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SHORT COMMUNICATION

Usefulness of CHROMagar Candida Medium, Biochemical Methods – API ID32C and VITEK 2 Compact and Two MALDI-TOF MS Systems for *Candida* spp. Identification

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Abstract

This study was conducted to compare of the yeasts identification results obtained with two new systems using the MALDI-TOF MS technique with the ones obtained using the routine identification methods of *Candida* spp. in clinical microbiology laboratories. All 124 *Candida* spp. isolates were recovered from the routine examination of clinical specimens in microbiological laboratories and collected in the Centre of Quality Control in Microbiology in Warsaw (Poland). Our findings confirm the high agreement (98%) of fungal identification using the standard, biochemistry laboratory methods and mass spectrometry technique.

Key words: *Candida* spp. – identification, chromogenic medium and biochemical methods, MALDI-TOF MS

The rise of fungal infections correlates with the widespread use of broad-spectrum antibacterial agents, prolonged hospitalization of critically ill patients and the increased number of immunocompromised patients (Bassetti *et al.*, 2006; 2009). *Candida albicans* is still the most frequently isolated, but the frequency of isolation of non-albicans species such as *Candida krusei*, *Candida glabrata, Candida kefyr*, *Candida tropicalis* and *Candida parapsilosis* is steadily increasing globally (Levy *et al.*, 1998; Marklein *et al.*, 2009). It is crucial to identify the *Candida* isolates to the species level in order to make therapeutic decisions. The species such as *C. glabrata* and *C. krusei*, when compared with *C. albicans*, are generally less susceptible to commonly used antifungal drugs such as fluconazole. The majority of *Candida dubliniensis* clinical isolates examined so far are susceptible to commonly antifungal drugs (Sullivan and Coleman, 1998). However, oral *C. dubliniensis* isolates with significantly reduced susceptibility to fluconazole have been recovered from immunocompromised patients who had previously been treated with fluconazole (Ruhnke *et al.*, 2000; Martinez *et al.*, 2002). Commercially available chromogenic agar media as well as biochemical and enzymatic panels are widely used for rapid identification of yeasts (Graf *et al.*, 2000; Freydiere *et al.*, 2001; Campanha *et al.*, 2005). These chromogenic media are species-specific, enabling the microorganism identification to the species level by their colour and colonial characteristics after 48-hour incubation at 37°C. As an alternative to the conventional and biochemical methods, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) has emerged as a rapid and accurate tool for identifying pathogens, including bacteria, mycobacteria, moulds and yeasts (Prod'hom *et al.*, 2010; Pulcrano *et al.*, 2013).

The aim of the study was to compare of the identification results obtained with two new systems using the MALDI-TOF MS technique: MALDI Biotyper (Bruker Daltonics, Germany) and VITEK MS (bioMérieux, France) with the ones obtained using the routine identification methods of *Candida* spp. in clinical microbiology laboratories: API ID32C and VITEK 2 YST (bioMérieux, France) and CHROMagar Candida Medium (Becton Dickinson).

The same set of strains was used in all methods compared in the study. All methods were duplicated. All 124 *Candida* spp. isolates were recovered from the routine examination of clinical specimens in microbiological laboratories throughout Poland, and submitted to the

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Centre of Quality Control in Microbiology (CQCM) located in Warsaw (Poland) for further evaluation and deep frozen. They were deposited in the CQCM collection. The strains used during the study were transferred from storage at –70°C onto the non-selective Sabouraud agar medium (bioMérieux, France) and incubated for 48 h at 30°C.

The biochemical identification of the strains was carried out at the same time using the API ID32C as based method of yeasts identification in routine laboratory and YST card system, according to the manufacturer's instructions. In parallel with the biochemical identification the study strains were inoculated on CHROMagar Candida Medium which shows different colour colonies for *C. albicans* (green), *C. tropicalis* (dark blue, with a pink halo), *C. krusei* (pink and downy appearance).

All tested *Candida* strains were simultaneously identified to species level by MALDI-TOF MS Biotyper and VITEK MS in line with the manufacturer's instructions. The results of the pattern-matching process were expressed as log score values ranging from 0 to 3 using the MALDI-TOF MS Biotyper application and software. The score values ranging from 2.300 to 3.000 meant "highly probable species identification"; 2.000–2.299 – "secure genus identification, probable species identification"; 1.800–1.999 – "identification to the genus level", and a score of ≤ 1.800 was interpreted as "no reliable identification".

