Original Article

Clinico-mycological analysis and antifungal resistance pattern in human immunodeficiency virus-associated candidiasis: An Indian perspective

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Abstract

Objectives: Candidiasis is a common human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome-associated opportunistic mycoses. The present study ascertained the species spectrum of *Candida* strains recovered from different clinical samples from symptomatic HIV-positive individuals and determined the antifungal susceptibility profile of the isolates. **Materials and Methods:** A variety of specimens were collected from 234 symptomatic HIV seropositive individuals depending on their clinical manifestations and subjected to direct microscopic examination. Blood samples were inoculated in biphasic blood culture medium and all other specimens on Sabouraud dextrose agar with chloramphenicol and incubated at 35°C–37°C. Species identification of the recovered *Candida* isolates was attempted on the basis of germ tube production, micromorphology on corn meal agar, color and morphology on HiCrome Candida Differential agar, and carbohydrate fermentation and assimilation tests. Susceptibility testing of the isolates was performed employing the VITEK 2 system. **Results:** A total of 167 *Candida* isolates were obtained; *Candida albicans* (136), *Candida tropicalis* (13), *Candida krusei* (8), *Candida parapsilosis* (5), *Candida glabrata* (4), and *Candida kefyr* (1). Fluconazole resistance was more frequent among nonalbicans species, and significantly higher 5-fluorocytosine resistance compared to *C. albicans* was also observed. Eight *Candida* strains (six *C. krusei*, one *C. kefyr,* and one *C. albicans*) were multidrug resistant. **Conclusion:** Although *C. albicans* continues to be the leading etiological agent of candidiasis, the incidence of nonalbicans species among HIV-positive Indian individuals is rising. Antifungal resistance was higher among nonalbicans *Candida* species. Another issue of therapeutic concern is the possible emergence of multidrug-resistant *Candida* strains among these patients.

Key words: *Candida*, candidiasis, drug resistance, human immunodeficiency virus/acquired immunodeficiency syndrome

INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) caused by human immunodeficiency virus (HIV) is a major public health challenge of the present century. The virus selectively destroys the CD4 population of T cells, the declining numbers of which herald the onset of a spectrum of opportunistic infections (OIs).[1] These OIs are a major cause of mortality and morbidity among the HIV infected.[2] Tuberculosis is the most common

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OI seen in HIV/AIDS patients in India, followed by candidiasis.[3]

Candida species are the most common cause of fungal infections, with the spectrum of manifestations ranging from asymptomatic colonization to severe clinical forms. They can cause a panorama of clinical infections including the protean manifestations of oropharyngeal candidiasis, esophageal candidiasis, vulvovaginal disease, cutaneous candidiasis, and systemic or invasive forms such as candidemia.[4,5] A state of cell-mediated immunodeficiency and T-cell dysfunction as seen in HIV infection is pivotal in creating a state of host susceptibility to *Candida* infections.[6] HIV/AIDS patients show increased rates of colonization by *Candida* species compared to HIV-negative individuals.[7,8] Oropharyngeal colonization by *Candida* species is seen in nearly 90% of HIV/AIDS patients at some point during the course of HIV disease, and a CD4 count <200 cells/μl is associated with a significantly higher frequency of *Candida* species colonization as well as subsequent development of oropharyngeal candidiasis.[8-11] This implies that a timely diagnosis of this condition, that is often the sentinel event indicative of progression to AIDS, and the mere initiation of highly active antiretroviral therapy in these patients could obviate the need for specific antifungal treatment.[12]

A gradual change in the distribution of *Candida* species causating candidiasis has been noted in recent years, with several nonalbicans *Candida* species being implicated with a greater frequency.[13,14] Furthermore, the common practice of treating mucosal and deep-seated forms of candidiasis with fluconazole, as well as the extensive chemoprophylactic use of azole antifungals in HIV-infected patients, has led to an increase in the number of *Candida* isolates that are azole-resistant.[15] There have been previous published reports of azole-resistant isolates of *Candida albicans* being recovered with increasing frequency in HIV/AIDS patients.[16] In addition, nonalbicans *Candida* species such as *Candida krusei* and *Candida glabrata* that exhibit intrinsic reduced susceptibility to the commonly used azole antifungals are isolated in high frequency among HIV-positive patients.[17,18]

