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Three Clustered Cases of Candidemia Caused by *Candida quercitrusa* and Mycological Characteristics of This Novel Species

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We investigated three nosocomial *Candida quercitrusa* candidemia cases occurring within 2 months in a Chinese hospital. Isolates were identifiable only by DNA sequencing of the rRNA genes. Genetic (via random amplified polymorphic DNA [RAPD]) and protein mass spectral (via matrix-assisted laser desorption ionization–time of flight mass spectrometry [MALDI-TOF MS]) analyses yielded identical profiles suggesting an outbreak. The fluconazole MICs of all the strains were 16 to 32 μg/ml.

Candidemia in hospitalized patients causes substantial mortality and has high costs (1, 2). Some studies have suggested that up to one-third of cases may occur as nosocomial clusters (3, 4). Furthermore, uncommon or novel *Candida* species are increasingly recognized to cause candidemia (5–8). Here, we describe the clinical characteristics of three candidemia cases caused by the novel species *Candida quercitrusa*, which occurred within a 2-month period in a single hospital's intensive care unit (ICU) in Harbin, China (9).

Patient 1 was admitted to the hospital's ICU in May 2010 with acute upper gastrointestinal bleeding. On day 7 of the admission, peripheral blood cultures (Bactec Myco/F Lytic; Becton, Dickinson, Sparks, MD, USA) grew a strain identified as *Candida pulcherrima* (strain 10H1064), as identified by the Vitek 2 YST system (bioMérieux, Marcy l'Etoile, France) (identification accuracy, 89%) (Fig. 1A). Patient 2 presented to the same ICU in April 2010 with major trauma. On days 50 and 59 of the admission, blood cultures from a central venous catheter (CVC) grew a strain identified as *C. pulcherrima* (strain 10H1067, initially identified as for patient 1) (Fig. 1B). Patient 3 was admitted to the ICU in July 2010 with fever, seizures, and obtundation. A strain identified as *Candida lusitaniae* (strain 10H1076) by the API 20C AUX system (bioMérieux) (identification accuracy, 86.2%) was recovered from peripheral blood cultures on day 10 (Fig. 1C). Table 1 summarizes the clinical details of the three cases. Patients 2 and 3 had central lines *in situ* and were receiving total parenteral nutrition (TPN).

The isolates identified as *C. pulcherrima* and *C. lusitaniae* were forwarded to the reference mycology laboratory at the Peking Union Medical College Hospital for further mycological studies, including observing their appearance on chromogenic media and species identification by molecular-based approaches (see below). This practice was in place for all suspected yeast strains causing invasive infection, per protocol, for a national survey of invasive fungal infections in China (the China Hospital Invasive Fungal Surveillance Net [CHIF-NET] study) (9).

Specifically, DNA sequencing of the isolates was performed by amplifying the internal transcribed spacer (ITS) region and the D1/D2 domain of the rRNA gene as previously described (9, 10). A comparison of all available *C. quercitrusa* sequences, including those of the isolates from the current study, was then performed using the maximum-parsimony (MP) method (11). The isolates were also analyzed by matrix-assisted laser desorption ionization–time of flight mass spectroscopy (MALDI-TOF MS) using Bruker Biotyper version 3.1 software (Bruker Daltonics, Bremen, Germany), and protein profiles were further studied by use of main spectra projections (12). The primers RAPD24 and RAPD1283 were then used for random amplified polymorphic DNA (RAPD) analysis (13). *In vitro* susceptibility to amphotericin B, fluconazole, voriconazole, itraconazole, and caspofungin was determined by Clinical and Laboratory Standards Institute M27–A3 methodology (14).

On Sabouraud dextrose agar and chromogenic media, the isolates grew well at 25°C and 30°C. They grew slowly at 37°C over 5 days (cf. with the type strain *C. quercitrusa* CBS 4412, which failed to grow at 37°C) but did not grow at 42°C. The colonies were dark blue on CHROMagar *Candida* medium (CHROMagar Company, Paris, France) (Fig. 2A) and dark green on Brilliance *Candida* agar (Oxoid Ltd., Hampshire, United Kingdom) (Fig. 2B).

