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Molecular Identification and Susceptibility of *Trichosporon* Species Isolated from Clinical Specimens in Qatar: Isolation of *Trichosporon dohaense* Taj-Aldeen, Meis & Boekhout sp. nov.▽

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Trichosporon **species have been reported as emerging pathogens and usually occur in severely immunocompromised patients. In the present work, 27 clinical isolates of *Trichosporon* species were recovered from 27 patients. The patients were not immunocompromised, except for one with acute myeloid leukemia. Sequence analysis revealed the isolation of *Trichosporon dohaense* Taj-Aldeen, Meis & Boekhout sp. nov., with CBS 10761^T as the holotype strain, belonging to the Ovoides clade. In the D1–D2 large-subunit rRNA gene analysis, *T. dohaense* is a sister species to *T. coremiiforme*, and in the internal transcribed spacer analysis, the species is basal to the other species of this clade. Molecular identification of the strains yielded 17 *T. asahii*, 3 *T. inkin*, 2 *T. japonicum*, 2 *T. faecale*, and 3 *T. dohaense* isolates. The former four species exhibited low MICs for five antifungal azoles but showed high MICs for amphotericin B. *T. dohaense* demonstrated the lowest amphotericin B MIC (1 mg/liter). For the majority of *T. asahii* isolates, amphotericin B MICs were high (MIC at which 90% of isolates were inhibited [MIC_{90}], ≥16 mg/liter), and except for fluconazole (MIC_{90}, 8 mg/liter), the azole MICs were low: MIC_{90}s were 0.5 mg/liter for itraconazole, 0.25 mg/liter for voriconazole, 0.25 mg/liter for posaconazole, and 0.125 mg/liter for isavuconazole. The echinocandins, caspofungin and anidulafungin, demonstrated no activity against *Trichosporon* species.**

Trichosporon species are yeast-like fungi, widely distributed in nature and commonly isolated from soil and other environmental sources, which have been involved in a variety of opportunistic infections and have been recognized as emerging fungal pathogens in immunocompromised hosts (19, 79, 80). Disseminated *Trichosporon* infections are potentially life-threatening and are often fatal in neutropenic patients (7, 22). Although uncommon, pathogenic species of this genus have been reported increasingly, mostly in patients with malignant diseases (3, 6, 9, 10, 11, 20, 32, 44, 47, 48, 63, 77), neonates (18, 56, 84), a bone marrow transplant recipient (22), a solid organ transplant recipient (50), and patients with human immunodeficiency virus (34, 35, 46). *Trichosporon* has also been reported to cause fungemia (5, 9, 25, 29, 30, 33, 53, 62). Members of the genus *Trichosporon* have occasionally been implicated as nail pathogens (16, 28, 74) and in subcutaneous infections (66). *Trichosporon* is considered an opportunistic agent, and therefore, recovery of *Trichosporon* species capable of growing at 37°C, especially from immunocompromised patients, should be regarded as potentially significant. Several reports have addressed the difficulty of identifying *Trichosporon* to the species level by physiological and biochemical characteristics (2, 64); therefore, molecular methods based on the sequencing of the internal transcribed spacer (ITS) have been developed (15, 69, 71, 72).

In the present paper, we report the isolation of *Trichosporon* species from clinical specimens over a 4-year period in Qatar, the poor performance of biochemical identification methods, the significance of molecular identification, and the antifungal susceptibility data for the isolates. While investigating the molecular identification of *Trichosporon* species, we found three strains that do not match any of the published strains in the literature. We describe this organism as *Trichosporon dohaense* Taj-Aldeen, Meis & Boekhout, sp. nov., the name proposed for this species.

MATERIALS AND METHODS

Patients. Twenty-seven patients from different regions and with various clinical symptoms presented at Hamad Hospital, Doha, Qatar. The demographic data, clinical specimens, and fungal etiology are reported in Table 1.

Isolation and identification of *Trichosporon* species. A total of 27 clinical specimens positive for *Trichosporon* species were recorded over a 4-year period. *Trichosporon* species were isolated and identified according to standard laboratory procedures. The clinical specimens were generally cultured on either Sabouraud dextrose agar (SDA; Difco Laboratories, Detroit, MI) plus 40 U/ml streptomycin and 20 U/ml penicillin (SDA+SP), SDA without antibiotics, or brain heart infusion plus 40 U/ml streptomycin and 20 U/ml penicillin. Blood cultures were performed using the Bactec automated culturing system (BD Diagnostic Systems). For culturing of urine, cysteine lactose electrolyte-deficient agar (Mast Diagnostics, United Kingdom) was added for isolation and enumer-

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TABLE 1. Clinical data and *Trichosporon* species recovered from clinical specimens

