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Identification of Uncommon *Candida* Species Using Commercial Identification Systems

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Recently, several studies have revealed that commercial microbial identification systems do not accurately identify the uncommon causative species of candidiasis, including *Candida famata*, *Meyerozyma guilliermondii*, and *C. auris*. We investigated the accuracy of species-level identification in a collection of clinical isolates previously identified as *C. famata* (*N* = 38), *C. lusitaniae* (*N* = 12), and *M. guilliermondii* (*N* = 5) by the Vitek 2 system. All 55 isolates were re-analyzed by the Phoenix system (Becton Dickinson Diagnostics), two matrix-assisted laser desorption ionization-time of flight mass spectrometry analyzers (a Vitek MS and a Bruker Biotyper), and by sequencing of internal transcribed spacer (ITS) regions or 26S rRNA gene D1/D2 domains. Among 38 isolates previously identified as *C. famata* by the Vitek 2 system, the majority (27/38 isolates, 71.1%) were identified as *C. tropicalis* (20 isolates) or *C. albicans* (7 isolates) by ITS sequencing, and none was identified as *C. famata*. Among 20 isolates that were identified as *C. tropicalis*, 17 (85%) were isolated from urine. The two isolates that were identified as *C. auris* by ITS sequencing originated from ear discharge. The Phoenix system did not accurately identify *C. lusitaniae*, *C. krusei*, or *C. auris*. The correct identification rate for 55 isolates was 92.7% (51/55 isolates) for the Vitek MS and 94.6% (52/55 isolates) for the Bruker Biotyper, as compared with results from ITS sequencing. These results suggest that *C. famata* is very rare in Korea, and that the possibility of misidentification should be noted when an uncommon *Candida* species is identified.

Keywords: *Candida*, identification, MALDI-TOF MS, sequencing

Introduction

Fungal infections pose an increasing risk for human health, especially in immunocompromised and critically ill patients. The most common fungal pathogens are *Candida* species, and up to 40 distinct *Candida* species have been shown to infect humans [16]. In general, *Candida albicans* is the most common species, followed by *C. glabrata*, *C. tropicalis*, and *C. parapilosis* [13, 17]. There has been an increase in invasive candidiasis caused by non-*albicans Candida* species over the last three decades [4]. Several non-*albicans Candida* species have presented different antifungal drug susceptibility patterns. Therefore, there is a need to accurately identify *Candida* species for the proper management of patients [17].

Technologies for the identification of *Candida* species have improved continuously over the past several decades, with methods ranging from conventional biochemical methods (manual and automated) to nucleic acid-based methods [12, 15]. In recent years, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS)-based methods have presented a promising alternative for routine identification of clinically relevant *Candida* species [1, 6, 11, 19].

Several studies have revealed that commercial microbial identification systems do not accurately identify the uncommon causative species of candidiasis, including *C. famata* (formerly *Torulopsis candida*, telemorph *Debrayomyces hansenii*), *Meyerozyma guilliermondii* (*Pichia guilliermondii*), and *C. auris* [1, 3, 7–9]. Some reports have suggested that

isolates initially identified as *C. famata* by conventional phenotypic methods were based on molecular identification methods [1, 3, 8]. Indeed, *C. famata* and *M. guilliermondii* are extremely difficult to differentiate by phenotypic characterization [3].

In this study, we investigated the accuracy of species-level identification in a collection of clinical isolates previously identified as *C. famata*, *M. guilliermondii*, and *C. lusitaniae* by the Vitek 2 system. We used another conventional phenotypic method (the Phoenix system), sequencing of the internal transcribed spacer (ITS) regions or 26S rRNA gene D1/D2 domains, and two commercially available MALDI-TOF MS systems (Vitek MS and Bruker Biotyper) for our identifications.

Materials and Methods

Yeast Isolates

A total of 55 clinical isolates from individual patients were included. These isolates were previously identified as *C. famata* (*N* = 38), *C. lusitaniae* (*N* = 12), and *M. guilliermondii* (*N* = 5) by the Vitek 2 system (bioMérieux Inc., USA) using a YST card in a single tertiary care hospital in Seoul, South Korea, from 2010 to 2013. The isolates were obtained from urine (*N* = 35), blood (*N* = 7), sputum (*N* = 4), ear discharge (*N* = 2), vaginal discharge (*N* = 2), or other areas (*N* = 5). All isolates were stored in 50% glycerol at −70ºC until testing. Prior to testing, the isolates were subcultured twice on Sabouraud dextrose agar plates at 35ºC. The study was reviewed and approved by the institutional review board of Chung-Ang University Hospital.

