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INVESTIGATING THE ANTIFUNGAL SUSCEPTIBILITY PATTERN OF *CANDIDA ALBICANS* ISOLATED FROM DIFFERENT CLINICAL SAMPLES BY KIRBY-BAUER DISC DIFFUSION AND BROTH MICRODILUTION METHOD TO FLUCONAZOLE AND AMPHOTERICIN B

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ABSTRACT

Introduction: Candidiasis is a severe fungal infection caused mostly by *Candida albicans* in humans. *Candida albicans* is an opportunistic infection in immunocompromised persons that has proven lethal despite antifungal treatment. The rise in antibiotic resistance in *C. albicans* is alarming, as it is in the human microbiome.

Aim and Objective: To detect the antifungal sensitivity pattern of *Candida albicans* isolated from various clinical samples to Fluconazole and Amphotericin B using the Kirby Bauer disc diffusion method and the Broth microdilution method.

Materials and Methods: This was a cross sectional study carried out in the Department of Microbiology at a tertiary care centre, Uttar Pradesh for a period of 1 year i.e, January 2023 to January 2024. A total of 75 isolates of Candida species from different clincal specimens like blood, BAL, urine, Pus, Et secreation and vaginal secreation were evaluated for its susceptibility against Fluconazole and Amphotericin B using Kirby Bauer disc diffusion method and Broth micro dilution method according to the CLSI guidelines 2023.

Results: In the present study out of 75 isolates of Candida species 30 (40%) isolates were confirmed to be *C.albicans.* The ratio of Males 19 (63.3%) was more as compared to that of the Females 11(36.6%) with the maximum age of 31-40 being affected the most followed by 41-50 and least in the age group above 61 years of age. The number of isolates was maximum in the urine sample with

50%.
Out of 75 isolates a total of 30 isolates of *Candida albicans* were isolated. A total of 13 (43.3%) samples of Candida were sensitive and 17 (56.6%) samples were resistant to Amphotericin B and 27(90%) samples of Candida were sensitive and 3(10%) samples were resistant to Fluconazole by Kirby bauer disc diffusion method.93.3% were sensitive whereas 2 (6.6%) were resistant against Amphotericin B and 25 isolates(83.3%) were sensitive and 5 isolates (16.6%) were resistant to Fluconazole by broth microdilution method.

Conclusion:

The Antifungal susceptibility testing by broth microdilution method revealed that fluconazole was exceedingly resistant against *Candida albicans* (16.6%) and increasingly susceptible to Amphotericin B(93.3%). Antifungal susceptibility testing could be used to predict clinical response or management failure. As a result, proper antifungal drug administration should only be prioritised after susceptibility testing.

Keywords: *Candida albicans*, Antifungal Susceptibility**,** Antifungal resistance, , Disc diffusion method, Microdilution method

INTRODUCTION

Candida albicans is a yeast fungus that is normally present on the skin and mucous membranes such as oral cavity, vagina, and rectum. It can travel through the blood stream and cause infection in any part of the body. *C. albicans* is the major cause of infection in humans [1] it is also an important part of the normal microbial flora in the oral cavity, gastrointestinal tract, and vagina in healthy humans. Mediate adhesion, biofilm formation, invasion into host cells, yeast-to-hypha transition (phenotypic switching), secretion of hydrolases, contact sensing, and thigmotropism are the pathogenic potentials of *C. albicans* [2].

There are Several factors increase the incidence rate of systemic candidiasis in colonized patients such as weakened immune system, mucosal and cutaneous barrier disruption, neutrophil dysfunction (quantitative or qualitative), metabolic disorders, and advanced age [3].

