mycoses
Diagnosis, Therapy and Prophylaxis of Fungal Diseases

Case report

Pseudozyma aphidis fungaemia with invasive fungal pneumonia in a patient with acute myeloid leukaemia: case report and literature review

Hyonsoo Joo,[1] Yeon-Geun Choi,[1] Sung-Yeon Cho,[1,2] Jae-Ki Choi,[1,2] Dong-Gun Lee,[1,2,3] Hee-Je Kim,[3] Irene Jo,[4] Yeon-Joon Park[4] and Kyo-Young Lee[5]

[1]*Division of Infectious Diseases, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea,* [2]*Vaccine Bio Research Institute, College of Medicine, The Catholic University of Korea, Seoul, Korea,* [3]*The Catholic Blood and Marrow Transplantation Centre, College of Medicine, The Catholic University of Korea, Seoul, Korea,* [4]*Department of Laboratory Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea and* [5]*Department of Hospital Pathology, College of Medicine, The Catholic University of Korea, Seoul, Korea*

Summary

Pseudozyma species rarely cause invasive diseases in humans, which are usually isolated from plants. There have been anecdotal reports regarding *Pseudozyma* species infections in patients with underlying diseases or in neonates. However, clinical data and the pathogenicity in humans are still insufficient. We experienced a case of *Pseudozyma aphidis* fungaemia with invasive fungal pneumonia that developed during reinduction chemotherapy in a 51-year-old male with acute myeloid leukaemia (AML). *P. aphidis* was suspected based on the morphology of the yeast isolated from the blood and was confirmed via rDNA gene sequencing analysis. The patient successfully underwent stem cell transplantation with continuing antifungal treatment and finally completely recovered from both the AML and infectious complications. Here, we report a case of *P. aphidis* infection that developed during neutropenia in an AML patient and review the global literature.

Key words: Acute myeloid leukaemia, fungaemia, neutropenia, pneumonia, *Pseudozyma aphidis* yeasts.

Introduction

Rare fungi have recently been implicated in human infections ranging from colonisation to invasive fungal infections (IFIs) in immunocompromised patients, accounting for <10% of all isolated fungal pathogens.[1] *Pseudozyma* species (spp.) are basidiomycetous yeast classified in the family Ustilaginaceae, and its members are close relatives of *Ustilago maydis* and other smut fungi. At least 20 *Pseudozyma* spp. are recognised, most of which are environmental pathogens.[2] *Pseudozyma* spp. infections in humans have rarely been reported after the first description as a human pathogen in 2003.[3] Data regarding the clinical characteristics and pathogenicity in humans remain insufficient. Recently, we experienced a case of *Pseudozyma aphidis* fungaemia with invasive fungal pneumonia after reinduction chemotherapy for acute myeloid leukaemia (AML). Here, we describe this case and review the global literature. The Institutional Review Board at Seoul St. Mary's Hospital approved this case report and waived the need for patient consent (No. KC15RISI0570).

Correspondence: S.-Y. Cho, MD, Division of Infectious Diseases, Department of Internal Medicine, Vaccine Bio Research Institute, College of Medicine, Seoul St. Mary's Hospital, The Catholic University of Korea, Banpo-daero 222, Seocho-gu, Seoul 137-701, Korea.
Tel: 82-2-2258-7578. Fax: 82-2-535-2494.
E-mail: yeon3120@naver.com

Submitted for publication 22 August 2015
Revised 22 October 2015
Accepted for publication 23 October 2015

Case report

A 51-year-old man who was diagnosed with AML and had not experienced remission after the first induction

chemotherapy started reinduction chemotherapy with 100 mg m^{-2} cytarabine for 7 days and 90 mg m^{-2} daunorubicin for 3 days. On day seven after reinduction chemotherapy (D7), neutropenic fever (up to 38.0 °C) developed as measured using an axillary thermometer. No other symptoms were reported. Laboratory data included a white blood cell count of 220 μl^{-1} (absolute neutrophil count, 0 μl^{-1}) and C-reactive protein level of 8.60 mg dL^{-1}. Empirical antibiotic therapy with ceftazidime (2 g twice a day) and isepamicin (400 mg once a day) was initiated after performing blood cultures. On D8, the results of the blood culture revealed presumptive growth of gram-positive cocci (GPC). Teicoplanin (12 mg kg^{-1} a day after loading with 12 mg kg^{-1} every 12 h for three doses) was added based on the culture results.

