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Case Report

First case of *Aspergillus caelatus* airway colonization in a Chronic Obstructive Pulmonary Disease patient

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ABSTRACT

Background: During a cross-sectional study on allergic aspergillosis in Chronic Obstructive Pulmonary Disease patients in Bogotá, Colombia, we reported the case of a 65-year-female patient with GOLD 2011 D classification, presenting dyspnea at the time of visit and aspergillus in repeated sputum cultures.
Methods: The isolate was identified at the section level based on macroscopic and microscopic characteristics and gene sequencing was used for precise molecular identification. Antifungal sensibility was determined by Sensititre YeastOne™ while virulence was assessed using a *Galleria mellonella* larvae model.
Results: The clinical isolate was first identified as Aspergillus section Flavi and sequencing of β-tubulin and calmodulin genes, in addition to the identification of *alfR* (aflatoxin regulator) gene, allowed the undoubted identification of the clinical isolate as *Aspergillus caelatus*. It exhibited virulence in *G. mellonella* similar to *A. flavus* and a high *in vitro* susceptibility against all antifungals except for amphotericin B.
Conclusion: This is the first human case of airway colonization attributed to *A. caelatus*. Resistance pattern justified the interest to discriminate this cryptic species.

Introduction

Among known *Aspergillus* species, *A. fumigatus* is the most common one implicated in human infections except in India, Sudan, and other tropical countries including Latin America where *A. flavus* is as prevalent as *A. fumigatus* (Paulussen et al., 2017). Traditionally, identification of *Aspergillus* species in clinical microbiology laboratories is based on macroscopic and microscopic characteristics (Rath, 2001). Recently gene sequencing allowed microbiology laboratories to identify molds which are scarce in clinical settings (Glass and Donaldson, 1995; Hong et al., 2005). In parallel, novel fungal species associated with clinical manifestations have been reported improving epidemiology knowledge of *Aspergillus* and aspergillosis (Siqueira et al., 2018; Barrs et al., 2013; Sugui et al., 2012; Guinea et al., 2015). In this study on allergic aspergillosis in Chronic Obstructive Pulmonary Disease (COPD) patients, one strain firstly reported as *A.* section *Flavi* was undoubtedly identified as *A. caelatus* (Tamari clade) after sequencing of partial β-tubulin and calmodulin genes, in addition to the amplification of *aflR* gene implicated in aflatoxin synthesis. *Tamari* clade shows subtle differences under the microscope which require expertise for adequate identification, expertise which is

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usually not available in most microbiology laboratories. *A. caelatus* has been described from groundnut seeds in USA, peanuts samples from Argentina and Kenya, and Brazilian nuts in different regions of Brazil (Horn, 1997; Pildain et al., 2008; Mutegi et al., 2009; Calderari et al., 2013).

Case report

During a cross-sectional study on allergic aspergillosis in COPD patients conducted between 1 January 2015 and 30 March 2016 in Bogotá, Colombia, we identified the case of a 65-year-female patient with Chronic Obstructive Pulmonary Disease (COPD) GOLD D (GOLD 2011 classification), heart failure, sleep apnea and chronic kidney disease. At the time of the visit she had not suffered from recent acute COPD exacerbations but was found to be symptomatic with dyspnea categorized as mMRC (modified medical research council) 3 and a COPD assessment test (CAT) of 27 as marker of compromised quality of life. She was receiving medical treatment with tiotropium bromide 5 μg/day, beclomethasone 1000 μg/day, and oxygen, and she had not received antibiotic or antifungal treatment in the three previous months. No chest X-ray abnormalities were described. The patient was admitted to the hospital one month after isolation due to pulmonary thromboembolism. She has continued in follow-up for 18 months without presenting episodes of exacerbation that have required hospitalization.

Aspergillus was isolated twice from sputum culture and identified as *Aspergillus* section *Flavi* on macroscopic characteristics on potato-dextrose agar after 7 days incubation. When cultivated on Sabouraud agar at 37 °C, the strain developed a yellow-green color being very similar to *A. flavus* except the tuft characteristic of this species. Lactophenol cotton blue wet mount preparation exhibits radiate conidial heads, 500–600 μm in diameter. Heads were often biseriate but uniseriate heads were also present. Vesicles were supported by a roughened conidiophore just beneath the globose vesicle, classic for this species. However, in contrast to *A. flavus* which possessed slightly echinulate conidia (4–5 μm diam.), in this case, globose or subspherical larger conidia (7–8 μm diam.) were revealed with prominent tubercles and a thick brown wall, as described in *Tamari* clade (Figure 1).

