Candida famata as a Cause of Invasive Candidiasis: Misidentification in Two Global Surveillance Systems

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ASM 2012
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AMENDED ABSTRACT

Background: *Candida famata* (CFAM; now *Debaryomyces hansenii*) has been described as a medically important yeast and this species has been included in many commercial identification systems that are currently in use in clinical laboratories. We evaluated isolates submitted as CFAM to two global antifungal surveillance programs using molecular methods and comparing with a commercial system.

Methods: A total of 53 strains previously identified as CFAM using Vitek and other commercial methods were evaluated. These isolates were collected during the SENTRY and ARTEMIS surveillance programs. Isolates were subcultured onto CHROMagar, identified (IDed) using Vitek 2 (V2), MALDI-TOF, amplified using primers for intergenic transcriber region (ITS), 28S ribosomal portion (D1/D2; few strains) and IGS (only *Debaryomyces* spp.[DSP]). Amplicons were sequenced on both strands and compared with available sequences.

Results: V2 IDed only 2 isolates as CFAM with excellent/very good/good confidence and in 15 instances low confidence IDs included CFAM (additional low confidence species were *C. parapsilosis* [CPRP] and *C. guilliermondii* [CGU]). Other species IDed with good confidence were CPRP (13 isolates); *C. lusitaniae* (CLUS; 6), CGU (5); other *Candida* spp. (3) and *Kodomaea ohmeri* (1). One isolate was unknown and one had low discrimination between *C. tropicalis* (CTRO) and CPRP. Sequencing methods demonstrated that 19 strains were CGU (only 5 accurately IDed by V2), 13 were CPRP, including cryptic species of *C. orthopsilosis* (1) and *C. metapsilosis* (1), and 5 were CLUS. Two isolates were IDed DSP by ITS, but were found to be *D. nepalensis* and *D. fabryi* by IGS sequencing. Six isolates belonged to other *Candida* spp. (*C. fermentati* [2], one each of *C. intermedia* [CIN], *C. pelliculosa* [CPEL], *Pichia fabianni* and CTRO), only two of which were correctly IDed by V2 (CPEL and CIN). MALDI results had 9 discordances with sequencing.

Conclusions: In a collection of 50 putative CFAM strains submitted to two global fungal surveillance studies, only 2 were correctly IDed as such by phenotypic methods, suggesting that the occurrence of this species in fungal infections is much lower than previously appreciated. V2 and molecular methods demonstrated agreement of only 56.6% when evaluating these problematic strains. The majority of strains categorized as CFAM by phenotypic methods were incorrect.

INTRODUCTION

Candida famata (formerly *Torulopsis candida*; teleomorph, *Debaryomyces hansenii*) is an ascomycetous yeast commonly found in foods, including dairy products. *C. famata* is a rare human pathogen that has been the putative etiologic agent in cases of bloodstream infections, peritonitis, ocular and bone infections. In the decade long ARTEMIS survey, *C. famata* was ranked 9th among 31 different species, accounting for 0.3% of 256,882 clinical isolates of *Candida*. Recent publications from reference laboratories have identified 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 methods. Such findings suggest that *C. famata* may be much less common as an etiologic agent of invasive candidiasis than previously reported. Our recent experience was similar in that five isolates originally identified as *C. famata* in the 2010 SENTRY Antifungal Surveillance Program were found by molecular methods to be three different species of *Candida* (*C. guilliermondii* [1], *C. lusitaniae* [1], and *C. parapsilosis* [3]), none of which were *C. famata*. With this background, we investigated the true prevalence of *C. famata* among 53 isolates originally submitted as such in two global surveillance programs, the ARTEMIS Program and the SENTRY Antimicrobial Surveillance Program for the time period from 2005 through 2011.

MATERIALS AND METHODS

Isolates. A total of 53 clinical isolates from individual patients previously identified as *C. famata* and submitted to the ARTEMIS (24 isolates) or the SENTRY (29 isolates) Programs were included in the study. The isolates represented 33 different study sites in 22 different countries; 34 were obtained from blood or deep tissue sites of infection.

Phenotypic characterization of isolates. Isolates were subcultured on CHROMagar (Becton-Dickinson and Company) and colony morphology and color were observed after 48h incubation at 35°C. Species identification was performed using the Vitek 2 yeast identification card (bioMérieux; Hazelwood, Missouri, USA) according to the manufacturer's instructions. Isolates were assessed for the presence or absence of pseudohyphae after growth for 24h of incubation at 35°C on corn meal agar (Remel; Lenexa, Kansas, USA).