Although the patient had no respiratory symptoms, infiltration was suspected in the right lower lung field on the chest radiograph on D9. Low-dose computed tomography of the lung was performed, which showed consolidation with surrounding ground glass opacity at the medial segment of the right middle lobe (RML) (Fig. 1). The serum galactomannan assay performed twice a week was negative. The patient received fluconazole instead of posaconazole as the primary antifungal prophylaxis; because he had participated in a clinical trial using a thrombopoietin receptor agonist during the same period, a drug interaction was possible. Then, the antifungal prophylaxis was empirically changed to caspofungin (50 mg a day after the loading dose)

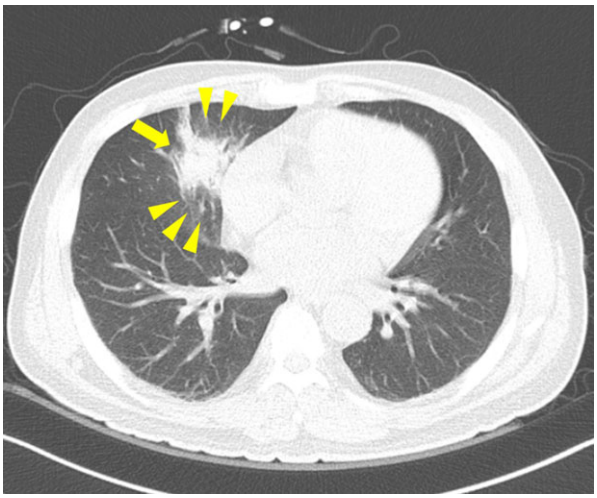

Figure 1 Low dose computed tomography of the lung. Consolidation with surrounding ground glass opacity at the medial segment of the right middle lobe.

for the possibility of fungal pneumonia according to the revised definition of IFI from the European Organization for the Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG).[4]

The GPC from the blood culture was identified as vancomycin-resistant *Enterococcus faecium* (3 of 4 bottles), using Vitek 2 (bioMérieux, Hazelwood, MO, USA). Teicoplanin was changed to linezolid (600 mg twice a day). The follow-up blood culture performed on D11 revealed negative conversion of vancomycin-resistant *Enterococcus* bacteraemia. However, the neutropenic fever persisted, and yeast-like organisms were noted from central blood culture (1 of 2 bottles) performed on the same day (D11). This culture result was reported after 2 days of incubation, using BD BACTEC FX blood culture system (BD Diagnostics, Sparks, MD, USA).

Colonies observed on the blood agar plate were white, dry or wrinkled (Fig. 2a). Microscopically, they appeared as yeast with branching pseudohyphae (Fig. 2b). The species could not be identified using either Vitek 2 or API 20C (bioMérieux). Therefore, further identification was performed using rDNA gene sequencing analysis with the following primers: internally transcribed spacer (ITS)-1 (forward primer [5′-TCC GTA GGT GAA CCT GCG G-3′] and reverse primer [5′-GCT GCG TTC ATC GAT-3′]), ITS-2 (forward primer [5′-GCA TCG ATG AAG AAC GCA-3′] and reverse primer [5′-TCC TCC GCT TAT TGA TAT-3′]), and D1/D2 domain (forward primer [5′-GCA TAT CAA TAA GCG GAG-3′] and reverse primer [5′-GGT CCG TGT TTC AAG ACG G-3′]).[5] The isolated gene sequence showed 100% concordance with the *P. aphidis* strain (GenBank accession number: KF443199.1 and KF443201.1). Because the fungaemia developed during the caspofungin therapy and pneumonia was aggravated with persistent neutropenic fever, caspofungin was changed to liposomal amphotericin B (5 mg kg^{-1} per day) on D15. The Hickman catheter was removed. The culture result of the tip of the removed Hickman catheter showed no growth.

