

Prevalence of Antifungal Resistant Strains of *Candida* Species in Immunocompromised Patients

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Abstract: The invitro susceptibilities of 50 *Candida* species isolates were tested against two antifungal agents by a disc diffusion of CLSI M44-A2 method for fungal susceptibility testing. The *Candida* species shows resistance towards Amphotericin B and fluconazole. According to the results obtained, *Candida albicans* was the, most frequently isolated with 86%, this was followed by *C. tropicalis* 4%, *C. krusei* 2% and *C. parapsilosis* 2%. In the current study it is found that a number of *Candida* isolates resistant to amphotericin B were 46.5% where as 11.9% strains were interpreted as semi dose dependent and non-*albicans Candida* all showed 100% resistance to Amphotericin B. However, the study with fluconazole shows 65% of *Candida* isolates were resistant whereas, non-*albicans candida* all showed 100% resistance.

Keywords: *Candida* species, fungal susceptibility, immunocompromised

1. Introduction

In recent years, the incidences of opportunistic fungal infections have increased tremendously, even though we are advancing in health care and therapeutic methods. Moreover, most of these pathogens are the antifungal drug-resistant, resulting in a high rate of fatality due to fungal infections. Yeasts such as *Candida* species have been claimed as opportunistic fungal pathogens. *Candida* species are the most common human commensal that able to cause a broad spectrum of disease in the hosts (Williams DW.*et.al*, 2000, Kim J. *et.al*, 2011).

Advances in medicine such as improvement in organ transplantation, intensive care, Chemotherapy, and antibiotics continue to create a growing population with substantially suppressed immune function. Immunocompromised individuals have increased susceptibility to serious fungal infections. The individuals at risk include intensive care and post surgical patients; human immunodeficiency virus (HIV) infected hosts, patients with hematological malignancies, elderly patients, and premature infants (Caugant.A.*et.al*, 1993, Dean DA. *et.al*, 1996).

Clear differences among the various non-*albicans Candida* in their susceptibility to specific drugs. Rapid, reliable identification to species is now needed more than ever for clinicians to make treatment choices (Hospenthal DR.*et.al*, (2006).

Antifungals used for prolonged periods, has led to acquired resistance among previously susceptible strains or species. That is why, it is necessary to evaluate the existing antifungal susceptibility pattern due to the emergence of resistant strains (Chander.J.,(2009) and Rattan. A., (1999).

With this background, the current study has been planned to determine the distribution of *Candida* species and *in vitro* susceptibilities of antifungal agents against the *Candida* isolated from the immunocompromised patients.

2. Materials and Methods

A total of 50 immunocompromised subjects were selected. Out of which 47 clinical *Candida* isolates were identified and taken to study their antifungal susceptibility. These included 43 *Candida albicans* ,02 *Candida tropicalis* ,01 *Candida krusei* and 01 *Candida parapsilosis*. Species identification was done with germ-tube test, colonial study on Corn meal agar (CMA), on *Candida* Chromogenic media, carbohydrate assimilation, and fermentation tests.

The inoculum for each fungal strain was prepared by taking four or five pure colonies from an overnight growth on SDA using a sterile inoculation loop. These colonies were emulsified in sterile normal saline. Gentle dilution was performed, till the turbidity was comparable visually to 0.5 McFarland turbidity standards with inoculum density approximately 10^8 cfu/ml. For antifungal susceptibility Kirby Bauer methods for susceptibility were used. The method is used as per CLSI M44-A2 for fungal susceptibility testing. The disks of Amphotericin B, and fluconazole were supplied by Hi Media Mumbai. The plates were examined after the incubation. The diameters of the zones of complete inhibition were measured to the nearest whole numbers in millimeters using zone scale and recorded.

3. Results

To determine whether the isolates tested against Amphotericin B and fluconazole were susceptible, intermediate or resistant; the diameters of the zones of inhibition obtained were compared with the standard zones interpretive breakpoints published by CLSI M44-A2 guidelines. Zone diameters in the disk diffusion assay were measured to the nearest whole millimeter at the point where there was a prominent reduction of growth after 24 and 48 hours of incubation. Interpretation of antifungal susceptibility testing for antifungals according to assigned susceptible, intermediate, and resistant categories for 47 *Candida* isolates was noted in table number 01.and

fig.no.01&02. In the present study susceptibility of *Candida albicans* to Amphotericine B was 53.5% in immunocompromised patients, whereas, non-*albicans Candida* all showed 100% resistance to Amphotericine B in the study groups. In the current study it is found that a number of *Candida* isolates resistant to amphotericine B were 46.5% where as 11.9% strains were interpreted as semi dose dependent. However, the study with fluconazole shows 65% of *Candida* isolates were resistant whereas, non-*albicans candida* all showed 100% resistance.

