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Speciation of Candida using CHROMagar from Various Clinical Specimens and their Antifungal Susceptibility Pattern at a Tertiary Care Hospital

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ABSTRACT

Background: Candida albicans remains the most common and are responsible for various clinical infections ranging from mucocutaneous infection to life threatening invasive diseases. But recent epidemiological data shift from C.albicans to non albicans Candida species and also increased resistance to antifungal drugs made the scenario a serious concern.

Methods: A total of 156 Candida isolates from various clinical specimens received in the department of Microbiology were taken up for the study over a period of one year i.e. from March 2019 to February 2020. The Candida were grown on Sabouraud dextrose agar to be evaluated for colony appearance, macroscopic examination, Gram staining, germ tube, urea hydrolysis etc. The Candida isolates were speciated by using CHROMagar medium. Antifungal susceptibility testing was performed as recommended by Clinical and Laboratory Standards Institute (CLSI) M44-A document.

Results: The isolation of non albicans Candida (54.5%) predominated over Candida albicans (45.5%). Non albicans Candida isolated were Candida tropcalis 40(25.6%), Candida krusei 21(13.4%), Candida glabrata 17(10.8%) and Candida dublinensis 07(4.4%) each. Candida species were all susceptible to Amphotericin B, followed by fluconazole (67.4%), miconazole (51.9%) and ketoconazole (22.5%).

Conclusions: The accurate species identification of Candida is important for the treatment because not all species respond to the same treatment and also because of the increasing antifungal resistance. CHROMagar is a convenient and rapid method of identification of Candida species specially in resource limited poor settings.

Keywords: antifungal susceptibility testing; Candida albicans; CHROMagar; non albicans Candida.

INTRODUCTION

Candida spp are the members of the normal flora of the skin, mucous membranes and gastrointestinal tract. They are endogenous opportunists which cause secondary infections in individual with some underlying immunocompromised conditions. Candidiasis is a common fungal disease found in humans affecting mucosa, skin, nails and internal organs of the body. Candida albicans is generally considered the major pathogen among the candida species. An increase in the prevalence of non-albicans species has been noted during the last decades.[1-3] It has become important to identify yeast isolated from various specimens to the species level.[4]

Species identification of Candida isolates is conventionally done by germ tube test, inoculation on corn meal agar, sugar assimilation and fermentation tests. Newer methods which have been developed for yeast identification include CHROM agar, API systems, Vitek 2 ID system and molecular methods.[5-7] Study of colony morphology on cornmeal agar, sugar fermentation and assimilation tests are time consuming and labour intensive.[8,9] Clinical laboratories may need to expand their yeast identification capabilities in order to facilitate these surveillance efforts.[10] Chromogenic media contains chromogenic substrates that reacts with enzymes secreted by microorganisms producing colonies with various pigmentation. These enzymes are species specific, allowing organisms to be identified to the species level by their color and colony characteristics.[11]

Though molecular techniques in yeast detection are highly sensitive and specific, their implementation in routine diagnostic is limited due to complex nature of tests and affordability.[12] Over the last few decades, there has been an increase in the incidence of candidiasis caused by other Candida species (non-albicans Candida) such as Candida

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dublinensis, Candida glabrata, Candida krusei, Candida tropicalis and Candida Parapsilosis.[3] The commonly used antifungal drugs show significant variation in the susceptibility pattern among the types of Candida species. Non albicans candida are less susceptible to azoles particularly fluconazole.[13]

Several studies reported the emergence of drug resistance Candida species in different parts of globe.[14,15] Thus the change in the susceptibility pattern of Candida species in clinical isolates and introduction of newer antifungal drugs has made the in vitro susceptibility testing of antifungal agents more relevant for using specific and sensitive drugs. Thus, the present study was undertaken for species identification of Candida isolates using CHROM agar and to evaluate the susceptibility pattern of Candida isolates from clinical specimens.

METHODS

A laboratory based cross sectional study was carried out in the Department of Microbiology, College of Medical Sciences, Bharatpur after approval by Institutional Review Commitee from March 2019 to February 2020. A total of 156 Candida species were isolated from various clinical specimens received in the Department of Microbiology. A total of 2,018 different clinical specimens (urine, sputum, foleys, high vaginal swab, endotracheal tube, pus, blood) were proceeded for investigation. The preliminary diagnosis of specimens were performed by wet mount, Gram stain, culture on Sabouraud dextrose agar (SDA) and urea hydrolysis test. For the clinical significance of Candida isolates from sputum and urine, the specimens were analyzed by as well for the evidence of budding yeast cell with pseudohyphae along with significant pus cells.[16,17] All samples were inoculated on SDA slants supplemented with chloramphenicol and aerobically incubated at 37^0 C for 24-48 hrs. For blood culture, 8-10 ml venous blood was collected aseptically and inoculated in 45 ml Brain heart infusion (BHI) broth. It was then incubated at 37^0C for upto 96 hours before reporting as no growth.

