ANALYSIS OF FUNGAL CONTAMINATION AND DISINFECTION OF TOOTHBRUSHES

Mitra Mobin [1], Cíntia De M. Borba [2], Carlos A.M. Filho [1], Fabrício I. Tapety [1], Iraci De M.S. Noleto [1], João B.M. Teles [1]

[1] University of Human Health Sciences and Technology of Piauí – NOVAFAPI, Teresina, PI, Brazil.

[2] Laboratory of Fungal Taxonomy, Biochemistry and Bioprospection – Oswaldo Cruz. Foundation, Rio de Janeiro, RJ, Brazil.

ABSTRACT

The aim of this study is to determine the fungal species in the toothbrushes of residents of a neighborhood on the east side of Teresina – PI, and to assess the efficiency of a disinfection method based on 2% sodium hypochlorite. Fifty toothbrushes were divided into two groups: group A comprised 30 toothbrushes used by the residents and group B (control group) 20 new toothbrushes. Fungal evaluation was conducted in Sabouraud culture medium containing chloramphenicol and CHROMagarTM Candida. Later, group A was divided into two subgroups (A1 and A2), which were submitted to disinfection by immersion in 2% sodium hypochlorite and once again screened for the presence of fungi. Seventeen fungal species were identified in group A before the disinfection. Fungal growth was not observed in subgroups A1 and A2, or group B after disinfection. All fungal species isolated from the toothbrushes were considered opportunistic and may cause health problems mainly in immunocompromised patients. The species most frequently found were: Candida albicans, Aspergillus niger, Penicillium citrinum, Geotrichum candidum, Aspergillus fumigatus and Cladosporium oxysporum. Fungal growth did not occur after toothbrush disinfection with 2% sodium hypochlorite, suggesting this is an efficient, low-cost method that can therefore be used by low income populations.

Key words: Toothbrushing, Fungi, Disinfection.

ANÁLISE DA CONTAMINAÇÃO FÚNGICA E DESINFECÇÃO DAS ESCOVAS

RESUMO

O presente estudo objetivou conhecer as espécies fúngicas existentes em escovas dentais de moradores de vila residencial na zona leste de Teresina – PI, bem como avaliar a eficácia de um método de desinfecção utilizando hipoclorito de sódio a 2%. Utilizou-se 50 escovas dentais divididas em dois grupos: grupo experimental A, com 30 escovas usadas pelos moradores e grupo B (controle) com 20 escovas novas. A análise fúngica foi realizada em meios de cultura Sabouraud acrescido de cloranfenicol e CHROMagarTM Candida. Posteriormente, o grupo A foi dividido em subgrupos (A1 e A2) que foram submetidos à desinfecção com hipoclorito de sódio a 2%, e procedeu-se novamente a análise fúngica. No grupo A foram identificadas 17 espécies de fungos. Nos subgrupos A1 e A2, bem como no grupo B, não foi observado crescimento fúngico. Todas as espécies isoladas das escovas dentais utilizadas pelos moradores são consideradas oportunistas podendo acarretar problemas, principalmente em pacientes imunocomprometidos, sendo as mais frequentes Candida albicans, Aspergillus niger, Penicillium citrinum, Geotrichum candidum, Aspergillus fumigatus e Cladosporium oxysporum. Não houve crescimento de fungos nas escovas dentais após a desinfecção com hipoclorito de sódio a 2% o que sugere ser um método eficaz e de baixo custo para populações carentes.

Palavras-chaves: escovação, fungos, desinfecção.

INTRODUCTION

Toothbrushes are important in daily oral hygiene; however they may retain residues that may favor the growth of bacteria and fungi, causing health problems mainly to immunocompromised individuals[1]. Thus, the development of simple, low-cost disinfection methods is of the utmost importance in preventing possible dental problems.

Historically, humans developed a culture of body care and practices which includes oral hygiene. Manuscripts found in Babylonia, dated 3.500 B.C., indicate the use of gold picks for tooth cleaning. Over the years, many other materials were used for oral hygiene. Most probably, the toothbrush that originated current ones was produced in England, in 1780, by Addis, and was made of a bone handle and natural bristles placed in holes drilled in one end and tied with wire. However, the first American industrial patent was by Wadsworth, in 1857, but innovations of the industrial process for toothbrush

production only appeared as from 1880, when plastic was used for the handles, then celluloid was used in 1900 and cellulose acetate in 1930, and in 1938 nylon bristles substituted natural ones made of animal hair[2]. However, the cleaning process of the toothbrush itself was left aside. The *American Dental Association* (ADA) recommends changing toothbrushes every 3 to 4 months and cleaning them with tap water after brushing to remove any residue that may have been left[3].

