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ORIGINAL ARTICLE

Bacteria in the apical root canals of teeth with apical periodontitis

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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.

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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canal, immediately placed into Gifu anaerobic medium (Nissui Pharmaceutical Co., Nissui, Japan) in a culture tube,[3] and sent to Department of Laboratory Medicine, Taipei City Hospital, Renai Branch for further handling.

Bacterial species determination and antibiotic sensitivity tests

The bacterial species determination and antibiotic sensitivity tests were routinely performed in the Department of Laboratory Medicine, Taipei City Hospital, Renai Branch. In brief, the paper point with bacterial sample in the Gifu anaerobic medium of a culture tube was incubated at 37°C for 2–3 days for the growth of aerobic bacteria and for 4–7 days for the growth of anaerobic bacteria. If turbidity was observed in the culture medium, this meant that there was bacterial growth in the culture medium. After culturing for 4–7 days, colonies of bacteria were seeded on anaerobic culture biplate of brucella blood esculin agar and kanamycin vancomycin lactose bile as well as on anaerobic blood agar plate using the media, such as brucella, Columbia, or Schaedler agar base supplemented with 5% sheep blood, additional vitamin K_1 and hemin to support the growth of anaerobes in the Concept Plus anaerobic culturing box (Concept Plus ONCP 01; Ruskinn Technology Ltd, Bridgend, Mid Glamorgan, UK).[8] For Gram-positive anaerobic cocci (GPAC), nalidixic acid–Tween and neomycin–vancomycin agar or nalidixic acid–Tween and neomycin blood agar was the best combination to support the growth of GPAC. Anaerobic bacteria were mainly identified using Gram stain, colony morphology, antibiotic susceptibility tests, and biochemical tests including catalyst and presumptive identification. *Peptoniphilus asaccharolyticus* decolorize readily with Gram stain and can be confused with Gram-negative anaerobes such as *Veillonella*.[8,9] However, *Veillonella* can be distinguished from GPAC by special potency disks (containing vancomycin 5 g, kanamycin 1000 g, and colistin 10 g). The cell morphology of older cultures of GPAC can be very irregular, with many coccobacillary and rod-like forms. To distinguish GPAC from microaerophilic organisms, such as *Streptococcus* spp., a simple and reliable test is to apply a 5 g metronidazole disk to the edge of an inoculum; GPAC show a zone of inhibition of 15 mm or greater, whereas microaerophilic strains show no zones after incubation for 48 hours.[8–10]

Aerobic bacteria were subcultured on phenylethyl alcohol agar plate or blood agar/eosin methylene blue agar biplate until the bacteria grew. Gram-negative bacteria were identified using Gram stain, colony morphology, catalyst test, and oxidase test. For example, *Neisseria diplococcus* can be identified by growing on commercial blood culture media using colony morphology, microscopic morphology, oxidase test, or carbohydrate utilization assays.[11] Gram-positive cocci (e.g., *Staphylococci*) were identified using Gram stain, colony morphology, catalase test, cytochromes, agglutination assay using rabbit or human plasma.[12] *Streptococci* were identified using colony morphology, the presence or absence of motility, and serologic, presumptive and hemolysis tests.[13,14]

Aerobic Gram-negative bacteria including Enterobacteriaceae and glucose nonfermenter bacilli (e.g. *Acinetobacter* and *Pseudomomas*) were identified according to the following characteristics: cell morphology, colony color/pigment/morphology, hydrogen sulfide production, pyoverdin production, oxidase, trypsin or benzyl–arginine arylamidase activity, pyrrolidonyl aminopeptidase activity, and susceptibility to desferrioxamine.[15,16] Eight biochemical tests including triple sugar iron agar, sulfide indole mobility semisolid medium, citrate agar, Voges–Proskauer semisolid medium, ornithine decarboxylase medium, urease agar, arginine dihydrolase medium, and lysine decarboxylase medium were also used to identify the Enterobacteriaceae. Triple sugar iron agar was used to identify the *Acinetobacter* and *Pseudomomas*. *Acinetobacter* was oxidase-negative[17] and *Pseudomomas aeruginosa* revealed glucose oxidase fluorescent pigment.[18] Aerobic Gram-negative bacteria were recently identified with Phoenix ID machine (Phoenix Co, Phoenix, AZ, USA).

