Evaluation of CHROMagar Candida, VITEK2 YST and VITEK® MS for identification of Candida strains isolated from blood cultures

Valutazione di CHROMagar Candida, VITEK2 YST e VITEK® MS per l’identificazione di isolati di Candida da emocoltura

Fatma Mutlu Sariguzel¹, Elife Berk², Ayse Nedret Koço³, Hafize Sav³, Gonca Aydemir³
¹Department of Microbiology, Kayseri Education and Research Hospital, Kayseri, Turkey; ²Department of Microbiology, Erciyes University Medical School, Kayseri, Turkey

INTRODUCTION

Candidemia is an increasingly common problem in hospitalized patients, especially among immunocompromised patients. C. albicans is the predominant cause of candidemia, with a crude mortality rate of approximately 40% [1-5]. However, non-albicans Candida species such as C. parapsilosis, C. glabrata, C. krusei and C. tropicalis are emerging as opportunistic pathogens [2]. C. parapsilosis is the second most frequently isolated Candida species from blood cultures in Europe, Latin America and Canada [2-6]. Non-albicans Candida species have decreased susceptibility to antifungal agents [2]. For this reason, fast accurate diagnosis and treatment are very important for the prognosis of patients with candidemia [6]. Several phenotypic tests based on colony morphology have been developed to distinguish Candida strains. In clinical laboratories, these methods are used as reference identification procedures [7, 8]. However, phenotypic tests are not practical for the clinical laboratory because they are time-consuming and labor-intensive. Therefore, Vitek 2 YST card automated systems and chromogenic media for the identification of Candida species have been developed [8-17]. MALDI-TOF MS technology was seen as a revolution in microbiology laboratories for the identification of Candida species [18-23]. CHROMagar Candida (Oxoid Brilliance™ Candida agar, England) is based on the formation of various colored colonies which result from the use of chromogenic substrates by species specific enzymes. These enzymes allow organisms to be identified at species level by their color and colony characteristics [8]. This media has been used to identify Candida spp. in several studies. Most studies related to this medium were carried out with stock isolates and direct clinical specimens [9-13]. In these studies, the percentage of correct identification by using CHROMagar Candida were reported as 99.4-95% [10, 11, 13]. The VITEK2 YST system (bioMérieux, France) is a fully automated instrument for the identification and susceptibility testing of microorganisms [14]. The ID-YST database of VITEK2 YST for yeast identification comprises 54 different taxa, including newly described species and takes into account recent advances in taxonomy. The time to identification by the VITEK2 YST system was 18 hours, compared to 48 to 72 hours by the API 20C AUX method [15]. Matrix-assisted laser desorption ionisation time of flight mass spectrometry (MALDI-TOF MS) is based on the detection of specific proteins released from microbial cell’s surface and has also

Corresponding author
Fatma Mutlu Sariguzel
Email: fmutluguzel@gmail.com
been applied recently for the rapid and accurate identification of strains isolated from blood cultures including *Candida spp.* [18-20]. Most studies related to this system were carried out with stock isolates and direct positive blood cultures bottles. Direct identification from positive blood cultures bottles should decrease the time to obtaining the result, with the exception of polymicrobial samples [24-27]. VITEK®MS (bioMérieux, France) is an automated microbial identification system based on MALDI-TOF MS technology.

In this study, we aimed to compare the conventional methods, CHROMagar Candida, VITEK2 YST card and VITEK®MS system for the identification of *Candida* strains isolated from blood cultures.

## MATERIALS AND METHODS

A total of 54 non-repetitive strains isolated from the positive blood cultures of intensive care unit patients were included in the study. All of these strains were considered as the aetiological agents. The isolated strains were stored frozen for one year until runtime and strains were subcultured onto Sabouraud dextrose agar for 48 hours at 37°C prior to test.

Conventional methods: Yeast colonies were identified by using a commercial system based on the reference method according to macroscopic morphology on Sabouraud dextrose agar, the assimilation of carbohydrate by API 20C AUX (bioMérieux, France) kits, with respect to the microscopic morphology on corn meal agar, germ tube test, capability of growing at 37°C, urea hydrolysis and sensitivity for cycloheximide. Quality control strain was *C. albicans* ATCC 90028.

