Antibiotic Susceptibility of Bacteria Associated with Endodontic Abscesses

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Antibiotics to treat endodontic infections are routinely prescribed based on previously published susceptibility tests. There is increased concern that bacteria have increased resistance to the currently recommended antibiotics. The purpose of this investigation was to perform antibiotic susceptibility tests on a panel of bacteria recently isolated from endodontic infections. The bacteria in this study were aseptically aspirated with a needle from endodontic abscesses, cultivated, and identified at the species level. Each of the 98 species of bacteria was tested for antibiotic susceptibility to a panel of six antibiotics using the Etest. The antibiotics were penicillin V, amoxicillin, amoxicillin + clavulanic acid, clindamycin, metronidazole, and clarithromycin. The percentages of susceptibility for the 98 species were penicillin V: 83/98 (85%), amoxicillin: 89/98 (91%), amoxicillin + clavulanic acid: 98/98 (100%), clindamycin: 94/98 (96%), and metronidazole: 44/98 (45%). Metronidazole had the greatest amount of bacterial resistance; however, if it is used in combination with penicillin V or amoxicillin, susceptibility of the combination with penicillin V or amoxicillin increased to 93% and 99%, respectively. Clarithromycin seems to have efficacy, but it is still considered an antibiotic under investigation because the minimum inhibitory concentration has not been established.

Endodontic infections occur when bacteria gain access to the normally sterile dental pulp or periradicular tissues and produce disease as opportunistic pathogens. Endodontic infections are polymicrobial with several species of predominately anaerobic bacteria cultured from each infection (1). Antibiotics (antimicrobials) are often prescribed for the adjunctive treatment of endodontic infections. The choice of antibiotic is usually based on previously published susceptibility testing and previous clinical success. There is concern that bacteria have increased resistance to the currently prescribed antibiotics.

Susceptibility testing is the determination of the bacterial pattern of resistance to a number of antibiotics. It would be ideal if susceptibility testing could always be undertaken before the prescription of antibiotics. Unfortunately, it usually takes from several days to weeks to cultivate and do susceptibility tests on anaerobic bacteria. Penicillin V has been the antibiotic of choice, because it has been effective against many of the facultative and strict anaerobes commonly found in polymicrobial endodontic infections (2–5).

Reports have shown that some species of bacteria (especially Gram-negative anaerobes) have become resistant to penicillin (6, 7). The prevalence of penicillin resistance for bacteria commonly found in endodontic infections and acute dental abscesses has been reported to be approximately 5% to 20% (2, 4, 8–13).

Previously the most common methods of susceptibility testing were the disk diffusion test, agar dilution, and both micro- and macro-broth dilution. Disadvantages of these systems include: nonquantitative interpretation, inconsistent application for slow growing bacteria and anaerobes, limited use for direct testing of clinical material, and very time consuming (14). The Etest (AB Biodisk, Culver City, CA) is based on the diffusion of a continuous, exponential concentration gradient of the antimicrobial from a plastic strip containing the antibiotic. The Etest overcomes several of the above disadvantages while producing an accurate, reproducible reference minimum inhibitory concentration (MIC), which has been used for susceptibility testing of endodontic isolates (3, 15). Each plastic strip is 5- $\times$ 50-mm with the dried antibiotic in a concentration gradient on one side and an MIC interpretive scale on the other side (Fig. 1). The MIC scale corresponds to 15 two-fold dilutions. After incubation of the Etest strip on agar media with a lawn of bacteria, an ellipse of inhibition is formed around the strip. The point where the ellipse intersects the strip is where the MIC is read from the interpretive scale. The Etest is technically simple to use. In the future, Etest strips may be developed for use with fungi, investigational antimicrobials, antimicrobial combinations, and as a surveillance tool around the world.

The purpose of this study was to determine the antibiotic susceptibility of 98 strains of bacteria recently isolated from 12 endodontic abscesses using the Etest. The antibiotics tested were penicillin V, amoxicillin, amoxicillin + clavulanic acid, clindamycin, metronidazole, and clarithromycin.