Antifungal resistance is a major concern in clinical practice and becoming a major problem. Intensive and long term use of antifungal drugs lead to decline in susceptibility and resistance patterns of *Candida* species [2]. Recently, resistance to common antifungals has been reported in different *Candida* species [3]. The polyenes, azoles, echinocandins, nucleoside analogs, and allylamines are used with varying efficacy depending on the type and site of infection and the sensitivity of the *Candida* species. Azole antifungal drugs are the mainstay of management of infections with *Candida* species. The most commonly prescribed antifungal used for most *C. albicans* infections is fluconazole, a member of the azole class of antifungals [4]. There is an extensive use of fluconazole for chemoprophylaxis and treatment of fungal infections due to their favorable oral bioavailability and safety. Moreover the environmental stress with exposure to antifungal drugs can mediate resistance. With the increased incidence of Candida infections, there has also been development of resistance to antifungal agents specially the azole group [5].

Antibiotic susceptibility testing is one of the ways to determine the resistance of the organism towards the antimicrobial agents and determination of minimum inhibitory concentration is the best choice to understand the actual degree of susceptibility or resistance towards the antimicrobial agents [6]. Some species of the Candida like *C. glabrata* and *C. krusei* are intrinsically resistant to Fluconazole . It has also been reported that these Fluconazole resistant *C. albicans* strains appear to be cross-resistant to other azoles .Similarly, rare but reported case of Amphotericin B resistance of Candida has been attributed to the alterations in the cell membrane, including reduced amounts of ergosterol, and were isolated following prolonged treatment. With the increasing number of clinical isolates' resistance towards the commonly used antifungal agents, more specifically, by the production of biofilm in case of *C. albicans*, there is a growing need for antifungal susceptibility testing of the biofilm-producers which can contribute towards the pool for therapeutic approaches [7].

In this regard, the present study was undertaken to investigate the antifungal sensitivity pattern of

Candida albicans isolated from various clinical samples against Fluconazole and Amphotericin B using the Kirby Bauer disc diffusion method and the Broth microdilution method.

MATERIAL AND METHODS

This was a cross sectional study carried out in the Department of Microbiology at a tertiary care centre for a period of 1 year i.e, January 2023 to January 2024. The Ethical clearance was duly obtained from the Institutional Ethical Committee. The Demographic details and clinical history along with the relevant clinical investigations was recorded after the informed consent.

Inclusion Criteria:

Candida isolates from all clinical specimen in pure culture were included in our study

Exclusion Criteria:

Repeated isolates from same clinical specimen of same patient and isolation of Candida species from mix culture were excluded from the study.

All clinical samples were cultured on both 5% Blood agar and MacConkey agar. Gramme staining was done on all positive cultures, and those with yeast-like budding cells were subcultured on SDA and HiChrome agar to identify the species. A germ tube test was used to discriminate between Candida albicans and NACA. Chrom agar was used for further identification, sugar absorption tests were performed using commercially manufactured sugar discs sucrose, maltose, dextrose, trehalose, lactose and dulcitol from HiMedia, and micromorphology was studied on maize meal agar.

A total of 75 isolates of Candida species from different clincal specimens like blood,BAL,Urine, Pus ,Et secreation and Vaginal secreation were included in our study.

