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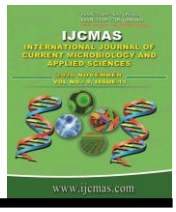

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Identification of Candida species and Antifungal Sensitivity Testing by Disc Diffusion Method

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A B S T R A C T

Candida is an opportunistic fungi responsible for causing Candidiasis when the patients immunity is low. Emergence of Nonalbicans Candida along with *Candida albicans* is also increasing. Increased use of antifungal drugs, use of antibiotics, long term use of catheters has contributes to resistance to antifungal drugs. Isolation of Candida species by conventional and Hichrome agar was done. Antifungal sensitivity testing was done by disc diffusion method. Aim of the study is to identify the *Candida* species from various samples by conventional method and on Hichrome agar; To do the antifungal sensitivity of Candidial species by disc diffusion method. Total samples received in mycology section in microbiology laboratory were 138. Out of these 102 shows Candida species. All these *Candida* species were grown on Conventional methods and Hichrome agar. Among the 102 *Candida* isolates, *Candida albicans* were 47(46%) and Non albicans Candida were 55 (53.9%). In non albicans Candida, *Candida tropicalis* 33 (32.3%) *Candida glabrata* 13(12.7%), *Candida krusei* 5(4.9%), *Candida parapsilosis* 3(2.9 %) and *Candida dublinesis* 1(0.9 %) were found. All candida species grown on Conventional method equally grow on Hichrome agar *Candida albicans* was commonest isolates in Urine and sputum samples. Whereas *Candida tropicalis* was commonest isolate in Blood cultures, Pus, Tracheal aspirate and CSF. Anifungal sensitivity of Candida species was done by Disc diffusion method against Amphoterecin B, Flucanozole and Itracanozole. Amphoterecin showed 100% sensitivity to Candida species. Resistant was seen in *Candida albicans* and *Candida tropicalis* to antifungal drugs. Other species does not show resistance. *Candida albicans* showed resistance to Itracanozole 22(47%) and Flucanozole 17(36%). Candida tropicalis showed resistance to Itracanozole 6(18%) and Flucanozole 5(15.1%). *Candida albicans* was the commonest isolate followed by Candida tropicalis. Nonalbicans Candida was reported at higher rate than *Candida albicans*. Hichrome agar is rapid method for identification of Candida species .Resistance to antifungal agents is seen in Candida species. Resistance to antifungal drugs for Itracanozole and Flucanozole by *Candida albicans* and *Candida tropicalis* is noted. The other Candidial species does not show resistance. Identification of correct Candida species and antibiotic sensitivity testing is necessary for correct treatment. *Candida albicans* and Nonalbicans Candida are also responsible for causing hospitalized infection. Hichrome agar gives early identification of Candida species. Resistance to azole group of antifungal is noted.

Introduction

Candida is opportunistic fungi, normally present as a part of human anatomical flora, however they are capable of causing infection in presence of opportunistic as low immunity (Roopa *et al.,* 2015). Candidiasis is the most common fungal disease in humans caused by Candida, yeast like fungi that produce pseudohyphae. Production of Pseudohyphae indicates active and invasive infection. It affect skin, mucosa and various internal organs of body. Different species of Candida are known such as *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, *Candida dubliniensis*, *Candida kefyr*, *Candida guilliermondii* and *Candida. viswanathii*. (Shivaprakash *et al.,* 2007) *Candida albicans* is the commonest species causing Candidiasis uptill now, but the trends have changed to non-albicans Candida (NAC) is increasing (Sardi *et al.,* 2013) and (Lewis *et al.,* 2009).

Immunity is lowered by various factors like indiscriminate use of antibiotics, AIDS and other immunosuppressive conditions have led to significant rise in Candida and other fungal infections (Ishan *et al.,* 2019).

Identification of Candida species can be done by conventional methods and by using HiCHROME agar. Isolation of Candida species is very important from an infection in order to administer appropriate therapy to reduce mortality, to control outbreaks and to carry out epidemiological investigation (Sanglard *et al.,* 2016).

