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Original Research Article

A retrospective investigation of *Candida spp*. and its antifungal susceptibility profile in cancer patients at a South Indian tertiary care hospital

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ABSTRACT

Background: Among the most frequently recognized pathogenic fungi, *Candida spp*. from Clinical samples plays a vital role. In recent decades, poor antifungal therapy usage critically in immunocompromised patients has led to antifungal drug resistance among *Candida*. Hence, expeditious and dependable species identification is crucial for the effective implantation of treatment strategies and the management of diseases.

Aim & Objectives: The purpose of the study was to look at the presence of *Candida* in various Clinical samples taken from cancer patients who are being treated at a tertiary care hospital in South India. The primary objective of this investigation is to ascertain the susceptibility patterns shown by antifungal drugs. This retrospective investigation was undertaken between January 2022 to April 2023.

Materials and Methods: The *Candida* isolates were subjected to antifungal susceptibility (AFST) by both Conventional (disc diffusion assay) and Automated methods (VITEK-2 compact - AST YSO1). The susceptibility pattern was recorded against antifungal agents like Fluconazole (FLU), Voriconazole (VOR), Amphotericin B (AMB), and Caspofungin. *Candida spp*. was identified by conventional method (Chrome ID) as per standard laboratory protocols and by an automated method using MALDI – TOF.

Results: Among 215 samples received from cancer patients suggestive of Candidiasis, *Candida* albicans (64.6%) was the predominant isolate, and the lowest one was *C. parapsilosis* (2.25%). Male patients had a higher prevalence of *Candida* (55.8%). Similarly, the prevalence was higher in patients over 60, with male patients having an incidence of 38.3% and female patients having 23%. By disk diffusion assay *C. albicans* and *C. tropicalis* showed 100% sensitivity to all antifungals used in this study.

Conclusion: We conclude that the disc diffusion assay is a more cost-effective, user-friendly, and effective screening test than the VITEK 2, and it has the potential to be used as a workable method for Antifungal susceptibility test. The use of Chrome ID for identification of *Candida spp*. is cost-effective and reliable. However, MALDI TOF has the advantage of rapidity, providing correct findings promptly. The timely delivery of diagnostic reports facilitates early diagnosis of patients. Therefore, it is recommended in this research to use MALDI TOF for the identification of *Candida spp*. and the disc diffusion technique for antifungal susceptibility testing (AFST).

1. Introduction

Candida non-albicans (NAC) have mainly replaced *C. albicans* in most *Candida* infections, while the prevalence of species varies depending on geographical location

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and patient demographic. Previous study reports by[1–3] suggested that fungal infections particularly those attributed to *Candida spp.* have been the most common cause of death in hospitalized patients, particularly in critical care units. *Candida spp*. contributes to a 30-60% mortality rate of hospitalized patients. Immunocompromised individuals are at increased risk due to graft transplantation, broad-spectrum antibiotic medication, catheter insertion, or digestive surgery.[4] According to earlier research *C. albicans* contributes to 50 -70% of infections caused by *Candida spp*. indicating that it may have an endogenous origin.[5] An elevated rate of Candidemia is a result of the patients in critical care units. This varies from 60 - 80% of those who are chemically sick patients, compared to healthy people (or) people with less severe diseases.[6] According to retrospective analysis, outbreaks of *C.rugosa*, *C.guillermondi*, *C.famata*, and *C.lusitaniae* were associated with a high death rate of up to 43%. The pattern of susceptibility to antifungal drugs has shown a very high degree of variation among *Candida spp*.[7] Antifungal testing has recently become a global standard through the CLSI to detect sensitivity patterns and the emergence of resistant strains, a global concern.[8] Antifungal resistance, however, has resulted in epidemiological instability, raising the difficulty of identifying and obtaining susceptibility patterns for later application and precise medications for resistant strains.[9] Conventional methods of identification are less popular among physicians due to the lengthier turnaround time, as early diagnosis is critical for commencing appropriate therapy. Several chromogenic substrate culture mediums have been created to aid in quick identification.[10] In the automated method, MALDI TOF for species identification is used to fetch the results on time with 100% accuracy.[11] As a result, the goal of this retrospective investigation was to assess and determine the Antifungal susceptibility testing profile of *Candida spp*. isolated from Patients in Cancer therapy.

