A prospective randomized trial to reduce oral *Candida* spp. colonization in patients with hyposalivation

Ensaio clínico aleatório para reduzir a colonização oral de *Candida* spp. em pacientes com hipossalivação

Sandra Regina Torres[a]
Camila Bernardo Peixoto[b]
Daniele Manhães Caldas[b]
Tiyomi Akiti[c]
Maria Glória Carvalho Barreiros[c]
Milton de Uzeda[d]
Marcio Nucci[e]

[a]Assistant Professor, Department of Oral Pathology and Diagnosis, School of Dentistry; [b]Dentists, School of Dentistry; [c]Micology Laboratory technicians, University Hospital; [d]Professor, Department of Medical Microbiology, Institute of Microbiology; [e]Associate Professor, Department of Internal Medicine, University Hospital – Federal University of Rio de Janeiro.

Corresponding author:
Sandra Regina Torres
Rua Almirante Gomes Pereira, 130,
apto. 202, Urca. Rio de Janeiro - RJ - Brazil
CEP: 22291-170
E-mail: *sandratorres@ufrj.br*

Received for publication on Mar 09, 2006
Sent for alterations on May 08, 2006
Accepted for publication on Dec 04, 2006

Abstract: Low salivary flow rates are associated with higher oral *Candida* spp. counts, which may predispose to oral candidiasis. The aim of this study was to compare the effect of stimulating salivary flow rates with that of a regimen of chlorhexidine mouth rinse on the intensity of *Candida* colonization in patients with reduced salivary flow rates. Thirty-one outpatients were randomized to stimulate salivary output (group 1) or to receive chlorhexidine mouth rinses (group 2). Evaluations were performed at baseline (T_0), at end of treatment (T_1), and 15 days after last day of treatment (T_2). Chewing-stimulated whole saliva samples were collected at each visit. Group 1 showed a constant reduction in median cfu counts, although the difference was significant only between T_0 and T_2 ($p = 0.004$). Group 2 showed a reduction in median *Candida* cfu counts between T_0 and T_1 ($p = 0.01$), but the counts increased at T_2 ($p = 0.01$), and the difference between T_0 and T_2 was not significant ($p = 0.8$). In conclusion, patients who received salivary stimulation showed reductions of *Candida* cfu counts in saliva and a trend for increasing salivary flow rates between baseline and end of study evaluations. The use of chlorhexidine mouth rinses dramatically reduced *Candida* cfu counts, but when patients discontinued treatment, intensity of colonization rose again.
Descriptors: Saliva; *Candida*; Xerostomia; Homeostasis; Colony count, microbial.

Resumo: O fluxo salivar reduzido está associado a maior quantidade de *Candida* spp. na boca, predispondo a candidíase. O objetivo deste estudo foi comparar o efeito da estimulação salivar ao efeito do uso de bochechos de clorexidina sobre a intensidade de colonização por *Candida* em pacientes com fluxo salivar reduzido. Trinta e um pacientes de ambulatório foram aleatoriamente incluídos nos protocolos de estimulação salivar (grupo 1) ou de bochecho com clorexidina (grupo 2). As avaliações foram realizadas no dia inicial (T_0), ao final do tratamento (T_1) e 15 dias após o final do tratamento (T_2). A cada consulta foram coletadas amostras de saliva total estimulada. O grupo 1 mostrou uma redução constante nas contagens medianas de UFC de *Candida*, embora a diferença estatística tenha sido apenas entre T_0 e T_2 ($p = 0{,}004$). O grupo 2 mostrou redução nas contagens de UFC de *Candida* entre T_0 e T_1 ($p = 0{,}01$), mas a contagem de UFC aumentou em T_2 ($p = 0{,}01$), sendo a diferença entre T_0 e T_2 não significante ($p = 0{,}8$). Concluiu-se que os pacientes que realizaram procedimentos de estimulação salivar apresentaram a quantidade de UFC de *Candida* salivar reduzida, além de apresentarem tendência ao aumento do fluxo. O uso de bochechos de clorexidina reduziu drasticamente a quantidade de UFC de *Candida* salivar, mas após o final do tratamento houve novo aumento.
Descritores: Saliva; *Candida*; Xerostomia; Homeostase; Contagem de colônia microbiana.