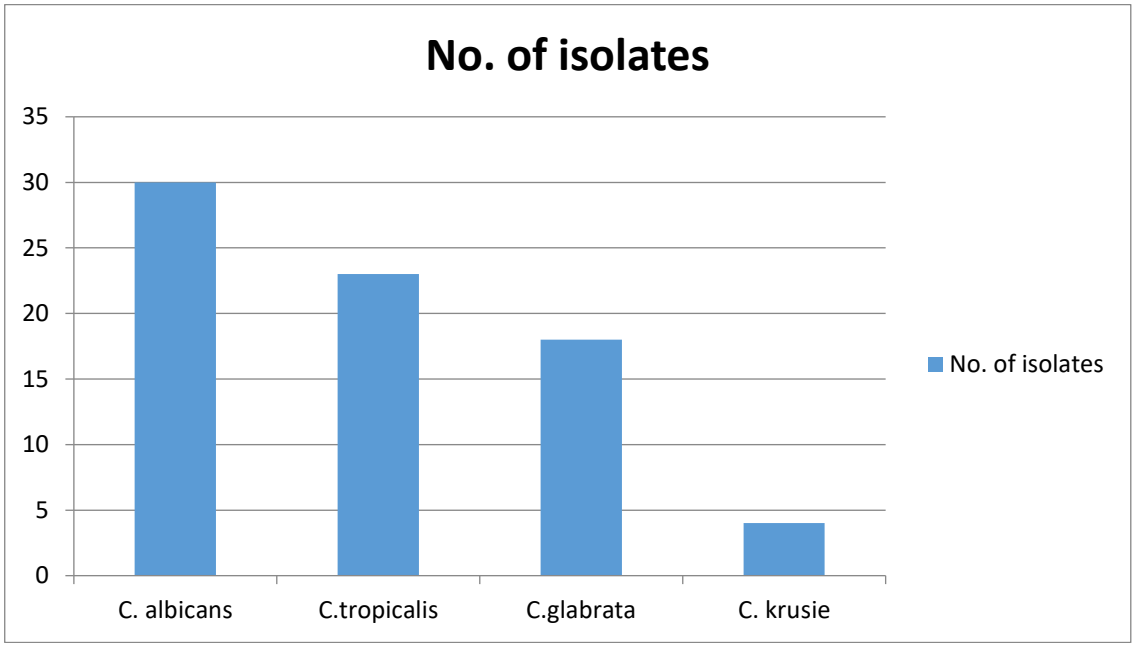
Antifungal sensitivity of Candida isolates was done by Kirby-Bauer disc diffusion method. Mueller Hinton agar supplemented with 0.2% glucose and 0.5µg/ml methylene blue dye medium (MH-GMB) was used for this purpose against azole group Fluconazole 25ug and Amphotericin B 25ug procured from Hi-media Laboratories Pvt Ltd India. The broth micro dilution method was done to determine the minimum inhibitory concentrations (MICs) according to the CLSI guidelines 2018 [7].

RESULTS

A total of 75 isolates of Candida species was included in the present study out of which 30 (40%) isolates were confirmed to be *C.albicans* followed by *C.tropicalis* with 30.6% , *C.glabrata* with 24% and least for *C. krusie* with 5.3%.

Type of Fungal isolates	**Number of Isolates**	**Percentage**
C. albicans	30	40 %
C.tropicalis	23	30.6 %
C.glabrata	18	24 %
C. krusie	4	5.3 %

Table No. 1 : The Type of Candida species isolates



Graph No. 1 : The Graphical Representation of Type of Candida species isolates

Gender	Total no. of Cases studies (N=30)	Percentage
Male	19	63.3%
Female	11	36.6%

Table No. 2 : Genderwise distribution of the *Candida albicans*

The ratio of Males 19 (63.3%) was more as compared to that of the Females 11(36.6%) [Table No. 2] with the maximum age of 31-40 being affected the most followed by 41-50 and least in the age group above 61 years of age [Table No. 3].

S.No.	Age (in years)	No. of Cases	Percentage
1.	0- 10	-	-
2.	11-20	3	10%
3.	21-30	4	13.3%
4.	31-40	11	36.6%
5.	41-50	9	30 %
6.	51-60	2	6.6 %
7.	≥61	1	3.43%

Table No.3 : Age wise distribution of *Candida albicans* patients from the study

Type of Sample	Number of Isolates	Percentage
BAL	1	3.3%
Urine	15	50%
Pus	6	20%
Et secretion	3	10%
Vaginal secretion	4	13.3%
blood	1	3.3%

Table No. 4 : Type of Sample Isolated from *Candida albicans*

The maximum number of isolates was found in the urine sample 50% followed by the pus 20% and least in the BAL 3.3% and the blood sample 3.3% [Table No. 4].

