Tropical Journal of Pharmaceutical Research October 2013; 12 (5): 821-825
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v12i5.24

Original Research Article

Investigation of Association between Slime Production by *Candida Spp* and Susceptibility to Fluconazole and Voriconazole

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Received: 17 November 2012 *Revised accepted:* 4 June 2013

Abstract

Purpose: *To determine the susceptibilities of fluconazole and voriconazole based on slime production by Candida spp.*
Methods: *Candida strains (115) isolated in the period between January 2011 and January 2012 were included in this study. Conventional methods were used for the identification. Candida albicans and non-C. albicans isolates were tested for slime production with modified tube adherence test and antifungal resistance with disk diffusion method.*
Results: *Slime positivity was 31.3 % in all Candida species. Slime positivity in non-C.albicans isolates (44.89 %) was higher than in C. albicans species (21.21 %). All C. albicans isolates were sensitive to fluconazole and voriconazole. The highest resistance to fluconazole (40 %) and voriconazole (5%) was by C. glabrata strains.*
Conclusion: *Species definition and determination of antifungal susceptibility patterns are advised for the proper management and treatment of patients.*

Keywords: *Candida, Fluconazole, Voriconazole, Antifungal susceptibility, Slime*

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INTRODUCTION

Candida spp. are members of normal flora and are also oppurtunistic pathogens that can cause serious systemic infections especially in immuncompromised patients. *Candida* infections have increased in the last two decades because of immunosuppresive treatments, long-term catheterisation, prolonged use of broad-spectrum antibiotics,cancer treatments and HIV infections. Various factors play a role in the pathogenesis of *Candida* infections, and slime production in Candida species is an important virulance factor which is associated with adherence to the surface of catheters and biomedical devices, and thus protects microorganisms from host defences. Slime (biofilm)-producing *Candida* species are known to be more resistant to immune response and antifungal agents which leads to treatment failure [1,2].

The aim of this study was to determine the relationship between slime production by *Candida spp* and susceptibility to fluconazole and voriconazole.

EXPERIMENTAL

Media/chemical agents

Sabouraud dextrose agar (SDA, Merck), Brain Heart Infision Broth (Plasmatec), Safranin (Merck) were used in this study.

Strains

A total of 115 *Candida* species, isolated from various clinical samples in the Clinical Microbiology Laboratory of Ankara Dışkapı Yıldırım Beyazıt Training Hospital, were included in this study. *Candida* species isolated from the same patient were excluded. Prior to being tested, all strains were subcultured twice on Sabouraud dextrose agar (SDA) to ensure viability and purity. For the identification of the isolates, conventional methods were used such as germ tube formation, microscopic morphology on cornmeal-Tween 80 Agar as well as commercial methods such as CHROMagar Candida. *Candida dubliniensis* isolates were identified on the basis of their initial dark green colony color on CHROMagar. If the species couldn't be identified by these methods they were classified as *Candida spp.* *C.albicans* ATCC 10231,reference strain was also included in this study.

Slime production

Slime production was determined using a modified tube adherence test. A loopful of organisms from the surface of a Sabouraud dextrose agar plate was inoculated into a polystyrene falcon tube containing 10 ml of Sabouraud broth supplemented with glucose (final concentration, 8%). The tubes were incubated at 35 °C for 24 h. The cell suspension in the tubes were poured out and washed with distilled water two times. After dying, 1 % safranine, the tubes were examined for the presence of the viscid slime layer. Slime production by each isolate was scored as negative, weak positive (1+), moderate positive (2+ or 3+) and strong positive(4+). Each isolate was tested at least three times and each tube was scored independently by two observers.

Antifungal agents and susceptibility test

Fluconazole (25 µg, Oxoid) and voriconazole (1 µg, Oxoid) disks were used for antifungal susceptibility tests. Antifungal susceptibility testing of *Candida* strains was performed according to the guidelines and criteria of Clinical and Laboratory Standards Institute (CLSI/M44-A) using disk diffusion method [3]. Inoculum was prepared from 24 h cultures in SDA. Plates containing Mueller-Hinton agar supplemented with 2 % glucose and 0.5 µg/ml methylene blue at a depth of 4.0 mm were used. The agar surface was inoculated by using a swab dipped in a cell suspension adjusted to the turbidity of a 0.5 McFarland standard. Fluconazole and voriconazole disks were placed on the surfaces of the plates. After incubation at 35 °C for 24 h, the inhibition diameters around the disks were measured.

