

# Utility of VITEK2 in Rapid Speciation and Antifungal Susceptibility Testing of Yeasts in a Resource Constrained Setting

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## ABSTRACT

**Introduction-** Incidence of invasive Candidiasis is on a rise. *C.albicans* is the most common species implicated, however recently increase in infection due to Non Albicans Candida species has also been noted. Empirical use of antifungal agents has led to emergence of resistance. The newer Non albicans *Candida* species are known to be inherently resistant to some of the antifungal agents. Hence it is necessary to speciate and determine the antifungal susceptibility pattern of pathogenic Candida species. The conventional methods of identification and antifungal susceptibility are labour intensive and time consuming Newer automated methods are easy to perform and help reduce the turnaround time.

**Aim and Objectives-** This study was carried out to access the usefulness of VITEK2 in identification and AFST of yeast in a resource constrained setting with respect to TAT and cost.

**Materials and Method-** Species identification and antifungal susceptibility profile of 100 yeast isolates recovered was determined using conventional methods and VITEK2. The results obtained by both methods were analysed for Concordance, TAT and Cost

**Results-** 95% essential agreement was observed between VITEK2 and conventional method. For fluconazole the essential agreement was found to be 96% and for amphotericin B, it was 98%. TAT by VITEK2 was significantly lower compared to conventional method. The cost per test was higher for VITEK2.

**Conclusion-** VITEK2 is a reliable, feasible, time saving alternative for identification and antifungal susceptibility of the yeasts.

**Keywords-** Non Albicans Candida species, Antifungal resistance, VITEK2, reliable, feasible

## INTRODUCTION

There has been a rise in the incidence of invasive fungal infections especially those caused by *Candida spp.* This can be attributed to a number of factors some of which include increase in the absolute number of immunocompromised patients, patients on cancer chemotherapy and post organ transplant patients on immunosuppressant. Prolonged use of antibiotics in hospitalized patients is major

risk factor for development of fungemia.(1)(2)(3)

*Candida albicans* is the most common species isolated. It the fourth most common cause of blood stream infections in hospitalized patients.(4) The other *Non-albicans Candida* species previously considered to be non-pathogenic are now increasing in frequency and gaining a foot hold. These include *C.tropicalis*, *C.parapsilosis*, *C.glabrata*, *C.krusie*,

*C.kefyr*, *C.lusitanaie*, *C. guilliermondii*, *C.famata*, *C.rugosa*, *C. inconspicua*, *C. rugosa*, *C. dubliniensis*, and *C. Norvegensis* (5)(6) The isolation rate of some of the rarely isolated NAC has increased between 2- and 10-fold over the last 15 years.(3)(4). The most recent one posing a threat to the health care system is *C.auris*.(6) Apart from *Candida* species other yeasts that are less commonly found include *Cryptococcus spp*, *Rhodotorula spp*, *Malassezia*, *Blastochizomyces spp*, *Trichosporon species*. (7)

Increasing use of antifungals for empirical treatment of fungal infections has led to development of resistance to the existing antifungals agents in some of the previously sensitive *Candida* species. The newer emerging *Candida* species are known to be inherently resistance to antifungal agents. Acquired resistance to azoles is commonly seen with *Candida glabrata* and intrinsic resistance azoles is found in *C. krusei*. *C. lusitaniae*, *C. haemulonii* are known to be intrinsically resistant to Amphotericin(5). *C.auris*, known to be multi-drug resistant demonstrates increased MIC to azoles as well as echinocandins like capsosfungins.(6)(8)

Appropriate and timely administration of antifungal agents helps to reduce mortality. Starting the patient on empiric antifungal therapy however does not guarantee treatment success. One of the reasons attributed is interspecies variations in susceptibility pattern and developing resistance in previously susceptible species. (1)(9) The specificity in the antifungal profile of different *Candida* species makes it necessary to speciate and determine the antifungal susceptibility pattern for improving patient care.(10)

The conventional methods of identification of yeasts include gram stain, germ tube morphology on corn meal agar, colour on CHROM agar, sugar assimilation test. The turnaround time for these tests are 48-72 hrs. The various methods for performing antifungal susceptibility test include dilution method, disc diffusion, E-

test.(4) These tests are not only cumbersome but also time taking resulting in delayed reporting of results. (9)(11) The gold standard for identification remains DNA sequencing of the D1/D2 region .