Introduction

Candida spp. are frequent colonizers of the oropharynx in humans, and high salivary *Candida* counts may predispose to oral candidiasis.[6,28,29] It has been shown that low salivary flow rates (SFR) are associated with higher oral *Candida* counts.[23,28,29] Therefore, increasing salivary output in subjects with low SFR could reduce oral *Candida* counts. Attempts to increase SFR include the use of sialogogue medications,[13,27] as well as clinical procedures such as encouraging chewing[14] and gustatory exposure.[27] Other measures to reduce colonization by *Candida* include use of antimicrobial mouth rinses.[12,20] In this study we evaluated the effect of stimulating SFR on the intensity of *Candida* colonization in patients with reduced SFR and high salivary *Candida* colony forming units (cfu) counts, and compared this strategy with a regimen of chlorhexidine mouth rinse, in a prospective randomized fashion.

Material and Methods

Patients' population

This was a randomized trial in which two methods for reducing *Candida* spp. oral colonization were compared. Outpatients from the Dental School and from the University Hospital, Federal University of Rio de Janeiro (UFRJ), were randomly selected to answer a questionnaire about xerostomia.[29] Patients who answered "yes" to at least one of the questions of the questionnaire were invited to participate in the study. Clinical and laboratory evaluations were performed, and patients who presented SFR < 1.0 ml/min[25] and *Candida* spp. cfu counts ≥ 400 cfu/mL[6] were included in the study. Exclusion criteria were: patients with oral candidiasis; patients with chewing-stimulated SFR ≥ 1.0 ml/min; patients with *Candida* cfu counts in saliva < 400 cfu/ml, and patients who received corticosteroids and antifungal agents. There were 124 patients evaluated, and 39 fulfilled the entry criteria. Twenty-three patients were randomized to group 1, and 16 patients to group 2. After randomization, 8 patients were excluded for the following reasons: in group 1, one patient started antifungal therapy and 4 patients dropped the study before second evaluation; in group 2, three patients dropped the study before second evaluation. Characteristics of the 31 evaluable patients (18 patients in group 1 and 13 patients in group 2) are shown in Table 1. All patients signed an informed consent. The study was approved by institutional ethical committee.

Table 1 - Baseline clinical characteristics of 31 patients randomized in the two groups.

Variables		Group 1 n (%)	Group 2 n (%)
Number of patients		18 (58)	13 (42)
Median age (range)		59 (40-77)	48 (30-81)
Gender* 6 Males: 25 Females		1 (6): 17 (94)	5 (38): 8 (62)
Dental prosthesis		10 (56)	7 (54)
Underlying diseases	Cardiovascular	13 (72)	7 (54)
	Gastrointestinal	5 (28)	2 (15)
	Allergy	3 (17)	3 (23)
	Neurological	4 (22)	1 (8)
	Diabetes	3 (17)	-
	HIV	-	3 (23)
	Hepatitis C	1 (6)	4 (31)
	Sjögren's Syndrome	1 (6)	2 (15)
	Cancer	2 (11)	1 (8)
	Osteoporosis	2 (11)	-
	Thyroid alterations	1 (6)	-
	Other	8 (44)	6 (46)
Concomitant medications	Antihipertensive	6 (33)	4 (31)
	Diuretics	6 (33)	3 (23)
	Antiaggregants	3 (17)	1 (8)
	Betablockers	4 (22)	1 (8)
	Antibiotics	2 (11)	2 (15)
	Antivirals	-	3 (23)
	Tranquilizers	3 (17)	1 (8)
	Analgesics	4 (22)	-
	Other	8 (44)	4 (31)
	No medications	2 (11)	2 (15)
	Median salivary flow rates, mL/min (range)	0.50 (0.06-0.96)	0.36 (0.01-0.78)
	Median *Candida* spp. counts, cfu/mL** (range)	1,905 (500-82,000)	21,700 (600-85,200)

Note: Patients had more than one underlying disease and some used more than one medication. *p = 0.05; **p = 0.02; p values non significant for all other comparisons.

Study therapies

Patients were randomly assigned to one of two groups:

- Group 1 - Patients were instructed to stimulate salivary output during 15 days by drinking 2 L of water daily, chewing meals intensely, chewing sugarless gum[1,14,27] (Trident©, São Paulo, SP, Brazil) three times a day, and chewing ginger flakes[27] (Ardrak©, Hidrolândia, GO, Brazil) three times a day. Patients with a past history of gastritis were asked to use sugarless candies (Flópi©, Lajeado, RS, Brazil) instead of gum,[24] and those who were hypertensive received raw ginger root instead of the salted ginger flakes.[11]
- Group 2 - Patients were given non-labeled 300 ml of a 0.12% chlorhexidine solution[12,20] (Periogard©, São Paulo, SP, Brazil), and were asked to rinse twice a day with 10 ml of the solution, during 1 minute, after 30 minutes of having performed oral hygiene procedures, after breakfast and supper, for 15 days.

