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Original Article

Microbiology Section

Caspofungin MIC Distribution amongst Commonly Isolated *Candida* Species in a Tertiary Care Centre - An Indian Experience

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ABSTRACT

Introduction: Emergence of *Candida* species resistant to Amphotericin B and triazole has led to use of echinocandins, mostly caspofungin in the management of invasive candidiasis. There are some published reports of caspofungin resistance in *Candida* species yet no studies on caspofungin susceptibility pattern of *Candida* species exist in Indian setup.

Aim: To carry out the antifungal susceptibility of *Candida* isolates against caspofungin.

Materials and Methods: In a retrospective study at a tertiary care teaching hospital, 60 preserved *Candida* isolates from inpatients of invasive candidiasis obtained over a period of 6 months from January 2015 to June 2015 were subjected to antifungal susceptibility to caspofungin and the Minimum Inhibitory Concentrations (MICs) of *Candida* species to caspofungin were determined by Epsilometer test (E-test).

Results: Thirty *Candida albicans* and 30 Non *albicans Candida* mainly *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis* were tested for caspofungin susceptibitity by E-test. Caspofungin resistance was detected in 6.67% *Candida* albicans isolates. Caspofungin resistance was not observed in *Candida parapsilosis*, *Candida glabrata* and *Candida tropicalis*. This shows that caspofungin resistance is still rare. Further elaborate studies with clinical correlation data are needed to detect prevalence of caspofungin resistance.

Conclusion: Emergence of resistance in our study warrants need of elaborate studies with clinical correlation data to detect prevalence of resistance to caspofungin. E-test method proved to be an easy and simple technique for testing susceptibility of Candida to caspofungin.

Keywords: Anti-fungal susceptibility, E-test, Fluconazole

INTRODUCTION

Candida species, a genus of ubiquitous yeasts and the commonest cause of fungal infections in humans is associated with a wide disease spectrum ranging from superficial mucocutaneous candidiasis to invasive candidiasis [1]. In the last three decades, rapid surge of immunocompromised population primarily HIV-AIDS and diabetes, indiscriminate use of broad spectrum antibiotics, malignancies and use of immunosuppressants for solid organ transplants has increased the problem of mucosal and systemic candidiasis [1-3].

Candida albicans being the commonest *Candida* affecting human has now been replaced by non albicans species such as *Candida tropicalis*, *Candida parapsilosis, Candida krusei, Candida glabrata, Candida kefyr, Candida guillermondii, Candida lusitaniae and Candida haemulonii* [4,5].

Taking into consideration route of administration, bioavailability, half life, side effects, Cerebrospinal Fluid (C.S.F) penetration and cost of treatment, fluconazole is the triazole of choice in invasive candidiasis [6-8].

Few *Candida* species have intrinsic resistant to triazoles [9,10]. Sub-therapeutic and prolonged triazole exposure might induce resistance in *Candida*. Concomitant tuberculosis in People Living with HIV-AIDS (PLWHA) warrants the co-administration of fluconazole with rifampicin. Rifampicin decreases the blood levels of fluconazole by inducing its hepatic metabolism [9]. Thus, strains which are susceptible in-vitro might not be exposed to required MIC in-vivo. This low level of drug promotes drug resistance [9,10].

With emergence of *Candida* species resistant to Amphotericin B in clinical isolates has led to the wider use of fluconazole, thus exposing sensitive strains to fluconazole more often, thus, promoting resistance [11].

With emerging triazole resistance, echinocandins are last resort drugs in the management of invasive candidiasis [12]. Till date *Candida* species exhibits significant sensitivity to echinocandins [12].

Cross-resistance between echinocandins is an area of concern too. Studies show that, activity of all echinocandins may be reduced in a setting of caspofungin (an echinocandin antifungal) resistance [13]. This suggests that, for prediction of echinocandidn susceptibility, we might use caspofungin as a surrogate marker.

There has been several published reports on the occurence of caspofungin resistance in *Candida* species especially *Candida glabrata* [12-16]. But in the Indian set-up no substantial report on echinocandin susceptibility pattern in *Candida* species exists.

A study was thus initiated to perform anti-fungal susceptibility of *Candida* isolates to caspofungin and determine the Minimum Inhibition Concentration (MICs) of *Candida* species to caspofungin by Epsilometer test (E-test).

MATERIALS AND METHODS

A retrospective study was conducted at a tertiary care teaching hospital. Sixty preserved *Candida* isolates from invasive candidiasis cases over a period of 6 months from January 2015 to June 2015 were tested for caspofungin susceptibility.

Sample size calculation: Due to lack of echinocandin susceptibility data in Indian set-up, proper sample size calculation was not possible. Hence, all preserved *Candida* isolates obtained from invasive clinical specimens of inpatients over the said period was included in the study.

Since, the study was conducted on preserved *Candida* isolates waiver of consent was obtained from Institutional Ethics committee. Clinical outcome data was unavailable.

