

Rapid Identification of Yeast Isolates from Clinical Specimens in Critically Ill Trauma ICU Patients

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ABSTRACT

Purpose: The purpose was to evaluate the performance of a commercially available chromogenic *Candida* speciation media and the Vitek 2 ID system for the identification of medically important yeasts and yeast-like organisms in a routine clinical microbiology laboratory.

Materials and Methods: A total of 429 non duplicate, consecutive yeast strains were included during the 3.5-year study period. The performance of the Vitek 2 ID system and a chromogenic agar medium was evaluated against the gold standard conventional phenotypic and biochemical identification method for speciation of yeast isolates from trauma patients.

Results: *Candida tropicalis* (64%) was the most common *Candida* species, followed by *Candida albicans* (14%), *Candida rugosa* (7%), and *Candida parapsilosis* (6.5%). Of the 429 isolates, 183 could be identified to species level by all the three methods. Agreement between the chromogenic agar method and conventional methods was 80% for *Candida tropicalis*, 100% for *Candida rugosa*, 89% for *Candida albicans*, and 77% for *Candida parapsilosis*. Vitek 2 had lower sensitivity, with agreement of 49% for *Candida tropicalis*, 100% for *Candida rugosa*, 39% for *Candida albicans*, and 31% for *Candida parapsilosis*.

Conclusion: Thus, in long-term ICU patients, an increasing trend of isolating nonalbicans *Candida* spp. continues. The chromogenic agar medium is a convenient and economic method to identify commonly isolated species in busy clinical microbiology laboratories.

Key words: *Candida*, chromogenic agar medium, identification, trauma, vitek

INTRODUCTION

Despite substantial advances in trauma care, critical traumatic injuries remain one of the leading causes of death in the below 44 years age group.^[1] Increased mortality in the critically ill adult trauma population is not solely due to the direct effects of trauma. Patients who survive the initial injury acquire nosocomial infections, which are the leading cause of death in them.^[1,2] Trauma patients usually have prolonged ICU stay and therefore are more

prone to acquire hospital-acquired infections (HAIs). These patients are usually treated with broad-spectrum antibiotics, making them more susceptible for *Candida* infections. In ICUs, the common infections due to *Candida* sp. are catheter-related blood stream infections (CR-BSIs), intra-abdominal infections, and urinary tract infections (UTIs). Invasive candidiasis is one of the major causes of morbidity and mortality in both immunocompetent and immunocompromised patients, the crude and attributable mortality being high as 40% and 20% respectively.^[3,4] Therefore better management of these infections solely depends on rapid identification and reporting of results.

In the past decade, hospitals across the world have noticed a change in the epidemiology of *Candida* infections, characterized by a progressive shift from *Candida albicans* to a predominance of nonalbicans

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Candida Spp.^[4-6] There is growing evidence of the increasing use of azoles causing this epidemiological shift.^[4,7] Characterization to species level helps to identify those strains which might be intrinsically resistant to some of the antifungal agents.

Identification of yeast pathogens by traditional methods requires several days and specific mycological media. These methods are thus labor intensive and time consuming. Several brands of chromogenic media are available for rapid identification of *Candida* Spp. The chromogenic media contain substrates which react with enzymes secreted by the target microorganisms to yield colonies of varying colors.^[8-10]

Commercial automated identification systems have also been developed and are being used in some routine clinical microbiology laboratories. These systems assert for accurate and rapid identification of medically relevant bacteria and yeast.^[11-15] The Vitek 2 system (Biomérieux, France) is a fully automated system, dedicated to the identification and susceptibility testing of microorganisms. The ID-YST database of Vitek 2 for yeast identification comprises 51 different taxa, including newly described species, taking into account recent advances in taxonomy.

In this study, we evaluated the performance of a commercially available chromogenic *Candida* speciation media and the Vitek 2 ID system for the identification of medically important yeasts and yeast like organisms in a routine clinical microbiology laboratory.

MATERIALS AND METHODS

The study was conducted at the Microbiology laboratory of the JPNA Trauma Centre of All India Institute of Medical Sciences (AIIMS) and the Mycology laboratory of the AIIMS hospital. AIIMS is a 2500-bedded, tertiary care, referral, and teaching hospital, where patients are referred from all over India. Its Trauma Centre is a 152-bedded, level 1 Trauma care hospital. The study was conducted from January 2007 to June 2010.

All the consecutive, nonduplicate isolates of yeast recovered from the clinical samples of patients admitted to the trauma center were included in the study.

All the *Candida* isolates were identified to the species level.

The following strains were taken as controls for evaluation of the various methods: *Candida albicans* ATCC 10231, *Candida tropicalis* ATCC 13803, *Candida krusei* ATCC 14243,

and *Candida glabrata* 15126, *Candida kefyr* ATCC 204093, *Candida guilliermondii* ATCC 6260, *Cryptococcus neoformans* ATCC 14116.

