Susceptibility of Endodontic Pathogens to Antibiotics in Patients with Symptomatic Apical Periodontitis

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Abstract

Introduction: The aim of this study was to evaluate susceptibility of predominant endodontic pathogens isolated from teeth with symptomatic apical periodontitis to most commonly prescribed antibiotics. **Methods:** Among 58 patients with symptomatic apical periodontitis, 47 and 11 cases were caused by primary and secondary root canal infection, respectively. The microbial samples were taken either from the root canals (35 cases) or by aspiration from apical abscesses (23 cases). Culture methods were used to identify the microorganisms present in the samples. Antibiotic susceptibilities of all isolates were evaluated by using the E-test method. **Results:** Microorganisms were isolated from 49 of the 58 samples studied and included facultative and obligate anaerobes. Streptococci and obligate anaerobes were the predominant microorganisms in cases of primary infection. *Enterococcus faecalis* dominated in cases of secondary infection. All tested microorganisms were highly sensitive to penicillin G, amoxicillin, and ampicillin. Susceptibilities to clindamycin and erythromycin were 73.8% and 54.7%, respectively. About 40% of the isolates were resistant to tetracycline. More than 50% of all anaerobes were resistant to metronidazole. All *E. faecalis* isolates were resistant to clindamycin. **Conclusions:** Based on the study results, penicillin and amoxicillin are suitable antibiotics for treatment of endodontic infection when conventional root canal treatment alone is insufficient. Clindamycin could be advised for penicillin-allergic patients with primary endodontic infections. *(J Endod 2010;■:1–6)*

Key Words

Antibiotics, antimicrobial susceptibility, apical periodontitis

Endodontic disease is caused by polymicrobial infections (1). Variation in the microorganisms isolated as a result of differences in diagnostic, sampling, and laboratory procedures have been shown in a number of studies (2–5). Symptomatic apical periodontitis (SAP) is defined as acute inflammation in the periradicular tissues that is associated with pain. It can be caused by primary infection as a result of pulp necrosis. Alternatively, it can develop because of an exacerbation of a secondary root canal infection in cases of failed endodontic treatment (6). In cases of primary and secondary/persistent root canal infection, a variety of microorganisms have been described in the literature (4, 5, 7). Certain species of facultative bacteria and anaerobes are associated with the symptoms of periradicular disease (8, 9). In addition, the microbial flora that colonize the root canals vary with the geographic location of the patients studied (10, 11).

The main treatment strategy for SAP is elimination of the intraradicular infection during a root canal treatment procedure (6). In more severe cases, surgical treatment could be indicated. The role of systemic antibiotic therapy in endodontics is limited. Usually, it is applied when patients present with progressive, diffuse swelling and systemic signs of infection including fever, malaise, and lymphadenopathy. Systemic antibiotics may also be used as a prophylactic measure for medically compromised patients (12). Despite these treatment guidelines, dental practitioners tend to overprescribe antibiotics in their practice, often without sufficient rationale for choosing a particular drug (13, 14).

The resistance of oral microflora to antibiotics has increased during the past decades, possibly because of the empiric use of antibiotics for a variety of pathologies (12, 15, 16). The antibiotic use and rates of microbial resistance are not uniform around the world (15, 17). Thus, it is prudent to study changes in the antimicrobial susceptibilities of endodontic pathogens to facilitate the choice of an appropriate antibiotic when indicated for the treatment of infections. The aim of this study was to evaluate susceptibilities of endodontic pathogens isolated from teeth with symptomatic apical periodontitis to the most commonly prescribed antibiotics.

Materials and Methods

Patient Selection

The Ethical Committee of Kaunas University of Medicine approved the study protocol, and all of the patients gave informed consent before the sampling procedure was performed. Fifty-eight adult patients with symptoms of SAP participated in the study. The diagnosis of SAP was based on the presence of pain and positive percussion and palpation tests associated with localized or diffuse swelling (18). The age of the patients ranged between 20 and 73 years old (mean = 36.8 years). Radiographic examination was performed to evaluate the root canal treatment status and the presence of periapical

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pathology. Information about the patients' use of antimicrobial drugs was collected before the sampling procedure. Medically compromised patients as well as those who presented with open access to the root canal system were excluded from the study.

Microbial Sampling

Microbial samples were obtained either directly from the root canal of the involved tooth (35 cases) or by puncture of the periapical tissues (23 cases). Direct sampling from the root canal was performed in cases when incision of periapical tissues was contraindicated because of the beginning of well-localized swelling. In cases when a periapical abscess was present and, therefore, incision and drainage was indicated, puncture of the apical abscess was performed before the incision.

