Mycopathologia (2016) 181:67–73
DOI 10.1007/s11046-015-9944-8

Comparison of Vitek Matrix-assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry Versus Conventional Methods in Candida Identification

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Received: 18 July 2014 / Accepted: 8 September 2015 / Published online: 23 September 2015

Abstract Candida species are generally identified by conventional methods such as germ tube or morphological appearance on corn meal agar, biochemical methods using API kits and molecular biological methods. Alternative to these methods, rapid and accurate identification methods of microorganisms called matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDİ–TOF MS) has recently been described. In this study, Candida identification results by API Candida kit, API 20C AUX kit and identifications on corn meal agar (CMA) are compared with the results obtained on Vitek–MS. All results were confirmed by sequencing internal transcribed spacer (ITS) regions of rDNA. Totally, 97 Candida strains were identified by germ tube test, CMA, API and Vitek–MS. Vitek–MS results were compatible with 74.2 % of API 20C AUX and 81.4 % of CMA results. The difference between the results of API Candida and API 20C AUX was detected. The ratio of discrepancy between Vitek–MS and API 20C AUX was 25.8 %. *Candida* species mostly identified as *C. famata* or *C. tropicalis* by and not compatible with API kits were identified as *C. albicans* by Vitek–MS. Sixteen *Candida* species having discrepant results with Vitek–MS, API or CMA were randomly chosen, and ITS sequence analysis was performed. The results of sequencing were compatible 56.2 % with API 20C AUX, 50 % with CMA and 93.7 % with Vitek–MS. When compared with conventional identification methods, MS results are more reliable and rapid for Candida identification. MS system may be used as routine identification method in clinical microbiology laboratories.

Keywords Candida · Identification methods · MALDI–TOF

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Introduction

The prevalence of Candida infections especially in immunocompromised patients and the patients who had invasive procedures was attempted [1]. For *Candida* species identification, besides morphological tests such as germ tube test and spore and hypha formation on CMA, several commercial biochemical tests based on enzymatic reactions (ex: API Candida, API 20C AUX and API ID 32C) were suggested as reference methods [2]. However, the diversity between the results of these tests is commonly encountered [3]. In addition, automatized enzymatic/biochemical methods (ex: VITEK 2 ID Yeast or VITEK Yeast Biochemical Card) have the useful test criteria in Candida identification with high sensitivity ratio [2]. In some institutes, molecular biological tests

such as ITS sequence analysis are used as "reference standard" method [4–6]. Sequencing the clinical isolates is time-consuming and not standardized. Therefore, this method can not be used routinely in Candida identification. Alternatively to these DNA-dependent approaches, recently, mass spectral analysis in Candida identification has been increasingly common [7, 8].

In several hospitals of our country, MALDİ–TOF (matrix-assisted laser desorption/ionization time-of-flight) mass spectrometry system that has recently been started to be used is one of the rapid automatized systems for Candida identification [9, 10]. This system identifies Candida by determining the highest protein load and behaving as a taxon-specific biomarker of which mass spectra are between 2 and 20 kDa. In comparison with genetic and morphological methods, the biggest advantage of mass spectrometry is being simple, easy sample preparation and short time of analysis. The sample preparation and evaluating the data take only several minutes.

In this study, the performance of Vitek–MS was evaluated in Candida identification and the results were compared with the results obtained from germ tube test, morphological appearance on CMA, API Candida and API 20C AUX system. All identification results were compared with the results of sequencing internal transcribed spacer (ITS) regions of rDNA as gold standard method [11].

Materials and Methods

Phenotypic Identification and Characterization

Candida Strains

In this study, totally 97 clinical *Candida* isolates stocked at −80 °C were included. After they were thrawn, they were cultured onto Saboraoud dextrose agar (SDA) and reidentify using the methods described below.

Germ Tube Test

All isolates were tested for germ tube formation. Colonies from each isolates were incubated in human serum at 37 °C for 2–3 h. Germ tube positive isolates were identified as *C. albicans* or *C. dubliniensis. C. dubliniensis* identification was also done by observation of cluster-type chlamydospore formation on CMA. Suşların tamamı germ tube oluşumu açısından test edildi.

API Kits

All isolates were reidentified with API 20C AUX test. Twenty-five of 50 isolates identified as non-albicans Candida using API Candida kit were reidentified with both API Candida and API 20CAUX kits (bioMerieux, France) according to manufacturer's suggestions.

Corn Meal Agar (CMA)

All isolates were inoculated onto CMA agar (Oxoid) with 1 % Tween 80, and then, all plates were incubated at 26 °C for 72 h. Then, isolates were identified by investigating under microscope (40X objective) according to their chlamydospore, blastospore and/or hypha formation.

