Rare Invasive Yeast Infections in Greek Neonates and Children, a Retrospective 12-Year Study

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Abstract: Although Candida species remain the leading cause of invasive fungal infections (IFI), the list of other isolated fungal pathogens is increasing. The aim of the study was to report cases of IFI caused by rare yeasts in the largest tertiary Greek pediatric hospital. A retrospective study was performed from 6/2008–6/2020 regarding IFI caused by rare species. Identification of isolates was attained by conventional, molecular, and MALDI TOF MS methods, and susceptibility testing was performed according to the Clinical and Laboratory Standards (CLSI) methodology. During a 12-year period, 14 different rare fungal species in 33 neonates and children with IFI hospitalized in intensive care and oncology units were isolated from blood, central catheters, peritoneal, pleural, or pericardial fluid specimens. It is the first time for IFI caused by Wickerhamomyces anomalus (Candida pelliculosa), Pichia fermentans (Candida lambica), Yarrowia (Candida) lipolytica, Pichia (Hansenula) kluyveri, Rhodotorula mucilaginosa, Wickerhamiella (Candida) pararugosa and Cyberlindnera (Candida) fabianii in Greek neonates and children to be reported. For most of these rare fungal species isolated in the present study, no official antifungal breakpoints have been defined, and there are no guidelines for their treatment. Clinical laboratories should be aware of uncommon and emerging yeast pathogens and be able to detect them with molecular and proteomic methods.

Keywords: fungi; invasive; children; rare

1. Introduction

Invasive fungal infections (IFI) occur mainly in neonates and children who are immunocompromised or in a critically ill condition [1,2]. These infections are characterized by high rates of morbidity and mortality, and thus, timely diagnosis and initiation of appropriate antifungal therapy is always considered crucial [3].

Candida species remain the leading cause of IFI among pediatric population [4]. Several less frequently isolated yeast species have been also implicated in IFI such as species that belong to the yeast genera Crypococcus, Rhodotorula, Malassezia, Geotrichum, etc. [5,6]. Some of them are considered frequent colonizers of skin or mucosal surfaces. Their clinical significance varies from superficial infections in normal hosts to invasive infections in immunocompromised individuals. Because of the rare incidence of IFI, published data mostly consist of case reports and small case series, and thus,
management recommendations are derived mainly from clinical experience [6]. Given the complexity of the patients at risk for infection and the increasing array of rare fungal pathogens with intrinsic resistance to antifungal agents, treatment remains challenging.

The aim of the study was to present epidemiological data of IFI caused by uncommon species in the largest tertiary Greek pediatric hospital over a 12-year period.

2. Materials and Methods

2.1. Study Design

A retrospective cohort study of IFI caused by rare fungal species was performed from June 2008 to June 2020 at “Aghia Sophia” Children’s Hospital in Athens. This is a 750-bed tertiary hospital that is a referral center for almost 75% of the Greek pediatric population and includes 3 neonatal intensive care units (NICU), 1 pediatric ICU (PICU), 2 hematology–oncology units (HOU), 1 bone marrow transplantation unit, 5 surgical units (SU), and 1 department of cardiovascular surgery (DCS). All records from the Department of Microbiology were reviewed as well as patients’ medical records when available. All the non-Candida yeasts were defined as rare fungal species.

IFI was defined according to the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group consensus revised definitions [7]. Proven fungal infection was defined as the isolation of fungi in blood, central catheters, peritoneal, pleural, or pericardial fluid specimens. Date of infection was defined as the date of clinical disease connected to the first positive culture for fungi that met disease criteria [7]. If multiple episodes of invasive fungal infection occurred in a single patient, episodes separated by clinical and microbiological resolution (defined by ≥14 days) were treated as a new episode.

2.2. Mycology and Antifungal Susceptibility Test

Cerebrospinal fluid (CSF) and Bronchoalveolar Lavage (BAL) were inoculated directly on Sabouraud Dextrose Agar (SDA) (Bioprepare, Keratea, Greece) and in Sabouraud Dextrose Broth (SDB) (prepared in house), whereas blood, peritoneal, and pleural fluid incubation in the BD BACTEC FX™ system (Becton Dickinson, Franklin Lakes, NJ, USA). Yeast pathogens from positive cultures were sub-cultured onto SDA, Candida Chrom Agar (CCA) (Bioprepare), and on selective mDixon agar (prepared in house), for Malassezia isolates, processed for identification by micromorphology and carbohydrate assimilation testing with API® 32 C, (BioMérieux, Craponne, France), enzymatic RapID™ YeastPlus System (ThermoScientific™–Remel, Lenexa, KS, USA), and Vitek2 (BioMerieux).

Identification of non-identified and confirmation of identified isolates was done by matrix-assisted laser desorption/ionization time-of-flight MALDI-TOF MS (Bruker, Karlsruhe, Germany) and where appropriate by sequencing of the ITS region, D1/D2 domain of the Large Subunit (LSU) rDNA gene, while Trichosporon species were genotyped by sequencing the Intergenic Spacer 1 (IGS) region and the D1/D2 domain [8]. Following identification, the pure isolates were frozen at −70 °C.

For susceptibility testing, isolates were freshly subcultured and analyzed. Minimal inhibitory concentrations (MICs) values were measured for amphotericin B, flucytosine, fluconazole, itraconazole, posaconazole, voriconazole, and caspofungin by broth microdilution method Micronaut-AM (Merlin Diagnostic, Bornheim, Germany) according to the manufacturers’ instructions and also by gradient concentration MIC method of the antifungal drug in strips (Liofilchem, Roseto degli Abruzzi, Italy), and for some cases, E-test (BioMérieux), applied on inoculated 0.5 McFarland yeast suspension turbidity 90 mm diameter RPMI agar plates with MOPS in a 1.5% agar base, supplemented with 2% glucose (Liofilchem,) and incubation at 35 °C for 24–48 h. Susceptibility to isavuconazole was determined by strips only. MICs were read upon the intersection point of the growth with concentration scale, taking into consideration the “trailing” effect where appropriate. Regarding Malassezia species,
MICs were determined only by gradient MIC method on modified RPMI 1640 agar according to previously published protocol [9].

