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Original Article

The biofilm production (SLIME) and fluconazole sensitivity of the C*andida* strains isolated from the mouth flora of newborns and adults

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Abstract. In this study we planned to investigate the differences of the Candida species in the newborns' and adults' mouth flora, their slime production and antimicrobial susceptibility. They were identified with conventional methods (germ tube production) and carbohydrate fermentation characteristics were evaluated by Api 20 C AUX (bioMerieux-France) kits. Stock solution of fluconazole was used for antibiotic susceptibility test. A total of 18 Candida isolates; 14 *C. albicans*, 2 *C. tropicalis*, 1 *C. kefyr* and1 *C. lusitaneae* were isolated from newborns and 18 Candida strains consisting of 13 *C. albicans*, 4 *C. tropicalis* and 1 *C. ciferii* were isolated from the adults with decayed teeth. Slime production and fluconazole susceptibility tests were performed on these strains. As a result, 4 of 18 (22%) *Candida* species from newborns and 2 of 18 (11%) strains from adults teeth were observed to be potent slime producer. None of the Candida strains isolated were resistant to fluconazole.

Keywords: Candida, biofilm, fluconazole, mouth flora

1. Introduction

Candida species are known as a member of the normal mouth flora and opportunistic pathogens. Candidiasis is an important and frequently seen disease which affects the mouth flora. Recently some of the Candida strains are accepted to be a cause of tooth decay (1).

It can be seen with several local and systemic diseases. Insufficiency of saliva secretion, poor hygiene of the mouth, prosthesis, inhalation sprays with corticosteroid, long term antibiotic treatment, diabetes, systemic steroid treatment, HIV infection, lymphoma, leukemia, and anemia are predisposition factors for the onset of oral candidiasis (2, 3, 4).

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Candidas are also members of the normal flora of the skin, throat, gastrointestinal tract and vagina. If for any reason the circumstances change in their environment and they increase in numbers or transfer to another environment without a flora they could invade and cause a disease. The colonized *Candida* elicits a granulomatous inflammation after penetrating in the tissue with pseudohyphae. The slime production in *Candidas* is an important pathogenity factor which plays a role for the adherence to the tissue and colonization of the C*andida* and protects the microorganism from host defences (2, 5).

Limited data exist on biofilm formation by *C. albicans* on dental surfaces. Enamel, cementum and dentine, in the absence or presence of a smear layer, are readily colonized by this microorganism. Microbial (slime) and host factors (fibrin and fibroinectn) both contribute in biofilm formation (6,7).

Although several species of *Candida* can infect the oral mucosa, the most commonly encountered oral fungal agent is *C. albicans*, which may be

highly infective because of its greater level of pathogenicity and adherence properties. *C. albicans* is an oral commensal in as many as 40% to 65% of healthy adults. The papillated dorsal surface of the tongue and palatal mucosa beneath a maxillary denture are favored reservoir sites (8).

Fluconazole is used in treatment against most *Candida* spp. and *Cryptococcus neoformans*. Among *Candida* spp., *C.krusei* is intrinsically resistant to fluconazole. While some of the *C. glabrata* strains may be dose-dependent susceptible to fluconazole, as many as 15% of *C. glabrata* strains may exhibit true resistance. Strains of *C. tropicalis*, *C. norvegensis*, *C. dubliniensis*, and *C. inconspicua* occasionally generate high fluconazole MICs. In addition, fluconazole-resistant strains of any *Candida* spp. are occasionally observed. Acquired resistance to fluconazole even among *C. albicans* isolates may occur following long-term fluconazole prophylaxis (9).

The purpose of this study is to detect and identify the *Candida* species found in the mouth flora of newborns and on decayed teeth surfaces of adults to indicate their slime production and to detect their resistance to fluconazole.

Table 1

The Slime test results of the *Candida* species isolated from newborns

Candida species	(n)	Slime positivity		
		4 (+)	2(+)	negative
C. albicans	(14)	3	8	3
C. tropicalis	(2)	-	1	1
C. kefyr	(1)	-	-	1
C. lusitaneae	(1)	1	-	-
Total	(18)	4	9	5

2.Material and method

The specimens were obtained from oral secretions of newborns from Pediatrics Unit of Yuzuncu Yil University Medical Faculty, and from the surface of decayed teeth of 18 years and adults from the Dental Care Unit of Van State Hospital. The patients from whom the specimens were collected have not been exposed to prior fluconazole.