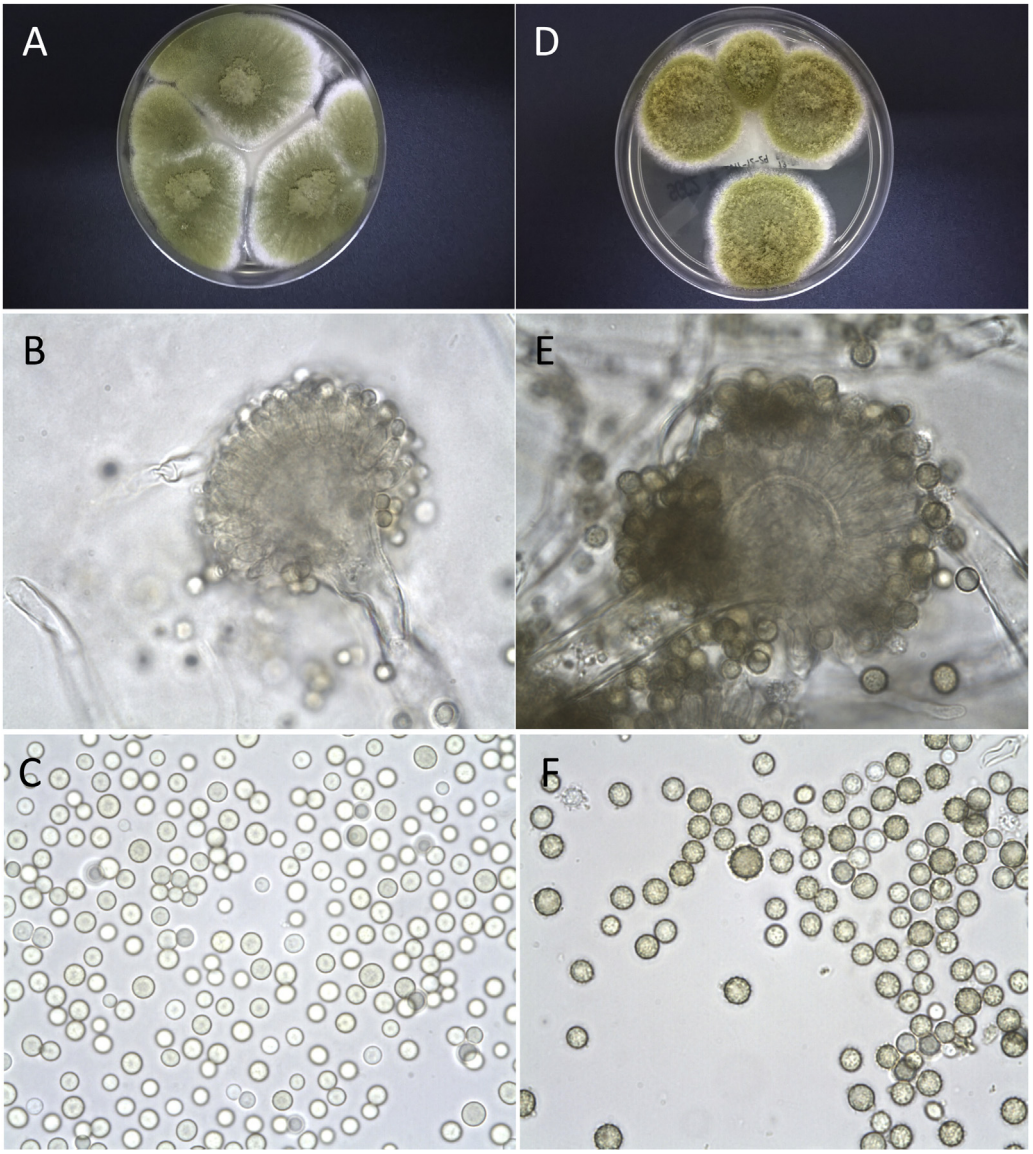

Figure 1. Macroscopic colonies on Sabouraud agar at 37 °C after 4 days of incubation: *Aspergillus flavus* (A) and *A. caelatus* (D). Wet mount preparation with lactophenol cotton blue exhibits microscopic elements, conidiophores, vesicles, phialides and conidia: *Aspergillus flavus* (B, C) and *A. caelatus* (E, F). Magnification: 200× (B, E); 400× (C, F).

