

## EVALUATION OF VITEK 2 SYSTEM FOR CLINICAL IDENTIFICATION OF CANDIDA SPECIES AND THEIR ANTIFUNGAL SUSCEPTIBILITY TEST

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### ABSTRACT

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#### OBJECTIVES

1. To evaluate the Vitek 2 system for clinical identification of Candida species and their antifungal susceptibility test; 2. To study the incidence of various types of Candida species in this part of Tamilnadu.

#### METHODS

Samples collected from different wards were subjected for culture, isolation and identification of Candida Species and Antifungal Susceptibility testing by Vitek System. Vitek 2 test was carried out in Apollo Specialty Hospital Lab Services, Madurai. The cost per test is Rs. 200 (Subsidized rate). The expenses for the lab tests (Vitek) were borne by the author himself.

#### RESULTS

124 samples were collected from urine, sputum, blood, pus and wounds. Candida albicans formed 43% of the samples. Among the 57% of Non-Candida albicans, Candida tropicalis formed 42%, Candida krusei formed 6%, Candida guilliermondii formed 4%, Candida inconspicua, Candida parapsilosis, Candida glabrata, Candida rugosa and Candida lusitaniae formed 1% each. Candida albicans and C. tropicalis showed high sensitivity to Voriconazole, Flucytosine, Amphotericin B and Fluconazole.

#### CONCLUSION

Candida tropicalis was identified as the most common Candida non-albicans species. Candida albicans and C. tropicalis showed high sensitivity to Voriconazole, Flucytosine, Amphotericin B and Fluconazole. This study was helpful to treat Candida albicans and Non-Candida albicans species patients accurately and earlier by Vitek method.

#### KEYWORDS

Candida Albicans, Non-Candida Albicans, Vitek 2, Chrome Agar.

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#### INTRODUCTION

Candida is a genus of yeasts and is the most common cause of fungal infections worldwide.

The Candida species are the 4th most common organisms causing blood stream infection and constitute 8% of all nosocomial infections.<sup>1</sup> Candidiasis is mainly caused by C. albicans, while there has been striking increase in the frequency with Non C. albicans. The most important species which are considered pathogenic to humans are C. albicans, C. tropicalis, C. krusei, C. glabrata, C. lusitaniae, Candida dubliniensis, Candida guilliermondii, Candida parapsilosis, Candida kefyr.<sup>2</sup> HiCrome Candida Differential Agar is a selective and a differential medium, which allows differentiation of Candida species namely C. albicans, C. krusei, C. tropicalis and C. glabrata on the basis of colouration and colony morphology.<sup>3</sup> The Vitek 2 system accommodates the reagent cards that are incubated and interpreted automatically.<sup>4</sup> The Candida Species sensitivity was observed with Voriconazole, Flucytosine Amphotericin B, Fluconazole, Micafungin and Caspofungin.

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#### MATERIALS AND METHODS

##### Type of Study

Cross sectional, observational, prospective study.

##### Settings

The study was conducted in a tertiary care teaching hospital, Vinayaka Mission's Medical College Hospital, Karaikal. Vitek 2 test was carried out in Apollo Specialty Hospital Lab Services, Madurai. The cost per test is Rs. 200 (Subsidized rate). The expenses for the lab tests (Vitek) were borne by the author himself.

##### Sample Size

124 samples were collected from urine, sputum, blood, pus and wounds.

##### Study Period

From March 2015 to March 2016.

##### Inclusion Criteria

Patients not treated with any antifungal treatment.

##### Exclusion Criteria

Patients already treated with any antifungal treatment.

#### METHODOLOGY

Present study was carried out in a tertiary care hospital at Vinayaka Mission's Medical College Hospital, Karaikal, for a period of one year from March 2015 to March 2016. The

clinical samples isolated from blood, urine, pus, endotracheal secretions and wounds were submitted to the laboratory. Those specimens suspected of fungal infection during the study period were processed. The samples were transported to the laboratory and streaked on Blood Agar medium and plate of Sabouraud Dextrose Agar (SDA) with chloramphenicol and gentamicin.

