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Research Article

Antifungaldrug sensitivity of *Candida* isolated from oral cavities of human immunodeficiency virus infected patients in a tertiary care centre

Rajeswari.M.R.[1], Mahendraswamy B Hiremath[2], Mohit.M[3],Hanumanthappa.A.R.[4]

[1]Associate Professor, Department of Microbiology, J.J.M Medical College
[2]AssistantProfessor, Department of Microbiology, CDSIMER DayanandaSagar University
[3]Medical student, Phase three, CDSIMER DayanandaSagar University
[4]Professor, Department of Microbiology, J.J.M Medical College

Corresponding Author

Rajeswari. M.R
Associate Professor, Department of Microbiology, J.J.M Medical College

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Abstract

Introduction: Human immunodeficiency virus (HIV) infected individuals frequently suffer from oropharyngeal candidiasis (OPC) has an opportunistic infection. *Candida albicans* is the often isolated species from the oropharyngeal candidiasis among *Candida* species. Non-*albicans Candida* (NAC) species are also being recovered increasingly. Intensiveantifungal use both for therapy and prophylaxis has favored the emergence of resistant strains.
Aims: The aims of the study were
1) To document the clinical types of candidiasis in HIV patients.
2) To identify the spectrum of *Candida* species in oropharyngeal candidiasis.
3) To identify the drug resistance pattern.
Materials and methods:150 oropharyngeal swabs were collected from 75 HIV seropositive individuals with clinical confirmation of oropharyngealcandidiasis.The clinical type of candidiasis were noted,standard microbiological techniques were performed for species identification and determination of drug resistance pattern.
Results:Out of 75 study subjects, *Candida albicans* was the leading species isolated from 70% cases, with non-*albicans Candida* species (NAC) being isolated from 30% cases. Regardless of the*Candida* species, drug resistance was observed in 12(24%), 10(20%), 7(14%) and5(10%) of the isolates to the drugs, Fluconazole, Itraconazole, Clotrimazole and Nystatin, respectively.
Conclusion:A rising trend of non-*albicansCandida*(NAC)along with varying levels of drug resistancerecommends the need for routine culture and antifungal drug susceptibility testof *Candida* species for effective controlling.

Key-words: *Candida*, HIV, non-*albicans Candida* (NAC)

INTRODUCTION

Candida species inhabit the skin and mucosal surfaces of the genital and intestinal tracts as well the oral cavity under normal healthy environments[1].However, under immunocompromised conditions, such as when a person is sick with human immunodeficiency virus (HIV), the colonization may grow out of control and quickly turn into a symptomatic infection [2].

Oropharyngeal candidiasis (OPC) is one of the opportunistic*Candida* infections which remain to be a common opportunistic infection in patients infected by the human immunodeficiency virus (HIV) [3]. It occurs in up to 90% of the patients at some point during the progression of HIV disease and is concomitant with high viral loads, low CD4+ T cell count (< 200 cells/mm3) and disease progression[3].

Though *Candida* albicans is the most often isolated species as a colonizer and pathogen of the oral mucosa, othernonalbicansCandidaspecies (NAC), such as *C. tropicalis, C. krusei, C. glabrata, C. dubliniensis, C. guilliermondii, C. parapsilosis,* and*C. kefyr*, have become a substantial cause of infection in patients with HIV infection [4].The prolonged progression of HIV infection predisposes these patients to recurrent episodes of OPC that can increase in frequency and severity through the course of HIV disease progression[1].

The development of antifungal resistance has been interrelated to the use of antifungal agents to treat recurrent infections in patients with HIV where the appropriate doses, prescribed for the usual duration, become ineffective [5].The azoles, particularlyfluconazole, remains the most common antifungal drug, but their frequent clinical use for both therapy and prophylaxis has favoured the advent of resistant strains [6].The clinical importance of non-*albicans Candida*species lies in the fact that they are usually less susceptible to the more commonly used azole antifungal drugs, a factor that poses significant difficulties in actual treatment [4].

