Title	Clinical characteristics, laboratory identification, and in vitro antifungal susceptibility of Yarrowia (Candida) lipolytica isolates causing fungemia: a multicenter, prospective surveillance study
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Citation	Journal of Clinical Microbiology, 2015, v. 53 n. 11, p. 3639-3645
Issued Date	2015
URL	http://hdl.handle.net/10722/223327
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Clinical Characteristics, Laboratory Identification, and *In Vitro* Antifungal Susceptibility of *Yarrowia* (*Candida*) *lipolytica* Isolates Causing Fungemia: a Multicenter, Prospective Surveillance Study

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Our case series showed that uncomplicated *Yarrowia lipolytica* fungemia might be treated with catheter removal alone. The Vitek 2 YST identification (ID) card system, matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), and internal transcribed spacer and 25S nuclear ribosomal DNA (nrDNA) gene sequencing provided reliable identification. All isolates had low MICs to voriconazole, echinocandins, and amphotericin B.

A growing number of non-albicans *Candida* spp. have emerged in recent years as important human pathogens among the growing, heterogeneous population of immunocompromised and critically ill patients (1–3). *Yarrowia lipolytica* (*Candida lipolytica*) is an ascomycetous yeast found ubiquitously in the environment and meat products, including sausages and dairy products, especially cheese (4). It may occasionally be found as a colonizer in the feces, oropharyngeal swabs, sputa, and skin swabs of asymptomatic persons (5). Its intense secretory activity is widely employed in the food, detergent, and pharmaceutical industries to produce aroma compounds, organic acids, polyalcohols, emulsifiers, and surfactants (4, 6, 7). Although *Y. lipolytica* was previously considered to be of low virulence, it has been increasingly recognized to cause sporadic cases and nosocomial clusters of human infections, especially catheter-related suppurative thrombophlebitis and fungemia associated with biofilm formation in immunocompromised or critically ill patients who experience prolonged hospitalization and who required long-dwelling intravascular catheterization and broad-spectrum antibacterial agents (5, 8–10). Other forms of clinical disease, including noncatheter-related fungemia, traumatic ocular infection, and acute exacerbation of chronic sinusitis, have also been reported (5, 8, 11). A major limitation of the literature on *Y. lipolytica* infection is that most studies were case reports or small case series focusing only on clinical characteristics (5, 8–17). Recently, Trabelsi and colleagues reported the epidemiological risk factors and clinical outcomes of 55 cases of *Y. lipolytica* fungemia in Tunisia and provided some data on the *in vitro* susceptibility test results of the isolates to a few antifungal drugs (6). However, details about the correlative microbiological characteristics, including phenotypic and genotypic identification and *in vitro* susceptibility test results for newer antifungal drugs such as the echinocandins and posaconazole, were lacking in this larger case series. In this multicenter, prospective surveillance study in China, we described the epidemiological and clinical characteristics of 14 cases of *Y. lipolytica* fungemia in correlation with the comparative performance of several commonly employed phenotypic and genotypic identification methods, and we also described the *in vitro* susceptibility of this emerging fungal pathogen to nine antifungal drugs, including the newer azoles and echinocandins.

This study was approved by the Institutional Review Boards of The University of Hong Kong/Hospital Authority Hong Kong West Cluster and the Peking Union Medical College Hospital. As described previously, the National China Hospital Invasive Fungal Surveillance Net (CHIF-NET) study was a prospective, laboratory-based, multicenter study of invasive yeast infections that started on 1 August 2009 (18). The present study included all of the *Y. lipolytica* blood culture isolates identified in the CHIF-NET study from 1 August 2009 to 31 July 2012. These isolates were identified as *Y. lipolytica* using the Vitek 2 YST identification (ID) card system (bioMérieux, France) as previously described (19). For isolates that had a single identification with a confidence value of <99.9%, multiple identifications, or no identification, internal transcribed spacer (ITS) region sequencing was used for confirmation (19). Additionally, we compared the performance of the API 20C Aux system (bioMérieux) and matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) for the identification of *Y. lipolytica* with that of the Vitek 2 YST ID Card system (see the supplemental material).

