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SPECIATION AND ANTIFUNGAL SUSCEPTIBILITY PROFILE OF CLINICAL CANDIDA ISOLATES

Microbiology

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ABSTRACT

INTRODUCTION: The epidemiological shift towards increase in prevalence of non-*albicans Candida* species, emergence of new Candida species and development of antifungal drug resistance to Candida species in global scenario made it relevant to identify Candida species with their antifungal susceptibility pattern. Hence, the present study was carried out.

MATERIALS & METHODS: Present prospective study was conducted at microbiology department of M.G.I.M.S. Sevagram, Maharashtra, India from January 2010 to December 2011. A total of 128 Candida strains, from various clinical specimens were identified by standard diagnostic techniques and with HiCrome Candida differential agar. All the identified Candida strains were tested for susceptibility against four antifungal agents amphotericin B, clotrimazole, ketoconazole and fluconazole by broth microdilution method (M27-A2) as per CLSI guidelines.

RESULTS: *C. albicans* (60.15%) were predominated over non-*albicans Candida* (39.85%). 3.90% isolates showed resistance to amphotericin-B, 7.03 % noted resistance to fluconazole while 3.90% isolates exhibited resistance to clotrimazole. *C. guillermondii* found susceptible to all antifungal agents and none of Candida isolate found resistant to ketoconazole.

CONCLUSION: Candida speciation and antifungal resistance surveillance must be essential for studying the trend of antifungal resistance in a particular area and thereby to develop rational antifungal therapy.

KEYWORDS

Candida species, antifungal susceptibility, broth microdilution method

INTRODUCTION

Candida is unique among mycotic pathogens as it causes a broad spectrum of clinical manifestations ranging from mere mucocutaneous overgrowth to life threatening systemic infections [1]. Invasive Candida infections are one of major causes of morbidity and mortality in immunocompromised as well as critically ill immunocompetent patients [2]. Candidiasis has emerged itself as an alarming opportunistic disease due to increase in the number of immunocompromised, aged, receiving prolonged antibacterial and aggressive cancer chemotherapy or undergoing invasive surgical procedures and organ transplantation patients [3]. Though *C. albicans* is generally considered as the major pathogen, during last decades the epidemiological shift towards increase in the prevalence of non-*albicans Candida* species has been noted along with emergence of new Candida species [3]. The drug resistance scenario has been also increasing due to over growing use of random antifungal agents [4]. The commonly used antifungal drugs show significant variation in the susceptibility pattern among the different Candida species. Several previous studies reported the emergence of antifungal drug resistance Candida species in global scenario [5]. With this background, the present study was undertaken to speciate the Candida isolates and to know their antifungal susceptibility profile for using specific and sensitive drugs for better therapeutic outcome.

MATERIALS AND METHODS

The present laboratory based prospective study was conducted at microbiology department of Mahatma Gandhi Institute of Medical Sciences, Sevagram, Maharashtra, India from January 2010 to December 2011. A total of 128 Candida strains, from various clinical specimens were identified by standard diagnostic techniques (germ tube test, chlamydospore formation on corn meal agar, sugar fermentation, sugar assimilation tests and temperature tolerance at 45^{0}C) and with HiCrome Candida differential agar (HiMedia, Mumbai, India). All the identified Candida strains were tested for susceptibility against four antifungal agents amphotericin-B (HIMEDIA), clotrimazole (SIGMA), ketoconazole (HIMEDIA) and fluconazole (HIMEDIA) by broth microdilution method (M27-A2) as per CLSI guidelines [6]. The results of antifungal susceptibility for fluconazole were interpreted as per CLSI interpretative criteria and for amphotericin B, ketoconazole as per Chakrabarti et al [7]. For clotrimazole, the results were interpreted as per Pelletier et al [8] and Martel et al [9].

RESULTS

Table 1: Different Candida species (n=128)

Candida Species	Number (%)
Candida albicans	77 (60.15)
Candida tropicalis	23 (17.96)
Candida parapsilosis	18 (14.06)
Candida krusei	09 (7.03)
Candida guillermondii	01 (0.78)

Of the 128 Candida isolated, *C. albicans* (77/128; 60.15%) were predominated over non-*albicans Candida* (51/128; 39.84%). The non-*albicans Candida* species isolated were *C. tropicalis* (17.96%), *C. parapsilosis* (14.06%), C. krusei (7.03%) and *C. guillermondi* (0.78%).

