DOI: 10.4149/BLL_2013_151

Bratisl Lek Listy 2013; 114 (12)
716 – 720

CLINICAL STUDY

Enterococcus faecalis and *Candida albicans* in the dental root canal and periapical infections

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Abstract: *Objective:* The aim of the present study was to examine the prevalence of *Enterococcus faecalis* and *Candida albicans* in endodontic infections.
Methods: Samples for microbiological examination were collected from 32 patients with deep dental caries, infected dental root canal, or periapical infection.
Results: Cultivation of the dental samples yielded four strains of *Enterococcus faecalis* (12.5 %), and three strains of *Candida albicans* (9.4 %). All *Enterococcus faecalis* isolates were susceptible to ampicillin, one isolate was resistant to tetracycline, two to erythromycin and azithromycin (additional 2 had intermediate susceptibility), and one strain had intermediate susceptibility to ciprofloxacin and moxifloxacin.
Conclusion: We conclude that *Enterococcus faecalis* and *Candida albicans* can participate in the dental root canal and periapical infections, and the use of effective irrigant solutions and intracanal medicaments active against these microbes is important in order to prevent endodontic therapy failures. Unexpected was the isolation of *C. albicans* from a nine-year-old child with periodontitis apicalis. This finding must draw attention to the possibility that even at such a young age, this microorganism could be a potential etiological agent in endodontic infections *(Tab. 2, Ref. 34)*. Text in PDF *www.elis.sk.*
Key words: *Enterococcus faecalis*, *Candida albicans*, endodontic therapy, endodontic infections, endodontic microbiology.

Facultatively anaerobic bacterium *Enterococcus faecalis* and diploid fungus *Candida albicans* are considered by many to be the most resistant species in the oral cavity, and a possible cause of root canal treatment failure (1).

Enterococci belong to natural inhabitants of intestinal and genital mucosa of humans. On the other hand, they have been recognized as potentially pathogenic for humans since the turn of the last century, and they are also isolated from infections of the dental root canal system (2, 3, 4). The source of enterococcal dental infection is probably exogenous, as they are only transient colonizers of the oral cavity, but with an ability to adhere to and survive in the dental plaque, from where they can invade the damaged dental root spaces (5, 6, 7). Enterococci make up only a small proportion of the initial dental root canal flora, which is dominated by anaerobic Gram-negative species, but they are frequently isolated from obturated root canals of teeth with chronic periapical pathological processes (8, 9). The most frequent *Enterococcus* species isolated from infected root canals, apical periodontitis and periradicular abscesses is *Enterococcus faecalis* (10), which is commonly the only species recovered from the obturated root canal. Several virulence factors of *E. faecalis* have already been recognized to facilitate the colonization of dentin and necrotic or improperly filled dental root canal, as well as to stimulate the inflammatory response with potential damage to periapical tissues (11, 12). Enterococcal biocines with their suppressing activity towards the other root canal colonizers, together with the ability of enterococci to survive long-term starvation, as well as with their natural resistance to several antimicrobial drugs including lincosamides, may result in their selection in this special locality, and consequential development of infection (11, 13). The high abilities of enterococci to adapt, survive and persist in a variety of adverse environments without the support of other bacteria (e.g., in root canals, where nutrients are scarce and means of escape from root canal medicaments are limited), as well as to resist many kinds of intracanal medicaments, support the hypothesis about their pathogenic role in chronic endodontic treatment failure (8, 10).

Candida albicans is the most commonly isolated fungus from the oral cavity of both healthy and medically compromised individuals. The frequency of oral *C. albicans* varies from 47 % to 75 %, while *C. krusei*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata* and *C. guilliermondii* represent less than 10 % of yeast species isolated from the mouth (14, 15). The dorsum of the tongue is the

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Acknowledgments: The study was supported by the Comenius University Research Grant No. UK/432/2009. The authors declare that they have no conflict of interests.

primary habitat of the yeasts in the mouth. Nevertheless, these microorganisms can be found at other oral sites such as the jugal mucous membrane (cheek mucous membrane), palate mucosa, tooth surfaces and saliva (16).

The colonization rate increases with age, as well as with denture wearing. As opportunistic oral pathogens, *Candida* species are responsible for mucosal infections in predisposed patients (17). The occurrence of fungi in root canal infections has been reported by culture, molecular methods and electron microscopy *in situ*. In addition, recent studies have confirmed the presence of fungi in therapy-resistant endodontic infections (18).

