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Kneiffiella palmae: A non-*Aspergillus* fungal infection isolated from a pulmonary nodule in a child with chronic granulomatous disease

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ABSTRACT

We report the first known human case of *Kneiffiella palmae* in the medical literature. *K. palmae* was isolated from a pulmonary nodule in a 7-year-old male with chronic granulomatous disease. The mold was identified as *K. palmae* at a national reference laboratory, where 17 other human respiratory samples tested positive for *K. palmae* from 2013 to 2021. Optimal antimicrobial treatment is unknown, but azoles and amphotericin B demonstrated *in vitro* activity against each tested isolate.

1. Introduction

Chronic granulomatous disease (CGD) is an inherited primary immunodeficiency caused by genetic mutations that result in defective neutrophil NADPH oxidase complexes. Patients with CGD are particularly vulnerable to recurrent and severe infections caused by catalase positive microorganisms (namely *Staphylococcus aureus, Burkholderia cepacia, Serratia marcescens, Nocardia* spp. and *Aspergillus* spp.). *Aspergillus* species are recognized as the most common molds isolated from CGD patients, and invasive aspergillosis is associated with high mortality in this patient population [1,2]. However, an emerging topic in the CGD literature is the increasing prevalence of non-*Aspergillus* fungal infections (NAFI) and the diagnostic challenges associated with these opportunistic filamentous fungi that many times are not initially recognized as true pathogens [3]. A particular challenge in the diagnosis of NAFI is the tendency for these fungi to form sterile mycelia with low levels of sporulation [4]. Prompt diagnosis of NAFI in CGD is crucial to improve patient outcomes.

This case report describes a 7-year-old boy with CGD and follows the course of diagnosis and management of pulmonary nodules found incidentally on imaging. Lung tissue samples grew mold, which was eventually identified as *Kneiffiella palmae* at a reference laboratory. We also present information from seventeen other respiratory samples that tested positive for *K. palmae* at the reference mycology laboratory from 2013 to 2021. *K. palmae* has not been previously attributed to human disease and introduces another NAFI to be considered in immunodeficient patients.

2. Case presentation

A 7-year-old male with X-linked CGD presented to the Children's Mercy Infectious Diseases Clinic for a post-hospital follow-up visit (day 0). Two months prior (day −60), he was admitted to the hospital for fever and abdominal pain and was diagnosed with *Serratia marcescens* hepatic and intra-abdominal abscesses. During his 4-week inpatient stay he was treated with cefepime and transitioned to levofloxacin for outpatient management. He then received a one-month steroid taper as adjuvant therapy for refractory liver abscesses. In addition, he continued his home regimen of itraconazole prophylaxis and interferon-gamma therapy for CGD.

In clinic, computed tomography (CT) of the abdomen was ordered to evaluate the status of the previously described liver lesions. Multiple pulmonary nodules were incidentally found. The patient did not have respiratory symptoms or new fevers. He subsequently underwent core needle biopsy of the nodules performed by interventional radiology on day +10. The biopsy report demonstrated multiple necrotizing granulomas with chronic inflammation surrounded by reactive fibroblast proliferation. Gram stain, acid fast bacilli (AFB), Grocott methenamine

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silver (GMS), and periodic acid-Schiff (PAS) stains were negative for microorganisms. On day +18, itraconazole prophylaxis was discontinued and replaced with empiric posaconazole while awaiting results of the biopsy culture. Broad-range aerobic/anaerobic bacterial, fungal, and AFB polymerase chain reactions (PCR) performed at the University of Washington Molecular Diagnosis Microbiology Laboratory were all negative.

A repeat CT of the chest performed 7 weeks later (day +53) showed larger pulmonary nodules with early cavitation of one of the pulmonary nodules despite empiric posaconazole therapy (Fig. 1). The patient underwent right thoracoscopic wedge resection with removal of the two pulmonary nodules on day +59. After the surgical intervention, the patient was admitted to the hospital to start empiric therapy for possible *Nocardia* infection with amikacin and imipenem-cilastatin in addition to antifungal therapy with posaconazole. Lung tissue review showed multiple necrotizing granulomas and negative Gram stain, AFB, GMS, and PAS stains for microorganisms. On day +65, lung tissue fungal and aerobic cultures turned positive for mold. Broad-range bacterial, fungal, and AFB PCRs were again negative. He was started on liposomal amphotericin B and other antimicrobials were discontinued.