Then, partial β-tubulin (*benA*) and calmodulin (*CaM*) genes were sequenced for precise identification of the clinical isolates. Total DNA was extracted by mechanical lysis using MagNA Lyser (Roche, Meylan, France) and green beads (1.4-mm ceramic beads, Roche, Meylan, France) with commercial plant DNA rapid lysis kit (Macherey-Nagel, Hoerdt, France) following manufacturer's instructions. PCR was carried out with β-tubulin and calmodulin primers as previously described (Balajee et al., 2005). The resulting amplicons were sequenced using the ABI PRISM BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). Sequences were assembled with Bioedit version v7.0.5 and compared with the search NCBI BLAST tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequencing of β-tubulin (571 bp) assigned the isolate to *Aspergillus caelatus* (isolate NRRL 26104) with an identity of 100%. The next matching species was *A. pseudoacaelatus* (strain CBS 117616) which demonstrated 99.5% similarity. Calmodulin sequence (559 bp) exhibited 99% identity with *Aspergillus caelatus* isolates (NRRL 26100, NRRL 25528, CBS 763.97) (Peterson, 2008) but also with *A. pseudocaelatus* (CBS 117616) (Samson et al., 2014).

Nucleotide sequence accession numbers. The partial β-tubulin and calmodulin gene sequences of this isolate have been deposited in GenBank under accession numbers MK409741 and MK 409742. β-tubulin and calmodulin dendrograms were generated by neighbour-joining method on MEGA version 7 (http://www.megasoftware.net/) to show taxonomic relatedness of the clinical isolates. Sequences of closely related species of Tamari clade *Aspergillus tamarii* (CBS 104.13), *A. fumigatus* (NRRL 163), and *A. flavus* (NRRL 1957) strains were retrieved from GenBank and included in phylogenetic tree (Figs. 2 and 3). In view of sequencing results which do not clearly differentiate *A. caelatus* from *A. pseudocaelatus*, *aflR* gene, which is implicated in aflatoxin biosynthesis by *A. flavus*, was used for differentiation between *A. caelatus* and *A. pseudocaelatus*, since the last is an aflatoxin producing species (Varga et al., 2011). Two *A. flavus* and *A. fumigatus* clinical isolates from the same study were used as PCR positive and negative controls, respectively. DNA sample from the clinical isolated did not carry a homolog of *alfR*, as well as those from both *A. fumigatus* clinical isolates. On the other hand, both clinical isolates of *A. flavus* carried *aflR* homologs (Figure 4). Taken together, these data allowed us to definitively identify the clinical isolate as *A. caelatus*.

Venous blood was collected for peripheral blood differential cell counts and sera analysis for detection of total of IgE (T-IgE), and anti-*A. fumigatus* specific IgE and IgG levels, according to the procedures previously standardized in the Hospital of Nantes. Briefly, T-IgE and *A. fumigatus* specific IgE against crude M3 antigen were assayed by ImmunoCAP (ImmunoCap® IgE, Phadia, Upssala, Sweden) and *A. fumigatus* specific IgG were detected by *A. fumigatus* ELISA kit (Bordier Affinity Products, Crissier, Switzerland) according to the manufacturer's recommendations. Cut-off value for T-IgE was considered 114 kIU/liter, *A. fumigatus* specific IgE >0.1 kIU/liter and >0.35 kIU/liter abnormal and positive, respectively. OD index values of ≥ 1 were considered positive for ELISA *A. fumigatus* IgG. The results were negative for the three parameters with a T-IgE value of 44.3 kIU/liter, *A. fumigatus* specific IgE <0.1 kIU/liter and OD index values of 0.4 for ELISA *A. fumigatus* IgG. This patient was not considered as a case of allergic bronchopulmonary aspergillosis because it does not meet the criteria established for it.

