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Original Research Article

Retrospective Analysis of Yeast Isolates: Emerging Mycological Trends

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Abstract

Introduction: Candida infections are increasing. Broad spectrum antibiotic use in critically ill patients, immunocompromised status of the patient, long ICU stay has led to increase prevalence of Candida infection.

Purpose: This study was undertaken to find the prevalence of Candida species in tertiary care hospital. Retrospective analysis of clinical samples received in microbiology laboratory from April 2017 to November 2019 were analyzed for the prevalence of various Candida species. The samples included urine, SSTI, blood, respiratory sample, sterile body fluid, high vaginal swab, and nasal and throat swab. The inclusion criteria for the samples were implemented.

Method: Samples fulfilling inclusion criteria and yielding candida species were processed manually as well as by automated system Vitek 2C. Both the results were compared and in case of discrepancy Vitek result were considered as final. The isolates obtained in various locations viz ICU, emergency, ward and OPD and samples were analyzed.

Result: A total of 1441 candida isolates were obtained from various clinical samples submitted in Lab. Candida tropicalis was the most common isolate obtained (47.19%) followed by *C. albicans* 24.84%. ICU (58.1%) was most common location where candida species was isolated. Urine (56%) was the most common sample yielding candida sp. rare pathogens (C. *parapsilosis*) was obtained in ICU.

Conclusions: This study shows that brief knowledge of the prevalent isolate helps in initiating and chosing the right antifungal therapy. It also highlights the role of implementing antifungal stewardship program.

Keywords: Candia infections, Fungal infection, Non albicans candida (NAC), Rare pathogens

Introduction

Fungal infection has been associated with significant mortality and morbidity in critically ill patients. Among fungal infections; Candida species have emerged as the most common cause of infection ranging from mucocutaneous to invasive devastating

diseases. Candidiasis represents the fifth leading cause of nosocomial infections (NI) ranging from 8% to 10%. and mortality associated with 15%-75% of mortality depending on the infective species of Candida. These patients have several underlying medical and surgical risk factors, and are frequently exposed to high-end medications.[1-3]

A brief knowledge on the prevalence of species at institute level can help the treating consultant in initiating antifungal therapy at right time. A well-equipped laboratory can help in right diagnosis. There should be ample resources for isolation and identification the organism to species level. Studies on candidemia from India report incidence ranging from 6.51 cases per 1,000 ICU admissions to 7.76 per 1000 ICU admissions. 4,5

An estimated 33-55% of all episodes of candidemia occurs in Intensive Care Units (ICU) it increases the cost to the patient and hospital stay. Patients admitted to ICU are more at risk of getting candida infections. Risk factors include high end antibiotics, immunosuppressive state, parenteral nutrition, invasive devices and procedures. Recent epidemiological studies have reported an increasing incidence of non-albicans candidemia. *Candida albicans* is the predominant species in outpatient department or where the patients are not exposed to any treatment or procedures.; however, Non albicans candida (NAC), which is less susceptible to antifungal drugs, ranks first in the India. [5,6] Candidemia has been frequently reported in literatures whereas other clinical samples from where candia sp has been isolated is not reported or limited reported. The present study was therefore undertaken to determine the epidemiology of candida sp in our hospital and to identify and speciate candida isolates from clinical samples submitted to the laboratory. i.e. urine; respiratory samples; samples of Skin and soft tissue (SSTI) infections which includes pus, drain fluid, tissue; blood; sterile body fluid; throat swab; high vaginal swab; nasal swab.

Material and Methods

This study was a retrospective analysis of samples submitted to lab and yielding candida species. Data from April 2017 to November 2019 were included in the study. The samples included urine, SSTI, blood, respiratory sample, sterile body fluid, high vaginal swab, and nasal and throat swab. The samples yielding candida sp and fulfilling inclusion criteria were included in the study. The inclusion and exclusion criteria are as follows

Inclusion Criteria

1) Repeated isolation of same species in non catheterised urine with pyuria was taken as inclusion criteria for all the urine isolates.

2) For respiratory isolate where quality score of respiratory samples was >2 with Candida isolate was processed. [7]

3) Any candida species grown in sterile samples.

4) Candida isolates obtained from pus/Tissue samples.

5) Candida species isolated from Throat swab of immunocompromised patients.