The follow-up blood culture performed on D14 showed no growth. However, pneumonia was aggravated despite the broad-spectrum antifungal agent. The findings of the bronchoscopy performed on D23 showed no endobronchial lesion. Transbronchial lung biopsy and bronchial washing and brushing were performed at the RML bronchus to identify the pathogens of fungal pneumonia. Pathology specimens showed inflammatory changes with necrosis and dichotomous hyphae with a septum, suggestive of organising

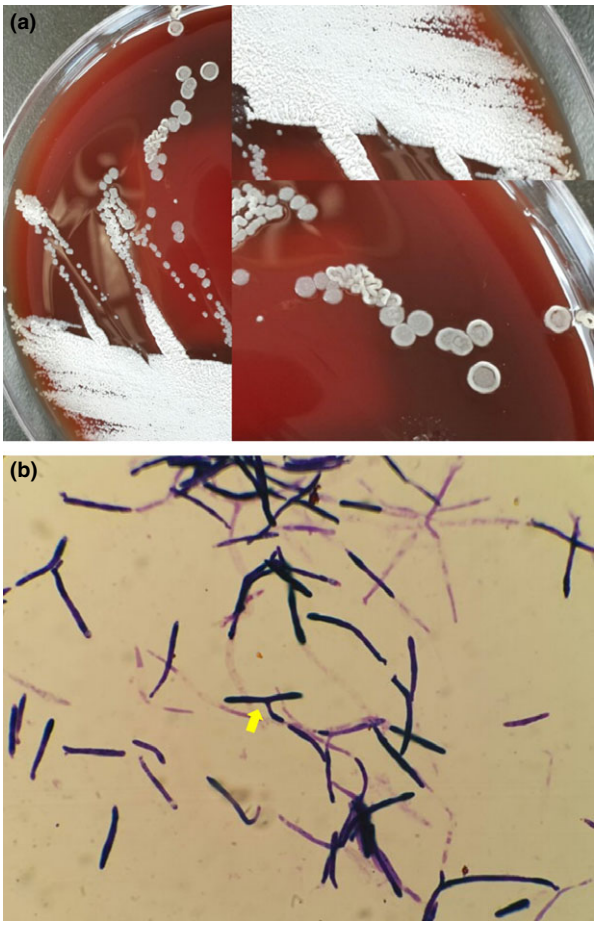

Figure 2 Colonies of yeast on blood agar and microscopic morphology. (a) Yeast-like colonies that are dry, creamy, brightly coloured, and glabrous in texture; (b) Gram stain showing yeast with branching pseudohyphae (arrow).

pneumonia with a fungal infection (Fig. 3). However, we could not identify the genus level due to tissue damage. Culture with bronchial washing fluid revealed no growth of fungal organisms. As the neutropenia recovered, the patient improved clinically and was discharged with itraconazole capsules (200 mg twice a day). At the outpatient clinic, chest radiography revealed little change in the RML consolidation despite 3 weeks of continued itraconazole. Considering the possibility of both proven invasive pulmonary aspergillosis and fungal pneumonia due to *Pseudozyma* spp., itraconazole was changed to voriconazole (4 mg kg^{-1} twice a day). The patient sequentially received consolidation chemotherapy and allogeneic stem cell transplantation (SCT) while continuing the voriconazole. During the voriconazole treatment, serum level of voriconazole was within the therapeutic range. The pneumonia resolved after 4 months of voriconazole treatment, with complete remission of AML after successful SCT.