Periogard©, Trident© sugarless gum, and Ardrak© ginger flakes were obtained from the manufacturer.

Evaluation

Baseline evaluation consisted of medical history, clinical examination, sialometry and microbiological analysis. Samples of chewing-stimulated whole saliva were obtained under standard conditions.[29] Saliva samples were collected between 9:00 AM and 11:00 AM, and no feeding, drinking, smoking or hygienic habits were allowed for 120 minutes prior to test section. Only the liquid component (not the foam) of saliva was measured. The SFR were determined as milliliters per minute. The samples of saliva were kept in a refrigerated recipient and taken to the Oral Microbiology Laboratory, UFRJ, within 2 hours.[28] The samples where heated at 55°C for 2 minutes to disaggregate whole saliva components and facilitate microbial recovery, and were homogenized in a vortex (Supermixer©, Melrose Park, IL, USA).[28] A 0.1 ml sample of saliva was plated onto CHROMagar Candida© (Paris, France) and incubated at 37°C for 72 hours. Total and colony-color-specific cfu were counted. One representative cfu of *Candida* of each color was isolated and *Candida albicans* was identified on the basis of germ tube formation, chlamydospore formation in cornmeal agar, and growth at 37.8°C and 45.8°C on Sabouraud agar.[21] The identification of other *Candida* species was performed at the Mycology Laboratory, University Hospital, UFRJ, and based on characteristic patterns of fermentation and assimilation of carbohydrates.[30]

Evaluations of SFR and *Candida* cfu counts in saliva were performed at baseline (T_0), at end of treatment (T_1), and at end of study (15 days after the last day of treatment - T_2). To measure the reduction in *Candida* cfu counts we used the differences (Δ) between median cfu counts at each period of collection, for each study group ($\Delta_1=T_0-T_1$; $\Delta_2=T_1-T_2$; $\Delta_3=T_0-T_2$). The same procedures were done to measure the differences in SFR.

Statistical analysis

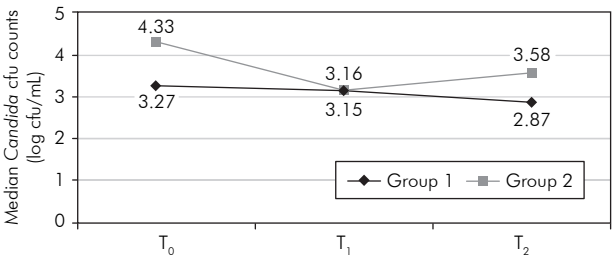
Categorical variables were analyzed by the chi-square or Fisher's exact test. The Wilcoxon test was used for comparison of unpaired continuous variables. Registration and analysis of the data were done using Epi-Info 6.0 software (Centers for Disease Control, Atlanta, GA, USA) and SPSS 10.0 for Windows (SPSS Inc., 1989-1999, Chicago, IL, USA).

Results

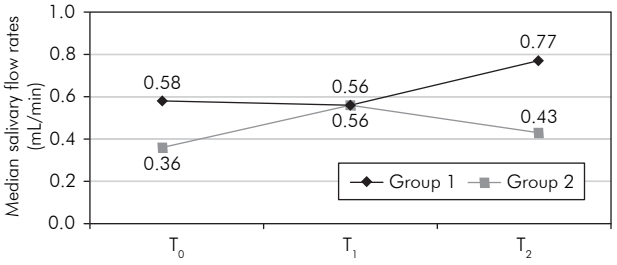
There were no significant differences between the two groups with regard to age, underlying diseases, use of concomitant medications, dental prosthesis wearing, and salivary flow rates. There were more females in group 1 ($p = 0.05$). Group 2 presented significantly higher cfu *Candida* counts ($p = 0.02$).

The three most frequent species found at baseline were *C. albicans* (87%), *Candida parapsilosis* (39%), and *Candida tropicalis* (13%). There were no significant differences in the number of patients colonized by each species.

As shown in Graph 1, there was a constant reduction in the median cfu counts from T_0 to T_1 and T_2, in group 1, although the difference reached statistical significance only between T_0 and T_2 (Δ_3 $p = 0.004$). On the other hand, group 2 showed a marked reduction in the median cfu counts between T_0 and T_1 (Δ_1



Graph 1 - Median *Candida* cfu counts (log cfu/mL) at the three periods of collection.