Table 2: Candida species susceptibility to antifungal agents

Candida species	Amphotericin B			Clotrimazole			Ketoconazole			Fluconazole		
	S	I	R	S	I	R	S	I	R	S	SD	R
C. albicans (77)	68 (88.31)	6 (7.79)	3 (3.89)	74 (96.10)	-	3 (3.89)	73 (94.80)	4 (5.19)	-	72 (93.50)	2 (2.59)	3 (3.89)
C. tropicalis (23)	19 (82.60)	3 (13.04)	1 (4.34)	22 (95.65)	-	1 (4.34)	21 (91.30)	2 (8.69)	-	21 (91.30)	1 (4.34)	1 (4.34)
C. parapsilosis (18)	18 (100)	-	-	17 (94.44)	-	1 (5.55)	13 (72.22)	5 (27.7)	-	18 (100)	-	-
C. krusei (9)	8 (88.88)	-	1 (11.11)	9 (100)	-	-	9 (100)	-	-	4 (44.44)	-	5 (55.55)
C. guillermondii (1)	1 (100)	-	-	1 (100)	-	-	1 (100)	-	-	1 (100)	-	-
Total (128)	**114 (89.06)**	**9 (7.03)**	**5 (3.90)**	**123 (96.09)**	**-**	**5 (3.90)**	**117 (91.40)**	**11 (8.59)**	**-**	**116 (90.62)**	**3 (2.34)**	**9 (7.03)**

Among all Candida isolates tested, 3.90% *Candida* isolates showed resistance to amphotericin-B comprising *C. albicans* (3.89 %), *C. tropicalis* (4.34 %) and C. *krusei* (11.11 %). Among the 3.90% Candida isolates exhibited resistance to clotrimazole, 3.89 % were *C. albicans*, 4.34 % were *C. tropicalis* while 5.55 % were *C. parapsilosis.* 7.03 % Candida isolates noted resistance to fluconazole representing *C.*

albicans (3.89 %), *C. tropicalis* (4.34 %) and C. *krusei* (55.55 %). None of the Candida isolate was found to be resistant to ketoconazole. The isolate of *C. guillermondii* was found to be susceptible to all the antifungal agents.

DISCUSSION

Comparative study of different Candida species isolated in their studies by different workers [7,10,11,12,13,14] showed that *C. albicans* isolation was highest in each of them except Chakrabarti et al [8], who reported *C. tropicalis* was highest (42 %) and *C. albicans* was 25 %. In our study also, the most frequently isolated species was *C. albicans* accounting for 60.15%. In the study of Baradkar et al [10], *C. albicans* isolation rate was higher (70%) than our study while Pfeller et al[11], Prasad et al [12], Vijaya et al [13] and Grace et al [14] were reported 52%, 47.6%, 46% and 43.15% respectively which were lower than that our study. In the present study, most common non-*albicans Candida* species was *C. tropicalis* (17.96%) which approximates to that reported by Baradkar et al [10] (17.94%), but isolation rate lower than that showed by Vijaya et al [13] (35.29 %). Other non-*albicans Candida* species representing *C. parapsilosis* (14.06%), *C. krusei* (7.03%) and *C. guillermondi* (0.78%) found in this study were also comparable with other workers [7,10,11,12,13,14]. In India, amphotericin-B is the drug of choice for invasive candidiasis with low or no resistance reports. However Chakrabarti et al [7] in his study reported the emergence of resistance to amphotericin-B in 15.4 % of *C. albicans* strains, 8.1 % in *C. tropicalis* strains and 33.3 % in *C. krusei* strains. In our study also, 3.9% Candida isolates showed resistance to amphotericin-B representing *C. albicans* (3.89 %), *C. tropicalis* (4.34 %) *and* C. *krusei* (11.11 %). Narang et al [15], in a study of neonatal systemic candidiasis found 24% resistance against fluconazole. In our study, 6.25% Candida isolates showed resistance to fluconazole including *C. albicans* (1.56 %), *C. tropicalis* (4.34 %) and C. *krusei* (55.55 %). Narain et al [16] reported 18.75 % strains of *C. albicans,* 14.5% strains of *C. tropicalis* and all the strains of *C. krusei* resistant to fluconazole. In the study of Madhu Sharma et al [17], resistance for fluconazole was found in *C. albicans* (14.28%), *C. tropicalis* (14.28%), *C. parapsilosis* (11.11%) and *C. krusei* (100%). None of the Candida isolate found resistant to ketoconazole in present study was exactly paralleled to findings of G. Sasikala et al [18]. However, Changdeo S. Aher [19] in his study reported 37.2 % Candida isolates resistant to ketoconazole while Khadka et al [20] reported it as 86%. 3.90% Candida isolates found resistant against clotrimazole in our study can be comparable with Khadka et al and Jayachandran AL et al [21] who reported it as 6% and 7.69% respectively. The resistant Candida isolates to clotrimazole found in our study were *C. albicans* (3.89 %), *C. tropicalis* (4.34 %) *and C. parapsilosis* (5.55%). Khadka et al noted 7.2% *C. albicans* while Jayachandran AL et al reported 7.52% *C. albicans*, 13.33% *C. tropicalis* and 9.09% *C. krusei* resistant against clotrimazole in their studies.

CONCLUSION

In our study, *C. albicans* was predominant species and antifungal resistance to amphotericin-B, fluconazole & clotrimazole was also found less. However, Candida speciation and antifungal resistance surveillance must be essential for studying the trend of antifungal resistance in a particular area and thereby to develop rational antifungal therapy.

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