2. Materials and Methods

2.1. Sample collection

The retrospective investigation was carried out in the Microbiology department in Cancer Institute (WIA) (Regional Cancer Centre), Adyar, Chennai. In the time frame spanned between January 2022 to April 2023, various biological specimens were collected from cancer patients. Our retrospective analysis included 215 cancer patients with suggestive of candidiasis, of which 120 were males and 95 were females. All the samples were processed as per microbiology standard operating procedure (CLSI, 2021).[12]

2.2. Ethical statement

The Institutional Ethical Committee IEC / 2023 / July 01, Cancer Institute (WIA), Tamil Nadu, approved this retrospective study.

2.3. Isolation of fungal cultures

All samples were obtained with strict adherence to aseptic procedures. The samples received at the microbiology laboratory were subjected to inoculation on both Blood and MacConkey agar. Subsequently, the plates were incubated at a temperature of 37°C for a duration of 48 to 72 hours. Colony morphology was studied based on size, color, and shape. *Candida* colonies were suspected to be smooth, pasty, opaque, white, or beige. The gram staining procedure was performed to validate the identity as *Candida spp*. To check the existence of gram-positive yeast and pseudo hyphae direct gram staining was done. HI Chrome agar and Sabouraud Dextrose agar (SDA) plates were inoculated with isolates that exhibited gram-positive budding yeast cells (Bernal et al., 1996 & Odds Bernerts 1994).[13,14]

2.4. Antifungal susceptibility test by conventional method (Disk diffusion assay)

The susceptibility of *Candida spp*. to antifungal agents was evaluated using disc diffusion assay utilizing the Kirby Bauer method. The antifungal discs include Voriconazole (1μg), Fluconazole (25 μg), and Amphotericin – B (20 μg). The testing procedure adhered to the guidelines set out by CLSI, specifically the M44-A guideline. Briefly, a disk containing these antifungal agents was applied to the surface of Muller Hinton agar plate (MHA) on which lawn culture of the clinical isolate had been done following incubation at 37°C for 24 - 48h, plates were examined, and zone of inhibition surrounding the disc measured and compared with established zone size ranges for individual antifungal drugs.

2.5. Antifungal susceptibility test by automated method (Vitek -2)

Based on the Kirby Bauer results using disk diffusion assay -the maximum zone size recorded was picked up for further study. Out of 215 strains of *Candida spp*. only 43 isolates were selected for the AST Vitek test. The susceptibility assessment for antifungal agents was conducted using a fungal susceptibility card (VITEK 2). The BACT ALERT automated system, manufactured by bioMerieux in France, was used for the Blood and Cerebrospinal fluid (CSF) cultures. The suspension of inoculum for the VITEK was done in a sterile saline solution, achieving a turbidity level equivalent to 2.0 McFarland's inoculum suspension was introduced into the Vitek -2 cassette, accompanied with a yeast susceptibility test card, and sterile polystyrene test tube for every individual organism. The loaded cassettes were inserted into Vitek-2 equipment, where the necessary yeast solution was diluted before the cards were automatically filled, incubated, and read. The duration of

incubation ranged from 10 to 26 hours depending on the rate of growth seen in the control well without the presence of drugs. The outcomes were then qualified as MICs in microorganisms per milliliter. The system offers a total of 64 well cards that contain aliquots for four antifungal drugs: Amphotericin B (AMB), Fluconazole (FLU), Voriconazole (VOR), and Caspofungin. The broth dilution technique is used in this system, which incorporates a software program to authenticate and analyze susceptibility in accordance with CLSI clinical breakpoints based on MIC values. The assurance of quality control has been achieved by testing the control strains ATCC 2209 *C. parapsilosis* and ATCC 6258 *C. krusei* as recommended by CLSI.[15]

