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Genotyping and Antifungal Susceptibility of *Dipodascus capitatus* Isolated in a Neonatal Intensive Care Unit of a Sicilian Hospital

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Abstract

In August 2015, *Dipodascus capitatus* was isolated from two patients admitted to the neonatal intensive care unit. Nosocomial acquisition of the fungus was suspected and epidemiological studies were undertaken. The patients were simultaneously hospitalized, and the comparison of the two isolates by two independent molecular typing methods have confirmed clonal dissemination of a single strain of *D. capitatus*. Antimicrobial susceptibility testing was useful for identifying the appropriated antifungal therapy in micafungin. To our knowledge these are the first described cases of neonatal *D. capitatus* infection and also the first report of successful treatment by micafungin.

Keywords

Dipodascus capitatus • Nosocomial Acquisition • Genotyping • Antifungal Susceptibility

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1 Introduction

Dipodascus capitatus, the teleomorph form of *Geotrichum capitatum,* also known as *Blastoschizomyces capitatus* or *Magnusiomyces capitatus*, is ubiquitary in nature, and is occasionally part of the normal human microbiota (Guého et al. 1987; DeHoog and Vitale 2007). Phylogenetically this yeast is considered as an Ascomycota, and morphologically as Trichosporon spp. by its ability to produce anelloconidia and arthoconidia

It has been described as cause of invasive fatal infection in immunocompromised patients with hematological malignances and severe neutropenia (Girmenia et al. 2005, Lafayette et al. 2011). In these patients an invasive infection is rarely caused by *D. capitatus*, but if it occurs it is associated with unfavorable outcome and mortality rate exceeding 50% (Saghrouni et al. 2012).

The acute disseminated infection may be associated with skin lesions characterized initially by purpuric nodules progressing to centrally necrotic lesions, then early diagnosis and appropriate management will improve its prognosis (Trabelsi et al. 2015).

It may resemble invasive candidiasis but is associated with high bloodstream recovery rates, deep organ involvement, and an awful prognosis. In the disseminated infection the galattomannan antigen could be positive suggesting a false diagnosis of invasive aspergillosis (Özkaya-Parlakay et al. 2012). This type of geotrichosis is reported more frequently in Europe than in the USA (85% and 10% of cases, respectively). Furthermore, 87% of the European cases occurred in Italy (Central and Southern), Spain and France suggesting that climatic factors can influence the epidemiology of geotrichosis (Girmenia et al. 2005).

The most favorable therapy is not established because of limited data on antifungal susceptibility and on different therapeutic strategies. The efficacy of treatment for systemic therapy remains controversial: multidrug combination is considered a more efficient regime, even if there is no evidence that this is more effective than single drug regime (Özkaya-Parlakay et al. 2012). In vitro activities of amphotericin B, fluconazole, itraconazole and voriconazole have been investigated. High activity of amphotericin B was observed and poor susceptibility of some strains to fluconazole and itraconazole have been described (Girmenia et al. 2003, Venditti et al. 1991). In some cases, voriconazole or caspofungin are used as first-line therapy, or amphotericin B in combination with voriconazole (Giacchino et al. 2006).

In the present report, we emphasize the importance of achieving appropriate diagnosis through the investigation of rarely encountered fungi, such as *D. capitatus*, the difficulties of identification, the role of molecular typing and susceptibility testing.

2 Case Reports

2.1 Patient 1

A 28 weeks-gestation extremely low birth weight (600 g) male infant, with severe intrauterine growth restriction (IUGR) and mild respiratory distress, was referred to our Neonatal Intensive Care Unit (NICU) on day 9 of life for surgical management of suspected necrotizing enterocolitis, already in therapy with sulbactam/ampicillin, amikacin, metronidazole, ranitidine, and insulin. Blood culture obtained at admission was negative. Fluconazole prophylaxis (3 mg/kg/48 h) was started and dedicated total parenteral nutrition, via peripherally inserted central catheter (PICC) positioned in the birth unit, was maintained. He received platelets and blood transfusions for persistent thrombocytopenia and anemia, but never presented neutropenia.