Every tooth was isolated with a rubber dam, and the field was disinfected with 96% ethanol (19). Sterile burs were used to gain access to the pulp cavity. Sterility of the operative field and access cavity was verified by taking a swab sample. The working length of the root canal was established radiographically. In multirooted teeth, the largest root canal was chosen for sampling. Initial enlargement was performed with minimal instrumentation and without any irrigation. In cases of endodontic retreatment, old filling material from the root canals of the involved tooth was removed without irrigation before the sampling procedure, which cleared a pathway for exudation from the periapical area. Three sterile paper points were introduced to the predetermined working length and held in place for 60 seconds to absorb the contents of the canal. Afterwards, the paper points were immediately transferred to the sterile swab transport system (AMIES, Copan, Italy) in a transport medium of protective agar gel, which contains scavengers that eliminate dissolved oxygen, super oxide, and free radicals. The swab transport system had previously been flushed with nitrogen gas to prevent oxidation of the transport medium.

In cases in which contents of periapical swelling were to be sampled, puncture of the apical swelling was performed under local anesthesia with articaine 4% (Ubistesin Forte; ESPE, St. Paul, MN). The anesthetic was placed about 3 cm further away from the puncture site. The patients were asked to rinse the area with 0.2% chlorhexidine mouthwash for disinfection of the oral mucosa. Aspiration of the exudate from the swollen mucosa was performed using a sterile 16-G needle with a disposable syringe. The aspirate was injected into a sterile swab transport system (AMIES). All obtained samples were transported within 2 hours of collection to the laboratory of Microbiology of the University Clinics for identification of the predominant bacteria and evaluation of their antimicrobial susceptibilities.

Microbiological Analysis

The samples were inoculated on Columbia agar with 5% horse blood (BBL; Becton, Dickinson and Company, Sparks, MD), chocolate

