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Full Length Research Paper

Prevalence of *Candida* species in the oral cavity of patients with periodentitis

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During the past two decades, there has been a significant increase in the prevalence of fungal infections caused by *Candida* species. Oral candidiasis is a common opportunistic infection of the oral cavity caused by yeast fungi of the genus *Candida* on the mucous membranes of the mouth. To isolate and determine the incidence rate of oral *Candida* species in periodentitis and gingivitis patients referred to school of dentistry, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, this study was carried out in 172 patients with periodentitis and gingivitis aged 11 to 72 years. Swabs samples were taken from salivary secretion, the palate mucosa and dentine carious lesions and were cultured directly on Sabouraud dextrose agar medium. Isolated yeasts were identified by CHROMagar *Candida*, germ tube test and Clamidoconidia formation (corn meal agar plus Tween 80 medium). Results showed the prevalence of *Candida albicans* (n = 120, 75%), *Candida glabrata* (n = 20, 12.5%), *Cadida tropicalis* (n = 10, 6.5%), *Candida dubliniensis* (n = 6, 4.0%) and *Cadida krusei* (n = 3, 2.0%). In this investigation, germ tube-test and chlamydospore formation were positive in the isolates that produced dark-green colonies and were considered as *C. dubliniensis* and light-green colonies were identified as *C. albicans.* CHROMagar *Candida* is a satisfactory isolation medium for oral and dental specimens. It is a satisfactory method for correct and rapid identification of common yeast species and easy recognition of mixed cultures in clinical samples.

Key words: Periodentitis, gingivitis, oral candidiasis, CHROMagar.

INTRODUCTION

The oral cavity is inhabited by more than 700 microbial species and many intrinsic and extrinsic factors affect the composition, metabolic activity and pathogenicity of the highly diversified oral microflora (Samaranayake et al., 2002; Aas et al., 2005). This fact has been correlated mainly to the use of broad-spectrum antibacterials, corticosteroids, anti-tumoral agents, oral contraceptives and increase in the number of immunocompromised patients (Eggimann et al., 2003). Yeasts, especially *Candida* spp. are the normal oral flora and their isolation from the mouth can be investigated in excessive consumption of fermentable carbohydrates (Samaranayakeet al., 1986), dental caries risk and denture-wearing status (Beightonet et al., 1991). *Candida* is not harmful in healthy hosts, but may cause opportunistic infections in immunocompromised hosts, such as patients suffering from AIDS, leukemia and diabetes. Oral candidiasis, which is frequently caused by *Candida albicans*, is one of the most common fungal opportunistic infections in immunocompromised patients (Klein et al., 1984). In the majority of clinical investigations, yeasts are routinely cultured on Sabouraud dextrose agar (SDA). This media is reliable for isolation of yeasts but overall, the colonies on SDA media are very similar in appearance and their subsequent identification in laboratory is required. CHROMagar *Candida* (CaC) is a selective and differential chromogenic medium for rapid screening of clinical specimens for identification of *Candida* spp. CaC contains various substrates for the enzymes of yeast species. It has been demonstrated that β-N-acetylgalactosaminidase which was produced by *C. albicans* enables the chromogenic substrates to be incur-

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Table 1. Detection of *Candida* species in 160 surveillance cultures using CHROM.

Number of positive culture	Colony color on CHROMagar *Candida*	GTT	GFT	Growth at 45°C	*Cadida* spp. Identification
120 (75%)	Light green	+	+	Good	*C. albicans*
20 (12.5%)	Pale edges on dark pink (purple)	–	–	ND	*C. glabrata*
10 (6.5%)	Dark blue with halo	–	–	ND	*C. tropicalis*
6 (4.0%)	Dark green	+	+	ND	*C. dubliniensis*
3 (2.0%)	Fuzzy, rough, large, pink	–	–	ND	*C. krusei*
23 (14.5%)	mixtures				*C. albicans* plus *C. tropicalis*
7 (4.5%)	mixtures				*C. albicans* plus *C. glabrata*

GTT, Germ tube test; CFT, clamidoconidia formation test; ND, not determined.

porated into the medium. Another potential advantage of chromogenic media is the easy identification of mixed yeast infections (Hospenthal et al., 2002; Lopez-Ribot et al., 1999; Willinger and Lewis, 2001). Importantly, many non-*albicans Candida* have decreased susceptibility to antifungal agents. Specifically, *Candida krusei* and *Candida glabrata* demonstrate decreased susceptibility to fluconazole (Lynnet al., 2003). Clinicians now depend on identification of *Candida* species for accurate selection of antifungal agent and to provide the best treatment possible to the patient. The aim of this study was to evaluate the prevalence of *Candida* spp. among oral isolates from patients with periodentitis and gingivitis using CaC (CHROMagar, Paris, France) medium to study the yeast populations of the microbiological samples obtained.