MATERIALS AND METHODS

Patients in this study were treated using a protocol approved by the Institutional Review Board at Oregon Health & Science University. A total of 98 strains of bacteria were cultivated from 12
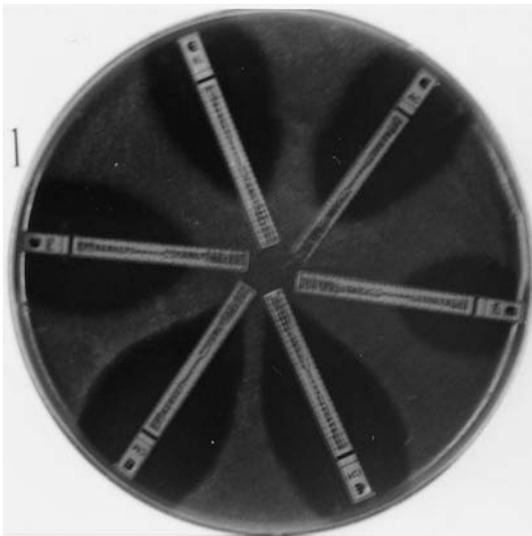
FIG 1. Etest strip with inhibition ellipse on a blood-agar plate.

TABLE 1. Minimum inhibitory concentrations (MICs) (μg/ml)

Antimicrobial agent	Susceptible	Intermediate	Resistant
Penicillin V	≤0.5	≤1	≤2
Metronidazole	≤8	≤16	≤32
Amoxicillin*	≤0.5	≤1	≤2
Amoxicillin/clavulanate	≤4	≤8	≤16
Clindamycin	≤2	≤4	≤8

* Amoxicillin is considered to have an MIC similar to ampicillin.

abscesses. The clinical samples were aseptically aspirated with a needle from each abscess and transported to the laboratory in an anaerobic container (BBL, Cockeysville, MD). All culturing of facultative and strict anaerobes was performed at 36°C in a Bactron II anaerobic chamber (Sheldon Manufacturing Inc. Cornelius, OR) with an atmosphere of 85% N2, 10% H2, and 5% CO_2. Each strain of bacteria was grown in broth to turbidity of 0.5 McFarland density and streaked on Brucella blood-agar plates 150 mm in diameter (Anaerobe Systems, San Jose, CA) with a cotton swab to obtain confluent growth. Six different Etest strips with the antimicrobials, penicillin V, amoxicillin, amoxicillin + clavulanic acid, clindamycin, metronidazole, and clarithromycin, were placed on the plates, which were incubated in the anaerobic chamber to produce a confluent growth of bacteria (Fig. 1). After incubation, an ellipse of inhibition around each antibiotic strip was read to determine the MIC calibrated in μl/ml. The National Committee for Clinical Laboratory Standards (NCCLS) has established MIC interpretive standards for resistance of anaerobes: penicillin G (penicillin V) ≤2 μl/ml, amoxicillin/clavulanic ≤16 μl/ml, metronidazole resistance at ≤32 μg/ml, clindamycin ≤8 μl/ml, and amoxicillin (ampicillin) ≤2 μg/ml. Clarithromycin is considered investigational for use with anaerobes.

RESULTS

The results using *Bacteroides fragilis* (ATCC 25285) as a reference strain conformed to the values for quality control as established by the NCCLS. Thirty-three (34%) strains of bacteria isolated from these endodontic abscesses were facultative anaerobes and 65 (66%) strains were strict anaerobes. Table 1 gives the number of species of bacteria with susceptible, intermediate, and resistant end points using the Etest for the 98 strains of bacteria in this study. Susceptible and intermediate MIC end points are both considered amenable to antibiotic therapy by the NCCLS (Table 2). The percentages of susceptible/intermediate treatment for each antibiotic in this study were penicillin V: 83/98 (85%), metronidazole: 44/98 (45%), amoxicillin: 90/98 (91%), amoxicillin + clavulanic acid: 98/98 (100%), and clindamycin: 94/98 (96%) (Table 3). If combination antibiotic therapy had been used to treat the bacteria isolated from these 12 abscesses, the percentage of

susceptible/intermediate for the combination of penicillin V/metronidazole would have been 91/98 (93%), and the combination of amoxicillin/metronidazole would have been 97/98 (99%).

Clarithromycin is considered an investigational antibiotic by the NCCLS for treatment of anaerobes and is without an established MIC. If the susceptibility MIC end point was determined to be ≤8 μg/ml, the level of susceptibility in this study would be 87/98 (89%). If the susceptibility MIC end point was determined to be ≤2 μg/ml, the level of susceptibility in this study would be 77/98 (78%).

