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Fungaemia caused by *Candida pulcherrima*

LASZLO BERECZKI*, N. BARTHA*, S. KOCSUBÉ†, J. SÓKI*, GY. LENGYEL‡, GY. TÁLOSI§, K. MÁDER§, J. DEÁK* & I. DÓCZI*

**Institute of Clinical Microbiology, Albert Szent-Györgyi Medical Centre, †Department of Microbiology, Faculty of Science and Informatics, §Department of Paediatrics, Albert Szent-Györgyi Clinical Centre, University of Szeged, Szeged, and ‡Research Institute of Military Health Centre, Hungarian Defence Forces Budapest, Budapest, Hungary*

Although neonatal bloodstream infections may be caused by a variety of fungi, invasive fungaemia due to *Candida pulcherrima* in a premature neonate has not been previously reported. We describe such a case in which antifungal susceptibility test data led to successful therapy. A colonized catheter used for parenteral nutrition is presumed to have been the main source of this persistent infection.

Keywords neonate, fungaemia, parenteral catheter, opportunistic pathogen, *Candida pulcherrima*

Introduction

Statistical data from different reports point to a gradual increase in the number of cases of fungal infections per year, including the presence of different or rare yeasts and filamentous fungi being recovered from clinical specimens [1]. The rates of morbidity and mortality of fungal bloodstream infections are significant in immunocompromised patients. *Candida pulcherrima* is occasionally able to cause life-threatening infections [2–7] and seems to be one of the opportunistic non-*C. albicans Candida* species which can colonize some parts of the human body. Invasive candidiasis in neonates is a severe and relatively common cause of sepsis associated with high mortality. The increasing number of non-*C. albicans* yeast infections emphasizes the need for the accurate identification of rare yeasts in routine diagnostics for adequate therapy.

Case report

A premature newborn Caucasian girl born as a gemini B twin in the 36th week of gestation, with a birth weight of 2,840 g, was admitted to the Neonatal Intensive Care Unit at the University of Szeged with severe respiratory distress syndrome (RDS). Her mother had gestational diabetes mellitus (GDM), but no other pathological disorder was found. After delivery, the baby was intubated in a moderately serious condition. Her laboratory results demonstrated a low white blood cell count, a moderately elevated haematocrit and elevated procalcitonin levels. Physical examination revealed diffuse respiratory sounds and acrocyanosis of the limbs. On the basis of these findings, intrauterine infection was suspected, and mechanical ventilation and parenteral empirical antibiotic therapy were started. On day 4 of extrauterine life, her condition deteriorated rapidly because of bleeding from the lungs. After improvement, on day 7, she was extubated, and enteral and partial parenteral nourishment was initiated. Blood cultures incubated in the Bactec 9240 Automated Blood Culture System (Becton Dickinson, Franklin Lakes, NJ, USA) were positive for methicillin-resistant *Staphylococcus epidermidis*, and teicoplanin therapy was therefore commenced. After a week, her condition deteriorated again with jaundice and tetraplegia occurring on day 14, and on day 15 cultures inoculated with blood samples became positive for fungi after 15.5 h of incubation. Microbiological investigation revealed the presence of slow-growing yeast colonies on Sabouraud glucose agar (SGA) plates. The isolate was at this stage unidentified and its *in vitro* minimal inhibitory concentrations (MICs) for fluconazole, voriconazole and amphotericin B were 2, 0.032, and 0.004 mg/l, respectively [8]. Fluconazole therapy (6 mg/kg/d) was carried out for three days after the isolate was interpreted as susceptible.

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Correspondence: Laszlo Bereczki, Institute of Clinical Microbiology, Albert Szent-Györgyi Medical Centre, University of Szeged, Semmelweis utca 6, H-6725 Szeged, Hungary. E-mail: bereczki.laszlo1@gmail.com

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On day 21, blood culture positivity reappeared for the same yeast (after 20 h of incubation), and the same MIC results were found. To overcome the suspected *in vivo* lower susceptibility or resistance to fluconazole, amphotericin B lipid complex therapy (5 mg/kg/d) was initiated. After 6 days, her general condition had improved, but abdominal ultrasound imaging revealed the development of a hepatic abscess. After 15 days of amphotericin B therapy, the blood cultures became negative (on day 33), and after a few days the hepatic abscess was observed to be smaller on ultrasound examination. With regard to her improving general condition, the girl was discharged from the hospital. The MIC values determined by Etest (bioMérieux, Marcy l'Etoile, France) on four occasions during the infection (days 15, 20, 25 and 29), showed the same results, i.e., fluconazole – 2 mg/l; voriconazole – 0.032 mg/l; and amphotericin B – 0.004 mg/l [8].

