Severe pneumonia with a massive pleural effusion in a child caused by *Tilletiopsis minor*: the first case from Saudi Arabia

Ibrahim A. Al-Zaydani, Martin R. P. Joseph, Ali Mohd Suheel, Ahmed M. Al-Hakami, Mohamed E. Hamid

From the Department of Pediatrics, Aseer Central Hospital, Abha, Saudi Arabia; Department of Microbiology, College of Medicine, King Khalid University, Abha, Saudi Arabia

Correspondence: Dr. Mohamed E. Hamid · Department of Microbiology, College of Medicine, King Khalid University, PO Box 641, Abha, Saudi Arabia · M: 966-509-773-687 F: 966-77-224-7570 · mehamid2@yahoo.com

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*Tilletiopsis* minor, a blastoconidia-forming yeast, was isolated from a 4-year-old boy suffering from severe pneumonia. Chest x-rays revealed the progression of widespread and multiple nodular lesions, nonsymmetrical interstitial and airspace infiltrates, and consolidations. Creamy yellow, irregular, wrinkled yeastlike organisms were isolated from the pleural fluid specimens when cultured on Sabouraud dextrose agar for 5 days and incubated at 30°C. Microscopically, the organisms showed broad, irregular filaments with blastoconidia but no budding cells. Manual bench tests and automated phenotypic analyses failed to recognize the organism. This unique and rare organism (AB7-11; DSM 29469) was identified using the sequence analysis of the internal transcribed spacer region of the nuclear ribosomal DNA. It showed a precise alignment with the type strains of *T minor*. Subsequent to this diagnosis, and the earlier nonresponse to vancomycin and meropenem, the patient was put on liposomal amphotericin. However, the condition continued to deteriorate, and then, intravenous voriconazole was added to control the infection. Finally, the patient's condition improved, and he was discharged in good condition after 1 month of stay in the hospital.

*Tilletiopsis* is a fungus that is categorized with a group of dimorphic yeasts in the class Exobasidiomycetes, which are characterized by the formation of blastoconidia but no budding cells. The genus contains few species renounced for generating potential biological control agents against a number of powdery mildew diseases. The genus *Tilletiopsis* was first described by Derx in 1930 when examining infected plant leaves; he suggested that the infection was due to *Tilletia* species, a smut fungus belonging to the family Tilletiaceae.

Apart from the original description of the species *Tilletiopsis minor* by Ramani et al., no other report is available to incriminate this species as a causal agent of human or animal diseases. Ramani et al. isolated *T minor* from cystic lesions in an immunocompromised patient. These authors observed yeasty growth with small satellite colonies at the periphery of the culture plate. When grown on potato dextrose agar at 30°C, *T minor* showed delicate, hyaline hyphae bearing curved conidia, which turned out into dark hyphae with scattered, curved conidia and globose chlamydospores with age. *Tilletiopsis* species, in general, were found to resemble *Entyloma* species in morphological terms, but blastospores of *Entyloma* do not bud in a yeastlike manner. The biochemical tests for differentiation of *Tilletiopsis* species have been studied by Ramani et al. *T minor* was found positive for arbutin, lactose, and phenylalanine but negative for mannitol and ammonium sulfate. *T minor* was found sensitive to amphotericin B (MIC, 0.06 mg/mL), fluconazole (MIC, 8 mg/mL), and itraconazole (MIC, 0.5 mg/mL). *Tilletiopsis* species, for example *T washingtonensis*, were found to have a unique ability to assimilate a number of compounds including ethanol, butanol, acetate, propionate, butyrate, and ethyl acetate.

A genetic study of *Tilletiopsis* species concluded that group I introns can be transferred horizontally among
species, and subsequently, when inherited, they can be diverged vertically. Four yeastlike fungi with blastococnidia, which were isolated from the plants in Thailand, were allocated to the genus *Tilletiopsis* based on morphological and chemotaxonomical characteristics. The 18S rDNA sequence analysis and DNA–DNA reassociation analyses indicated that the strains merited new species ranks, namely *Tilletiopsis derogia*, *Tilletiopsis oryzicola*, and *Tilletiopsis pennisi*.

This report aimed to describe a serious pulmonary illness in a child caused by a rare yeast fungus.

**CASE**

A previously healthy 4-year-old male patient from Abha region, Saudi Arabia, presented to Asser Central Hospital (a tertiary hospital in the southwest of Saudi Arabia) with fever lasting 4 weeks. The fever was associated with cough and shortness of breath in the last 2 weeks prior to admission to the hospital. The patient’s history was unremarkable with no evidence of immunodeficiency, neither primary immunodeficiency disorders nor immunosuppressive medications. The child received the scheduled vaccination. His maternal grandfather died of pulmonary tuberculosis 1 year back.

On clinical examination, the patient was found febrile (39°C); respiratory rate, 35/min; pulse rate, 25/min; blood pressure, 107/68 mm Hg; and oxygen saturation 93% in room air. The mucous membranes were neither pale nor jaundiced; however, palpable, small bilateral auxiliary lymph nodes were observed. He had a bacillus Calmette Guerin scar on the left upper arm. The chest examination revealed markedly diminished breath sound on the right side and a bilateral crepitation. He was conscious with an unsteady gait. Other systemic examinations were ordinary. Chest x-ray showed bilateral pulmonary infiltrates with a right-sided pleural effusion. A bilateral lower lobe partial opacity was prominent on the right side (**Figure 1**). No pulmonary nodules or cavitation was noted. The mediastinal lymph node was normal.

