*Original Article*

Journal of Microbiology and Related Research
Volume 10 Number 1, January - June 2024
DOI: http://dx.doi.org/10.21088/jmrr.2395.6623.10124.2

# Clinical Identification & Anti-fungal Susceptibility of *Candida Species* by Vitek-2 System

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**How to cite this article:**

Sheetal Sharma, Vipin Kathuria, Kuldeep Yadav *et al.* Clinical Identification & Anti-fungal Susceptibility of *Candida Species* by Vitek-2 System. J Microbiol Relat Res. 2024;10(1):15–20.

**Abstract**

***Introduction:*** Among fungal infections, invasive candidiasis is often associated with increased number of morbidity and death rate. Candida species causing so many infections range from non fatal mucocutaneous infections to fatal blood stream infections, so the aim of this study is isolate and speciate the Candida species and their anti-fungal susceptibility to avoid unnecessarily consumption of anti-fungal drugs.

***Material & Methods:*** A Prospective study which was carried out in the Department of Microbiology, Pacific Institute of Medical Sciences Udaipur. Specimen collected from the various sites were collected and cultured on SDA agar and incubated. Identification of Candida species was done through Hi-Crome Candida Differential Agar and anti-fungal susceptibility was evaluated by VITEK-2 automated system and results were calculated through statistical analysis.

***Results:*** Out of 64 *Candida* isolates, 34.38% were *Candida albicans*, followed by *Candida tropicalis* (31.25%) *Candida krusei* (20.31%), *Candida kefyr* (12.50%) and *Candida glabrata* (1.56%). The most common *Candida* species isolated from urine were *Candida albicans* (34.24%) followed by *Candida tropicalis* (31.25%). Voriconazole (92.19%) and Amphotericin B (89.06%) were found the most sensitive drugs against the Candida isolates followed by Flucytosine (79.69%), Caspofungin (76.56%), Micafungin (70.31%) and Fluconazole (62.50%). All the *Candida kefyr* isolates were susceptible to Voriconazole, Amphotericin B and Flucytosine and resistant to Fluconazole. all *Candida glabrata* isolates were susceptible to all the 4 drugs except Micafungin and Caspofungin.

***Conclusion:*** The present study shows the distribution of *Candida species* in various clinical specimens and also revealed that *Non-albicans Candida* species are emerging as the predominant species. The increased resistance of *Candida* isolates towards common anti-fungal drugs which is a concern around all over the world.

**Keywords:** Composting; Plastic Bag; Plant Growth study; Physio-Chemical Properties; Nutrient Loss.

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**Received on:** 02.09.2024

**Accepted on:** 18.10.2024

## INTRODUCTION

The primary cause of the elevated rate of mortality and morbidity is fungus infections in the patients who are immunocompromised and in ICU patients. Among fungal infections, Candida is the commonest pathogenic organism causing invasive infections resulting in increased hospitalization and life threatening conditions.[1] Candida is yeast like fungus that produces pseudo-hyphae. *Candida* species are very common residents of skin, gut and genitals area but occasionally these fungi causes variety of infections, which ranging from the non fatal mucocutaneous infections to invasive Blood Stream Infections (BSI) or systemic infections in immunocompromised patients. From several studies of last few years, it is found out that non-albicans Candida species have now become predominant.[2]

There are about the 20 different species of the *Candida* that are known to cause infections in the humans.[3] Invasive *Candida* (IC) infections are broadly reported in critical patients, admitted in the ICU. The important risk factors for invasive Candidiasis included organ transplants, over consumption of broad-spectrum antimicrobial agents, prolonged Hospitalization, surgery, advanced life support and pugnacious chemotherapy, older age (over 60 years), chronic renal failure and diabetes mellitus, gastrointestinal or cutaneous colonization.[4] In recent years, the epidemiology of invasive candidiasis (IC) has gradually changed all over the world.[5] The *non-albicans Candida* species emphasized the significance of identification of the *Candida* isolate's infecting species for commencement of prompt and efficient therapy, particularly when anti-fungal susceptibility results are not readily available.[6] In these medical conditions, the commensal Candida may convert into opportunistic pathogenic microorganisms causing candidiasis in host.[7,8] The potential of clinical significance in the speciation has been acknowledged as Candida species shows dissimilarity in the expression of putative virulence factors and anti-fungal susceptibility.[9] Surveillance of fungal ecology and the anti-fungal resistance either within patients in ICU or within Candida species is necessary for the superintendence of invasive fungal infections.[10]