Most microorganisms found in toothbrushes form part of the native oral microbiota. However, the toothbrush may be an intra- and inter-individual source of contamination and may act as vehicle to reintroduce microorganisms of intra or extra-oral origin to the oral cavity[1]. Sogi et al.[4] showed experimentally that in addition to cleaning the oral cavity, toothbrush hygiene is necessary in order to ensure oral hygiene.

Sato et al.[5] assessed bacteria survival rate in toothbrushes after brushing and the efficacy of their decontamination by spraying with antimicrobial solutions, and verified a significant decrease in toothbrush contamination.

Little is known about the type of fungal species found in toothbrushes and if those contaminated toothbrushes might be source of microorganisms that cause infection. Studies have been published focusing only aspects of the contamination by *Candida* species, mainly in full or partial removable orthodontic appliance wearers[6,7].

The scarcity of information on fungus isolation from toothbrushes and toothbrush disinfection, and the need of further raising awareness on toothbrush care encouraged the present work.

MATERIAL AND METHODS

This quantitative experimental research was conducted from January to August, 2009. The neighborhood on the east side of Teresina city-PI was chosen because it is difficult for its low-income residents to reach oral health systems. They are treated at the comprehensive health clinic of the NOVAFAPI University and most of them have oral health problems.

This research followed ethical principles, according the declaration of Helsinki of 2000 and Resolution number 196 of the National Health Council, Brazil.

Fifty toothbrushes were studied. Thirty of them were supplied to the experimental group of male and female neighborhood residents aged 10 to 40 years, who used them for 30 days. The control group consisted of 20 new toothbrushes which were examined in the condition they were acquired in. The 50 toothbrushes were of the same brand, size and hardness.

The exclusion criterion was applied to those volunteers who did not accept the terms of free agreement proposed.

The toothbrushes were classified into two groups: Group A - experimental, with 30 toothbrushes supplied and used by the residents for one month. After that time, the toothbrushes were collected and submitted to fungal determination. Then they were randomly subdivided into two groups of 15 (A1 and A2) and disinfection processes were applied. Group B (control group) consisted of 20 new toothbrushes which were submitted to fungus determination immediately after removal from their packages at NOVAFAPI Microbiology Laboratory.

Group A toothbrushes were collected and placed in test tubes containing sterile saline solution. Then 100µl of the dirty solution were collected from each test tube and placed in Petri dishes with BBL™ CHROMagar™ Candida (BD-Difco, New Jersey, USA), incubated at 37ºC, and Sabouraud Dextrose agar (HiMedia Laboratories PVT. Ltd., Mumbai, India) with chloramphenicol (0.05g/l), and incubated at room temperature until colony growth, according methodology developed by Santos[8].

Group B toothbrushes (control group), all new, were removed from their packages and submitted to the same tests as group A.

Microcultures according Ridell[9] were obtained for better visualization of filamentous fungi structures and subsequent identification of their genus and species.

After observing the morphological characteristics of each colony and the microscopic structures of the fungi, species were identified following Pitt[10] and Hoog et al.[11] identification keys. A selective culture medium for *Candida* species was used for yeast identification.

Toothbrushes from subgroups A1 and A2 were submitted to disinfection processes using 2% sodium hypochlorite. The toothbrushes from subgroup A1 were immersed for 3 minutes and the ones from subgroup A2 for 5 minutes. Then fungi were determined following the procedure described above.

The statistical analysis was of the descriptive type from the percentages found and the Chi-square test with a 5% significance level verified the existence of associations of fungi presence before and after disinfection and between the experimental and control groups.

RESULTS

Fungal presence was determined in all the toothbrushes from group A. Seventeen species of fungi were identified and the most frequent were: *Candida albicans*, 26.7%; *Aspergillus nig*er, 16.7%; *Penicillium citrinum*, 13.3% and *Geotrichum candidum, Aspergillus fumigatus, Cladosporium oxysporum* 10% as shown in Fig. 1. There was no fungal colony growth in group B (control) toothbrushes.

No yeast or filamentous fungi colony growth was obtained from toothbrushes from subgroups A1 and A2 that were submitted to disinfection with 2% sodium hypochlorite for two different times.

The mean of colony forming unit (CFU) per ml of fungal species isolated from toothbrushes group A, before and after the disinfection process, is shown in Fig. 2.

