

Original Research Article

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Antifungal Susceptibility Profile of *Candida* and its Comparison in Various *Candida* Species

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ABSTRACT

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Candida spp. can cause a wide variety of infections in immunosuppressed patients of long term broad – spectrum antibiotics, steroids or other immunosuppressive agents, diabetes mellitus, AIDS, Malignancy, Neutropenia. Although the majority of infections are caused by *Candida albicans*, Non-*C. albicans Candida* (NCAC) species are emerging as important pathogen in humans. Our study included 130 clinical isolates which showed gram positive budding yeasts cells with or without pseudohyphae. KOH mount, germ tube, Carbohydrate assimilation test and fermentation test and antifungal susceptibility by disc diffusion method was done. 65% of the total isolates were sensitive to Fluconazole and 82% of the total isolates were sensitive to Voriconazole

Introduction

With the increase in the population of patients with immunodeficiency or undergoing immunosuppressive therapy, mycoses have become a growing problem in modern medical care. In addition, the diagnosis of these diseases can be problematic, drug resistance is of great concern, and fewer drugs are available compared to bacterial or viral diseases (1,2,3).

Candida is the most prevalent fungal pathogen in humans. *Candida* is a part of the normal flora in healthy individuals, and is

usually confined to the skin and mucosal surfaces of the oral cavity, gastrointestinal and urogenital tracts, and vagina. Although the majority of infections are caused by *Candida albicans*, Non-*C. albicans Candida* (NCAC) species are emerging as important pathogen in humans. *Candida* spp. is also one of the most frequent pathogens isolated in bloodstream infections, associated with significant morbidity and mortality in hospitalized patients (4-6). Fluconazole is an azole group of antifungal agent, been widely used for both treatment and prophylaxis of fungal infections. Due to its limited spectrum of antifungal activity and resistance noticed in

immunocompromised hosts, second generation triazoles like voriconazole have been developed. Possibility of innate or acquired resistance of *Candida spp* to antimycotic drugs has emphasized invitro antimycotic susceptibility testing.

Extensive use of antimycotic drugs led to change in prevalence of *Candida spp* with a decrease in proportion of *C. albicans* and increase in proportion of non albicans such as *C glabrata*, *C krusei*.

The present study included 130 clinical isolates which showed gram positive budding yeasts cells with or without pseudohyphae. Our study aimed to determine the *in vitro* susceptibilities of various *Candida* species isolates to fluconazole and Voriconazole and to compare both.

Materials and Methods

A total of 130 *Candida* species isolated from clinical samples like urine, pus, sputum, blood, oral swabs, vaginal swabs, endotracheal aspirates, endotracheal tips, were included in this study. The study was conducted from November 2012 to April 2014 in the department of Microbiology, ESIC MC PGI MSR Hospital Bangalore. Data of patients regarding the use of long term broad – spectrum antibiotics, steroids or other immunosuppressive agents, diabetes mellitus, AIDS, Malignancy, Neutropenia were collected at enrolment.

Examination of specimen was done by Gram staining, 10% and 40 % KOH mount, Cultural characteristics were noted on SDA with chloramphenicol, Germ tube test, chlamydospore formation on cornmeal agar with 1% tween 80 (by Dalmau technique) speciation was done by Carbohydrate assimilation test by Auxanographic plate method (Haley and standard modification)

using Yeast nitrogen base and 20 % sugars. Sugar fermentation test with 6% sugars was done for speciation.

Antifungal susceptibility test was done by Kirby- Bauer's disc diffusion method on glucose methylene blue Mueller Hinton agar (GM-MH) using commercially available antifungal discs [Hi Media, Mumbai, India] fluconazole (25µg), Voriconazole 1 µg.(Fig 3). Zone diameters were interpreted as per the approved NCCLS (M44-A) guidelines. Antifungal susceptibility test: Antifungal susceptibility test was done by the disc diffusion method on glucose methylene blue Mueller Hinton agar (GM-MH). GM-MH was prepared by addition of 2% glucose and 0.5 µg/ml of methylene blue to Mueller Hinton agar. The agar medium should have a pH of 7.2-7.4 at room temperature after gelling.

The inoculum was prepared by picking five distinct colonies of approximately 1mm from 24 hours old cultures grown on Sabouraud's dextrose agar (SDA). Colonies were suspended in 5ml of sterile 0.85% saline. This suspension was adjusted to turbidity of 0.5 Mc Farlands yielding 1×10^6 - 5×10^6 cells/ml

Preparation of GM-MH Agar (appendix)

Inoculation of Test plates-Within 15 minutes of inoculum preparation, the swab dipped in suspension was streaked on the entire surface of GM-MH agar. The antifungal discs were placed 24mm apart from each other. The plates were then incubated at 37⁰C for 24 hours.

