Species distribution and in vitro antifungal susceptibility patterns of Candida

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Abstract

Introduction: Due to advance in medical and surgical management nosocomial fungal infection rate has been increased. Recently Candididiasis has become one of the major fungal infections among hospitalized patients. Several Candida species can give rise to same clinical presentations but their antifungal susceptibility patterns may be different. The aim of the study is species identification of Candida, isolated from different clinical samples and to carry out their in vitro antifungal susceptibility tests.

Methods: From different clinical samples several Candida species were isolated. Speciation of Candida was done by using Corn meal agar, Chrom agar and Hi-Candida identification kit. In vitro antifungal susceptibility to fluconazole, ketoconazole and amphotericin B was performed by disc diffusion method as per CLSI document M44A.

Results: From 108 samples, most common species isolated was C. albicans (42.5%), followed by C. tropicalis, C. glabrata, C. parapsilosis and C. krusei. Overall 71.87% of isolates were found to be fluconazole sensitive. Fluconazole resistant rate is seen higher in Candida krusei, Candida glabrata, and Candida tropicalis. Ketoconazole was found to be sensitive against 87% of isolates. Amphotericin B was effective against 100% of isolates, although reduced zone size to Amphotericin B was seen by 10 isolates.

Conclusion: Speciation of Candida and antifungal susceptibility testing should be done routinely to prevent therapeutic failures.

Keywords: Antifungal sensitivity, amphotericin B, Candida albicans, fluconazole,

Introduction

Nosocomial fungal infection has been increased nowadays due to advancement in medical management, and change in patient profile. Ubiquitous in nature, Candida inhabit the gastrointestinal tract including the mouth, oropharynx, female genital tract and skin. The genus Candida includes several species implicated in human pathology. Candida albicans is by far the most common species causing infections in humans[1]. The emergence of non-albicans Candida spp. as significant pathogens has however been well recognized during the past decade.[2,3] Candida spp. have been shown to cause a similar spectrum of disease ranging from oral thrush to invasive disease, yet differences in disease severity and susceptibility to different antifungal agents have been reported[4]. Amphotericin B, a polyene fungicidal agent, has been used for the treatment for invasive Candidal infections, but cost and dose related side effects limit its use.[5] Azole group of drugs, are commonly used in treating many forms of Candidal infections for a long time, however, their prolonged use has led to the development of drug resistance in C. albicans and other

species. Azole resistance is more seen in non Candida albicans spp. compared to Candida albicans[6,7]. Antifungal susceptibility test was done by disc diffusion method, as per CLSI guideline in M44-A document[8]. By doing early speciation of Candida and performing antifungal susceptibility tests, it will be helpful in guiding physicians to select the proper antifungal drug and thus therapeutic failures can be prevented.

Methods

The study was carried out in the department of Microbiology, R.G. Kar medical college, India during January 2011 to December 2011. The aims of the study were to isolate and identify Candida species from different clinical samples and to determine the antifungal susceptibility pattern to fluconazole, ketoconazole, and amphotericin B.

A total of 108 Candida isolates from various clinical samples (oropharyngeal swabs, high vaginal swabs, blood, respiratory specimens, skin and nail scrapings, and synovial fluid) were taken up for species identification. All the samples were inoculated on Sabouraud's Dextrose Agar (SDA) slants supplemented with chloramphenicol and incubated at 37°C for 24-48 hrs. Any growth found on SDA slope was processed for identification of the species. From the isolated colony, Gram staining, and germ tube test were performed. Speciation of Candida was done by Dalmau plate[9,10] method, using Corn meal agar. From pure isolated colony heavy inoculum of yeast was streaked across Corn meal agar plate and cover slip was placed over it and incubated for 48hrs at 25°C in BOD incubator. The arrangement of hyphae, pseudohyphae, blastospores, and chlamydiospores were noted after incubation of 72 hours.

CHROM agar Candida[11,12,13] (HiMedia, Mumbai, India) was used to differentiate several Candida spp. by growth of different coloured colonies in it. This is based on the direct detection of specific enzymatic activities by adding certain substrates of fluorochromes to the media. A subculture was made from primary isolation media in CHROM agar Candida media, and it was incubated at 37°C for 24 hours. After 24 to 48 hours colony morphology and colour of the colony was noted.

Hi Candida Identification Kit[6] was used to detect several sugar utilization by the Candida isolates. The kit contains 12 sterile medias for urease test and 11 different carbohydrates utilization tests . The tests are based on the principle of pH change and substrate utilization.