Case no.	Clinical specimen	Clinical finding(s)[a]	Patient age/sex[b]	Patient origin	Identification of isolate by the Vitek II yeast ID/API ID 32 C system	Closest hit (BLAST)[c]	No. of identical nucleotides/total nucleotides based on the rDNA sequence		Identification
							LSU	ITS	
1	Urine	Pyuria, RBC	26/M	India	*T. asahii*	*T. asahii*	556/556	439/440	*T. asahii*
2	Urine	Pyuria	63/M	Qatar	*T. asahii*	*T. asahii*	555/555	458/459	*T. asahii*
3	Urine	Pyuria	32/M	Egypt	*Trichosporon* species	*T. faecale*	576/577	490/490	*T. faecale*
4	Urine	Pyuria,	23/F	Egypt	*T. asahii*	*T. asahii*	571/571	550/550	*T. asahii*
5	Urine	Pyuria	68/M	Palestine	*T. asahii*	*T. asahii*	555/555	469/471	*T. asahii*
6	Urine	Pyuria	77/F	Qatar	*T. asahii*	*T. asahii*	554/554	469/471	*T. asahii*
7	Urine	Pyuria	41/M	Nepal	*T. asahii*	*T. asahii*	576/577	558/558	*T. asahii*
8	Toenail	Distolateral onychomycosis, whitish discoloration	72/M	United States	*T. asahii*	*T. asahii*	557/557	498/499	*T. asahii*
9	Toenail	Onychomycosis	30/M	Egypt	*Trichosporon* species	*T. asahii*	556/556	467/469	*T. asahii*
10	Toenail	Onychomycosis	50/M	Sudan	*T. inkin*	*T. asahii*	533/533	490/490	*T. asahii*
11	Toenail	Onychomycosis	25/M	Pakistan	*Trichosporon* species	*T. asahii*	515/515	335/336	*T. asahii*
12	Fingernail	Onychomycosis, whitish-yellowish, (subungual hyperkeratosis)	22/M	Nepal	*T. asahii*	*T. asahii*	567/567	550/550	*T. asahii*
13	Nail	Onychomycosis	25/M	Philippines	*T. asahii*	*T. asahii*	587/588	565/565	*T. asahii*
14	Toenail	Onychomycosis	70/F	Qatar	*Trichosporon* species	*T. coremiiforme*/ *T. asahii*	560/561	556/566	***T. dohaense***
15	Ear discharge	Pus discharge, ear pain	15/M	Qatar	*T. asahii*	*T. asahii*	557/557	469/470	*T. asahii*
16	Ear discharge	Pus discharge, ear pain	26/F	India	*Trichosporon* species	*T. asahii*	556/556	467/469	*T. asahii*
17	Catheter	Catheter site infection	41/M	Bangladesh	*Trichosporon* species	*T. coremiiforme*/ *T. asahii*	545/546	651/662	***T. dohaense***
18	Skin/intertrigo	Tinea pedis of the diabetic foot	31/M	Qatar	*T. asahii*	*T. japonicum*	571/571	466/466	*T. japonicum*
19	Scalp hair	White piedra	28/F	Qatar	*Trichosporon* species	*T. inkin*	600/600	498/502	*T. inkin*
20	Skin scraping	Tinea pedis	34/M	India	*Trichosporon* species	*T. coremiiforme*/ *T. asahii*	560/561	549/560	***T. dohaense***
21	Tissue	Leg cellulitis	18/M	Pakistan	*Trichosporon* species	*T. asahii*	581/582	541/541	*T. asahii*
22	Skin	Tinea pedis	33/M	Qatar	*Trichosporon* species	*T. japonicum*	621/621	541/541	*T. japonicum*
23	Bone	Osteomyelitis	65/M	India	*Trichosporon* species	*T. asahii*	585/586	557/559	*T. asahii*
24	Blood	Fungemia	6/F	Qatar	*Trichosporon* species	*T. faecale*	620/621	522/522	*T. faecale*
25	Swab	Urethral discharge	28/M	Kenya	*Trichosporon* species	*T. inkin*	603/603	539/539	*T. inkin*
26	Swab	Balanitis	39/M	India	*T. asahii*	*T. inkin*	526/526	508/512	*T.* cf *inkin*
27	Bronchial lavage	Trauma, cough	52/M	Unknown	*T. asahii*	*T. asahii*	424/424	498/498	*T. asahii*

[a] RBC, red blood cells.
[b] M, male; F, female.
[c] Valid for both the LSU and the ITS, except for cases 14, 17, and 20, where the first name is the hit for the LSU and the second name is the hit for the ITS.

ation of the organism. According to standard laboratory guidelines, total colony counts in the range of 10^4 to 10^5 CFU/ml or more were considered significant, and the presence of yeast cells observed by direct microscopy of urine, with associated clinical symptoms such as pyuria, suggested urinary tract infection. Organisms isolated from specimens that did not meet such criteria were excluded from the study. *Trichosporon* species isolated from nails were considered significant after two successive isolations from a patient, direct microscopy showing compatible fungal cells, and the absence of dermatophytes in culture, according to the diagnostic criteria of Gupta et al. (26). Culture plates were incubated at 28°C and 37°C and were observed daily for growth up to 5 days. Colonies appearing yeast-like in consistency were examined in a lactophenol cotton blue wet mount for microscopic characteristics. *Trichosporon* showed budding yeast cells, hyphae, and arthroconidia.

