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Wooden Sticks as the Source of a Pseudoepidemic of Infection with *Rhizopus microsporus* var. *rhizopodiformis* among Immunocompromised Patients

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Wooden sticks used to suspend feces obtained for surveillance cultures were found to be the source of *Rhizopus microsporus* var. *rhizopodiformis* causing a pseudo-outbreak among 17 immunocompromised patients cared for in three different wards. Nonsterile wooden products should therefore not be used for collecting, handling, and processing specimens for microbiological examination.

Nosocomial infections caused by *Rhizopus microsporus* are infrequently reported (6, 7), and among the four varieties known, only *R. microsporus* var. *microsporus* and *R. microsporus* var. *rhizopodiformis* have been reported to cause infections in humans. A recently reported outbreak of cutaneous infection by *R. microsporus* among four preterm infants has drawn attention to the risks of using wooden devices infested with fungal spores in clinical practice (5). The source of that outbreak was found to be wooden tongue depressors which were used as splints for intravenous and arterial cannulation sites. We report here a pseudo-outbreak of intestinal colonization with *R. microsporus* var. *rhizopodiformis* among immunocompromised patients which was shown to have been due to laboratory contamination only by full identification of the fungus to species level and variety.

Description of the outbreak. Between July and November 1996, *R. microsporus* var. *rhizopodiformis* was cultured from the fecal samples that had been obtained for surveillance from 17 immunocompromised patients (Table 1). The first patient had been nursed in hematology unit I, where he had received cytotoxic treatment for non-Hodgkin lymphoma. The organism was cultured from a single fecal specimen after bone marrow recovery. The fungus was then cultured from the feces of a patient in the pediatric oncology ward and from a second patient in the same hematology unit. The latter patient was granulocytopenic at the time (Table 1, patient 3) and had remained febrile for 5 days despite treatment with ceftazidime and teicoplanin. Fungal infection was suspected, and amphotericin B desoxycholate (1 mg/kg of body weight per day) was started empirically. Invasive zygomycosis due to *R. microsporus* was seriously considered after the fungus was cultured a second time from this patient's feces. After the third case (Table 1, patient 4) had been identified in the hematology unit I within 15 days of the first positive culture, a thorough review of the cases was undertaken, but no explanation for the apparent outbreak was found. The outbreak seemed to subside in August, as only one additional case was identified in hematology unit I. However, in October and November, *R. microsporus* was recovered once more from the feces of a further four patients

in hematology unit I (Table 1, patients 9 to 12), four patients in the pediatric oncology ward (patients 13 to 16), and one patient in hematology ward II (patient 17). Sporadic cases had also been observed in these departments previously (patients 2, 6, and 8, respectively). *R. microsporus* var. *rhizopodiformis* was cultured from a single fecal sample in all but two cases (Table 1) but not from any other clinical material. Nor was there any evidence of a defined fungal infection. Furthermore, all the specimens were processed in the same section of the laboratory, suggesting that this was, in fact, a pseudo-outbreak caused by laboratory contamination.

An extensive investigation of laboratory procedures was therefore undertaken, and suspicion fell upon the wooden sticks used to suspend fecal material in sterile saline before plating out for culture. This suspicion was confirmed when *R. microsporus* var. *rhizopodiformis* was cultured from sawdust collected from the packet that had been used during the pseudo-outbreak as well as directly from a sample of wooden sticks from another unopened packet (Fig. 1). The sticks were therefore immediately withdrawn from use, and since then, there have been no further positive fecal samples.

Culture techniques and mycology. Fecal samples were obtained for routine surveillance twice weekly from all patients expected to become neutropenic after receiving cytotoxic chemotherapy for hematological malignancy. A loopful of fecal material was suspended in 5 ml of sterile saline and allowed to settle for 10 min. Specimens of various foodstuffs, including bread, biscuits, preserves, and condiments, were obtained from the wards and homogenized in sterile saline. Ten microliters of fecal supernatant and 1 ml of food suspension or of various drinks, including milk, yogurt, and fruit juices, were plated onto a Sabouraud glucose agar plate containing 10 mg of chloramphenicol per liter. Thermometers were sampled by using a swab moistened in sterile saline and plated directly onto the same medium, as were sawdust and scrapings of the wooden sticks. All plates were incubated at 30°C in air for up to 4 days.

Cultures of food and drink and of swabs from the thermometers yielded no growth, whereas a fungus growing in dark grayish-brown colonies with cottony aerial mycelia was isolated from feces and material from the wooden sticks. No other fungi were recovered from culture. Microscopic examination showed that the colonies were composed of broad nonseptate hyphae, consistent with members of the genus *Rhizopus*. The

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TABLE 1. Patient characteristics and details of fecal surveillance specimens from which *R. microsporus* var. *rhizopodiformis* was recovered

Patient	Underlying disease and/or condition ^a	Ward ^b	Date(s) of positive feces culture(s) ^c	No. of positive fecal samples/total no.
1	NHL	H I	27/06/96	1/6
2	B-cell lymphoma	PO	03/07/96	1/2
3	AML, BMT	H I	04/07/96, 09/07/96	2/10
4	NHL	H I	12/07/96	1/6
5	Myelodysplasia	H I	15/07/96	1/8
6	ALL	H II	18/07/96	1/17
7	AML	H I	19/08/96	1/15
8	Aplastic anemia	PO	26/09/96	1/22
9	NHL	H I	17/10/96, 24/10/96	2/4
10	AML	H I	17/10/96	1/6
11	NHL	H I	18/10/96	1/10
12	Burkitt lymphoma	H I	01/11/96	1/3
13	ALL	PO	04/11/96	1/6
14	ALL	PO	10/11/96	1/1
15	Myelodysplasia	PO	11/11/96	1/11
16	ALL, BMT	PO	11/11/96	1/9
17	Myelofibrosis	H II	12/11/96	1/11

^a NHL, non-Hodgkin lymphoma; AML, acute myeloid leukemia; BMT, bone marrow transplantation; ALL, acute lymphoblastic leukemia.

