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

Saad J. Taj-Aldeen,1* Nasser Al-Ansari,1 Sittana El Shafei,1 Jacques F. Meis,2 Ilse Curfs-Breuker,2 Bart Theelen,3 and Teun Boekhout4,†

Microbiology Division, Department of Laboratory Medicine and Pathology, Hamad Medical Corporation, P.O. Box 3050, Doha, Qatar,1 and Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, Nijmegen,2 CBS Fungal Diversity Centre, Utrecht,3 and Department of Internal Medicine and Infectious Diseases, University Medical Centre, Utrecht,4 The Netherlands

Received 19 November 2008/Returned for modification 13 February 2009/Accepted 20 March 2009

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 10761T 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 [MIC90], ≥16 mg/liter), and except for fluconazole (MIC90, 8 mg/liter), theazole MICs were low: MIC90s 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-
The susceptibilities of all the strains to amphotericin B 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).

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

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Clinical specimen</th>
<th>Clinical finding(s)</th>
<th>Patient age/sex</th>
<th>Patient origin</th>
<th>Identification of isolate by the Vitek II yeast ID/API 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>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Urine</td>
<td>Pyuria, RBC</td>
<td>26/M India</td>
<td>T. asahii</td>
<td>T. asahii</td>
<td>556/556 439/440</td>
<td>T. asahii</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Urine</td>
<td>Pyuria</td>
<td>63/M Qatar</td>
<td>T. asahii</td>
<td>T. asahii</td>
<td>555/555 458/459</td>
<td>T. asahii</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Urine</td>
<td>Pyuria</td>
<td>32/M Egypt</td>
<td>Trichosporon</td>
<td>Trichosporon</td>
<td>576/577 490/490</td>
<td>T. faeae</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Urine</td>
<td>Pyuria,</td>
<td>23/F Egypt</td>
<td>T. asahii</td>
<td>T. asahii</td>
<td>571/571 550/550</td>
<td>T. asahii</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Urine</td>
<td>Pyuria,</td>
<td>68/M Palestine</td>
<td>T. asahii</td>
<td>T. asahii</td>
<td>555/555 469/471</td>
<td>T. asahii</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Urine</td>
<td>Pyuria</td>
<td>77/F Qatar</td>
<td>T. asahii</td>
<td>T. asahii</td>
<td>554/554 469/471</td>
<td>T. asahii</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Urine</td>
<td>Pyuria</td>
<td>41/M Nepal</td>
<td>T. asahii</td>
<td>T. asahii</td>
<td>576/577 558/558</td>
<td>T. asahii</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Toenail</td>
<td>Distolateral onychomycosis, whitish discoloration</td>
<td>72/M United States</td>
<td>T. asahii</td>
<td>T. asahii</td>
<td>557/557 498/499</td>
<td>T. asahii</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Toenail</td>
<td>Onychomycosis</td>
<td>30/M Egypt</td>
<td>Trichosporon</td>
<td>Trichosporon</td>
<td>556/567 467/469</td>
<td>T. asahii</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Toenail</td>
<td>Onychomycosis</td>
<td>50/M Sudan</td>
<td>T. inkin</td>
<td>T. inkin</td>
<td>533/533 490/490</td>
<td>T. asahii</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Toenail</td>
<td>Onychomycosis</td>
<td>25/M Pakistan</td>
<td>Trichosporon</td>
<td>Trichosporon</td>
<td>515/515 353/356</td>
<td>T. asahii</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Nail</td>
<td>Onychomycosis</td>
<td>25/M Philippines</td>
<td>T. asahii</td>
<td>T. asahii</td>
<td>587/588 565/565</td>
<td>T. asahii</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Toenail</td>
<td>Onychomycosis</td>
<td>70/F Qatar</td>
<td>Trichosporon</td>
<td>Trichosporon</td>
<td>560/561 556/566</td>
<td>T. dohaense</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Ear discharge</td>
<td>Pyus discharge, ear pain</td>
<td>15/M Qatar</td>
<td>T. asahii</td>
<td>T. asahii</td>
<td>557/557 469/470</td>
<td>T. asahii</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Ear discharge</td>
<td>Pyus discharge, ear pain</td>
<td>26/F India</td>
<td>Trichosporon</td>
<td>Trichosporon</td>
<td>556/556 467/469</td>
<td>T. asahii</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Catheter</td>
<td>Catheter site infection</td>
<td>41/M Bangladesh</td>
<td>Trichosporon</td>
<td>Trichosporon</td>
<td>545/546 651/662</td>
<td>T. dohaense</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Skin/intertrigo</td>
<td>Tinea pedis of the diabetic foot</td>
<td>31/M Qatar</td>
<td>T. asahii</td>
<td>T. japonicum</td>
<td>571/571 466/466</td>
<td>T. japonicum</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Scalp hair</td>
<td>White piedra</td>
<td>28/F Qatar</td>
<td>Trichosporon</td>
<td>Trichosporon</td>
<td>600/600 498/502</td>
<td>T. inkin</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Skin scraping</td>
<td>Tinea pedis</td>
<td>34/M India</td>
<td>Trichosporon</td>
<td>Trichosporon</td>
<td>560/561 549/560</td>
<td>T. dohaense</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Tissue</td>
<td>Leg cellulitis</td>
<td>18/M Pakistan</td>
<td>Trichosporon</td>
<td>Trichosporon</td>
<td>581/582 541/541</td>
<td>T. asahii</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Skin</td>
<td>Tinea pedis</td>
<td>33/M Qatar</td>
<td>Trichosporon</td>
<td>Trichosporon</td>
<td>621/621 541/541</td>
<td>T. japonicum</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Bone</td>
<td>Osteomyelitis</td>
<td>65/M India</td>
<td>Trichosporon</td>
<td>Trichosporon</td>
<td>585/586 557/559</td>
<td>T. asahii</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Blood</td>
<td>Fungemia</td>
<td>6/F Qatar</td>
<td>Trichosporon</td>
<td>Trichosporon</td>
<td>620/621 522/522</td>
<td>T. faeae</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Swab</td>
<td>Urethral discharge</td>
<td>28/M Kenya</td>
<td>Trichosporon</td>
<td>Trichosporon</td>
<td>603/603 539/539</td>
<td>T. inkin</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>Swab</td>
<td>Balanitis</td>
<td>39/M India</td>
<td>T. asahii</td>
<td>T. asahii</td>
<td>526/526 508/512</td>
<td>T. cf inkin</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>Bronchial lavage</td>
<td>Mucoid tracheal</td>
<td>52/M Unknown</td>
<td>Trichosporon</td>
<td>Trichosporon</td>
<td>424/424 498/498</td>
<td>T. asahii</td>
<td></td>
</tr>
</tbody>
</table>

