Send Orders for Reprints to reprints@benthamscience.ae

Open Biological Sciences Journal, 2018, *4*, 1-6

Open Biological Sciences Journal

Content list available at: www.benthamopen.com/BIOLSCI/

DOI: 10.2174/2352633501804010001

RESEARCH ARTICLE

Antifungal Susceptibility Pattern of *Candida Albicans* in Human Infections

Kamal Uddin Zaidi, Abin Mani*, Richa Parmar and Vijay Thawani

Biotechnology Pharmacology Laboratory and Human genetics Laboratory, Centre for Scientific Research and Development, People's University, Bhopal 462037, India

Received: November 22, 2017 Revised: January 26, 2018 Accepted: January 27, 2018

Abstract:

Introduction:

Candida albicans is an opportunistic pathogen under immune-compromised conditions and despite anti-fungal therapies, it has become lethal. Increase in the antimicrobial resistance in *C. albicans* is a matter of concern since it is in the human microbiome.

Aims and Objectives:

This study was conducted to estimate the incidence of *C. albicans* in infections, and evaluate its antifungal susceptibility in clinical samples.

Methods and Materials:

Two hundred isolates of *C. albicans* from different clinical samples were analyzed against its susceptibility towards four antifungal agents (fluconazole, ketoconazole, itraconazole and amphotericin-B) using well diffusion and MIC by microdilution assay.

Results:

All isolates in the study were sensitive to amphotericin-B and ketoconazole and a high frequency of fluconazole and itraconazole resistance was observed. Oral and catheter tips were observed to be the major sites of *C. albicans* infections. Significant resistance aganist fluconazole (56.5%) and itraconazole (64.5%) was observed with MIC at 16, 32 to 64µg/ml. All isolates were observed to be sensitive for ketoconazole and amphotericin-B at 0.5µg/ml and 1µg/ml.

Conclusion:

The study shows a higher antibiotic resistance in the clinical samples which proves the risk in *C. albicans* management program.

Keywords: Antifungal resistance, Fluconazole, Microdilution assay, Minimum inhibitory concentration.

1. INTRODUCTION

Antifungal resistance is a major concern in clinical practice. The advance of therapy has increased the survival rate but the risk factors have accumulated with an increase in cases of infectious diseases [1, 2]. In fungus, *C. albicans* is the most vital opportunistic pathogen where it normally resides in oral, conjunctival, gastrointestinal and genitourinary tracts Moreover, infection caused by *Candida* species is called as candidiasis [3]. About 75% women have this fungus without it causing infection [4, 5]. The factors that predispose women to vaginal candidiasis are changes in pH, use of oral contraceptives, tight clothing, and personal hygiene [6, 7].

There is an extensive use of fluconazole and itraconazole for chemoprophylaxis and treatment of fungal infections

* Address correspondence to this author at the Biotechnology Pharmacology Laboratory and Human genetics Laboratory,Centre for Scientific Research and Development, People's University, Bhopal 462037, India; Tel: 02554004084; E-mails: abinmani@gmail.com, zaidi.kamal92@gmail.com

due to their favorable oral bioavailability and safety. Hence it has led to fluconazole resistance in a high percentage [8]. Azole-resistant *C. albicans* is frequently observed in oropharyngeal candidiasis of HIV patients [9]. Apart from albicans, species of Candida like *C. glabrata, C. krusei,* and *C. lusitaniae* have been reported with reduced its susceptibility to fluconazole [10, 11]. Pfaller*et al.,* 2004 reported that changes in the structure of azoles are the factors responsible for cross-resistance patterns among Candida species. Moreover the environmental stress with exposure to antifungal drugs can mediate resistance [13]. The present study evaluates the prevalence of antifungal resistance in *C. albicans* isolated from catheter tip, oral, urine and high vaginal swab. Where, antifungal resistance against amphotericin B, ketoconazole, itraconazole and fluconazole was analyzed. The antifungal resistance against infected location was correlated to visualize the development of drug resistance and to estimate the prevalence of resistant strains towards the site of infection which will help to known the sensitive site procedure. The study was conducted to assess the *C. albicans* management, as it is one of the normal human floras and its alternation to antifungal resistance is a concern for human health with the development of drug resistance.