The isolate (UTHSCSA DI20-343) was sent to the Fungus Testing Laboratory (FTL) at the University of Texas Health Science Center at San Antonio for identification and antifungal susceptibility testing. Hyphae without the presence of reproductive structures were observed within the culture. DNA sequencing of the internal transcribed spacer region of the nuclear rDNA (ITS) was used for species identification. BLASTn results of the ITS sequence in GenBank showed 98.2–99.4% identity with authentic strains of *Kneiffiella palmae* (previously *Hyphodontia palmae*). Seventeen other samples submitted to the FTL from 2013 to 2021 were also identified as *K. palmae* (Fig. 2). Of note, all isolates were obtained from respiratory samples such as bronchoalveolar lavages, lung tissue, and sputum. A phylogenetic analysis was also performed as previously described [5–7] with reference strains of *Kneiffiella* to confirm the identity of these isolates (Fig. 2). Antifungal susceptibility for this patient's isolate and between 12 and 14 other *K. palmae* isolates were performed by the CLSI broth microdilution method [8]. (Table 1)

By the time fungal species identification and susceptibility data were available, the patient had completed a 4-week course of liposomal amphotericin B. A repeat CT of the chest and abdomen showed stable liver findings and no new pulmonary nodules were discovered. He was discharged with posaconazole 300mg daily and levofloxacin 250mg daily on day +94. He was closely followed outpatient. CT of the chest obtained after one year (day +459) did not indicate new nodules or residual infection. Currently, he continues prophylactic posaconazole and levofloxacin.

3. Discussion

In the last two decades, NAFI have been increasingly recognized among CGD patients. However, due to the need for invasive procedures and specialized diagnostic technology to identify these opportunistic filamentous fungi, NAFI continue to be an under-represented cause of invasive disease in CGD patients. Moreover, pulmonary fungal infections in CGD patients are difficult to diagnose due to their uncommon clinical manifestations. Salvator et al. reported that most patients with CGD and pulmonary fungal infections were asymptomatic and about 37% of patients did not have respiratory symptoms or fever [9]. Approximately 70–80% of NAFI have been reported in pediatric CGD patients aged 0–18 years [3,4,10]. It is thought that the wide use of itraconazole prophylaxis may have contributed to the emergence of breakthrough infections caused by non-*Aspergillus* molds (e.g., *Chrysosporium* spp., *Aspergillus* (*Neosartorya*) spp., *Phaeoacremonium* spp., *Paecilomyces* spp., *Phellinus* spp., *Rasamsonia* spp., *Scedosporium* spp., etc.) particularly the ones associated with azole resistance [3,4,10–14].

Kneiffiella palmae (previously *Hyphodontia palmae*) [15] is a relatively unstudied member of the Schizoporaceae family in the Hymenochaetales order. This white wood rot fungus has a smooth odontoid hymenial surface with ellipsoid shaped basidiospores [5], and up to this point has only been studied in a botanical context [16]. In culture, *K. palmae* grow as non-sporulating white to cream colonies. The genus *Hyphodontia sensu lato* is a species-rich described in 1958 and belonging to the order Hymenochaetales [6]. *Hyphodontina sensu lato* currently comprises over 120 species; however large molecular phylogenetic assessments have shown that *Hyphodontia sensu lato* concept was polyphyletic and comprises six genera, namely *Fasciodontia, Hastodontia, Hyphodontia, Lyomyces, Kneiffiella,* and *Xylodon*, where many taxa are still taxonomically doubtful [7]. Recently Haider et al. reported seven CGD patients with invasive infections caused by *Phellinus* spp., another wood decay fungus from the Hymenochaetales order [11].