Preserved isolates at -20°C were revived and subcultured twice on Sabouraud's Dextrose Agar (SDA) to ensure purity and viability. The identification of the isolates was confirmed up to species level using standard identification protocol (gram staining, germ tube test, morphologic appearance by Dalmau technique and sugar assimilation and fermentation tests) [17]. Susceptibility testing was done on fresh sub-cultures made 24 hours prior to testing.

MIC determination by Caspofungin E-test was performed using RPMI 1640 agar medium supplemented with 2% glucose and 3-morpholinopropane-1-sulfonic acid (MOPS) buffer and the results were interpreted after 24 hours. The E-test method was performed according to the manufacturer's instructions (AB Biodisk, Sweden). The strips contain a pre-defined and continuous gradient of drug which enables quantitative MIC determination. A cotton-tipped, sterile swab was used to inoculate *Candida* from a 0.5 McFarland density standard yeast suspension onto a 90-mm agar plate containing RPMI 1640 medium supplemented with 2% glucose and buffered with MOPS to pH 7.0. Excess moisture was allowed to be fully absorbed into the agar. E-test strips were applied to the inoculated surface. The plates were incubated at 35°C and read at 24 hours. The MIC was read as the lowest concentration at which the border of the elliptical zone of growth inhibition intersected the scale on the test strip. An 80% inhibition in growth was used as the MIC cut-off (microcolonies were ignored). The E-test MICs were rounded up to the next even log 2 concentrations.

The results were interpreted with revised clinical breakpoints for echinocandins, determined by CLSI broth dilution method, published by CLSI as CLSI M27-S4 in 2012 [18]. New CLSI breakpoints, 2012 defining susceptibility to caspofungin are as follows [Table/Fig-1].

Isolate	MIC values (µg/ml)		
	Susceptible (S)	Intermediate (I)	Resistant (R)
Candida albicans	≤0.25	0.5	≥1
Candida glabrata	≤0.12	0.25	≥0.5
Candida parapsilosis	≤2	4	≥8
Candida tropicalis	≤0.25	0.5	≥1

[Table/Fig-1]: MIC breakpoint values for Caspofungin against *Candida* species (as per CLSI document M27-S4, 2012).

Quality control strains and MIC quality control ranges were used as per manufacturer's instructions [19]. They were 0.064µg/ml - 0.25µg/ml for *Candida albicans* ATCC 90028, 0.25µg/ml - 1µg/ml for *Candida krusei* ATCC 6258, and 0.25µg/ml - 2µg/ml for *Candida parapsilosis* ATCC 22019 [19].

In this study, only the prevalence of caspofungin resistance among Candida isolates was determined. No comparisons were made with any other parameters. Hence, no statistical tests were employed in the analysis of data.

RESULTS

Sixty isolates of *Candida* species were evaluated. It included 30 isolates of *Candida albicans*, 12 isolates of *Candida glabrata*, 10 isolates of *Candida parapsilosis* and 8 isolates of *Candida tropicalis.* Forty eight (80%) isolates were susceptible, 10(16.67%) isolates were intermediate and 2(3.33%) isolates were resistant [Table/Fig-2].

Number of susceptible strains as per new CLSI breakpoint criteria 2012 were 24(80%) for *Candida albicans*, 8(66.67%) for *Candida glabrata*, 10(100%) for *Candida parapsilosis* and 6(75%) for *Candida tropicalis*. Number of intermediate strains as per new CLSI breakpoint criteria 2012 were 4(80%) for *Candida albicans*, 4(75%) for *Candida glabrata* and 2(75%) for *Candida tropicalis* [Table/Fig-2].

Isolate	Number of isolates			
	Susceptible	Intermediate	Resistant	Total
Candida albicans	24	4	2	30
Candida glabrata	8	4	0	12
Candida parapsilosis	10	0	0	10
Candida tropicalis	6	2	0	8
Total	48	10	2	60

[Table/Fig-2]: Susceptibility pattern of *Candida* species as determined by E-test.

Isolate	MIC values (µg/ml)									Total no. of isolates
	MIC = 0.06 µg/ml	MIC= 0.12 µg/ml	MIC = 0.25 µg/ml	MIC= 0.5 µg/ml	MIC = 1 µg/ml	MIC = 2 µg/ml	MIC = 4 µg/ml	MIC = 8 µg/ml	No Zone on Etest	
Candida albicans	6	18	0	4	0	0	0	0	2	30
Candida glabrata	0	8	4	0	0	0	0	0	0	12
Candida parapsilosis	0	0	0	3	7	0	0	0	0	10
Candida tropicalis	0	3	3	2	0	0	0	0	0	8
Total	6	29	7	9	7	0	0	0	2	60

[Table/Fig-3]: Number of *Candida* species isolates with various MIC values to caspofungin.

Two (6.67%) isolates of *Candida albicans* were resistant as they showed no zone of inhibition [Table/Fig-3].

DISCUSSION

Caspofungin, a member of a novel echinocandin family, is a potent fungicidal agent against all strains of *Candida.* In our study, E-test was used to detect caspofungin susceptibility in *Candida* species.

