Prevalence, Biofilm Forming Ability and Antifungal Susceptibility of Candida Species in Primary Root Canal Infections

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Abstract

This study aimed to identify the prevalence of Candida species in primary endodontic infections in addition to its biofilm forming ability and antifungal susceptibility.

After consenting of 100 patients, microbiological samples were obtained using paper points from the main canal in single-rooted teeth and largest canal or canal with periapical radiolucency in multi-rooted teeth with primary endodontic infections. Samples were cultured onto Sabouraud's dextrose agar with chloramphenicol and CAN2 chromogenic agar plates and tested for culture and sensitivity. Congo red agar plate was used to test for biofilm formation. Antifungal susceptibility testing was then performed against Amphotericin B, Fluconazole, Voriconazole, Caspofungin, Micafungin, and Flucytosine to measure the minimal inhibitory concentration by the automated VITEK-2 System for yeast according to CLSI.

Candida species was present in 15% of the patients. Candida albicans showed significantly higher biofilm forming ability than candida tropicalis. Candida albicans and candida tropicalis were susceptible to all antifungal drugs tested. Candida Krusie was resistant to fluconazole.

C. albicans might play a role in primary endodontic infection and intracanal biofilm formation.

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Introduction

Success of endodontic therapy depends mainly on microorganisms elimination from the root canal system [1]. Caries is considered the major rout of entry of microorganisms to the root canal system [2]. Different fungal species have been isolated from secondary endodontic infections [3]. *Candida albicans* is the most frequently isolated species from secondary endodontic infections. *Candida albicans* has been shown to play a significant role in endodontic treatment failure [4,5,6].

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Unlike *Candida glabrata*, *Candida tropicalis*, and *Candida parapsilosis*, *Candida albicans* has the ability to form biofilms on different surfaces [7,8]. *Candida albicans* in a mature biofilm is about 100 folds resistant to antifungals than are planktonic cells [9,10,11].

Other *candida species* such as *Candida glabrata, Candida guilliermondii, Candida parapsilosis, Candida krusei, Candida inconspicua, Candida dubliniensis*, and *Candida tropicalis* have also been isolated from secondary endodontic infections [12]. These species have the ability to accommodate with different environmental conditions and to attach to several surfaces including root dentin and root filling materials [12].

Candida albicans is recognized by dental pulp and periradicular tissue immune cells and can evoke immune responses. Yet, it can evade host defenses and bind to root dentin forming biofilms. *Candida albicans* is also shown to invade the dentinal tubules resisting intracanal disinfectants and endodontic cleaning

procedures[13]. *Candida albicans* is proven to be resistant to most of the conventional antimicrobials and intracanal medications used [14]. Waltimo et al. showed that *Candida albicans* is resistant to endodontic treatment and stated that *Candida albicans* need 16 hours incubation with saturated calcium hydroxide solution in order to be deactivated [15].

Prevalence of *Candida species* in infected root canals is shown to be ranging from 0.5% to 55% [12]. Although the prevalence of *Candida albicans* is about 18% of secondary endodontic infections [3], minimal data could be found about the prevalence of *Candida albicans* in primary endodontic infections. Hence, this study aimed to identify the prevalence of *Candida species*, especially *Candida albicans*, in primary endodontic infections in addition to its biofilm forming ability and antifungal susceptibility.

Materials and methods

After approval from the local Ethics Committee (Protocol No 037-07-20), a total of 100 patients were included in this study according to the following criteria.

a. *Inclusion criteria:*
i. Patient's age between 20-40 years.
ii. Both male and female.
iii. Patients who are medically free.
iv. Patients who are diagnosed as necrotic pulp

b. *Exclusion Criteria:*
i. Patients with previous root canal treatment
ii. Pregnant females.
ii. Patients with systemic diseases
iii. Patients taking systemic antifungal drugs.
iv. Patients having systemic fungal infections.

Patients signed a consent explaining the aim of study. Demographic data, medical and dental history were obtained. Chief complaint and history of chief complaint were recorded.

