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Identification and Antifungal Susceptibility of Penicillium-Like Fungi from Clinical Samples in the United States

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Penicillium **species are some of the most common fungi observed worldwide and have an important economic impact as well as being occasional agents of human and animal mycoses. A total of 118 isolates thought to belong to the genus *Penicillium* based on morphological features were obtained from the Fungus Testing Laboratory at the University of Texas Health Science Center in San Antonio (United States). The isolates were studied phenotypically using standard growth conditions. Molecular identification was made using two genetic markers, the internal transcribed spacer (ITS) and a fragment of the β-tubulin gene. In order to assess phylogenetic relationships, maximum likelihood and Bayesian inference assessments were used. Antifungal susceptibility testing was performed according to CLSI document M38-A2 for nine antifungal drugs. The isolates were identified within three genera, i.e., *Penicillium*, *Talaromyces*, and *Rasamsonia*. The most frequent species in our study were *Penicillium rubens*, *P. citrinum*, and *Talaromyces amestolkiae*. The potent *in vitro* activity of amphotericin B (AMB) and terbinafine (TRB) and of the echinocandins against *Penicillium* and *Talaromyces* species might offer a good therapeutic alternative for the treatment of infections caused by these fungi.**

Penicillium is one of the largest fungal genera. It comprises some of the most commonly known filamentous fungi and can be found on numerous substrates, as well as in very diverse habitats (1). Apart from the species included in this genus, many other fungi, such as those included in the genera *Hamigera*, *Paecilomyces*, *Rasamsonia*, *Sagenomella*, *Talaromyces*, and *Trichocoma*, also show penicillium-like "little brush" structures. In spite of the morphological similarity of these fungi, recent phylogenetic studies have classified these genera into well-established families, i.e., *Aspergillaceae* (*Hamigera*, *Penicillium*), *Thermoascaceae* (*Paecilomyces*), and *Trichocomaceae* (*Rasamsonia*, *Sagenomella*, *Talaromyces*, *Trichocoma*) (2).

Despite the ubiquity of these fungi in air and in human habitats, their clinical significance is not well understood. Penicillium-like fungi are commonly recovered from clinical samples and in routine hospital air surveys; however, they are often discounted as mere contaminants. In addition, their identification to the species level is rarely made in routine laboratories due to the complexity of the phenotypic methods required for their *in vitro* study. Further, the high number of species currently accepted in these genera makes this task even more difficult (1, 3). The use of molecular methods does, however, represent a rapid and relatively simple approach for the identification of *Penicillium* species, as well as for species in other, closely related genera (4, 5).

Partly due to the aforementioned difficulties, the role of penicillium-like fungi in human pathology has been considered relatively unimportant. However, one species, *Talaromyces* (formerly *Penicillium*) *marneffei*, is notable for its clinical relevance as an agent of fatal systemic mycosis, mostly in HIV-infected patients, and mainly in southeast Asia, India, and China (6). A few other penicillium-like fungi are seen in the clinical setting, but with a considerably lower incidence. Some species of *Penicillium*, such as *P. chrysogenum*, *P. citrinum*, *P. commune*, *P. decumbens*, *P. piceum*, and *P. purpurogenum* (currently *Talaromyces picesus* and *Talaromyces purpurogenus*, respectively), have been reported only rarely (7).

Clinical manifestations due to *Penicillium* species include superficial and invasive infections, as well as allergies (8). Infections in humans are mainly related to host immunity (9). There are very few data on animal infections by *Penicillium* species, and the few cases reported have been restricted to systemic diseases and fungal osteomyelitis in dogs. *Penicillium brevicompactum*, *P. purpurogenum*, and, recently, *P. canis* have also been reported in fungal infections in dogs (10–12). Antifungal susceptibility data for clinically available antifungal agents and treatment options for infections caused by *Penicillium* species are also poorly understood, apart from data published for *T. marneffei* (13).

The main goal of the present study was to identify, by molecular means, a large set of clinical and environmental isolates of *Penicillium* and related genera that had been isolated in the United States. The results can provide much-needed information on the diversity of species in that part of the world. Additionally, this study was designed to provide antifungal susceptibility data for the species identified, which will allow more-appropriate patient management of these infections.