Mass Spectrometry Analysis

Sample preparation for mass spectrometry analysis has been described elsewhere [11]. Candida colonies taken from 24 h incubated culture on SDA. All isolates were identified with Vitek–MS (bioMerieux, France) system according to manufacturer's suggestions.

ITS Sequencing

Randomly chosen 16 isolates having discrepant identification results by Vitek-MS, API or CMA were tested for rDNA sequencing. First, DNA extraction was done from the colonies on SDA using 502 MagCore® Genomic DNA bacterial kit (RBC Bioscience, Taiwan). Sequence analysis was done with primers specific for internal transcribed spacer regions ITS1 (F; 5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (R; 5′-TCCTCCGCTTATTGATATGC-3′) [12]. Samples were sequenced using ABI Prism 310 Genetic Analyzer and ABI PRISM BigDye Terminator Cycle Sekans kit (Applied Biosystems). The obtained electropherograms are analyzed and evaluated using ChromasPro version1.5 (Technelysium Pty Ltd, Queensland, Australia). Identification of nucleotide sequences was done with BLAST (BLAST N 2.2.28) analysis. For this aim, Genbank nükleotid data bank was used [13].

Results

Vitek–MS results were compatible in 72 isolates (74.2 %) with API 20C AUX and in 79 isolates (81.4 %) with CMA (Table 1). Candida identification results with API or CMA that are compatible with MS are shown in Table 1. Incompatibility between API and MS results was determined in 25/97 (25.8 %) (Table 2).

The comparison of the results of 25 isolates with API Candida and API 20C AUX kits and MS identification results of these same 25 isolates is shown in Table 3. Candida isolates identified as *C. famata* with both API 20C AUX and API Candida kits could not be identified as *C. famata* in MS. The comparison of identification results of 25 isolates with both API kits, MS, CMA and germ tube test is shown in Table 4. Incompatibility between API kits was detected in 40 % (10/25) isolates. MS results were determined incompatible with both API kits in 12 isolates. All these isolates were identified as *C. albicans* with MS, and 4 of them were identified as *C.tropicalis* with both API kits. Two isolates identified as *C. albicans* with MS were identified as *C. famata* with both API kits.

Since the discordant results were obtained with both API kits, MS results were compared with any of API Candida or API 20C and AUX results in order to evaluate the performance of MS. MS results were compatible in 8 with API Candida and in 12 isolates with API 20C AUX (Table 4).

The compatibility of MS with CMA was determined in 16 of 25 isolates (Table 4). Six of 9 MS–CMA incompatible isolates were identified as *C. albicans* and *C. tropicalis.* CMA–API 20C AUX incompatibility was determined in 12 isolates. Three of them were between *C. famata* and *C. albicans,* whereas 6 of them were between *C. albicans* and *C.tropicalis*. In all *C. glabrata* (n:15, 15.4 %) isolates, the results were compatible between API 20C AUX and MS.

Forty of 49 (81.6 %) isolates and 10 of 48 (20.8 %) non-albicans isolates identified as *C.albicans* with MS had positive germ tube tests. Germ tube test was negative in 4 of 6 isolates identified as *C. famata* with API 20C AUX and *C. albicans* with MS, whereas germ tube test was positive in the rest two isolates.

Only 16 isolates having discrepant results by MS, API or CMA were identified by sequence analysis. Of these, 16 isolates were incompatible in 8 with CMA, in 6 with API, in 2 with both API and CMA. After sequencing, 7 (43.7 %) isolates were incompatible with API results. The results of sequence analysis and their comparison with API 20C AUX, MS and CMA are shown in Table 5.

ITS sequence analysis results were compatible 56.2 % (9/16) with API, 50.0 % (8/16) with CMA and 93.7 % (15/16) with MS. Only one incompatible sequence analysis result with MS was identified as *C. tropicalis* and as *C. albicans* with API, and this isolate was determined as germ tube positive.

