CASE REPORT

Disseminated Nosocomial Fungal Infection by *Aureobasidium pullulans* var. *melanigenum*: a Case Report

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We report on a rare case of disseminated nosocomial fungal infection due to *Aureobasidium pullulans* var. *melanigenum* in a severely traumatized patient. Repeated blood and urine cultures yielded multicellular filamentous hyphal structures of varying size accompanied by budding yeast-like-cells of ellipsoidal morphology. The patient became asymptomatic after fluconazole therapy.

CASE REPORT

The patient, a 28-year-old male, was admitted to the intensive care unit of the Ospedali Riuniti of Reggio Calabria, Reggio Calabria, Italy, following multiple traumas due to a road accident. The patient suffered a head trauma and had a Glasgow coma scale rating of III-IV. After initial treatment with standard life support measures to stabilize hemodynamic and respiratory parameters, the patient underwent a total-body computed tomography scan, which showed diffuse cerebral edema, hemoperitoneum, multiple fractures of the left femur and tibia, a suspected pelvic fracture, and multiple ruptures of the liver's right lobe, which made immediate surgery mandatory. After surgery, the patient underwent mechanical ventilation and prolonged total parenteral nutrition.

Following the isolation of a methicillin-resistant strain of *Staphylococcus epidermidis* from a blood culture (BCB-Slide system; Roche Products Pty. Ltd.) antibiotic therapy was started, with teicoplanin at 800 mg/day plus vancomycin at 2 g/day, divided into two doses daily. Four days later, following the onset of dyspnea and fever (37.5 to 38°C), a pulmonary infection was suspected. Laboratory data included a total leukocyte count of 10.7×10^9 /liter with 70% neutrophils, 26% lymphocytes, and 4% monocytes. Microbiological examination of a bronchial aspirate showed the presence of *Acinetobacter anitratus* var. *anitratus*. Antibiotic therapy was therefore changed, replacing teicoplanin with ceftazidime pentahydrate at 4 g/day. After an initial decrease in body temperature lasting 3 days, fever increased again to 40°C. Therefore, a new blood culture was carried out, leading to the isolation of *Pseudomonas aeruginosa*. On the basis of antibiogram results, antibiotic treatment was changed again, with aztreonam at 6 g/day plus tobramycin at 300 mg/day, divided into three doses daily. Six days later, since fever was stable at around 38°C and the pa-

tient's condition was not improving, new cultures from blood and urine were made. After 72 h of incubation, the blood culture became diffusely turbid and several smooth and mucoid colonies appeared on agar growth media (chocolate agar and malt agar) enclosed in the BCB-Slide Roche system (Fig. 1). The urine culture, in contrast, was negative.

Microscopic examination of fungal cultures revealed multicellular filamentous hyphal structures of varying size accompanied by budding ellipsoidal yeast-like cells, both in the blood culture and in the colonies growing on agar media. These fungal isolates were subsequently classified as *Aureobasidium pullulans*.

Immediately before starting antifungal therapy, a new set of blood and urine cultures and a culture in liquid medium of the catheters used for parenteral nutrition (BCB-Slide system) were set up. Blood samples were collected through both an intravenous catheter and the median elbow (antecubital) vein. The same fungal strain was isolated, this time from all cultured samples (Fig. 2).

Initial antifungal treatment was intravenous fluconazole at 400 mg/day, divided into two doses. Two subsequent samples from both blood and urine, taken at weekly intervals, were still positive for the fungus. Both blood and urine samples from the third week, however, scored negative. Maintenance therapy with fluconazole at 200 mg/day for a further 4 weeks was therefore administered.

After another 90 days of hospitalization, the patient eventually recovered from coma without relapse into *A. pullulans* infection and was discharged with the necessary prescriptions for home care and rehabilitation. During maintenance therapy, the patient was monitored by culture at weekly intervals, while during further hospitalization, he was monitored at fortnightly intervals. In all cases both blood and urine cultures were negative. Antimycotic therapy was interrupted after five negative cultures.

All positive cultures were plated in duplicate on petri dishes containing Sabouraud dextrose agar culture medium (Oxoid) with 500 µg of chloramphenicol/ml and were incubated at 30 and 37°C, respectively. After 72 h, culture dishes incubated at

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FIG. 1. Growth of mucoid fungal colonies on chocolate agar-BCB-Slide system (left) and cultures obtained from blood (center) and the catheter used for parenteral nutrition (right), both positive for *A. pullulans*.