Received 21 July 2015 **Returned for modification** 6 August 2015
Accepted 24 August 2015

Accepted manuscript posted online 26 August 2015

Citation Zhao Y, Chan JF-W, Tsang C-C, Wang H, Guo D, Pan Y, Xiao Y, Yue N, Chen JH-K, Lau SK-P, Xu Y, Woo PC-Y. 2015. Clinical characteristics, laboratory identification, and *in vitro* antifungal susceptibility of *Yarrowia* (*Candida*) *lipolytica* isolates causing fungemia: a multicenter, prospective surveillance study. J Clin Microbiol 53:3639–3645. doi:10.1128/JCM.01985-15.

Editor: D. W. Warnock

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Supplemental material for this article may be found at http://dx.doi.org/10.1128/JCM.01985-15.

TABLE 1 Clinical characteristics of 13 patients with *Yarrowia lipolytica* fungemia[a]

Case	Isolate	Sex/Age (yr)	Reason for admission (comorbidities)	i.v. catheters (days)[b]	i.v. antibiotics	ICU	Specimen(s)	Treatment	Outcome
1	PW3298	M/65	Road traffic accident with traumatic subdural hematoma, multiple fractures, and gastrectomy	PVC (9)	Yes	No	Blood (peripheral)	Catheter removal and i.v. fluconazole 200 mg daily	Discharged after 14 days of hospitalization
2	PW3299	M/46	Postoperative fever (CRHD with valvular replacement)	PVC (11)	Yes	Yes	Blood (peripheral)	Catheter removal	Discharged after 20 days of hospitalization
3	PW3300	M/27	Fever with hypotension (cervical spinal fracture with paraplegia)	PICC (44)	Yes	Yes	Blood (PICC)	Catheter removal	Transferred to another hospital after 55 days of hospitalization
4	PW3301	M/67	Ischemic stroke with hemorrhagic transformation (ESRF)	CVC (7)	Yes	Yes	Blood (peripheral and CVC)	Catheter removal and i.v. itraconazole 350 mg daily	Discharged after 30 days of hospitalization
5	PW3302	M/73	Recurrent peritonitis (carcinoma of pancreas)	CVC (40)	Yes	Yes	Blood (peripheral and CVC)	Catheter removal and i.v. amphotericin B 25 mg	Discharged against medical advice after 45 days of hospitalization
6	PW3303	M/1	Apnea of prematurity	PVC (15)	Yes	No	Blood (peripheral)	Catheter removal	Discharged after 16 days of hospitalization
7	PW3304	M/3	Respiratory distress (congenital heart disease)	CVC (75)	Yes	Yes	Blood (peripheral and CVC)	Catheter removal and oral fluconazole 150 mg daily for 12 days	Discharged after 4 mo of hospitalization
8	PW3305	M/63	Bronchiectactic exacerbation (bronchiectasis)	PVC (6)	Yes	Yes	Blood (peripheral)	Catheter removal and i.v. fluconazole 400 mg daily for 5 days	Died after 10 days of hospitalization
9	PW3306 and PW3307	M/75	Intracerebral hemorrhage	CVC (30)	Yes	Yes	Blood (peripheral and CVC, respectively)	Catheter removal and i.v. fluconazole 400 mg daily for 14 days	Died after 35 days of hospitalization
10	PW3309	M/43	Pneumonia (brainstem death)	PVC (8)	Yes	No	Blood (peripheral)	Catheter removal	Discharged after 10 days of hospitalization
11	PW3310	M/45	Intestinal obstruction	CVC (38)	Yes	Yes	Blood (peripheral and CVC)	Catheter removal and i.v. fluconazole 400 mg daily for 27 days	Discharged after 49 days of hospitalization
12	PW3311	M/73	Open skull fracture	PVC (31)	Yes	Yes	Blood (peripheral)	Catheter removal and i.v. fluconazole 200 mg daily for 37 days	Discharged after 2 mo of hospitalization
13	PW3312	F/82	Right hip fracture	CVC (38)	Yes	Yes	Blood (peripheral and CVC)	Catheter removal and i.v. fluconazole 400 mg daily for 40 days	Died after 45 days of hospitalization

[a] The clinical data of 1 of the 14 cases in this study was not available. CRHD, chronic rheumatic heart disease; CVC, central venous catheter; ESRF, end-stage renal failure; F, female; i.v., intravenous; ICU, intensive care unit; M, male; PVC, peripheral venous catheter; PICC, peripherally inserted central catheter.

