



SPECIATION AND ANTIFUNGAL SUSCEPTIBILITY PATTERN OF CANDIDA ISOLATED FROM HIV PATIENTS

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Abstract : BACKGROUND AND OBJECTIVES - Candidiasis is a common opportunistic infection in HIV infected patients. Though *C. albicans* species is the major cause of infection, there is a gradual shift towards non *albicans* *Candida* species, particularly in patient with advanced immunosuppression. Several non *albicans* *Candida* species are inherently resistant to commonly used antifungal drugs. The objective of the present study was to speciate *Candida* isolated from HIV infected patients and to evaluate its antifungal susceptibility pattern. MATERIALS AND METHODS - From the period of October 2014 to December 2014, various samples were collected from HIV patients from ART OP with clinical feature suggestive of candidiasis. *Candida* thus isolated was speciated by germ tube test, growth at 45 degree celsius, morphology on corn meal agar and Hicrome *Candida* agar. Antifungal susceptibility was performed by disc diffusion method. RESULTS - Out of 108 HIV positive patients with clinical features suggestive of Candidal infection, *Candida* was isolated from 48 samples. *Candida albicans* (59 percentage) was the predominant species followed by *Candida tropicalis* (27 percentage). Antifungal susceptibility testing showed 36 percentage of the species were resistant to Fluconazole. CONCLUSION - Though *Candida albicans* was the major isolate, non *albicans* *Candida* species were also implicated more in number. Hence it is essential to know the changing pattern of *Candida* species and its antifungal susceptibility for appropriate antifungal therapy.

Keyword : HIV Patients, *Candida* speciation, Hicrome *Candida* agar, Non *albicans* *Candida* species, Antifungal susceptibility testing

INTRODUCTION:

Candidiasis is a common infection caused by yeast like fungus. 1 Two major medical events have revived the interest in fungal disease in general and *Candida* species in particular. The first was the introduction of antibacterial drugs and second was the increase of immunosuppressed patients due to chemotherapy or acquired immuno deficiency disease. 2 The spectrum of *Candida* infection is diverse, starting from asymptomatic colonization to pathogenic forms. 3 Though *C. albicans* species is the most common causative agent of infection, there is a gradual trend toward change in the *Candida* species with non *albicans* *Candida* being associated with HIV/AIDS. 4,5 During the course of HIV infection, the rate of *Candida* infection is inversely related to the CD4 counts, which in turn depends on

the use of Antiretroviral treatment. Eventhough the introduction of antiretroviral therapy (ART) had a major impact on the infectious complications of AIDS, candidiasis still remains a common opportunistic infection in HIV infected patients. 6 The prolonged use of drugs for oral candidiasis in HIV patients might cause the development of drug resistance candidiasis. 7 Several non *albicans* *Candida* species are inherently resistant to commonly used antifungal drugs. *Candida* species also differ in their virulence factor. 8 Thus identification of *Candida* upto species level along with antifungal susceptibility has become very essential.

AIM AND OBJECTIVES:

1. To speciate *Candida* isolated from various clinical samples of HIV patients using Hicrome *Candida* Agar.
2. To evaluate their Susceptibility to Antifungal drugs by using Glucose Methylene Blue – Mueller Hinton Agar by Disc Diffusion Method.

MATERIALS AND METHODS:

Study period was from October 2014 to December 2014. According to their clinical presentation, samples (oral swabs, vaginal swab, sputum, skin and nail scrapings) were collected from HIV patients from ART OP with clinical features suggestive of candidiasis and their CD4 count were recorded. Direct smears were prepared and Gram Staining was done to look for Gram positive budding yeast cells and pseudo hyphae. Samples were inoculated on SDA and incubated at 25o C in incubator for one week. SDA bottles were observed daily for growth. SDA with creamy pasty colonies were taken for further processing and speciated by germ tube test, growth at 45oC, morphology on corn meal agar and Hicrome *Candida* agar M1297A from HIMEDIA. The color produced in Hicrome *Candida* agar by each of the isolates of *Candida* were noted and interpreted according to manufacturer's guidelines. Antifungal Susceptibility Testing by Disc Diffusion Method was done for the isolates of *Candida* species using Glucose Methylene Blue-Mueller Hinton Agar and Himedia Hexa Antimyc- 01 containing Amphotericin B (100 units), Clotrimazole (10mcg), Fluconazole (25 mcg), Itraconazole (10 mcg), Ketoconazole (10mcg) and Nystatin (100 units).

