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Microflora of root filled teeth with apical periodontitis in Latvian patients

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SUMMARY

Objective. The aim of the present study was to investigate the microbial flora of root filled teeth with apical periodontitis and to determine the prevalence of β-lactamase producing strains in isolated bacteria in Latvian patients.

Materials and methods. 33 root filled teeth with asymptomatic persisting periapical lesions were selected for the present study. During nonsurgical endodontic retreatment, the root filling material was removed and canals were sampled. Determination of microbial species was based on series of biochemical tests using identification kits. All strains of bacteria were tested for β-lactamase production by using chromogenic nitrocefin-impregnated slides.

Results. Bacteria were found in 32 (97%) of initial specimens from the teeth. The number of isolated microbial strains in the specimens ranged from one to six (mean 2.7). 79% of the isolated microbial species were Gram-positive bacteria. The most common isolates were *Streptococcus* (27%), *Actinomyces* (27%), *Staphylococcus* (18%), *Enterococcus* (18%) and *Lactobacillus* (18%) spp. Yeasts were found as four isolates in 3 cases (9%). β-lactamase-producing bacterial strains were detected in 12 specimens, 36% of the patients. The most common enzyme-producing bacteria belonged to *Actinomyces* and *Staphylococcus* spp.

Conclusions. The microbial flora in previously treated root canals with apical periodontitis is limited to a small number of predominantly Gram-positive microbial species. The most common isolates are *Streptococcus, Actinomyces, Staphylococcus, Enterococcus and Lactobacillus* spp. A moderately high prevalence of β-lactamase producing bacterial strains was detected in patients with root filled teeth with apical periodontitis.

Key words: apical periodontitis, microflora, root fillings, β-lactamase.

INTRODUCTION

Controlled clinical studies have demonstrated that successful endodontic treatment may be obtained in over 90% of treated cases while results from epidemiological studies indicate success-rates from 35 to 78% (1). The presence of microorganisms in the root canal system is considered to be the major cause of periapical pathology and treatment failure (2).

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Studies indicate differences in the composition of the flora in retreatment cases compared with primary necrotic cases (3, 4). The microbiota associated with persistent secondary infections is usually composed of a single species or at least by a low number of species. Gram-positive bacteria are predominant (3-6) and *Enterococcus faecalis* is frequently isolated from retreatment cases (6, 7).

Previously treated teeth with persistent periapical lesions might be preserved with nonsurgical retreatment or endodontic surgery. Evidence-based dentistry recommends selection of alternate treatment options on the basis of the best available evidence (8). The outcome of retreatment of teeth with apical periodontitis is inferior to the success rate of primary treatment (9, 10). Systematic review of nonsurgical endodontic retreatment results demonstrates success rate from 70.9% to 83.0% depending on the duration of observation period (11). This lower

prognosis in root canal retreatment cases may be associated with difficulties in the elimination of the specific microflora (3, 12). Microorganisms may persist in the apical part of a root canal, in invaginations and lateral canals. In addition, the environmental conditions and nutritive conditions in retreated root canals differ from those in untreated cases.

Microorganisms exist under conditions of starvation in filled root canals. To survive under such conditions, bacteria activate complex mechanisms of molecular regulations (13, 14). Such factors as dentine buffer capacity, bacterial invasion into dentine and biofilm also contribute to difficulties of infection elimination. The biofilm structure on infected root canal walls is identified by means of transmission electron microscopy (15). Microorganisms are also discovered in the biofilm on the apical surface of endodontically treated teeth (16). In the biofilm, microorganisms show resistance to antimicrobial agents up to 1000 times higher than in the form of planctonic cells (17).

β-lactam antibiotics are the antimicrobial agents most commonly used in treatment of many infectious diseases (18). Bacterial resistance to the antibacterial agents has been a clinically significant problem for over 40 years. Development of resistance is often complex and may involve a variety of bacterial adaptations, including barriers to antibiotic penetration, alteration of drug-target sites and inactivation of antimicrobial agents. The major mechanism of β-lactam resistance is bacterial production of β-lactamase (19). β-lactamase is a group of enzymes that catalyze the hydrolysis of the beta-lactam ring of the antibiotics yielding inactive products. Antibiotic resistance is the result of genetic changes in the microbe, either by mutation or genetic transfer. Although genes for bacterial resistance may have existed before the clinical use of antimicrobial agents, selection of new resistant strains is driven by the wide-spread use of antimicrobial agents (20).