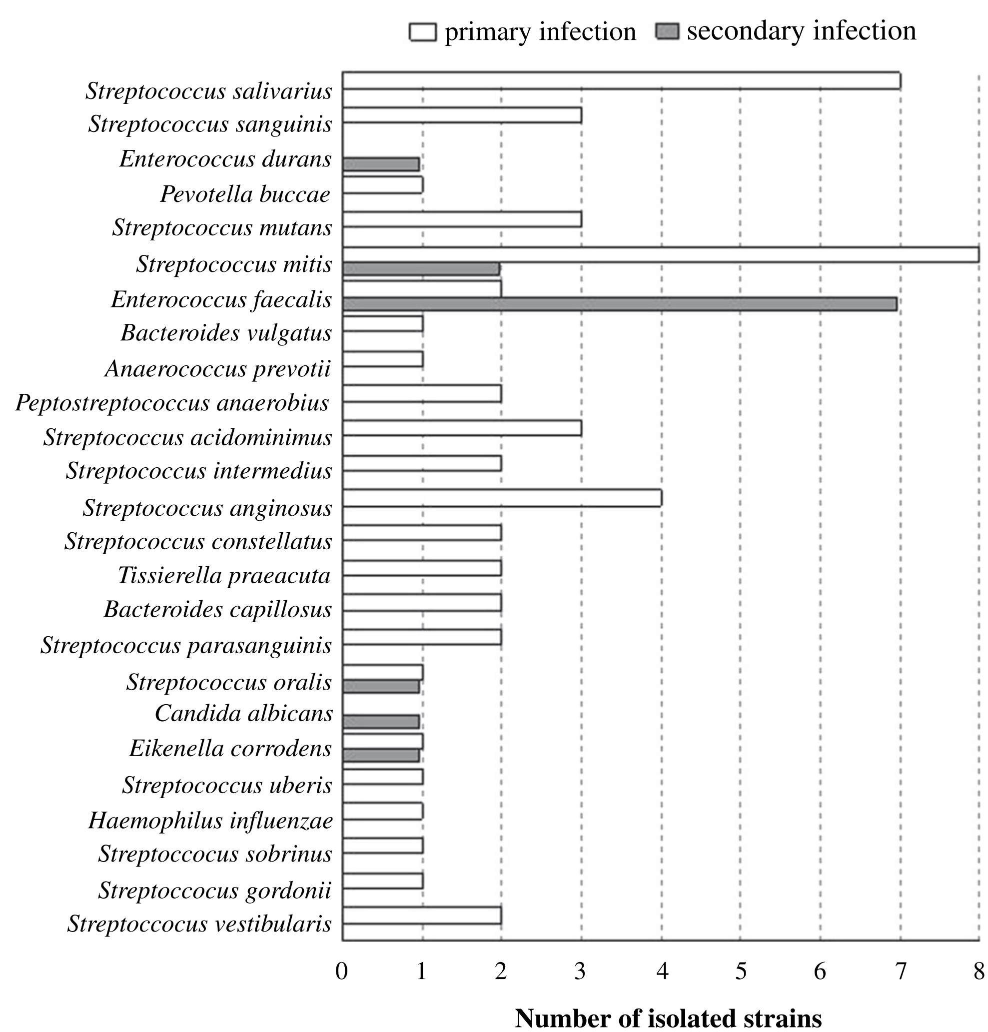

Figure 1. The distribution of bacterial species isolated in cases of symptomatic apical periodontitis according to root canal infection type (primary or secondary).

agar (BBL; Becton, Dickinson and Company, Sparks, MD), Schaedler agar with vitamin K3 and 5% horse blood (BBL; Becton, Dickinson and Company, Sparks, MD), and thioglycollate broth. Inoculated Columbia blood agar and chocolate agar plates were incubated at 35°C in 5% carbon dioxide for 48 to 72 hours. After inoculation, Schaedler agar was immediately incubated at 35°C in an anaerobic atmosphere chamber consisting of 10% hydrogen, 10% carbon dioxide, and 80% nitrogen (Bug Box, West Yorkshire, UK). The cultures were then inspected after 24 hours and 48 hours of incubation. An aerotolerance test was performed on each isolate recovered from the primary anaerobe medium plate. The thioglycollate broth was examined daily for 5 days. For each colony grown on the anaerobic plate and for growth in the thioglycollate broth, Gram-stained smears were prepared to identify a pathogen. The results of the Gram staining from the thioglycollate broth dictated the need for further growth in the same or another type of media. The isolated bacteria and strict anaerobic bacteria were identified with the Phoenix ID system (Becton, Dickinson and Company, Sparks, MD) and the BBL crystal ID system, respectively.

Antimicrobial Susceptibility Test

The antibiotic susceptibility/resistance of all isolated microorganisms was measured. The following antibiotics were tested: penicillin G, ampicillin, amoxicillin, amoxicillin clavulanate, clindamycin, metronidazole, erythromycin, tetracycline, and vancomycin. The antimicrobial susceptibilities of the isolates were determined by minimal inhibitory concentration values using the E-test System (AB Biodisk, Solna, Sweden) or the Phoenix ID system and interpreted according to the Clinical and Laboratory Standards Institute guidelines (20).

Statistical analyses included the chi-square test (Fisher exact test and Kendall tau correlation coefficient) for analyzing differences between the isolated microorganisms according to diagnosis (primary or secondary infection), bacterial sampling technique (directly from root canal or by abscess puncture), and prior antimicrobial therapy (presence or absence).

Results

Microorganisms were isolated from 49 of the 58 samples studied. The number of bacterial species per sample varied from one to four. The molars were the teeth mostly frequently associated with SAP in this study. Forty-seven teeth had necrotic pulps (primary infection), and 11 had been endodontically treated previously (secondary infection). All swab samples, took for sterility control, were negative, indicating a disinfection of the operation field.

Of the 66 cultivable isolates, 25 different bacterial species, including facultative and obligate anaerobes, were identified. *Enterococcus faecalis* dominated in cases of secondary infection compared with the cases of primary infection ($p < 0.001$). Streptococci and obligate anaerobes were the predominant types of microorganisms in cases