Biochemical identification. A small inoculum from an isolated colony of each isolate was inoculated onto SDA+SP plates, incubated at 37°C for 48 h, and used to prepare inocula for substrate assimilation profiles employing the Vitek II yeast identification (ID) system (Biomerieux, France), as recommended by the manufacturer. The yeast suspension was automatically inoculated into a Vitek II ID yeast card, which was programmed to identify only three *Trichosporon* species, viz., *Trichosporon asahii*, *T. inkin*, and *T. mucoides.* The isolates were also identified using the API ID 32 C system (Biomérieux).

Morphology, physiology, and rRNA gene sequencing. The morphology of the isolates was investigated using line inoculations onto the following media: YPGA (1% yeast extract–0.5% bacteriological peptone L37 [Oxoid]–4% glucose agar), yeast malt extract agar (Difco), yeast morphology agar (YMoA; Difco), malt extract agar (Oxoid), SDA (Difco), and potato dextrose agar (PDA; Difco). The formation of blastoconidia was also investigated using these media. Growth at different temperatures (25, 30, 35, 37, 40, and 42°C) was evaluated using inoculated YPGA slants placed in incubators at the appropriate temperatures. The nutritional requirements, fermentative capabilities, reactions to diazonium blue B, and urease activities of the yeast strains were assessed according to the work of Yarrow (83).

Genomic DNA was extracted as described by Bolano et al. (8), with minor adjustments. Molecular identification of the isolate was performed by sequence analysis of the D1–D2 domains and the ITS1 and ITS2 regions of ribosomal DNA according to the method of Okoli et al. (54). The sequences generated were compared to the available data in the NCBI database with the Basic Local Alignment Search Tool (BLASTn) (4). Trees were generated with PAUP (version 4.0b10) using the neighbor-joining algorithm with Kimura 2 as a distance measure and 1,000 bootstrap replicates (75).

Susceptibility testing. The susceptibilities of all the strains to amphotericin B (Bristol-Myers Squibb, Woerden, The Netherlands), itraconazole (Janssen Research Foundation, Beerse, Belgium), fluconazole, voriconazole, anidulafungin (Pfizer Central Research, Sandwich, United Kingdom), caspofungin (Merck Sharp & Dohme BV, Haarlem, The Netherlands), posaconazole (Schering-Plough, Utrecht, The Netherlands), and isavuconazole (Basilea Pharmaceutica, Basel, Switzerland) were tested using the standard broth microdilution method as described in NCCLS (now CLSI) document M27-A2 (49).

Each isolate was grown on SDA at 35°C for 24 to 48 h, and a stock inoculum suspension was prepared according to the recommendations of the NCCLS (49). This suspension was then adjusted with a spectrophotometer to 75 to 77% transmittance at a wavelength of 530 nm. The working suspension was made by a 1:1,000 dilution of the suspension in RPMI 1640 (GIBCO BRL, Life Technologies, Woerden, The Netherlands) to produce the final test concentration of 1×10^3 to 5×10^3 CFU/ml. Aliquots (100 μl) of the diluted suspension were inoculated into 96-well flat-bottom microtiter plates (Costar; Omnilabo International BV, Breda, The Netherlands) with antifungals by using a multichannel pipette. The concentrations of amphotericin B, itraconazole, voriconazole, and

posaconazole ranged from 0.016 to 16 mg/liter, that of fluconazole from 0.063 to 64 mg/liter, those of anidulafungin and caspofungin from 0.008 to 8 mg/liter, and that of isavuconazole from 0.004 to 4 mg/liter.

MIC end points were determined after 48 h of incubation at 35°C with an Anthos HT3 spectrophotometer (Salzburg, Austria) and the MikroWin 2000 program at 450 nm. The MIC of amphotericin B was taken from the well with the lowest concentration with 100% inhibition of growth (compared to the growth control well), while the MICs of fluconazole, itraconazole, voriconazole, posaconazole, isavuconazole, caspofungin, and anidulafungin were taken from the wells with prominent decreases in turbidity (approximately ≤50% growth inhibition) from that of the growth control well. Ranges for MICs and for MICs at which 50 and 90% of the isolates of *T. asahii* tested were inhibited (MIC_{50} and MIC_{90}) were calculated. The MICs for the quality control strains *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were all within the reference ranges (data not shown).

Nucleotide sequence accession numbers. The *T. dohaense* strains identified in this study were assigned the following GenBank accession numbers: FJ228473 for the large subunit (LSU) and FJ228476 for the ITS of the case 14 isolate; FJ228472 for the LSU and FJ228474 for the ITS of the case 17 isolate; and FJ228471 for the LSU and FJ228475 for the ITS of the case 20 isolate.

RESULTS

Twenty-seven isolates of *Trichosporon* species originating from 27 patients were obtained. Information pertaining to the source of isolation and the clinical symptoms of the patients yielding these isolates is provided in Table 1. Low discernible differences were observed in the microscopic morphologies of the strains. The assimilation profiles of the strains by the Vitek II and/or the API ID 32 C system were not discriminatory for some strains. When the clinical isolates were subjected to identification by these two biochemical methods, 13 isolates were identified as *T. asahii* and 1 as *T. inkin*, while 13 isolates yielded variable results, but the systems were not discriminatory, and hence, these isolates are referred to as "*Trichosporon* species," indicating the inability of biochemical methods to discriminate among various *Trichosporon* species. When conventional identification methods were employed, *Trichosporon* species such as *T. japonicum* and *T. inkin* were misidentified as *T. asahii*, or *T. asahii* was misidentified as *T. inkin* (Table 1).