2.3. Ethical Approval and Informed Consent

The study was reviewed and approved (5878/07-03-2014) by The Hospital’s Research Ethics Board.

3. Results

During the study period, 49 incidences of rare fungi species were detected in 45 neonates and children with IFI and non-IFI infections. Fourteen different rare fungal species—cases of filamentous fungi have not been included—were isolated in 33 out of 416 neonates and children (7.9%) with IFI hospitalized in oncology and intensive care units. These fungi were isolated from blood, central catheters, peritoneal, pleural, or pericardial fluid specimens. Yeast species that were isolated for bronchial samples were not included in the analysis. Epidemiological and clinical characteristics of neonates and children with IFI and fungal species isolated are presented in Table 1.

IFI caused by *Trichosporon* species were diagnosed in eight children. *Trichosporon asahii* was isolated in six and *Trichosporon mucoides* in two patients. Two patients were hospitalized in the Bone marrow transplant unit (BMTU), two in HOU, one in SU, one in DCS, one in PICU, and one in NICU. *T. mucoides* exhibited lower MIC values for amphotericin B than *T. asahii* (Table 2). Both species showed increased MIC values for echinocandins and flucytosine. Regarding azoles, most *Trichosporon* species showed low MIC values, except for fluconazole.

*Saccharomyces cerevisiae* (*Candida robusta*) caused IFI in six patients. Two patients were hospitalized in the NICU, two in the PICU, one in the DCS, one in the HOU, and all of them were females. All isolates were found susceptible to amphotericin B, echinocandins, voriconazole, isavuconazole, and flucytosine (Table 2). Isolates exhibited a posaconazole MIC value from 1.0 to 6.0 mg/dL, while increased MIC for fluconazole were detected in most cases. Repetitions were performed with posaconazole and constant differences were observed both with strips and microdilution method. Thus, it was hypothesized that the gradient MIC method with strips may not be the most appropriate, mainly in terms of *S. cerevisiae* growth and posaconazole activity in media. Nevertheless, despite the differences, the MICs remain low and comparable to those in other studies. Since the microdilution is the reference method, and in absence of clinical breakpoint, all but one isolates considered sensitive to posaconazole [10,11].

Among the six IFI cases caused by *Cryptococcus* and former *Cryptococcus* species that were detected, the responsible isolated species were *Naganishia albida* (*n* = 3), *Cryptococcus uniguttulatus* (*n* = 2), and *Cryptococcus terreus* (*n* = 1). *Cryptococcus* species were isolated from blood cultures of three patients and *N. albida* from two blood cultures and a pleural fluid sample of one patient with congenital heart disease. Three patients were hospitalized in the HOU, one in the DCS, one in the NICU, and one in the BMTU. All of them were males. Isolates exhibited an amphotericin B MIC value from 0.38 to 1.5 mg/L. Flucytosine, fluconazole, and as expected, echinocandins showed limited in vitro activity against *N. albida*, *C. uniguttulatus*, and *C. terreus* (Table 2).

Regarding *Malassezia furfur* isolates, they were detected in blood cultures and one central catheter of five patients. Three of them were hospitalized in the NICU, one patient in the HOU, and one in the BMTU. The lowest MIC values were found for amphotericin B, itraconazole, posaconazole, and isavuconazole (Table 2). Interestingly, all isolates were found resistant to echinocandins and flucytosine, while three isolates exhibited increased MIC values for fluconazole.

*Wickerhamomyces anomalus* (*Candida pelliculosa*) caused IFI in two patients who were hospitalized in the HOU and in the DCS. Both strains showed low MIC values to many antifungal agents including itraconazole, voriconazole, posaconazole, echinocandins, and flucytosine (Table 2).
Table 1. Epidemiological and clinical characteristics and yeasts species isolated from neonates and children diagnosed with invasive fungal infections (IFI) in Athens, 6/2008–6/2020.

| Case | Month/Year | Yeasts Species                  | Age           | Gender | Unit  | Sample                  | Medical History                                             |
|------|------------|--------------------------------|---------------|--------|-------|-------------------------|------------------------------------------------------------|
| 1    | 8/2019     | *Saccharomyces cerevisiae*     | 2.5 months    | female | DCS   | blood, central catheter | congenital heart disease, renal failure, surgical history  |
| 2    | 5/2019     | *Saccharomyces cerevisiae*     | 14 months     | female | PICU  | blood                   | pneumococcal meningitis                                     |
| 3    | 12/2012    | *Candida robusta*              | 7 years       | female | HOU   | blood                   | acute lymphoblastic leukemia                                |
| 4    | 4/2010     | *Cryptococcus uniguttulatus*   | 9 months      | female | PICU  | blood                   | myeloblastoma                                               |
| 5    | 4/2010     | *Cryptococcus uniguttulatus*   | less than 12 months | female | NICU  | blood                   | NK                                                         |
| 6    | 3/2010     | *Cryptococcus uniguttulatus*   | 7 months      | female | NICU  | blood                   | jejunal atresia, surgical history                           |
| 7    | 10/2017    | *Trichosporon asahii*          | 2.5 years     | female | HOU   | blood                   | malignancy of the yolk sac, surgical history               |
| 8    | 3/2015     | *Trichosporon asahii*          | 11 years      | male   | SU    | peritoneal fluid        | peritonitis due to appendicitis, surgical history          |
| 9    | 4/2020     | *Trichosporon asahii*          | 1 month       | female | NICU  | catheter                | congenital heart disease, pacemaker insertion, hypotonia, surgical history |
| 10   | 7/2018     | *Trichosporon asahii*          | 14 years      | male   | BMTU  | blood                   | acute lymphoblastic leukemia relapse after BMT              |
| 11   | 12/2014    | *Trichosporon asahii*          | 10 years      | female | BMTU  | pleural fluid           | acute lymphoblastic leukemia                               |
| 12   | 6/2011     | *Trichosporon asahii*          | 10 years      | female | PICU  | blood, bronchial secretion, pericardial fluid | Blackfan-Diamond anemia, renal failure                     |
| 13   | 12/2009    | *Trichosporon mucoides*        | 2 months      | male   | DCS   | pericardial fluid       | congenital heart disease                                   |
| 14   | 9/2009     | *Trichosporon mucoides*        | 10 years      | male   | HOU   | peritoneal fluid        | acute lymphoblastic leukemia                               |
| 15   | 1/2018     | *Naganishia albida*            | 2 years       | male   | HOU   | blood                   | acute lymphoblastic leukemia                               |
| 16   | 6/2016     | *Naganishia albida*            | 5 years       | male   | BMTU  | blood                   | acute lymphoblastic leukemia                               |
| 17   | 1/2016     | *Naganishia albida*            | 24 days       | male   | DCS   | pleural fluid           | congenital heart disease                                   |
| 18   | 10/2016    | *Cryptococcus uniguttulatus*   | 15 years      | male   | HOU   | blood                   | osteosarcoma, surgical history                             |
| 19   | 10/2016    | *Cryptococcus uniguttulatus*   | 13 years      | male   | HOU   | blood                   | acute lymphoblastic leukemia                               |
| 20   | 4/2009     | *Cryptococcus terreus*         | 6 days        | male   | NICU  | blood                   | necrotizing enterocolitis, surgical history                |
Table 1. Cont.