Evidence exists that antibiotic resistance has increased in the oral microflora over the last 10-20 years. The prevalence of β-lactamase producing microorganisms has been examined in different ecological niches of the oral cavity. Studies conducted 25 years ago demonstrate low levels of β-lactamase producing bacteria (21, 22). During the last 10-15 years, a high level of β-lactamase producing bacteria (53-74%) has been discovered in periodontal pathology (23, 24) while 38.5 % is discovered in samples of acute purulent infections of the oral cavity (25). Different microbial species, especially Gram-negative microorganisms, have become resistant to penicillin (22, 26). Recent use of β-lactam antibiotics has a tendency to increase the emergence of β-lactamase-producing bacteria (27, 28).

The aim of the present study is to investigate the microbial flora of root filled teeth with apical periodontitis in Latvian patients and to determine the prevalence of β-lactamase producing bacteria associated with such lesions.

MATERIALS AND METHODS

Clinical material

33 patients in need of non-surgical endodontic retreatment were selected from those who attended the Endodontic Department at the Institute of Stomatology of Riga Stradins University or an extensive private dental clinic (ARK) in Riga, Latvia.

Criteria of inclusion: 1) radiolucency seen on radiographs (PAI = 4 and 5) (29) at the apex of endodontically treated teeth, 2) improper quality of root canal filling (voids, pores, distance from the filling depth to the radiological apex equals 0-5 mm), 3) time from previous endodontic treatment more than 4 years. Criteria of exclusion: 1) antibiotic treatment during last 3 months, 2) general diseases, 3) signs of acute periapical pathology, 4) teeth having sinus tracts, 5) teeth with temporary filling or with missing restoration.

Information on the patients' age, sex, time passed from previous canal treatment, distance from the filling depth to radiological apex, filling materials, use of antibiotics etc. were obtained by a clinical investigation and a questionnaire.

Endodontic retreatment and bacterial sampling

One specialist carried out retreatment (AM) both at the Endodontic Department of the Institute of Stomatology of Riga Stradins University and at the ARK clinic. The treatment was performed under aseptic conditions. After preparation of access cavities, the teeth were isolated with rubber dam and disinfected with 5.25% NaOCl solution. The solution was inactivated with 5% sodium thiosulphate. After opening of the root canal entrance the type of filling material was estimated and appropriate method of removal chosen. In order to collect samples from different parts of root canal after partial removal of the filling material and rinsing with sterile physiological saline solution, the first microbiological sample was taken. One sterile paper point was introduced into canal and left for 1 minute. Removal of root canal filling was continued without any antibacterial treatment. Sterile files were used to remove the last part of previous root filling materials and repeated samplings using one sterile paper point were carried out from

the apical parts. Before microbiological sampling, the root canals were treated with endodontic files to generate dentine shavings. The paper points were immediately placed in transportable tubes (Port-A-cul-tube, Becton Dickinson, USA). After microbiological sampling, the removal of the filling was finished using solvents, rinsing agents (2.5% NaOCl solution, 2% chlorhexidine solution) and lubricants. In all cases the complete root canal preparation was carried out during one session.

Microbiological procedures

Low nutrient medium R2A (LABMTM) and sheep blood agar (National Diagnostic Centre, Latvia) culture media were used for cultivation of microorganisms. Samples were incubated under aerobic as well as anaerobic conditions in CO2 atmosphere (BBL Gas PakTM, Becton Dickinson, USA) and 37°C for up to 14 days. Morphology of grown microorganisms was studied by means of a microscope (Leica, BME, 40×15 magnifications). To identify the pure cultures of microorganisms, test systems (BBL, CrystalTM, Becton Dickinson, USA) with Gram-positive, Enteric/Nonfermenter and Anaerobe identification kits were used. In addition, Gram-staining as well as indole, catalase and oxidase reactions were carried out. All strains of bacteria were tested for β-lactamase production by using chromogenic nitrocefin-impregnated slides. The bacterial pure culture was placed onto a Nitrocefin slide (Dry SlideTM Nitrocefin, Becton Dickinson, USA). In case of β-lactamase production, change of color from yellow to red was manifested. Yeasts were identified according to micromorphologic signs using Fungiscreen 4H (Sanofi Diagnostics Pasteur, France).

RESULTS

Microorganisms were isolated in 32 cases (97%) from 33 teeth included in the project. From 1 to 6 species (mean 2.7) were isolated from each sample. In one sample microorganisms were not detected, in 6 cases monoinfection was found, 2 species were isolated in 13 cases and 3 species were isolated in 3 cases. Four or more species were isolated in 10 cases. Streptococcus intermedius, Bacteroides caccae, Streptococcus constellatus, Streptococcus intermedius, Esherichia coli and Actinomyces viscosus were isolated as monoinfection. Eighty-five isolates pertained to 29 genera of microorganisms. The main parts of identified bacteria (79%) were Gram-positive. Facultative anaerobes were found in 47 isolates (56%) including yeasts, obligate anaerobes in 25 isolates

