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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

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Candida famata **(teleomorph *Debaryomyces hansenii*) has been described as a medically relevant yeast, and this species has been included in many commercial identification systems that are currently used in clinical laboratories. Among 53 strains collected during the SENTRY and ARTEMIS surveillance programs and previously identified as *C. famata* (includes all submitted strains with this identification) by a variety of commercial methods (Vitek, MicroScan, API, and AuxaColor), DNA sequencing methods demonstrated that 19 strains were *C. guilliermondii*, 14 were *C. parapsilosis*, 5 were *C. lusitaniae*, 4 were *C. albicans*, and 3 were *C. tropicalis*, and five isolates belonged to other *Candida* species (two *C. fermentati* and one each *C. intermedia*, *C. pelliculosa*, and *Pichia fabianni*). Additionally, three misidentified *C. famata* strains were correctly identified as *Kodomaea ohmeri*, *Debaryomyces nepalensis*, and *Debaryomyces fabryi* using intergenic transcribed spacer (ITS) and/or intergenic spacer (IGS) sequencing. The Vitek 2 system identified three isolates with high confidence to be *C. famata* and another 15 with low confidence between *C. famata* and *C. guilliermondii* or *C. parapsilosis*, displaying only 56.6% agreement with DNA sequencing results. Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) results displayed 81.1% agreement with DNA sequencing. One strain each of *C. metapsilosis*, *C. fermentati*, and *C. intermedia* demonstrated a low score for identification (<2.0) in the MALDI Biotyper. *K. ohmeri*, *D. nepalensis*, and *D. fabryi* identified by DNA sequencing in this study were not in the current database for the MALDI Biotyper. These results suggest that the occurrence of *C. famata* in fungal infections is much lower than previously appreciated and that commercial systems do not produce accurate identifications except for the newly introduced MALDI-TOF instruments.**

Candida famata (formerly *Torulopsis candida*; teleomorph, *Debaryomyces hansenii*) is an ascomycetous yeast commonly found in foods, including dairy products (1, 2). *C. famata* is a rare human pathogen that has been the putative etiologic agent in cases of bloodstream infection (BSI) (3–9) peritonitis (10, 11) and ocular (12, 13) and bone (14) infections. In the 10.5-year ARTEMIS DISK survey (15), *C. famata* was ranked ninth among 31 different species.

Recent publications from reference laboratories have suggested that isolates initially identified as *C. famata* by phenotypic methods were found to include strains of *C. guilliermondii*, *C. lusitaniae*, *C. fermentati*, *C. intermedia*, and *C. palmioleophila* when subjected to molecular identification (5, 16). Such findings suggest that *C. famata* may be much less common as an etiologic agent of invasive candidiasis (IC) than previously reported (5, 16). Our recent experience has been similar in that five isolates originally identified as *C. famata* in the 2010 SENTRY antimicrobial surveillance program were found by molecular methods to be three different species of *Candida* (*C. guilliermondii*, *C. lusitaniae*, and *C. parapsilosis*) (17). With this background, we investigated the true prevalence of *C. famata* among 53 isolates originally submitted as such in two very large global surveillance programs, the ARTEMIS and SENTRY programs, for the time interval from 2005 through 2011.

MATERIALS AND METHODS

Yeast isolates. Fifty-three clinical isolates from individual patients previously identified as *C. famata* and submitted to the ARTEMIS (24 isolates) or the SENTRY (29 isolates) program were included in the study. The yeast identification methods employed in the submitting laboratories included the Vitek yeast identification system (32 isolates [60%]), MicroScan (4 isolates [8%]), API (4 isolates [8%]), and AuxaColor (2 isolates [4%]). The identification system was not specified for 11 isolates (20%). The isolates represented 33 different study sites in 22 countries; 34 were obtained from blood or deep tissue sites of infection. These isolates represented all (100.0%) of the *C. famata* isolates submitted to the University of Iowa (Iowa City) and JMI (North Liberty, IA) reference laboratories during the time period from 2005 through 2011. Isolates were subcultured upon receipt and stored until further testing.