Molecular identification of the strains yielded 17 *T. asahii* isolates and 10 isolates of other *Trichosporon* species: 3 *T. inkin*, 3 *T. dohaense*, 2 *T. faecale*, and 2 *T. japonicum* isolates. Of the specimens examined, seven were from urine, seven from nails, two from ear discharges, four from superficial sites (three from skin and one from hair), and one each from tissue, bone, urethral discharge, blood, respiratory, balanitis, and a catheter. There were 21 males and 6 females, aged 6 to 77 years (median age, 38.5 years). The apparent bias toward male patients can be explained by the fact that the majority of the immigrant workers in Qatar who form the main patient groups are males. All patients had one or more preexisting clinical manifestations, such as pyuria, onychomycosis, skin infection, fungemia, or a respiratory problem.

Phylogenetically, three strains (viz., those from case 14 [CBS 11017; also called IHEM 22874], case 17 [CBS 10333; also called IHEM 22872], and case 20 [CBS 10761^T; also called IHEM 22873]) of *T. dohaense* sp. nov. belonged to the Ovoides clade but did not match any known species (Table 1). In the D1–D2 LSU rRNA gene analysis, *T. dohaense* is a sister species to *T. coremiiforme*, and in the ITS analysis, *T. dohaense* is basal to the other species of this clade, viz. *T. aquatile*, *T. asahii*, *T. asteroides*, *T. caseorum*, *T. coremiiforme*, *T. faecale*, *T. inkin*, *T. japonicum*, *T. lactis*, and *T. ovoides* (Fig. 1) (67). Based on the sequencing analysis, the closest relative to the three strains of *T. dohaense* is *T. coremiiforme*, with 97.8% similarity for the ITS and 99.8% similarity for the D1–D2 region. The percentages of similarity and numbers of mismatches with other species in the Ovoides cluster are given in Table 2. *T. inkin* can be differentiated by its growth at 42°C; *T. caseorum* utilizes lysine; and the other species, except for *T. lactis*, do not assimilate glucosamine, which is slowly and weakly assimilated by *T. dohaense*. *T. dohaense* differs from *T. lactis* by growth on melezitose, starch, methanol, glucosamine (N source), and 0.01% cycloheximide; growth at 37°C; and lack of growth on sorbose, mannitol, lactate, and nitrite (67). On the basis of these data, we propose the following description of the new species *T. dohaense*.

Latin description of *Trichosporon dohaense* Taj-Aldeen, Meis & Boekhout, sp. nov. In medio liquido cellulae zymoideae globosae vel ellipsoideae, 5.5 ad 9.0 per 3.5 ad 5.0 μm, polariter gemmantes. In agaro YMoA post 10 dies 25°C, coloniae variabiles, ca. 20 mm diametro, leves vel modice irregulares vel verrucosae, marginem versus sulcatae, butyrosae, cremeae vel pallide isabellinae, marginem versus albidae, glabrae vel mycelio aerio albido obtectae. Cellulae zymoideae sicut sopra, nonnumquam etiam e latere, e basi lata gemmantes; cellulae maiores, 5 ad 11 μm diameter et filamenta ad 90 per 2.0 ad 3.0 μm praesentes; hyphae vel pseudohyphae nonnumquam praesentes; arthroconidia cylindrica, magnitudine variabilia, 5 ad 20 per 2 ad 4 μm. Non fermentat. D-Glucosio, D-galactosio, D-glucosamino (d,w), D-ribosio (+,d), D-xylosio, D-arabinosio (d), L-arabinosio, sucrosio, maltosio, trehalosio (+,dw), methyl-α-D-glucosido, cellobiosio, salicino (+,dw), arbutino (+,dw), lactosio (dw), raffinosio (w), melezitosio, amylo solubili, glycerolo, *meso*-erythritolo, *myo*-inositolo (w), 2-keto-D-gluconato, D-gluconato, D-glucuronato, succinato, methanolo, ethanolo, propane 1,2 diolo (dw), butane 2,3 diolo (dw), acido galacturonico (+,d) utitur; neque L-sorbosio, melibiosio, galactitolo, D-galacturonato, DL-lactato, citrato, acido quinico, saccharato, vel verosimile L-rhamnosio (−,w), inilino (−,w), ribitolo (−,w), L-arabinitolo (−,w), D-glucitolo (−,w), D-mannitolo (−,w). Ethylamino, L-lysino, cadaverino, et D-tryptophano utitur, neque nitrato et glucosamino. Vitaminis absentibus crescere potest an non. Substantia amyloidea vix formatur. Temperaturis 25 ad 40°C crescere potest, neque 42°C; 0.01% cycloheximido addito haud crescit, neque in medio 50% glucosii addito; reactiones urei et diazonium blue B positivae. Holotypus CBS 10761^T (CBS H-20142), isolatus ex cute humana; depositus in collectione herbario CBS Fungal Biodiversity Centre, Utrecht, The Netherlands.