| Case | Month/Year | Yeasts Species                   | Age    | Gender | Unit      | Sample  | Medical History                                                                 |
|------|------------|---------------------------------|--------|--------|-----------|---------|--------------------------------------------------------------------------------|
| 21   | 10/2012    | Malassezia furfur                  | 13 years | male   | HOU       | blood   | acute myeloidleukemia, total parenteral nutrition | primary immunodeficiency, VP shunt |
| 22   | 4/2020     | Wickerhamomyces anomalus          | 4 years | male   | BMTU      | central catheter | prematurity, Hirschsprung disease, colostomy | congenital heart disease, duodenal atresia |
| 23   | 4 month    | NICU                             | 4 month | male   | NICU      | blood   | necrotizing enterocolitis, surgical history, total parenteral nutrition          |                                              |
| 24   | 12/2016    | Wickerhamomyces anomalus          | 3 months | male   | NICU      | blood   | acute lymphoblastic leukemia, prematurity, congenital heart disease, respiratory distress syndrome |
| 25   | 12/2013    | Pichia fermentans                | 12 days | female | NICU      | blood   | acute lymphoblastic leukemia, prematurity, congenital heart disease, respiratory distress syndrome |
| 26   | 7/2013     | Wickerhamomyces anomalus          | 3 years | male   | HOU       | peritoneal fluid |                                             |                                              |
| 27   | 2/2019     | Pichia fermentans                | 4 months | female | DCS       | blood, central catheter |                                             |                                              |
| 28   | 5/2010     | Pichia fermentans                | less than 12 months | female | PICU      | blood   | NK                                                                                       |                                              |
| 29   | 6/2008     | Pichia (Hansenula) kluyveri      | 8 years | male   | HOU       | blood   | acute lymphoblastic leukemia                                                       |                                              |
| 30   | 1/2020     | Rodotorulamucilaginosa           | 12 years | male   | BMTU      | blood   | acute lymphoblastic leukemia                                                       |                                              |
| 31   | 1/2018     | Cyberlindnera (Candida) fabianii  | 1 month | male   | NICU      | blood   | pulmonary dysplasia                                                               |                                              |
| 32   | 1/2014     | Yarrowia (Candida) lipolytica    | 12 months | female | PICU      | pleural fluid |                                              |                                              |
| 33   | 10/2008    | Wickerhamiella                   | 3 years | female | HOU       | blood   | acute lymphoblastic leukemia                                                       |                                              |

BMTU, bone marrow transplant unit; DCS, department of cardiovascular surgery; HOU, hematology and oncology unit; NICU, neonatal intensive care unit; PICU, pediatric intensive care unit; SU, surgical unit; NK, not known; VP, ventriculoperitoneal.
Table 2. Antifungal minimal inhibitory concentrations for isolated species.