Any visible growth seen on SDA slope were further speciated by standard protocol that include Gram stain, germ tube test, urea hydrolysis test etc. Gram positive budding yeast cells with pseudohyphae on microscopic examination and negative urea hydrolysis test were further inoculated on CHROMagar and incubated at 37^0 C for 24 to 48 hrs. Candida species were differentiated based on the type of growth and colour of isolates on CHROMagar (HiMedia, Mumbai,India).[18,19] Appearance of Candida species on CHROM agar were (C. albicans-light green, C. glabrata-cream to white, Candida krusei-purple, C. tropicalis-blue to purple, C. dublinensis-dark green. Antifungal susceptibility testing was performed and interpreted for all the isolates of Candida using disc diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI) M44-A document guidelines.[20] The inoculum was prepared and compared the turbidity to 0.5 Mc Farland Standard. Mueller Hinton agar supplemented with 2% glucose and 5µg/ml methylene blue.[21,22] We used ATCC strains of Candida albicans ATCC 10231, Candida glabrata ATCC 15126, Candida krusei ATCC 14243 and Candida tropicalis ATCC 750 as control. Antifungal disc containing fluconazole (25µg), miconazole (10 µg), ketoconazole (15µg), amphotericin B (100 units) were used. Zone of inhibition around the disc was measured after incubating the media at 37^0 C for 24 hrs.

RESULTS

A total of 156 Candida species were isolated from various clinical specimens processed during the study period. Candida albicans was the commonest species isolated 71(45.5%) followed by Candida tropcalis 40(25.6%), Candida krusei 21(13.4%), Candida glabrata 17(10.8%) and Candida dublinensis 07(4.4%). Isolation rate of non albicans Candida (NAC) was higher 85(54.5%) as compared to Candida albicans 71(45.5%) (Table 1).

Table 1. Distribution of Candida albicans and non albicans candida isolates. (n=156)

Candida isolates	Number of isolates	Percentage
Candida albicans	71	45.50
Non albicans candida	85	54.50

Out of a total of 156 Candida species isolated, organisms isolated from various clinical samples were: urine(44.8%), sputum (23.7%), catheter tip (19.8%), high vaginal swab (7.6%), endotracheal tube (2.5%), pus (0.6%), blood (0.6%) as shown in (Table 2).

Table 2. Distribution frequency of Candida species obtained from various clinical specimens.

Specimen	C.albicans	C. dublinensis	C.krusei	C.glabrata	C.tropicalis	Total
Urine	25	3	10	12	20	70(44.8)
Sputum	27	0	4	0	6	37 (23.7)
Catheter tip	8	3	4	3	13	31(19.8)
High vaginal swab	10	1	0	0	1	12(7.6)
Endotracheal tube	0	0	2	2	0	4(2.6)
Pus	0	0	1	0	0	1(0.8)
Blood	1	0	0	0	0	1(0.7)
Total	71	7	21	17	40	156 (100)

Maximum Candida albican was isolated from sputum sample but non from endotracheal tube and pus. All the isolates were 100% sensitive to amphotericin B. Candida species were 22.5% susceptible to ketoconazole, 67.4% susceptible to fluconazole and 51.9% susceptible to miconazole.

Candida dublinensis and Candida krusei were most sensitive to ketoconazole with susceptible rate of (71.4%) each. Candida albican was most susceptible to fluconazole (87.3%), followed by Candida tropicalis with a susceptible rate of (75%). Candida glabrata was most susceptible to miconazole (76.4%) followed by Candida albicans (67.6%). Susceptibility rate of amphotericin B is 100% by all the Candida isolates. Antifungal susceptibility profile of various Candida species is shown in (Table 3).

Table 3: Antifungal susceptibility testing of various Candida spp.

