# Fixed Orthodontic Appliances, Clinical Aspects of Gingival Tissue and Enzymatic Activity of Candida spp.

**Hélcio Aparecido Bianchi[1], Cyra Maria Pires de Carvalho Bianchi[1], Diniz Pereira Leite-JR[1,4], Tomoko Tadano[2], Claudete Rodrigues de Paula[3], Vanessa Krummer Perinazzo-Oliviera[3], Hugo Dias Hoffmann-Santos[1,4], Rosane Christine Hahn[1,2]**

[1]Laboratory of Investigation-Laboratory of Mycology - Faculty of Medicine, Federal University of Mato Grosso (UFMT), Cuiabá, Mato Grosso, Brazil, [2]Laboratory of Mycology – University Hospital Júlio Muller (HUJM), Cuiabá, Mato Grosso, Brazil, [3]Laboratory of Pathogenic Yeasts -Faculty of Odontology/Institute of Biomedical Sciences, University of São Paulo (USP), São Paulo, Brazil, [4]University Center Várzea Grande (UNIVAG), Cristo Rei Várzea Grande, Mato Grosso, Brazil

**Abstract**

Objectives: The purpose of this study was to evaluate orthodontic appliances, the clinical appearance of gingival tissue and virulence of yeasts isolated in 80 patients. Of these, 40 belonged to the control group and 40 used orthodontic appliances. Material and methods: Yeasts were identified by both classic and automated methods (VITEK 2). Enzymatic activity (proteinases and phospholipases) were determined. Results: Among the 80 patients, *Candida spp.* was isolated in 27 (64.3%) among those who used orthodontic appliances and 15 (35.7%) among non-users. A statistically significant correlation was determined between the two groups (isolation of yeast in relation to the use of an appliance) ($p<0.05$ and OR=3.4). *Candida albicans* was the most frequent isolate (31 isolates), 17 (42.5%) in cases from the orthodontic appliances group and 14 (35.0%) in the control group. A statistically significant association was determined between the clinical appearance of the patients' gingival tissue and the presence of orthodontic appliances ($p<0.05$). Control group patients were more likely to present clinically healthy gingiva (OR=0.2). Proteinases were present in 100% of the strains from both groups, while for phospholipases, positivity was 22.5% for patients using an appliance and 15.0% for the control group. Conclusion: The use of orthodontic appliances may predispose patients to alterations in the oral microbiota, resulting in greater probability of clinically unhealthy gingiva.

*Key Words: Orthodontic appliances, Candida spp., Enzyme assays*

## Introduction

Candida yeasts are commonly detected in the oral cavity of healthy individuals without the development of any signs or symptoms of yeast. However, in certain individuals and under certain conditions, Candida spp. can become pathogenic, causing infection [1]. This pathogenicity is facilitated by the ability of some species of the genus to produce virulence factors, such as hydrolytic enzymes (proteases and phospholipases), contributing to the invasion and destruction of host tissue [2-4].

The species *C. albicans* is considered to be dominant in oral microbiota, constituting 60-70% of all isolates, followed by *C. tropicallis* and *C. glabrata* [5].

The insertion of orthodontic appliances is the most important environmental change in the oral cavity after tooth eruption, which can lead to qualitative and quantitative changes in the equilibrium of the oral microbiota, thereby promoting an increase in the population of Candida species in saliva buccal mucosa and the number of *Lactobacillus spp.* and *Streptococcus mutans* in saliva and dental plaque [6,7].

Knowing the correct methods of orthodontic appliance and oral cavity hygiene and how to disinfect appliances is fundamental. Caring for the patient, together with good dental work that conforms to biosecurity guidelines reduces the risk of opportunistic infections [8,9].

Orthodontic appliances consist of individual components that include: brackets, generally stainless steel with and without nickel, titanium; welded metal connectors, silver brazing and copper spot welding electrodes; orthodontic arches made of stainless steel, twisted steel wire, cobalt-chromium alloys, titanium alloys, beta alloys, nickel-titanium; elastomer links; and metal bands (stainless steel) for bracing [10].

These structures are ideal locations for the adhesion and proliferation of microorganisms, increasing the accumulation of plaque and calculus, which makes the region more acidogenic and can contribute to increased proliferation of colonies of Candida spp. [11-13].