Curved and spiral-shaped *Campylobacter* was identified using the following tests or characteristics: catalase; H_2 requirement; urease; H_2S (triple sugar iron); hippurate hydrolysis; indoxyl acetate hydrolysis; aryl sulfatase; selenite reduction; growth in 1% glycine; and growth at 25°C.[19] Oral spirochetes were identified using oral bacterial growth medium containing 10% heat-inactivated rabbit serum at 37°C in an AS-580 anaerobic chamber (Concept plus; Ruskinn Technology) with an atmosphere of 85% nitrogen, 5% carbon dioxide, and 10% hydrogen.[20] The direct fluorescent antibody test was adopted to demonstrate the presence of spirochetes by the darkfield microscopy.[21,22]

Another molecular analytic tool, MALDI-TOF MS biotyper (Bruker, Sancordon Inc., Bremen, Germany) was used to confirm all bacterial species included anaerobes and aerobes.[6,7] Colonies were picked from the nonselective sheep blood agar plate for aerobic bacteria, from the CDC anaerobic sheep blood agar for anaerobic bacteria, from chocolate agar for *Haemophilus* spp., and from Karmali medium for *Campylobacter*. In brief, MALDI target plates were inoculated by picking a freshly grown overnight colony with the tip of a sterile toothpick and smearing the specimen directly onto a ground steel MALDI target plate in a thin film. The microbial film was then overlaid with 1.5 μL of a MALDI matrix (a saturated solution of α-cyano-4-hydroxy-cinnamic acid in 50% acetonitrile/2.5% trifluoroacetic acid) and allowed to dry at room temperature. Mass spectra were acquired using the MALDI-TOF spectrometer in a linear positive mode (Microflex; Bruker Daltonics, Bremen, Germany). Measured mass spectra ranged from 2000 Da to 20,000 Da and comparisons of spectra were entirely automated. Extraction of the peaks from the generated mass spectra and their matching against the reference spectra (*main spectra*) of the integrated database provided by the manufacturer was performed with MALDI Biotyper software (Bruker Daltonics). The score value is defined by three components: the matches of the unknown spectrum against the main spectrum; the matches of the main spectrum peaks against the unknown spectrum; and the correlation of intensities of the matched peaks. This leads to a first score, from 0 (no match) to 1000 (perfect identity), which is converted into a log score from 0 to 3. When the score was $\geq$1.7, the identification was considered high confidence based on communication from the manufacturer. When the score was $<$1.7, a second attempt was performed on

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another run, and the higher of the two scores was used for final analysis. Final scores of <1.7 were considered ambiguous identifications.[6,7] In this study, only the bacterial species that were initially identified by conventional cultures/biochemical methods and subsequently confirmed by MALDI-TOF MS methods were reported.

Disk diffusion technique was used for antibiotic susceptibility test for aerobes.[23] Otherwise, the BD Phoenix antimicrobial susceptibility testing system (Phoenix Co, Phoenix, AZ, USA) was adopted for antibiotic susceptibility test for aerobes.[23,24] The colony was seeded in brain heart infusion or thioglycollate broth for enrichment, and then swabbed in Mueller Hinton agar plate. However, *Streptococci* or *Enterococci* did not need enrichment, and the colony was seeded in 5% blood agar plate until microbial inhibition zone was found. The size of inhibition zone was measured and compared with that from National Committee for Clinical Laboratory Standards. The commercial antimicrobial susceptibility testing system was introduced into clinical microbiology laboratories in the 1980s and has been used in the majority of laboratories since the 1990s.[23–26] In addition, the VITEK instrument developed for the provision of rapid microbial inhibitor concentration results was introduced in the 1980s. VITEK 1 is still used in a number of laboratories. The more automated VITEK 2 received Food and Drug Administration clearance in 2000. In this study, BBL crystal identification system anaerobe ID Kit (Becton Dickinson Company, Sparks, MD, USA) was adopted in antimicrobial susceptibility testing for anaerobes.[25,26]

Results

In 62 teeth with apical periodontitis, bacterial growth was found in 50 (80.6%) teeth. However, only 34 bacterial species (phylotypes) were identified from a total of 118 bacterial isolates. Of the 50 bacteria-positive teeth, 32 had two species and 18 had three species of bacteria cultured from the apical portion of the root canal. Of the 118 bacterial isolates, 83 (70.3%) were anaerobes, and 35 (29.7%) were aerobes. Among the 34 bacterial species in 50 bacteria-positive teeth, *Prophyromonas endodontalis* was found in 10 (20%) teeth; *Bacteroides*, *Dialister invisus*, and *F. nucleatum* were found in 9 (18%) teeth; *Treponema denticola* and *Enterococcus faecalis* were found in 8 (16%) teeth; *Peptostreptococcus* and *Olsenella uli* were found in 6 (12%) teeth; *Veillonella* was found in 5 (10%) teeth. Other species were discovered in fewer than 4 teeth (Table 1).

