**Rhodotorula** spp. isolated from blood cultures: clinical and microbiological aspects

GISELE M. DUBOC DE ALMEIDA*, SILVIA FIGUEIREDO COSTA†, MARCIA MELHEM‡, ADRIANA L. MOTTA*, MARIA WALDEREZ SZESZ§, FUMIKO MIYASHITA‡, LIGIA C. PIERROTTI§, FLÁVIA ROSSI* & MARCELO N. BURATTINI*

*Microbiology Laboratory, Division of Central Laboratory, LIM 03 Department of Pathology, University of São Paulo, †LIM 54 Department of Infectious Diseases, University of São Paulo, ‡Adolfo Lutz Institute, Secretary of Health, São Paulo, and §Department of Infectious Diseases, University of São Paulo, São Paulo, Brazil

The emergence of less common fungal pathogens has been increasingly reported in the last decade. We describe 25 cases of *Rhodotorula* spp. isolated from blood cultures at a large Brazilian tertiary teaching hospital from 1996–2004. We also investigated the *in vitro* activity of four antifungal drugs, using a standardized method. The median age of patients was 43 years. The majority of patients (88%) had a central venous catheter (CVC) and 10 (40%) were recipients of a bone marrow transplant. The episode was classified as a bloodstream infection (BSI) in 80% of the patients. Amphotericin B deoxycholate was the most common antifungal used and CVC was removed in 89.5% of the patients. Death occurred in four patients (17.4%), all classified as BSI. All strains were identified as *R. mucilaginosa* by conventional methods. Misidentification of the species was observed in 20% and 5% of the strains with the Vitek Yeast Biochemical Card and API 20C AUX systems, respectively. Amphotericin B demonstrated good *in vitro* activity (MIC₅₀/ₙ₉₀, 0.5 μg/ml) and the MICs for fluconazole were high for all strains (MIC₅₀/ₙ₉₀ > 64 μg/ml).

**Keywords** *Rhodotorula*, bloodstream infections, clinical characteristics, antifungal susceptibility testing

---

**Introduction**

Fungal infections related to healthcare assistance have become a major medical issue in the last decades. Although the majority of these infections are caused by *Candida* and *Aspergillus* species, an increasing number of infections are caused by less common pathogens [1–4]. These fungi have been referred to by some authors as ‘emerging pathogens’. Many of them have been traditionally considered as laboratory contaminants and/or of low virulence. These pathogens include yeasts other than *Candida* species and a wide variety of dematiaceous and hyaline moulds.

*Rhodotorula* spp. have been increasingly recognized as opportunistic pathogens, capable of causing invasive infections in the appropriate setting. Over the past 40 years, over a hundred cases of *Rhodotorula* infections have been described [5–11].

*Rhodotorula* is classified in the family *Sporidiobolaceae* sharing many characteristics with yeasts from the genus *Cryptococcus*. It typically produces mucoid colonies with a distinct carotenoid pigment and is widely distributed in the environment. It has been isolated from soil, air, aquatic ecosystems, plants and fruits. In the household environment, it was cultured from swabs of toothbrushes and shower curtains. In humans, this yeast has been isolated from the skin, nails and respiratory, gastrointestinal and genital tracts usually representing harmless colonization [1,12–17].
The most frequent infections caused by these yeasts are bloodstream infections (BSI) which occur mainly in immunosuppressed patients and in the presence of central venous catheters (CVC) [18,19]. However, it is important to emphasize that well designed studies addressing specific risk factors for Rhodotorula-associated BSI have not yet been conducted.

Few publications have addressed the susceptibility of Rhodotorula to antifungal agents using standardized methodology. Previous studies demonstrated amphotericin B and flucytosine to have good in vitro activity whereas fluconazole and echinocandins displayed limited activity [20,21]. The purpose of this study was to describe the occurrence of Rhodotorula isolated from blood cultures in our institution, to present the clinical profile of affected patients and to determine the MIC of common antifungal agents against the isolates.

Materials and methods

Setting

The study took place at Hospital das Clinicas, a 2200 bed tertiary teaching complex, located in the city of Sao Paulo, Brazil. It is comprised of six buildings attached to the University of Sao Paulo Medical School providing care for a diverse population including bone marrow and solid organ transplant and other immunosuppressed patients. In the outpatient building, there are two day hospital units: one unit for cancer patients under chemotherapy (AQTDH) and other (ATMO) for bone marrow transplant (BMT) patients. The protocol was approved by the local institutional review board.

Case finding

Records from the microbiology laboratory served to identify patients with positive blood cultures for Rhodotorula species from 1 January 1996 to 31 December 2004.