The common thread among various case reports of NAFI is the prediagnostic suspicion for a non-*Aspergillus* cause of illness; isolates initially disregarded as contaminants in the laboratory due to lack of diagnostic sporulating structures; need for surgical resection; and the necessity of molecular testing for definitive diagnosis. In this case, we also initially questioned if *K. palmae* played a significant clinical role in this patient's pulmonary disease or was simply a contaminant due to the absence of diagnostic reproductive structures, fungal elements on the lung tissue biopsy, and information in the medical literature. However, the lack of hyphae visualization on lung tissue could have been related to sampling bias with the initial core needle biopsy and/or low burden of infection due to the use of prophylactic itraconazole, later replaced by posaconazole. Moreover, an exuberant granulomatous inflammatory response with paucity of fungal elements has been described in CGD

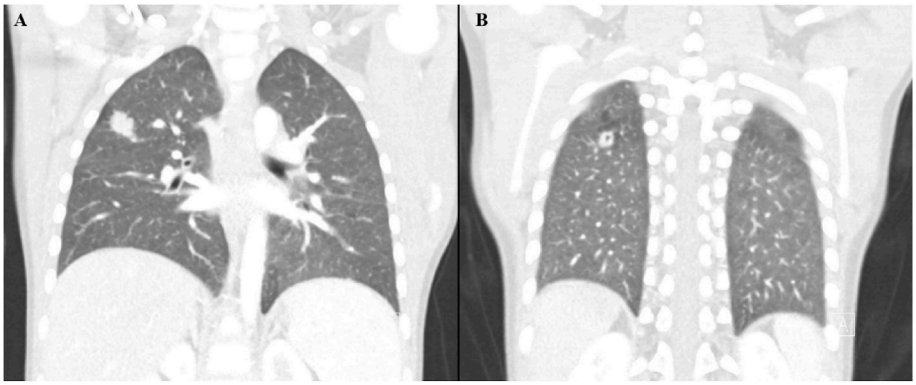

Fig. 1. Computed tomography of the chest with contrast of patient with enlarging pulmonary nodules. A) Pulmonary nodule (1.3 cm x 1.2 cm x 1.3 cm) in the posterior segment of right upper lobe nodule. B) Pulmonary nodule (1.1 cm x 0.9 cm x 1.0 cm) in the superior segment of the right lower lobe with a small focus of gas seen within it.