Blood agar and SDA agar were incubated at 37°C. After incubating for 48 hours, typical creamy pasty colonies from which “feet” extending out from the margins into the surrounding agar appeared on the blood agar and SDA.

On observing Gram staining from SDA and Blood Agar plates, Candida species appeared as gram positive budding yeast cells with Pseudohyphae, Chlamyospore and blastoconidia.

The germ tube test was done for identifying *C. albicans*; 0.5 mL of human serum was taken into a small tube. The colony of yeast was touched with a Pasteur pipette. It was emulsified in the serum and incubated at 37°C for 2 hours. A drop of this serum was transferred to a slide for examination. With the coverslip on, slide was examined under a microscope using low and high power objectives.

Candida isolates isolated from blood agar, SDA plates were streaked in CHROMagar™ Candida agar and incubated at 37°C for 48 hrs. The CHROMagar™ allows identifying colonies of *C. albicans*, *C. dubliniensis*, *C. tropicalis* and *C. krusei* by morphology and colour reaction.

*C. albicans* or *C. dubliniensis* appeared as green colonies and *C. tropicalis* as steel blue colonies. *C. krusei* colonies showed rose colour. The other species formed white to rose colonies.

The speciation of Candida species was done with automated methods. The Vitek 2 was used to identify the Candida Species with Vitek 2 YST Card and anti-fungal susceptibility Card with Vitek 2 AST-YS07 Card. The cost per test is Rs. 200 (Subsidized rate). The expenses for the lab tests (Vitek) were borne by the author himself.

**For Sample Preparation**

24 hr. old culture was tested on the instrument. Vitek 2 compact cassette with labelled barcode was selected. Cassette number was defined. The polystyrene tubes were placed in the cassette; 3 mL of sterile Vitek saline was taken in the polystyrene tubes. The colonies were isolated from the plate and suspended in Vitek saline (3 mL) with help of sterile loop. The suspension was made uniform thoroughly. The density of the inoculums was checked by the Densichek plus. McFarland standard (Vitek 2.20 YST and Vitek 2 YST Card) was checked by the Densichek plus provided by the BioMerieux. The cards were then ready for inoculation. Vitek 2.20 YST and Vitek 2 YST Cards pre-inserted were transferred from the respective suspensions into the selected polystyrene tubes. All the inoculums in cassette were ready to be loaded into the instrument in filler section.

**Sample Loading**

The cassette was loaded into the Filler section. The door was closed. The Fill on the User Interface Screen was pressed. It was (Filling) completed in 70 seconds cycle. When the filling cycle was finished, the blue indicator light on the instrument flashed. The cassette was loaded into the Load section. (It was done within 10 minutes of removing from the Filling section). Barcodes were scanned and checked against the Maintain

Virtual Cassette electronic Work list. Straws were cut and sealed. Cards were loaded into the carousel. Cassette waste discarded. It was indicated by flashing blue arrow on the instrument. The loading was thus finished.

**Result Reading**

In this case, Vitek 2 YST was evaluated using 150 isolates of both commonly and rarely observed species of Candida. The result generated as genus-level, group-level identification, while the Vitek 2 AST-YS07 Card evaluated the sensitivity and resistance of Amphotericin B, Fluconazole, Micafungin, Caspofungin, Voriconazole, and Flucytosine.

The final profile results were compared with the database and the identification of the unknown organism was obtained. A final identification of “excellent,” “very good,” “good,” “acceptable” or “low discrimination” was considered to be correct.