[b] Duration of intravenous catheter *in situ* before onset of fungemia.

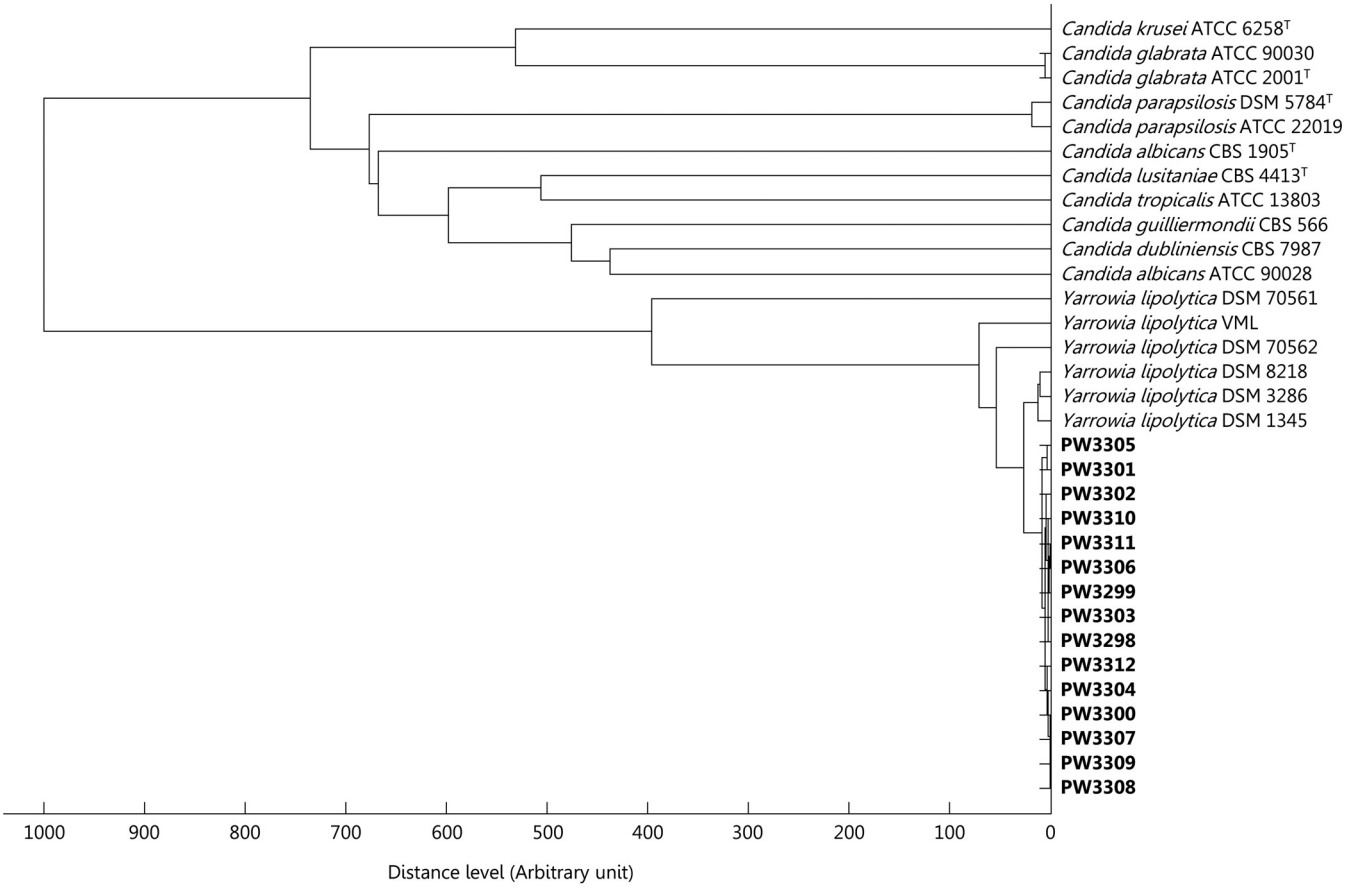

FIG 1 Dendrogram generated from the hierarchical cluster analysis (HCA) of the MALDI-TOF mass spectra of the case isolates, *Y. lipolytica*, and other *Candida* spp. showing their relative relatedness.