Table 1 The number of *Candida* species identified with Vitek–MS and the number of isolates of which identification results were compatible with API 20C AUX and CMA

Candida species identified with Vitek–MS (*n*)		The number of isolates compatible with Vitek–MS	
		API 20C AUX (*n*)	Corn Meal Agar (*n*)
C. albicans	49	35	39
C. glabrata	13	12	13
C. tropicalis	13	10	10
C. parapsilosis	6	5	5
C. kefyr	5	5	4
C. krusei	4	2	4
C. guilliermondii	4	2	2
C. dubliniensis	2	1	2
C. famata	1	–	–
Total	97	72	79

Table 2 The comparison of 25 incompatible results of Vitek–MS with API 20C AUX

Vitek–MS	API 20C AUX
C. albicans ($n = 13$)	*C. tropicalis*
C. albicans ($n = 9$)	*C. famata*
C. krusei ($n = 2$)	*C. famata*
C. guilliermondii ($n = 1$)	*C. famata*

Discussion

In this study, most of the isolates identified as non-albicans Candida with API kits were reidentified as *C. albicans* with Vitek–MS. Moreover, most of the

Table 3 The number of *Candida* species identified as non-albicans with API Candida and the comparison of identification results of the same isolates with API 20C AUX and MS

Candida species	VİTEK–MS (*n*)	API Candida (*n*)	API 20C AUX (*n*)
C. albicans	15	–	4
C. famata	–	5	4
C. glabrata	3	5	3
C. guilliermondii	1	1	–
C. krusei	–	1	–
C. parapsilosis	2	3	1
C. tropicalis	4	10	13
Total	25	25	25

Table 4 The distribution of *Candida* species identified as non-albicans with API Candida and their reidentification results with other tests: germ tube test, CMA, API 20C AUX and Vitek–MS

Vitek–MS	API *Candida*	API 20C AUX	CMA	Germ tube
C. albicans	*C. famata*	*C. famata*	*C. albicans*	–
C. albicans	*C. tropicalis*	*C. tropicalis*	*C. tropicalis*	–
C. albicans	*C. tropicalis*	*C. tropicalis*	*C. albicans*	–
C. albicans	*C. tropicalis*	*C. tropicalis*	*C. tropicalis*	–
C. albicans	*C. parapsilosis*	*C. tropicalis*	*C. albicans*	+
C. albicans	*C. famata*	*C. famata*	*C. albicans*	+
C. albicans	*C. krusei*	*C. tropicalis*	*C. albicans*	+
C. albicans	*C. tropicalis*	*C. tropicalis*	*C. albicans*	+
C. albicans	*C. glabrata*	*C. tropicalis*	*C. tropicalis*	–
C. albicans	*C. famata*	*C. famata*	*C. albicans*	+
C. albicans	*C.glabrata*	*C. albicans*	*C. glabrata*	+
C. albicans	*C. tropicalis*	*C. albicans*	*C. albicans*	+
C. albicans	*C. glabrata*	*C. tropicalis*	*C. tropicalis*	+
C. albicans	*C. guilliermondii*	*C. albicans*	*C. albicans*	+
C. albicans	*C. tropicalis*	*C. albicans*	*C. tropicalis*	+
C. guilliermondii	*C. famata*	*C. famata*	*C. tropicalis*	–
C. tropicalis	*C. tropicalis*	*C. tropicalis*	*C. albicans*	+
C. glabrata	*C. famata*	*C. glabrata*	*C. glabrata*	–
C. tropicalis	*C. tropicalis*	*C. tropicalis*	*C. tropicalis*	–
C. parapsilosis	*C. parapsilosis*	*C. parapsilosis*	*C. parapsilosis*	–
C. glabrata	*C. glabrata*	*C. glabrata*	*C. glabrata*	–
C. tropicalis	*C. tropicalis*	*C. tropicalis*	*C. tropicalis*	+
C. tropicalis	*C. tropicalis*	*C. tropicalis*	*C. tropicalis*	+
C. parapsilosis	*C. parapsilosis*	*C. tropicalis*	*C. albicans*	–
C. glabrata	*C. glabrata*	*C. glabrata*	*C. glabrata*	–

isolates identified as *C. albicans* with MS were identified as *C.tropicalis* and as *C. famata* with API 20C AUX. Again, most of *C. famata* isolates identified with API were identified as *C. albicans* with both MS and sequencing. It was reported that *C. famata* identified with conventional methods were identified as *C. guilliermondii, C. lusitaniae* and *C. parapsilosis* with sequence analysis [14]. In our study, a germ tube-negative isolate identified as *C. famata* was identified as *C. krusei* with CMA and sequence analysis. This result shows that *C. famata* result should be confirmed with morphological tests.

