Novel cyp51A mutation profile in Aspergillus fumigatus from a patient with systemic lupus erythematosus and limited systemic sclerosis: first clinical case of acquired azole-resistance in Colombia

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Infectología

Universidad del Rosario

Bogotá - Colombia

2026

Identificación del proyecto

Institución académica: Universidad del Rosario

Dependencia: Infectología

Título de la investigación: Novel cyp51A mutation profile in Aspergillus fumigatus from a patient with systemic lupus erythematosus and limited systemic sclerosis: first clinical case of acquired azole-resistance in Colombia

Instituciones participantes: Hospital Universitario Mayor – Mederi

Tipo de investigación: Reporte de caso

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Tabla de contenido

Running title: Acquired azole-resistance *Aspergillus* in Colombia

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Abstract

Background. *Aspergillus fumigatus* is a saprophytic filamentous fungus that causes infection mostly in patients with underlying diseases, including those that alter the immune system. Despite progress in the diagnosis and treatment of this mycosis, mortality remains very high, particularly after hematogenous spread. Additionally, azole resistance can be developed in patients with long-term use of voriconazole, worsening their prognosis. However, this issue of acquired resistance has never been reported in Colombia. In fact, data on azole-resistant *A. fumigatus* is very limited in South America. **Case presentation.** Here we report a fatal case of a 29-year-old woman with both systemic lupus erythematosus and limited systemic sclerosis, who was initially diagnosed with chronic pulmonary aspergillosis, was treated with voriconazole for over a year, and subsequently developed invasive aspergillosis. The isolate, recovered from bronchoalveolar lavage, was identified as an azole-resistant *A. fumigatus* (MIC of >32 µg/ml for voriconazole and >1 µg/ml for itraconazole). Even though the patient started treatment with caspofungin, she was admitted to the intensive care unit and died of respiratory failure. Amplification and sequencing of the *cyp51A* gene, revealed the new *cyp51A* mutation profile TR_{46}/F46Y/Y121F/M172V/E427K. **Conclusions**. While azole-resistant *A. fumigatus* occurs globally, we describe the first clinical case of acquired resistance in Colombia, where resistant isolates have been found in the soil of flower and vegetable fields, but never from a patient. This case highlights one of the major global public health concerns, which is the development of resistance in *A. fumigatus*, particularly in patients receiving long-term therapy with voriconazole, encouraging enhanced surveillance of resistance trends in clinical settings.

Keywords: Aspergillosis; azole resistance; overlap syndrome; *Aspergillus fumigatus*; Colombia

Background

Aspergillus fumigatus is a saprophytic filamentous fungus isolated mainly from soil and decaying vegetation [1]. Although daily inhalation of hundreds of *Aspergillus* conidia is common [2], infection mostly occurs in patients with underlying diseases. In fact, the large burden of aspergillosis is sustained by immunocompromised individuals, including cancer patients, stem cell and solid organ transplant recipients, patients with congenital immune deficiencies, and those receiving treatments that alter the immune system [1, 3, 4]. While the lower respiratory tract and lungs are the most commonly infected, hematogenous spread may cause systemic involvement, with mortalities that remain very high despite progress in the diagnosis and treatment. Additionally, development of azole resistance can occur in patients who undergo long-term use of voriconazole, the first-line therapeutic option, worsening their prognosis [5]. While the prevalence of azole-resistance in *A. fumigatus* in the clinical setting is difficult to establish, considering that aspergillosis is not a notifiable disease and that antifungal susceptibility testing is not performed in most countries, a few surveillance studies have been done. In Germany, the UK, the Netherlands, and France, resistant *A. fumigatus* strains account for about 20 to 30% of cases, whereas in other European countries, India, the USA, Australia, Brazil, Argentina, and Peru, the prevalence of resistance is less than 10% [6]. Here, we report a fatal case of a female patient with overlap syndrome, who was diagnosed with chronic pulmonary aspergillosis, was treated with voriconazole, and subsequently developed invasive aspergillosis caused by an azole-resistant *A. fumigatus*, the first clinical case of acquired resistance reported in Colombia. Remarkably, the *cyp51A* gene variant TR_{46}/F46Y/Y121F/M172V/E427K identified in this study adds to the novel drug-resistant polymorphism combinations observed in *A. fumigatus*, in which, globally, TR_{34}/L98H and TR_{46}/Y12F/T289A have been identified as the predominant mechanisms of resistance in both clinical and environmental isolates [5-7]. Our report contributes data to the epidemiology of azole-resistant *A. fumigatus* in South America, which, while it is beginning to emerge, is still limited.

