in 5 teeth. The other 25 bacterial species were detected in fewer than five teeth. Approximately 80—95% of bacterial isolates of anaerobes were sensitive to ampicillin/sulbactam (Unasyn), amoxicillin/clavulanate (Augmentin), cefoxitin, and clindamycin. For *E. faecalis*, 85—90% of bacterial isolates were sensitive to gentamicin and linezolid.

Conflicts of interest: The authors have no conflicts of interest relevant to this article.
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Conclusion: Root canal infections are usually caused by a mixture of two or three species of bacteria. Specific kinds of antibiotic can be selected to control these bacterial infections after antibiotic sensitivity testing.
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Introduction

Knowledge of microbial location and organization within the root canal system is important for understanding the disease process and establishing effective antimicrobial therapeutic strategies.[1] Apical periodontitis is essentially an inflammatory disease of microbial etiology primarily caused by infection of the pulp and root canal system.[2] Every species of bacterium observed in the root canal system may be an endodontic pathogen. Moreover, bacterial profiles of the endodontic microbiota vary between individuals. This indicates that apical periodontitis has a heterogeneous etiology, and multiple bacterial combinations in the root canal can cause apical periodontitis. To achieve a successful endodontic treatment, it is important to know the specific bacterial species harbored in each root canal and the specific antibiotics that can kill the harbored bacteria or at least inhibit their growth.

Early studies of the microbiota in the root canals of teeth with apical periodontitis were conducted using broad-range culture/biochemical methods.[3] However, some species of bacteria are difficult to cultivate. Later, molecular detection methods such as species-specific polymerase chain reaction and the original checkerboard DNA—DNA hybridization assay were used for identification of bacterial species.[4,5] The adoption of 16S ribosomal RNA gene clone library analysis allows a more comprehensive broad-range study of bacterial communities in endodontic infections. By these techniques, not only cultivable species but also as-yet-uncultivated and uncharacterized bacteria can be identified.

Recently, proteomic techniques have achieved a relevant role in the identification of microorganisms in the field of clinical microbiology. Matrix-assisted laser desorption ionization—time of flight mass spectrometry (MALDI-TOF MS) was launched in the late 1980s.[6,7] It uses peptides or proteins present in a bacterial sample to form mass spectral peaks. These spectra can generate pathognomonic patterns that provide unbiased identifications of particular bacterial species and even genotypes within species. MALDI-TOF MS has been reported to possess the advantages of time saving, low cost, and high accuracy for bacterial identification.[6,7]

In this study, bacterial samples were taken from the apical third of root canals of 62 pulp-necrosis single-canal teeth with apical periodontitis. Selective media plating and biochemical tests were used first to identify the bacterial species in each sample. The specific bacterial colonies were further confirmed by MALDI-TOF MS.[6,7] Only the bacterial species that were initially identified by conventional cultures/biochemical methods and subsequently confirmed by MALDI-TOF MS methods are reported in this study. The purposes of this study were to provide the data on bacteria existing in the apical root canals of teeth with apical periodontitis in Taiwanese patients and to evaluate whether these identified bacterial species were susceptible to specific kinds of antibiotics using antimicrobial susceptibility tests. We hoped that the bacterial data could provide information on the causative bacteria that induce apical periodontitis. Furthermore, the data from antimicrobial susceptibility tests could provide a useful clinical guide for the selection of antibiotics for treatment of patients with exacerbated apical periodontitis or its associated cellulitis, especially in the period waiting for the results of antimicrobial susceptibility tests.

Materials and methods

Tooth samples

In this study, 62 pulp-necrosis single-canal teeth (48 incisors and 14 canines) with apical periodontitis were collected at the dental clinic of the Renai Branch of Taipei City Hospital. These 62 teeth included 9 traumatized teeth with intact crown, 7 teeth with type I dens invaginatus and dental caries in the lingual invagination, and 46 teeth with the relatively intact crown and proximal caries or secondary caries at the margin of composite resin restorations. In addition, 8 of the 62 teeth had sinus tracts at the alveolar mucosa near the tooth apex regions. However, none of our 62 teeth had acute symptoms and signs. For taking bacterial samples from the apical third of the root canal, all instruments and the operation field were sterilized. The selected tooth was isolated with a rubber dam, scrubbed with povidone—iodine aqueous solution (1% Aqua Better Iodine; K.Y. Co., Taipei, Taiwan), sterilized with 75% alcohol, and accessed by high speed drill to find the root canal. When the pulp chamber of the sample tooth was accessed, all the soft dentin was removed as completely as possible. The exposed pulp chamber was carefully sterilized again as before, and then a No. 15 sterile paper point with apex soaked with normal saline was inserted into the deepest part of the apical root canal for 1 minute with the help of the endodontic microscope. Because all our sample teeth were single-canal teeth with relatively intact crown and large pulp chamber and the thin paper point was inserted into the root canal of the sample tooth cautiously with the help of the endodontic microscope, it was possible to insert the thin sampling paper point into the apical portion of the root canal without contacting the pulp chamber and the coronal two-thirds of the root canal. After sampling, the paper point was removed from the root

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