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Identification of Candida Species and Its Antifungal Susceptibility from Various Clinical Samples

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Abstract:

Background:-
Candida species are true opportunistic pathogens that exploit recent technological advances to gain access to the circulation and deep tissues. Candida species produce a wide spectrum of diseases, ranging from superficial mucocutaneous disease to invasive illness.

Objective:-
To isolate and identify candida species from various clinical samples and determined their antifungal susceptibility

Materials and Methods: -
A cross-sectional study was conducted in the department of Microbiology at Santosh Medical College, Ghaziabad from March 2024- June 2024. 383 various clinical samples including pus, urine, fluid, blood and high vaginal swab were collected from patients suspected of candidiasis as per inclusion criteria patients above 5 years age both IPD and OPD patients included and patients below 5 years age were excluded from the study, All particulars of the patients, major complaints and past history including immune status of patients filled in predesigned proforma. All samples were processed in microbiology laboratory for following tests KOH Mount, Direct Gram stain, Culture on SDA, Chrome Agar for species identification, germ tube test, Sugar fermentation test, sugar assimilation test for species identification and antifungal susceptibility test.

Results:-
Out of 383 patients 54% were male and 46% were females. 41.8% maximum cases were from age group 41-60 years and 11.7% minimum numbers of cases from above 60 years of age. Out of 383 samples, 41.8% urine samples, 33.4% pus samples, 12.5% blood samples, 12.3% high vaginal swab 2.6% sterile fluid samples. Candida species were isolated from 80 (20.8%) samples. Out of 80 Candida species, 25% C. albicans and 75% nonalbicans species. 47.5% C. tropicalis, 18.7% C. parapsilosis and 8.7% were

C. glabrata. Isolates were 100% sensitive to Amphotericin B and Voriconazole. C. albicans were 90% sensitive to Fluconazole antifungal drug.

Conclusion:-

Correct identification along with correct antifungal drug treatment as per isolated species needed to solve the problem of antifungal drug resistance and to take part in antifungal stewardship.

Key Word: C. albicans, Non-albicans candida, Germ tube techniques, Chromogenic media, Antifungal susceptibility test

1. Introduction

Candida species are mainly responsible for life threatening invasive infections in immunocompromised or severely ill patients. The increase in numbers of cases of mycoses has been favored by the increase in number of immunocompromised individuals and species that were previously not associated with human disease.[1]. Candida is a ubiquitous fungi organism. It has been one of the many eukaryotic organisms. The term Candida have been originated from the Latin word "Candid", meaning white. Candida spores are a commensal, benign form of a dimorphic fungus that, in the event of a disruption in the flora's equilibrium or host debilitation, transform into invasive, harmful pseudo hyphae.

Candida albicans and non-albicans species are closely related but differ from each other with respect to epidemiology, virulence characteristics, and antifungal susceptibility. All Candida species cause diseases ranging from superficial infections such as oral thrush to invasive disease, yet they show differences in disease severity and susceptibility to different antifungal agents.

Depending on the type of infection, the location and stage of the illness, and the host's reaction, the ability of *Candida* species to cause infections might vary. While phenotypic switching or coating with platelets can be utilized to elude the immune system, enzymes that damage or degrade cell membranes and extracellular proteins once contact is achieved enhance adherence and allow the yeast to infiltrate the host."

"Candidiasis" describes a variety of illnesses brought on by certain species of *Candida*. Infections can be systemic, affecting key body organs, or superficial, affecting the skin, nails, and mucosal membranes. If a diagnosis is delayed, candidiasis poses a serious risk to life and has a high death rate.[2]. In the Asia-Pacific region, *Candida* albicans is the predominant *Candida* species causing invasive candidiasis and candidemia in Australia, Japan, Korea, Hong Kong, Malaysia, Singapore and Thailand whereas *C. tropicalis* is the most frequently encountered *Candida* species in India and neighboring countries.[3]. The global change in spectrum of *Candida* species is also observed in India. However, the higher prevalence of candidemia due to *C. tropicalis* instead of *C. glabrata* or *C. parapsilosis* is well documented. Thus there is a need of good diagnostic mycology laboratories, rapid diagnosis and refinement of antifungal strategies.[1,3]. Speciation helps to understand the epidemiology of *Candida* species particularly the source and mode of transmission. This in turn facilitates the development of effective measures to prevent and control transmission of resistant pathogens.

