Prevalence of Oral *Candida* Species in a North American Pediatric Population

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Oral candidiasis caused by species other than Candida albicans has been observed. This study evaluated the prevalence of oral yeast species among 196 children during routine oral exam. Based on standard mycological testing, 130 (66%) subjects had fungal growth. Candida albicans isolates were recovered in 56% of children, but an extensive diversity in the non-albicans species was observed. Intrinsic differences in the pediatric population may favor the presence of yeast species other than C. albicans

Key words: children, candida albicans, candida dubliniensis, North American

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INTRODUCTION

Candida albicans is the fungal species most frequently isolated as an oral colonizer and pathogen.[1] However, among the immunocompromised population, a drastic increase in the incidence of oral candidiasis caused by other less pathogenic *Candida* species has been observed over the last decade including the newly identified species *Candida dubliniensis*.[2-8]

Candida albicans is a member of the indigenous microbial flora present in small numbers in the oral cavity of a large proportion of normal individuals where its growth is normally suppressed by other microorganisms.[1,9] However, *C. albicans* is an opportunistic pathogen capable of causing a variety of infections ranging from the common such as denture stomatitis and thrush to the more serious systemic infections.[6,10-14]

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The prevalence and persistence of *Candida* in the oral cavity have been associated with increased carbohydrate intake and increased levels of salivary steroids, especially glucocorticoids.[15-17] Oral candidiasis, on the other hand, has been associated with a deficiency of many dietary factors (e.g. iron, zinc, vitamin K and several of the water-soluble vitamins).[15-17] Other predisposing factors to oral candidiasis include infancy and old age, xerostomia, poor oral hygiene, orthodontic appliances, mouth breathing, endocrine disturbance, antibiotics and steroids, HIV and immunosuppressive drugs.[18-21] In addition, candidiasis can sometimes manifest as an adverse effect of certain drug therapies such as the use of topical corticosteroids in the treatment of bronchial asthma, a condition relatively common in children.[22] Malnutrition specifically, particularly in the young has been hypothesized as playing a role in the development of *Candida* infections, in that a higher carrier rate of yeasts was detected in the mouths of subjects with a poor diet.[3,16] In general, children have been shown to have high percentage of yeast possibly reflecting the effect of poor diet among the studied population groups.[3,23] In this study, the prevalence and diversity of *Candida* carriage among a seemingly healthy group of children in the United States at the University of Maryland pediatric dental clinic was evaluated.

MATERIALS AND METHODS

Pediatric patients of low socio-economic status that attended routine oral exams were included in the study. Following IRB approval, informed consent from the parents or legal guardians and clinical data was obtained on each of the children participating in the study. Over a period of one and a half year, 196 children were evaluated. The children ranged in age from 1 to 3 years and were equally divided between females and males. The patients' charts indicated past histories of diaper rash (7%), oral candidiasis (6%), anemia (2%), facial rash (2%), and isolated cases of neutropenia, tracheomalacia, rickets, eczema. Although antibiotic use was reported, no antibiotic therapy was administered within two weeks prior to sampling. No current health

problems were reported at the time of sample collection.

Oral samples were obtained from the mid-dorsum of the tongue with a sterile swab and immediately used to inoculate Sabouraud's Dextrose Agar (SDA; DIFCO Laboratories, Detroit, MI) plates. Cultures were incubated at 37ºC for 48 h and evaluated daily for growth of yeast colonies. Yeast isolates growing on original cultures were speciated based on standard mycological methods including germ tube formation in serum at 37ºC for 3 h, chlamydospore production on corn meal agar and ability to grow at 45ºC to screen for *C. dubliniensis* (unlike *C. albicans*, *C. dubliniensis* fails to grow at 45ºC). Isolates were grown on CHROMagar Candida medium (Becton Dickinson, Baltimore, MD), for speciation based on colony color.

