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Antifungal susceptibility pattern of candida species among cancer patients attending B.P Koirala Memorial Cancer Hospital, Chitwan

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Abstract

Background: Cancer is considered as a second most common cause of human death worldwide. The sufferers with most cancers are ongoing remedies for cell destruction which immediately lead them to at risk of infections because of their immune- compromised state. Among cancer patients, the fungal flora of the body imposes a greatest threat for own self. Antifungal resistance has been evolving lately as a burgeoning health care problem among *Candida* species. So, this study is aimed to explore the antifungal susceptibility pattern of *Candida* species among cancers patient.

Methodology: This study was a cross-sectional study carried out in the Microbiology laboratory of B.P Koirala Memorial Cancer Hospital, Bharatpur, Chitwan, Nepal. Standard microbiological techniques were used to identify *Candida* isolates, and HiCrome differential media were used for *Candida* speciation. Antifungal susceptibility testing was determined by using the Clinical and Laboratory Standards Institute (CLSI) disk diffusion method.

Results Out of 680 processed samples, 50 *Candida* positive samples were found, with the majority (48.0%) isolated from urine. *Candida albicans* was the most predominant species (38%), followed by *Candida glabrata* (24%), *Candida tropicalis* (22%), and *Candida krusei* (16%). All isolates were highly sensitive to voriconazole (96%), with Clotrimazole 40 showing the highest resistance (80%), followed by ketoconazole and amphotericin B.

Conclusion *Candida albicans* is the most common cause of *Candida* infections, with most isolates exhibiting resistance to voriconazole and clotrimazole. This suggests the need for ongoing antifungal susceptibility surveillance to monitor trends in *Candida* species among cancer patients.

Keywords: Antifungal drug; Cancer; *Candida*; Drug resistance

1. Introduction

Candidiasis is the most prevalent fungal infection in humans, affecting the mucosa, skin, nails, and internal organs. It is caused by a variety of yeast-like fungus in the genus *Candida*, with *Candida albicans* serving as the main species. *Candida tropicalis, Candida krusei, Candida glabrata, Candida guilliermondii, Candida parapsilosis, Candida lusitaniae, Candida kefyr, Candida rugosa, Candida dubliniensis*, and *Candida viswanathii* are among the other harmful species (1).

Cancer is a disorder of excessive cell growth that is caused by a series of inherited mutations that are harmful to both the parent cell and offspring cells (2). In cancer patients, body cells were developed in an uncontrolled way and procreate in other parts of the body (3). Cancer was the second main reason for loss of life globally, accounting for a predicted 9.6 million deaths, or one in six deaths, in 2018 (4).

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Patients with cancer are more susceptible to *Candida* infections due to altered immunological mechanisms (5). Chemotherapy weakens cellular and humoral immune responses and directly damages mucosa (3). The host immune response is frequently changed by this course of treatment. For example, the study found that cancer patients had myeloperoxidase (MPO) deficiency and were at an increased risk of *Candida* infections (6). Another study discovered that thrombospondin-1 causes an imbalance in the host immune system, which reduces the ability of the fungus to be cleared up, spreads, and raises mortality (7).

There have been concerns raised about the indiscriminate and excessive use of antifungal agents, which increases the emergence of antifungal resistance (8) .As a result of the change in drug susceptibility pattern of Candida species and the introduction of newer antifungal medications, in vitro susceptibility testing of antifungal agents has become more important for using specific and sensitive treatments. So that it is now more crucial than ever to isolate, characterize, identify, and test for *Candida* species in clinical specimens in order to control fungal infections. Four different species of *Candida* can be separated and isolated using the CHROMagar medium, which is a simple, quick, and reliable technique (9).Therefore this study will evaluate the Candida species and antifungal susceptibility pattern among cancer patients.

2. Material and methods

In the Department of Microbiology at the BP Koirala Memorial Cancer Hospital, a laboratory-based cross-sectional study was conducted from May 2022 to October 2022.A total of 680 distinct clinical specimens, from OPD and indoor patient was taken including blood, bronchoalveolar lavage (BAL), catheter tip, urine and sputum, were sent for laboratory analysis.

Wet mount, Gram stain, Sabouraud dextrose agar (SDA) culture, and urea hydrolysis testing were used to make preliminary diagnoses on the specimens (10). The investigation excluded isolates that were found to be different species of fungus than Candida. The samples were examined under a microscope for evidence of budding yeast cells with pseudohyphae and significant pus cells in order to determine the clinical significance of Candida isolates from sputum and urine(11). All samples were plated onto SDA slants and incubated aerobically at 37 °C for 24-48 hours.

