ORIGINAL ARTICLE

# Comparison of the VITEK 2 Yeast Antifungal Susceptibility Testing with CLSI Broth Microdilution Reference Method for Testing Four Antifungal Drugs against *Candida* species Isolated from Blood Samples

*Vijaya S. Rajmane[1*], Shivaji T. Mohite[1]*

*[1]Department of Microbiology, Krishna Institute of Medical Sciences "Deemed to be University", Karad-415110 (Maharashtra) India*

**Abstract**

*Background*: Although the patients in the Intensive Care Unit (ICU) are at risk for candidemia resulting in complication, rapid diagnosis of aetiologic agent will reduce the delay in initiating the appropriate therapy with adequate dosage of antifungals thus improving the outcome. Due to reduced susceptibility of non-*albicans Candida* species to antifungal agents; testing the antifungal susceptibility pattern has become important both epidemiologically and for patient management. *Aim and Objectives*: To compare the VITEK 2 Yeast antifungal susceptibility testing with Clinical and Laboratory Standards Institute (CLSI) Broth Microdilution (BMD) reference method against isolates of *Candida* species and assess the Categorical Agreement (CA) and errors. *Material and Methods*: *Candida* species isolated from blood samples of patients admitted under Medical and Surgical ICU of Krishna Hospital and Medical Research Centre, Karad were subjected to antifungal susceptibility testing by CLSI reference BMD and VITEK 2 method. *Results*: Compared to the reference BMD method, the VITEK 2 system yielded highly reproducible and accurate MIC results and excellent overall categorical agreement at 100% for amphotericin B, flucytosine, fluconazole, and voriconazole against *C. albicans*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, *C. lusitaniae* and *C. guillermondii* except *C. rugosa* and *C. glabrata*. *Conclusion*: The VITEK 2 method demonstrated excellent reproducibility and standardization. It provides a fully automated method for fungal identification, determining the MICs against *Candida* spp. based on spectrophotometry, thus eliminating the inherent bias of manual MIC determination.

**Keywords:** CLSI BMD, VITEK 2, Categorical Agreement, Errors

## Introduction:

*Candida* is one of the most frequent cause of Bloodstream Infections (BSIs) leading to significant morbidity and mortality, especially in non-neutropenic critically ill patients. Between 1995 and 2002, the frequency of nosocomial candidemia rose significantly from 8% to 12% of all reported bloodstream infections [1].

This shift in *Candida* species causing BSIs is mainly attributed to use of azoles in the prophylactic treatment of immuno compromised patients. This in turn has led to reduced susceptibility of non-*albicans Candida* species to antifungal agents either due to intrinsic resistance to fluconazole, biofilm production or acquired resistance to azoles during therapy [2-3]. The epidemiology of candidemia varies in different regions and even from one hospital to another within the same locality. Thus, *Candida* species distribution and susceptibility profile varies in different regions both at local and worldwide level.

The Clinical and Laboratory Standards Institute (CLSI) has developed standardized procedure for

antifungal susceptibility testing of yeasts, but it may not be the most efficient and convenient procedure for everyday use in clinical laboratory. Therefore, commercial methods like Sensititre Yeast One Antifungal Panel, E test, (VITEK 2) system, etc. for in vitro susceptibility testing have been recently developed. Among this VITEK 2 system (BioMerieux France) is a fully automated method which determines growth spectro-photometrically and is also feasible for using in the clinical laboratories [4].

The purpose of this study is to assess the suitability of VITEK 2 system to determine the susceptibility of yeasts to antifungal agents. Categorical Agreement (CA) between the two methods was determined by comparing the MIC results obtained using AST-YS06 fungal susceptibility card with those obtained using the reference method (CLSI M27-A3, USA, 2008) [5].

**Material and Methods:**

This longitudinal hospital based observational study was carried out in the department of Microbiology, Krishna Institute of Medical Sciences, Karad during a period of two years from January 2011 to December 2012. The statement of approval for the study was taken from the ethical committee. After taking informed consent, the blood samples were collected from patients admitted in Medical and Surgical Intensive Care Unit (ICU) of Krishna Hospital and Medical Research Centre, Karad. Blood samples were processed and isolation and identification of Candida species were done according to standard protocol. Antifungal susceptibility testing of the Candida species isolated was carried out by broth microdilution method as per CLSI M27-A3, USA, 2008 guidelines [5] and VITEK 2 system (BioMerieux, France).