The results of antibiotic susceptibility test are shown in Table 2. Approximately 80–95% of bacterial isolates of anaerobes were sensitive to cefoxitin (FOX, cephalosporin second generation), clindamycin, amoxicillin/clavulanate (Augmentin), and ampicillin/sulbactam (Unasyn). About 80–85% of *Campylobacter* isolates were sensitive to metronidazole, chloramphenicol, and clindamycin. Approximately 80–90% of *Streptococcus* isolates were sensitive to penicillin, clindamycin, linezolid, levofloxacin, and cefepine (cephalosporin fourth generation). Approximately 80–90% of *Staphylococcus* isolates were sensitive to sulfoamide, linezolid, amoxicillin/clavulanate, and cefoxitin (cephalosporin second generation). About 85–90% of *E. faecalis* isolates were sensitive to high-level gentamicin (120 μg/mL) and linezolid (30 μg/mL). Approximately 80–90% of *Pseudomonas* and *Acinetobacter* isolates were sensitive to gentamicin, ceftazidime (cephalosporin third generation), ciprofloxacin and levofloxacin. In addition, 80–85% of Enterobacteriaceae isolates were sensitive to ceftazidime and ciprofloxacin.

Discussion

This study found concomitant presence of 2 (32 teeth) or 3 species (18 teeth) of bacteria in the apical root canals of 50 (80.6%) out of 62 teeth with apical periodontitis. Ten isolates of *Prophyromonas endodontalis*, nine isolates of *Bacteroides*, *Dialister invisus*, or *F. nucleatum*, eight isolates of *Treponema denticola* or *E. faecalis*, six isolates of *Peptostreptococcus* or *O. uli*, and five isolates of *Veillonella* were detected in ≥10% of 50 teeth with apical periodontitis. Rocas and Siqueira[27] studied the presence of 83 oral bacterial species in the samples taken from infected root canals of 43 teeth with chronic apical periodontitis using a 16S ribosomal RNA gene-based reverse-capture checkerboard hybridization assay.[27] They found that all the 43 samples contain at least two oral bacterial species. All our nine isolated bacterial species mentioned above apart from *E. faecalis* and *Peptostreptococcus* could be identified in ≥10% of 43 teeth with chronic apical periodontitis in the study of Rocas and Siqueira.[27] Siqueira et al[28] also used a 16S ribosomal RNA gene-based reverse-capture checkerboard hybridization assay to examine the bacterial DNA in the apical root canal of 20 teeth with apical periodontitis. They found bacterial DNA in 19 out of 20 teeth. Seven bacterial species including *Pseudoramibacter alactolyticus*, *Bacteroidetes* clone X083, *Streptococcus* spp., *O. uli*, *Synergistes* clone BA121, *F. nucleatum*, and *Porphyromonas endodontalis* were detected in ≥10% of 19 teeth with apical periodontitis. These findings indicate that our results are comparable with those of the latter two studies.[27,28] We suggest that the inconsistency in frequencies of detection of several oral bacterial species in these three studies were mainly due to the use of different methods for bacterial identification, the use of different teeth for study, and the use of different techniques for bacterial sampling.[27,28]

Enterococci are considered to be the most abundant Gram-positive cocci colonizing the intestine, with *E. faecalis* being one of the most common bacteria associated with different forms of periradicular diseases.[29] Several culture studies revealed that *E. faecalis* is the most frequent species in root canals of endodontically-treated teeth, with the prevalence reaching up to 90% of cases. Root canal treated-teeth are approximately nine times more likely to harbor *E. faecalis* than those teeth with primary infections.[30] *E. faecalis* has been commonly recovered from teeth treated in multiple visits and/or teeth left open for drainage. Thus, *E. faecalis* may be a secondary invader that succeeds in colonizing the root canal, resists treatment, and causes a secondary infection that then becomes persistent. *E. faecalis* is usually the most frequent species isolated from human clinical

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Table 1 Frequency of detection of 34 species of bacteria in 50 bacteria-positive teeth with apical periodontitis.

