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ANTIFUNGAL SUSCEPTIBILITY PATTERN OF *CANDIDA SP.* ISOLATES FROM CASES OF CANDIDIASIS

Kavitha M. K.* and Dr. Arun B.

School of Health Sciences, Kannur University, Kerala, India.

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***Corresponding Author**
Kavitha M. K.
School of Health Sciences,
Kannur University, Kerala.
India.

ABSTRACT

Candidiasis is one of the most prevalent and important opportunistic fungal infections caused by *Candida* species. Many studies state that even after introduction of new antifungal agents, drug resistance of *Candida s*p. will continue to grow and may become crucial, especially to fluconazole. The present study was conducted during a period of one year from September 2016 to September 2017 at a Tertiary care hospital in North Kerala. The clinical samples obtained were isolated and identified as different species of *Candida*. Antifungal Disk Diffusion Susceptibility Testing was performed using Mueller-Hinton Agar + 2% Glucose and 0.5 µg/mL Methylene Blue Dye (GMB) Medium – with pH between 7.2 and 7.4 and antimicrobial Disks for fluconazole, voriconazole, itraconazole and amphoterecin B. A total of 180 *Candida* isolates were obtained. 47% of the isolates were identified as *C.albicans*, 51% identified as *C.tropicalis* and 2% as *C.glabrata*. All the *C.albicans* and *C.glabrata* strains (100%)isolated were shown as sensitive to the drugs tested. 62% isolates of *C.tropicalis* were obtained as sensitive to fluconazole, voriconazole and amphoterecin B and the remaining 38% were obtained as resistant to these drugs. All the *C.tropicalis* isolates (100%) were sensitive to itraconazole. The study emphasizes on the increased occurrence and emergence of drug resistant strains of *C.tropicalis* isolates from cases of Candidiasis.

KEYWORDS: *C.albicans, C.tropicalis*, *C.glabrata,* antifungal disk diffusion susceptibility test, Mueller – Hinton agar.

INTRODUCTION

Fungi are eukaryotic organisms. Approximately 1.5 million species of fungi exist on earth. Yeasts are common on plant leaves and flowers, soil and salt water. Yeasts are also found on

the skin surfaces and in the intestinal tracts of warm-blooded animals. The common "yeast infection" is typically Candidiasis and is caused by the yeast-like fungus *Candida.* It mainly causes vaginal infections and thrush of the mouth and throat. It also causes severe disease in persons with immunosuppression.[1]

Candida is rounded and oval-shaped yeast measuring 3-30 µm in diameter. It reproduces asexually through a budding process. Candidiasis is one of the most prevalent and important opportunistic fungal infections caused by *Candida* species. The pathogenicity of *Candida* species is attributed to certain virulence factors, such as the ability to evade host defences, adherence, biofilm formation (on host tissue and on medical devices) and the production of tissue-damaging hydrolytic enzymes.[2]

Chakrabarti. A (2011)[3] and Pfaller M A (2012)[4] states that even after introduction of new antifungal agents, drug resistance will continue to grow and may become crucial. Katiraee F *et al* (2015)[5] also states resistance of *Candida sp*. to azole antifungals, especially fluconazole, has been increased.

The present study aims to find out the antifungal susceptibility pattern of *Candida* sp.isolates from candidiasis.

MATERIALS AND METHODS

The present study was conducted during a period of one year from September 2016 to September 2017 at School of Health Sciences, Kannur University. The clinical samples were obtained from the patients having the symptoms of candidiasis, attending a Tertiary care hospital in North Kerala. The clinical samples collected include nail scrapings, skin scrapings, vaginal swabs and oral swab.

In the laboratory, the samples (except vaginal swab) were first treated with a solution made from 20% potassium hydroxide (KOH) and examined directly under the low power and then high power objectives of the microscope for yeast morphology.

All the samples were then inoculated onto Sabouraud's Dextrose agar (Himedia M 063) and incubated at room temperature. Examined the colonies developed after 2-3 days and the species were identified by standard methods and HiCrome Candida Differential Agar (Himedia M1297A).[6]

Antifungal Disk Diffusion Susceptibility Testing was performed using Mueller-Hinton Agar (Himedia M173) + 2% Glucose and 0.5 µg/mL Methylene Blue Dye (GMB) Medium – with pH between 7.2 and 7.4. Antimicrobial Disks for fluconazole (FLC 25 mcg/disc), voriconazole (VRC 1 mcg/disc), itraconazole (IT 10 mcg/disc) and amphoterecin B (AP 100 units/disc) were used. The plates were inverted and placed in an incubator set to 35°C. Examined each plate after 24 hours of incubation.[7]

RESULT

During the study period, a total of 325 samples were collected. From these samples, 180 *Candida* isolates were obtained. Out of which, 84 isolates (47%) were identified as *C.albicans*, 92 isolates (51%) were identified as *C.tropicalis* and 2% identified as *C.glabrata*.

