ORIGINAL RESEARCH PAPER

Volume - 9 | Issue - 6 | June - 2020 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

PRESENCE OF CANDIDA ALBICANS IN PRIMARY ENDODONTIC INFECTION AND ASSESSING ITS SUSCEPTIBILITY TO COMMON ROOT CANAL MEDICAMENTS -A PCR BASED STUDY.

Dental Science

Dr. Namrata Jain Senior Lecturer, Kusum Devi Sunderlal Dugar Jain Dental College and Hospital, Kolkata

Dr. B.V Sreenivasa Murthy* Dean, Faculty of Dental Sciences, Ramaiah University of Applied Sciences, Bengaluru *Corresponding Author

Dr. Beena Associate professor at Department of Microbiology, M.S. Ramaiah Medical College, Bengaluru

Dr. Sylvia Mathew Professor at Department of Conservative Dentistry and Endodontics, Faculty of Dental Sciences, Ramaiah University of Applied Sciences, Bengaluru

ABSTRACT

Background: Microbial infection of the dental pulp and the periapical area, often sequelae of dental caries is the most common prelude to endodontic treatment. Successful endodontic therapy depends upon the reduction or elimination of these microorganisms and there should be knowledge about the role of specific species involved.

Aim: To assess the presence of Candida albicans from primary endodontic infection using polymerase chain reaction and evaluate its susceptibility to common root canal medicaments.

Methodology: Thirty-five root canal samples from primary endodontic infections were collected using file and paper points following disinfection protocol. Samples were inoculated in Sabouraud Dextrose Agar and incubated for 2-3 days. Taxonomy was evaluated using Gram-staining and germ tube test by macroscopic examination and optical microscopy. The growth was subjected to polymerase chain reaction. The positive samples were assessed for susceptibility to Calcium Hydroxide and 2% Chlorhexidine using Agar Diffusion test.

Statistical Analysis used: ANOVA using SPSS version 20.0

Results: Out of thirty-five samples only one was positive for Candida albicans, four were positive for polymerase chain reaction. When susceptibility of Candida albicans to intracanal medicaments was assessed, Calcium Hydroxide showed larger zone of inhibition compared to 2% Chlorhexidine.

Conclusion: Prevalence of Candida albicans in primary endodontic infection in this study was 11.4% using polymerase chain reaction. Calcium Hydroxide produced larger inhibition zone than Chlorhexidine against Candida albicans. Further researches utilizing modern molecular diagnostics and disinfection protocols needed for managing root canal infections.

KEYWORDS

Candida albicans, Calcium Hydroxide, Chlorhexidine, Intracanal medicament, Polymerase chain reaction

Introduction

Pathoses of the pulp and periapical tissues are mainly caused by microorganisms. Root canal infections are polymicrobial in nature **(Attia et al., 2015).** The majority of researches deals with bacteria, but there are studies reporting the association of viruses and fungi with endodontic infection **(Baumgartner et al. 2000)**. Increasing concern has arisen about Candida albicans in failed root canal infections and primary endodontic infections.

Candida is a resistant microorganism. It can remain in the root canals even after thorough mechanical instrumentation and irrigation procedures **(Nair et al., 1990).**The understanding of the role of Candida albicans in infection will enable an effective strategy for the management of infection.

Isolation and identification of the organism can be done in two ways – Traditional culture methods and Molecular diagnostic methods**.** The major drawback of culture based method is impossibility of cultivating and identifying a large number of bacterial species **(Siqueira & Rôças, 2005).** Whereas, a molecular identification technique depends on the genes that are unique for each species. Thus, these methods can be used to combat the limitations of the conventional technique.

After isolating, identifying and understanding the role of microorganisms in the disease process, efforts need to be made for the elimination of the same. Dressing the canal with intracanal medicament can help in reducing the residual microorganisms to provide a conducive environment for periapical tissue repair **(Jeison B et al, 2013)**. Calcium Hydroxide and Chlorhexidine are effective as an antibacterial intracanal medicament. In this study the antifungal activity of medicament is tested for elimination of Candida albicans from root canal.