## MATERIAL AND METHODS

**Study place:** Department of Microbiology, Pacific Institute of Medical Sciences Udaipur.

**Study design:** Prospective study.

**Study duration:** One year

Total of 230 patients were tested for *Candida* infection. Patient's clinical samples including urine sample from mid-stream urine, nasal swab, End tracheal tube, Stool, Central line tip, Pus, Pleural fluid, Throat swab or sputum sample, skin scrapings from the infected part, blood samples and vaginal swabs etc. were collected as per SOP. All the specimens were cultured on Nutrient Agar, Blood Agar and MacConkey Agar for the primary inoculation. After incubation of 24-72 hours, the colonies grown on culture stained with gram stains and microscopy is done. If the mix growth is obtained than the colonies were separated by subculture on Saboraud's Dextrose Agar with antibacterial antibiotics incubated at 25°C and 37° C. Colonies are appeared in 1-3 days. Identification was done on the basis of colony morphology (On Sabouraud's Dextrose agar colonies were creamy white, smooth and with a yeasty odour) and microscopy. In microscopy purple budding yeast cells are seen. Further the identification of Candida species was done with various methods. The method of Candida species identification was Hi-Crome Candida Differential Agar. Inoculation was done for differentiating the Candida species based on the colour pigmentation on Hi-Crome Candida Differential Agar.

### *Anti-fungal susceptibility testing by automated method VITEK-2 (BioMerieux)*

We used the VITEK-2 to check the susceptibility of fungal agents against different fungal drugs. 3 ml of sterile normal saline taken in polystyrene tubes, picked a colony from culture of Candida isolates and it was mixed in the saline. It was mixed properly. The Densichek plus instrument provided by the BioMerieux was used to check the density of prepared inoculums up-to McFarland standard 2.0 for (VITEK-2 YST cards). Now the 280 ml of prepared 2.0 MacFarland suspension was picked with pipettes and mixed it with another tube with 3 ml of saline and mixed it properly by pipetting. VITEK-2 AST cards were placed into tubes. Tubes were placed with VITEK-2 AST cards in the cassettes and after inserting the cassettes into the VITEK-2 device, the corresponding yeast AST cards were loaded, incubated and automatically read by the device. The growth rate in the drug-free control well determined the length of incubation, which ranged from 18 to 20 hours. The outcomes were reported as MICs, or micrograms per millilitre. The density of the inoculums was checked by the

Densichek plus instrument was used to check the density of prepared inoculums.

**Fig. 1:** Automated Vitek-2 System (BioMerieux)

**Fig. 2:** SDA showing smooth colonies of *Candida albicans*

## RESULT

### Distribution of Candida species according to sample type

The most common sample from which Candida were isolated was urine (53.12%). Then other samples were sputum (20.30%) and vaginal swab (12.50%). Percentage of Candida species isolated from nasal swab were (4.68%), central line tip (3.12%), blood (1.57%), Et. Tube (1.57%), stool (1.57%) and pus (1.57%).

**Table 1:** Candida species distribution based on sample type

| No. | C. Species | C. albicans | C. tropicalis | C. krusei | C. kefyr | C. glabrata |
|---|---|---|---|---|---|---|
| 1 | Urine | 13 (38.24%) | 11 (32.35%) | 8 (23.53%) | 1 (2.94%) | 1 (2.94%) |
| 2 | Nasal swab | – | – | – | 3 (100%) | – |
| 3 | Vaginal swab | 4 (50%) | – | 3 (37.5%) | 1 (12.5%) | – |
| 4 | Blood | – | – | 1 (100%) | – | – |
| 5 | Et. Tube | – | 1 (100%) | – | – | – |
| 6 | Sputum | 5 (38.46%) | 5 (38.46%) | 1 (7.69%) | 2 15.38%) | – |
| 7 | Stool | – | 1 (100%) | – | – | – |
| 8 | Central line tip | – | 2 (100%) | – | – | – |
| 9 | Pus | – | – | – | 1 (100%) | – |
| 10 | Pleural fluid | – | – | – | – | – |
| | **Total** | **22 (34.38%)** | **20 (31.25%)** | **13 (20.31%)** | **8 (12.50%)** | **1 (1.56%)** |