According to Calderone et al. [14] and Giolo, Svidzinski et al. [15], the main virulence factors of yeasts are: self-expression of extracellular enzymes, phospholipases and proteases, which degrade host tissues; the production of toxic substances that cause cell injury; adhesion to cells and tissues; the formation of biofilms on cells and inanimate surfaces; the production of germ tubes by certain species of Candida spp.; the production of hemolysin; cell surface hydrophobicity; and resistance to hydrogen peroxide.

Several studies have demonstrated the relation between increased synthesis and the activity of extracellular enzymes, phospholipases and proteinases, with increases in the pathogenic potential of yeasts, leading to more serious clinical signs of candidiasis [15-18]. This study presents unpublished data from the central-western region of Brazil and its relevance is based on investigation into the use of orthodontic appliances, their association with the clinical appearance of patients' gingiva, and assessment of the virulence (production of proteinases and phospholipases) of yeast strains.

Corresponding author: Rosane Christine Hahn, Federal University of Mato Grosso - Faculty of Medicine - Research Laboratory. Avenue Fernando Correa da Costa, 2367 – Boa Esperança - Cuiabá/MT, Tel: +556536158809; E-mail: rchahn@terra.com.br

## Materials and Methods

### Location and study design, data collection period

This descriptive analytic cross-sectional study was conducted at the Dental Clinic of the Faculty of Dentistry, University of Cuiabá (UNIC) and *Três Américas Clinic*, both located in the City of Cuiabá, MT, in the central-western region of Brazil from July 2011 to March 2012.

### Ethical considerations

The study was approved by the Research Ethics Committee of the Júlio Müller University Hospital (IRB/HUJM/UFMT) under protocol number 953/CEP-HUJM/2010.

### Population and patients

The population consisted of 80 patients all of whom had permanent dentition and no dentures or dental implants. Selection was not based on age, sex or race. Patients were not being administered antibiotic therapy and had not used mouthwash in the 10 days prior to the trial. No special care was taken concerning guidance on diet and tooth brushing. Group A consisted of 40 patients, 42.5% males (n=17) and 57.5% females (n=23) who used fixed orthodontic appliances and Group B (control group), 40 patients, 30.0% males (n=12) and 70.0% females (n=28) who did not use orthodontic appliances.

### Inclusion criteria

Patients who were under regular orthodontic treatment.

### Exclusion criteria

Patients who did not have complete permanent dentition, prosthetic patients being administered antibiotic or immunosuppressive medication and frequent users of mouthwash.

### Sample collection

Material was collected with sterile swabs from predetermined regions: the tongue, mucous membranes inside the mouth and spontaneously secreted saliva.

### Microbiological procedures

The samples were immediately duplicate plated in sterile test tubes containing Sabouraud dextrose agar (DifcoTM, Becton, Dickinson and Company, EUA) supplemented with 100mg/L of chloramphenicol and in duplicate in test tubes containing sterile agar Mycosel (DifcoTM, Becton, Dickinson and Company, EUA) and subsequently incubated under BOD (Biochemical Oxygen Demand) for 48-72 h at 25°C. The yeast colonies were isolated and tested for purity by plating on chromogenic medium (CHROMagar®, Becton, Dickinson and Company, EUA). To identify the yeasts present, both classic (germ tube test, micromorphology, and auxanogram zymogram) and automated methods (VITEK 2 Compact Systems, Copyright 2007, bioMérieux Inc., Marcy l'Etoile, France) were used.

The strains were subjected to tests to determine *in vitro* production of exoenzymes, including proteinases and phospholipases. Protein production was assessed using the method described by Ruchel et al. [19] and phospholipase production in accordance with the recommendations of Price et al. [20].

### Analysis of clinical aspects of the gingiva

The clinical appearance of the gingiva was assessed in accordance with biological criteria for normal gingival health to determine and classify patient hygiene, as follows: unhealthy - presence of gingival exudate, pus, swelling gums, spontaneous bleeding, swelling, irregular gingival contour and the presence of substantia alba; healthy - pale, smooth, pink gums, well-bonded to the teeth and bones and lack of food deposits.

### Statistical analysis

Pearson's chi-square test was used to check for associations between categorical variables and the two proportions test to check equality of prevalence; and the Student t test for unpaired compare two independent means or its analogue nonparametric Mann-Whitney test when necessary, considering significant p value $<0.05$ in the two-tailed test. Nonparametric Spearman's Rho test was used to verify correlations.

