

Facultative gram-negative enteric rods in persistent periapical infections

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A bacteriological investigation has been made in two cases of persistent periapical infections. Neither of the two infections responded to root canal therapy including penicillin or penicillin and erythromycin. Samples were taken aseptically for bacteriological examination through the root canals. Chloroform-washed, sterile paper points were used for sampling. Cultivation was performed immediately at chairside on Kanamycin-Vancomycin laked blood agar (KVLB) and blood agar supplemented with menadione, cysteine and glucose for anaerobic incubation and chocolate agar for aerobic incubation. *Enterobacter cloacae* was only isolate in case 1; *Klebsiella pneumoniae* and enterococci were found in case 2. Obligately anaerobic bacteria were not found. The treatment was successful after antibiotic therapy based on susceptibility testing of the isolates. The route of infection by facultative enteric rods is discussed. □ *Microbiological examination; endodontics*

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Facultative gram-negative rods are only occasionally found in periapical infections. Kaufman & Henig (12) isolated them in 5% of periapical infections, but usually they have not been detected (3, 10, 11, 19, 22). Possibly because of their low frequency and the fact that they usually are in minority in mixed dental infections, little attention has been paid to this group of bacteria. However, there are a few reports of periapical infections, where *Klebsiella pneumoniae* or related strains have been isolated either as a single pathogen or together with other bacteria (5, 12, 13, 15, 25). *Enterobacter cloacae* appears to be a rare microorganism in dental infections, and there are no previous reports where *E. cloacae* has been the only pathogen.

In this paper we report two complicated endodontic cases, one with *E. cloacae* as the only pathogen isolated and a mixed infection yielding growth of *K. pneumoniae* and enterococci. In both cases it was only after selection of antimicrobials on the basis of bacterial susceptibility that the conventional root canal treatment was successful. The importance of facultative gram-negative microorganisms in periapical infections, the need for microbiological examination and antibiotic susceptibility testing are discussed.

Materials and methods

Bacteriological procedures

Specimens were obtained through opened root canals with chloroform-washed sterile paper points, applying the precautions presented by Möller (16) to avoid contamination, and cultured within 30 sec as follows. The specimens in the paper point were cultured on prereduced Kanamycin (75 µg/ml)–Vancomycin (7.5 µg/ml) laked blood agar plate (KVLB) (14) and blood agar plate supplemented with menadione (0.5 mg/l), cysteine (500 mg/l) and glucose (2 g/l). The plates were immediately placed in an anaerobic jar (BBL, Baltimore, USA) and incubated at 37°C. In addition, the specimens were also cultured on chocolate agar plates and incubated in an atmosphere of 5% CO₂ in air at 37°C. Finally, the paper point was inserted into thioglycollate broth.

The growth on chocolate agar was recorded on day 2, and the subcultures required for the identification and testing of antimicrobial sensitivity were performed. Anaerobic jars were opened after 7 days of culture and subcultured as above. Subcultures were tested also for aerotolerance. The identification of bacteria was based on the appearance of bacteria in gram-stained

smears, colony morphology and fermentation patterns (14). Antibiotic susceptibility of isolates was performed using the disc diffusion method (Rosco, Taastrup, Denmark) (1).

Root canal treatment

The mechanical preparation was based on the standardized technique described by Ingle (9). As an irrigant physiological saline (Orion Pharmaceuticals Ltd, Helsinki, Finland) was used. The canals were filled with either $\text{Ca}(\text{OH})_2$ paste or dexamethasonesodiumphosphate-dequanilinium acetate (Triodent®, Orion).

Results

Patient 1

In a routine roentgenological examination of a 32-year-old woman an inadequate root filling of d 25 was found. A week after the old root filling had been removed she complained of local tenderness. The root canal was opened and then filled with Triodent® and the patient was given phenoxymethylpenicillin, 10^6 units three times a day. As the symptoms continued, penicillin was replaced by erythromycin, 250 mg four times a day for 7 days, and the canal was left open overnight for drainage. No samples for microbiological examination were taken at this moment. On the fourth day of erythromycin treatment only a small hard periapical nodule was found. The patient had no pain and the canal was filled permanently with gut-tapercha. Three days later she had severe local pain with excessive pus formation. The filling was removed and the antibiotic therapy interrupted for two days before the microbiological specimens were taken.

The bacteriological cultures revealed no growth on KVLB-agar. *E. cloacae* was isolated as the only organism from both supplemented blood agar (anaerobic) and chocolate agar (aerobic) plates. The isolate was sensitive to ampicillin, tetracycline and sulfa-trimetoprim and resistant to penicillin and erythromycin. The patient was given ampicillin, 500 mg four times a day for 7

days. Tenderness and swelling were absent on day three and the canal was filled 10 days after the beginning of ampicillin therapy. When checked 3 months later the patient was symptomless and no local swelling or pain was observed.