**CLSI Broth Microdilution Method (M27-A3):**

Antifungal agents used were amphotericin B, fluconazole, voriconazole and 5-flurocytosine. The antifungal powders were obtained in the form of pure salts from Sigma-Aldrich. Antifungal stock solutions were prepared at concentration of at least 1280 µg/mL, or ten times the highest concentration to be tested, whichever is greater. Antifungal agents like amphotericin B, voriconazole were dissolved in Dimethyl Sulfoxide (DMSO) (HiMedia) and series of dilutions at 100 times the final concentration was prepared from antifungal stock solution in the same solvent. Each intermediate solution was further diluted to final strength in the test medium. The drug concentration ranges used were 0.0313 to 16 µg/mL for amphotericin B and voriconazole and 0.125 to 64 µg/mL for flucytosine and fluconazole. The reference strains of *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6528 were used for Quality Control.

The interpretative criteria for amphotericin B have not yet been defined. Experience to date using this standard procedure it has been concluded that if an amphotericin B Minimum Inhibition Concentration (MIC) of >1g/ml is obtained for a *Candida* spp. isolate, then that isolate is likely to be resistant to amphotericin B.

The interpretative breakpoints for fluconazole with MIC $\leqslant$ 8 g/ml was considered Susceptible (S), 16-32 g/ml as Susceptible Dose Dependent (SDD) and $\geqslant$ 64 g/ml as Resistant (R).

For flucytosine, MIC $\leqslant$ 4 g/ml was considered susceptible, 8-16 g/ml as Intermediate (I) and $\geqslant$ 32 g/ml as resistant. For voriconazole MIC $\leqslant$ 1 g/ml was considered susceptible, 2 g/ml as SDD and $\geqslant$ 4 g/ml as resistant.

**VITEK 2 Yeast Susceptibility Test:**

The inoculum suspensions were prepared from 24 hr old cultures of Candida spp. grown on Sabouraud Dextrose Agar (SDA) adjusted to a turbidity equivalent to 2 McFarland units using a spectrophotometer. This suspension was placed into a VITEK 2 instrument and read automatically. A yeast susceptibility card was used to test each organism. The drug concentration ranged from 0.25-16 µg/mL for amphotericin B, 1-64 µg/mL for fluconazole and flucytosine and 0.125-16 µg/mL for voriconazole.

**Statistical Analysis:**

In the present study, the antifungal susceptibility was done by both conventional BMD method and automated VITEK 2 system. The CA percentages and the errors were calculated by comparing the MICs determined with both the methods [6]. CLSI interpretative breakpoints for the antifungal drugs were used to determine categorical agreement percentages. CAs were calculated at end points of 24 h for amphotericin B and fluconazole and 48 h for flucytosine and voriconazole of the comparative methods [7].

Very Major Errors (VME) were defined as instances when the reference MIC indicates resistance and the VITEK 2 system MIC indicated susceptibility.

Major (ME) errors were defined as the instances when the isolate was classified resistant by VITEK 2 system and susceptible by the reference method.

minor Errors (mE) were defined as instances when an isolate was either susceptible or resistant by one method and SDD by the other [6-7].

**Results:**

A total of 36 *Candida* isolates were included in the present study; out of which 12 were *C. albicans*, 10 *C. tropicalis*, 4 isolates each of *C. rugosa* and *C. krusei*, 2 isolates each of *C. glabrata* and *C. parapsilosis*, 1 isolate each of *C. guillermondii*.

In the present study as per the recommendations of CLSI [5] the MIC readings have been taken after 24 h of incubation for amphotericin B and fluconazole and 48 h of incubation for flucytosine and voriconazole. The data is presented accordingly.

The CAs between the VITEK 2 system and CLSI method among *C. albicans*, *C. tropicalis, C. krusei, C. parapsilosis, C. lusitaniae* and *C. guillermondii* for all four antifungal agents were excellent i.e 100% with no errors [Table1-5].

