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Effectivity of Caspofungin on the Resistant Isolates of *Candida albicans*

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Abstract

Objective: To compare the performance of Caspofungin with the conventional antifungal drugs on the *Candida albicans* isolates.

Materials and methods: Cases and Samples: The present study was carried out in the Department of Microbiology J. N. Medical College, AMU. Total of 6000 patients included in the study. Samples were collected according to their clinical presentation.

Identification of Candida species: It was done as per the standard protocol on 6000 samples,103 Candida spp. were isolated. Among which 68 were *Candida albicans* and 35 were Non-albicans Candida.

Evaluation and comparision of antifungal activity of Caspofungin: a. Disc diffusion, and b. Broth mico dilution method.

Results: The susceptibility testing of Caspofungin and other conventional antifungal agents by broth dilution method revealed very low MIC of Caspofungin (0.062-1 µg/ml) as compared to fluconazole (1-64 µg/ml). Thus, Caspofungin proved to be more potent than other antifungals *in vitro*.

Conclusion: We found Caspofungin to be more potent on the resistant isolates of *Candida albicans in vitro*.

Keywords: *C. albicans*; Caspofungin; Fluconazole; Disc diffusion; Broth microdilution

Introduction

In comparison to bacterial pathogens, fungi were less frequently the cause of infectious diseases in humans. However, with the increased number of immunosuppressed patients, fungal infections have gained enormous medical importance. Today Candida spp. have become common nosocomial pathogens frequently leading to death and represents a serious public health challenge with increasing medical and economic importance due to the high mortality rates and increased costs of care and duration of hospitalization [1,2].

In recent years, a rapid increase in microbes that are resistant to conventional antibiotics has been observed worldwide. In India, there is a lack of multicentric studies regarding antifungal susceptibility pattern.

There have been a few reports of strains of *C. albicans* showing resistance to amphotericin B and azoles [3]. But irrespective of their resistance to azoles and amphotericin B, results of a global surveillance which dealt with trends in the susceptibility of Candida spp. to Caspofungin found no evidence for a shift in the Caspofungin MIC distribution [4]. Caspofungin is a fungicidal echinocandins and is active against many species of Candida. Echinocandin is a new class of antifungal agents, which acts through noncompetitive inhibition of 1,3-β D glucan synthesis of the fungal cell wall. Caspofungin and other echinocandins exhibit potent activity against fluconazole resistant Candida spp. The emerging trend of resistance to fluconazole and other triazoles among Candida isolates from Blood Stream Infections(BSI) has made Caspofungin very important. Another advantage is its less drug related toxicity as compared to amphotericin B and Azoles.

Taking into consideration the above mentioned facts, the present study was undertaken with the following Aims and Objectives:

- To determine the prevalence of *Candida albicans* infection in and around Aligarh region.
- To assess the drug resistance pattern in the clinical isolates of *Candida albicans*.
- To compare the antifungal effects of Caspofungin with the conventional antifungal drugs.

Materials and Methods

Study group

The present study was carried out in the Department of Microbiology J. N. Medical College, AMU. Duration of study was One and half year from Jan 2015 to August 2016. The cases selected for the study are all clinically important immunocompromised patients susceptible to Candida infection, irrespective of age and sex which includes:

- Neonates and infants,
- Patients having endotracheal intubation,
- Patients on parenteral nutrition,
- Patients having central venous catheter,
- Recipients of intravenous lipid emulsion,
- Patients on systemic steroid use,
- Patients having high or low glucose level, insulin use, etc,
- Patients having low platelet count,
- Patients having complicated intra abdominal surgery,
- Patients on broad-spectrum antibiotics.

Various clinical specimens including skin swab, nails, oral

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swab, cervical swab, urine, sputum, BAL (Bronchoalveolar lavage), Endotracheal aspirate CSF, pus and blood culture were collected. The specimens were obtained using standard microbiological techniques for fungal organisms.

Direct microscopy

Specimens like endotracheal aspirate, urine, oral swab etc. were subjected to direct microscopy by making a KOH mount and/or a Gram stained smear.

Fungal culture

The culture was done on two Sabouraud dextrose agar (SDA) slants containing chloramphenicol (0.05 mg/ml) by rolling over the surface and subsequently in BHI broth also. One tube was incubated at 25°C and the remaining tube and BHI broth were incubated at 37°C. The isolates were identified in accordance to (i) Colony characteristics, (ii) Germ-tube test (GTT test), (iii) growth at 42°C, (iv) morphology on CMA, (v) Sugar fermentation tests and (vii) Sugar assimilation tests [5-7].

Antifungal susceptibility testing

Antifungal susceptibility testing was performed by the disc diffusion and broth microdilution methods.

Disc diffusion method: Antifungal susceptibility by the disc diffusion method was performed by modification of the method described by Chakrabarti et al. The antifungal tested were Amphotericin B, Nystatin, Ketoconazole, Clotrimazole, Fluconazole, Itraconazole and Caspofungin (HiMedia Laboratories, Mumbai, India). Test strain was considered Sensitive - when the zone diameter was ≥ 80% of the zone diameter of the control strain. Intermediate - when the zone diameter was <80% but there is visible zone of inhibition. Resistant - when there was no zone of inhibition.

