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CASE REPORT

First Case of Fungemia Due to *Pseudozyma aphidis* in a Pediatric Patient with Osteosarcoma in Latin America

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We report the first case of blood infection due to *Pseudozyma aphidis* in Latin America. We contribute evidence showing this organism to be a potential human pathogen, and we provide new data about its identification, drug susceptibility, and treatment outcome.

CASE REPORT

A 6-year-old female from Córdoba, Argentina, was diagnosed with osteosarcoma of the left tibia and lung metastasis and started chemotherapy administered through a long-term indwelling central line (a central venous catheter [CVC]) on 2 September 2013 at the Hospital de niños de la Santísima Trinidad. On 2 October, she presented with febrile neutropenia and was empirically treated with ceftazidime, vancomycin, and amikacin; blood and urine samples were submitted for laboratory testing, and both cultures were negative. On 4 October, she received clindamycin due to facial edema. From 6 October to 13 November, she was transfused with platelets and red blood cells several times due to thrombocytopenia and anemia. On 5 November, she developed severe mucositis and erythroderma and was treated with ceftazidime, nystatin, amikacin, vancomycin, and fluconazole. On 13 November, she received a 1-week treatment with meropenem and liposomal amphotericin B. On 18 November, she developed conjunctival hyperemia and was treated with tobramycin. On 28 November, she underwent supracondylar amputation of the left lower limb. On 20 December, the patient presented with febrile neutropenia (white blood cells [WBCs], 1,030/mm^3) and was admitted to the intensive care unit (ICU) in the same hospital. Table 1 summarizes the clinical data and evolution of the case since admission. A blood sample (BS1) obtained by venipuncture and a hub blood sample (drawn through the catheter hub) (HBS1) were taken and sent to the microbiology laboratory. Yeast cells (*Pseudozyma aphidis*; isolate Y1) were detected in the hub blood sample after 2.6 days of incubation in a Bact/ALERT PF (bioMérieux Inc., Durham, NC). However, the patient did not show clinical signs of illness on day 3 after admission; therefore, the isolate was considered not significant and she was discharged. Four days later, the patient presented with vomiting, dehydration, and febrile neutropenia (WBCs, 200/mm^3) and was admitted once again at the ICU in the same institution. At that point, an empirical antibiotic treatment with ceftazidime and amikacin was initiated and a new blood sample and hub blood sample (BS2 and HBS2) were taken and processed. After 18 h of incubation, both cultures were positive for *Pseudomonas aeruginosa*. Four days after readmission, the patient presented with fever and severe neutropenia (WBCs, 80/mm^3) and showed high (233 mg/liter) C-reactive protein levels. A blood sample and a hub blood sample (BS3 and HBS3) were taken and processed. After 12 h of incubation, the blood sample culture was positive for *Escherichia coli*, and 2 h later, the hub blood sample culture was positive for yeast (Y2). This isolate (Y2) had the same phenotypic features as the first yeast isolate (Y1). Six days after readmission, the patient presented with mouth bullous lesions and acute tubular necrosis. Since antibiotic susceptibility testing showed the presence of extended-spectrum-beta-lactamase-producing *E. coli*, the antibiotic treatment was changed to meropenem and amikacin. Moreover, in light of the isolation of Y2, the CVC of the patient was removed and sent to the microbiology laboratory together with a new blood sample (BS4). The catheter was processed by the method of Brun-Buisson et al. (1). After 3.5 days of incubation, the blood sample was positive for yeast (Y3) and the catheter yielded $<$100 CFU ml^{-1}. Ten days after readmission, the patient presented with fever and severe neutropenia (WBCs, 130/mm^3) and showed high (178 mg/liter) C-reactive protein levels. Antifungal treatment with liposomal amphotericin B was initiated. A new blood sample and a hub blood sample were taken (BS5 and HBS4) and processed. After 3.7 days of incubation, the hub blood sample was positive for yeast (Y4). On day 15 after readmission and after 5 days of antifungal treatment, a blood sample and a hub blood sample (BS6 and HBS5) and a peripheral venous catheter (PVC) sample were taken and processed. All these cultures were negative. After 14 days of treatment, the patient did not show clinical signs of illness and she was discharged.

