International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

In Vitro Antifungal Susceptibility Pattern of Oropharyngeal and Oesophageal *Candida* Species in HIV Infected Patients

Poonam Chanderlal Sharma[1], Sanjaykumar Rajaram More[1*], Sharmila Sanjay Raut[1], Vimal Shriram Rathod[1]

[1]Department of Microbiology,
Dr. Shankarrao Chavan Government Medical College, Nanded- 431 601, Maharashtra, India

[*]Correspondence Email: dr_sanjaymore@yahoo.co.in

Received: 25/02//2013 *Revised: 29/03/2013* *Accepted: 15/04/2013*

ABSTRACT

Background: The widespread use of the antifungal agent fluconazole for therapy and prophylaxis in HIV infected patients has resulted in fluconazole resistant strains of *C. albicans* and an increased frequency of non-albicans *Candida* strains, especially among patients with late-stage AIDS.
Aim: To isolate, identify and to do the antifungal susceptibility testing *Candida* species (oropharyngeal and oesophageal) in HIV infected patients.
Method:Two hundred HIV positive cases were selected for this study. Identification of *Candida* species was done by conventional standard techniques and the Kirby Bauer disk diffusion method was used to test the susceptibility of *Candida* isolates.
Findings: The resistance pattern of *C. albicans* was 34.07% resistant to Fluconazole,10.99% resistant to Voriconazole, 7.69% resistant to Ketoconazole, 6.59% resistant to Itraconazole, 2.19% resistant to Clotrimazole and 1.09% resistant to Amphotericin B. While resistance pattern of Non *Candida albicans* was 16.95% resistant to Fluconazole,10.17% resistant to Voriconazole, 13.56% resistant to Ketoconazole, 13.56% resistant to Itraconazole, 13.56% resistant to Clotrimazole and 1.69% resistant to Amphotericin B.
Conclusion: Identification of *Candida* to the species level has become compulsory to assist the selection of appropriate antifungal agents in treatment of invasive candidiasis because most of the Non *Candida albicans* exhibit reduced Fluconazole susceptibility.

***Keywords**:* Fluconazole, *C. albicans*, Non *Candida albicans*

INTRODUCTION

Candidiasis is undoubtedly the most common fungal infection in HIV infected individuals. It manifests most commonly as thrush, oropharyngeal candidiasis and may also present as oesophagitis and even skin rash. [1]

C. albicans is the predominant species causing candidiasis and may be found as a sole pathogen or mixed with other non-albicans species. Some data suggest that non-albicans strains of *Candida* are less clinically relevant than strains of *C. albicans* when they are present simultaneously. The widespread use of the

antifungal agent fluconazole for therapy and prophylaxis in HIV infected patients has resulted in fluconazole resistant strains of *C. albicans* and an increased frequency of non-albicans *Candida* strains, especially among patients with late-stage AIDS. In one prospective study conducted among HIV-infected adults the estimated rate of carriage of fluconazole resistant *C. albicans* declined since the introduction of highly active antiretroviral therapy (HAART). [2]

Aims and objectives

- To isolate and identify *Candida* species (oropharyngeal and oesophageal) in HIV infected patients.
- To do the antifungal susceptibility testing of *Candida* isolates.

MATERIALS AND METHODS

Two hundred HIV positive cases with clinically suspected oropharyngeal and oesophageal candidiasis of all age group and both sexes attending ART Centre of tertiary care hospital were selected for this study. Patients were explained about the study with informed consent. Clinical details were noted in the proforma and samples were obtained.

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.

The Kirby Bauer disk diffusion method was used to test the susceptibility of Candida isolates following the Clinical and Laboratory Standards Institute (CLSI) guidelines M44-A for antifungal disk diffusion susceptibility testing of yeasts. Plates were incubated for 24 hours.

Figure 1: Antifungal sensitivity testing by Kirby Bauer disc diffusion method on Glucose methylene blue mueller hinton agar.