Phenotypic characterization of isolates. Isolates were subcultured on CHROMagar (Becton, Dickinson and Company), and colony morphology and color were observed after 48 h of incubation at 35°C. Species identification was performed using the Vitek 2 yeast identification card (bioMérieux, Hazelwood, MO) according to the manufacturer's instructions. Isolates were assessed for the presence or absence of pseudohyphae after growth for 24 h at 35°C on cornmeal agar (Remel, Lenexa, KS). The MIC values for amphotericin B, flucytosine, fluconazole, posaconazole, voriconazole, anidulafungin, caspofungin, and micafungin were deter-

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mined by broth microdilution as described in Clinical and Laboratory Standards Institute (CLSI) document M27-A3 (18).

DNA sequencing-based identification. Isolates were subjected to DNA extractions using the QIAquick extraction kit (Qiagen, Hilden, Germany) in the QiaCube automated system (Qiagen). Amplification and sequencing were performed for the intergenic transcribed spacer (ITS) region for all strains (19). Additionally, the 28S ribosomal subunit (D1/D2) (19, 20) was used for assisting in the identification of *C. fabianii* and *C. intermedia/pseudointermedia*, and the intergenic spacer (IGS) was sequenced for *Debaryomyces* spp. (21). Amplicons were sequenced on both strands and compared with available reference sequences through BLAST. Results were considered acceptable if homology with other entries in the databases used for comparison was >99.5%. Available sequences that were considerably different from the majority of entries for one species were considered outliers and discarded in the analysis. Additionally, if no match was found in the database, the identification was based on species complex, genus, family, or order, according to the most current classifications systems.

MALDI-TOF MS. All isolates were tested via matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) using the Bruker Daltonik MALDI Biotyper (Fremont, CA) by following the manufacturer's instructions and previously described guidelines for yeast identification (22, 23).

RESULTS AND DISCUSSION

DNA-sequencing identification results compared to those of Vitek 2 and MALDI-TOF MS (MALDI). Among the 53 isolates originally identified as *C. famata* by the submitting laboratory, the majority (33/53; 62.3%) (Table 1) were identified as *C. guilliermondii* (19 isolates) or *C. parapsilosis* (14 isolates), with two identified as the cryptic species *C. orthopsilosis* (one isolate) and *C. metapsilosis* (one isolate), by ITS sequencing. An additional seven *Candida* species were identified among 17 remaining isolates, with five being *C. lusitaniae*, four *C. albicans*, three *C. tropicalis*, and two *C. fermentati* and three isolates belonging to other species. The latter three isolates included one strain each of *C. intermedia/pseudointermedia*, *C. pelliculosa*, and *C.* (*Cyberlidnera/Pichia*) *fabianni*. Additionally, one *Kodomaea ohmeri* isolate was identified using ITS sequencing, and two isolates displaying low discrimination on the ITS and 28S rRNA sequencing between *D. hansenii* and *D. nepalensis* or *D. castellii* were identified as *Debaryomyces nepalensis* and *Debaryomyces fabryi* using IGS sequencing (21).

Only three isolates (5.7%) were identified as *C. famata* with high confidence using the Vitek 2 yeast identification card (Table 1). However, these isolates were confirmed neither by ITS sequencing nor by MALDI-TOF MS. The most frequent Vitek 2 result was a low discrimination between *C. famata* and *C. guilliermondii* (14 isolates), for which 12 were identified as *C. guilliermondii* and two as *C. fermentati* by ITS sequencing. MALDI was not able to produce reliable results for two *C. guilliermondii* strains or for any of the *C. fermentati* strains (one was identified as *C. guilliermondii* with a low score value [1.70], and one had no reliable identification) (Table 1).

Among 13 isolates that were identified with high confidence as *C. parapsilosis* by the Vitek 2 and one that had low discrimination between *C. famata* and *C. parapsilosis*, 11 were confirmed as belonging to this species by both ITS sequencing and MALDI. Two isolates were identified as the cryptic species *C. orthopsilosis* and *C. metapsilosis* (one each) by sequencing, but among those the MALDI was able to produce reliable results only for *C. orthopsilosis* (Table 1).

The Vitek 2 system identified five of the putative *C. famata* isolates as *C. lusitaniae*, five as *C. guilliermondii*, and one as *C. pelliculosa*. These results were confirmed by ITS sequencing and MALDI-TOF MS, except for one *C. guilliermondii* isolate which was not identified by the MALDI Biotyper. One isolate had low discrimination in the Vitek 2 between *C. tropicalis* and *C. parapsilosis*, but ITS sequencing and MALDI identified it as *C. tropicalis*. Four isolates received as *C. famata* were identified as *C. albicans* and two as *C. tropicalis* by all methods used.