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MATERIALS AND METHODS

Sample collection. A total of 118 isolates identified morphologically as a *Penicillium* spp. were received from the Fungus Testing Laboratory at the University of Texas Health Science Center in San Antonio (UTHSCSA). The isolates were from different locations in the United States and comprised 108 clinical specimens that were isolated from humans, 6 that were isolated from animals, 1 that was isolated from a clinical environment, and 3 that were of unknown origin (see Table S1 in the supplemental material).

Phenotypic characterization. The isolates were subcultured onto malt extract agar (MEA; Difco Laboratories, Detroit, MI). Phenotypic identification was carried out using standard growth conditions as described previously (1). For microscopic observation, slides were made with Shear's solution using 7-to-10-day-old cultures. In addition, we evaluated the ability of the isolates to grow at 37°C.

DNA extraction, amplification, and sequencing. The isolates were grown on MEA for 7 to 14 days at 25°C prior to DNA extraction. DNA was extracted using a FastDNA kit and the kit protocol (MP Biomedicals, Solon, OH) with the homogenization step using a FastPrep FP120 cell disrupter (Thermo Savant, Holbrook, NY) according to the manufacturers' instructions. The DNA regions selected for sequencing were those recommended by Visagie et al. (1) for *Penicillium* identification. PCR was performed to amplify the internal transcribed spacer (ITS) of the ribosomal DNA (rDNA) and a fragment of the β-tubulin gene. The primer pairs used were ITS5/ITS4 for the ITS region (14) and Bt2a/Bt2b for β-tubulin (15).

Single-band PCR products were purified and sequenced at Macrogen Corp. Europe 104 (Amsterdam, The Netherlands) with a 3730XL DNA analyzer (Applied Biosystems, Foster City, CA). Sequence assembly and editing were performed using SeqMan v. 7.0.0 (DNASTAR, Madison, WI).

Phylogenetic reconstructions. Preliminary identification of the isolates to the genus level was performed by analysis of ITS sequences, using the BLAST algorithm implemented in the GenBank, CBS-KNAW, and MycoBank databases. Isolates were identified to the species level using the ITS and β-tubulin sequences. Multiple-sequence alignments were performed for each locus in MEGA v 6.0 software (16), using the CLUSTALW algorithm (17), refined with MUSCLE (18) and manually adjusted using the same software platform.

Phylogenetic analyses were made with the individual loci and combined genes using maximum likelihood (ML) in MEGA v. 6.0 (16) and Bayesian inference (BI) under MrBayes version 3.1.2 (19). For ML, support for internal branches was assessed by 1,000 ML bootstrapped pseudoreplicates of data. A bootstrap support (bs) value of ≥70 was considered significant. The phylogenetic reconstruction by BI was performed using 5 million Markov chain Monte Carlo (MCMC) generations, with two runs (one cold chain and three heated chains), and samples were stored every 1,000 generations. The 50% majority-rule consensus tree and posterior probability (pp) values were calculated after discarding the first 25% of the samples. A pp value of ≥0.95 was considered significant. The best substitution model for all gene matrices was estimated using jModelTest v.2.1.3 (20, 21).

Antifungal susceptibility testing. Antifungal susceptibility of the isolates was determined according to the Clinical and Laboratory Standards Institute (CLSI) broth microdilution M38-A2 method for filamentous fungi (22). The *in vitro* activities of amphotericin B (AMB), voriconazole (VRC), itraconazole (ITC), posaconazole (PSC), terbinafine (TRB), anidulafungin (AFG), caspofungin (CFG), micafungin (MFG), and 5-fluorocytosine (5FC) were determined for those species with five or more isolates. The MIC was defined as the lowest concentration to inhibit 100% of growth on visual inspection for AMB, ITC, PSC, and VRC and to reduce growth by 80% for TRB compared to the drug-free control well. The minimal effective concentration (MEC) was defined as the lowest concentration seen to produce short, stubby, abnormally branched hyphae for the echinocandins. Both the MIC and the MEC parameters were determined at 48 h.

Candida krusei ATCC 6258, *Candida parapsilosis* ATCC 22019, *Paecilomyces variotii* ATCC MYA-3630, and *Aspergillus fumigatus* ATCC MYA-3626 were used as quality control strains for all tests.

Nucleotide sequence accession numbers. The nucleotide sequences obtained in this work were submitted to GenBank under accession numbers LT558856 to LT558973 for the ITS and LT558974 to LT559090 for β-tubulin (see Table S1 in the supplemental material).