On day 21 of life, because of persistent abdominal distension, a gastrografin enema was performed and demonstrated meconium ileum. After 5 days, when baby's weight reached 800 g, an exploratory laparotomy and a temporary ileostomy were performed. In the 48 h after surgery, the baby developed clinical symptoms

of infection with fever and pulmonary floccular infiltration at chest X-rays, treated with teicoplanin and meropenem; PICC was then removed and replaced on day 28 of life. PICC culture developed fungal growth which was 5 days later further identified as *D. capitatus*. Because of initial evidence of in vitro resistance to amphotericin B (by Kirby-Bauer method), and limited spectrum of tested antimycotic drugs suitable for neonates, fluconazole was continued at treatment dosage (6 mg/kg/die) until results of another antimicrobial susceptibility testing. On day 38 of life, intravenous micafungin (8 mg/kg/die) was started, according to the second antimicrobial susceptibility testing (colorimetric microdilution test). Targeted antifungal treatment appeared to be effective with regression of clinical symptoms and full recovery of X-ray pattern. *D. capitatus* was not further detected in the subsequent samples (PICC and blood culture). On day 60 of life, patient was back-transferred to birth NICU for laser treatment of retinopathy of prematurity and died at 5 months of life for complications of severe prematurity.

2.2 Patient 2

A male term neonate with prenatal diagnosis of left-sided congenital diaphragmatic hernia (CDH) was born by caesarean section in our unit in the same week (Assumption day holiday period) when patient 1 claimed the onset of clinical symptoms of infection. He presented a polymalformative condition with CDH, narrow thorax, pulmonary hypertension, severe IUGR, postaxial polydactyly, dysmorphic facial features, blue sclerae, congenital hypothyroidism, no hematological abnormalities. Further investigations showed normal karyotype, array comparative genomic hybridization and faciogenital dysplasia 1 gene analyses. Blood culture collected at birth was negative. An umbilical venous catheter (UVC), inserted at birth to guarantee parenteral nutrition, was replaced on second day by PICC. The baby was permanently depending on assisted ventilation and antibiotics (ampicillin/sulbactam and gentamicin) were administered since birth.

On day 3 of life, surgical correction of CDH was performed and fluconazole prophylaxis (3 mg/kg/48 h) was started. Because of funguria on the next day, fluconazole dosage was increased to 6 mg/kg/die and intravesical transcatheter instillation of amphotericin B was performed.

After 5 days of incubation, fungal growth from UVC culture was observed, and the isolates were further identified as *D. capitatus*. On day 15 of life, targeted antifungal treatment with intravenous micafungin (8 mg/kg/die) was started, according to antimicrobial susceptibility testings (Kirby- Bauer and colorimetric microdilution test). The patient died two days later because of severe pulmonary hypertension as a complication of his multiple congenital malformations. On cultures performed at death, *D. capitatus* was not isolated from PICC and pleural fluid but only from endotracheal tube, likely as a sign of environmental contamination.

3 Methods

PICC from patient 1 and UVC from patient 2 were routinely cultured on Columbia blood agar and Sabouraud dextrose agar. The colonies grown on plates were further inoculated to CHROMagar-Candida (Beckton Dickinson, Heidelberg, Germany), and were identified by microbiological investigations and sequence analysis.

Sequencing analysis of the Internal Transcribed Spacer (ITS) of 18S rDNA in the rRNA gene was performed to ensure identification of these organisms according to (Subramanya Supram et al. 2016).

The sequence data were analyzed using the National Center for Biotechnology Information (Bethesda, Md., USA) BLAST system (available at http://www.ncbi.nlm.nih.gov/BLAST/).