Molecular identification. Isolates were submitted to DNA extractions using QIAquick Extraction kit (Qiagen, Hilden, Germany) in the QiaCube automated system (Qiagen). Amplification and sequencing of was performed for the intergenic transcribed spacer region (ITS) for all strains. Additionally, 28S ribosomal subunit (D1/D2) was used for a few strains and IGS was sequenced for *Debaryomyces* spp.. Amplicons were sequenced on both strands and compared with available reference sequences through the internet using a threshold for similarity >99.5%.

MALDI-TOF MS. All isolates were subjected to Matrix-Associated Laser Desorption Ionization-Time Of Flight mass spectrometry (MALDI-TOF MS) using the Bruker Daltonik MALDI Biotyper (Fremont, California, USA) by following the instructions of the manufacturer.

RESULTS

- Among the 53 isolates originally identified as *C. famata* by the submitting laboratory, only three (5.7%) were identified as such with confidence using the Vitek 2 yeast identification card (Table 1). These isolates were identified as *C. guilliermondii* (2) by ITS sequencing and MALDI-TOF (MALDI) and as *D. nepalensis* (1) using ITS (low discrimination among *D. hansenii* and *D. nepalensis*) and IGS sequencing. This isolate was not reliably identified by the MALDI Biotyper.
- The Vitek 2 most frequent ID 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 and none of the one *C. fermentati* (one was identified as *C. guilliermondii* with a low score value [1.70]).
- Among 14 isolates that were identified with high confidence as *C. parapsilosis* by the Vitek 2, 11 were confirmed by both ITS sequencing and MALDI. Two isolates were identified as the cryptic species *C. orthopsilosis* and *C. metapsilosis* (one each) by sequencing, but MALDI was only able to produce reliable results for *C. orthopsilosis*.
- 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 one *C. guilliermondii* 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 (95.0-98.5% 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* 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. One isolate was not reliably identified by the Vitek 2 and the MALDI and was identified as *Pichia fabianii* by ITS sequencing.
- The MALDI Biotyper system displayed 81.1% agreement with DNA sequencing whereas the Vitek 2 displayed only 56.6% agreement (Table 2).

Table 1. Identification by DNA sequencing, Vitek 2, MALDI-TOF MS and phenotypic methods for 53 clinical isolates sent to the ARTEMIS and/or SENTRY Surveillance Programs between 2005 and 2011 as *Candida famata*.