All patient isolates were identified as *C. quercitrusa* by querying their ITS region and D1/D2 domain sequences against the GenBank database (accessed 31 December 2013) (Table 2). The ITS and D1/D2 sequences of all three study isolates were identical and shared 98.9 and 99.8% sequence similarity, respectively, to those of *C. quercitrusa* type strain CBS 4412 (Table 2). The GenBank sequences of the remaining *C. quercitrusa* isolates also shared high sequence similarity (95.8 to 100%) with those of *C. quercitrusa* CBS 4412 (Table 2), but at least moderate intraspecies sequence variation was indicated within the ITS region and D1/D2 domain

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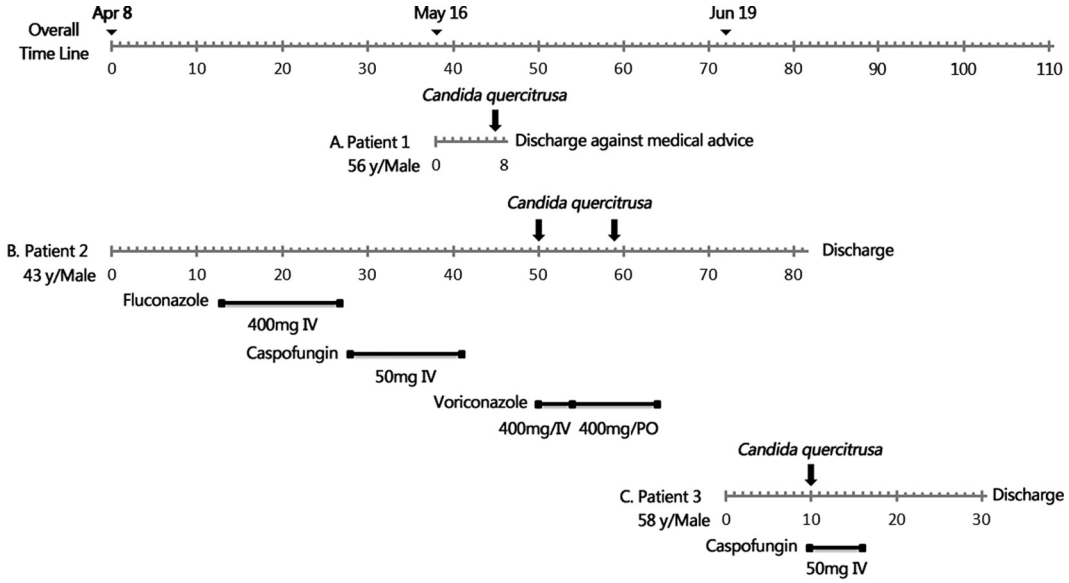

FIG 1 Clinical features, treatment regimens, and outcomes of patients with *Candida quercitrusa* candidemia. Time axis in each case (A, B, and C, respectively) indicates period of hospital stay, with numbers of days since the beginning of hospitalization. Lines with black squares, period and dose per day of antifungal treatment; down-facing black arrows, dates of positive *C. quercitrusa* blood culture.

for this species. MP analysis of the ITS region revealed genetic heterogeneity among the *C. quercitrusa* strains analyzed; there was clustering of the three patient isolates (strains 10H1064, 10H1967, and 10H1076), which were clearly separated from the nonclinical strains (Fig. 3). MALDI-TOF MS assigned no identification to the patient isolates (spectral score, <1.5). Their RAPD profiles were identical, as were their MALDI-TOF MS spectra (see Fig. S1A and S1B in the supplemental material).

TABLE 1 Clinical features of three patients with *Candida quercitrusa* candidemia