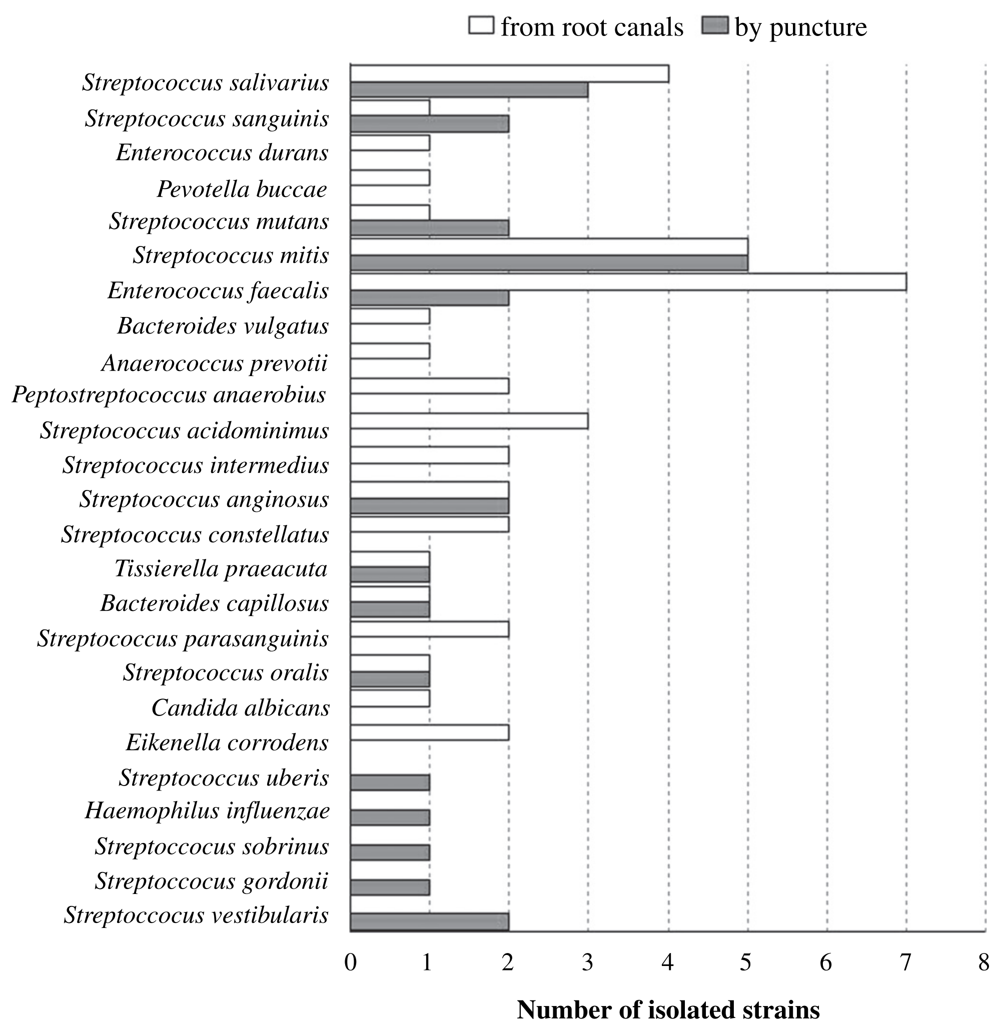

Figure 2. The distribution of bacterial species isolated in cases of symptomatic apical periodontitis according to the technique of bacterial sampling.

of primary infection compared with the cases of secondary infection ($p < 0.001$) (Fig. 1). There were no significant differences between the isolated bacterial species when comparing different sampling techniques ($p > 0.05$) (Fig. 2).

Among the study participants, 18 patients had received antibiotic therapy before the sampling procedure. Of those, 8 individuals had used amoxicillin, 6 had used doxycycline, 2 had used metronidazole and erythromycin, and 2 patients did not know the name of antibiotic they had used. In the samples obtained from those 18 patients, bacterial types were identified in 32.6% of all isolated streptococci, 20% of isolated enterococci, and none of the strict anaerobes (Fig. 3). No statistically significant differences were found between facultative anaerobes when correlated with the presence or absence of antimicrobial therapy before sample collection ($\chi^2_3 = 4.3$, $p = 0.2$; Kendall tau $r = 0.2$, $p = 0.06$). In addition, no correlation was found between antibiotic susceptibilities and previous antibiotic use ($p > 0.05$).

All of the microorganisms tested were highly sensitive to penicillin G, amoxicillin, and ampicillin (Table 1). The percentage of the bacteria susceptible to clindamycin and erythromycin was 73.8% and 54.7%, respectively. Among all tested isolates, 39.6% were resistant to tetracycline. A total of 55.6% of all anaerobes were resistant to metronidazole; however, 100% of them were susceptible to clindamycin. Enterococci showed 100% resistance to clindamycin, 30% to tetracycline, and intermediate (90%) susceptibility to erythromycin (Table 1).