30°C developed several smooth, mucous, creamy white colonies with irregular borders and traces of a darker pigmentation, especially near the dish borders. Colonies darkened in color after a few days and became completely black after a week (Fig. 2). Cultures incubated at 37°C were similar to those incubated at 30°C, although young colonies were pinkish and had a slightly lower growth rate, a lighter basal pigmentation, and a delay in the appearance of the black pigmentation. Microscopic examination of fungal cultures revealed, in all cases, hyaline, ellipsoidal unicellular smooth conidia, variable in size (8 to 12 μm by 4 to 6 μm), sometimes budding, and originating from rare septate hyphae consisting of both hyaline and darker cells. The appearance of the microorganism, at both macro-

scopic and microscopic levels, rapidly changed within the first week of incubation and was characterized by the growth of black colonies with many hyphal structures (range, 10 to 16 μm by 8 to 25 μm), thick-walled chlamydospores, and small clusters of hyaline ellipsoidal blastoconidia, variable in size, all characteristic features of *A. pullulans* (Fig. 2).

All microscopic features of the fungus were observed with a Leica DMR/HCS microscope with a Leica Quantimed COHU High Performance CCD Q550I W camera and Leica-Qwin software.

Slide cultures on malt agar (Difco) and potato dextrose agar (Oxoid) showed synchronous formation of blastoconidia, thus excluding the possibility that the microorganism belonged to the genus *Hormonema* (12).

The isolated fungal strain was classified as *Aureobasidium pullulans* var. *melanigenum* on the basis of the early black pigmentation of the colonies (7).

A. pullulans is classified among dematiaceous fungi because of its capacity to produce a dark, melanin-like pigment that accumulates in the cell wall (12). It is a ubiquitous microorganism that can be easily isolated from the phyllosphere and from plant residues, soil, wood, air, and even stone (1, 4, 11, 18). Its isolation from clinical specimens is generally considered as a contaminant. Of the 14 species of *Aureobasidium* known to date, *A. pullulans* is the best known to clinical mycologists, since it has been indicated as a rare etiologic agent of phaeohyphomycosis, keratomycosis, septicemia, peritoneal sepsis, and dermatological infections (6, 9, 11, 12, 13, 14, 15, 16).

Nosocomial infections caused by *A. pullulans* are rare and have been described only in some cases of peritonitis involving patients undergoing peritoneal dialysis and in one case of severe infection where the fungus was isolated from a splenic abscess (2, 3, 9, 17). Even more uncommon are infections where *A. pullulans* can be isolated from blood cultures (5, 10). Interestingly, for our patient, who was neither immunosuppressed nor neutropenic, *A. pullulans* was isolated from both blood and urine. Isolation from urine was not achieved initially and became possible only after isolation from blood, thus suggesting a progressive colonization of the patient's organs. To our knowledge, therefore, this is the first reported case of disseminated nosocomial fungal infection by *A. pullulans* var. *melanigenum*.

The strain isolated from this patient was able to grow on artificial media at 37°C, to survive and proliferate in vivo, and to produce high levels of melanin pigments in culture, thus suggesting that some strains of this species should be considered potential pathogens. Indeed, growth at high temperatures, production of melanin pigments, and survival in vivo within animal tissues have long been considered important virulence factors for fungi (8).

With regard to the possible cause and source of infection, this patient underwent several surgical procedures that might have provided, to different extents, potential entry sites for the microorganism. On the other hand, the patient suffered severe and multiple traumatic injuries, which might have led to accidental inoculation of the pathogen into the host. Although the mechanism of infection remains unknown, it is likely that in-

FIG. 2. (Top) Appearance of *A. pullulans* var. *melanigenum* cultures on Sabouraud dextrose agar plus chloramphenicol, after 3 (left) and 7 (right) days of incubation at 30°C. (Bottom) Microscopic appearance of cultures showing *A. pullulans* var. *melanigenum* after 3 (left) and 7 (right) days of incubation at 30°C, shown by phase-contrast microscopy. Magnification, $\times 40$.

fection occurred through traumatic inoculation during the road accident. Indeed, the patient had open fractures and came repeatedly in contact with the soil, which is known to be a rich reservoir of microorganisms that can become pathogenic in compromised hosts. Another likely source of infection in our patient may have been accidental contamination of the temporary catheters used during the hospital stay.

With advances in medicine, fungal nosocomial infections are becoming more and more important. The increased frequency of organ transplantations, the development of more effective therapies for severe diseases such as cancer, and new drugs and surgical techniques have increased the chances of recovery for many patients otherwise destined not to survive. It is therefore not surprising that the rates of nosocomial infections, opportunistic or not, have increased together with the number of microbial species very virulent for severely ill patients. Indeed, there are no limits to the number of fungal species in the environment that can potentially invade and disseminate in host tissues, increasing the already high number of opportunistic microorganisms.