Disk diffusion method was employed for screening the susceptibility pattern of the yeasts. To standardize the disk diffusion test, Clinical and Laboratory Standards Institute (CLSI) subcommittee on antifungal susceptibility tests has developed recommendations in M44-A document[8].

A total of 94 pure isolates were selected for antifungal susceptibility test. Muller Hinton Agar supplemented with glucose and methylene blue, was prepared[14,15](Muller Hinton Agar + 2% Glucose + 0.5µg/ml Methylene Blue Dye) for carry out antifugal susceptibility tests.

Fluconazole (25µg), ketoconazole (15µm) and amphotericin B (100 units) discs were purchased from a commercial source. Antifungal susceptibility test was performed as per CLSI recommendations in document M44-A[8]. Results were compared with that of the results of C. albicans ATCC 90028, C. tropicalis ATCC 750, C. parapsilosis ATCC 90001.

Results

A total of 108 Candida isolates from different clinical samples including oropharyngeal swabs(33), high vaginal swabs (35), blood(18), respiratory samples(12), skin/nail scrapings (9) and synovial fluid(1) were processed for species identification as shown in table 1.

Table 1: Distribution of clinical samples in the study

Clinical specimens	Number of isolates
1.Oropharyngeal swabs	33
2.High vaginal swabs	35
3.Blood	18
4.Respiratory specimens	12
5.Nail and skin scrapings	09
6.Synovial fluid	01
Total	108

Candida albicans was the commonest species isolated (42.5%), followed by Candida tropicalis (27.7%), Candida glabrata (17.5%), Candida parapsilosis (7.4%) and Candida krusei (4.6%). Isolation rate of non Candida albicans spp. was higher (57.5%) compared to Candida albicans (42.5%) [Table 2]. A higher number of Candida albicans were found in all clinical samples except blood where Candida tropicalis was found to be predominant (44.4%). From synovial fluid Candida tropicalis was isolated. The second common isolated spp. from oropharyngeal candidosis was Candida tropicalis (24.2%) and that from high vaginal swabs was Candida glabrata (25.7%) as depicted in table 2.

Table 2: Species distribution of Candida among various clinical samples

Species	Specimen						
	OPC (n = 33)	HVS (n = 35)	Blood (n = 18)	Resp (n = 12)	Nail (n = 9)	SkinScraping	Synovial Fluid (n = 1)	Total (n = 108)
C. albicans	17(51.5)*	14(40)	4(22.2)	6(50)	5(55.5)		0	46(42.5)
C. tropicalis	8(24.2)	6(17.1)	8(44.4)	4(33.3)	3(33.3)		1	30 (27.7)
C. glabrata	5(15.1)	9(25.7)	2(11.1)	2(16.6)	1(11.1)		0	19 (17.5)
C. parapsilosis	3(9.0)	4(11.4)	1(5.5)	0(0)	0(0)		0	8 (7.4)
C. krusei	0(0)	2(5.7)	3(16.6)	0(0))	0(0)		0	5 (4.6)

OPC: Oropharyngeal candidosis, HVS: High vaginal swab, Resp: Respiratory specimens

In the present study Candida tropicalis was the most commonly isolated spp. from blood 44.4%), followed by Candida albicans (22.2%), Candida krusei (16.6%), Candida glabrata (11.1%), and Candida parapsilosis (5.5%). Overall 70.2% of isolates were susceptible to luconazole, 11.7% were susceptible dose dependent, whereas 18 % of isolates were resistant to fluconazole. Among the isolates of Candida albicans, 84.2% were susceptible to luconazole, and 10.5% isolates showed resistance to it. Fluconazole resistance rate is higher in Candida krusei (60%) followed by, Candida glabrata (29.4%) and Candida tropicalis (19.2%) as shown in table 3.

In the present study, 73.4% of the total isolates were susceptible to ketoconazole, 11.7 % were resistant to it, whereas 14.8% were susceptible dose dependent. Higher rate of ketoconazole resistance was seen in Candida krusei (20%), followed by Candida glabrata (17.6%), and Candida tropicalis (15.2%) (Table 3). All the isolates were susceptible to amphotericin B, although 10 isolates were susceptible dose dependent as depicted in table 3.