One isolate showed low discrimination among *C. intermedia/C. pseudointermedia* using ITS sequencing analysis (95.0 to 98.5% homology). The Vitek 2 and the MALDI identified this strain as *C. intermedia*, with very good confidence and a score value of 1.97, respectively. One *C. sphaerica* isolate identified by the Vitek 2 had high ITS homology with *Debaryomyces* spp. and was identified as *Debaryomyces fabryi* by IGS sequencing. No reliable identification was produced using the MALDI.

The Vitek 2 showed a good identification for a *Kodamaea ohmeri* isolate that was in agreement with ITS sequencing, and this isolate was identified with a score of 1.78 as *C. guilliermondii* by the MALDI. One isolate was not reliably identified by the Vitek 2 and MALDI and was identified as *C. fabianii* by ITS sequencing.

Thus, within the entire collection of 53 putative *C. famata* isolates, none were confirmed as such by DNA-sequencing-based methods. The Vitek 2 system gave *C. famata* as a possible identification for a total of 18 isolates (three *C. famata* isolates and 15 isolates with low discrimination between *C. famata* and *C. guilliermondii/C. parapsilosis*), but the MALDI Biotyper did not identify any of these isolates as *C. famata*.

Whereas Jensen and Arendrup (16) demonstrated that the type strain of *C. famata* (CBS 796) did not form pseudohyphae on cornmeal agar and produced light red pigmented colonies on CHROMagar, our collection of putative *C. famata* isolates showed variable production of pseudohyphae (55%) and a colony color on CHROMagar that ranged from white to green to blue and purple. None of the strains in the present collection produced a red or light red colony on CHROMagar.

Accuracy of Vitek 2 and MALDI versus sequencing-based identification. Clearly, this collection of predominantly non-*C. albicans Candida* species poses problems for yeast identification systems. Using ITS and/or 28S ribosomal gene sequencing, this collection of 53 isolates previously identified in clinical laboratories worldwide as *C. famata* was shown to contain 14 different species (Table 2). By comparison, the Vitek 2 yeast identification card identified eight different species, and MALDI identified six species. Several of these species are distinctly uncommon as causes of IC and are quite difficult to identify with any degree of accuracy (17, 24). Among the more common species as determined by reference sequencing (*C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. guilliermondii*, and *C. lusitaniae*), 58.1% (25/43) were correctly identified by the Vitek 2 and 88.4% (38/43) were correctly identified by MALDI (Table 2). The accuracy of the Vitek 2 for the identification of these five species is considerably lower than reported previously (89.8% [one choice] and 99.1% [including low discrimination]) (25), but this would improve to 97.6% in the present study if the low-discrimination results of *C. famata/C. guilliermondii* and *C. famata/C. parapsilosis* were considered acceptable for the identification of *C. guilliermondii* and *C. parapsilosis*, respectively (Table 2). Likewise, the cryptic species *C. fermentati*, *C. metapsilosis*, and *C. orthopsilosis* are not expected to be differentiated from *C. guilliermondii* and *C. parapsilosis*, respec-

TABLE 1 Identification by DNA sequencing methods, the Vitek 2, and mass spectroscopy for 53 clinical isolates sent to the ARTEMIS and/or SENTRY surveillance program between 2005 and 2011 as *Candida famata*