|                     | Amphotericin B | Fluconazole | Itraconazole | Voriconazole | Caspofungin | Anidulafungin | Micafungin | Flusytosine | Isavuconazole |
|---------------------|----------------|-------------|--------------|--------------|-------------|---------------|------------|-------------|---------------|
|                     | Microdilution Strips | Microdilution Strips | Microdilution Strips | Microdilution Strips | Microdilution Strips | Microdilution Strips | Microdilution Strips | Microdilution Strips | Microdilution Strips |
| **Saccharomyces cerevisiae** | 0.125 | 0.016 | 2 | 12 | 1 | 4 | 0.015 | 0.064 | 0.125 | 1.5 | 0.125 | 0.125 | 0.031 | 0.008 | 0.0625 | 0.16 | 0.0625 | 0.016 | 0.094 |
| **Candida robusta** | 0.125 | 0.125 | 128 | 256 | 2 | 32 | 0.25 | 0.38 | 4 | 6 | 0.125 | 0.19 | 0.015 | 0.064 | 0.031 | 0.032 | 0.0625 | 0.016 | 0.0625 | 0.016 | 0.019 |
| **Trichosporonoides** | 0.0125 | 0.047 | 3 | 12 | 4 | 4 | 0.19 | 0.047 | 1.5 | 0.125 | 0.25 | 0.125 | 0.023 | 0.125 | 0.023 | 0.0625 | 0.047 | 0.125 |
| **Cryptococcus uniguttulatus** | 0.125 | 0.125 | 0.125 | 0.5 | 4 | 8 | 4 | 3 | 0.031 | 0.25 | 0.25 | 0.25 | 0.125 | 0.125 | 0.036 | 0.25 | 0.016 | 0.0625 | 0.016 | 0.047 |
| **Cryptococcus terreus** | 0.125 | 0.25 | 2 | 3 | 4 | 4 | 0.015 | 0.032 | 0.25 | 1.5 | 0.125 | 0.25 | 0.125 | 0.016 | 0.125 | 0.047 | 0.0625 | 0.008 | 0.023 |
| **M. furfur** | 0.0125 | 0.047 | 3 | 12 | 4 | 4 | 0.19 | 0.047 | 1.5 | 0.125 | 0.25 | 0.125 | 0.023 | 0.125 | 0.023 | 0.0625 | 0.047 | 0.125 |
| **Malassezia furfur** | 0.125 | 0.25 | 2 | 3 | 4 | 4 | 0.19 | 0.047 | 1.5 | 0.125 | 0.25 | 0.125 | 0.016 | 0.125 | 0.047 | 0.0625 | 0.008 | 0.023 |
| **Wickerhamiella pararugosa** | 0.0125 | 0.125 | 0.25 | 0.38 | 4 | 4 | 0.015 | 0.032 | 0.25 | 1.5 | 0.125 | 0.25 | 0.125 | 0.016 | 0.125 | 0.036 | 0.25 | 0.016 | 0.125 |
Pichia fermentans (Candida lambica) and Yarrowia (Candida) lipolytica were isolated from patients’ samples who were hospitalized in ICU. Both species exhibited low MIC values against the majority of antifungal agents, except fluconazole, which showed limited in vitro activity against Yarrowia (Candida) lipolytica. Pichia (Hansenula) kluyveri, Rhodotorula mucilaginosa, and Wickerhamiella (Candida) pararugosa were isolated from patients’ blood samples with history of malignancy. P. kluyveri and W. pararugosa exhibited low MIC values for all antifungals except fluconazole. Regarding R. mucilaginosa, the fungus was susceptible to amphotericin B, flucytosine, and isavuconazole. Finally, Cyberlindnera (Candida) fabianii was isolated from a neonate who was hospitalized in the NICU for a long period due to pulmonary dysplasia and the fungus was isolated in blood samples as well as in bronchial secretions and in rectal carriage. The fungus exhibited low MIC values to all antifungal agents (Table 2).

4. Discussion

Identification of rare and emerging causes of fungaemia and disseminated infections in neonates and children, during an extended 12-year period in the largest tertiary Pediatric Hospital of Greece, is presented in the present study. This is the first report of IFI in Greek neonates and children caused by very rare species such as W. anomalus, P. fermentans, Y. lipolytica, P. kluyveri, R. mucilaginosa, W. pararugosa and C. fabianii. The study provides useful antifungal susceptibility data that may guide selection of appropriate antifungal therapy in the future.

Trichosporon species can colonize many different systems of the human body including the skin, gastrointestinal system, and respiratory system and are capable of causing both superficial and invasive infections [12]. Regarding children population, invasive disease is predominantly found in patients with hematologic disorders as well as in premature neonates with T. asahii being the leading cause [13]. Azoles are considered as the primary choice among antifungal agents for the treatment of invasive trichosporonosis, while several species, including T. asahii, are resistant in vitro to amphotericin B, flucytosine, and echinocandins [6]. As expected, isolates exhibited increased echinocandin MIC values.

S. cerevisiae can be found as a harmless and asymptomatic colonizer of mucosal surfaces in normal individuals. However, it might also be responsible for IFI particularly in vulnerable patients receiving probiotics or critically ill patients with severe underlying diseases such as cancer [14]. Few cases in the literature refer to pediatric population, and all were treated effectively [15–17]. The antifungal agent of choice has not been established. S. cerevisiae is usually susceptible to amphotericin B and flucytosine, while MICs for fluconazole and itraconazole present a wide range [6]. Regarding our detected isolates, all were susceptible to amphotericin B and flucytosine and exhibited increased MIC values for fluconazole. The clinical experience with echinocandins is limited, however there have been reports from adults with successful treatment based on these agents [17,18].

The genus Cryptococcus comprises approximately 70 species, which are able to cause infections in humans as well as in animals [5]. Cryptococcus neoformans and Cryptococcus gatti are considered the major pathogens. However, other Cryptococcus species (e.g., C. uniguttulatus) and N. albida, former Cryptococcus albidus, are prevalent worldwide and have emerged as opportunistic pathogens over the last few years [19]. Epidemiological studies suggest that patients with impaired cell-mediated immunity or invasive devices are predisposed to these infections [20]. Non-neoformans cryptococci have been reported to cause infection in many organ systems, particularly in bloodstream and central nervous system. The choices and duration of treatment for non-neoformans cryptococci infections depend on the anatomical sites of involvement, the host-immune status, response to therapy, and the severity of infection [21]. In general, amphotericin B is a mainstay of the treatment, while echinocandins are inactive against Cryptococcus species [6]. Azole agents are reasonable alternatives for patients with less severe infection. Interestingly, our cases were found resistant to fluconazole and flucytosine. According to the literature, MICs values of flucytosine, fluconazole, and other azoles are elevated in many reports [22–24]. Regarding fluconazole, resistance is more common in patients who had already received azole prophylaxis [20,25]. Although this might indicate reduced susceptibility, it is not known if this observation correlates with a worse outcome [26]. In view of the different susceptibility
profiles of Cryptococcus species, characterization at species level of clinical isolates and detailed drug susceptibility testing are considered compulsory.