Patient 2

A 27-year-old woman had been treated earlier for an acute pulpitis in d 26. At that time the pulp was extirpated, but the root canals were not filled. Five months later, when first examined at the Department of Cariology, she had local swelling and pain. The root canals were prepared and treated with Triodent®, but no antibiotics were given. She visited the clinic only irregularly, and as the symptoms continued the root canal medicament was renewed on every occasion. As the local symptoms persisted, she was given phenoxymethylpenicillin, 10^6 units three times a day for 7 days.

On the fourth day of the penicillin treatment the patient developed severe local pain, and microbiological samples were taken. *K. pneumoniae* and enterococci were obtained from all media, except KVLB-agar, which gave no growth. Both of the isolated strains were resistant to phenoxymethylpenicillin and erythromycin. Tetracycline and cephalosporine were effective only against *K. pneumoniae* and ampicillin only against enterococci. Sulfa-trimetoprim was effective against both bacteria, and sulfa-trimetoprim (400 mg + 80 mg) 2×2 for 10 days was started. All symptoms disappeared within 2 days. The root canals were filled 3 weeks after the sulfa-trimetoprim treatment was over. Late filling was performed because the patient failed to keep the appointments. Four months later she was symptomless and there were no radiological changes.

Discussion

The recent development of new microbiological techniques, especially of anaerobic culturing methods and improved identification of anaerobic bacteria, has opened a new era in the investigation and treatment of

odontogenic infections. Facultatively anaerobic bacteria constitute the minority of the flora reported to be present in root canals and in periapical infections. The presence of anaerobic bacteria of the genera *Bacteroides*, *Fusobacterium*, *Peptostreptococcus*, *Peptococcus*, *Veillonella*, *Eubacterium*, *Bifidobacterium* and *Actinomyces* in infected root canals has been reported (3, 10, 11, 16, 19, 22). Of facultatively anaerobic microorganisms, streptococci and lactobacilli are most frequently found (19, 22). The bacteria in infected root canals and in periapical infections are members of the normal oral flora, particularly of the dental plaque (21, 22). Anaerobes such as *Bacteroides fragilis* and *Clostridium* sp. are not usually found in the oral flora. On the other hand, *B. fragilis* has recently been isolated from severe orofacial infections involving fractures or large abscesses of the mandible (3, 10).

The reported frequency of facultative gram-negative enteric rods in specimens taken from infected root canals usually does not exceed 5% (5, 12, 15, 25). Whenever isolated these bacteria seem to be associated with persistent periapical infections (12, 15). Their route of invasion and whether they are primary or secondary invaders have not been discussed. Engström (4) isolated enterococci from root canal specimens in 9% of his cases and considered their presence to cause prolonged periods of treatment.

The search for pathogens in infected root canals has given valuable information of the correlation between clinical symptoms and the presence of certain bacteria, especially *B. melaninogenicus* (20, 22). From the clinician's point of view it would be even more important to know the relationship of the bacteriological findings to the prognosis. It is clear, however, that several other factors, such as root canal anatomy and host defence, in addition to the bacteria isolated, influence the prognosis of the patient.

Gram-negative facultative enteric rods are occasionally found in the oral flora. Their relative proportion may rise during prolonged antibiotic therapy and among the aged (18, 24). In addition, root canal irrigants or the dentist's hands can be contaminated and mediate the infection (17). In

hospitals, infections caused by gram-negative rods can spread via infusions or local disinfectants. The irrigants and local anti-inflammatory agents used in these two cases were, however, found to be sterile.

The absence of anaerobic bacteria could be explained by prior penicillin therapy, as these bacteria generally are penicillin-sensitive (2, 23). The resistance of gram-negative facultative enteric rods is known to be based on the poor penetration of the drug through the bacterial cell wall rather than on betalactamase production, which, if present, could also defend penicillin-sensitive bacteria (8). Moreover, penicillin may have offered gram-negative facultative enteric rods and enterococci a possibility to take over, if present in minute amounts prior to the treatment, or it may have allowed their secondary invasion (6). Using the same procedure when taking samples for bacterial culture, we constantly find 2–10 anaerobic and 0–2 aerobic species in specimens from infected root canals without previous antimicrobial therapy (7). Frequently isolated anaerobic strains include such highly oxygen- and penicillin-sensitive organisms as *B. melaninogenicus* and *F. nucleatum*.

Epidemiological and controlled clinical follow-up studies are needed to clarify the suggested relevance of different bacterial species in root canal infections (3–6, 10, 12, 15, 20, 22). In persisting infections, isolation of bacteria and testing their sensitivity to antimicrobial agents may be useful to the clinician, helping him to choose adequate treatment.

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