Thereby, the present study aimed to investigate the presence of Candida albicans in the root canal of teeth with primary endodontic infection using polymerase chain reaction and assessing its susceptibility to two common root canal medicaments. Null Hypothesis was formulated that, there is no effect of Calcium Hydroxide and Chlorhexidine on Candida albicans associated with primary endodontic infection.

Methodology

Source of Data: 35 Subjects for the study were selected from patients visiting Dept of Conservative Dentistry and Endodontics, Faculty of Dental Sciences, Ramaiah University of Applied Sciences (FDS, RUAS). Patients requiring Root Canal Treatment either single or multiple rooted teeth with radiographic evidence of apical periodontitis, periodontally sound and possible post endodontic restoration were included. Patient receiving antibiotic or antifungal treatment during previous six months, history of Systemic disease and teeth that could not be isolated using rubber dam were excluded from the study. The study protocol describing specimen collection was approved by the Ethics committee of Ramaiah University. Patient who met the inclusion criteria and agreed to take part in the study were asked to sign the written informed consent form. Detailed medical and dental history was obtained from each patient. Control strains of Candida albicans (ATCC 90028) were obtained from the culture collection at Dept. Of Microbiology of Ramaiah Medical College and Teaching Hospital, Bangalore.

Root canal Sampling

Microbial samples from the root canals of 35 patients were obtained under strict asepsis. Next disinfection, the endodontic access with a sterile endoaccess bur (Prima Dental India Pvt. Ltd.) was made. Sample were taken from the widest canal or root with periapical radiolucency seen through radiograph. The sample was obtained using sterile No. 10K or No. 15 K type file, introduced to a level approximately 1 mm short of the tooth apex, based on diagnostic radiographs, with a discrete filing motion. Afterwards sterile absorbent paper cones were placed inside the root canal for 1 minute for absorbing the fluid present in the canal. These paper points and K files were then transferred to the test tubes containing peptone water.

The samples were then transferred to the Microbiology Lab within 4 hours of sample collection and were left in the incubator at 37°C for 24 hours to facilitate the growth of the organism. The samples were then streaked on the Sabouraud dextrose agar plate and incubated at 37°C for 2 days.

Those plates exhibiting macroscopic changes (cream coloured, raised, smooth, and butyrous colonies) indicating the growth of fungi, was further subjected to gram staining and germ tube test to confirm the presence or absence of Candida albicans *(Fig.1A and 1B).*

Fig. 1A and 1B Colonies formed on SDA plate A. Control Strain B. Clinical Strain

Gram staining test:
A loopful of the broth culture taken with a sterile loop, was placed on the slide. The slide was heat fixed and the smear was flooded with Basic Fuchsin (Nice chemicals, India) and then was gently rinsed with water. Gram's iodine (Nice chemicals, India) was let to stand for one minute followed by rinsing. Ethyl alcohol was used to decolorize. The slide was again rinsed with water and stained with Safranin (Nice chemicals, India) for 45 seconds. The slide was rinsed with water and was blotted dry with bibulous paper.

Germ tube testing:
Germ tube testing was further done to confirm that the gram-positive fungal organism was indeed Candida albicans. The yeast was lightly inoculated into 1 ml of sterile serum and incubated for 2.5 hours at 37°C. After incubation, suspensions were placed on a glass slide, mounted under a cover slip and examined for the presence of germ tubes (Fig. 2)

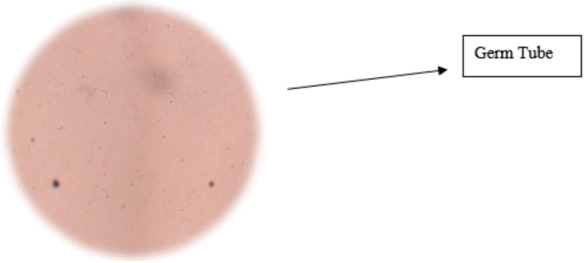

Fig.2 Germ Tube Test positive confirming the presence of Candida albicans

DNA Extraction: Control strains of Candida albicans (ATCC 90028) were obtained. DNA extraction from the clinical sample was done using HiPurA™ Mammalian Genomic DNA Purification Kit (HIMEDIA). The lysis solution and proteinase K solution was added to the isolates. They were mixed by vortexing and was incubated at 55°C until the tissue was completely digested. After which lysis solution was again added followed by vortexing thoroughly for 15 seconds. A homogenous mixture was obtained indicating efficient cell lysis. The sample was incubated at 70°C for 10 minutes.

