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# Rapid Species Identification and Antifungal Susceptibility Testing of *Candida* Isolated from Different Hospital Acquired Infections by VITEK 2 System

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**A B S T R A C T**

Hospital acquired *Candida* infection is a major cause of morbidity and mortality especially in critically ill and immunocompromised patients. Therefore, an accurate and early identification is necessary for the management of patients. The aim of our study was rapid identification of *Candida* species and their antifungal susceptibility testing (AST) by VITEK 2 system in hospital acquired fungal infections. A total of 50 *Candida* isolates were identified by both conventional methods and by Vitek-2 system. Antifungal susceptibility of each isolate was determined by broth microdilution method and Vitek-2 system. Out of these 50 *Candida* isolates, *C. albicans* (n = 29) were most commonly isolated, followed by *C. tropicalis* (n = 9), *C. krusei* (n = 6), *C. glabrata* (n = 4), and *C. parapsilosis* (n = 2). *C. albicans*, *C. tropicalis* and *C. krusei* showed resistance to Flucytosine. *C. albicans* and *C. glabrata* showed resistance to Voriconazole. *C. krusei* showed resistance to Amphotericin B. All the correlation coefficient indices were statistically significant between Vitek-2 system and broth microdilution method in antifungal susceptibility testing of different *Candida* species. Sensitivity and specificity of Vitek2 system method in antifungal susceptibility testing for Flocytosine were 84%, 86% respectively, for Voriconazole were 94%, 96% respectively, and for Amphotericin B were 96%, 98% respectively. Our study revealed that Vitek-2 system reduces the period required for identification and antifungal susceptibility of *Candida* species. So, Vitek-2 system appeared to be a rapid reliable method for identification and AST for the *Candida* species to prescribe appropriate antifungal agents for early and better management of fungal infections especially in critically ill and immunosuppressed patients.

## Introduction

Hospital acquired Yeast infections has been markedly increasing resulting in high morbidity and mortality (Espinel-Ingroff *et al.*, 2005).This is particularly important in cancer patients who are undergoing chemotherapy, especially if neutropenic, and these infections can lead to bad prognosis (Vento et al., 2003). The most common causative pathogens for hospital acquired fungal infections were *Candida* species especially in both immunocompromised and seriously ill patients. In spite that the most commonly isolated species in clinical laboratories is *Candida albicans*, non-albicans species has been increasing in the frequency (Barbara Graf *et al.*, 2011). The most common non-albicans species were *C. tropicalis*, *C. parapsilosis* and *C. glabrata* which were considered major causative pathogens of candidemia (Meyer *et al.*, 2009).

Fungal candidemia prognosis depends on the host immunological status, the yeast species virulence, the antifungals resistance of the causative yeast, and the antifungal therapy efficacy. Fungal infection especially inimmunocompromised patients can be rapidly fatal if not early and accurately treated. Thus, early identification of species and antifungal susceptibility testing in cases of critical infections is crucial (Pereira *et al.*, 2010).Azole resistance has emerged in many *Candida* species, like *C. glabrata* which is known to have acquired resistance to fluconazole and other azole drugs. On the other hand, *C. krusei* showed intrinsic resistance to older azoles antifungals.

Amphotericin resistance has been detected in species, such as *C. lusitaniae* and *C. haemulonii* (Rodriguez-Tudela *et al.*, 2008).This emerged antifungal resistance, particularly with azole drugs, amphotericin B and echinocandins (which is a new class of antifungal) necessitates the accurate *in vitro* antifungal susceptibility testing. The empiric therapy for treatment of hospital acquired fungal infections caused by unknown *Candida* spp. should be avoided (Clinical and Laboratory Standards Institute, 2008; Diekema *et al.*, 2009). Identification of *Candida* isolates by either classical or conventional methods is still typically done by biochemical, morphological and physiological tests.