2. MATERIAL AND METHODS

2.1. Sample Collection

Pathological strains of *C. albicans* from five different sources viz., blood (B), catheter tip (CT), high vaginal swab (HVS), oral (O) and urine (U) were collected from the Department of Microbiology, People's College of Medical Sciences and Research centre(PCMS&RC) and Peoples Dental Academy, People's University, Bhopal. The culturing of *C. albicans* from CT, HVS, O and U and was done on Sabouraud Dextrose Agar (SDA) medium and primarily B samples on blood culturing media and transfer to SDA. The strains were confirmed based on its culture characteristics and microscopic observation.

2.2. Antifungal Susceptibility

Well diffusion antifungal susceptibility assay was carry out by the method proposed by Magaldi [14]. SDA plates with inoculums were assayed in the wells made using sterilized cork borer, to which, antifungal agents amphotericin B (100 U), ketoconazole (20 μg/mL), itraconazole (20μg/mL), and fluconazole (20 μg/mL) were loaded. The plates were incubated at 37 for 48 hours and the zone of inhibition was recorded.

2.3. Minimum Inhibitory Concentration

Using microdilution assay, the minimal inhibitory concentration (MIC) against fluconazole, itraconazole, ketoconazole and amphotericin-B was determined based on the method of Eloff [15]. 96 well plates with 100 µl of *C. albicans* inoculums in sabouraud dextrose broth were cultured for 48 hours at 37˚C. The stock solution (100µl/ml) of antifungal agents amphotericin B, ketoconazole, itraconazole and fluconazole was serially diluted (0.5 to 64µg/ml) and added to separate wells along with 100µ/ml sabouraud dextrose broth. The plates were incubated at 37˚C for 48 hours and the MIC value was determined by reading the intensity of the colour produced in the wells after the addition of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), defining the cell concentration in the well.

3. RESULT AND DISCUSSION

In this study, 200 clinical *C. albicans* were isolated; of which 10.6% were of high-risk patients. The age group of patients in the study was from 18 to 90 years. Slight female preponderance was observed in the study with M: F ratio of 0.86:1.14.The number of *C. albicans isolates* (Table **1**) ranged from U (20%), HVS (22.5%) CT (22.5%), O (22.5%) to B (12.5%). It was observed that there is considerable variation among *C.albicans* between the site and patients. It is also reported a high degree of variability among their isolates [16].

Table 1. Antifungal sensitive patternwith respect to the rute of pathogen.

Route of Pathogen	Sensitive Strains: 158		Resistant Strains: 42	
	Fluconazole	Itraconazole	Fluconazole	Itraconazole
O: 45 (22.5%)	18 (46.7%)	21 (46.7%)	03 (6.7%)	02 (4.4%)
CT: 45 (22.5%)	21 (46.7%)	16 (35.6%)	10 (22.2%)	09 (20.0%)
HVS: 45 (22.5%)	17 (37.8%)	14 (31.1%)	06 (13.3%)	04 (8.9%)
U 40: (20%)	22 (50%	10 (25%)	05 (12.5%)	03(7.5%)

(Table 1) contd.....

Route of Pathogen	Sensitive Strains: 158		Resistant Strains: 42	
	Fluconazole	Itraconazole	Fluconazole	Itraconazole
B 25: (12.5%)	8 (32%	10 (40%)	0	0

The clinical isolates were identified up to the genus level. Hyphal morphology of isolates and characteristics of the spores were observed using lactophenol cotton blue stain under the light microscope. Morphological and physiological characteristics of the isolates were used for the identification of yeasts based on the Buschelman *et al.* [17], morphological identification of Candida spp.

In the present study, we examined the antifungal susceptibility of antifungal agents. Using *C. albicans* in disk diffusion and a micro-dilution method, comparative efficacies of ketoconazole, amphotericin B, fluconazole and itraconazole were examined *in vitro*. The zone of inhibition of a different antifungal agent against *C. albicans* (mean±SD) was observed at a concentration of 100µg/ml (Fig. **1**).The susceptibility pattern of the isolates showed 100% sensitivity to ketoconazole and amphotericin-B, moreover Fluconazole (81 isolates) and itraconazole (63 isolates) resistances were observed. Antifungal susceptibility test revealed that all isolates (200) were sensitive to amphotericin-B and ketoconazole. However, significant resistance of 56.5% and 64.5% was observed for fluconazole and itraconazole, respectively. It was observed that 158 strains were sensitive and 42 strains were resistant to fluconazole and itraconazole. CT showed high resistant strains being 42.2% followed by HVS strains. Strains isolated from blood were sensitive to both the antibiotics (Table **1**).Our findings are in accordance with the study conducted by Fadda *et al.,*2008 [18] where decreased susceptibility to azoles in *C. albicans*. was observed. It is also reported similar susceptibility of *C. albicans* [19].