Newer automated method that not only reduces the turnaround time but is also cost-effective is the need of hour.API -ID, Pheonix BD, MicroScan, VITEK2, MALDI-TOF are some of the newer methods that have help overcome the fallacies of conventional methods.

BioMerieux developed VITEK2 is commercially available automated method that works on the principle of spectrophotometry and uses visible light to directly measure organism growth. It uses several parameters based on the growth characteristics observed are used to provide appropriate input for the MIC calculations of anti-fungal agents. (12) The system is highly reproducible and accurate.(11)(13)

This study was carried out to access the usefulness of VITEK2 in identification and AFST of yeast in a resource constrained setting with respect to TAT and cost.

## MATERIALS AND METHODS

**Study design-** After obtaining permission from the institutional ethics committee, a prospective study was carried out in Dept of Microbiology of a Tertiary care teaching institute over a period of one year July 2014- June 2015.

Consecutive, non-duplicate 100 isolates of yeast obtained from various clinical specimen, during the study period was included. The yeast isolate was identified using conventional method and Automated VITEK 2.

The yeast isolates obtained were subcultured on Sabouraud Dextrose agar (SDA). Gram positive round to oval budding cells were identified as yeasts and processed for further speciation. Germ tube test was performed as per the standard technique. Germ tube test was considered to be positive if the isolate showed presence of true hyphae characterized by apical elongation, long terminal cell, no

constriction and absence of septum. *Candida albicans* and *Candida dubliniensis* give a positive germ tube test result and all other *Candida spp* are negative. The yeasts were further speciated based on the morphological arrangement of blastoconidia and pseudohyphae on corn meal agar using the Dalmau technique. Sugar assimilation tests was performed using modified Wickerham plate technique.

Antifungal susceptibility testing was performed using the conventional micro broth dilution method and automated VITEK 2. The isolates were tested for the susceptibility to Fluconazole (0.25µg to 64 µg) and Amphotericin B (0.03 µg to 16 µg) as per CLSI M27-A2 by micro broth dilution. Minimum inhibitory concentration of each of the drugs were noted. Ten concentrations were tested for each of the antifungal agents testing. Minimum inhibitory concentration is the lowest concentration of the antimicrobial agent that inhibits visible growth.

All the reagents and media required to perform the test were procured from Himedia, Mumbai India. The antifungal powders were procured from Sigma-Aldrich, Mumbai. The stock solution of the antifungal agent was prepared by weighing appropriate amount of antifungal agent and dissolving it in appropriate diluent eg. fluconazole being soluble was dissolved in water and amphotericin B in Dimethylsulphoxide.

0.5 McFarland inoculum was prepared and diluted 1:50 in RPMI, which was further diluted to 1:20 giving a final inoculum concentration of  $10^3$  cells /ml. 100µl of inoculum was added along with 100 µl of drug suspension of varying concentration in each well. One well each was put up as growth control and drug control. The microtitre plates were incubated at 37°C for 24hrs. First reading was taken at the end of 24 hrs. The plates were further incubated for an additional 24 hrs and a reading was taken at the end of 48 hrs. The readings were considered only if there was growth detected in the drug free

well at the end of 24 hrs and 48 hrs. In cases where there was no growth in the drug free well at the end 48 hrs, the test was considered invalid and repeated. The first visibly clear well was considered as MIC.

### Identification and susceptibility testing using Vitek2

2 McFarland inoculum suspension was prepared and as per the requirement ID/AST cards were inserted in the inoculum suspension and the cassette along with the suspension was loaded into the instrument. The Vitek2 instrument was automatically filled, sealed, and incubated by individual test cards with prepared culture suspension. Cards were held at 35.5°C for 18 h, with optical density readings taken automatically at every 15 min. Based on these readings, an identification profile was established and interpreted according to a specific algorithm.