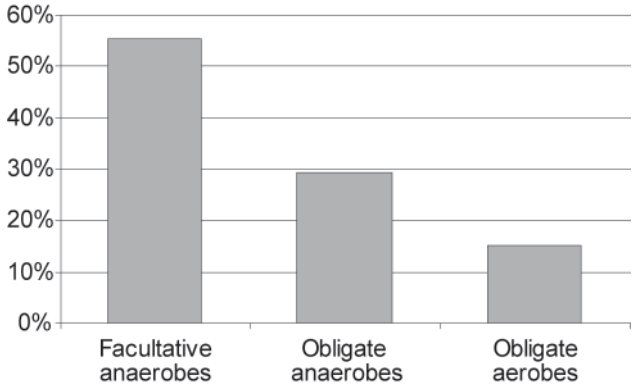

Fig. 1. Distribution of isolated microorganisms by aerotolerance

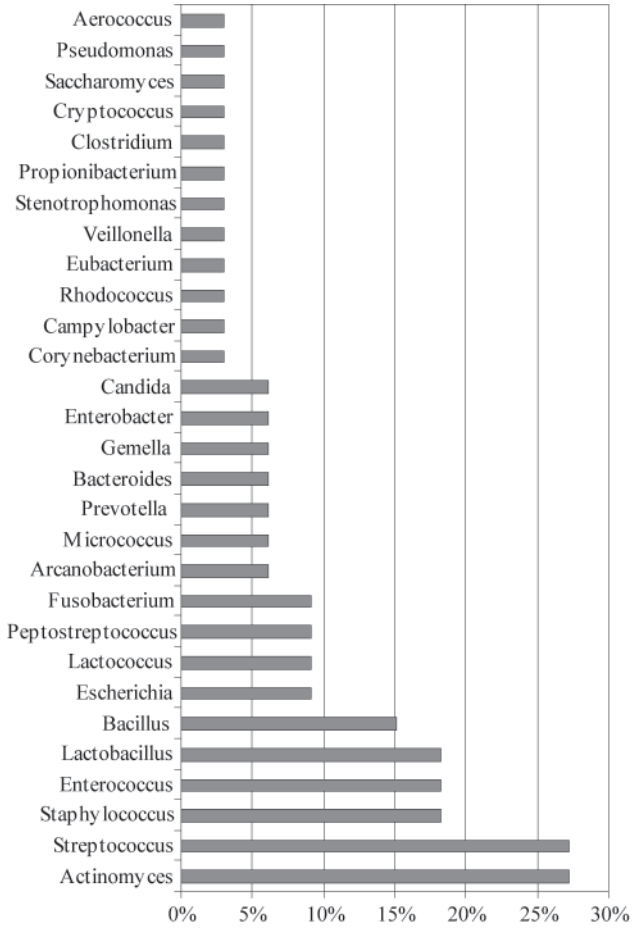

Fig. 2. Prevalence of isolated microorganisms in root filled teeth with apical periodontitis

(29%) and obligate aerobes were found in 13 isolates (15%) (Figure 1).

More frequently isolated microorganisms were *Streptococcus* (27%), *Actinomyces* (27%), *Staphylococcus* (18%), *Enterococcus* (18%) and *Lactobacillus* (18%) *spp. Enterococcus faecalis* was isolated in 5 cases (16%). *Enterococcus faecalis* was the single species belonging *Enterococcus spp.* Yeasts were found as four isolates in 3 cases (9%). Two species of yeasts were found in one root canal sample. *Candida albicans* was isolated in 2 cases. Other isolates

Table. Identity and prevalence of β-lactamase producing bacterial strains in patients with root filled teeth with apical periodontitis

Bacterial strains	No. of samples
Actinomyces odontolyticus	1
Actinomyces israelii	1
Actinomyces viscosus	2
Bacillus licheniformis	1
Bacteroides caccae	1
Corynebacterium aquaticum	1
Enterobacter cloacae	2
Esherichia coli	2
Propionibacterium avidum	1
Rhodococcus equi	1
Staphylococcus capitis	1
Staphylococcus epidermidis	1
Staphylococcus lentus	1

belonged to *Saccharomyces* and *Cryptococcus spp.* (Figure 2).

β-lactamase producing strains were discovered in 12 of the 32 patients with cultivable bacterial populations. Four patients had all isolated microorganisms as β-lactamase producing ones. Identity and prevalence of β-lactamase producing bacterial strains were determined in patients with cultivable microflora (Table 1). Thirteen β-lactamase producing microbial strains were found in the retreated root canals. Most isolated β-lactamase producing microorganisms were Actinomyces and Staphylococcus species.