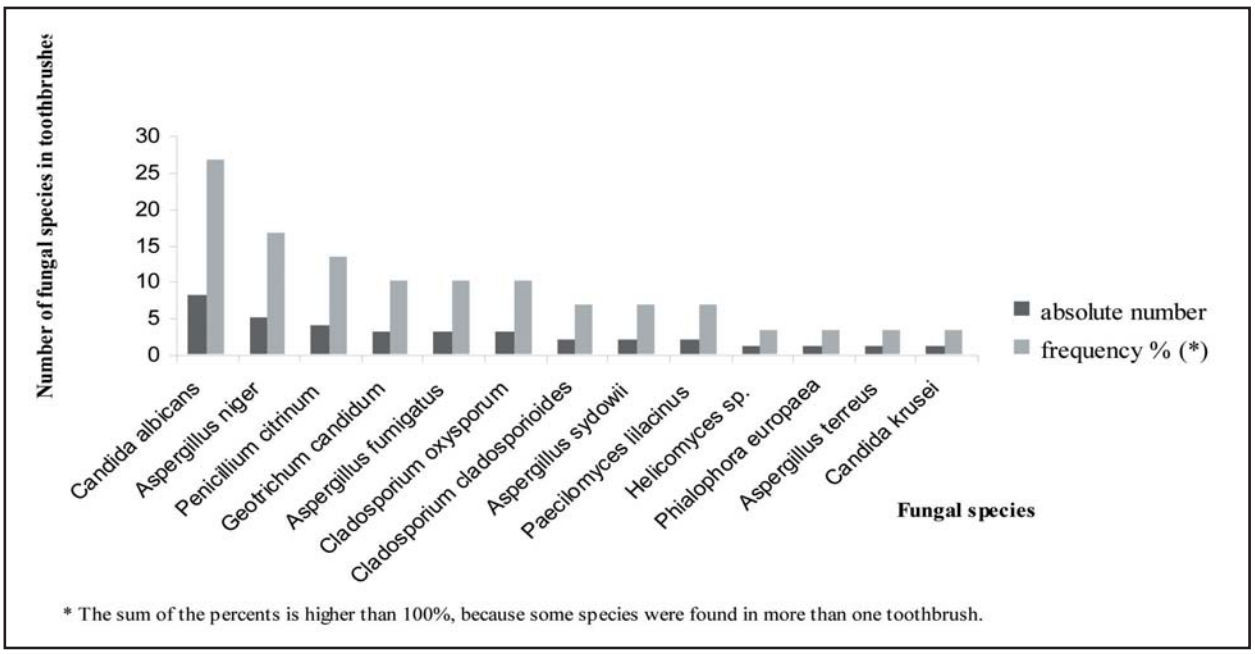

Fig. 1.Quantitative analysis of fungal species isolated from toothbrushes of residents of a neighborhood on the east side of Teresina-PI.