DNA Isolation: Ethanol (96%-100%) was added to the lysate and was vortexed for 5-10 seconds. The lysate obtained was subjected for centrifugation at 10000rpm for 1 minute. The flow was discarded and diluted Wash Solution was added to the column and then centrifuged at 10000rpm for 1 minute. The flow was again discarded and the same collected tube was reused with the column. The procedure was repeated and centrifuged at 13,000-16,000rpm for 3 minutes and the column was dried. Elution buffer was pipette directly on the column and was incubated for 1 minute at room temperature (15-25°C). Centrifuged at ≈10000rpm for 1 minute to elute the DNA. Re-elution was done by repeating the same step.

DNA Amplification: Forward Primer (GCC GGT GAC GAC GCT CCA AGA GCT G) and Reverse Primer (CCG TGT TCA ATT GGG TAT CTC AAG GTC) were used. PCR cycle was repeated 35 times to amplify the target DNA (Table 1)

Table. 1 PCR cycle conditions

Pre denaturation	Denaturation	Annealing	Extension	Final Extension
95°C	95°C	90°C	72°C	72°C
5 min	30 sec	55 sec	2 min	7 min
	35 cycles			

Gel Electrophoresis: PCR products were analysed by 2% agarose gel. The gel was stained with 0.5 µg/mL of ethidium Bromide and visualized under ultraviolet light and 200-bp DNA ladder digest was used as a molecular weight marker.

Susceptibility tests of intracanal medicament
The susceptibility of candida strains to intracanal dressing medicaments was tested by the Agar Diffusion method. Incubation wells (4*4mm) were punched in agar plate and were filled with saline, 2% GLUCOCHeX (chlorhexidine) and DENTOCAL (calcium hydroxide paste) Following which the positive culture from the plate was streaked onto the agar diffusion plate and then incubated at 37°C for 3days. The zones of inhibition for each medicament used against candida albicans was measured using a transparent ruler at the end of seven days.

Statistical analysis
The statistical analysis of the acquired data was carried out using the IBM SPSS Statistics 20. O software. To assess the prevalence of Candida albicans, descriptive analysis was done. In order, to evaluate statistical significance for the antifungal property of the intracanal medicament Kruskal-Wallis was performed. Inter-group comparison was determined using Mann-Whitney test. The significance level was set at 0.05.

Results
Out of thirty-five samples which were cultured only one (2.85%) was positive for Candida albicans, whereas four out of thirty-five samples (11.4%) were positive through polymerase chain reaction (Fig. 3A, 3B and 4)

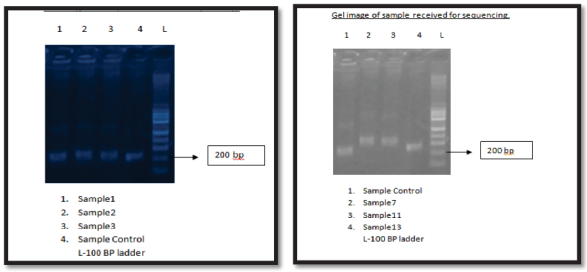

Fig 3A and 3B Gel Electrophoretic image of sample

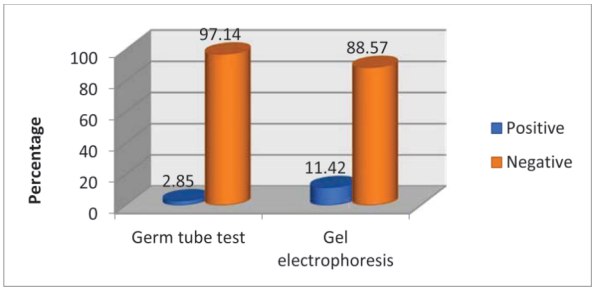

Fig. 4 Graph showing the presence of Candida albicans in root canals identified through culture and PCR technique

medicaments. The inhibition zones were evaluated after 7 days. Calcium Hydroxide paste (DENTOCAL) and 2% Chlorhexidine gel (GLUCO CHeX 2%) used in the present study exerted antifungal activity. The diameters of inhibition zones of Calcium Hydroxide paste