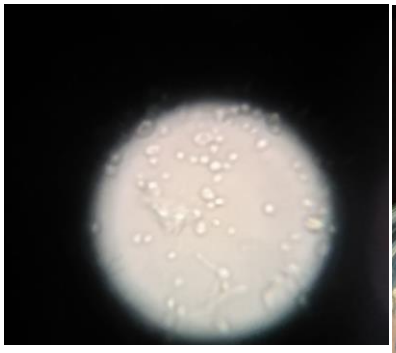


Fig No. 1:*Candida albicans* on Sabouraudextrose agar

Fig No. 2:*Candida albicans* on germ tube formation

Fig No. 3: *Candida albicans* on Hichromagar

Out of 75 isolates a total of 30 isolates of *Candida albicans* were isolated. A total of 13 (43.3%) samples of Candida were sensitive and 17 (56.6%) samples were resistant to Amphotericin B and 27(90%) samples of Candida were sensitive and 3(10%) samples were resistant to Fluconazole by Kirby bauer disc diffusion method.
93.3% were sensitive whereas 2 (6.6%) were resistant against Amphotericin B and 25 isolates(83.3%) were sensitive and 5 isolates (16.6%) were resistant to Fluconazole by broth microdilution method.

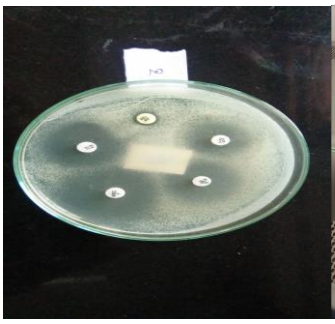


Fig. No.4: Sensitivity pattern by Kirby-Bauer disc diffusion method

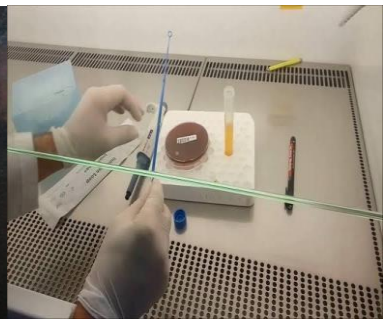


Fig No. 5 (a)

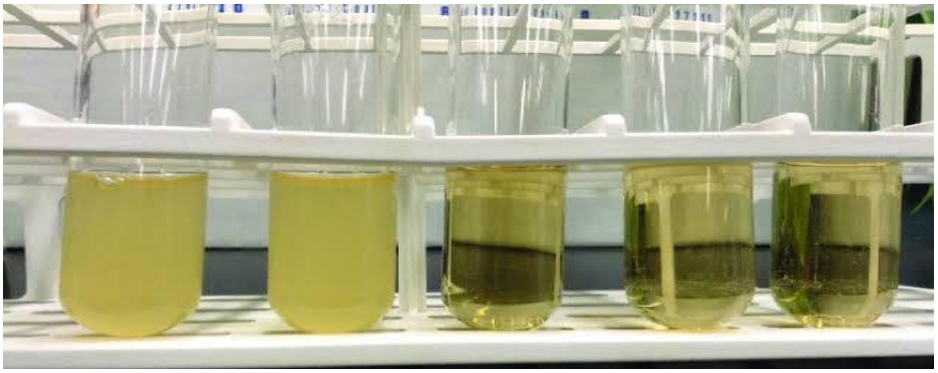


Fig No. 5 (b) : The Antifungal sensitivity pattern by broth microdilution method

Antifungal-Fluconazole	**Number of isolates N=30**	**Percentage of isolates**
Sensitive	27	90 %
Resistant	3	10 %

Table No. 5 : Antifungal Sensitivity pattern of *Candida albicans* against fluconazole by Kirby Bauer disc diffusion method according to the CLSI guidelines

A total of 27(90%) samples of Candida were sensitive and 3(10%) samples were resistant to Fluconazole by Kirby bauer disc diffusion method [Table No. 5].

Antifungal-Fluconazole	**Number of isolates N= 30**	**Percentage of isolates**
Sensitive	25	83.3%
Resistant	5	16.6%

Table No. 6: Antifungal Sensitivity pattern of *Candida albicans* by CLSI broth Microdilution method

In the Table No. 6 it was illustrated that out of 30 isolates of *C.albicans* tested for susceptibility pattern by CLSI broth microdilution method 25 isolates(83.3%) were sensitive and 5 isolates (16.6%) were resistant to Fluconazole showing Mic>/=64ug/ml.