Thus the present study was undertaken to find the prevalence of *Candida* infections at our set up isolate and speciate *Candida* species from patients having *Candida* infections by using conventional methods and to determine their antifungal susceptibility patterns.

2. Material and Methods

The present cross-sectional study was conducted in the Department of Microbiology at Santosh Medical College and Hospital, Ghaziabad Uttar Pradesh from May 2024 to July 2024 after taking ethical clearance from Institutional Ethical Committee.

Study Design: Cross-sectional study

Study Location: This study was a tertiary care teaching hospital based study done in Microbiology department at Santosh Medical College and Hospital, Ghaziabad Uttar Pradesh.

Study Duration: May 2024 to July 2024.

Sample Size: 383 clinical samples including pus, blood, urine, high vaginal swab and sterile fluid.

Sample Size Calculation: The sample size was estimated on the basis of a single proportion design. We assumed that the confidence interval of 10% and confidence level of 95%. The sample size actually obtained for this study was 383 patient's clinical samples.

Sample Selection Method: The patients suspected for candidiasis, above 5 years of age, both IPD and OPD patients and all samples including pus, blood, urine, high vaginal swab and sterile fluid included in the study. Patient below 5 years of age, leaky container, samples not reached laboratory timely and patient on antifungal treatment were excluded from the study. 383 clinical samples including pus, blood, urine, high vaginal swab and sterile fluid were collected from patients as per inclusion and exclusion criteria.

Inclusion criteria:
1. The patients suspected for candidiasis, above 5 years of age,
2. Both IPD and OPD patients,
3. All samples including pus, blood, urine, high vaginal swab and sterile fluid included in the study.

Exclusion criteria:
1. Patient below 5 years of age,
2. Leaky container,
3. Samples not reached laboratory timely, and
4. Patient on antifungal treatment

Procedure methodology:

Patient was explained about the procedure of sample collection. An informed consent was taken from the patients. All particulars of the patient, chief complaints and past history including immune-compromised status, were filled in a set proforma. Samples were collected in a sterile universal container, pus samples by a sterile cotton swab and blood sample were collected in sterile glucose broth bottles. The samples were properly labelled and immediately transported to Microbiology lab for processing. One swab was used for direct microscopy by KOH mount and Gram stain and another swab was used for cultured on Sabouraud's Dextrose Agar. Candida Species identification done by Hi-chrome candida agar, Germ tube test for albicans and nonalbicans species, sugar fermentation and sugar assimilation test. Antifungal susceptibility was performed on by mixing 38 gram of Muller Hinton agar Himedia M173 with 20 gram glucose with 500 µg of methylene blue in 1000 ml of distil water autoclave at 121 degree centigrade for 15 minutes cool to 45 to 50 degree centigrade and pour into sterile petri dish. Antifungal discs used for susceptibility testing were Fluconazole, Voriconazole and Amphotericin B.

3. Result

The present study was carried out in the Department of Microbiology Santosh Medical College Ghaziabad. A total of 383 Samples were collected from various wards and intensive care units (ICUs). Out of the total 383 samples, *Candida* species were isolated from 80 samples (20.8%). *Candida* tropicalis was by far the most common species followed by *Candida* albicans.