Clinical feature	Patient 1	Patient 2	Patient 3
Age (yr)	56	43	58
Gender	Male	Male	Male
Reason for hospital admission	Acute upper gastrointestinal bleeding, high fever	Fracture dislocation caused by a falling	Clonic spasm, high fever, meningitis
Underlying disease	Hypertension, coronary heart disease, diabetes	Aspiration pneumonia	Hypertension
Total no. of blood cultures taken	1	9	4
Date(s) (day/mo/yr) of *C. quercitrusa* isolation[a]	23/05/2010 (7)	06/06/2010 (50), 28/05/2010 (59)	29/06/2010 (10)
Clinical status at time of positive culture			
Immunosuppressive state	No	No	No
Neutropenia ($<10^9$ per liter)	No	No	No
Presence of CVC	No	Yes	Yes
Broad-spectrum antibiotics	Yes	Yes	Yes
Total parenteral nutrition	No	Yes	Yes
Surgery within 30 days	No	Yes	No
Intensive care	Yes	Yes	Yes
Previous antifungal agents within 30 days	No	Yes	No
Concomitant bacteremia	No	No	No
Concomitant candidemia	No	Yes (*Candida lipolytica*)	No
Indwelling urinary catheter	No	Yes	Yes
Therapy			
Antifungal	No antifungal therapy commenced	Voriconazole 400 mg daily, 14 days	Caspofungin 50 mg daily, 8 days
CVC removal[a]	Not applicable	Yes (64)	Yes (10)
Antifungal, after culture	Yes	Yes	Yes
Outcome	Unknown[b]	Recovered	Recovered

[a] Numbers in parentheses indicate days since beginning of hospitalization.
[b] Patient discharged against medical advice and was lost to further follow-up.

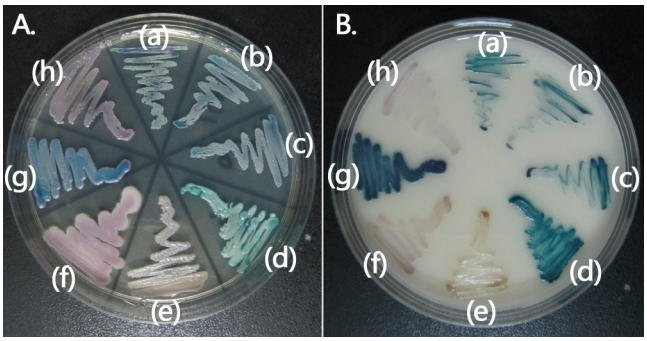

FIG 2 Phenotypic characteristics of *C. quercitrusa* isolates on CHROMagar *Candida* medium (A) and Brilliance *Candida* agar (B). (a) *C. quercitrusa* 10H1064; (b) *C. quercitrusa* 10H1067; (c) *C. quercitrusa* 10H1076; (d) *C. albicans* ATCC 90028; (e) *Candida parapsilosis sensu stricto* ATCC 22019; (f) *C. krusei* ATCC 6258; (g) *C. tropicalis* 10H1048; and (h) *C. glabrata sensu stricto* 10H1043.

All the isolates had fluconazole MICs of 16 to 32 μg/ml. The MIC ranges for itraconazole and voriconazole were 0.25 to 0.5 and 0.125 to 0.25 μg/ml, respectively. The MICs for caspofungin and amphotericin B were low (MIC ranges, 0.5 to 1 and ≤0.5 μg/ml, respectively).

This report details, for the first time, candidemia due to the novel species *C. quercitrusa* in three Chinese patients identified through surveillance (9). Although *C. quercitrusa* isolates have been recovered from plant, water, and insect material in a number of countries (15–18), the species has not been reported to cause human infection (Table 2). The cases herein are also notable for their clustering in the same hospital within only a 2-month period.

Of interest, an analysis of the ITS and D1/D2 region sequences of the available *C. quercitrusa* isolates in the GenBank database to date suggests that this species may be genetically diverse, with intraspecies sequence heterogeneity in these gene regions (Table 2). However, the ITS and D1/D2 sequences of the patient isolates

TABLE 2 Summary of *C. quercitrusa* isolates from published literature or GenBank