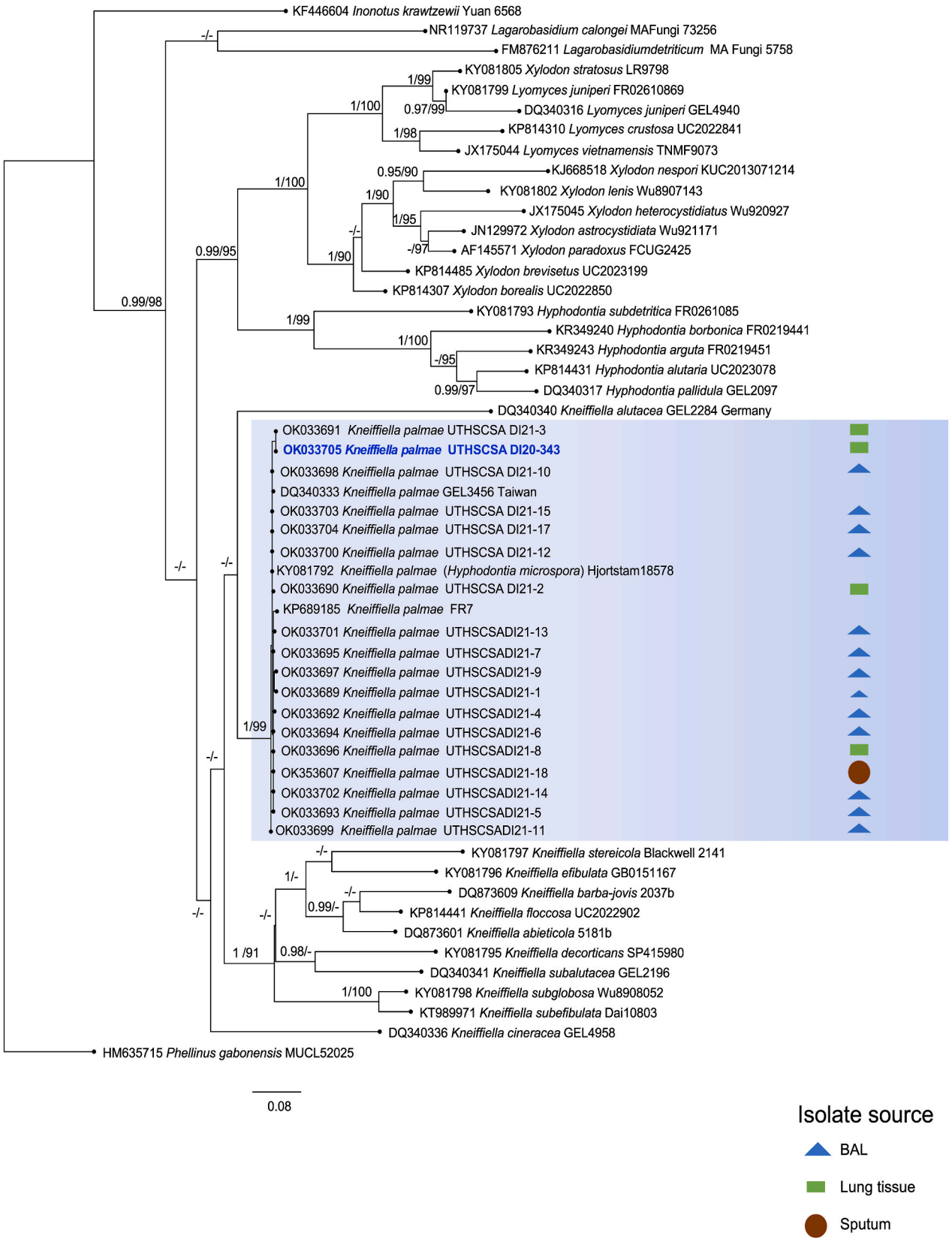

Fig. 2. A phylogenetic tree inferred from ITS using maximum likelihood (substitution model = GTR+G+I) implemented in IQ-Tree, showing relationship of isolate UTHSCSA DI20-343 and 17 others with representative species of *Kneiffiella* in the family Schizoporaceae. Bayesian posterior probabilities using MrBayes v3.2, ≥ 0.95 and bootstrap values ≥80% are shown at the nodes. GenBank accession numbers are shown in front of the species names and isolate numbers are shown after the names. UTHSCSA, University of Texas Health Science Center at San Antonio. Isolate sources are denoted by symbols: rectangle = lung tissue, triangle = bronchoalveolar lavage, circle = sputum.

Table 1
Antifungal susceptibilities against *Kneiffiella palmae* identified at a reference laboratory in the United States from 2013 to 2021[a].

Antifungal	Amphotericin	Itraconazole	Posaconazole	Voriconazole	Isavuconazole	Terbinafine	Micafungin
Case isolate MIC, μg/ml	≤0.03	0.06	0.06	0.06	0.125	0.5	>8
No. isolates tested	15	14	15	13	14	14	13
MIC, μg/ml (range)	≤0.03–0.5	≤0.03–0.25	≤0.03–0.06	≤0.03–0.25	≤0.03–0.25	0.125 - >2	≤0.015 - >8
MIC_{50}, μg/ml	0.06	≤0.03	≤0.03	≤0.03	0.06	1	0.03
MIC_{90}, μg/ml	0.25	0.25	0.06	0.125	0.25	>2	4
GM, μg/ml	0.052	0.047	0.033	0.054	0.061	1.16	0.187
Mode, μg/ml	0.06	≤0.03	≤0.03	≤0.03	≤0.03	>2	≤0.015

GM, geometric mean; MIC_{50}, concentrations required to inhibit 50% of organisms; MIC_{90}, concentrations required to inhibit 90% of organisms.
[a] Minimum inhibitory concentrations for amphotericin B, itraconazole, posaconazole, voriconazole, isavuconazole, and terbinafine, and the minimum effective concentration for micafungin.

patients with invasive fungal infections, contrary to the abundant fungal growth with angioinvasion described in neutropenic patients [17,18]. Given *K. palmae* was the only microorganism isolated in two separate tissue cultures after an extensive negative diagnostic work up and referral laboratory confirmed correct identification of fungus, we attributed the pulmonary findings to be secondary to *K. palmae* infection. Interestingly, the eighteen total *K. palmae* isolates identified at the reference laboratory were all obtained from respiratory samples. Host immune status and histological information for these additional isolates are not available; however, basic demographic information is presented in Table 2.