Graph 2 - Median salivary flow rates (mL/min) at the three periods of collection.

Table 2 - Median cfu *Candida* spp. counts (cfu/ml) of the 31 patients, at the three periods of collection.

Species	Group 1						Group 2					
	T_0		T_1		T_2		T_0		T_1		T_2	
	n	cfu	n	cfu	n	cfu	n	cfu	n	cfu	n	cfu
C. albicans	14	1,890	13	1,690	11	1,310	13	3,300	10	1,245	8	1,895
C. parapsilosis	9	420	7	520	3	630	3	1,400	1	140	1	40
C. tropicalis	1	20	1	30	1	60	3	13,360	2	2,025	2	113,610
C. krusei	2	95	-	-	1	30	1	300	-	-	-	-
C. norvegensis	-	-	-	-	-	-	1	2,240	-	-	-	-
C. glabrata	-	-	1	5,020	-	-	1	400	1	4,400	1	29,200

n = number of patients colonized. Some patients were colonized by more than one species. Differences for cfu counts: Group 1: *C. albicans*: Δ_1 p = 0.24; Δ_2 p = 0.11; Δ_3 p = 0.13. *C. parapsilosis*: Δ_1 p = 0.62; Δ_2 p = 0.60; Δ_3 p = 0.07. Group 2: *C. albicans*: Δ_1 p = 0.01; Δ_2 p = 0.04; Δ_3 p = 0.32. *C. parapsilosis*: Δ_1 p = 0.27; Δ_2 p = 0.32; Δ_3 p = 0.14.

p = 0.01), but at T_2, there was an increase in median cfu counts (Δ_2 p = 0.01), and the difference in median cfu counts at baseline (T_0) and end of study (T_2) was not statistically significant (Δ_3 p = 0.8).

Differences in SFR were also measured at each period of collection, for each group. Graph 2 shows SFR at the three periods of collection. In group 1, no statistical differences were seen in median SFR from T_0 to T_1 (Δ_1 p = 0.15) and from T_1 to T_2 (Δ_2 p = 0.72), but there was a trend for increasing SFR between the baseline and end of study evaluations (Δ_3 p = 0.07). In group 2, median SFR were higher from T_0 to T_1 (Δ_1 p = 0.33) and reduced from T_1 to T_2 (Δ_2 p = 0.40). Comparing T_0 to T_2, there was a trend for higher SFR at end of study in this group (Δ_3: p = 0.07).

We analyzed the counts of most frequent *Candida* species (Table 2). In group 1, there were no statistically significant differences in cfu counts of *C. albicans* at the three periods, whereas for *C. parapsilosis*, there was a trend for increasing the intensity at end of study comparing to baseline. In group 2, there was a significant reduction in *C. albicans* counts at T_1 comparing to T_0, but like total cfu counts, it rose again at end of study. Regarding *C. parapsilosis*, there was no significant difference comparing the three evaluations.

Discussion

This study showed that the use of chlorhexidine mouth rinses dramatically reduced *Candida* cfu counts, but after stopping the rinses, there was an increase in cfu counts, and comparing baseline and end of study cfu counts, the difference was not statistically significant. On the other hand, salivary stimulation (group 1) resulted in a constant reduction in *Candida* cfu counts, although less intense than in group 2 (Graph 1).

Regarding SFR, patients who were instructed to stimulate salivary output had an increase in SFR ($p = 0.07$) comparing baseline and end of study evaluations. Surprisingly, patients assigned to receive chlorhexidine mouth rinses also had an increase in SFR ($p = 0.07$) comparing baseline and end of study evaluations. Therefore, the significant and long-lasting reduction in *Candida* cfu counts observed in group 1 may be explained by an increase in salivary flow rates.

Xerostomia has been reported as a side effect of chlorhexidine use, but no study evaluated the effect of chlorhexidine on SFR.[2] We don't have a clear explanation for the increase in SFR observed at end of study in group 2, but we suppose that these patients may have changed their habits, incorporating practices that increase SFR, such as chewing gums or candies, drinking more water, even if they were not instructed to do so. Unfortunately we did not evaluate this possible bias.