Study design

A retrospective study was conducted and medical charts of all patients identified were reviewed using a standardized data extraction form. The following variables were evaluated: demographic features, underlying disease, presence and type of transplantation, patient location at the time of positive blood culture (intensive care unit, medical or surgical unit, ATMO or AQTDH), acute physiology and chronic health evaluation (APACHE) II score (at the index blood culture) [22]; presence, duration and type of CVC, polymicrobial positive blood cultures; parenteral nutrition and use of corticosteroids, other cytotoxic agent and chemotherapy in the previous month. Presence and duration of neutropenia, exposure to antimicrobials and antifungal agents were recorded considering the two weeks prior to the isolation of Rhodotorula from blood culture. In the follow-up period, data whether or not CVC was removed (including time of removal), antifungal treatment (institution, type and duration) as well as outcome (survival or death) were also recorded.

Definitions

- Index blood culture was defined as the first positive blood culture for Rhodotorula sp.
- BSI was defined as the isolation of Rhodotorula sp. in ≥1 blood cultures from a patient with signs and symptoms of infection in the absence of any other likely source of infection [23]. The isolation of Rhodotorula from blood cultures >30 days of the index blood culture from a patient with clinical signs of infection was defined as a new case. Cases occurring either prior to or within 2 days of hospital admission were considered outpatient acquired.
- Neutropenia was defined as an absolute neutrophil count less than 500 cells/mm³.
- Use of corticosteroids: use of ≥20 mg prednisone per day for ≥30 days (or equivalent doses of other steroids) or cumulative dose >600 mg in the previous 30 days.
- Polymicrobial blood culture was defined as the isolation of other organisms (bacteria or other fungal species) in addition to Rhodotorula, either from the same blood culture or from multiple blood cultures performed during a single episode of fever or chills.
- Follow-up period was defined as the time elapsed between the index blood culture and the next thirty days, or death or until patient was discharged.

Microbiological analyses

Blood and CVC tip cultures. Blood cultures were collected from a peripheral vein and/or from a CVC at the discretion of the attending physician and processed according to standard microbiologic techniques. Cultures were monitored using an automated culture system (Bactec 9240 System, Becton, Dickinson and Company, Sparks, MD, USA). Catheter tips were cultured by the semiquantitative roll-plate method [24].

Organism identification. All fungal isolates were identified according to the routine method used in the mycology laboratory. An automated system (Vitek...
System, bioMerieux, Durham, NC, USA) was used according to manufacturer instructions. Supplementary standard morphological and biochemical testing were performed as needed [25]. Isolates were stored frozen in 20% glycerol at either −20°C or −70°C until the study was performed. Strains stored in the mycology bank were further subcultured onto potato dextrose agar (Remel, Lenexa, KS, USA) and CHROMagar™ Candida (Hardy Laboratories, Santa Maria, CA, USA) to ensure viability and purity. Confirmation of species identification was performed using Vitek Yeast Biochemical Card (YBC) and API 20C AUX systems (bioMerieux) as recommended by the manufacturer. Results were compared with conventional methods (carbohydrate/nitrate assimilation tests, colonial morphology, absence of ballistoconidia and pseudohyphae, urease production and growth at 37°C). These conventional methods were used as the reference method. Discrepancies between commercial and conventional methods were subjected to a repeat test.

Susceptibility testing

(a) Antifungal agents and microdilution panels. Standard powders of fluconazole and voriconazole (Pfizer, New York, NY, USA), itraconazole (Janssen, Beerse, Belgium) and amphotericin B (Bristol-Myers Squibb, Princeton, NJ, USA) were obtained from their respective manufacturers. Stock solutions were prepared in water (fluconazole) or dimethyl sulfoxide (amphotericin, voriconazole, itraconazole). Reference microdilution trays containing serial dilutions of the antifungal agents in MOPS (morpholinepropanesulfonic acid)-buffered RPMI 1640 medium (Sigma Chemical Co., St Louis, MO, USA) were prepared according to the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) [26]. Trays containing an aliquot of 0.1 ml in each well were sealed and frozen at −70°C until they were used in the study.

(b) Antifungal susceptibility test methods. Antifungal susceptibility testing was performed by the reference broth microdilution method as outlined in CLSI document M27-A2 [26]. Final concentrations of the antifungal agents were 0.015–8 μg/ml for amphotericin B, 0.016–8 μg/ml for itraconazole and voriconazole, and 0.0125–64 μg/ml for fluconazole. The yeast inoculum was adjusted to match the turbidity of a 0.5 McFarland standard (OXOID, Basingstoke, UK) by using a Vitek colorimeter (bioMerieux). This yeast suspension was further diluted 1:1000 in RPMI 1640 medium to obtain a 2 × test concentration and an aliquot of 0.1 ml was added to each well of the microdilution tray (final inoculum, 0.5 × 10³ to 2.5 × 10³ cells/ml). The trays were incubated at 35°C, and the MICs were determined spectrophotometrically at 530 nm after 72 h of incubation. For voriconazole, fluconazole and itraconazole, the MIC endpoint was defined as the lowest concentration which produced 50% or more reduction of growth compared with that of the growth control well. For amphotericin, the MIC endpoint was defined as 90% of reduction of the growth.