Exclusion Criteria

1) Insignificant Candida sp. growth in urine and respiratory cultures.

A pure growth of isolated yeast colony from clinical samples was further processed for species identification. Samples received in the laboratory were inoculated on CLED agar, Macconkey agar, Blood agar (BA) and chocolate agar and incubated at 37^0C overnight for 24hrs to 48hrs. Yeast colonies obtained were subcultured onto Sabaurauds Dextrose agar and BA medium. Growth from SDA plate was used for Germ tube test; production of colour on chromogenic medium (TTC) and corn meal agar (CMA) and incubated for 24 h at37^0C.[8] Chromogenic medium was read after 24 hours, Morphology on CMA was seen

after 48 hours of incubation. From BA plate growth was subjected to identification by Vitek 2 compact system. In case of discrepancy Vitek 2C result was accepted as final identification.[9]

Quality controls used were *C. albicans* ATCC 10231, C. parapsilosis ATCC 22019

Result

A total of 1441 candida isolates were obtained from various clinical samples submitted in Lab. These isolates were identified and analyzed for their species. It was found that most common location where candida was grown was ICU followed by emergency department as shown in Fig 1.

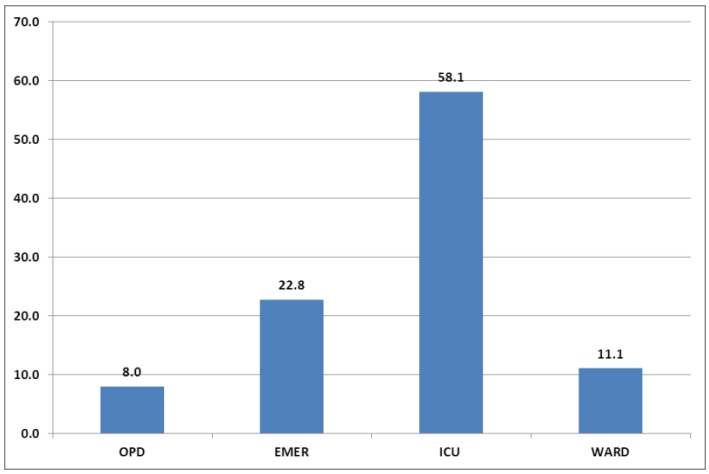

Figure 1: showing distribution of candida species in various samples.

Figure 2shows that isolates of non albicans candida comprised of 75.16% (1083) of the total isolates (1441). *Candida tropicalis* was the most common isolate obtained (47.19%) followed by *C. albicans* 24.84% as shown in Figure 2.

