CHARACTERISATION OF YEASTS ISOLATED FROM VARIOUS CLINICAL SAMPLES WITH EMPHASIS ON RISK FACTORS AND CLINICAL OUTCOME OF CRYPTOCOCCAL INFECTION IN A TERTIARY CARE HOSPITAL

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ABSTRACT

BACKGROUND

Over the past decade, there has been a significant increase in the number of reports of systemic and mucosal yeast infections. These infections have a direct impact on the choice of empiric antifungal therapy and clinical outcome.

The aim of the study is to determine the risk factors and characterisation of the yeasts from various clinical specimens.

MATERIALS AND METHODS

In a prospective study, a total of 200 yeasts isolated from various clinical specimens were processed and identified up to species level by germ tube test, growth on corn meal agar, sugar fermentation and assimilation test, India ink preparation, urease test and Candida differential agar. The demographic data and risk factors were recorded.

Statistical Analysis- The data was analysed in terms of frequency percentage.

RESULTS

Candida species was the most predominant (97%) among the yeasts. Majority of the isolates were C. tropicalis (44%) followed by C. albicans (34%), C. glabrata, C. krusei, C. parapsilosis, Cryptococcus neoformans, C. dubliniensis, C. kefyr and Trichosporon asahii. Diabetes, broad-spectrum antibiotic therapy, prematurity, malignancy, steroids and AIDS were the risk factors.

CONCLUSION

There is increase in prevalence of non-albicans Candida species and increase in incidence of disseminated cryptococcosis in HIV seropositive patients. Thus, early isolation and speciation will aid the clinicians to institute proper antifungal therapy, thus decreasing morbidity and mortality.

KEYWORDS

Yeast, Candida, Risk factors, Cryptococcus.

HOW TO CITE THIS ARTICLE: Furtado ZV, Dias M. Characterisation of yeasts isolated from various clinical samples with emphasis on risk factors and clinical outcome of cryptococcal infection in a tertiary care hospital. J. Evid. Based Med. Healthc. 2017; 4(94), 5784-5788. DOI: 10.18410/jebmh/2017/1165

BACKGROUND

In the past decade, there is increased vulnerability to opportunistic mycotic infections, of which majority are caused by yeasts and yeast-like fungi. This increase is due to the consequence of frequent usage of antibiotics, immunosuppressive drugs, organ transplantations, lymphomas, leukaemias, HIV infections, myelosuppression, neutropenia, intravascular devices and also among seriously ill and injured patients such as those with malignancies, diabetes, severe burns or open wounds.[1,2] The introduction of fluconazole and itraconazole has resulted in significant increase of emerging pathogens like C. tropicalis, C. glabrata, C. krusei and others. This transition has had a major clinical impact due to decreased susceptibility of these non-albicans yeasts to antifungal agents.[3] C. glabrata and C. krusei are intrinsically resistant to azoles.[4] Rhodotorula, Saccharomyces cerevisiae, Geotrichum candidum and Trichosporon species recently has been reported as causative agent of opportunistic mycoses. Rhodotorula have been implicated as a cause of meningitis, endocarditis, fungaemia, keratitis and peritonitis.[5] The incidence of cryptococcal meningitis has also increased and continues to be a major cause of significant morbidity and mortality in immunocompromised as well as immunocompetent patients.[6] Therefore, early identification of yeasts to the species level is essential; hence, this study was taken up to find out the risk factors and characterisation of yeasts from various clinical samples in order to initiate prompt and correct antifungal treatment.

Financial or Other, Competing Interest: None.
Submission 28-11-2017, Peer Review 05-12-2017,
Acceptance 12-12-2017, Published 13-12-2017.
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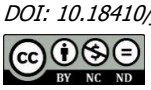
DOI: 10.18410/jebmh/2017/1165



MATERIALS AND METHODS

This prospective study was conducted for a period of 2 years from July 2010 to July 2012 in the Department of Microbiology of a tertiary care hospital after obtaining the institutional ethics committee approval. A total of 200 yeast

species isolated from blood, urine, sputum, CSF, body fluids, catheter tips, genital specimens and wound swab were included in the study. Clinical significance of yeast isolate was determined based on presence of pus cells, repeated isolation, presence of pseudohyphae, supportive clinical features and risk factors. The demographic data like age, sex, immunocompromised status and underlying risk factor were noted.