The genus Malassezia is classified into at least 17 species. M. furfur is a frequent colonizer of human skin, which can cause various types of skin infections including folliculitis and seborrheic dermatitis. It may, also, lead to severe systemic conditions such as catheter-related fungaemia [27]. Colonization of the skin and subsequent extension to central venous catheters seems to happen more often in neonates than adults. Among the most important risk factors are exposure to lipid-rich intravenous infusions, low birth weight, and prolonged duration of antimicrobial exposure [28]. Systemic infections may, also, occur in patients with various forms of immunosuppression and underlying diseases such as hematological malignancies [29]. Therapeutic management is based mainly on amphotericin B and fluconazole. In vitro resistance to flucytosine and echinocandins seems to be a common finding and was detected in our isolates as well [6,30,31].

W. anomalus is an opportunistic pathogen that rarely causes fungaemia. Although many cases have been reported as sporadic, epidemics have also been detected in pediatric intensive care units [32–34]. As there is still no consensus on breakpoints for antifungal agents, amphotericin B seems optimal for such infections as most of reported cases have shown good sensitivity to this agent [34,35]. Regarding azoles, many published studies have reported low in vitro susceptibilities to itraconazole, fluconazole, and voriconazole [35,36]. However, Barchiesi et al. reported that 46 strains isolated in 37 patients had low sensitivity to azoles [37].

R. mucilaginosa is the most common species of the genus Rhodotorula, which consist of 46 species. It is widely distributed in the environment and is a constituent of the normal respiratory system, gastrointestinal, genital flora, and skin. It has emerged as an important cause of nosocomial and opportunistic infections, particularly in immunocompromised patients [38]. Predisposing factors for Rhodotorula infection are the presence of central venous catheter and underlying malignancy [39]. Amphotericin B is the drug of choice for the treatment of serious fungaemia followed by flucytosine. As there are reports of moderate susceptibility to echinocandins and fluconazole, these agents should not be considered as appropriate therapy [40].

C. fabianii has been described as a yeast with low virulence [41]. However, recently, few case reports have highlighted its pathogenic activity in high-risk patients, thus recognizing that fungus is an emerging pathogen associated with fungaemia, sepsis, and multiple organ dysfunction syndrome. Interestingly, half of reported cases occurred in pediatric patients, while an outbreak of fungaemia involving preterm neonates has also been reported [42,43]. Although data on antifungal susceptibility of C. fabianii are limited, it appears that fluconazole, itraconazole, and posaconazole are less active compared to voriconazole, 5-flucytosine, and echinocandins [44]. However, according to previous reports, the fungus has the ability to develop resistance to different antifungals such as azoles, echinocandins, and amphotericin B [45]. To our knowledge, we report the first case of fungaemia caused by C. fabianii in Greek children.

Y. lipolytica is an ascomycetous yeast found ubiquitously in the environment and meat products [46]. Although it was considered to be of low virulence, it has been also recognized to cause nosocomial clusters of infections [47]. The fungus has the ability to adhere and colonize CVC lines through slime production leading subsequently to candidemia. The major knowledge gap is the optimal management strategy [46]. Although the accepted guidelines for candidiasis recommend catheter removal and intensive treatment with antifungal agents, some clinicians suggest that catheter removal alone might be sufficient for Y. lipolytica catheter-related infection [47–49].

P. fermentans has rarely been implicated in invasive infections and only few cases have been published up to now [50,51]. Regarding our case, the fungus was detected in a blood sample of an infant who was hospitalized in the ICU and was susceptible to many antifungal agents including amphotericin B, flucytosine, and voriconazole, which is in line with the already published data [52].

W. pararugosa are uncommon species, which have not been described in detail in the literature. Until today, only four cases have been reported with bloodstream infection caused by this fungus [53–55].
Two of them were reported in Qatar and were isolated from blood samples of a 6-month-old infant with a history of intrauterine growth restriction and a 5-year-old patient. In our case, the fungus was susceptible to most of antifungal agents, while it exhibited high MIC for fluconazole which had, also, been detected in cases reported from Qatar.

Finally, *P. kluveyeri* has been reported recently as the responsible pathogen for causing oral infection in an Iranian patient with hematological malignancy [56]. The fungus showed low MICs to fluconazole, amphotericin B, and anidulafungin and showed increased MIC to caspofungin. In our case, this fungus caused fungaemia in a male child with hematological malignancy. The isolate was found resistant to fluconazole, while it showed low MICs to all the other antifungal agents.

Conventional laboratory techniques usually cannot identify rare fungal species, such as those described in the present study. Use of novel molecular methods such as MALDI-TOF and sequencing of multiple genomic regions are very useful for the identification of uncommon fungal pathogens [57]. Regarding the antifungal susceptibility testing, it was observed that MIC values were approximately the same by broth microdilution method and also by gradient concentration of the antifungal drug in strips except the difference in MIC values of amphotericin B that was detected in one Trichosporon isolation. However, broth microdilution method is considered as the gold standard method.

Limitation of the present study is that it is a retrospective single-center study, although it represents data from the largest Greek tertiary pediatric center. Due to the extended study period, certain clinical parameters and outcomes were not feasible to be documented from the children’s records.

5. Conclusions

As IFI are associated with high mortality, it is crucial to include fungi in our differential diagnosis and identify the specific fungal species. The list of emerging fungal pathogens is growing, and thus, both microbiologists and clinicians should be aware epidemiology and possible approaches to therapy.