Identification of all clinical isolates was done to the species level by means of conventional mycological methods, chromogenic *Candida* speciation media, and the Vitek 2 ID system.

For the purpose of comparison, the conventional methods were taken as the gold standard method. The conventional identification was carried out in the Mycology laboratory of AIIMS hospital. All isolates were sent from the Trauma center to that laboratory with a coded number. The identification by other two methods was done at the Microbiology laboratory of the Trauma Centre. Results were compared only when the final report was generated by all the three methods.

Conventional mycological methods

All the *Candida* isolates obtained from clinical specimens were initially subcultured onto sabouraud dextrose agar (SDA) medium and incubated at 37°C till growth appeared. Colonies from the SDA were then plated onto corn meal agar with tween 80 for morphological examination^[16,17] and triphenyltetrazolium chloride (TTC) medium for determination of pigment production. A germ tube test was performed on yeast-like colonies for presumptive identification of *Candida albicans*. The strains were further characterized by manual sugar assimilation methods.^[16,18] The tests were performed according to standard methods and using the above control strains.

Identification on chromogenic agar medium

Growth from SDA slants was plated onto Chrom agar (BD, USA). The plates were incubated at 37°C for 48 hours and the colony morphology (color, size, and texture) were assessed to interpret the identification of species. The interpretation was based on published appearance of various species on chromogenic agar: Parrot green colonies of *C. albicans*; steel blue colonies of *C. tropicalis* accompanied by purple pigmentation which diffuses into surrounding agar by growth; larger, fuzzy, rose-colored colonies with white edges of *C. krusei*; smooth white to light pink colonies of *C. glabrata* which later became pink; pink to lavender colonies of *C. parapsilosis*; gray to pale pink of *Cryptococcus neoformans* and green color with pale borders of *C. rugosa* [Figure 1]. The quality of the media was checked every time with the ATCC strains. Species were identified when the isolates conformed unequivocally



Figure 1: Colonies grown on CHROMagar Candida for 48 hours at 30°C: (a) *C. neoformans*, (b) *C. albicans*, (c) *C. tropicalis*, (d) *C. guilliermondii*, (e) *C. krusei*, (f) *C. kefyr*, (g) *C. glabrata*

to these morphological features. Two different observers observed the morphology of each isolates. All isolates which gave doubtful morphology or which did not conform to the accepted morphological features were taken as “unidentified” or “misidentified.”

Vitek 2 ID system

All the strains were subjected to identification by the Vitek 2 system. The tests were performed according to the manufacturer’s instructions. Species which were identified as “high discrimination” were taken as the identification of an isolate. The isolates which were “unidentified” or were identified as more than one species (“low discrimination”; for example 50 % probability of identification for three different species) were all taken as “unidentified” or “misidentified” respectively and excluded from analysis.

RESULTS

During the 3.5-year study period, a total of 429 nonduplicate yeast isolates were obtained from the clinical samples of trauma patients. Of these, 238 (55%) were from urine, 174 (40.5%) from blood, 8 (2%) from CVC tips, 4 (1%) from pus, 3 (0.6%) from peritoneal fluid, and 2 (0.4%) from tracheal aspirates. The distribution and sources of *Candida* species, as identified by the gold standard conventional methods, are shown in Table 1. The ratio of *C. albicans* to nonalbicans candida isolates was 1: 6.

Of these 429 isolates, complete identification to the species level by all the three methods could be obtained in 183 (43%) isolates.

Table 1: Sources and distribution of Candida species in clinical samples

| Candida species | Source | | | Total (%) |
|-------------------------|------------|-------------|------------|-------------|
| | Blood (%) | Urine (%) | Others (%) | |
| <i>C. tropicalis</i> | 72 (41.38) | 189 (79.41) | 15 (88.23) | 276 (64.33) |
| <i>C. albicans</i> | 32 (18.39) | 29 (12.18) | 0 | 61 (14.21) |
| <i>C. rugosa</i> | 28 (16.09) | 01 (0.4) | 0 | 29 (6.75) |
| <i>C. parapsilosis</i> | 23 (13.21) | 04 (1.68) | 01 (5.8) | 28 (6.52) |
| <i>C. glabrata</i> | 8 (4.59) | 02 (0.84) | 01 (5.8) | 11 (2.56) |
| <i>C. krusei</i> | 7 (4.02) | 01 (0.4) | 0 | 8 (1.86) |
| <i>C. lusitanae</i> | 1 (0.57) | 0 | 0 | 1 (0.23) |
| <i>Trichosporon sp.</i> | 3 (1.72) | 12 (5.04) | 0 | 15 (3.49) |
| Total | 174 | 238 | 17 | 429 |

Of these 183 strains, the comparison between chromogenic medium and the gold standard method is shown in Table 2. There was agreement in identification by the chromogenic agar medium in 150 (82%) strains. The performance of the Vitek 2 system against the gold standard is shown in Table 2. Of the 183 strains, only 98 (53%) showed agreement in identification.