Discussion

Microorganisms that are able to invade the pulp tissue and survive in a root canal system are the causative pathogens in primary endodontic infections. The bacteria remaining in the root canal system after endodontic treatment cause secondary, or persistent, infections (1). Bacterial cultures and molecular studies have confirmed that *E. faecalis* is one of the most prevalent bacteria found in the root canal after endodontic treatment (4, 21). The reports about differences of species isolated from teeth with primary or secondary infections were mainly based on studies of asymptomatic apical periodontitis or pulp necrosis (22, 23). Data on the differences in microorganisms isolated from symptomatic teeth with the presence of primary or secondary infection are limited (4). We experienced that SAP was less frequent compared with asymptomatic cases in dental practice. Therefore, a small number of participants could be a limitation of this study. In order to overcome this problem, the statistical methods designed to find a relationship between the samples with very small expected frequencies have been applied (Fisher exact test).

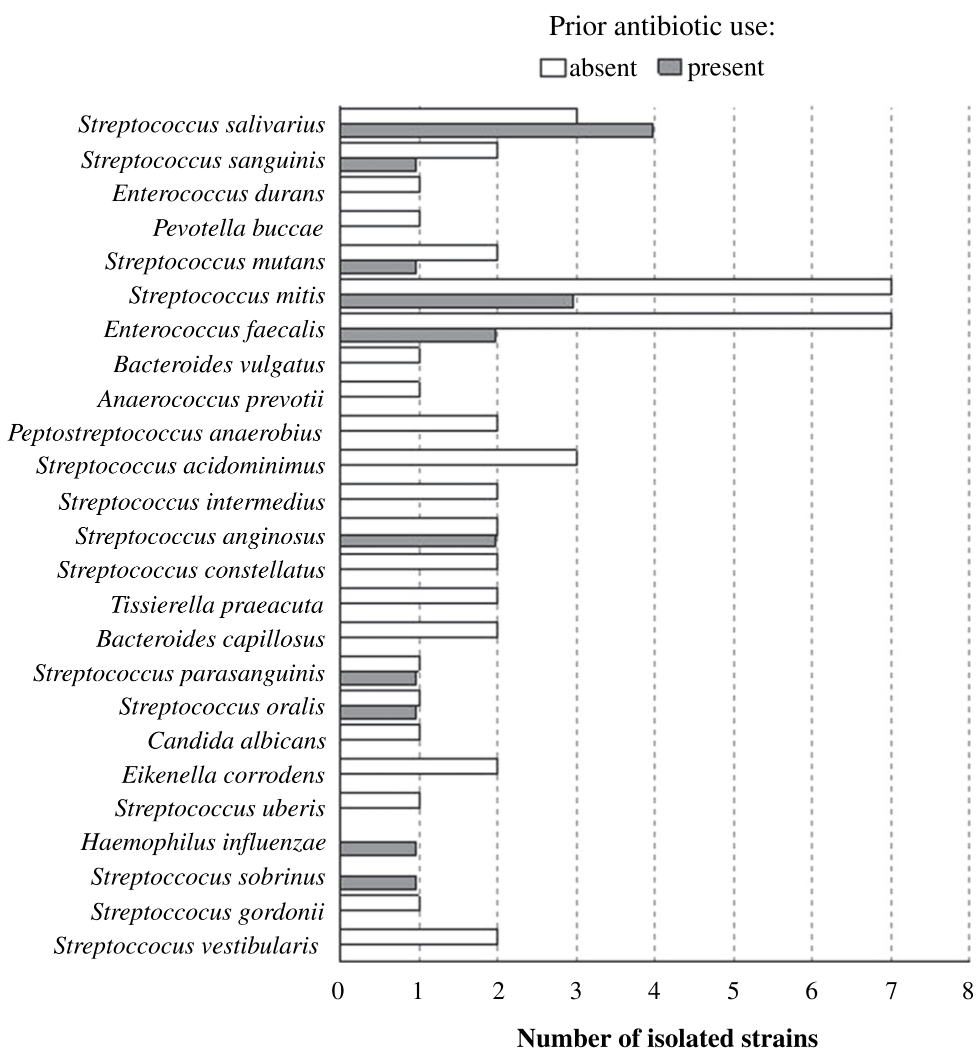

Figure 3. The distribution of bacterial species isolated in cases of symptomatic apical periodontitis according to prior antibiotic use.