(DENTOCAL) was greater than 2% GLUCO CHeX gel. (Fig.5)

Fig.5 Zones of Inhibition formed by Calcium Hydroxide paste and 2% Chlorhexidine gel

TABLE 2 and Fig 6: Comparison of the antifungal efficacy of the intracanal medicaments against Candidal isolates by Agar Diffusion Test.

N	Minimum	Maximum	Mean	Std. Deviation
Saline 4	0	0	.00	.000
Calcium Hydroxide 4	14	17	15.75	1.258
Chlorhexidine 4	10	12	11.00	1.155

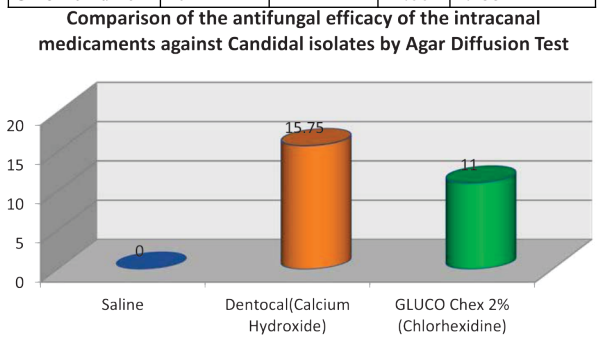

TABLE 3: Comparison of the groups using Kruskal-Wallis

Kruskal Wallis	10.315
p value	.006*

The result obtained showed statistically significant values (p value=0 .006). The inhibition of growth in the CH group was statistically significant in comparison to the 2% CHX group.

TABLE 4: Inter-group comparison using Mann-Whitney test

Groups	P value
Saline * Dentocal	0.013*
Saline * Chlorhexidine	0.013*
Dentocal* Chlorhexidine	0.019

Inter- group comparison showed value of 0.019 between Calcium Hydroxide and Chlorhexidine which is more than 0.016 thus not statistically significant.

DISCUSSION

In the present study the prevalence of Candida albicans was found to be in the range of 2.8% - 11.4% in primary endodontic infection using culture and PCR technique. This result is in context with the several findings by different authors where prevalence of CA identified through culture and PCR technique fall within the range of 3-21 % **(Jr et al., n.d.).** The incidence of Candida albicans from infected root canals ranges from 1% - 17% **(Baumgartner et al. 2000)**. The presence of yeast in treatment resistant cases ranges from 3-18% as shown by culture and PCR techniques **(Siqueira Jr & Sen, 2004)**. The study reported the fungi prevalence to be as high as 21% by polymerase chain reaction **(Dumani et al., 2012).** The prevalence of Candida albicans in primary infections using culture technique was 7% **(Lana et al., 2001)** and 8% **(Shah 2017)** respectively**.**

In this study, 2.8% of sample were positive for Candida albicans through culture technique and 11.4% were positive through PCR technique respectively (Fig. 4). A possible explanation for the difference in the results obtained could be because of the technique involved. Culture based techniques are less sensitive, less specific, time consuming , laborious and is dependent on mode of sample transport and experience of the microbiologist **(Siqueira & Rôças, 2005)**. Whereas, molecular techniques have a higher sensitivity and specificity than conventional culture techniques and also yields accurate identification of microbes **(Id et al., 2016).**Therefore, the choice of methods depends on the goal of the investigation. Identification of the causative agents warrant molecular method approach whereas culture method can be preferred for effective treatment planning **(Waltimo et al., 2004).**

Candida albicans , commonly isolated from the oral cavity **(Chandra et al., 2010)** can grow in many morphologic forms **(Odds, 1988)**. They exhibits a variety of virulence factors such as adherence, thigmotrophism, phenotypic switching and secretes "aspartyl protease" that degrades dentinal collagen **(Waltimo et al., 2004).** Candida albicans can grow on the dentinal surfaces and penetrates into the dentinal tubules (**Chandra et al., 2010).**Candida are dentinophilic microorganism **(Şen et al., 1997).** Thus, the sample were obtained by filing the dentinal walls of the canals using K file.