Mycological cultures and antifungal susceptibility testing of *Candida* species are not routinely performed in Indian laboratories. Further, there is a paucity of literature on the yield and diversity of *Candida* species recovered from sites other than oropharynx in HIV-positive individuals. The present study was, therefore, designed to define the epidemiology of *Candida* infection in HIV/AIDS patients including its correlation with CD4 counts; to identify and characterize the species of *Candida* isolates and ascertain their distribution in various specimen types; and to determine the *in vitro* susceptibility profile of the prevalent isolates to the four common antifungal agents-fluconazole, voriconazole, amphotericin-B, and 5-fluorocytosine.

MATERIALS AND METHODS

Study setting and design

The present descriptive, cross-sectional study was conducted prospectively over a period of 3 years from October 2008 to September 2011, at the Department of Microbiology of a tertiary care health center in New Delhi, India. A total of 234 symptomatic HIV seropositive patients with varied clinical manifestations and presenting to the antiretroviral center or admitted in the ward were recruited for the study.

Enrollment and evaluation of patients

Before enrollment and specimen collection, a written informed consent was obtained from each participant which was duly explained to him in the Hindi language. HIV serostatus of the participants was confirmed employing the algorithm as described under strategy III of National AIDS Control Organization guidelines.[19] Specific, structured, and predesigned questionnaires were used to gather information regarding sociodemographic profile, risk factors, and clinical history of the patients. A detailed clinical examination was performed by the physicians, and routine laboratory investigations including chest radiographs were obtained. CD4 counts of all enrolled HIV-positive patients were measured using the fluorescence-activated cell sorting count system, Becton Dickinson, Singapore, BD. The institutional ethics committee approved the protocol for the study.

Specimen collection

Specimens were collected depending on the clinical manifestations of the patients. These included oral swabs, sputum, blood, cerebrospinal fluid, stool, urine (midstream), vaginal swabs and skin scrapings. Standard precautions and strict asepsis were followed throughout sample collection, and the specimens were immediately transported to the laboratory for further processing.

Isolation and identification of *Candida* species

An aliquot of the specimens was transferred to a clean, grease-free microscope slide with a drop of 10% potassium hydroxide, and a coverslip

was placed over it. The preparation was slightly warmed and examined under ×40 objective of the microscope for fungal elements. In addition, smears were Gram-stained and examined under oil immersion objective of the microscope for the presence of Gram-positive budding yeast cells and pseudohyphae.

Blood sample for fungal culture as collected by venipuncture was inoculated in biphasic blood culture medium (brain heart infusion agar and broth) and incubated at 37°C. All other specimens were inoculated on sabouraud dextrose agar (SDA) with chloramphenicol and incubated at 35°C–37°C for 48 h. Culture tubes were examined after 48 h of incubation and those with no growth were further incubated for a week before they were reported as sterile. Any pasty, opaque, and cream-colored colonies on SDA were identified by Gram stain. Once the Gram-stain confirmed the identification of the isolate as Gram-positive budding yeast cells, speciation of *Candida* isolates was done by their ability to produce germ tubes, micromorphology on corn meal agar with Tween 80 (Hi-Media, India), color and morphology on HiCrome Candida Differential agar (Hi-Media, India); and biochemical profile including carbohydrate fermentation and assimilation tests using yeast nitrogen base agar (Difco, Becton Dickinson, India). All tests were performed as per standard recommended procedures.[20,21] The following strains were used as controls: *C. albicans* ATCC90028, *Candida parapsilosis* ATCC22019, *C. krusei* ATCC6258, *C. glabrata* ATCC90030, and *Candida tropicalis* ATCC750.