Bacterial species	Gram stain	Anaerobe or aerobe	Number of teeth (%)
1. *Prophyromonas endodontalis*	−	Anaerobe	10 (20%)
2. *Bacteroides* clone X083	−	Anaerobe	9 (18%)
3. *Dialister invisus*	−	Anaerobe	9 (18%)
4. *Fusobacterium nucleatum*	−	Anaerobe	9 (18%)
5. *Treponema denticola*	−	Anaerobe	8 (16%)
6. *Enterococcus facalis*	+	Aerobe	8 (16%)
7. *Peptostreptococcus*	+	Anaerobe	6 (12%)
8. *Olsenella uli*	+	Anaerobe	6 (12%)
9. *Veillonella*	−	Anaerobe	5 (10%)
10. *Eikenella corrodens*	−	Anaerobe	4 (8%)
11. *Bifidobacterium eriksonii*	+	Anaerobe	3(6%)
12. *Peptococcus*	+	Anaerobe	3 (6%)
13. *Streptococcus*	+	Aerobe	3 (6%)
14. *Prophyromonas gingivalis*	−	Anaerobe	3 (6%)
15. *Prevotella denticola*	−	Anaerobe	3 (6%)
16. *Prevotella intermedius*	−	Anaerobe	3 (6%)
17. *Acinetobacter anitratus*	−	Aerobe	2 (4%)
18. *Pseudomonas aeruginosa*	−	Aerobe	2 (4%)
19. *Escherichia coli*	−	Aerobe	2 (4%)
20. *Klebsiella pneumoniae*	−	Aerobe	2 (4%)
21. *Proteus mirabilis*	−	Aerobe	2 (4%)
22. *Enterobacter cloacae*	−	Aerobe	2 (4%)
23. *Proprionibacterium acnes*	+	Anaerobe	2 (4%)
24. *Serratia liquefaciens*	−	Aerobe	2 (4%)
25. *Staphylococci epidermidis*	+	Aerobe	1 (2%)
26. *Nesseris*	−	Aerobe	1 (2%)
27. *Citrobacter freundii*	−	Aerobe	1 (2%)
28. *Klebsiella oxytoca*	−	Aerobe	1 (2%)
29. *Klebsiella ozaenae*	−	Aerobe	1 (2%)
30. *Enterobacter gergoviae*	−	Aerobe	1 (2%)
31. *Proteus vulgaris*	−	Aerobe	1 (2%)
32. *Pseudomonas stutzeri*	−	Aerobe	1 (2%)
33. *Campylobacter curvus*	−	Aerobe	1 (2%)
34. *Campylobacter rectus*	−	Aerobe	1 (2%)

specimens, representing 80—90% of the clinical significant enterococcal isolates.[29,30] This leads to a growing concern regarding the role of the *Enterococci* in endodontic and implanted medical device-associated infections reported by Teixeira et al.[31] Although *E. faecalis* was not the most commonly isolated species in the present study, it was still the fifth most common species among a total of 34 species. Many strains of *Enterococci* are instrinsically resistant to β-lactam antibiotics.[32] The last therapeutic remedy for *Enterococci* was vancomycin until Leclercq et al[33] described a strain of *E. faecium* that contains a plasmid mediating resistance to the glycopeptide antibiotics such as vancomycin and teicoplanin. Vancomycin-resistant isolates of *E. faecalis* have also been described.[34] Effective bactericidal activity against *Enterococci* by combination of ampicillin, penicillin or vancomycin, and high-level gentamicin or streptomycin was also reported.[34,35] Furthermore, the new oxazolidinone drugs (e.g., linezolid), which have a unique mechanism of inhibiting bacterial protein synthesis and display effect on vancomycin-resistant *E. faecalis*,[36,37] gentamicin (120 μg/mL) and linezolid were also found to be very effective for inhibition of *E. faecalis* in this study.