These phenotypic systems are usually less accurate and time-consuming. In addition, they can't identify the *Candida* at the species level. To have a reliable system for species identification, the performance of classical methods should be reassessed (Maurizio Sanguinity *et al.*, 2007). The Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) considered broth microdilution methods for antifungal Susceptibility Testing as the standard reference methods for antifungal susceptibility testing (Verweij *et al.*, 1999; Rodriguez-Tudela *et al.*, 2008). Although a number of AST systems are commercially available, their performance is variable and time consuming (Buchaille *et al.*, 1998; Cuenca-Estrella *et al.*, 2005; Hata *et al.*, 2007; and Zaragoza *et al.*, 2011).

The Vitek 2 antifungal susceptibility system is a fully automated commercial system that determines growth of yeast spectrophotometrically and simultaneously allowed both identification of fungal species and antifungal susceptibility testing. The species identification by the Vitek 2 cardsis performed through comparison of the biochemical profile with an extensive database (Al-Sweih *et al.*, 2005 and Mokaddas *et al.*, 2007). AST-YS01 cardis used and can detect antifungal susceptibility for amphotericin B (AMB), fluconazole (FLC), flucytosine (5FC), and voriconazole (VRC) antifungal drugs. The minimum inhibitory concentration (MIC)can be determined by microdilution methodology in µg/ml (Pfaller *et al.*, 2007). The Vitek 2 system has been reported by many scientists to have high reproducibility and an excellent agreement with the CLSI microdilution reference procedure (>95%) for fluconazole and, more recently, for amphotericin B, flucytosine, and voriconazole (Posteraro *et al.*, 2009). Antifungal susceptibility in MIC results can be determined the after 9.1 to 27.1 h of incubation (mean, 12 to 14 h) (Pfaller *et al.*, 2007). Thus, the U.S. FDA approved in 2006 the clinical use of the Vitek 2 system to detect antifungal susceptibility (Pfaller *et al.*, 2007). Early and rapid identification and drug susceptibility testing of *Candida* infections can help prompt optimization of antimicrobial therapy and save the life of many patients (Sood *et al.*, 2000).

## Materials and Methods

### Patients

This study was carried out at the Medical Microbiology and Immunology Department, Faculty of medicine, Tanta University, Egypt.

Samples were collected from patients who were admitted to Tanta University Hospitals over a period of 6-9 months. Inclusion criteria of patients were immunocompromised patients such as cancer patients receiving chemotherapy especially with neutropenia or cell mediated immunodeficiencies, patients under corticosteroid therapy, and diabetetic patients that are at high risk of fungal infection. Exclusion criteria were all samples with laboratory confirmed isolates other than *Candida* infections, and patients under antifungal treatment.

## Materials and Methods

Patients' demographics were recorded followed by clinical examination to determine the type of infection, and Microbiological investigations as follows:

### Samples collection, transport and isolation of *Candida* species

Different samples including oral, vaginal, anorectal, urine, stool, respiratory tract specimens, endotracheal aspiration and blood samples were collected under aseptic precautions. Samples were transported as soon as possible to the medical Microbiology and Immunology Department, faculty of medicine, Tanta university and were subjected to the following: direct smear examination, culture on Sabouraud's Dextrose agar (Oxoid). Blood samples were cultured on blood culture bottles (Oxoid), and then subcultured on Sabouraud's Dextrose agar. Arising colonies were identified by colony morphology and stained films, germ tube test, and sugar fermentation.

### Antifungal susceptibility testing by broth microdilution method

Antifungal susceptibility testing was performed according to CLSI broth microdilution (BMD) reference method (Clinical and Laboratory Standards Institute, 2008; Verweij, 1999). The MICs for flucytosine, voriconazole, and amphotericin B were determined. The following antifungal compounds were included in our assay: Amphotericin B (0.03-16 µg/mL, Sigma-Aldrich), flucytosine (0.12-64 µg/mL, Sigma-Aldrich), and voriconazole (0.015-8 µg/mL, Pfizer S.A., NY). A stock solution of each antifungal agent was prepared in two-milliliter aliquots in either dimethyl-sulfoxide (amphotericin B and voriconazole) or in distilled water (flucytosine). The media used for the final drug dilutions was RPMI 1640 with potassium bicarbonate and without L-glutamine, buffered to pH 7 using 165 mM MOPS buffer (Sigma-Aldrich). The media were prepared as 2x stocks, and 100 µL was added to each well of the microdilution plates. The plates were sealed and were stored at -80 ºC until use. The amphotericin B MIC was read as the lowest concentration that produced the complete inhibition of growth, the flucytosine and voriconazole MICs were read as the lowest concentrations that produced a prominent decrease in turbidity (an approximately 50% reduction in growth) relative to the growth for the drug-free control (National Committee for Clinical Laboratory Standards, 2002).