In vitro antifungal susceptibility testing

Antifungal susceptibility testing of isolates was performed employing the VITEK 2 system. For each strain, a standardized suspension was prepared in sterile saline and its turbidity adjusted to a 2.0 McFarland standard. Each inoculum was placed in a VITEK 2 cassette along with a sterile polystyrene test tube and an antifungal susceptibility test card. The cassettes were then loaded into the VITEK 2 instrument, and dilution of yeast suspensions, as well as filling, incubation, and reading of cards was performed automatically by the instrument. With each run of cards, appropriate quality control strains were also included in this study.

Data analysis

All the data were entered in a Microsoft Excel sheet and analyzed at a 95% confidence interval using the Epi-info software, version 3.5.3, CDC, Atlanta, GA, USA. Quantitative data were summarized as arithmetic mean and standard deviation for central tendencies and median for nonnormal/skewed distributions. Categorical variables were expressed in proportions and percentages. Chi-square and Fisher's exact test were applied to analyze the differences between proportions. A two-tailed P value < 0.05 was considered as statistically significant.

RESULTS

A summary of the sociodemographic and immunological profile of study individuals is depicted in Table 1. The various clinical manifestations seen in these patients were as follows:

Table 1: Demographic characteristics and CD4 profile of study population (*n*=234)

Variable	Values
Age (years)	
Range	13-68
Mean age	34.09±9.58
Median age	32
CD4 count (cells/μl)	
Range	16-1033
Mean	237.05±165.06
Median	209
Variable	***n* (%)**
Age group (years)	
≤20	10 (4.3)
21-30	92 (39.3)
31-40	85 (36.3)
41-50	35 (15)
≥51	12 (5.1)
Gender	
Male	158 (67.5)
Female	70 (29.9)
Transgender	6 (2.6)
Marital status	
Married	192 (82.1)
Unmarried	42 (17.9)
Type of patient	
Inpatient	55 (23.5)
ART clinic	179 (76.5)
Mode of HIV transmission	
Heterosexual promiscuous	181 (77.4)
Injection drug use	23 (9.8)
Blood transfusion	11 (4.7)
Homosexual	6 (2.6)
Not known	13 (5.5)
HIV status of partner	
Positive	111 (47.4)
Negative	40 (17.1)
Not tested	83 (35.5)
CD4 count (cells/μl)	
<200	107 (45.7)
201-500	111 (47.4)
>500	16 (6.8)

HIV=Human immunodeficiency virus; ART=Antiretroviral therapy

weight loss (183; 78.2%), oral ulcers (175; 74.8%), fever (157; 67.1%), anorexia (103; 44%), cough (93; 39.7%), diarrhea (66; 28.2%), dyspnea (63; 26.9%), neck rigidity (62; 26.5%), night sweats (37; 15.8%), dysphagia (36; 15.4%), generalized lymphadenopathy (24; 10.3%), burning micturition (20; 8.5%), vaginal discharge (3; 1.3%), and cutaneous manifestations (2; 0.9%). A history of past tubercular disease or evidence of coexisting active tuberculosis was discernible in 116 (49.6%) of 234 enrolled individuals.

A total of 395 clinical samples comprising of 232 oral swabs, 71 sputa, 32 blood cultures, 21 cerebrospinal fluids, 21 stool samples, 13 urine samples, three vaginal swabs and two skin scrapings were processed in the present study. *Candida* species were isolated in 137 (58.5%) patients, and a total of 167 isolates of *Candida* species were obtained. The specimen-wise yield of *Candida* species was as follows: isolation rate was highest with oral swabs (55.2%; 128/232), followed by sputum (38%; 27/71), vaginal discharge (33.3%; 1/3), urine (23.1%; 3/13), blood (15.6%; 5/32), stool (9.5%; 2 of 21) and cerebrospinal fluid (4.8%; 1 of 21). None of the two skin scrapings yielded any *Candida* growth.