Antifungal-AMP B	**Number of isolates N= 30**	**Percentage of isolates**
Sensitive	13	43.3 %
Resistant	17	56.6 %

Table No.7 : Antifungal Sensitivity pattern of *Candida albicans* against Amphotericin B by Kirby Bauer disc diffusion method

A total of 13 (43.3%) samples of Candida were sensitive and 17 (56.6%) samples were resistant to Amphotericin B [Table No. 7]

Antifungal-AMP B	**Number of isolates N= 30**	**Percentageof isolates**
Sensitive	28	93.3%
Resistant	2	6.6 %

Table No. 8: Antifungal Sensitivity pattern of *Candida albicans* against Amphotericin B by CLSI broth microdilution method

In the Table No. 8 it was illustrated that 93.3% were sensitive whereas, 6.6% were resistant against Amphotericin B by broth microdilution method.

Antifungal	**Kirby bauer disc diffusion Method**		**Broth microdilution method**	
	Sensitive	**Resistant**	**Sensitive**	**Resistant**
Fluconazole	27(90%)	3(10%)	25(83.3%)	5(16.6%)
Amphotericin b	13(43.3%)	17(56.6%)	28(93.3%)	2(6.6%)

Table No. 9: Antifungal Sensitivity pattern of *Candida albicans* against Amphotericin B by CLSI broth microdilution method

Type of Sample	Fluconazole	Amphotericin B
BAL	0	0
Urine	2	13
Pus	0	2
Et secretion	0	1
Vaginal secretion	1	1
blood	0	0

Table No. 10: Sample wise resistance pattern of *C.albicans* against Fluconazole and Amphotericin B by CLSI Kirby bauer disc diffusion method

Type of Sample	Fluconazole	Amphotericin B
BAL	0	0
Urine	4	2
Pus	0	0
Et secretion	0	0
Vaginal secretion	1	0
blood	0	0

Table No. 11 : Sample wise resistance pattern of *C.albicans* against Fluconazole and Amphotericin B by CLSI broth microdilution method

In the present study it was observed that by Kirby bauer disc diffusion method 3(10%) were resistant to Fluconazole whereas, 5(16.6%) showed resistance to Fluconazole by Broth microdilution method. It was also observed that 17(56.6%) showed resistance against Amphotericin B by Kirby bauer disc diffusion method and 2 (6.6%) was found to be resistant by Broth microdilution method.
In the study it was also observed that the maximum number of sample observed resistant was found in the urine sample.

DISCUSSION

The incidence of fungal infection as well as candidemia has increased significantly, contributing to morbidity and mortality in the developed countries. The alarming increase in infections with multidrug resistant bacteria is due to overuse of a broad spectrum antimicrobials, which leads to over growth of *Candida* spp.; thus, enhancing its opportunity to cause the disease. A shift has been observed in the relative frequency of each *Candida* spp. Antifungal drugs used to treat systemic and invasive candidiasis are limited to polyenes, allylamines, azoles, and the recently discovered echinocandin class of compounds [6] . Candida spp. resistance to antifungal treatment has quickly increased in recent decades, raising severe concerns among healthcare experts.
A total of 75 isolates of Candida species was included in the present study out of which 30 (40%) isolates were confirmed to be *C.albicans.* This study was in support with the study performed by L. Sherry et al.,[8] where the rate of *Candida albicans* was found to be maximum. In the present study it was observed that the ratio of Males 19 (63.3%) was more as compared to that of the Females 11(36.6%) with the maximum age of 31-40 being affected the most followed by 41-50 and least in the age group above 61 years of age. There were other studies which were parallel to our study where the male was more common. R A Kashid et al.,[9] reported the isolation of Candida species was higher in males (55.10%) with male to female ratio of 1:0.81. In another study by Amar CS et al., more Candida isolates from male and the male female ratio was reported as 0.66:1[10]. The study by B S G Sailaja et al.,[11] was similar to our study where the maximum age of 31-40 being affected the most but in contrast with the study by Arasi et al.,. which reported that more Candida strains in age group >60 years [12]. The maximum number of isolates was found in the urine sample 15 (50%). This finding were similar to the study by Alvarez-Lerma et al.,[13]. and CA Kauffmann et al., [14]. Another study by Sankarankutty Jay and Vipparti Harita [15] also reported that more strains were

isolated from Urine.