Identification by BD Phoenix

All 55 isolates of *Candida* species were identified by the Phoenix system (Becton Dickinson Diagnostics, USA) using the BD Phoenix Yeast ID panel according to the manufacturer's instructions [18].

MALDI-TOF MS

We analyzed the 55 isolates using two MALDI-TOF MS systems, the Vitek MS (bioMérieux) and the Bruker Biotyper (Bruker Germany). The formic acid extraction procedure was followed according to the manufacturer's instructions for the identification of yeast isolates. Briefly, a single colony was transferred to a target plate and overlaid with 1 µl of formic acid and matrix solution, respectively. Once air dried, the loaded plate was applied to the instrument and analyzed. We repeated the test with the ethanol-formic acid extraction method if no result was obtained. Yeast identification was achieved by analyzing the spectra with those on the MALDI-TOF MS database. The peaks from these spectra were compared with the characteristic pattern for the species or genus of yeast, leading to identification.

DNA Sequencing

Sequencing analysis of the ITS and D1/D2 regions was performed to obtain accurate identifications. After lyticase-based cell lysis, DNA of isolates was extracted using the Viral Gene-spin Viral DNA/RNA Extraction Kit (Intron, Korea). DNA from all isolates was amplified and sequenced directly with the ABI PRISM 3130 genetic analyzer (Applied Biosystems, USA) using the ITS-1 (forward, 5′-TCCGTAGGTGAACCTGCGG-3′) and ITS-4 (reverse, 5′-TCCTCCGCTTATTGATATGC-3′) primers, which amplify the ITS region [5]. The D1/D2 sequencing was performed using the NL-1 (forward, 5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL-4 (everse, 5′-GGTCCGTGTTTCAAGACGG-3′) primers for assisting in the identification of two isolates from ear discharge [5]. Sequence similarity searches were performed using the BLAST tool in the NCBI database.

Results

Isolates Previously Identified as *C. famata* by the Vitek 2 System

None of the 38 isolates that had previously been identified as *C. famata* by the Vitek 2 system was identified as *C. famata* by ITS or D1/D2 sequencing. The 38 isolates were instead identified as *C. tropicalis* (20/38 isolates, 52.6%), *C. albicans* (7/38 isolates, 18.4%), *C. parapsilosis* (4/38 isolates, 10.5%), *M. guilliermondii* (2/38 isolates, 5.3%), *C. auris* (2/38 isolates, 5.3%), or others (3 isolates, including one strain each of *C. lusitaniae*, *C. krusei*, and *Pichia fabianii*). Two isolates from ear discharge that were identified by additional D1/D2 sequencing were *C. auris*. Among the 20 isolates that were identified as *C. tropicalis* by ITS sequencing, 17 (85%) were isolated from urine (Table 1).

Among the 38 isolates previously identified as *C. famata* by the Vitek 2 system, 31 (81.6%) were correctly identified by the Phoenix system compared with results from ITS sequencing. The remaining five isolates (2 *C. auris*, *M. guilliermondii*, *C. krusei*, and *P. fabianii*) were misidentified, and two isolates (*C. lusitaniae* and *C. tropicalis*) were unidentified (Table 1).

The correct identification rate for the 38 isolates was 89.5% (34/38 isolates) for the Vitek MS and 92.1% (35/38 isolates) for the Bruker Biotyper, as compared with results from ITS sequencing. The Vitek MS and the Bruker Biotyper misidentified two isolates and one isolate of *C. tropicalis* from urine, respectively. Both systems failed to correctly identify the two isolates of *C. auris* from ear discharge (Tables 1 and 2).

Isolates Previously Identified as *C. lusitaniae* by the Vitek 2 System

The 12 isolates that had been previously identified as *C. lusitaniae* by the Vitek 2 system were identified as

Table 1. Identification results by DNA sequencing, the Phoenix system, and two MALDI-TOF MS systems (Vitek MS and Bruker Biotyper) for 55 clinical isolates previously identified as *Candida famata*, *C. lusitaniae*, and *Meyerozyma guilliermondii* by the Vitek 2 system.