Case presentation

A 29-year-old female with the overlap syndrome, experiencing symptoms of both systemic lupus erythematosus (SLE) and limited systemic sclerosis (SSc), diagnosed in June 2022,

was admitted in December 2024 to a fourth-level hospital, with a five-day history of fever, dyspnea on minor exertion, and dry cough. From the date of diagnosis of SLE and SSc, she was on daily oral treatment with corticosteroids (prednisolone 5 mg) and hydroxychloroquine (200 mg), as well as on weekly immunosuppressants (methotrexate 500 mg) administered intramuscularly. The patient was severely underweight (BMI of 15.4 Kg/m^2), receiving 1 mg of folic acid daily. On physical examination, she presented generalized diminished breath sounds with bilateral crackles. Previous hospitalizations were recorded in June, July, and October 2022, in March 2023, and in June 2024. In the first hospitalization, she was diagnosed with hospital-acquired pneumonia (HAP) and empirically treated with ampicillin/sulbactam. A month later, she presented to the hospital with a wet cough, dysphonia, dyspnea, and pleuritic chest pain. Clinical tests reported leukocytosis (14,330 cells/µl; normal count 4,500-11,000 cells/µl), neutrophilia (12,190 cells/µl: normal count 1;500-8;000 cells/µl), and elevated C-reactive protein (CRP) (24.12 mg/L; normal level 1.0 to <10.0 mg/L). A high-resolution computed tomography (HRCT) reported a rounded consolidation in the left lobe's basal segment, with cavitation. On suspicion of pneumonia, empiric cefepime therapy was given for eight days. Tuberculosis, nontuberculous *Mycobacteria* infection, histoplasmosis, and pulmonary cryptococcosis were ruled out. Blood and bone-marrow cultures were negative, but the fever persisted; therefore, a combination of meropenem and vancomycin, a broad-spectrum antibiotic regimen, was prescribed. The third hospitalization was due to lupus exacerbation. In March 2023, the patient presented again with respiratory symptoms with leukocytosis (23,760 cells/µl), neutrophilia (21,650 cells/µl), elevated CRP (21.1 mg/L), and elevated D-dimer levels (1.98 µg/mL; normal level <0.5 µg/mL). The patient had a serum *Aspergillus* galactomannan (GM) antigen of 1.65 (negative value <0.5), and *Aspergillus fumigatus*, identified by macro and micromorphology, was recovered from bronchoalveolar lavage (BAL). In this instance, the antifungal susceptibility of the isolate was not determined, considering that this is not routinely performed in most clinical microbiology laboratories. With a diagnosis of chronic pulmonary aspergillosis (CPA), the patient started treatment with intravenous voriconazole (6 mg/Kg every 12 hours the first day, plus 4 mg/Kg every 12 hours for 6 days). Despite treatment, the serum *Aspergillus* GM antigen increased (3.83) after 11 days of the first measurement; therefore, the patient started oral voriconazole (200 mg) for 12 additional

weeks. By September 2023, the serum GM antigen had decreased but persisted positive (0.57). In June 2024, the patient was admitted due to the exacerbation of chronic respiratory symptoms associated with persistent fever. A chest computed tomography (CT) scan showed increased apical cavitation (Figure 1A), and a fiberoptic bronchoscopy (FBS) showed purulent endobronchitis. Empirical treatment of meropenem was given for 10 days. In addition, given that a BAL GM antigen was positive (>1.0), the patient initiated oral voriconazole for an additional 12 months. In the last hospitalization, in December 2024, she was readmitted due to respiratory difficulty. Chest X-ray showed evidence of alveolar-occupying opacities with multilobe involvement, and chest CT showed incipient consolidation of the lateral segment of the middle lobe. Blood cultures were negative after 72 hours. Tuberculosis was ruled out. The patient continued with voriconazole treatment. A month later, a CT scan revealed a 10 mm pulmonary nodule in the lateral segment of the left lower lobe and ground-glass opacities. In February 2025, a new BAL culture reported *A. fumigatus* resistant to voriconazole (MIC >32 µg/ml) and itraconazole (MIC >1 µg/ml), as determined by an E-test. Considering the microbiological findings, host factors, and clinical and radiological evidence (Figure 1A), the patient was diagnosed with invasive aspergillosis (IA) by a multi-azole-resistant *A. fumigatus*; therefore, she started treatment with intravenous caspofungin (50 mg per day). Unfortunately, a few days after diagnosis, the patient was admitted to the intensive care unit (ICU) and died of respiratory failure (Figure 1B).