RESULTS

On the basis of the results of the analysis of the ITS region, we discovered that the 118 isolates investigated corresponded to species belonging to *Penicillium* ($n = 85$), *Talaromyces* ($n = 31$), or *Rasamsonia* ($n = 2$). Identification of the isolates at the species level through phylogenetic analysis with the combination of ITS and β-tubulin sequences is summarized in Table 1.

We carried out a phylogenetic study for each of the three genera involved. The first objective was to identify *Penicillium* isolates by grouping them into their respective sections (see Fig. S1 in the supplemental material). The aligned data set was 919 bp long (ITS, 521 bp; β-tubulin, 398 bp), and Kimura's two-parameter (K2) model with gamma distribution (+G) was the model selected for each fragment. This analysis showed that our isolates corresponded to 23 species belonging to the following 10 sections: *Chrysogena* ($n = 28$), *Citrina* ($n = 17$), *Fasciculata* ($n = 11$), *Lanata-Divaricata* ($n = 11$), *Aspergilloides* ($n = 7$), *Exilicaulis* ($n = 4$), *Roquefortorum* ($n = 3$), *Brevicompacta* (n = *2*), *Penicillium* ($n = 1$), and *Sclerotiora* ($n = 1$). The most frequently identified taxa were *P. rubens* (22.4%; $n = 19$; section *Chrysogena*) and *P. citrinum* (16.5%; $n = 14$; section *Citrina*). Additionally, within section *Citrina*, seven *Penicillium* sp. isolates could not be identified at the species level.

A second phylogenetic reconstruction was performed to identify the *Talaromyces* isolates (see Fig. S2 in the supplemental material). The aligned data set was 847 bp long (ITS, 474 bp; β-tubulin, 373 bp), and the model selected was the Tamura three-parameter (T92) model with gamma-distributed rates and the presence of invariant sites (G + I) for ITS and K2 + G + I for β-tubulin. In this genus, 10 species were identified belonging to four sections, i.e., *Talaromyces* ($n = 25$), *Trachyspermi* ($n = 3$), *Islandici* ($n = 2$), and *Helici* ($n = 1$). The most prevalent species were *T. amestolkiae* (22.6%; $n = 7$) and *T. purpurogenus* (16.1%; $n = 5$), both in section *Talaromyces*. A total of seven isolates could not be identified at the species level and are referred to here using the following six designations: *Talaromyces* sp. strain I ($n = 1$; section *Talaromyces*), *Talaromyces* sp. strain II ($n = 2$; section *Talaromyces*), *Talaromyces* sp. strain III ($n = 1$; section *Talaromyces*), *Talaromyces* sp. strain IV ($n = 1$; section *Helici*), *Talaromyces* sp. strain V ($n = 1$; section *Islandici*), and *Talaromyces* sp. strain VI ($n = 1$; section *Trachyspermi*).

The third phylogenetic analysis (see Fig. S3 in the supplemental material) was carried out to identify the *Rasamsonia* isolates. The aligned data set was 1,078 bp long (ITS, 599 bp; β-tubulin, 479 bp), and the selected models for each fragment were T92 and K2, with uniform rates used for ITS and β-tubulin, respectively. The isolates were identified as *R. argillacea* and *R. eburnea*.

The 118 isolates were mainly from the respiratory tract (72.9%), usually from human bronchoalveolar lavage (BAL) fluid

TABLE 1 Molecular identification and growth at 37°C of the isolates included in the study