Gram stain of the specimens showing gram-positive yeast cells were subjected to further identification tests. CSF samples from all clinically-suspected meningitis cases was subjected to preliminary microscopic examination comprising of India ink and Gram's stain. The samples were cultured on Sabouraud's dextrose agar, 5% sheep blood agar and chocolate agar, incubated at 37˚C and examined daily for growth of cream coloured pasty colonies. Isolated yeast was identified to genus and species level by standard mycological procedures- (a) Germ tube production; (b) Capsule production by India ink preparation; (c) Growth on corn meal agar at 25˚C; (d) Sugar fermentation using glucose, sucrose, maltose, lactose and trehalose; (e) Yeast Nitrogen Base Agar (HiMedia) for assimilation tests using glucose, sucrose, maltose, lactose, dextrose, galactose, melibiose, cellobiose, inositol, xylose, raffinose, trehalose, dulcitol and starch; and (f) Urease test.[7,8] They were also inoculated on chromogenic agar (HiCrome-HiMedia Candida Differential Agar).

Statistical Analysis- Descriptive statistical analysis was done in terms of frequency percentage.

RESULTS

Out of the 200 yeast isolates, Candida species was the most predominant group with 194 isolates (97%). Among Candida, C. tropicalis 88 (44%) was the commonest, followed by C. albicans 68 (34%), C. glabrata 21 (10.5%), C. krusei and C. parapsilosis 3.5% each. C. dubliniensis and C. kefyr were least common 1% and 0.5% each. Cryptococcus neoformans was seen in 5 (2.5%) and Trichosporon asahii in 1 (0.5%) sample (Figure 1).

In this study, most of the yeast isolates were predominant in the age group of 51-60 years (Figure 2) and the risk factor associated with this age group was diabetes followed by malignancy, usage of broad-spectrum antibiotics and others. We noticed a male preponderance.

The most significant risk factor for yeast infections was diabetes, followed by treatment with broad-spectrum antibiotics, prematurity, malignancy, steroids, AIDS and others comprising of indwelling catheter, radiation therapy, renal insufficiency, burns, history of antifungal prophylaxis, immunosuppressive agents and major GI surgery (Table 1 and 2).

All patients with cryptococcosis were HIV seropositive, Trichosporon asahii was isolated in a patient on broad-spectrum antibiotics.

Candida tropicalis was the predominant isolate in urine, blood and body fluids followed by C. albicans, C. glabrata, C. krusei, C. kefyr and C. dubliniensis. C. albicans was seen predominantly in pus, vaginal swab and catheter tips (Table 3).

Cryptococcus neoformans was seen in CSF (4 isolates) and blood (3 isolates) and Trichosporon asahii was isolated in one urine sample.