The sensitivity of identification by chromogenic agar for most commonly isolated species was 80% for *C. tropicalis*, 100% for *C. rugosa*, 89 % for *C. albicans*, and 77% for *C. parapsilosis* (77%) as shown Table 2. Misidentification or nonidentification of *C. tropicalis* was usually seen due to the difficulty in interpretation of green color, which was misidentified as *C. albicans* in 11 isolates. In 10 isolates of *C. tropicalis*, no color was obtained on chromogenic agar medium.

Of the 50 isolates of *C. tropicalis* misidentified by Vitek 2, 36 were identified as two or three different probable species, 8 were identified with a probability of >90%, but as a different species than conventional methods and 6 had low discrimination of identification. In general, the most common misidentification by Vitek 2 was *C. tropicalis* or *C. albicans* identified as *Cryptococcus laurentii* or *Streptomyces ceferrii*.

We compared the efficiency of Vitek 2 and chromogenic agar for identification of yeast isolates [Table 2]. Chromogenic agar was certainly the more efficient method over Vitek 2 for identification of most commonly isolated species from our center. Both the methods were apt for identification of some species such as *Candida rugosa*. But they were not found to be expeditious in identification of less encountered species - *Candida krusei*, *Candida glabrata*, *Candida lusitanae*.

DISCUSSION

In this study consistent with the published report from different part of the world, Nonalbican *Candida* species

Table 2: Comparison of CHROMagar with Vitek 2

| Spp. name | CHROMagar | | Vitek 2 | | P value |
|------------------------|---------------|------------------------------------|---------------|------------------------------------|---------|
| | Agreement (%) | Misidentification/unidentified (%) | Agreement (%) | Misidentification/unidentified (%) | |
| <i>C. tropicalis</i> | 86 (80.37) | 21 (19.63) | 52 (48.29) | 55 (51.71) | <0.0001 |
| <i>C. rugosa</i> | 29 (100) | 0 | 29 (100) | 0 | =1.0 |
| <i>C. albicans</i> | 16 (88.88) | 2 (11.12) | 7 (38.88) | 11 (61.12) | =0.0045 |
| <i>C. parapsilosis</i> | 10 (76.92) | 3 (23.08) | 4 (30.76) | 9 (69.24) | =0.0472 |
| <i>C. glabrata</i> | 2 (66.67) | 1 (33.33) | 1 (33.33) | 2 (66.67) | =1.000 |
| <i>C. krusei</i> | 1 (50) | 1 (50) | 1 (50) | 1 (50) | =1.000 |
| <i>C. lusitaniae</i> | 1 (100) | 0 | 0 | 1 (100) | =1.000 |
| <i>Trichosporon</i> | 5 (50) | 5 (50) | 4 (40) | 6 (60) | =1.000 |
| Total | 150 (82) | 33 (18) | 98 (54) | 85 (46) | <0.0001 |

had predominance over *C. albicans*.^[4,6] *C. tropicalis* was most common isolate in all samples, followed by *C. albicans*. A relatively greater proportion of *C. tropicalis* isolates in our study is concordant with other studies from India.^[19,20]

We also isolated a large number of *C. rugosa* from patients admitted to the ICUs, which occurred as a small outbreak.^[21] *C. rugosa* appears to typically produce a readily identifiable and unique color/colony type on chromogenic agar medium. It has been shown in clinical reports and by *in vitro* testing to be less susceptible to amphotericin B.^[21,22] Rapid identification of this nonalbican *Candida* is of great importance to allow provision of appropriate therapy to patients. Also, since it has a tendency to for rapid cross-transmission, early identification is vital.^[21]

In our study we evaluated two rapid methods, chromogenic agar medium and fully automated Vitek 2 system for identification of yeast and yeast-like organism. The strains were simultaneously tested by the conventional method. The results indicate that CHROMagar is able to correctly identify majority of common clinical isolates. The apparent lack of accuracy of Vitek 2 ID in our study is in contrast with previous studies which had shown higher sensitivity.^[14,23-25]

We observed that for those strains which could not be resolved by chromogenic agar medium alone, sensitivity increased when we used both chromogenic agar and Vitek 2 from 80% to 94% for *C. tropicalis* and up to 100% for some of the organisms. Thus Vitek 2 ID could be an additional help in those situations where CHROMagar could not give identification.^[26] However there were certain limitations in our study such as there was no isolate of *Candida dublinensis* which is difficult to distinguish from *Candida albicans* on chromogenic agar and very few or no isolates of *Candida lusitaniae*, *Candida krusei*, *Candida glabrata*, and *Cryptococcus neoformans* for comparison.

In conclusion, the chromogenic agar medium supported by Vitek 2 ID is a valuable method for identification

of commonly encountered, medically important yeast species. The method is easy to interpret and gives rapid results.

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