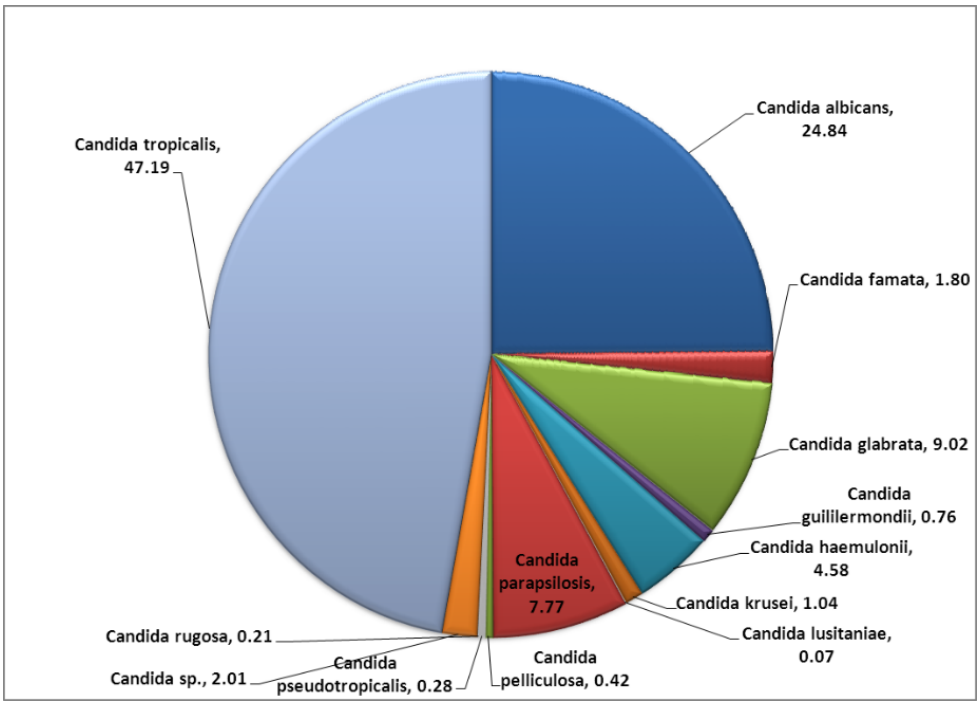

Figure 2: showing distribution (%) of candida species

Table 1: Candida species distribution in all the locations

Species	OPD		EMR		ICU		Ward	
	n	%	n	%	n	%	n	%
Candida *albicans*	47	40.9	61	18.6	218	26.0	31	19.4
Candida *famata*		0.0	3	0.9	18	2.2	5	3.1
Candida *glabrata*	5	4.3	28	8.5	80	9.6	17	10.6
Candida *guililermondii*	1	0.9	2	0.6	7	0.8	1	0.6
Candida *haemulonii*	1	0.9	6	1.8	47	5.6	12	7.5
Candida *krusei*	6	5.2	3	0.9	5	0.6	1	0.6
Candida *lusitaniae*	1	0.9		0.0	0	0.0	0	0.0
Candida *parapsilosis*	9	7.8	22	6.7	69	8.2	12	7.5
Candida *pelliculosa*	0	0.0	0	0.0	6	0.7		0.0
Candida *pseudotropicalis*	0	0.0	1	0.3	2	0.2	1	0.6
Candida *rugosa*	1	0.9	0	0.0	2	0.2	0	0.0
Candida *sp.*	4	3.5	7	2.1	11	1.3	7	4.4
Candida *tropicalis*	40	34.8	195	59.5	372	44.4	73	45.6
Total	115	100	328	100	837	100	160	100

Distribution of individual candida species in four locations is shown in Table 1. It can be seen from the figure that ICU has the maximum number of isolations of candida species followed by emergency.

Distribution of species among all the candida isolates obtained is shown in Figure 3. The most common non-albicans candida isolated is *C. tropicalis* in each location. *C. albicans* was isolated in 40.5% of OPD cases followed by *C. tropicalis* which was only 35.3%. Sample distribution and location wise sample distribution is shown in Figure 4 and Figure 5

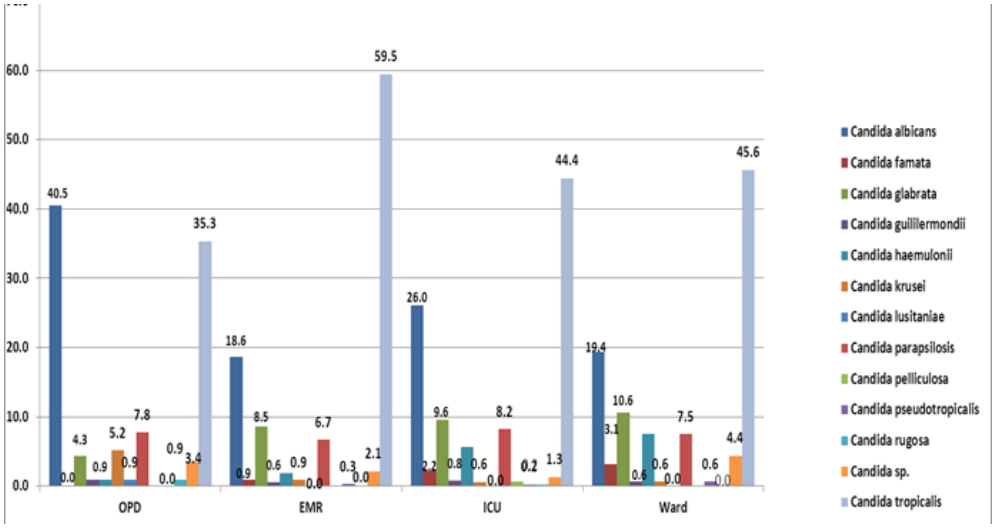

Figure 3: Distribution of species among all the candida isolates (%)