THE AIMS OF THE STUDY WERE

1) To document the clinical types of candidiasis in HIV patients**.**
2) To identify the spectrum of *Candida* species in oropharyngeal candidiasis**.**
3) To identify the drug resistance pattern with respect to commonly available antifungal drug**.**

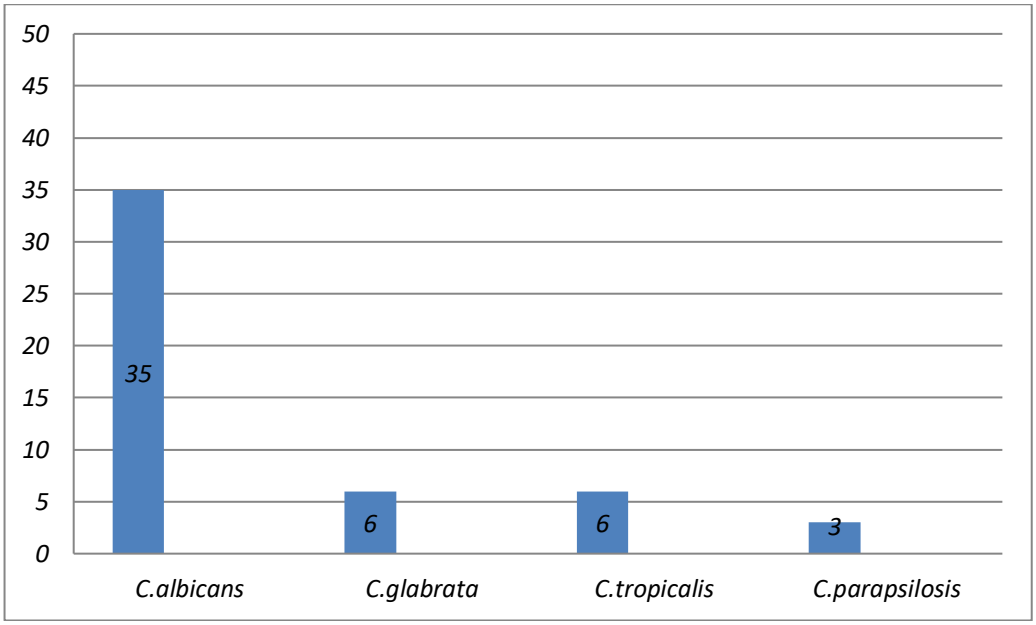
MATERIAL AND METHODS

A prospective cross sectional study was carried out on patients attending Bapuji hospital, a tertiary care center attached to J.J.M. medical College for a period of 1.5 years, between july 2022 to December 2023.Institutional ethical committee clearance (IECC) was obtained. The study was carried out on 75 patients with HIV presenting with features suggestive of oropharyngeal candidiasis (OPC). Forty-five were male patients and thirty were female patients with age ranging from 5 to 70 years. 150 oropharyngeal swabs were collected from 75 patients. The samples were brought to the microbiology laboratory immediately, and processed as per standard microbiological methods.All the samples were subjected for Gram's staining and10% potassium hydroxide(KOH) mount to look for yeast and psedohyphae, after which they were inoculated onto Sabouraud's dextrose agar (SDA) supplemented with chloramphenicol and incubated for 48 hours. If required, incubation was done for up to 1 week. The growthof *Candida* was confirmed by the presence of creamy white (yeast like) colonies.Species identification was done by grams staining,germ tube production test, sugar fermentation test, sugar assimilation test, chlamydospore production test on cornmeal agar(CAM) and further, the colonies were grown on *Candida* CHROM agar (Hi-chrome *Candida* Hi-Media, Mumbai).The antifungal susceptibility pattern of *Candida*isolates to four antifungal agents, Fluconazole, Itraconazole, Clotrimazole, and Nystatinwas determined by disc diffusion method (CLSI M44-A document). Discs used were Fluconazole(10mcg), Itraconzaole (10mcg), Clotrimazole (10mcg) and Nystatin (100mcg).