	Identification by:					
Strain	DNA sequencing	Vitek 2	MALDI-TOF (score value if <2.00)	Pseudohyphae	Country	Specimen type
1	*C. albicans*	*C. albicans*	*C. albicans*	NT[a]	Brazil	Blood
2	*C. albicans*	*C. albicans*	*C. albicans*	NT	Poland	Blood
3	*C. albicans*	*C. albicans*	*C. albicans*	NT	Italy	Unknown
4	*C. albicans*	*C. albicans*	*C. albicans*	Positive	Germany	Blood
5	*C. fabianii*	Unidentified	No reliable identification	Negative	Slovakia	Blood
6	*C. fermentati*	Low-discrimination *C. famata/C. guilliermondii*	*C. guilliermondii* (1.70)	Negative	United States	Blood
7	*C. fermentati*	Low-discrimination *C. guilliermondii/C. famata*	No reliable identification	Negative	Colombia	Blood
8	*C. guilliermondii*	Low-discrimination *C. famata/C. guilliermondii*	*C. guilliermondii*	Negative	Italy	Blood
9	*C. guilliermondii*	Low-discrimination *C. famata/C. guilliermondii*	*C. guilliermondii*	Positive	Mexico	Blood
10	*C. guilliermondii*	Low-discrimination *C. famata/C. guilliermondii*	*C. guilliermondii*	Negative	Turkey	Unknown
11	*C. guilliermondii*	Low-discrimination *C. famata/C. guilliermondii*	*C. guilliermondii*	Negative	Brazil	Blood
12	*C. guilliermondii*	Low-discrimination *C. guilliermondii/C. famata*	*C. guilliermondii*	Negative	United States	Blank
13	*C. guilliermondii*	Low-discrimination *C. guilliermondii/C. famata*	*C. guilliermondii*	Positive	United States	Bronchoalveolar lavage fluid
14	*C. guilliermondii*	Low-discrimination *C. guilliermondii/C. famata*	No reliable identification	Negative	Argentina	Blood
15	*C. guilliermondii*	Low-discrimination *C. guilliermondii/C. famata*	*C. guilliermondii*	Negative	France	Other
16	*C. guilliermondii*	Low-discrimination *C. guilliermondii/C. famata*	*C. guilliermondii*	Negative	United States	Blood
17	*C. guilliermondii*	Low-discrimination *C. guilliermondii/C. famata*	*C. guilliermondii*	Positive	Argentina	Blood
18	*C. guilliermondii*	Low-discrimination *C. guilliermondii/C. famata*	*C. guilliermondii*	Negative	Italy	Unknown
19	*C. guilliermondii*	*C. guilliermondii/C. famata*	*C. guilliermondii*	Positive	Switzerland	Unknown
20	*C. guilliermondii*	*C. guilliermondii*	*C. guilliermondii*	Positive	Brazil	Blood
21	*C. guilliermondii*	*C. guilliermondii*	*C. guilliermondii*	Positive	Colombia	Urine
22	*C. guilliermondii*	*C. guilliermondii*	*C. guilliermondii*	Positive	Australia	Blood
23	*C. guilliermondii*	*C. guilliermondii*	No reliable identification	Negative	Argentina	Blood
24	*C. guilliermondii*	*C. guilliermondii*	*C. guilliermondii*	Positive	Australia	Blood
25	*C. guilliermondii*	*C. famata*	*C. guilliermondii*	Positive	South Korea	Blood
26	*C. guilliermondii*	*C. famata*	*C. guilliermondii*	Positive	Germany	Blood
27	*C. intermedia/C. pseudointermedia*	*C. intermedia*	*C. intermedia* (1.97)	Positive	China	Blood
28	*C. lusitaniae*	*C. lusitaniae*	*C. lusitaniae*	Negative	Mexico	Unknown
29	*C. lusitaniae*	*C. lusitaniae*	*C. lusitaniae*	Positive	Mexico	Unknown
30	*C. lusitaniae*	*C. lusitaniae*	*C. lusitaniae*	Positive	Mexico	Unknown
31	*C. lusitaniae*	*C. lusitaniae*	*C. lusitaniae*	Negative	France	Other
32	*C. lusitaniae*	*C. lusitaniae*	*C. lusitaniae*	Negative	Hungary	Tissue
33	*C. metapsilosis*	*C. parapsilosis*	No reliable identification	Positive	United States	Blood
34	*C. orthopsilosis*	*C. parapsilosis*	*C. orthopsilosis*	Positive	Venezuela	Abscess
35	*C. parapsilosis*	Low-discrimination *C. parapsilosis/C. famata*	*C. parapsilosis*	Positive	South Africa	Blood
36	*C. parapsilosis*	*C. parapsilosis*	*C. parapsilosis*	Positive	Mexico	Blood
37	*C. parapsilosis*	*C. parapsilosis*	*C. parapsilosis*	Positive	Turkey	Unknown
38	*C. parapsilosis*	*C. parapsilosis*	*C. parapsilosis*	Positive	Brazil	Blood
39	*C. parapsilosis*	*C. parapsilosis*	*C. parapsilosis*	Positive	Mexico	Blood
40	*C. parapsilosis*	*C. parapsilosis*	*C. parapsilosis*	Positive	Ireland	Unknown
41	*C. parapsilosis*	*C. parapsilosis*	*C. parapsilosis*	Positive	Italy	Blood
42	*C. parapsilosis*	*C. parapsilosis*	*C. parapsilosis*	Positive	Turkey	Unknown