Antifungal drug susceptibility testing was performed by Sensititre YeastOne (Thermo Fisher Scientific, Cleveland, OH) colorimetric assay. Antifungal drug panels were inoculated according to the manufacturer's instructions. MICs for the clinical isolates were determined after 48 h incubation at 35 °C. Fungal growth was evidenced as a change in the colorimetric growth indicator, resazurin from blue (negative) to pink (positive). MICs for azoles and amphotericin B were interpreted as the well with the lowest drug concentration in which the growth indicator remained blue. MECs for echinocandins were read after 36 h incubation as the lowest drug concentration in which the growth indicator changed to purple. In addition, wells were also observed under light microscopy to detect any abnormal growth (short branched hyphal clusters) when compared to the growth control well. *A. fumigatus* ATCC 204305 was used as quality control (QC) strains. Antifungal drug susceptibility testing revealed susceptibility to posaconazole (0.12 mg/L), voriconazole (0.5 mg/L), itraconazole (0,25 mg/L) and high MICs to fluconazole (256 mg/L), amphotericin B (4 mg/L) and 5-Flucytosine (8 mg/L). The strain was also susceptible to caspofungin (0.12 mg/L) and micafungin (0.12 mg/L).

To assess the virulence of *A. caelatus*, *Galleria mellonella* (Lepidoptera) larvae were used as host model (Slater et al., 2011; Binder et al., 2016). The larvae in the 5th instar (200–300 mg) were selected and starved for 24 h. Groups of ten larvae were inoculated in the last right proleg with 10 μL of spores' suspensions in PBS with 0.1% Tween 20 (1×10^4, 1×10^5, and

Figure 2. Neighbour-joining tree based on beta-tubulin sequence data of the clinical isolate (CLI50) and reference strains.

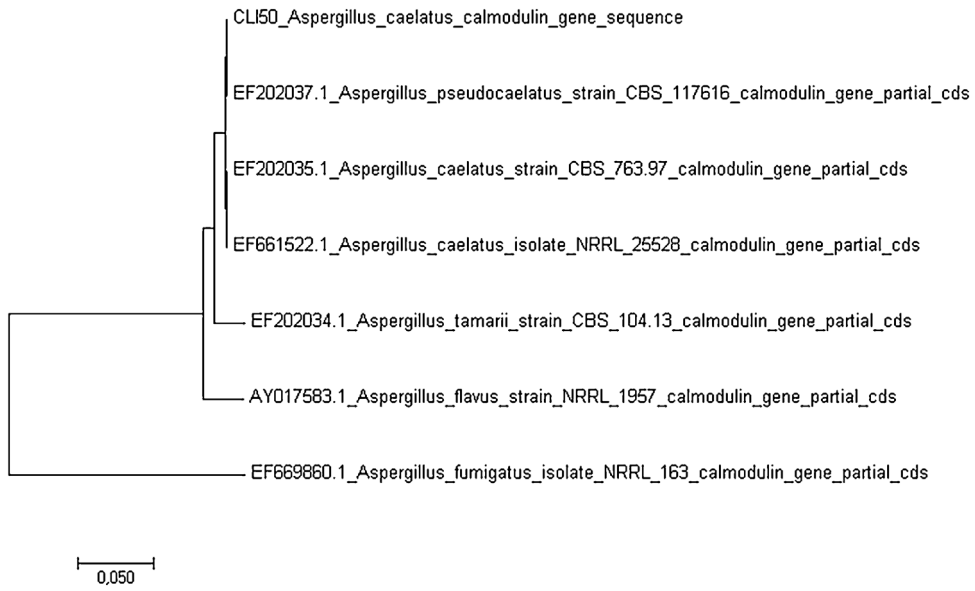

Figure 3. Neighbour-joining tree based on calmodulin sequence data of the clinical isolate (CLI50) and reference strains.