There is emerging resistance of antifungal drugs to candida species. Indiscriminate use of azole group of drugs leads to resistance. Therefore Antifungal sensitivity testing is essential for administrating accurate antifungal drugs (Morrell *et al.,* 2005).

Materials and Methods

The study was conducted in Department of Microbiology in Mycology section of Government Medical College Aurangabad from July 2019 to July 2020.Samples of all age group with Clinically suspected Candidal infection were collected in OPD and ward and were send to Mycology section for processing. Also the samples processed in Bacteriology laboratory showing growth of Candida were also included in our study. Various samples which were processed were Urine, Blood, Sputum and Pus, Nail scraping and tracheal aspirate.

From samples direct microscopy using 10% KOH and Gram stain shows gram positive oval yeast cells. Samples was Inoculated on Sabourauds dextrose agar (SDA). Next day on SDA colonies of Candida species were small, 1-2mm creamy white smooth, pasty were seen. Again from colonies gram stain was done (gram positive oval budding yeast with or without pseudohyphae). Candidial species were identified by germ tube test and on corn meal agar. If Germ tube is Positive than presumptively identify as *Candida albicans* and Candida dubliniensis. If germ tube is negative than other Candidial species are suspected. Identified Candida species were verified by growing them on Hichrome agar by characteristic colony color. Chromogenic media contain chromogenic substrates which react with enzymes secreted by target microorganisms to yield colonies of varying colors. Hichrome medium can be used as selective medium for growing Candida species in less than 48 hours.

Colonies from SDA were inoculated on Hichrome agar and kept for incubation at 37degree centrigrade. The species were identified by characteristic colony color. Identification of *Candida* species by seeing the color of colonies as follows.

Candida.albicans – Light green colored smooth colonies.

Candida.tropicalis - Blue to metallic blue colored raised colonies.

Candida.glabrata - cream to white smooth colonies.

Candida. krusei - Purple color.

Candida. dubliniensis- dark green. (HiMedia Laboratories Pvt. Ltd. HiCrome Candida Differential Agar M1297A; 2011.)

Antifungal susceptibility testing was done by disc diffusion method as recommended by CLSI M-44 A2 guidelines.9. (CLSI, 2009).

Muller Hinton agar with 2% glucose and 0.5µg/ml methylene blue was used.

Following antifungal discs were used

1. Fluconazole 10µg / discs, (Himedia lab.Pvt Ltd)

2. Itraconazole 10µg / discs (Himedia lab.Pvt Ltd)

3. Amphotericin B 20 µg / discs (Himedia lab.Pvt Ltd)
The standard strains used were *Candida albicans* ATCC 90028, *Candida tropicalis* ATCC 750, *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258. Candida glabrata ATCC.

Procedure- For antifungal sensitivity testing Inoculum suspension was prepared by taking Five colony from Sabourauds dextrose agar and suspended in 5ml of sterile normal saline. The resulting suspension was vortexed for 15 seconds. The cell density was adjusted with spectrophotometer by adding sufficient sterile saline to increase the transmittance to that produced by a 0.5 Mcfarland standard at 530 NM wavelengths.

Inoculation of test plates

The plates were inoculated as per standard CLSI guidelines and incubated at 35 degree C. Zone diameters in millimeter for the zone of complete inhibition were determined after 24 hours of incubation. When insufficient growth was encountered at the hour reading the plates were re-evaluated after a further 24 hours.

Results and Discussion

Total 138 samples were received. Out of 138 total samples Candida isolates were seen in 102 (73%) and 36 (26%) sample were sterile.

Table 1 shows the age groups of patients showing Candida isolates

As shown in Table 1 maximum Candida isolates were found in above 50 years of age group patients that is 39 (38.2%) and less in 10 to 30 years of age that is 10(9.8%).
In babies and age groups of 40 to 50 years Candida isolates were 12.7 %.

Among the nonalbicans Candida, *Candida tropicalis* was 33(32.3%) in maximum numbers followed by *Candida glabrata* 13(12.7%), *Candida krusei* 5(4.9%), *Candida parapsilosis* 3(2.9 %) and *Candida dublinesis*1 (0.9 %).

