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PHENOTYPIC CHARACTERIZATION AND ANTIFUNGAL SUSCEPTIBILITY PATTERN OF CANDIDA SPECIES IN TERTIARY CARE CENTER

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ABSTRACT The number of non albicans Candida species isolated from clinical samples is increasing continuously every year. The present study is prospective, which aimed to phenotypically characterize the candida species and their antifungal susceptibility pattern. All samples were subjected to KOH wet mount, gram's staining and culture on Sabouraud's dextrose agar. Further speciation was done by germ tube test, corn meal agar inoculation and carbohydrate utilization. Susceptibility testing was done by disc diffusion method (fluconazole, itraconazole and ketoconazole disc). Among total 317 Candida isolates, non albicans C. tropicalis (57) was predominant isolate. Resistance to fluconazole was maximum 59.93% and resistance to ketoconazole was 40.7%.

Summary: In present study non albicans Candida species showed relatively higher resistance to itraconazole. Species-level identification of Candida and their antifungal sensitivity testing should be performed to achieve better clinical result.

KEYWORDS : Non albicans Candida, Fluconazole, Ketoconazole, Itraconazole

INTRODUCTION: Over the past few years the incidence of mycotic infection has progressively increased. Fungi once considered as nonpathogenic or less virulent are now recognized as a primary cause of morbidity and mortality in immune-compromised and severely ill patient.(1) Candidal infection constitutes the major fungal infection of human in our country and it is a part of normal microflora of human body; to act as pathogens, interruption of normal host defense is necessary.(2,3) Etiology for the emergence of Candida species as important health care associated pathogens includes - prolonged use of antimicrobial agents, chronic use of steroid, organ transplantation, malignancy, indwelling medical devices for prolonged duration, total parenteral nutrition, acquired immunodeficiency disease (HIV) etc.The spectrum of disease caused by Candida is extensive, including mucosal candidiasis, cutaneous candidiasis, mucocutaneous candidiasis and systemic candidiasis. Thus the range of manifestations extends from simple mucosal colonization to multiple organ invasion or invasive candidiasis.[3,4]

Antifungal prophylaxis is indicated in the prevention of colonization and multiplication of Candida spp. in patients susceptible to primary and recurrent infections.(4) There is growing evidence of the increasing use of azoles so there is concern over the increase in azole resistance. Change in drug susceptibility of different species of Candida and the introduction of newer antifungal agents has made the in vitro susceptibility testing of antifungal agents more important which helps in rational use of the same.[5] In the current study, we aimed to identify the Candida spp. among various samples and to investigate the susceptibility pattern of these species to antifungal agents.

MATERIAL AND METHODS: The prospective study was carried out in department of Microbiology, G. R. Medical College, Gwalior. All specimen received from in patient of the various department of J. A. group of Hospitals from April 2016 to March 2017 were processed for isolation and identification of Candida species, according to the standard microbiological method. This work was executed after approval from the ethical committee of GRMC Gwalior.The medical history was taken from each patient; including age gender and underlying chronic disease. Mean and median age was calculated. All the samples were collected and transported under strict aseptic precaution in a sterile container. The various material collected were initially spread onto a labeled slide for KOH wet mount and gram stain. Gram staining smear were examined for the presence of gram positive budding yeast cells with pseudohyphaeand then inoculated on Saboraud's dextrose agar and blood agar. Media were incubated at 37º C for 24 hours and observed following the day but extended to week if no growth in 24 hour. Identification of fungal growth was finally based on its macroscopic and microscopic features. Isolated colonies were subjected to gram staining and germ tube test, corn meal agar inoculation (Dalmau culture plate technique) and carbohydrate utilization.(6,7) Corn meal agar morphology of few Candidia species is depicted in images 1 to 4. Antifungal susceptibility testing was carried out on isolated and identified colonies using commercially prepared antifungal disc fluconazole, itraconazole, ketoconazole on Muller Hinton Agar with 2% glucose & 0.5 µg/ml (MHA-GMB) as per CLSI guidline.(8) Candida albicans ATCC 90028 and Candida krusei ATCC 6258.

Result: Among total 317 Candida isolates, 134 (42.27%) were identified as C. albicans and 183 (57.7%) were identified as non albicans Candida species. The majority of Candida spp. were Candida albicans 134 (42.27%) followed by Candida tropicalis 57 (18%). Distribution of Candida species is depicted in graph 1. The isolation rate of Candida species was found to be highest among male 185 (58.40%) followed by female 132 (41.6%). 45.74 % Candida species were positive for germ tube test. 91.53% and 92.74% Candida isolates were identified by sugar fermentation test and sugar assimilation test respectively. 95.53% Candida isolates were identified by corn meal agar morphology pattern. Department wise distribution showed in table 1. Maximum isolates were obtained from intensive care unit, which explained that isolation rate were maximum in immune-compromised patients and patients on prolonged indwelling medical devices. Age wise distribution is depicted in table 2. In female maximum Candida isolates were obtained in age group 26-50 years. Antifungal resistance pattern showed in table 3. Resistance to fluconazole was maximum 59.93%, non albicans Candida species showed relatively higher resistance to itraconazole.