Figure 1 : GRAM STAINING OF CANDIDA

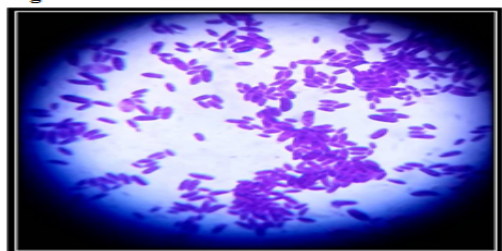


Figure 2: GERM TUBE FORMATION



Figure 3 : CHLAMYDOSPORE FORMATION ON CORN MEAL AGAR

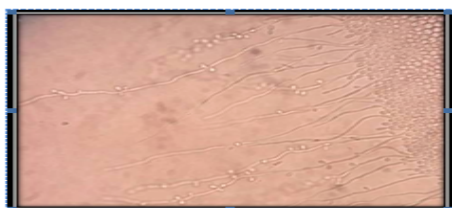
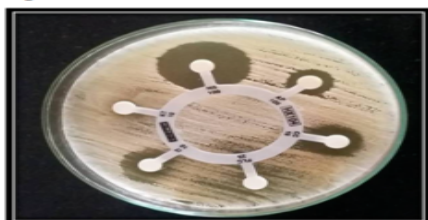


Figure 4 : SPECIATION ON MICROME CANDIDA AGAR

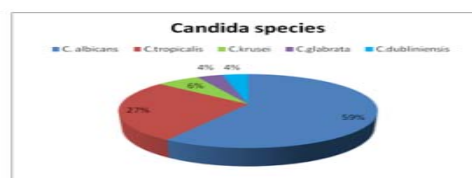


Figure 5 : ANTIFUNGAL SUSCEPTIBILITY TESTING



RESULTS:

Out of a total 108 HIV positive patients with clinical features suggestive of candidal infection, Candida was isolated from 48 samples. Most common presentation was oral candidiasis 35 (73%), followed by vaginal candidiasis 10 (21%), cutaneous candidiasis 2 (4%), pulmonary candidiasis 1 (2%). Among the 48 isolates, 26 (54%) were males and 22 (46%) were females. Among the 48 isolates 21 (44%) were in the age group of 31-45 years, 19 (40%) were in the age group of 16-30 years and 8 (16%) were in the age group of 46-60 years. With reference to immune status, 30 (63%) patients with candidiasis had CD4 count less than 200 cells/mm³ and 14 (29%) had CD4 count between 200 - 500 cells/mm³ and 4 (8%) had CD4 count more than 500 cells/mm³. The predominant species isolated was *Candida albicans* 28 (59%), followed by *Candida tropicalis* 13 (27%), *Candida krusei* 3 (6%), *Candida dubliniensis* 2 (4%) and *Candida glabrata* 2 (4%). Among the 48 isolates of *Candida*, 44 (92%) were sensitive to Nystatin, 41 (85%) isolates were sensitive to Amphotericin B, 34 (71%) were sensitive to Itraconazole, 33 (69%) isolates were sensitive to Ketoconazole, 32 (67%) were sensitive to Clotrimazole and 29 (60%) were sensitive to Fluconazole.