Microbiological studies. All yeast isolates showed rough, cream-colored colonies on Sabouraud dextrose agar (Difco; Becton Dickinson and Company, Baltimore, MD, USA) and on CHROMagar Candida (CHROMagar Company, Paris, France).

Received 23 April 2015 **Returned for modification** 16 June 2015
Accepted 17 August 2015

Accepted manuscript posted online 19 August 2015

Citation Orecchini LA, Olmos E, Taverna CG, Murisengo OA, Szuzs W, Vivot W, Córdoba S, Bosco-Borgeat ME, Montanaro PC. 2015. First case of fungemia due to *Pseudozyma aphidis* in a pediatric patient with osteosarcoma in Latin America. J Clin Microbiol 53:3691–3694. doi:10.1128/JCM.01095-15.

Editor: G. A. Land

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TABLE 1 Clinical data, evolution, and treatment of the case after first admission at intensive care unit[a]

Day after admission or readmission (treatment)	Clinical event(s)	Sample	Time to positivity	Isolate
Initial admission				
1	Admitted at ICU; fever	BS1		
		HSB1	2.6 days	Yeast (Y1)
3	No clinical signs of illness; discharged			
Readmission: 4 days after initial admission				
1 (ceftazidime and amikacin)	Fever, vomiting, and dehydration; WBCs, 200/mm^3	BS2	18 h	*P. aeruginosa*
		HSB2	18 h	*P. aeruginosa*
4	Fever; WBCs, 80/mm^3; CRP, 233 mg/liter	BS3	12 h	*E. coli*
		HSB3	14 h	Yeast (Y2)
6 (meropenem and amikacin [for extended-spectrum-beta-lactamase-producing *E. coli*])	Mouth bullous lesions and acute tubular necrosis			
8 (meropenem and amikacin [for extended-spectrum-beta-lactamase-producing *E. coli*])		BS4	3.5 days	Yeast (Y3)
		CVC		Yeast (<100 CFU/ml)
10 (liposomal amphotericin B [for 14 days])	Fever; WBCs, 130/mm^3; CRP, 178 mg/liter	BS5		
		HSB4	3.7 days	Yeast (Y4)
15		BS6		
		HSB5		
		PVC		
24	No clinical signs of illness			

[a] WBCs, white blood cells; CRP, C-reactive protein; BS, blood sample; HBS, catheter hub blood sample; PVC, peripheral venous catheter.

Fusiform blastoconidia and the presence of hyphae were observed on 5% malt extract broth (2). Germ tube test results were negative. API 20 AUX (bioMérieux, Marcy l'Etoile, France) yielded the same nonconclusive profile in all yeast isolates.

Two isolates (Y2 and Y3) were sent to the national reference center, the Mycology Department of the Instituto Nacional de Enfermedades Infecciosas (INEI) Dr. Carlos G. Malbrán, for further analysis. Morphological, physiological, and biochemical tests were performed according to the standard methodology (2). These characteristics were similar for the two isolates. The results of the tests were as follows. For morphological characterizations, after 7 days of growth at 28°C on yeast extract-malt extract (YM) agar (2), the colonies were white to pale yellowish-cream-colored, irregular, slightly raised, folded in the surface with a fringed margin, and of butyrous consistency; after 3 days of growth on 5% malt extract broth at 28°C, fusiform spindle-shaped elongated blastoconidia and the presence of hyphae were observed. With respect to physiological and biochemical characteristics, fermentation was absent; galactose, L-sorbose, sacarose, maltose, cellobiose, trehalose, lactose, melibiose, raffinose, melezitose, D-xylose, L-arabinose, D-ribose, D-mannitol, myoinositol, erythritol, and citrate were assimilated; and L-rhamnose and nitrate were not assimilated. The urease test result was negative. Isolates grew well at 28, 35, and 37°C.