(Continued on following page)

TABLE 1 (Continued)

Strain	Identification by: DNA sequencing	Vitek 2	MALDI-TOF (score value if <2.00)	Pseudohyphae	Country	Specimen type
43	*C. parapsilosis*	*C. parapsilosis*	*C. parapsilosis*	Positive	Mexico	Unknown
44	*C. parapsilosis*	*C. parapsilosis*	*C. parapsilosis*	Positive	Brazil	Unknown
45	*C. parapsilosis*	*C. parapsilosis*	*C. parapsilosis*	Negative	Brazil	Blood
46	*C. parapsilosis*	*C. parapsilosis*	*C. parapsilosis*	Positive	South Africa	Blood
47	*C. pelliculosa*	*C. pelliculosa*	*C. pelliculosa*	Negative	South Korea	Blood
48	*C. tropicalis*	*C. tropicalis*	*C. tropicalis*	NT	United States	Blood
49	*C. tropicalis*	*C. tropicalis*	*C. tropicalis*	NT	Brazil	Tissue
50	*C. tropicalis*	Low-discrimination *C. tropicalis/C. parapsilosis*	*C. tropicalis*	Positive	Israel	Blood
51	*Debaryomyces fabryi*	*C. sphaerica*	No reliable identification	Negative	France	Blood
52	*Debaryomyces nepalensis*	*C. famata*	No reliable identification	Negative	China	Unknown
53	*Kodamaea ohmeri*	*Kodamaea ohmeri*	*C. guilliermondii* (1.78)	Positive	Brazil	Unknown

[a] NT, not tested.

tively, by the Vitek 2 or any other phenotypic identification system.

An incorrect species identification (major error) occurred for two isolates (3.8%) with the MALDI Biotyper (one *C. fermentati* and one *K. ohmeri* misidentified as *C. guilliermondii*); however, the misidentification of the *C. fermentati* can be explained by the fact that this organism, a cryptic species within the *C. guilliermondii* species complex (26), was not in the current database. Generally, MALDI has the advantage of having a "no reliable result" response rather than making an erroneous identification (Table 2). Among the 10 discrepancies or low-score results observed between MALDI and the molecular reference method, six were due to a report of no reliable identification by the MALDI Biotyper, two of which were species not included in the MALDI database (*C. fermentati* and *C. fabianii*). *C. metapsilosis* and *C. orthopsilosis*, newly recognized as members of the *C. parapsilosis* species complex, are frequently misidentified by phenotypic methods as *C. parapsilosis sensu stricto* (27). Previous studies show that these two species can be identified by MALDI (28, 29); however, in two instances (2, 7, 11, 13, 28, 29) the original manufacturer spectral database library was secondarily modified to include these rare yeast species. Interestingly Hendrickx et al. (30) were able to identify both of these cryptic species by MALDI-TOF MS without any database modifications. Overall, our results are comparable with those obtained in previously published studies where MALDI was compared to a reference molecular identification method (22, 29, 31).

***In vitro* susceptibility profile of *C. famata* and other species of *Candida* identified by molecular methods.** In general the MIC values obtained for the eight antifungal agents tested conform to the wild-type (WT) MIC distributions for *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. guilliermondii*, *C. lusitaniae*, *C. orthopsilosis*, and *C. pelliculosa* described previously for each species (32–38). Notable exceptions include decreased susceptibility to flucytosine (MIC > WT) among *C. guilliermondii* (MIC > 1 μg/ml [one isolate]) and *C. lusitaniae* (MIC > 0.5 μg/ml [two isolates]) strains, to fluconazole among *C. guilliermondii* (MIC > 8 μg/ml [three isolates]) and *C. parapsilosis* (MIC > 2 μg/ml [two isolates]) strains, to posaconazole among *C. guilliermondii* (MIC > 0.5 μg/ml [six isolates]), *C. albicans* (MIC > 0.06 μg/ml [one isolate]), and *C. tropicalis* (MIC > 0.12 μg/ml [one isolate]) strains, and to voriconazole among *C. guilliermondii* (MIC > 0.25 μg/ml [two isolates]) and *C. tropicalis* (MIC > 0.06 μg/ml [one isolate]) strains (Table 3).