Species (number of isolates)					
Vitek 2	Specimen type	Sequencing	Phoenix ID	Vitek MS	Bruker Biotyper
C. famata (38)	Urine (28)	*C. tropicalis* (17)	*C. tropicalis* (14)	*C. tropicalis* (14)	*C. tropicalis* (14)
			C. tropicalis (2)	***C. albicans* (2)**	*C. tropicalis* (2)
			C. tropicalis (1)	*C. tropicalis* (1)	***C. parapsilosis* (1)**
		C. albicans (6)	*C. albicans* (6)	*C. albicans* (6)	*C. albicans* (6)
		C. parapsilosis (2)	*C. parapsilosis* (2)	*C. parapsilosis* (2)	*C. parapsilosis* (2)
		C. lusitaniae (1)	**No ID[a] (1)**	*C. lusitaniae* (1)	*C. lusitaniae* (1)
		M. guilliermondii (1)	*M. guilliermondii* (1)	*M. guilliermondii* (1)	*M. guilliermondii* (1)
		P. fabianii (1)	***C. melibiosica* (1)**	*P. fabianii* (1)	*P. fabianii* (1)
	Blood (4)	*C. tropicalis* (2)	*C. tropicalis* (2)	*C. tropicalis* (2)	*C. tropicalis* (2)
		C. parapsilosis (1)	*C. parapsilosis* (1)	*C. parapsilosis* (1)	*C. parapsilosis* (1)
		M. guilliermondii (1)	***C. parapsilosis* (1)**	*M. guilliermondii* (1)	*M. guilliermondii* (1)
	Ear discharge (2)	*C. auris* (2)	***C. catenulata* (1)**	***C. albicans* (1)**	***N. meningitidis*[b] Serogroup A (1)**
			***C. haemulonii* (1)**	***C. haemulonii* (1)**	***P. rhizosphaerae*[c] (1)**
	Vaginal discharge (1)	*C. albicans* (1)	*C. albicans* (1)	*C. albicans* (1)	*C. albicans* (1)
	Bile (1)	*C. parapsilosis* (1)	*C. parapsilosis* (1)	*C. parapsilosis* (1)	*C. parapsilosis* (1)
	Hemo vac (1)	*C. tropicalis* (1)	**No ID (1)**	*C. tropicalis* (1)	*C. tropicalis* (1)
	Sputum (1)	*C. krusei* (1)	***C. neoformans* (1)**	*C. krusei* (1)	*C. krusei* (1)
C. lusitaniae (12)	Urine (7)	*C. lusitaniae* (3)	**No ID (2)**	*C. lusitaniae* (2)	*C. lusitaniae* (2)
			***C. melibiosica* (1)**	*C. lusitaniae* (1)	*C. lusitaniae* (1)
		C. albicans (2)	*C. albicans* (2)	*C. albicans* (2)	*C. albicans* (2)
		C. tropicalis (2)	*C. tropicalis* (2)	*C. tropicalis* (2)	*C. tropicalis* (2)
	Blood (3)	*C. lusitaniae* (2)	**No ID (1)**	*C. lusitaniae* (1)	*C. lusitaniae* (1)
			***C. melibiosica* (1)**	*C. lusitaniae* (1)	*C. lusitaniae* (1)
		C. tropicalis (1)	*C. tropicalis* (1)	*C. tropicalis* (1)	*C. tropicalis* (1)
	Joint fluid (1)	*C. lusitaniae* (1)	**No ID (1)**	*C. lusitaniae* (1)	*C. lusitaniae* (1)
	Pus (1)	*C. krusei* (1)	***Z. bailli*[d] (1)**	*C. krusei* (1)	*C. krusei* (1)
M. guilliermondii (5)	Sputum (3)	*M. guilliermondii* (2)	*M. guilliermondii* (1)	*M. guilliermondii* (1)	*M. guilliermondii* (1)
			No ID (1)	*M. guilliermondii* (1)	*M. guilliermondii* (1)
		C. glabrata (1)	*C. glabrata* (1)	*C. glabrata* (1)	*C. glabrata* (1)
	Central cath tip (1)	*C. parapsilosis* (1)	*C. parapsilosis* (1)	*C. parapsilosis* (1)	*C. parapsilosis* (1)
	Vaginal discharge (1)	*C. glabrata* (1)	*C. glabrata* (1)	*C. glabrata* (1)	*C. glabrata* (1)

[a]No identification.

[b]*Neisseria meningitidis*.

[c]*Pseudomonas rhizosphaerae*.