Kneiffiella spp. pathogenesis has not been described. However, the important role of reactive oxygen species in other filamentous basidiomycetes has been studied [19]. In patients with CGD, the absence of functional NADPH oxidase complex and derived microbicidal reactive oxygen species have an impact on the control of the lung-damaging inflammatory response and activation of effective immune responses to limit the spread of fungal infections such as neutrophil extracellular trap formation, autophagy and other intracellular signaling pathways [20,21].

Non-*Aspergillus* fungal infections in CGD, especially those caused by basidiomycetous species, are not easily identified with standard microbiologic methods (i.e., phenotypic characterization) because these species are mostly nonsporulating in culture; thus, their true incidence remains unknown. Today, DNA sequencing has become the gold standard in the diagnosis of these fungi, particularly in cases where the morphology of the fungus is nondefinitive or has been altered due to the use of antifungal medications. Singh et al. utilized DNA sequencing of the internal transcribed spacer region and the large subunit of the nuclear ribosomal rDNA gene to identify many basidiomycetes causing respiratory ailments [22]. Their work is a catalog of data which proved helpful for identification of this fungus. The use of advanced molecular diagnostics is of upmost importance in these cases to establish a prompt diagnosis and to better understand the association of less known fungal species and human disease.

Currently, there is no data to guide the empiric or definitive treatment of *K. palmae*. Based on the antifungal susceptibility presented in Table 1, the azoles and amphotericin B demonstrated potent *in vitro* activity against each isolate tested. In contrast, marked variability was observed with both micafungin and terbinafine. Our patient was treated empirically with posaconazole followed by amphotericin B. However, he also underwent surgical resection of the two pulmonary nodules for diagnostic purposes, but this most likely played a significant therapeutic role as well.

We hope this information will prove helpful for future clinicians encountering a diagnosis of *Kneiffiella palmae* infection in an immunocompromised patient and encourage further study into non-*Aspergillus* fungal infections.

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None to declare.

Table 2
Demographic information of the additional seventeen samples positive for *Kneiffiella palmae* identified at a reference laboratory in the United States from 2013 to 2021.

Accession number	Sex	Site of culture	Geographic location
UTHSCSA DI21-1	Male	BAL	South Carolina
UTHSCSA DI21-2	Male	Lung tissue	Florida
UTHSCSA DI21-3	Female	Lung tissue (right middle lobe)	Missouri
UTHSCSA DI21-4	Male	BAL	Massachusetts
UTHSCSA DI21-5	Male	BAL	Florida
UTHSCSA DI21-6	Female	Lung tissue (right upper lobe)	Florida
UTHSCSA DI21-7	Female	BAL	Texas
UTHSCSA DI21-8	Male	Lung tissue (right lower lobe)	Massachusetts
UTHSCSA DI21-9	Male	BAL	Unknown
UTHSCSA DI21-10	Female	BAL	Virginia
UTHSCSA DI21-11	Female	BAL	Florida
UTHSCSA DI21-12	Unknown	BAL	Unknown
UTHSCSA DI21-13	Male	BAL	Florida
UTHSCSA DI21-14	Male	BAL	South Carolina
UTHSCSA DI21-15	Male	BAL	Kentucky
UTHSCSA DI21-17	Male	BAL	Louisiana
UTHSCSA DI21-18	Male	Sputum	Florida
[a]UTHSCSA DI20-343	Male	Lung tissue (right upper lobe)	Missouri

BAL, bronchoalveolar lavage.
[a] Case Report Patient.

Consent

Written informed consent was obtained from the patient or legal guardian(s) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

Conflict of interest

Authors Ituarte B, Canete-Gibas C, and Olarte L have no relevant financial or non-financial interests to disclose. Wiederhold N has received research funding from Astellas, bioMerieux, Maxell Biosciences, F2G and Sfunga; consultant honorarium from Terranova Medica; and drug powders from Cidara, F2G, Mycovia, and Pfizer.

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