**RESULTS**

Age Group	Total No.=124	Percentage
1-10 yrs.	6	5
11-20 yrs.	1	1
21-30 yrs.	12	9
31-40 yrs.	22	18
41-50 yrs.	24	19
51-60 yrs.	22	18
61-70 yrs.	20	16
71 and above	17	14

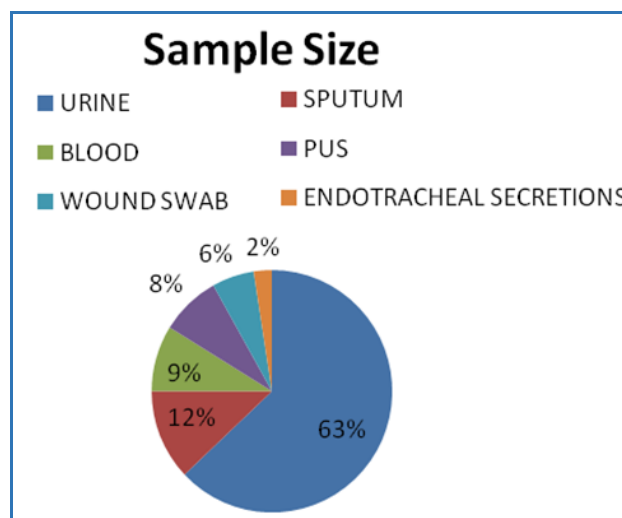
**Table 1: Age Wise Isolation of Candida Species (n=124)**

Table 1 shows out of 124 isolates, 6% isolates were from patients below 20 yrs., 64% were from patients between 21-60 yrs. and 30% were from patients above 60 yrs.

Sex	Total No. of Isolates (n=124)	Percentage of Isolates
Female	78	63
Male	41	33
Male children	4	3
Female Children	1	1
<b>Total</b>	<b>124</b>	<b>100</b>

**Table 2: Gender Wise Isolation of Candida Species (n=124)**

Table 2 shows out of 124 isolates, 63% of isolates were from females, 33% were from males, 3% were from male children and 1% was from female children.



**Fig. 1: Sample Wise Isolation n=124**

Fig. 1 shows out of 124 isolates, 63% were from urine samples, 12% were from sputum, 9% were from blood, 8% were from pus samples, 6% were from wound swabs and 2% were from endotracheal secretions.

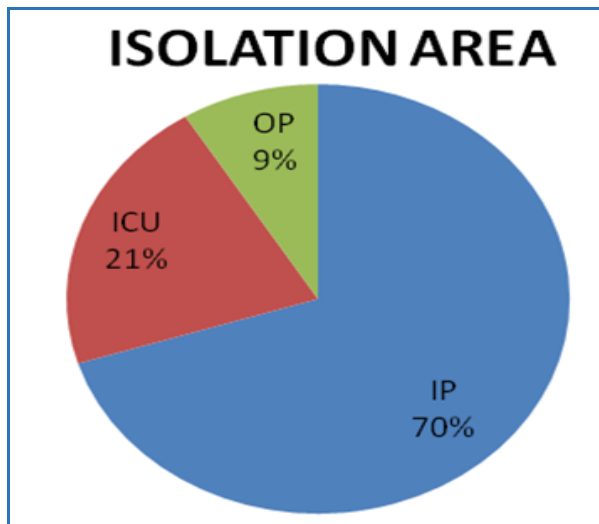


Fig. 2: Area Wise Isolation (n=124)

Fig. 2 shows out of 124 isolates, 70% were from In-patients, 21% were from ICU, 9% were from Out-patients.

Candida Species	Total No. of Isolates n=124	Percentage of Isolates
Candida albicans	53	43
Candida tropicalis	52	42
Candida krusei	7	6
Candida guilliermondii	5	4
Candida inconspicua	2	1
Candida parapsilosis	1	1
Candida glabrata	1	1
Candida rugosa	1	1
Candida lusitaniae	2	1
<b>Total</b>	<b>124</b>	<b>100</b>

Table 3: Isolation of Candida Species (n=124)

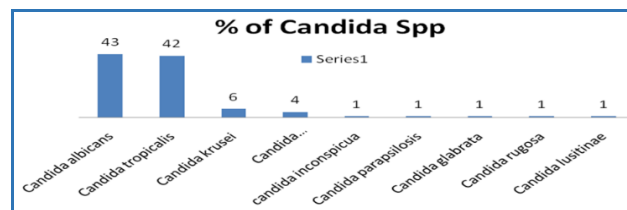


Fig. 3: Isolation of Candida Species (n=124)

Table 3, Fig. 3 shows out of 124 isolates isolated Candida albicans were 43%, Candida tropicalis were 42%, Candida krusei were 6%, Candida guilliermondii were 4%, Candida inconspicua, Candida parapsilosis, Candida glabrata, Candida rugosa and Candida lusitaniae were 1% each.