For antifungal susceptibility testing the loaded cassettes were placed into the Vitek2 instrument, and the respective inoculum suspensions were diluted appropriately by the instrument, after which the cards were filled, incubated, and read automatically.

The results obtained by the conventional method and automated VITEK 2 system were compared and analysed. The essential and categorical agreement, very major error, major error and minor error was calculated for fluconazole(11). For amphotericin B since the interpretative breakpoints have not been defined by CLSI, the essential agreement and, the substantial and non-substantial difference was calculated.(13) The statistical significance between the MIC value obtained at 24 hrs and 48 hrs for fluconazole and amphotericin B was calculated using McNemar test. The statistical significance between the difference in TAT by conventional and VITEK 2 for identification and AFST was calculated using paired t test.

## RESULTS

100 yeast isolates were processed for identification and antifungal susceptibility to Fluconazole and Amphotericin B by both conventional and VITEK 2 system. Of the 100 isolates tested all (100%) were identified unequivocally to species level by in conventional phenotypic methods. VITEK 2™ identified two isolates with low discrimination and one isolate remained unidentified. 44% isolates were identified as *C.tropicalis*, 29% as *C.albicans*, 7% as *C.glabrata*, 12% as *C.parapsilosis*, 1% each of *C.guilliermondii* and *C.lusitanaie*, 6% as *Trichosporon spp*, by conventional method. In addition to the strains identified by conventional method, 2% of the isolates were identified as *C.famata* and *C.haemulonii*.

93 % isolate identification was concordant by both techniques and 7% were discordant.

Two isolates identified with low discrimination by VITEK 2 (one *C.tropicalis* and one *C.parapsilosis*), were resolved using additional testing methods based on morphological arrangement of blastoconidia, and were in concordance with

the phenotypic method. Total 95% isolates were concordant by two techniques.

Table 1 – Comparison of the results of discordant isolates

Conventional Identification	No. of isolates	Identification by VITEK 2™
<i>C.parapsilosis</i>	1	<i>C.famata</i>
<i>C.glabrata</i>	1	<i>C.famata</i>
<i>C.glabrata</i>	2	<i>C.haemulonii</i>

Four discordant species that remained unidentified by VITEK2 were further identified by VITEK MS.

Table 2- Comparison of identification results

Conventional	VITEK 2™	VITEK MS™
<i>C.parapsilosis</i> (1)	<i>C.famata</i>	<i>Kodamea ohmeri</i>
<i>C.glabrata</i> (1)	<i>C.famata</i>	<i>C.famata</i>
<i>C.glabrata</i> (2)	<i>C.haemulonii</i>	<i>C.haemulonii</i>
<i>C.albicans</i> (1)	Unidentified	<i>C.albicans</i>

Of the 99 isolates identified by VITEK 2, 51 isolates showed excellent identification, 18 very good, 14 each were acceptable and good. 2 isolates were identified with low discrimination.

### Antifungal susceptibility testing-

50 isolates were tested for susceptibility to fluconazole and amphotericin by conventional broth dilution and VITEK 2.

Table 3- Comparison of AFS test result by Conventional BMD and VITEK 2™ (AST-YS07)

	Fluconazole		Amphotericin B		
	Conventional	VITEK 2™		Conventional	VITEK 2™
Total number tested	50	50	Total number tested	50	50
Susceptible (MIC ≤8µg/ml)	34(68%)	34(68%)	Susceptible (MIC≤1 µg/ml)	32(64%)	34(68%)
Susceptible dose dependent (SDD) (MIC 16-32 µg/ml)	6(12%)	8(16%)	--	--	--
Resistant (MIC≥ 64 µg/ml)	10(20%)	8(16%)	Resistant (MIC≥1 µg/ml)	18(36%)	16(32%)

For fluconazole, MIC value obtained by VITEK 2™ of 47 isolates tested was either the same (20 isolates) or within two dilutions of the reference value obtained by broth microdilution method (27 isolates). The essential agreement was 96% at 24 hrs and 94% at 48 hrs. There was no statistical difference between the essential agreement calculated at 24 hrs and 48 hrs. Two isolates showed category discrepancy; thus, the categorical agreement was 96%. There was no very major error and major error in the

result generated by VITEK 2™.Two isolates (one isolate of *C.tropicalis* and one isolate of *C.albicans*) showed minor error. Both the isolates were resistant by conventional broth dilution method and susceptible dose dependent (SDD) by VITEK 2™. Minor error rate was 4%.