(c) Quality controls. Quality control was ensured by testing the strains recommended by CLSI Candida krusei ATCC 6258 and Candida parapsilosis ATCC 22019 in each batch of MIC testing.

Statistical analysis

To calculate incidence rates, the numbers of hospital admissions were collected. For BMT patients, incidence was calculated by using the number of BMT performed during the same period as a denominator. The incidence rates were calculated as the number of Rhodotorula-associated BSI per 1000 admissions and per 1000 transplants. Chi-square test for linear trend was used. P value ≤ 0.05 was considered to be statistically significant. Registration and analysis of data were performed by EpiInfo 6.04 software (Centers for Disease Control and Prevention).

Results

Rhodotorula spp. were isolated from blood cultures of 28 patients from 1996–2004. The percentage of Rhodotorula isolates from blood cultures during the study period related to the total number of fungal isolates, most frequent genera, other yeasts, and filamentous fungi can be observed in Table 1. The clinical charts of three patients could not be found and they were excluded from the study.

Clinical characteristics

The clinical characteristics of the 25 patients with Rhodotorula isolated from blood cultures are shown in Tables 2 and 3. They were predominantly adults, with a median age of 43 years (range: 22 days to 70 years). Seven of these (28%) patients were younger than 15 years of age including a newborn. The majority of patients had cancer as the underlying disease (72%, 18/25). Of these, 94.4% had a diagnosis of hematological malignancy with non-Hodgkin’s lymphoma being the most frequent type. At the time of fungemia, 33% of patients were hospitalized in different surgical and clinical units. The length of hospitalization before isolation of Rhodotorula varied from 1–41 days.
(mean, 14.7 days). The remaining patients were being followed at one of the two day hospital units (50% in the ATMO). The APACHE II score of 12 patients calculated at the index blood culture was 11 ± 4.7 (mean ± standard deviation).

Bone marrow transplant in the previous year or within two weeks after the first positive blood culture was present in ten patients (40%), being 30% allogeneic and 70% autologous. The time between BMT and isolation of *Rhodotorula* on blood culture ranged from

---

**Table 1** Number of fungal isolates from blood cultures at Hospital das Clinicas from January 1996 to December 2004 and percentage of *Candida* spp., *Cryptococcus neoformans*, *Rhodotorula* spp., *Pichia anomala*, other yeasts and filamentous fungi.

<table>
<thead>
<tr>
<th>Year</th>
<th>Total†</th>
<th><em>Candida</em> spp. N(%)</th>
<th><em>C. neoformans</em> N(%)</th>
<th><em>P. anomala</em>† N(%)</th>
<th><em>Rhodotorula</em> spp. N(%)</th>
<th>Other yeasts N(%)</th>
<th>FF N(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996</td>
<td>128</td>
<td>102 (79.7)</td>
<td>11 (8.6)</td>
<td>5 (3.9)</td>
<td>5 (3.9)</td>
<td>2 (1.6)</td>
<td>3 (2.3)</td>
</tr>
<tr>
<td>1997</td>
<td>129</td>
<td>102 (79.0)</td>
<td>14 (10.9)</td>
<td>1 (0.8)</td>
<td>4 (3.1)</td>
<td>4 (3.1)</td>
<td>4 (3.1)</td>
</tr>
<tr>
<td>1998</td>
<td>130</td>
<td>98 (75.4)</td>
<td>11 (8.5)</td>
<td>8 (6.2)</td>
<td>2 (1.5)</td>
<td>9 (6.9)</td>
<td>2 (1.5)</td>
</tr>
<tr>
<td>1999</td>
<td>108</td>
<td>91 (84.2)</td>
<td>10 (9.3)</td>
<td>1 (0.9)</td>
<td>1 (0.9)</td>
<td>2 (1.9)</td>
<td>3 (2.8)</td>
</tr>
<tr>
<td>2000</td>
<td>128</td>
<td>110 (85.9)</td>
<td>6 (4.7)</td>
<td>1 (0.8)</td>
<td>8 (3.1)</td>
<td>7 (5.5)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>2001</td>
<td>131</td>
<td>109 (83.3)</td>
<td>7 (5.3)</td>
<td>7 (5.3)</td>
<td>3 (2.3)</td>
<td>2 (1.5)</td>
<td>3 (2.3)</td>
</tr>
<tr>
<td>2002</td>
<td>121</td>
<td>105 (86.8)</td>
<td>5 (4.1)</td>
<td>2 (1.7)</td>
<td>4 (3.3)</td>
<td>1 (0.8)</td>
<td>4 (3.3)</td>
</tr>
<tr>
<td>2003</td>
<td>139</td>
<td>122 (87.8)</td>
<td>9 (6.4)</td>
<td>0 (0.0)</td>
<td>3 (2.2)</td>
<td>3 (2.2)</td>
<td>2 (1.4)</td>
</tr>
<tr>
<td>2004</td>
<td>181</td>
<td>158 (87.3)</td>
<td>6 (3.3)</td>
<td>7 (3.9)</td>
<td>2 (1.1)</td>
<td>5 (2.8)</td>
<td>3 (1.6)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1195</td>
<td>997 (83.4)</td>
<td>79 (6.6)</td>
<td>32 (2.7)</td>
<td>28 (2.3)</td>
<td>35 (3.0)</td>
<td>24 (2.0)</td>
</tr>
</tbody>
</table>

†Number of fungal isolates, duplicates excluded; †Outbreak of *Candida* spp. occurred in 2004; *Outbreaks of *P. anomala* occurred on 1998, 2001 and 2004. Other yeasts, distinct genera or the same species with less than two isolates per year. FF, filamentous fungi.

