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IDENTIFICATION OF VARIOUS SPECIES OF CANDIDA AND THEIR ANTIFUNGAL SUSCEPTIBILITY FROM CLINICAL SAMPLES OF PATIENTS ATTENDING GGH, ANANTAPURAMU.

Microbiology

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ABSTRACT

Aims: Our study aimed to identify the distribution of Candida species among clinical isolates and their sensitivity pattern for common antifungal drugs.

Place and Duration of Study: Department of Microbiology, Government Medical College Anantapuramu, Andhra Pradesh, India, from January 2019 to October 2019.

Methodology: A total of 164 Candida species were isolated from different clinical samples received from both outpatients and inpatients attending GGH Anantapuramu. Repeat samples were taken whenever colonization was suspected. Routine samples were processed following the department's standard operating procedure. Culture positive yeasts were confirmed by Gram stain and subcultured on Sabouraud dextrose agar. For identification of yeast, standard identification protocol was followed using the Germ tube Test, Dalmau Plate Culture Method, Sugar Fermentation Tests, and CHROM agar. Antifungal susceptibility test was done by Kirby-Bauer disc diffusion method using 2% glucose and 0.5µg/ml methylene blue.

Results: Among the 164 culture-positive isolates, most predominant species isolated was C. tropicalis 82(50%) followed by 34 (20.73%) C.albicans, C.parapsilosis 22(13.41%) C.glabrata 15(9.14%) C.krusei 11(6.70%).In our study, NAC 130 (79.27%) were predominant isolates. Candida spp. were isolated from urine 61 (37.19%), Sputum 42 (25.60%), High vaginal swab 21(12.80%), Blood Culture 32(19.53%), and wound swabs 8 (4.88%). A larger number of females were affected than males, and the age group of > 60 years was more susceptible to Candidiasis.
All Candida isolates were 100% sensitive to amphotericin B. Voriconazole was the next effective drug with 86.6% susceptibility. 62.19% of strains were resistant to fluconazole.

Conclusion: Routine Speciation and antifungal susceptibility to be done to avoid the use of resistant antifungals.

KEYWORDS

Antifungal susceptibility Candida spp, Non-albicans Candida.

INTRODUCTION:

The genus Candida comprises about 200 species, of which close to 20 have been associated with various diseases in humans. [1] *Candida* spp.are the fourth leading cause of nosocomial bloodstream infections in modern hospitals. The most challenging clinical problem is the increased rate of non-*Candida albicans* isolation and the rapidly growing resistance of *Candida* species(2).In recent years, there is a gradual increase in the incidence of fungal infections. This is because of the risk factors such as increased use of broad-spectrum antibiotics, improper use of antifungals, underlying malignant diseases, HIV/AIDS, organ transplantation, prolonged hospital stay, and exposure to invasive procedures. [1,3] Non-albicans species of Candida are currently the pathogens most frequently recovered from adults and children in tertiary care medical centers. [4] The dominant pathogenic species are Candida albicans, Candida glabrata, Candida krusei, Candida lusitaniae, Candida parapsilosis, and Candida tropicalis. Pathogenesis of candidiasis depends on the expression of virulence factors like germ tube formation, adhesions, phenotypic switching, biofilm formation, and the production of hydrolytic enzymes [5].

Aims & Objectives:

Aim of the present study is to identify Candida albicans, various species of Non-Albicans Candida (NAC), and to know the Antifungal susceptibility of the different Candida species isolated.

MATERIAL & METHODS:

The study was conducted at Department of microbiology Government Medical College Anantapuramu, during January 2019 to October 2019, for a period of 10 Months, The total Number of cases studied were 164. The *Candida* species were isolated from different clinical samples received from outpatient and inpatients attending GGH, Anantapuramu. Blood culture bottles were processed using the conventional method. Preliminary identification was made by Gram staining of the culture isolates from routine culture samples received into the Microbiology laboratory. Subculture was done on Sabouraud Dextrose Agar and incubated at 25^{0}C and 37^{0}C for further Identification. Based on the colony characteristics on SDA and Grams staining of the smear from SDA, the Identification of the genus Candida was made. Species-level identification is made using **t**he germ tube test dalmau plate culture for chlamydospore. Identification based on the color of the colonies on CHROM agar (HiMedia, Mumbai) plates and Confirmation of species was done based on sugar fermentation tests (6).