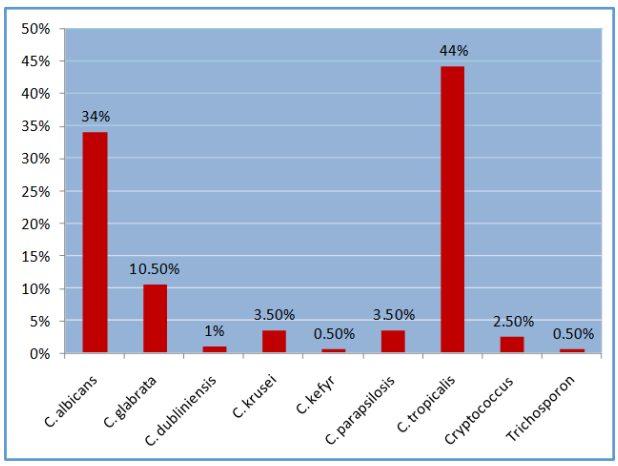


Figure 1. Shows the Species of Yeasts in Clinical Specimens

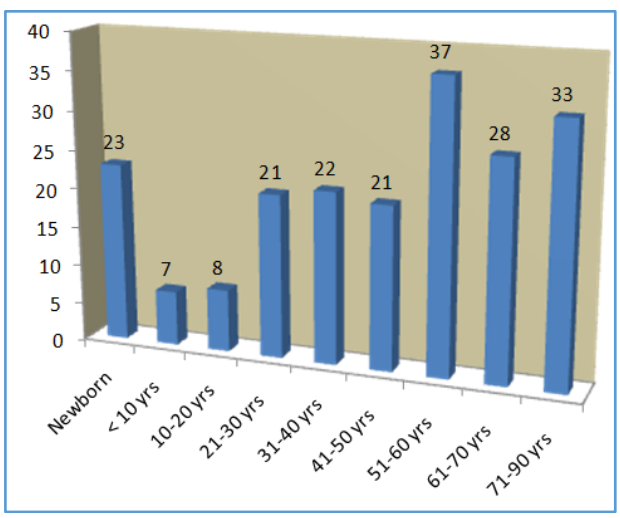


Figure 2. Shows the Age Distribution

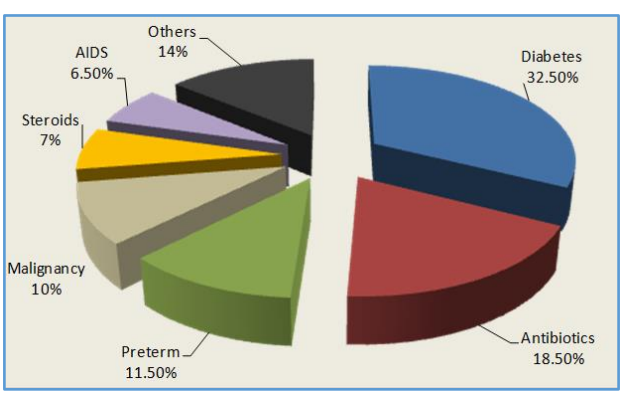


Figure 3. Shows the Risk Factors for Yeast Infection

	Candida albicans	Candida glabrata	Candida dubliniensis	Candida krusei	Candida kefyr	Candida parapsilosis	Candida tropicalis
Diabetes	23	4	1	2	0	1	38
Antibiotic	11	3	0	2	0	2	18
Preterm	10	4	0	1	1	0	7
Malignancy	4	5	0	0	0	2	9
Steroids	6	2	0	0	0	1	5
AIDS	4	0	0	0	0	1	3
Others	10	3	1	2	0	0	8

Table 1. Shows the Distribution of Yeast Isolates Among the Various Risk Factors

Specimen (No)	Candida albicans	Candida glabrata	Candida dubliniensis	Candida krusei	Candida kefyr	Candida parapsilosis	Candida tropicalis
Urine (91)	25	10	2	5	0	4	44
Blood (45)	11	7	0	1	1	2	22
PUS (34)	17	2	0	0	0	0	15
Vaginal swab (13)	9	0	0	0	0	0	4
Catheter TIP (10)	5	2	0	1	0	1	1
CSF (4)	0	0	0	0	0	0	0
Body fluids (2)	0	0	0	0	0	0	2
Sputum (1)	1	0	0	0	0	0	0

Table 2. Shows the Distribution of Yeasts in Clinical Specimens

*Cryptococcus neoformans was seen in CSF (4 isolates) and blood (3 isolates) and Trichosporon asahii was isolated in one urine sample.

