ORIGINAL ARTICLE

SUSCEPTIBILITY OF CANDIDA SPECIES TO ANTIFUNGAL DRUGS IN WESTERN INDIA

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ABSTRACT

Introduction: The increase in candidaemia is associated with high mortality. A shift has been observed in the relative frequency of each *Candida* spp. isolated from blood. Options of the antifungal drugs available for treatment of systemic & invasive candidiasis are restricted to polyenes, allylamines, azoles and recently developed echinocandin class of molecules. A rise in the incidence of antifungal resistance to *Candida* spp. has also been reported over the past decade. Studies on prevalence of infections and antifungal susceptibility testing are useful in deciding clinical strategies.

Aims: To do species level identification and detect resistance, if any, among Indian clinical isolates of *C. albicans.*

Methodology: From total 135 patients from a tertiary care hospital of Gujarat, *Candida* species were isolated from different clinical specimens. The growth of *Candida* on Sabouraud's dextrose agar was confirmed by Gram staining in which gram positive budding fungal cells were observed. Then its growth was examined for colony morphology on Sabouraud's dextrose agar and chlamydospore production on Corn meal tween 80 agar. Germ tube tests and other biochemical tests like sugar fermentation, sugar assimilation and urease test were performed to identify the species of Candida. Antifungal susceptibility testing was performed by NCCLS M44-A Disc diffusion method.

Results: Out of total 135 samples, C. Albicans were isolated from 52 (38.5%). Among Non Albican Candid (NAC), *Candida glabrata* was 36 (26.7%) followed by *Candida tropicalis 25*(18.5%). C. albicans was found resistant to Fluconazole, Itraconazole and Amphotericine B in 3.8%, 3.8% and 1.9% cases respectively. For NAC, resistance of Fluconazole, Itraconazole and Amphotericine B was found in 4.8%, 3.6% and 2.4% cases respectively.

Keywords: Candida Albicans, Fluconazole, Itraconazole

INTRODUCTION

Candidiasis, the infection caused by species of genus *Candida*, can be acute or chronic, superficial or deep, and its clinical spectrum is so wide that a more specific definition cannot be made. There has been an increase in the incidence of candidaemia over the last two decades in different parts of the world including India, USA, Europe and Australia.[1-4] The increase in candidaemia is associated with high mortality. Growing population of immunocompromised patients and advances in medical and surgical managements has contributed an increase in candidaemia.[3,4,5] Other associated risk factors include exposure to broad spectrum anti-microbial agents, mucosal colonization by *Candida* spp., indwelling vascular catheters and premature infants.[6,7]

A shift has been observed in the relative frequency of each *Candida* spp. isolated from blood. There are published data from various centres regarding the incidence and relative frequency of *Candida* spp.[8] Non-albicans *Candida* species (NAC) are also being implicated in recent years.[9,10,11]

Options of the antifungal drugs available for treatment of systemic & invasive candidiasis are restricted to polyenes, allylamines, azoles and recently developed echinocandin class of molecules.[12,13] Fluconazole is the antifungal agent which is most commonly used for prophylaxis as it can be orally administered and is comparatively cheaper than other

antifungal agents. Fluconazole is the drug of choice whereas amphotericinB is given intra-venous in critical patients.

However, selection of appropriate empiric therapy is complicated by increasing prevalence of NAC species.[14] Undesirable side effects, toxicity and emergence of drug resistance are the limitations for use of these drugs. Emergence of drug resistance in *C. albicans* is reported from all over the world.[12,13]

A rise in the incidence of antifungal resistance to *Candida* spp. has also been reported over the past decade.[15,16]

Studies on prevalence of infections and antifungal susceptibility testing are useful in deciding clinical strategies.[17] Aim of this study was to do species level identification and detect resistance, if any, among Indian clinical isolates of *C. albicans.*

METHODOLOGY

Present study was done in a tertiary care hospital of Gujarat between year 2011 to 2013. Permission from ethical committee of the institute was obtained. Informed written consent from all patients was taken before enrolling in the study. From total 135 patients, *Candida* species were isolated from different clinical specimens.

Specimen collection: Specimens were collected under complete aseptic precautions The set of two swabs were collected for each specimen. Out of that one was subjected for direct smear examination and other was inoculated on Sabouraud's dextrose agar and incubated at 37°C aerobically. Direct smear examination was done by 10 % KOH preparation and Gram staining.