---

**Table 2** Summary of 25 cases of *Rhodotorula* isolated from blood cultures at Hospital das Clinicas, from 1996-2004.

<table>
<thead>
<tr>
<th>Patient; year</th>
<th>Sex/age</th>
<th>Underlying Disease</th>
<th>Unit</th>
<th>BMT</th>
<th>CVC</th>
<th>Antifungal therapy</th>
<th>CVC removed</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1; 1996</td>
<td>M/53</td>
<td>NH Lymphoma</td>
<td>ATMO</td>
<td>Yes</td>
<td>Yes</td>
<td>Amphotericin</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>2; 1996</td>
<td>F/8</td>
<td>Aplastic Anemia</td>
<td>ATMO</td>
<td>Yes</td>
<td>Yes</td>
<td>?</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>3; 1996</td>
<td>M/7</td>
<td>CNS tumor</td>
<td>Clinical</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>4; 1996</td>
<td>M/47</td>
<td>Cirrhosis</td>
<td>Surgical</td>
<td>No</td>
<td>Yes</td>
<td>Amphotericin</td>
<td>Yes</td>
<td>Died</td>
</tr>
<tr>
<td>5; 1996</td>
<td>F/39</td>
<td>Acute Leukemia</td>
<td>AQTDAH</td>
<td>No</td>
<td>Yes</td>
<td>Amphotericin</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>6; 1997</td>
<td>M/58</td>
<td>Burkitt Lymphoma</td>
<td>Clinical</td>
<td>Yes</td>
<td>Yes</td>
<td>Amphotericin</td>
<td>Yes</td>
<td>Died</td>
</tr>
<tr>
<td>7; 1997</td>
<td>M/43</td>
<td>NH Lymphoma</td>
<td>AQTDAH</td>
<td>No</td>
<td>Yes</td>
<td>Amphotericin</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>8; 1997</td>
<td>F/21</td>
<td>Hodgkin's Lymphoma</td>
<td>AQTDAH</td>
<td>No</td>
<td>Yes</td>
<td>Amphotericin</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>9; 1998</td>
<td>M/19</td>
<td>_ – *</td>
<td>Surgical</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>10; 1999</td>
<td>M/3</td>
<td>Falciform Anemia</td>
<td>Clinical</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>11; 2000</td>
<td>M/70</td>
<td>Short Bowel</td>
<td>Surgical</td>
<td>No</td>
<td>Yes</td>
<td>Fluconazole</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>12; 2000</td>
<td>M/53</td>
<td>NH Lymphoma</td>
<td>ATMO</td>
<td>No</td>
<td>Yes</td>
<td>#</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>13; 2000</td>
<td>M/53</td>
<td>Acute Leukemia</td>
<td>AQTDAH</td>
<td>No</td>
<td>Yes</td>
<td>Amphotericin</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>14; 2000</td>
<td>M/14</td>
<td>NH Lymphoma</td>
<td>Clinical</td>
<td>Yes</td>
<td>Yes</td>
<td>Amphotericin</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>15; 2001</td>
<td>M/51</td>
<td>Acute Leukemia</td>
<td>AQTDAH</td>
<td>Yes</td>
<td>Yes</td>
<td>Amphotericin</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>16; 2001</td>
<td>M/50</td>
<td>NH Lymphoma</td>
<td>ATMO</td>
<td>Yes</td>
<td>Yes</td>
<td>Amphotericin</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>17; 2002</td>
<td>M/49</td>
<td>Acute Leukemia</td>
<td>ATMO</td>
<td>Yes</td>
<td>Yes</td>
<td>Amphotericin</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>18; 2002</td>
<td>F/41</td>
<td>Hodgkin's Lymphoma</td>
<td>AQTDAH</td>
<td>No</td>
<td>Yes</td>
<td>Amphotericin</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>19; 2002</td>
<td>F/48</td>
<td>Acute Leukemia</td>
<td>ATMO</td>
<td>Yes</td>
<td>Yes</td>
<td>Amphotericin</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>20; 2002</td>
<td>M/22 days</td>
<td>Congenital liver disease</td>
<td>ICU</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Died</td>
</tr>
<tr>
<td>21; 2003</td>
<td>M/7</td>
<td>Aplastic Anemia</td>
<td>ATMO</td>
<td>Yes</td>
<td>Yes</td>
<td>?</td>
<td>Yes</td>
<td>Died</td>
</tr>
<tr>
<td>22; 2003</td>
<td>F/47</td>
<td>Acute Leukemia</td>
<td>Clinical</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>23; 2003</td>
<td>M/55</td>
<td>NH Lymphoma</td>
<td>ATMO</td>
<td>Yes</td>
<td>Yes</td>
<td>Fluconazole</td>
<td>Yes</td>
<td>Survived</td>
</tr>
<tr>
<td>24; 2004</td>
<td>M/67</td>
<td>NH Lymphoma</td>
<td>AQTDAH</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Survived</td>
</tr>
<tr>
<td>25; 2004</td>
<td>M/37</td>
<td>NH Lymphoma</td>
<td>AQTDAH</td>
<td>No</td>
<td>Yes</td>
<td>Fluconazole Amphotericin</td>
<td>Yes</td>
<td>Survived</td>
</tr>
</tbody>
</table>

