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RESEARCH ARTICLE

STUDY OF OROPHARYNGEAL CANDIDIASIS IN HIV INFECTED PATIENTS

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ABSTRACT

Candida occurs as normal oral commensal in 20-50% of Indian population. But in HIV infection the colonization rate increases dramatically to 81.3% on an average Oropharyngeal candidiasis occurs when CD4 count is 200-500 cell/mm3, with fall in CD4 count to <200 cells/mm3 oesophageal candidiasis occur in HIV infected patients Aim- To identify and characterize Candida species (oropharyngeal and oesophageal) in HIV infected patients and to correlate them with CD4+ T lymphocyte count. Method- Two twenty two hundred HIV positive cases were selected for this study. Identification of Candida species was done by conventional standard techniques using Gram's stain, cultural character on Saboraud dextrose agar, germ tube test, morphology on corn meal agar, colour difference on CHROM agar and sugar assimilation test. Findings- The prevalence of candidiasis was 75.91%. with majority of the patients belonged to age group 31 - 45 years. Male to female ratio was 1.93: 1 . 63.47% of patients belonged to Stage 4 of WHO clinical staging. Candida albicans was the most common species isolated 52.31%. Non Candida albicans isolates were 47.69% of which most common was C. tropicalis 32.61%, C. krusei 5.17%, C. parapsilosis 3.45%, C. glabrata 2.87%, C.kefyr 1.72%, C.dubliniesis 1.72% and C. guillermondii 1.15%. Conclusion- Oropharyngeal Candida colonization remains common in HIV-infected individuals, even with ART. Candida albicans continues to be the common and important pathogen among the Candida species there is a increase in the incidence of non-Candida albicans species like Candida dublinienses, Candida krusei, Candida tropicalis and Candida glabrata.

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INTRODUCTION

By the end of 2013, an estimated 35 million (33.1 million - 37.2 million) people were infected with HIV globally. The virus targets the cells with CD4 surface molecules, majority being in the T-lymphocytes. With waning CD4 cell number immunodeficiency emerges. The progressive decline in immunological responses makes them susceptible to opportunistic infections (Chander, 2002; NACO, 2017 and Cook, 2003). Though HIV is the causative agent of AIDS, most morbidity and mortality in AIDS patients results from opportunistic infections; approximately 80% of these patients are seen to die as a result of such an infection rather than from HIV (Sivaraman, 1992). There are major differences in the spectrum of opportunistic infections in India and in the West. (White, 1992 and Aquinas, 1996). National AIDS Control Organisation (NACO) data reveal that the tuberculosis is the commonest infection in AIDS patients followed by candidiasis, cryptosporidiosis, and others (NACO, 2017; Cook, 2003 and Sivaraman, 1992). The major cause of morbidity and mortality in HIV infected persons are different opportunistic infections.

These infections, responsible for morbidity and mortality, vary from region to region (Alemayehu, 2009). *Candida* occurs as normal oral commensal in 20-50% of Indian population.But in HIV infection the colonization rate increases dramatically to 81.3% on an average (Chander, 2002). Candidiasis can involve any site of the gastrointestinal tract, with the oesophagus and the small bowel being the commonest sites. These lesions are clinically significant because they may progress to haematogenous infection. The usual presentation of oropharyngeal and oesophageal infections is in the form of white, "cottage cheese" patches, redness or soreness, loss of taste and cracking and redness at the corners of the mouth. Oesophageal candidiasis usually includes pain when swallowing and difficulty swallowing. These all causes negatively impacts on quality of life and threatens the general wellbeing of HIV positive individuals (Centers for Disease Control and Prevention, 2017). Erythematous candidiasis is the most common type of oral candidiasis encountered in HIV infected/AIDS patients (Segal, 2008 and Maria, 2003). In HIV infection, candidiasis occurs as angular chelitis in stage 2, pseudomembranous/flat erythematous candidiasis in oral cavity in stage 3, oesophageal candidiasis in stage 4 (Cook, 2003 and Frimpong, 2017). With normal or nearly normal CD4 count vaginal candidiasis occur, oral candidiasisoccurs

when CD4 count is < 300 cells/mm^3 with fall in CD4 count to <100 cells/mm^3 oesophageal candidiasis occur CD4+ counts below 200 cells/mm^3 or a high viral load (>10,000 copies/ml) is a major risk factor for development of OPC in such patients. Recurrent episodes of OPC are seen in HIV/AIDS patients and they are also more prone to develop invasive candidiasis (WHO, 2017 and NACO, 2017). Though *Candida albicansis* the most frequently isolated species as a colonizer and pathogen of the oral mucosa, other Candida species, such as *C. tropicalis, C. krusei, C. glabrata, C.dubliniensis, C. guilliermondii, C. parapsilosis, C.kefyr,* and *C.pelliculosa* have become a significant cause of infection in patients with HIV infection (Chander, 2002).