A total of 14 cases of *Y. lipolytica* fungemia were identified during the study period. The clinical characteristics of 13 patients with available information are listed in Table 1. One patient (case 9) had two *Y. lipolytica* isolates identified from the blood cultures obtained from the peripheral vein and central venous catheter simultaneously. Twelve (92.3%) were males. Their median age was 63 years (range, 1 to 82 years). Similar to previous reports, all had risk factors for *Y. lipolytica* fungemia, including prior use of broad-spectrum antibacterials (100.0%), prolonged hospitalization and admission to the intensive care unit (84.6%), and intravascular catheterization (100.0%). Seven (53.8%) had a central venous catheter. The time interval between the setup of the intravenous catheter and the onset of fungemia was longer in those with central venous catheters (mean, 38.9 days) than those with peripheral venous catheters (mean, 13.3 days). All patients had catheter removal after *Y. lipolytica* was isolated in blood culture, but the use of antifungal treatment was variable. Four (30.8%) did not receive any antifungal treatment, but they all survived after catheter removal. The remaining nine (69.2%) received systemic antifungal treatment, including fluconazole, itraconazole, and amphotericin B for a range of 5 to 40 days. Three (23.1%) died during hospitalization.

A major knowledge gap in the literature on *Y. lipolytica* catheter-related fungemia is the optimal management strategy. While the clinical practice guideline for the management of candidiasis by the Infectious Diseases Society of America recommends the use of systemic antifungal treatment and catheter removal, some have suggested that catheter removal alone might be sufficient in treating *Y. lipolytica* catheter-related infection (5, 14, 20). Catheter removal without systemic antifungal treatment has rendered the blood culture negative in some cases of *Y. lipolytica* catheter-related fungemia, including those involving neutropenic patients (5, 14). *Y. lipolytica*-associated suppurative thrombophlebitis may also be successfully treated with catheter removal alone (5). Nearly one-third (cases 2, 3, 6, and 10) of our patients did not receive systemic antifungal treatment, but their fungemia resolved and their clinical conditions improved after catheter removal. Our data suggested that *Y. lipolytica* catheter-related infection may be treated with catheter removal without systemic antifungal therapy, provided that there is clinical improvement with resolution of systemic and/or local symptoms and signs, microbiological documentation of clearance of fungemia, and an absence of persistent infective foci such as endocarditis, abscess, and endophthalmitis.

Rapid and accurate identification is especially important for *Y. lipolytica*, as the need and choice of systemic antifungal treatment may differ between infections caused by *Y. lipolytica* and other *Candida* spp. The Vitek 2 YST ID Card system identified all 15 (100.0%) isolates as *Y. lipolytica* with probabilities of 94.0% to 99.0% (see Table S1 in the supplemental material). None of the isolates were identified as *Y. lipolytica* by the API 20C Aux system.

