tigation supports the need for ongoing epidemiological surveillance and the rapid involvement of infection control specialists in Asian-Pacific outbreaks, to target prevention and control of emerging and reemerging infectious pathogens. Furthermore, our data emphasize the importance of compliance with infection control practices, especially hand hygiene, to help limit the transmission of communicable diseases.

Acknowledgments

Potential conflicts of interest. All authors: no conflicts.

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Clinical Infectious Diseases 2005;41:1361–2 © 2005 by the Infectious Diseases Society of America. All rights reserved. 1058-4838/2005/4109-0028$15.00

Touched by Dr. Butler’s Wisdom

Sir—I read with particular interest the article by Murray et al. [1] regarding the situation of Dr. Thomas Butler. I completely agree with the main ideas of the article. Dr. Butler, who has spent most of his career studying diseases of underdeveloped countries, has been removed from his post and convicted of charges not related to his original arrest, in what has been considered an unprecedented, unfair, and disproportionate treatment for such a reputable physician and scientist.

Let me tell you that I did not have the privilege of meeting Dr. Butler in person, but I was touched by his wisdom 12 years ago. At that time, I was working as a research fellow at the Clinical Sciences Division of the International Centre for Diarrhoeal Diseases Research in Bangladesh. My colleagues and I were looking for new antibiotics to test in clinical trials against multidrug-resistant *Shigella*, highly prevalent in the country at that time. Our interest was concentrated on azithromycin, but few data had been published on this drug’s effect on enteric infections. I contacted Dr. Butler by fax, requesting advice on the idea of using an azalide against an enteropathogen. His response was immediate; he not only encouraged me to go on but also provided in vitro data from his lab on the matter. With that information, we persuaded the center’s scientists to conduct the study. We performed the trial and published the results [2], but more importantly, we added to ciprofloxacin and pivmecillinam a new antimicrobial with which to treat shigellosis in the region. I am sure that none of that would have been possible without Dr. Butler’s help.

Dr. Butler has surely helped many fellows like me during his very productive academic career. Our contact was so brief that he might not remember the very valuable advice he provided via fax in 1993, but I would like to support him in this very difficult time of his life. If common sense prevails, he should be back to his previous position so he may continue to help us to fight infectious diseases around the world.

Acknowledgments

Potential conflicts of interest. C.S.: no conflicts.

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References


Diagnosis of Cutaneous Mucormycosis Due to *Rhizopus microsporus* by an Innovative PCR–Restriction Fragment-Length Polymorphism Method

Sir—Mucormycosis is an opportunistic infection caused by saprophytic fungi belonging to the class Zygomycetes and the order Mucorales. These molds live in soil, air, decaying matter, and substrates such as fruits, cereals, and breads, and infections have been reported in rhinocerebral, pulmonary, gastrointestinal, cutaneous,
and disseminated manifestations [1, 2]. Host risk factors include diabetes, leukemia, bone marrow transplantation, immunosuppressive chemotherapy, broad-spectrum antibiotic use, and breakdown of the cutaneous barrier [2–4]. Recently, the number of such breakthrough infections has significantly increased, with associated mortality rates of 70%–100% [5]. We report a study that suggests the use of polymerase chain reaction (PCR) and restriction fragment–length polymorphism analysis is a promising tool for an early diagnosis and targeted treatment of mucormycosis.

A 63-year-old woman presented to the medical intensive care unit with septic shock in the context of aggressive B lymphoma, diagnosed a month before. She was sedated, underwent intubation and mechanical ventilation, and she received vasoactive support. Antineoplastic chemotherapy against lymphoma was rapidly initiated. Microbiologic findings revealed Pseudomonas aeruginosa, rare Candida parapsilosis, and cytomegalovirus, and there was a significant inflammatory syndrome (the C-reactive protein level was 80 mg/L, and the procalcitonin level was 7.92 ng/mL).

Ten days after admission to the intensive care unit, the patient developed rapidly expanding lesions on her back that evolved toward a necrotic aspect. Because of the patient’s profound immunosuppression, liposomal amphotericin B (10 mg/kg) was given intravenously. Findings of a mycological examination of a first incisional biopsy specimen were consistent with mucormycosis. A wide local surgical incision of the necrotic lesions was then performed. Numerous large and aseptate fungal hyphae were identified in epidermal and dermal structures by histological staining. Subsequent cultures of the surgically excised tissue yielded an aerobic mycelium that grew rapidly on Sabouraud media. This mold was microscopically classified as a Rhizopus species on the basis of the presence of stolons, brown pigmented rhizoids, sporangiophores, and globose sporangia—both apophysate and columellate (figure 1).

The diagnosis of primary cutaneous mucormycosis seemed likely because respiratory and blood samples tested negative for Zygomycetes. However, despite an adequate antifungal treatment and use of vasopressive amines, the patient experienced multisystem organ failure, and died after 30 days in the intensive care unit.

Using PCR and restriction fragment–length polymorphism analysis, we retrospectively confirmed the identification of the Rhizopus genus and found the specific species to be Rhizopus microsporus. Primers targeted a 830-bp sequence in the 18S fungal ribosomal gene with exclusion of human DNA, and then digestion with restriction enzymes enabled a specific identification at genus and species level. The amplicon was then digested using the restriction enzymes PpuMI, AflIII, BmgBI, and AciI, respectively specific for the genera Rhizomucor, Mucor, and Rhizopus, and for Absidia corymbifera. As expected from mycological examination findings, the digestion with BmgBI resulted in 2 fragments (600 bp and 230 bp in length). The PCR product was not digested with PpuMI, AflIII, and AciI, because the target amplicon does not possess any restriction site for these enzymes. These results confirmed the presence of a Rhizopus species, and a second round of digestion with AseI revealed features specific for R. microsporus or R. azygosporus (figure 2). On the basis of epidemiological and clinical data, R. microsporus was found to be likely implicated in this infection [6, 7].