Genus (total no. of isolates)	Species	Section	No. of isolates	Growth at 37°C (mm/7 days)
Penicillium (85)	*P. rubens*	*Chrysogena*	19	19
	P. citrinum	*Citrina*	14	14
	Penicillium sp.	*Chrysogena*	7	7
	P. oxalicum	*Lanata-Divaricata*	7	7
	P. glabrum	*Aspergilloides*	5	4
	P. crustosum	*Fasciculata*	4	0
	P. polonicum	*Fasciculata*	4	1
	P. roqueforti	*Roquefortorum*	3	0
	P. chrysogenum	*Chrysogena*	2	2
	P. citreonigrum	*Exilicaulis*	2	0
	P. rolfsii	*Lanata-Divaricata*	2	2
	P. brevicompactum	*Brevicompacta*	2	0
	P. sumatrense	*Citrina*	1	0
	P. pancosmium	*Citrina*	1	0
	P. roseopurpureum	*Citrina*	1	0
	P. decumbens	*Exilicaulis*	1	1
	P. rubefaciens	*Exilicaulis*	1	1
	P. allii	*Fasciculata*	1	0
	P. echinulatum	*Fasciculata*	1	0
	P. palitans	*Fasciculata*	1	0
	P. brasilianum	*Lanata-Divaricata*	1	1
	P. singorense	*Lanata-Divaricata*	1	1
	P. adametzioides	*Sclerotiora*	1	0
	P. coprophilum	*Penicillium*	1	0
	P. frequentans	*Aspergilloides*	1	0
	P. rudallense	*Aspergilloides*	1	1
Talaromyces (31)	*T. amestolkiae*	*Talaromyces*	7	7
	T. purpureogenus	*Talaromyces*	5	5
	T. pinophilus	*Talaromyces*	3	3
	T. aurantiacus	*Talaromyces*	2	2
	T. ruber	*Talaromyces*	2	2
	Talaromyces sp. I	*Talaromyces*	2	2
	Talaromyces sp. II	*Talaromyces*	1	1
	Talaromyces sp. III	*Talaromyces*	1	0
	T. cnidii	*Talaromyces*	1	1
	T. funiculosus	*Talaromyces*	1	1
	Talaromyces sp. IV	*Helici*	1	1
	T. columbinus	*Islandici*	1	1
	Talaromyces sp. V	*Islandici*	1	1
	T. atroroseus	*Trachyspermi*	1	1
	T. diversus	*Trachyspermi*	1	1
	Talaromyces sp. VI	*Trachyspermi*	1	0
Rasamsonia (2)	*R. argillacea*		1	1
	R. eburnea		1	1

(see Table S1 in the supplemental material). We carried out antifungal susceptibility testing of the most frequent species, i.e., a total of 51 isolates (39 *Penicillium* isolates and 12 *Talaromyces* isolates) representing seven species (Tables 2 and 3). Overall, TRB and the echinocandins showed the best *in vitro* activity against *Penicillium* species, with modes of <0.03 μg/ml for TRB, 0.06 μg/ml for CFG and AFG, and 0.125 μg/ml for MFG. Amphotericin B showed intermediate antifungal activity, with an overall mode of 2 μg/ml, while the azoles showed various levels of activity, with wide MIC ranges and modes of 0.5 μg/ml for PSC and ITC and 2 μg/ml for VRC. The highest MIC values were observed for 5FC (Table 2). Terbinafine, the echinocandins, and AMB showed *in vitro* activity against *Talaromyces* species similar to that seen with the *Penicillium* species, while 5FC, with a mode of 0.125 μg/ml, showed good *in vitro* activity compared to the results seen with *Penicillium* species. In contrast, the azoles showed poor *in vitro* activity, with wide MIC ranges and modes of >16 μg/ml for PSC, VRC, and ITC (Table 3).

The results of growth at 37°C (Table 1) showed that 71.7% of the *Penicillium* isolates ($n = 61$) were able to grow at this temperature. All the isolates belonging to both section *Chrysogena* ($n = 28$) and section *Lanata-Divaricata* ($n = 11$) grew at 37°C, whereas, among the isolates belonging to section *Citrina*, only those identified as *P. citrinum* managed to grow at this physiologically relevant temperature. On the other hand, practically all isolates of the genus *Talaromyces* (99.6%; $n = 29$), as well as the two isolates of the genus *Rasamsonia*, grew at 37°C (Table 1).

DISCUSSION

Despite the uncertainty concerning the true role that penicillium-like fungi play in human pathology, there are several reports of infections that seem to have involved these fungi in the clinical setting, in particular, isolates from respiratory samples (23, 24). However, to our knowledge, the species diversity of a large collection of penicillium-like fungi from clinical origins has never been explored. Thus, this was the first study to investigate more than 100 isolates from clinical sources and to demonstrate, by using combined sequence analyses of the ITS region and β-tubulin gene, that three different genera of penicillium-like fungi (i.e., *Penicillium*, *Talaromyces*, and *Rasamsonia*) are, in fact, associated with these types of samples. As expected, *Penicillium* species were the most common (72%).

The most frequently identified species within *Penicillium* were *P. citrinum* and *P. rubens*. *P. citrinum* has been reported to be a human-opportunistic pathogen responsible for keratitis, cutaneous infections, and pneumonia (25–28).