Discussion: In recent years, Candida spp. has emerged as principal pathogens in a variety of human infections. This study isolated a total of three hundred and seventeen Candida isolates from various clinical sources. Out of these maximum Candida isolates were obtained from urine which were 93 (29.3%), 74 (23.30%) from blood, 58 (19.0%) from high vaginal swab. This was concordance with study of S. C. Deorukharet al and P. Mnge et al in many clinical sample isolates. (1, 9) In our study mean age was 47.07 year, whereas in study of Anupriya et

al and Nadeem Jeddy et al mean age were less than our study. (10, 11) In the Abhishek Mehta et al. study median age was less than our study.(12) In our study distribution of male was more than female which is similar to other studies (Bhattacharjee P. and Dutta et al).(13, 14) R. Mehta et al. identified 59.13% non albicans Candida isolates which was more than Candida albican sisolates 40.87%. They showed increasing trend of non albicans Candida than Candida albicans. (15) This is concordance with present study.

In study of Omar JM et al. among non-albicans Candida isolates, C. glabrata was the predominant isolate accounting 6.8% and C. albicans were 84.5% identified.(16) In study of C. Roopa et al. and S. C. Deorukhkar et al. among non-albicans Candida isolates C. tropicalis was the predominant isolate. (1, 17) In our study 42.27% isolates were identified as C. albicans and among non albicans Candida spp. C.tropicalis 18%, followed by C. guilliermondii 9.8% were predominant isolates. In our study 100% of C. albicans showed germ tube formation which is correlated well with study of D. Kim et al. [18]

In study of Singh K et al. and Arul Sheeba Malar S. et al. 100% chlamydospores formation was seen with Candida species. (19, 20) In our study 91.03% of C. albicans showed chlamydospores which are much lower than other studies. This may be due to atypical strains of C. albicans. This method is helpful in identification of germ tube test negative C. albicans. Few C. albicans may not form chlamydospores, those strains were identified by using rice extract agar, potato dextrose agar or husk seed agar.

In study of Tumbarello et al. & Elham Baghdadi et al. fluconazole and itraconazole resistance was correlated with our study.(21, 22) In study of S. C. Deorukhkar et al obtained 44.74% resistance to ketoconazole which were higher than fluconazole resistance 35.75% and itraconazole resistance 40.72%. (1) The study of Arul Sheeba Malar S. et al showed 100% itraconazole resistance, which were higher than our study.(23) Our study also confirms the increasing problem of azole resistance in the course of different infection as shown by other authors. [21,22]

Conclusion: Non albicans Candida species showed relatively higher resistance to itraconazole. Species-level identification of Candida and their antifungal sensitivity testing should be performed to achieve better clinical result and to select an appropriate and effective antifungal therapy. High resistance to antifungal agents is an alarming sign to the healthcare professionals.

Table 1: Department wise distribution of various Candida species isolates

Candida Species	Department Wise Distribution of Isolate					Total
	Medical ward	Surgical ward	Intensive Care Unit	Pediatrics ICU	Obs and Gyane ward	
C. albicans	38	18	44	5	29	134
C. tropicalis	13	13	23	2	6	57
C. guilliermondii	5	4	18	3	1	31
C. krusei	16	2	5	2	3	28
C. kefyr	3	4	11	3	3	24
C. parapsilosis	4	4	2	1	6	17
C. glabrata	1	1	5	1	7	15
C. dubliniensis	1	0	2	3	5	11
Total	**81**	**46**	**110**	**20**	**60**	317

Table 2: Age wise distribution of males and females

Age group	Male		Female		Total
	Number	(%)	Number	(%)	
1-25	23	12.43 %	35	26.5 %	**58**
26-50	57	30.81 %	59	44.7 %	**116**
51-75	72	38.91 %	24	18.2 %	**96**
76-100	33	17.85 %	14	10.6 %	**47**
Total	**185**	**58.4%**	**132**	**41.64%**	**317**