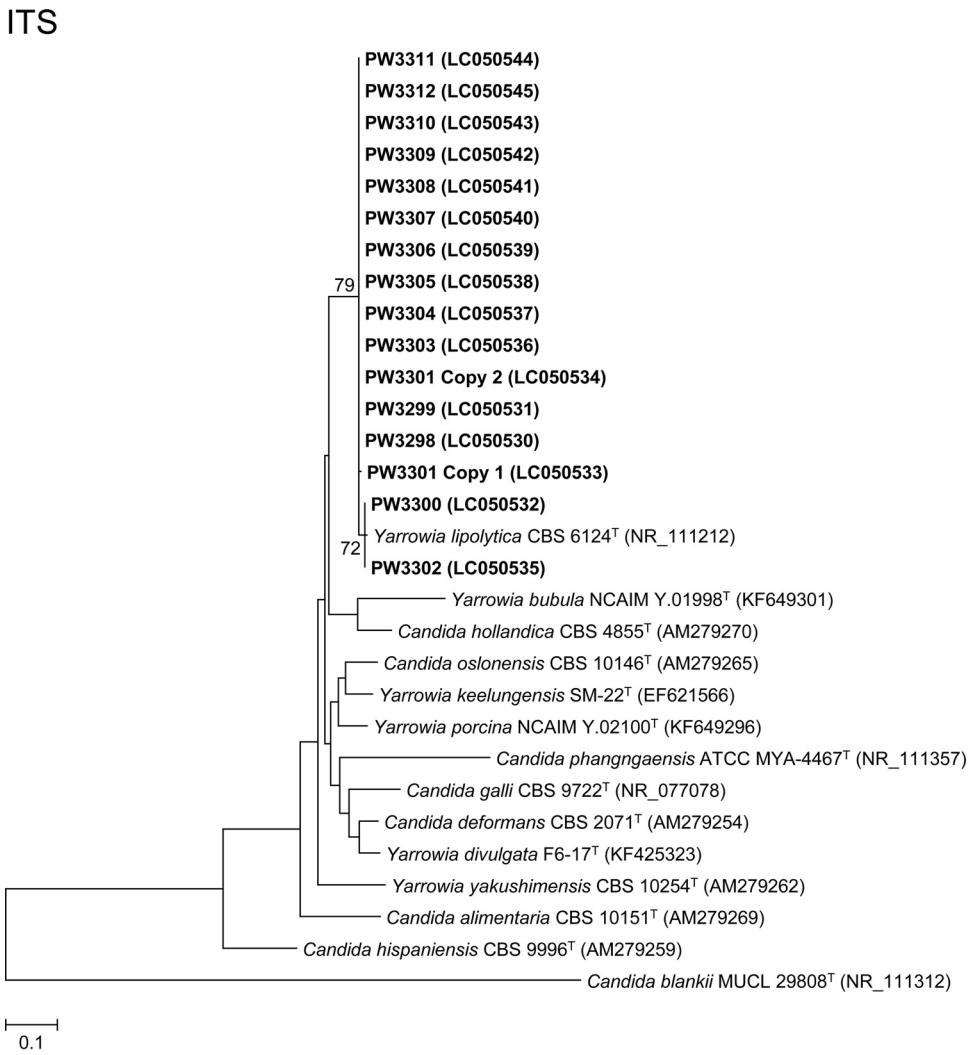

FIG 2 Phylogenetic trees showing the relationship between the 15 fungal isolates and other members of the *Yarrowia* clade. The trees were inferred from ITS and 25S nrDNA sequence data by the maximum likelihood method with the substitution models T92 (Tamura 3-parameter model) + G (gamma-distributed rate variation) and K2 (Kimura 2-parameter model) + G, respectively. The numbers of nucleotide positions of the trimmed, aligned sequences included for phylogenetic analyses are 293 and 488, respectively. The trees were rooted using *Candida blankii* strains MUCL 29808^T and NRRL Y-17068^T, respectively. The scale bars indicate the estimated numbers of substitutions per base. The numbers at the nodes, expressed in percentages, indicate levels of bootstrap support calculated from 1,000 trees, and bootstrap values lower than 70 are not shown. All accession numbers (in parentheses) are given as cited in the DDBJ/ENA/GenBank databases. The case isolates reported in this study are highlighted in bold type.

Nine (60.0%) were unidentifiable and the other six (40.0%) were misidentified as *Candida magnoliae*, *Candida zeylanoides*, *Candida dubliniensis*, or *Sporobolomyces salmonicolor* with probabilities of 42.9% to 99.5%. In contrast, MALDI-TOF MS accurately identified all (100.0%) isolates as *Y. lipolytica* with top match scores of 1.884 to 2.250. Hierarchical cluster analysis of the MALDI-TOF mass spectra of all 15 isolates also unambiguously showed that they were clustered with other *Y. lipolytica* isolates (Fig. 1).