If insufficient growth was observed after 24 hours, the plates were read after 48 hours. Zone diameters were interpreted as per the approved NCCLS (M44-A) guidelines. The quality control test was performed by using *C. parapsilosis* (ATCC 22019), and *C. albicans* (ATCC 90028).

Interpretation of Disk diffusion test results- Zone of inhibition of uniform circular semi-confluent growth is considered. The zone diameter to the nearest whole mm at the point at which there is prominent reduction in growth is measured.

Results and Discussion

The chart shows that 65 % of the isolates were sensitive to Fluconazole and 35% resistant. 82% were sensitive and 18% resistant to Voriconazole.

The chart shows that 100% of *C. dubliniensis* were sensitive to Fluconazole, 93.75% of *C.*

tropicalis, 88.8% of *C. albicans*, 75% of *C parapsilosis*, 66.6 % of *C. guilliermondi*, 50 % of *C. lusitanae*, 40% of *C. krusei*, 20% of *C. glabrata* were sensitive to Fluconazole.

100 % of *C. albicans* and *C. dubliniensis* were sensitive to Voriconazole.80% of *C. guilliermondi* and *C. krusei*, 75% of *C. lusitanae*, 60 % of *C. glabrata*, 80 % of *C. parapsilosis* and 95 % of *C. tropicalis* were sensitive to Voriconazole

6 (4.6%) of the total isolates were resistant to both Fluconazole and Voriconazole.4 out of 6 isolates belonged to *C. glabrata* and 2 belonged to *C tropicalis* (Table 1–3).

Zone diameter interpretive standards

Antifungal agent	Disk content	Zone Diameter, Nearest whole		
		R	S-DD	S
Fluconazole	25 µg	≤14	15-18	≥19
Voriconazole	1 µg	≤13	14-16	≥17

Table.1 Antifungal susceptibility test

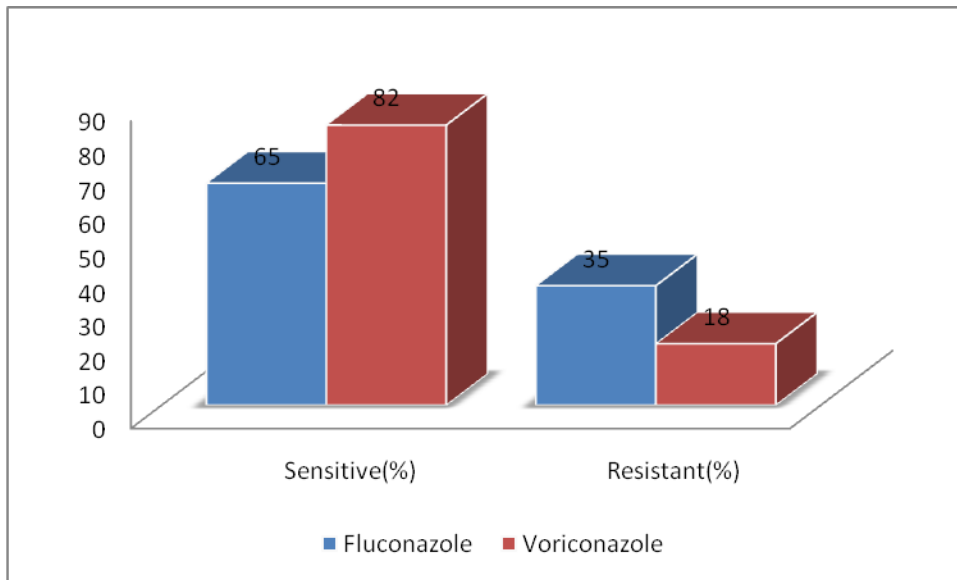
Drug	Sensitive (%)	Resistant (%)
Fluconazole	65	35
Voriconazole	82	18