Description of *Trichosporon dohaense* Taj-Aldeen, Meis & Boekhout sp. nov. (i) Etymology. The specific epithet *dohaense* is derived from Doha, the capital of Qatar, where the isolates were recovered.

(ii) Morphological characterization. After 2 weeks at 25°C in 2% glucose broth in yeast nitrogen base, a ring, flocks, and sediment are present. Yeast cells are globose or subglobose to ellipsoidal, 5.5 to 9.0 by 3.5 to 5.0 μm in size, and show polar budding. On YMoA after 10 days at 25°C, colonies are somewhat variable, ca. 20 mm in diameter, slightly convex, smooth to somewhat irregular to warty, and transversely ridged toward

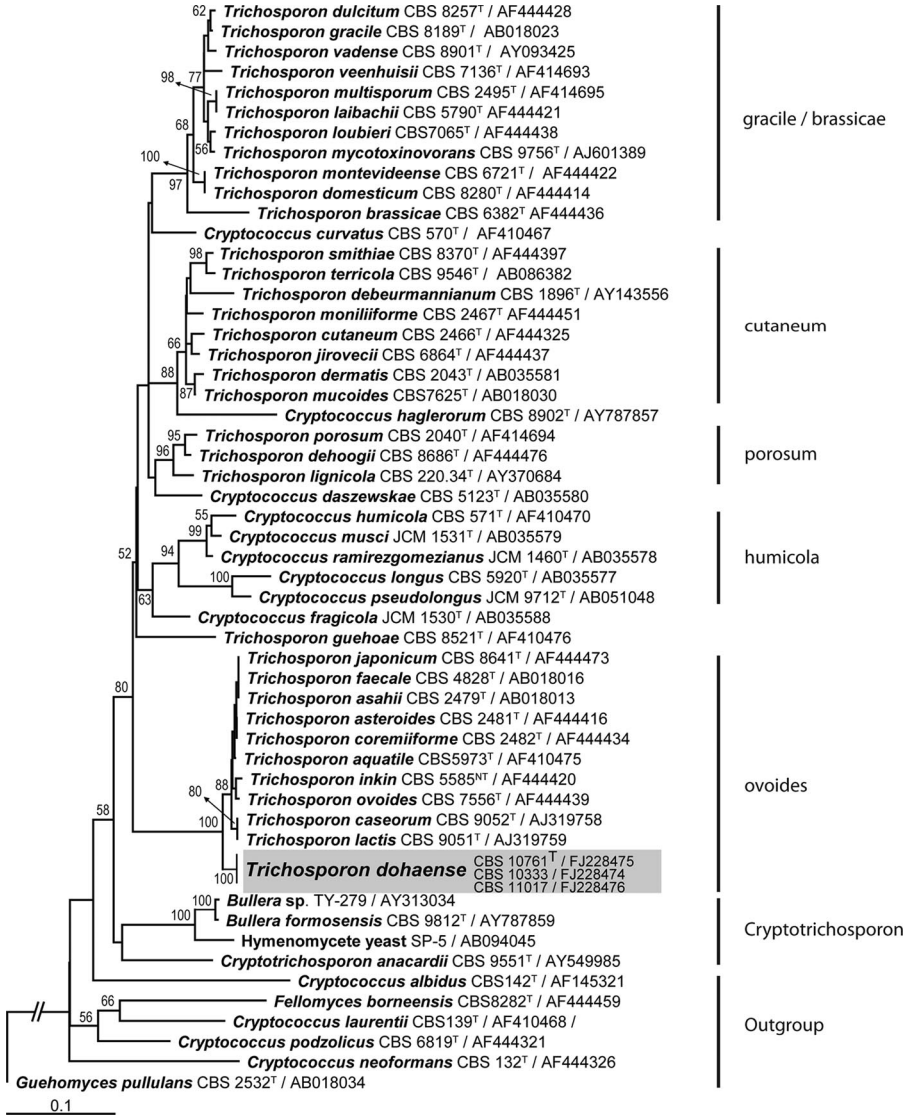

FIG. 1. Phylogenetic position of *Trichosporon dohaense* in the Ovoides clade of the Trichosporonales, based on phylogenetic analysis of the ITS regions of the rRNA gene using PAUP, version 4.0b10, for Macintosh (74). Neighbor-joining analysis was performed with the Kimura 2 substitution model. Strain names and GenBank accession numbers are given after each species name. Next to the tree branches, bootstrap support values after 1,000 replications are given. Clade names are given on the right.

the margin. They are butyrous, cream to pale café au lait (isabella), but toward the margin they become whitish, dull to shiny, glabrous, or covered with a whitish aerial mycelium. The margin is entirely or locally submerged with hyphal growth. On SDA, colonies are *Candida*-like, smooth with a mucoid texture (Fig. 2A), and they become irregular to warty in older cultures (Fig. 2B) Yeast cells are globose to ellipsoidal, or somewhat irregularly shaped, 5.5 to 8.0 (or 12.0) by 3.5 to 6.5 μm, with polar or occasionally lateral budding on a rather broad base; somewhat bigger and refractive cells, 5.0 to 11.0 μm in diameter, are present (Fig. 3A and B). Filaments as large as ca. 90 by 2.0 to 3.0 μm are present. Hyphae or pseudohyphae may be present or absent. Arthroconidia are cylindrical and somewhat variable in size, 5.0 to 20.0 by 2.0 to 4.0 μm (Fig. 3C and D). Extensive hyphae are present in Dalmau plate culture on YMoA. On malt extract agar, the surface of the colony may be covered with tapered synnemata.