Microbiological investigations have shown that yeasts may be present in the microflora of apical periodontitis. They may enter the pulp through dentinal tubules, deep caries lesion or fracture or as contaminants from oral microflora during root canal treatment. Natural factors such as ecological changes in an infected root canal, as well as iatrogenic factors such as the use of canal medicaments allowing yeasts to survive whilst suppressing other microorganisms, may operate in favor of root canal yeast infection. Almost all yeasts isolated from these spaces belong to the genus of *Candida* with *C. albicans* being the most frequently isolated species (19).

The isolation of yeasts from dental root canal of patients with necrotic pulp and persistent endodontic infections, frequently in pure culture, indicates that also these microbes may play a role of an important endodontic pathogen, with *C. albicans* being the leading species (20, 21).

The aim of the present study was to examine the prevalence of *Enterococcus faecalis* and *Candida albicans* in endodontic infections of patients of the Department of Stomatology and Maxilofacial Surgery, Faculty of Medicine, Comenius University, and the St. Elisabeth's Hospital, Bratislava, Slovakia.

Material and methods

Biological samples

Samples for microbiological examination were collected from 32 patients with deep dental caries, infected dental root canal, or periapical infection (Tab. 1). The affected hard dental tissue was collected by manual excavator, and the infected content of the root canals was sampled by pulpextractor and root canal instrument Hedstroem File (VDW GmbH, Germany). In the cases with clinical status and radiographic examination resulting in the necessity of tooth extraction, the whole tooth was sent for examination. All samples were placed into nutrient broth (Imuna Pharm, Slovakia) and immediately transported to the microbiological laboratory.

Cultivation and identification of Enterococcus faecalis and Candida albicans strains

The cultivation of samples was performed in nutrient broth for 24 hours at 35 ºC in ambient atmosphere and subcultured on blood agar and on Sabouraud dextrose agar pH 5.6±0.2 (Oxoid, Great Britain), at the same conditions for 24 and 48 hours, respectively. The suspected enterococcal strains were preliminarily identified according to the standard diagnostic methods (22), and their species-level identification was performed using commercial biochemical identification set ENCOCCUS test (Erba Lachema, Czech Republic). The isolated yeast strains were submitted to identification according to their growth properties on BBL CHROMAGAR Candida (BD, France), cultivated for 48 hours at 30±2 ºC according to germ-tube test results.

Tab. 1. Aerobic culture results of patients with dental infections.

Patients	Age	Diagnosis	Aerobic culture results
1	38	Pulpitis	Viridans streptococci
2	7	Dentoalveolar abscess*	Viridans streptococci
3	31	Periodontitis apicalis	Viridans streptococci
4	32	Dentoalveolar abscess*	Viridans streptococci
5	40	Dentoalveolar abscess*	Viridans streptococci
6	37	Periodontitis apicalis	Negative
7	9	Periodontitis apicalis	Viridans streptococci, Candida albicans
8	32	Caries dentis profunda	Candida albicans
9	43	Periodontitis apicalis	Enterococcus faecalis, viridans streptococci
10	16	Dentoalveolar abscess*	Viridans streptococci
11	49	Periodontitis apicalis	Enterococcus faecalis, Gram-negative rods, Neisseria sp.
12	72	Periodontitis apicalis	Staphylococcus sp. cogulase-negative
13	72	Periodontitis apicalis	Staphylococcus sp. cogulase-negative
14	57	Dentoalveolar abscess*	Staphylococcus sp. cogulase-negative
15	66	Dentoalveolar abscess*	Viridans streptococci
16	57	Periodontitis apicalis	Viridans streptococci
17	63	Periodontitis apicalis	Viridans streptococci
18	39	Dentoalveolar abscess*	Viridans streptococci
19	77	Dentoalveolar abscess*	Viridans streptococci
20	51	Periodontitis apicalis	Viridans streptococci
21	28	Periodontitis apicalis	Negative
22	33	Periodontitis apicalis	Candida albicans
23	36	Dentoalveolar abscess*	Staphylococcus sp. cogulase-negative
24	40	Periodontitis apicalis	Viridans streptococci
25	28	Periodontitis apicalis	Negative
26	10	Periodontitis apicalis	Negative
27	17	Periodontitis apicalis	Viridans streptococci
28	62	Periodontitis apicalis	Viridans streptococci
29	23	Periodontitis apicalis	Enterococcus faecalis, viridans streptococci
30	24	Periodontitis apicalis	Enterococcus faecalis, viridans streptococci
31	27	Periodontitis apicalis	Viridans streptococci
32	45	Periodontitis apicalis	Enterococcus dispar, viridans streptococci