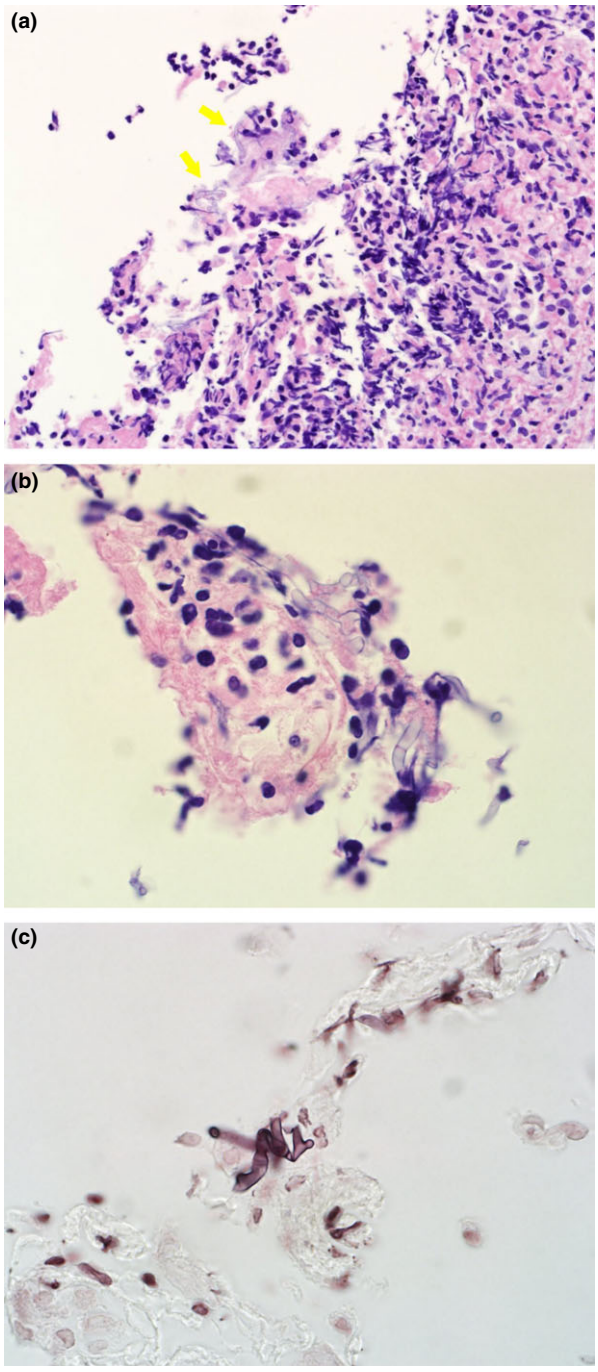

Figure 3 Histopathology of lung tissue from a transbronchial lung biopsy specimen. Fungal organisms on the background of necrosis and inflammatory infiltration. (a) Haematoxylin and eosin stain, ×100; (b) Haematoxylin and eosin stain, ×400; (c) Silver stain, ×400.

Discussion

Invasive fungal infections remain a major cause of significant morbidity and mortality in immunocompromised patients, especially those with hematologic malignancies. Of the IFIs, a rare fungus is difficult to treat in the aspects of the early diagnosis and appropriate treatment, which might be related to the severity of the patient's condition and often challenging intrinsic susceptibility pattern of the pathogens.[6] *Pseudozyma* spp. was first described as a possible human pathogen in 2003, when it was identified from blood cultures from three Thai patients, as *P. antarctica*, *P. parantarctica*, and *P. thailandica*.[3] The first case

Table 1 Literature review of *Pseudozyma* species infections in human.