MATERIALS AND METHODS

Clinical samples

One hundred and seventy two samples of oral swabs were taken from salivary secretion of the palate mucosa and dentine carious lesions from patients with periodentitis referred to the educational clinics of Dentistry school, Ahvaz Jundishapur University of Medical Sciences and Dentistry clinics, Ahvaz, Iran. Of the tested oral samples, 37.5% were from males (n = 64) and 62.5% were from females (n = 108) with an age range of 11 to 72 years. All oral swabs were placed into test tubes containing 2 ml of sterile normal saline solution.

Culture media

A 100-µl aliquot of the undiluted sample (David et al., 1995) was spread onto agar plates containing SDA (Merck, Germany) supplemented with chloramphenicle (SC) prepared according to the manufacturer's instructions and uniformly spreading by using a sterile bent glass rod. Plates were incubated at 37°C for 48 h. All isolated yeasts were identified by CHROMagar *Candida*, CaC medium (CHROMagar, Paris, France). Both colony color evaluation and phenotypic identification included germ tube formation at 37°C in bovine serum, clamidoconidia formation growth in corn meal-Tween 80 agar (Sandven, 1990; Williams and Lewis, 2000) and growth at 45°C. It is important to notice that *Candida dubliniensis* is unable to grow at 45°C temperature, according to Venitia, et al. (2002). According to the description given by Kathrin et al. (2000), Chlamydoconidia formation in *C. dubliniensis* occurs in clusters or pairs on short branching pseudohyphae, while in *C. albicans*, it occur singly on elongated pseudohyphae. Germ tube-positive isolates that produced dark-green colonies were suspiciously considered for *C. dubliniensis*, while light-green colonies were presumptively identified as *C. albicans* (Schoofs et al., 1997).

RESULTS

One hundred and sixty samples obtained from the oral cavities of 172 patients with periodentitis and gingivitis was positive for oral candidal infection (93.0%). Candidal infection was registered both in females (n = 110; 64%) and in males (n = 62; 36%). Among these specimens, identification of yeasts revealed *C. albicans* (120, 75%), *C. glabrata* (20, 12.5%), *Cadida tropicalis* (10, 6.5%), *C. dubliniensis* (6, 4.0%) and *C. krusei* (3, 2.0%). All *C. dubliniensis* cases (6, 4.0%) demonstrated negligible or no growth at 45°C. It was found that 30 (18.5%) of 160 positive cultures contained mixtures of *Candida* species and the most common mixtures observed in the present study were either *C. albicans* plus *C. glabrata* or *C. albicans* plus *C. tropicalis* (Table 1 and Figures 1 and 2).

DISCUSSION

In agreement with findings of others (Back-Brito et al., 2009), the majority of yeast isolates from oral cavity swabs were *C. albicans* (75%), but it was often recovered in association with other yeasts. The higher prevalence of isolates of *C. glabrata* which is the second most common yeast isolated in this survey, is similar with reports of the literature (Houang et al., 1997). Overall, chromogenic media have been reported to enable the identification of mixed cultures (Pfaller et al., 1996; Willinger and Manafi, 1999). Cultures that contained mixtures of *Candida* species were found and this has also been reported by other researchers (Louwagie et al., 1995; Moyer et al., 1995; Odds and Bernaerts, 1994). The most common mixtures observed in the present study were *C. albicans* plus *C. glabrata* or *C albicans* plus *C. tropicalis*. Thus,