The prevalence ratio (PR) was used to estimate the strength of association, because the odds ratio as it may overestimate the PR interfering with the inference analysis. Poisson regression with robust variance was adopted to adjust the covariates and determine the independent effect of explanatory variables on the response variable. The variables selected for this model had $\leq 0.20$ p value in the univariate analysis or biological plausibility. The validity of the model was verified by the goodness-of-fit test of Hosmer & Lemeshow. All analyzes were performed using Stata Statistical Software®, version 12.0 (College Station, TX).

## Results

There was no significant difference between the average age of Group A patients (22.7 years; CI 95%=20.2-25.2 years) and that of Group B patients (26.2 years; CI 95%=23.3-29.1 years). The average daily brushing of Group A patients (M=2.7; CI 95%=2.5-2.9) was significantly less frequent (p=0.04) than that of Group B patients (M=3.0; CI 95%=2.9-3.2). Furthermore, patients in Group A had a significantly lower frequency of average daily tongue cleaning (M=1.8; CI 95%=1.5-2.2; p=0.001) than those in Group B (M =2.6; CI 95%=2.3-2.9). Nonetheless, the frequency of toothbrush replacement among Group A patients was significantly higher (M=3.2 months; CI 95%=2.4-3.9 months) than that among Group B patients (M=3.4 months; CI 95%=3.0-3.8 months; p=0.02). There were no significant differences evident in the number of average daily flossing of between groups A and B ($p>0.05$).

Group B patients showed a moderate, positive correlation ($r=0.536$; $p<0.05$) between the number of brushings per day and the number of daily tongue brushings. A weak, but statistically significant, negative correlation (r=-0.343;

p<0.05), between the number of daily brushings and the time until replacement of toothbrushes was observed among Group A patients. In this group, there was a moderate, positive correlation (r=0.538; p<0.05) between the number of flossings per day and the number of daily tooth brushings.

Candida species were isolated in 22 patients (59.5%) who used orthodontic appliances and in 15 patients (40.5%) from the control group. A greater variation of species was also detected in the group who used orthodontic appliances. Factors associated with the use of orthodontic appliances are detailed in *Table 1*. In this univariate analysis, the incidence of *C. albicans* infection was 49% lower among Group A patients than among Group B patients (PR=0.51; p=0.01). The incidence of angular cheilitis was 143% higher among men than among women (PR=2.43; p=0.002).

***Table 1.*** *Univariate analysis of factors associated with the use of orthodontic appliances in patients selected from two dental clinics of Cuiabá, Mato Grosso, Brazil: 2011-2012.*

| **Associated factors** | **Total** | **n (%) Group A** | **n (%) Group B** | **p** |
|---|---|---|---|---|
| **Sex** | | | | |
| Female | 51 | 23 (45.1) | 28 (54.9) | |
| Male | 29 | 17 (58.6) | 12 (41.4) | 0.24 |
| **Species** | | | | |
| *C. albicans* | 26 | 12 (46.1) | 14 (53.9) | |
| *C. parapsilosis* | 4 | 3 (75.0) | 1 (25.0) | |
| *C. dubliniensis* | 6 | 6 (100.0) | 0 (0.0) | |
| *C. tropicalis* | 1 | 1 (100.0) | 0 (0.0) | 0.07 |
| **Education** | | | | |
| Elementary school | 11 | 8 (72.7) | 3 (27.3) | |
| High school | 36 | 14 (38.9) | 22 (61.1) | |
| Higher education | 33 | 18 (54.5) | 15 (45.5) | 0.11 |
| **Oral hygiene** | | | | |
| Poor | 14 | 13 (92.9) | 1 (7.1) | |
| Average | 38 | 19 (50.0) | 19 (50.0) | |
| Good | 28 | 8 (28.6) | 20 (71.4) | <0.001 |
| **Proteinase** | | | | |
| Non-producing strain | 0 | 0 (0.0) | 0 (0.0) | |
| Producing strain | 2 | 1 (50.0) | 1 (50.0) | |
| Highly producing strain | 35 | 21 (60.0) | 14 (40.0) | 0.78 |
| **Phospholipase** | | | | |
| Non-producing strain | 23 | 14 (60.9) | 9 (39.1) | |
| Producing strain | 10 | 7 (70.0) | 3 (30.0) | |
| Highly producing strain | 4 | 1 (25.0) | 3 (75.0) | 0.29 |
| Gingival bleeding | 22 | 14 (63.6) | 8 (36.4) | 0.13 |
| Angular cheilitis | 18 | 11 (61.1) | 7 (38.9) | 0.28 |
| Group A: Users of orthodontic appliances. Group B: Non-users of orthodontic appliances. | | | | |

Proteinases were present in 100% of the strains from both groups, while for phospholipases, positivity was 37.8% (n=14) of the strains. *Table 2* shows the final result of the multivariate analysis of the characteristics of the patients belonging to the two groups.