**CHROMagar Candida medium:** The CHROMagar Candida (Oxoid Brilliance™ Candida agar, England) was used according to the manufacturer’s guidelines. Green colonies on CHROMagar Candida were identified as *C. albicans*. Blue colonies on CHROMagar Candida were identified as *C. tropicalis*. Colonies of other *Candida* spp., including *C. glabrata* and *C. parapsilosis*, appear as a variety of beige/brown/yellow colors, due to the mixture of natural pigmentation and some alkaline phosphatase activity. These strains were differentiated according to color and colony morphology. Brown colonies on CHROMagar Candida were identified as *C. parapsilosis*. Yellow colonies on CHROMagar Candida were identified as *C. glabrata*.

**VITEK2 YST card system:** Inoculum suspensions for use with the VITEK2 YST cards (bioMérieux, France) were obtained from overnight cultures, with the turbidity being adjusted to a 1.8 to 2.2 McFarland Standard using the bioMérieux DenSicheck instrument, according to the manufacturer’s recommendations.

**MALDI-TOF VITEK®MS:** The strains were tested by depositing one yeast colony on a steel MALDI target slide by loop and a drop of formic acid was dropped onto the slide. The spot was dried and then overlaid with 1 µl MALDI matrix solution (VITEK MS-CHCA) and air-dried. The prepared slide was inserted into the VITEK®MS system. Identification of yeast was analysed by the VITEK® MS database. The peaks from these spectra were compared to the characteristic pattern for a particular species, genus or family of microorganisms and this resulted in the organism identification. Quality control strains were *C. albicans* ATCC 90028. *E. coli* ATCC 8739 was used to calibrate the instrument for each run.

**Sequencing analysis:** DNA extraction was performed by Fluorion i12 nucleic acid isolation device (Iontek, Turkey). Species identification of *Candida* isolates was confirmed by sequencing the ITS1-5.8S-ITS2 region as described by White et al. [24]. The ITS region was amplified using the ITS1 forward primer 5’- TCCGTAGGTGAACCTGCGG-3’ and the ITS4 reverse primer 5’-TCCTC- CGCTTATTGATATGC -3’. Sequence data were generated using the ABI PRISM® BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) and sequenced using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). Sequences entered into a BLASTN search were provided by GenBank for species identification.

## RESULTS

The 54 strains were identified as *C. parapsilosis* (n=32), *C. albicans* (n=19), *C. glabrata* (n=1) and *C. tropicalis* (n=2) according to the reference identification procedures used in this study. All isolates identification obtained by conventional methods were concordant with sequencing analysis. The distribution of the results obtained by differ-
ent methods to identify the 54 Candida strains is shown in Table 1. One C. albicans and one C. glabrata isolates were misidentified as C. parapsilosis by CHROMagar Candida. In microscopic morphology of these strains, C. albicans had chlamydospores, blastospores and pseudohypha and C. glabrata had only blastospores. Two C. parapsilosis and three C. albicans isolates were misidentified by VITEK2 YST. In microscopic morphology of these strains, C. parapsilosis had curved pseudohyphae, with blastoconidia, large giant cells and C. albicans had chlamydospore, blastospore and pseudohypha. The CHROMagar Candida and VITEK2 YST automated card system correctly identified 96.2% and 90.7% of all strains, respectively. VITEK®MS system correctly identified 100% of the strains.

<table>
<thead>
<tr>
<th>Candida species</th>
<th>Methods (%)</th>
<th>Conventional methods</th>
<th>CHROMagar Candida</th>
<th>VITEK2 YST card</th>
<th>VITEK®MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. parapsilosis</td>
<td>(n=32)</td>
<td>32/32 (100%)</td>
<td>32/32 (100%)</td>
<td>30/32 (93.7%)</td>
<td>32/32 (100%)</td>
</tr>
<tr>
<td>C. albicans</td>
<td>(n=19)</td>
<td>19/19 (100%)</td>
<td>18/19* (94.7%)</td>
<td>16/19* (84.2%)</td>
<td>19/19 (100%)</td>
</tr>
<tr>
<td>C. glabrata**</td>
<td>(n=1)</td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>C. tropicalis**</td>
<td>(n=2)</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
</tr>
</tbody>
</table>

*One C. albicans isolate was misidentified as C. parapsilosis by CHROMagar Candida.
†One C. glabrata isolate was misidentified as C. parapsilosis by CHROMagar Candida.
‡One C. parapsilosis isolate were misidentified as C. glabrata by VITEK2 YST card.
§Three C. albicans isolates were misidentified as C. parapsilosis, C. lusitaniae and C. famata by VITEK2 YST card.
**Because of the small number of C. glabrata and C. tropicalis isolates, the identification rates of these strains has not shown.