(iii) Assimilation. Fermentation is absent. Growth is positive on D-glucose, D-galactose, D-glucosamine (d,w), D-ribose (+,d), D-xylose, D-arabinose (d), L-arabinose, sucrose, maltose, trehalose (+,dw), methyl-α-D-glucoside, cellobiose, salicin (+,dw), arbutin (+,dw), lactose (dw), raffinose (w), melezitose, soluble starch, glycerol, *meso*-erythritol, *myo*-inositol (w), 2-keto-D-gluconate, D-gluconate, D-glucuronate, succinate, methanol, ethanol, propane-1,2-diol (dw), butane-2,3-diol (dw), and galactonic acid (+,d). Growth is absent in L-sorbose, melibiose, galactitol, D-galacturonate, DL-lactate, citrate, quinic acid, and saccharate. Growth is absent or latent in L-rhamnose (−,w), inulin (−,w), ribitol (−,w), L-arabinitol (−,w), D-glucitol (−,w), D-mannitol (−,w). Ethylamine, L-lysine, cadaverine,

TABLE 2. Percentages of similarity and numbers of mismatches of the closest relatives from the Ovoides clade with the type strain of *T. dohaense* in the ITS regions and the D1–D2 domains of the LSU of the rRNA gene in GenBank

Strain[a]	GenBank accession no.	Species	ITS (405[b])		LSU (517[b])	
			% Similarity	No. of mismatches	% Similarity	No. of mismatches
CBS 2482	AF444434	*Trichosporon coremiiforme*	97.8	9	99.8	1
CBS 2481	AF444416	*Trichosporon asteroides*	97.8	9	98.6	7
CBS 8641	AF444473	*Trichosporon japonicum*	97.5	10	98.8	6
CBS 4828	AB018016	*Trichosporon faecale*	97.5	10	98.8	6
CBS 2479	AB018013	*Trichosporon asahii*	97.5	10	98.8	6
CBS 5973	AF410475	*Trichosporon aquatile*	97.0	12	98.3	9
CBS 5585	AF44420	*Trichosporon inkin*	96.3	15	99.0	5
CBS 7556	AF444439	*Trichosporon ovoides*	96.3	15	98.8	6
CBS 9051	AJ319759	*Trichosporon lactis*	96.3	15	98.3	9
CBS 9052	AJ319758	*Trichosporon caseorum*	96.3	15	97.7	12

[a] Strains are listed in descending order with regard to the percentage of similarity, based first on the ITS and second on the LSU values.
[b] Query length (base pairs).

and D-tryptophan are assimilated, but nitrate and glucosamine (N source) are not. Growth without vitamins is variable (Myco 194 is negative and Myco 483 is positive). Formation of starch-like compounds is absent or weak (in both regular and acidified glucose fermentation media). There is growth between 25 and 40°C, but no growth at 42°C. There is no growth with 0.01% cycloheximide and no growth on 50% glucose. Results of urea and diazonium blue B tests are positive.

(iv) Type strain. The type strain is Myco 483 (CBS 10761^T).

(v) Origin of strains. Myco 483 (CBS 10761^T; IHEM 22873; MycoBank accession number 513091) was isolated from infected skin (tinea pedis), Myco 194 (CBS 10333; IHEM 22872) from an infected catheter site, and Myco 643 (CBS 11017; IHEM 22874) from a patient with onychomycosis.

(vi) Clinical origin. *T. dohaense* was isolated from cutaneous specimens. Strain Myco 483 (CBS 10761^T) was isolated from a 34-year-old male patient from India with tinea pedis. The patient had irritated, erythematous scaly lesions on the left lower limb (dorsal and plantar) for 4 years. The patient was successfully treated with oral terbinafine tablets, local econazole cream, and Whitfield's ointment (salicylic acid and benzoic acid).

Antifungal susceptibility testing. Table 3 demonstrates the MIC ranges of amphotericin B and five azole antifungals for 25 *Trichosporon* species isolates. For 15 *T. asahii* isolates, MIC_{50}s and MIC_{90}s are also given. For the majority of isolates, amphotericin B MICs were high and azole MICs were low. The new species *T. dohaense* demonstrated the highest susceptibility to amphotericin B (MICs, 0.5 to 1 mg/liter) and the azoles posaconazole and isavuconazole. There was one *T. asahii* isolate for which the fluconazole MIC was 64 mg/liter and the voriconazole MIC was also higher (2 mg/liter). The new azole isavuconazole was the most potent drug, with the lowest MICs for all species. The echinocandins, caspofungin and anidulafungin (both with MICs of >8 mg/liter), demonstrated no activity against *Trichosporon* species (not shown).