Curiously, to date, *P. rubens* has not shown any link to clinical isolates, although it is a recently resurrected species, closely related to *P. chrysogenum* (29). In contrast, *P. chrysogenum* has already been identified as a human pathogen associated with cutaneous and invasive infections (8, 30, 31). A total of 28 of our isolates were classified within section *Chrysogena*, including 19 identified as *P. rubens*, 2 identified as *P. chrysogenum*, and 7 that were very similar to those but which will require additional phylogenetic markers to distinguish them properly (29). Although we were not able to demonstrate the pathogenic role of *P. rubens* and *P. chrysogenum*, the high number of strains of this species recovered and their ability to grow at 37°C highlight the clinical importance of these species.

Other *Penicillium* species found in our study were *P. glabrum* and *P. oxalicum*, which were the most frequent species after *P. rubens* and *P. citrinum*. While *P. glabrum* has not been associated with human infections, Chowdhary (32) reported three cases of invasive infections by *P. oxalicum* in patients with acute myeloid leukemia, diabetes mellitus, and chronic obstructive pulmonary disease. The lung was theorized to be the portal of entry for this pathogen in those three cases.

Several reports have recognized *P. decumbens* as the cause of a disseminated infection, a perioperative paravertebral infection, and a fungus ball (7). However, only one isolate of *P. decumbens*

TABLE 2 Results of *in vitro* antifungal susceptibility testing of 39 isolates of *Penicillium* species

Species (no. of isolates tested)	Antifungal[a]	No. of isolates with antifungal MIC (μg/ml) of:										
		≤0.03	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	>16
P. citrinum ($n = 10$)	CFG		2	7	1								
	AFG		4	5	1								
	MFG		1	1	7	1							
	TRB	3	7										
	PSC				1			7	2				
	VRC												10
	ITC						1	4	1	1			3
	AMB							3	7				
	5FC									2	4	3	1
P. rubens ($n = 10$)	CFG		1	2	4	3							
	AFG		1	4	1	4							
	MFG		1	2	5	2							
	TRB	8	1	1									
	PSC				2	2	6						
	VRC					1		2	4	1			2
	ITC			2		1	7						
	AMB				1		1	4	4				
	5FC							1	2	4	2		1
Penicillium sp. ($n = 7$)	CFG			1	6								
	AFG		1	1	1	4							
	MFG				3	3		1					
	TRB	3	4										
	PSC				1	1	4	1					
	VRC					1	1	2	3				
	ITC				2	2	2	1					
	AMB							1	6				
	5FC												7
P. oxalicum ($n = 7$)	CFG			2	1	2			1	1			
	AFG		1	2	1		1			2			
	MFG				2	2		1	1	1			
	TRB	5	2										
	PSC					4	2	1					
	VRC								7				
	ITC				1	1		1	4				
	AMB					2	4	1					
	5FC							1		1	4		1
P. glabrum ($n = 5$)	CFG		5										
	AFG		4		1								
	MFG		3	2									
	TRB	2		3									
	PSC				2	1	2						
	VRC							1	3	1			
	ITC			1	1		1	2					
	AMB			1	2	2							
	5FC							3	1	1			

[a] CFG, caspofungin; AFG, anidulafungin; MFG, micafungin; TRB, terbinafine; PSC, posaconazole; VRC, voriconazole; ITC, itraconazole; AMB, amphotericin B; 5FC, flucytosine.

was identified in our study; that isolate represents another rare species in the clinical setting (32). Some other *Penicillium* species, including many, such as *P. rubefaciens*, *P. brasilianum*, *P. singarense*, and *P. rudallense*, represented by only one isolate each in our study, have not been recognized previously in isolates from clinical specimens, but their ability to grow at 37°C suggests a potential pathogenicity.

It is relevant that a considerable number of our isolates were identified as *Talaromyces* species. Apart from *T. marneffei*, which is the most clinically important member, this genus contains some opportunistic species of clinical importance such as *T. amestolkiae*, *T. indigoticus*, *T. piceus*, *T. purpurogenus*, *T. radicus*, *T. ruber*, *T. rugulosus*, *T. stollii*, and *T. verruculosus*. Most of these species were part of the genus *Penicillium* (3, 33). Nearly 80% of our isolates identified as *Talaromyces* species belong to section *Talaromyces*, which is the only section that includes both animal-pathogenic and human-pathogenic species (33). *T. amestolkiae* and *T. purpurogenus* were the species most frequent identified