For spirochetes, only *Treponema denticola* was identified in this study. This finding was consistent with that reported by Moter et al[38] and further strengthened the hypothesis that *Treponema* are opportunistic pathogens. *Olsenella* species shows disease associations similar to those observed for lactobacilli in the oral cavity.[39,40] *Olsenella* is a new genus to constitute anaerobic lactobacilli (the other is *Atopobium*).[40] *O. uli*, formerly called *Lactobacillus uli*, has been found in endodontic infections.[41] It has also been reported as one of the causative organisms that are isolated from oral cavity and pulp chamber and can result in significant clinical bacteremia.[42]

Campylobacter spp. are primarily zoonotic with a variety of animals implicated as reservoirs for human infection.[19] Curved and spiral-shaped *Campylobacter rectus* and *Campylobacter curvus* were susceptible to chloramphenicol, clindamycin, and metronidazole in this study. This finding was consistent with the findings reported by Wexler et al.[43]

Although chemical and physical factors can induce periradicular inflammation, a large body of scientific evidence indicates that endodontic infection is essential to the

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Table 2 Antimicrobial susceptibility for anaerobes, *Campylobacter*, *Streptococcus*, *Staphylococcus*, *Enterococcus*, glucose nonfermenter bacilli, and Enterobacteriaceae using disk diffusion technique.

Organism	Anaerobes	*Campylobacter*	*Streptococcus*	*Staphylococcus*	*Enterococcus*	Glucose nonfermenter bacilli		Enterobacteriaceae					
					E. faecalis	*Pseudomonas*	*Acinetobacter*	*Citrobacter freundii*	*Escherichia coli*	*Klebsiella*	*Serratia*	*Enterobacter*	*Proteus*
Tested isolates	$n = 83$	$n = 2$	$n = 3$	$n = 1$	$n = 8$	$n = 3$	$n = 2$	$n = 1$	$n = 2$	$n = 2$	$n = 2$	$n = 2$	$n = 2$
Tetracycline			70	70									
Vancomycin				75 *	75 *								
Chloramphenicol		85						70	75	70	70	75	70
Ampicillin		40		75	65			25	25	30	25	25	30
Cefazolin (I)		50						25	25	25	30	25	30
Gentamicin[#]				65	85[#]	80	80						
Ampicillin/ sulbactam	95					55	40	50	50	45	40	40	45
Amoxicillin/ clavulanate*	90*			90*									
Cefoxitin (II)	80			95									
Ceftazidime (III)						85	80	75	85	80	85	80	85
Cefepime (IV)			90										
Clidamycin	85	85	80	75									
Metronidazole	75	80											
Penicillin	70		80 *	75	70								
Ciprofloxacin						85	80	80	85	80	80	85	80
Linezolid			85 *	90	90								
Sulfoamide			75	80									
Levofloxacin			90			90	85						

* Using minimal inhibitory concentration method; [#]for *Enterococcus faecalis* using gentamicin 120 μg/mL, for others using gentamicin <30 μg/mL; vancomycin: 30 μg/mL, cefepime 30 μg/mL, linezolid: 30 μg/mL; I, II, III, and IV: cephalosporin first, second, third, and fourth generation.

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progression and perpetuation of the different forms of apical periodontitis.[44] Several previous studies found that primary root canal infection in untreated root canals is a polymicrobial mix with approximately equal proportions of Gram-positive and Gram-negative species but dominated by obligate anaerobes.[45–48] Our results were comparable with the findings that primary endodontic infections are usually characterized by a mixed infection with the anaerobic bacteria being the predominant species.[1,46] The bacterial species and prevalence were also consistent with the results reported by both Rocas and Siqueira[49] and Siqueira et al.[28] The anaerobic Gram-negative bacteria are part of the normal flora of the mouth, upper respiratory tract, intestinal tract, and urogenital tract in humans and animals.[50] In this study, the leading 16 species, except *E. faecalis* and *Streptococcus*, isolated from the root canals of teeth with apical periodontitis were anaerobes. These results were consistent with those reported in several previous studies.[41,47,48,51]

The difficulties in culturing or identifying all microbial species exiting in the root canal are of special concern. Because the nutritional and physiologic needs of most microorganisms are still unknown, not all microorganisms can be cultivated under artificial conditions. Furthermore, 50–80% of bacterial species composing the microbiota associated with diverse human sites, including the oral cavity, are still uncultivated.[52] Therefore, the growth of bacteria in 50 (80.6%) of 62 teeth with apical periodontitis and the successful culture of 83 isolates of anaerobes in this study were reasonable results.