For the blood culture, 8–10 ml of venous blood were aseptically collected and cultured in 45 ml of Brain Heart Infusion (BHI) broth. It was then incubated at 37 °C for up to 96 hours before being reported as no growth.

Any visible growth seen on the SDA slope was examined for species identification. A morphological examination, Gram staining, germ tube test, and urea hydrolysis test were carried out on an isolated colony. On CHROMagar, the yeasty, pasty, and creamy colony that underwent microscopic analysis and revealed Gram-positive budding yeast cells with pseudohyphae and a negative urea hydrolysis test was further processed for Candida speciation (12). Based on the growth pattern and color of the isolates using CHROMagar *Candida* (HiMedia, Mumbai, India), different species of *Candida* were identified (13) .

The color of colonies on CHROMagar was observed after 24-48 hours of incubation at 37 °C (*C. albicans*—light green, *C. glabrata*—cream to white, *Candida krusei*—purple, fuzzy, and *C. tropicalis*—blue to purple).

All *Candida* isolates were tested for antifungal susceptibility using the disc diffusion method, as indicated by the Clinical and Laboratory Standards Institute (CLSI) M44-A document guidelines(14).The turbidity was measured against the 0.5 McFarland Standard after the inoculum was made by suspending colonies of growth in 5 ml of sterile saline (15). Mueller-Hinton agar containing 2% glucose and 0.5 g/ml methylene blue was streaked evenly with a cotton swab dipped in the inoculum suspension (16).*C. albicans* ATCC 90028, *C. tropicalis* ATCC 750 were used as controls.

Fuconazole (10 mg), ketoconazole (10 mg), clotrimazole (10 mg), voriconazole(1µg) and Amphotericin B(100µg), itraconazole (10mg). Antifungal discs were applied to the inoculation media. After the medium had been incubated at 37 °C for 24 hours, the zone of inhibition surrounding the disc was measured(17).

3. Results

3.1. Distribution of organisms isolated

Out of 680 samples, 630 (92.6%) had no growth, whereas 50 (7.4%) had positive for *Candida* species. These distributions were shown in table 1. Here two types of *Candida* species were seen they are *C. albicans* and Non *albicans*. Non *albicans* were further classified into three type's namely *C. krusei*, *C. glabrata* and *C. tropicalis*. Out of 4 *Candida* isolated, *C. albicans* was reported highest of all contributing 19(38.0%) of total growth followed by *C. glabrata* of 12(24.0%). *C. tropicalis* was 11(22.0%) and *krusei* was 8(16.0%). Thus here *C. albicans* species were 19(38.0%) and Non *albicans* species were 31(62.0%) of total growth. These distributions were shown in table no 1. Now further results and discussion was based on total positive samples (50 positive samples among total 680samples).

Table 1 Distribution of organisms isolated

SN.	Organisms isolated	Frequency	Percent
1	*C. albicans*	19	38.0%
2	*C. glabrata*	12	24%
3	C. *tropicalis*	11	22%
4	*C. krusei*	8	16%
5	Total	50	100%

3.2. Distribution of Candida species among sample

Candida species were mostly isolated from Urine sample 24(48%) followed by BAL sample 10(20%), Pus sample 6(12%) and sputum sample 5(10%) .*Candida* species Were reported from Blood sample 4(8%) and only 1(2%) isolated was report from Catheter Tube sample. In urine sample *C. albicans* was reported on 9(37.5%) followed by *C. tropicalis* 7(29.17%) and *C. glabrata* 5(20.83%). *C. krusei* was least reported only on 3(12.5%) in urine sample. In BAL samples *C.albican, C. glabrata* and *C. krusei* was reported similar contributing 3(30%) of total Bal sample. *C. tropicalis* was isolated only in 1(10%) of Bal sample.In Blood samples *C. albicans* was contributing highest 2 (50%) among all spp. of *Candida* followed by *C. krusei* and *C. tropicali s* were equally isolated up to 1(25%) of each. None of blood samples were positive for *C.glabrata*. In Pus sample *C. albicans* was reported on 3(50%) followed by *C. glabrata* of 2(33.33%) and *C. krusei* 1(16.67%). None of Pus sample was positive for *C. tropicalis.* In sputum sample both *C. albicans* and *C. glabrata* were equally isolated up to 2(40%) followed by *C. tropicalis* of 1(20%). None of sputum sample was positive for *C. krusei.* In Catheter Tube only one *C. tropicalis* was isolated. those distribution were shown in Table no 2.