2.6. Identification of yeasts by MALDI –TOF MS

The species identification of *Candida* was performed using MALDI-TOF-MS. Yeast cells were cultivated on Sabouraud dextrose agar plates for a duration of 24 hours at a temperature of 37°C. Using a disposable plastic loop, single yeast colonies were directly transferred from the culture medium onto each location of the steel target plate. After that, 1μl of formic acid with a concentration of 70% was applied to each sample that had been spotted on a steel target plate and then dried at room temperature. For the positive control bacterial test, a standard was used. One microliter of a matrix solution containing α-cyano-4-hydroxycinnamic acid is applied uniformly to all the test spots. The yeast identification process was conducted using a 3.0 system that relied on mass spectra produced by micro flex LT software. These spectra were then compared with 2 databases. The identification scores were evaluated in accordance with standards specified by the manufacturer. The cut-off for confident genus identification is a log score of 1.999 or above is required. An accurate species identification was indicated by a log score value of > 2.0 and the identification is considered non-reliable when it is <1.7 score value. If at least one duplicate site yielded high confidence (score >1.7) that was consistent with the sequencing result, then the identification was also deemed accurate.[16–18]

3. Result

3.1. Prevalence and distribution of the study population in Candida spp .

In this retrospective research, an analysis was conducted on a cohort of 215 patients who were presented with different types of malignancies. The data was gathered over the course of 15 months, from January 2022 to April 2023. The research population consisted only of IP (in-patients) from tertiary care hospital. Among the total cases, 9 cases of each were reported to be head and neck cancer and in hematological malignancies, 4 cases of each were recorded in the gastrointestinal tract, genitourinary tract, and gynecological cancer respectively. Three CNS cases and only 1 case of Osteosarcoma were reported. A total of 34 types of malignancies were recorded among 215 patients. Out of 215 patients tested the highest prevalence was *C. albicans* at 64.6% and the lowest prevalence was *C. parapsilosis* at 3.25% (Table 1). The age and sex for the patients were studied within the range of 0-90 years. 55.8% of the patients were males followed by females at 44.1%. Among patients under the age of 20, 21 positive cases were observed in female patients, while male patients accounted for 15 positive cases. There were 45 positive instances in the 20 - 40 age range including 22 male and 23 female patients. In the age group of 41 -60, 66 males & females tested positive for *Candida*. Male patients had a 38.3% incidence and female patients had a 23.1% incidence among patients over the age of 60 making this a most notable prevalence (Table 2). Most of the clinical isolates were from sputum (n=110) subsequently followed by urine (72), Blood (12), Bronchial wash (9), oral swab (5), wound swab (3), Catheter tip (2), and ETT (2) respectively (Figure 4). 90% of cases were gram-positive (yeast pseudo hyphae). (Figure 2). All *Candida* isolates grew effectively on both differential agar and HI chrome media after 72h at 37°C (Figure 1). In this study, 64.6% of the 215 *Candida spp.* were *C. albicans*, the most isolated spp. and 35% were non-albicans. The unpaired t-Test showed a p-value of 0.0154 this distribution was found to be statistically significant (Table 3). *C.albicans* and *C. tropicalis* were 100% sensitive to fluconazole, Amphotericin B and Voriconazole by disc diffusion technique. Whereas *C. parapsilosis* was 85.7% sensitive to all the 3 antifungal drugs. C.glabarata was only 70% sensitive to Amphotericin B. *C. glabarata*, *C. auris* showed higher resistance of 83.3% and 30% against Fluconazole respectively (Table 4) (Figure 3).

3.2. Antifungal susceptibility of isolated Candida spp. by an automated method

A susceptibility test for antifungal drugs was conducted on a total of 43 isolates. *C. krusei* had the lowest MIC value of 0.12 μg/ml for voriconazole, while *C. auris* and *C. parapsilosis* had the highest MIC value of 32 μg/ml for fluconazole resistance. The results were interpreted according to CLSI guidelines. In the same way, all four antifungal drugs worked very well with all 43 isolates against *C. albicans* and *C. tropicalis*. Amphotericin B was found to work on all 43 isolates of *Candida spp*. with MIC values that ranged from 0.047 to 2μg/ml. In the same way, *C. auris* showed resistance to fluconazole, voriconazole, and Caspofungin, while *C. glabarata* and *C. parapsilosis* showed only resistance to fluconazole (Table 5).