[d]*Zygosaccharomyces bailli*.

Misidentified results are depicted in bold letters.

C. lusitaniae (6 isolates), *C. tropicalis* (3 isolates), *C. albicans* (2 isolates), and *C. krusei* (1 isolate) by ITS sequencing (Table 1).

Using the Phoenix system, 5 isolates (41.7%) were correctly identified, whereas 7 isolates (6 *C. lusitaniae* and 1 *C. krusei*) were unidentified or misidentified compared with results

Table 2. Identification results for 55 clinical isolates according to two MALDI-TOF MS systems (Vitek MS and Bruker Biotyper).

Sequencing	Vitek MS		Bruker Biotyper	
Species	Species	Confidence value	Species	Score value
C. albicans	*C. albicans*	99.9	*C. albicans*	2.14
C. albicans	*C. albicans*	99.9	*C. albicans*	2.40
C. albicans	*C. albicans*	99.9	*C. albicans*	2.28
C. albicans	*C. albicans*	98.1	*C. albicans*	2.20
C. albicans	*C. albicans*	99.9	*C. albicans*	2.17
C. albicans	*C. albicans*	99.9	*C. albicans*	2.05
C. albicans	*C. albicans*	99.9	*C. albicans*	2.02
C. albicans	*C. albicans*	99.9	*C. albicans*	2.22
C. albicans	*C. albicans*	99.9	*C. albicans*	2.24
C. auris	***C. albicans***	**86.0**	***N. meningitidis* Serogroup A**	**1.06**
C. auris	***C. haemulonii***	**99.9**	***P. rhizosphaerae***	**1.41**
C. glabrata	*C. glabrata*	99.9	*C. glabrata*	2.28
C. glabrata	*C. glabrata*	99.9	*C. glabrata*	2.32
M. guilliermondii	*M. guilliermondii*	99.9	*M. guilliermondii*	1.82
M. guilliermondii	*M. guilliermondii*	99.9	*M. guilliermondii*	2.16
M. guilliermondii	*M. guilliermondii*	99.9	*M. guilliermondii*	2.12
M. guilliermondii	*M. guilliermondii*	99.9	*M. guilliermondii*	1.99
C. krusei	*C. krusei*	99.9	*C. krusei*	2.01
C. krusei	*C. krusei*	99.9	*C. krusei*	2.21
C. lusitaniae	*C. lusitaniae*	99.9	*C. lusitaniae*	2.11
C. lusitaniae	*C. lusitaniae*	99.9	*C. lusitaniae*	2.11
C. lusitaniae	*C. lusitaniae*	99.9	*C. lusitaniae*	2.16
C. lusitaniae	*C. lusitaniae*	99.9	*C. lusitaniae*	2.05
C. lusitaniae	*C. lusitaniae*	99.9	*C. lusitaniae*	2.07
C. lusitaniae	*C. lusitaniae*	99.9	*C. lusitaniae*	2.07
C. lusitaniae	*C. lusitaniae*	99.9	*C. lusitaniae*	1.90
C. parapsilosis	*C. parapsilosis*	99.9	*C. parapsilosis*	1.76
C. parapsilosis	*C. parapsilosis*	99.9	*C. parapsilosis*	1.91
C. parapsilosis	*C. parapsilosis*	99.9	*C. parapsilosis*	1.87
C. parapsilosis	*C. parapsilosis*	99.9	*C. parapsilosis*	1.98
C. parapsilosis	*C. parapsilosis*	99.9	*C. parapsilosis*	1.90
C. tropicalis	***C. albicans***	**99.9**	*C. tropicalis*	2.00
C. tropicalis	***C. albicans***	**81.4**	*C. tropicalis*	1.85
C. tropicalis	*C. tropicalis*	99.9	***C. parapsilosis***	**1.89**
C. tropicalis	*C. tropicalis*	99.9	*C. tropicalis*	2.06
C. tropicalis	*C. tropicalis*	99.9	*C. tropicalis*	1.77
C. tropicalis	*C. tropicalis*	99.9	*C. tropicalis*	1.83
C. tropicalis	*C. tropicalis*	99.9	*C. tropicalis*	2.20
C. tropicalis	*C. tropicalis*	99.9	*C. tropicalis*	2.01
C. tropicalis	*C. tropicalis*	99.9	*C. tropicalis*	1.90
C. tropicalis	*C. tropicalis*	99.9	*C. tropicalis*	1.88
C. tropicalis	*C. tropicalis*	99.9	*C. tropicalis*	2.07

Table 2. Continued.