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF) identification was performed by using a MALDI Microflex LT system (Bruker Daltonics, Bremen, Germany). Protein extraction and sample preparation were carried out using a formic acid extraction procedure and α-cyano-4-hydroxycinnamic acid (CHCA) as a matrix solution according to the manufacturer's instructions. Mass spectra were processed using MALDI Biotyper software (Bruker Daltonics, Bremen, Germany) with default settings and were matched against reference libraries. The results by MALDI-TOF yielded score values of <1.7 and were therefore considered unreliable. Molecular characterization was performed by sequencing two different variable regions of ribosomal DNA (rDNA) widely used in the identification of yeast (3–5): the internal transcribed spacer 1 (ITS1)–5.8S–ITS2 region with primers ITS1/ITS4 (6) and the D1/D2 domain of the 26S rRNA gene with primers NL1/NL4 (6). DNA extraction, amplification, and sequencing procedures were done as described before (7). The sequence data obtained were compared to sequences published in the GenBank database (National Center of Biotechnology Information, National Library of Medicine, Bethesda, MD) using the BLASTN program. rDNA regions were identical for the two isolates, and BLAST analysis of the sequences revealed a 100% similarity to *Pseudozyma aphidis* type strain CBS517.

Isolates were introduced in the Culture Collection of the Mycology Department of the INEI Dr. Carlos G. Malbrán (DMic) with the following collection numbers: DMic144752 (Y3) and DMic144761 (Y2).

To investigate the antifungal drug susceptibility, MIC values were determined according to the E.Def 7.2 reference document of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (8). Amphotericin B, flucytosine, and itraconazole (Sigma-Aldrich, Quimica, Argentina), fluconazole and voriconazole (Pfizer, SA, Argentina), and posaconazole (Merck Co.) were used as antifungal drugs and were provided as standard powders of known potency. *Candida parapsilosis* ATCC 22019, *C. krusei* ATCC 6258, and *C. neoformans* ATCC 90112 were used as quality control strains. For amphotericin B, the MIC endpoint was defined as the lowest drug concentration that caused a prominent reduction (MIC of 0 mg/liter or >90% growth reduction) in growth compared with the growth in the drug-free well. Meanwhile, for azoles and flucytosine, the MIC endpoint was defined as the lowest drug concentration at which the growth of the isolates was reduced by 50% or more compared with that of the control

(MIC of 2 mg/liter or ≥50%). MIC values were expressed in milligrams per liter and were as follows: 128 for flucytosine, 0.13 for amphotericin B, 2 for fluconazole, 0.03 for itraconazole and voriconazole, and 0.015 for posaconazole.

Pseudozyma spp. belong to the phylum *Basidiomycota*, subphylum *Ustilaginomycotina*, class *Ustilaginomycetes*, and order *Ustilaginales* (9). *Pseudozyma* spp., as well as the closely related *Ustilago* spp., are well-known plant pathogens, although these organisms have been implicated occasionally in human infections. Due to this, they have been poorly studied and their mechanism of pathogenesis remains unknown. Until recently, very few cases had been reported worldwide. The first report of human infection was in 2003 and accounted for the isolation of three *Pseudozyma* species (*Pseudozyma antarctica*, *Pseudozyma parantarctica* sp. nov., and *Pseudozyma thailandica* sp. nov.) from blood samples of Thai patients with spontaneous pneumothorax, leptospirosis, and aseptic meningitis; acute asthmatic attack; and respiratory failure, respectively (10). Since then, other cases of human infection due to *Pseudozyma* species have been reported: a CVC-associated infection due to *P. aphidis* in a child with short gut syndrome (11), a brain abscess associated with a *Pseudozyma* sp. in a patient with astrocytoma (12), a case of mycetoma associated with *Nocardia otitidiscaviarum* and *P. aphidis* (13), a fungemia due to *P. aphidis* in a neonate (14), a pulmonary infection due to *P. aphidis* in a patient with Burkitt lymphoma (15), and a new report that shows the isolation of three new additional *Pseudozyma* species (*Pseudozyma alboarmeniaca* sp. nov., *Pseudozyma crassa* sp. nov., and *Pseudozyma siamensis* sp. nov.) from blood of Thai patients (16). According to this, *P. aphidis* seems to be the *Pseudozyma* species most frequently isolated from human samples.