When the pulp-necrosis tooth is treated with root canal therapy, prescriptions of antibiotics are usually not necessary and antibiotic are given to the patients in the condition of exacerbated apical periodontitis or its associated cellulitis to prevent the further spread of infection or in the condition of patients who have immunocompromised or severe systemic diseases.[53,54] The precise antibiotics were given after we obtained the information from the antimicrobial susceptibility tests. However, in the waiting period, the results of our study could provide reference information to select and prescribe the tentative antibiotics to cover the diseases of the exacerbated apical periodontitis or its associated cellulitis. Moreover, the apical lesions are predominantly due to the direct invasion of the bacteria or their associated toxin, by-products or enzymes from the infected apical root canal to the periapical region.[55] Because the apical root canal and periapical region are infected by the same source of bacteria, they should have some overlapping of bacterial species culturing from both places, even though the apical root canal and periapical region have different microenvironments. In addition, the root canal system is a relatively closed system and the periapical region is an open system, thus it is easier for the clinicians to take bacterial samples without the chance of contamination from the apical root canal than from the periapical tissue. In case of carious tooth-related oral infection, culturing of bacteria from the infected oral site may sometimes be replaced by culturing of bacteria from the apical root canal.[55]

This study also found that ampicillin/sulbactam, amoxicillin/clavulanate, cefoxitin and clindamycin were four most effective drugs for cellulitis associated with anaerobes infection from teeth with apical periodontitis. In addition, vancomycin-resistant isolates of *E. faecalis* were susceptible to linezolid and high-level gentamicin.

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References

1. Jose F, Siqueira Jr JF, Rocas IN. Microbiology and treatment of endodontic infections. In: Cohen S, Hargreaves KM, editors. *Pathway of pulp*. 10th ed. Baltimore: CV Mosby Co; 2011. p. 572–8.
2. Siqueira Jr JF. Microbiology of apical periodontitis. In: Qrstavik D, Pitt Ford T, editors. *Essential endodontology*. 2nd ed. Oxford: Blackwell Munksgaard Ltd; 2008. p. 135.
3. Lee LW, Lan WH. A clinical evaluation of antimicrobial effectiveness of EDTA as an endodontic irrigant. *Chin Dent J* 1988;**7**:106–12.
4. Foschi F, Cavrini F, Montebugnoli L, Stashenko P, Sambri V, Prati C. Detection of bacteria in endodontic samples by polymerase chain reaction assay and association with defined clinical signs in Italian patients. *Oral Microbial Immunol* 2005;**20**:289–95.
5. Chen CC, Teng LJ, Kaiung S, Chang TC. Identification of clinically relevant viridans streptococci by an oligonucleotide array. *J Clin Microbiol* 2005;**43**:1515–21.
6. Marvin LF, Roberts MA, Fay LB. Matrix assisted laser desorption/ionization time-of-flight mass spectrometry in clinical chemistry. *Clin Chim Acta* 2003;**337**:11–21.
7. Baar van BL. Characterization of bacteria by matrix-assisted laser desorption/ionization and electrospray mass spectrometry. *FEMS Microbiol Rev* 2000;**24**:193–202.
8. Song Y, Finegold SM. *Peptostreptococcus, Finegoldia, Anaerococcus, Peptoniphilus, Veillonella*, and other anaerobic cocci. In: Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, et al., editors. *Manual of clinical microbiology*. 11th ed. Washington DC: ASM Press; 2015. p. 909–19.
9. Wren MWD. Anaerobic cocci of clinical importance. *Br J Biomed Sci* 1996;**53**:294–301.
10. Jousimies-Somer HR, Summanen P, Ctron D, Baron E, Wexler HM, Finegold SM. *Wadsworth–KTL anaerobic bacteriology manual*. Belmont: Star Publishing; 2002.
11. Elias J, Frosch M, Vogel U. Nesseria. In: Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, et al., editors. *Manual of clinical microbiology*. 11th ed. Washington DC: ASM Press; 2015. p. 635–51.
12. Becker K, Skov RL, Von Eiff C. *Staphylococcus, Micrococcus* and other catalase-positive cocci. In: Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, et al., editors. *Manual of clinical microbiology*. 11th ed. Washington DC: ASM Press; 2015. p. 354–82.
13. Chew TA, Smith JM. Detection of diacetyl (caramel odor) in presumptive identification of the "*Streptococcus milleri*". *J Clin Microbial* 1992;**30**:3028–9.
14. Spellerberg B, Brandt C. Streptococcus. In: Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, et al., editors. *Manual of clinical microbiology*. 11th ed. Washington DC: ASM Press; 2015. p. 383–402.