Table 3: Antifungal susceptibility patterns to fluconazole, ketoconazole, amphotericinB

Species	Antifungal drugs								
	Fluconazole			Ketoconazole			Amphotericin B		
	S	SDD	R	S	SDD	R	S	SDD	R
C. albicans (n = 38)	32 (84.2)	2 (5.2)	4 (10.5)	31 (81.5)	4 (10.5)	3 (7.8)	35 (92.1)	3 (7.8)	0
C.tropicalis (n = 26)	17 (65.3)	4 (15.3)	5 (19.2)	17 (65.3)	5 (19.2)	4 (15.3)	24 (92.3)	2 (7.6)	0
C. glabrata (n = 17)	9 (52.9)	3 (17.6)	5 (29.4)	10 (58.8)	4 (23.5)	3 (17.6)	14 (82.3)	3 (17.6)	0
C. parapsilosis (n = 8)	8(100)	0	0	7(87.5)	1(12.5)	0	8(100)	0	0
C. krusei (n = 5)	0	2 (40)	3 (60)	4 (80)	0	1 (20)	3 (60)	2 (40)	0
Total (n = 94)	66 (70.2)	11 (11.7)	17 (18.0)	69 (73.4)	14 (14.8)	11 (11.7)	84 (89.3)	10 (10.6)	0

S= susceptible, SDD = Susceptible dose dependent, R = resistant

Discussion

Candidiasis varies in acuteness and severity from oral thrush and Candidal diaper dermatitis to fungemia and may be associated with sepsis and shock indistinguishable from bacterial sepsis. Although C. albicans remain the most common pathogen in oropharyngeal candidiasis (OPC), non-albicans species are increasingly associated with invasive Candidiasis[16]. Non Candida –albicans Candida (NCAC) spp. are closely related to Candida albicans, but differ from each other with respect to epidemiology, virulence characteristics, and antifungal susceptibility.

In the present study from a total of 108 clinical samples Candida albicans was found to be most commonly isolated species, as seen in studies by different authors[2,17]. However in the present study the isolation rate of non albicans Candida was higher (57.5%) compared to Candida albicans (42.5%). In the study by Chakrabati A et al.,[18] and Mokaddas EM et al.,[19] it is seen that non-albicans Candida had a higher isolation rate than C. albicans from different clinical samples. These findings seem to suggest that non-albicans Candida are emerging as important pathogens. Frazer, Victoria J and co-workers[20] in their studies showed that in patients with sustained Candidaemia, the mortality which was associated with the non-albicans Candida species was higher, with a statistical significance. In the present study, Candida tropicalis was the commonest spp. (44.4%), isolated from blood samples, as seen in studies by Shivaprakasha S et. al and Xess I, Jain N et al.,[21,22] C. tropicalis is the most virulent of the NCAC species, this may be due to its ability to adhere to epithelial cells in vitro and its ability to secrete moderate levels of proteinase[23]

In the present study 18% of total isolates were found to be resistant to fluconazole by disc diffusion method. Highest rate of fluconazole resistant was seen in Candida krusei (60%), followed by Candida glabrata (29.4%), Candida tropicalis (19.2%). The resistance to fluconazole is of great concern, because it is the most commonly used azole for superficial as well as deep candidiasis. All the isolates are sensitive to amphotericin B, which is in agreement with the study by Mendirratta DK et al.[24] In another study by Deorukhkar SC et al.,[25] it was seen that the resistant rate of Candida to fluconazole and amphotericin B was 27.3% and 5.8% respectively.

In the present study higher rate of ketoconazole resistance was seen in Candida krusei (20%), followed by Candida glabrata (17.6%), and Candida tropicalis (15.2%). Higher rate of ketoconazole resistance in Candida glabrata (16.6%) was also seen in several other studies[26].

Among the different methods disc diffusion method is one of the simple, cost effective method that can give result within 42 hrs. In the studies of Pfaller M A et al.,[14] and Lee S C et al.,[15] it was seen that prepared MH-GMB agar plates provide acceptable performance for disk diffusion testing for at least 30 days when stored at 5°C.

Conclusion

An increase in the predisposing conditions has resulted in an increasing incidence of non albicans Candida infections. Some of the non albicans Candida spp. is intrinsically resistant to commonly used antifungal drugs. Therefore, early speciation of Candida isolates along with their antifungal susceptibility tests not only will restrict the empirical use of antifungal agents but also greatly influence the treatment options for the clinicians and thus will be beneficial for the patients.

Conflict of interest: The authors declare that they have no conflict of interests.

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