For phylogenetic analysis, DNA extraction, PCR amplification, and DNA sequencing of the ITS region for the isolates were performed according to our previous publications (21–28) using the primer pairs ITS1/ITS4 (29) for the ITS and NL1/NL4 (30) for the 25S nuclear ribosomal DNA (nrDNA) (see the supplemental material). ITS and partial 25S nrDNA gene sequencing identified 14 and all 15 isolates as *Y. lipolytica*, respectively, confirming the results of the Vitek 2 YST ID card system and MALDI-TOF MS. Since the electropherogram obtained from direct PCR product sequencing for the ITS of isolate PW3301 was uninterpretable where double or multiple peaks were observed frequently in the sequence trace, the PCR product of the ITS of isolate PW3301 was cloned into plasmids for another sequencing trial (see the supplemental material). Two of the four clones selected for sequencing showed one ITS copy while the other two clones showed another ITS copy, indicating the presence of intrastrain ITS heterogeneity in the isolate PW3301. Pairwise alignment of the two ITS copies of PW3301 showed that they differed by a single nucleotide insertion of an A residue at the ITS2 region. This is the first report of intrastrain ITS heterogeneity in *Y. lipolytica*. Pairwise alignment also showed that the ITS and 25S nrDNA sequences of the 15 isolates possessed 98.3% to 99.3% and 99.8% to 100% identities to that of *Y. lipolytica* strain CBS 6124^T, respectively. Phylogenetic analyses