3.3. Maldi- TOF

A MALDI-TOF-MS analysis was conducted for 50 isolates to validate the species identification. According to our

Table 1: Incidence of different *Candida spp*. among various cancer patients during

Site of Cancer	*C.albicans*	*C.tropicalis*	*C.krusei*	*C.glabarata*	*C.auris*	*C.parapsilosis*
Pancreatic	2	1	2	1	1	0
Lung	11	1	2	1	1	1
Breast	4	1	1	0	0	0
Acute lymphoblastic Leukemia (ALL)	5	0	0	2	1	1
Tongue -Oropharynx	7	1	0	3	0	0
Bladder	8	1	1	0	0	0
Right colon	10	0	0	0	0	0
Stomach	1	1	2	1	0	1
Lymphoma	2	1	2	0	0	0
Oesophagus	0	0	2	0	1	0
Ovary	4	1	0	2	0	0
Bone - Osteosarcoma	2	0	1	0	0	0
Thyroid	0	1	1	0	1	0
Oral cavity	4	0	1	0	0	0
Lymphoreticular System- Myeloma	10	0	0	5	0	0
Brain-Astrocytoma	2	0	3	0	0	0
Rectum	3	2	0	1	4	0
Hypopharynx	10	1	1	0	0	3
Cervix	10	0	1	0	0	0
Uterus	1	0	0	0	0	0
Gall bladder	0	0	0	2	0	0
Lymphoreticular Lymphoma	4	1	0	0	0	0
Larynx	10	1	0	0	0	0
Prostrate	0	1	1	0	0	0
Brain-Vestibular	0	0	0	0	1	0
Acute Myeloid Lymphoma (AML)	10	1	1	0	0	0
Multiple Myeloma	10	0	0	0	0	0
Myeloma	3	0	0	0	0	0
Buccal Mucosa	0	0	0	0	0	0
Multiple Lymphoreticular Lymphoma	1	1	1	0	0	0
Neuroendocrine	1	0	0	0	0	0
Chronic lymphocytic Leukaemia	2	0	0	0	0	1
Gingiva carcinoma	1	0	0	0	0	0
Kidney	1	1	0	0	0	0
Total	139 (64.6%)	18 (8.37%)	23(10.6%)	18(8.37%)	10(4.65%)	7(3.25%)

Head & Neck Cancer – Oral to Oesophagus - Tongue- Oropharynx, Larynx, Hypopharynx, Oral cavity, Buccal mucosa, Gingivum carcinoma, Lung, Thyroid, Oesophagus; Hematological Malignancies – Lymphoma, Lymphoreticular Lymphoma, Lymphoreticular Myeloma, ALL, AML, Multiple Myeloma, Myeloma, Multiple lymphoreticular lymphomas, Chronic lymphocytic Leukaemia (CLL); Gastro Intestinal Tract - Right colon, Stomach, Rectum, pancreatic; Genito Urinary tract Cancer – Bladder, Prostrate, Gall Bladder, Kidney; Gynecological Cancer – Uterus, Cervix, Ovary, Breast; CNS – Brain -Astrocytoma, Vestibular, Neuroendocrine; Osteosarcoma - Bone.

Table 2: Age and gender-wise distribution of *Candida Spp*. with cancer

Age	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	Total
Male	2	13	15	7	19	18	32	13	1	120 (55.8%)
Female	2	19	20	3	10	19	16	6	0	95 (44%)

Table 3: Distribution of *C. albicans* and non - *Candida* albicans spp.

Species	**No. of Isolates (%)**	**P- Value**
Candida albicans	139 (64%)	
Non - *Candida* albicans spp.	76 (35%)	0.0154
Total	215	

Table 4: Antifungal susceptibility pattern of *candida* isolates by disk diffusion assay

Candida spp .	**Total n =215**					
	Fluconazole		**Voriconazole**		**Amphotericin-B**	
C. albicans (n= 139)	Sensitive 139 (100%)	Resistant -	Sensitive 139 (100%)	Resistant -	Sensitive 139 (100%)	Resistant -
C.tropicalis (n= 18)	18 (100%)	-	18 (100%)	-	18 (100%)	-
C.krusei (n= 23)	1 (4.34%)	*—	1 (4.34%)	-	1 (4.34%)	-
C. glabarata (n= 18)	3 (16.6%)	15 (83.3%)	3 (16.6%)	-	3 (16.6%)	-
C.parapsilosis (n= 7)	6 (85.7%)	1 (14.28)	6 (85.7%)	-	6 (85.7%)	-
C.auris (n= 10)	-	3 (30%)	-	-	7 (70%)	-

As per CLSI guidelines- Fluconazole (25μg) > 19mm (Susceptible); <14mm (Resistant); Amphotericin B (20μg) > 10mm (Susceptible); <10mm (Resistant). Voriconazole (1 μg) 17mm (Susceptible); 13mm (Resistant).
* *C. krusei* are assumed to be intrinsically resistant to fluconazole and their MICs should not be interpreted using this scale.