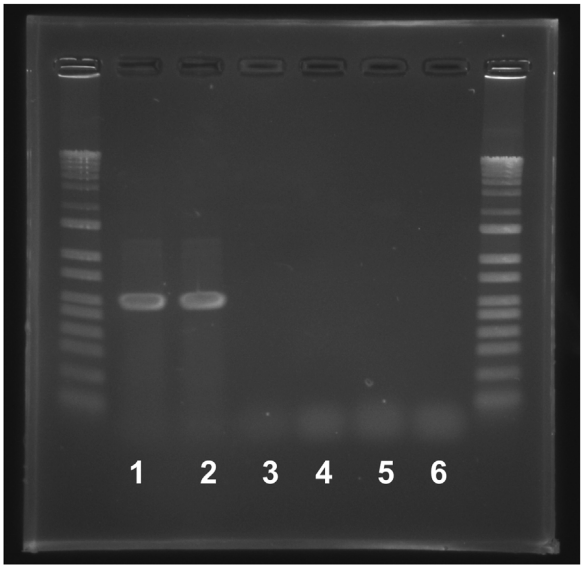

Figure 4. PCR results using *aflR* primers for *A. flavus* 32 (1), *A. flavus* 42 (2), *A. caelatus* CLI50 (3), *A. fumigatus* 11 (4), *A. fumigatus* 17 (5) and control (water) (6).

1×10^6 spores/larvae) of *A. caelatus*, one clinical isolate of *A. flavus* from the same study, and *A. fumigatus* ATCC 204305. The control groups were performed in parallel: untouched larvae, ethanol 70% cleaned larvae, and ethanol 70% cleaned larvae inoculated with 10 μL of PBS with 0.1% Tween 20. Mortality was recorded every 24 h for 7 days. Results are shown as survival curves (Kaplan-Meier plots) and analyzed by Mantel-Cox test in GraphPad Prism 7.00 software, with p set at 0.01 Both *A. caelatus* and *A. flavus* showed similar virulence patterns with 100% mortality rates after 48 h when larvae were inoculated with 1×10^5 and 1×10^6 spores ($p = 0.022$ and 0.317, respectively). The smaller inoculum size (1×10^4 spores/larvae) induced 90% and 80% mortality after 7 days for *A. caealatus* and *A. flavus*, respectively ($p = 0.406$). *A. fumigatus* was shown to be less virulent than both ($p < 0.001$), with 100% mortality at 5 days with 1×10^6 spores/larvae, and 80% and 40% mortality at 7 days with inoculum of 1×10^5 and 1×10^4 spores/larvae, respectively (Figure 5).

Discussion

Aspergillus spp. within the section *Flavi* have a worldwide distribution occurring mainly in tropical environments. *A. flavus*, the most prevalent species in the section *Flavi*, causes a broad spectrum of disease in humans ranging from hypersensitivity reactions to invasive infections (Krishnan et al., 2009). *Aspergillus tamarii* which as *A. caelatus* belong to the Tamarii clade (which encompasses species with tuberculate large spores) has only been reported in a keratitis, a burn wound infection, and some cutaneous lesions cases (Aries et al., 2018; Cuadros et al., 2017; Sharma et al., 2013). However, phenotypic methods to distinguish species in this section showed limits since the species only exhibited slight microscopic differences (Rath, 2001). Indeed, *A. caelatus* and its related species *A. pseudocaelatus* can be easily misidentified as *A. flavus* since they display a yellow-green color and possess a roughed conidiophore and biseriate vesicles. Genotypic methods that have been used for typing *A. flavus* isolates include RFLP (James et al., 2000), RAPD (Heinemann et al., 2004) and microsatellite polymorphism analysis (Guarro et al., 2005). Because of tedious and labor-intensive approaches, they are not suitable methods for rapid discrimination of species of *A.* section *Flavi*. In the last decade, more widespread use of sequencing of internal transcribed spacer rRNA genes and other conserved DNA regions such as β-tubulin, calmodulin, and RPP2 genes has provn to be useful for identifying most of these filamentous fungi. Based on one of the last revisions which combined morphology, molecular data, and toxin analysis, this section encompasses seven yellow-green spored, biseriate clades: *A. flavus*, *A. tamarii*, *A. nomius*, *Petromyces alliaceus*, *A. togoensis*, *A. leporis*, and *A. avenaceus* (Varga et al. 2011). Sequencing and phylogenetic analysis of the β-tubulin and calmodulin showed that our strain was clustered with other reference strains of *A. caelatus* and *A. pseudocaelatus* which belong to *Tamarii* clade. Comparison of this isolate with *A. flavus* as control for aflatoxin production through amplification of the *aflR* gene definitively distinguishes between *A. caelatus* and *A. pseudocaelatus*. This difficulty to identify *A. caelatus* could explain why this species had not been previously mentioned by clinical laboratories.