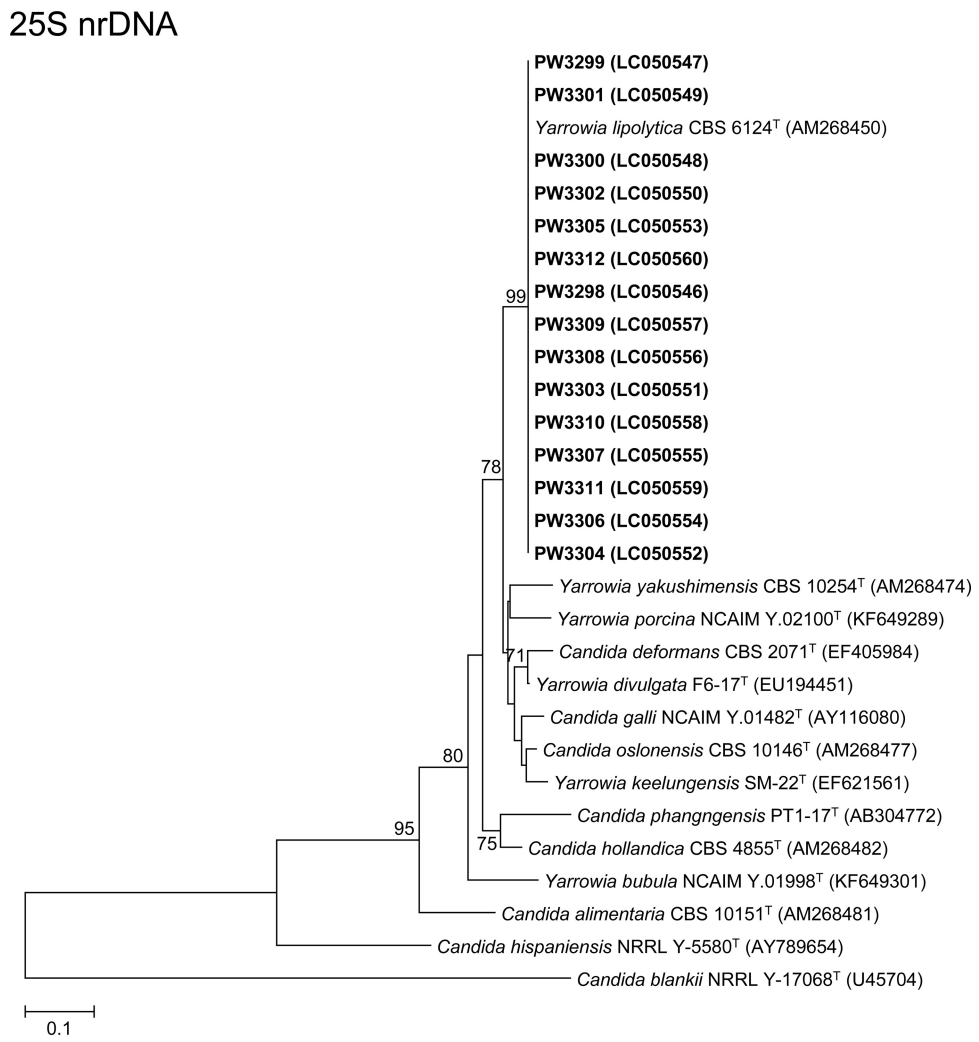

FIG 2 continued

based on the ITS and partial 25S nrDNA sequences showed that all 15 isolates were clustered with *Y. lipolytica* CBS 6124^T (Fig. 2). The low intraspecies sequence variability for ITS and 25S nrDNA for *Y. lipolytica* makes these two gene loci good DNA markers for the identification of this fungus. In addition, ITS and 25S nrDNA sequencing confirmed that isolates PW3306 and PW3307 obtained from the same patient (case 9) were identical (100% sequence identities for both loci).

The susceptibilities of the isolates to nine different antifungal drugs were tested by the broth microdilution method using Sensititre YeastOne plates (Trek Diagnostic Systems, United Kingdom) according to the manufacturer's instructions for *Candida* spp. and Pfaller et al. (31). The MIC values and ranges were calculated using WHONET 5.6 (World Health Organization Collaborating Center for Surveillance of Antibiotic Resistance, Boston, MA, USA). The susceptibilities of the isolates to fluconazole and voriconazole were also evaluated using Sensi-Disc antimicrobial susceptibility test disks (BBL; BD Diagnostic Systems, Sparks, MD, USA) by the disk diffusion method according to the Clinical and Laboratory Standards Institute M44-A2 standard (32). We performed antimicrobial susceptibility tests for only one of the two isolates obtained in case 9 because they were identified to be the same strain. All 14 (100.0%) *Y. lipolytica* isolates had MICs of ≤2.00 μg/ml to voriconazole by the broth microdilution method (Table 2). The MICs to the other azoles were less consistent, with many of them having MICs of ≥4.00 μg/ml to fluconazole, itraconazole, or posaconazole by the broth microdilution method. The broth microdilution and disk diffusion methods demonstrated that MICs and zone sizes were in general agreement with each other for voriconazole and fluconazole, with the isolates having lower MICs in the broth microdilution method also showing larger zone sizes in the disk diffusion method. As for the other antifungal agents, all 14 (100.0%) isolates had MICs of ≤2.00 μg/ml to caspofungin, micafungin, anidulafungin, and amphotericin B, whereas 10/14 (71.4%) isolates had MICs of ≥4.00 μg/ml to flucytosine.

Fluconazole was the most commonly used antifungal drug in the nine patients who received systemic antifungal treatment. This was based on previous observations that most *Y. lipolytica* isolates were susceptible to fluconazole *in vitro* (6, 8, 10). However, three of our patients (cases 8, 9, and 13) who received fluconazole died despite treatment and catheter removal. Their blood culture iso-

TABLE 2 *In vitro* antifungal susceptibilities of *Yarrowia lipolytica* isolates in this study