The CA between VITEK 2 and CLSI method results among *C. rugosa* was excellent at 100% for voriconazole. The CA for amphotericin B was 75% with one (25%) VME. The CA for fluconazole was 75% and was low for flucytosine i. e, 50% without any VME. The mE were one (25%) and two (50%) for fluconazole and flucytosine respectively [Table 6].

The CAs between VITEK2 and CLSI method results among *C.glabrata* were excellent at 100% for amphotericin B and flucytosine. The CAs for fluconazole and voriconazole were low i.e 50% with one (50%) mE, one (50%) and VME respectively [Table 7].

The VITEK2 method demonstrated excellent reproducibility and standardization. Furthermore, in vitro antifungal susceptibility of *Candida* species are obtained in less than 23.00 h (mean, 17.23 h).

**Table 1: Categorical Agreement and Errors for 12 Isolates of *C. albicans***

| Antifungal Agent | Test Method | No. of MICs by Category | VITEK 2 | | | % CA with VK2 | % of VK2 errors | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | S | SDD/I | R | | VME | ME | mE |
| Amphotericin B (24 h) | BMD | S | 12 | - | 0 | 100% | 0 | 0 | 0 |
| | | R | 0 | - | 0 | | | | |
| Flucytosine (48 h) | BMD | S | 12 | 0 | 0 | 100% | 0 | 0 | 0 |
| | | I | 0 | 0 | 0 | | | | |
| | | R | 0 | 0 | 0 | | | | |
| Fluconazole (24 h) | BMD | S | 12 | 0 | 0 | 100% | 0 | 0 | 0 |
| | | SDD | 0 | 0 | 0 | | | | |
| | | R | 0 | 0 | 0 | | | | |
| Voriconazole (48 h) | BMD | S | 12 | 0 | 0 | 100% | 0 | 0 | 0 |
| | | SDD | 0 | 0 | 0 | | | | |
| | | R | 0 | 0 | 0 | | | | |

*BMD-Broth Microdilution, S-Sensitive, R-Resistant, SDD-Susceptible dose-dependent, I-Intermediate*

**Table 2: Categorical Agreement and Errors for 10 Isolates of *C. tropicalis***

| Antifungal Agent | Test Method | No. of MICs by Category | VITEK 2 | | | % CA with VK2 | % of VK2 errors | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | S | SDD/I | R | | VME | ME | mE |
| Amphotericin B (24 h) | BMD | S | 7 | - | 0 | 100% | 0 | 0 | 0 |
| | | R | 0 | - | 3 | | | | |
| Flucytosine (48 h) | BMD | S | 10 | 0 | 0 | 100% | 0 | 0 | 0 |
| | | I | 0 | 0 | 0 | | | | |
| | | R | 0 | 0 | 0 | | | | |
| Fluconazole (24 h) | BMD | S | 10 | 0 | 0 | 100% | 0 | 0 | 0 |
| | | SDD | 0 | 0 | 0 | | | | |
| | | R | 0 | 0 | 0 | | | | |
| Voriconazole (48 h) | BMD | S | 10 | 0 | 0 | 100% | 0 | 0 | 0 |
| | | SDD | 0 | 0 | 0 | | | | |
| | | R | 0 | 0 | 0 | | | | |

*BMD-Broth Microdilution, S-Sensitive, R-Resistant, SDD-Susceptible dose-dependent, I-Intermediate*

**Table 3: Categorical Agreement and Errors for 4 Isolates of *C. krusei***

| Antifungal Agent | Test Method | No. of MICs by Category | VITEK 2 | | | % CA with VK2 | % of VK2 errors | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | S | SDD/I | R | | VME | ME | mE |
| Amphotericin B (24 h) | BMD | S | 4 | - | 0 | 100% | 0 | 0 | 0 |
| | | R | 0 | - | 0 | | | | |
| Flucytosine (48 h) | BMD | S | 4 | 0 | 0 | 100% | 0 | 0 | 0 |
| | | I | 0 | 0 | 0 | | | | |
| | | R | 0 | 0 | 0 | | | | |
| Fluconazole (24 h) | BMD | S | 0 | 0 | 0 | 100% | 0 | 0 | 0 |
| | | SDD | 0 | 0 | 0 | | | | |
| | | R | 0 | 0 | 4 | | | | |
| Voriconazole (48 h) | BMD | S | 4 | 0 | 0 | 100% | 0 | 0 | 0 |
| | | SDD | 0 | 0 | 0 | | | | |
| | | R | 0 | 0 | 0 | | | | |