Similarly, human infections due to *Ustilago* spp. have been reported in patients with cutaneous disease (17), chronic leptomeningitis and ependymitis (18), CVC-associated infections (19), chronic skin rash (20), and central-line-related infections (21).

It is worthy of note, as mentioned before (14), that almost all cases of *Pseudozyma* infections are invasive, and it has been proposed that, in those cases, hand-related transmission (11), inoculation by needle biopsy (12), vaginal or nosocomial transmission during birth (14), inhalation of conidia from the environment (15), and translocation of fungus across mucosal gastrointestinal barriers in patients with compromised integrity and anatomy of the gastrointestinal tract (11) represent potential portals of entry for CVC contamination. Since *Pseudozyma* spp., as well as *Ustilago* spp., are associated with corn, dietary history is important in evaluating patients with invasive disease caused by these yeasts (11). Unfortunately, we have no record of the patient dietary history. It is also worth noting that, as in the present work, three of the four cases in which *P. aphidis* were isolated involved pediatric patients (11, 13–15).

Recently, a panel of experts of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Fungal Infection Study Group (EFISG) and the European Confederation of Medical Mycology (ECMM) included *Pseudozyma* spp. in their clinical guidelines for the diagnosis and management of "rare invasive yeast infections" (8). However, they remarked that these fungal organisms are not rare *per se* since they are encountered in food items or in the environment. Still, as predicted from their low pathogenicity, invasive *Pseudozyma* infections are reported at low numbers in severely immunocompromised hosts. Risk factors associated with them are similar to those of non-albicans *Candida* spp., i.e., extremes of age, cancer chemotherapy, neutropenia, presence of a CVC, and severe thrombocytopenia (8).

Morphological, physiological, and biochemical features were consistent with those of yeasts of the order *Ustilaginales* (9) and similar to those of *P. aphidis* isolates previously reported. However, on CHROMagar Candida, our isolates developed cream-colored colonies, in contrast with the findings of Prakash et al. (14), and could not be identified using an API 20 AUX system. Due to this, sequencing of ITS and/or D1/D2 domains was necessary for proper identification, in agreement with the literature (8, 11–15). Also, isolates could not be identified by MALDI-TOF. However, it is important that the performance of this technique, as well as of DNA sequencing, depends on the quality of the databases employed (8). In our case, only one *Pseudozyma aphidis* strain was present in the reference database, but this could be improved by the creation of an in-house database and the addition of different strains belonging to the same species (22, 23).

Overall, *Pseudozyma aphidis* isolates exhibited low (≤0.13 mg/liter) drug MIC values for most antifungal drugs tested. However, the fluconazole MIC value was 2 mg/liter, and flucytosine was unable to inhibit yeast growth. It is worth remarking that, to date, no MIC breakpoints have been established for antifungal agents against *Pseudozyma* spp.; thus, no categorical interpretation was done. Interestingly, our findings are in agreement with previous reports of Prakash et al. (14) and Lin et al. (11).

Although the available data are too limited to permit firm recommendations, first-line treatment options include amphotericin B, voriconazole, and posaconazole, whereas fluconazole and flucytosine should be avoided (8). On the other hand, as *Pseudozyma* spp. belong to the phylum *Basidiomycota*, candins (such as caspofungin, anidulafungin, and micafungin) are not indicated for treatment given that members of this phylum lack the target protein for these antifungal drugs.

Although *Pseudozyma* spp. are today clearly opportunistic human pathogens, little is known about the pathogenesis and management of invasive infections caused by these organisms. This article discusses the first case of blood infection due to *Pseudozyma aphidis* in Latin America and provides new data about identification, drug susceptibility, and treatment outcomes.

Nucleotide sequence accession numbers. The rDNA region sequences of the isolates were submitted to GenBank (GenBank accession numbers KM610219 and KM610218).

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