Strain	Country	Origin	Reference or source	ITS		D1/D2[a]	
				Accession no.	Identity[b]	Accession no.	Identity[b]
Type strain CBS 4412	Australia	Insect	22	AM158924	Reference	U45831	Reference
Clinical isolates							
10H1064	Mainland China	Human blood	This study	KF220648	625/632 (98.9)	KF220651	564/565 (99.8)
10H1067	Mainland China	Human blood	This study	KF220649	625/632 (98.9)	KF220652	564/565 (99.8)
10H1076	Mainland China	Human blood	This study	KF220650	625/632 (98.9)	KF220653	564/565 (99.8)
Isolates from other sources							
BJ50	Mainland China	Apple orchard	Unpublished	NA[c]	NA	JQ219335	565/565 (100)
JM36	Mainland China	Coffee	Unpublished	NA	NA	KC510074	548/551 (99.4)
LYSJLFL-1	Mainland China	Food	Unpublished	NA	NA	JX049432	554/560 (98.9)
JHSb	Mainland China	Marine	Unpublished	DQ665264	566/591 (95.8)	EF375703	555/566 (98.1)
PH-M32	Mainland China	Wastewater	Unpublished	NA	NA	GU373802	548/551 (99.4)
TA256	Mainland China	Apple orchard	Unpublished	NA	NA	JQ219336	565/565 (100)
WG1	Mainland China	Wine grape	17	GU237045	537/558 (96.2)	NA	NA
339	Taiwan	Plant	Unpublished	NA	NA	JN544056	565/565 (100)
NU9L75	Taiwan	Leaf	Unpublished	NA	NA	HM461728	537/538 (99.8)
NRRL Y-27941	America	Insect	18	NA	NA	DQ655691	543/544 (99.8)
B176	America	Sugar beet root	Unpublished	NA	NA	EU196383	294/294 (100)
UNC MB27	America	Flower	15	NA	NA	JN642539	473/474 (99.8)
HA 1669	Australia	Insect	Unpublished	AM160627	606/606 (100)	AM160627	565/565 (100)
N17	Brazil	Grape	16	GQ999840	457/465 (98.3)	NA	NA
17a/3	Germany	Root tip	Unpublished	HQ680959	443/445 (99.6)	NA	NA
129	Ghana	Cocoa bean	23	NA	NA	AY529522	561/563 (99.6)
G4	Ghana	Cocoa bean	24	NA	NA	DQ466526	555/556 (99.8)
NCL 6	India	Grape	25	FJ231428	538/549 (98.0)	NA	NA
3.1	Italy	Beverage	Unpublished	NA	NA	JN417623	503/503 (100)
EGV28	Mexico	Zea mays	Unpublished	JX455761	566/582 (97.2)	NA	NA
EGV70	Mexico	Zea mays	Unpublished	JX455759	518/523 (99.0)	NA	NA
DMKU-RK1	Thailand	Rice/Corn/Sugarcane	Unpublished	NA	NA	AB773291	562/567 (99.1)
DMKU-RK14	Thailand	Rice/Corn/Sugarcane	Unpublished	NA	NA	AB772038	564/565 (99.8)
DMKU-RK2	Thailand	Rice/Corn/Sugarcane	Unpublished	NA	NA	AB773292	564/565 (99.8)
DMKU-RK4	Thailand	Rice/Corn/Sugarcane	Unpublished	NA	NA	AB773294	562/567 (99.1)
DMKU-RK5	Thailand	Rice/Corn/Sugarcane	Unpublished	NA	NA	AB773295	563/565 (99.6)
DMKU-RK504	Thailand	Rice/Corn/Sugarcane	Unpublished	NA	NA	AB773375	562/567 (99.1)
DMKU-RK506	Thailand	Rice/Corn/Sugarcane	Unpublished	NA	NA	AB773377	539/546 (98.7)
DMKU-RK516	Thailand	Rice/Corn/Sugarcane	Unpublished	NA	NA	AB773384	562/567 (99.1)
EC4	Thailand	Water	Unpublished	NA	NA	AB436403	564/565 (99.8)

[a] D1/D2, D1/D2 domain of the 26S ribosomal DNA.
[b] Number of nucleotides identical/number of nucleotides compared (%) between the indicated *C. quercitrusa* strain and type strain CBS 4412.
[c] NA, not available.