*BMD-Broth Microdilution, S-Sensitive, R-Resistant, SDD-Susceptible dose-dependent, I-Intermediate*

**Table 4: Categorical Agreement and Errors for 2 Isolates of *C. parapsilosis***

| Antifungal Agent | Test Method | No. of MICs by Category | VITEK 2 | | | % CA with VK2 | % of VK2 errors | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | S | SDD/I | R | | VME | ME | mE |
| Amphotericin B (24 h) | BMD | S | 2 | - | 0 | 100% | 0 | 0 | 0 |
| | | R | 0 | - | 0 | | | | |
| Flucytosine (48 h) | BMD | S | 2 | 0 | 0 | 100% | 0 | 0 | 0 |
| | | I | 0 | 0 | 0 | | | | |
| | | R | 0 | 0 | 0 | | | | |
| Fluconazole (24 h) | BMD | S | 2 | 0 | 0 | 100% | 0 | 0 | 0 |
| | | SDD | 1 | 0 | 0 | | | | |
| | | R | 0 | 0 | 0 | | | | |
| Voriconazole (48 h) | BMD | S | 2 | 0 | 0 | 100% | 0 | 0 | 0 |
| | | SDD | 0 | 0 | 0 | | | | |
| | | R | 0 | 0 | 0 | | | | |

*BMD-Broth Microdilution, S-Sensitive, R-Resistant, SDD-Susceptible dose-dependent, I-Intermediate*

**Table 5: Categorical Agreement and Errors for One Isolate Each of *C. lusitaniae* and *C. guillermondii***

| Antifungal Agent | Test Method | No. of MICs by Category | VITEK 2 | | | % CA with VK2 | % of VK2 errors | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | S | SDD/I | R | | VME | ME | mE |
| **Amphotericin B (24 h)** | **BMD** | **S** | 2 | - | 0 | 100% | 0 | 0 | 0 |
| | | **R** | 0 | - | 0 | | | | |
| **Flucytosine (48 h)** | **BMD** | **S** | 2 | 0 | 0 | 100% | 0 | 0 | 0 |
| | | **I** | 0 | 0 | 0 | | | | |
| | | **R** | 0 | 0 | 0 | | | | |
| **Fluconazole (24 h)** | **BMD** | **S** | 2 | 0 | 0 | 100% | 0 | 0 | 0 |
| | | **SDD** | 0 | 0 | 0 | | | | |
| | | **R** | 0 | 0 | 0 | | | | |
| **Voriconazole (48 h)** | **BMD** | **S** | 2 | 0 | 0 | 100% | 0 | 0 | 0 |
| | | **SDD** | 0 | 0 | 0 | | | | |

*BMD-Broth Microdilution, S-Sensitive, R-Resistant, SDD-Susceptible dose-dependent, I-Intermediate*

**Table 6: Categorical Agreement and Errors for 4 Isolates of *C. rugosa***

| Antifungal Agent | Test Method | No. of MICs by Category | VITEK 2 | | | % CA with VK2 | % of VK2 errors | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | S | SDD/I | R | | VME | ME | mE |
| **Amphotericin B (24 hrs)** | **BMD** | **S** | 2 | - | 0 | 75% | 25 | 0 | 0 |
| | | **R** | 1 | - | 1 | | | | |
| **Flucytosine (48 hrs)** | **BMD** | **S** | 2 | 0 | 0 | 50% | 0 | 0 | 50 |
| | | **I** | 0 | 0 | 2 | | | | |
| | | **R** | 0 | 0 | 0 | | | | |
| **Fluconazole (24 hrs)** | **BMD** | **S** | 3 | 0 | 0 | 75% | 0 | 0 | 25 |
| | | **SDD** | 1 | 0 | 0 | | | | |
| | | **R** | 0 | 0 | 0 | | | | |
| **Voriconazole (48 hrs)** | **BMD** | **S** | 4 | 0 | 0 | 100% | 0 | 0 | 0 |
| | | **SDD** | 0 | 0 | 0 | | | | |
| | | **R** | 0 | 0 | 0 | | | | |