Table 5: Antifungal susceptibility results and Minimum Inhibitory Concentration (MIC) range against 4 antifungal drugs as evaluated by the VITEK test and distribution of isolates of *Candida spp*.

Candida spp.	**Total number of isolates**	**MIC Range (μg/ml)**	**Range - Sensitive (%)**	**Resistant (%)**
C.albicans	18 (41.8%)			
Fluconazole		< 2 to >8	18 (100%)	0
Voriconazole		< 0.12 to >1	18 (100%)	0
Caspofungin		< 0.25 to >1	18 (100%)	0
Amphotericin B		< 0.5 to >2	18 (100%)	0
C. tropicalis	4 (93%)			
Fluconazole		< 2 to >8	4 (100%)	0
Voriconazole		< 0.125 to >1	4 (100%)	0
Caspofungin		< 0.25 to >1	4 (100%)	0
Amphotericin B		< 0.5 to >2	4 (100%)	0
C. parapsilosis	2 (4.6%)			
Fluconazole		< 2 to >8	0	2(100%)
Voriconazole		< 0.12 to >1	2(100%)	0
Caspofungin		< 2 to >8	2(100%)	0
Amphotericin B		< 0.5 to > 2	2(100%)	0
C. auris	2 (4.6%)			
Fluconazole		<4 to > 64	0	2(100%)
Voriconazole		<0.03 to > 16	0	2(100%)
Caspofungin		<0.25 to >8	0	2(100%)
Amphotericin B		>0.125 to >8	2(100%)	0
C. glabarata	6 (13.9%)			
Fluconazole		0.50 to >64	0	6(100%)
Voriconazole		-	6(100%)	0
Caspofungin		<0.12 to >0.5	6(100%)	0
Amphotericin B		<0.5 to > 2	6(100%)	0
C. krusei	11 (25.5%)			
Fluconazole		<1 to >64	11(100)	-
Voriconazole		< 0.5 to >2	11(100%)	0
Caspofungin		<0.25 to >1	11(100%)	0
Amphotericin B		<0.5 to > 2	11(100%)	0

Table 6: Matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF) analyzed with bruker daltonics.

Candida spp .	**No. of analysed isolates n=50**	**MALDI- TOF Score**		
		>2.0	**1.7 - 2.0**	**<1.7**
Candida albicans	16	9	5	2
Candida tropicalis	15	11	2	2
Candida krusei	0	0	0	0
Candida glabrata	6	3	3	0
Candida parapsilosis	2	1	1	0
Candida auris	4	4	0	0
Species not included in Databases	7	-	-	-
Total	50	28	11	4