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The colonies on SGA were slow-growing, convex and cream-coloured, with smooth surfaces and entire margins. Growth was adequate at 25–30°C but insufficient at 37°C on every agar medium. The micromorphology on Difco rice starch agar (RSA; Becton Dickinson) revealed ovoid vegetative cells (4–6 μm) without pseudohyphal growth with faint droplets of oil after 24 h at 25°C. On CHROMagar Candida (Becton Dickinson), pale-purple, waxy colonies were formed, as found with many other yeasts. After 48 h under the same conditions, the micromorphology on RSA demonstrated numerous subspherical chlamydospores with a relatively large cell size (7–10 μm), thick cell walls and large drops of oil. After 1 week, no considerable changes in micromorphology were detected as compared with the cultures after 48 h (Fig. 1). The macromorphology of colonies on SGA exhibited characteristic features after 48 h, i.e., a faint halo of diffusible wine-red pigment (pulcherrimin) on the reverse side of larger isolated colonies. After 1 week at 25°C, the red halo expanded beyond the colony and extended to some millimetres.

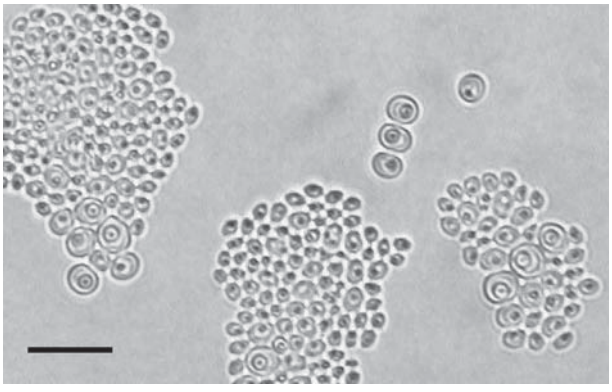

Fig. 1 Micromorphology of *Candida pulcherrima* isolate on RSA at 48 h. Chlamydospores were formed among the crowd of budding cells. No pseudohyphal growth was seen. Magnification × 400; bar denotes 20 μm.

The biochemical pattern of the isolate was determined with an AUXACOLOR 2 (Bio-Rad, Hercules, CA, USA) assimilation kit and additional tests. Assimilation studies at 30°C revealed that after 48 h the isolate was positive for glucose, maltose, sucrose, galactose, cellobiose, trehalose, adonitol, melezitose and xylose and negative for lactose, raffinose, inositol, hexosaminidase, phenoloxidase, prolinearylaminidase, arabinose, nitrate and urease.

Molecular characterization was carried out by sequencing a fragment of internal transcribed spacer (ITS) region amplicon (18S, 5.8S and 26S rDNA), the primers were ITS1 (5′-TCCGTAGGTGAACCTGCG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) [9]. Blast analysis in GenBank indicated a nearly complete sequence alignment with *Metschnikowia pulcherrima* strains (with 99% identity). The production of pulcherrimin and examinations on diluted V8 juice agar [10] revealing the lack of ascospore production indicated the asexual anamorphic stage of the isolate, and it was therefore identified as *C. pulcherrima*. The results of sequencing (358 bp) of the ITS region of the isolated *C. pulcherrima* strain have been deposited under the accession number JN229413 in GenBank.

Discussion

Candida pulcherrima, a common environmental yeast, is a rare opportunistic pathogen in humans. The species is the anamorphic stage of the ascosporic fungus *M. pulcherrima*, a non-pathogenic member of the surface flora of some fruits, and especially noble rotting botrytized grapes. *Candida pulcherrima* is a member of the oral cavity flora in adults, but veterinary isolations have also been reported. Most of the human pathogenic reports involved onychomycosis, root caries lesions, respiratory diseases and a few cases of bloodstream infections [2–7].

In our case, numerous risk factors for invasive fungal infection were present simultaneously, i.e., a premature newborn patient, GDM of the mother, a parenteral medical device, basic RDS and prophylactic antibiotic therapy. It is important to emphasize the *in vivo* ineffectiveness of fluconazole in the treatment of the patient. The *in vivo* and *in vitro* findings from *in vitro* susceptibility tests were completely different because of the long-lasting fungal presence in the bloodstream. Only limited data are available in the literature but they indicate that *C. pulcherrima* is susceptible *in vitro* to fluconazole, voriconazole and anidulafungin [11,12]. Neither intrinsic resistance, nor lowered

susceptibility to any antimycotic is known for this species. It is also necessary to emphasize the importance of the colonization of medical devices such as parenteral nutritional catheters, as demonstrated in similar cases where, besides the fluconazole treatment of a *C. pulcherrima* bloodstream infection, removal of a biofilm-colonized parenteral catheter was a key step in the successful therapy [3,7].

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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