Table 2 Distribution of Candida species among sample

Isolation						
SN.	**Sample type**	***C. albicans***	***C. glabrata***	***C. krusei***	**C. *tropicalis***	***Total***
1	Bal	3(30%)	3(30%)	3(30%)	1(10%)	10(20%)
2	Blood	*2(50%)*	0(0%)	1(25%)	1(25%)	4(8%)
3	Catheter Tube	0(0%)	0(0%)	0(0%)	1(100%)	1(2%)
4	Pus	3(50%)	2(33.3%)	1(16.67%)	0(0%)	6(12%)
5	Sputum	2(40%)	2(40%)	0(0%)	1(20%)	5(10%)
6	Urine	9(37.5%)	5(20.8%)	3(12.5%)	7(29.17%)	24(48%)
7	Total					50(100%)

3.3. Antifungal pattern of Candida species

Voriconazole was shown the most sensitive against *Candida* out of 50 positive samples, accounting for 48(96%) of cases, followed by itraconazole 31(62%), fluconazole 26(52%), and amphotericin B 23(46%). The least sensitive drugs were

ketoconazole, Clotrimazole with 13(26%) and 9(18%), respectively. Clotrimazole was discovered the most resistant against *Candida*, accounting for 40(80%), followed by ketoconazole 35(70%) and amphotericin B 27(54%). These distributions are shown in Figure 3.

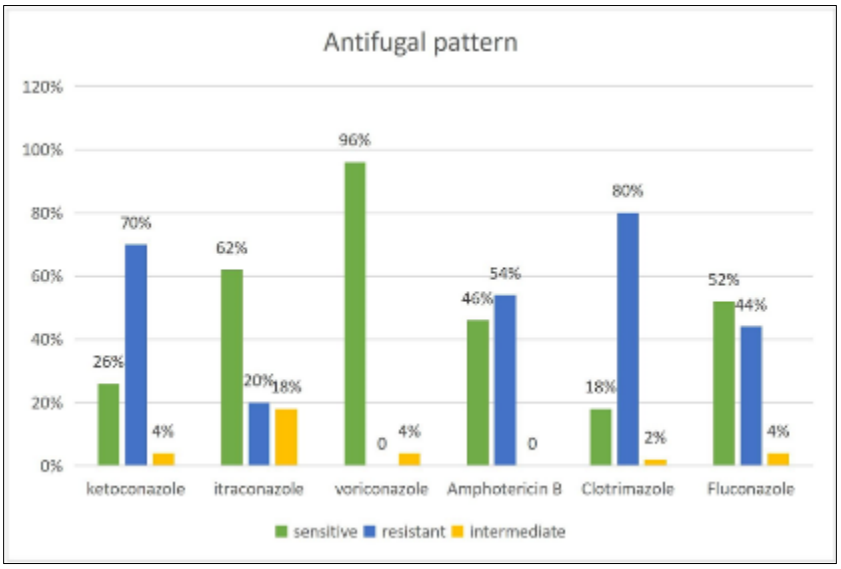

Figure 1 Antifungal pattern of *Candida* species

4. Discussion

In recent years, there has been a striking rise in the prevalence of opportunistic yeast infections, particularly those brought on by endogenous human commensal *Candida* species, which has raised severe concerns in the medical community all over the world. Although *Candida albicans* has historically been the most common infection- causing agent, other species, including *C. tropicalis*, *C. glabrata*, *C. krusei*, and *C. parapsilosis*, have also been linked to systemic and mucosal candidiasis. *C. albicans*'s antifungal susceptibility profile and pathogenicity factors a need for accurate and quick species because of variety identity since this directly influences the decision of treatment (18).

It was a hospital based cross-sectional, quantitative type of study. 680 Samples were collected and processed in the Microbiology laboratory of BPKMCH, Bharatpur, Chitwan, Nepal. The samples were collected from the patients attending OPD or admitted to the hospitals. Among them 280 (41.17%) were male and 400 (58.82%) were females. In our study, out of total 680 samples processed, 92.64%(n=630) were reported no growth, while 7.3% (n=50) had growth respectively.NACs 31(62%) was the main pathogen in our investigation when compared to *C.* ablican 19(38%) which is almost opposite to prior studies done by Maheronnaghsh et al. they found that out of 74 *Candida* isolates, with 75.5% *Candida albicans* and 24.5% non-*albicans* species (19). In our investigation, the prevalence of NACs 31(62%) was in the following order: *C. glabrata* 12(38.70%) *C. tropicalis* 11(35.48%) and *C. krusei* 8(25.80%) of the 680 total sample processed. This finding was contrariety to studies done by Maheronnaghsh *et al* which was found that *C. tropicalis* was the most common Non-*Albicans* species (9.4%), followed by *C. krusei* (7%), *C. glabrata* (5.4%), and *C. kefyr* (2.7%) (20). The most prevalent species of *C. glabrata* among NACs in clinical samples is an important finding of our investigation. Because of the high occurrence of increasing resistance of this species to commonly used antifungal drugs, this might be a serious threat.