MS results were more compatible with API 20C AUX results when same isolates with both API kits

Table 5 The comparison of sequence analysis results with identification results with other tests

Isolate no.	CMA	API 20 C AUX	Vitek–MS	Sequence analysis
1	*C. tropicalis*	*C. tropicalis*	*C. albicans*	*C. albicans*
2	*C. guilliermondii*	*C. tropicalis*	*C. tropicalis*	*C. tropicalis*
3	*C. guilliermondii*	*C. tropicalis*	*C. tropicalis*	*C. tropicalis*
4	*C. glabrata*	*C. albicans*	*C. albicans*	*C. albicans*
5	*C. glabrata*	*C. glabrata*	*C. famata*	*C. famata*
6	*C. glabrata*	*C. kefyr*	*C. kefyr*	*C. kefyr*
7	*C. tropicalis*	*C. albicans*	*C. albicans*	*C. albicans*
8	*C. albicans*	*C. famata*	*C. albicans*	*C. albicans*
9	*C. albicans*	*C. tropicalis*	*C. albicans*	*C. albicans*
10	*C. albicans*	*C. famata*	*C. albicans*	*C. albicans*
11	*C. tropicalis*	*C. albicans*	*C. albicans*	*C. albicans*
12*	*C. albicans*	*C. albicans*	*C. tropicalis*	*C. albicans*
13	*C. dubliniensis*	*C. albicans*	*C. dubliniensis*	*C. dubliniensis*
14	*C. albicans*	*C. famata*	*C. albicans*	*C. albicans*
15	*C. guilliermondii*	*C. guilliermondii*	*C. guilliermondii*	*C. guilliermondii*
16	*C. krusei*	*C. famata*	*C. krusei*	*C. krusei*

* This isolate was the only isolate which causes incompatibility between MS and sequence analysis

compared were tested. In a study evaluating the commercial identification systems (Vitek, API ID 32C, API 20C AUX, Yeast Star, Auxacolor, RapID Yeast Plus system, and API Candida), it was observed that API Candida, in comparison with other tests, identifies germ tube-negative isolates 93 % accurately. However, it could not identify some germ tube negatives [15]. The disadvantage of API Candida is that 26 *Candida* species exists in its database, whereas 43 species exist in database of API 20C AUX. Moreover, *C. dubliniensis* could not be identified in API Candida. As shown in our study, an isolate identified as *C.albicans* with API 20C AUX was identified as *C. dubliniensis* with MS and sequence analysis.

MS results were more compatible with CMA results (81.4 %) when they were compared to API 20C AUX results. Therefore, it is our opinion that API results are needed to be confirmed with morphological tests like colony morphology, germ tube test and the formation of chlamydospores, pseudohypha, arthroconidia or blastoconidia. In our study, API compatibility with sequencing was found low as 56.2 %. In studies comparing API with reference molecular tests, the ratio of compatibility was 84.5 % by Seyfarth et al. [16] and 76.8 versus 85 % by Freydiere et al. [17].

When compared to conventional identification methods, MS had the most compatible method with sequence analysis (93.7 %) in our study. When ITS sequence analysis was accepted as reference method in several studies, the ratio of accurate identification of MS was determined as 94 % [16], 92.5 % [18], 97.6 and 96.1 % [19], 99 % [20] and 85.2 % [21]. In a recently published study, it was reported that accurate identification ratio of MALDİ–TOF MS and conventional methods was 98.3 and 96.5 %, respectively, when rDNA sequence analysis was the reference. It was proposed that MS was able to identify rare and hardly identified *Candida* species that could not be determined by conventional methods [22]. In addition, it was put forwarded that MS was helpful to identification of related subspecies (*C. parapsilosis, C. orthopsilosis and C. metapsilosis*) [23]. In our study, it was shown that *Candida* species identified less in comparison with *C. albicans* such as *C. krusei, C. dubliniensis* and *C. guilliermondii* were accurately identified.

Since the identification with API kits takes 48–72 h, whereas it takes 15–24 h with VİTEK ID Yeast system, it causes loosing of time. Molecular biological methods (PCR or sequence analysis) are tedious, hard and time-consuming. Therefore, MS

gives more rapid and more accurate Candida identifications compared to conventional methods. Moreover, it is possible to identify *Candida* species directly from positive blood culture bottles using MALDİ–TOF MS within minutes [24]. The identification with MS is always possible if the mass spectra are within its database. This is the main problem in all databases. However, species selection in the database of API 20C AUX is much more limited. Update of API is more inappropriate compared to mass spectra database because MS databases are updated continuously taking ITS sequence analysis as the reference method [18].

In conclusion, it was observed that a single identification test was not adequate for the identification of *Candida* species. The use of MS as routine laboratory test seems to have several advantages such as giving rapid identification results, requiring less material and lower costs, leading to easy interpretation of results and storing the results in a wide database. Early identification with MS would save time for determination of antifungal susceptibility and proper treatment strategy.

Acknowledgments We thank ION-TEK, a commercial agency (Istanbul, Turkey) for their assistance with ITS sequence analysis. Many thanks to Dr. Murat Sayan for his evaluations of electropherograms.

Compliance with Ethical Standards

Conflict of interest There is no conflict of interest.

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