First Case of Bloodstream Infection Due to *Candida magnoliae* in a Chinese Oncological Patient

Lo Cascio G.¹ Dalle Carbonare L.³, Maccacaro L.², Caliari F.³, Ligozzi M.², Lo Cascio V.³, Fontana R.²

¹Servizio di Microbiologia, Ospedale G.B, Rossi, Verona, Italy
²Dipartimento di Patologia, Sezione di Microbiologia, Università di Verona, Italy
³Dipartimento di Scienze Biomediche e Chirurgiche, Università di Verona, Italy
Abstract

We report a case of fungemia caused by Candida magnoliae, a yeast never associated to human disease. The infection occurred in a 43-year-old Chinese patient with gastric cancer complicated by peritoneal carcinosis. Multiple blood culture were positive for yeast; the species was excellent identified with biochemical and molecular methods. The phylogenetic analysis showed a closely relationship of C. magnoliae to Candida krusei.
A 42-year-old male patient of Chinese origin who had previously undergone surgery for gastric cancer, subsequently complicated by peritoneal carcinosis, presented to the Verona University Hospital Casualty Department for anorexia, vomiting, asthenia, and weight loss. Physical examination revealed ascites and clinical signs of intestinal subocclusion. Nineteen months earlier he had undergone a subtotal distal gastrectomy and lymphadenectomy for a diffuse-type gastric carcinoma with positivity for Helicobacter pylori (pT3-pN9/34-M0). A central venous catheter had been positioned, after which he had been treated with 2 cycles of an ECF chemotherapy protocol (epirubicin, cisplatin and fluorouracil), followed by combined radiotherapy (45 Gy in 25 sessions) and chemotherapy (4 cycles of 5-fluorouracil and folinic acid). Four months later peritoneal carcinosis was detected, so the patient was treated with a further 8 chemotherapy cycles of oxaliplatin, fluorouracil (5-FU) and folinic acid; the last cycle was administered 2 months prior to presentation to the Casualty Department.

Physical examination showed a cachectic, pyretic patient with ascites and clinical signs of intestinal subocclusion. His blood test showed Ht 28.9%, Hb 9.7 g/dL, RBC 3.2 E+12/L, WBC 5.49 E+09/L with 4,900 neutrophils/mm$^3$ and 810 lymphocytes/mm$^3$, K$^+$ 3.1 mmol/L, total protein 63.4 g/L with albumin 33.9 g/L, and ESR 40 mm/h. Chest x-ray findings were normal. Computed tomography confirmed peritoneal carcinosis.

During his stay in hospital the patient was treated with levofloxacin 500 mg i.v. once daily for 12 days for a suspected urinary tract infection, cefotaxime 1 g i.v. three times daily for 7 days, later replaced by meropenem 1 g i.v. three times daily for 5 days because of suspected bacterial peritonitis, and mouth-cleansing nystatin for 15 days until discharge. During his hospitalization, the patient underwent evacuative paracentesis 5 times, with emission of about 2,200 ml of serum-haematic fluid each time. No microbiological culture was done.
On day 21 the patient was febrile (38.2°C) and 6 blood cultures (BactAlert FAN, bioMérieux) were performed over a 4-day period. After a 48-h incubation, 4 blood cultures became positive for a yeast. The patient was given amphotericin 1 mg/kg i.v. for three days, then replaced by fluconazole 200 mg i.v. for fourteen days. After about 2 days his temperature reverted to normal and the patient was discharged 10 days later. He died one month later as a result of cancer progression and deterioration.

The morphological, physiological, and biochemical characteristics of the 4 strains isolated from the patient’s blood on days 23 and 25, respectively, were examined by standard methods commonly used in yeast taxonomy (1). The ID 32C panel (bioMérieux) and the YST card for Vitek 2 (bioMérieux) were used for identification of the yeast strains, according to the manufacturer’s instructions. The yeast isolates from the patient’s blood cultures grew after 24 to 48 h of incubation at 37°C in the Bact/Alert System (bioMérieux). Colonies developed on Sabouraud’s destrose agar over 7 days of incubation at 25°C and were white to cream coloured, soft and smooth. The germ tube test was negative. No hyphae or pseudohyphae were observed. Microscopic morphology on a Dalmau plate (Fig. 1) showed globose to oval cells, single or budding, with thick-walled chlamydospores. No pseudomycelium was seen. The yeast failed to grow on cycloheximide-containing media, and was negative at the urease test and positive at the nitrate assimilation test. It fermented glucose and sucrose only. All strains gave excellent identification for Candida magnoliae using ID 32C (profile 4020110211, 99.4% agreement) and the YST card for Vitek 2. One of these strains, C. magnoliae SL040806, was selected for further study.

To confirm the biochemical identification, genomic DNA was isolated from fungal colonies grown on Sabourad agar plates, as described (8), and sequence analysis of the 18S ribosomal DNA (a highly conserved region in Candida species) was performed, as described by Suzuki et
al. (14); sequences alignment was done using the CLUSTAL W program (1). Comparative analysis with the C. magnoliae sequence deposited in GenBank (accession number AB018145) revealed strong identity (99.4%).

The phylogenetic relationship of C. magnoliae to other Candida spp was investigated by sequencing the internal transcribed spacer (ITS) region located between the 18S and 26S rRNA genes and subdivided into the ITS1 region, between the 18S and 5.8S rRNA genes, and the ITS2 region, between the 5.8S and 26S rRNA genes. This region is more divergent and distinctive than the highly conserved rRNA genes for Candida species (10, 17) and has never been examined in C. magnoliae. Primers and conditions used to amplify the ITS region were as described (17). The ITS sequences of the C. magnoliae SL040806 strain and those of the reference strains obtained from GenBank were aligned using the CLUSTAL W program (1) and a phylogenetic tree was generated (Fig. 2). Phylogenetic analysis was performed according to Suzuki et al. (15) using the neighbour-joining method and the two-parameter model; confidence values for individual branches were determined by bootstrap analysis. The ITS region sequences of the strain C. magnoliae SL040806 have been released in GenBank (accession no. AM408497). The accession numbers of the reference sequences are indicated in the tree shown in Figure 2.