*BMD-Broth Microdilution, S-Sensitive, R-Resistant, SDD-Susceptible dose-dependent, I-Intermediate*

**Table 7: Categorical Agreement and Errors for 2 Isolates of *C. glabrata***

| Antifungal Agent | Test Method | No. of MICs by Category | VITEK 2 | | | % CA with VK2 | % of VK2 errors | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | S | SDD/I | R | | VME | ME | mE |
| **Amphotericin B (24 h)** | **BMD** | **S** | 2 | - | 0 | 100% | 0 | 0 | 0 |
| | | **R** | 0 | - | 0 | | | | |
| **Flucytosine (48 h)** | **BMD** | **S** | 2 | 0 | 0 | 100% | 0 | 0 | 0 |
| | | **I** | 0 | 0 | 0 | | | | |
| | | **R** | 0 | 0 | 0 | | | | |
| **Fluconazole (24 h)** | **BMD** | **S** | 1 | 0 | 0 | 50% | 0 | 0 | 50 |
| | | **SDD** | 1 | 0 | 0 | | | | |
| | | **R** | 0 | 0 | 0 | | | | |
| **Voriconazole (48 h)** | **BMD** | **S** | 1 | 0 | 0 | 50% | 50 | 0 | 0 |
| | | **SDD** | 0 | 0 | 0 | | | | |
| | | **R** | 1 | 0 | 0 | | | | |
| | | **R** | 0 | 0 | 0 | | | | |

*BMD-Broth Microdilution, S-Sensitive, R-Resistant, SDD-Susceptible dose-dependent, I-Intermediate*

**Discussion:**

The categorical agreement in the present study against amphotericin B for *C. albicans*, *C. krusei*, *C. guillermondii*, *C. parapsilosis* and *C. glabrata* was similar to the study conducted by Gonzalez Cejudo *et al* i.e 100%. However for *C. tropicalis* and *C. lusitaniae* it was slightly lower with 9.5% very major error and 16.7% minor error respectively [4].

Lower percentages of categorical agreement were detected for *C. glabrata* against fluconazole and voriconazole which was consistent with the findings obtained by other authors [4, 7]. The present study shows very major error of 50% for *C. glabrata* against voriconazole and minor error of 50% against fluconazole which was inconsistent with other studies [4, 7, 8]. The overall categorical agreement for flucytosine among most of our isolates was high which was similar to results obtained by Cejudo *et al.* and Pfaller *et al*. [4, 8]. In the present study the very major error was seen in *C. rugosa* against amphotericin B, however we could not find this species in other studies for comparison [4, 8, 9]. In the present study excellent categorical agreement was observed for comparison of the VITEK 2 system with BMD method for all the isolates against all four antifungal drugs except for *C. rugosa* and *C. glabrata*. There was not a single isolate with major error against any of these drugs. The mean time required to obtain susceptibility results with VITEK 2 method in our study was 17:23 h with a range of 11:46 h to 23:00 h which was consistent with the findings in other studies [9, 1, 11].

**Conclusion:**

The surveillance of antifungal susceptibility profiles provides a useful tool for hospitals to validate empiric treatment regimes. Though the cost of each test by BMD method is less comparable to VITEK 2 system; it is a tedious and time consuming procedure. Overall, the VITEK 2 system provides a rapid, fully automated method for determining the MICs against *Candida* spp. thus eliminating the subjectivity of manual MIC determination methods. Thus, the VITEK 2 method demonstrated excellent reproducibility and standardization and it also confines the time necessary for optimizing antifungal treatment decisions. However, more studies are necessary to evaluate the ability of VITEK 2 method to identify the resistant isolates.

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[*] ***Author for Correspondence:*** *Dr. Mrs. Vijaya S. Rajmane, Department of Microbiology, Krishna Institute of Medical Sciences, Karad- 415110 Email: drvsrajmane@yahoo.com Cell: +91 9860299944*