Another interesting observation of the present study is the increase in SFR that occurred after discontinuation of salivary stimulation in group 1 patients. A possible explanation for this result is the possibility that once salivary glands are stimulated, the output continues to increase even after ceasing the stimulus. Indeed, some studies have shown a long-term effect of gum-chewing in SFR.[1,14]

Stimulating the output of SFR seems to enhance oral homeostasis, and thus promote natural protection. Continuous salivary flow protects by its cleansing effect and by the antimicrobial action of salivary proteins.[5] Many salivary proteins have activity against *Candida*.[15,19] Histatin is a peptide that shows potent candidacidal effect.[16] Moreover, secretory IgA inhibits *Candida* adherence to oral mucosa.[4] It has been demonstrated that chewing increases the secretion of IgA, as well as other salivary proteins.[22] Therefore, it is possible that patients who stimulated salivary output had an increase in salivary IgA and proteins, and this exerted protection against *Candida*. Further studies evaluating sialochemistry should be carried out in order to support our hypothesis.

Ginger (*Zingiber officinale*), one of the gustatory stimulants of group 1, is used mainly for oral and gastric disturbances.[8] Some studies have investigated antimicrobial *in vitro* activities of ginger,[10,18] but there has been no clinical trial conducted to investigate its antimicrobial and salivary stimulant properties.

Candida susceptibility to chlorhexidine has been evaluated in recent studies.[3,17,20] In denture plaque biofilms, *Candida* has shown better response to chlorhexidine than to fluconazole and miconazole.[17] However, clinical studies using chlorhexidine for prophylaxis of oral candidiasis in chemotherapy or radiotherapy patients have shown conflicting results.[7,9] Resistance to chlorhexidine has been reported in some phenotypic resistant subpopulations of *C. albicans*.[5,26] In the present study, the effect of chlorhexidine was evaluated in the two most frequent species of *Candida*, but the small number of patients hampers any conclusion regarding this issue.

These results may have important clinical and experimental implications. First, health care workers may apply these measures in order to stimulate salivary output and to reduce *Candida* colonization. Furthermore, clinical and laboratory research must be performed to study the effects of salivary stimulation on sialochemistry.

Conclusion

Patients with reduced salivary flow rates and high *Candida* cfu in saliva that received salivary stimulation showed reduction of *Candida* cfu counts in saliva and a trend for increasing SFR between baseline and end of study evaluations. The use of chlorhexidine mouth rinses dramatically reduced *Candida* cfu counts, but when patients finished treatment, the intensity of colonization rose again.

Acknowledgements

We wish to thank the staff personnel of the University Hospital and Dental School, Federal University of Rio de Janeiro (UFRJ), for referring patients; the Dental School students, who applied the questionnaires; and Fernando A. C. Magalhães, for the laboratorial support.