Graph 1: Distribution of various Candida species isolates

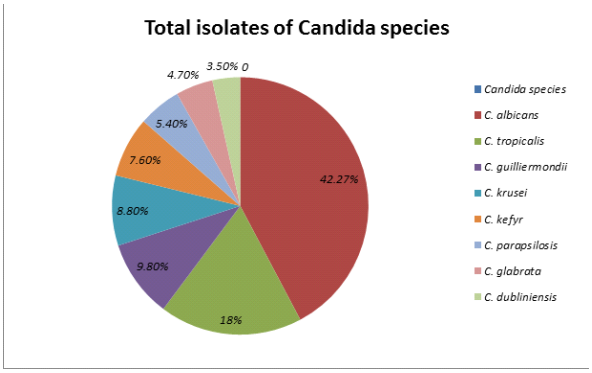
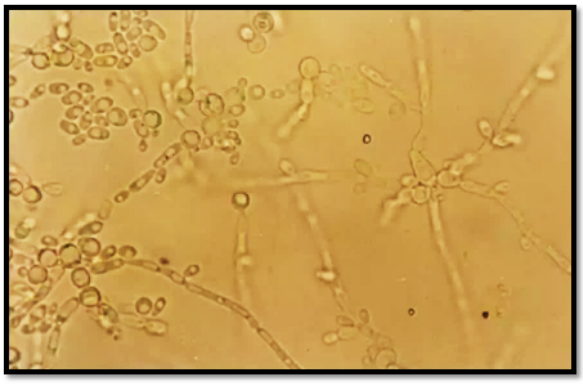


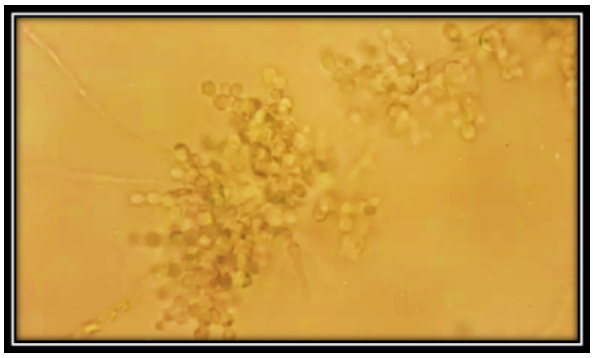
Table 3:Antifungal susceptibility pattern seen with respect to species

Candida Species	Antifungal drug		
	Fluconazole R	Ketoconazole R	Itraconazole R
C. albicans	63 47.01%	31 23.13%	45 33.6%
C. tropicalis	34 59.64%	21 36.84%	26. 45.6%
C. guilliermondii	17 54.83%	13 41.93%	9 29.03%
C.krusei	15 53.6%	7 25%	15 53.6%
C. kefyr	13 54.2%	10 41.7%	8 33.33%
C. parapsilosis	12 70.6%	10 58.82%	6 35.3%
C. glabrata	9 60%	11 73.33%	7 46.7%
C. dubliniensis	6 54.5%	9 81.81%	4 36.4%
Total	169 53.30%	112 35.33%	120 37.85%

Images 1-Terminal Chlamydospores formation of Candida albicanson corn meal agar



Images 2- Microscopic appearance of C. guilliermondii on CMA



Images3- Microscopic appearance of C. krusei on CMA

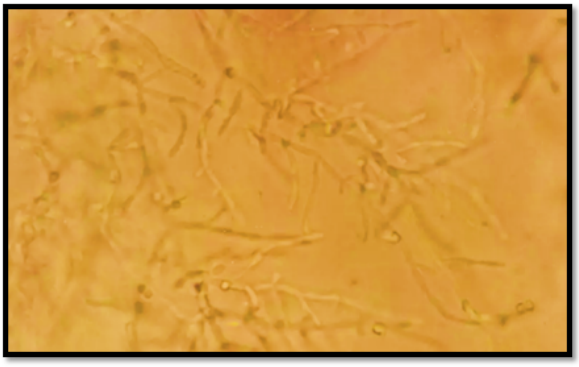
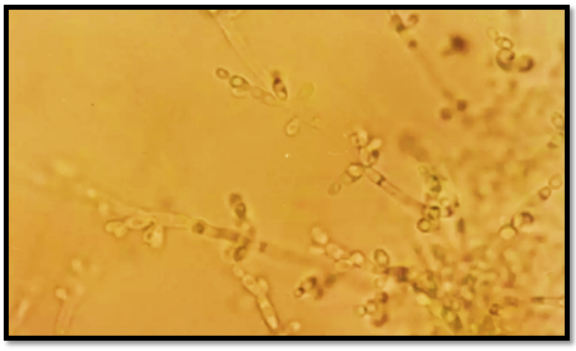


Image 4 Microscopic appearance of C .tropicalis on CMA



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