Nosocomial fungal infections can occur when patients' conditions deteriorate, such as after major surgical interventions, debilitating drug treatments, and/or high-dose antibiotic therapies. In these situations, high-risk patients should be carefully monitored mycologically, to prevent their condition being exacerbated by a nosocomial fungal infection. It is also necessary that clinicians, in seeking the most effective therapies for their patients, and laboratory microbiologists, in attempting to isolate the diverse etiological agents of infectious diseases, both develop greater awareness of and closer collaboration on the emerging problem of systemic nosocomial fungal infections.

Moreover, antifungal drug regimens based on low-toxicity molecules, such as fluconazole, may be better for critically ill patients, who may require long-term antifungal treatment and may suffer severe damage from other, more efficient but highly toxic substances, such as amphotericin B.

REFERENCES

1. Ajello, L. 1977. The black yeasts as disease agents: historical perspective, p. 9–16. In *Proceedings of the Fourth International Conference on the Mycoses*. PAHO scientific publication no. 356. Pan-American Health Organization, Washington, D.C.
2. Caporale, N. E., L. Calegari, D. Perez, and E. Gezeule. 1996. Peritoneal catheter colonization and peritonitis with *Aureobasidium pullulans*. *Peritoneal Dial. Int.* **16**:97–98.
3. Clark, E. C., S. M. Silver, G. E. Hollick, and M. G. Rinaldi. 1995. Continuous ambulatory peritoneal dialysis complicated by *Aureobasidium pullulans* peritonitis. *Am. J. Nephrol.* **15**:353–355.
4. de Hoog, G. S., and J. Guarro. 1995. Atlas of clinical fungi. Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.
5. Girardi, L. S., R. Malowitz, G. T. Tortora, and E. D. Spitzer. 1993. *Aureobasidium pullulans* septicemia. *Clin. Infect. Dis.* **16**:338–339.
6. Gupta, V., R. Chawla, and S. Sen. 2001. *Aureobasidium pullulans* scleritis following keratoplasty: a case report. *Ophthalmic Surg. Lasers* **32**:481–482.
7. Hermenides-Nijhof, E. J. 1977. *Aureobasidium* and allied genera. *Stud. Mycol.* **14**:144–176.
8. Hogan, L. H., B. S. Klein, and S. M. Levitz. 1996. Virulence factors of medically important fungi. *Clin. Microbiol. Rev.* **9**:469–488.
9. Ibanez Perez, R., J. Chacon, A. Fidalgo, J. Martin, V. Paraiso, and J. L. Munoz-Bellido. 1997. Peritonitis by *Aureobasidium pullulans* in continuous ambulatory peritoneal dialysis. *Nephrol. Dial. Transplant.* **12**:1544–1545.
10. Kaczmarek, E. B., J. A. Liu Yin, J. A. Tooth, E. M. Love, and I. W. Delamore. 1986. Systemic infection with *Aureobasidium pullulans* in a leukaemic patient. *J. Infect.* **13**:289–291.
11. Kwon-Chung, K. J., and J. E. Bennett. 1992. Medical mycology, p. 396–446. Lea & Febiger, Philadelphia, Pa.
12. McGinnis, M. R., I. F. Salkin, W. A. Schell, and L. Pasarel. 1991. Dematiaceous fungi, p. 644–658. In A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), *Manual of clinical microbiology*, 5th ed. American Society for Microbiology, Washington, D.C.
13. Petril, M. G., J. Konig, H. P. Moecke, et al. 1997. Epidemiology of invasive mycosis in ICU patients: a prospective multicenter study in 435 non-neutropenic patients. *Intensive Care Med.* **23**:317–325.
14. Polonelli, L., L. Ajello, and G. Morace. 1993. *Micologia medica*, p. 11–20. Società Esculapio Editrice, Bologna, Italy.
15. Rolston, K. 2001. Overview of systemic fungal infection. *Oncology* **15**(Suppl.):1–4.
16. Salkin, I. F., M. A. Gordon, and W. B. Stone. 1976. Cutaneous infection of a porcine (*Erethizon dorsatum*) by *Aureobasidium pullulans*. *Sabouraudia* **14**:47–49.
17. Salkin, I. F., J. A. Martinez, and M. E. Kemna. 1986. Opportunistic infection of the spleen caused by *Aureobasidium pullulans*. *J. Clin. Microbiol.* **23**:828–831.
18. Urzi, C., F. De Leo, P. Salamone, and G. Criseo. 2001. Airborne fungal spores colonising marbles exposed in the terrace of Messina Museum, Sicily. *Aerobiologia* **17**:11–17.