DNA Sequencing	VITEK 2	MALDI-TOF (score value [if <2.00])	Pseudohyphae	Country	SpecimenType
C. albicans	*C. albicans*	*C. albicans*	NT	Brazil	Blood
C. albicans	*C. albicans*	*C. albicans*	NT	Poland	Blood
C. albicans	*C. albicans*	*C. albicans*	NT	Italy	Unknown
C. albicans	*C. albicans*	*C. albicans*	pos	Germany	Blood
Pichia fabianii	Unidentified	No reliable ID	neg	Slovakia	Blood
C. fermentati	Low discrimination *C. famata/C. guilliermondii*	*C. guilliermondii* (1.70)	neg	United States	Blood
C. fermentati	Low discrimination *C. guilliermondii/C. famata*	No reliable ID	neg	Colombia	Blood
C. guilliermondii	Low discrimination *C. famata/C. guilliermondii*	*C. guilliermondii*	neg	Italy	Blood
C. guilliermondii	Low discrimination *C. famata/C. guilliermondii*	*C. guilliermondii*	pos	Mexico	Blood
C. guilliermondii	Low discrimination *C. famata/C. guilliermondii*	*C. guilliermondii*	neg	Turkey	Unknown
C. guilliermondii	Low discrimination *C. famata/C. guilliermondii*	*C. guilliermondii*	neg	Brazil	Blood
C. guilliermondii	Low discrimination *C. guilliermondii/C. famata*	*C. guilliermondii*	neg	United States	Blank
C. guilliermondii	Low discrimination *C. guilliermondii/C. famata*	*C. guilliermondii*	pos	United States	Broncho lavage
C. guilliermondii	Low discrimination *C. guilliermondii/C. famata*	No reliable ID	neg	Argentina	Blood
C. guilliermondii	Low discrimination *C. guilliermondii/C. famata*	*C. guilliermondii*	neg	France	Other
C. guilliermondii	Low discrimination *C. guilliermondii/C. famata*	*C. guilliermondii*	neg	United States	Blood
C. guilliermondii	Low discrimination *C. guilliermondii/C. famata*	*C. guilliermondii*	pos	Argentina	Blood
C. guilliermondii	Low discrimination *C. guilliermondii/C. famata*	*C. guilliermondii*	neg	Italy	Unknown
C. guilliermondii	*C. guilliermondii/C. famata*	*C. guilliermondii*	pos	Switzerland	Unknown
C. guilliermondii	*C. guilliermondii*	*C. guilliermondii*	pos	Brasil	Blood
C. guilliermondii	*C. guilliermondii*	*C. guilliermondii*	pos	Colombia	Urine
C. guilliermondii	*C. guilliermondii*	*C. guilliermondii*	pos	Australia	Blood
C. guilliermondii	*C. guilliermondii*	No reliable ID	neg	Argentina	Blood
C. guilliermondii	*C. guilliermondii*	*C. guilliermondii*	pos	Australia	Blood
C. guilliermondii	*C. famata*	*C. guilliermondii*	pos	South Korea	Blood
C. guilliermondii	*C. famata*	*C. guilliermondii*	pos	Germany	Blood
C. intermedia/C. pseudointermedia	*C. intermedia*	*C. intermedia* (1.97)	pos	China	Blood
C. lusitaniae	*C. lusitaniae*	*C. lusitaniae*	neg	Mexico	Unknown
C. lusitaniae	*C. lusitaniae*	*C. lusitaniae*	pos	Mexico	Unknown
C. lusitaniae	*C. lusitaniae*	*C. lusitaniae*	pos	Mexico	Unknown
C. lusitaniae	*C. lusitaniae*	*C. lusitaniae*	neg	France	Other
C. lusitaniae	*C. lusitaniae*	*C. lusitaniae*	neg	Hungary	Tissue
C. metapsilosis	*C. parapsilosis*	No reliable ID	pos	United States	Blood
C. orthopsilosis	*C. parapsilosis*	*C. orthopsilosis*	pos	Venezuela	Abscess
C. parapsilosis	Low discrimination *C. parapsilosis/C. famata*	*C. parapsilosis*	pos	South Africa	Blood
C. parapsilosis	*C. parapsilosis*	*C. parapsilosis*	pos	Mexico	Blood
C. parapsilosis	*C. parapsilosis*	*C. parapsilosis*	pos	Turkey	Unknown
C. parapsilosis	*C. parapsilosis*	*C. parapsilosis*	pos	Brazil	Blood
C. parapsilosis	*C. parapsilosis*	*C. parapsilosis*	pos	Mexico	Blood
C. parapsilosis	*C. parapsilosis*	*C. parapsilosis*	pos	Ireland	Unknown
C. parapsilosis	*C. parapsilosis*	*C. parapsilosis*	pos	Italy	Blood
C. parapsilosis	*C. parapsilosis*	*C. parapsilosis*	pos	Turkey	Unknown
C. parapsilosis	*C. parapsilosis*	*C. parapsilosis*	pos	Mexico	Unknown
C. parapsilosis	*C. parapsilosis*	*C. parapsilosis*	pos	Brazil	Unknown
C. parapsilosis	*C. parapsilosis*	*C. parapsilosis*	neg	Brazil	Blood
C. parapsilosis	*C. parapsilosis*	*C. parapsilosis*	pos	South Africa	Blood
C. pelliculosa	*C. pelliculosa*	*C. pelliculosa*	neg	South Korea	Blood
C. tropicalis	*C. tropicalis*	*C. tropicalis*	NT	USA	Blood
C. tropicalis	*C. tropicalis*	*C. tropicalis*	NT	Brazil	Tissue
C. tropicalis	Low discrimination *C. tropicalis/C. parapsilosis*	*C. tropicalis*	pos	Israel	Blood
Debaryomyces fabryi	*C. sphaerica*	No reliable ID	neg	France	Blood
Debaryomyces nepalensis	*C. famata*	No reliable ID	neg	China	Unknown
Kodamaea ohmeri	*Kodamaea ohmeri*	*C. guilliermondii* (1.78)	pos	Brazil	Unknown

a. NT = not tested

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 ID (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 ID (1)
C. parapsilosis (12)	Vitek 2	11 (91.7)	*C. famata/C. guilliermondii* 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 ID (1)
Pichia fabianii (1)	Vitek 2	0 (0.0)	Unidentified (1)
	MALDI	0 (0.0)	No reliable ID (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 ID (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 ID (1)
D. fabryi (1)	Vitek 2	0 (0.0)	*C. famata* (1)
	MALDI	0 (0.0)	No reliable ID (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)	

CONCLUSIONS

- *C. famata* appears to be far less common as a cause of infection 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 documented that a wide range of *Candida* species may be similarly misidentified.
- MALDI-TOF seems to be a rapid, accurate, and cost-effective identification system for both common and uncommon species of *Candida*. The present study confirms the capabilities of this technology against a very challenging collection of *Candida* species and in all instances a lack of result was given rather than an incorrect identification. Future supplementation of the existing database with additional strains of these rare species should result in even greater performance.

ACKNOWLEDGEMENTS

The antifungal global surveillance programs which served as the source of data used in the development of this manuscript was, in part, supported by Pfizer Inc. and Astellas Ltd. We would also like to acknowledge the contributions of the participants in the SENTRY Program.

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