** DISCUSSION **

Because of the need to rapidly and accurately identify Candida isolates at the species level for treatment of candidemia, we evaluated the CHROMagar Candida agar, fully automated VITEK2 YST and VITEK®MS systems. All isolates were simultaneously compared with conventional methods and sequencing analysis, this latter being used as reference method. CHROMagar Candida has been used to identify C. albicans, C. tropicalis, and C. krusei with a high degree of accuracy in several studies [9-11]. In a Turkish study, Cetinkaya et al. reported that the sensitivity ratio of this medium was found as 97.7% including 255 Candida isolates (e.g., C. parapsilosis, C. albicans, C. tropicalis, and C. glabrata) [11]. In another study including C. albicans, C. tropicalis, C. glabrata, and C. krusei, Pfaller et al. found 95% sensitivity ratio using CHROMagar [13]. The researchers concluded that CHROMagar Candida also allowed the identification of C. glabrata. In our study, two Candida isolates were misidentified as C. parapsilosis by CHROMagar Candida. But, the other isolates were identified accurately by CHROMagar Candida. Although we detected high concordance between the reference system and CHROMagar, identification of C. glabrata was not concordant with the reference method. This discrepancy may be explained by the small number of C. glabrata isolates in our study. Most studies reported that the VITEK2 YST card system was shown as a reliable method in identification of yeasts and this system correctly identified 93-99% of common and uncommon yeasts [7, 15, 16, 18]. Meurman et al. have investigated the concordance between the VITEK2 YST card system and conventional methods among 151 clinical specimens and 16 known culture types [17]. In this study, an unequivocal identification was obtained for 155 (92.8%) isolates and all isolates of C. albicans, C. glabrata and C. krusei were identified correctly. One out of 22 C. parapsilosis isolates had a low discrimination result. In the Graf et al. study, C. albicans, C. tropicalis and C. glabrata were correctly identified in 100% of isolates without additional tests; C. parapsilosis was identified in 96.8% of isolates without additional tests [14]. In our study, five Candida isolates were misidentified by VITEK2 YST. The C. albicans identification rate was lower as compared to the above mentioned studies, whereas all of C. tropicalis and C. glabrata isolates were identified accurately. The databases of most commercially available identification software associated with a MALDI device include the reference spectra of the most commonly encountered yeasts in the laboratory,
such as C. albicans, C. parapsilosis, C. glabrata, C. tropicalis and C. krusei [21]. In other comparative studies with MALDI-TOF, conventional methods and sequencing of the ITS regions of ribosomal DNA were found similar according to correct identification rates. Lavergne et al. evaluated the performance of the VITEK®MS and reported that the concordance between MALDI-TOF MS and conventional identification was 97% for yeasts [23]. Iriart et al. [22] reported that the concordance between MALDI-TOF MS, conventional methods and VITEK2 YST card was 97.9% for yeasts. In our study, we shown that the correct identification rate of VITEK®MS system was 100%.

In the present study, the identification with CHROMagar Candida and API 20C AUX required an incubation at 37°C for at least 48 hours, while the VITEK2 YST card required an incubation at 35°C for at least 15 hours. But, identification performed by means of VITEK®MS required only 2 minutes. Among these automated systems, VITEK®MS has the highest identification rate and it allows a faster identification compared with other methods.

In conclusion, conventional methods are still considered as the reference standard for identification of yeast isolates, but they are time-consuming and labor-intensive. The CHROMagar Candida, VITEK2 YST card and VITEK®MS automated systems appear to be excellent alternative methods for yeast identification in clinical microbiology laboratories. However, conventional identification methods should be used together with CHROMagar Candida and VITEK2 YST card to avoid misdiagnosis.

Conflict of interest: The authors declare no conflict of interest.

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Keywords: Candida, identification, CHROMagar Candida, VITEK2 YST, VITEK®MS.
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