Two techniques were used for molecular typing, the random amplification of polymorphic DNA (RAPD) method and PCR fingerprinting. The RAPD primer selected for genotyping was OPE-4 (5- GTGACATGCC-3). The PCR fingerprinting technique was performed with the

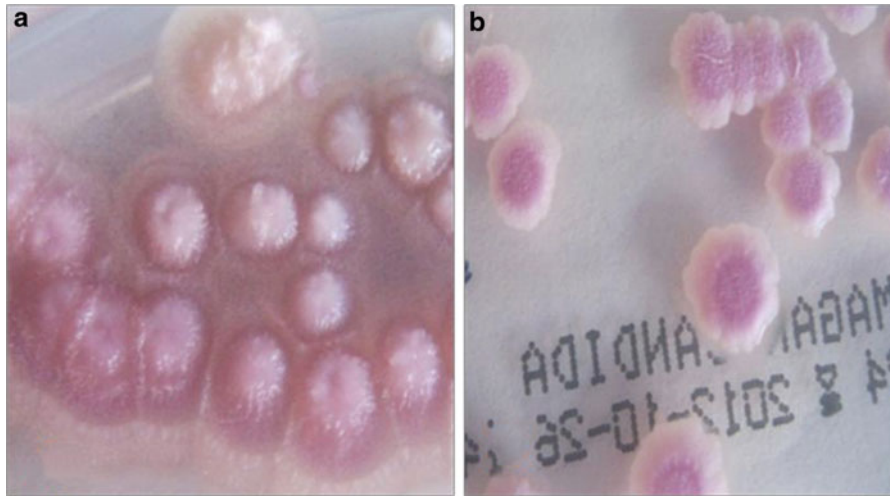

Fig. 1 *D. capitatus* (**a**) and *C. krusei* (**b**) on CHROMagar-candida

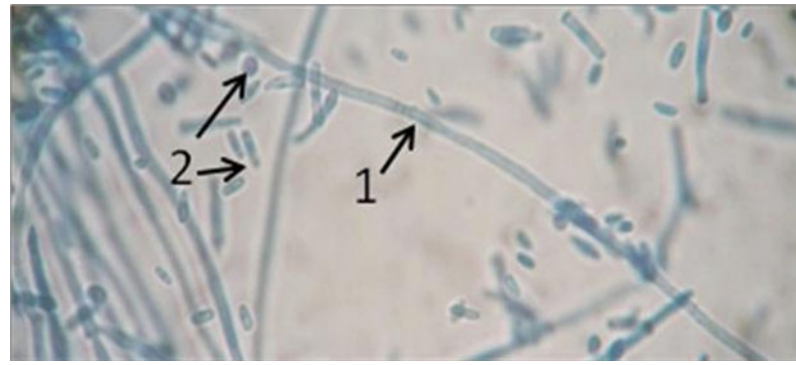

Fig. 2 Presence of septate hyphae (*1*) and cylindrical or clavate conidia (*2*) observed in lactophenol cotton blue preparation

core sequence of phage primer M13-GAGGGTGGCGGTTCT-' as a single primer according to Gadea and Erzoz. (Gadea et al. 2004; Ersoz et al. 2004).

Typing techniques were performed at least two times on separate days. Band patterns were electrophoresed through 1.2% agarose gels (Pronadisa, Madrid, Spain), stained with ethidium bromide (Sigma Aldrich Química), and photographed under UV light by Gel Doc BIORAD. Finally, antifungal susceptibility of the yeasts was determined both by Kirby-Bauer method that by colorimetric microdilution test (SENSITITRE® YEASTONE®), and the results were interpreted according to the breakpoints of CLSI guidelines M27-S4 (CLSI 2012).

4 Results

After 5 days at 30 °C the colonies on Sabouraud dextrose agar were whitish, glassy, and funiculose with a smooth expanding zone. The morphotype on CHROMagar-Candida showed white-pink and wrinkled and fimbriate colonies that were easily distinguishable in color and morphology from those of *C. krusei* (Fig. 1).