Identification: The growth of *Candida* on Sabouraud's dextrose agar was confirmed by Gram staining in which gram positive budding fungal cells were observed. Then its growth was examined for colony morphology on Sabouraud's dextrose agar and chlamydospore production on Corn meal tween 80 agar. Germ tube tests and other biochemical tests like sugar fermentation, sugar assimilation and urease test were performed to identify the species of Candida.

Antifungal susceptibility testing: Antifungal susceptibility testing was performed by NCCLS M44-A Disc diffusion method.[18]

Inoculum was prepared by picking five distinct colonies of approximately 1 mm in diameter from a 24 h old culture of *Candida* species. Colonies were suspended in 5 ml of sterile saline and its turbidity was adjusted visually with the transmittance to that produced by a 0.5 McFarland standard.

Inoculation of test plates were done with a sterile cotton swab dipped into the suspension. The dried surface of a sterile Mueller-Hinton + GMB (glucose and methylene blue) agar plate was inoculated by evenly streaking the swab over the entire agar surface. Anti fungal disks of Fluconazole,Iitraconazole and Amphotericin B were dispensed onto the surface of the inoculated agar plate. The plates were inverted and placed in an incubator set to 35° C within 15 minutes after the anti fungal disks were applied. The inoculated plates were examined after 20 to 24 hours of incubation. The zone of inhibition was measured and the result is recorded as susceptible. The susceptible category implies that an infection due to the strain may be appropriately treated with the dose of antimicrobial agent recommended for that type of infection and infecting species.

Susceptible-dose dependent (S-DD): The susceptible-dose dependent category includes isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates.

Resistant (R): Resistant strains are those that are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules.

RESULTS

All the 135 *Candida* sp. were found with microscopy and culture positive on both blood agar and SDA for *Candida* and were only considered and subjected to the tests for further identification. Out of total 135 samples, C. Albicans were isolated from 52 (38.5%) and remaining i.e 83 (61.5%) were having positive for Non Albican Candid (NAC). Among Non Albican Candid (NAC), *Candida glabrata* was 36 (26.7%) followed by *Candida tropicalis 25*(18.5%) which was isolated and the other non-*albicans Candida* species are summarized in Table 1.

Table 1: Species distribution of Candida

Species	No. (%)
C. albicans	52 (38.5)
C. glabrata	36 (26.7)
C. tropicalis	25 (18.5)
C. parapsilosis	15 (11.1)
C. guilliermondi	7 (5.2)

Table 2: Age & Gender wise Distribution of *C. albicans* and non-*albicans Candida*

Age	Candida Albicans		Non-Albicans Candida		Total
	Male	Female	Male	Female	
0-10	4	4	2	10	20
11-20	3	7	1	2	13
21-30	4	5	4	26	39
31-40	6	7	3	9	25
41-50	1	3	5	0	9
51-60	2	2	2	7	13
>60	1	3	1	11	16
Total	**21**	**31**	**18**	**65**	**135**

Table 2 shows age & gender wise distribution of patients. Out of total 52 patients having isolates of C. albicans, 31 (59.6%) were female and 21 (40.4%) were male. Whereas, among patients having NAC isolates, 65 (78.3%) were female and 18 (21.7%) were male. For C. albicans, maximum number of patients were from age group of 31 to 40 years. Whereas, for NAC, maximum number of patients were from age group of 21 to 30 years.

Table 3 shows susceptibility pattern of candida speices to Fluconazole, Itraconazole and Amphotericine B. Out of all species of Candida, resistance to Fluconazole, Itraconazole and Amphotericine B in 4.4%, 3.7% and 2.2% cases respectively.

C. albicans was found resistant to Fluconazole, Itraconazole and Amphotericine B in 3.8%, 3.8% and 1.9% cases respectively. For NAC, resistance of Fluconazole, Itraconazole and Amphotericine B was found in 4.8%, 3.6% and 2.4% cases respectively.

Resistance to Fluconazole and Itriconazole were found maximum in C. parapsilosis. For all antifungal drugs, C. albicans had showed maximum number of sensitive cases.

