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RESEARCH ARTICLE OPEN ACCESS

Antifungal Susceptibility Pattern of *Candida* Isolates: A Comparison in H.I.V. Positive and Negative Patients from A Tertiary Care Hospital of Northern India

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Abstract

Candidiasis is recognized as a significant cause of morbidity, especially in immunocompromised individuals. An epidemiologic change in *Candida* species and emergence of resistance can impact the usage of antifungal agents as empirical therapy for Candidiasis in patients with or without AIDS. The present study was done to find out: i) The species of *Candida* isolated from H.I.V. and Non-HIV infected patients. ii) The resistance pattern of these *Candida* isolates to antifungal agents. A total of 160 *Candida* species isolates (80 isolates each from H.I.V. and Non-HIV infected patients) were characterized. Identification of yeast isolates was made by standard procedures including morphology (Staib agar, cornmeal agar, CHROMagar), germ tube test, fermentation, and assimilation of sugars and growth at 42°C. In addition, sensitivity testing was done using the broth microdilution method (M27-A2) as per the C.L.S.I. guidelines against amphotericin B, nystatin, voriconazole, fluconazole, ketoconazole, and itraconazole. In both the groups, i.e., H.I.V. and Non-HIV infected patients, *Candida albicans* was the most common species (61.2 % and 85 % respectively), followed by *Candida guilliermondi* (16.2 % and 5 %), *Candida tropicalis* (5 % and 3.7 %), *Candida krusei* (5% and 2.5 %), *Candida dubliniensis* 1(5 % and 1.2 %) and others. Among HIV infected patients fluconazole resistance was 16.25%, ketoconazole 13.5%, clotrimazole 12.5%, itraconazole 6.25 %. In the non-HIV infected group, fluconazole resistance was 8.75% and itraconazole 1.25%. For the appropriate treatment of *Candida* infections, antifungal susceptibility has become an essential tool, especially in the present scenario of increasing resistance.

Keywords: *Candida*, HIV-Positive, Antifungal, resistance, oral Candidiasis

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INTRODUCTION

Candidiasis has been recognized as a significant cause of morbidity, especially in immunocompromised individuals. The use of highly active antiretroviral therapy (HAART) causes inhibition of viral replication, which eventually causes the recovery of CD4 T+ lymphocytes in HIV-positive patients. The prolonged course of H.I.V. infection further puts at risk these patients to repeated episodes of various opportunistic infections like Candidiasis that can increase in severity with the progression of H.I.V. disease. In addition, candidiasis' management causes the development of antifungal resistance amid the course of treatment[1-3]. Infection by resistant *Candida* strains does not respond to antifungal therapy even with standard doses for an appropriate time duration[4,5].

Another scenario is that multiple exposures to antifungal agents can cause a shift to *Candida* species other than *albicans*, which will eventually lead to hard to treat, refractory, and recurrent infections[6,7]. Thus, an epidemiologic change in *Candida* species and the emergence of resistance could cause a significant impact on the use of antifungal agents as empirical therapy for Candidiasis in patients with or without AIDS. Opportunistic infections, including Candidiasis, aggravate morbidity and decrease the span and quality of life of HIV-infected patients and thus require accurate diagnosis and adequate treatment. In-vitro sensitivity testing is clinically helpful in foreseeing whether the patients are going to improve with treatment or not. However, in India, in the same way as other developing nations, in-vitro antifungal testing is not performed as routine testing. Accordingly, not much is known about the in-vitro antifungal sensitivity of *Candida* species cultured from HIV-infected patients with Candidiasis. This study was done to find out the species of *Candida* isolated from H.I.V. Negative and H.I.V. Positive patients and their resistance pattern to antifungal agents.

MATERIALS AND METHODS

Patients and setting

One hundred and sixty patients with Candidiasis (80 each, with and without H.I.V. infection) were included in this study. Cases were enrolled from the outpatient department (O.P.D.), In-patient wards, Anti-Retroviral Treatment clinic, and I.C.T.C. of the Department of Microbiology, J.N.M.C.H., Aligarh Muslim University. A complete physical examination and clinical history were elicited to know any similar disease episodes, use of antifungal drugs, and past medical history. In addition, specimens for mycological investigations were collected based on the system involved, including blood, oral and vaginal swabs, esophageal biopsy, skin scrapping, and feces.

The serostatus (H.I.V.) of the patients was screened and confirmed at H.I.V. Testing Laboratory (I.C.T.C.) as per the recommendation of the National AIDS Control Organization (N.A.C.O.) (Guidelines for H.I.V. testing, March 2018). All tests were performed manually according to the manufacturer's recommendations. Flow-Cytometry was used for the estimation of the absolute CD4 cell counts of the patients.