RESULTS

Among the 196 children tested, 130 (66%) were colonized by a variety of yeast species varying from light to very heavy fungal growth. A striking observation, however, was the extensive diversity in the yeast species recovered. While the majority of the isolates consisted mainly of *C. albicans* (73) (56%), other less frequently encountered species were seen: 30 (23%) *Candida glabrata*, 19 (14.6%) *Candida tropicalis*, 5 (3.8%) *Candida krusei*, 5 (3.8%) *Candida parapsilosis*, 1 (<1%) *Candida lusitainiae*, 2 (1.5%) *Saccharomyces cerevisiae*, with one patient harboring *C. dubliniensis*. Nine of the yeast positive children simultaneously harbored two or more different species; 2 patients had *C. albicans* and *C. topicalis*, 1 had *C. albicans* and *C. glabrata* and 1 had *C. albicans*, *C. glabrata* and *C. tropicalis* recovered from the same culture.

Three of the positive patients demonstrating heavy *C. albicans* growth had a history of topical antifungal therapy, one with *C. albicans* and *C. tropicalis* recovered had a history of antibiotic therapy and one patient had *C. albicans dermatidis*. More interestingly, however, was the recovery of *C. dubliniensis*, a species almost exclusively associated with an immunocompromised state. The patient with heavy colonization of *C. dubliniensis* was a 2 and a half year old child with documented history of thrush.

DISCUSSION

Sixty-six percent of the 196 children aged 1 to 3 years old, had fungal growth during routine oral exam. Most of the isolates were *C. albicans*, but there were 64 mixed or isolated cases of a variety of other *Candida* species, including the newly identified *C. dubliniensis*. This high prevalence and diversity in the species recovered suggest that intrinsic differences in the pediatric population may favor the presence of yeast species other than *C. albicans*.

Published epidemiological findings so far report the detection of *C. dubliniensis* isolates at low incidence levels in normal healthy individuals (< 3%).[4,24] In previous studies in our laboratory, a large-scale screening of 202 healthy adult population only one individual was found to harbor *C. dubliniensis* (<1%), confirming the low incidence level of this species among healthy populations.[4] Screening of normal pediatric population, on the other hand, *C. dubliniensis* was recovered from 4 of the 30 children screened (13.3 %).[3, 23] The increased prevalence of *C. dubliniensis* among healthy children in comparison to the adult population could be attributed to an immature immune system.

A similar study compared the presence and prevalence of yeast species in the oral cavities of 64 malnourished Nigerian children to that in 30 HIV+ and non-HIV+, age and sex-matched group of American children.[3] In that study, the frequency of yeast in the Nigerian group was found to be considerably higher than that of the United States group, similar to what was noted by Aldred *et al.*[25] The yeast recovered from the American children, both HIV+ and non-HIV+, consisted mainly of *C. albicans* and *C. dubliniensis* and only one *C. glabrata* whereas the Nigerian group harbored more of the less pathogenic and less frequently encountered yeast species, with six children having a combination of different species simultaneously.[3] In a similar study by Basu *et al.* (26), 77% of malnourished Indian children were colonized with yeast compared to 27% of children who were not suffering from malnutrition, suggesting that malnutrition might favor the presence of yeasts and *Candida* species other than *C. albicans*.

Several factors are considered to play an important role in oral colonization and infection by *Candida* species. HIV infections and AIDS, however, remain the main predisposing factors to candidiasis in both adult and pediatric populations. Numerous studies have shown candidiasis to be one of the most common oral lesions in children.[23,27,28] In fact, in HIV infected individuals, the onset of oral candidiasis is considered a marker for the beginning of AIDS.[27,28]

In addition, a study evaluating the most common diseases of the oral mucosa in children attributed 6% of lesions to oral candidiasis.[29] *Candida* species were also frequently present in breastfeeding children probably due to their immature immune system and their passage through the birth canal.[30] In a study by Alamoudi *et al.*, the presence of yeast in resting and stimulated saliva was high in general, with > 50% of children having high fungal counts.[18,20,31-35] These observations suggest that intrinsic differences between different populations including factors such as malnutrition, vitamin deficiency and high corticosteroid levels[15] might favor the presence of the less frequently recovered and less pathogenic yeast species.