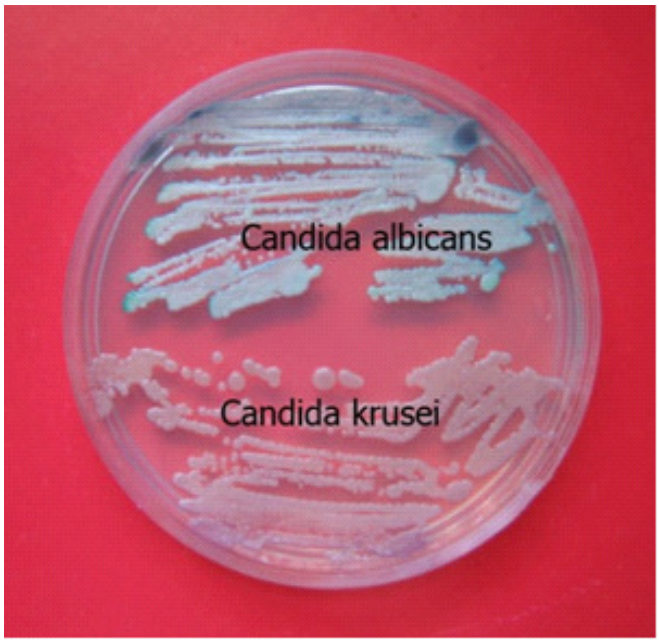

Figure 1. *C. albicans* showing characteristic light green color and *C. krusei* showing fuzzy rose-colored agar on CHROMagar.

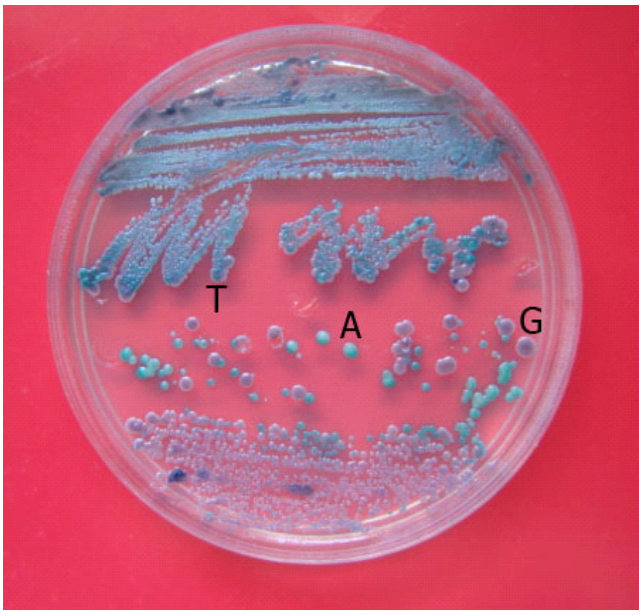

Figure 2. Colony characterized of T: *C. tropicalis*, A: *C. albicans* and G: *C. glabrata* on CHROMagar.

CHROMagar not only facilitates the detection of mixed cultures but also detects species of isolates within the mixture without the need of additional subcultures.

In this investigation, the most common association was *C. albicans* plus *C. tropicalis*, which was detected in 14.5% of the samples containing mixed fungal population, while the most common association was *C. albicans* plus *C. glabrata* (46.5% according to Pfaller et al., 1996). In this study, 6 isolates were seen as dark green colonies and identified as *C. dubliniensis*. Willinger et al. (2001) also reported that some of *C. dubliniensis* isolates yielded a dark green color. This is an important point to note, however, this species is very rarely encountered in clinical specimens. In this study, twenty isolates of *C. glabrata* produced pink, glossy colonies with pale edges on the pink colonies. Pfaller and Houston (1996) and Willinger et al. (2001) concluded that CaC also allowed the identification of *C. glabrata*.

In conclusion, the use of this medium could allow mycology laboratories to rapidly identify *Candida* spp. in clinical samples (Ainscough and Kibbler, 1998). More importantly, this capability will also enable clinicians to more rapidly make appropriate antifungal choices, decreasing patient morbidity and mortality.

In spite of its greater cost in comparison with SDA, CHROMagar *Candida* medium is a satisfactory isolation medium for oral cavity specimens, allowing rapid and correct identification of yeast colonies and easy recognition of mixed cultures. Thus, it is easy to use, would save staff time and is suitable for routine use in clinical mycology laboratories.

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