DISCUSSION

The prescription of antibiotics should be adjunctive to appropriate clinical treatment. Antibiotics are indicated when signs and symptoms are associated with systemic involvement, for patients with progressive infections, or for patients who are immunocompromised (16). Selection of an antibiotic regimen should be based on knowledge of the efficacy of an antibiotic for the bacteria most often associated with severe infections. It should also be remembered that endodontic infections are ecosystems of bacteria in which by-products of one species of bacteria may be nutrients for other species of bacteria (17). Thus, if an antibiotic is effective against some species of bacteria in a polymicrobial infection, it may indirectly affect other bacteria in that ecosystem.

Resistance to penicillin is usually by three mechanisms. They are barriers to bacterial cell wall penetration, inability to bind to the penicillin binding proteins, and production of β-lactamase. Whether the production of β-lactamase by one species of bacteria can protect other non-β-lactamase-producing bacteria in a polymicrobial infection is controversial.

Penicillin V is still considered by most authorities to be the antibiotic of choice for orofacial and endodontic infections (12, 16, 18). A study by Lewis et al. (12) recently assessed the prevalence of penicillin resistance in the United Kingdom. Lewis et al. (12) determined that 23% of their isolates were resistant to penicillin (MIC > 1 mg/L), whereas only 5% were resistant to amoxicillin/clavulanic acid. In this study, 15/98 (15%) total strains of bacteria were resistant to penicillin V, whereas 9/98 (9%) were resistant to amoxicillin and none of the 98 isolates were resistant to amoxicillin/clavulanic acid. It has been shown that recent administration of β-lactam antibiotics, such as penicillin V, does increase the emergence of β-lactamase-producing bacteria (19). When penicillin was only taken for 1 or 2 days, few β-lactamase-producing bacteria emerged, but after several days, over 50% of the cases acquired β-lactamase-producing bacteria (19). Thus, if a patient has recently taken penicillin V or other β-lactam antibiotic, the prescription of amoxicillin/clavulanic acid or a β-lactamase stable antibiotic is recommended for unresolved infections.

Clindamycin had efficacy against 94/98 (96%) of the bacteria in this study. Previous studies have also demonstrated the efficacy of

TABLE 2. Efficacy of each antibiotic for the 98 strains of bacteria in this study

Antibiotic*	Pen V	Met	Pen V/Met	Amox	Amox/Met	Amox/Clav	Clind
End point							
Susceptible	77	43	89	89	97	98	94
Intermediate	6	1	2	—	—	—	—
Susceptible/Intermediate*	**83/85%**	**44/45%**	**91/93%**	**89/91%**	**97/99%**	**98/100%**	**94/96%**
Resistant	15	54	7	9	1	—	4
Total Bacteria	98	98	98	98	98	98	98

Pen V = penicillin V; Met = metronidazole; Pen V/Met = combination of penicillin V and metronidazole; Amox = amoxicillin; Amox/Met = combination of amoxicillin and metronidazole; Amox/Clav = amoxicillin/clavulanate; Clind = clindamycin.
* Susceptible/intermediate end points are considered amenable to therapy.

TABLE 3. Number of resistant strains to each of the antibiotics for each of the 12 abscesses

Abscess Sample	Pen V	Met	Pen V/Met	Amox	Amox/Met	Amox/Clav	Clind
1	2/6	6/6	2/6	0/6	0/6	0/6	0/6
2	1/7	6/7	1/7	2/7	1/7	0/7	0/7
3	2/6	3/6	0/6	2/6	0/6	0/6	0/6
4	2/10	8/10	1/10	1/10	0/10	0/10	0/10
5	0/8	2/8	0/8	0/8	0/8	0/8	0/8
6	1/9	4/9	0/9	1/9	0/9	0/9	1/9
7	0/9	4/9	0/9	0/9	0/9	0/9	0/9
8	0/7	0/7	0/7	0/7	0/7	0/7	0/7
9	1/8	3/8	0/8	1/8	0/8	0/8	0/8
10	2/8	8/8	2/8	1/8	0/8	0/8	1/8
11	1/10	5/10	0/10	0/10	0/10	0/10	1/10
12	3/10	5/10	1/10	1/10	0/10	0/10	1/10

Pen V = penicillin V; Met = metronidazole; Pen V/Met = penicillin V/metronidazole; Amox = amoxicillin; Amox/Met = amoxicillin/metronidazole; Amox/Clav = amoxicillin/clavulanate; and Clind = clindamycin.

clindamycin, and it is often recommended for the treatment of serious odontogenic infections when penicillin is contraindicated or in cases in which penicillin therapy has failed (16, 18, 20–23).