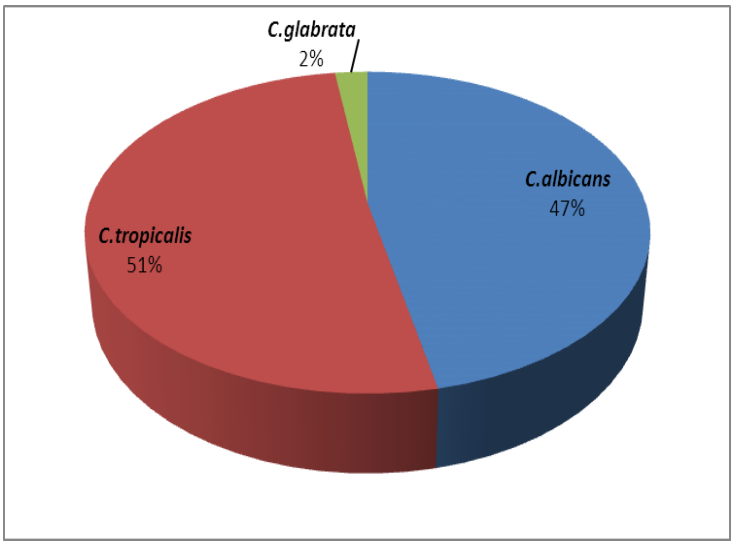

Figure. 1: Graphic representation of the species distribution of *Candida sp*. Isolates from cases of candidiasis.

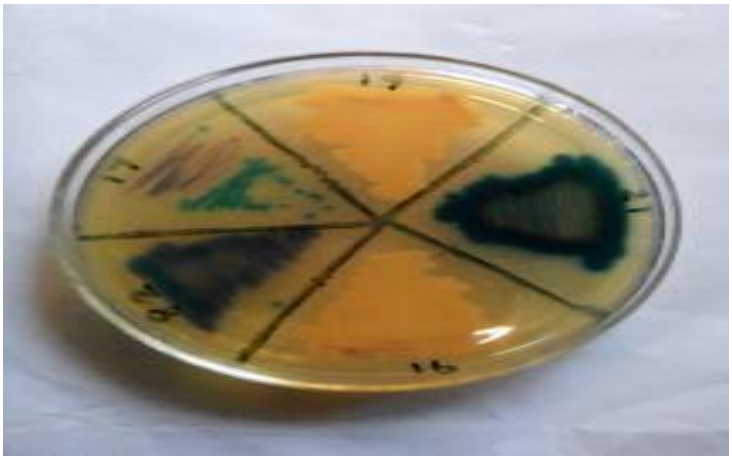

Figure 2. Growth of *Candida sp.* On Hicrome *Candida* Differential agar.

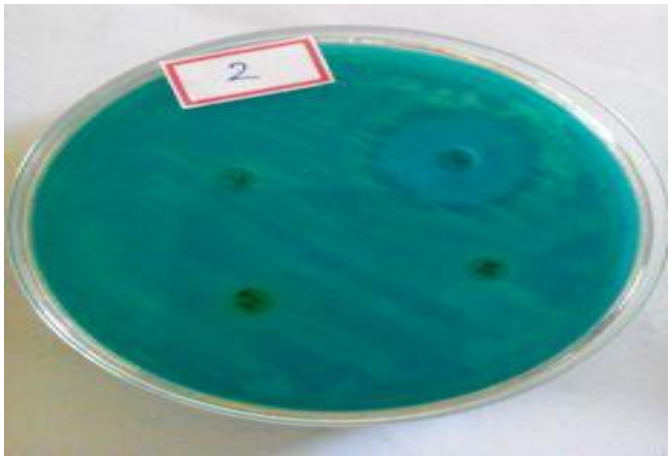

Figure 3. Antifungal sensitivity pattern shown by *C.tropicalis.*

The sensitivity pattern of all the isolates against the frequently used anti fungal drugs is shown in the Table. 1.

Table. 1. Sensitivity pattern shown by different species of *Candida* against antifungal agents.

Species isolated	Voriconazole VRC1mcg/disc		Fluconazole FLC 25 mcg/disc		Itraconazole IT 10 mcg/disc		Amphoterecin B AP 100 units/disc	
	S	R	S	R	S	R	S	R
C.albicans (84)	84	Nil	84	Nil	84	Nil	84	Nil
C.tropicalis (92)	57	35	57	35	92	Nil	57	35
C.glabrata (4)	4	Nil	4	Nil	4	Nil	4	Nil

All the *C.albicans* and *C. glabrata* strains (100%)isolated were shown sensitive to the drugs tested. 62% isolates of *C.tropicalis* were obtained as sensitive to fluconazole, voriconazole and amphoterecin B. and the remaining 38% were obtained as resistant to these drugs. All the *C.tropicalis* isolates (100%) were sensitive to itraconazole.

DISCUSSION

Out of the 180 *Candida* isolates obtained, 47% were *C.albicans*, 51% identified as *C.tropicalis* and 2% identified as *C.glabrata*. 38% of the *C.tropicalis* isolated was shown to be resistant to fluconazole, voriconazole and amphoterecin B. The rest of the isolates (62%) showed sensitivity to these drugs and all the isolates of *C. tropicalis* were sensitive to itraconazole. All the *C.albicans* and *C.glabrata (*100%) were sensitive to the drugs tested.