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**References**

1. Lehrnbecher, T.; Schöning, S.; Poyer, F.; Georg, J.; Becker, A.; Gordon, K.; Attarbaschi, A.; Groll, A.H. Incidence and Outcome of Invasive Fungal Diseases in Children with Hematological Malignancies and/or Allogeneic Hematopoietic Stem Cell Transplantation: Results of a Prospective Multicenter Study. *Front. Microbiol.* 2019, 10, 681. [CrossRef] [PubMed]
2. Brissaud, O.; Guichoux, J.; Harambat, J.; Tandonnet, O.; Zaoutis, T. Invasive fungal disease in PICU: Epidemiology and risk factors. *Ann. Intensive Care* 2012, 2, 6. [CrossRef] [PubMed]
3. Arendrup, M.C.; Fisher, B.T.; Zaoutis, T.E. Invasive fungal infections in the paediatric and neonatal population: Diagnostics and management issues. *Clin. Microbiol. Infect.* 2009, 15, 613–624. [CrossRef]
4. Noni, M.; Stathi, A.; Vaki, I.; Veleglaki, A.; Zachariadou, L.; Michos, A. Changing Epidemiology of Invasive Candidiasis in Children during a 10-Year Period. *J. Fungi* 2019, 5, 19. [CrossRef] [PubMed]
5. Pfaller, M.A.; Diekema, D.J. Rare and emerging opportunistic fungal pathogens: Concern for resistance beyond Candida albicans and Aspergillus fumigatus. *J. Clin. Microbiol.* 2004, 42, 4419–4431. [CrossRef] [PubMed]
6. Arendrup, M.C.; Boekhout, T.; Akova, M.; Meis, J.F.; Cornely, O.A.; Lortholary, O. ESCMID and ECMM joint clinical guidelines for the diagnosis and management of rare invasive yeast infections. *Clin. Microbiol. Infect.* 2014, 20 (Suppl. 3), 76–98. [CrossRef]
7. De Pauw, B.; Walsh, T.J.; Donnelly, J.P.; Stevens, D.A.; Edwards, J.E.; Calandra, T.; Pappas, P.G.; Maertens, J.; Lortholary, O.; Kauffman, C.A.; et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin. Infect. Dis. 2008, 46, 1813–1821. [CrossRef]

8. Arakatsis, M.; Abel, P.; Kanellopouli, M.; Adamou, D.; Alexandrou-Athanasoulis, H.; Stathi, A.; Platsouka, E.; Milioni, A.; Pangalis, A.; Velegkri, A. Sequence-based identification, genotyping and EUCAST antifungal susceptibilities of Trichosporon clinical isolates from Greece. Clin. Microbiol. Infect. 2014, 20, 777–783. [CrossRef]

9. Velegkri, A.; Alexopoulos, E.C.; Kritikou, S.; Gaitanis, G. Use of fatty acid RPMI 1640 media for testing susceptibilities of eight Malassezia species to the new triazole posaconazole and to six established antifungal agents by a modified NCCLS M27-A2 microdilution method and Etest. J. Clin. Microbiol. 2004, 42, 3589–3593. [CrossRef]

10. Pfaller, M.A.; Messer, S.; Jones, R.N. Activity of a new triazole, Sch 56592, compared with those of four other antifungal agents tested against clinical isolates of Candida spp. and Saccharomyces cerevisiae. Antimicrob. Agents Chemother. 1997, 41, 233–235. [CrossRef]

11. Barchiesi, F.; Arzeni, D.; Fothergill, A.W.; Di Francesco, L.F.; Caselli, F.; Rinaldi, M.G.; Scalise, G. In vitro activities of the new antifungal triazole SCH 56592 against common and emerging yeast pathogens. Antimicrob. Agents Chemother. 2000, 44, 226–229. [CrossRef] [PubMed]

12. De Almeida Júnior, J.N.; Hennequin, C. Invasive Trichosporon Infection: A Systematic Review on a Re-emerging Fungal Pathogen. Front. Microbiol. 2016, 7, 1629. [CrossRef] [PubMed]

13. Foster, C.E.; Edwards, M.S.; Brackett, J.; Schady, D.A.; Healy, C.M.; Baker, C.J. Trichosporonosis in Pediatric Patients With a Hematologic Disorder. J. Pediatr. Infect. Dis. Soc. 2018, 7, 199–204. [CrossRef] [PubMed]

14. Muñoz, P.; Bouza, E.; Cuenca-Estrella, M.; Eiros, J.M.; Caselli, F.; Rinaldi, M.G.; Scali, G. In vitro activities of the new antifungal triazole SCH 56592 against common and emerging yeast pathogens. Antimicrob. Agents Chemother. 2000, 44, 226–229. [CrossRef] [PubMed]

15. Romanio, M.R.; Coraine, L.A.; Maielo, V.P.; Abramczyc, M.L.; de Souza, R.L.; Oliveira, N.F. Saccharomyces cerevisiae Fungemia in a pediatric patient after treatment with probiotics. Rev. Paul. Pediatr. 2017, 35, 361–364. [CrossRef]

16. Belet, N.; Dalgiç, N.; Oncel, S.; Çiftçi, E.; Ince, E.; Güriz, H.; Barlas, M.; Doğru, U. Catheter-related fungemia caused by Saccharomyces cerevisiae in a newborn. Pediatr. Infect. Dis. J. 2005, 24, 1125. [CrossRef]

17. Roy, U.; Jessani, L.G.; Rudramurthy, S.M.; Gopalakrishnan, R.; Dutta, S.; Chakravarty, C.; Jillwin, J.; Chakrabarti, A. Seven cases of Saccharomyces fungaemia related to use of probiotics. Mycoses 2017, 60, 375–380. [CrossRef]

18. Choi, G.; Meijer, S.L.; Hazenberg, M.D. Disseminated bread yeast fungaemia in a baker’s wife with acute myeloid leukaemia. Br. J. Haematol. 2012, 158, 298. [CrossRef]

19. McCurdy, L.H.; Morrow, J.D. Infections due to non-neoformans cryptococcal species. Compr. Ther. 2003, 29, 95–101. [CrossRef]

20. Khawcharoenporn, T.; Apisarnthanarak, A.; Mundy, L.M. Non-neoformans cryptococcal infections: A systematic review. Infection 2007, 35, 51–58. [CrossRef]

21. Perfect, J.R.; Dismukes, W.E.; Dromer, F.; Goldman, D.L.; Graybill, J.R.; Hamill, R.J.; Harrison, T.S.; Larsen, R.A.; Lortholary, O.; Nguyen, M.H.; et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the infectious diseases society of america. Clin. Infect. Dis. 2010, 50, 291–322. [CrossRef] [PubMed]