For amphotericin B, MIC value obtained by VITEK 2™ of 48 isolates was either the same (18 isolates) or within two dilutions of the reference value obtained by broth microdilution method (30 isolates).

The essential agreement was 98% at 24 hrs and 96% at 48 hrs. There was no significant difference in essential agreement at 24 hrs and 48 hrs ( $p>0.05$ ). No isolate showed an MIC value which was discrepant by four or more, two fold dilutions. The substantial difference was 0%. Two isolates showed an MIC value which was discrepant by three,

two fold dilutions. The non-substantial difference was 4%

MIC results of 46\* isolates were used to calculate the species wise essential agreement for Fluconazole and Amphotericin B at 24 hrs and 48 hrs. 4 Isolate one of *C.parapsilosis*, two of *C.glabrata*, the identifications of which were not concordant, were not included.

Table 4- Comparison of Species wise essential agreement for Fluconazole AFST

Species	No.of isolates Tested (46)	Test method	Range (µg/ml)	Essential agreement		%Resistance (n)	
				24 hrs	48 hrs	24 hrs	48 hrs
<i>C.tropicalis</i>	20	VITEK 2™	≤1->64	95% (19)	90% (18)	10% (2)	5% (1)
		24hrs BMD	0.125->64				
		48 hrs BMD	0.5->64				
<i>C.albicans</i>	17	VITEK 2™	≤1->64	95% (16)	95% (16)	11.76% (1)	5.88% (1)
		24hrs BMD	0.5->64				
		48hrs BMD	0.5->64				
<i>C.parap-silosis</i>	4	VITEK 2™	≤1-2	100%	100%	25% (1)	25% (1)
		24hrs BMD	0.5-2				
		48 hrs BMD	1-2				
<i>C.glabrata</i>	4	VITEK 2™	16->64	100%	100%	75% (3)	75% (1)
		24hrs BMD	16->64				
		48 hrs BMD	32->64				
<i>C.lusitaniae</i>	1	VITEK 2™	≤1	100%	100%	0	0
		24hrs BMD	1				
		48 hrs BMD	1				

Table 5 :Species wise essential agreement for Amphotericin B AFST Comparison betweenBMD and VITEK 2™

Species	No.of isolates Tested N=46	Test method	Range (µg/ml)	Essential agreement		Resistance% (n)	
				24 hrs	48 hrs	VITEK2™	BMD
<i>C.tropicalis</i>	20	VITEK 2™	0.25-16	95% (19)	95% (19)	25% (5)	20% (4)
		24hrs BMD	0.06-4				
		48 hrs BMD	0.5-4				
<i>C.albicans</i>	17	VITEK 2™	0.5-8	100%	95% (16)	11.76% (2)	11.76% (2)
		24hrs BMD	0.125-8				
		48 hrs BMD	0.5-8				
<i>C.parapsilosis</i>	4	VITEK 2™	0.5	100%	100%	20% (1)	20% (1)
		24hrs BMD	0.5				
		48 hrs BMD	1				
<i>C.glabrata</i>	4	VITEK 2™	1-16	100%	100%	75% (3)	75% (3)
		24hrs BMD	1-4				
		48 hrs BMD	1-8				
<i>C.lusitaniae</i>	1	VITEK 2™	0.5	100%	100%	0%	0%
		24hrs BMD	0.5				
		48 hrs BMD	0.5				

The cost per test included only the cost of consumable. The cost of labour and equipment was not included. Total cost for Identification and AFST by Conventional was Rs.457.75 and by VITEK 2™ it was Rs.622.