Fungal culture and species identification

One Hundred and sixty *Candida* isolates (80 isolates each from H.I.V. and Non-HIV infected patients) were characterized. Identification of yeast isolates was made by standard procedures including morphology on CHROMagar, Cornmeal agar, and Staib agar (HiMedia® Laboratories Pvt. Ltd, India), germ tube formation, fermentation and assimilation of sugars and growth at 42°C [8].

In vitro antifungal susceptibility testing

The determination of the M.I.C. (minimum inhibitory concentration) was done using a broth microdilution test as per the C.L.S.I. guidelines (Clinical and Laboratory Standard Institute) M27-A3 and M27-S4). The following antifungal agents were used- amphotericin B, nystatin, ketoconazole, fluconazole, itraconazole, and voriconazole (HiMedia® Laboratories Pvt. Ltd, India)[9,10]. Aliquots of 100 microliters of each antifungal drug at a double concentration of the final concentration were put in the wells of 96-well microtiter plates. Before testing, fungal strains were subcultured and incubated at 30°C for 24 hours. Next, 100 µl of the inoculum was added to each microdilution well containing 100 µl of the serial dilution of the antifungal agents to reach the final concentration; the plates were then incubated at 35°C for 48 hours. The minimum inhibitory concentrations (M.I.C.s) were calculated after 48 hours. American Type Culture Collection (A.T.C.C.) strains recommended by C.L.S.I., *C. albicans* (ATCC

64550), *C. parapsilosis* (ATCC 22019), and *C. krusei* (ATCC 6258) were used as controls.

RESULTS

Demographically, both the groups, ie. H.I.V. Negative and Positive patients presenting with Candidiasis were comparable in terms of age and sex. The HIV-positive patients with Candidiasis constituted 47 (59%) males and 33 (41%) females with a mean age of 32 years. The HIV-negative patients with Candidiasis constituted 41 (51%) males and 39 (49%) females with a mean age of 29 years (Table-1). Heterosexual contact was the most common mode of the transmission of H.I.V. infection (Table-2)

Most of the patients with Candidiasis in the H.I.V. positive group presented with oral Candidiasis (51 patients), followed by vulvovaginal Candidiasis, Cutaneous Candidiasis, oesophageal Candidiasis, candidal diarrhea, and candidemia. In the HIV-negative group, 31 patients presented with oral Candidiasis, and 28 patients were diagnosed with vulvovaginal Candidiasis. In addition, five patients were confirmed with candidemia and were of the pediatric age group (Table 3).

In H.I.V. Negative patients, *Candida albicans* was the commonest species isolated (85 %), followed by *Candida guilliermondi* in 5%, *Candida parapsilosis* in 2.5 %, *Candida tropicalis* in 3.7 %, *Candida krusei* in 2.5 %, and *Candida dubliniensis* 1.2 % [Table-4].

In HIV-positive patients, *Candida albicans* was isolated from 61.2 %, followed by *Candida guilliermondi* (16.2 %), *Candida parapsilosis* (7.5%) *Candida tropicalis* (5 %), *Candida krusei* (5%), and *Candida dubliniensis* (5 %).

Among HIV infected patients fluconazole resistance was 16.25%, ketoconazole 13.5%, clotrimazole 12.5%, itraconazole 6.25 %. Higher M.I.C. against the azoles was also recorded in

Table 1. Demographic Profile of Study Groups

		HIV Negative (n=80)	HIV Positive (n=80)
Sex	Male	41	47
	Female	39	33
Age (Years)	Mean (±SD)	29 (±9.31)	32 (±11.26)
Marital status	Married	52	39
	Unmarried	41	28
Social background	Rural	51	35
	Urban	42	32
Occupation	Farmer	20	19
	Driver	00	04
	Laborer	10	02
	Business	24	11
	Army/police	02	00
	Others	22	46

Table 2. Risk profile (mode of transmission of H.I.V. Infection) of H.I.V. positive patients with Candidiasis

Mode of H.I.V. infection	HIV positive (n=80)	HIV Negative (n=80)
Sexual	60	-
Blood and products	7	-
Injection drug user	2	-
Not specified	11	-

Table 3. Distribution of Various presentations of Candidiasis in H.I.V. Positive and H.I.V. Negative patients

Disease	HIV positive (n=80)	HIV Negative (n=80)
Oral Candidiasis only	51	31
Vulvovaginal candidiasis	10	28
Cutaneous Candidiasis	07	14
Esophageal Candidiasis	05	01
Candida diarrhea	04	01
Candidemia	03	05

Table 4. Distribution of various species of Candida isolated from H.I.V. positive and H.I.V. Negative Patients

Candida Species	HIV Positive (%)	HIV Negative (%)
Candida albicans	49 (61.2)	68 (85)
Candida guilliermondi	13 (16.2)	4 (5)
Candida parapsilosi	6 (7.5)	2 (2.5)
Candida tropicalis	4 (5)	3 (3.7)
Candida krusei	4 (5)	2 (2.5)
Candida dubliniensis	4 (5)	1 (1.2)

many isolates rendering them intermediately sensitive. A significantly higher number of non-*albicans Candida* isolates were resistant to azoles (6 out of 31). In H.I.V. Negative group fluconazole resistance was found in 8.75% and itraconazole 1.25%. [Table-5].

