Journal of Cardiovascular Disease Research
ISSN: 0975-3583, 0976-2833 VOL15, ISSUE 01, 2024

Original Article

EVALUATING THE ANTIFUNGAL SUSCEPTIBILITY PATTERN OF *CANDIDA ALBICANS* BY KIRBY-BAUER DISC DIFFUSION AND BROTH MICRODILUTION METHOD AGAINST FLUCONAZOLE AND AMPHOTERICIN B FROM DIFFERENT CLINICAL ISOLATES

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ABSTRACT

Introduction: Candidiasis is a serious fungal infection mostly caused by *Candida albicans* in humans. *Candida albicans* is an opportunistic infection in immunocompromised people that has proved fatal despite antifungal treatment. The rise in antibiotic resistance in *C. albicans* is concerning, 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, February 2023 to February 2024. A total of 100 isolates of Candida species from different clinical 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 100 isolates of Candida species 40 (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%. The ratio of Males 26 (65%) was more as compared to that of the Females 14 (35%) 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 maximum number of isolates was found in the urine sample 50% followed by the pus 20% and least in the BAL and the blood sample with 2.5% .
A total of 36(90%) samples of Candida were sensitive and 4(10%) samples were resistant to Fluconazole whereas 17 (42.5%) samples of Candida were sensitive and 23 (57.5%) samples were resistant to Amphotericin B by Kirby bauer disc diffusion method.
Out of 40 isolates of *C.albicans* tested for susceptibility pattern by CLSI broth microdilution method 33 isolates(83%) were sensitive and 7 isolates (17.5%) were resistant to Fluconazole showing Mic>/=64ug/ml whereas 37 (92.5%) were sensitive and 3(7.5%) were resistant against Amphotericin B by broth microdilution method.

Conclusion:
The Antifungal susceptibility testing by broth microdilution method revealed that fluconazole was exceedingly resistant against *Candida albicans* (17.5%) and increasingly susceptible to Amphotericin B(92.5%). 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: Antifungal Susceptibility**,** Antifungal resistance, Disc diffusion method, Microdilution method, CLSI

INTRODUCTION

It is extremely important to know the factors and mechanisms of the pathogenicity of *C. albicans* precisely because of their wide range, from dimorphism, biofilm formation, thigmotropism, expression of adhesion proteins, and secretion of extracellular hydrolytic enzymes. *C. albicans* is able to cause infections ranging from superficial to systemic and life-threatening. *Candida albicans* is a yeast fungus that lives on the skin and mucous membranes like the oral cavity, vagina, and rectum. It can spread through the bloodstream and infect any region of the body. *C. albicans* is the leading source of infection in humans [1], but it is also an essential part of the natural microbial flora in the oral cavity, gastrointestinal system, and vagina in healthy people. *C. albicans'* pathogenic potential includes mediate adhesion, biofilm formation, penetration of host cells, yeast-to-hypha transformation (phenotypic switching), hydrolase secretion, contact sensing, and thigmotropism [2].
Several variables contribute to the higher frequency of systemic candidiasis in colonised patients, including a compromised immune system, mucosal and cutaneous barrier disruption, neutrophil malfunction (quantitative or qualitative), metabolic problems, and advanced age [3].
Antifungal resistance is a serious worry in clinical practice, and it is becoming a major issue. Intensive and long-term antifungal medication treatment reduces Candida species susceptibility and

resistance patterns [2]. Recently, resistance to common antifungals has been found in various Candida species [3]. Polyenes, azoles, echinocandins, nucleoside analogues, and allylamines are employed with varied degrees of efficiency depending on the nature and location of infection, as well as the Candida species' sensitivity. Azole antifungal medications are the most often used treatments for Candida infections. Fluconazole, an azole-class antifungal, is the most often recommended antifungal for the majority of *C. albicans* infections [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 method for determining an organism's resistance to antimicrobial agents, and determining the minimum inhibitory concentration is the best way to understand the true degree of susceptibility or resistance to antimicrobial agents [6]. Some Candida species, such as *C. glabrata* and *C. krusei*, are innately resistant to Fluconazole. Fluconazole-resistant *C. albicans* strains have also been reported to be cross-resistant to other azoles.