Clinical examination was performed. Pulp status was assessed by electric pulp tester (Analytic Technology, Redmond, WA), thermal stimulation and confirmed clinically thereafter following access cavity preparation. Response to palpation and percussion was also recorded to assess the condition of the periapical area. Periodontal evaluation was done. All data were recorded in a diagnostic chart. Two-dimensional periapical radiographic examination was performed.

Following proper pain control, surface disinfection of the tooth and the surrounding field was performed using 30 % hydrogen peroxide for one minute. This was followed by 5% iodine dye (Wokadine Solution 5% w/v, Wockhardt Ltd., Mumbai, India) then neutralized using 5% sodium thiosulfate (Nice Chemicals, Kerala, India).

Rubber dam isolation was performed, and coronal access cavity preparations were performed using sterilized round burs (Dia-bur, Mani, Japan). Patency of the canal was checked using sterile K-file #10 (Mani Inc, Tochigi, Japan) up to the working length established using electronic apex locator (J Morita Corp, Tokyo, Japan).

Root canals were then irrigated using sterile saline to moisten the canal before sample selection. Microbiological samples were obtained by inserting paper points into the root canal for one minute. Samples were obtained from the main canal in single-rooted teeth and largest canal or canal with periapical radiolucency in multi-rooted teeth.

Each sample was inserted immediately in a separate sterile screw-capped tube containing 3 ml of sterile Sabouraud's broth as a transport media. Samples were transported to the microbiology laboratory for culture and sensitivity. Samples were cultured onto Sabouraud's dextrose agar (SDA) with chloramphenicol and CAN2 chromogenic agar plates (bioMérieux Diagnostics, Lyon, France). SDA and CAN2 chromogenic agar plates were incubated at 36 ± 1 °C for 24-48 hours. All isolates were identified according to conventional methods of De Hoog et al. [16] as follows; identification of *C. species* with colonial morphology on SDA, color of the colonies on CAN2, gram staining, germ tube test, and sugar assimilation profiles obtained using the automated VITEK-2 System (bioMérieux Diagnostics, Lyon, France).

Each Congo red agar plate was inoculated with identified *Candida species* and incubated aerobically at 37 °C for 24-48 hours for biofilm formation detection as described by Freeman et al. [17] and Dag et al. [18]. Black colonies with a dry crystalline consistency indicated biofilm formation.

Antifungal susceptibility testing was performed against Amphotericin B, Fluconazole, Voriconazole, Caspofungin, Micafungin, and

Flucytosine to measure the minimal inhibitory concentration (MIC) by the automated VITEK-2 System for yeast according to CLSI.

The significance level was set at $P \leq 0.05$. Statistical analysis was performed using SPSS version 20 software for windows (SPSS Inc, Chicago, USA).

Results

Out of the 100 cases studied, 15 (15%) showed *candida species* as detailed in table 1. Cases with periapical lesions showed significantly more *candida species* than cases without periapical lesion (P<0.001). *Candida albicans* was significantly more prevalent than *Candida krusie* (P<0.001) and *candida tropicalis* (P<0.001) in primary root canal infections.

Candida albicans showed significantly higher biofilm forming ability than candida *tropicalis* (P<0.001). *Candida krusie* failed to demonstrate any biofilm forming ability as shown in table 2.

Candida albicans and *Candida tropicalis* were susceptible to all antifungal drugs tested. *Candida Krusie* was resistant to fluconazole as shown in table 3.

	With periapical lesion	Without periapical lesion	Total
Candida albicans	8 (8%)	2 (2%)	10 (10%)
Candida krusei	1 (1%)	1 (1%)	2 (2%)
Candida tropicalis	3 (3%)	0 (0%)	3 (3%)
Total	12 (12%)	3 (3%)	15 (15%)

Table 1. Number and percentage of different types of *Candida species* in primary root canal infections with and without periapical lesion.