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15. Wauters G, Vaneechoutte M. Approaches to the identification of aerobic Gram-negative bacteria. In: Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, et al., editors. *Manual of clinical microbiology*. 11th ed. Washington DC: ASM Press; 2015. p. 613–34.
16. Vaneechoutte M, Nemec A, Kampfer P, Cools P, Wauters G. *Acinetobacter, Chryseobacterium, Moraxella*, and other non-fermentative Gram-negative rods. In: Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, et al., editors. *Manual of clinical microbiology*. 11th ed. Washington DC: ASM Press; 2015. p. 813–37.
17. Forbes BA, Sahm DF, Weissfeld AS. Enterobacteriaceae. In: *Bailey & Scott's diagnostic microbiology*. 12th ed. Houston: Mosby Co; 2007. p. 323–33.
18. Hoiby N, Ciofu O, Bjarnsholt T. Pseudomonas. In: Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, et al., editors. *Manual of clinical microbiology*. 11th ed. Washington DC: ASM Press; 2015. p. 773–90.
19. Fitzgerald C, Nachamkin I. *Campylobacter* and *Arcobacter*. In: Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, et al., editors. *Manual of clinical microbiology*. 11th ed. Washington DC: ASM Press; 2015. p. 998–1012.
20. Hook 3rd EW, Roddy RE, Lukehart SA, Hom J, Holmes KK, Tam MR. Detection of *Treponema pallidum* in lesion exudate with a pathogen specific monoclonal antibody. *J Clin Microbiol* 1985;**22**:241–4.
21. Ito F, Hunter EF, George RW, Pope V, Larsen SA. Specific immunofluorescence staining of pathogenic treponemes with a monoclonal antibody. *J Clin Microbiol* 1992;**30**:831–8.
22. Bian J, Liu X, Cheng YQ, Li C. Inactivation of cyclic Di-GMP binding protein TDE0214 affects the motility, biofilm, and virulence of *Treponema denticola*. *J Bacteriol* 2013;**195**: 3897–905.
23. Jorgensen JH, Turnidge JD. Susceptibility test methods: dilution and disk diffusion methods. In: Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, et al., editors. *Manual of clinical microbiology*. 11th ed. Washington DC: ASM Press; 2015. p. 1253–73.
24. Koreman EW, Allen SD, Dowell VR, Sommers HM. Antimicrobial susceptibility testing. In: *Koreman's color atlas and textbook of diagnostic microbiology*. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2006. p. 981–3. 1011–2.
25. Karlowsky JA, Richter SS. Antimicrobial susceptibility testing systems. In: Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, et al., editors. *Manual of clinical microbiology*. 11th ed. Washington DC: ASM Press; 2015. p. 1274–85.
26. Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. *Clin Infect Dis* 2009;**49**:1749–55.
27. Rocas N, Siqueira Jr JF. Root canal microbiota of teeth with chronic apical periodontitis. *J Clin Microbiol* 2008;**46**: 3599–606.
28. Siqueira Jr JF, Rocas IN, Alves FR, Silva MG. Bacteria in the apical root canal of teeth with primary apical periodontitis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009;**107**: 721–6.
29. Rocas IN, Siqueira Jr JF, Santos KR. Association of *Enterococcus faecalis* with different forms of periradicular diseases. *J Endod* 2004;**30**:315–20.
30. Siren EK, Haapasalo MP, Ranta K, Salmi P, Kerosuo EN. Microbiological findings and clinical treatment procedures in endodontic cases selected for microbiological investigation. *Int Endod J* 1997;**30**:91–5.
31. Teixeira LM, Carvalho MGS, Facklam RR, Shewmaker PL. Enterococcus. In: Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, et al., editors. *Manual of clinical microbiology*. 11th ed. Washington DC: ASM Press; 2015. p. 403–21.
32. Winn WC, Allen SD, Janda WM. Antimicrobial susceptibility testing. In: *Koreman's color atlas textbook of diagnostic microbiology*. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2006. p. 1011–2.
33. Leclercq R, Derlot E, Duval J, Courvalin P. Plasmid-mediated resistance to vancomycin and teicoplanin in *Enterococcus faecium*. *N Eng J Med* 1988;**319**:157–61.
34. Nicas TI, Cole CT, Preston DA, Schabel AA, Nagarajan R. Activity of glycopeptides against vancomycin-resistant Gram-positive bacteria. *Antimicrob Agent Chemother* 1989;**33**: 1477–81.