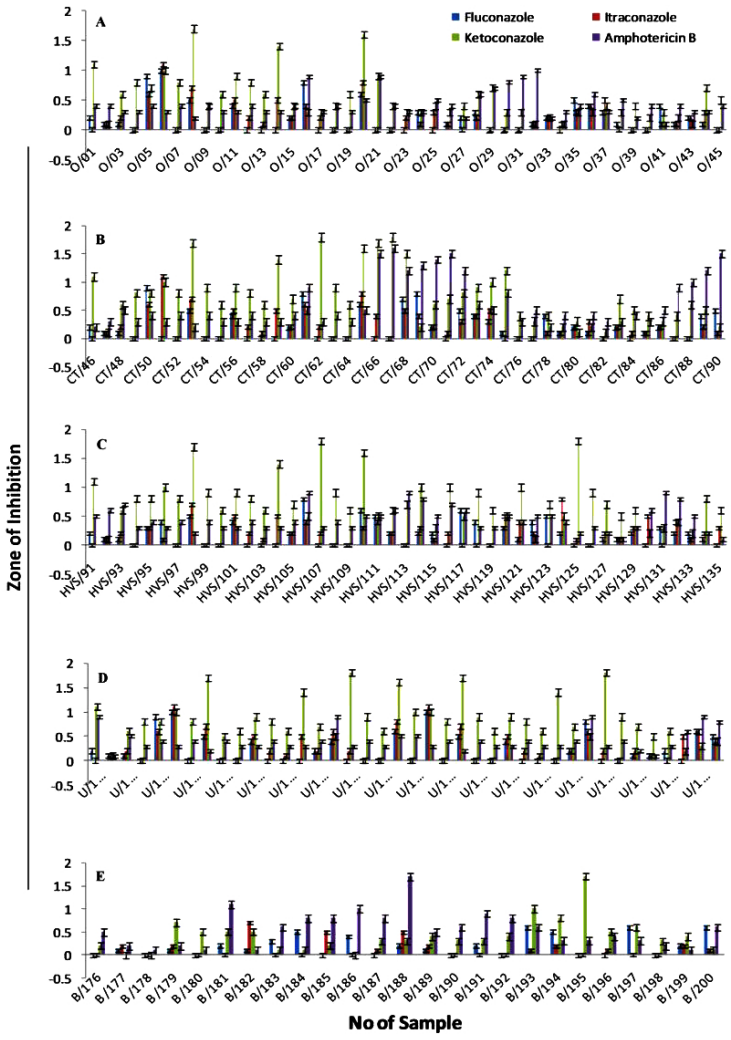

Fig. (1). Antifungal susceptibility in *Candida albicans*; [A] Oral, [B] Catheter tip, [C] High vaginal swab, [D] Urine, [E] Blood.

Minimum inhibitory concentration was tested at the final concentrations ranging from0.5 µg/ml to 64µg/ml. The wells that showed no color (pink) of the reagent (MTT) indicate the concentration at which the fungal growth was inhibited and the lowest concentration showing no colour was recorded in terms of the MIC value. The MIC values of fluconazole for the isolates ranged from 16µg/ml, 32µg/ml to 64µg/ml with 18.63%, 32.4% and 27.5% susceptibility, respectively. In case of itraconazole, the isolates showed susceptibility of 30.9%, 11.34%and 19.53% towards 16, 32 and 64µg/ml of antibiotic, respectively. Moreover, all isolates were observed to be sensitive for ketoconazole and amphotericin-B with 25% and 60% at 0.5µg/ml and 75% and 40% at 1µg/ml susceptibility, respectively (Table **2**). Early detection of drug susceptibility to the organism has to be carried out for a successful treatment of any infectious disease [20, 21]. In immunodeficient or immune suppressed patients, early therapies can alter the course of fungal infections. It was stated that antifungal susceptibility testing is useful to know the susceptibility in correlation with 48- and 24-h fluconazole MICs in Candida isolates showed good correlation to visual MICs [12, 15, 22, 23] Comparative evaluation of 24-h visualization and 48-h spectrophotometric MIC end points to 48-h microdilution broth visual MICs of fluconazole, itraconazole, voriconazole, and posaconazole in Candida spp. reported that both the MIC endpoints results were similar. Moreover it was stated that spectrophotometric MICs are more effective than visual MICs [22]. This study carries significance duce to an increase in the antifungal resistance of *Candida* which is a lethal threat to immune compromised and hospital acquired infections. Intrinsic resistance to antifungal therapy had been reported . on various Candida species towards commonly used antifungal agents. It was also observed that the antifungal resistances are developed due to the treatment. It is very important to understand the mechanisms of drug resistance to improve the efficiency of treatment since Candida infections have high impact on immune compromised patients.