Candida sp.	**Number of Biofilm former**	**Percentage**
Candida albicans	**7/10**	**70%**
Candida krusei	**0/2**	**0%**
Candida tropicals	**1/3**	**33.3%**

Table 2. Number and percentage of biofilm forming *Candida species* in primary root canal infections with and without periapical lesion.

	Amphotricin B	Fluconazole	Caspofungin	Flucytosine	Voriconazole	Micafungin
C. albicans	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
C. tropicalis	3 (100%)	3 (100%)	3 (100%)	3 (100%)	3 (100%)	3 (100%)
C. krusei	2 (100%)	0 (0)	2 (100%)	2 (100%)	2 (100%)	2 (100%)

Table 3. Susceptibility of different *Candida species* in primary root canal infections to different antifungal drugs.

Discussion

Primary root canal infection is considered as a mixed infection as it is caused by a combination of different microorganisms. Fungi, viruses, gram positive cocci and obligate anaerobes have been isolated from primary root canal infections. Recently, research in endodontic microbiology has shown interest in archaea, virus and fungi in addition to bacteria [12].

Grossman was the first to report the presence of fungi in root canal infections [19]. However, most of the research focused on its presence in secondary rather than primary root canal infections. *Candida species* is prevalent in the oral cavity as a commensal in more than 50% of the world's population [20,21]. Yet, when it becomes infected and invade the host tissues, it can cause a disease [22]. This transition is dependent on conditional changes that cause the expression of a variety of virulence factors including adherence, protease secretion, hyphal formation, thigmotropism, and phenotypic switching phenomenon [23].

Results of the current study showed that *Candida species* are prevalent in 15% and *Candida albicans* is prevalent in 10% of the primary root canal infections. This is in full agreement with the previous studies showing prevalence of 1-17% [1924]. Baumgartner et al [25] and Dumani et al [26] showed higher prevalence of 21% using molecular technique for detection. This higher prevalence is attributed to the study design, as they used polymerase chain reaction rather than culture method using selective Sabouraud's Dextrose agar. Molecular methods are highly sensitive in the identification of the causative microorganism whereas culture methods are best applied for determination of the most effective treatment plan [23]. *Candida tropicalis* is the second most prevalent species in the current study [3%]. This is a logical finding as *C. tropicalis* is the second most common *Candida species* prevalent in the oral cavity of diabetic and non-diabetic humans [27].

Candida albicans was shown to form biofilm in 70% of the cases containing *Candida albicans*. This comes in full agreement with previous studies demonstrating the biofilm forming ability of *Candida albicans* [282930]. This biofilm forming ability allows *Candida albicans* to evade intracanal procedures of disinfection. In a mature multilayered biofilm, the extracellular

matrix prevents irrigant penetration into deeper layers in sufficient concentrations to exert antimicrobial effects. Although root canal instrumentation disrupts biofilm structure, about 30% of the root canal walls are left untouched after proper instrumentation. *Candida tropicalis* also showed biofilm forming ability in 33% of the cases containing *Candida tropicalis*. This finding contradicts that of Nikawa et al [30] who showed candida tropicalis not to have any biofilm forming ability. This could be attributed to geographical variances among patients.

Similar to Sen et al [31], our results showed that *Candida albicans* and *Candida tropicalis* are susceptible to all antifungals tested. In the clinical setting, *Candida albicans* is shown to resist antifungals and disinfectants used. This could be explained by the biofilm forming ability, the extracellular matrix prevents further penetration of the antifungals and disinfectants into deeper layers in optimal concentrations.

In conclusion, *Candida albicans* might play a role in primary endodontic infections, especially in cases with periapical lesions. *Candida albicans* shows biofilm forming ability which is considered as one of its virulence factors that help it evade the immune response and antimicrobials.

Conclusions

Candida albicans might play a role in primary endodontic infections and biofilm formation.

Acknowledgments

The authors deny any conflicts of interest related to this study.

Clinical Relevance

Candida albicans might play a role in primary endodontic infections, especially in cases with periapical lesions. *Candida albicans* shows biofilm forming ability.

Declaration of Interest

The authors report no conflict of interest.

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