In this study, the occurrence of *C. tropicalis* (51%) was found to be greater than *C.albicans* (47%). Mousavi S A A *et al* (2012)[8] has reported most frequent isolate as *C.albicans* (82.2%), *C.glabrata* (7.29%), *C.parapsilosis* and *C.kefyr* (4.1%) and *C.tropicalis* (2%). But

Marinho S A *et al*. (2010)[9] has reported 13.2% as *C.tropicalis*. Shokohi T *et al*. (2010)[10] also has reported *Candida albicans* as the most common species (77.5%), followed by *C. glabrata* (15%), *C. tropicalis* (5%) *and C. krusei* (2.5%). According to Sumitra Devi L and Megha Maheshwari (2014)[11] and Kaup S *et al*. (2016)[12], the most common species isolated was *Candida albicans* (50%-52%) followed by *Candida tropicalis* (25%- 27.08%). Pahwa N *et al* (2014)[13] has reported *C. albicans* (42.2%), *C. tropicalis* (22.4%) and other non albicans *candida*. Golia S *et al* (2013)[14], Kaur R *et al*. (2016)[15] and Vaghela G M *et al* (2015)[16] has reported 35.7%, 36.7% and 38.5% isolates as *C.albicans* respectively and 26.7%, 41.1% and 18.5% respectively as *C.tropicalis*. Other non albicans *Candida* like *C.parapsilosis* (19.6% and 6.7%), *C.glabrata* (13.6% and 10%) and *C.krusei* (4.4% and 3.3%) have also been reported by them. Occurence of *C.kefyr* (2.2%) has also been reported by Kaur R *et al*. (2016)[15], but in the present study no other NAC (Non albicans *candida)* were obtained other than *C.tropicalis* and *C.glabrata*.

The overall resistance in *Candida* spp. to fluconazole and voriconazole is considered to be around 3-6% (Chakrabarti. A, 2011).[3] ElFeky D S *et al* (2016)[17] has reported 1.6% resistant to amphoterecin B, 7.9% resistant to voriconazole and 11.1% resistant to fluconazole. Roopa C and Sunilkumar Biradar (2015)[18] have reported 44.2% isolates resistant to fluconazole. In the present study, the overall resistance in *Candida sp*. is 19%.

Similar to the results found in this study, none of the *Candida albicans* isolated was found to be resistant to voriconazole by Fattouh. M *et al*, 2015,[19] whereas non albicans *candida* were resistant to voriconazole by 4.9% and to fluconazole by 2.2%, which is in contrast to the 38% resistance shown by *C.tropicalis* in this study.

C.albicans has shown resistance to fluconazole in 3.8%- 12.12% (Das P P *et al* (2016),[20] Dr. Jayalakshmi L *et al* (2014),[21] Fattouh. M *et al* (2015)[19] and Vaghela G M *et al*, (2015)[16] and also in higher frequencies 35.3% to 56.7% (Katiraee F *et al* (2015),[5] Kaur R *et al.* (2016)).[15] Whereas, the present study could not find any resistance to fluconazole by *C.albicans*. Similarly, Giri S and Anupma Jyoti Kindo (2014)[22] and Sobel J D *et al.* (2004)[23] found all the isolates of *C.albicans* as sensitive to fluconaazole. Resistance of *C. tropicalis* to fluconazole was high 68.2%- 71.4% (Dr. Jayalakshmi L *et al* (2014),[21] Kaur R *et al*. (2016)[15] but Das P P *et al* (2016)[20] reported none of the non albicans *Candida* as resistant to fluconazole.

In the present study, all the isolates of *C.albicans* and *C.glabrata* were sensitive to amphoterecin B and 38% of *C.tropicalis* was resistant to the drug. Das P P *et al* (2016)[20] and Roopa C and Sunilkumar Biradar (2015)[18] had reported all the isolates as susceptible to amphoterecin B. According to Katiraee F *et al* (2015)[5] and Vaghela G M *et al* (2015),[16] 1.7% - 1.9% of *C.albicans* was resistant to amphoterecin B. Kaur R *et al.* (2016)[15] has reported 28.6% *C.albicans* and 60% of *C.tropicalis* as resistant to amphoterecin B. *C. albicans* was found resistant to Itraconazole in 3.8% cases and NAC were found to be resistant to Itraconazole in 3.6% cases (Vaghela G M *et al* (2015),[16] whereas in the present study, none of the isolates were resistant to itraconazole.

Shyamala R and Parandekar P K (2014)[24] in their study have reported decreased susceptibility to Fluconazole and Voriconazole among NAC than *C. albicans* which is matching with the result found in our study.

CONCLUSION

It can be concluded that not only the occurrence of *C.tropicalis* in clinical condition is increased but also their resistance to various frequently used antifungal drugs like voriconazole, fluconazole and amphoterecin B. The study emphasizes on the increased occurrence and emergence of drug resistant strains of *C.tropicalis* isolates from cases of Candidiasis.

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