Aims and Objectives

To identify and characterize Candida species in HIV infected patients. To correlate occurrence of candidiasis with CD4+ T lymphocyte count.

MATERIALS AND METHODS

Source of data: Two twenty two hundred HIV positive cases with clinically suspected oropharyngeal and oesophageal candidiasis of all age groups and both sexes attending ART Centre of our tertiary care hospital were selected for this study.

Collection of sample: Two sterile cotton tipped wooden swabs moistened with saline were used to swab and scrap the lesion in the mouth without touching any other structure after rinsing the mouth. Swabs kept in sterile container with cotton plugs and transported immediately to microbiology laboratory. One swab for Gram stain. Second swab inoculated on Sabouraud dextrose agar and 5% blood agar. Identification of Candida species was done by conventional standard techniques using Gram's stain, cultural character on Saboraud dextrose agar, germ tube test, morphology on corn meal agar, colour difference on Hichrome Candida differential agar (CHROM agar) and sugar assimilation test by using HIMEDIA candida identification kit KB006.

RESULTS AND OBSERVATIONS

Out of 220 samples collected and processed 167 samples showed growth on SDA, the prevalence being 75.91%.

Table 1. Age-wise distribution of oropharyngeal candidiasis (culture positive)

Age group (years)	Number of cases(n=167)	Percentage
0-15	3	1.80%
16-30	44	26.35%
31-45	88	52.69%
46-60	28	16.77%
61-75	4	2.39%
Total	167	100%

As per table 1, out of 167 culture positive study subjects 88 (52.69%) are from age group 31- 45 years, while 44 (26.35%) are from age group 16- 30 years followed by 28 (16.77%) of age group 46-60 years . Mean age of study subjects is 37.17 years with standard deviation of 11.67. Table no 2 shows that 106 (63.47%) of the cases belong to stage 4, while 40 (23.95%) of the cases belong to stage 3 followed by 11 (6.59%) in stage 1. Less number of patients belong to stage 2 i.e.10 (5.99%).

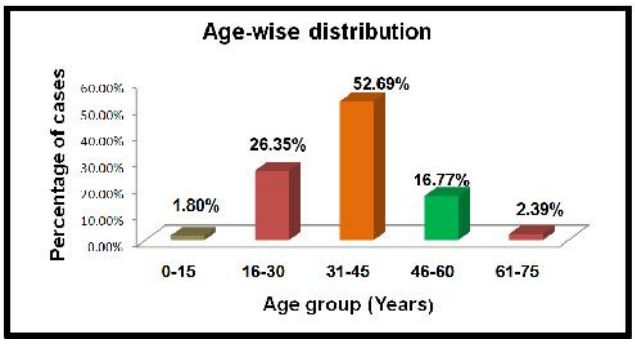


Table 2: Distribution of oropharyngeal candidiasis as per WHO clinical staging (n=167)

WHO clinical staging	Number of cases	Percentage
Stage 1	11	6.59%
Stage 2	10	5.99%
Stage 3	40	23.95%
Stage 4	106	63.47%
Total	167	100%

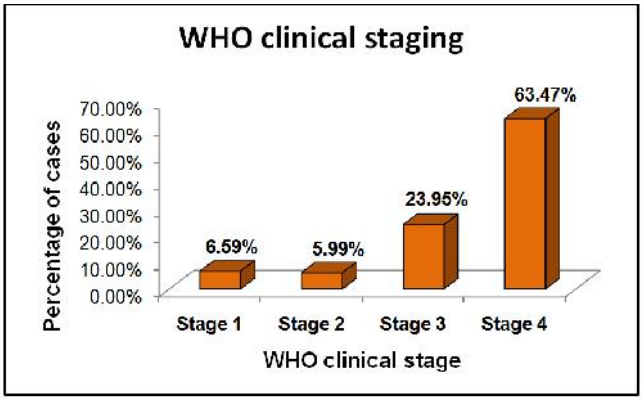


Table 6. Distribution of oropharyngeal candidiasis as per Antiretroviral therapy (ART) mode (n=167)

ART mode	Number	Percentage
On ART	156	93.41%
No ART	11	6.59%
Total	167	100%

Table no 6 shows out of 167 culture positive samples, 156 (93.41%) of the HIV seropositive cases in the study group were on anti-retroviral therapy and 11 (4.19%) were not on ART therapy.