The resulting slides were then processed in the VITEK MS device with MYLA software offering the automated analysis of the obtained mass spectra against the built-in database. A single identification is displayed (green), with a confidence value (% probability) from 60.0 to 99.9 (good confidence level), when one significant yeast or yeast group is retained. "Low-discrimination" identifications are displayed (red) when two or four significant yeasts or yeast groups are retained. When no match is found, the yeast is considered unidentified (orange).

The results of analysis are shown in Table I. The growth characteristics of 124 *Candida* spp. strains on CHROMagar Candida Medium enabled the unambiguous identification of isolates belonging to three species: green colonies as *C. albicans* (n = 68; 55%), steel-blue colonies – *C. tropicalis* (n = 21; 17%) and pink colonies – *C. krusei* (n = 35; 28%). Green colony colour suggested *C. albicans* strains, but different intensity of colour was difficult to evaluate objectively. Thirty five of strains (28.2%), which grew on chromogenic medium, formed pink/violet to pink colour colonies: pink colonies (n = 1), pink/violet-pink colonies (n = 24), white large glossy pink colonies (n = 2), matt pink colonies (n = 3), pink colonies with white "halo" (n = 3) or ivory to lavender colonies (n = 2). According to manufacturer instructions, pink colony colour suggested *C. krusei* strains.

Biochemical analysis with the API ID 32C system identified 123 (99,2%) *Candida* isolates. The isolates, which grew on chromogenic medium forming pink/violet-pink colonies (n = 24) and white large glossy pink colonies (n = 2), were identified by API ID 32C as *C. glabrata* (n = 26). All isolates growing as matt pale pink colonies (n = 3) were identified as *C. krusei*. Only four from five isolates which grew forming pink with a white "halo" or ivory colonies were identified as *C. parapsilosis*. The green colour colonies were identified by API ID 32C as *C. albicans* (n = 67) and *C. dubliniensis* (n = 1).

The identification levels of VITEK 2 YST were: excellent – 58.1%, very good (34.7%), good (4.8%) and acceptable (only 2.4%). The results obtained with VITEK 2 YST card were correct with API identification for the majority of common clinical yeasts, *C. albicans*

Table I
Cumulative results of *Candida* strains identification

CHROMagar Candida Medium		Identification (no strains)			
Colony colour (no strains)		API ID 32C	VITEK 2 YST	MALDI Biotyper	VITEK® MS
Study strains (n = 124)					
Dark green, green or light green colonies (n = 68)		*C. albicans* (n = 67)	*C. albicans* (n = 66)	*C. albicans* (n = 66)	*C. albicans* (n = 66)
		C. dubliniensis (n = 1)	*C. dubliniensis* (n = 2)	*C. dubliniensis* (n = 2)	*C. dubliniensis* (n = 2)
Dark blue colonies (n = 21)		*C. tropicalis* (n = 21)	*C. tropicalis* (n = 21)	*C. tropicalis* (n = 21)	*C. tropicalis* (n = 21)
Pink/violet colonies (n = 35)	Pink/violet-pink colonies (n = 24)	*C. glabrata* (n = 26)	*C. glabrata* (n = 26)	*C. glabrata* (n = 26)	*C. glabrata* (n = 26)
	White large glossy pink colonies (n = 2)				
	Pink colonies (n = 1)	*C. kefyr* (n = 1)	*C. kefyr* (n = 1)	*C. kefyr* (n = 1)	*C. kefyr* (n = 1)
	Matt pale pink colonies (n = 3)	*C. krusei* (n = 3)	*C. krusei* (n = 3)	*C. krusei* (n = 3)	*C. krusei* (n = 3)
	Pink colonies with white halo (n = 3)	*C. parapsilosis* (n = 3)	*C. parapsilosis* (n = 5)	*C. parapsilosis* (n = 5)	*C. parapsilosis* (n = 5)
	Ivory to lavender colonies (n = 2)	*C. parapsilosis* (n = 1)			
		Not ID (n = 1)			

(n = 66), *C. glabrata* (n = 26) and *C. krusei* (n = 3). The problematic isolate with light-green colonies was identified with API ID 32C as *C. albicans*, whereas VITEK 2 system identified it as *C. dubliniensis* (n = 1). The only strain unidentified by API ID 32C was later identified by YST card as *C. parapsilosis*.

The MALDI Biotyper correctly identified 71.8% and 26.6% and 1.6% of isolates at the species level using scores of ≥ 2.300 and ≥ 2.000 and ≥ 1.800, respectively. The MALDI Biotyper performed better than the routine methods for identification of *C. kefyr*, *C. dubliniensis* and *C. parapsilosis*. *C. dubliniensis* (n = 2) was correctly identified using the MALDI Biotyper (score ≥ 2.000), while it was misidentified as *C. albicans* (n = 1) using the traditional methods (API ID 32C), likely due to the close phylogenetic relationship between the two species. VITEK MS offered a good identification to the species level of all *Candida* isolates (n = 124). The confidence interval for this *Candida* identification method ranged from 95.6% to 99.9%.