Table 5. Sensitivity profile of isolated candida species to various antifungal agents

Antifungal Resistance	HIV Positive (%)	HIV Negative (%)
Fluconazole	13 (16.25)	7 (8.75)
Ketoconazole	11 (13.5)	6 (7.5)
Clotrimazole	10 (12.5)	6 (7.5)
Itraconazole	5 (6.25)	1 (1.25)
Amphotericin B	0 (0)	0 (0)
Nystatin	2 (2.5)	2 (2.5)

DISCUSSION

In this study, oropharyngeal Candidiasis was the most common presentation in HIV-positive (63.7%) and HIV-negative patients (38.7%), similar to other studies' results. Khedri et al[1] reported Oropharyngeal Candidiasis as the most common mucocutaneous infection in HIV-positive individuals with an incidence of 59.3%. Shilpa et al. et al.[11] documented Candidiasis in 49 % of HIV-positive patients, Nagalingeswaran K et al[12] in 70 %, A. Singh et al[13] in 65 % and Anupriya Wadhwa et al[14] reported Candidiasis in 50% HIV-positive patients. Other studies have documented the prevalence of Candidiasis in 23 to 27% of patients co-infected with H.I.V.[15,16].

In 80 cases of Candidiasis who were H.I.V. positive, *Candida albicans* were isolated from 49 (61.2%) while non- *candida albicans*, including *C. guilliermondi, C. tropicalis, C. parapsilosis, C. dubliniensis,* were isolated from 31 (38.7%) patients. Khedri et al.[1] reported *C. albicans* in 52.9% HIV-positive cases with Oropharyngeal Candidiasis. Even Though *Candida albicans* persists as a significant causative species, the frequency of non-albicans species of *Candida* has modestly risen. Oral Candidiasis in HIV/AIDS patients caused by non-*albicans Candida* is well documented[14,15,17]. A study by Ismail H Sahand et al.[17] on the presence of *Candida* in oral swabs of HIV-infected individuals reports the isolation rate of *candida albicans* from 52% of patients and non-albicans *Candida* from the 48%[14,17] reported that 40% of all *Candida* isolates to be Non-*albicans*[14].

Sixteen Percent of the *candida* species isolated showed resistance to fluconazole, similar to a study that reported fluconazole resistance in 21% isolates[18]. Fluconazole resistance is mainly due to past fluconazole (azoles), specifically multiple and long-term use[19]. Extended-term and repeated antifungals are sometimes required in AIDS patients and are at greater risk of developing an infection with resistant strains. Additionally, resistance in *C. albicans* is associated with a steady increase in non-*albicans* species as a causative agent of resistant mucosal Candidiasis, especially in patients with high immunosuppression[12]. Fluconazole resistance was relatively lower (8.7%) in the patients without H.I.V. infection, and maybe this is because of Non-recurrent Candidiasis and minimal prior exposure to antifungal drugs. The development of resistance to antifungal agents, especially to azoles in the *candida albicans* and non-*albicans* species, is an area of concern. This species can expand its resistance repertoire with the development of stable resistance to Azoles, including fluconazole. It is well documented that cross-resistance happens between the antifungal drugs[2], and when these resistant strains infect a majority of patients, this will limit our choices for treating Candidiasis in such patients.

Although enough literature has accumulated in the last years regarding the prevalence of vaginal colonization and vulvovaginal Candidiasis in HIV-Negative and HIV-Positive women, the deficit in our knowledge remains, mainly related to the pathophysiology of the disease, as the frequency of vulvovaginal Candidiasis does not increase with the H.I.V.

positivity status[13]. In our study, 28 females who were H.I.V. negative were diagnosed with *candida* vaginitis as compared to 10 females with H.I.V. coinfection, the frequency of vulvovaginal Candidiasis in H.I.V. positive patients is less as compared to the H.I.V. negative group as other forms of Candidiasis develop, present, and get diagnosed earlier[13].

As the number of patients with H.I.V. infection continues to increase in number due to the increase in survival rates, the problem of opportunistic infection also grows in parallel. A few years back, C. albicans was the significant species implicated in Candidiasis in immunocompromised conditions; although it still is, the incidence of Non-*albicans Candida* and its rise is a matter of concern. Therefore, for the appropriate management of *Candida* infections, antifungal susceptibility has become an essential tool, especially in the present scenario of increasing resistance.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors designed the experiments. PAK. performed the experiments. PAK and NF, analysed the data. PAK and NF wrote the manuscript. HMK gave his invaluable inputs at all the stages of this work. AA helped in proof-reading and editing the manuscript. MAK, SS did the technical work and helped in editing the manuscript. All authors read and approved the manuscript.

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DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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