Six different *Candida* species were identified, of which *C. albicans* (136/167; 81.4%) was the most prevalent species isolated, while nonalbicans *Candida* species (31) constituted 18.6% of the isolates. The nonalbicans *Candida* species recovered included *C. tropicalis* (13), *C. krusei* (8), *C. parapsilosis* (5), *C. glabrata* (4), and *Candida kefyr* (1). The distribution of *Candida* species in different specimen types is shown in Table 2.

The majority (68 of 137; 49.6%) of HIV-positive patients with a positive culture for *Candida* species had CD4 counts between 201 and 500 cells/μl; 59 (43.1%) had CD4 counts below 200 cells/μl and 10 (7.3%) had CD4 counts more than 500 cells/μl. Further, the differential prevalence of *Candida* species isolation did not vary significantly across different CD4 categories ($P = 0.62$).

A graphical representation comparing susceptibility profile of *C. albicans* and nonalbicans *Candida* strains to the four antifungals is provided in Figure 1. It was observed that fluconazole resistance was significantly higher among nonalbicans *Candida* species versus *C. albicans* (29% vs. 0.7%; $P < 0.0001$). Likewise, significant differences were also observed between nonalbicans *Candida* species and *C. albicans* with regard to resistance to 5-fluorocytosine (6.5% vs. 0%; $P = 0.03$). The species-wise susceptibility profile of *Candida* strains to all the four antifungals is summarized in Table 3.

Considering an intermediate susceptibility and susceptible dose dependence to a given antifungal agent also as resistance, 4 (2.4%), 6 (3.6%), and 2 (1.2%) *Candida* strains were found to be resistant to one, two, and three of the antifungal agents, respectively. Eight strains of *Candida* (six *C. krusei*, one *C. kefyr,* and one *C. albicans*) were found to be multidrug resistant, that is, resistant to two or more antifungals.

DISCUSSION

Prompt and rapid diagnosis of candidiasis is essential. Past few years have witnessed a rising number of reports of *Candida* infections from the Indian subcontinent. High isolation rates of *Candida* species have been reported in HIV-infected individuals with oral ulceration, which is in agreement with our study.[22,23] In addition, similar to previous reports, *C. albicans* was the predominant *Candida* species isolated from oral cavity of HIV-positive patients.[24,25] However, recent literature has highlighted a shift in the distribution profile of *Candida* species and the emerging role of nonalbicans *Candida* species in the causation of oral candidiasis. Kwamin *et al*. have documented nearly 30.5% of *Candida* isolates recovered from HIV-positive patients with oropharyngeal candidiasis to be nonalbicans *Candida* species.[23] We also found 15.6% (20/128) of *Candida* isolates from oral cavities of HIV-positive individuals to be nonalbicans species. With regard to species distribution, Maninder and

Table 2: Distribution of various *Candida* species (*n*=167) in different specimen types

Candida species	Clinical specimen							
	Oral swab	Sputum	Blood	Stool	Urine	Cerebrospinal fluid	Vaginal swab	Skin scrapings
C. albicans (*n*=136)	108	21	3	1	2	1	0	0
C. tropicalis (*n*=13)	8	3	1	0	1	0	0	0
C. krusei (*n*=8)	4	1	1	1	0	0	1	0
C. parapsilosis (*n*=5)	4	1	0	0	0	0	0	0
C. glabrata (*n*=4)	3	1	0	0	0	0	0	0
C. kefyr (*n*=1)	1	0	0	0	0	0	0	0

C. albicans=Candida albicans; *C. tropicalis=Candida tropicalis*; *C. krusei=Candida krusei*; *C. parapsilosis=Candida parapsilosis*; *C. glabrata=Candida glabrata*; *C. kefyr=Candida kefyr*