Concerning pathology, the size of *A. flavus* spores could explain their preferential deposition in the upper respiratory tract. Larger size of *A. caelatus* spores could be another reason why this species had not been previously reported in human diseases. However,

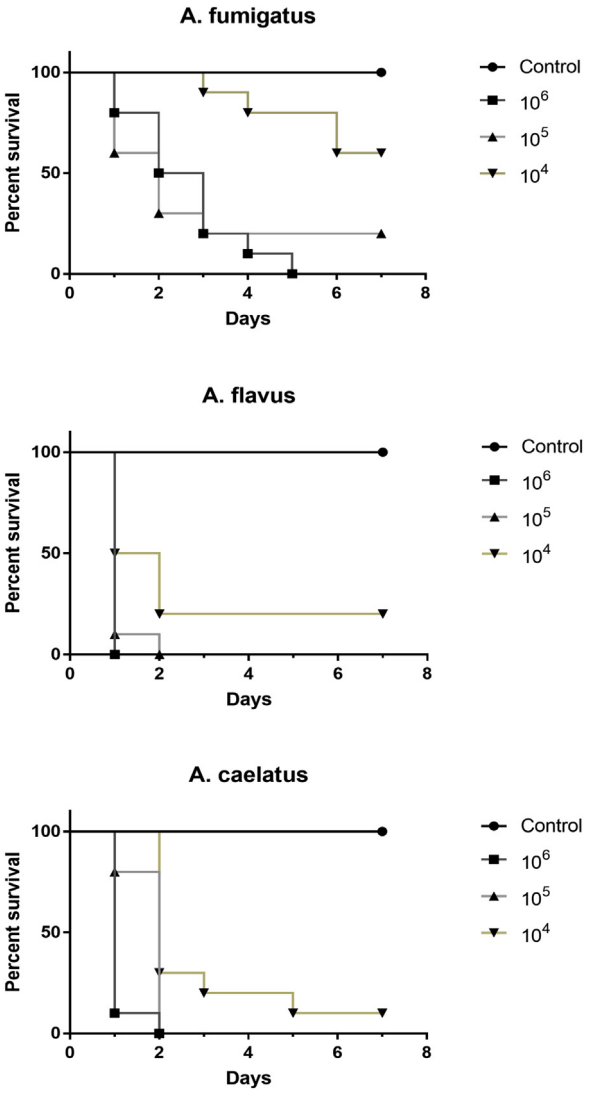

Figure 5. Comparative virulence among *Aspergillus fumigatus*, *A. flavus*, and *A. caelatus* assessed in *Galleria mellonella* larvae. Kaplan–Meier survival curves of larvae infected with different inoculum sizes (10 μL of spores' suspensions in PBS with 0.1% Tween 20) (1×10^4, 1×10^5, and 1×10^6 spores/larvae).

pathogenicity of *A caelatus* seems to be more elevated than *A. flavus* in the *G. mellonema* larvae model. Not all *Aspergillus* spp. are virulent in *G. mellonella* larvae. The virulence of *A. fumigatus* and *A. flavus*, the most important *Aspergillus* human pathogens, in *G. mellonella* larvae is dependent on inoculum size. However, *A. flavus* is about 100 times more pathogenic to *G. mellonella* and mice than *A. fumigatus*, despite the latter being responsible for most invasive fungal infection cases in humans (St Leger et al., 2000). The spores of both species are phagocytosed to a similar extent, but only *A. flavus* spores germinate inside larvae haemocytes, probably due to the bigger spore size of *A. flavus*. This correlation might be extended to *A. caelatus*, which has the biggest spores among the three *Aspergillus* species evaluated here (Binder et al., 2016). Following the CLSI breakpoints used for both *A. fumigatus* and *A. flavus* species, our *A. caelatus* isolate was found to be resistant to amphotericin B. Because of emergence of *Aspergillus* resistance to first line azole drugs, this pattern must be highlighted since liposomal amphotericin B is becoming a therapeutic alternative and it is often considered as one choice in an empirical therapy for suspected invasive fungal infection. (Hamprecht et al., 2018; Ullmann et al., 2018).

