Molecular Identification and Susceptibility of *Trichosporon* Species Isolated from Clinical Specimens in Qatar: Isolation of *Trichosporon dohaense* Taj-Aldeen, Meis & Boekhout sp. nov.\(^7\)

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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\(^1\) 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. faeae*, 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\(_{90}\)], \(\geq 16\) mg/liter), and except for fluconazole (MIC\(_{90}\), 8 mg/liter), the azole MICs were low: MIC\(_{90}\) for 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), a bone marrow 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, cystine lactose electrolyte-deficient agar (Mast Diagnostics, United Kingdom) was added for isolation and enumer...
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. Yeast malt extract agar (Difco), yeast morphology agar (YMoA; Difco), malt extract agar (BAP), and potato dextrose agar (PDA; Difco) were used to prepare inocula for substrate assimilation profiles employing the Vitek II yeast ID/32 C system placed in incubators at the appropriate temperatures. The susceptibilities of all the strains to amphotericin B, itraconazole, voriconazole, and isavuconazole (Basilea Pharmaceutica, Basel, Switzerland) were tested using the standard broth microdilution method. The yeast suspension was automatically inoculated into a Vitek II ID/3 system (Biomerieux, France), as recommended by the manufacturer. The yeast suspension was adjusted with a spectrophotometer to 75 to 77% transmittance at 540 nm. A small inoculum from an isolated colony of each isolate was grown on SDA at 35°C for 24 to 48 h, and a stock inoculum was prepared.

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.

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<th>Case no.</th>
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<th>Clinical finding(s)</th>
<th>Patient age/sex</th>
<th>Patient origin</th>
<th>Identification of isolate by the Vitek II yeast ID/32 C system</th>
<th>Closest hit (BLAST)</th>
<th>No. of identical nucleotides/total nucleotides based on the rDNA sequence</th>
<th>Identification</th>
<th>LSU</th>
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<td>T. asahii</td>
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<td>T. japonicum</td>
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<td>T. coremiiforme</td>
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<td>72/M United</td>
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<td>603/603 539/539</td>
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<td></td>
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<tr>
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<td>72/M United</td>
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<td>T. inkin</td>
<td>526/526 508/512</td>
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<td>T. inkin</td>
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</table>

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

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.
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. The patients had one or more preexisting clinical manifestations, such as pyuria, onychomycosis, skin infection, fungemia, or 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 attributed to gender-related factors, such as male patients being more likely to seek medical attention for infections of the skin, nails, and hair. Male patients had one or more preexisting clinical manifestations.

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 skin, 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.

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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]; 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. coremiforme*, 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. coremiforme*, *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. coremiforme*, 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 of *T. dohaense*.

Latin description of *Trichosporon dohaense* Taj-Aldeen, Meis & Bockhout, sp. nov. In medio liquido cellularum zyamoidea globosae vel ellipsoidae, 5.5 ad 9.0 per 3.5 ad 5.0 µm, polaris germinantes. In agar YM oA post 10 dies 25°C, coloniae variabiles, ca. 20 mm diametro, leves vel modice irregularres vel verrucosae, marginem versus sucatae, butyrosae, cremeae vel pallide isabellinae, marginem versus albidae, glabrae vel mycelio aerio albidoe obtectae. Cellularae zyamoidea sicut sopra, nonnumquam etiam et latere, e basi latae germinantes; cellularae maiorae, 5 ad 11 µm diamet 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 fermentant. D-Glucosio, D-galactosio, D-glucosamino (d,w), D-ribosio (+,-d), D-xylosio, D-arabinosio (d), L-arabinosio, succrino, maltosio, trehalosio (+,dw), methyl-D-glucosidio, cellobiosio, salicino (+,dw), arbutino (+,dw), lactosio (dw), raffinosiso (w), melezitosio, amylo solubili, glycero1, meso-erythritol, myo-inositolo (w), 2-keto-D-gluconato, D-gluconato, D-gluconato, succinato, methanol, ethanol, propane 1,2 diolo (dw), butane 2,3 diolo (dw), acido galacturonic (w) utitur; neque L-sorbosio, melibiosio, galactito, D-galacturono, DL-lactato, citrato, acido quinico, saccharato, vel verosimile L-rhamnoso, (w), inilino (w), ribitolo (w), L-arabinonitolo (w), D-gluconitolo (w), D-mannitolo (w), Ethylamino, L-lysino, cadaverino, et D-tryptophano utitur, neque nitrito 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 crescitur, neque in medio 50% glucosi addito; reactiones urei et diazonium blue B positivae. Holotypus CBS 10761T (CBS H-20142), isolatus ex cute hu- manae; depositus in collectione herbario CBS Fungal Biodiversity Centre, Utrecht, The Netherlands.

Description of *Trichosporon dohaense* Taj-Aldeen, Meis & Bockhout 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 globosse or subglobosse to ellipsoidale, 5.5 to 9.0 by 3.5 to 5.0 µm in size, and show polar budding. On YM oA 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...
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-xyllose, D-arabinose (d), L-arabinose, sucrose, maltose, trehalose (+,dw), methyl-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, 

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**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.
and D-tryptophan are assimilated, but nitrate and glucosamine (N source) are not. Growth without vitamins is variable (Mycobacterium 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 10761T).

(v) Origin of strains. Myco 483 (CBS 10333; 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 10761T) 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} and MIC_{90} 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).

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. 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.](image)

![FIG. 3. T. dohaense. (A) Yeast cells (CBS 10671T). (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 10671T) on cornmeal agar after 10 days at 25°C. Phase-contrast optics were used. Bar, 5 μm.](image)
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. beigeli. Several new taxa have recently been proposed for inclusion in the genus (17, 21, 38, 39, 40–42, 43, 67, 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
azole, posaconazole, and isavuconazole are active against MIC of 64 mg/liter, with a simultaneous increase in the voriconazole 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 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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REFERENCES


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