Candida Species	C. albicans (n=53)	C. tropi calis (n=52)	C. guiller mondi (n=5)	Candida krusei (n=7)	C. lusitaniae (n=2)	C. parap silosis (n=1)	C. rugosa (n=1)	C. inconspicua (n=2)	C. glabrata (n=1)
Voriconazole	49(92%)	48(92%)	3(60%)	6(85%)	2(100%)	1(100%)	1(100%)	2(100%)	0%
Caspofungin	40(75%)	38(73%)	3(60%)	6(85%)	2(100%)	1(100%)	1(100%)	2(100%)	0%
Micafungin	42(78%)	41(79%)	3(60%)	6(85%)	2(100%)	1(100%)	1(100%)	2(100%)	0%
Amphotericin B	47(89%)	44(85%)	4(80%)	6(85%)	2(100%)	1(100%)	1(100%)	2(100%)	0%
Flucytosine	49(92%)	44(85%)	4(80%)	1(15%)	2(100%)	1(100%)	1(100%)	2(100%)	0%
Fluconazole	44(81%)	40(77%)	4(80%)	1(15%)	2(100%)	1(100%)	1(100%)	2(100%)	0%

Table 4: Antifungal Sensitivity Pattern of Candida Isolates (n=124)

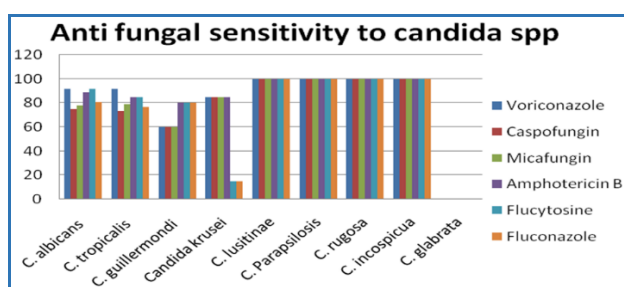


Fig. 4: Antifungal Sensitivity Pattern of Candida Isolates (n=124)

Table 4, Fig. 4 shows out of 53 isolates, Candida albicans which showed sensitivity to Voriconazole were 92%, Flucytosine were 92%, Amphotericin B were 89%, Fluconazole were 81%, Micafungin were 78%, Caspofungin were 75%.

Out of 52 isolates, Candida tropicalis showed sensitivity to Voriconazole were 92%, Amphotericin B were 85%, Flucytosine were 85%, Micafungin were 79%, Fluconazole were 77%, Caspofungin were 73%.

Out of 7 isolates, Candida krusei showed sensitivity to Voriconazole, Caspofungin, Micafungin and Amphotericin B were 85% each, Flucytosine and Fluconazole were 15% each.

Out of 5 isolates C. guilliermondii showed sensitivity to Voriconazole, Caspofungin and Micafungin were 60%; Amphotericin B, Flucytosine and Fluconazole were 80% each.

Out of 2 isolates C. lusitaniae showed 100% sensitivity to Caspofungin, Micafungin, Voriconazole, Amphotericin B, Flucytosine and Fluconazole each.

One isolate of C. parapsilosis, C. rugosa, C. inconspicua showed 100% sensitivity to Caspofungin, Micafungin, Voriconazole, Amphotericin B, Flucytosine and Fluconazole each.

One isolate of Candida glabrata showed 100% resistance to Caspofungin, Micafungin, Voriconazole, Amphotericin B, Flucytosine and Fluconazole each.

