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Lentinus squarrosulus: precision identification through molecular method in challenging fungaemia case

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Background

Fungal identification poses a formidable challenge in medical diagnostics. This case study unveils the first documented case of *Lentinus squarrosulus* fungemia in a 30-year-old male afflicted by retroviral disease and undergoing Highly Active Antiretroviral Therapy (HAART).

Case(s) description

Here we report a retroviral disease patient who exhibited symptoms of persistent fever, headache, and blurred vision over a month, along with papilloedema but no neurological deficits. Brain scans ruled out brain parenchymal lesions or abnormal meningeal enhancements, and serum cryptococcal antigen tests returned negative. Despite the clinical indication for a lumbar puncture, the patient adamantly refused the procedure. In the quest for answers, serum fungal cultures indicated the presence of non-sporulating hyaline fungal filaments. Nevertheless, both routine blood culture and *Mycobacterium tuberculosis* (MTB) culture tests proved negative. With limited treatment options, the patient was initiated on Amphotericin B therapy, which continued for a period of 10 days before a subsequent transition to Itraconazole. Acknowledging the complexity of the case, a fungal culture was expedited to the Institute Medical Research for advanced and precise identification using state-of-the-art Sanger sequencing technology.

Discussion

Through the application of internal transcribed spacer (ITS) gene sequencing, we have conclusively identified a fungal culture in the patient's serum as *Lentinus squarrosulus*—a species not previously reported in human cases and notably unrecognized as a human pathogenic organism. The identification was achieved through PCR amplification and sequencing, as the culture remained sterile, with microscopic examination revealing non-sporulating hyaline hyphae. *Lentinus squarrosulus* is an edible mushroom classified under Polyporales, primarily consumed in Thailand, as well as other parts of Asia and central Africa. Despite its historical use as a food source, this mushroom has garnered recent attention for its diverse bioactive properties, including immunomodulation, antimicrobial effects, antiproliferative qualities, and antiulcer properties, as demonstrated in *in vitro* studies. In summary, the significance of precise diagnosis in challenging cases of fungemia is underscored by the identification of *Lentinus squarrosulus*, a fungus not previously recognized as pathogenic to humans.

Figure 1.

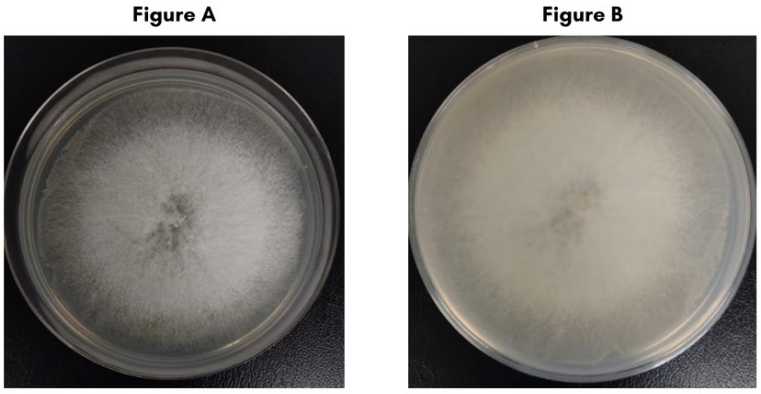

Figure A (surface view) & B (reverse view): Whitish, mycelial growth of Lentinus squarrosulus at 30°C, day 13 on potato dextrose agar (PDA).

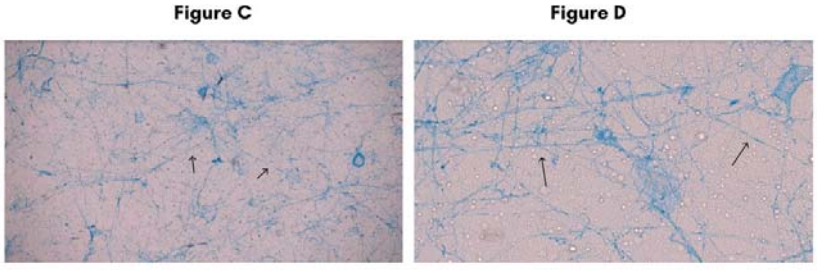

Figure C & D. The above photomicrographs were taken from a direct colony smear stained with LPCB dye and viewed at 100x and 400x magnifications respectively. As shown by arrows only hyphal structures were seen. No fungal basidiospore is seen.

Table 1.

Table: Hit BLAST results (top 3)

	BLAST match	Identity (%)	GenBank Accession No.
1.	*Lentinus squarrosulus*	100%	ON715779
2.	*Lentinus squarrosulus*	99.84%	MG719283
3.	*Lentinus squarrosulus*	99.84%	MH053154

References
1. Basic local alignment search tool (2020) (Accessed June 2020) https://blast.ncbi.nlm.nih.gov/Blast.cgi

O0959 | 02230

Big ulcers in a tiny preterm baby

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Background
Invasive fungal disease other than candidiasis is very uncommon in neonates, even in those born with extreme prematurity. One such entity is primary cutaneous aspergillosis (PCA).

Case(s) description
This female neonate was born at 23 5/7 weeks of gestation with 580 g after preterm labour. She was intubated, received surfactant, and was started on amoxicillin and amikacin. Antibiotics were stopped after three days with negative blood cultures. Because of severe dysbalance of blood electrolytes, potentially a consequence of underestimated perspiratio insensibilis, air humidification in the incubator was increased from 70% to 80%. On day 18, multiple, deep, yellowish, round, sharpy demarcated abdominal skin ulcers appeared and cultures yielded Aspergillus fumigatus. A tissue biopsy was withheld due to the risk of accidental perforation of the abdominal wall. The ventilator system and incubator were changed. There were no signs of systemic infection. Even before culture results were available, the prophylactic fluconazole had been changed to empiric caspofungin. She showed a quick response to treatment and therefore no further change of antifungals was deemed necessary. Treatment was stopped on day 39.

Discussion
Two comprehensive publications on PCA provide detailed information on 53 cases. PCA typically evolves around the second to third week of life with the most relevant risk factor being extreme prematurity. Most of the reported cases had previously received corticosteroids, a known risk factor for altered skin colonisation.(1,2) In our case no corticosteroids were administered prenatally, however, hydrocortisone was administered postnatally for low blood pressure. Humidified incubators further promote fungal growth and have been associated with outbreaks in NICUs.(3,4) Hospital infection prevention and control did investigate the NICU but could neither find a reservoir nor were secondary cases identified. Histopathology would have been desirable, however, data from the largest review shows that in only half of the cases identification of hyphae by staining was available.(1) The lesions showed prompt response to caspofungin, therefore, antifungals were not switched to the first line (liposomal amphotericin B) or alternative (voriconazole) treatment.(5) The increasing numbers of extremely premature neonates may lead to rising numbers of opportunistic infections in this population.

Figure 1. Skin_DOL_18