The time taken for identification and AFST by conventional was 24 hrs and 48 hrs respectively and that by VITEK was 18 hrs and 15 hrs identification.

## DISCUSSION

The conventional methods for identification and antifungal susceptibility testing of yeasts do not adequately cater to accurate and early identification of the newer yeasts which have the capacity for inherent and acquired resistance.(14) Also, these methods are not only time consuming but also labour intensive. Commercially available automated systems provide a good option.(11) However their use in a resource constrained setting has not been extensively studied.



In the present study, we evaluated the utility of commercially available VITEK2 for identification and antifungal susceptibility testing of 100 yeast isolates. Identification results obtained by conventional phenotypic techniques and broth microdilution (BMD) as per CLSI M27-A2 were taken as standards for comparison. The parameters evaluated included degree of agreement, error rate, turnaround time (TAT) and cost

Of the 100 isolates, all isolates were identified by the conventional method as compared to VITEK2, that identified 97 isolates unequivocally, and three isolates with low discrimination, that were later resolved using additional testing. One isolate remained unidentified.

The possible reason for the isolate remaining unidentified could be low reactivity because of poor growth.(15) The other reason attributed could technical error arising out of the inability to prepare a uniform suspension usually seen with *Trichosporon species*. Cultures older than 24 hrs can be another probable reason for non-identification.(16)

When the results of identification of VITEK 2™ were compared with the conventional phenotypic tests, 95 isolates' identification were in agreement.(15)The essential agreement for correct identification between VITEK 2™ and conventional method in the present study was found to be 95% and 93% with or without additional test respectively.

The results of the present study are similar to those reported in literature. (5)(9)(15)(17)(18)(19) The agreement in other studies varies from 54% to 93.5% and improved to 100% with additional testing. The standard for comparison was different in different studies.

The discordance in identification, in the present study, (5%) was higher as compared to that observed in other studies where the discordance was 1.7% and 1.8% and was primarily related to *C.glabrata*. (15)(19).

A species wise analysis demonstrated that the four commonly identified species *C.tropicalis*, *C.albicans*, and *C.parapsilosis* and *C.glabrata* showed an initial concordance of 97.27%, 96.55%, 83.83% and 57.14% respectively. When the isolates identified with low discrimination were retested using additional testing methods, the concordance rates increased for *C.tropicalis* and *C.parapsilosis* to 100% and 91% respectively.

In other studies, a concordance rate of 100% has been reported for *C.albicans*, *C.tropicalis* and *C.glabrata*.(15,20,21)The concordance rate for *C.parapsilosis* was lower in a study reported by Graf B *et al* and Muerman O *et al* , 95% and 96% respectively.(15,21)In another study carried out by Jain *et al*, the concordance rate for *C.albicans*, *C.tropicalis*, *C.parapsilosis*, *C.glabrata* was only 39%, 49%, 31% and 33% respectively. This study showed an overall low concordance for the commonly isolated species.(9)

In the present study, concordance for *C.glabrata* was low. Of the seven isolates identified as *C.glabrata* by conventional, VITEK 2™ identified four isolates as *C.glabrata*, two as *C.haemulonii* and one as *C.famata*. Similar finding was noted in the study carried out by Jain *et al*. Two of the three isolates were misidentified or unidentified by VITEK 2™.(9)This finding in the present study is in contrast with other studies where *C.glabrata* has been identified with 100% concordance.(15,20,21)The identification of isolates of *C.glabrata* as *C.famata* and *C.haemulonii* by VITEK 2™ was supported by the results of VITEK MS™. Misidentification by conventional methods could be due to similar morphological features shown by *C.glabrata* ,*C.famata* and *C.haemulonii* on corn meal agar. All three appear as oval, budding yeast cells with no pseudohyphae. However, they can be differentiated based on the results of sugar assimilation tests. (22–24) However, in the present case, sugar assimilation test results were also similar.

Conventional methods proved to be inadequate for identifying *C.famata* and *C.haemulonii*. Since, identification by VITEK 2™ is based on 51 different biochemical tests, these isolates were identified correctly when compared to conventional.