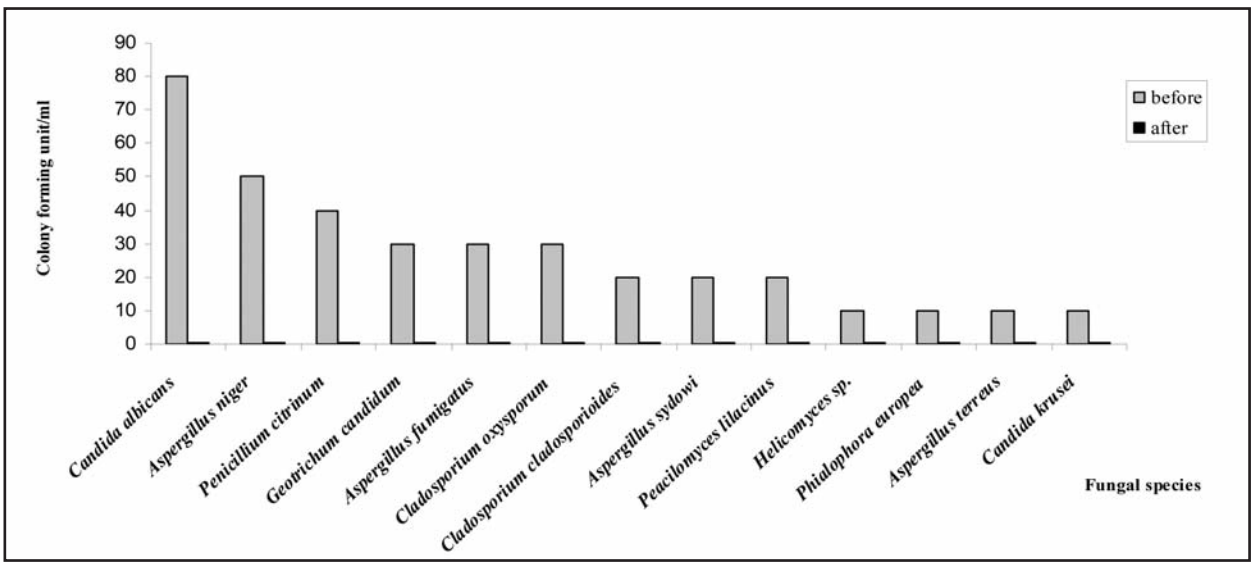

Fig. 2.Mean number of colony forming units per milliliter of fungal species isolated from toothbrushes of residents of Teresina-PI before (■) and after (■) the disinfection procedures with 2% sodium hypochlorite.

DISCUSSION

Toothbrushes are excessively contaminated by microorganisms during their everyday use and contaminated bristles may become an instrument of transmission and inoculation through gingival abrasion or pre-existent lesions[12]. Microorganisms can multiply and their number increase in toothbrushes, which may become a potential hazard, mainly for immunocompromised individuals, diabetics, individuals with vascular diseases and elderly people[13]. Our results show that different species of fungi are present in toothbrushes after their use and many of them are described as opportunistic[11]. According JADA[3] although several studies have shown that microorganisms can grow in toothbrushes after their use, there is not enough clinical evidence to conclude that microorganism growth in toothbrushes may cause adverse oral or systemic effects. However, it should be considered that most studies were conducted on bacteria and very few on fungi[13-18]. Therefore, it is not known how fungi behave, how the host-fungus relationship is established in the oral cavity or how the transition from a commensal to a parasite takes place.

Candida albicans was the species most frequently isolated from toothbrushes in this study. Although its presence in the mouth is currently considered as a normal commensalism condition, *Candida* is known to cause most of the fungal infections in immunocompromised patients[14] and is involved in periodontitis and caries[15-17].

It should be highlighted that an *in vitro* analysis of the retention and survival of three pathogenic microorganisms, *Porphyromonas gingivalis*, *Streptococcus mutans* and *C. albicans*, in three different types of toothbrushes and different times, showed that *C. albicans* was the only fungal species able to survive in all the times and toothbrushes assessed[13]. The authors suggest that the fact that *C. albicans* cells survived in all three toothbrush types indicates that the mouth could be inoculated from a contaminated toothbrush, that existing lesions could be aggravated, and that this fungus could be disseminated from an oral focus.

Thus the importance of the knowledge on fungus present in toothbrushes and their identification to the species level. Malmberg et al.[18] researched the microflora in 44 toothbrushes after use and showed that fungi and yeasts were present in 50% of the samples; however they did not identify the species.

In our study, *Aspergillus niger* was the most frequently found species after *C. albicans.* According Sidrim and Moreira [19], *Aspergillus* species are cosmopolitan and widely present in nature and can be found in organic waste, soil, air, the surface of living beings, etc. They produce a large number of conidia that are carried by the air and cause respiratory problems. The conidia might colonize lesions and penetrate tissues during surgical incisions. Invasive aspergillosis of immunocompromised patients has a high mortality rate, reaching 74%. The most frequent clinical presentation of the infection is pulmonary, followed by sinusitis and invasion of the CNS. The same authors describe *A. niger*, one of the species isolated from toothbrushes in this study, as a fungus that may cause external otitis and pulmonary diseases. The fact that *Aspergillus* sp. are widely distributed in nature and can easily grow on organic waste, and the isolation of *A. niger* in addition to *A. fumigatus* from toothbrushes, add to the importance of toothbrush sanitization.

In this work*, Penicillium citrinum* was the third most frequent species found colonizing toothbrush bristles. Mobin[20] mentions that *P. citrinum* is extremely common and found in the air. It causes infections of the urinary tract, lungs, ceratitis and a lethal infection in a leukemia patient is described in the literature [11].

It is important to point out that the species found with lower frequencies during this study are also capable of causing infections in immunocompromised patients and even in some immunocompetent patients. They may cause cutaneous, pulmonary and even disseminated infections [11].