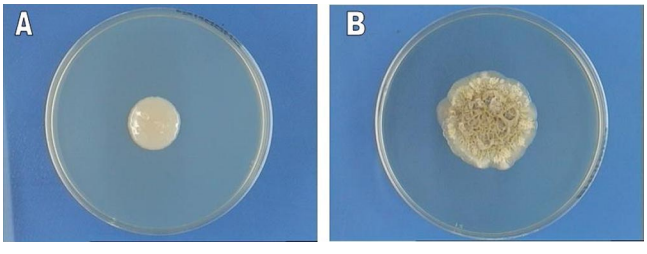

FIG. 2. Colony morphology of *Trichosporon dohaense* strain Myco 194 (CBS 10333), grown on SDA+SP at 37°C. (A) Mucoid appearance at an early stage (96 h) of growth; (B) irregular warty growth after 45 days.

DISCUSSION

The reported clinical cases caused by opportunistic fungal infections are constantly rising, and new species within the genus *Trichosporon* are emerging. Cases of *Guehomyces pullulans* (*T. pullulans*) (17) infection of patients with chronic granulomatous disease (45) or the isolation of this species from the oral cavities of AIDS patients (52) have been reported. *T. mucoides* has been reported to cause infection in a heart and

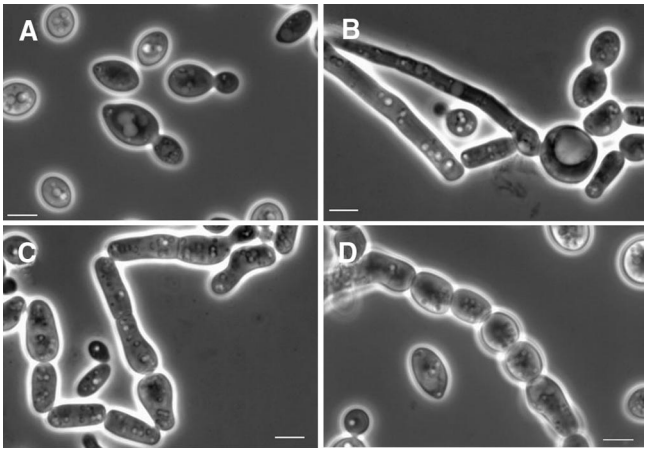

FIG. 3. *T. dohaense*. (A) Yeast cells (CBS 10671^T). (B) Globose cells with hyphae (CBS 10333) in liquid medium (2% glucose in yeast nitrogen base) after 5 days at 25°C. (C) Arthroconidia and yeast cells (CBS 10333) after 5 days in liquid medium (2% glucose in yeast nitrogen base) at 25°C. (D) Arthroconidia (CBS 10671^T) on cornmeal agar after 10 days at 25°C. Phase-contrast optics were used. Bar, 5 μm.

TABLE 3. Results of antifungal susceptibility testing of clinical isolates of *Trichosporon* species

Trichosporon species (no. of isolates)	MIC (mg/liter) of:														
	Amphotericin B			Fluconazole			Itraconazole			Voriconazole			Posaconazole			Isavuconazole		
	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%
T. asahii (15)[a]	2–≥16	8	≥16	0.25–64	4	8	0.063–1	0.25	0.5	0.016–2	0.125	0.25	0.063–0.25	0.25	0.25	0.008–0.5	0.125	0.125
T. faecale (2)	2			4–8			0.25–0.5			0.5			0.25			0.125		
T. inkin (3)	1–4			0.25–4			0.031–0.125			0.031–0.063			0.063–0.25			0.002–0.016		
T. japonicum (2)	4–16			4			0.125			0.063–0.125			0.125–0.25			0.031–0.063		
T. dohaense (3)	1			1–4			0.031–0.25			0.016–0.25			0.031–0.125			0.063–0.25		

[a] Antifungal susceptibility testing was done for 15 of 17 *Trichosporon asahii* isolates.

kidney transplant recipient (51). *T. dermatis* has been reported to cause fungemia in a 13-month-old male with a history of autoimmune enteropathy (25). Five species of *Trichosporon* were reported during this study. *T. asahii* is the most common species associated with clinical specimens in Qatar, representing 62.9% of the cases, while *T. inkin* and *T. dohaense* together account for 11.1%, and *T. faecale* and *T. japonicum* account for 7.4% each. It is worth noting that several species belonging to the Ovoides clade are well-known human pathogens, namely, *T. asahii*, *T. asteroides*, *T. coremiiforme*, *T. faecale*, *T. inkin*, *T. japonicum*, and *T. ovoides* (67). *T. asahii* is isolated mainly from the blood, lung tissue, and urine of patients suffering from deep-seated trichosporonosis (23, 67), but it is also isolated from skin (23) or white piedra (14, 78). *T. asahii* is the most common species, isolated in the present work from a variety of specimens, including urine, nail, skin, tissue, and bone (Table 1). *T. asahii* is thought to be much more common in cases of systemic infection than other *Trichosporon* species (81). Our study supports the view that *T. asahii* is the most common species associated with human clinical specimens and has a wide geographical distribution (2).