Procedures for performing the disk diffusion test

All identified isolates were subcultured onto Sabouraud dextrose agar to ensure purity and viability. Inoculum was prepared by picking five distinct colonies of approximately 1 mm in diameter from a 24-hours culture of *Candida* isolates. Colonies were suspended in 5 mL of sterile 0.145 mol/L saline (8.5 g/L NaCl; 0.85% saline). The resulting suspension was vortexed for 15 s and its turbidity was adjusted visually by adding sufficient sterile saline or more colonies to adjust the transmittance. Within 15 min of adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the suspension. The swab was rotated several times and pressed firmly against the inside wall of the tube above the fluid level. This helped to remove excess fluid from the swab. The dried surface of a sterile Glucose Methylene Blue Mueller-Hinton agar plate was inoculated by evenly streaking the swab over the entire agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum and the rim of the agar was swabbed. The lid was left ajar

for 3-15 min to allow for any excess surface moisture to be absorbed before applying the drug-impregnated disks. Variations in inoculum density were avoided by ensuring that the degree of turbidity level was as similar as possible.

Application of disks to inoculated agar plates

Discs applied were Fluconazole (25 µg), Voriconazole (1 µg), Amphotericin B (20 µg), Itraconazole (10 µg), Clotrimazole (10 µg) and Ketoconazole (30 µg). Antimicrobial disks were dispensed onto the surface of the inoculated agar plate with a forceps. Each disk was pressed down to ensure complete contact with the agar surface and they were distributed evenly so that they were no closer than 24 mm from center to center. Because the drug diffuses almost instantaneously, a disk was not removed once it had come into contact with the agar surface. Instead, a new disk was placed in another location on the agar. The plates were inverted and incubated at 35-37°C for 15 min after which the disks were applied.

Reading the plates and interpreting the results

Plates were examined after 20-24 hours of incubation. For satisfactorily streaked plates with the correct inoculums, the resulting zones of inhibition were uniformly circular and there was a semi confluent lawn of growth. The plate was held a few inches above a black, nonreflecting background illuminated with reflected light. The zone diameters were measured to the nearest whole millimeter at the point at which there was a prominent reduction in growth. Pinpoint micro colonies at the zone edge or large colonies within a zone were encountered frequently and these were ignored. Whenever these colonies were sub cultured and retested, identical results were usually obtained, i.e. a clear zone with micro colonies at the zone edge or large colonies within the zone. Plates were read at 48 hours only when insufficient growth was observed after 24 hours incubation.

Interpretation of the disk diffusion test results

A susceptible category implied that an infection due to the strain may be appropriately treated with the dose of antimicrobial agent recommended for that type of infection, unless otherwise contraindicated. This was obtained after measurement of the zone diameter to the nearest whole mm and compared with reference values. The susceptible-dose dependent (intermediate) category included isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates. Susceptibility was dependent on achieving the maximal possible blood level. This was obtained after measurement of the zone diameter to the nearest whole mm and compared with reference values. Resistant strains were those that were not inhibited by the usually achievable concentrations of the agent with normal dosage schedules or when zone diameters have been in a range at which clinical efficacy has not been reliable in treatment studies. This was obtained after measurement of the zone diameter to the nearest whole mm and compared with reference values.

RESULTS AND OBSERVATIONS

From 200 samples, the total number of *Candida* species isolated was 150.

Of 150 species isolated, *Candida albicans* was the most common species isolated 60.67%. Non *Candida albicans* isolates were 39.33% of which most common was *C. dublinienses* 25.33%, *C. krusei* 12.00%, *C. tropicalis* 1.33% and *C. glabrata* 0.67%. Occurrence of different species of *Candida* in isolates is shown in Table I.