Likewise, a rare but documented case of Amphotericin B resistance in Candida has been linked to changes in the cell membrane, such as decreased ergosterol levels, and was isolated after extended therapy. There is a growing need for antifungal susceptibility testing of the biofilm-producers, which can contribute to the pool for therapeutic approaches, due to the growing number of clinical isolates that are resistant to the commonly used antifungal agents, specifically because *C. albicans* produces biofilm [7].

In light of this, the current study was conducted to examine, using the Kirby Bauer disc diffusion method and the Broth microdilution method, the antifungal sensitivity pattern of *Candida albicans* isolated from different clinical samples against Fluconazole and Amphotericin B.

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, February 2023 to February 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:

Pure cultured Candida isolates from every clinical specimen were used.

Exclusion Criteria:

The investigation excluded isolates of Candida species from mix cultures and multiple isolates from the same clinical specimen belonging to the same patient.

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.

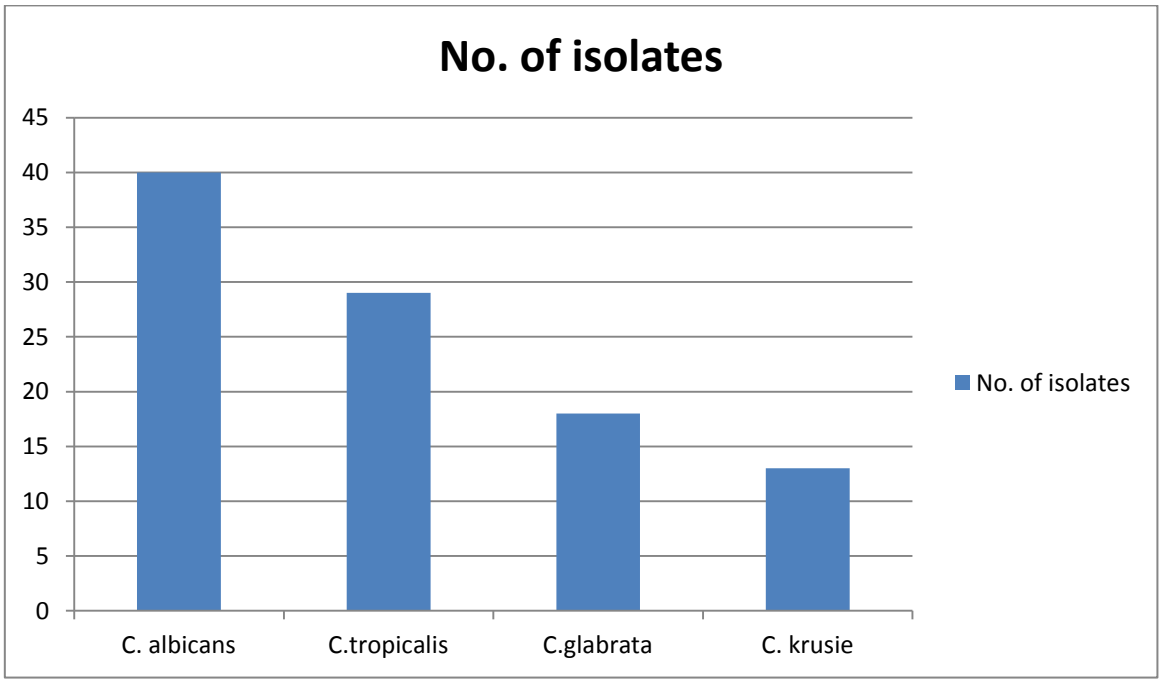
A total of 100 isolates of Candida species from different clincal specimens like blood,BAL,Urine, Pus ,Et secreation and Vaginal secreation were included in our study.
Antifungal sensitivity of Candida isolates was done by Kirby-Bauer disc diffusion method. Mueller Hinton agar supplemented with 0.2% 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 2023 [7].