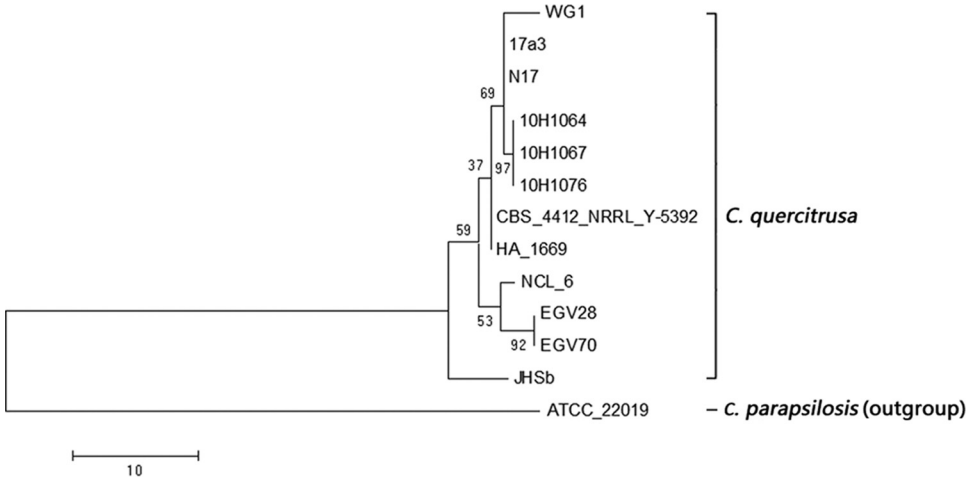

FIG 3 The maximum-parsimony tree generated from *C. quercitrusa* ITS sequences available in GenBank (see Table 2), with *C. parapsilosis* ATCC 22019 (GenBank accession no. FJ872015) as outgroup.

in the present report were identical. Taken together with their identical RAPD and MALDI-TOF MS protein profiles, the findings suggest that the isolates may have originated from a common source. Furthermore, MP analysis of their ITS sequences indicated that they are genetically distinct from previously isolated strains from nonclinical sources. A limitation of the present study is that it was performed retrospectively. Given the initial misidentification of the isolates and the resultant delay in accurate species identification, we were consequently unable to investigate for a possible environmental source or the potential for human-to-human transmission, including that through hospital staff. Although definite clustering of cases cannot be confirmed, the proximity in time and close similarity of the genetic and protein profiles suggest a minioutbreak.

The transmission of infection through the contamination of blood culture vials or of intravenous preparations such as TPN bags remains another possibility, suggesting a pseudo outbreak (19). The TPN bags were not available for culture. However, as not all the patients received TPN, this was unlikely to be the source. The blood culture bottles were of two different batches (lot 9320384 for patients 1 and 2, lot 0055634 for patient 3). There were no other patients with *Candida* in their blood at the time that our patients were ill with candidemia.

Note that phenotypic identification methods, including the use of chromogenic media, failed to correctly identify this species. MALDI-TOF MS, while not being able to assign species, did not misidentify the organism. Analyses of spectra from other clinical isolates are important in creating a robust spectral library for this species (20, 21). The fact that DNA sequencing of the ITS region and/or the D1/D2 domain remains necessary for species identification emphasizes the need to be vigilant for unusual species assignments by standard identification methods.

Curiously, all three *C. quercitrusa* strains grew very slowly at 37°C yet were able to cause candidemia in humans. The fungal load necessary to result in clinical infection is unknown. If infection is hypothesized to originate from contaminated intravenous fluids/materials, it is possible that a relatively large fungal load was inoculated into the bloodstream and resulted in candidemia.

The relatively high MICs (16 to 32 μg/ml) to fluconazole suggest that this antifungal agent should not be used to treat *C. quercitrusa* infections. However, the species was more susceptible to amphotericin B, the newer azoles, and caspofungin. Patients 2 and 3 recovered with no relapse after receiving voriconazole and caspofungin therapy, respectively.

In conclusion, we detail *C. quercitrusa* as a human pathogen, for the first time, with a clustering of three *C. quercitrusa* candidemia cases in a single ICU. Molecular methods are needed for the accurate identification of this species. The species is less susceptible to fluconazole.

Nucleotide sequence accession numbers. Nucleotide sequences for the ITS regions and D1/D2 domains of *C. quercitrusa* 10H1064, *C. quercitrusa* 10H1067, and *C. quercitrusa* 10H1076 were deposited in GenBank under accession numbers KF220648 to KF220653.

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We have no conflicts of interest to declare.

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