None of the 53 isolates were confirmed to be *C. famata* (*D. hansenii*); however, the two clinical isolates that were confirmed to be *Debaryomyces* spp. (*D. nepalensis* and *D. fabryi*) displayed low MIC values against echinocandins (MIC values of 0.25 and 0.06 μg/ml for anidulafungin, 0.06 μg/ml for caspofungin, and 0.25 μg/ml for micafungin) and were less susceptible to amphotericin B (MIC, 2 μg/ml), flucytosine (MIC range, ≤0.5 to 2 μg/ml), fluconazole (MIC range, 0.5 to 16 μg/ml), and posaconazole (MIC range, 0.25 −1 μg/ml). In contrast to the other triazoles, voriconazole was quite active (MIC range, 0.015 to 0.12 μg/ml) against these species (Table 3). Notably, the two species in this collection that were most frequently misidentified as *C. famata*, *C. parapsilosis* and *C. guilliermondii*, both show elevated echinocandin MIC values.

The MIC values for the triazoles and the echinocandins against the very rare species of *Candida* were generally low (<2 μg/ml and <0.5 μg/ml, respectively), with the exception of *C. fermentati* (fluconazole, anidulafungin, and micafungin), *C. fabianii* (fluconazole), and *C. orthopsilosis* (anidulafungin).

The results of this study are similar to the previous reports by Desnos-Ollivier et al. (5) and Jensen and Arendrup (16) in that the vast majority of isolates identified as *C. famata* by conventional phenotypic methods cannot be confirmed as such by either molecular or proteomic methods of identification. Among 26 isolates sent to the laboratory of Desnos-Ollivier (Paris, France) with an identification of *C. famata* (*D. hansenii*), only three were confirmed as such using ITS sequence analysis; six were found to be *C. fermentati* (*P. caribbica*), 10 were *C. guilliermondii* (*P. guilliermondii*), two were *C. haemulonii*, two were *C. lusitaniae*, and three were *C. palmioleophila*. Jensen and Arendrup (16) analyzed 11 isolates in a Danish collection of clinical isolates previously identified as *C. famata* and found that none could be confirmed using either ITS sequencing or MALDI. They found that four isolates were *C. palmioleophila*, one was *C. guilliermondii*, three were *C. lusitaniae*, and two were *C. intermedia*. In the present study, we found isolates of *C. guilliermondii*, *C. fermentati*, *C. lusitaniae*, and

TABLE 2 Species identification of isolates previously identified as *C. famata* by the Vitek 2 and MALDI-TOF methods in comparison with reference molecular methodology

Reference identification (no. of isolates)	Test method	No. (%) correct	Discrepancies (no. of isolates)
C. guilliermondii (19)	Vitek 2	5 (26.3)	*C. famata/C. guilliermondii* low discrimination (12), *C. famata* (2)
	MALDI	17 (89.5)	*C. guilliermondii* with low score value (1), no reliable identification (1)
C. parapsilosis (12)	Vitek 2	11 (91.7)	*C. famata/C. parapsilosis* low discrimination (1)
	MALDI	12 (100.0)	
C. lusitaniae (5)	Vitek 2	5 (100.0)	
	MALDI	5 (100.0)	
C. albicans (4)	Vitek 2	4 (100.0)	
	MALDI	4 (100.0)	
C. tropicalis (3)	Vitek 2	2 (66.7)	*C. tropicalis/C. parapsilosis* low discrimination (1)
	MALDI	3 (100.0)	
C. fermentati (2)	Vitek 2	0 (0.0)	*C. famata/C. guilliermondii* low discrimination (2)
	MALDI	0 (0.0)	*C. guilliermondii* with low score value (1), no reliable identification (1)
C. fabianii (1)	Vitek 2	0 (0.0)	Unidentified (1)
	MALDI	0 (0.0)	No reliable identification (1)
C. intermedia (1)	Vitek 2	1 (100.0)	
	MALDI	0 (0.0)	*C. intermedia* with low score value (1)
C. metapsilosis (1)	Vitek 2	0 (0.0)	*C. parapsilosis* (1)
	MALDI	0 (0.0)	No reliable identification (1)
C. orthopsilosis (1)	Vitek 2	0 (0.0)	*C. parapsilosis* (1)
	MALDI	1 (100.0)	
C. pelliculosa (1)	Vitek 2	1 (100.0)	
	MALDI	1 (100.0)	
D. nepalensis (1)	Vitek 2	0 (0.0)	*C. sphaerica* (1)
	MALDI	0 (0.0)	No reliable identification (1)
D. fabryi (1)	Vitek 2	0 (0.0)	*C. famata* (1)
	MALDI	0 (0.0)	No reliable identification (1)
K. ohmeri (1)	Vitek 2	1 (100.0)	*K. ohmeri* (1)
	MALDI	0 (0.0)	*C. guilliermondii* (1)
Total (53)	Vitek 2	30 (56.6)	
	MALDI	43 (81.1)	