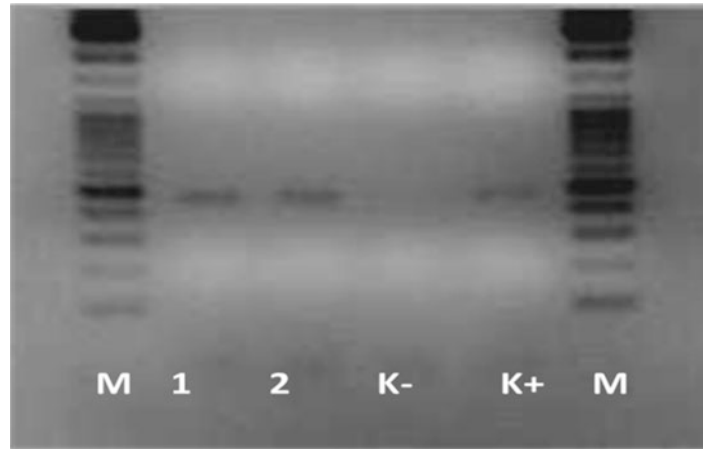

Fig. 3 PCR for 18S rDNA, M ladder 100 bp Fermentas, lane 1 for patient 1, and lane 2 for patient 2, K- and K+ control negative and positive

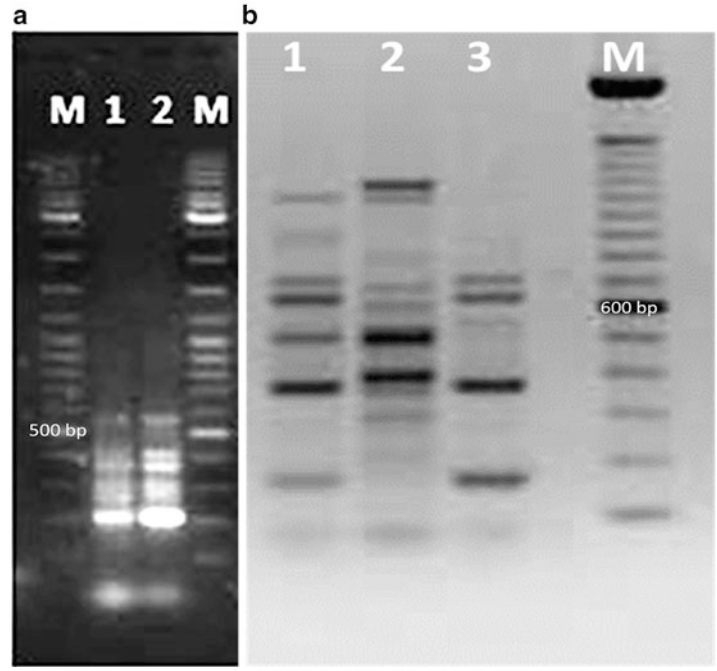

Fig. 4 (**a**) Pattern obtained with primer M13: lane M ladder 100 bp Fermentas, lane 1 for patient 1, and lane 2 for patient 2. (**b**) Pattern obtained with primer M13 (ladder 100 bp Invitrogen) of strains isolated from three adult patients in the same hospital in 2012

Microscopic examination, with lactophenol cotton blue preparation, revealed hyphae and conidiophores creeping or ascending, profusely branched at acute angles with conidiogenous cells which formed long, cicatrized stems of hyphal growth (rachides) on which the conidia were born. Conidia were cylindrical to clavate, with a rounded apex and flat base. Also, arthroconidia and endoconidia were often present (Yarrow 1998) (Fig. 2).

The PCR for gene ITS revealed a template with molecular weight equal to 480 bp as showed in Fig. 3

The DNA sequences of all the isolates were completely matched to that of *D. capitatus* (*G. capitatum*) from the Gen Bank DNA database (Accession number AY788305).