RESULTS

A total of 100 isolates of Candida species was included in the present study out of which 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	40	40 %
C.tropicalis	29	29 %
C.glabrata	18	18 %
C. krusie	13	13 %

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=40)	Percentage
Male	26	65%
Female	14	35%

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

The ratio of Males 26 (65%) was more as compared to that of the Females 14 (35%) [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	7.5%
3.	21-30	4	10%
4.	31-40	20	50%
5.	41-50	10	25%
6.	51-60	2	5%
7.	≥61	1	2.5%

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

Type of Sample	Number of Isolates	Percentage
BAL	1	2.5%
Urine	20	50%
Pus	8	20%
Et secretion	3	7.5%
Vaginal secretion	7	17.5%
blood	1	2.5%

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 and the blood sample with 2.5% [Table No. 4].

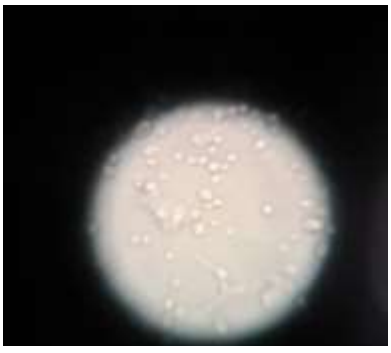


Fig No. 1:*Candida albicans* on Sabouraudextrose agar

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

Fig No. 3: *Candida albicans* on Hichromagar

Out of 100 isolates a total of 40 isolates of *Candida albicans* were isolated. A total of 36(90%) samples of Candida were sensitive and 4(10%) samples were resistant to Fluconazole whereas 17 (42.5%) samples of Candida were sensitive and 23 (57.5%) samples were resistant to Amphotericin B by Kirby bauer disc diffusion method.

Out of 40 isolates of *C.albicans* tested for susceptibility pattern by CLSI broth microdilution method 33 isolates(83%) were sensitive and 7 isolates (17.5%) were resistant to Fluconazole showing Mic>/=64ug/ml whereas 37 (92.5%) were sensitive and 3(7.5%) were resistant against Amphotericin B by broth microdilution method.

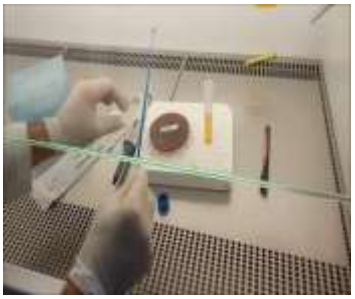


Fig No. 4 (a) Kirby-Bauer disc diffusion method

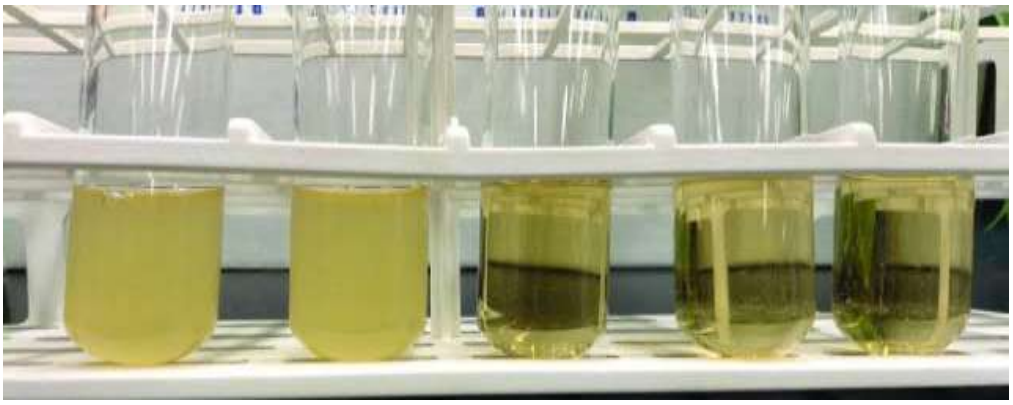


Fig No. 4 (b) : The Antifungal sensitivity pattern by broth microdilution method

Antifungal-Fluconazole	**Number of isolates N=40**	**Percentage of isolates**
Sensitive	36	90 %
Resistant	4	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 36(90%) samples of Candida were sensitive and 4(10%) samples were resistant to Fluconazole by Kirby bauer disc diffusion method [Table No. 5].

Antifungal-Fluconazole	Number of isolates N= 40	Percentage of isolates
Sensitive	33	83%
Resistant	7	17.5%

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 40 isolates of *C.albicans* tested for susceptibility pattern by CLSI broth microdilution method 33 isolates(83%) were sensitive and 7 isolates (17.5%) were resistant to Fluconazole showing Mic>/=64ug/ml.