In table 2, out of 102 showing Candida isolates urine samples were 56 (54.9%) , blood were 19 (18.6%), sputum were 11 (10.7%), Pus 8 (7.8%) and 4 (3.9%) were from CSF and Tracheal aspirate. *Candida albicans* was maximally isolated species in urine and sputum samples. In urine sample *Candida albicans* was isolated in 29 (52%) followed by *Candida tropicalis, Candida*

glabrata, Candida krusei, Candida parapsilosis and Candida dublinensis. Most of the patients were catheterized. Among the total 102 samples 48 (47%) patients were catheterized and 58(56.8%) patients were not having catheter. In11 total sputum samples *Candida albicans* 7 (64%) were in maximum.

In Blood cultures, Pus, Tracheal aspirate and CSF most common species isolated was *Candida tropicalis*. In blood cultures out of 19 maximum isolates were *Candida tropicalis* 7 (36%).

Among the positive isolates *Candida albicans* was the most common species identified. Among the 102 Candida isolates, *Candida albicans* were 47(46%) in numbers followed by non albicans Candida were 55 (53.9%)as shown in Table 3.

Out of 102 Candida isolates 52 (51%) patients presented with fever on the other hand 50 (49%) patients did not manifest fever.

Candida species were identified by Conventional methods those species were confirmed by growing them on HICHROME agar. All species were grown on Hichrome. Table 4 shows different Candida species on Hichrome agar. HiChrome agar was used as differential medium for speciation of Candida isolates. HiChrome agar is an effective and fast screening agar for speciation of Candida. Both of the medium shows growth on both medium.

Table 5 shows antifungal sensitivity testing of *Candida albicans*. All strains of *Candida albicans* were susceptible to Amphotericin B. While out of 47 *Candida albicans* only 30(63.8%) shows sensitivity to Fluconazole and 25 (71.4%) shows sensitivity to Itraconazole. Resistant to *Candida albicans* by Fluconazole 17(36%) and Itraconazole 22(47%) were noted. Itracanozole were found to be more resistant than fluconazole.

Table 6 shows showing antifungal sensitivity of *Candida tropicalis*. All strains of *Candida tropicalis* were susceptible to Amphotericin B. While out of 33 *Candida tropicalis* 28 (84.8%) shows sensitivity to Fluconazole and 27 (82%) shows sensitivity to Itraconazole. Resistant to *Candida tropicalis* by Fluconazole 5 (15.1%) and Itraconazole 6 (18%) were noted less in *Candida tropicalis* than *Candida albicans.*

Table.1 Shows Age groups of positive candida isolates

Sr.no	Age groups	Total no. of patients	Percentage
1	Baby	13	12.7%
2	10 -20yrs	10	9.8%
3	20-30 yrs	10	9.8%
4	30 -40 yrs	17	16.6%
5	40 -50 yrs	13	12.7%
6	50 above	39	38.2%
		102	

Table.2 Distribution of isolated *Candida* species in different samples

Sr.no	Name of Candida species	Urine	Blood	Sputum	CSF	Tracheal aspirate	Pus	Total
1	*Candida.albicans*	29	6	7	2	1	2	47
2	*Candida.tropicalis*	15	7	3	2	3	3	33
3	*Candida.glabrata*	6	3	1	0	0	3	13
4	*Candida. Krusei*	3	2	0	0	0	0	5
5	*Candida .parapsilosis.*	2	1	0	0	0	0	3
6	*Candida.dublinensis*	1	0	0	0	0	0	1
	Total	56	19	11	4	4	8	102

Table.3 The distribution of *Candida albicans* and non albicans Candida

Sr.no	Candida isolates	No. of isolates	Percentage
1	*Candida albicans*	47	46%
2	Non albicans Candida	55	53.9%
	Total	102	

Table.4 Comparison of conventional method of *Candida* species on SDA and Hichrome agar