Sequencing	Vitek MS		Bruker Biotyper	
Species	Species	Confidence value	Species	Score value
C. tropicalis	*C. tropicalis*	99.9	*C. tropicalis*	1.80
C. tropicalis	*C. tropicalis*	99.9	*C. tropicalis*	1.90
C. tropicalis	*C. tropicalis*	99.9	*C. tropicalis*	2.15
C. tropicalis	*C. tropicalis*	99.9	*C. tropicalis*	2.16
C. tropicalis	*C. tropicalis*	99.9	*C. tropicalis*	1.69
C. tropicalis	*C. tropicalis*	99.9	*C. tropicalis*	1.86
C. tropicalis	*C. tropicalis*	99.9	*C. tropicalis*	1.84
C. tropicalis	*C. tropicalis*	99.9	*C. tropicalis*	1.82
C. tropicalis	*C. tropicalis*	99.9	*C. tropicalis*	1.99
C. tropicalis	*C. tropicalis*	99.9	*C. tropicalis*	2.01
C. tropicalis	*C. tropicalis*	99.9	*C. tropicalis*	1.71
C. tropicalis	*C. tropicalis*	99.9	*C. tropicalis*	2.00
P. fabianii	*P. fabianii*	99.9	*P. fabianii*	2.01

Misidentified results are depicted in bold letters.

from ITS sequencing (Table 1).

The Vitek MS and the Bruker Biotyper correctly identified all isolates that had been previously identified as *C. lusitaniae* by the Vitek 2 system (Tables 1 and 2).

Isolates Previously Identified as *M. guilliermondii* by the Vitek 2 System

Among the five isolates previously identified as *M. guilliermondii* by the Vitek 2 system, two were identified as *M. guilliermondii*, and the remaining three isolates were identified as *C. glabrata* (2 isolates) and *C. parapsilosis* (1 isolate) by ITS sequencing (Table 1).

Using the Phoenix system, four of the five isolates (80.0%) were correctly identified (with no identification for *M. guilliermondii*) compared with the results from ITS sequencing (Table 1).

The Vitek MS and the Bruker Biotyper correctly identified all isolates that previously had been identified as *M. guilliermondii* by the Vitek 2 system (Tables 1 and 2).

Discussion

C. famata is a rare human pathogen, previously reported as the ninth-ranking among 31 different *Candida* species [17]. Recent reports suggest that *C. famata* is easily misidentified and might be much less common than previously reported [1, 3, 7–9]. In this study, among 38 isolates initially identified as *C. famata* by the Vitek 2 system, none was identified as *C. famata.* The majority (27/38 isolates, 71.1%) were identified as *C. tropicalis* (20 isolates) or *C. albicans* (7 isolates) by ITS sequencing (Table 1). Among 20 isolates that were identified as *C. tropicalis*, 17 (85%) were isolated from urine. In a previous report three of the 26 isolates initially identified as *C. famata*, only were confirmed as such; 10 were identified as *M. guilliermondii* [3]. In two other studies, among 27 isolates identified as *C. famata* by the Vitek 2 system, all were eventually identified as *M. guilliermondii* by ITS sequencing [1, 9]. In our study, only two isolates (2/38, 5.3%) were ultimately identified as *M. guilliermondii* by ITS sequencing. In Korea, the most frequently recovered *Candida* species from urine samples are *C. albicans* and *C. tropicalis* [10, 21]. *M. guilliermondii* is also a common isolate, ranking fifth in the blood and 10th in the urine [21]. Two isolates in our study that were ultimately identified as *C. auris* by ITS sequencing were obtained from ear discharge. *C. auris*, isolated from the external ear canal, was described as a new species in 2009 and was recently recognized as an emerging multidrug-resistant yeast that can cause a wide spectrum of infections [7, 20]. *C. auris* is reported to be misidentified as *C. haemulonii* and *C. famata* by the Vitek 2 system. Because of its reduced susceptibility to fluconazole, voriconazole, and caspofungin, correct identification of *C. auris* is important [7, 14]. In contrast, 50% (6/12 isolates) and 40% (2/5 isolates) of *C. lusitaniae* and *M. guilliermondii,* respectively, were correctly identified by the Vitek 2 system, and the remaining isolates were confirmed to be more common *Candida* species (*C. albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, and *C. krusei*) by ITS sequencing. These results suggest that