Confirmation of the species of the resistant *A. fumigatus* isolate was done by sequencing of the internal transcribed spacer (ITS) region [8] (GenBank accession number PV935454). By amplifying and sequencing the *cyp51A* gene [9], a 46 bp tandem repeat in the promoter region (TR_{46}) together with the mutations F46Y, Y121F, M172V, and E427K, which confer multi-azole resistance, were identified in the voriconazole and itraconazole-resistant isolate (GenBank accession number PX262267).

Discussion and conclusions

IA is one of the most frequent mycoses affecting patients treated with steroids and/or immunosuppressive drugs, including those with autoimmune diseases. Corticosteroids, particularly, profoundly weaken the antifungal response by promoting a Th2-skewed immune profile and by dysregulating neutrophil recruitment, which contributes to tissue

injury instead of effective fungal conidia clearance from lungs [10]. More often reported in patients with SLE, *Aspergillus* infections can also occur in patients with SSc. However, individuals with overlap syndrome of SLE and SSc have an even higher risk of opportunistic infections due to the immunomodulation and increased immune dysregulation associated with the syndrome itself, therefore raising mortality rates [3]. Previous episodes of bacterial pneumonia, as it occurred with our patient, or any other lung structural abnormalities, are additional underlying conditions for IA [4]. Clearly, the diversity of biological agents that target the immune system, along with the increase in lung illnesses, underscores the challenges and further complications to manage aspergillosis.

To aggravate the situation, azole resistance in *A. fumigatus* is being reported globally at increasing frequency, in both clinical and environmental isolates [5, 6]. In fact, several voriconazole and itraconazole-resistant *A. fumigatus* isolates have been found in the soil of flower and vegetable fields in Colombia [11, 12]. To our knowledge, however, this is the first time that a multi-azole-resistant isolate has been reported from a clinical case in the country. Cross-resistance can develop in the environment after the fungus comes into contact with azole fungicides used in agriculture, which leads to an environmental-to-clinical transmission. This was first postulated after azole-naïve patients developed aspergillosis caused by resistant isolates, and since then, numerous cases have been described [13]. The other pathway that contributes to the development of resistance in *A. fumigatus* is the long-term azole therapy, particularly in patients with weakened immune systems [5, 13]. This last scenario very likely occurred with our patient, given that she was on prolonged voriconazole therapy in the setting of CPA and was severely immunocompromised. While newly acquired infection by a resistant environmental strain remains plausible, long-term azole therapy has long been recognized as a powerful predictor for the emergence of resistance, particularly in patients with chronic infections, not only associated with clinical or radiological deterioration, but also coupled with circulating biomarkers and positive cultures while the patient is on therapeutic doses of an azole [14], as it occurred with our patient. Considering that voriconazole resistance has significant implications for patient survival [1, 5], routine testing of clinically relevant isolates should be performed, which is not currently done in most institutions and countries. Importantly, when pan-azole resistance is developed in an individual patient, switching therapy to an alternative intravenous agent such as micafungin

or liposomal amphotericin B is strongly recommended, although evidence that supports the use of either antifungal is currently lacking, and monitoring of adverse events should be done [15]. In the particular case of our patient, caspofungin was chosen as the alternative for salvage therapy due to the unavailability of micafungin in Colombia.