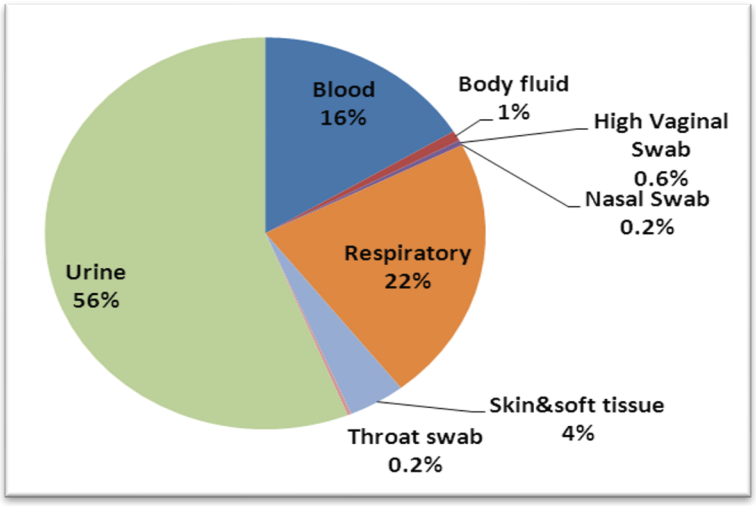

Figure 4: Showing various samples isolating Candida Species (%)

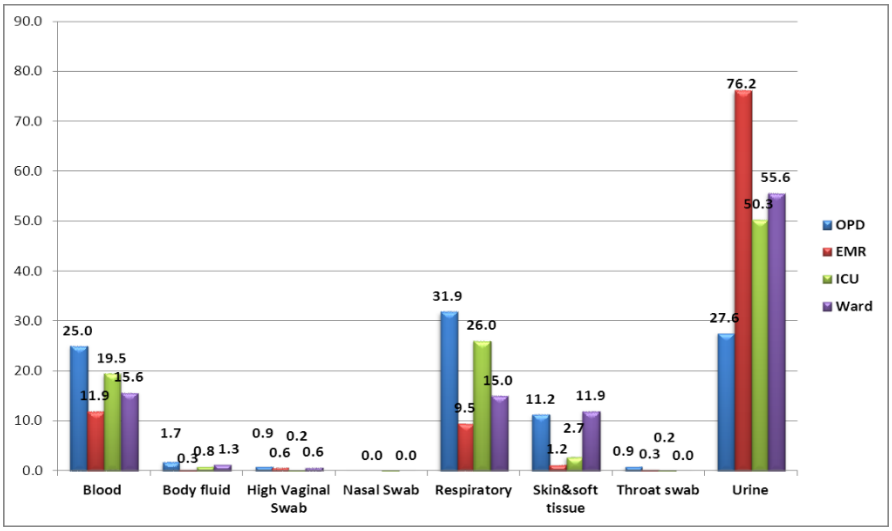

Figure 5: Location wise sample distribution (%)

Discussion

Isolation of candida has been increasing with the advent of new life saving technologies and procedures.Critical patients admitted in ICU are at high risk to get Candida infection. Candida infection can be categorized as "normal commensal", "colonizer or contaminant", or "pathogenic".

Empirical treatment or targeted therapy needs judicious evaluation of patient by consultant. Some exclusion or inclusion criteria as presented in this paper can help in saving time and unnecessary exposure to antifungals. [10]

Recently, Azoulay et al [11] have observed that patients with Candida airway colonization, defined as recovery of Candida from the respiratory tract, had a significantly longer period of MV (13 vs 6 days, P b.0001), ICU stay (17 vs 9 days, Pb.0001), and hospital stay (36 vs 22days, P b.0001) as compared to patients not colonized with Candida from the respiratory tract. In another study It has been observed that colonization with candida is associated with increase Mechanical ventilation days and ICU stay. [12]

The incidence of fungal infections due to Candida sp. has been increasing in recent years profoundly affecting public health.[13] Currently, Candida yeasts are the most common opportunistic pathogens in humans and are associated with almost 80% of all nosocomial fungal infections.[14]