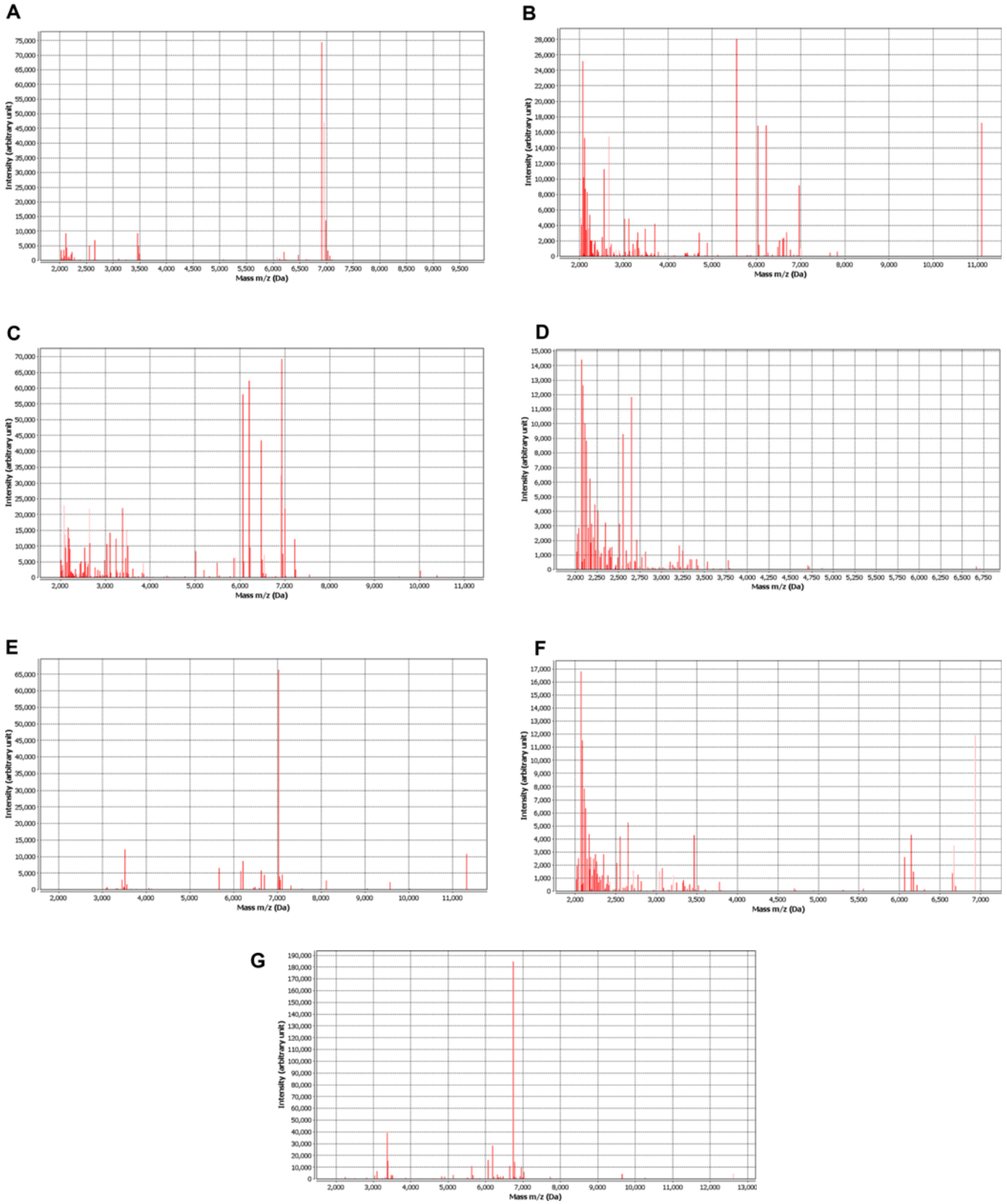

Fig. 1. Spectra of *Candida* species generated by MALDI-TOF MS, the Vitek MS (bioMérieux).
(**A**) *C. albicans;* (**B**) *C. glabrata;* (**C**) *C. tropicalis;* (**D**) *C. parapsilosis;* (**E**) *C. krusei;* (**F**) *C. lusitaniae;* (**G**) *Meyerozyma guilliermondii.*