Another factor that may favor fungal survival and proliferation besides lack of hygiene and subsequent residue accumulation is keeping the toothbrush wet in a closed environment. According Mialhe et al. [21] a bathroom closet is not the best place to keep a toothbrush, and boxes or bristle protectors should not be used, since they maintain a warm, moist environment that favors the growth of microorganisms, especially fungi. Toothbrushes should be kept clean, without residues, in a place where they can dry quickly and without contact with other toothbrushes [2].

Several studies have suggested the use of products to eliminate the bacteria that accumulate and proliferate in toothbrush bristles [22- 24].

Junior and Pallos [25] used a domestic microwave oven and different times of heat exposure to evaluate *in vitro* decontamination of toothbrushes previously

contaminated with three bacteria species and *C. albicans*. The results showed that all microorganisms were eliminated with a seven-minute exposure.

Chaves et al.[1] studied the bacteria survival rate and the efficacy of disinfection after spraying antibacterial solutions on the toothbrushes of kindergarten children, and concluded that 1% sodium hypochlorite provided a significant reduction of bacteria survival, more efficient than acetic acid.

The results obtained in the present study show that there was fungal growth in used toothbrushes that were not disinfected and that all isolated species are considered opportunistic and may cause problems, especially in immunocompromised patients. The fungi most frequently identified were: *Candida albicans*, *Aspergillus niger*, *Penicillium citrinum*, *Geotrichum candidum, Aspergillus fumigatus* and *Cladosporium oxysporum*. No toothbrush presented fungal growth after decontamination for at least 3 minutes.

Immersion in 2% sodium hypochlorite proved to be an efficient, low-cost method for toothbrush disinfection. Sodium hypochlorite can be easily bought at drugstores or obtained free at health centers. After disinfection, toothbrushes should be rinsed with filtered tap water to remove excess sodium hypochlorite and its taste. Many studies have described cytotoxic effect of sodium hypochlorite at different concentrations, mainly when it is used in endodontic practices[26-28]. However, an evaluation of direct cytotoxic and genotoxic effects of four commercial brands of 2 – 2.5% sodium hypochlorite solutions showed cytotoxic and genotoxic properties, which did not appear to have intensity sufficient to cause undesirable effects to the host tissues after transient contact[29]. In addition, the Health Ministry of Brazil[30] recommends the use of 2.5% sodium hypochlorite to disinfection of fruits and vegetables before their ingestion. Thus, our recommendation is safe and does not represent a limitation of this method.

ACKNOWLEDGEMENTS

The authors would like to thank the NOVAFAPI University for its support, biomedicine academics Danielle Costa Rodrigues and Ayslan Batista Barros for their help and the staff at the Microbiology laboratory.

CORRESPONDENCE

Dra.Mitra Mobin
Rua Vitorino Orthiges Fernandes, 6123 - Bairro Uruguai
64073-505, Teresina, Piauí, Brasil.
mitramobin@novafapi.com.br