Notes:

- **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.

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, econazole (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 1:3,000 dilution of the suspension in RPMI 1640 (GIBCO BRL, Life Technologies, Wroclaw, The Netherlands) to produce the final test concentration of 1 × 10³ to 5 × 10³ CFU/ml. Aliquots (100 µl) of the diluted suspension were inoculated into 96-well flat-bottom microtiter plates (Costar; Omnilabo Internation 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 Anthon HT3 spectrophotometer (Salzburg, Austria) and the MicroWin 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 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 10761T; 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 nitrate (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 & Bockhout, sp. nov.
In medio liquido cellulae zymoideae globosae vel ellipsoideae, 5.5 ad 9.0 et 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 irregularres vel verrucosae, margine versus sulcatae, butyrosea, cremae vel pallide isabellinae, margine versus albidae, gliabae vel meycelio aerio albido obtectae. Cellulae zymoideae sicut sorspra, nonnumquam etiam e latere, e basi latae gemmantes; coloniae maxores, 5 ad 11 μm diametet et filamenta ad 90 per 2.0 ad 3.0 μm praesentes; hyphae vel pseudohyphae nonnumquam praeentes; arthroconidia cylindrica, magnitudine variabilis, 5 ad 20 per 2 ad 4 μm. Non fermentant. D-Glucosio, D-galactosio, D-glucosaminio (dw), D-ribobio (+,-d), D-xylolbio, D-arabinosio (d), L-arabinosio, sucroisio, maltosio, trehalosio (+,-dw), methyl-D-glucosidio, cellubiosio, salicino (+,-dw), arbutino (+,-dw), lactosio (dw), raffinosio (w), melezitosio, amylo solubili, glycerolo, meso-erythritolio, myo-inositolio (w), 2-keto-D-glucanato, D-glucanato, D-glucuronato, succinato, methanolo, ethanololo, propante 1,2 dioio (dw), butane 2,3 diio (dw), acido galactuconico (+,-d utitur; neque L-sorbosio, melibiosio, galactitolo, D-galacturonato, DL-lactato, citrato, acido quinico, saccharato, vel versusimile L-rhamnosio (w), inilino (w), ribitolo (w), L-arabinolito (w), D-glucolito (w), D-mannitolito (w), Ethylamino, L-lysino, cadaverino, et D-tryptophano uti tur, neque nitrato et glucosaminio. Vitaminis absentibus crescere potest an non. Substantia amyloidea vix formatur. Temperaturis 25 ad 40°C crescere potest, neque 42°C; 0.01% cycloheximido ad medio haud crescit, neque in medio 50% glucosii adido; reaciones urei et diazoniolumo blue positiae. Holotypus CBS 10761T (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 & 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 globosa or subglobosa to ellipsoida, 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