C. intermedia, as well as seven additional species (Tables 1 and 2), all misidentified as *C. famata*. Clearly these studies demonstrate that the vast majority of isolates identified as *C. famata* by phenotypic methods are likely to be a different species of *Candida*, and they call into question numerous reports of this species as an etiologic agent of IC.

The diversity of MIC values for the triazoles, and especially the echinocandins, in this so-called *C. guilliermondii*/*C. famata* group (16) is impressive and again emphasizes the importance of accurate species identification if one chooses to initiate therapy based on such information. Whereas *C. famata*, along with *C. albicans*, *C. tropicalis*, *C. fabianii*, *C. intermedia*, and *C. pelliculosa*, is shown to be highly susceptible to the echinocandins (Table 3), isolates within the *C. parapsilosis* and *C. guilliermondii* species complexes are well known to show reduced susceptibility to echinocandins due to a naturally occurring polymorphism within the *FKS* locus that reduces susceptibility (39, 40). Echinocandins are not recommended as first-line agents for the treatment of the latter two species but would likely be effective in the treatment of IC due to the more highly susceptible species, including *C. famata* (41).

The difficulty in differentiating *C. famata* from *C. guilliermondii* by phenotypic methods is well known (5, 16, 42, 43). This is shown very clearly to be the case for the Vitek 2 yeast identification card in the present study. Within this collection of 53 clinical isolates previously identified as *C. famata*, 19 (35.8%) were found to be *C. guilliermondii* by ITS sequence analysis, of which only 5 showed an acceptable result by the Vitek 2 (Table 2). The remaining 14 isolates were classified either as having low discrimination

TABLE 3 Selected *in vitro* susceptibility results for 53 isolates of *Candida* identified by molecular methods as determined by 24-h CLSI broth microdilution methods