Candida albicans can survive the challenging conditions of the root canal ecosystem **(Peciuliene et al., 2000)**. They rapidly gain resistance to antimicrobial agents owing to its phenotypic switching property **(Odds, 1997**).Therefore, suitable intracanal medicament that could be potentially efficacious against Candida albicans should be used. The widely used intracanal medicaments Calcium Hydroxide and Chlorhexidine were used in this study.

Calcium Hydroxide have moderate bactericidal activity due to its high pH (11-12.5) and its dissociation into the highly interactive hydroxyl ions which kills bacterial cells by damaging the cytoplasmic membrane, protein denaturation and damaging of DNA **(Tang et al., 2004).** CH in solution form loses its high pH after interaction with organic and inorganic substances and may become ineffective. Thus, paste form of CH (DENTOCAL) is recommended **(Turk et al., 2009).** CH requires a minimum period of 7 days to act as an effective intracanal agent, therefore in the present study the specimen was kept for 7 days to evaluate its antifungal efficacy **(Sjögren et al., 1991).**

Chlorhexidine is positively charged molecule that interacts with negatively charged phosphate groups present on the bacterial cell wall, allowing the Chlorhexidine molecule to penetrate the bacteria and leading to intracellular toxicity **(Estrela et al., 2003).**Chlorhexidine also exhibit Substantivity which makes it a durable intracanal medicament. **(Mohammadi & Abbott, 2009).**

Chlorhexidine gluconate used in concentration of 0.002-2% presents a wider antimicrobial spectrum, lower toxicity and better diffusion through dentinal tubules **(Estrela et al., 2003).** 2% concentration of CHX may increase the diffusion of the medicament into the dentinal tubules **(Vaghela et al., 2011)**. The gel formulation can yield low toxicity on periapical tissues and viscosity of the gel keeps the active agent in contact with the root canal walls and dentinal tubules **(Ferraz et al., 2001).** Therefore, gel form of chlorhexidine 2% was preferred for the study.

Culture medium used in this study was Sabouraud Dextrose Agar which is a selective media and allowed only Candida organisms to grow. The presence of Candida albicans was confirmed by the germ tube test and polymerase chain reaction. Agar diffusion test is the generally accepted procedure for determining in vitro sensitivity under routine laboratory conditions **(Sen et al., 2000;).** In this study, Agar diffusion method was performed since it is simple, standard, and non-reproducible.

The present study showed that CH was more effective on Candida albicans than Chlorhexidine but both the intracanal medicament exhibited zone of inhibition indicating antifungal nature. Thus, null hypothesis was rejected. The possible reason for this result could be the ability of calcium hydroxide to inactivate the cytotoxic effects of the endotoxin released by microorganisms. Secondly, the usage of Calcium Hydroxide in a paste form (DENTOCAL) acted as a backup reservoir for the medicament thus enhancing its efficacy. Calcium ions has a very important role to play in the regulation of *Candida albicans* morphogenesis. Calcium ions can inhibit the mycelial growth of

Candida albicans. The antimicrobial effect of calcium hydroxide, due to the release of hydroxyl ions, might be enhanced due to the inhibition of *Candidal* growth by calcium ions***(Holmes et al., 1991).***

The paste form of CH was very effective in killing CA, probably because the medicament was placed in direct contact with the tested microorganism in the agar diffusion method **(Ferguson et al. 2002).** The Study reported the combination of calcium hydroxide and chlorhexidine was better than calcium hydroxide alone against Candida albicans **(Ercan et al., 2006).** Calcium hydroxide preparation with glycerine and chlorhexidine demonstrated more antifungal activity than calcium hydroxide preparations with cetrimide and distilled water **(Turk et al., 2009).**

The effect of calcium hydroxide against Candida albicans is very controversial in the literature. In contrast, it has been previously found that Calcium hydroxide is not an effective agent on Candida albicans **(Waltimo et al., 2004).**The difference in the results obtained from the study could be because of some methodologic differences or presence of the buffering agent in the culture medium, that prevent the pH of calcium hydroxide to reach sufficient level to give enough antimicrobial activity regardless of the diffusion of calcium hydroxide through the agar plates **(de Almeida Gomes et al., 2006).** With respect to this study, Chlorhexidine was not as effective as calcium hydroxide.