* Tooth extraction, when the clinical state made the conservative preservation impossible

Antimicrobial susceptibility testing

The susceptibility of enterococcal strains to ampicillin, amoxicillin-clavulanic acid, erythromycin, azithromycin, ciprofloxacin, moxifloxacin, tetracycline, gentamicin, chloramphenicol, and vancomycin, as well as the susceptibility of the isolated *Candida albicans* strains toward amphotericin-B, nystatin, clotrimazole, fluconazole, itraconazole, and voriconazole were performed by

Tab. 2. Antimicrobial susceptibility of *Enterococcus faecalis* strains.

The tested drug	Enterococcus faecalis strains			
	1	2	3	4
Ampicillin	S	S	S	S
Co-amoxicillin	S	S	S	S
Tetracycline	S	S	S	R
Chloramphenicol	S	S	S	S
Erythromycin	I	I	R	R
Azithromycin	I	I	R	R
Ciprofloxacin	S	S	S	I
Moxifloxacin	S	S	S	I
Gentamicin (HL)	S	S	S	S
Vancomycin	S	S	S	S

S – susceptible, R – resistant, I – intermediately susceptible, HL – high level

the disk-diffusion method according to CLSI recommendations (23, 24, 25).

Results

Aerobic cultivation of 32 dental samples (11 from male and 21 from female patients) yielded only four *Enterococcus faecalis* strains (12.5 %), two from middle-aged males, and two from young females suffering from periodontitis apicalis. In three cases, viridans streptococci were isolated together with *E. faecalis* strain. In the forth case, gram-negative rods and *Neisseria sp*. were isolated as well (Tab. 1).

All *E. faecalis* isolates were susceptible to ampicillin, which is the drug of choice for patients with non-life-threatening enterococcal infections. One isolate was resistant to tetracycline, two to erythromycin and azithromycin (additional two had intermediate susceptibility), and one strain had intermediate susceptibility to ciprofloxacin and moxifloxacin. There was detected neither vancomycin-resistant, nor high-level gentamicin-resistant strain (Tab. 2).

From the examined samples, only three *Candida albicans* strains were isolated (9.4 %); one from a nine-year-old child with periodontitis apicalis (together with viridians streptococci), and two from young adults with periodontitis apicalis, and with deep dental caries (both in pure culture) (Tab. 1). All three strains were susceptible to all of the tested antifungal agents.

Discussion

Root canals of infected teeth have a complex microbial flora consisting of anaerobic or facultatively anaerobic cocci and rods, anaerobic fusiform bacteria, spirochaetes, and sometimes fungi. All of them come from the oral microflora and may contribute to endodontic infections (2, 18). However, only few of them have a remarkable potential to survive adverse conditions during root canal treatment, which leads to eventual treatment failure. As *Enterococcus faecalis* and *Candida albicans* seem to be able to resist various endodontic treatment procedures and survive in the nutrient-deficient environment of a treated dental root canal (13), the study was focused on detecting these microbes in the samples of patients suffering from endodontic infections. No anaerobic cultivation was performed in spite of the important role of oral anaerobic bacteria in the dental infections pathogenesis, as they are highly susceptible to a properly performed endodontic treatment (17).

The common recovery of *E. faecalis* from the root canals of teeth in which previous treatment has failed is notable; this microbe is frequently isolated from secondary infections of root canal space with periapical pathology (9). In root canals of patients with therapy-resistant endodontic infections, Fouad et al (26) detected *E. faecalis* strains with 22 % prevalence, which approximately corresponds to our prevalence results, namely 12.5 %. The smaller percentage of *E. faecalis* positivity in our study may reflect the fact, that not only patients with secondary root canal infections were included in our study.