Table I: Occurrence of different species of *Candida* in isolates

Candida species	Number of species	Percentage
C. albicans	91	60.67%
C. dubliniensis	38	25.33%
C. krusei	18	12%
C. tropicalis	02	01.33%
C. glabrata	01	00.67%
Total	150	100%

The resistance pattern of *C. albicans* was 34.07% resistant to Fluconazole,10.99% resistant to Voriconazole, 7.69% resistant to Ketoconazole, 6.59% resistant to Itraconazole, 2.19% resistant to Clotrimazole and 1.09% resistant to Amphotericin B. While resistance pattern of Non *Candida albicans* was 16.95% resistant to Fluconazole,10.17% resistant to Voriconazole, 13.56% resistant to Ketoconazole, 13.56% resistant to Itraconazole, 13.56% resistant to Clotrimazole and 1.69% resistant to Amphotericin B. Antifungal sensitivity pattern of *Candida* species isolated is shown in table II.

Table II: Antifungal sensitivity patterns of Candida species isolated

***Candida* species**		**Antifungal drugs tested**					
		Flu	**Vor**	**Ket**	**Itr**	**Clo**	**Amp B**
C. albicans	R	31	10	7	6	2	1
	%	34.07	10.99	07.69	06.59	02.19	01.09
C. dubliniensis	R	6	3	4	4	5	1
	%	15.79	07.89	10.53	10.53	13.16	02.63
C. krusei	R	3	2	2	2	2	0
	%	16.67	11.11	11.11	11.11	11.11	0
C. tropicalis	R	1	1	1	1	0	0
	%	50	50	50	50	0	0
C. glabrata	R	0	0	1	1	1	0
	%	0	0	100	100	100	0
Non Candida albicans	R	10	6	8	8	8	1
	%	16.95	10.17	13.56	13.56	13.56	1.69

Flu → Fluconazole
Vor → Voriconazole
Ket → Ketoconazole
Itr → Itraconazole
Clo → Clotrimazole
Amp B → Amphotericin B

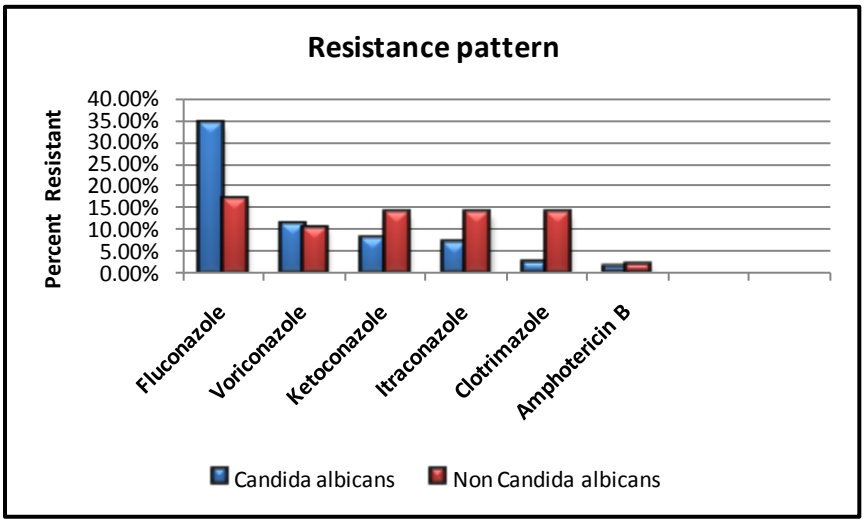

Figure 2: The resistance pattern of C. albicans and Non candida albicans.

DISCUSSION

In our study resistance of *C. albicans* to Fluconazole(34.07%) was similar to other studies by Usharani D M et al. [3] (37.57%), Deorukhkar et al. [4] (5.5%), Njunda et al. [5] (74.2%) and Nweze et al. [6] (16.7%).