Although in our case the presence of *A. caelatus* cannot be related to an active infectious process, it is of importance to consider the *in vitro* antifungal susceptibility pattern of *Aspergillus* from the section *Flavi* and their predominant role as pathogens in tropical and subtropical countries (Taghizadeh-Armaki et al., 2017; Hagiwara et al., 2016). Therefore, it is necessary to know the human pathogenic role of each of them and to develop rapid and easy identification techniques and susceptibility tests.

In conclusion, because of the increase of non-section *Fumigati Aspergillus* spp. isolation and spreading of resistance to azole drugs, it is still recommended to perform accurate fungal identification and *in vitro* susceptibility testing for optimal patient management.

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Conflict of interest

The authors declare they do not have any conflict of interest to disclose.

Ethical approval

This study was approved by the Ethics Committee of Pontifícia Universidad Javeriana

249 Bogotá (FM-CIE-8414-15).

CAM, PLP, RMX designed the study; JI, JA, CS, AM, CAM selected the participants of the study and conducted the clinical evaluation; PLP, CAM, ER, BA, RMX carried out the experiments and analyzed the data. CAM, PLP, JA, RMX analyzed the global data, and wrote the paper.

References

Aries P, Hoffmann C, Schaal JV, Leclerc T, Donat N, Cirodde A, et al. *Aspergillus tamarii*: an uncommon burn wound infection. J Clin Pathol 2018;71(4):379–80.
Balajee SA, Gribskov JL, Hanley E, Nickle D, Marr KA. *Aspergillus lentulus* sp. nov., a new sibling species of *A. fumigatus*. Eukaryot Cell 2005;4(3):625–32.
Barrs VR, van Doorn TM, Houbraken J, Kidd SE, Martin P, Pinheiro MD, et al. *Aspergillus felis* sp. nov., an emerging agent of invasive aspergillosis in humans, cats, and dogs. PLoS One 2013;8(6)e64871.
Binder U, Maurer E, Lass-Flörl C. *Galleria mellonella*: an invertebrate model to study pathogenicity in correctly defined fungal species. Fungal Biol 2016;120(2):288–95.
Calderari TO, Iamanaka BT, Frisva JC, Pitt JI, Sartori D, Pereira JL, et al. The biodiversity of *Aspergillus section* Flavi in brazil nuts: from rainforest to consumer. Int J Food Microbiol 2013;160(3):267–72.
Cuadros J, Gros-Otero J, Gallego-Angui P, Scheu AK, Montes-Mollón Á, Pérez-Rico C, et al. *Aspergillus tamarii* keratitis in a contact lens wearer. Med Mycol Case Rep 2017;19:21–2.
Glass NL, Donaldson GC. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol 1995;61(4):1323–30.
Guarro J, Sole M, Castany R, Cano J, Teixido A, Pujol I, et al. Use of random amplified microsatellites to type isolates from an outbreak of nosocomial aspergillosis in a general medical ward. Med Mycol 2005;43(4):365–71.
Guinea J, Sandoval-Denis M, Escribano P, Peláez T, Guarro J, Bouza E. *Aspergillus citrinoterreus*, a new species of section Terrei isolated from samples of patients with nonhematological predisposing conditions. J Clin Microbiol 2015;53(2):611–7.
Hagiwara D, Watanabe A, Kamei K, Goldman GH. Epidemiological and genomic landscape of azole resistance mechanisms in *Aspergillus* fungi. Front Microbiol 2016;7:e1382.
Hamprecht A, Morio F, Bader O, Le Pape P, Steinmann J, Dannaoui E. Azole resistance in *Aspergillus fumigatus* in patients with cystic fibrosis: a matter of concern?. Mycopathologia 2018;183(1):151–60.
Heinemann S, Symoens F, Gordts B, Jannes H, Nolard N. Environmental investigations and molecular typing of *Aspergillus flavus* during an outbreak of postoperative infections. J Hosp Infect 2004;57(2):149–55.
Hong SB, Go SJ, Shin HD, Frisvad JC, Samson RA. Polyphasic taxonomy of *Aspergillus fumigatus* and related species. Mycologia 2005;97(6):1316–29.