Enterococci are resistant to many antimicrobial drugs used in dentistry. The intrinsic resistance of enterococci to lincosamides or cephalosporins may be combined with an acquired resistance mechanisms to penicillins, tetracyclines, chloramphenicol, glycopeptides, or high-level resistance to aminoglycosides. This allows them to survive in an environment in which antimicrobial agents are used (10). In periodontitis refractory to conventional treatment, an increased prevalence of bacteria resistant to antibiotics may be found. However, *E. faecalis* strains isolated in the present study were highly susceptible to the tested antimicrobial drugs, which can be explained by lack of previous antimicrobial therapy in patients with positive cultures. In all cases, ampicillin which is the drug of first choice for non-invasive enterococcal infections could be used. Tetracycline, another commonly used drug in the dentistry, would be effective in all but one case.

Candida is another refractory microbe isolated from patients with primary or secondary endodontic infections. Miranda and coworkers (20) reported isolation of yeasts from necrotic root canals associated with primary apical periodontitis with a 22.6 % frequency, in comparison with the 45 % incidence of yeasts on the tongue dorsa of the same group of patients. *Candida albicans* was the most frequently isolated species at both investigated sites but other species were also found. The 9.4 % frequency of yeasts isolation from endodontic infections of patients in our study corresponds more closely to the findings of Waltimo et al (27) who detected Candida in 7–18 % of infected root canals, being commonly associated with persistent cases of apical periodontitis. *Candida albicans* was the only species isolated in our study, which is consistent with results of other studies identifying *C. albicans* as the most common yeast isolated from root canals in both primary and secondary endodontic infections (20, 21, 27, 28, 29, 30).

Interesting, yet unexpected, was the isolation of *C. albicans* from a nine-year-old child with periodontitis apicalis, as in children, the oral cavity is scarcely colonized by this microorganism. This finding must draw attention to the possibility that even at such a young age, this microorganism could be a potential etiological agent in endodontic infections.

Antifungal drugs are generally not used in endodontic infections therapy; therefore, no antifungals-resistant strains were expected. Consistently, all isolated *C. albicans* strains in our study

were highly susceptible to the tested antimycotic drugs used in clinical practice.

The main goal of root canal treatment is based on removing the irritants from infected root canals both mechanically and chemically, and then obstructing the root canal system to eliminate or reduce the microorganisms, as well as on preventing the reinfection. Residual microbes of the root canal system are the major cause of apical periodontitis persisting after either poor or proper treatment (31). However, a complete elimination of microorganisms is not always achieved in clinical practice due to anatomical complexities of root canals and consequent limitations in accessibility by using instruments and irrigators, as well as due to the fact that the use of antimicrobial medication has been advocated to disinfect the root canal system whilst root tissues are closely related to the periodontal apparatus (32). Therefore, it is important to consider the possible presence of *E. faecalis* and *C. albicans* in selection of the most appropriate agents with reliable activity also against these two microbes.

Additional important aspect of endodontic infections caused by *E. faecalis* and *C. albicans* is their potential role in infective endocarditis (33). Both microbes, surviving in root canal system and spreading to the periodontal tissues, may under certain circumstances invade the bloodstream and cause infections of endocardium in predisposed patients (34). Moreover, this fact should be taken in account when planning the antimicrobial prophylaxis during dental invasive procedures in patients at risk (those with cardiac deformities, artificial devices in the circulatory system, and those suffering from immunocompromising conditions).

Conclusions

The present study underscores the possible role of two of the most refractory microbes in oral cavity, *Enterococcus faecalis* and *Candida albicans*, in the etiopathogenesis of endodontic infections. Even if they were not isolated with very high frequencies (12.5 % and 9.4 %, respectively), during the therapy of endodontic infections it is important to take into account their possible presence in the infected teeth structures. The isolation of *C. albicans* from the sample of a nine-year-old child is evidence that the possible role of Candida in endodontic infection should be considered also in children.

The primary goal of endodontic therapy is to reduce significantly or eliminate microorganisms and their by-products from the root canal system. As both examined microorganisms tend to be resistant to many environmental factors and antimicrobial agents, it is important to pay attention to their possible presence in the treated root canal, and to use effective irrigant solutions and intracanal medicaments in order to prevent endodontic therapy failure due to *E. faecalis* or *C. albicans*.

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Received February 7, 2012.
Accepted September 20, 2013.