C. famata is less common in Korea than previously thought.

Of 55 total isolates, the Phoenix system correctly identified 40 (72.7%), whereas 15 (27.3%) were misidentified or not identified (Table 1). The Phoenix system did not accurately identify all isolates of *C. lusitaniae* (7 isolates), *C. krusei* (2 isolates), *C. auris* (2 isolates), and *P. fabianii* (1 isolate). Some isolates of *M. guilliermondii* (2/4) and *C. tropicalis* (1/23) were misidentified or not identified by the Phoenix system. Despite previous reports that the Phoenix system is superior to the Vitek 2 system for species-level identification of yeasts [2,18], the Phoenix system could not accurately identify uncommon *Candida* species.

Table 2 shows the species identifications by two MALDI-TOF MS systems (the Vitek MS and the Bruker Biotyper) for the 55 clinical isolates studied. As previously reported [11], the Bruker Biotyper requires an additional tube-based extraction step (ethanol/formic acid extraction), whereas the Vitek MS does not. Previous studies have reported identification rates of 87.3–93.0% for the Vitek MS [2,6] and 81.1–98.6% for the Bruker Biotyper [1,2,6,11]. In this study, the correct identification rate for 55 isolates was 92.7% (51/55 isolates) for the Vitek MS and 94.6% (52/55 isolates) for the Bruker Biotyper compared with results from ITS or D1/D2 sequencing. The Vitek MS and Bruker Biotyper failed to identify four isolates (2 *C. auris* and 2 *C. tropicalis*) and three isolates (2 *C. auris* and 1 *C. tropicalis*), respectively (Tables 1 and 2). Two *C. auris* isolates were misidentified as *C. albicans* (confidence value 86.0) and *C. haemulonii* (confidence value 99.9) by the Vitek MS and as *Neisseria meningitidis* Serogroup A (score value 1.06) and *Pseudomonas rhizosphaerae* (score value 1.41) by the Bruker Biotyper. Kathuria *et al*. [7] reported that all of 90 *C. auris* isolates were identified as *C. auris* with score values >2.00 (for 77/90 isolates) and 1.70–1.99 (for 13/90 isolates) by the Bruker Biotyper.

Some uncommon species of *Candida* are highly virulent and show reduced antifungal drug susceptibility. Thus, accurate identification of these species is critical. The performance of MALDI-TOF MS was superior to that of conventional automated identification systems such as Vitek 2 and Phoenix, in particular regarding performance with *Candida* species uncommonly isolated in clinical settings. However, MALDI-TOF MS still could not accurately identify some uncommon *Candida* species. It seems that the specific spectra of most of the uncommon *Candida* species were not included in the databases or had insufficient and phenotypic variation. Currently, MALDI-TOF MS is optimized for use, but many clinical microbiology laboratories still rely on conventional automated identification systems for routine identification of *Candida* isolates. The present study indicates that *C. famata* is less common in Korea than previously thought, and that the possibility of misidentification should be noted when an uncommon *Candida* species is identified.

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References

1. Castanheira M, Woosley LN, Diekema DJ, Jones RN, Pfaller MA. 2013. *Candida guilliermondii* and other species of *Candida* misidentified as *Candida famata*: assessment by Vitek 2, DNA sequencing analysis, and matrix-assisted laser desorption ionization-time of flight mass spectrometry in two global antifungal surveillance programs. *J. Clin. Microbiol.* **51:** 117-124.
2. Chao QT, Lee TF, Teng SH, Peng LY, Chen PH, Teng LJ, Hsueh PR. 2014. Comparison of the accuracy of two conventional phenotypic methods and two MALDI-TOF MS systems with that of DNA sequencing analysis for correctly identifying clinically encountered yeasts. *PLoS One* **9:** e109376.
3. Desnos-Ollivier M, Ragon M, Robert V, Raoux D, Gantier JC, Dromer F. 2008. *Debaryomyces hansenii* (*Candida famata*), a rare human fungal pathogen often misidentified as *Pichia guilliermondii* (*Candida guilliermondii*). *J. Clin. Microbiol*. **46:** 3237-3242.
4. Diekema D, Arbefeville S, Boyken L, Kroeger J, Pfaller M. 2012. The changing epidemiology of healthcare-associated candidemia over three decades. *Diagn. Microbiol. Infect. Dis.* **73:** 45-48.
5. Clinical and Laboratory Standards Institute. 2008. Interpretive criteria for identification of bacteria and fungi by DNA target sequencing; approved guideline. CLSI document MM18-A. Clinical and Laboratory Standards Institute, Wayne, PA.
6. Jamal WY, Ahmad S, Khan ZU, Rotimi VO. 2014. Comparative evaluation of two matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) systems for the identification of clinically significant yeasts. *Int. J. Infect. Dis.* **26:** 167-170.
7. Kathuria S, Singh PK, Sharma C, Prakash A, Masih A, Kumar A, *et al*. 2015. Multidrug-resistant *Candida auris* misidentified as *Candida haemulonii*: characterization by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), and DNA sequencing