### Identification of *Candida* species and antifungal susceptibility by VITEK 2 system using ID-YST card

Before testing, a suspension of each isolate was inoculated onto Sabouraud dextrose agar

slants to ensure the purity and the viability of the cultures. The inoculum suspensions for the VITEK 2 were prepared in sterile saline at turbidity equal to a 2.0 McFarland standard. The individual test cards were automatically filled with the prepared culture suspension, sealed, and incubated by the VITEK 2 instrument. The cards were incubated at 35.5ºC for 18 h, and optical density readings were taken automatically every 15 min. 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. For antifungal susceptibility test: The VITEK 2 cards containing serial two fold dilutions of amphotericin B, flucytosine, and voriconazole were provided by the manufacturer. *Candida* inocula were prepared in sterile distilled water from a 24-h culture and were incubated on Sabouraud dextrose agar at 35 ºC or 30 ºC. The inocula for the VITEK 2 were prepared in sterile saline to turbidity equal to a 2.0 McFarland standard. Each standardized inoculum suspension was placed into a VITEK 2 cassette along with a sterile polystyrene test tube and a yeast susceptibility test card. The cassettes were placed in the VITEK 2 instrument and the respective yeast suspensions were diluted appropriately, after which the cards were filled, incubated, and read automatically by the VITEK 2.

The time of incubation varied from 10 to 30 h based on the growth rate in the drug-free control well, and the results were expressed as MICs in micrograms per milliliter.

## Results and Discussion

The present study was done on 50 *Candida* isolates identified into species level by Vitek-2 system. *C. albicans* (n = 29) (58%) were most commonly isolated, followed by *C. tropicalis* (n = 9) (18%), *C. krusei* (n = 6) (12%), *C. glabrata* (n = 4) (8%), and *C. parapsilosis* (n = 2) (4%).

As shown in table 1, AST for *Candida albicans*, showed that all of them were susceptible for Amphotericin B by both Vitek-2 system and BMD method, 27 isolates (93.1%) were susceptible for Voriconazole by Vitek-2 system while 28 (96.6%) were susceptible for the same drug by BMD method.26C.albicans (89.7%) were susceptible for Flucytosine by Vitek-2 system and 28 (96.6%) were susceptible for Flucytosine by BMD method but 3 (10.3%), 2 (6.9%) were resistant to both Flucytosine and Voriconazole by Vitek-2 system respectively, 1 (3.4%) was resistant to both Flucytosine and Voriconazole by BMD method. As regards to *C. tropicalis* (n=9), AST showed that all of them were susceptible for Amphotericin B and Voriconazole by both Vitek-2 system and BMD method, eight (88.9%) were susceptible for Flocytocine by both Vitek-2 system BMD method and only one (11.1%) was resistant to Flocytocine by both Vitek-2 system BMD method. Among *C. krusei* (n=6), all of them were susceptible for Voriconazole by both Vitek-2 system and BMD method, 4 (66.7%) were susceptible for Amphotericin B by Vitek-2 system and 5 (83.3%) were susceptible for Amphotericin B by BMD method, 2 (33.3%) were susceptible for Flocytocine by Vitek-2 system and 1(16.7%) was susceptible for Flucytosine by BMD method and 4 (66.7%), 2 (33.3%) showed resistance to both Flucytosine and Amphotericin B by Vitek-2 system respectively, 5 (83.3%), 1 (16.7%) showed resistance to both Flucytosine and Amphotericin B by BMD method respectively. All of *C. glabrata* isolates (n=4) were susceptible for Flucytosine and Amphotericin B by both Vitek-2 system and BMD method, 3 (75%) were susceptible for Voriconazole by both Vitek-2 system and

BMD method and 1 isolate (25%) was resistant to Voriconazole. Lastly, *C. parapsilosis* (n=2) were susceptible for Voriconazole, Flucytosine, and Amphotericin B by both Vitek-2 system and BMD method.