TABLE 1. Antimicrobial Susceptibility of All Isolated Microorganisms

Antibiotics	Antimicrobial susceptibility Anaerobes (*n* = 9) %	Streptococci (*n* = 43) %	Enterococci (*n* = 10) %	Others (*n* = 3) %	Total* (*n* = 65) %
Penicillin G					
S	100	72.1	100	100	81.0
I	0	25.6	0	0	17.4
R	0	2.3	0	0	1.6
Ampicillin		_____			
S	100		100	100	100
Amoxicillin					
S	_____	83.7	_____	_____	83.7
I		16.3			16.3
Amoxicillin – clavulanate					
S	_____	_____	100	_____	100
Clindamycin					
S	100	85.7	0	_____	73.8
I	0	4.8	0		3.3
R	0	9.5	100		23.0
Metronidazole					
S	44.4	_____	_____	_____	44.4
R	55.6				55.6
Erythromycin					
S	_____	65.1	10.0	_____	54.7
I		0	90.0		17.0
R		34.9	0		28.3
Tetracycline					
S	_____	58.1	70.0	_____	60.4
R		41.9	30.0		39.6
Vancomycin					
S	_____	100	100	_____	100

S, susceptible; I, intermediate susceptibility; R, resistant.

*Candida albicans was not included.

The findings of our study show that pathogens isolated from teeth affected by SAP in cases of persistent infection differed from those isolated from teeth with primary infection, which is in concordance with the findings reported by de Sousa et al (5). Enterococci, particularly *E. faecalis,* were identified (alone or in combination with other microorganisms) only in those patients who had previously undergone root canal treatment for SAP. In contrast, in cases of primary infection, facultative and obligate anaerobes were identified as the causative microorganisms, which is consistent with previous findings (8, 23–25).

The goal of the treatment of SAP is to control the spread of infection and provide symptom relief, which can usually be achieved without the use of antibiotics. However, systemic antimicrobial therapy may be useful for preventing the spread of pathogens to other anatomic sites in high-risk patients. In such cases, systemic antimicrobial therapy should be directed against the most common pathogens (12). According to the results of our study, *E. faecalis* should be the target of antibiotic therapy for SAP caused by secondary infection, whereas streptococci and strict anaerobes should be targeted in cases of primary infection. Dental practitioners sometimes find it difficult to determine when antimicrobial treatment is indicated and to choose an appropriate drug. Essentially, the treatment strategy should be to prescribe a drug with the smallest possible spectrum of activity to minimize the development of antibiotic-resistant strains of bacteria (12). All positive cultures in this study were tested for susceptibilities to antibiotics with the intention of determining the most effective drug for all isolated microorganisms. Bacterial sensitivity was evaluated according to the Clinical and Laboratory Standards Institute guidelines (20).

All tested obligate anaerobes were susceptible to penicillin G and ampicillin, as has been seen in other studies (5, 8). Only 2.3% of the isolated streptococci were resistant to penicillin, and all were amoxicillin susceptible to some extent (Table 1). The susceptibility of streptococci to penicillin was higher in our study than in reports from Japan or the United States (5, 25). All tested enterococci (100%) were susceptible to penicillin, ampicillin, and amoxicillin clavulanate, which is in contrast to studies that have shown the presence of enteroccoci that are resistant to penicillin and ampicillin (26, 27).

Until now, the question of whether penicillins should be the first choice of antibiotic has remained unanswered. Resistance of the bacteria found in endodontic infections to penicillin has been reported as 9% to 19% (23, 28). In our study, however, only 1.6% of all isolated bacteria were resistant to penicillin.

Although it has been suggested that prior antimicrobial therapy with β-lactam antibiotics selects for β-lactamase–producing bacteria, penicillins remain effective and suitable as a first-choice antibiotic for the treatment of odontogenic infections (29). β-Lactamase inhibitors, such as clavulanic acid, have been recommended for use in combination with amoxicillin to extend the spectrum of coverage in cases when treatment with β-lactam antibiotics alone was not effective (23). The results of this study showed that extended-spectrum antibiotics, rather than amoxicillin alone, were not needed in cases of symptomatic apical periodontitis because the bacteria isolated from those cases had high susceptibilities to penicillin G and to amoxicillin.

Good efficacy of clindamycin against the bacteria isolated in teeth affected by SAP has been reported (23, 24, 28). As a result, clindamycin has often been recommended for the treatment of endodontic infections in cases of an allergy to penicillin or when penicillin was not effective. The findings of this study showed a high susceptibility of anaerobes (100%) and streptococci (85.7%) to clindamycin (Table 1). Resistance of all tested enteroccoci confirmed the previously reported intrinsic resistance of enterococci to clindamycin (30). Therefore, clindamycin may only be suitable for the treatment of primary infections because enterococci were the predominant microorganisms in isolates from secondary infections. For patients allergic to penicillin, antibiotics for the treatment of SAP with persistent infection should not be prescribed empirically without testing for microbial sensitivity.