HOD, Hodgkin's Lymphoma; NH, non-Hodgkin's; AQTDAH, chemotherapy day hospital unit; ATMO, bone marrow outpatient clinic; ICU, Intensive Care Unit; *patient 9 with liver abscess due to *S. aureus*; discharged 24 hours after withdrawing blood cultures; # patient number 12 was transferred to another hospital; ? Unknown.

© 2008 ISHAM, Medical Mycology, 46, 547–556
Transplant; CVC, central venous catheter.

Table 3 Main characteristics of 25 patients with Rhodotorula isolates from blood cultures at Hospital das Clinicas from 1996–2004.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (years)</td>
<td>43 (9)</td>
</tr>
<tr>
<td>Male sex</td>
<td>76 (30/25)</td>
</tr>
<tr>
<td>Underlying disease</td>
<td>96 (43/25)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>44 (17/25)</td>
</tr>
<tr>
<td>Leukemia</td>
<td>24 (10/25)</td>
</tr>
<tr>
<td>Others</td>
<td>28 (11/25)</td>
</tr>
<tr>
<td>BMT</td>
<td>40 (16/25)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>13 (05/23)</td>
</tr>
<tr>
<td>&lt;100 cels /mm³</td>
<td>13 (03/23)</td>
</tr>
<tr>
<td>CVC</td>
<td>88 (22/25)</td>
</tr>
<tr>
<td>Duration of CVC</td>
<td></td>
</tr>
<tr>
<td>1–45 days</td>
<td>26 (05/19)</td>
</tr>
<tr>
<td>46–90 days</td>
<td>32 (16/19)</td>
</tr>
<tr>
<td>&gt;90 days</td>
<td>42 (08/19)</td>
</tr>
<tr>
<td>Parenteral nutrition</td>
<td>12 (03/25)</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>43 (10/23)</td>
</tr>
<tr>
<td>Cytotoxic drugs</td>
<td>43 (10/23)</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>52 (12/23)</td>
</tr>
<tr>
<td>Use of antibiotics</td>
<td>48 (11/23)</td>
</tr>
<tr>
<td>Use of antifungal</td>
<td>08 (02/23)</td>
</tr>
</tbody>
</table>

Except for age, data are expressed as the percentage of patients with the variable (n=number of patients with the variable/total patients with available information of the variable); BMT, bone marrow transplant; CVC, central venous catheter.

—11 to +240 days (mean, 61.4 days). In only one case, Rhodotorula isolation occurred on the 470th day after BMT.

Central venous catheter was present in 88% of the patients (14 had the semi-implantable Hickman type). The average time that the catheter was inserted prior to isolation of Rhodotorula in blood culture was 86.5 days (range, 4–261 days).

Neutropenia was present in three patients with a mean duration of seven days preceding the index blood culture. Eleven patients were on previous antibiotic therapy; third generation cephalosporin and glycopeptides were the most commonly used drugs. The mean duration of antibiotic therapy was 9.5 days. Only two patients were using parenteral antifungal drugs before the index blood culture (i.e. fluconazole 200 mg for three days and prophylactic amphotericin B for seven days).

The average number of positive blood cultures per patient was 2.6 (range, 1–4). Polymicrobial blood culture occurred in 28% of patients (7/25). The following microorganisms were isolated concurrently with Rhodotorula sp. from blood cultures: Acinetobacter spp. + Staphylococcus aureus (1), Candida albicans (1), Enterobacter cloacae (1), Staphylococcus aureus (1), Staphylococcus haemolyticus (1), Pseudomonas aeruginosa+viridans Streptococcus (1) and Kodamaea ohmeri (1).