Sr.no	Candida species	Conventional method	Hichrome agar
1	*Candida.albicans*	47	47
2	*Candida.tropicalis*	33	33
3	*Candida.glabrata*	13	13
4	*Candida .krusei*	5	5
5	*Candida .parapsilosis.*	3	3
6	*Candida.dublinenesis*	1	--
		102	

Table.5 Showing Antifungal sensitivity pattern of *Candida albicans*

Sr.no	Antifungals	Sensitive	Resistant
1	Fluconazole	30(64%)	17(36%)
3	Amphotericin B	47	---
4	IT	25 (53%)	22 (47%)
	Total	47	

Table.6 Showing antifungal sensitivity of *Candida tropicalis*

Sr.no	Antifungals Agents	Sensitive	Resistant
1	Fluconazole	28 (84.8%)	5 (15.1%)
2	Amphotericin B	33	-
4	Itraconazole	27 (82%)	6 (18%)
	Total	33	

Table.7 Showing antifungal sensitivity of *Candida glabrata*

Sr.no	Antifungals	Sensitive	Resistant
1	Fluconazole	12 (92.3%)	1 (7.6%)
2	Amphotericin B	13	-
3	Itraconazole	10 (77%)	3 (23%)
	Total	13	

Table 7 showing antifungal sensitivity of *Candida glabrata.* All strains of *Candida glabrata* were susceptible to Amphotericin B. While out of 13 *Candida glabrata* 12 (92.3%) shows sensitivity to Fluconazole and 10 (77%) shows sensitivity to Itraconazole. Resistant to *Candida glabrata* by Fluconazole 1(7.6%) and Itraconazole 3(23%) were noted less in *Candida glabrata than Candida albicans. and Candida tropicalis.*

All species of *Candida krusei, Candida parapsilosis and Candida dublinenesis* were sensitive to Amhotericin B, Fluconazole and Itraconazole.

In the present study *Candida albicans* is the predominant species isolated from patients in urine and sputum samples similar to the study by Jayapriya *et al* (2012).

Along with *Candida albicans* the other Non albicans Candida species were also isolated. When taken into consideration both non albicans Candida was reported at a higher rate than *Candida albicans.* Among non-albicans Candida, *Candida tropicalis* was the most common species isolated in blood and pus in our study which is similar to study Ishita pandit *et al.,* Increased use of antifungal drugs, use of antibiotics, long term use of catheters has contributes to emergence of nonalbicans Candida species. Deoruhkar *et al* (2013).

Candida isolates were seen in maximum in the age group above 50 years of age similar age group was also affected in study by Bhattacharjee *et al.,* (2016). Advanced age also contributes to major risk factors associated with Candida.

Out of 102 isolates most of Candida isolates were from urine samples, followed by Blood, Sputum, Pus, Tracheal aspirate and CSF. *Candida albicans* (52%) was isolated in maximum number in Urine and Sputum samples. In urine samples nonalbicans Candida were also isolated which were *Candida tropicalis*, Candida *krusei* and *Candida glabrata.* Most of the patients were catheterized. Insertion of in dewelling catheter for longer period might have been associated with Candidial infections.

In the present study, Conventional method and Hichrome differential agar were used for identification of Candida isolates. All species grown on SDA agar, by germ tube, on corn meal agar were accurately identified by Hichrome agar similar findings were shown by study Mangalkar *et al*., (2015). Among the newer test Hichrome agar is easy, effective and rapidly detecting medium identifying Candida species in 24 -48 hours.

Antifungal resistance to Itracanozole and Fluconazole were noted to Candida species in our study. More over Itraconazole showed more resistance than Fluconazole. Therefore Antifungal sensitivity testing is essential for administrating accurate antifungal drugs. By Morrell *et al* (2005)

In conclusion the candidial infection occurs in hospitalized patients. Non albicans Candida specially *Candida tropicalis* is also the causative agent followed by other species. Resistance to antifungal drugs among various Candida species is also noted. Indiscriminate use of azole group of drugs leads to resistance. Conventional method takes many days for

Candida species identification. HiChrome agar can be used for rapid identification. Earlier detection of species and antifungal sensitivity testing can be used for earlier treatment and can save patients life.

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