The occurrence of mucormycosis in
immunocompromised patients is increasing, and physicians who treat patients in intensive care units must be aware of these fatal emerging infections [8, 9]. Today, the diagnosis of mucormycosis relies on analyses of clinical samples by microscopy and culture. Unfortunately, the recovery of Zygomycetes from tissues can be problematic, and fungal detection is often hindered by the absence of fungal growth in culture [1]. Therefore, the diagnosis is often made after a too-long delay or even postmortem.

The only effective treatment for these infections consists of high doses of amphotericin B, combined with surgical debridement and control of the underlying disease. Moreover, differences have been noted in the in vitro susceptibilities of genera and species in the class Zygomycetes to available antifungals [10]. Thus, amphotericin B seems less effective against Rhizopus than it is against the Absidia or Mucor genera. This heterogeneity may require an improved identification of Zygomycetes genera and species. The discrimination between Mucorales species belonging to a single genus usually must be done by a reference laboratory. Thus, frequently, when attempting to identify fungi, clinicians take an alternative approach and use panfungal primers for DNA sequencing [11]. Recently, Kontoyiannis et al. [12] reported a 21% discordance rate between results of the morphological and sequence-based methods used to determine the fungal genera.

Therefore, we propose use of a molecular test that enables rapid identification of Mucorales at the genus and the species level. Further studies are now clearly warranted to prospectively assess the accuracy of the PCR and restriction fragment–length polymorphism on clinical samples in comparison to classical methods. PCR and restriction fragment–length polymorphism analysis could then become an easy-to-use diagnostic tool to help clinicians establish more-targeted therapeutic or preemptive strategies to use against this life-threatening infection at an earlier stage.

**Acknowledgments**

We thank Dr. Quentin Vicens for his precious help in checking our English.

**Potential conflicts of interest.** All authors: no conflicts.

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**References**

Candida kefyr as an Emerging Pathogen Causing Nosocomial Bloodstream Infections in Neutropenic Leukemia Patients

Sir—Nosocomial candidemia occurs predominantly in patients who have hematological malignancies and/or have undergone stem cell transplantation and is associated with a high mortality rate [1]. Although *Candida albicans* fungemia is most common, non-albicans species of *Candida*, which are often resistant to antifungal agents, are increasingly observed as pathogens (*Candida parapsilosis*, 20%–40% of cases; *Candida tropicalis*, 10%–30% of cases; *Candida krusei*, 10%–35% of cases; *Candida glabrata*, 5%–40% of cases; *Candida lusitaniae*, 2%–8% of cases; *Candida guilliermondii*, 1%–5% of cases; followed by *Candida rugosa*, *Candida stellatoidea*, *Candida norvegensis*, and *Candida famata*, <1% of cases) [1, 2].

Here we describe *Candida kefyr* bloodstream infections in 3 neutropenic leukemia patients after chemotherapy and/or stem cell transplantation. The infections were diagnosed using the Bactec 9240 system (Becton Dickinson) and the ID32C yeast identification system (bioMérieux) and on the basis of Gram-staining of culture isolates.

Patients 1 and 2 (a 41-year-old woman and a 54-year-old man) developed fever during neutropenia and after receipt of mitoxantrone, topotecan, and cytarabine salvage chemotherapy for relapsed acute myeloid leukemia 10 months and 9 months after autologous peripheral blood stem cell transplantation, respectively. *C. kefyr* was detected in 3 blood cultures (1 aerobic and 1 anaerobic), on a central venous catheter, and in mouth washings from patient 1; *C. kefyr* was found in 2 blood cultures (1 aerobic and 1 anaerobic) from patient 2. Patient 1 had several pulmonary infiltrates, and patient 2 had several hepatic lesions, but no other organs were involved. Blood culture results were negative after therapy with amphotericin B (patient 1) or caspofungin (patient 2).

The patient 3 (a 63-year-old woman) developed fever during neutropenia after undergoing human leukocyte antigen-matched unrelated allogeneic peripheral blood stem cell transplantation for relapsed Philadelphia chromosome–positive acute lymphoblastic leukemia. Over 19 days, *C. kefyr* was detected in blood cultures (9 aerobic and 3 anaerobic), 2 mouth washings, 3 stool specimens, and on a central venous catheter, reflecting colonization. Unspecific arthritis of the talocalcanean joints was observed, suggesting *Candida* arthritis, but no other organs were involved. Therapy with fluconazole and amphotericin B was immediately started and granulopoiesis was stimulated. Despite hematopoietic regeneration, can-

**Figure 1.** Response of *Candida kefyr* bloodstream infection in patient 3 to combination antifungal therapy that included caspofungin. *Staphylococcus epidermidis* was also detected in 2 blood cultures but disappeared after antibiotic treatment, according to the antibiogram. Day 0 was the day of allogeneic peripheral blood stem cell transplantation (PBSCT). *a,* Graph depicting recovery of the leukocyte and the granulocyte counts. *b,* Serum levels of C-reactive protein (CRP). +, Blood cultures positive for *C. kefyr.* *b,* Blood cultures positive for *S. epidermidis.* *c,* Graph showing the highest daily body temperature. *d,* Schematic representation of the antifungal therapy given after PBSCT. Dosages were as follows: nystatin, 1.5 million IU/day orally; caspofungin, 50 mg/day intravenously; fluconazole, 600 mg/day intravenously; amphotericin B, 3 mg/kg/day intravenously.