The isolation of Rhodotorula sp. from blood culture was classified as BSI in 20 patients (80%). No patient had more than one episode of BSI. The infection was considered outpatient-acquired in 70% of the cases. The overall incidence of Rhodotorula BSI during the study period was 0.059 cases per 1000 admissions. In the BMT population, the overall incidence of BSI was 9.58 cases per 1000 transplants. During the study period, no trending towards an increase of Rhodotorula-associated BSI was observed (p = 0.55; for BMT patients, p = 0.29).

Follow-up period

Catheter removal and antifungal use in patients classified as non-BSI. Among the non-BSI (five patients), two had a CVC and were recently hospitalized for BMT (Table 2, patients 22 and 23). These patients had blood cultures drawn from their central line in the absence of signs and symptoms of infection as a routine protocol of the Hematology Service. Although the episodes were not considered BSI, both patients had their CVC removed (5 and 8 days after the index blood culture) and one received 12 days of parenteral fluconazole therapy followed by amphotericin B (total dose of 350 mg).

Catheter removal and antifungal use in patients classified as BSI. Of the 20 patients with BSI, all patients had a CVC at the onset of infection. Information about CVC removal was available for 19 patients. Of these, only two patients did not have their central line removed. One patient had no other possible access site due to local venous thrombosis and the other patient was a neonate who died four days after the positive blood culture. The infant had concomitant candidemia without the physician’s knowledge of culture results. For all remaining patients, the time between the first positive blood culture and catheter removal ranged from 1–37 days. The catheter tip was positive in only two cases.

Information about antifungal therapy was available for 17 of 20 patients. Fifteen (88%) patients received antifungal treatment. The delay between the first positive blood culture and the start of therapy varied from 1–27 days (mean, 9.1 days) and 93% were treated with amphotericin B; liposomal formulation was administered to only one patient. The average duration of therapy was 13.4 days and the total dose varied from 60–930 mg (mean, 450 mg). One patient was treated with 400 mg of parenteral fluconazole for five days and discharged with a recommendation to complete 10 more days of oral fluconazole (Table 2, patient 11).
Outcome

Two patients were lost to follow-up. Of the remaining 23 patients, 4 (17.4%) died within two weeks of a positive blood culture. All were considered to have a BSI. They also had a CVC inserted 4–261 days prior to the isolation of the yeast (mean, 73.2 days). Three of them had their CVC removed after 3, 10 and 19 days of the index blood culture, respectively. One was the neonate previously described, with concomitant C. albicans, who did not receive antifungal treatment. Disseminated fungal infection was found on his necropsy. The second was a cirrhotic patient with nosocomial pneumonia.

The third was a 46-year-old man who died on the 25th day after auto-BMT. Rhodotorula was isolated from peripheral blood cultures starting a week before BMT and repeatedly after the transplant. He became afibrile only after the catheter removal (19 days after the index blood culture), despite the use of a broad spectrum of antibiotics and a cumulative dose of 750 mg of amphotericin and complete recovery of neutropenia. Acinetobacter sp. was isolated from blood three days before CVC removal. He died a week after CVC removal with shock and respiratory distress syndrome. The last case was a seven-year-old boy on the 240th day after auto-BMT, not neutropenic, who died five days after isolation of Rhodotorula. He had an earlier CVC removal but no information about antifungal treatment. The results of the bronchoalveolar lavage performed a day before he died were positive for Rhodotorula and Pneumocystis (carinii) jiroveci.

Microbiology identification

Initially, the Vitrek YBC identified 15 (60%) strains as R. mucilaginosa, 7 (28%) as R. glutinis and 3 (12%) as Rhodotorula sp. Only 20 isolates were available for further re-identification. Of these, all had demonstrated growth at 37°C, absence of ballistoconidia and some heterogeneity regarding macroscopic morphological colonial aspects. All 20 strains were identified as R. mucilaginosa by conventional methods (carbohydrate assimilation/ negative nitrate tests). Misidentification of the species as R. glutinis was observed in 20% and 5% of the strains with the Vitrek YBC and the API 20C AUX systems, respectively. Discrepancies between commercial and conventional methodologies remained after a repeat test.

Susceptibility testing

In vitro susceptibility results for the 20 strains of Rhodotorula mucilaginosa tested with amphotericin B, fluconazole, itraconazole and voriconazole are described below. Minimal inhibitory concentration (MICs) at which 50% (MIC50) and 90% (MIC90) of the isolates tested were inhibited for each drug after 72 h at 35°C were obtained. No differences were observed in MIC results when antifungal testing was performed at 30°C and with agitation of the microplates (data not shown). For all isolates, amphotericin B demonstrated good in vitro activity (MIC50/90, 0.5 μg/ml; range, 0.25–1 μg/ml) contrasting with what was observed for the azoles. Itraconazole and voriconazole displayed variable in vitro results with MICs ranging from 0.03 to >8 μg/ml for itraconazole (MIC50/90, 0.25/2 μg/ml) and from 0.06–4 μg/ml for voriconazole (MIC50/90, 1/4 μg/ml). The MICs of fluconazole were high for all strains (MIC50/90 >64 μg/ml).