Guého et al. (23, 24) significantly revised the taxonomy of the genus *Trichosporon* on the basis of partial 26S rRNA sequences, combined with a reanalysis of morphological and biochemical properties and an analysis of the coenzyme Q system. The genus *Trichosporon* was delineated as containing six clearly differentiated opportunistic pathogens of humans (23): *T. asahii* and *T. mucoides* are known to cause deep invasive infections; *T. asteroides* and *T. cutaneum* cause superficial skin infections; *T. ovoides* causes white piedra of the scalp; and *T. inkin* causes white piedra of the pubic hair. Unfortunately, most of the literature on serious opportunistic trichosporonosis refers to the older nomenclature of *T. beigelii*. Several new taxa have recently been proposed for inclusion in the genus (17, 21, 38, 39, 40–42, 43, 70, 73). The genus *Trichosporon* now comprises 36 species. The number of *Trichosporon* species causing disseminated disease is expanding; *T. asteroides*, *T. loubieri*, and *T. dermatis* have recently been shown to cause disseminated trichosporonosis (25, 33, 38, 55). *Trichosporon* has been reported to be the most common cause of non-*Candida* yeast infections in patients with hematological malignancies, and the infections were associated with high mortality rates, despite antifungal therapy (59). Accurate identification of *Trichosporon* species is important, since different species may have different antifungal susceptibilities (57, 59, 64); *T. asahii*, *T. faecale*, and *T. coremiiforme* exhibited high MICs for amphotericin B, while other species showed lower MICs (64, 65). For most of the *Trichosporon* isolates in our study, high amphotericin B MICs were found, confirming previous results. Although echinocandins are increasingly regarded as the preferred treatment choice for candidemia in patients with severe sepsis and septic shock (58), clinical failure and breakthrough infections with *Trichosporon* have been reported with the use of caspofungin and micafungin (3, 7). In this study, caspofungin and anidulafungin demonstrated no in vitro activity against *Trichosporon* species. The general conclusion is that polyenes and echinocandins should not be used to treat *Trichosporon* infections. The five azoles tested in our series were all active in vitro, confirming previous reports on voriconazole and itraconazole (64). Only one *T. asahii* isolate exhibited a fluconazole

MIC of 64 mg/liter, with a simultaneous increase in the voriconazole MIC (2 mg/liter). In general, itraconazole, voriconazole, posaconazole, and isavuconazole are active against *Trichosporon* species in vitro, with the most potent agent being the new azole isavuconazole.

The assimilation of a large number of carbon and nitrogen compounds traditionally forms the basis for the species identification of yeasts, although the inconsistency of assimilation may cause misleading identification results. The Vitek II and API ID 32 C systems are programmed to identify only three species of *Trichosporon*, namely, *T. asahii*, *T. inkin*, and *T. mucoides.* Consequently, strains may be misidentified, and genetically distinct species could be overlooked (Table 1). The application of modern molecular methods, including the sequencing of rRNA genes, offers a reliable means of overcoming this difficulty (17, 43, 64).

T. inkin is frequently isolated from clinical specimens (66), such as white piedra (13, 23, 76), but also from patients with peritonitis (12, 36, 37), endocarditis (61), lung abscesses (60), subcutaneous nodules (rheumatoid arthritis patients receiving corticosteroid therapy) (65), sternal surgical wound infections (13), and invasive infections (31, 82). In the present study, *T. inkin* was isolated in cases of white piedra, balanitis, and urethral discharge. *T. asteroides* is known to have been isolated from skin (2, 23) and from a patient with a nosocomial bloodstream infection (33). *T. ovoides* is not reported to cause systemic infections but is known to cause white piedra (14), and the species is isolated from the homes of patients with summer-type hypersensitivity pneumonitis (67, 68, 72). The isolation of *Trichosporon* species from various clinical specimens in the present work further suggests that the species-specific patterns of infection previously delineated in *Trichosporon* infection (23) need reconsideration.

Although *T. coremiiforme*, *T. faecale*, and *T. japonicum* have been isolated from the houses of patients with summer-type hypersensitivity pneumonitis (64, 67, 68), they have occasionally been isolated from clinical specimens. More recently, *T. faecale* was isolated from the skin of a tinea pedis patient (27), and *T. japonicum* was isolated from a sputum specimen (1). During this study, *T. japonicum* was isolated in two cases of tinea pedis. *T. faecale* was isolated from two patients: a 32-year old male patient with pyuria and a 6-year old female patient with fungemia (Table 1). To our knowledge, this report describes the first case of fungemia caused by *T. faecale*, which was successfully treated with liposomal amphotericin B (5.8 mg/kg of body weight/day) for 2 weeks. The present study reports the emergence of *T. japonicum* and *T. faecale* as potent human pathogens.

The new species, *T. dohaense* was isolated three times from cutaneous sites (tinea pedis, onychomycosis, and an infected catheter site) during the past 4 years in Doha, Qatar. The emergence of *T. dohaense* as a human pathogen supports the idea that *Trichosporon* species are potent opportunistic human pathogens, and therefore, the recovery of a *Trichosporon* species from a clinical specimen should be regarded as potentially significant. Moreover, the currently available Vitek II yeast identification and API ID 32 C systems are not reliable enough to identify correctly all the clinically relevant *Trichosporon* species, and molecular analysis is required to achieve an accurate identification of the species.

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