Table 1 Minimum inhibitory concentration (μg/ml) of antifungal drugs displayed against isolates obtained by colorimetric microdilution test and Kirby-Bauer

	1G	2C
MIC (μg/ml)		
AB ≤1 (S)	1	1
5FY ≤ 1 (S)	0,5	0,5
AN ≤ 2 (S)	2	2
CAS ≤ 2 (S)	3	3
MIC ≤ 2 (S)	2	2
FLC 16–32 (SD)	32	32
ITC ≤ 2 (S)	1	1
POS ≤ (S)	1	1
VRC ≤ 2 (SD)	1	2
Kirby-Bauer Alone size		
AB (20 μg)	8	8
KCA (10 μg)	22	22
ECN (10 μg)	24	24
NY (100 μg)	24	22
MCL (10 μg)	22	27
FLC (100 μg)	12	12

AB amphotericin B, *5FY* 5 fluorocytosine, *AN* anidulafungin, *CAS* caspofungin, *MIC* micafungin, *FLC* fluconazole, *ITC* itraconazole, *POS* posaconazole, *VRC* voriconazole *KCA* Ketoconazole, *ECN* econazole, *NY* nystatin, *MCL* miconazole, *S* susceptible, *SD* susceptible dose dependent

Therefore, all the isolates were identified as *D. capitatus*. The Patterns obtained with primer M13 are shown in Fig. 4. The identifying of the isolated strains was also confirmed by RAPD PCR with primer OPE-4. The discriminatory power was comparable for each of the two primers. The profiles were reproducible between different DNA preparations from the same strain as well as between runs when samples were run a second or a third time. This would indicate that the two cases of disseminated infection caused by *D. capitatus* could be related epidemiologically.

In addition, the genome profiles were compared with those of three other *D. capitatus* isolates, that acted as control organism, performing RAPD PCR and PCR fingerprinting. These controls were not temporally related and were isolated from sputum of adult patients that were hospitalized in the hematologic and surgical wards of the same hospital in 2012 (Fig. 4b).

The susceptibility results for *D. capitatus* strains performed by Kirby-Bauer (K-B) method showed the two strains resistant to amphotericin B but susceptible for micafungin, econazole, ketoconazole, and nystatin. Fluconazole showed intermediated sensitivity. The results of the colorimetric microdilution test, used as confirmation test, are displayed in Table 1. Both *D. capitatus* isolates showed homogeneous results in antifungal susceptibility testing. Differences in voriconazole MICs were not clinically significant. While the resistance to amphotericin B obtained by K-B method was not confirmed.

5 Discussion

D. capitatus infection is rare but can cause life-threatening invasive infections with high morbidity and mortality (Trabelsi et al. 2015). The septicemia is frequently not responsible for clinical signs and symptoms of yeast infection, and respect to candidemia it develops more frequently deep organ dissemination as secondary localization (60–80% vs 10–20% of patients) (Miglietta et al. 2015).

During septicemia, *D. capitatus* produces a soluble antigen cross-reactive with the enzyme immunosorbent assay for *Aspergillus* that offers high sensitive diagnostic tool in managing invasive infection, but determines false-positive results versus invasive aspergillosis (Trabelsi et al. 2015).

The diagnosis of *D. capitatus* invasive infection appears to be easier compared with other fungal infections because hemoculture is positive in more than 70% of invasive cases versus 50% of *Candida* spp. and 10% of *Aspergillus* spp. cases (Girmenia et al. 2005).

The molecular typing is relevant in producing information on genetic variability of isolates and useful in recognizing the source of infection.

We report the up to date first isolation of *D. capitatus* in neonatal age. Our patients were critically ill newborns in NICU with severe general conditions characterized by fetal malnutrition, poor immune defenses, need of empirical broad spectrum antibiotics, intensive use of

multiple invasive devices, and moreover had undergone high-risk neonatal surgery. These characteristics present some overlapping with those of other pediatric and adult patients reported in the literature (Girmenia et al. 2005) and may be triggering factors for the onset of *D. capitatus* infection.