Antifungal susceptibility test was done by Kirby-Bauer disc diffusion method on MHA agar supplemented with 2% glucose and 0.5µg/ml methylene blue dye. Agar plates were inoculated with a suspension of yeast cells as lawn culture whose turbidity was adjusted to 0.5 McFarland standards (106 CFU/ml) Antifungal disks were placed incubated at 37°C for 24–48 hours.) the diameter of the zone of inhibition was measured. Results were interpreted as per CLSI guidelines [7,8].Readymade antifungal disc (HiMedia, Mumbai, India) of Amphotericin B (20mcg), Fluconazole (10mcg), Itraconazole (10mcg), Ketoconazole (30 mcg) and Voriconazole. (1mcg) were used for antifungal susceptibility tests.

RESULTS

During the study period of 10 months between Jan 2019- Oct 2019,a total of 164 candida species were isolated from Various Clinical samples, reported in the department of microbiology, GMC ATP was studied. Urine is the most common sample type from which Candida has been isolated.

Table 1 Shows Various *Candida* Spp. Isolated From Clinical Specimens

Sample Distribution (n=164)		
Sample type	**No Of Samples**	**%**
Wound Swab	8	4.88%
Sputum	42	25.60%
Vaginal Swab	21	12.80%
Urine	61	37.19%

Blood Culture	32	19.53%
Total	164	

Table 2 Showing Age Wise And Sex Wise Distribution Of Candida Species.

	C.albicans n=34		Non albicans Candida n=130	
Age in Years	**n=15 Male**	**n= 19 Female**	**n=51 Male**	**n=79 Female**
0-10	2	0	9	8
11-20	0	1	1	6
21-30	2	7	8	17
31-40	2	0	5	6
41-50	2	4	6	13
51-60	2	0	9	5
>60	5	7	13	24

In the present study, 66 *Candida* spp. were obtained from males and 98 from females as shown in Table 2.Most common age group affected was > 60yrs.

Table 3 Showing Various Candida Species Distribution

Species Distribution (n=164)		
Isolates	**Number**	**%**
C. albicans	34	20.73%
Non albicans Candida (n=130)		
C.tropicalis	82	50%
C.glabrata	15	9.14%
C.krusei	11	6.70%
C.parapsilosis	22	13.41%
Total	130	

Among the 164 Candida Species isolated, the predominat species was *C. tropicalis* 82(50%) *followed by C.albicans*34 *(20.73%),* C.parapsilosis 22(13.41%) *C.glabrata 15(9.14%) C.krusei 11(6.70%).I*n our study NAC 130 (79.27%) were predominant isolate compared to C.albicans 34(20.73%)

Fig 1: CHROMagar Plate **Fig 2:** Showing Sugar Fermentation showing various species of reactions differentiating various Candida. Candida species

Table No 4 Showing Distribution of Co morbid Factors and Species Distribution.

Co-morbid factors and Species distribution					
Co-morbid factors	**No of Patients**	**C.albicans**	**%**	**NAC**	**%**
Indwelling urinary catheter	23	9	39.13%	14	60.86
Long term Antibiotic therapy	14	2	14.28%	12	85.72%
Surgery	2	0	0	2	100
IV line	12	2	16.66%	10	83.4%
Ventilator	2	2	100	0	0
Pregnancy	10	2	20%	8	80%
T2DM	34	6	17.6%	28	82.3%
Chronic kidney disease	8	2	25%	6	75%
Preterm delivery	17	3	17.65%	14	82.35%
Low birth weight	21	2	9.52%	19	90.47%
Antitubercular therapy	2	0	0	2	100
Dyspnoea	8	2	20%	6	80%
Menopause	11	2	18.18%	9	81.81%
Total	**164**	**34**		**130**	

Among the various Co morbid Conditions, C.albicans was associated with Type 2 Diabetes Mellitus,34 (20.73%), Indwelling urinary catheter 23(14.24%). NAC was more frequently associated with Type 2 Diabetes Mellitus, Indwelling urinary catheter, Preterm delivery, Low birth weight.

Fig.3. Showing Antifungal Susceptibility Testing Using KIRBY BAUER Method

DISCUSSION

Candida can be a commensal, and can act as opportunistic pathogen. As there is an increase in the number of patients who are immunocompromised, aged, receiving antibacterial and aggressive cancer chemotherapy, or undergoing invasive surgical procedures and organ transplantation, Candidiasis has emerged as an alarming opportunistic disease [9].