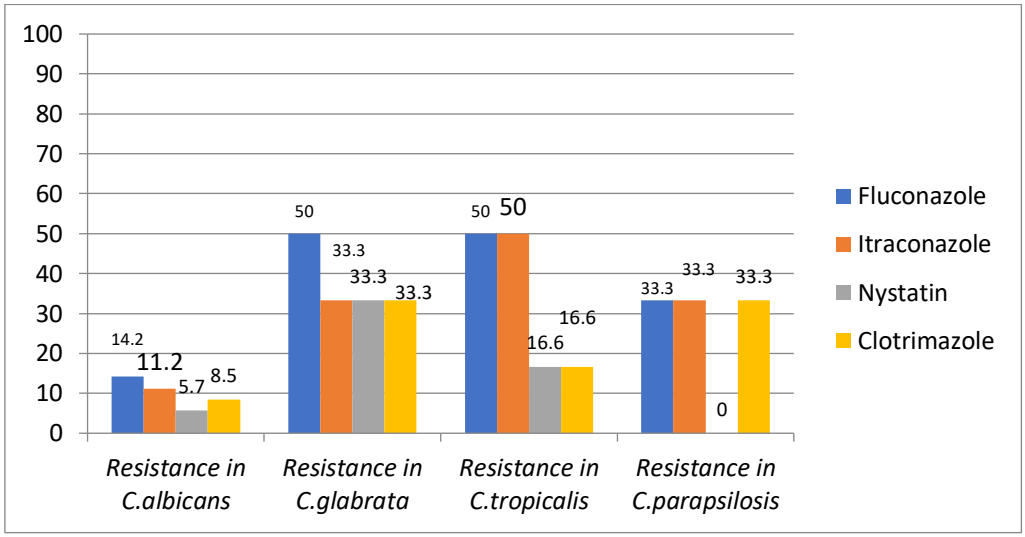
RESULTS

Out of 75 study subjects, 50 yeast isolates were recovered with a prevalence rate of 66.7%. The clinical pattern of candidiasis was documented with the commonest variant noted being the pseudomembranous type which was observed in 30(60%) patients. The other variants documented were angular cheilitis, hyperplastic and atrophic types in 10(20%), 5(10%) and 5(10%) patients, respectively.Isolation and identification of species was done, with the commonest species isolated being *Candida* albicans. The results have been compiled and presented in Table/Figure 1.Antifungal susceptibility pattern of the isolates to the commonly used antifungals, Fluconazole, Itraconazole, Nystatin, and Cotrimazole, were assessed and are presented in Table/ Figure 2.

TABLES /FIGURES:



Table/Figure 1. Distribution of *Candida* species



Table/Figure2. Drug resistance to *Candida* species

DISCUSSION

Oropharyngeal candidiasis is the furthermost common opportunistic infection among HIV-seropositive patients and in those with AIDS, and it represents a major treatment challenge[7].There are quite a few classes of drugs that are used to treat oropharyngeal candidiasis with the most frequent being azoles. Widespread and repeated use of azole drugs has led to resistance to antifungal therapies; a problem that is apparently spreading widely[8].

In our study, the predominant species recovered was *Candida albicans*. This is compatible with the study carried out by Menon et al., [9] in India and was relatively lower when compared to studies carried out by Mulu et al., (82.3%)[3], Latiff et al.,(86%)[10], and Maurya et al., (90.5%)[11]. The non-albicans*Candida* species predominantly identified in our cohort was *Candida glabrata*, *Candida parapsilosis*and *Candida tropicalis*.In our study, *Candidaalbicans* demonstrated resistance to all four drugs, ie Fluconazole, Itraconazole, Nystatin and Clotrimazole. Among the 35 isolates of *Candida albicans*, resistance to Fluconazole, Itraconazole, Nystatin and Clotrimazole was seen in 5(14.2%), 4(11.4%), 2(5.7%) and 3(8.5%) isolates respectively.