All the correlation coefficient indices were statistically significant between Vitek-2 system and broth microdilution method in antifungal susceptibility testing of different *Candida* species (Table 2). Sensitivity and Specificity of Vitek2 system method in antifungal susceptibility testing for Flucytosine were 84%, 86% respectively, Sensitivity and Specificity for Voriconazole were 94%, 96% respectively, and Sensitivity and Specificity for Amphotericin B were 96%, 98% respectively. We used broth microdilution method as reference method (Table 3).

**Table.1** Antifungal susceptibility testing of different *Candida* species by Vitek2 system and broth microdilution method (BMD)

| Species name (n=50) | Identification method | Flucytosine | | Voriconazole | | Amphotericin B | |
|---|---|---|---|---|---|---|---|
| | | S | R | S | R | S | R |
| *C. albicans* (n=29) | Vitek 2 | 26 (89.7%) | 3 (10.3%) | 27 (93.1%) | 2 (6.9%) | 29 (100%) | 0 (0%) |
| | BMD | 28 (96.6%) | 1 (3.4%) | 28 (96.6%) | 1 (3.4%) | 29 (100%) | 0 (0%) |
| *C. tropicalis* (n=9) | Vitek 2 | 8 (88.9%) | 1 (11.1%) | 9 (100%) | 0 (0%) | 9 (100%) | 0 (0%) |
| | BMD | 8 (88.9%) | 1 (11.1%) | 9 (100%) | 0 (0%) | 9 (100%) | 0 (0%) |
| *C. krusei* (n=6) | Vitek 2 | 2 (33.3%) | 4 (66.7%) | 6 (100%) | 0 (0%) | 4 (66.7%) | 2 (33.3%) |
| | BMD | 1 (16.7%) | 5 (83.3%) | 6 (100%) | 0 (0%) | 5 (83.3%) | 1 (16.7%) |
| *C. glabrata* (n=4) | Vitek 2 | 4 (100%) | 0 (0%) | 3 (75%) | 1 (25%) | 4 (100%) | 0 (0%) |
| | BMD | 4 (100%) | 0 (0%) | 3 (75%) | 1 (25%) | 4 (100%) | 0 (0%) |
| *C. parapsilosis* (n=2) | Vitek 2 | 2 (100%) | 0 (0%) | 2 (100%) | 0 (0%) | 2 (100%) | 0 (0%) |
| | BMD | 2 (100%) | 0 (0%) | 2 (100%) | 0 (0%) | 2 (100%) | 0 (0%) |

S= susceptible R=Resistant

N.B: The CLSI interpretive breakpoints for flucytosine (susceptible less or equal to 4 µg/mL, resistant more or equal to 32 µg/mL), forvoriconazole (susceptible less or equal to 1 µg/mL, resistant more or equal to 4 µg/mL) and for amphotericin B, isolates with MICs of ≥1 µg/ml were categorized as resistant.

**Table.2** Comparison between Vitek2 system and broth microdilution method in antifungal susceptibility testing of different *Candida* species

| | | Vitek 2 | | | | | |
|---|---|---|---|---|---|---|---|
| | | Flucytosine | | Voriconazole | | Amphotericin B | |
| | | r | P.value | r | P.value | r | P.value |
| BMD | *C. albicans* | 0.574 | < 0.05* | 0.619 | < 0.05* | 0.924 | < 0.05* |
| | *C. tropicalis* | 0.854 | | 0.863 | | 0.863 | |
| | *C. krusei* | 0.523 | | 0.831 | | 0.523 | |
| | C. Glabrate | 0.812 | | 0.819 | | 0.924 | |
| | *C. parapsilosis* | 0.803 | | 0.803 | | 0.924 | |