Antifungal susceptibility testing was initially performed by the broth microdilution method, according to the guidelines outlined in Clinical and Laboratory Standards Institute document M37A (13). RPMI medium and an inoculum size of 5 x 10^3 CFU were used. Since no growth was detected under the standard incubation conditions, the susceptibility test was repeated using incubation at 35°C in air, supplemented or not with 10% CO₂ until growth occurred. Under these conditions the yeast showed apparent turbidity in four days’ incubation with CO₂ and in 14 days in air. A change in turbidity equal to or greater than 90% compared to drug-free control
results was used to establish MIC breakpoints under both conditions. The results are summarized in Table 1. Fluconazole resistance was detected both after 14 days’ incubation in ambient air and after 4 days in air supplemented with 10% CO₂. *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258, used as a quality control, gave MICs within the expected range in both conditions, thus excluding the possibility that drug inactivation may have given false resistance results.

To the best of our knowledge, *Candida magnoliae* has been implicated in just one case of human disease (tenosynovitis in an immunocompetent child) (7). Our report describing a bloodstream infection by *C. magnoliae* in a terminal oncological patient is the first to indicate the role of this yeast also in candidemia.

The incidence of bloodstream infection caused by *Candida* spp. has increased progressively over the past two decades (6,14), and among the *Candida* species identified to date, *Candida albicans* ranks first, though recent studies show a shift towards an increasing prevalence of non-*albicans* species. This change has been attributed in part to the widespread use of antifungal agents and to the increasing number of immunocompromised hosts; however, it may also reflect the increasing awareness of non-*C. albicans* *Candida* species as important opportunistic pathogens (3). It has also become apparent that species once considered to be only of industrial importance or innocuous inhabitants of the environment are capable of attacking the human host. These organisms may vary greatly in their susceptibility to the current antifungal agents, thus escaping the effect of empirical treatments.

*Candida magnoliae* is a yeast isolated from magnolia flowers and from the bumblebee gut. It is also an industrially important yeast with substantial erythritol-producing ability. Erythritol has been used as functional sugar substitute for various foods because it is non-carcinogenic, a low-
calorie sweetener and safe for diabetics (18). It is also used in biotechnology to produce mannitol from glucose.

A worrying feature of the infection caused by *C. magnoliae* was the very slow growth of the yeast under the conditions recommended by the CLSI (formerly NCCLS) guidelines for antifungal susceptibility tests. Apparently, the yeast was resistant to fluconazole under the modified conditions used by us and the possibility that this resistance was innate was suggested by the strict phylogenetic relationship we found to *C. krusei* (Fig. 2). We do not know whether the resistance detected under these conditions was clinically significant since the patient was first treated with amphotericin to which the yeast was “susceptible” under both conditions. Since the shift to fluconazole therapy occurred after three days of amphotericin use, the drop in fever could have been linked to the activity of the first drug used.

Considering the patient’s Chinese origins we formulated different speculative hypotheses as to how he may have contracted this type of *Candida*: (i) he may have consumed dairy products such as yoghurt or cheese or honey which contain yeasts such as *Candida magnoliae*, which, in view of his condition, may have entered the bloodstream; (ii) he may have taken a magnolia-based medicine, used in Chinese medicine to treat gastrointestinal disorders; or (iii) he may have used sugar-free products with traces of this yeast. Language difficulties made it impossible to investigate any of these hypotheses in depth.

Clinical microbiology laboratories and physicians will often be involved in identifying and treating infections with unusual yeasts, previously not considered to be pathogens. The great variability of these species in terms of their susceptibility to the current antifungal agents entails further difficulty with regard to the empirical treatment of these potential infections. *Candida magnoliae* should be added to the long list of yeasts capable of causing bloodstream infections in immunocompromised or critically ill patients.
REFERENCES


### Table 1: Antifungal susceptibility testing

<table>
<thead>
<tr>
<th>Drug</th>
<th><em>Candida magnoliae</em> SL040806 MIC (µg/ml) under condition</th>
<th><em>Candida parapsilosis</em> ATCC 22019 MIC (µg/ml) under condition</th>
<th><em>Candida krusei</em> ATCC 6258 MIC (µg/ml) under condition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>Amphotericin</td>
<td>0.016</td>
<td>0.125</td>
<td>0.5</td>
</tr>
<tr>
<td>Flucytosine</td>
<td>&lt;0.03</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>4</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>0.06</td>
<td>0.06</td>
<td>0.5</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>0.03</td>
<td>0.03</td>
<td>0.125</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>0.25</td>
<td>0.5</td>
<td>0.06</td>
</tr>
</tbody>
</table>

- **A**: incubation at 37°C for 4 days in air supplemented with 10% CO₂
- **B**: incubation at 37°C for 14 days in air
Fig. 1: Morphologic features of *Candida magnoliae* on cornmeal agar at 7 days of growth at ambient temperature: globose to oval cells, single or budding. No pseudomycelium is seen.
Fig 2. Phylogenetic tree generated from neighbour-joining analysis of the ITS region sequences depicting the relationships of *Candida magnoliae* to closely related yeast taxa. Reference sequences were from the type strains of the species and were retrieved from GenBank under the accession numbers indicated.