Sample wise distribution

| Sample (n = 108) | Candida isolated (n = 48) |
|-------------------|---------------------------|
| Oral (64) | 35 |
| Vaginal (26) | 10 |
| Skin & nails (11) | 2 |
| Sputum (7) | 1 |

Age & Sex wise distribution

| Age group | Males (n=26) | Females (n=22) |
|-----------|--------------|----------------|
| 16 - 30 | 10 | 9 |
| 31 - 45 | 11 | 10 |
| 46 - 60 | 5 | 3 |

Distribution of Candida according to CD4 count

| CD4 count | No of candida isolates |
|-----------|------------------------|
| < 200 | 30 |
| 200 - 500 | 14 |
| > 500 | 4 |

Antifungal Susceptibility Pattern

| Species | AP | | | KT | | | CC | | | NS | | | IT | | | FLC | | |
|----------------------------|----|---|---|----|---|----|----|---|----|----|---|---|----|---|----|-----|---|----|
| | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R |
| <i>C. albicans</i> (28) | 25 | 1 | 2 | 22 | 1 | 5 | 21 | 1 | 6 | 26 | 1 | 1 | 21 | 1 | 6 | 20 | 1 | 7 |
| <i>C. tropicalis</i> (13) | 11 | 1 | 1 | 9 | 1 | 3 | 8 | 1 | 4 | 12 | 0 | 1 | 9 | 0 | 4 | 8 | 1 | 4 |
| <i>C. krusei</i> (3) | 2 | 0 | 1 | 1 | 0 | 2 | 1 | 1 | 1 | 2 | 0 | 1 | 2 | 0 | 1 | 0 | 0 | 3 |
| <i>C. glabrata</i> (2) | 2 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 2 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 2 |
| <i>C. dubliniensis</i> (2) | 1 | 0 | 1 | 0 | 0 | 2 | 1 | 0 | 1 | 2 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 |
| Total (48) | 41 | 2 | 5 | 33 | 2 | 13 | 32 | 3 | 13 | 44 | 1 | 3 | 34 | 1 | 13 | 29 | 2 | 17 |

AP-Amphotericin B(100units), KT-Ketoconazole(10mcg), CC-Clotrimazole(10mcg), NS-Nystatin(100units), IT Itraconazole(10mcg), FLC-Fluconazole(25mcg). S-Sensitive, I (SDD)-Intermediate Sensitive (Susceptible-Dose Dependent), R-Resistant.

DISCUSSION:

The first step in the development of a *Candida* infection is colonization of the mucocutaneous surface. HIV infection is not only associated with increased colonization rates but also with the development of overt disease.⁹ In the present study oral candidiasis (73%) was the most common presentation. It is similar to Anwar Khan et al³ study. The study period was only for three months and samples were collected from the ART out patients. Patients with symptoms of esophageal candidiasis were referred to gastroenterology department. So prevalence of esophageal candidiasis was not calculated in this study. Male:female ratio was 1.2:1 and the most common age group affected was 30-45 years. Most of the patients from whom *Candida* was isolated possessed CD4 count < 200 cells/mm³. Candidiasis is caused by *Candida albicans* 28 (59%) and by non *albicans* *Candida* species 20 (41%). Among non *albicans* *Candida* species, *Candida tropicalis* was the major species isolated. Sachin Deorukkar et al⁵ and Anaparthi Usharani et al² have also reported *Candida tropicalis* predominance among non *albicans* *Candida* species which is similar to the present study. *Candida tropicalis* is becoming an emerging pathogen globally. In vitro susceptibility testing provides a measure of the activities of two or more drugs and monitor the development of resistance. In this study the most sensitive drug was Nystatin followed by Amphotericin B. 36% of the species were resistant to Fluconazole. Fluconazole is widely used as prophylactic drug because of its high oral bio-availability, minimum drug interaction and minimal adverse effects. Hence resistance to Fluconazole is increasing.¹⁰ Resistance to azole antifungals continues to be a significant problem in the common fungal infection like candidiasis.

CONCLUSION:

In the present study though *Candida albicans* was the major isolate, non *albicans* *Candida* species were also implicated more in number. *Candida tropicalis* was the next most common species after *Candida albicans*. The antifungal susceptibility testing showed more resistance to Fluconazole and high sensitivity to Nystatin followed by Amphotericin B. Hence periodic surveillance of fungal infection in HIV infected patient is essential to know the changing pattern of *Candida* species and its antifungal susceptibility.

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