Regarding the mutations associated with multiple azole resistance, the TR_{46}/Y121F/T289A and TR_{34}/L98H genotypes are the most frequently detected in environmental samples in Colombia [5, 11, 12]. Additionally, TR_{46}/Y121F/T289A has been reported in clinical isolates from various countries, particularly those recovered from azole-naïve patients [5], which supports environmental transmission. Notoriously, in the isolate from our patient, the mutation T289A was not identified. While the TR_{46} promoter is commonly found in conjunction with Y121F and T289A, in intermediate to highly voriconazole-resistant isolates, the combination TR_{46}/Y121F (without T289A) has already been reported in China, exclusively in clinical *A. fumigatus* isolates with high voriconazole MICs [16]. Even the unique Y121F substitution was reported in an *A. fumigatus* that was not susceptible to voriconazole but was susceptible to itraconazole and posaconazole, recovered from an azole-naïve patient in France [17]. Apart from Y121F, three other mutations (F46Y, M172V, and E427K) were identified in the isolate reported herein. F46Y and M172V, specifically, with or without E427K, have been associated with voriconazole preexposure of patients and with high itraconazole MICs [18]. Together, our findings show that different mutations in the *cyp51A* gene, as well as different combinations of these genetic alterations, can occur, therefore contributing to diverse resistant profiles in *A. fumigatus*. In addition, identifying isolates with uncommon and/or multiple resistant mutations strongly suggests that resistance originated within the patient under prolonged antifungal therapy [15], which reinforces the idea that, with our patient, we were facing acquired resistance.

In summary, this case report highlights the need for a comprehensive diagnosis workup and personalized therapy plans, given the diverse medical presentations of aspergillosis. In addition, considering that infections remain one of the leading causes of death in patients with autoimmune diseases, mainly due to the emergence of resistance during therapy, our case displays the importance of promptly detecting resistant isolates to guide effective antifungal therapy and to mitigate complications. Azole resistance in *A. fumigatus* is a major global public health concern; therefore, enhanced surveillance of resistance trends in clinical

settings must be encouraged worldwide, particularly among patients receiving long-term therapy with voriconazole. Finally, our report contributes to the limited clinical and genotypic data on azole resistance in the region, which, to date, has been solely described in Argentina, Brazil, and Peru, not only from patients with prior azole exposure but also from those that were azole-naïve [5].

List of abbreviations

BAL: bronchoalveolar lavage
BMI: Body mass index
CPA: Chronic pulmonary aspergillosis
CRP: C-reactive protein
CT: Computed tomography
FBS: Fiberoptic bronchoscopy
GM: galactomannan
HAP: Hospital-acquired pneumonia
HRCT: High-resolution computed tomography
IA: Invasive aspergillosis
ICU: Intensive care unit
ITS: Internal transcribed spacer
MIC: Minimal inhibitory concentration
SLE: Systemic lupus erythematosus
SSc: Limited systemic sclerosis
Th2: T helper 2
TR: Tandem repeat

Declarations

Ethics approval

Patient's identification was anonymized for this study, which was carried out in accordance with the Mederi Research Ethics Committee on Human Beings protocol CEISH-2025102, Bogota, Colombia, and in accordance with the Helsinki Declaration as revised in 2013.

Consent to participate

Written informed consent for publication was obtained from the patient's next of kin.

Data Availability

The authors confirm that the data supporting the findings of this study are available within the article. The DNA sequences obtained in this study were deposited in GenBank under the accession numbers PV935454 and PX262267.

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Funding

This work was funded by the School of Medicine and Health Sciences, Universidad del Rosario, and by Centro de Investigaciones de Méderi - CIMED.

Author Contributions

CMP collected clinical case data, discussed findings and edited the first draft of the manuscript; MAV, XCL and EM collected and analyzed clinical case data, and edited the first draft of the manuscript; JAC revised and analyzed diagnostic images, and edited the first draft of the manuscript; KLV collected clinical case data and edited the first draft of the manuscript; CF performed DNA amplification and sequencing, sequence analysis, and wrote the first draft of the manuscript. All authors have read and approved the final version of the manuscript.

Acknowledgments

To the Sequencing Core Facility (GenCore), from Universidad de los Andes, Bogota, Colombia, for generating and subsidizing part of the sequencing results. The GenCore laboratory receives support from the Vice-Presidency for Research and Creation at Universidad de los Andes. We thank all personnel involved in this patient's care.