References

1. Aagaard A, Godiksen S, Teglers PT, Schiodt M, Glenert U. Comparison between new saliva stimulants in patients with dry mouth: a placebo-controlled double-blind crossover study. J Oral Pathol Med. 1992;21(8):376-80.
2. Albandar JM, Gjermo P, Preus HR. Chlorhexidine use after two decades of over-the-counter availability. J Periodontol. 1994;65(2):109-12.
3. Bhende S, Spangler D. *In vitro* assessment of chlorhexidine gluconate-impregnated polyurethane foam antimicrobial dressing using zone of inhibition assays. Infect Control Hosp Epidemiol. 2004;25(8):664-7.
4. Cannon RD, Nand AK, Jenkinson HF. Adherence of *Candida albicans* to human salivary components adsorbed to hydroxylapatite. Microbiology. 1995;141(Pt 1):213-9.
5. Chandra J, Mukherjee PK, Leidich SD, Faddoul FF, Hoyer LL, Douglas LJ *et al*. Antifungal resistance of candidal biofilms formed on denture acrylic *in vitro*. J Dent Res. 2001;80(3):903-8.
6. Epstein JB, Pearsall NN, Truelove EL. Quantitative relationship between *Candida albicans* and the clinical status of human subjects. J Clin Microbiol. 1980;12(3):475-6.
7. Epstein JB, Vickars L, Spinelli J, Reece D. Efficacy of chlorhexidine and nystatin rinses in prevention of oral complications in leukemia and bone marrow transplantation. Oral Surg Oral Med Oral Pathol. 1992;73(6):682-9.
8. Felippe Junior J. Ansiedade. Estratégia Biomolecular. Disponível em: *http://www.medicinacomplementar.com.br/pagina_include/ansiedade.shtm*
9. Ferretti GA, Raybould TP, Brown AT, Macdonald JS, Greenwood M, Maruyama Y *et al*. Chlorhexidine prophylaxis for chemotherapy- and radiotherapy induced stomatitis: A randomized double-blind trial. Oral Surg Oral Med Oral Pathol. 1990;69(3):331-8.
10. Ficker C, Smith ML, Akpagana K, Gbeassor M, Zhang J, Durst T *et al*. Bioassay-guided isolation and identification of antifungal compounds from ginger. Phytother Res. 2003;17(8):897-902.
11. Fodor JG, Whitmore B, Leenen F, Larochelle P. Lifestyle modifications to prevent and control hypertension. 5. Recommendations on dietary salt. Canadian Hypertension Society, Canadian Coalition for High Blood Pressure Prevention and Control, Laboratory Centre for Disease Control at Health Canada, Heart and Stroke Foundation of Canada. CMAJ. 1999;160(9 Suppl):S29-34.
12. Giuliana G, Pizzo G, Milici ME, Giangreco R. *In vitro* activities of antimicrobial agents against *Candida* species. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1999;87(4):44-9.
13. Grisius MM. Salivary gland dysfunction: A review of systemic therapies. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2001;92(2):156-62.
14. Jenkins GN, Edgar WM. The effect of daily gum-chewing on salivary flow rates in man. J Dent Res. 1989;68(5):786-90.
15. Jorge AOC, Totti MAG, Almeida OP, Scully C. Oral candidiasis established in the sialoadenectomised rat. J Oral Pathol Med. 1993;22(2):54-6.
16. Kavanagh K, Dowd S. Histatins: antimicrobial peptides with therapeutic potential. J Pharm Pharmacol. 2004;56(3):285-9.
17. Lamfon H, Al-Karaawi Z, McCullough M, Porter SR, Pratten J. Composition of *in vitro* denture plaque biofilms and susceptibility to antifungals. FEMS Microbiol Lett. 2005;242(2):345-51.
18. Lopez P, Sanchez C, Batlle R, Nerin C. Solid- and vapor-phase antimicrobial activities of six essential oils: susceptibility of selected foodborne bacterial and fungal strains. J Agric Food Chem. 2005;53(17):6939-46.
19. Mandel ID. The role of saliva in maintaining oral homeostasis. J Am Dent Assoc. 1989;119(2):298-304.
20. Meiller TF, Kelley JI, Jabra-Rizk, DePaola LG, Abdullahel Baqui AAM, Falkler WA. *In vitro* studies of the efficacy of antimicrobials against fungi. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2001;91(6):663-70.
21. Pinjon E, Sullivan D, Salkin I, Shanley D, Coleman D. Simple, inexpensive, reliable method for differentiation of *Candida dubliniensis* from *Candida albicans*. J Clin Microbiol. 1998;36(7):2093-5.
22. Proctor GB, Carpenter GH. Chewing stimulates secretion of human salivary secretory immunoglobulin A. J Dent Res. 2001;80(3):909-13.
23. Radfar L, Shea Y, Fischer SH, Sankar V, Leakan RA, Baum BJ *et al*. Fungal load and candidiasis in Sjogren's syndrome. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003;96(3):283-7.
24. Soreide E, Holst-Larsen H, Veel T, Steen PA. The effects of chewing gum on gastric content prior to induction of general anesthesia. Anesth Analg. 1995;80(5):985-9.
25. Sreebny LM, Baum BJ, Edgar WM, Epstein JB, Fox PC, Larmas M. Saliva: Its role in health and disease. Int Dent J. 1992;42(4)(Suppl. 2):291-304.
26. Suci PA, Tyler BJ. A method for discrimination of subpopulations of *Candida albicans* biofilm cells that exhibit relative levels of phenotypic resistance to chlorhexidine. J Microbiol Methods. 2003;53(3):313-25.
27. Tarzia O. Halitose. Rio de Janeiro: EPUC; 1996.
28. Torres SR, Peixoto CB, Caldas DM, Silva EB, Akiti T, Nucci M *et al*. Relationship between salivary flow rates and *Candida* counts in subjects with xerostomia. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2002;93(2):149-54.
29. Torres SR, Peixoto CB, Caldas DM, Silva EB, Magalhaes FA, Uzeda M *et al*. Clinical aspects of *Candida* species carriage in saliva of xerotomic subjects. Med Mycol. 2003;41(5):411-5.
30. Warren NG, Hazen KC. *Candida*, *Cryptococcus* and Other Yeasts of Medical Importance. *In*: Murray PR, Barron EJ, Pfaller MA, Tenover FC, Yolken RH. Manual of Clinical Microbiology. Washington: ASM Press; 1995. p. 723-32.