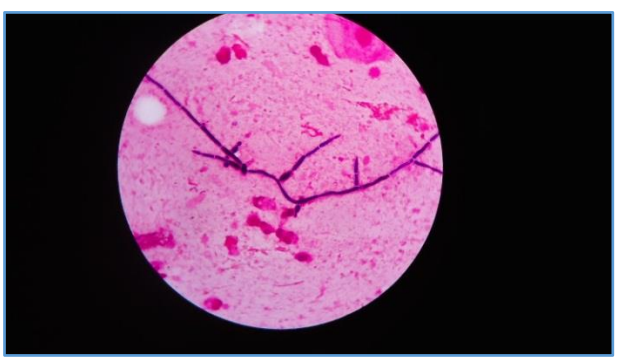


Figure 4. Gram's Stain of Candida Species

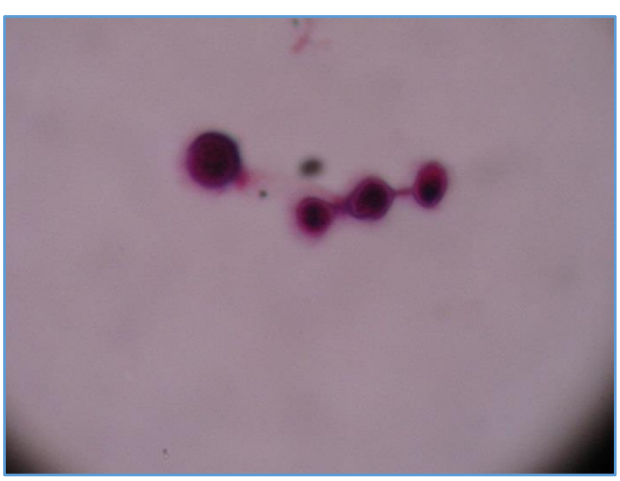


Figure 5. Gram's Stain showing Cryptococcus Neoformans

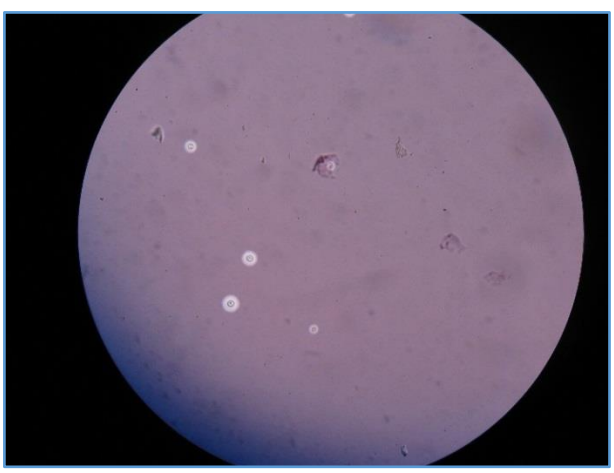


Figure 6. India Ink Preparation showing Capsule of C. Neoformans

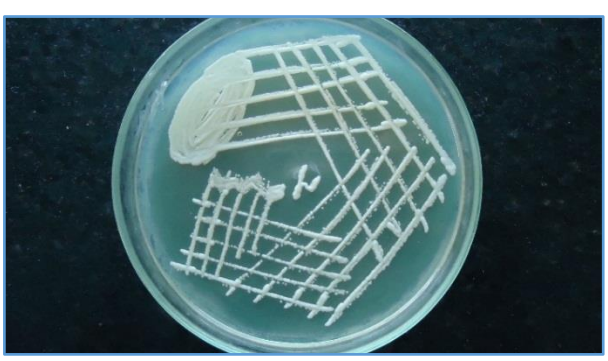


Figure 7. Growth of Candida on SDA Plate

Figure 8. Growth of C. Neoformans on SDA

Figure 9. CHROMagar showing Colonies of C. Tropicalis (Top) and T. Asahii (Below)

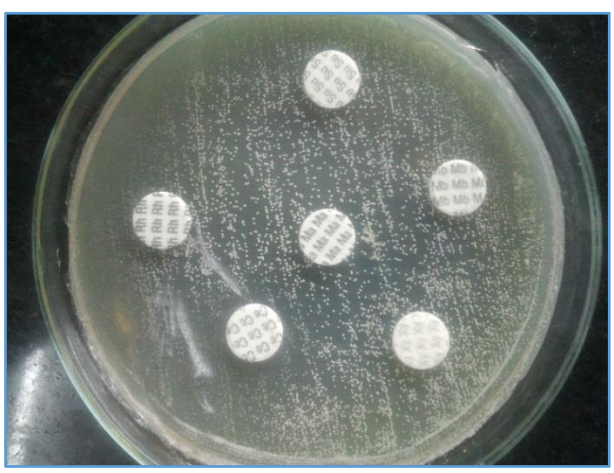


Figure 10. Yeast Nitrogen Base showing Assimilation Patterns of T. Asahii

DISCUSSION

Over the past decade, there has been a significant increase in the number of reports of systemic and mucosal yeast infections. Infections with these yeast species also have a direct impact on the choice of empiric antifungal therapy and clinical outcome. The potential clinical importance of species level identification has been recognised as Candida species differ in the expression of virulence factors and antifungal susceptibility.

In our study, Candida species was the most predominant (94.6%) yeast. Among the yeast isolates, C. tropicalis was the commonest, followed by C. albicans, C. glabrata, C. krusei, C. parapsilosis, C. dubliniensis and C. kefyr. Cryptococcus neoformans was isolated in five specimens, which included blood and CSF. Trichosporon asahii was isolated from urine. In a study conducted by Sumita Rajeevan et al[9] also showed that C. tropicalis was the predominant isolate followed by C. albicans, C. glabrata, C. parapsilosis, C. krusei, C. guilliermondii, C. kefyr and C. lusitaniae.