The distribution of oral yeast species and etiological patterns of invasive *Candida* spp infections were also shown to have geographical specificity.[20,35] For example, *C. glabrata* was most often found in North America, while *C. tropicalis* and *C. parapsilosis* more frequently isolated in South America.[11,36] Therefore, species identification and awareness of epidemiological trends of the various *Candida* species are essential for the selection of appropriate therapeutic strategies.[11,37]

The chromogenic medium CHROMagar Candida is currently routinely used in the clinical microbiology laboratories for speciation of yeast directly from patient samples. Studies evaluating its use as a differential medium for species identification demonstrated rates of mixed colonization varying between 26.3% and 60%.[20,38-40] Therefore, in addition to consideration of the characteristics of the populations evaluated, the method used for processing patient samples may influence the frequency of detection of mixed colonization.

In our sample population, no oral lesions consistent with oral thrush were documented at the time of sampling in any of the patients and no major underlying conditions predisposing to oral candidiasis were reported in their medical charts. No correlation

was observed in regard to variables like age, gender or any other conditions reported in the medical charts between the patients with and without fungal colonization. Although no apparent predisposing factors are noted, the fact that our pediatric population consisted mainly of inner city children of low socio-economic status, supports the notion that malnutrition and poor oral hygiene may have contributed to the high prevalence and diversity of fungal colonization in this sample population.[3,15-17] A similar study in a matched pediatric population of higher socio-economic status would validate these speculations.

Candidal colonization of the oral cavity is a prerequisite to candidiasis (thrush). Colonization of the oral tissues depends on several factors which alter the oral microbiota increasing the risk for opportunistic infections.[9,25,40] The disparity in the observed levels of candidal colonization and species diversity warrants furthering depth investigations into the factors that influence oral *Candida* colonization particularly in pediatric populations. Recognizing the various factors and conditions that play a role in candidal colonization and the progression of colonization to infection by the various *Candida* species will greatly contribute to our understanding of fungal pathogenesis. Such crucial information will have important clinical implications as it aids in the identification and the design of novel therapeutic strategies aimed at the prevention and/or treatment of fungal infections.