Both our patients seem to confirm that the *D. capitatus* acts as an opportunistic agent responsible for invasive infection only if host defenses are insufficient. As well as reported in adults with haemato-oncological or other severe diseases, critical general conditions which determine acidosis, cardiopulmonary insufficiency, poor immune response and low physical and biological defenses may predispose to *D. capitatus* invasive infection (Viscoli et al. 1999).

In our patients, we identified the isolates performing both phenotypic and genotypic evaluation, and we demonstrated the same molecular profile of these two isolates at migration patterns.

History of both patients demonstrated a likely epidemiological relationship. Patient 1 (outborn) being the index case was transferred from a NICU of another hospital and then patient 2 (inborn) has acquired the same microorganism in our NICU soon after birth, which occurred 15 days after admission of patient 1.

We suppose that patient 1 has been exposed to a pre-transfer PICC contamination by *D. capitatus* in the birth NICU, without classical sign and symptom of infection until a trigger factor (such as main abdominal surgery in an 800 g newborn and its general consequences) allowed invasive infection with emergence of systemic signs (fever, acidosis, pulmonary floccular infiltrations) that lead to diagnosis and identification of *D. capitatus* as the responsible etiologic agent. It is possible that in the pre-clinical period *D. capitatus* has been cross-transmitted from patient 1 to patient 2, likely through instruments or hands of health care workers. Many other concurrent factors may have played a role in allowing such transmission: the first-line use of fluconazole in neonatal unit which demonstrated to be not protective versus *D. capitatus* infection that can therefore easily spread, and the concomitant period of overcrowding (with many newborns in the NICU) and understaffing (because of personnel shortage during the Assumption week holidays in August) that increases the risk of cross-infections. After evidence of isolation of *D. capitatus*, infected newborns were immediately isolated and contact precautions, hand hygiene and sterile procedures have been implemented in the NICU. No other cases of infection by *D. capitatus* were registered.

We cannot exclude that *D. capitatus* has been imported from the former hospital but we do not have any clear evidence because no microbiological investigations have been performed prior to transfer. By typing the two cases confirm the emergence of *D. capitatus* as an opportunistic agent in which the use of central vascular catheters can be identified as a major risk factor of infection. Other cases of geotrichosis by contamination of central venous catheter were described but in adult patients with acute leukemia or polytraumatism (D'Antonio et al. 1994; Trabelsi et al. 2015).

D. capitatus was not responsible for the outcome of our patients. Patient 1 fully recovered from acute abdomen and fungal infection after introduction of adequate antimicrobial treatment, therefore he was back-transferred to the former hospital and died at 5 months of age for complications related to severe prematurity and IUGR. Patient 2 died at 18 days of life because of multiple congenital anomalies and subsequent pulmonary hypertension, before adequate antimicrobial treatment could demonstrate its efficacy.

There is no general optimal treatment strategy for *D. capitatus* infections. In vitro antifungal susceptibility findings are reported contradictory to those observed in the clinical practice (Arendrup et al. 2014). However, the evidence of in vitro intermediate sensitivity to fluconazole suggests that prophylaxis with fluconazole (whose efficacy and safety in preventing Candida infection in high risk newborns has been largely demonstrated by Kaufman 2010) is not protective versus *D. capitatus* infection, that can therefore easily spread.

6 Conclusion

Therapeutic choice against *D. capitatus* in newborns is very limited because most antifungal drugs cannot be used at this stage of life. Amphotericin B and at least in our cases micafungin appear to be the most appropriate and safe drugs in newborns and should be used as first choice treatment.

Low number of clinical reports do not allow to establish appropriate therapeutic protocols, and further studies are necessary to acquire data and to identify efficient target therapy.