TABLE 3 Results of *in vitro* antifungal susceptibility testing of 12 isolates of *Talaromyces* species

Species (no. of isolates tested)	Antifungal[a]	No. of isolates with antifungal MIC (μg/ml) of:											
		≤0.03	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	>16
T. amestolkiae (7)	CFG			2	3	1		1					
	AFG		1	3	3								
	MFG			3	2	2							
	TRB	6	1										
	PSC						2		1				4
	VRC									1			6
	ITC									1			6
	AMB				1	2	2	2					
	5FC			2	3		1		1				
T. purpurogenus (5)	CFG					1				1	1	2	
	AFG			1	3							1	
	MFG			2	1	2							
	TRB	1	2	1								1	
	PSC								1	1		1	2
	VRC								1	2	2		
	ITC												5
	AMB								3	2			
	5FC			3	2								

[a] CFG, caspofungin; AFG, anidulafungin; MFG, micafungin; TRB, terbinafine; PSC, posaconazole; VRC, voriconazole; ITC, itraconazole; AMB, amphotericin B; 5FC, flucytosine.

among our isolates. These, together with *T. ruber* and *T. stollii*, were recovered from pulmonary and invasive infections in humans (7, 9) and animals (8, 12). The production of a red diffusible pigment by the colonies of *T. purpurogenum* is a feature shared with *T. marneffei* and can lead to misidentification of those two species in diagnostic laboratories. Although the former can grow at 37°C, it is unable to develop a yeast morphology such as *T. marneffei* can (9).

We also identified two isolates belonging to *Rasamsonia*, one as *R. argillacea* and the other as *R. eburnea* (formerly *Talaromyces eburneus*). Recently, rates of infections by isolates within this genus appear to have increased in humans and animals, and those species are considered emerging pathogens (34–36). In 2011, nine cases of invasive infections by *Rasamsonia argillacea* were reported in patients with chronic granulomatous disease (37, 38) and, more recently, in a patient with graft-versus-host disease (39). Houbraken et al. (35) identified clinical isolates of *R. eburnea* from blood cultures, sputum, and peritoneal dialysis fluid from a patient with peritonitis. *Rasamsonia argillacea* and *R. eburnea* are phylogenetically close and have similar phenotypic characteristics, except that *R. eburnea* shows a blackish brown reverse (40).

Several cases have been reported in which the respiratory tract was the portal of entry of infections by penicillium-like fungi, with or without systemic dissemination. Although the majority of isolates in this study were obtained from respiratory specimens, it was not possible to establish the true pathogenic role of the identified species because of the nature of the samples and the absence of clinical data.

In vitro antifungal susceptibility profiles of penicillium-like species of fungi other than *T. marneffei* are currently based on very few studies and are mainly taken from case reports that have shown differing results (8, 27, 32). Our results show that TRB and the echinocandins are highly active *in vitro* against *Penicillium* and *Talaromyces* spp. However, these antifungals are not widely used for treating invasive infections by these fungi (41). Terbinafine was chosen as a good alternative for long-term maintenance therapy for treatment of an infection associated with one isolate from a dog with osteomyelitis (11). In the present study, AMB also had intermediate antifungal activity, agreeing with previous studies (42, 43); however, the clinical experience reported in two cases of infections by species within this group revealed that the patients did not respond to this drug (8). Our susceptibility results show that the azoles have variable activity against *Penicillium* species and high MICs for *Talaromyces* species. In fact, ITC has been used as a prophylactic treatment for infections by *T. marneffei* (42). Chowdhary et al. (32) reported resistance to VRC in three cases of invasive infections by *P. oxalicum*, where the successful alternative treatment was PSC. We observed intermediate MIC values for VRC in our isolates of *P. oxalicum*, while the *P. citrinum* isolates showed resistance to this drug. This confirms the observations of Mok et al. (27), who reported high MIC values for the azoles against one isolate of *P. citrinum* from an acute leukemia patient with pneumonia and pericarditis.

In conclusion, although human and animal infections caused by penicillium-like fungi are infrequent, this study revealed that a relative wide range of species, all able to grow at 37°C, should be taken into account in the diagnosis of such infections. Identification at the species level remains difficult on the grounds that species of various genera share similar morphological characteristics. This supports the relevance of using DNA sequence data to identify them. More data are needed from both *in vitro* susceptibility studies and clinical outcomes in order to determine an effective treatment for infections caused by penicillium-like fungi.

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