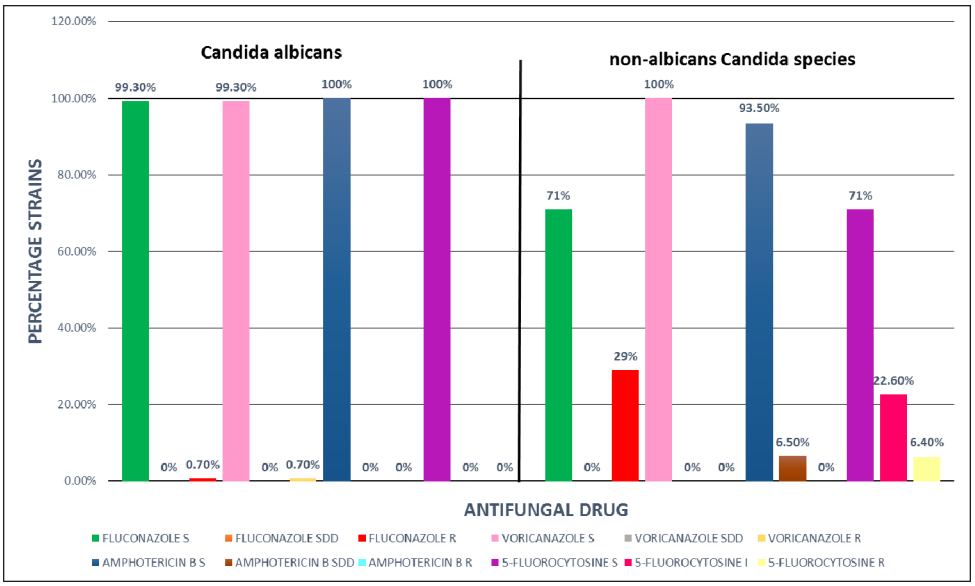

Figure 1: Susceptibility of albicans versus nonalbicans *Candida* species to various antifungal agents

Table 3: Antifungal susceptibility profile of various *Candida* species (*n*=167)

Candida species	Fluconazole			Voriconazole			Amphotericin B			5-fluorocytosine		
	Susceptible	SDD	Resistant	Susceptible	SDD	Resistant	Susceptible	SDD	Resistant	Susceptible	Intermediate	Resistant
C. albicans (*n*=136)	135 (99.3)	0 (0)	1 (0.7)	135 (99.3)	0 (0)	1 (0.7)	136 (100)	0 (0)	0 (0)	136 (100)	0 (0)	0 (0)
C. tropicalis (*n*=13)	13 (100)	0 (0)	0 (0)	13 (100)	0 (0)	0 (0)	13 (100)	0 (0)	0 (0)	13 (100)	0 (0)	0 (0)
C. krusei (*n*=8)	0 (0)	0 (0)	8 (100)	8 (100)	0 (0)	0 (0)	6 (75)	2 (25)	0 (0)	2 (25)	6 (75)	0 (0)
C. parapsilosis (*n*=5)	5 (100)	0 (0)	0 (0)	5 (100)	0 (0)	0 (0)	5 (100)	0 (0)	0 (0)	5 (100)	0 (0)	0 (0)
C. glabrata (*n*=4)	4 (100)	0 (0)	0 (0)	4 (100)	0 (0)	0 (0)	4 (100)	0 (0)	0 (0)	2 (50)	0 (0)	2 (50)
C. kefyr (*n*=1)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)
Total (*n*=167)	157 (94)	0 (0)	10 (6)	166 (99.4)	0 (0)	1 (0.6)	165 (98.8)	2 (1.2)	0 (0)	158 (94.6)	7 (4.2)	2 (1.2)

SDD=Susceptible dose dependent; *C. albicans=Candida albicans*; *C. tropicalis=Candida tropicalis*; *C. krusei=Candida krusei*; *C. parapsilosis=Candida parapsilosis*; *C. glabrata=Candida glabrata*; *C. kefyr=Candida kefyr*