The other less common isolates *C.lusitanaie*, *C.guillermondii* were identified with 100% concordance. However, the number of isolate identification compared being low, a conclusion cannot be made.

One isolate of *C.parapsilosis* was identified as *C.famata* by VITEK 2™. Other studies have also shown a similar discordance in identification of *C.parapsilosis*. (25,26) According to one of the study, phenotypic methods almost always identify *C.famata* incorrectly.(27) The reason for this incorrect identification could not be explained. The identification of the four discordant and one unidentified isolates was further ratified using VITEK MS™. The isolate identified as *C.parapsilosis* by conventional was identified as *C.famata* by VITEK 2™, but VITEK MS™ identified the same isolate as *Kodamea ohemeri*. The reason for this discrepancy could not be explained. One isolate of *C.albicans* which was unidentified by VITEK 2™, was identified as *C.albicans* by VITEK MS™. Similar finding has been observed in a study carried out by P Mathur et al, where six isolates of *C.albicans* remained unidentified by VITEK 2™.(19) VITEK 2™, does not perform well for starch, inulin, rhamnose, peptone and peptone assimilation.(28) This could have been a probable reason for the isolate remaining unidentified.

In the present study, the antifungal susceptibility results of fluconazole and amphotericin B obtained by VITEK 2™ using AST-YS07 were compared with conventional BMD method for 50 *Candida* species.

CLSI M27-A2 interpretative standards were followed in the present study. Isolates with MIC ≤ 8 µg/ml were

considered to be sensitive, isolates with MIC 16-32 µg/ml were susceptible dose dependent (SDD) and isolates with MIC ≥ 64 µg/ml were taken as resistant for Fluconazole.(29) The interpretative breakpoints for amphotericin B has not been described by current CLSI standards. Considering that, MIC values of most of the susceptible isolates fall below 1 µg/ml, isolates with MIC values ≥ 1 µg/ml, can be taken as resistant.(29,30)

Of the 50 isolates tested for Fluconazole susceptibility by conventional broth microdilution, 12% were susceptible dose dependent (SDD) and 20% were resistant. Using VITEK 2™ 16 % were found to be susceptible dose dependent (SDD) and 16% were found to be resistant. Conventional broth microdilution detected 18% of the total isolates tested as resistant for Amphotericin B, whereas 16 % were resistant by VITEK 2™.

*C.glabrata* demonstrated the highest resistance to both fluconazole and Amphotericin B by both methods. *C.parapsilosis* was the second most common fluconazole resistant isolate by both systems. In other studies a reduced susceptibility has been shown by *C.glabrata* to amphotericin B.(32–34) For amphotericin B, *C.tropicalis* was the second most common resistant isolate. Yang et al noted in their study noted a higher resistance to *C.tropicalis* in their study.(35)

The essential agreement between VITEK 2™ and conventional BMD for fluconazole in different studies range from 95-97.9 % and Categorical agreement ranged from 92-95.5 %. The essential agreement in the present study was found to be 96% and the categorical agreement was found to be 96%. The essential agreement in the present study at 24 hrs was in accordance with other studies in literature. However, the essential agreement at the end of 48 hrs was lower than that at the 24hrs. The difference in the MIC value obtained at the end of 24 hrs and 48 hrs was not statistically significant (p>0.05).

Considering species wise essential agreement for fluconazole at the end of 24 hrs and 48 hrs, all isolates were in 100% agreement, except two isolates of *C.tropicalis* which showed an essential agreement of 95% and 90% at the 24 hrs and 48 hrs respectively. The low agreement at the end of 48hrs was because of high MIC value obtained by BMD as compared to VITEK 2™. Similar findings were noted by Pfaller *et al.*(38) High MIC value at the end of 48 hrs could be because of trailing phenomenon which was noted in these isolates. Trailing is a technical artefact observed with fungistatic drugs. It contributes to false positive results for antifungal resistance due to residual growth above the MIC level (partial inhibition of growth over an extended range of antifungal concentration, observed in 5% of isolates). This trailing growth can affect the MIC so much, so as to make an isolate that appears susceptible after 24 hrs appear resistant at 48 hrs.(29,39,40) In the present study, trailing did not have any effect on the categorical agreement.