Broth micro dilution method: Broth micro dilution method was adopted in this study as per CLSI Guidelines based on Document No. M-27A3. The antifungal tested were Fluconazole, Ketoconazole and Caspofungin. MICs were calculated as the lowest concentrations at which there was 80% inhibition of growth compared with that in a drug free control.

Results

6000 samples were collected in one and half yr of study. Out of 6000 samples, Candida was isolated in 103 (1.7%) as per the standard protocol of identification. Among the total Candida isolates, 68 (66.02%) were *C. albicans* and remaining 35 (33.98%) were non-albicans Candida. Among the non-albicans Candida, 12 were *C. glabrata*, 11 were *C. tropicalis*, 5 were *C. krusei*, 5 were *C. dubliniensis* and 2 were *C. gulermondii* according to the chrome agar results. But as this study was concerned with *C. albicans* only so, further confirmation for non-albicans was not done.

Resistance pattern of *Candida albicans* isolates with different antifungals via disc diffusion method is mentioned in Table 1. By broth microdilution, 22.2% isolates of *Candida albicans* were resistant to fluconazole and 5.6% isolate were dose dependent sensitive (Table 2). But no isolates of *Candida albicans* were resistant to Caspofungin (Table 3). The susceptibility testing of Caspofungin and other azoles by broth dilution method revealed very low MIC of Caspofungin (0.062-1 µg/ml) as compared to fluconazole (1-64 µg/ml).

Discussion

Overall the rate of Candida isolation from various specimens in our study group was 1.7%. *C. albicans* formed the largest group (66.02%) of Candida species isolated in this study. This observation is consistent with that of various works which reports nearly identical reports. Pfaller had reported 50 to 70% *Candida albicans* isolation, Wingard reported 54%, 65% by Roilides et al. and 66% by Pfaller et al. in 2007 [8-11]. Indian studies which also reported similar findings were Narain et al. (53.3%) and Kaur et al. (50%). However, Kotwal et al. in 2011 noted a much higher prevalence of *C. albicans* (78.1%) [12-14].

However certain other studies showed non albicans Candida as the most frequently isolated species. This species variation may be due to the differences in empiric or prophylactic practices.

In our study, resistance was observed in 22.2% isolates to fluconazole, 27.9% isolates to ketoconazole and clotrimazole, 23.5% isolates to itraconazole and 11.8% isolates to amphotericin B. 5% isolates to nystatin and none to caspofungin. These findings affirm with the study conducted by Narang et al. and Kotwal et al. who found a higher rate of fluconazole resistance (24% and 26% respectively) [15]. The study was in contrast to the study by Xess et al., Belet et al. and Rizvi et al. who reported 11.7%, 8.5% and 10.3% resistance to fluconazole respectively [16-18].

In this study, all the isolates (100%) were susceptible to Caspofungin. This finding is similar to the study of Pfaller et al. who determined the *in vitro* activity of Caspofungin against 351 fluconazole resistant Candida

Antifungal agent	Sensitive	Resistant
Clotrimazole	49(72.1)	19(27.9)
Fluconazole	50(73.5)	18(26.5)
Amphotericin B	60(88.2)	8(11.8)
Nystatin	67(98.5)	1(1.5)
Ketoconazole	49 (72.1)	19(27.9)
Itraconazole	52(76.5)	16(23.5)
Caspofungin	68(100)	0(0.0)
Figures in parenthesis indicate percentage		

Table 1: Susceptibility pattern of Candida isolates to various antifungal agents.

	MIC of fluconazole (µg/ml)										Total
	0.125	0.25	0.5	1	2	4	8	16	32	≥ 64	
C. albicans		-	-	13(72.2)	-	-	-	-	1(5.6)	4(22.2)	18(100)
Figures in parenthesis indicate percentage (S, <8 µg/ml; S-DD, >8 µg/ml and ≤ 32 µg/ml; R, >32 µg/ml)											

Table 2: MIC values for fluconazole by broth dilution method.

	MIC of Caspofungin (µg/ ml)										Total
	0.125	**0.062**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥ 64**	
C. albicans	-	16(88.8)	1(5.6)	1(5.6)	-	-	-	-	-	-	18(100)
Figures in parenthesis indicate percentage (S, <0.25 µg/ml; S-DD,0. 25 µg/ml and ≤ 16 µg/ml; R, >16 µg/ml)											

Table 3: MIC values for Caspofungin by broth dilution method.

isolates and reported that 99% were susceptible to Caspofungin [19]. Bachmann et al. in 2002 reported that caspofungin was equally active against fluconazole-susceptible and fluconazole-resistant isolates [20].

Antifungal drug resistance is a rapidly changing problem especially in the immunocompromised patients. Treatment failure, attributable to the development of azole resistant *C. albicans* strains appears to become common nowadays. Most of the antifungal agents are limited in clinical applications because of their complications. For example, Amphotericin B has bad effect on kidneys and leads to renal failure, fever, nausea, diarrhea after using these drugs. The Azoles family like Fluconazole cause liver toxicity and inhibits testosterone synthesis. So, new drugs with less side effects are the need of the hour. Caspofungin is an echinocandin antifungal agent exhibiting significant *in vitro* activity against the Candida spp. Caspofungin is very potent and effective against Candida spp. including some azoles-resistant isolates. Caspofungin is well tolerated by most patients and drug-related toxicity is minimal. The excellent safety profile evident for patients with candidiasis permits the usage of caspofungin for the treatment of complicated infections in immunocompromised patients [21,22].

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