Out of 383 patients, males were predominant 207 (54%) in comparison to female patients which were 176 (46%). Among male patients only 37 (46%) had *Candida* species infection while among female patients *Candida* species were isolated from 43 (54%) patients. In the present study, the patients belonged to a wide age group ranging from 5 years to above 60, i.e. the geriatric age group. The mean age of the patients was 34.9 ±1.6 years. Out of 383 patients, the maximum cases 160 (41.8%) were from adult age group from years 41 to 60, followed by 129 (33.7%) from 21 to 40 years of age and 49 (12.8%) from 5 to 20 years age group. However, the number of cases from old age group i.e 60 years of age was less 45 (11.7%). Out of 383, 283 (74.5%) were from hospital wards and ICUs while 100 (26.3%) were from out patients. Out of 80 *Candida* spp. 21 (26.2%) were from ICUs,42 (52.5%) was from IPD patients and 17 (21.25%) were from community patients (OPD).

Table 1 - Among the samples, maximum number of samples were urine 160 (41.8%), followed by pus samples 128 (33.4%), which included skin sample, frank discharge from wounds, eye samples and ear samples, followed by blood 48 (12.5%), high vaginal swab 47 (12.3%) and sterile fluids 10 (2.6%).

Maximum number of *Candida* isolates were from pus samples 30 (7.8%), followed by urine 23 (6%), and high vaginal swabs 19 (4.9%). However, in blood and sterile fluids the prevalence of *Candida* infections were 6 (1.5%) and 2 (.5%) respectively.

Sample	Total Sample Received	Culture positive for *Candida*	Culture negative for *Candida*
Pus (including skin samples, eye discharge, ear discharge)	128 (33.4%)	30(7.8%)	98 (25.6%)
Blood	48 (12.5%)	6(1.5%)	42 (10.9%)
Urine	160 (41.8%)	23 (6%)	137 (35.7%)
High Vaginal Swab	47 (12.3%)	19 (4.9%)	28 (7.3%)
Sterile fluids	10 (2.6%)	2 (.5%)	8 (2%)
Total	383	80	303

Table 2 - In the present study, on direct microscopy budding yeast cells were seen in 60 samples, out of which 59 were culture positive and 1 was culture negative. Around 21 samples were missed during KOH mount which produced growth on culture media. In Gram staining 72 were positive for Gram positive budding yeast cells which also grow on SDA whereas 8 samples were negative for Gram staining.

Table 2: *Direct microscopy and Gram staining in relation to culture positivity.*

	KOH		GRAM STAINING	
	Positive	**Negative**	**Positive**	**Negative**
Culture positive	59	21	72	8
Culture negative	1	302	-	303
Total	60	323	72	311

Among the culture positives for yeast, 20 (25%) *Candida* albicans were isolated and 60 (75%) were Non albicans *Candida*. The speciation of *Candida* species included 38 (47.5%) *Candida* tropicalis, followed by 20 (25%) *Candida* albicans,15 (18.7%) were *Candida* parapsilosis and
7 (8.7%) were *Candida* glabrata. On the basis of germ tube test 20 (25%) showed production of germ tube while 60 (75%) isolates were negative for the test. On culture on Hichrome agar for differential diagnosis of *Candida* dark blue colour was seen in 38 samples, bluish green was seen in 20 samples,15 showed cream coloured growth and 7 showed pale purple. The isolates were tested for antifungal susceptibility by disc diffusion method. *Candida* albicans were 90% sensitive to Flucanazole and 100% sensitive for voriconazole and amphotericin B. However, C. tropicalis, C. parapsilosis and C. glabrata were 100% sensitive to Flucanazole, Voriconazole and Amphotericin B.

5. Discussion

One of the significant opportunistic infections that must be identified as soon as possible is *Candida* albicans. Mycological technique detection and identification is restricted in environments with low resources. Changes in virulence determinants in *Candida* albicans strains lead to the replacement of commensals with disease- causing colonizing strains, which can cause a variety of diseases ranging from mucosal or superficial infections to widespread candidiasis. The infections can be mild to severe. Timely identification and early treatment help reduce mortality and morbidity in patients.
Hence the study was undertaken to estimate the prevalence of *Candida* infections at our set up, determine the species of *Candida* in these infections and their antifungal susceptibility pattern.