The frequency of poor oral hygiene was 286% higher among Group A patients than among Group B patients. Group A patients had an average oral hygiene that was 274% higher relative to that of Group B patients. Compared with the control group, users of orthodontic appliances showed good oral hygiene, which was 73.7% lower. The prevalence of

gingival bleeding was 66% lower among Group A patients than among Group B patients.

*Table 2. Result of Poisson regression and robust variance in relation to the use of orthodontic appliances in patients selected from two dental clinics of Cuiabá, Mato Grosso, Brazil: 2011-2012.*

| Associated factors | PR | CI 95% | p |
|---|---|---|---|
| **Oral hygiene** | | | |
| Poor | 3.86 | 1.54 - 9.66 | 0.004 |
| Average | 3.74 | 1.44 - 9.76 | 0.007 |
| Good | 1 | - | - |
| Gingival bleeding | 1.66 | 1.01 - 2.79 | 0.05 |
| PR: Prevalence Ratio. Model adjusted for sex, etiologic agent, education, proteinase, phospholipase, and cheilitis. | | | |

## Discussion

In our study, analysis of the results revealed that patients who used orthodontic appliances presented greater isolation of Candida species than non-users and that those with better oral hygiene tended to present less frequent establishment of microorganisms.

Similar results were reported by Jorge et al. [11], who investigated the presence of *C. albicans* in saliva samples collected from 266 patients, aged 7 to 25 years-old. The authors sought to associate the type of orthodontic appliance used, the patient's sex and the extent of oral health care. Their results also demonstrated greater isolation of *C. albicans* in patients who used orthodontic appliances in the control group and a lower percentage of isolation of this fungal organism in patients with better oral hygiene. We should highlight that all the patients in their study used orthodontic appliances [11].

In 1982, Addy et al. [8] affirmed that orthodontic appliances may predispose the patient to the proliferation of Candida yeasts in the oral cavity; however, the results obtained by the authors prevented them from definitively concluding that the appliances could change the patient's status from non-infected to that of positive for candidiasis.

No statistically significant differences were determined among the three groups of adolescents (12-16 years-old) studied by Addy et al. The first group consisted of adolescents who did not use appliances, the second, of those who used fixed devices and the third, of those who used removable appliances. However, the prevalence of Candida species isolation in certain sites in the oral cavity was significantly increased in two groups, those who used fixed and removable appliances [8].

A study conducted by Hägg et al. [21] investigated the accumulation of microorganisms during the use of fixed orthodontic appliances and indicated that their use can promote changes in the bacterial population, possibly due to ecological changes. The patients selected by Hägg et al. comprised a cohort including 50 Chinese individuals and of these, 27 (13 males and 14 females) were evaluated to determine whether there were any important differences in the increase of Candida yeasts and enterobacteria.

The design of the study conducted by Hägg et al. because they observed that following the placement of fixed appliances, the number of Candida species increased, determined by the imprint technique ($p<0.001$).

Even though patients submitted to treatment with orthodontic appliances were provided orientation regarding good oral health practices, researchers of the Department of Orthodontics and Oral Biosciences (University of Hong Kong) decided they required a cohort study involving a more specific group of patients to confirm their preliminary findings. Analysis of the results verified that following the insertion of orthodontic appliances, a transient increase in the population of Candida species and species of coliforms occurred during orthodontic treatment, indicating that even after oral hygiene instructions were given to the patients, no reduction in accumulated microorganisms occurred, leading to the undesirable consequences observed in the study [21].

In another study, conducted by Arslan et al. [22] in Turkey, the authors aimed to analyze the possible qualitative and quantitative changes in Candida species in adolescent patients and investigate the effects of the insertion of fixed appliances in relation to colony counts of the same at one year follow-up. Initially, 72 patients were studied in order to determine the frequency of Candida species in the oral cavity, in samples collected from the dorsal surface of the tongue, the middle region of the palate and saliva. In the second phase, fixed orthodontic appliances were inserted in 42 patients (58.5%) presenting oral carriage of Candida yeasts and new samples of saliva and from tooth surfaces adjacent to orthodontic were collected after 1, 6 and 12 months of treatment.