4. Discussion

The study identified the specific species of *Candida* that causes infections among patients who were immunocompromised. The study also determined the in vitro susceptibility of *Candida* species isolated from the oral samples. The antifungal agents tested include Amphotericin B and fluconazole. In the present study susceptibility of *Candida albicans* to Amphotericine B was 53.5% in immunocompromised patients, whereas, non-*albicans Candida* all showed 100% resistance to Amphotericine B in the study groups. In the current study it is found that a number of *Candida* isolates resistant to amphotericine B were 46.5% where as 11.9% strains were interpreted as semi dose dependent. However, the study with fluconazole shows 65% of *Candida* isolates were resistant whereas, non-*albicans candida* all showed 100% resistance. The findings of the present study more or less correlate with those of Anaissie and co-workers (1996) in which fluconazole shows 66 % of efficacy and amphotericine B 64%, respectively.

Findings from the study indicate that isolates of *Candida krusei* were resistant to fluconazole. This was not surprising because it is well established that *Candida krusei* is intrinsically resistant to fluconazole (Berrouane *et al.*, 1999). This was also confirmed in a study by Hamza *et al.* (2008) who reported in Tanzania that all isolates of *Candida krusei* tested were resistant to fluconazole.

Table 1: Interpretation of antifungal susceptibility testing for Amphotericine B and fluconazole.

Sr. No	Antifungals and <i>Candida</i> isolates	Number of <i>Candida</i> Isolates	Interpretation category		
			S	I	R
1	Amphotericine B (AP)				
i	<i>Candida albicans</i>	43	23	05	15
ii	<i>Candida krusei</i>	01	-	-	01
iii	<i>Candida parapsilosis</i>	01	-	-	01
iv	<i>Candida tropicalis</i>	02	-	-	02
2	Fluconazole (FLC)				
i	<i>Candida albicans</i>	43	16	-	27
ii	<i>Candida krusei</i>	01	-	-	01
iii	<i>Candida parapsilosis</i>	01	-	-	01
iv	<i>Candida tropicalis</i>	02	-	-	02

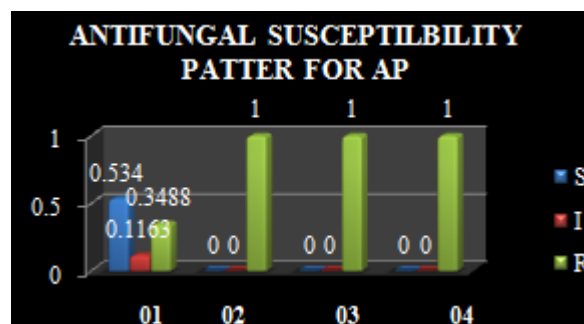


Figure 1: Interpretation of antifungal susceptibility testing for Amphotericine B (AP).

01. *C. albicans*; 02. *C. krusei*; 03. *C. tropicalis*; 04. *C. parapsilosis*

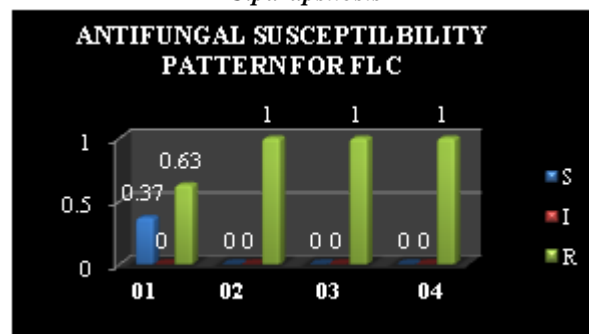


Figure 2: Interpretation of antifungal susceptibility testing for fluconazole (FLC).

01. *C. albicans*; 02. *C. krusei*; 03. *C. tropicalis*; 04. *C. parapsilosis*

Ruhnke and co-workers (1994), observed resistance to fluconazole in *Candida* species isolated from HIV symptomatic patient having oropharyngeal *Candidiasis*. These workers have shown that there is a regular increase in MIC to fluconazole in *Candida albicans*. White and co-workers (1998) suggested that mutations in certain genes of *Candida* lead to the emergence of antifungal drug resistance. Pfaller and co-workers (2003) tested 7,837 isolates of *Candida* against fluconazole and found 351 isolates resistant to this drug. The finding from the current study shows the fluconazole is resistant to most of the *Candida* species, which correlates with the other studies.

In the present study, *Candida* isolates demonstrated more resistance to an azole group of antifungal agents as compared to amphotericine B. Azole resistance in *Candida* species is of concern because these drugs are frequently used as therapeutic alternatives to amphotericine B (Deorukhkar and Saini, 2013 a). Amphotericine B has a rapid cidal action on the most strain of *Candida* species, but due to nephrotoxicity associated with it, amphotericine B is not the first choice of treatment (Giri and Kindo, 2012). An azole group of antifungal agents is preferred because they are easy for administration and are less toxic (Deorukhkar and Saini, 2013 a). Azole resistance was more common in non-*albicans candida* species as compared to *Candida albicans*, which similar to the observation of Deorukhkar et al. (Deorukhkar et al., 2012 a).

The successful treatment of oral candidiasis in immunocompromised patients depends on the early

identification of the species and sensitivity patterns to antifungal agents. The high growing rate of non *albicans* *Candida* resistant to azole confirms the importance of monitoring changes in the distribution of pathogenic *Candida* species. The sensitivity pattern of *Candida* species as revealed in this study shows that *Candida* species isolated were more resistance to an azole group of antifungal agents as compared to amphotericine B.

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