	Antifungal agents							
Isolates	**Ketoconazole**		**Fluconazole**		**Miconazole**		**Amphotericin (B)**	
	(S)	(R)	(S)	(R)	(S)	(R)	(S)	(R)
C.albicans	8 (11.2%)	63 (88.7%)	62 (87.3%)	9(12.6)	48 (67.6%)	23 (32.3%)	71 (100%)	0 (0%)
C.dublinensis	5 (71.4%)	2 (28.5%)	4 (57.1%)	3 (42.8%)	1 (14.2%)	6 (85.7%)	7 (100%)	0 (0%)
C.Krusei	15 (71.4%)	6 (28.5%)	6 (28.5%)	15 (71.4%)	12 (57.1%)	9 (42.8%)	21 (100%)	0 (0%)
C.glabrata	2(11.7%)	15 (88.2%)	3 (17.6%)	14 (82.3%)	13 (76.4%)	4 (23.5%)	17 (100%)	0(0%)
C.tropicalis	5 (12.5%)	35 (87.5%)	30(75%)	10 (25%)	7 (17.5%)	33 (82.5%)	40 (100%)	0 (0%)
Total C.spp	35 (22.5%)	121 (77.5%)	105(67.4%)	51 (32.6%)	81 (51.9%)	75 (48.1%)	156 (100%)	0(0%)

DISCUSSION

In the present study NAC (54.5%) was isolated at a higher rate than Candida albican as reported by other workers.[23-26] But in a different study by Vijaya et al[27] and Khadka et al[28], Candida albican was the predominant organisms that were isolated and is not in accordance with our study. The majority of Candida isolates were obtained from urine (44.8%) and sputum (23%). This indicates the higher incidence and distribution of Candida species causing urinary tract and respiratory tract infections. This is in accordance with a study done by Khadka et al[28] who reported candida species the most prevalent cause of urinary and respiratory infections. However, in a study by Vijaya et al[27] most of the candida spp were isolated from stool sample which is in contrary to our study.

Among the candida isolates, the most prevalent was C. albicans (45.5%) followed by C. tropicalis (25.6%) and C. Krusei (13.4%) respectively. This pattern is similar with other studies done by other workers.[28,29] As far as NAC species is concerned, the most prevalent was C. tropicalis (25.6%) followed by C. krusei (13.4%) and C. glabrata (10.8%). Similar data had been shown by different studies done in different parts of the world.[28,30,31]

Speciation of Candida species by CHROMagar on the basis of color differentiation has the advantage of rapid identification, technically simple, cost effective as well as reliable when compared with technically demanding, time consuming and expensive conventional methods. In the under developed and developing countries like ours, CHROMagar can be considered as a simple phenotypic test alternative to molecular assay. CHROMagar has high sensitivity as well as specificity for the identification of Candida species.[19,32] CHROMagar facilitates identification between yeast spp from specimens containing mixture of yeast spp and do not affect the viability on subsequent subcultures.[27,33] Though the results on CHROMagar exactly parallel that of conventional method, it is superior to SDA in terms of suppressing the bacterial growth.[19]

In this study, all the Candida isolates were found to be(100%) susceptible to Amphotericin B. This finding is similar with the studies done by other authors.[25,34] In our study, Candida species were found to be (67.4%) susceptible to fluconazole, miconazole (51.9%) and to ketoconazole (22.5%) respectively. Candida albicans is the most susceptible species to fluconazole(87.3%) in the present study, whereas , C. Glabrata is the most resistant (82.3%) to this antifungal agent. Our finding is very close to the findings reported by Mondal et al[35] which showed only (18%) resistant by C. albicans and 19.2% resistant by C. tropicalis respectively, whereas, C. Glabrata showed maximum resistant rate of (42.9%) to fluconazole.

In the present study, the most resistant antifungal agent among the four antifungals used was ketoconazole with a resistant rate of (77.5%) by the Candida species isolated. Among the Candida species, the most resistant pattern was shown by C. albicans with a rate of (88.7%), followed by C, tropicalis (87.5%) and C. glabrata (88.2%). The higher resistant rate to ketoconazole by C. albicans and C. Glabrata is similar with the study done by Khadka et al[28] but is contrary to the findings reported by Mondal et al[35] and Binesh et al[36] which showed only (2.1%) resistant by C. albicans. This high resistance rate of ketoconazole might be due to overuse of this antifungal agent and also their empirical therapy in the present scenario. These findings suggests the need for speciation and also to perform antifungal susceptibility tests before treatment with any antifungal drug in view of the increasing resistance among various Candida species.

CONCLUSIONS

The present study highlights the fact that CHROMagar differential media is useful in early identification of the Candida to species level. It is time saving as well as cost effective when compared to other conventional methods like sugar fermentation, assimilation, morphology on corn meal agar etc. This will help the clinicians to make early decision regarding antifungal therapy thereby decreasing patient morbidity and mortality. In our study, C. albicans was the predominant among all the Candida spp. isolated and this predominant isolate is sensitive to Amphotericin B and to fluconazole. Ketoconazole is the most resistant drug among all the four antifungal agents used in our study.

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