The group of users of orthodontic appliances presented greater isolation for all species of yeast, except *C. albicans*, suggesting the existence of other factors involved and not just the presence of the device itself (*Table 1*). The prevalence of *C. albicans* yeast isolation was 72% among patients who did not use an orthodontic appliance (PR=0.58, 95% CI=0.39-0.87, p=0.01) in the bivariate analysis.

The literature addressing this topic is scarce and we observed that the studies available generally have different designs and use different isolation and testing techniques, precluding the comparison of equivalent results [21]. However, they do show a consensus regarding the increase in the number of Candida species when fixed orthodontic appliances are used, despite recommendations regarding good oral health practices by dentists to patients before initiating treatment [23,24].

More recently, Lee et al. [25] evaluated 112 patients who used orthodontic appliances and based on multiple samples obtained over 12 months, 32% of patients were considered carriers prior to their treatment and this percentage gradually increased to 50% by month 5, remaining stable for the remainder of the observation period. However, among orthodontic patients, 11% were infected by Candida species and 25% were non-infected, while in 14% of patients, the infection had resolved during the study period.

Even though few works have studied this problem and focus on different aims, certain factors that could influence data regarding the conversion to transient carrier status in

patients who use fixed or removable appliances should be highlighted: (a) the sample size may not be adequate to observe significant differences; (b) the observation period should include sample collection prior to, during and post-treatment, rather than on a single occasion; and (c) the methods chosen for qualitative and quantitative detection may not be sufficiently sensitive to detect low density Candida species, thus leading to possible exclusion of relevant patients.

In this study, 100% of the strains isolated from patients presented proteinase enzymatic activity, independent of the use of orthodontic appliances, while phospholipases were detected in 36.4% (positive and strongly positive activity) in the group of patients who used orthodontic appliances and 40.0% (positive and strongly positive activity) in the non-user group. Previous studies have shown that 30 to 100% of strains of *C. albicans* are phospholipase secretory, with varying degrees of activity (1, negative; 2, positive; 3, strongly positive) [24-26].

The results for proteinase activity were positive for all the strains tested in this work. These findings are similar to those reported for other countries and markedly similar to those reported by Candido [27] 96.6%, and Shimizu [25], 100%. The variations observed could be related to the diversity of strains in the studies conducted [28-30].

In this study, poor oral hygiene and gingival bleeding behaved as factors associated with the use of orthodontic appliances in patients with oral candidiasis.

## Conclusion

In conclusion, the use of orthodontic appliances has an influence on alterations in the oral microbiota and on the establishment of pathogenic populations of Candida, corroborating the general consensus of the limited literature available; however, no direct influence was determined, suggesting that other factors are involved and that the use of orthodontic appliances may not even be the most important.

## Conflict of Interest

The authors declare that there are no conflicts of interest.

## References

1. Farah CS, Ashman RB, Challacombe SJ. Oral candidosis. *Clinics in Dermatology.* 2000; **18**: 553-562.

2. Penha SS, Birman EG, Silveira FRX, Paula CR. Frequency and enzymatic activity (proteinase and phospholipase) of *Candida albicans* from edentulous patients, with and without denture stomatitis. *Pesquisa Odontológica Brasileira.* 2000; **14**: 119-122.

3. Lacaz SC, Porto E, Martins JEC, Heins-Vaccari EM, Melo NT (2000) Tratado de Micologia Médica. (9thedn), São Paulo: Sarvier.

4. Moris DV, Melhem MSC, Martins MA, Mendes RP. Oral Candida spp.. colonization in human immunodeficiency virus-infected individuals. *Journal of Venomous Animals and Toxins including Tropical Diseases.* 2008; **14**: 224-257.

5. Stenderup A. Oral Mycology. *Acta Odontologica Scandinavica.* 1990; **48**: 3-10.

6. Yang Z. Phylogenetics as applied mathematics. *Trends in Ecology and Evolution.* 2003; **18**: 11.

7. Trabulsi LR, Alterthum F (2008) Microbiologia. (5thedn), São Paulo: Atheneu.

8. Addy M, Shaw WC, Hansford P, Hopkins M. The effect of orthodontic appliances on the distribution of Candida and plaque in adolescents. *Journal of Orthodontics.* 1982; **9**: 158-163.

9. Gray D, Ivicintyre G. Does oral health promotion influence the oral hygiene and gingival health of patients undergoing fixed appliance orthodontic treatment? A systematic literature review. *Journal of Orthodontics.* 2008; **35**: 262-269.

10. Mclaughlin RP, Bennett JC, Trevisi H (2004) Mecânica sistematizada de tratamento ortodôntico. (2ndedn), Artes Médicas: São Paulo.