The interpretive criteria for the fluconazole and voriconazole disk diffusion tests were those of the CLSI: susceptible(S), zone diameters of ≥ 19 mm fluconazole and ≥ 17 mm voriconazole; intermedier (I) zone diameters of 15 to 18 mm fluconazole and 14 to 16 voriconazole; and resistant (R), zone diameters of ≤ 14 mm fluconazole and ≤ 13 mm voriconazole.

Statistical analysis

The data were analyzed using SPSS program, version 17.0 and subjected to Chi-square test. At 95% confidence interval, $P < 0.05$ was considered statitically significant.

RESULTS

Slime activity and antifungal susceptibility of 115 *Candida* strains isolated from clinical samples were investigated in this study. The most common species recovered were *C. albicans* (57.4 %) followed by *C. glabrata* (17.4 %), *C.tropicalis* (12.2 %), *C. parapsilosis* (5.21 %), *C. dubliniensis* (5.21 %) and *Candida spp.* (2.60 %) A total of 36 (31.3 %) out of 115 *Candida* isolates were slime producers. The highest slime production was found among *C. parapsilosis* isolates (66.66 %). It was also shown that non-*C. albicans* strains (44.89 %) produced significantly higher slime factor than *C. albicans* strains (21.21 %) ($p = 0.007$). Slime production results are reported in Table 1.

A total of eight *C.glabrata* isolates were resistant to fluconazole and one *C.glabrata* isolate was intermedier (I) to this antifungal. All *Candida* isolates were susceptible to voriconazole except one resistant and one intermedier *C. glabrata* isolates. These isolates were also resistant to fluconazole. Antifungal susceptibility data are listed in Table 2.

Table 1: Slime production by *Candida* isolates

Yeast (n)	Negative	Weak positive	Moderate positive	Strong positive	Total
C. albicans (66)	52	5	1	8	14
C. glabrata (20)	15	4	1	-	5
C. tropicalis (14)	5	-	1	8	9
C. parapsilosis (6)	2	-	-	4	4
C. dubliniensis (6)	4	-	2	-	2
Candida spp.(3)	1	-	1	1	2
*Total non-C.albicans (*49)	27	4	5	13	22

Table 2: Antifungal susceptibility of *Candida spp*.

Species	S n(%)		I n(%)		R n(%)	
	Flu	Vor	Flu	Vor	Flu	Vor
C. albicans	66 (100)	66 (100)	-	-	-	-
C. glabrata	11 (55)	18 (90)	1 (5)	1 (5)	8 (40)	1 (5)
C. tropicalis	14 (100)	14 (100)	-	-	-	-
C. parapsilosis	6 (100)	6 (100)	-	-	-	-
C. dubliniensis	3 (50)	6(100)	3 (50)	-	-	-
Non-*C. albicans*	37 (75.5)	47 (96)	4 (8.2)	1 (2)	8 (16.3)	1 (2)
Candida spp.	3 (100)	3 (100)	-	-	-	-
Total	103(89.6)	113 (98.2)	4 (3.4)	1 (0.9)	8 (7)	1(0.9)

We investigated the correlation between slime activity and antifungal susceptibility of the two antifungal agents. For all *Candida* species no correlation was detected between slime and antifungal susceptibility.

DISCUSSION

Candida spp. can cause both superficial and serious systemic diseases and are now recognized as one of the major agents of nosocomial infections. Recent data from the US National Nosocomial Infections Surveillance System [1] rank these organisms as the fourth most common cause of bloodstream infection. Biofilms are the structured microbial communities that are attached and encased in the matrix of exopolimeric material and are important for the development of clinical infection. Many candida infections involve the formation of biofilms on implanted devices. When bacteria exist in the biofilm form they are 10 - 1000 times more resistant to antibiotics than are planktonic cells [1,4]. In the present study, we investigated the correlation between slime production and resistance to two antifungal agents.

Various rates of slime production have been reported in a number of studies. While Mohandas and Ballal reported high rates of 51 and 90.32 % in 2011 for *C. albicans* and non-*C. albicans* isolates, respectively [5], in other studies which were supported by our results as well, lower resistance rates were found [6,7]. In this study total slime positivity rate was 31.31 % (21.21 % in *C. albicans* and 44.89 % in non-*C. albicans* isolates).