Adults' teeth were sampled with sterile toothpicks with tapered ends and the newborns' whole unstimulated saliva samples about one-two ml were collected in sterile disposable syringe without needle. All the samples were processed within two hours in the laboratory. Samples were centrifuged at vortex mixer for 20 seconds for homogenization. All the samples were inoculated to brain hearth infusion broth in order to transport after then the samples were inoculated to Sabouraud's Dextrose Agar (BBL-USA) medium. After 24-48 hours of incubation at 35^{o}C, the isolated *Candida* species were evaluated for their carbohydrate fermentation and germ tube forming abilities (10). *C. albicans* strains were identified with germ tube production, and carbohydrate fermentation characteristics were detected using API 20 C AUX (bioMerieux-France) kits. These identified strains were all from the strains that colonized in the mouth flora.

Slime Production: Slime production was determined by using a modified tube adherence test (2). A loopful of organisms from the surface of Sabouraud-Dextrose-Agar plates was inoculated into the tubes containing 10 ml of Sabouraud-Broth supplemented with glucose (final concentration 8%). The tubes were incubated at 35^{o}C for 24 hours, the cell suspension in the tubes were poured out and washed with distilled water two times. After dying with 1 % safranine, the viscid layer produced on the walls of the tubes were interpreted as slime production (positive) (7).

Stock solution of 5,120 µg/ml fluconazole (Pfizer Inc. UK-O49858) in sterile water was used for antibiotic susceptibility test. Serial dilutions were prepared in RPMI 1640 medium. Test concentrations were diluted from 64µg/ml to 0.125 µg/ml. M27-A broth microdilution method of NCCLS (National Committee for Clinical Laboratory Standards) was used as the susceptibility test procedure. At least 5 colonies of *Candida* were suspended in 0.9 % NaCl. 0.5 McFarland standard (from $1x10^6$ to $5x10^6$) was adjusted using Becton Dickonson colorimetric device. The stock solution was diluted first in 1:50 then in 1:20 in RPMI 1640 and 2x test concentrations were obtained. 100 µl of *Candida* solutions with last concentrations of 0.5×10^3 to $2.5x10^3$ CFU/ml were added to microplates so the final volume was adjusted to be 200 µl. The microplates were incubated at 35^{o}C for 48±2 hours and after incubation the results of any recovery were recorded. The MIC of fluconazole was defined as the lowest concentration at which there was 80% inhibition of growth compared with that in the drug-free control well (11).

Table 2

The Slime test results of the *Candida* strains isolated from the surfaces of the decayed teeth of 18 years and older adults

Candida strains	(n)	Slime positivity		
		4(+)	2(+)	negative
C. albicans	(13)	-	10	3
C. tropicalis	(4)	2	1	1
C. ciferii	(1)	-	-	1
Total	(18)	2	11	5

3.Results

A total of 18 *Candida* isolates; 14 *C. albicans*, 2 *C. tropicalis*, 1 *C.kefyr* and 1 *C. lusitaneae* were isolated from 50 samples obtained from newborns. Another 18 *Candida* isolates consisting of 13 *C. albicans*, 4 *C. tropicalis* and 1 *C. ciferii* were isolated from 50 samples from the surfaces of decayed teeth of 18 years and older age group. First the Slime test and afterwards fluconazole susceptibility test were performed on the species. The test results were shown in Tables 1, 2, 3.

In the newborns group, the MIC values of the 16 *Candida* isolates (88%) out of 18, indicated that these species were susceptible to fluconazole (MIC<16) and 2 species (12%) were fluconazole dose-dependent susceptible (MIC=16 to 64) and none of the species were resistant to fluconazole, MIC_{50}:0.125, MIC_{90} :8.

In the adults group, the MIC values indicated that all the *Candida* species were susceptible to fluconazole, MIC_{50} :0.125, MIC_{90}:0.125. As a conclusion none of the *Candida* strains isolated from both newborns and adults groups were resistant to fluconazole.