---

Vol. 47, 2009
Molecular Identification of Trichosporon Species

1793

---

http://jcm.asm.org/content/47/10/1793/abstract

Received October 2, 2017. Accepted by guest

Downloaded from http://jcm.asm.org on October 2, 2017 by guest
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-D-glucoside, cellobiose, salicin (+,dw), arbutin (+,dw), lactose (d), 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 (+,d), inulin (w), ribitol (w), L-arabinitol (w), D-glucitol (w), D-mannitol (w). Ethylamine, L-lysine, cadaverine,
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 10761T).

(v) Origin of strains. Myco 483 (CBS 10333; IHEM 22872; MycoBank accession number 513091) was isolated from infected skin (tinea pedis), Myco 194 (CBS 10333; IHEM 22873) 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<sub>50</sub> and MIC<sub>90</sub> 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...
TABLE 3. Results of antifungal susceptibility testing of clinical isolates of Trichosporon species

<table>
<thead>
<tr>
<th>Species</th>
<th>MIC (mg/liter) Range</th>
<th>50%</th>
<th>90%</th>
<th>MIC (mg/liter) Range</th>
<th>50%</th>
<th>90%</th>
<th>MIC (mg/liter) Range</th>
<th>50%</th>
<th>90%</th>
<th>MIC (mg/liter) Range</th>
<th>50%</th>
<th>90%</th>
<th>MIC (mg/liter) Range</th>
<th>50%</th>
<th>90%</th>
<th>MIC (mg/liter) Range</th>
<th>50%</th>
<th>90%</th>
<th>MIC (mg/liter) Range</th>
<th>50%</th>
<th>90%</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. asahii</td>
<td>50%  16–8</td>
<td>25</td>
<td>0.25</td>
<td>90%  0.063–0.25</td>
<td>0.125</td>
<td>0.25</td>
<td>50%  0.031–0.063</td>
<td>0.063</td>
<td>0.25</td>
<td>90%  0.008–0.016</td>
<td>0.0125</td>
<td>0.0125</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. faecale</td>
<td>50%  4–8</td>
<td>25</td>
<td>0.25</td>
<td>90%  0.002–0.016</td>
<td>0.008</td>
<td>0.0125</td>
<td>50%  0.003–0.0063</td>
<td>0.0063</td>
<td>0.0125</td>
<td>90%  0.003–0.0063</td>
<td>0.0063</td>
<td>0.0125</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. inkin</td>
<td>50%  1–4</td>
<td>25</td>
<td>0.25</td>
<td>90%  0.003–0.0063</td>
<td>0.0063</td>
<td>0.0125</td>
<td>50%  0.003–0.0063</td>
<td>0.0063</td>
<td>0.0125</td>
<td>90%  0.003–0.0063</td>
<td>0.0063</td>
<td>0.0125</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. japonicum</td>
<td>50%  4–16</td>
<td>25</td>
<td>0.25</td>
<td>90%  0.063–0.125</td>
<td>0.125</td>
<td>0.25</td>
<td>50%  0.063–0.125</td>
<td>0.125</td>
<td>0.25</td>
<td>90%  0.063–0.125</td>
<td>0.125</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. dohaense</td>
<td>50%  0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>90%  0.031–0.063</td>
<td>0.063</td>
<td>0.25</td>
<td>50%  0.031–0.063</td>
<td>0.063</td>
<td>0.25</td>
<td>90%  0.031–0.063</td>
<td>0.063</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Antifungal susceptibility testing was done for 15 of 17 T. 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.

Gueho 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, 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 distinct species, and molecular analysis is required to achieve an accurate identification of the species.

ACKNOWLEDGMENT

We gratefully thank Walter Gams, CBS Fungal Biodiversity Institute, The Netherlands, for the Latin text.

REFERENCES

22. Guèho, E., M. T. Smith, G. S. de Hoog, G. Billo-Grand, R. Christen, and


Sugita, T., A. Nishikawa, R. Ikeda, and T. Shinoda. 1999. Identification of medically relevant Trichosporon species based on sequences of internal tran-