Similarly to our results, more recent studies showed slime production is common especially in non-*C. albicans* strains. In Tumbarello et. al study, they reported 22.6% slime positivity rate in *C. albicans* and 33.3% in non-*C. albicans* isolates [6]. Yıldırım et. al found 17% in *C. albicans*, 33% in non- *C. albicans* [7]. In contrast to this findings, Dag *et al* found slime positivity 39.3 and 37.7 % in *C. albicans* and non-*C. albicans*, respectively [8]. We found that there was a statistically significantly higher slime production in non-*C. albicans* strains than in *C. albicans* isolates. The highest slime production was found in *C. tropicalis* among all non-*C. albicans* isolates including *C. glabrata*, *C. parapsilosis*, *C. dubliniensis*.

Despite the widespread use of fluconazole for more than two decades, we found no evidence that *C. albicans* has developed increased resistance to fluconazole. All *C. albicans* species were sensitive to both antifungal agents. However, resistance rates to fluconazole in *C. albicans* are different in the other studies [9-11]. It is known that non-*C. albicans* species increase in candida infections and these species have a resistance to antifungal drugs. Non-*C. albicans* species have various degrees of susceptibility to the frequently used antifungal drugs while *C. krusei* is intrinsically resistant to fluconazole, *C. glabrata* is less susceptible or has higher minimal inhibitory concentrations (MICs) than other *Candida* species [9] *C. glabrata* is an

oppurtunistic pathogen that has become increasingly frequent in bloodstream and mucosal infections in immunocompromised patients .The increasing use of azole antifungals for the treatment of *C. glabrata* infections has resulted in emergence of resistance strains [11]. In the present study, there was a statistically significant higher fluconazole resistance in non-*C. albicans* than *C. albicans* isolates (p = 0.005). Resistance to fluconazole was observed relatively high, mainly in isolates of *C. glabrata*. Eight (40 %) *C. glabrata* isolates were found resistant to fluconazole and one *C. glabrata* was found intermedier. The resistance rates for fluconazole in *C. glabrata* are varried in the other studies.

All *Candida spp.* were susceptible to voriconazole except one *C. glabrata* strain which was also resistant to fluconazole. In addition, one *C. glabrata* isolate was found intermedier and it was also resistance to fluconozole too. Cross resistance between fluconazole and voriconazole is described among isolates of *C.glabrata*. The results of our antifungal susceptibility test are generally consistent with the findings from other studies. Similarly in Gültekin *et al*'s study conducted with 46 *Candida spp* isolated from blood samples, all Candida isolates were determined to susceptible to fluconazole and voriconazole [12]. However, rates of resistance tofluconazole (5 %) and voriconazole (7.7 %) have been reported for *C. albicans* isolates [10].

In the present study which was conducted with 66 *C. albicans* and 49 non-*C. albicans* strains, we investigated the association between slime activity and susceptibility to fluconazole and variconazole. The results showed that some *Candida* species produced slime but the antifungal susceptibility test performed indicate that some of these species were susceptible to fluconazole and voriconazole. For *C. albicans* and non-*C. albicans*, no correlation was detected between slime activity and the susceptibility of the two antifungal agents because all *C. albicans, C. tropicalis* and *C. parapsilosis* strains were sensitive to both antifungal agents. Similar results have been found in other studies In one of them, conducted with 19 *C. parapsilosis* and 35 *C. albicans* strains, the authors investigated whether slime activity patterns correlated with the strains' susceptibility to fluconazole, ketoconazole and amphothericine B. They did not find correlation between slime activity and the MIC of all three antifungal agents [13]. Also, Shin *et al* did not find any significant association between biofilm production and clinical characteristics of candidemia due to *C. albicans*, since only two of 30 blood isolates of *C.albicans* in their study were biofilm-positive [2]. Yücesoy *et al* investigated the biofilm production of various *Candida* strains with tube adherence method and compared this activity with fluconazole and amphotericin B susceptibility. They found no statistically significant difference between biofilm activity and susceptibility to amphotericin B. However, statistically significant difference was found between biofilm activity and susceptibility to fluconazole (p = 0.03) [9]. These divergent results underlines the need for further studies.

CONCLUSION

Candida spp. isolated from various clinical samples were highly susceptible to the tested antifungals, namely, fluconazole and voriconazole. Since voriconazole exhibits higher efficacy than fluconazole in non-*C. albicans* isolates it may be appropriate to prefer voriconazole in the treatment of fungal infections caused by fluconazole resistant non-*C. albicans* strains.

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