Very major or major error rates were not observed for fluconazole. The minor error rate was 4%, which is similar to those found in other studies.(13) Minor error was noted with one isolate each of *C.tropicalis* and *C.albicans*. Both the isolates were categorized as resistant by conventional broth dilution and susceptible dose dependent (SDD) by VITEK 2™. This error rate is in accordance with study carried out by Posteraro *et al* where the error rate was 5%.(11) The minor error obtained did not affect the essential agreement as the MIC values obtained by VITEK 2™ were within two two fold dilutions.

The overall essential agreement for Amphotericin B in the present study between VITEK 2™ and BMD was found to be 98% at the end of 24 hrs 96% at the end of 48 hrs which is lower than that found by Pfaller in their study (99.1%.) *C.parapsilosis*, *C.lusitaniae*, *C.glabrata*, showed 100% essential agreement by the two methods. The discrepancy in agreement

was found in one isolate of *C.albicans* and one isolate of *C.tropicalis*(95% at the end of 24hrs and 48 hrs) .A lower MIC value was obtained by BMD as compared to VITEK 2™. One of the probable reasons for this discrepancy could be that the RPMI media used for broth microdilution in detection of amphotericin B is less reliable. Use of antibiotic medium 3 supplemented with 2% glucose, permits better detection of amphotericin B resistant isolate. (36)In case of *C.albicans* the essential agreement was 100% at the end of 24 hrs, but the agreement at the end of 48hrs was lower (95%). Similar finding was noted by Pfaller *et al.*(37) Though *C.glabrata* showed 100% essential agreement, the MIC obtained were higher by VITEK 2 as compared to broth microdilution. This finding is again similar to that observed by Pfaller *et al.*(37) There have been reports of developing resistance to Amphotericin B reported in *C.glabrata*, reporting of higher MIC value by VITEK 2™ will be of help in early detection of resistant isolates.

Nonsubstantial difference was seen in two isolates (MIC results in three or four, two fold dilution) whereas there was no isolate showing substantial difference (more than four two fold dilution) in MIC. Cuenca – Estrella *et al* in their study found no substantial difference and one non substantial difference in their study which correlates well with the present study.(13)

In the present study, turnaround time ranged between 24-72 hrs for the identification of the isolates by conventional method, median TAT being 48 hrs. The time required to identify *C.glabrata* was more as compared to other isolates (72 hrs).The turnaround time for identification by VITEK 2™ ranged from 18-18.5, the average turnaround time being 18.25 hrs, which is significantly less compared to that required by conventional. (p<0.01) This is in accordance with studies carried out Melhem *et al* and Graf *et al*, where the TAT by VITEK 2™ was 18 hrs and 15 hrs respectively.(15,39) The turnaround time for antifungal susceptibility testing by BMD



ranged between 24- 48 hrs with a median of 24 hrs, whereas with VITEK 2™ it ranged from 11.75 hrs to 28.25 hrs. The mean turnaround time was found to be 15.42 hrs which is again less than that required by conventional method. When compared statistically, the lower TAT by VITEK 2™ was significant ( $p < 0.01$ ) which is consistent with other studies.(13,20)

The cost of identification of one isolate by conventional method was approximately Rs.330 per test isolate and that for antifungal susceptibility testing of Fluconazole and Amphotericin B was found to be approximately Rs.128 for one isolate. The cost for identification of one isolate by VITEK 2™ was approximately Rs.311 and that for antifungal susceptibility testing it was another Rs.311. However, with VITEK 2™, along with fluconazole and amphotericin, results of other antifungal agents were also provided which included voriconazole, itraconazole, micafungin and caspofungin.

## CONCLUSION

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.

## Study limitation

The main limitation of the study was that certain yeast species which pose problem in identification such as *C.krusei*, *C.kefyr* were not included because of the inclusion of 100 consecutive non duplicate isolates.(3) Due to cost constraints, a convenient sample size of 100 yeast isolates was included.

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