Table 1 shows that the most common *Candida* species isolated were *Candida albicans* (34.38%), followed by *Candida tropicalis* (31.25%). Other Candida species isolated were Candida krusei (20.31%), Candida kefyr (12.50%) and *C. glabrata* (1.56%). It shows the most common *Candida* species isolated were *Candida albicans* (34.28%) from urine (38.24%) followed by sputum (38.46%). The 2nd most common isolated *Candida* species were *Candida tropicalis* (31.25%) from urine (32.35%) followed by sputum (38.46%). The other species isolated were *Candida krusei* most commonly from Urine (23.53%) followed by vaginal swab (37.5%). *Candida kefyr* (12.50%) were isolated most commonly from nasal swabs (100%). *Candida glabrata* were isolated from urine (2.94%). According to results the *non-albicans Candida* species are predominant in all the clinical samples.

**Table 2:** Antifungal susceptibility pattern of Candida isolates by automated method (VITEK-2)

| Drugs | Sensitive | Intermeduate | Resistance | TRM |
|---|---|---|---|---|
| **Fluconazole** | 40 (62.50%) | 0 | 24 (37.5%) | 0 |
| **Flucytosine** | 51 (79.69%) | 0 | 10 (15.63%) | 3 (4.69%) |
| **Voriconazole** | 59 (92.19%) | 0 | 0 | 5 (7.81%) |
| **Amphotericin B** | 57 (89.06%) | 0 | 0 | 7 (10.94%) |
| **Micafungin** | 45 (70.31%) | 0 | 0 | 19 (29.69%) |
| **Caspofungin** | 49 (76.56%) | 3 (4.69%) | 0 | 12 (18.75%) |

Table 2 shows the total sensitivity pattern by Automated Method and it revealed that Voriconazole (92.19%) and Amphotericin B (89.06%) were found the most sensitive drugs against the Candida isolates. The antifungal sensitivity patterns showed by other drugs were Flucytosine (79.69%), Caspofungin (76.56%), Micafungin (70.31%) and Fluconazole (62.50%). 4.69% isolates show intermediate sensitivity for Caspofungin. Some drugs Micafungin in 29.69% isolates and Caspofungin in 18.75%, Amphotericin B in 10.94%, Voriconazole in 7.81% and Flucytosine in 4.69% isolates were terminated by Machine.

**Table 3:** Antifungal susceptibility pattern in various Candida species by automated method VITEK-2.

| Drugs | Candida Albicans | | Candida Tropicalis | | Candida Krusei | | Candida Kefyr | | Candida Glabrata | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sensitive | I/R/ TRM | Sensitive | I/R/ TRM | Sensitive | I/R/TRM | Sensitive | I/R/TRM | Sensitive | I/R/TRM |
| **Fluconazole** | 17 (77.27%) | 5 R (22.73%) | 19 (95%) | 1 R (5%) | 2 (18.18%) | 11 R (81.82%) | 0 | 8 R (100%) | 1 (100%) | 0 |
| **Flucytosine** | 20 (90.91%) | 2 TRM (9.09%) | 20 (100%) | 0 | 3 (23.08%) | 10 R (76.92%) | 8 (100%) | 0 | 1 100%) | 0 |
| **Voriconazole** | 17 (77.27%) | 5 TRM (22.73%) | 20 (100%) | 0 | 13 (100%) | 0 | 8 (100%) | 0 | 1 100%) | 0 |
| **Amphotericin-B** | 20 (90.91%) | 2 TRM (9.09%) | 17 (85%) | 3 TRM (15%) | 12 (92.31%) | 1 TRM (7.69%) | 8 (100%) | 0 | 1 100%) | 0 |
| **Micafungin** | 20 (90.91%) | 2 TRM (9.09%) | 20 (100%) | 0 | 6 (46.15%) | 7 TRM (53.85%) | 0 | 8 TRM (100%) | 0 | 1 TRM (100%) |
| **Caspofungin** | 20 (90.91%) | 2 TRM (9.09%) | 20 (100%) | 0 | 9 (69.23%) | 3 I/1 TRM | 0 | 8 TRM (100%) | 0 | 1 R (100%) |