Case no.	Age/sex	Underlying condition	Predisposing factors			Clinical presentation	Isolated specimen
			CVC	TPN	Plant/crop		
1	N/A	N/A	N/A	N/A	N/A	N/A	Blood
2	N/A	N/A	N/A	N/A	N/A	N/A	Blood
3	N/A	N/A	N/A	N/A	N/A	N/A	Blood
4	7/F	Short gut syndrome	+	+	+[1]	Fever, chill, malaise, fatigue	Blood
5	78/M	Astrocytoma	N/A	N/A	N/A	Fever after brain surgery	Abscess of brain
6	51/M	Farmer, chronic leg swelling	–	–	+	Chronic mycetoma of leg	Leg[2]
7	17/M	Burkitt lymphoma, chemotherapy	+	N/A	N/A	Neutropenic fever, lung infiltrates	Pleural fluid
8	0/M	Hemolytic jaundice	N/A	N/A	N/A	Lethargy, poor feeding	Blood
9	52/F	Crohn's disease, total colectomy state	+	+	–	Fever, headache, weakness	Blood
10	N/A	N/A	N/A	N/A	N/A	N/A	Blood
11	N/A	N/A	N/A	N/A	N/A	N/A	Blood
12	N/A	N/A	N/A	N/A	N/A	N/A	Blood
13	68/F	Adenocarcinoma of ampulla of Vater	+	N/A	N/A	Fever, chill	Blood
14	6/F	Osteosarcoma with lung metastasis	+	N/A	N/A	Neutropenic fever	Blood
15	51/M	AML, reinduction chemotherapy	+	+	–	Neutropenic fever, lung infiltrates[3]	Blood

Case no.	*Pseudozyma spp.*	MIC (mg dL^{-1})						Treatment			
		FLC	ITC	VRC	AMB	CAS	5FC	Antifungal agent	Duration	Outcome	Country, year [reference]
1	*P. antarctica*	N/A	N/A	N/A	N/A	N/A	R[4]	N/A	–	N/A	Thai, 2003 [3]
2	*P. parantarctica*	N/A	N/A	N/A	N/A	N/A	R[4]	N/A	–	N/A	Thai, 2003 [3]
3	*P. thailandica*	R[4]	R[4]	N/A	N/A	N/A	R[4]	N/A	–	N/A	Thai, 2003 [3]
4	*P. aphidis*	4	0.125	N/A	0.25	N/A	N/A	FLC → ITC	N/A	Improved	USA, 2008 [8]
5	*Pseudozyma* spp.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	–	Death	Korea, 2010 [9]
6	*P. aphidis*	N/A	N/A	N/A	N/A	N/A	N/A	ITC[5]	1 year	Improved	China, 2011 [10]
7	*P. aphidis*	4	0.25	0.03	0.25	4	N/A	LAMB → VRC	25 day	Improved	Brazil, 2013 [11]
8	*P. aphidis*	8	0.03	0.06	0.03	8	>64	AMB → VRC	14 day	Improved	India, 2013 [7]
9	*Pseudozyma* spp.	N/A	N/A	N/A	N/A	N/A	N/A	FLC → VRC	N/A	Improved	USA, 2014 [12]
10	*P. alboarmeniaca*	32	4	2	0.25	>16[6]	>64	N/A	–	N/A	Thai, 2014 [13]
11	*P. crassa*	>64	4	2	0.25	>16[6]	>64	N/A	–	N/A	Thai, 2014 [13]
12	*P. siamensis*	32	4	2	0.125	>16[6]	>64	N/A	–	N/A	Thai, 2014 [13]
13	*P. aphidis*	16	0.19	0.032	0.19	>32	>32	LAMB	14 day	Improved	France, 2015 [14]
14	*P. aphidis*	2	0.03	0.03	0.13	N/A	128	LAMB	14 day	Improved	Argentina, 2015 [15]
15	*P. aphidis*	N/A	N/A	N/A	N/A	N/A	N/A	LAMB → VRC	4 month	Improved	[This case]

AMB, amphotericin B; AML, acute myeloid leukaemia; CAS, caspofungin; CVC, central venous catheter; FLC, fluconazole; ITC, itraconazole; LAMB, liposomal amphotericin B; MIC, minimal inhibitory concentration; N/A, not available; TPN, total parenteral nutrition; VRC, voriconazole; 5FC, flucytosine.