Metronidazole has been reported to be highly effective against anaerobes (5, 24), and in combination with penicillin, it has been recommended for the treatment of symptomatic infections of endodontic origin (12). However, in a study by Khemaleelakul et al (23), 12% of strict anaerobes were resistant to metronidazole. We found that the isolated anaerobes were highly resistant (55.6%) to metronidazole, which is in agreement with Baumgartner and Xia (28). Perhaps a combination of metronidazole with penicillin could be more effective compared with the administration of single medicaments as shown by several studies (23, 28).

The findings of our study show low antimicrobial susceptibility of the isolated bacteria to erythromycin. Almost 35% of the isolated streptococci were resistant to this antibiotic, which is similar to the resistance rates reported by other authors (24, 25). In contrast, our study found that most enterococci (90%) had intermediate sensitivity to erythromycin, suggesting greater susceptibility than was seen in other studies (26, 31). Tetracycline appeared to be the least effective antibiotic against the microorganisms isolated from teeth affected by SAP (Table 1).

Typically, the microorganisms found in root canals and on external surfaces of root tips do not represent a single species, and they tend to form biofilms (32). Consequently, their response to antimicrobial treatment may differ from the response seen with a single species of bacteria (33–35). Further investigation of microbial biofilm resistance to antibiotics in cases of endodontic infections is needed.

Conclusions

Because all predominant endodontic pathogens found in this study were highly susceptible to penicillin and amoxicillin, these two antibiotics seem to be suitable choices for endodontic treatment when conventional root canal treatment alone is not sufficient. In the present study, differences between microbial species in primary and secondary root canal infections were determined. Clindamycin should be advised for penicillin-allergic patients in cases of primary SAP; tetracycline and erythromycin are of limited antimicrobial value and, therefore, should not be prescribed empirically for patients with SAP.