22. Averbuch, D.; Boekhout, T.; Falk, R.; Engelhard, D.; Shapiro, M.; Block, C.; Polacheck, I. Fungemia in a cancer patient caused by fluconazole-resistant Cryptococcus laurentii. Med. Mycol. 2002, 40, 479–484. [CrossRef] [PubMed]

23. McCurdy, L.H.; Morrow, J.D. Ventriculitis due to Cryptococcus uniguttulatus. South. Med. J. 2001, 94, 65–66. [CrossRef] [PubMed]

24. Bernal-Martinez, L.; Gomez-Lopez, A.; Castelli, M.V.; Mesa-Arango, A.C.; Zaragoza, O.; Rodriguez-Tudela, J.L.; Cuenca-Estrella, M. Susceptibility profile of clinical isolates of non-Cryptococcus neoformans/non-Cryptococcus gattii Cryptococcus species and literature review. Med. Mycol. 2010, 48, 90–96. [CrossRef]

25. Garelick, J.M.; Khodabakhsh, A.J.; Lopez, Y.; Bami, M.; Lister, M. Scleral ulceration caused by Cryptococcus albidus in a patient with acquired immune deficiency syndrome. Cornea 2004, 23, 730–731. [CrossRef]
26. Choe, Y.J.; Blatt, D.B.; Yalcindag, A.; Geffert, S.F.; Bobenchik, A.M.; Michelow, I.C. Cryptococcus albidus Fungemia in an Immunosuppressed Child: Case Report and Systematic Literature Review. *J. Pediatr. Infect. Dis. Soc.* 2020, 9, 100–105. [CrossRef]

27. Gaitanis, G.; Magiatis, P.; Hantschke, M.; Bassukas, I.D.; Velegraki, A. The Malassezia genus in skin and systemic diseases. *Clin. Microbiol. Rev.* 2012, 25, 106–141. [CrossRef]

28. Devlin, R.K. Invasive fungal infections caused by Candida and Malassezia species in the neonatal intensive care unit. *Adv. Neonatal Care* 2006, 6, 68–77. [CrossRef]

29. Tragiannidis, A.; Bisping, G.; Koehler, G.; Groll, A.H. Minireview: Malassezia infections in immunocompromised patients. *Mycoses* 2010, 53, 187–195. [CrossRef]

30. Ashbee, H.R. Update on the genus Malassezia. *Med. Mycol.* 2007, 45, 287–303. [CrossRef]

31. Theelen, B.; Cafarchia, C.; Gaitanis, G.; Bassukas, I.D.; Boekhout, T.; Dawson, T.L., Jr. Malassezia ecology, pathophysiology, and treatment. *Med. Mycol.* 2018, 56, S10–S25. [CrossRef] [PubMed]

32. Pasqualotto, A.C.; Sukiennik, T.C.; Severo, L.C.; de Amorim, C.S.; Colombo, A.L. An outbreak of Pichia anomala fungemia in a Brazilian pediatric intensive care unit. *Infect. Control Hosp. Epidemiol.* 2005, 26, 553–558. [CrossRef] [PubMed]

33. Yılmaz-Semerci, S.; Demirel, G.; Ta¸stekin, A. Wickerhamomyces anomalus blood stream infection in a term newborn with pneumonia. *Tirk. J. Pediatr.* 2017, 59, 349–351. [CrossRef] [PubMed]

34. Lin, H.-C.; Lin, H.-Y.; Su, B.-H.; Ho, M.-W.; Ho, C.-M.; Lee, C.-Y.; Lin, M.-H.; Hsieh, H.-Y.; Lin, H.-C.; Li, T.-C.; et al. Reporting an outbreak of Candida pelliculosa fungemia in a neonatal intensive care unit. *J. Microbiol. Immunol. Infect.* 2013, 46, 456–462. [CrossRef] [PubMed]

35. Da Matta, V.L.R.; de Souza Carvalho Melhem, M.; Colombo, A.L.; Moretti, M.L.; Rodero, L.; Duboc de Almeida, G.M.; dos Anjos Martins, M.; Costa, S.F.; Souza Dias, M.B.G.; Nucci, M.; et al. Antifungal drug susceptibility profile of Pichia anomala isolates from patients presenting with nosocomial fungemia. *Antimicrob. Agents Chemother.* 2007, 51, 1573–1576. [CrossRef]

36. Kalkanci, A.; Dizbay, M.; Turan, O.; Fidan, I.; Yalçın, B.; Hirfanoğlu, I.; Kuşımur, S.; Aktas, F.; Sugita, T. Nosocomial transmission of Candida pelliculosa fungemia in a pediatric intensive care unit and review of the literature. *Tirk. J. Pediatr.* 2010, 52, 42–49.

37. Barchiesi, F.; Tortorano, A.M.; Di Francesco, L.F.; Rigoni, A.; Giacometti, A.; Spreghini, E.; Scalise, G.; Viviani, M.A. Genotypic variation and antifungal susceptibilities of Candida pelliculosa clinical isolates. *J. Med. Microbiol.* 2005, 54, 279–285. [CrossRef]

38. Pasqualotto, G.C.; Copetti, F.A.; Meneses, C.F.; Machado, A.R.L.; Brunetto, A.L. Infection by Rhodotorula sp. in children receiving treatment for malignant diseases. *J. Pediatr. Hematol. Oncol.* 2005, 27, 232–233. [CrossRef]

39. Tuon, F.F.; Costa, S.F. Rhodotorula infection. A systematic review of 128 cases from literature. *Rev. Iberoam. Micol.* 2008, 25, 135–140. [CrossRef]

40. Duggal, S.; Jain, H.; Tyagi, A.; Sharma, A.; Chugh, T.D. Rhodotorula fungemia: Two cases and a brief review. *Med. Mycol.* 2011, 49, 879–882. [CrossRef]