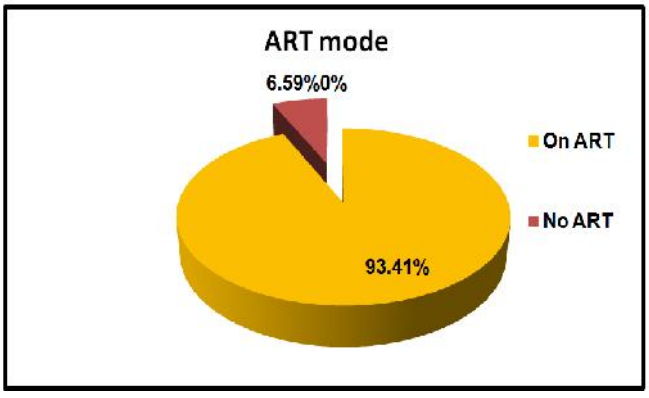


Table 3. Distribution of oropharyngeal candidiasis according to CD4+ count (n=167)

CD4+ count (cells/µl)	Number	Percentage
≤ 150	83	49.70%
151-200	45	26.95%
> 200	39	23.35%
Total	167	100%

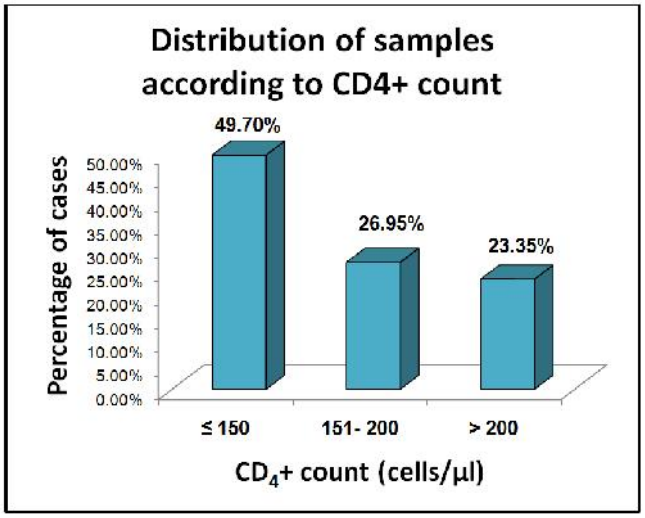
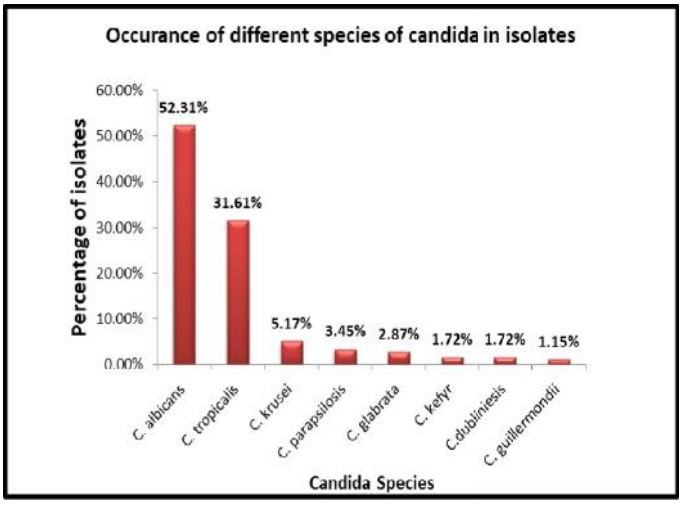


Table no 9 shows that , more number of Candida are isolated when the CD4+ counts goes on decreasing. 83 (49.70%) of the cases had CD4+ counts ≤ 150 cells/µl, while 45 (26.95%) had CD4+ counts between 151-200 cells/µ1 and 39 (23.35%) of cases had CD4+ counts > 200 cells/µ1. The mean CD4+ count was 177.43 ± 117.16 cells/µ1.