11. Jorge AOC, Almeida NQ, Unterkircher CS, Shimizu MT. Influência do uso de aparelhos ortodônticos sobre a presença de *Candida albicans* na cavidade bucal. *Revista Da Associacao Paulista De Cirurgioes Dentistas.* 1987; **41**: 308-310.

12. Jorge AOC, Koga-Ito CY, Gonçalves CR, Fantinato V, Unterkircher CS. Presença de leveduras do gênero Candida na saliva de pacientes com diferentes fatores predisponentes e de indivíduos controle. *Revista de Odontologia da Universidade de São Paulo.* 1997; **11**: 279-285.

13. Tommasi AF (2014) Diagnóstico em Patologia Bucal. (4thedn), São Paulo: Brazil.

14. Calderone RA, Fonzi WA. Virulence factors of Candida albicans. *Trends in Microbiology.* 2001; **9**: 327-335.

15. Giolo MP, Svidzinski TIE. Fisiopatogenia, epidemiologia e diagnóstico laboratorial da candidemia. *Brazilian Journal of Pathology and Laboratory Medicine.* 2010; **46**: 225-234.

16. Ivanovska N. Phospholipases as a factor of pathogenicity in microorganisms. *Journal of Molecular Catalysis B: Enzymatic.* 2003; **22**: 357-361.

17. Mohan V, Ballal M. Proteinase and phospholipase activity as virulence factors in Candida species isolated from blood. *Revista Iberoamericana de Micología.* 2008; **25**: 208-210.

18. Furlaneto-Maia L, Specian AF, Bizerra FC, de Oliveira MT, Furlaneto MC. *In vitro* evaluation of putative virulence attributes of oral isolates of Candida spp.. obtained from elderly healthy individuals. *Mycopathologia.* 2008; **166**: 209-217.

19. Ruchel RF, de Bernardis F, Ray TL, Sullivan PA, Cole GT. Candida acid proteinases. *Journal of Medical and Veterinary Mycology.* 1992; **30**: 123-132.

20. Price MF, Wickinson ID, Gentry LO. Plate method for detection of phospholipase activity in Candida albicans. *Journal of Medical and Veterinary Mycology.* 1982; **20**: 7-14.

21. Hägg U, Kaveewatcharanont P, Samaranayake YH, Samaranayake LP. The effect of fixed orthodontic appliances on the oral carriage of Candida species and Enterobacteriaceae. *European Journal of Orthodontics.* 2004; **26**: 623-629.

22. Arslan SG, Akpolat N, Kama JD, Ozer T, Hamamci O. One-year follow-up of the effect of fixed orthodontic treatment on colonization by oral Candida. *Journal of Oral Pathology & Medicine.* 2008; **37**: 26-29.

23. Hadeel Mazin, Suzan Ali, Rasha Salah. The Effect of Fixed Orthodontic Appliances on Gingival Health. *Journal of Dental and Medical Sciences.* 2016; **15**: 82-88.

24. Goes P, Dutra CS, Lisboa MRP, Gondim DV, Leitão R, et al. Clinical efficacy of a 1% Matricaria chamomile L. mouthwash and 0.12% chlorhexidine for gingivitis control in patients undergoing orthodontic treatment with fixed appliances. *Journal of Oral Science.* 2016; **58**: 569-574.

25. Lee W, Low BK, Samaranayake LP, Hagg U. Genotypic variation of *Candida albicans* during orthodontic therapy. *Front Bioscience.* 2008; **1**: 3814-3824.

26. Freitas AOA, Marquezan M, Nojima MCG, Alviano DS, Maia LC. The influence of orthodontic fixed appliances on the oral microbiota: A systematic review. *Journal of Orthodontics.* 2014; **19**: 46-55.

27. Samaranayake LP, Raeside JM, Mcfarlane TW. Factors affecting the phospholipase activity of Candida species *in vitro*. *Sabouraudia.* 1984; **22**: 201-207.

28. Shimizu MT. Fosfolipase em espécies de Candida. *Revista de Microbiologia.* 1989; **20**: 338.

29. Ray TL, Payne CD. Comparative production and rapid purification of Candida acid proteinase from protein-supplemented cultures. *Infect Immunity.* 1990; **58**: 508-514.

30. Candido RC, Azevedo RVP, Komesu MC. Enzimotipagem de espécies do gênero Candida isoladas da cavidade bucal. *Revista da Sociedade Brasileira de Medicina Tropical.* 2000; **33**: 437-442.