Usha, in their study on oropharyngeal candidiasis in HIV-infected patients, found *C. tropicalis* and *C. krusei* to be the commonest nonalbicans *Candida* species isolated.[26] We also observed *C. tropicalis* to be the leading nonalbicans *Candida* species isolated from oral swabs of HIV-infected individuals. Furthermore, several studies have attempted to analyze the association between candidiasis and CD4 counts. Maurya *et al*. reported a strong correlation between *Candida* colonization/candidiasis of the oral cavity and CD4 counts <200 cells/μl.[8] On the other hand, similar to our report, Costa *et al*. reveal contradictory findings with no significant correlation noted between CD4 cell counts and *Candida* isolation rates.[27]

In our study, recovery rate of *Candida* was highest with oral swabs, followed by sputum and vaginal swabs. The yield was lowest with cerebrospinal fluid samples and skin scrapings. While the highest yield of *Candida* species seen with oral swabs is consistent with other studies, blood cultures have generally yielded the lowest number of *Candida* isolates in HIV-positive patients.[11,28] A comparative summary of specimen-wise *Candida* species prevalence profile (other than those from oral swabs) in our study versus similar reports from India and the Western world is summarized in Table 4.

Fluconazole is the most widely employed drug for the management of candidiasis. We report 94% of *Candida* strains isolated in our study to be susceptible and 6% to exhibit *in vitro* resistance to fluconazole. Similar figures were reported by Lattif *et al*. in their study on Indian HIV/AIDS patients with oropharyngeal lesions.[22] A plausible hypothesis for the low fluconazole resistance rates as documented in our study is that it was only a

Table 4: Studies undertaken on human immunodeficiency virus-associated candidiasis in recent years

Study group	Study site	Study duration	Sample size	*Candida* species isolated
			Sputum	
Present study	New Delhi, India	October 2008 to September 2011	71 sputum samples	21 *C. albicans* 3 *C. tropicalis* 1 *C. krusei* 1 *C. parapsilosis* 1 *C. glabrata*
Ogba *et al.*[29]	Calabar, Nigeria	May 2009 to July 2010	272 sputum samples from HIV positive subjects	32 *C. albicans* 5 *C. tropicalis* 2 *C. dubliniensis* 1 *C. guilliermondii*
Bharathi and Rani[30]	Visakhapatnam, India	June-July 2010	100 sputum samples from HIV reactive patients	26 *C. albicans* and 29 nonalbicans *Candida* species
Shailaja *et al.*[31]	Hyderabad, India	2004 (published)	100 sputa from HIV reactive patients	6 *C. albicans* (pathogenic) 18 nonalbicans *Candida* species (colonizers)
			Blood	
Present study	New Delhi, India	October 2008 to September 2011	32 blood cultures	3 *C. albicans* 1 *C. tropicalis* 1 *C. krusei*
Anwar *et al.*[11]	Aligarh, Uttar Pradesh	2010-2011	Total samples tested not specified	1 *C. albicans* 2 *C. tropicalis*
			Stool	
Present study	New Delhi, India	October 2008 to September 2011	Stool samples from 21 HIV-reactive patients with diarrhea	1 *C. albicans* 1 *C. krusei*
Uppal *et al.*[32]	New Delhi, India	April-July 2007	50 stool samples each from HIV seropositive cases with and without diarrhea	*Candida* species isolated in 18 cases with diarrhea and 6 cases without diarrhea; speciation of *Candida* isolates not attempted
Lehman *et al.*[33]	Cameroon, Africa	January-December 2011	37 stool samples from HIV seropositive patients with diarrhea and 164 stool samples from those without diarrhea	*Candida* species isolated in 7 cases with and 23 cases without diarrhea; speciation of *Candida* isolates not attempted
			Urine	
Present study	New Delhi, India	October 2008 to September 2011	Urine samples from 13 HIV seropositive symptomatic patients	2 *C. albicans* 1 *C. tropicalis*
Pignato *et al.*[34]	Sicily, Italy	2009 (published)	Urine samples from 46 HIV seropositive asymptomatic subjects	2 *C. albicans* 1 *C. glabrata*
Esebelahie *et al.*[35]	Benin City, Nigeria	2013 (published)	urine samples of 100 asymptomatic HAART-naïve HIV-reactive individuals	20 *C. albicans* 2 *C. krusei* 1 *C. parapsilosis*
			urine samples of 100 asymptomatic HIV reactive individuals on HAART	7 *C. albicans* 1 *C. krusei*
			Cerebrospinal fluid	
Present study	New Delhi, India	October 2008 to September 2011	21	1 *C. albicans*
Deorukhkar and Saini[36]	Maharashtra, India	2012 (published)	16	1 *C. albicans*
			Vaginal swabs	
Present study	New Delhi, India	October 2008 to September 2011	Vaginal swabs from 3 HIV infected women	1 *C. krusei*
Oliveira *et al.*[37]	Salvador, Brazil	May 2006 to May 2007	Vaginal swabs from 64 HIV infected women	9 *C. albicans* 3 *C. parapsilosis* 3 *C. glabrata* 1 *C. dubliniensis* 1 *C. albicans* + *C. glabrata*