REFERENCES

1. Calderone, R. A. *Candida* and candidiasis. ASM Press, Washington, D.C. 2002.
2. Jabra-Rizk, M. A., Baqui, A. A. M. A., Kelley, J. I., Falkler, W. A., Jr., Merz, W. G., and Meiller, T. F. Identification of *Candida* dubliniensis in a prospective study of patients in the United States. J Clin Microbiol 37: 321-326, 1999.
3. Jabra-Rizk, M. A., Falkler, W. A., Jr., Enwonwu, C. O., Onwujekwe, D. I., Merz, W. G., and Meiller, T. F. Prevalence of yeast among pediatric populations in Nigeria and the United States. Oral Microbiol Immunol 16: 383-385, 2001.
4. Jabra-Rizk, M. A., Falkler, W. A., Jr., Merz, W. G., Baqui, A. A. M. A., Kelley, J. I., and Meiller, T. F. Retrospective identification of *Candida* dubliniensis among Candida albicans clinical laboratory isolates from HIV and non-HIV individuals. J Clin Microbiol 38: 2423-2426, 2000.
5. Jabra-Rizk, M. A., Ferreira, S. M. S., Sabet, M., Falkler, W. A., Merz, W. G., and Meiller, T. F. Recovery of *Candida* dubliniensis and other yeast from human immunodeficiency virus-associated periodontal lesions. J Clin Microbiol 39: 4520-4522, 2001.
6. Jabra-Rizk, M. A., Johnson, J. K., Forrest, G., Mankes, K., Meiller, T. F., and Venezia, R. A. Prevalence of *Candida dubliniensis* fungemia at a large teaching hospital. Clin Infect Dis 41: 1064-1067, 2005.
7. Sullivan, D. J. Presentation: molecular epidemiological analysis of Candida dubliniensis, a newly described pathogen. 98th General Meeting of the American Society of Microbiology. Atlanta, GA, 1998.
8. Wingard, J., Merz, W., and Saral, R. Candida tropicalis: a major pathogen in immunocompromised patients. Ann Intern Med 91: 529-543, 1979.
9. Cannon, R. D., and Chaffin, W. L. Oral colonization by *Candida albicans*. Crit Rev Oral Biol Med 10: 359-383, 1999.
10. Blumberg, H. M., Jarvis, W. R., Soucie, J. M., Edwards, J. E., Patterson, J. E., Pfaller, M. A., Rangel-Frausto, M. S., Rinaldi, M. G., Saiman, L., Wiblin, R. T., Wenzel, R. P., and Group, N. S. Risk factors for candidal bloodstream in fections in surgical intensive care unit patients: the NEMIS Prospective Multicenter Study. Clin Infect Dis 33: 177-186, 2001.
11. Colombo, A. L., and Guimaraes, T. Epidemiology of hematogenous infections due to *Candida* spp. Rev Soc Bras Med Trop 36: 599-607, 2003.
12. Meis, J. F. G. M., Ruhnke, M., De Pauw, B. E., Odds, F. C., Siegert, W., and Verweij, P. E. *Candida dubliniensis* candidemia in patients with chemotherapy-induced neutropenia and bone marrow transplantation. Emerg Infect Dis 5: 150-153, 1999.
13. Mukherjee, P. K., Zhou, G., Munyon, R., and Ghannoum, M. A. Candida biofilm: a well-designed protected environment. Med Mycol 43: 191-208, 2005.
14. Walsh, T. J., and Groll, A. H. Emerging fungal pathogens: evolving challenges to immunocompromised patients for the twenty-first century. Transpl Infect Dis 1: 247, 1999.
15. Enwonwu, C. O., and Meeks, V. I. Oral candidiasis, HIV, and aliva glucocorticoids. Am J Pathol 148: 1313-1318, 1996.
16. Reed, B. D., Slattery, M. L., and French, T. K. The association between dietary intake and reported history of *Candida* vulvovaginitis. J Family Pract 29: 509-515, 1989.
17. Samaranayake, L. P. Nutritional factors and oral candidosis. J Oral Pathol 15: 61-65, 1986.
18. Jorge, A. O. C., Koga-Ito, C. Y., Goncalves, C. R., Fantinato, V., and Unterkircher, C. S. Presence of *Candida* genus yeast in the saliva of patients with different predisposing factors and of control individuals. Rev Odontol Univ Sao Paulo 11: 279-285, 1997.
19. Oksala, E. Factors predisposing to oral yeast infections. Acta Odontol Scand 48: 71-74, 1990.
20. Torres, S. R., Peixoto, C. B., Caldas, D. M., Silva, E. B., Magalhaes, F. A. C., Uzeda, M., and Nucci, M. Clinical aspects of *Candida* species carriage in saliva of xerotomic subjects. Med Mycol 41: 411-415, 2003.
21. Torres, S. R., Peixoto, C. B., Caldas, D. M., Silva, L. R., Akiti, T., Nucci, M., and Uzeda, M. Relationship between salivary flow rates and *Candida* counts in subjects with xerostomia. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 93:149-154, 2002.
22. Buhl, R. Local oropharyngeal side effects of inhaled corticosteroids in patients with asthma. Allergy 61: 518–526, 2006.
23. Brown, D. M., Jabra-Rizk, M. A., Falkler, W. A., Baqui, A. A. M. A., and Meiller, T. F. Identification of Candida dublin- iensis in a prospective study of HIV-seropositive pediatric dental patients. J Pediatr Dent 22: 234-238, 2000.
24. Sullivan, D. J., Moran, G., Donnelly, S., Gee, S., Pinjon, E., McCartan, B., Shanley, D. B., and Coleman, D. C. *Candida dubliniensis*: an update. Rev Iberoam Micol 16: 72-76, 1999.