The blood hematological and chemical tests of the patient revealed the following results: white blood cells (WBC), 24 000/mm³; hemoglobin, 13.6 gm/dL; platelet, 219 000/mm³. Na, 132 mmol/L; K, 4.2 mmol/L; urea, 18 mg/dL; glucose, 91 mg/dL; albumin, 3.4; gamma-glutamyl transferase, 41 IU/L; alanine aminotransferase, 34 IU/L; and erythrocyte sedimentation rate, 42 mm Hg. The pleural fluid analysis showed the following results: WBC, 200/mm³; neutrophil, 75%; lymphocytes, 25%; protein, 5.3 gm/L; glucose, 43 mg/dL; and lactate dehydrogenase, 594 IU/L.

The cytological examination showed the presence of acute and chronic inflammatory cells with few mesothelial cells, but no evidence of granulomatous inflammation. No malignant cells were seen. Serology for human immunodeficiency virus was negative, and the investigations revealed no underlying defect of humoral or cell-mediated immunity.

The patient was initially started on broad-spectrum antibiotics that included intravenous vancomycin and meropenem. No response to these medications was reported, and the patient's condition worsened with progression of widespread lesions. The computed tomography scan of the chest revealed a moderate right pleural effusion that was noted particularly with pleural drainage.

On day 18 of hospital admission, the pleural fluid specimens were submitted to microbiology laboratory and processed following standard methods. A creamy yellow, irregular, wrinkled yeastlike organism was isolated from the pleural fluid specimens when cultured on Sabouraud dextrose agar (SDA; Difco, Becton, Dickinson and Company, Sparks, Maryland) for 5 days and incubated at 30°C (**Figure 2**). The suspected fungal growth was subcultured on a fresh SDA plate to improve the growth and appearance of distinguished fungal elements. The organism was labeled AB7-11 (DSM 29469) and identified on the basis of colony morphology appeared on SDA and on the basis of microscopic features following recommended guiding principles.

Microscopically, the organisms showed broad irregular filaments with blastoconidia but no budding cells. Manual bench tests and the automated phenotypic analyses failed to recognize the organism.

**Figure 1.** CT-scan of the chest of the 4-year-old male patient presented to Asser Central Hospital with severe pneumonia showing moderate right pleural effusion, bilateral lower lobe partial opacity prominent in the right side but no pulmonary nodules or cavitation was noted.
Gram-stained smears made from the culture revealed medium-sized irregular hyphae with the evidence of blastoconidia, but no budding yeast cells were visible (Figure 3).

The strain was found to have phenotypic properties (morphological, biochemical, and physiological) predictable of yeasts. Subsequently, a report of yeast fungal infection was submitted.

In vitro susceptibility assay was performed using the well method on SDA. The following drugs were tested: amphotericin B 100 mg/mL (Sigma, Missouri, USA), which revealed 15-mm inhibition zones; cotrimoxazole 25 µg (Liofil Chem, Italy), 16-mm inhibition zones; fluconazole 2 mg/mL (Diflucan I.V. Roerig/Pfizer Inc., France), 70-mm inhibition zones; fungizone 5 mg/mL (E. R Squibb & Sons Ltd, England), 21-mm inhibition zones; itraconazole 10 mg/mL (Janssen Biotech N. V, Belgium), 70-mm inhibition zones; metronidazole 5 mg/mL (PSI Pharmaceutical Co., Jeddah, Saudi Arabia), 8-mm inhibition zones; nystatin 100 mg/mL (Sigma, Missouri, USA), 37-mm inhibition zones; and voriconazole 2 mg/mL (Vfend, Amgen technology, Ireland), 70-mm inhibition zones (Figure 2).

Given this diagnosis, and the earlier nonresponse to vancomycin and meropenem, the patient was put on liposomal amphotericin. However, the condition continued to deteriorate, and then, intravenous voriconazole was added to control the infection. The patient’s condition improved, and he was discharged in good condition after 1 month of stay in the hospital (Figure 4).

Definitive identification of this unique and rare organism was made using the sequence analysis of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA. A neighbor-joining phylogenetic tree based on the domains 1 and 2 (D1/D2) of the large subunit rDNA showed the joining of the strain AB7-11 to T. minor (Figure 5; arrow) and relatively remote connection to other closely related yeast species. Accordingly, the strain AB7-11 (DSM 29469) was identified as T. minor.

**DISCUSSION**

In the recent years, significant developments in diagnostic technologies have been made for detecting and identifying yeasts in clinical specimens. One of these technologies is the application of gene sequence analyses, especially in the D1/D2 of large subunit rDNA and the ITS. The increasing application of molecular and phylogenetic analyses has resulted in important changes in yeast systematics. These have led to redefining many old genera and defining new ones as per the present case (Figure 5).

Identification of yeasts based on phenotypic characterization is strenuous and time-consuming, and in many situations, it is not conclusive. In the present re-
case report

TILLETIOPSIS MINOR IN SAUDI ARABIA

Figure 5. Neighbor-joining phylogenetic tree based on the D1/D2 domain of the LSU rDNA showing the association of strain AB7-11 to Tilletiopsis minor (arrow) and to closely related yeast species. Bar, 0.1 substitution per nucleotide position.

In conclusion, it is advised not only to consider fungal infection in routine respiratory illnesses but also perform in vitro antimicrobial testing, since unique yeasts or molds may respond differently to empirical antifungal agents.

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Conflict of interest
None-declared.

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