*statistically significant

r=Correlation coefficient

**Table.3** Sensitivity and specificity of Vitek2 system method in antifungal susceptibility testing of different *Candida* species

| Antibiotic | Sensitivity | Specificity | PPV | NPV | Accuracy |
|---|---|---|---|---|---|
| **Flocytocine** | 84% | 86% | 85% | 84% | 85% |
| **Voriconazole** | 94% | 96% | 96% | 94% | 95% |
| **Amphotericin** | 96% | 98% | 98% | 96% | 97% |

*Candida* species is a major causative organism of hospital acquired systemic mycosis, morbidity and mortality worldwide, especially in critically ill and immunocompromised patients (Sardi *et al.*, 2013). Among *Candida* species, *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei* were the most common species recovered in clinical laboratories (Graf *et al.*, 2000). Our study was carried on 50 *Candida* isolates identified into species level by Vitek-2 system. *C. albicans* (58%) were most commonly isolated, followed by *C. tropicalis* (18%), *C. krusei* (12%), *C. glabrata* (8%), and *C. parapsilosis* (4%). These results are in agreement with a previous study of Jha *et al.*,2006 in which the majority of *Candida* species were *C. albicans* (70%) followed by *C. tropicalis* (13.33%), *C. krusei* (10%), *C. glabrata*, *C. parapsilosis* (3.33%), and *C. stellatoidea* (3.33%). Also Kumari *et al.*, 2014 found similar results. In our study, we also evaluated the Vitek-2 AST system with the CLSI broth microdilution method for antifungal susceptibility testing of *Candida* species. Most of *Candida* isolates were susceptible to both antifungal drugs tested by AST Vitek-2 cards and the CLSI BMD method, but some of *C. albicans*, *C. tropicalis* and *C. krusei* showed resistance to Flucytosine by both Vitek-2 system and BMD method. Some of *C. albicans* and *C. glabrata* strains showed resistance to Voriconazole. Similarly, Magill *et al.*, (2006) and Pfaller *et al.*, (2007) detected resistance to azole antifungal drugs in *C. albicans* and *C. glabrata* species. Four (66.7%) of the isolates of *C. krusei* were resistant to Flucytosine drug and 2 (33.3%) were resistant to amphotericin B by Vitek-2 system and 5 (83.3%) were resistant to Flucytosine and 1 (16.7%) was resistant to amphotericin B by CLSI broth microdilution method. This has noted by other workers, Pahwa *et al.*, (2014) and Zhang *et al.*, (2015) who found that *C. krusei* was the most resistant species to many antifungal drugs and had intrinsic resistance to azole drugs and poor susceptibility to other antifungals, including amphotericin B. For this reason, Flucytosine and amphotericin B should be avoided in treatment of *C. krusei* fungal infections. Vitek-2 system was the first commercially available automated approach to AST and provides optimal susceptibility test standardization (Alexander and Pfaller, 2006). In our study all the correlation coefficient indices between Vitek-2 system and broth microdilution method (reference method) in antifungal susceptibility testing of different *Candida* species were statistically significant and sensitivity and specificity of Vitek 2 system method in antifungal susceptibility testing for Flucytosine were 84%, 86% respectively, for Voriconazole were 94%, 96% respectively, and for Amphotericin B were 96%, 98% respectively. Our results were in parallel with that of Pfaller *et al.*, (2007). We could conclude that Vitek-2 system can be used as a reliable method for AST in addition to its reliability as a rapid method for *Candida* species identification.

The present study revealed that Vitek-2 system reduces the period required for identification and antifungal susceptibility of

*Candida* species isolates. So, Vitek-2 system appeared to be arapid reliable method for identification and AST for the *Candida* species to prescribe appropriate antifungal agents for the better management of hospital acquired fungal infections especially in critically ill and immunosuppressed patients.

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