25. Aldred, M. J., Arendorf, T. M., Wade, W. G., Tschoepe, G. A., and Brownlow, N. P. Frequency and density of yeasts in the mouths of malnourished children. Comm Dent Oral Epidem 17: 136-138, 1989.

26. Basu, R., Basu, N., and Banerjee, A. K. Incidence of *Candida* in the oral cavity. Bull Calcutta Sch Trop Med 9: 20-21, 1961.

27. Rioboo-Crespo, M. R., Planells del Pozo, P., and Rioboo-Garcia, R. Epidemiology of the most common oral mucosal diseases in children. Med Oral Patol Oral Cir Bucal 10: 376-387, 2005.

28. Ramos-Gomez, F. Dental considerations for the paedriatic AIDS/HIV patient. Oral Dis 8: 49-54, 2002.

29. Bessa, C. S., PJ. Aguiar, MC. do Carmo MA. Prevalence of oral mucosal alterations in children from 0 to 12 years old. J Oral Pathol Med 33: 17-22., 2004.

30. Zöllner, M. S. A. C., Jorge, A. O. C. *Candida* spp. occurrence in oral cav ities of breast feeding in fants and in their mothers' mouths and breasts. Braz Oral Res 17: 151-155, 2003.

31. Alamoudi, N., Farsi, N., Faris, J., Masoud, I., Merdad, K., and Meisha, D. Salivary characteristics of children and its relation to oral microorganism and lip mucosa dryness. J Clin Pediatr Dent 28: 239-248, 2004.

32. Stenderup, A. Oral mycology. Acta Odontol Scand 48: 3-10, 1990.

33. Kleinegger, C. L., SR. Vargas, K. Soll, DR. Frequency, intensity, species, and strains of oral Candida vary as a function of host age. J Clin Microbiol. , 34 2246–2254, 1996.

34. Main, B. E., Calman, K. C., Ferguson, M. M., Kaye, S. B., MacFarlane, T. W., Mairs, R. J., Samaranayake, L. P., Willox, J., and Welsh, J. The effect of cytotoxic therapy on saliva and oral flora. Oral Surg Oral Med Oral Pathol 58: 545-548, 1984.

35. Qi, Q. H., T. Zhou, XD. Frequency, species and molecular characterization of oral *Candida* in hosts of different age in China. J Oral Pathol Med 34: 352–356, 2005.

36. Redding, S. D., MC. Kirkpatrick, WR. Coco, BJ. Patterson, TF. Fothergill, AW. Rinaldi, MG. Thomas, CR Jr. *Candida glabrata* is an emerging cause of oropharyngeal candidiasis in patients receiving radiation for head and neck cancer. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 97: 47-52, 2004.

37. Bassetti, M., Righi, E., Costa, A., Fasce, R., Molinari, M. P., Rosso, R., Pallavicini, F. B., and Viscoli, C. Epidemiological trends in nosocomial candidemia in intensive care. BMC Infect Dis 10: 1-6, 2006.

38. Beighton, D., Ludford, R., Clark, D. T., Brailsford, S. R., Pankhurst, C. L., Tinsley, G. F., Fiske, J., Lewis, D., Daly, B., Khalifa, N., Marren, V., and Lynch, E. Use of CHROMagar Candida medium for isolation of yeasts from dental samples. J Clin Microbiol 33: 3025-3027, 1995.

39. Kindelan, S. A., Yeoman, C. M., Douglas, C. W. I., and Franklin, C. A comparison of intraoral carriage in Sjogren's syndrome patients with healthy xerostomic controls. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 85: 162-167, 1998.

40. Lockhart, S. R., Joly, S., Vargas, K., Swails-Wenger, J., Enger, L., and Soll, D. R. Natural defenses against Candida colonization breakdown in the oral cavities of the elderly. J Dent Res 78: 857-868, 1999.