Table 12. Distribution of different candida species in oropharyngeal candiadsis

Candida species	Number of species(n=174)			Percentage
	single	mixed	Total	
C. albicans	84	7	91	52.31%
C. tropicalis	50	5	55	31.61%
C. krusei	8	1	9	5.17%
C. parapsilosis	6	0	6	3.45%
C. glabrata	4	1	5	2.87%
C. kefyr	3	0	3	1.72%
C.dubliniesis	3	0	3	1.72%
C. guillermondii	2	0	2	1.15%
Total	160	14	174	100%



The total number of candida species isolated was (160 X 1) + (7X 2) = 174. Out of 174 isolates, *Candida albicans* was the most common species isolated 91 (52.31%). Non *Candida albicans* isolates were 83 (47.69%) of which most common was *C. tropicalis* 55 (32.61%), *C. krusei* 9 (5.17%), *C. parapsilosis*6 (3.45%), *C. glabrata* 5 (2.87%), *C.kefyr* 3 (1.72%), *C.dubliniesis* 3 (1.72%) and *C. guillermondii* 2 (1.15%).

DISCUSSION

The most common opportunistic fungal infection in HIVpositive patients is candidiasis, affecting mainly mucocutaneous system. *C. albicans*remains the most common species responsible for candidiasis, disease due to newer species like *C. dubliniesis*are also increasing.[14] A routine check for opportunistic infections including oropharyngeal candidiasis is important and should be carried out because oral lesions in HIV patients show the potency of immune system, prognosis of the disease and treatment response to ART medication .[15] Identifying candida to its species level is important because it helps guiding proper treatment. In the present study of 220 HIV seropositive patients with clinically suspected oropharyngeal attending ART Centre of our tertiary care hospital were selected randomly. With informed consent and brief history samples were obtained. All samples were subjected for gram staining and were inoculated on Saboraud dextrose agar, Blood agar and CHROM agar. Isolates were then subjected to germ tube test, Cornmeal agar morphology, Assimilation tests to differentiate various candida species and biofilm production by Microtitre plate method. Antifungal susceptibility testing was done by Kirby Bauer disc diffusion method. The CD4+ count of each patient was obtained, and the results were statistically analyzed. Out of 220 samples collected 167 samples (75.91%) showed growth of candida on SDA and 53 samples (24.09%) had candida negative culture. Hence the prevalence of oropharyngeal candidiasis in our study is 75.91% (167/220). Our finding is comparable with the study done by Konate A *et al.* [16]In his study of HIV/AIDS infected patients with OPC prevalence rate was 79.4% (227/286). While in study done by Mistry *et al.* [17]from Vadodara (India) found more prevalence of OPC i.e. 87.6%. Costa *et al.* [18]and F. Barchies*iet al.* [19] found less prevalence of oropharyngeal candidiasis than our study i.e. 62.6% (62/99) and 68% (68/102) respectively.

Age distribution: In the present study, we found that the most prevalent age group was 31–45 years (52.69%), followed by 15-30 years (26.35%). Mean age was 37.14 ± 11.67 years. This finding is similar to those reported by Costa *et al.* andF Barchies*iet al.* , i.e. 37.1 and 38 years respectively. [18,19] These age group being the most productive age group of the society are at higher risk of HIV infection. Lower incidence in lower age group may be due to fear of social stigma, low uptake of testing and consequently low uptake of treatment. [20]

Sex distribution: Our study showed a higher proportion of OPC in HIV-infected male 65.87% (110) compared to females 34.13% (57).Male: Female ratio being 1.93:1. Which is comparable with the study done by Tercas *et al.* [21] and Ranganathan K *et al.* [22] , who showed Male: Female ratio as 1.4:1 and 1.03:1 in their respectively studies. Whereas in the study by Anupriya *et al.* Male: Female ratio was high i.e. 4.8:1. [23] In contrast study done by *Butt FM et al.* found more female 59% (36/61) are infected than male 41%(25/61) .[24] National AIDS Control Organisation NACO (2015) India

has also showed higher prevalence of HIV infection in Male (0.30%) as compared to females (0.22%).[2] Preponderance of males may be due to their migration to the metropolitan cities in search of work. Staying away from their spouse for longer periods and the philandering habit of males being seen might have resulted in their acquiring HIV infection.While the males belonged to a wider age spectrum, the females were a considerably younger population, and most of them acquired infection from their spouses, reflecting the male dominance in Indian society and emphasizing an increased need for awareness and counselling of both the spouses.[23] Moreover, the male preponderance seen might have been due to the fact that in the existing social milieu in India females do not seek medical care because of fearing ostracism and loss of family support.[25]