HIV=Human immunodeficiency virus; HAART=Highly active antiretroviral therapy; *C. albicans=Candida albicans*; *C. tropicalis=Candida tropicalis*; *C. krusei=Candida krusei*; *C. parapsilosis=Candida parapsilosis*; *C. glabrata=Candida glabrata*; *C. kefyr=Candida kefyr*; *C. dubliniensis=Candida dubliniensis*; *C. guilliermondii=Candida guilliermondii*

single time analysis. Perhaps, repeated collection and susceptibility testing of isolates from the same patient at different stages of HIV disease and at different time intervals would provide a better insight into the evolution and emergence of azole-resistant *Candida* strains in HIV seropositive individuals. Moreover, the extensive prophylactic and therapeutic use of fluconazole in developed countries versus the

developing nations is additionally responsible for the lower incidence of fluconazole refractile *Candida* strains in the latter. Reports of fluconazole-resistant *C. albicans* from HIV seropositive individuals are common.[16,38] However, we encountered only one fluconazole-resistant *C. albicans* strain in our study, a finding similar to that of Kumar *et al*., who observed none of the *C. albicans* strains isolated in their study to be resistant to fluconazole.[39] On the contrary, another study examining oral *Candida* isolates from HIV-reactive populations of South Africa and Cameroon has documented nearly half of *C. albicans* isolates collected by the researchers to be resistant to all the azole antifungals tested.[40] Among nonalbicans *Candida* species, fluconazole resistance was highest among *C. krusei*, a finding well-supported by the previous published studies.[17] In addition, while cross-resistance to voriconazole was documented in the single fluconazole resistant *C. albicans* strain recovered in our study, all the eight strains of *C. krusei* and the single strain of *C. kefyr* that we isolated exhibited 100% susceptibility to voriconazole while being fluconazole resistant. This is in contrast to a previous published paper that reports a high level of cross-resistance between azole antifungals and recommends avoiding other azole agents for the treatment of fluconazole-resistant *Candida* strains.[41]

Despite the use of polyene antifungals for nearly 50 years, amphotericin B resistance among *Candida* strains is infrequently encountered. Likewise, none of the of *C. albicans* strains in our study was amphotericin B resistant, and among the nonalbicans, *Candida* species isolated, only two strains of *C. krusei* exhibited a susceptible dose-dependent response to this antifungal. While several researchers have reported high amphotericin B susceptibility among *Candida* isolates, there are reports of emerging amphotericin B resistance published by others.[16,38,42,43]