A total of 13 (43.3%) samples of Candida were sensitive and 17 (56.6%) samples were resistant to Amphotericin B and 27(90%) samples of Candida were sensitive and 3(10%) samples were resistant to Fluconazole by Kirby bauer disc diffusion method by Kirby bauer disc diffusion method.

93.3% were sensitive whereas 2 (6.6%) were resistant against Amphotericin B and 25 isolates(83.3%) were sensitive and 5 isolates (16.6%) were resistant to Fluconazole by broth microdilution method, which was incompatible with the study conducted by kamal Uddin Zaidi.et.al., [16] which showed 56.5% resistance and 43 % sensitivity to Fluconazole and which was in comparison with the studies conducted by Lulu Zhang.et.al., [17] which showed 10.6% resistance and 89.2% sensitivity to fluconazole and study conducted by shirshaklamsalet.al.,[18] showed 80.9% susceptibility and 9.1% resistance to fluconazole.

The susceptibility pattern obtained in the present study against azole antifungal fluconazole was also in agreement with a previous study by Rathod et.al.,[19] where higher susceptibility rates were observed against fluconazole. The development of resistance against azole antifungals can be due to alteration of the lanosterol 14 alpha demethylase target enzyme because of either overexpression or mutation in Erg11 gene encoding the enzymehenry et.al 2000 [20].

In the present study, we examined the antifungal susceptibility and resistance of antifungal agents of Fluconazole against *C. albicans* in disk diffusion and a micro-dilution method. The zone of inhibition of a different antifungal agent against *C. albicans* was observed at a concentration of 25µg/ml..Our findings were not in accordance with the study conducted by Fadda et al.,2008 [21] where decreased susceptibility to azoles in *C. albicans* was observed.

In the current study *candida albicans* sensitivity to amphotericin B was observed to be 96%sensitivity which was in comparison with the study by P.Badiee et.al which showed 99.5% sensitivity to amphotericin B. resistance rates of *C. albicans* to amphotericin B were reported to be 2.6% [22] and 7% [23] in Shiraz and Mazandaran which is incomparison with the present study which showed 4% resistance to Amphotericin B.

The changing epidemiology of candidemia highlights the need for close monitoring of *Candida* species distribution and susceptibility to optimize treatment and outcome [24].

Minimum inhibitory concentration was tested at the final concentrations ranging from 0.5 µg/ml to 256µg/ml. The dilution that showed no growth indicate the concentration at which the fungal growth was inhibited and the lowest concentration showing no colour was recorded in terms of the MIC value.. Early detection of drug susceptibility to the organism was carried out for a successful treatment of any infectious disease [25]. The diagnosis and identification of Candida species along with its antimicrobial susceptibility pattern in patients is very important for maintaining the rational use of antifungals. With various types of antifungal agents available in the market, performing antifungal susceptibility testing and reporting their therapeutic outcome seems to be necessary. Evaluation of the recent antifungal agents is required, as well

This discovery is significant since Candida's antifungal resistance has increased, posing a fatal threat to immune deficient patients and hospital acquired infections. There have been reports of intrinsic resistance to antifungal medication [17, 18]. Antifungal resistance has also been observed to develop as a result of treatment. Understanding the mechanisms of medication resistance is critical for improving treatment efficacy since Candida infections have a significant impact on immune impaired patients.

CONCLUSION

Antifungal susceptibility testing could be used to predict clinical response or management failure. Studies on prevalence of infections and antifungal susceptibility testing can help with deciding on clinical strategies to manage the problem of drug resistance. As a result, diagnosing and identifying Candida species in patients, as well as their antimicrobial susceptibility pattern, will assist clinicians in selecting the proper antifungal medication, so contributing to an overall reduction in treatment costs and hospital stay.

Declarations:
Conflicts of interest: There is no any conflict of interest associated with this study
Consent to participate: We have consent to participate.
Consent for publication: We have consent for the publication of this paper.
Authors' contributions: All the authors equally contributed the work**.**

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