REFERENCES

1. Chaves RAC, Ribeiro DML, Zaia JE, Alves EG, Souza MGM, Martins CHG, Mestriner SF. Avaliação de soluções antibacterianas na descontaminação de escovas dentais de pré-escolares. Rev Odontol UNESP 2007;36:29-33.
2. Barros OB, Pernambuco RA, Tomita NE. Escovas dentais. Pós-Grad Rev Fac Odontol São José dos Campos 2001;4:32-37.
3. JADA. Toothbrush care, cleaning and replacement. Am Dent Assoc 2006;137:415.
4. Sogi SH, Subbareddy VV, Kiran SN. Contamination of toothbrush at different time intervals and effectiveness of various disinfecting solutions in reducing the contamination of toothbrush. J Indian Soc Pedod Prev Dent 2002;20:81-85.
5. Sato, S, Ito IY, Lara EHG, Panzeri H, Albuquerque Junior RF, Pedrazzi V. Bacterial survival rate on toothbrushes and their decontamination with antimicrobial solutions. J Appl Oral Sci 2004;12:99-103.
6. Arendorf TM, Walker DM. Candida albicans: its association with dentures, plaque and the oral mucosa. J Dent Assoc S Afr 1980;2:563-568.
7. Barbeau J, Seguin J, Goulet JP, de Koninck L, Avon SL, Lalonde B, Rompré P, Deslauriers N. Reassessing the presence of Candida albicans in denture-related stomatitis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;95:51-59.
8. Santos LC. Laboratório ambiental. Paraná: Universidade Estadual do Oeste do Paraná; 1999.
9. Riddell RW. Permanent stained mycological preparations obtained by slide culture. Mycologia 1950; 42:265-70.
10. Pitt JI. A laboratory guide to common *Penicillium* species. Noth Ryde: Commonwealth Scientific and Industrial; 1985.
11. Hoog GS, Guarro J, Gené J, Figueras MJ. Atlas of clinical fungi. 2 ed. Washington: ASM Press; 2000.
12. Coutinho P G, Bittar P, Ditterich R G, Rastelli MC, Romanelli MCMOV, Wambier DS. Análise do acondicionamento e condições de escovas dentais utilizadas por pré-escolares. Rev Odonto Ciênc 2007;22:335-339.
13. Bunetel L, Tricot-Doleux S, Agnani G, Bonnaure-Mallet M. In vitro evaluation of the retention of three species of pathogenic microorganisms by three different types of toothbrush. Oral Microbiol Immunol 2000;15:313-316.
14. Zegarelli DJ. Fungal infections of the oral cavity. Otolaryngol Clin N Am 1993;26:1069-1089.
15. Kurnatowska AJ. Activity of hydrolytic enzymes of *Candida albicans* strains isolated from patients with periodontal and membrane mucosae of oral cavity diseases. Mycopathologia 1998;141:105-109.
16. Lamster IB, Grgic JT, Mitchell-Lewis DA, Begg MD, Mitchell A. New concepts regarding the pathogenesis of periodontal disease in HIV infection. Ann Periodontol 1998; 3:62-75.
17. Nikawa H, Hamada T, Yamamoto T. Denture plaque: past and recent concerns. J Dent 1998; 26:299-304.

18. Malmberg E, Birkhed D, Norvenius G, Nordn JG, Dahldn G. Microorganisms on toothbrushes at day-care centers. Acta Odontol Scand 1994;52:93-98.
19. Sidrim J JC, Moreira J LB. Fundamentos clínicos e laboratoriais de micologia médica. Rio de Janeiro: Guanabara Koogan; 1999.
20. Mobin M. Myxomycetes e fungos micófilos ocorrentes em palmeiras no Parque Nacional de Sete Cidades (Piripiri-Piauí-Brasil) 1994-1997. [Máster thesis]. Recife: Universidade Federal de Pernambuco; 1997;296.
21. Mialhe FL, Silva DD, Possobon RF. Avaliação dos cuidados relativos ao armazenamento e desinfecção das escovas dentais por acadêmicos de Odontologia. Rev Odontol da UNESP 2007;36:231-235.
22. Glass RT, Lare MM. Toothbrush contamination: a potential health risk? Quintessence Int 1986;17:39-42.
23. Caudry SD, Klitorinos A, Chan EC. Contaminated toothbrushes and their disinfection. J Can Dent Assoc 1995; 61: 511-516.
24. Neal RP, Rippin JW. The efficacy of a toothbrush disinfectant spray – an in vitro study. J Dent 2003; 31: 153-157.
25. Junior JC, Pallos D. Avaliação da esterilização de escovas dentais em forno de microondas (estudo in vitro). Rev Biociênc 2001;7:39-42.
26. Fidalgo TKS, Barcelos R, Petrópolis DB, Azevedo BR, Primo LG, Silva Filho FC. Citotoxidade de diferentes concentrações de hipoclorito de sódio sobre osteoblastos humanos. Rev Gaúcha Odontol 2009;57:317-321.
27. Kalil MV, Fidel RAS, Araujo HP, Boller MAA, Silva LE. Avaliação da citotoxicidade da solução aquosa de clorexidina a 5,0 por cento. JBE J Brás Endodontia 2004; 5: 33-37.
28. Huth KC, Jakob FM, Saugel B, Cappello C, Paschos E, Hollweck R, Hickel R, Brand K. Effect of ozone on oral cells compared with established antimicrobials. Eur J Oral Sci 2006;114:435-440.
29. Gahyva SMM, Siqueira Jr JF. Citotoxidade e genotoxicidade de quatro soluções comercias de hipoclorito de sódio avaliada pelo SOS Chromotest. Rev Paul Odontol 2001; 23:12-14.
30. Brasil, Ministério da Saúde, Agência de Vigilância Sanitária. Portaria CVS-6/99 de 10/03/99. Regulamento Técnico que estabelece os parâmetros e critérios para o controle higiênico-sanitário em estabelecimentos de alimento.
URL: http://www.sehal.com.br /vigilancia_sanitaria/legisla_cvs06_99.pdf