Who clinical Staging: As per WHO clinical staging based of opportunistic infection majority of patients belongs to either stage 3 or 4. In our study 63.47% (106/167) of the patients with candidiasis belonged to stage 4 and 23.95% (40/167) belonged to stage 3. Which is comparable with the study done in North India by Anupriya*et al*. (2007), where 73% patients belonged to stage 4 and 23.33% belonged to stage 3. [23] Study by Champa H *et al.* from South India found 54% (54/100) patients belonged to stage 4 and 36% (36/100) belonged to stage 3 .[26] Higher no of cases in advance stages may be due to decrease immunological status as the HIV advances in life .It may also due to lack of awareness regarding the clinical spectrum and the various presentations of AIDS, in addition to the lack of diagnostic facilities at the peripheral health centres, due to which patients present very late to the tertiary health centres.[23] Staging is important to start ART and monitor the response.

Correlation of OPC with CD4+ count: Worldwide there is a constant increase of HIV infection. With progression of the disease, there is a constant decrease in CD4+ count and this leads to the commensals becoming opportunistic. Hence as the CD4+ count goes on decreasing, the number of *Candida* infected patients goes on increasing. [27] In our study 83 (49.70%) of the cases had CD4+ counts ≤ 150 cells/µl, 45 (26.95%) had CD4+ counts between 151-200 cells/µl and 39 (23.35%) of cases had CD4+ counts > 200 cells/µl. Majority of patients in our study had CD4+ count less than 200 cells/µl i.e. 76.65% (49.70+26.95), which is similar to the study by Arora U *et al.* andChampa H et al., where it was 76.66% and 84.07% respectively .[28] Mean CD4+ count in our study was 177.43 ± 117.16 cells/µl. Studies by LattiffAA *et al.* and Anupriya*et al.* , showed mean CD4+ count 132 ± 60.45 cells/µl and 145 ± 52.1 cells/µl respectively.[29,23] While in study done by Fichtenbaum *et al.* it was high i.e. 233 cells/µl. [30] Hence in most of the studies CD4+ count is less than 200 at which oral candidiasis occurs.[23,29]

In contrast study by Costa *et al.* did not find a significant correlation between oral carriage and CD4+ cell count or HIV-1 RNA in plasma .In his study 14.5% (9/62) of the oral carriage had their CD4+ cell count low of 200 cells/mm^3 and 27.4% (17/62) had their viral load above 20000 copies/mL no patient had oral lesions by *Candida*.[18] The low absolute CD4+ T-lymphocyte count has traditionally been cited as the greatest risk factor for the development of Oropharyngeal Candidiasis and current guidelines suggest increased risk of OPC once CD4+ T lymphocyte counts fall below 200 cells/µL. [31]

Species isolates

Table: Rate of isolation of candida species in various studies

Studies	Year	Percentage isolation of	
		Candida albicans	*Non-Candida albicans*
F. Barchiesi*et al.*[109]	2002	93.00%	7.00%
Vargas KG *et al.*[46]	2002	84.00%	16.00%
Costa *et al.* [108]	2006	50.00%	50.00%
Patel M *et al.* [12]	2006	78.60%	21.40%
Anupriya*et al.*[112]	2007	59.30%	40.70%
Nadagir*et al.* [120]	2008	66.60%	22.40%
Ranganathan K *et al.* [77]	2008	85.00%	25.00%
Alborzi*et al.* [127]	2010	50.00%	50.00%
Champa H *et al.* [115]	2010	28.31%	71.69%
Jeddy Ne *et al.* [128]	2011	90.00%	10.00%
Kalpanadevi*et al.* [129]	2012	78.00%	26.00%
Mistry *et al.* [107]	2015	63.71%	36.29%
Krishnam SP*et al.* [130]	2015	84.60%	15.40%
Tercas*et al.* [111]	2017	56.00%	44.00%
Present study		52.31%	47.69%

The table shows a changing trend of occurrence of the *C. albicans*and Non *Candida albicans*species over the last 15 years in OPC cases. Studies from India or outside showed an increasing occurrence of non-Candida albicans as a leading cause of oral candidiasis. *C. albicans*still being the most common cause of oral candidiasis in HIV positive individuals.In our study among all *candida species* isolates *Candida albicans*was found to be maximum i.e. 52.31% (91/174) than Non*albicans candida* i.e. 47.69% (83/174). This is comparable with the studies done by Alborzi A et al. and Costa *et al.* , where 50% *C.albicans* was isolated in both studies.[32,18] A recent epidemiological trend is the emergence of less pathogenic species of Non-albicans Candida as significant opportunistic pathogens. [33] Among Non albicans Candida 8 different species i.e. *C. glabrata, C. tropicalis, C. krusei, C.parapsilosis, C .kefyr, C. gulliermondi and C.dubliniensis*were isolated in our study.