Horn BW. *Aspergillus caelatus*, a new species in section Flavi. Mycotaxon 1997;61:185–92.
James MJ, Lasker BA, McNeil MM, Shelton M, Warnock DW, Reiss E. Use of a repetitive DNA probe to type clinical and environmental isolates of *Aspergillus flavus* from a cluster of cutaneous infections in a neonatal intensive care unit. J Clin Microbiol 2000;38(10):3612–8.
Krishnan S, Manavathu EK, Chandrasekar PH. *Aspergillus flavus*: an emerging non-fumigatus *Aspergillus* species of significance. Mycoses 2009;52(3):206–22.
Mutegi CK, Ngugi HK, Hendriks SL, Jones RB. Prevalence and factors associated with aflatoxin contamination of peanuts from Western Kenya. Int J Food Microbiol 2009;130(1):27–34.
Paulussen C, Hallsworth JE, Pérez SA, Nierman WC, Hamill PG, Blain D, et al. Ecology of aspergillosis: insights into the pathogenic potency of *Aspergillus fumigatus* and some other *Aspergillus* species. Microb Biotechnol 2017;10(2):296–322.
Peterson SW. Phylogenetic analysis of *Aspergillus* species using DNA sequences from four loci. Mycologia 2008;100(2):205–26.
Pildain MB, Frisvad JC, Vaamonde G, Cabral D, Varga J, Samson RA. Two novel aflatoxin-producing *Aspergillus* species from Argentinean peanuts. Int J Syst Evol Microbiol 2008;58(3):725–35.
Rath PM. Phenotypic and genotypic characterization of reference strains of the genus *Aspergillus*. Mycoses 2001;44(3–4):65–72.
Samson RA, Visagie CM, Houbraken J, Hong S-B, Hubka V, Klaassen CHW, et al. Phylogeny, identification and nomenclature of the genus *Aspergillus*. Stud Mycol 2014;78:141–73.
Sharma S, Yenigalla BM, Naidu SK, Pidakala P. Primary cutaneous aspergillosis due to *Aspergillus tamarii* in an immunocompetent host. BMJ Case Rep 2013;2013: e010128.
Siqueira JPZ, Sutton DA, Gené J, García D, Wiederhold N, Guarro J. Species of *Aspergillus* section *Aspergillus* from clinical samples in the United States. Med Mycol [102_TD$DIFF]2018;56(5):541–50.
Slater JL, Gregson L, Denning DW, Warn PA. Pathogenicity of *Aspergillus fumigatus* mutants assessed in *Galleria mellonella* matches that in mice. Med Mycol 2011;49(Suppl. 1):S107–13.
St Leger RJ, Screen SE, Shams-Pirzadeh B. Lack of host specialization in *Aspergillus flavus*. Appl Environ Microbiol 2000;66(1):320–32.
Sugui JA, Peterson SW, Clark LP, Nardone G, Folio L, Riedlinger G, et al. *Aspergillus tanneri* sp. nov., a new pathogen that causes invasive disease refractory to antifungal therapy. J Clin Microbiol 2012;50(10):3309–17.
Taghizadeh-Armaki M, Hedayati MT, Ansari S, Omran SM, Saber S, Rafati H, et al. Genetic diversity and in vitro antifungal susceptibility of 200 clinical and environmental *Aspergillus flavus* isolates. Antimicrob Agents Chemother 2017;61: e00004–17.
Ullmann AJ, Aguado JM, Arikan-Akdagli S, Denning DW, Groll AH, Lagrou K, et al. Diagnosis and management of *Aspergillus* diseases: executive summary of the 2017 ESCMID-ECMM-ERS guideline. Clin Microbiol Infect 2018;(Suppl 1):e1–e38.
Varga J, Frisvad JC, Samson RA. Two new aflatoxin producing species: and an overview of *Aspergillus* section Flavi. Stud Mycol 2011;69(1):57–80.