35. Forbes BA, Sahm DF, Weissfeld AS. *Streptococcus, Enterococcus*, and similar organisms. In: *Bailey & Scott's Diagnostic Microbiology*. 12th ed. Houston: Mosby Co; 2007. p. 276–8.
36. Bonora MG, Solbiati M, Stepan E, Zorzi A, Luzzani A, Catania MR, et al. Emergence of linezolid resistance in the vancomycin-resistant *Enterococcus faecium* multilocus sequence typing C1 epidemic lineage. *J Clin Microbiol* 2006; **44**:1153–5.
37. Diekema DJ, Jones RN. Oxazolidinone antibiotics. *Lancet* 2001; **358**:1975–82.
38. Moter A, Riep B, Haban V, Heuner K, Siebert G, Berning M, et al. Molecular epidemiology of oral treponemes in patients with periodontitis and in periodontitis-resistant subjects. *J Clin Microbiol* 2006;**44**:3078–85.
39. Hall V, Copsey SD. *Propionibacterium, Lactobacilli, Actinomyces*, and other non-spore-forming anaerobic Gram-positive rods. In: Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, et al., editors. *Manual of clinical microbiology*. 11th ed. Washington DC: ASM Press; 2015. p. 920–39.
40. Preza D, Olsen I, Aas JA, Willumsen T, Grinde B, Paster BJ. Bacterial profiles of root caries in elderly patients. *J Clin Microbiol* 2008;**46**:2015–21.
41. Munson MA, Pitt-Ford T, Chong B, Weightman A, Wade WG. Molecular and cultural analysis of the microflora associated with endodontic infections. *J Dent Res* 2002;**81**:761–6.
42. Lau SK, Woo PC, Fung AM, Chan KM, Woo GK, Yuen KY. Anaerobic, non-sporulating, Gram-positive bacilli bacteremia characterized by 16S rRNA gene sequencing. *J Med Microbial* 2004;**53**:1247–53.
43. Wexler HM, Molitoris D, St John S, Vu A, Read EK, Finegold SM. *In vitro* activities of faropenem against 579 strains of anaerobic bacteria. *Antimicrobial Agent Chemother* 2002;**46**: 3669–75.
44. Sundqvist G. Taxonomy, ecology and pathogenicity of the root canal flora. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1994;**784**:522–30.
45. Sundqvist G, Figdor D. Life as an endodontic pathogen. *Endo Topics* 2003;**6**:3–28.
46. Sakamoto M, Siqueira Jr JF, Rocas IN, Benno Y. Bacterial reduction and persistence after endodntic treatment procedure. *Oral Microbiol Immunol* 2007;**22**:19–23.
47. Fouad AF, Barry J, Caimano M, Clawson M, Zhu Q, Carver R, et al. PCR-based identification of bacteria associated with endodontic infections. *J Clin Microbiol* 2002;**40**: 3223–31.
48. Gomes BP, Pinheiro ET, Gadê-Neto CR, Sousa EL, Ferraz CC, Zaia AA, et al. Microbiological examination of infected dental root canals. *Oral Microbiol Immunol* 2004;**19**:71–6.
49. Rocas IN, Siqueira Jr JF. Detection of novel oral species and phylotypes in symptomatic endodontic infections including abscess. *FEMS Microbial Lett* 2005;**250**:279–85.
50. Kononen E, Conrads G, Nagy E. *Bacteroides, Porphyromonas, Prevotella, Fusobacterium* and other anaerobic Gram-negative rods. In: Jorgensen JH, Pfaller MA, Carroll KC,

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Funke G, Landry ML, Richter SS, et al., editors. *Manual of clinical microbiology*. 11th ed. Washington DC: ASM Press; 2015. p. 967–93.
51. Khemaleelakul S, Baumgartner JC, Pruksakorm S. Identification of bacteria in acute endodontic infections and their antimicrobial susceptibility. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002;**94**:746–55.
52. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. *J Clin Microbiol* 2005; **43**:5721–32.
53. Dorn SO, Cheung GSP. Management of endodontic emergencies. In: Hargreaves KM, Berman LH, editors. *Cohen's pathways of the pulp*. 11th ed. St. Louis: Elsevier; 2016. p. 706–21.
54. Eleazer PD, Rosenberg PA. Endodontic emergencies and therapeutics. In: Torabinegad M, Walton RE, Fouad AF, editors. *Endodontics: principles and practices*. 5th ed. St Louis: Elsevier; 2015. p. 162–71.
55. Lin LM, Huang GTJ. Pathobiology of apical periodontitis. In: Hargreaves KM, Berman LH, editors. *Cohen's pathways of the pulp*. 11th ed. St. Louis: Elsevier; 2016. p. 630–59.

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