**DISCUSSION**

In our study out of 124 isolates isolated, 43% were Candida albicans and 57% were Non-Candida albicans. Non-Candida albicans were isolated more in numbers than C. albicans. Rachana Mehta et al, has reported that Candida albicans was

the commonest species isolated (40.9%). The isolation of Non-Candida albicans (59.1%) predominated over Candida albicans (40.9%).<sup>5</sup> This study correlates with our study.

In our study out of 124 isolates, isolated Candida albicans were 43%. "Among 57% of Non-Candida albicans, the Candida tropicalis were 42%, Candida krusei were 6%, Candida guilliermondii were 4%, Candida inconspicua, Candida parapsilosis, Candida glabrata, Candida rugosa and Candida lusitanae were 1% each." Dr. Jayalakshmi L et al,<sup>6</sup> has reported that the commonest Candida isolate was C. albicans (31.42%) followed by C. tropicalis (26.66%). Other species isolated were C. glabrata (19.04%), C. parapsilosis (10.47%), C. krusei (5.71%), C. kefyr (4.76%) and C. guilliermondii (1.9%). The most common Non-Candida albicans isolates were C. tropicalis, C. Krusei and C. guilliermondii. This study correlates with our findings.

Candida tropicalis species has emerged as the major Candida non-albicans species (43%) in our study. Our study correlates with the study by Patel et al (40.9%), and Kashid et al (46.25%) who have also reported Candida tropicalis as the most common Candida non-albicans species.<sup>7,8</sup>

In our study, out of 53 isolates Candida albicans which showed sensitivity to Voriconazole were 92%, Flucytosine were 92%, Amphotericin B were 89%, Fluconazole were 81%, Micafungin were 78%, Caspofungin were 75%. Gulgun Yenisehirli et al has reported that all the tested C. albicans isolates were found to be susceptible to amphotericin B; 85% of the isolates were classified as showing susceptibility to caspofungin. The sensitivity rates of C. albicans isolates to fluconazole and voriconazole were 74% and 86% respectively. The Candida albicans showed sensitivity to Amphotericin B, Fluconazole, Voriconazole.<sup>9</sup> This study bears a good correlation with our study.

In our study, out of 52 isolates Candida tropicalis showed sensitivity to Voriconazole were 92%, Amphotericin B were 85%, Flucytosine were 85%, Micafungin were 79%, Fluconazole were 77% and Caspofungin were 73%. Pasquale T et al, has reported that C. tropicalis is usually susceptible to the Voriconazole, Flucytosine and amphotericin B.<sup>10</sup> In our study Candida tropicalis showed high sensitivity to Voriconazole, amphotericin B and Flucytosine. Thus, our study has similar findings.

In our study, out of 7 isolates Candida krusei showed sensitivity to Voriconazole, Caspofungin, Micafungin and Amphotericin B were 85% each, Flucytosine and Fluconazole were 15% each. Orozco AS et al has reported that C. krusei is intrinsically resistant to fluconazole.<sup>11</sup> Our study correlates well with the study of Orozco AS et al.

In this study, out of 5 isolates C. guilliermondii showed sensitivity to Voriconazole, Caspofungin and Micafungin were 60%, Amphotericin B, Flucytosine and Fluconazole were 80% each. Girmenia C et al has reported that C. guilliermondii is an uncommon Candida species. This species has reduced susceptibility to Fluconazole.<sup>12</sup> However, C. guilliermondii is usually susceptible to Amphotericin B.

## CONCLUSION

The conventional methods of identification of Candida species are time consuming and difficult to perform. Candida tropicalis was identified as the most common Candida non-albicans species. Candida albicans and C. tropicalis showed high sensitivity to Voriconazole, Flucytosine, Amphotericin B and Fluconazole. This study was helpful to treat Candida

albicans and non-Candida albicans species patients accurately and earlier by Vitek method. Rapid, accurate susceptibility test results and resistance detection are clinically relevant. This purpose is well served by YST cards with VITEK® 2 instruments.

## LIMITATIONS

This is a single centered study.

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