CONCLUSION

Within the limitations of the current study it can be concluded that Candida albicans may be associated with primary root canal infections. It was more accurately identified through PCR analyses. The presence of this organism in the endodontic infection necessitates its elimination from the root canal using appropriate intracanal medicaments. The results of the present study also state that Calcium Hydroxide was more effective to eliminate Candida albicans. This is an invitro study, therefore to assess the clinical efficacy of the intracanal medicament, further long-term clinical studies with larger sample size can be planned.

REFERENCES

1. Antimicrobial effects of calcium hydroxide, chlorhexidine, and propolis on Enterococcus faecalis and Candida albicans. (2013). April. https://doi.org/10.1111/jicd.12041
2. Article, O. (2017). Identification of presence of Candida albicans in primary root canal infections : An in vitro study. 109–113. https://doi.org/10.4103/0970-7212.195440
3. Attia, D. A., Farag, A. M., Afifi, I. K., & Darrag, A. M. (2015). ScienceDirect Antimicrobial effect of different intracanal medications on various microorganisms. Tanta Dental Journal, 12(1), 41–47. https://doi.org/10.1016/j.tdj.2014.10.005
4. Baumgartner, J. C., Watts, C. M., & Xia, T. (2000a). Occurrence of Candida albicans in infections of endodontic origin. Journal of Endodontics, 26(12), 695–698.
5. Baumgartner, J. C., Watts, C. M., & Xia, T. (2000b). Occurrence of Candida albicans in Infections of Endodontic Origin. 5, 5–8.
6. Chandra, S. S., Miglani, R., Srinivasan, M. R., & Indira, R. (2010). an Antifungal Agent. Journal of Endodontics, 36(4), 675–678. https://doi.org/10.1016/j.joen.2010.01.015
7. de Almeida Gomes, B. P. F., Vianna, M. E., Sena, N. T., Zaia, A. A., Ferraz, C. C. R., & de Souza Filho, F. J. (2006). In vitro evaluation of the antimicrobial activity of calcium hydroxide combined with chlorhexidine gel used as intracanal medicament. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 102(4), 544–550.
8. Dumani, A., Yoldas, O., Yilmaz, S., Akcimen, B., Seydaoglu, G., & Kipalev, A. (2012). In vitro susceptibility of e . faecalis and c . albicans isolates from apical periodontitis to common antimicrobial agents , antibiotics and antifungal medicaments. 4(1).
9. Ercan, E., Dalli, M., & Dülgergil, Ç. T. (2006). In vitro assessment of the effectiveness of chlorhexidine gel and calcium hydroxide paste with chlorhexidine against Enterococcus faecalis and Candida albicans. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 102(2), e27–e31.
10. Estrela, C., Ribeiro, R. G., Estrela, C. R. A., Pécora, J. D., & Sousa-Neto, M. D. (2003). Antimicrobial effect of 2% sodium hypochlorite and 2% chlorhexidine tested by different methods. Brazilian Dental Journal, 14(1), 58–62.
11. Ferguson, J. W., Hatton, J. F., & Gillespie, M. J. (2002). Effectiveness of Intracanal Irrigants and Medications against the Yeast Candida albicans.
12. Holmes, A. R., Cannon, R. D., & Shepherd, M. G. (1991). Effect of calcium ion uptake on Candida albicans morphology. FEMS Microbiology Letters, 77(2 3), 187–194.
13. Id, A., Author, N. C., Dola, B., Dentrisry, C., Moinuddin, M. K., Surgery, D., Pradesh, A., & Pradesh, A. (2016). Polymerase chain reaction in exploring endodontic infections Polymerase chain reaction in exploring endodontic infections Gene Targets for microbial identification. 1–5.