[1]The patient consumed large amounts of tortilla corn chips.

[2]Histopathology of deep tissue from the foot showed grains surrounded by inflammatory cells, periodic acid-Schiff stain showed clustered yeast-like cells, and tissue cultures showed septate hyaline hyphae on a wet mount.

[3]Histopathology of lung tissue showed necrosis, inflammatory cells, and septate dichotomous hyphae. However, a fungus culture or sequencing using the tissue was not performed.

[4]Not reported MIC values.

[5]In addition to repeated debridement.

[6]MIC values for micafungin.

of *P. aphidis* human infection was reported in a 7-year-old girl with short gut syndrome in 2008.[7]

Pseudozyma spp. is classified under the family Ustilaginaceae, phylum Basidiomycota, subphylum Ustilaginomycotina, class Ustilaginomycetes, and order Ustilaginales.[2,8] Ustilaginales are plant pathogens that can infect corn plants to produce tumour-like galls. Of the *Pseudozyma* spp., *P. aphidis* is known as the most common human pathogen. However, due to the rare isolation of *P. aphidis* in human infections, this rare fungus species cannot be identified using commercial systems that are available in routine diagnostic laboratories. However, sequencing and phylogenetic analysis can help with the direct detection of a fungus from blood or tissues because yeast taxonomy is continually evolving.

We retrospectively reviewed the global literature to identify the possible risk factors, clinical presentation, and optimal treatment strategies for *Pseudozyma* infection; in addition to the present case, 14 case reports were found (Table 1).[3,7–15] Since 2014 when previous literature review was reported by Prakash *et al.* [8], there were seven additional cases including our case. Therefore, we present a updated literature review with some modifications and adding recent cases. Of the 15 cases, seven were identified as *P. aphidis*, two were *Pseudozyma spp.* without spp. level identification, and the remaining six cases were non-*aphidis Pseudozyma* isolates that were all different spp. from Thailand. Of the nine cases with reported underlying medical conditions, three had gastrointestinal (GI) tract problems such as short bowel syndrome or intestinal surgery,[4,9,13] and three had neutropenia[7,14,15] which can damage the gut mucosa. Of the seven patients for whom the presence or absence of a central venous catheter (CVC) was reported, six patients had a CVC. The risk factors are thought to be similar to those of other less common yeast infections including GI tract problems, the presence of a CVC, and neutropenia. We could not identify the exact social history or dietary history to determine potential exposure to plants or crops. However, crop exposure was identified in two cases: a farmer with mycetoma of the leg and a paediatric patient who had eaten corn chips.[8,10] In the present case, the possible port of entry for the *P. aphidis* fungaemia is uncertain. While the fungaemia met the definition of a catheter-related bloodstream infection because *P. aphidis* was isolated only from central blood, the origin of *P. aphidis* is unclear.

The major manifestation of *Pseudozyma* infection is fungaemia (12 of 15 cases, 80%); others (n = 3) have been isolated from a brain abscess that developed after brain surgery, deep biopsy of mycetoma of the leg, and pleural fluid.[9–11] In our case, we proved *P. aphidis* fungaemia with fungal pneumonia from lung tissue. However, our case has a limitation, in that we could not identify the fungal pathogen from the lung tissue. Fungus culture with fresh lung tissue and sequencing of the tissue was not performed. Galactomannan assay using bronchial washing fluid was not performed either. Antifungal susceptibility testing was performed in 11 cases of human infections caused by *Pseudozyma* spp., *P. aphidis* was susceptible to itraconazole, voriconazole, and amphotericin B; had varying susceptibility to fluconazole; and was resistant to echinocandines and flucytosine.[7,8,11,14,15] Non-*aphidis Pseudozyma* spp. seemed to have susceptibility to amphotericin B only.[13] In all reported cases, *Pseudozyma* spp. were resistant to echinocandins and flucytosine.