The identification methods used in this study were characterised by a favourable parameter profile. We analysed the number of identified strains of the same species. The degree of identification correspondence expressed as a percentage of the total number of tested strains was shown in Table II.

Quality control strains were obtained from the American Type Culture Collection (ATCC): *C. albicans* ATCC 14053, *C. glabrata* ATCC 2950, *C. krusei* ATCC 6258, *C. parapsilosis* ATCC 22019 and *C. tropicalis* ATCC 13803. *C. albicans*, *C. krusei* and *C. tropicalis* reference strains grew on the chromogenic medium in line with the manufacturer's description. The colonies of *C. parapsilosis* ATCC 22019 strain looked like *C. krusei* on this medium. *C. glabrata* ATCC 2950 grew as violet-pink colonies.

Several authors have reported difficulties in differentiation of *C. albicans* from *C. dubliniensis* due to the phenotypic similarity of the two species (Sullivan and Coleman, 1998; Raut and Varaiya, 2009). Both species produce the germ tube, chlamydospores and grow on CHROMagar Candida as green colonies. Although *C. dubliniensis* colonies are dark green unlike the green or light green colonies of *C. albicans*, this difference may be inadequate for primary identification and often lost after serial subculture (Kirkpatrick *et al*., 1998). *C. dubliniensis* may be underreported in clinical samples because most currently used isolation and identification methods fail recognize this yeast (Campanha *et al*., 2005; Pravin *et al*., 2015).

Our findings confirm the high agreement of fungal identification using the standard, biochemistry laboratory methods and mass spectrometry technique. Although our high 98% agreement is based on the analysis of 124 clinical, patient-derived strains only, these results remain in accordance with previous observations on larger numbers of clinical isolates (Van Veen *et al*., 2010; Sow *et al*., 2015).

Conventional methods including macroscopic/microscopic characteristic and biochemical profile by API identification system offer efficiency and accuracy to facilitate the diagnosis. We found that CHROMagar Candida Medium offers easy identification of several species based on colony colour and morphology, as well as an accurate differentiation between the three most common *Candida* species *i.e. C. albicans*, *C. tropicalis* and *C. krusei*. As shown in this paper, different shades of green or pink colour of colonies growing on a chromogenic media may indicate the same or different yeast species. This supports the need to use in a laboratory different diagnostic methods, such as biochemistry or mass spectrometry. In our study the API ID 32C identification had to be repeated twice in a few cases, which delayed the time until the results were available after an additional 24–48 hours.

The VITEK 2 YST card was able to reliably differentiate between *C. dubliniensis* and *C. albicans*. The percentage of correct identification is similar to the ones by other commercial identification systems (Graf *et al*., 2000; Freydiere *et al*., 2001; Sow *et al*., 2015). We believe that for microbial identification tests, there should be at least 90% agreement with the existing system or reference method before the new method is considered verified (Clark *et al*., 2009).

Table II
Compliance of *Candida* strains identification results using different methods

MALDI Biotyper		VITEK® MS		VITEK 2 YST		Compatibility with MS* results (%)	
Score/ID Level	No/% strains	Confidence interval (%)	No/ % strains	ID Level	No/ % strains		
2.300–3.000 / excellent	89/71,8%	99.9	11/8.9%	Excellent	72/58.1%	API ID 32C	98,4%
2.000–2.299 / very good	33/26,6%			Very good	43/34.7%	VITEK 2 YST	100%
1.800–1.999 / good	2/1,6%	95.6–99.9	113/91.1%	Good	6/4.8%	CHROMagar Candida Medium	72,6%
≤1.8 / acceptable	–			Acceptable	3/2.4%		

*MS – mass spectrometry method (MALDI Biotyper and VITEK® MS)

In summary, conventional methods of fungal identification are very useful in clinical microbiology laboratories. Identification by conventional microbiology methods takes at least 16 hours, if a culture is positive. The introduction of MALDI-TOF MS in the microbiology laboratory could mean a radical change in the identification accuracy, reducing time (10 minutes per a single isolate) and cost (about 5 times cheaper than a conventional identification) (Sow *et al*., 2015). MALDI-TOF clearly outperformed the diagnosis capacities of phenotypic methods by reducing the delay of results and giving accurate identification at species level (Van Veen *et al*., 2010; Marinez-Lamas *et al*., 2011). Moreover, this approach appears to be cost-effective and should be implemented especially in resource-poor context (Sow *et al*., 2015).

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