The present study showed a significant variation in the distribution of *Candida* spp in different clinical samples.The most predominant species was found to be *C. tropicalis 82(50%) followed by C.albicans* 34 *(20.73%),* C.parapsilosis 22(13.41%) *C.glabrata 15(9.14%) C.krusei 11(6.70%)*

Table No 5 Showing Comparative Studies Of Distribution Of Various Species Of Candida

Comparative studies of distribution of various species of candida					
	C. albicans	**C. tropicalis**	**C. parapsilosis**	**C. glabrata**	**C. krusei**
Present Study	20.73	50%	6.09	4.87%	7.13%
Gautam Panwar, (10) Vandana Sardana et.al	17.94%	43.58%	17.09%	8.54%	5.12%
Sardana V et al.(11)	20.10%	26.40%	14.50%	39%	
Sirinivas Rao MS et al.(12)	26.92%	36.53%		19.23%	
Mohammad Asadzadeh, Ziauddin Khan, et.al(13)	32.85%	13.57%	40.72%	10.4%	1.44%
Biranthabail Dhanashree, Munmun B. Marak et.al (14)	45.55%	28.89%	2.22%	3.33%	20%

Our findings are similar to the previously reported data by Gautam Panwar, Vandana Sardana et.al Sardana V et al.Sirinivas Rao MS et al., however, Ziauddin Khan Mohammad Asadzadeh et.al 2012-2017 Munmun B. Marak and Biranthabail Dhanashree et.al 2018 reported a lower prevalence rate (of *C. albicans.*

In the present study, it is found that candidiasis can occur at all ages and both sexes. The youngest being a oneyear old infant, while the oldest was 72 years. According to surveys conducted by Emeribe et al. [15] and Puri et al. [16] *Candida* infection was found the maximum in the age group of 21–40 years. In our study, maximum isolation of *Candida* spp was found in the age group of 51 to 60 years, this could be due to lower immunity, aging, predisposing factors such as diabetes mellitus and malignancies. In this age group more number of females were affected than males with an incidence of 98 (59.75%) and 66 (40.25%), respectively, which is similar to the study done by earlier workers. The rate of isolation of *Candida* spp. in females was about 61.2%, while in

males, it was only 38% [17].

One of the most frequent predisposing factors in our study was Type 2 Diabetes Mellitus, 20.73% of the total cases, followed by symptomatic urinary tract infection (24%), pregnancy (8.8%), and catheterized chronic kidney disease (14.02%), respectively which is comparable to the study done by munmun et.al.in which T2DM 28% catheterized chronic kidney disease (15.5%).(18)

Among the 164 *Candida* isolates tested, all were susceptible to amphotericin B in our study which is similar to the results reported by Arora et al. [19].13.4% strains were sensitive to fluconazole, and 86.6% were resistant. Among the nonsusceptible *Candida* spp.23.5% were *C. albicans*, 28.9% were *C. tropicalis,* and 0 % were *C. krusei*. 13.4% of the *Candida* spp. were resistant to voriconazole, among which 0 were *C.krusei* and 32.1% were *C. albicans* and 24.0% *C. tropicalis*.

However, Yenisehirli et al. reported 34% and 14% resistance rates of fluconazole and voriconazole among *C. albicans*, respectively [20].

Comparative studies of Sensitivity Patterns of various Candida species						
		C. albicans	**C. tropicalis**	**C. parapsilosis**	**C. glabrata**	**C. krusei**
Present Study	**Amphotericin B**	76.50%	92.60%	95.45%	86.60%	90.90%
	Fluconazole	8 (23.5%)	6 (28.9%)	8 (36.36%)	0 (0%)	0 (0%)
	Voriconazole	24 (67.9%)	68 (68.2%)	20 (90.90%)	13 (86.6%)	11 (100%)
Munmun B. and Birantha bail Dhanashree et.al	**Amphotericin B**	100	100	100	100	100
	Fluconazole	47.05	36.36	100	100	19.11
	Voriconazole	46.57	26.02	100	100	20.54
Saeid Mahdavi Omran, Mojtaba et all	**Amphotericin B**	100	100	100	100	
	Fluconazole	15.5	88.5	100	82.5	
	Voriconazole	100	92.4	91.9	81.3	

Resistance against fluconazole found highest with *C. tropicalis* (85.4%) and also showed highest azole resistance as compared to other species. Among all the one hundred sixty four isolates of *Candida*, 126 (76.82%) showed resistance to fluconazole, which is again highest number among the all antibiotics used. Fluconazole being the most widely used empirical antifungal drug, its decreasing susceptibility against various species is a matter of concern.

C. krusei is known for its innate resistance to fluconazole, [21] our study showed 72.7 % resistance. The emergence of species other than *C. albicans* is the selection of less susceptible species by the pressure of antifungal agents such as fluconazole [22].After extensive literature survey it has been found that resistance to fluconazole has increased. [23,24].

CONCLUSION

Species-level identification helps us to understand the1) Species distribution of Candida in different geographical areas. 2) The change in the distribution pattern of Candida species. 3) A better insight into the pathogenesis. 4) Antifungal susceptibility testing enables us to avoid unnecessary usage of resistant antifungals.

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