Resistance to fluconazole in our study (14.2%) was in accordance to that conducted by Anwar khan et al.,(10.34%)[12] and Mulu et al.,(16%)[3].But a study conducted by Menon et al.,(22.2%)[9] revealed higher resistance levels.Resistance to Itraconazole in our study(11.4%) was comparable to the value obtained by Mulu et al.,(8%)[3]but was in contrast to studies done by Menon et al.,[9], and Anwar Khan et al.,(6.8%)[12]. Resistance to Nystatin(5.7%) and Clotrimazole(8.5%) unlike in a study conducted by Jeddy et al., [13] where no resistance was observed to both drugs. Infections caused by *Candida albicans* are associated with varying levels of fluconazole resistance depending on the type of infection,with oropharyngeal candidiasis having higher resistance to fluconazole and this is dependent on upon previous fluconazole treatment and prior OPC infections[14]. The mechanisms of resistance to azole antifungal agents can be mainly categorized as (i) variations in the cell wall or plasma membrane, which lead to impaired azole uptake; (ii) changes in the affinity of the drug target Erg11p (lanosterol 14α-demethylase) to azoles or in the cellular content of Erg11p due to target site mutation or overexpression of the ERG11 gene; and (iii) the efflux of drugs facilitated by membrane transport proteins belonging to the ATP-binding cassette (ABC) transporter family (CDR1 and CDR2) or to the major facilitator superfamily (MDR1 and FLU1)[5].

Amongst the 6 isolates of *Candida glabrata* recovered, resistance was demonstrated by 3(50%) to Fluconazole and 2(33.3%) isolates each to the remaining 3 drugs included in the study. Resistance to Fluconazole in our study was comparable to studies done by Tercas et al., (50%) [7] but higher when contrasted against that done by Mulu et al.,(12%) [3]. Resistance to Itraconazole was higher in our study compared to Muluet al., (4%) [3] and Anwar Khan et al.,(6.9%)[12]. Resistance to Nystatin and Cotrimazole was higher in our study when compared a study conducted by Dar et al.,(11.1% to both drugs)[16].*C. glabrata* exhibits decreased susceptibility to the azole class of antifungals [14].Although primary in vitro resistance to fluconazole has been reported, acquired resistance to azoles is, by far, the most common form of resistance in *C. glabrata* and is most often seen for fluconazole. Development of azole resistance in clinical isolates of *C. glabrata* has been almost exclusively linked to the occurrence of activating mutations in the zinc cluster transcription factor Pdr1 [4].

Amongst the 6 isolates of *Candida tropicalis*, in our study, resistance was observed in 3(50%) isolates each to Flucanazole and Itraconazole. Both values were higher when compared to Muluet al.,(8% for Fluconazole and 4% for

Itraconazole)[3]. Resistance was also documented to Clotrimazole and Nystatin in our study, with 1(16.6%) isolate being resistant to each drug. Very little information is available regarding the mechanism of resistance in this species as very few studies have been done to determine this. One mechanism described for the decreased susceptibility to azoles of this isolate seemed to be due to an overexpression of ERG11gene, associated with a missense mutation of this gene [17,18].
Amongst the 3 isolates of *Candida parapsilosis*, 1(33.3%) isolate demonstrated resistance to each Fluconazole, Itraconazole and Clotrimazole. while no resistance was observed to Nystatin. A combination of molecular mechanisms, including the presence of point mutations in the ERG11 gene, overexpression of ERG11, and genes encoding efflux pumps, are involved in fluconazole resistance in *C. parapsilosis*[19].

CONCLUSION

In conclusion, resistance to azoles is a major obstacle hindering the physician in providing effective treatment to the patient. To overcome this issue, speciation as well as drug sensitivity testing must be done, especially in HIV patients, in whom non-*albicansCandida* species are being isolated at a higher frequency. Also, the commonly available and low costing Nystatin must also be considered for treatment as we noticed lower resistance to this drug when compared to standard azole drugs.

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Conflicting Interest: No conflicts of interest.

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