Isolate	MIC (μg/ml)[a] for:									Disk diffusion zone diameter (mm)	
	Azoles				Echinocandins			Others			
	FZ	IZ	VOR	PZ	CAS	MF	AND	AB	5-FC	FZ	VOR
PW3298	4	0.25	0.03	0.50	1	1	0.50	1	16	20	25
PW3299	4	0.50	0.06	0.50	0.25	0.12	0.03	1	4	20	28
PW3300	4	0.12	0.03	0.50	0.50	0.50	0.12	1	8	20	26
PW3301	16	0.50	0.25	0.03	0.02	0.03	0.03	0.25	0.12	6	6
PW3302	0.50	0.06	0.03	0.12	0.06	0.12	0.03	0.50	4	21	25
PW3303	2	0.25	0.03	0.50	0.50	0.50	0.12	0.50	8	25	30
PW3304	16	0.50	0.25	1	0.50	0.50	0.50	2	16	13	30
PW3305	>256	2	2	2	0.50	0.50	0.12	1	16	6	6
PW3307	64	1	1	2	0.25	0.50	0.25	0.25	0.50	6	6
PW3308	128	>16	2	>8	0.50	1	0.12	0.50	4	6	6
PW3309	128	>16	2	>8	0.50	1	0.12	0.50	8	6	6
PW3310	128	>16	2	>8	0.50	1	0.25	0.50	4	6	6
PW3311	64	1	1	2	0.25	0.25	0.12	0.25	0.50	6	6
PW3312	64	1	1	2	0.12	0.25	0.12	0.25	0.50	6	6
MIC data											
MIC range	0.50–>256	0.06–>16	0.03–2	0.03–>8	0.02–1	0.03–1	0.03–0.50	0.25–2	0.12–16		
MIC_{50}	16	0.50	0.25	1	0.50	0.50	0.12	0.50	4		
MIC_{90}	128	>16	2	>8	0.50	1	0.50	1	16		

[a] 5-FC, flucytosine; AB, amphotericin B; AND, anidulafungin; CAS, caspofungin; FZ, fluconazole; IZ, itraconazole; MF, micafungin; PZ, posaconazole; VOR, voriconazole.

lates had high MICs of 32 to >256 μg/ml to fluconazole. Importantly, all of these isolates also had MICs of ≥1 μg/ml to itraconazole and ≥2 μg/ml to posaconazole (Table 2). Thus, high MICs to fluconazole might help to predict high MICs to itraconazole and posaconazole, and infections caused by *Y. lipolytica* isolates with high MICs to fluconazole should not be treated with itraconazole and posaconazole. Our *in vitro* antifungal susceptibility data showed that voriconazole, caspofungin, micafungin, anidulafungin, and amphotericin B may be better treatment options.

Our study highlighted the need to establish standardized recommendations for determining *in vitro* antifungal susceptibility for *Y. lipolytica* using different testing methods. The *in vitro* susceptibility to fluconazole and voriconazole by the broth microdilution and disk diffusion methods differed markedly when the recommended MICs and zone sizes for other *Candida* spp. were used for interpretation. *Y. lipolytica* isolates with MICs of ≥16 μg/ml and ≥1 μg/ml against fluconazole and voriconazole, respectively, had zone sizes corresponding to the resistant category by the disk diffusion method. Furthermore, there are as yet no interpretative criteria for posaconazole and amphotericin B for *Candida* spp., although in general, an MIC of ≤1 μg/ml is considered to inhibit most clinical isolates (33). The *in vitro* antifungal susceptibility of more clinical isolates of *Y. lipolytica* with correlation to the patients' clinical response should be evaluated to determine the optimal breakpoints of this emerging fungal pathogen.

Nucleotide sequence accession numbers.

The ITS and partial 25S nrDNA sequences of the 15 *Y. lipolytica* isolates have been deposited in the DDBJ/ENA/GenBank databases with the accession numbers LC050530 to LC050560.

ACKNOWLEDGMENTS

This work is partly supported by the University Development Fund, the Committee for Research and Conference Grant, Cheng Yu Tung Fellowship, Mary Sun Medical Scholarship, Wong Ching Yee Medical Postgraduate Scholarship, and the University Postgraduate Scholarship, The University of Hong Kong, Hong Kong, the Croucher Senior Medical Research Fellowship, Croucher Foundation, Hong Kong, and by the donations of Larry Chi-Kin Yung and the Hui Hoy and Chow Sin Lan Charity Fund Limited. The CHIF-NET surveillance study is supported by an Investigator-Initiated Research Grant from Pfizer Corporation China. Jasper F. W. Chan has received travel grants from the Pfizer Corporation Hong Kong and Astellas Pharma Hong Kong Corporation Limited. The funding sources had no role in study design, data collection, analysis, interpretation, or writing of the report.

The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

We declare no conflicts of interest.

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