Table 2. Antifungal susceptibility and minimum inhibitory concentration of antifungal agent.

Antifungal Agent	Minimum Inhibitory Concentration (µ/ml)					No of Isolates
	0.5	1	16	32	64	
Fluconazole	-	-	23(18.63%)	40(32.4%)	24(27.5%)	87(100%)
Itraconazole	-	-	22(30.9%)	18(11.34%)	31(19.53%)	71(100%)
Ketoconazole	50(25%)	150(75%)	-	-	-	200(100%)
Amphotericin-B	120(60%)	80(40%)	-	-	-	200(100%)

CONCLUSION

Out of 200 clinical isolates from 5 routes of infection (blood, catheter tip, high vaginal swab, oral and urine), it was observed that oral and catheter tips are the major sites where *C. albicans* infection can occur. In the study, it was also observed that catheter tip strains have high antibiotic resistant strains when considering other regions; it can be due to the pathological conduction of the patients that mediates the infection of resistant strain and the development of drug resistance. Thus the study signifies that catheter can be a factor for the development of antibiotic resistance since the procedure is carried out mostly on patients under antimicrobial treatment or immune compromised. It was also observed that the Candida isolated from oral route has higher antibiotic resistance pattern than viginal followed by UTI which is the prominent candida infection site. In this study, C. albicans showed 100% susceptibility against ketoconazole and amphotericin-B at 1 µg/ml, whereas fluconazole and itraconazole resistance strains showed susceptibility upto16 to 64µg/ml, thus signifying an increase in resistance pattern. The study reveals higher antibiotic resistance in the clinical samples which proves the risk in *C. albicans* management program due to the risk of development of drug resistance.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

The authors are thankful to People's University, Peoples Group, Bhopal, for laboratory facilities, to carry out this research work.

REFERENCES

[1] Spampinato C, Leonardi D. Candida infections, causes, targets, and resistance mechanisms: Traditional and alternative antifungal agents. BioMed Res Int 2013; 2013: 204237.
[http://dx.doi.org/10.1155/2013/204237] [PMID: 23878798]

[2] Emerging Threats in Antifungal-Resistant Fungal Pathogens. Front Med 2016; 3: 10-1.

[3] Ako-Nai AK, Kassim OO, Adeniran MO, Taiwo O. A study of urinary tract infections at Ile-Ife, Nigeria: East. Afr Med 1993; 70: 110-4.

[4] Dan M, Poch F, Levin D. High rate of vaginal infections caused by non-C. albicans Candida species among asymptomatic women. Med Mycol 2002; 40(4): 383-6.
[http://dx.doi.org/10.1080/mmy.40.4.383.386] [PMID: 12230217]

[5] Ellepola AN, Samaranayake LP. The effect of limited exposure to antimycotics on the relative cell-surface hydrophobicity and the adhesion of oral Candida albicans to buccal epithelial cells. Arch Oral Biol 1998; 43(11): 879-87.
[http://dx.doi.org/10.1016/S0003-9969(98)00064-8] [PMID: 9821511]

[6] Carlsen G. The Candida Yeast Answer. Provo: Candida Wellness Center 2001.

[7] Enweani IB, Gugnani HC, Okobia R, Ojo SB. Effect of contraceptives on the prevalence of vaginal colonization with *Candida* species in Edo State, Nigeria. Rev Iberoam Micol 2001; 18(4): 171-3.
[PMID: 15496123]

[8] Redding SW, Kirkpatrick WR, Saville S, *et al.* Multiple patterns of resistance to fluconazole in Candida glabrata isolates from a patient with oropharyngeal candidiasis receiving head and neck radiation. J Clin Microbiol 2003; 41(2): 619-22.
[http://dx.doi.org/10.1128/JCM.41.2.619-622.2003] [PMID: 12574256]

[9] Skiest DJ, Vazquez JA, Anstead GM, *et al.* Posaconazole for the treatment of azole-refractory oropharyngeal and esophageal candidiasis in subjects with HIV infection. Clin Infect Dis 2007; 44(4): 607-14.
[http://dx.doi.org/10.1086/511039] [PMID: 17243069]