Caspofungin resistance in *Candida* species is rare [12,13]. This is probably due to limited use owing to high cost of echinocandin therapy especially in developing countries [12]. But, in the face of increasing azole resistance [9-12], use of echinocandins, namely caspofungin is expected to increase in the near future. Hence, knowledge about the caspofungin susceptibility pattern in the region will allow better patient management. In our study 80% (48/60) *Candida* species were caspofungin susceptible, 16.67%(10/60) were caspofungin intermediate while 3.33% (2/60) exhibited caspofungin resistance [Table/Fig-2]. Badiee et al., reported similar resistance rates of *Candida* species to caspofungin, which shows that caspofungin resistance is still a rarity among *Candida* isolates [12].

The standard method of antifungal susceptibility testing is MICs detected by CLSI broth dilution [18]. When compared, the result of CLSI broth dilution and E-test for caspofungin susceptibility testing. Arendrup and Pfaller found that MICs detected by E-test were 1 dilution step higher for *Candida albicans* and *Candida tropicalis*, 2 dilution steps higher for *Candida glabrata*, 3 dilution steps higher for *Candida krusei* and 1 dilution step lower for *Candida parapsilosis* as compared to MICs detected by CLSI broth dilution [20]. Considering these findings, if we analyse the MIC values obtained in our study [Table/Fig-3] it is possible that all intermediate isolates of *Candida albicans, Candida glabrata* and *Candida tropicalis* isolates might actually be susceptible.

Considering *Candida parapsilosis* isolates in our study, although susceptible, showed higher MICs as compared to other species [Table/Fig-3]. This finding is as expected of *Candida parapsilosis* which is known to exhibit low resistance yet have intrinsically higher MIC values to caspofungin due to amino acid polymorphisms in Fks 1 region [12,13].

Hence, in our clinical setup too caspofungin resistance was rare possibly attributed to limited use.

The two resistant isolates were *Candida albicans*, which constituted 6.67% (2/30) of all *Candida albicans* isolates [Table/Fig-2]. From the point of view of our study, it is imperative to consider the existence of caspofungin resistance in 6.67% *Candida albicans*. Resistance of *Candida albicans* to caspofungin is rare though reported by Badiee P et al., in 2011 in a meagre 1.8% isolates [12]. *Candida albicans* is clearly the commonest cause of candidiasis [1] and hence, this finding is alarming.

None of our *Candida glabrata* isolates were caspofungin resistant. Yet literature suggests that caspofungin resistance in *Candida* exists primarily among *Candida glabrata* isolates [12,14-16] owing to rapid acquisition of Fks1 hot spot mutations due to genomic plasticity of its haploid genome, during the course of prolonged caspofungin therapy [13,21]. Limited use of caspofungin in our set-up might explain the absence of caspofungin resistance in *Candida glabrata*, in our study.

At present, the only standard method for detecting caspofungin resistance is the CLSI broth dilution method interpreted with revised clinical breakpoints for echinocandins, published by CLSI as CLSI M27-S4 in 2012 [18]. Resistance to caspofungin can also be identified using molecular platforms for detecting Fks1 hot-spot mutations [22]. Both these techniques are considered to be accurate [18,22]. But, broth dilution is very labour intensive and cannot be used in routine laboratories [23]. On the other hand, molecular detection techniques are too expensive to be employed by all routine laboratories [24]. E-test however, is a relatively cheap and easy to perform alternative for caspofungin susceptibility testing [23]. Anna Serefko and Anna Malm in their study in 2007 showed that the E-test method was a reliable technique performing invitro susceptibility testing of *Candida albicans* to caspofungin [25].

At present no standard performance characteristics exist for the interpretation of caspofungin E-test in *Candida* species. Our study attempts to provide preliminary data which might be beneficial to other extensive studies for preparation of caspofungin E-test interpretive criteria.

Low resistance (3.33%) of *Candida* species to caspofungin is probably due to infrequent use of caspofungin in our setup. Occurence of caspofungin resistance in 6.67% *Candida albicans* isolates is alarming since, *Candida albicans* is the commonest cause of candidiasis and is rarely known to exhibit caspofungin resistance. Hence, there is a need of elaborate studies with clinical correlation data to detect prevalence of resistance to caspofungin.

LIMITATION

Sample size calculation was not possible because of lack of similar studies in the Indian set-up and hence, the results of this study cannot be extrapolated to a larger population. Due to a small sample size subgroup analysis of prevalence of caspofungin resistance based was not possible. Being a retrospective study, data on clinical outcome and duration of caspofungin therapy was not available.

CONCLUSION

Caspofungin resistance in 6.67% *Candida albicans* isolates is alarming and elaborate studies need to be conducted with clinical correlation data to detect prevalence of resistance to caspofungin. Echinocandins are the last resort drugs in the antifungal armamentarium. Judicious use of echinocandins is necessary to prevent emergence of resistance to them. E-test method proved to be an easy and simple technique for testing susceptibility of *Candida* to caspofungin.

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