A total of 383 different clinical samples were collected from the patients admitted in various wards, intensive care units (ICUs) and OPDs of our hospital. Out of the total 383 samples, *Candida* species were isolated from 80 samples (20.8%). In the present study it was found that *Candida* infections occurred in both sexes and at all ages. Distribution of patients according to gender showed that male patients were also suggests that there is male predominance 54% to 47% of female patients but for *Candida* growth the ratio interchanged and it was seen that 54% of patients were female and 46 % were male. In the present study, the patients belonged to a wide age group ranging from 5 years to above 60 i.e. the geriatric age group. The mean age of the patients was 34.9 ± 1.6 years. Out of 383 patients, most of the isolates were from adult age group i.e. 41 to 60 years of age 38 (47%), followed by old age above 60 years 20 (26%),18 (22%) in younger age group 21 to 40 years and least in 5 to 20 years of age 4 (5%). Similar findings were reported by Joseph K et al., and Goel R et al.[4,5]. Out of 383, 283 (74.5%) were from hospital wards and ICUs while 100 (26.3%) were from outpatients. Out of 80*Candida* spp. 21 (26.2%) were from ICUs, 42 (52.5%) were IPD patients 17 (21.25%) were community patients (OPD). Similar findings were reported by Sharma et al as 94% of the cases from hospital inpatients (IPD) and remaining 6% were outdoor patients.[6].
Higher prevalence of *Candida* in hospitalized patients may be due to prolonged stay in hospitals and increasing use of antibiotics. Also prolonged ICU stay of patients may lead prevalence of *Candida* infections in immunocompromised and patients on prolonged indwelling medical devices.
The most significant chronic illness which can act as a potential risk factor is diabetes followed by tuberculosis and HIV and respectively. Similarly, Kandhari K Cand Rama KM found higher occurrence of candidiasis in those individuals with diabetes and HIV.[7]

Maximum isolates in our study were pus specimens 30 (7.8%) which included wound pus, ocular swabs, auricular infections swab, oral thrush and skin swabs, followed by urine samples that were 23 (6%) and High Vaginal swabs that were 19 (4.9%). In contrast some studies have reported maximum cases from respiratory[8] and urine samples.[9]
Out of 80 samples, *Candida albicans* were 25%, while non-*albicans Candida* accounted for the majority 75%. Among non-*albicans*, *C. tropicalis* was the commonest yeast 38 (47.5%), followed by *C. albicans 20(25%), C. parapsilosis15 (18.7%),* and *C. glabrata were 7 (8.7%).*

Similar findings are reported by Urvashi et. al.[10] and other studies [10,11,12]. However some authors have reported the predominance of *Candida* albicans over Non albicans *Candida*.[13,14]

Antifungal susceptibility was determined by disc diffusion method using MHA with methylene blue. The results were interpreted using CLSI guidelines for yeast. The isolates were 100% sensitive to voriconazole and

amphotericin. However, *Candida* albicans were 90% sensitive to Flucanazole. Similar findings have been reported by Sabhapandit D et al.,[15] and Gade N et al.[16]

5. Conclusions

Candida albicans the most common species responsible for candidiasis, particularly in immunocompromised individuals non-albicans *Candida* species have been increasingly recognized in clinical samples, particularly in patients with long-term antifungal treatment, which can lead to resistance. C. albicans can develop antifungal resistance, especially in patients with recurrent infections or those on prolonged antifungal therapy. Non-albicans *Candida* species tend to show higher levels of intrinsic resistance to certain antifungal agents. For instance, C. glabrata often shows reduced susceptibility to azoles, and C. krusei is inherently resistant to fluconazole. Accurate identification of the *Candida* species is crucial for effective treatment, as the choice of antifungal therapy can vary depending on the species. Non-albicans *Candida* infections may require different or more aggressive treatment strategies due to their varying resistance.

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