DISCUSSION

Microorganisms were found in 32 cases (97%) from 33 teeth involved in the study. In similar studies microorganisms were isolated in 44% of the sampled cases by Sundqvist (4), 73% by Molander (3) and in 80% and 83% by Peciuliene (6, 30). The high recovery rate of intracanal bacteria might be explained by the sampling techniques used. Following suggestions dentin shavings were made in apical part of the root canal and were not rinsed out before sampling (3, 31). Absence of microorganisms observed in one case does not mean the canal has not been infected. Cultivability of microorganisms could be lost in the processes of sampling, transporting and laboratory operations, particularly it could result from their small quantity or they might be located in inaccessible parts of the root canal system. It could also be an infection of non-cultivable bacteria (32).

The obtained results demonstrate that monoinfection was isolated in 19% of the cases, 2 species were isolated in 41% of the cases and polymicrobial infection with 3 and more species was discovered in 41% of the cases. The present data differ from the ones obtained in other studies. In Scandinavian studies, monoinfection was discovered in 79% from positively cultivated teeth (4) or presence of 1 to 2 species in 85% teeth (3). In research conducted in the USA, 1 or 2 species were discovered in 85% of the cases if paper points had been used for sampling and in 89% of the cases if endodontic files had been used (33). In a study using polymerase chain reaction-based analysis the mean number of species in samples filled up to 2 mm short of the radiographic apex was 3, but cases in which the filling was more than 2 mm from apex yielded a mean of 5 species (34). The studies also discovered the connection to presence of polymicrobial infection due to improper root canal filling (7) and to poor quality of restoration (35). The different mean number of isolated microbial species may be explained by variation in inclusion criteria, definition of retreatment cases and differences of retreatment procedures in the studies.

The strong definition of "true retreatment cases" is stated in a Scandinavian study by Molander (3). To be included in the study, teeth were required to have been root canal treated more than 4 years previously. According to Strindberg criteria (36) healing cannot be expected. Also teeth have been without clinical signs of acute pathology and root-fillings should end within 5 mm of the radiographic apex. But chloroform had to be used to soften gutta-percha and this had a statistically significant impact on the recovery of intracanal bacteria (3).

From bacteria isolated in the present study 56% were facultative anaerobes and 79% were Gram-positive species. These indicators are similar to the discovered ones in previous studies; for example, Sundqvist et al. (4) and Molander et al. (3). They discovered 58% and 69% facultative anaerobes and 87% and 74% Gram-positive bacteria respectively. In the USA research 80% were Gram-positive bacteria (37), in Brazil 57% facultative anaerobes and 83% Gram-positive species (7) were detected. In the present research isolated facultative anaerobes pertain to *Streptococcus*, *Actinomyces*, *Enterococcus*, *Lactobacillus* and *Staphylococcus* genera most at all. In similar studies, *Streptococcus*, *Actinomyces* and *Enterococcus spp.* were more frequently isolated (4, 7).

Prevalence of *Enterococcus faecalis* (16%) in teeth with cultivable bacteria is lower than in other studies. *E. faecalis* is isolated from 22% up till 77% from rootfilled teeth with apical periodontitis (3, 4, 6, 7, 38). In our study *E. faecalis* was not isolated as a monoinfection in any case. In another study *E.*

faecalis as the single species was discovered in 18 (67%) out of 27 cases (7). In Lithuanian study *E. faecalis* was isolated as monoinfection in 11 teeth (33%) from 33 culture positive teeth (30). It is hypothesized that *E. faecalis* enters the root canal system during or after treatment (39). Depending on the coronal restoration, filled root canals may invariably have been exposed to the oral cavity. The different prevalence of *E. faecalis* might be explained by strong criteria for inclusion in the study, only teeth having permanent restoration were included.

β-lactamase producing microorganisms were found in 36% of the patients with cultivable bacteria. Similar prevalence of β-lactamase producing microorganisms (38%) was found in the samples of acute purulent infection in a study in Great Britain (25). More often β-lactamase producing bacteria (72%) were found in the bacterial population of refractory marginal periodontitis (40). β-lactamase producing microorganisms are potentially resistant to antibiotics. Although systemic antibiotics are not used in retreatment of teeth with apical periodontitis, the design of the study was used to collect information about antimicrobial resistance of the oral microflora in Latvian patients.

It is necessary to compare results with the data obtained in other studies analyzing prevalence of β-lactamase producing bacteria and antimicrobial resistance in root canal infection. There is an agreement that further information still is needed to get a thorough knowledge about residual post-treatment root infection and post-treatment apical periodontitis in order to improve quality of root canal treatment procedures (41).

CONCLUSIONS

The microbial flora in previously treated root canals with apical periodontitis is limited to a small number of predominantly Gram-positive bacterial species. The most common isolates are *Streptococcus*, *Actinomyces*, *Staphylococcus*, *Enterococcus*, and *Lactobacillus spp.* A moderately high prevalence of β-lactamase producing microorganisms is detected in patients with root filled teeth with apical periodontitis.

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