In our study resistance of *C. albicans* to Voriconazole was higher (10.99%) as compared to study by Nweze et al. [6] (1.9%). In our study resistance of *C. albicans* to Ketoconazole was 7.69%, while in studies by Deorukhkar et al. [4] and Njunda et al. [5] it was 8.33% and 17.2% respectively which was similar to our study.

6.59% of the *C. albicans* isolates were resistant to Itraconazole. While 2.5%, 4.16% and 11.1% were found resistant in studies by Usharani D M et al. [3] , Deorukhkar et al. [4] and Nweze et al.[6] respectively which correlated with our study.

In our study, 2.19% of the *C. albicans* isolates were resistant to Clotrimazole, which was in accordance to study by Usharani D M et al. [3] (2.5%). While it was higher (35.6%) in the study by Njunda et al. [5] Resistance of *C. albicans* to Amphotericin B was lower (1.09%) as compared to study by Njunda et al.[5] (54.4%) and Malar S et al.[7] (11.2%).

Similarly, non *Candida albicans* has also shown an increased resistance to azoles as in other studies conducted by Usharani D M et al. [3] and Deorukhkar et al. [4]

CONCLUSION

Though *Candida albicans* continues to the common & important pathogen among the *Candida* species, there is a drastic increase in the incidence of other species like *Candida dublinienses*, *Candida krusei*, *Candida tropicalis* and *Candida glabrata*. The increasing emergence of non-*Candida albicans* seems to be associated with HIV pandemic

Identification of *Candida* to the species level has become compulsory to assist the selection of appropriate antifungal agents in treatment of invasive candidiasis because most of the Non *Candida albicans* exhibit reduced Fluconazole susceptibility. The long-term use of azoles in the prophylaxis of systemic mycoses can result in the selection of *Candida* isolates that are more resistant to azole therapy.

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 antifungal susceptibility testing in most of the tertiary care laboratories.

ACKNOWLEDGEMENT

Staff of Microbiology Department, ART centre, Study patients.

REFERENCES

1. Chander J. Text book of Medical mycology. 2nd ed. New Delhi: Mehta Offset Pvt Ltd. Naraina II, 2002. pp 212 – 31.
2. Maria CD, Joseph SS, Elias JA. Clinical mycology. 1st ed. Chapter 8. In: Candida, Dignani MC, Solomkn JS, Elias J Annaissie, eds. Philadelphia: Churchill Livingstone Publication; 2003. pp. 195-239.
3. Usharani DM, Sulochana DK , Singh LH. A study on the prevalence, pattern of antifungal susceptibility and co-relation of CD4 + T lymphocytes amongst the candida isolates from HIV patients in Manipur. National J. of Basic Medical Sciences II (4): 329-322.
4. Deorukhkar S., Katiyar R., Saini S. Species Identification and Antifungal susceptibility pattern of Candida

isolates from oropharyngeal lesions of HIV infected patients. National Journal of Integrated Research in Medicine 2012; 3(4): 86-90.
5. Njunda AL, Nsagha SD, Assob JCN, Kamga LH, Teyim P. In vitro antifungal susceptibility patterns of Candida albicans from HIV and AIDS patients attending the Nylon Health District Hospital in Douala, Cameroon. Journal of Public Health in Africa 2012; 2(2): 4-7
6. Nweze E, Ogbonnaya U. Oral candida isolates among HIV-infected subjects in Nigeria. Jour of Microbiology, Immunology and Infection. June 2011, 4(3): 172-177.
7. Malar S, Viswanathan T, Malarvizhi A, Lavanya V, Moorthy K. Isolation, characterisation and antifungal susceptibility pattern of Candida albicans and non albicans Candida from Integrated counseling and testing centre (ICTC) patients. African Journal of Microbiology Research 2012; 6(31):6039-6048.

How to cite this article: Sharma PC, More SR, Raut SS et. al. In vitro antifungal susceptibility pattern of oropharyngeal and oesophageal *Candida* species in HIV infected patients. Int J Health Sci Res. 2013;3(5):1-6.

****