Metronidazole is a nitroimidazole compound, which was developed to treat protozoan infections but later was found to be effective against anaerobes. Metronidazole has been recommended for use in combined therapy with penicillin to treat odontogenic infections (16, 22). Of the antibiotics tested in this study, metronidazole had the greatest amount of bacterial resistance 54/98 (55%). Metronidazole is only active against anaerobes and should not be used alone for the treatment of endodontic infections, which also contain facultative bacteria. This may explain the high resistance of metronidazole in this study, because 33/98 (34%) was facultative bacteria. Metronidazole is only active when it is reduced to form an unstable intermediate that binds to microbial DNA and produces damage that prevents replication and transcription. In this study, metronidazole was effective against six strains of bacteria that exhibited resistance to penicillin V. If metronidazole were used in combination with penicillin V, the percentage of susceptible bacteria would increase from 85% to 93%. If metronidazole were used in combination with amoxicillin, the percentage of susceptible bacteria would increase from 92% to 99%. Thus, the combination of penicillin and metronidazole continues to have clinical efficacy even with the relatively high incidence of resistance to metronidazole.

Clarithromycin is a macrolide and analogue of erythromycin. It has been recommended as an alternative to erythromycin because it is effective against facultative and anaerobic bacteria, which are resistant to erythromycin (5, 21). In addition, food has no effect on the absorption of clarithromycin, and it only needs to be taken twice a day with less gastric upset than erythromycin. However, because the NCCLS has not established an MIC for clarithromycin, it is considered an investigational antibiotic. It is recom-

mended for the treatment of infections throughout the body, including the respiratory system (5).

Recent studies support our current practice of reserving antibiotic therapy for patients who have systemic signs and symptoms associated with an endodontic infection, patients with progressive infections, or patients who are immunocompromised (2, 24–28). Studies have shown that the prescription of penicillin or amoxicillin does not affect radiological healing or affect the rate of flare-ups for patients with chronic apical periodontitis (2, 27, 28). Studies also found that when patients receive appropriate local treatment, there is no significant difference between a placebo and penicillin prescribed for pain and localized swelling associated with teeth having a necrotic pulp (24, 25). In addition, the use of penicillin is not effective in reducing the symptoms or number of analgesic medications taken by patients for untreated irreversible pulpitis (26). It is also believed that the use of antibiotics to prevent posttreatment infections in healthy (nonimmunocompromised) patients is not medically or scientifically supported (16, 21–23, 29). The patient risk-benefit ratio must always be considered with the possibility of bacteria developing resistance and adverse drug reactions, including allergies, idiosyncratic reactions, and drug toxicity.

In conclusion, penicillin V seems to remain the antibiotic of choice because of its efficacy in polymicrobial infections, relatively narrow spectrum for bacteria found in endodontic infections, low toxicity, and low cost. Although metronidazole has relatively poor efficacy by itself, in combination with penicillin V the susceptibility of the bacteria in this study was virtually the same as amoxicillin. Amoxicillin and amoxicillin/clavulanate did have greater activity for the bacteria isolated in this study than penicillin V by itself. However, amoxicillin and amoxicillin/clavulanate have a wider spectrum of activity than penicillin V. This spectrum includes many species of bacteria found elsewhere in the body.

Amoxicillin and amoxicillin/clavulanate may increase the risk of selecting for resistant organisms outside of the oral cavity. Amoxicillin and amoxicillin/clavulanate are indicated for the treatment of immunocompromised patients, who may have odontogenic infections containing nonoral bacteria. Amoxicillin and amoxicillin/clavulanate may also be indicated for the most serious infections because of their more rapid and sustained plasma levels. Clindamycin remains an excellent alternative for patients allergic to the penicillins. Clarithromycin seems to be an alternative for erythromycin for mild infections when penicillin cannot be prescribed.

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