In the present study, majority of the yeast (68.5%) were recovered from urine and blood. We isolated predominantly Candida tropicalis (48.4%), followed by Candida albicans (27.5%), Candida glabrata (11%), Candida krusei (5.5%), Candida parapsilosis (4.4%), Candida dubliniensis (2.2%) and Trichosporon asahii (1.1%) in urine. Among blood isolates, Candida tropicalis (47.8%) was commonest, followed by Candida albicans (23.9%), Candida glabrata (15.2%), Cryptococcus neoformans (4.3%), Candida krusei (2.2%), Candida kefyr (2.2%) and Trichosporon asahii (2.4%). During the last 20 years, there has been an increasing incidence of invasive candidiasis worldwide, but differences in geographical epidemiology are emerging, in particular regarding a shift towards non-albicans species.[10] In neonates, Candida albicans was responsible for candidaemia followed by Candida tropicalis and Candida glabrata in agreement with results of Zaoutis et al.[11] The same trend was noticed in burns too. But, C. albicans was the main pathogen in vulvovaginal candidiasis.

The most significant risk factor for candida was diabetes followed by treatment with broad-spectrum antibiotics, prematurity, malignancy, steroids, burns, surgery and AIDS. Non-albicans candida like C. tropicalis and C. glabrata are the predominant pathogens in diabetes, malignancy and use of broad-spectrum antibiotics. Many of the patients were diabetic with cancer on broad-spectrum antibiotics and 89% had candiduria associated with urinary catheters in a study done by Arlene et al[12] in patients with urinary candidiasis. In a study by Arora et al[13] with candidaemia, most common risk factor was use of intravenous catheter (63%), followed by prolonged use of antibiotics (35%) and immunosuppression (23%).

A total of 161 samples of CSF were processed for bacterial and fungal culture during the study period comprising of 89 from known HIV positive cases. Out of the 89 samples from known HIV positive cases, 31 samples yielded C. neoformans in a study done by Manoharan et al.[14]

C. neoformans is an opportunistic fungal infection and cryptococcal meningitis is the presenting manifestation of AIDS. It is the fourth most commonly recognised cause of life-threatening infection among these patients.[14] In this study, Cryptococcus neoformans was isolated in five patients and all the patients were HIV seropositive. In our study, it was isolated from both blood and CSF indicating patients had disseminated cryptococcosis, which signifies bad prognosis and high mortality. Mortality rate was very high in case of cryptococcosis, 3 out of 5 patients succumbed to infection within 3 days of initiation of antifungal therapy.

There are very few case reports on isolation of Cryptococcus in blood. In a study done by Monaco et al[15] over a period of 10 years on 128 positive HIV patients, diagnosis was established in 116 episodes by CSF study

(Indian ink, culture, antigen detection), in 9 cases by Cryptococcus species recovery from blood cultures and in 3 cases by antigen detection in patient's serum with a latex reactive and mortality rate was 35.8%.

There are few case reports of infections caused by Trichosporon asahii.[16,17] We isolated Trichosporon asahii from a urine sample of a catheterised patient treated with broad-spectrum antibiotics.

The advantage of using CHROMagar is that it facilitates the rapid isolation and identification of Candida to species level. The CHROMagar is the simple, rapid and cost-effective method for the speciation of Candida compared to the time consuming, cumbersome conventional method.[18] We noticed C. glabrata, C. kefyr and C. parapsilosis produced similar coloured colonies on CHROMagar, which may be difficult to differentiate if used alone. These strains can be easily differentiated by morphology on cornmeal agar. We suggest combination of cornmeal agar and CHROMagar can be used for early identification. Even C. albicans and C. dubliniensis produce light green and dark green colour on CHROMagar and can be differentiated based on growth at 42-45° C. C. albicans grows at 42-45° C, but C. dubliniensis does not grow at 42-45° C.[7]

CONCLUSION

This study showed that there is increase in prevalence of non-albicans Candida species, especially C. tropicalis and C. glabrata.

The results of this study also showed increase in incidence of disseminated Cryptococcosis in HIV seropositive patients. Cryptococcosis has emerged as important cause of death in HIV seropositive patients. The systemic cryptococcal infection can resemble clinically and radiologically as tuberculosis, which is endemic in India and is usually not considered as first differential diagnosis. Hence, awareness and high index of suspicion will aid in early diagnosis and prompt treatment resulting in decreased mortality.

The significant observation in this study was the emergence of rare yeast species like Trichosporon as a nosocomial pathogen, which is widely distributed in nature. The successful treatment of yeast infections depends on the early identification of the species and sensitivity patterns to antifungal agents.

Thus, early isolation and speciation will help the clinicians/microbiologists to know the pathogen and institute proper antifungal therapy in appropriate time, thereby avoiding any treatment failures and mortality.

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