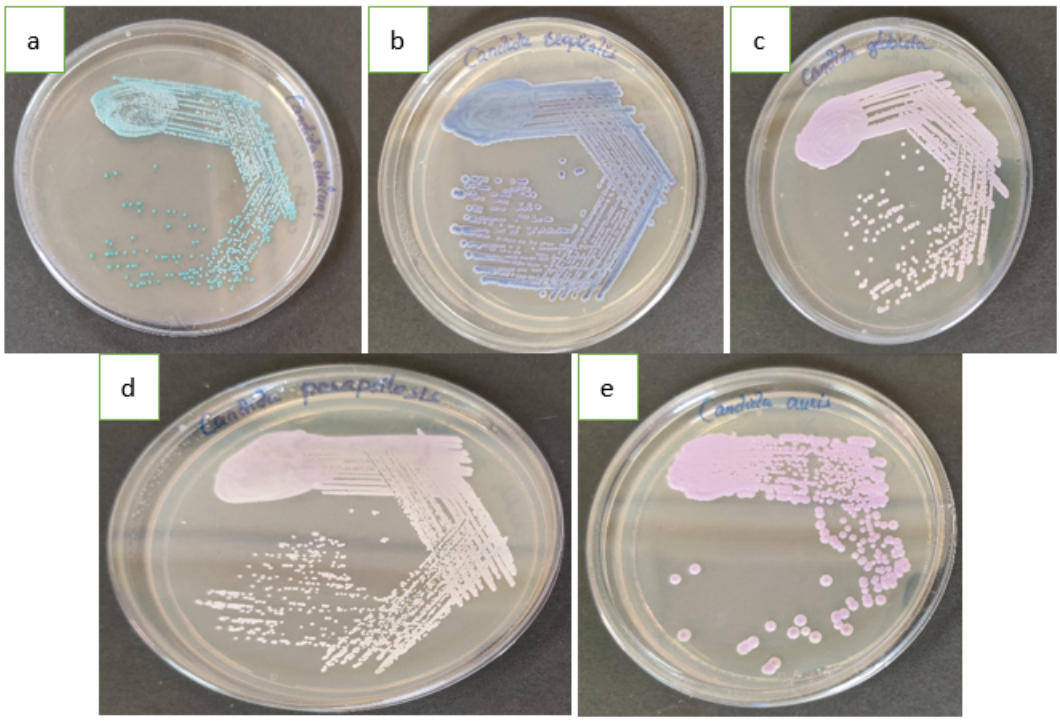


Fig. 1: ***Candida spp***

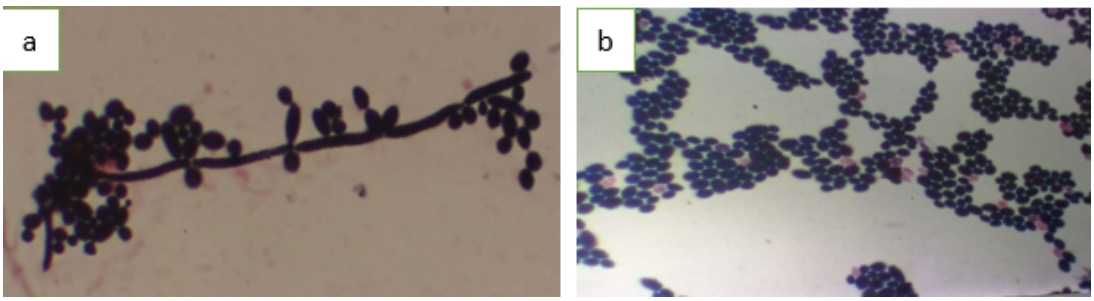
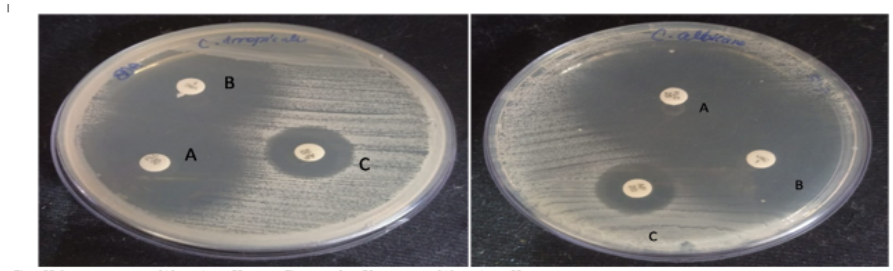


Fig. 2: Direct gram staining of *Candida spp*; **a:** Presence of pseudo hyphae; **b:** Round Blastocondia of *C. albicans*



***C. albicans* – sensitive to all** ***C. tropicalis* – sensitive to all** **(A. Fluconazole, B. Voriconazole, C. Amphotericin B)**

Fig. 3: Anti fungal susceptibility pattern of *candida* isolates by disk diffusion assay

Fig. 4: Distribution frequency of *candida spp*. obtained from various clinical samples

results, the MALDI-TOF-MS method exhibited a 90% accuracy rate in properly identifying the isolates. A score greater than 2.0 was indicative of a reliable identification of both the genus and species. Isolates with a score greater than 2.0 include *Candida* and non-*Candida spp*. Out of 215 isolates, 50 isolates were identified by MALDI-TOF-MS accounting for 23% of all isolates. *Candida albicans* 16 (7.44%) followed by *C. tropicalis* 15 (6.97%), C. glabrata 6 (2.79%), 4 (1.86%) isolated in *C. auris*, and 2 (0.93%) isolated in *C. parapsilosis* (Table 6).