[10] Vazquez JA, Peng G, Sobel JD, *et al.* Evolution of antifungal susceptibility among Candida species isolates recovered from human immunodeficiency virus-infected women receiving fluconazole prophylaxis. Clin Infect Dis 2001; 33(7): 1069-75.
[http://dx.doi.org/10.1086/322641] [PMID: 11528582]

[11] Safdar A, van Rhee F, Henslee-Downey JP, Singhal S, Mehta J. *Candida glabrata* and Candida krusei fungemia after high-risk allogeneic marrow transplantation: No adverse effect of low-dose fluconazole prophylaxis on incidence and outcome. Bone Marrow Transplant 2001; 28(9): 873-8.
[http://dx.doi.org/10.1038/sj.bmt.1703252] [PMID: 11781648]

[12] Pfaller MA, Messer SA, Boyken L, *et al.* Cross-resistance between fluconazole and ravuconazole and the use of fluconazole as a surrogate marker to predict susceptibility and resistance to ravuconazole among 12,796 clinical isolates of Candida spp. J Clin Microbiol 2004; 42(7): 3137-41.
[http://dx.doi.org/10.1128/JCM.42.7.3137-3141.2004] [PMID: 15243072]

[13] Zaidi KU, Mani A, Thawani V, Mehra A. Total protein profile and drug resistance in candida albicans isolated from clinical samples. Mol Biol Int 2016; 2016: 4982131.
[http://dx.doi.org/10.1155/2016/4982131] [PMID: 27478638]

[14] Magaldi S, Mata-Essayag S, Hartung de Capriles C, *et al.* Well diffusion for antifungal susceptibility testing. Int J Infect Dis 2004; 8(1): 39-45.
[http://dx.doi.org/10.1016/j.ijid.2003.03.002] [PMID: 14690779]

[15] Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med 1998; 64(8): 711-3.
[http://dx.doi.org/10.1055/s-2006-957563] [PMID: 9933989]

[16] Merz WG, Connelly C, Hieter P. Variation of electrophoretic karyotypes among clinical isolates of Candida albicans. J Clin Microbiol 1988; 26(5): 842-5.
[PMID: 3290238]

[17] Buschelman B, Jones RN, Pfaller MA, Koontz FP, Doern GV. Colony morphology of Candida spp. as a guide to species identification. Diagn Microbiol Infect Dis 1999; 35(1): 89-91.
[http://dx.doi.org/10.1016/S0732-8893(99)00051-6] [PMID: 10529886]

[18] Fadda ME, Podda GS, Pisano MB, Deplano M, Cosentino S. Prevalence of Candida species in different hospital wards and their susceptibility to antifungal agents: Results of a three year survey. J Prev Med Hyg 2008; 49(2): 69-74.
[PMID: 18847180]

[19] Hadley S, Martinez JA, McDermott L, Rapino B, Snydman DR. Real-time antifungal susceptibility screening aids management of invasive

yeast infections in immunocompromised patients. J Antimicrob Chemother 2002; 49(2): 415-9.
[http://dx.doi.org/10.1093/jac/49.2.415] [PMID: 11815592]

[20] Hospenthal DR, Murray CK, Rinaldi MG. The role of antifungal susceptibility testing in the therapy of candidiasis. Diagn Microbiol Infect Dis 2004; 48(3): 153-60.
[http://dx.doi.org/10.1016/j.diagmicrobio.2003.10.003] [PMID: 15023422]

[21] Meletiadis J, Melchers WJ, Meis JF, Van Den Hurk P, Jannes G, Verweij PE. Evaluation of a polymerase chain reaction reverse hybridization line probe assay for the detection and identification of medically important fungi in bronchoalveolar lavage fluids. Med Mycol 2003; 41(1): 65-74.
[http://dx.doi.org/10.1080/mmy.41.1.65.74] [PMID: 12627806]

[22] Espinel-Ingroff A, Barchiesi F, Cuenca-Estrella M, *et al.* International and multicenter comparison of EUCAST and CLSI M27-A2 broth microdilution methods for testing susceptibilities of Candida spp. to fluconazole, itraconazole, posaconazole, and voriconazole. J Clin Microbiol 2005; 43(8): 3884-9.
[http://dx.doi.org/10.1128/JCM.43.8.3884-3889.2005] [PMID: 16081926]

[23] Khosravi AR, Riazipour M, Shokri H, Mousavi ML, Mahmoudi M. Characterization of the similarity of protein patterns and virulence of clinical Candida albicans isolates. J Biol Sci 2008; 8: 760-6.
[http://dx.doi.org/10.3923/jbs.2008.760.766]