4.Discussion

Pathogenic fungi in the genus *Candida* can cause both superficial and serious systemic diseases, and are now recognized as one of the major agents of hospital-acquired infections. Many *Candida* infections involve the formation of biofilms on implanted devices such as indwelling catheters or prosthetic heart valves. Biofilms of *C. albicans* formed in vitro on catheter material consist of matrix-enclosed microcolonies of yeasts and hyphae, arranged in a bilayer structure (12,13).

Table 3

The Fluconazole MIC values

Newborns		Adults	
1. *C. albicans*	0.125	1. *C. albicans*	0.125
2. *C. kefyr*	0.125	2. *C. albicans*	0.125
3. *C. albicans*	0.125	3. *C. tropicalis*	0.125
4. *C. albicans*	8	4. *C. tropicalis*	0.125
5. *C. albicans*	16	5. *C. tropicalis*	0.125
6. *C. tropicalis*	0.125	6. *C. albicans*	0.125
7. *C. albicans*	32	7. *C. albicans*	0.125
8. C. albicans	1	8. *C. albicans*	0.125
9. *C. albicans*	0.125	9. *C. ciferii*	0.125
10. *C. lusitenae*	0.125	10. *C. albicans*	0.125
11. *C. albicans*	4	11. *C. albicans*	0.125
12. *C. albicans*	0.125	12. *C. albicans*	0.125
13. *C. albicans*	0.125	13. *C. albicans*	0.125
14. *C. albicans*	0.125	14. *C. albicans*	0.125
15. *C. albicans*	0.125	15. *C. tropicalis*	0.125
16. *C. tropicalis*	0.125	16. *C. albicans*	0.125
17. *C. albicans*	8	17. *C. albicans*	8
18. *C. albicans*	0.125	18. *C. albicans*	0.125

In a study of Yüce and colleagues (7), on 63 *Candida* strains isolated from hospitalized patients, potent slime positivity was reported to be 1.6%. In another study performed by Hilmioğlu and colleagues (5), the Slime production rates of *Candida* species were compared using various techniques and it was reported that from a total of 215 *Candida* isolates, 104 isolates (48%) were Slime positive with SGL (Sabouraud with glucose liquid) and BHISG (brain heart infusion solution with glucose) methods and 114 isolates were Slime positive with CRBHIL (Congo red brain heart infusion liquid) method.

In our study the Slime positivity was rated as 2(+) or 4(+). The 4(+) result indicates a potent Slime formation whereas 2(+) result indicate a slight Slime formation. From 4 of 18 (22%) *Candida* species isolated from mouth flora of the newborns in newborn intensive care unit and from 2 of 18 (11%) species isolated from the surfaces of decayed teeth of adults, a potent slime production was observed. The potent slime positivity rates obtained in our study were concordant with similar studies and considered to be an important finding because of the fact that

slime production is a special feature of *Candida* pathogenity (14).

The reported fluconazole resistance rates of *Candida* species isolated from different body regions from several studies are as follows; vulvovaginal 30%, intensive care unit equipments 27.31%, various sites of the body 6.8%, oropharingeal 5%, vaginal 3.6%, different sites of women 0.8%, blood 0.7%, urine 0% (15-23). In these studies fluconazole resistance rates were generally reported very low, changing between 0%-30%. In our study none of the *Candida* isolates were resistant to fluconazole.

In an earlier study dose-dependent susceptibility to fluconazole was found to be 1.6% and antifungal resistance was not detected (24).

Fluconazole has a strong effect so it was the most effective drug against the several *Candida* species studied (25). Compared with other treatment strategies, fluconazole was shown to be superior to nystatin suspension for the treatment of oral thrush in otherwise healthy infants (26).

In a study; fluconazole showed the greatest activity, and amphotericin B showed the least activity against biofilm cells. These findings were confirmed by scanning electron microscopy of the biofilms (27). Slime production renders a microorganism to be more resistant to external environment. In our study it was shown that some *Candida* species produced biofilm but the antibiogram performed indicated that these species were susceptible to fluconazole.

Our study shows that the *Candida* encountered mostly as opportunistic pathogens in various clinics and especially in intensive care units could be treated with fluconazole in adequate dose and time and although there is an increase in slime producing *Candida* species, fluconazole could be a reliable treatment choice for these microorganisms.

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