and its antifungal susceptibility profile variability by VITEK 2, CLSI broth microdilution, and E-test method. *J. Clin. Microbiol.* **53:** 1823-1830.
8. Kim HY, Huh HJ, Choi R, Ki CS, Lee NY. 2015. Three cases of candidiasis misidentified as *Candida famata* by the Vitek 2 system. *Ann. Lab. Med.* **35:** 175-177.
9. Kim SH, Shin JH, Mok JH, Kim SY, Song SA, Kim HR, *et al*. 2014. Misidentification of *Candida guilliermondii* as *C. famata* among strains isolated from blood cultures by the VITEK 2 system. *Biomed. Res. Int.* **2014:** 250408.
10. Kim T-H, Chung D-S, Lee M-K. 2007. Risk factors for hospital-acquired urinary tract infection due to *Candida* species. *Kor. J. Med. Mycol.* **12:** 156-162.
11. Lacroix C, Gicquel A, Sendid B, Meyer J, Accoceberry I, Francois N, *et al*. 2014. Evaluation of two matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) systems for the identification of *Candida* species. *Clin. Microbiol. Infect.* **20:** 153-158.
12. Leaw SN, Chang HC, Sun HF, Barton R, Bouchara JP, Chang TC. 2006. Identification of medically important yeast species by sequence analysis of the internal transcribed spacer regions. *J. Clin. Microbiol.* **44:** 693-699.
13. Lee MK, Yong D, Kim M, Kim MN, Lee K. 2010. [Species distribution and antifungal susceptibilities of yeast clinical isolates from three hospitals in Korea, 2001 to 2007]. *Kor. J. Lab. Med.* **30:** 364-372.
14. Lee WG, Shin JH, Uh Y, Kang MG, Kim SH, Park KH, Jang HC. 2011. First three reported cases of nosocomial fungemia caused by *Candida auris*. *J. Clin. Microbiol.* **49:** 3139-3142.
15. Marcos JY, Pincus DH. 2013. Fungal diagnostics: review of commercially available methods. *Methods Mol. Biol.* **968:** 25-54.
16. Miceli MH, Diaz JA, Lee SA. 2011. Emerging opportunistic yeast infections. *Lancet Infect. Dis.* **11:** 142-151.
17. Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Ellis D, Tullio V, *et al*. 2010. Results from the artemis disk global antifungal surveillance study, 1997 to 2007: a 10.5-year analysis of susceptibilities of *Candida* species to fluconazole and voriconazole as determined by CLSI standardized disk diffusion. *J. Clin. Microbiol.* **48:** 1366-1377.
18. Posteraro B, Ruggeri A, De Carolis E, Torelli R, Vella A, De Maio F, *et al*. 2013. Comparative evaluation of BD Phoenix and Vitek 2 systems for species identification of common and uncommon pathogenic yeasts. *J. Clin. Microbiol.* **51:** 3841-3845.
19. Putignani L, Del Chierico F, Onori M, Mancinelli L, Argentieri M, Bernaschi P, *et al*. 2011. MALDI-TOF mass spectrometry proteomic phenotyping of clinically relevant fungi. *Mol. Biosyst.* **7:** 620-629.
20. Satoh K, Makimura K, Hasumi Y, Nishiyama Y, Uchida K, Yamaguchi H. 2009. *Candida auris* sp. nov., a novel ascomycetous yeast isolated from the external ear canal of an inpatient in a Japanese hospital. *Microbiol. Immunol.* **53:** 41-44.
21. Won EJ, Shin JH, Lee W-K, Koo SH, Kim SY, Park Y-J, *et al*. 2013. Distribution of yeast and mold species isolated from clinical specimens at 12 hospitals in Korea during 2011. *Ann. Clin. Microbiol.* **16:** 92-100.