Table 3 shows, the pattern of anti-fungal susceptibility in different *Candida* species using VITEK-2. According to the pattern, Candida albicans was most responsive to fluconazole and voriconazole (77.27%), followed by flucytosine, amphotericin B, Micafungin and capsofungin (90.91%). Candida tropicalis was most sensitive to Flucytosine, Voriconazole, Micafungin, Caspofungin (100%), followed by Fluconazole (95%) and Amphotericin B (85%). Candida krusei was most sensitive to Voriconazole (100%) and Amphotericin B (92.31%), followed by Caspofungin (69.23%) and Micafungin (46.15%). Candida kefyr showed complete sensitivity (100%) against

Flucytosine, Voriconazole and Amphotericin B. Candida glabrata showed complete sensitivity (100%) against Fluconazole, Flucytosine, Voriconazole and Amphotericin B.

## DISCUSSION

*Candida* infections are among the most prevalent causes of the morbidity and mortality in the whole world. Among them *Candida* is also a major concern worldwide due to its increasing incidence and also the increased resistance towards drugs. Fast and very accurate identification of *Candida species* and their anti-fungal susceptibility pattern are of great importance for the selection of appropriate anti-fungal agent and for patient management. The very common *Candida species* isolated were *Candida albicans* (34.28%) from urine (38.24%) followed by sputum (38.46%) shows very similarity with the results of A. Rengaraj. *et al.* study in 2019.The present study reported that the burden of *Candida albicans* species and non-albicans species to be 34.38% and 65.62% respectively. In our study the *Non-albicans Candida* species showed dominance on *Candida albicans* species. Which is similar to Reshma Bhaskaran *et al.* study in 2020, also showed predominance of non-albicans *Candida* species over *Candida albicans* species.

In many previous studies *Candida albicans* was still the most predominant isolated species as showing in some recent studies also Seyoum E. *et al.* study in 2020 comparatively to non *albicans Candida* species which shows quite differences from our study and the reason behind differences occurred in the studies or this shifting the trend of *Candida species* could be the geographical area, age, different hospital settings, different departments, environmental conditions, co-morbidity, immunosuppression, underlying diseases and antibiotic therapies etc.

In our study Vitek-2 is used to evaluate the anti-fungal susceptibility of all candida isolates and it revealed that In Our study all the Candida isolates showed high susceptibility towards Voriconazole (92.19%) which is co-relatable with studies Singh R *et al.*[14], Sundaram M. *et al.*[16] and Seyoum *et al.* Amphotericin B (89.06%) were found the second most sensitive drugs against the Candida isolates and results of our study co-relate with Kaur R *et al.*[14] and Sundaram M. *et al.* For Micafungin and Caspofungin results are correlated well with the study Sundaram M. *et al.*In our study Fluconazole shows less sensitivity against Candida isolates (62.50%) which showed difference with the result in Elias Seyoum *et al.* study in 2020, in which 85.6% of Candida species were susceptible to Fluconazole.

Flucytosine (79.69%), Caspofungin (76.56%) and Micafungin (70.31%) also showed good sensitivity against all the candida isolates. The shifting pattern of Candida species' distribution among isolates and altered anti-fungal susceptibility patterns by the time is the major concern worldwide that's why to prevent these alterations, a diagnosis of the Candida species and an assessment of the susceptibility to antifungal are therefore essential.

## CONCLUSION

The current study demonstrated the distribution of different *Candida* species in a range of clinical samples. It also showed that non-albicans *Candida* species are becoming more common and that isolates of *Candida* are becoming more resistant to common anti-fungal medications, which is a global concern. The use of conventional methods for identification and anti-fungal testing of *Candida species,* these methods are not only time consuming but also labour intensive. Commercially available automated systems provide a good option. However their use in a resource constrained setting has not been extensively studied. The VITEK 2 system ensures that each test is performed in a standardized manner and provides quantitative MIC results are very reproducible and so accurate. Use of VITEK 2 will help reduce the turnaround time for identification and susceptibility and reduce labour. When the benefits in terms of cost, labour and ease of performance was considered, VITEK 2 can be a preferable option in resource constrained settings.

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