Antifungal-AMP B	Number of isolates N= 40	Percentage of isolates
Sensitive	17	42.5 %
Resistant	23	57.5 %

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

A total of 17 (42.5%) samples of Candida were sensitive and 23 (57.5%) samples were resistant to Amphotericin B [Table No. 7]

Antifungal-AMP B	Number of isolates N= 40	Percentage of isolates
Sensitive	37	92.5%
Resistant	3	7.5%

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 37 (92.5%) were sensitive whereas 3(7.5%) were resistant against Amphotericin B by broth microdilution method.

Antifungal	Kirby bauer disc diffusion Method		Broth microdilution method	
	Sensitive	Resistant	Sensitive	Resistant
Fluconazole	36(90%)	4(10%)	33(83%)	7(17.5%)
Amphotericin B	17(42.5%)	23(57.5%)	37(92.5%)	3(7.5%)

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	3	18
Pus	0	3
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	6	3
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 4(10%) were resistant to Fluconazole whereas, 7(17.5%) showed resistance to Fluconazole by Broth microdilution method. It was also observed that 23(57.5%) showed resistance against Amphotericin B by Kirby bauer disc diffusion method and 3 (7.5%) 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

Early diagnosis and commencement of appropriate antifungal therapy are vital to improve outcomes in invasive *Candida* infections, which continue to be associated with high rates of morbidity and mortality particularly in immunocompromised hosts.
In affluent nations, fungal infections and candidemia are becoming more common, which has led to a rise in morbidity and mortality. The concerning rise in infections involving bacteria resistant to drugs is a result of the abuse of wide spectrum antibiotics, which promotes the overgrowth of Candida spp. and increases the likelihood that the bacterium will cause illness. There has been a change in each Candida species' relative frequency. Antifungal medicines used to treat systemic and invasive candidiasis are confined to polyenes, allylamines, azoles, and the recently discovered echinocandin class of chemicals [6] . The rapid rise in Candida spp. resistance to antifungal treatment in recent decades has caused serious worries among medical professionals.

A total of 100 isolates of Candida species was included in the present study out of which 40 (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 26 (65%) was more as compared to that of the Females 14(35%) 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 20 (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 36(90%) samples of Candida were sensitive and 4(10%) samples were resistant to Fluconazole whereas 17 (42.5%) samples of Candida were sensitive and 23 (57.5%) samples were resistant to Amphotericin B by Kirby bauer disc diffusion method.
Out of 40 isolates of *C.albicans* tested for susceptibility pattern by CLSI broth microdilution method 33 isolates(83%) were sensitive and 7 isolates (17.5%) were resistant to Fluconazole showing Mic>/=64ug/ml whereas 37 (92.5%) were sensitive and 3(7.5%) were resistant against Amphotericin B 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 92.5% sensitivity which was in comparison with the study by P.Badiee et.al which showed 99.5% sensitivity to amphotericin B. The 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]. Maintaining the sensible use of antifungals requires accurate diagnosis and identification of the Candida species as well as knowledge of the antimicrobial susceptibility pattern in patients. Given the variety of antifungal medicines on the

market, it appears vital to test for antifungal susceptibility and report the therapy outcome. A review of the most recent antifungal medications is also necessary.

This finding is critical because hospital acquired infections and immunocompromised patients are at risk of death due to the growing antifungal resistance of Candida. Intrinsic resistance to antifungal medications has been reported [17, 18]. It has also been noted that treatment-induced development of antifungal resistance occurs. Since immune-compromised individuals are greatly affected by Candida infections, it is imperative to comprehend the mechanisms underlying medicine resistance in order to improve treatment efficacy [27,28].

CONCLUSION

Testing for antifungal susceptibility may be used to forecast management failure or clinical response. Selecting clinical approaches to address the issue of medication resistance can be aided by research on the prevalence of infections and antifungal susceptibility testing. Consequently, the diagnosis and identification of Candida species in patients, along with their pattern of antimicrobial sensitivity, can help clinicians choose the appropriate antifungal drug, thereby reducing treatment costs and length of 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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