^b H I, adult hematology unit I; H II, adult hematology unit II; PO, pediatric oncology ward.

^c Day/month/year.

sporangiophores bore elongated columellate, brown, spherical sporangia filled with sporangiospores, consistent with *Rhizopus microsporus* var. *rhizopodiformis*. The fungus also grew at 50°C, which differentiates it from *R. microsporus* var. *microsporus*.

We were initially afraid that we might have been experiencing an outbreak among some of our most vulnerable patients, particularly as a single strain that probably originated from a single source was involved. However, the temporal clustering of *Rhizopus* cultures and the distribution of positive specimens among three different departments located in two separate buildings were the most important clues that we were dealing with a pseudoepidemic. Moreover, the fungus was found only in fecal material, and although *Rhizopus* species are known to cause fatal gastrointestinal infection, the presence of the fungus in stools is more likely to reflect ingestion of spores or contamination rather than invasive disease (4, 8). Also, although one patient received antifungal treatment following the isolation of the fungus, there was no reason to suspect fungal infection in the other cases. *Rhizopus* species have also been cultured from elastoplast bandages (7) and a cotton stockinette (2) which had been in direct contact with the skin and had caused invasive cutaneous infections. This fungus therefore may be ubiquitous on materials originating from plants. The wooden sticks that we used were imported originally from China, where conditions favorable for growth of this organism are present.

Pseudoepidemics involving molds are rare (1, 9), but one such outbreak also involving *Rhizopus* species was traced to wooden tongue depressors used for collecting fecal specimens (1). In our case, suspensions of feces almost certainly became contaminated during processing in the laboratory as a result of using contaminated wooden sticks. Thus, the use of wooden products of any type should also be avoided when processing

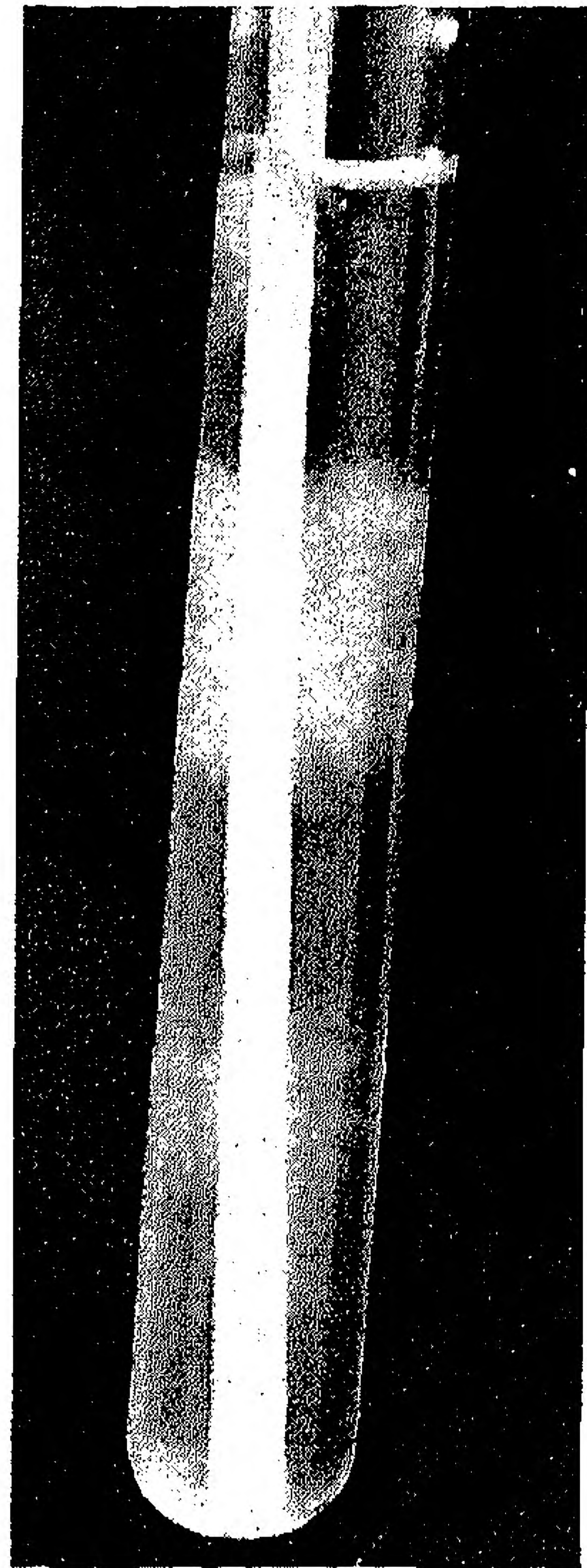


FIG. 1. Culture of *R. microsporus* var. *rhizopodiformis* from wooden sticks after 3 days of incubation at 30°C in Sabouraud glucose broth containing 10 mg of chloramphenicol per liter.

specimens for culture in the laboratory, especially as the microbiological quality of wood is difficult to control and sterilization by autoclaving may fail (3). Only sterilized or near-sterile alternatives such as plastic should be used, especially when dealing with clinical material from patients with compromised host defenses.

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