4. Discussion

The prevalence of fungal infections has experienced a significant increase in recent decades.[19] Infections associated with *candida* have become more prevalent in ICU. These infections have been linked to a mortality rate of 47%.[20] Various risk factors have been identified for the development of *Candida* infections, including broad-spectrum antibiotics treatment, hemodialysis, pancreatitis, and treatment with steroids or other immunosuppressive drugs.[21] In our study, we observed a higher incidence of *Candida* infections in males (55%) compared to females (44%). This predominance of males may be attributed to the larger sample size of male patients in our study. Similar findings were reported, the researcher's also identified males are a risk factor for the development of candidemia.[22–25] In contrast to our study, researchers reported that females comprised 74.7% of the study cohort.[26] In our investigation, most clinical samples were obtained from Sputum (51%) followed by Urine (33.4%). This is in concurrence with an earlier study they documented that most of the clinical samples were obtained from urine with 32.4%.[27] Interestingly, in our study, none of the *C. albicans* were resistant to any of the antifungals employed, but an increased sensitive pattern of *C. albicans* was observed. Several studies have documented that *C.albicans* susceptibility may suggest that the drug regimen for the species should be improved.[28,29] According to earlier reports *C. albicans* was shown to be the major species responsible for *candida* infection.[30] Our reports also indicated that *Candida* species often exhibit susceptibility to Amphotericin B as an antifungal drug. Similar reports revealed that AFST of all isolates were sensitive to Amphotericin B, Voriconazole, and echinocandins.[31] Few reports also suggested that there was an increase of resistance in echinocandins, and their study also recommends Amphotericin B as a first-line therapy for the potential infection.[27,31] In Contradictory to our study, search suggested that the majority (98%) of *C. albicans* were susceptible only to fluconazole.[32] Based on previous reports it has been shown that non-albicans exhibit a higher level of resistance to fluconazole in comparison to *C. albicans*.[33–35] In our investigation, it was determined that 64% of the species belonged to *C. albicans*, which was shown to be the most often isolated species. The remaining 35% of the spp. were classified as non-albicans *candida spp*. This distribution was found to be statistically significant, as indicated by a p value of 0.0154. Previous findings found that 53% of *C. albicans* and 47% of non-albicans *candida* species showed similar research patterns followed by another report with 53% of *C. albicans* and 14% of NAC in their study.[1,36] In our investigation, MALDI-TOF-MS recognized 90% of the isolates. This is in accordance with earlier reports, in their study on clinical isolates that could enable 94% of sample identification.[11] Few reports successfully achieved a 100% identification of *Candida* using this methodology.[12] Based on the results obtained, it can be inferred that MALDI-TOF-MS is the quick and precise method for identifying species of *Candida* in Clinical isolates.[37]

5. Conclusion

The timely and precise detection of *Candida spp*. is crucial for the proper choice of antifungal medications and the delivery of optimum healthcare to patients. The primary objective of this study is to investigate the *Candida spp*. that are prevalent across a diverse cohort of individuals diagnosed with Cancer and their susceptibility patterns. The use of chrome ID as a means of identifying *Candida spp*. is both cost-effective and acceptable. Nevertheless, MALDI-TOF has some notable benefits like enhanced precision, efficient data capture, and prompt reporting, hence enabling faster detection of medical diagnoses in patients. We have also done a comparison between the VITEK 2 system and the disc diffusion test, it is notable that the disk diffusion is more economically viable and user-friendly. *Candida* albicans exhibited the highest prevalence and demonstrated significant sensitivity to all antifungal agents used in this investigation. According to our results, the MALDI-TOF-MS method exhibited a 90% accuracy rate in properly identifying the isolates. Therefore, this research suggests the use of MALDI TOF for the identification of *Candida spp*. and the incorporation of AFST using the disc diffusion method.

6. Authors' Contribution

All the authors were responsible for the study conception, design, analysis, and interpretation of data. The authors participated in drafting and revising the article and gave final approval for the version to be submitted.

7. Conflict of Interest

All authors declare that they have no conflict of interest concerning the research, authorship, and or publication of this article.

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