41. Arastehfar, A.; Fang, W.; Al-Hatmi, A.M.S.; Afsarian, M.H.; Daneshnia, F.; Bakhtiar, M.; Sadati, S.K.; Badali, H.; Khodavaisy, S.; Hagen, F.; et al. Unequivocal identification of an underestimated opportunistic yeast species, Cyberlindnera fabianii, and its close relatives using a dual-function PCR and literature review of published cases. *Med. Mycol.* 2019, 57, 833–840. [CrossRef] [PubMed]

42. Al-Sweih, N.; Ahmad, S.; Khan, S.; Joseph, L.; Asadzadeh, M.; Khan, Z. Cyberlindnera fabianii fungaemia outbreak in preterm neonates in Kuwait and literature review. *Mycoses* 2019, 62, 51–61. [CrossRef] [PubMed]

43. Desai, M.; Nitta, B.; Dhanani, H.; Djurkovic, S.; Katugaha, S. Multiple organ dysfunction syndrome and death secondary to Cyberlindnera fabianii. *Med. Mycol. Case Rep.* 2019, 26, 1–4. [CrossRef] [PubMed]

44. Park, J.H.; Oh, J.; Sang, H.; Shrestha, B.; Lee, H.; Koo, J.; Cho, S.I.; Choi, J.S.; Lee, M.H.; Kim, J.; et al. Identification and Antifungal Susceptibility Profiles of Cyberlindnera fabianii in Korea. *Mycobiology* 2019, 47, 449–456. [CrossRef] [PubMed]

45. Hof, H.; Amann, V.; Tauber, C.; Paulun, A. Peritonitis in a neonate due to Cyberlindnera fabianii, an ascomycetic yeast. *Infection* 2017, 45, 921–924. [CrossRef]
46. Zhao, Y.; Chan, J.F.; Tsang, C.C.; Wang, H.; Guo, D.; Pan, Y.; Xiao, Y.; Yue, N.; Chen, J.H.; Lau, S.K.; et al. Clinical Characteristics, Laboratory Identification, and In Vitro Antifungal Susceptibility of Yarrowia (Candida) lipolytica Isolates Causing Fungemia: A Multicenter, Prospective Surveillance Study. *J. Clin. Microbiol.* 2015, 53, 3639–3645. [CrossRef]

47. Shin, J.H.; Kook, H.; Shin, D.H.; Hwang, T.J.; Kim, M.; Suh, S.P.; Ryang, D.W. Nosocomial cluster of Candida lipolytica fungemia in pediatric patients. *Eur. J. Clin. Microbiol. Infect. Dis.* 2000, 19, 344–349. [CrossRef]

48. Agarwal, S.; Thakur, K.; Kang, A.; Singh, G.; Gupta, P. Catheter-related candidemia caused by *Candida lipolytica* in a child with tubercular meningitis. *Indian J. Pathol. Microbiol.* 2008, 51, 298–300. [CrossRef]

49. Chang, C.L.; Park, T.H.; Lee, E.Y.; Lim, Y.T.; Son, H.C. Recurrent Self-Limited Fungemia Caused by *Yarrowia lipolytica* in a Patient with Acute Myelogenous Leukemia. *J. Clin. Microbiol.* 2001, 39, 1200–1201. [CrossRef]

50. Vervaeke, S.; Vandamme, K.; Boone, E.; De Laere, E.; Swinne, D.; Surmont, I. A case of Candida lamberca fungemia misidentified as Candida krusei in an intravenous drug abuser. *Med. Mycol.* 2008, 46, 853–856. [CrossRef]

51. Trowbridge, J.; Ludmer, L.M.; Riddle, V.D.; Levy, C.S.; Barth, W.F. Candida lambica polyarthritis in a patient with chronic alcoholism. *J. Rheumatol.* 1999, 26, 1846–1848. [PubMed]

52. Posteraro, B.; Spanu, T.; Fiori, B.; De Maio, F.; De Carolis, E.; Giaquinto, A.; Prete, V.; De Angelis, G.; Torelli, R.; D’Inzeo, T.; et al. Antifungal susceptibility profiles of bloodstream yeast isolates by Sensititre YeastOne over nine years at a large Italian teaching hospital. *Antimicrob. Agents Chemother.* 2015, 59, 3944–3955. [CrossRef] [PubMed]

53. El Helou, G.; Palavecino, E. Candida pararugosa: First Reported Bloodstream Infection in an Adult. *Cureus* 2017, 9, e1283. [CrossRef] [PubMed]

54. Taj-Aldeen, S.J.; AbdulWahab, A.; Kolecka, A.; Deshmukh, A.; Meis, J.F.; Boekhout, T. Uncommon opportunistic yeast bloodstream infections from Qatar. *Med. Mycol.* 2014, 52, 552–556. [CrossRef] [PubMed]

55. Oliveira, V.K.P.; Ruiz, L.d.S.; Oliveira, N.I.A.J.; Moreira, D.b.; Hahn, R.C.; Melo, A.S.d.A.; Nishikaku, A.S.; Paula, C.R. Fungemia caused by Candida Species in a Children’s public hospital in the city of São Paulo, Brazil: Study in the period 2007–2010. *Rev. Inst. Med. Trop. SÃ£o Paulo* 2014, 56, 301–305. [CrossRef]

56. Aslani, N.; Janbabaei, G.; Abastabar, M.; Meis, J.F.; Babaeian, M.; Khodavaisy, S.; Boekhout, T.; Badali, H. Identification of uncommon oral yeasts from cancer patients by MALDI-TOF mass spectrometry. *BMC Infect. Dis.* 2018, 18, 24. [CrossRef]

57. Kidd, S.E.; Chen, S.C.-A.; Meyer, W.; Halliday, C.L. A New Age in Molecular Diagnostics for Invasive Fungal Disease: Are We Ready? *Front. Microbiol.* 2020, 10, 2903. [CrossRef]