Here, we described a case of a 51-year-old male patient with AML who suffered from neutropenic fever during chemotherapy with a defined bacterial and fungal infection that was finally diagnosed as *P. aphidis* fungaemia and concurrent invasive fungal pneumonia without genus level identification. Many other fungal pathogens show subtle morphological differences between forms found in tissue and in culture. Further accumulation of data regarding rare fungi is needed. This case is worth reporting in the aspect that *P. aphidis* fungaemia developed during neutropenic fever with concurrent invasive fungal pneumonia in an AML patient.

Conflict of interest

We declare no conflicts of interest.

References

1 Azie N, Neofytos D, Pfaller M, Meier-Kriesche HU, Quan SP, Horn D. The PATH (Prospective Antifungal Therapy) Alliance(R) registry and invasive fungal infections: update 2012. *Diagn Microbiol Infect Dis* 2012; **73**: 293–300.

2 Howell SA, Hazen KC, Brandt ME. *Candida, Cryptococcus*, and other yeasts of medical importance. In: Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW (eds), *Manual of Clinical Microbiology*, 11th edn. Washington, DC: ASM Press, 2015: 1984–2014.

3 Sugita T, Takashima M, Poonwan N *et al.* The first isolation of ustilaginomycetous anamorphic yeasts, *Pseudozyma* species, from patients' blood and a description of two new species: *P. parantarctica* and *P. thailandica*. *Microbiol Immunol* 2003; **47**: 183–90.

4 De Pauw B, Walsh TJ, Donnelly JP *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**: 1813–21.

5 CLSI. *Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing; Approved Guidelines*. CLSI document MM18-A. Wayne, PA: Clinical and Laboratory Standards Institute, 2008.
6 Arendrup MC, Boekhout T, Akova M, Meis JF, Cornely OA, 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.
7 Lin SS, Pranikoff T, Smith SF *et al.* Central venous catheter infection associated with *Pseudozyma aphidis* in a child with short gut syndrome. *J Med Microbiol* 2008; **57**: 516–8.
8 Prakash A, Wankhede S, Singh PK *et al.* First neonatal case of fungaemia due to *Pseudozyma aphidis* and a global literature review. *Mycoses* 2014; **57**: 64–68.
9 Hwang S, Kim J, Yoon S *et al.* First report of brain abscess associated with *Pseudozyma* species in a patient with astrocytoma. *Korean J Lab Med* 2010; **30**: 284–8.
10 Chen B, Zhu LY, Xuan X *et al.* Isolation of both *Pseudozyma aphidis* and *Nocardia otitidiscaviarum* from a mycetoma on the leg. *Int J Dermatol* 2011; **50**: 714–9.
11 Parahym AM, da Silva CM, Domingos Ide F *et al.* Pulmonary infection due to *Pseudozyma aphidis* in a patient with Burkitt lymphoma: first case report. *Diagn Microbiol Infect Dis* 2013; **75**: 104–6.
12 Siddiqui W, Ahmed Y, Albrecht H, Weissman S. *Pseudozyma* spp catheter-associated blood stream infection, an emerging pathogen and brief literature review. *BMJ Case Rep* 2014; **2014**: doi: 10.1136/bcr-2014-206369.
13 Mekha N, Takashima M, Boon-Long J, Cho O, Sugita T. Three new basidiomycetous yeasts, *Pseudozyma alboarmeniaca* sp. nov., *Pseudozyma crassa* sp. nov. and *Pseudozyma siamensis* sp. nov. isolated from Thai patients. *Microbiol Immunol* 2014; **58**: 9–14.
14 Herb A, Sabou M, Delhorme JB *et al. Pseudozyma aphidis* fungemia after abdominal surgery: first adult case. *Med Mycol Case Rep* 2015; **8**: 37–39.
15 Orecchini LA, Olmos E, Taverna CG *et al.* First case of fungemia due to *Pseudozyma aphidis* in a pediatric patient with osteosarcoma in Latin America. *J Clin Microbiol* 2015; **53**: 3691–4.