References

Arendrup MC, Boekhout T, Akova M et al (2014) ESCMID and ECMM joint clinical guidelines for the diagnosis and management of rare invasive yeast infections. Clin Microbiol Infect Suppl 3:76–98

CLSI (2012) Reference method for Broth Dilution antifungal susceptibility testing of yeasts; fourth informational supplement, CLSI document M27-4, vol 32 (ISBN 1–56238–864-9). Clinical and Laboratory Standards Institute, Wayne

D'Antonio D, Piccolomini R, Fioritoni G et al (1994) Osteomyelitis and intervertebral discitis caused by Blastoschizomyces capitatus in a patient with acute leukemia. J Clin Microbiol 32:224–227

DeHoog GS, Vitale RG (2007) Bipolaris, Exophiala, Scedosporium, Sporothrix and other dematioceous fungi. In: Murray PR (ed) Manual of clinical microbiology, 9th edn. ASM Press, Washington, DC, pp 1898–1917

Ersoz G, Otag F, Erturan Z et al (2004) An outbreak of Dipodascus capitatus infection in the ICU: three case reports and review of the literature. Jpn J Infect Dis 57:248–252

Gadea I, Cuenca-Estrella M, Prieto E et al (2004) Genotyping and antifungal susceptibility profile of dipodascus capitatus isolates causing disseminated infection in seven hematological patients of a tertiary hospital. J Clin Microbiol 42:1832–1836

Giacchino M, Chiapello N, Bezzio S et al (2006) Aspergillus galactomannan enzyme-linked immunosorbent assay cross-reactivity caused by invasive Geotrichum capitatum. J Clin Microbiol 44:3432–3434

Girmenia C, Pagano L, Martino B et al (2005) Invasive infections caused by Trichosporon species and Geotrichum capitatum in patients with hematological malignancies: a retrospective multicenter study from Italy and review of the literature. J Clin Microbiol 43:1818–1828

Girmenia C, Pizzarelli G, D'Antonio D et al (2003) In vitro susceptibility testing of Geotrichum capitatum: comparison of the E-test, disk diffusion, and Sensititre colorimetric methods with the NCCLS M27-A2 broth microdilution reference method. Antimicrob Agents Chemother 47:3985–3988

Guého E, de Hoog GS, Smith MT et al (1987) DNA relatedness, taxonomy and medical significance of Geotrichum capitatum. J Clin Microbiol 25:1191–1194

Kaufman DA (2010) Challenging issues in neonatal candidiasis. Curr Med Res Opin 26:1769–1778

Lafayette TC, Oliveira LT, Landell M et al (2011) Dipodascus capitatus (Geotrichum capitatum): fatal systemic infection on patient with acute myeloid leukemia. Rev Soc Bras Med Trop 44:648–650

Miglietta F, Vella A, Faneschi ML et al (2015) *Geotrichum capitatum* septicaemia in a haematological patient after acute myeloid leukaemia relapse: identification using MALDI-TOF mass spectrometry and review of the literature. Infez Med 23:161–167

Özkaya-Parlakay A, Cengiz AB, Karadağ-Öncel E et al (2012) Geotrichum capitatum septicemia in a hematological malignancy patient with positive galactomannan antigen: case report and review of the literature. Turk J Pediatr 54:674–678

Saghrouni F, Abdeljelil JB, Youssef YB et al (2012) Geotrichum capitatum septicemia in patients with acute myeloid leukemia. Report of three cases. Med Mycol Case Rep 1:88–90

Subramanya Supram H, Gokhale S, Chakrabarti S et al (2016) Emergence of Magnusiomyces capitatus infections in Western Nepal. Med Mycol 54:103–110

Trabelsi H, Néji S, Gargouri L et al (2015) Geotrichum capitatum septicemia: case report and review of the literature. Mycopathologia 179:465–469

Venditti M, Posteraro B, Morace G et al (1991) In-vitro comparative activity of fluconazole and other antifungal agents against Blastoschizomyces capitatus. J Chemother 3:13–15

Viscoli C, Girmenia C, Marinus A et al (1999) Candidemia in cancer patients: a prospective, multicenter surveillance study by the Invasive Fungal Infection Group (IFIG) of the European Organization for Research and Treatment of Cancer (EORTC). Clin Infect Dis 28:1071–1079

Yarrow D (1998) Methods for the isolation, maintenance and identification of yeasts. Elsevier Science, Amsterdam