Table 3: Susceptibility of *Candida* species to antifungal drugs

Anti fungal drug		*C. albicans* (n=52) (%)	*C. glabrata* (n=36) (%)	*C. tropicalis* (n=25) (%)	*C. parapsilo-Sis* (n=15) (%)	*C. guillier-Mondi* (n=7) (%)	Total (n=135) (%)
Fluconazole	S	47 (90.4)	32 (88.9)	22 (88.0)	12 (80.0)	6 (85.7)	119 (88.1)
	SDD	3 (5.8)	2 (5.6)	2 (8.0)	2 (13.3)	1 (14.3)	10 (7.4)
	R	2 (3.8)	2 (5.6)	1 (4.0)	1 (6.7)	0 (0.0)	6 (4.4)
Itriconazole	S	48(92.3)	33 (91.7)	23 (92.0)	13 (86.7)	6 (85.7)	123 (91.1)
	SDD	2 (3.8)	2 (5.6)	1 (4.0)	1 (6.7)	1 (14.3)	7 (5.2)
	R	2 (3.8)	1 (2.8)	1 (4.0)	1 (6.7)	0 (0.0)	5 (3.7)
Amphotericine B	S	48 (92.3)	35 (97.2)	22 (88.0)	15 (100.0)	5 (71.4)	125 (92.6)
	SDD	3 (5.8)	1 (2.8)	2 (8.0)	0 (0.0)	1 (14.3)	7 (5.2)
	R	1 (1.9)	0 (0.0)	1 (4.0)	0 (0.0)	1 (14.3)	3 (2.2)

S = sensitive, SDD = sensitive dose dependent, R= resistance

DISCUSSION

Candidiasis is a prevalent opportunistic mucosal infection, caused predominantly by *C. albicans*, which affects a significant number of otherwise healthy men and women of childbearing age. A variety of local and systemic host factors and exogenous factors have been described to increase the prevalence of *Candida* infection. *C. albicans* remains the commonest isolate in all the studies. *C. glabrata* found commonest after *C. albicans* in most of the studies. Incidence of *C. albicans* varies from 43.1% to 87.5%. [19,20] In present study the incidence was 38.5%, which is quite comparable with the studies of Bauters et al. and Ritcher et al.[21,22]

The incidence of non-albicans species is also very similar to findings of Ritcher et al. and Corsello et al.[23,24] The predominant non-*albicans Candida* was *C. glabrata* (26.7%) followed by *C. tropicalis* (18.5%) and these are found to be associated with hospital acquired infection[25] and many reports showed the prevalent isolates as *C. tropicalis*.[16,25,26] In this study, there is single species difference between *C. glabrata* and *C. tropicalis*. Other non-*albicans Candida* species were *Candida guilliermondii(5.2%)* and *Candida parapsilosis (11.1%)*.

Antifungal susceptibility testing remains an area of intense interest. Susceptibility testing can be used for drug discovery and epidemiology, but this study was focused on use of antifungal susceptibility testing to predict therapeutic outcome. With the demonstration that susceptibility of Candida species to azole antifungal agents (particularly fluconazole) which generated correlations with clinical outcome of candidiasis that were qualitatively similar to that seen for antibacterial agents.[17] In present study 3.8 % isolates of *C. albicans* were re-

sistant to fluconazole; similar susceptibility pattern was reported by Ritcher et al.[22]

Most non-albicans *Candida* species have higher azole MICs and infections they cause are often difficult to treat.[22] In present study also higher resistance was observed in (6.7%). One of the possible explanations for more frequent isolation of non-albicans species may be the increased use of topical azole agents.[27]

The extended prophylactic use of fluconazole in suspected cases would be a pro-bable cause of high resistance pattern to fluconazole in our institute. Another established fact is that antifungal drug response *in vitro* may be dose dependent which is expressed as susceptible dose dependent (SDD), that is, although susceptible *in vitro* but resistance failure may be seen *in vivo* at the usual dose. In such situations, increase in dose of drug above the usual dose often results in clinical cure.[28] Wide spread use of fluconazole in various clinical conditions is the major cause of NAC dominance over *C. albicans*.[16]

Western data have shown that *Candida* species are reliably susceptible to polynes, azole and echinocandins. But Indian studies shown a very high resistance to fluconazole for all candidal isolates although the amphotericinB susceptibility is high.[29]

The increased prevalence of non-*albicans* species was found to be replacing *C. albicans* and this finding is correlation with a study by Jha et al.[30]

CONCLUSION

It can be concluded from present study that azoles can be used for empirical therapy of uncomplicated candidiasis as most of the isolates were found susceptible. However, culture should be done to detect non-albicans species and antifungal susceptibility testing is essential in recurrent cases of candidiasis.

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