References

1. Dahlen G. Culture-based analysis of endodontic infections. In: Fouad AF, ed. Endodontic microbiology. 1st ed. Ames, IA: Wiley-Blackwell; 2009:225–41.
2. Jacinto RC, Montagner F, Signoretti FGC, et al. Frequency, microbial interactions, and antimicrobial susceptibility of Fusobacterium nucleatum and Fusobacterium necrophorum isolated from primary endodontic infections. J Endod 2008;34:1451–6.
3. Yamane K, Ogawa K, Yoshida M, et al. Identification and characterization of clinically isolated biofilm-forming gram-positive rods from teeth associated with persistent apical periodontitis. J Endod 2009;35:347–52.
4. Foschi F, Cavrini F, Montebugnoli L, et al. Detection of bacteria in endodontic samples by polymerase chain reaction assays and association with defined clinical signs in Italian patients. Oral Microbiol Immunol 2005;20:289–95.
5. de Sousa ELR, Ferraz CCR, de Almeida Gomes BPF, et al. Bacteriological study of root canals associated with periapical abscesses. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;96:332–9.
6. Sigurdsson A. Clinical manifestations and diagnosis. In: Orstavik D, Pitt Ford T, eds. Essential endodontology: prevention and treatment of apical periodontitis. 2nd ed. Oxford, UK: Blackwell Munksgaard Ltd; 2008:235–62.
7. Gomes BPFA, Pinheiro ET, Jacinto RC, et al. Microbial analysis of canals of root-filled teeth with periapical lesions using polymerase chain reaction. J Endod 2008;34:537–40.
8. Jacinto RC, Gomes BPFA, Ferraz CCR, et al. Microbiological analysis of infected root canals from symptomatic and asymptomatic teeth with periapical periodontitis and the antimicrobial susceptibility of some isolated anaerobic bacteria. Oral Microbiol Immunol 2003;18:285–92.
9. Sakamoto M, Rôças IN, Siqueira JF Jr, et al. Molecular analysis of bacteria in asymptomatic and symptomatic endodontic infections. Oral Microbiol Immunol 2006;21: 112–22.
10. Rôças IN, Baumgartner JC, Xia T, et al. Prevalence of selected bacterial named species and uncultivated phylotypes in endodontic abscesses from two geographic locations. J Endod 2006;32:1135–8.
11. Machado de Oliveira JC, Siqueira JF Jr, Rôças IN, et al. Bacterial community profiles of endodontic abscesses from Brazilian and USA subjects as compared by denaturing gradient gel electrophoresis analysis. Oral Microbiol Immunol 2007;22:14–8.
12. Baumgartner JC. Antibiotics in endodontic therapy. In: Newman MG, Van Winkelhoff AJ, eds. Antibiotics and antimicrobial use in dental practice. 2nd ed. Carol Stream, IL: Quintessence Publishing Co, Inc; 2001:143–57.
13. Öcek Z, Sahin H, Baksi G, Apaydin S. Development of a rational antibiotic usage course for dentists. Eur J Dent Educ 2008;12:41–7.
14. Rodriguez-Núñez A, Cisneros-Cabello R, Velasco-Ortega E, et al. Antibiotic use by members of the Spanish Endodontic Society. J Endod 2009;35:1198–203.
15. Monnet DL, Kristinsson KG. Turning the tide of antimicrobial resistance: Europe shows the way. Euro Surveill 2008;13:19039.
16. Al-Haroni M. Bacterial resistance and the dental profesionals role to halt the problem. J Dent 2008;36:95–103.
17. Van Winkelhoff AJ, Herrera D, Oteo A, et al. Antimicrobial profiles of periodontal pathogens isolated from periodontitis patients in the Netherlands and Spain. J Clin Periodont 2005;32:893–8.
18. Iqbal M, Kim S, Yoon F. An investigation into differential diagnosis of pulp and periapical pain: a PennEndo database study. J Endod 2007;33:548–51.
19. Peters LB, Wesselink PR, van Winkelhoff AJ. Combinations of bacterial species in endodontic infections. Int Endod J 2002;35:698–702.
20. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests. Approved standard M2–A9. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
21. Peciuliene V, Reynaud AH, Balciuniene I, et al. Isolation of yeasts and enteric bacteria in root-filled teeth with chronic apical periodontitis. Int Endod J 2001; 34:429–34.
22. Jacinto RC, Gomes BPFA, Shah HN, et al. Incidence and antimicrobial susceptibility of Porphyromonas gingivalis isolated from mixed endodontic infections. Int Endod J 2006;39:62–70.
23. Khemaleelakul S, Baumgartner JC, Pruksakorn 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.
24. Kuriyama T, Karasawa T, Nakagawa K, et al. Bacteriology and antimicrobial susceptibility of gram-positive cocci isolated from pus spacements of orofacial odontogenic infections. Oral Microbiol Immunol 2002;17:132–5.
25. Kuriyama T, Karasawa T, Nakagawa K, et al. Bacteriologic features and antimicrobial susceptibility in isolates from orofacial odontogenic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000;90:600–8.
26. Pinheiro ET, Gomes BPFA, Drucker DB, et al. Antimicrobial susceptibility of Enterococcus faecalis isolated from canals of root filled teeth with periapical lesions. Int Endod J 2004;37:756–63.
27. Dahlen G, Samuelsson W, Molander A, et al. Identification and antimicrobial susceptibility of enterococci isolated from the root canal. Oral Microbiol Immunol 2000; 15:309–12.
28. Baumgartner JC, Xia T. Antibiotic susceptibility of bacteria associated with endodontic abscesses. J Endod 2003;29:44–7.
29. Kuriyama T, Nakagawa K, Karasawa T, et al. Past administration of β -lactam antibiotics and increase in the emergence of β-lactamase-producing bacteria in patients with orofacial odontogenic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000;89:186–92.
30. Singh KV, Weinstock GM, Murray BE. An *Enterococcus faecalis* ABC homologue (Lsa) is required for the resistance of this species to clindamycin and quinupristin-dalfopristin. Antimicrob Agents Chemother 2002;46:1845–50.
31. Pinheiro ET, Gomes BPFA, Ferraz CCR, et al. Evaluation of root canal microorganisms isolated from teeth with endodontic failure and their antimicrobial susceptibility. Oral Microbiol Immunol 2003;18:100–3.
32. Nair PNR. On the causes of persistent apical periodontitis: a review. Int Endod J 2006;39:249–81.
33. Sedgley CM, Lee EH, Martin MJ, et al. Antibiotic resistance gene transfer between Streptococcus gordonii and Enterococcus faecalis in root canals of teeth ex vivo. J Endod 2008;34:570–4.
34. de Paz LE, Bergenholtz G, Svensater G. The effects of antimicrobials on endodontic biofilm bacteria. J Endod 2010;36:70–7.
35. Williamson AE, Cardon JW, Drake DR. Antimicrobial susceptibility of monoculture biofilms of a clinical isolate of Enterococcus faecalis. J Endod 2009;35: 95–7.