We recovered 1441 Candida isolates in this study. ICU was the most common location where maximum candida was isolated. NAC were the most common isolates as seen in this study. Out of 1441 samples, 75.16% were NAC sp.; with *C. tropicalis* being highest among these (47.19%), whereas *C. albicans* was 24.84%. In ICU samples total 837 Candida sp were isolated, among these 619 were NAC with *C. tropicalis* 44.4%. Patient in ICU were at high end antibiotic, which contributes to the selection pressure and growth of Candida Sp. In OPD samples *C. albicans* (40.5%) were high in number, where patients were not on antibiotic. It was similar to the study done by D.P singh who recovered 137 Candida isolates, 55 (40.1%) were of *C. albicans* and 82(59.9%) isolates were NAC sp; with *C. tropicalis* (23.4%) contributing highest among NAC sp. 45.3% of 137 candida isolates were from urine Samples which was similar to the current study. [15]

In the current study maximum samples were of urine (56%), followed by Respiratory tract (22%), blood 16%, Skin soft tissue 4%, body fluid 1%, HVS 0.6%, Nasal swab 0.2%, Throat swab 0.2%. *C. albicans* were the common opportunistic pathogen in the OPD samples. Rare candida isolates were more commonly obtained in ICUs (*C. glabrata, C. hemulunii, C. parapsilosis*) location as shown in table 1. In this study male patients (850, 59%) were more affected than females (591, 41%). Most common age group affected were >50 yrs age (mean age was 53.46 yrs). Such results were similar to few other studies also. In a study done by Yankee C. Magalhaes [16] in which he reported maximum yeast isolates mainly from urine (54, 50%), the respiratory tract (21, 19.4%), blood (20, 18.6%), the catheter tip (7, 6.4%) and CSF (2, 1.8%), and the remaining four specimens (3.7%) were from wound secretions (1, 0.9%), wound fragments (1, 0.9%), vaginal secretions (1, 0.9%) and stools (1, 0.9%). Maximum samples were of urine and most predominant species in the urine samples were *C. albicans* 22.2%. the majority of yeast species were isolated from female patients (56%) and the most affected age group being over 60 yrs of age (51%). These patients were predominantly admitted to the Intensive care units (60%). In another study by Moen et al 2009 who also reported that *C. albicans* were the most commonly isolated yeasts from the urinary tract and was responsible for approximately 50% to 70% of cases of candiduria, followed by *C. glabrata* (5% to 33%) and other species of non-albicans yeasts (8% to 28%).

P Sengupta isolated 63 yeasts isolates on culture of 68 samples of oral mucosa, blood, urine, vaginal swabs, out of which 42 were germ tube test positive and also showed chlamydospores production in CMA and identified as *C. albicans*, which were isolated in most cases. [17] In the current study, 357 candida albicans were isolated, out of which 350 showed positive germ tube test with chlamydospores formation on CMA.

In the study by Satish T Pote, out of 176 isolates, 75 (42.61%) isolates were of *C. albicans*, 50 (28.6%) of *C. tropicalis*, 22(7.95%) of *C. glabrata* and other species were detected. [18] Out of 75 isolates of *C. albicans*, only 64 were identified by chromogenic media and all confirmed by Maldi ToF. In a study in study by Sanguinette et al. maximum samples were from urine followed by sputum, vaginal swabs, and blood [19], a total of 750 clinical yeast isolates were evaluated by two identification system (after doing microscopic morphology on cornmeal agar) VITEK 2C and RapID system that correctly identified 737 and 716 isolates respectively, ie: 98% by vitek 2C and 95 % by rapid system. We also identified clinical yeast isolates by Vitek 2C system and in case of

discrepancies, we accepted Vitek2C result as final identification. [20]

Conclusion

Incidence of opportunistic yeast infection has registered a tremendous increase in recent years. This is because of the improvement of methods for long term management of immunosuppressed, cancerous and other severely debilitated patients who are increasingly being put on steroids, cytotoxic drugs, irradiation and broad-spectrum antibiotics.

Fungal infections are on the rise due to changing practices in medical care; increase in the immunocompromised states, and due to increase in the use of antibiotics and steroid therapy. Currently, the awareness levels among the medical personnel regarding fungal diseases are lacking.

This study was done to analyze yeasts isolated from every possible biological specimen. This study was needed to know the fungal infection burden in the hospitalized patients for timely and judicious use of antifungals. Antifungal stewardship is need of the hour.

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