Discussion

Rhodotorula species were traditionally considered to be a contaminant but within the past two decades they have been progressively recognized as human pathogens. While of lower virulence than Candida or Trichosporon, reports have shown that Rhodotorula can cause severe and even fatal invasive infections [27–29].

Approximately 70 cases of Rhodotorula infection have been described in patients with bloodstream infections [18–20,30–35]. Twenty-three of these cases occurred in a single cancer institution in 1992; the largest case series published thus far [19]. In our country, 10 cases have been described, most of them in the past two years [33–35]. In this study, we document the occurrence of Rhodotorula species isolated from blood cultures of 25 patients at a large tertiary-care hospital over a nine-year period. These data represent the largest case series since the work presented by Kiehn et al. in 1992.

Prior to 1995, isolates of Rhodotorula from blood cultures in our hospital were rare. Although infrequent, representing only 2.3% of all the fungal isolates from blood during the study period, Rhodotorula was the most common non-Candida non-Cryptococcus yeast isolated from blood cultures. Its frequency was higher than Trichosporon spp. (data not shown) which has been often regarded as the most common non-Candida yeast infection causing invasive fungal infection [36–38]. The overall incidence of Rhodotorula BSI in this study was similar to that reported by Lunardi et al. [35] (0.059 cases per 1000 admissions vs. 0.056 cases per 1000 admissions) and lower than that reported for Candida BSI in Brazil (2.49 cases per 1000 admissions) [39], Europe (0.20–0.38 cases per 1000 admissions) [40].
and United States (0.46 cases per 1000 admissions) [41]. No trending towards an increase of Rhodotorula-associated BSI was observed over time (p = 0.55). Nevertheless, historical records from the microbiology laboratory suggest that in our hospital, Rhodotorula is here to stay. In the last two years, five more cases of fungemia have been observed, mostly in patients with hematological malignancies.

Consistent with the literature, the majority of the 25 patients described had an underlying disease (96%) with hematological malignancy being the most prevalent. They were mainly non-neutropenic adult outpatients with CVC that had been inserted for various reasons, mostly for treatment of cancer. The distinct temporal distribution of cases was not suggestive of an outbreak. We were surprised to observe that 40% of our patients were BMT recipients since Rhodotorula has been considered a rare complication of BMT transplants. In a series of 1186 bone marrow recipients, only one case was reported [42]. In addition, among cases of Rhodotorula fungemia previously described, only six patients had undergone BMT [8,20,35,43–45]. Further investigation is needed to understand the epidemiology and risk factors of Rhodotorula fungemia in this patient population.

Other relevant findings included the use of broad spectrum antibiotics, exposure to steroids, other immunosuppressive agents and chemotherapy in approximately half of the patients. The use of broad spectrum antibiotics and exposure to cytotoxic agents probably contributed to the increase in gastrointestinal colonization and to damage of the intestinal mucosa, respectively. Although it is known that Rhodotorula can colonize the gastrointestinal tract, additional studies are needed to investigate the true role of the gastrointestinal tract as a source of Rhodotorula fungemia. Surveillance cultures from a diverse patient population should be obtained, including tests for yeast density and sequence of colonization. Molecular-relatedness studies should also be performed.

The use of fluconazole as a prophylactic therapy has been associated with the emergence of resistant organisms [38,46,47]. Fluconazole became available at our hospital in 1996 and consumption has progressively increased over the years. In 2000, prophylactic use of this agent was officially recommended for afebrile neutropenia in BMT patients. Although only one patient was receiving fluconazole in the two weeks preceding the index blood culture, the degree of previous exposure might have been underestimated due to the short investigated period of drug exposure and the retrospective nature of the study.

Previous reports have suggested the presence of Rhodotorula as a major ‘risk factor’ for Rhodotorula fungemia [18–20]. Consistent with these findings, our study showed the presence of a CVC in 88% of the patients. The colonization of the catheter might occur as a result of skin colonization and/or due to transient fungemia secondary to gastrointestinal colonization. Environmental exposure may also play a role since the yeast is ubiquitous in nature. Exposure to tap water both in the hospital and in the community might contribute to yeast colonization and development of infection, especially for severely immunosuppressed patients with a CVC.

Regarding the clinical significance of the isolation of Rhodotorula from blood cultures, 80% of the cases were considered as BSI. Our definition of BSI was adapted from the CDC and transient fungemia was not considered as a category. Among the five cases considered as colonization, two patients were on the conditioning or early phase of BMT. They were subjected to catheter removal and antifungal therapy. Samonis et al. [48] reported a case of transient fungemia in a cancer patient with mucositis without the presence of CVC, who improved without any antifungal treatment. This could have been the case of two of our patients without a CVC.