Out of 50 *Candida* species, 24 (48%) of the urine samples, 10 (20%) of the BAL samples, 6 (12%) of the pus samples, 5(10%) of the sputum samples, and 4 (8%) of the blood samples had found in our study. which discovered that the majority of Candida species were present in urine samples. This finding is similar to the previous study by Patel et al, Out of 219, 78 (35.62%) of the urine samples, 10 (4.57%) of the pus, 20 (9.13%) of the BAL, 32 (14.61%) of the sputum, and 10 (4.57%) of the samples from other sites (21). *Candida* is demonstrated to be a prominent pathogen for UTI (urinary tract infection) under these circumstances because it has a stronger bond to the mucosal epithelial cells of the urinary tract than other fungi species (21).

Of the 50 positive isolated in our study, females had a greater prevalence of *Candida* infection than men 32(64%) than 18(36%); this finding was similar to the study done by Chung et al., which reported that 34829 positive sample female

were higher in number 31707(91%) than male 3122(8.92%) (22). As a result, we conducted additional research and focused on candidiasis to determine whether *Candida* infection association in female cancer patient.

Out of 50 positive samples in our investigation, Voriconazole was shown to be the most effective against *Candida*, accounting for 48(96%) of instances. Itraconazole 31(62%) was next, followed by fluconazole 26(52%), and amphotericin B 23(46%). The least sensitive drugs were ketoconazole and clotrimazole, with sensitivity ratings of 13 (26%) and 9 (18%) respectively. Which was similar in a research conducted by Jangla et al found that out 60 *Candida* species voriconazole was sensitive to 55(91.66%) followed by Itraconazole 37(61.67%), Fluconazole 29(49.8%), and Ketoconazole 28(47%), respectively (23). Our study showed Voriconazole is an effective drug 48(96%) where as 94% sensitive to Voriconazole was found in a similar study done by Badiee and Alborzi et al (24) .92% by Khan et al (25). 83% by Shafi FT et al (26) respectively. Voriconazole has broad-spectrum action against pathogenic yeasts, including isolates that are inherently fluconazole-resistant, such as *Candida krusei*. It has a high clinical efficacy in individuals with fluconazole-resistant *Candida* infections (27). Despite the fact that voriconazole was determined to be beneficial in the study, practicing the surveillance with a monitor is necessary.

In our study the drug shown to be the most resistant against *Candida* was clotrimazole, which accounted for 40(80%) of the resistance. Ketoconazole 35(70%), amphotericin B 27(54%) and fluconazole 22(44%) were next in line. Clotrimazole was reported to be the most resistant among *C.glabrata* at up to 11(91.7%) followed by *C.kruseri* 7(87.5%).Our findings significantly contrast with those of Mukesh Azad et al who found that just 15% of *Candida* isolated were resistant to clotrimazole (28) ,whereas khan M *et al* who found that just 59.5% of *Candida* isolates were resistant to clotrimazole (29). Clotrimazole is currently widely used as a first-line topical antifungal in immunocompromised patients for the prevention or treatment of mucosal candidiasis. Chronic and repeated usage of clotrimazole to treat immunocompromised people predisposes to developing resistance (30).These findings suggest the rapid increase in resistance among *Candida* species for clotrimazole and need for speciation and antifungal susceptibility before treatment with antifungal drug.

5. Conclusion

C. albicans was shown to be the most common species responsible for Candida infections in our investigation. Voriconazole and itraconazole, two regularly used antifungal medications, showed a high degree of sensitivity, whereas ketoconazole and clotrimazole were the least effective against *C. albicans* and NAC spp. Thus, the study highlights the requirement for *Candida* isolate speciation and suggests antifungal susceptibility testing for all clinical isolates. For the proper selection of antifungal medication, it is clinically crucial to identify different isolates of *Candida* to their species level along with antifungal sensitivity patterns.

Compliance with ethical standards

Acknowledgment

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Disclosure of Conflicts of Interest

The authors declare no conflict of interest.

Statement of Ethical approval

The research has complied with all the relevant national regulations and institutional policies and has been approved by the Institutional Research Committee (IRC) of BP Koirala Memorial Cancer Hospital (letter of approval Registration No 60/2022).

Statement of informed Consent

Written informed consent was taken from all the participants involved in the study.

Availability of Data

The datasets of the current study will be available from the corresponding author upon reasonable request.

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