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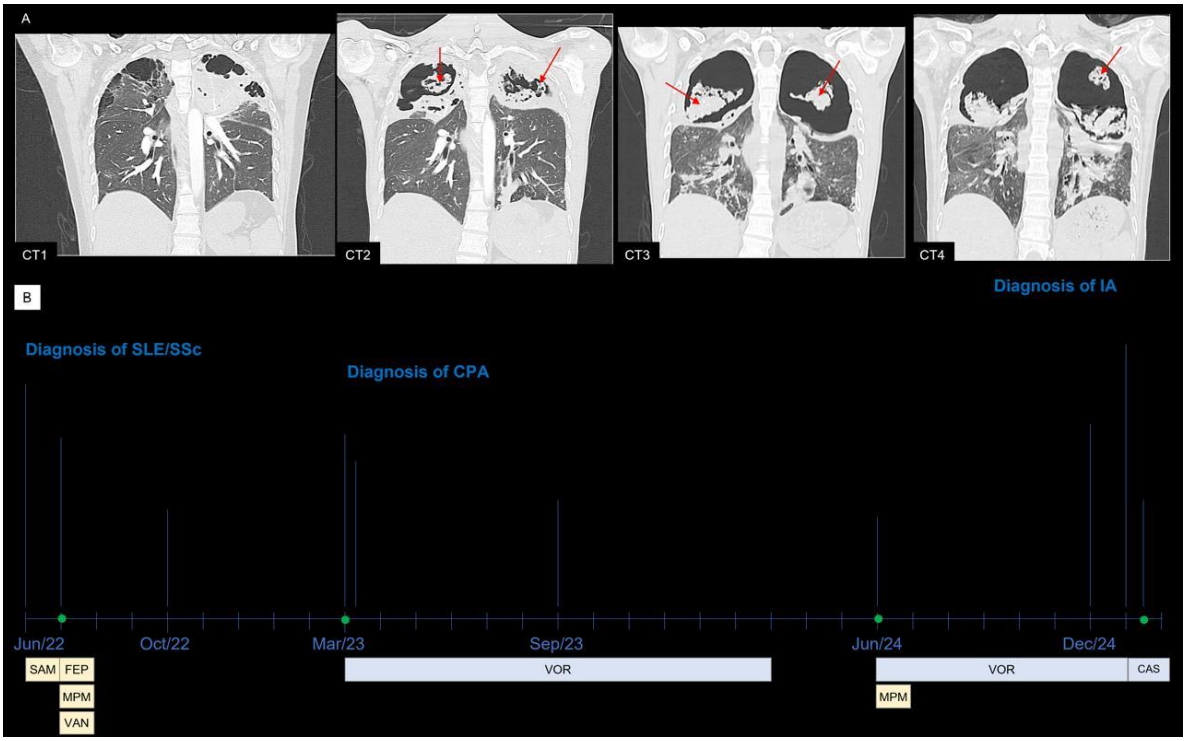

Figure 1. Computed tomography (CT) findings, and time course of diagnostic and therapeutic events. **A**. Evolution of CT features of pulmonary lesions at four different times from disease onset (green dots on the timeline). Volume loss in the upper lobes is observed. Consolidation and cavitation progression are evident over time. Round, hyperdense areas, suggesting fungal balls, are indicated (red arrows). Bronchial wall thickening and nodules are also observed. **B**. Timeline, per month, with the main events, including CT scans, and antibiotic and antifungal treatment in the clinical history of the patient. SLE: systemic lupus erythematosus; SSc: limited systemic sclerosis; HAP: hospital-acquired pneumonia; SAM: ampicillin-sulbactam; FEP: cefepime; MPM: meropenem; VAN: vancomycin; HRCT: high-resolution computed tomography; CPA: chronic pulmonary aspergillosis; GM: *Aspergillus* galactomannan; BAL: bronchoalveolar lavage; VOR: voriconazole; IA: invasive aspergillosis; CT: computed tomography; FBS: fiberoptic bronchoscopy; CAS: caspofungin; ICU: intensive care unit.