Unfortunately, we were unable to determine the source of the blood culture in many patients due to lack of information. Most of the patients have a central line (notably long-term devices) in place at the time of the index blood culture. For many of them, blood samples were mainly obtained through the central line. Only two patients had positive blood cultures obtained from the catheter as well as from a peripheral vein. Nevertheless, several previous reports have shown that Rhodotorula blood cultures from peripheral veins are usually negative in patients with CVC [18–20]. One hypothesis is related to the current limitations of the diagnostic tests for CVC-related BSI infections. The other possibility is that the CVC is not the true source of the fungemia. In this study, proper specimen collection and transportation to the clinical microbiology laboratory were not done in a controlled manner. In addition, the microbiological technique utilized was the semi-quantitative catheter segment culture which does not seem to be the most accurate for the diagnosis of such infections in patients with long-term devices [49].

The optimal therapy for these infections remains to be defined. Based on previous case reports, a definitive treatment recommendation cannot be made. Variable results with different treatment regimens have been described in the literature [8,18,19,30–32,47,48]. In our
study, most of the patients were subject to a combined approach (CVC removal and systemic antifungal therapy) with large variations related to treatment initiation, dose and duration of therapy.

The mortality rate due to Rhodotorula infection is estimated to be about 15% [36]. Of the 23 patients, four died. We considered that Rhodotorula fungemia may have contributed to death of patients number 20 and 21 (Table 2). Necropsy results were available only for patient 20, who also had positive blood culture for C. albicans. The necropsy results demonstrated systemic yeast infection. Slides were not available for further analyses and post-mortem cultures were not performed. Nevertheless, the possibility that both yeasts might have played a role in the child’s death could not be excluded.

Rhodotorula is a heterogeneous group of yeasts with currently 50 species described in the literature [50,51]. The most frequent human pathogen is R. mucilaginosa, which is the current name for the species formerly known as R. rubra, followed by R. glutinis and R. minuta [12]. Correct identification of species is important for proper treatment, epidemiological surveys and outbreak investigation. Although there is an increasing use of molecular biology-based diagnostic methods, routine identification of clinical yeast isolates in clinical microbiology laboratories, particularly those from developing countries, is still performed by biochemical, morphological and physiological tests. In our results, all strains were identified as R. mucilaginosa according to conventional methods. Misidentification of the isolates as R. glutinis was observed in 20% and 5% of the strains with the Vitek YBC and API 20C AUX systems, respectively. Care should be taken about the confirmation of the species using commercial systems and nitrate assimilation test should be performed in parallel to separate R. mucilaginosa (nitrate —) from R. glutinis (nitrate +).

There are a limited number of publications reporting the in vitro susceptibility testing of Rhodotorula strains with a standardized method. Similar to previous reports, amphotericin B yielded the lowest MICs (MIC90, 0.5 μg/ml) [20,21,52]. The MICs for fluconazole were very high in all isolates (MIC90 > 64 μg/ml) which has been already described. The resistance mechanism of Rhodotorula for fluconazole is not known but the repeated pattern of high MICs suggests the presence of intrinsic resistance. We were unable to perform antifungal susceptibility testing of 5-flucytosine (5-FC) and for new compounds such as posaconazole and echinocandins. Previous reports have suggested good in vitro activity of 5-FC but this drug is not readily available in Brazil. Itraconazole and voriconazole showed variable activity, whereas fluconazole displayed uniformly limited activity. Of the broad-spectrum azoles, ravuconazole seems to have a higher in vitro activity than posaconazole. However, more studies are needed to evaluate the role of the newer triazoles as an alternative option for the treatment of these infections. Other authors found poor in vitro activity of caspofungin and micafungin highlighting the lack of effectiveness of the echinocandins for heterobasidiomycetous yeasts [20,21].

In summary, Rhodotorula spp. are capable of causing invasive infections, especially in patients with impaired host defenses and/or in the presence of CVC. Rhodotorula infection remains an infrequent cause of fungemia. It should be emphasized that no case-controlled studies have been performed specifically for infections due to Rhodotorula. Some of the clinical findings described in our study as well as in the published literature are not different from other bloodstream fungal infections such as candidemia. The question whether or not all cases of Rhodotorula infection should always be subjected to systemic antifungal treatment (combined with CVC removal, if present) remains to be answered. Likewise, the appropriate antifungal agent and duration of treatment should be established. Carefully conducted prospective clinical studies should help in answering these questions. Until then, a conservative approach combining amphotericin B with catheter removal should always be considered to prevent more serious infection, especially for the severely immunosuppressed patients.

Acknowledgements

This study was presented, in part, at the 12th International Congress of Infectious Diseases, Lisbon, Portugal, 2006. The authors would like to thank Frank Preugschat for helpful suggestions.

Conflict of interest

The authors disclose that they have no potential conflict of interest relevant to this article.

References


© 2008 ISHAM, Medical Mycology, 46, 547–556
Rhodotorula spp. isolated from blood cultures

44 Alliot C, Desablens B, Garidi R, Tabuteau S. Opportunistic infection with Rhodotorula in cancer patients treated by che-

This paper was first published online on iFirst on 10 March 2008.