14. Jr, F. S., Sen, B. H., & Janeiro, R. De. (n.d.). Fungi in endodontic infections. 632–641. https://doi.org/10.1016/j.tripleo.2003.12.022
15. Lana, M. A., Ribeiro Sobrinho, A. P., Stehling, R., Garcia, G. D., Silva, B. K. C., Hamdan, J. S., Nicoli, J. R., Carvalho, M. A. R., & Farias, L. de M. (2001). Microorganisms isolated from root canals presenting necrotic pulp and their drug susceptibility in vitro. Oral Microbiology and Immunology, 16(2), 100–105.
16. Mohammadi, Z., & Abbott, P. V. (2009). The properties and applications of chlorhexidine in endodontics. International Endodontic Journal, 42(4), 288–302.
17. Molander, A., Reit, C., Dahlen, G., & Kvist, T. (1998). Microbiological status of root filled teeth with apical periodontitis. International Endodontic Journal, 31(1), 1–7.
18. Nair, P. N. R., Sjögren, U., Krey, G., Kahnberg, K.-E., & Sundqvist, G. (1990). Intraradicular bacteria and fungi in root-filled, asymptomatic human teeth with therapy-resistant periapical lesions: a long-term light and electron microscopic follow-up study. Journal of Endodontics, 16(12), 580–588.
19. Odds, F. C. (1988). Candida and candidosis 2nd edn. Bailliáere Tindall: London.
20. Odds, F. C. (1997). Switch of phenotype as an escape mechanism of the intruder. Mycoses, 40, 9–12.
21. Peciuliene, V., Balciuniene, I., Eriksen, H. M., & Haapasalo, M. (2000). Isolation of Enterococcus faecalis in previously root-filled canals in a Lithuanian population. Journal of Endodontics, 26(10), 593–595.
22. Sen, B. H., Akdeniz, B. G., & Denizci, A. A. (2000). ORAL SURGERY ORAL MEDICINE ORAL PATHOLOGY The effect of ethylenediamine-tetraacetic acid on Candida albicans. 90(5), 651–655. https://doi.org/10.1067/moe.2000.109640
23. Siqueira, J. F., & Rôças, I. N. (2005). Exploiting molecular methods to explore endodontic infections: Part 1--current molecular technologies for microbiological diagnosis. Journal of Endodontia, 31(6), 411–423. https://doi.org/http://dx.doi.org/10.1097/01.don.0000157989.44949.26
24. Siqueira Jr, J. F., & Sen, B. H. (2004). Fungi in endodontic infections. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 97(5), 632–641.
25. Sjögren, U., Figdor, D., Spångberg, L., & Sundqvist, G. (1991). The antimicrobial effect of calcium hydroxide as a short term intracanal dressing. International Endodontic Journal, 24(3), 119–125.
26. Tang, G., Samaranayake, L. P., & Yip, H. (2004). Molecular evaluation of residual endodontic microorganisms after instrumentation, irrigation and medication with either calcium hydroxide or Septomixine. Oral Diseases, 10(6), 389–397.
27. Turk, B. T., Sen, H., & Ozturk, T. (2009). In vitro antimicrobial activity of calcium hydroxide mixed with different vehicles against Enterococcus faecalis and Candida albicans. YMOE, 108(2), 297–301. https://doi.org/10.1016/j.tripleo.2009.03.029
28. Vaghela, D. J., Kandaswamy, D., Venkateshbabu, N., Jamini, N., & Ganesh, A. (2011). Disinfection of dentinal tubules with two different formulations of calcium hydroxide as compared to 2% chlorhexidine: As intracanal medicaments against Enterococcus faecalis and Candida albicans: An in vitro study. Journal of Conservative Dentistry: JCD, 14(2), 182.
29. Waltimo, T. M. T., Haapasalo, M., Zehnder, M., & Meyer, J. (2004). Clinical aspects related to endodontic yeast infections. Endodontic Topics, 9(1), 66–78.