Organism (no. of isolates)	MIC (μg/ml)							
	Amphotericin B	Flucytosine	Anidulafungin	Caspofungin	Micafungin	Fluconazole	Posaconazole	Voriconazole
C. guilliermondii (19)	1	≤0.5	2	0.12	1	2	0.5	0.06
	1	≤0.5	4	0.5	2	16	1	0.25
	1	≤0.5	2	0.12	1	2	0.5	0.12
	0.5	≤0.5	1	0.12	1	2	0.5	0.06
	0.5	≤0.5	2	0.5	1	8	0.25	0.12
	1	4	2	0.12		8	1	2
	0.5	≤0.5	2	1	1	2	0.5	0.06
	0.5	≤0.5	2	0.12	2	2	1	0.12
	1	≤0.5	2	0.12	1	2	0.5	0.06
	1	≤0.5	1	0.06	0.5	8	1	0.25
	1	≤0.5	2	0.25	1	2	0.25	0.06
	0.5	≤0.5	1	0.12	1	2	0.25	0.03
	1	≤0.5	1	0.25	1	16	1	0.5
	1	≤0.5	2	0.25	2	2	0.5	0.12
	0.5	≤0.5	2	0.5	1	4	0.5	0.12
	0.5	≤0.5	2	0.12	1	2	0.25	0.06
	0.5	≤0.5	2	0.25	1	2	0.25	0.06
	0.5	≤0.5	2	0.12	1	2	0.5	0.06
	1	≤0.5	2	0.25	1	16	1	0.25
C. parapsilosis (12)	1	≤0.5	2	0.12	1	0.25	0.12	0.015
	1	≤0.5	2	0.5	1	0.5	0.12	≤0.008
	1	≤0.5	4	0.5	2	16	0.25	0.25
	1	≤0.5	1	0.25	1	0.5	0.25	0.03
	1	≤0.5	2	0.25	1	0.5	0.25	0.03
	1	≤0.5	2	0.5	2	0.5	0.25	0.03
	1	≤0.5	2	0.5	2	0.5	0.12	0.03
	1	≤0.5	2	0.25	2	0.5	0.12	0.015
	1	≤0.5	0.5	0.25	1	1	0.25	0.03
	1	≤0.5	2	0.25	1	2	0.25	0.06
	0.5	≤0.5	2	0.5	1	2	0.25	0.06
	1	≤0.5	2	1	1	8	0.12	0.5
C. lusitaniae (5)	0.5	≤0.5	0.5	0.12	0.25	0.5	0.12	0.015
	1	≤0.5	0.25	0.12	0.25	0.5	0.12	0.015
	0.5	≤0.5	0.12	0.06	0.12	1	0.12	0.03
	1	16	0.5	0.25	0.5	0.12	0.12	≤0.008
	1	4	0.5	0.25	0.25	0.25	0.12	0.015
C. albicans (4)	0.5	≤0.5	0.015	0.12	0.03	≤0.5	≤0.06	≤0.06
	1	≤0.5	0.03	0.12	0.06	≤0.06	0.12	0.015
	0.25	≤0.5	0.03	0.03	0.03	≤0.06	0.015	≤0.008
	1	≤0.5	≤0.008	0.015	0.015	0.12	0.03	0.015
C. tropicalis (3)	1	≤0.5	0.015	0.06	0.015	0.5	0.12	0.03
	0.5	≤0.5	0.015	0.03	0.12	0.5	0.06	0.03
	1	≤0.5	0.03	0.03	0.03	2	0.5	0.12
C. fermentati (2)	1	≤0.5	2	0.25	0.5	8	0.5	0.25
	1	≤0.5	1	0.25	1	2	0.5	0.12
C. fabianii (1)	0.5	4	0.03	0.03	0.03	4	1	0.12
C. intermedia/pseudointermedia (1)	0.5	≤0.5	≤0.008	≤0.008	0.12	0.25	0.06	0.015
C. methapsilosis (1)	1	≤0.5	2	0.12	0.5	1	0.25	0.06
C. orthopsilosis (1)	1	≤0.5	0.25	0.25	0.5	1	0.25	0.03
C. pelliculosa (1)	0.5	≤0.5	0.015	0.03	0.06	2	0.5	0.12
Debaryomyces fabryi (1)	2	≤0.5	0.25	0.25	0.25	0.5	0.25	0.015
Debaryomyces nepalensis (1)	2	2	0.06	0.25	0.25	16	1	0.12
Kodamaea ohmeri (1)	0.5	1	1	0.5	0.5	4	0.25	0.06

between *C. famata* and *C. guilliermondii* (12 isolates) or as having excellent identification for *C. famata* (two isolates). The Vitek 2 identified three isolates as *C. famata* with either a good or excellent result, and these results were not confirmed by sequencing. At this time, given the extremely low prevalence of *C. famata* as an etiologic agent of IC relative to that of *C. guilliermondii*, coupled with the inability of this identification method to differentiate between the two, it would be prudent for the Vitek 2 software to be reconfigured to automatically select *C. guilliermondii* whenever the biochemical profile indicates *C. famata* or *C. famata/C. guilliermondii*. Based on the data shown in the present study, the probability of an erroneous result using this strategy would be very remote.

Previous studies have shown MALDI to be rapid, accurate, and cost-effective in the identification of both common and uncommon species of *Candida* (16, 31, 44). The present study confirms the capabilities of this technology against a very challenging collection of *Candida* species. MALDI showed a good degree of accuracy (88.4%) against the five most common species in this collection, including a number of isolates of *C. guilliermondii* that were unable to be definitively identified by the Vitek 2. Although the very rare species in this collection were not identified by the MALDI Biotyper, a result of "no reliable identification" was given rather than an incorrect organism identification. Future supplementation of the existing MALDI database with additional strains of these rare species should result in improved performance.

In conclusion, *C. famata* appears to be far less common as a cause of IC than was previously understood, to the extent that an identification of *C. famata* from a phenotypically based system is almost certainly incorrect. Although *C. guilliermondii* and *C. parapsilosis* are the species most likely to be misidentified as *C. famata*, we and others have now demonstrated that a wide range of *Candida* species may be similarly misidentified. The application of molecular and proteomic methods of fungal identification provide rapid, powerful and, in the case of proteomics, cost-effective alternatives to the currently applied phenotypic techniques.

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