Amongst them *C. tropicalis* i.e.31.61% (55/174) was most common specie isolated, which is consistence with the studies from the USA were *C. tropicalis* is reported to be most common non-albicans Candida spp. in oral thrush.[34]Champa H *et al.* and Deorukhkar S *et al.* also reported *C. tropicalis* predominance among Non albicans Candida i.e. 24.66% and 12.29% respectively.[26,35]*C. tropicalis*is becoming an emerging pathogen globally. Increasing number of immunocompromised patients along with increased use of an antifungal regimen and use of broad spectrum antibiotics are the major contributory factors for this. [34,35]

C. krusei was second most common isolates among non albicans in our study i.e. 5.17% (9/174) which is similar with the studies by Anupriya*et al.* andNadagir*et al.* , where it was 4.7% and 6.67% respectively.[23,36]

*C. parapsilosis*isolateswere 3.45% (6/174) which is in concordance with the studies by Nadagir andPatel M *et al.* who also isolated this specie in their studies as 3.79% and 4% respectively. [33,36]

C. glabrata isolates in present study was 2.87% (5/174), which is similar to study by Lattiff AA *et al.* , where he found 3% *C. glabrata* in OPC patients.[29] Our finding is less than the studies by Patel M *et al.* andAnupriya*et al.* where *C. glabrata* was 5.2% and 14.8% respectively.[23,33]

*C. kefyr*isolates in present study was 1.72% (3/174) which is less than the study by Champa H *et al*. who reported 3.53% *C. kefyr* in cases of OPC. In present study *C. dubliniensis*isolates was 1.72% (3/174), which is less than the finding by Patel M *et al*.andNadagir*et al.,* who showed *C. dubliniensis* 6.3% and 16.29% in their respective studies . [33,36] *C. guillermondii* was least isolated in our study i.e. 1.15% which is in comparable with study by Nadagir*et al*. and Ranganathan K *et al*., where *C. guillermondii* was the rarest isolate i.e. 1.5% and 2% respectively .[36,22] There is a difference in occurrence of non-*Candida albicans* species between South India and North India in HIV positive cases. In South Indian studies there is increase isolation of *C. dubliniensis*and no isolation of *C. glabrata*whereas studies from North India showed a very few isolates of *C. dubliniensis*with increased isolation of *C. glabrata*. *C. krusei*species was isolated in all the studies. [26] Such difference is not much seen in Central India as reported by More SR *et al.* and Deorukhkar*et al*., who showed*C. dubliniense*sand *C. tropicalis* as most common Non *albicans Candida* in their respective studies. [37,38]

Conclusion

Oropharyngeal *Candida* colonization remains common in HIV-infected individuals, even with ART. Studies monitoring the distribution and antifungal susceptibility of *Candida* isolates from these populations have crucial importance for choosing the correct antifungal therapy during candidiasis

*C. albicans*is the most common species, suggesting endogenous infection. non candida species are also emerging.

The increasing emergence of non- *Candida albicans* seems to be associated with HIV pandemic. Since *C. dubliniensis* closely resembles *C.albicans* phenotypically it is possible that it is being missed in most of laboratories where only germ tube is solely used of the identification of *C.albicans*. Emergence of C. dubliniensis infection in HIV seropositive patients is a matter of concern due to the emergence of resistance to commonly used azole antifungals. CHROM agar when used to speciate can give excellent results within short time. Presumptive identification becomes easier especially in case of non-*Candida albicans*. Hence Chromagar can be routinely used instead. If this is corroborated with additional tests like germ tube and sugar assimilation test, identification upto species level can be made appropriately. Decreased immunity (expressed in terms of CD4+ lymphocyte count) increases the fungal growth leading to frequent occurrence of candidiasis. Hence correlation with the CD4+ counts is necessary. Thus we conclude that the morbidity and risk associated with Candidiasis, along with increased incidence of treatment refractory Candidiasis as well as the high incidence of AIDS, makes it important that species identification of Candida isolates should be done along with correlation with the CD4+ counts is important for successful treatment of Candidiasis.

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