Furthermore, all the strains of *C. albicans* in our study were susceptible to 5-fluorocytosine while 75% strains of *C. krusei* and the single strain of *C. kefyr* isolated showed intermediate susceptibility and 50% strains of *C. glabrata* were 5-fluorocytosine resistant. In a study undertaken among late presenting AIDS patients in Ethiopia, one of the 25 strains of *C. albicans,* one of the 25 strains of *C. glabrata,* and one of the 25 strains of *C. tropicalis* were found to be 5-fluorocytosine resistant.[43]

Mulu *et al*. have reported multidrug resistance in eight of the 90 *Candida* isolates tested for antifungal susceptibility in their study.[43] Likewise, the authors of the present study also report multidrug resistance, with majority of the multidrug-resistant strains being nonalbicans *Candida* species. Emergence of multidrug-resistant *Candida* strains could be a matter of grave therapeutic concern in India, more so in the immunocompromised populations.

The present study was undertaken at one of the largest tertiary care centers in North India that caters to a large and diverse population base. The study, however, is fraught with certain limitations. It was not a case–control analysis and thus, we could not compare the isolation rates, species diversity, and antifungal susceptibility profile of *Candida* strains recovered from symptomatic HIV seropositive individuals with those from healthy HIV-negative controls. Second, the sample size of the study population was small, and the study was essentially a single-center analysis. Another shortcoming was that a history or documentation of prior fluconazole exposure was not a part of our study protocol and thus, we could not describe the azole resistance in *Candida* strains as being intrinsic or acquired. Furthermore, the lack of a longitudinal follow-up study design precluded an analysis of species distribution profile and evolution of antifungal resistance among *Candida* strains with reference to correlation with HIV disease progression.

CONCLUSION

Our study has showcased the present etiological trends of candidiasis in HIV-positive individuals in an Indian setting. It points toward a gradually evolving trend of *Candida* infections whereby, *C. albicans* continues to remain the predominant *Candida* species isolated from HIV-positive individuals, but with the incidence of nonalbicans species on the rise. Our study also highlights the current resistance patterns of various *Candida* species and points toward a possible emergence of multidrug-resistant *Candida* strains among HIV-infected populations of India. This could pose an imminent therapeutic challenge in the future.

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Conflicts of interest

There are no conflicts of interest.

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Mycoplasma genitalium

M. genitalium was first identified in the early 1980s and has become recognized as a cause of male urethritis, responsible for approximately 15%–20% of nongonococcal urethritis (NGU) cases, 20%–25% of nonchlamydial NGU, and approximately 30% of persistent or recurrent urethritis.

The pathogenic role of M. genitalium is less definitive in women than it is in men. M. genitalium infections in women are commonly asymptomatic

Clinical presentation

Male	Female
Symptoms	
Often asymptomatic but associated with similar symptoms to chlamydia	Often asymptomatic
Dysuria	Vaginal discharge
Urethral discharge	Pelvic pain
Possibly Proctitis	Intermenstrual bleeding
	Post-coital bleeding
	Possibly dysuria
Complications	
Significance in Epididymo-orchitis is unknown	pelvic inflammatory disease (PID), infertility and ectopic pregnancy Possible role in tubal factor infertility

Diagnostic Considerations

M. genitalium is a slow-growing organism. Culture can take up to 6 months, Therefore, NAAT is the preferred method for M. genitalium detection.

Samples for testing: urine, urethral, vaginal, and cervical swabs and endometrial biopsies

Treatment

M. genitalium lacks a cell wall, and thus antibiotics targeting cell-wall biosynthesis (e.g., beta-lactams including penicillins and cephalosporins) are ineffective against this organism. Given the diagnostic challenges, treatment of most M. genitalium infections will occur in the context of syndromic management for urethritis, cervicitis, and PID.

Treatment Options	
Urethritis & Cervicitis	1 gram single dose Azithromycin OR Moxifloxacin 400 mg daily for 7 to 14 days
Pelvic Inflammatory Disease (Considered where patients don't respond within 7 to 10 days)	Moxifloxacin 400 mg daily for 14 days