Table.2 Fluconazole susceptibility in Various *Candida* species isolated

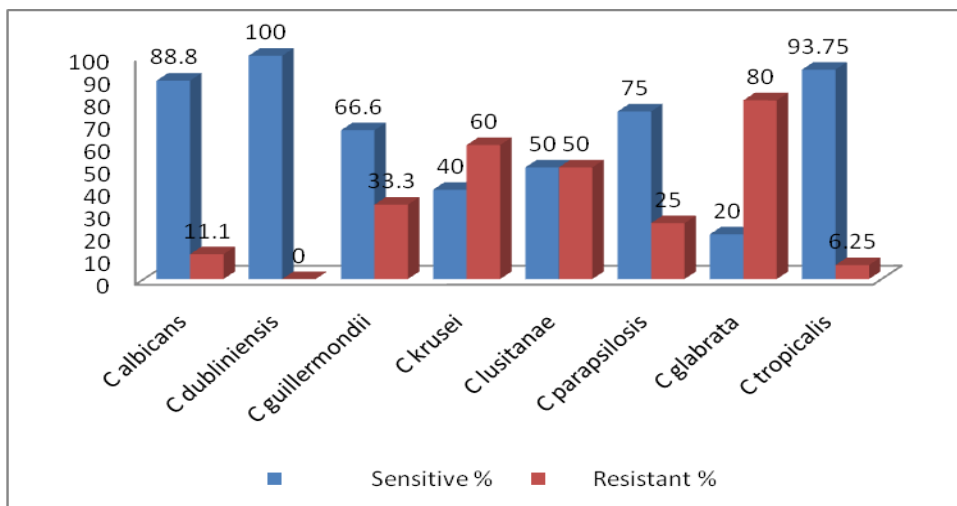
<i>Candida</i> species	Sensitive %	Resistant %
<i>C. albicans</i>	88.8	11.1
<i>C. dubliniensis</i>	100	0
<i>C. guilliermondii</i>	66.6	33.3
<i>C. krusei</i>	40	60
<i>C. lusitanae</i>	50	50
<i>C. parapsilosis</i>	75	25
<i>C. glabrata</i>	20	80
<i>C. tropicalis</i>	93.75	6.25

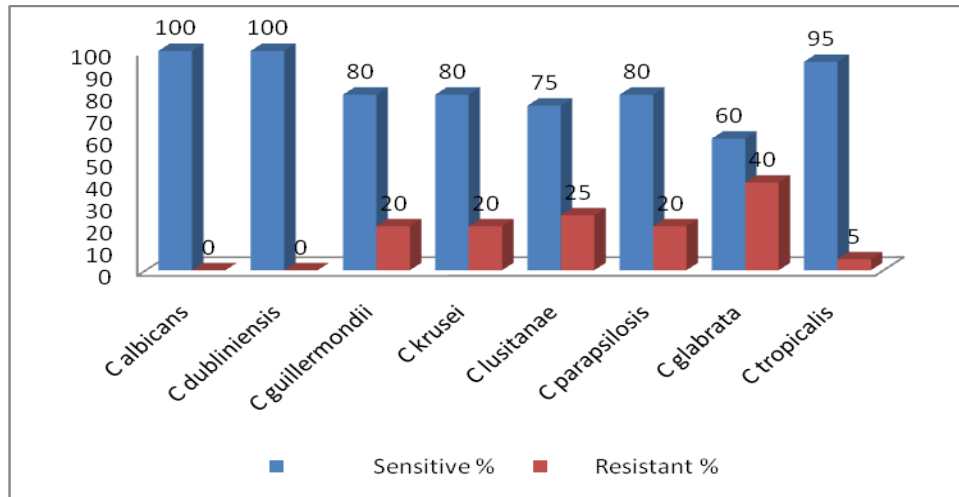
Table.3 Voriconazole susceptibility in Various *Candida* species isolated

<i>Candida</i> species	Sensitive %	Resistant %
<i>C. albicans</i>	100	0
<i>C. dubliniensis</i>	100	0
<i>C. guilliermondii</i>	80	20
<i>C. krusei</i>	80	20
<i>C. lusitanae</i>	75	25
<i>C. parapsilosis</i>	80	20
<i>C. glabrata</i>	60	40
<i>C. tropicalis</i>	95	5



Antifungal Susceptibility Test





6 (4.6%) isolates of the total 130 isolates were resistant to fluconazole but sensitive to Voriconazole. Of which *C. albicans*, *C. guilliermondii* (1each), *C. glabrata* and *C. krusei* (2 each). This was similar to a study conducted by Regha *et al.*, which showed that 3.2 % of total (95) isolates were fluconazole resistant but all were sensitive to voriconazole. 2.3% of *C. tropicalis* isolates and 9.5% of *C. parapsilosis* were fluconazole resistant but all were sensitive to voriconazole.

The triazoles remain active against many of isolates, Fluconazole is the first line antifungal agent for treating uncomplicated candidiasis (7-11). More fluconazole-resistant strains of endemic species may emerge whenever there are lapses in infection control precautions and extensive use of fluconazole. Voriconazole seemed to be superior to fluconazole with a better susceptibility in the fluconazole resistant strains.

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