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Note

First itraconazole resistant *Aspergillus fumigatus* clinical isolate harbouring a G54E substitution in Cyp51Ap in South America

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ABSTRACT

Background: A 27-year-old male rural worker was admitted with a fungal keratitis due to an injury involving plant detritus.
Materials and methods: Specimens were collected for microscopy examination and culture. The isolate was identified by morphological and molecular criteria. Susceptibility testing was performed using CLSI methods. *CYP51A* gene was PCR amplified and sequenced.
Results: An *Aspergillus fumigatus* strain resistant to itraconazole (MIC > 8 μg/ml) was isolated. The isolate was susceptible to amphotericin B, posaconazole, voriconazole and caspofungin. *CYP51A* sequencing showed two mutations leading on the G54E substitution. The patient received natamycin as treatment.
Conclusions: This is the first report in South America of a clinical *A. fumigatus* strain carrying the substitution G54E at Cyp51Ap associated with itraconazole resistance. Considering the patient was azole-naive, this resistant isolate may have been acquired from the environment.

Primer aislamiento clínico en Sudamérica de una cepa de *Aspergillus fumigatus* resistente a itraconazol con la sustitución G54E en Cyp51Ap

Palabras clave:
Aspergillus fumigatus
Resistencia a azoles
CYP51

RESUMEN

Antecedentes: Un trabajador rural de 27 años de edad fue hospitalizado con una queratitis fúngica debido a un traumatismo con un resto vegetal.
Materiales y métodos: Se tomaron las muestras para los exámenes de microscopía y cultivo. El aislamiento se identificó mediante criterios morfológicos y moleculares. Se realizaron pruebas de sensibilidad a los antifúngicos siguiendo el documento del CLSI. Se amplificó y secuenció el gen *CYP51A* de la cepa.
Resultados: Se aisló una cepa de *Aspergillus fumigatus* resistente a itraconazol (CIM > 8 μg/ml). El aislamiento resultó sensible a la anfotericina B, el posaconazol, el voriconazol y la caspofungina. La secuenciación del gen *CYP51* reveló 2 mutaciones que generan la sustitución G54E. El paciente fue tratado con natamicina oftálmica.
Conclusiones: Este es el primer caso informado en Sudamérica de una cepa clínica de *A. fumigatus* con la sustitución G54E en el Cyp51Ap, asociada con resistencia al itraconazol. Teniendo en cuenta que el paciente no había recibido nunca antes tratamiento alguno con azoles, podría haber adquirido esta cepa resistente del ambiente.

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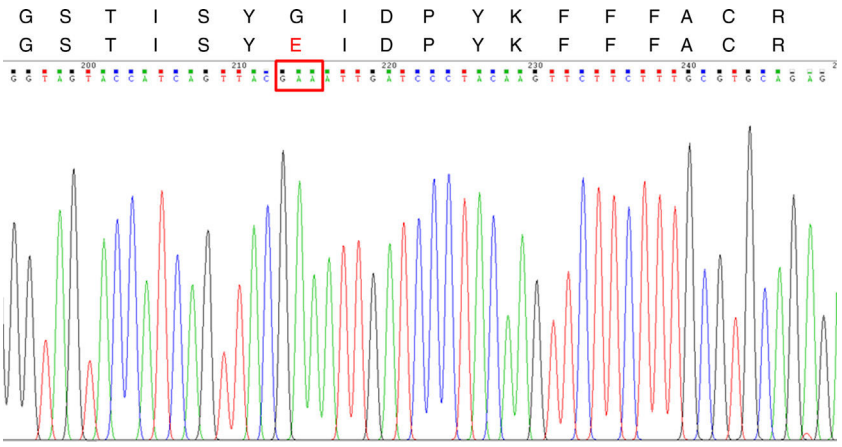

Fig. 1. *CYP51A* DNA sequencing chromatogram and Cyp51Ap amino acid sequence for the ITC-resistant *A. fumigatus* strains. Upper line: Segment of the wild type *A. fumigatus* Cyp51Ap (GenBank accession no. AAK73659.1) between the amino acid residues 48 and 65. Lower line: the same Cyp51Ap segment of the ITC-resistant strain, showing the amino acid substitution (G54E). The red box in the DNA sequencing chromatogram shows the mutated codon 54.

A 27-year-old male rural worker was admitted to the "Dr. José María Cullen" Hospital (Santa Fe – Argentina) in November 2013 with a corneal ulcer. The patient had suffered an ocular traumatism with a vegetal detritus while he was working in harvesting vegetables. At patient arrival, ocular samples were obtained at the Ophthalmology Department of the hospital. Conjunctival mucopurulent exudate and corneal surface samples were obtained by swabbing and by scrapping with a Kimura spatula, respectively. Samples were immediately sent to the Microbiology Laboratory for their processing. Samples were Giemsa stained and cultured in Sabouraud Dextrose Agar (SDA) with chloramphenicol (Britania, Argentina) and in Thioglycollate broth. Meanwhile, the patient received empirical treatment with oral fluconazole (400 mg/day) and ophthalmic natamycin (5% solution), two drops every 30 min during the first 24 h, together with intravenous ceftazidime and vancomycin. During patient's first day of hospitalization the laboratory informed that septate fungal hyphae were seen in the Giemsa stain, leading to the diagnosis of a keratitis due to a hialohyphomycete. With these data, fluconazole was suspended and natamycin and antibacterials were maintained. Natamycin dosage intervals were changed after the second hospitalization day to two drops every hour during 7 days. Then, it was slowly tapered following the good clinical response until the complete resolution of the infection. After the third hospitalization day, colonies of *Aspergillus fumigatus* grew in all the SDA slants.

The strain was identified as *A. fumigatus* by morphological criteria and by PCR amplification and sequencing of the β-tubulin gene as previously described.[1,2] Antifungal susceptibility testing was performed using the broth microdilution method according to the CLSI M38A2 document for itraconazole (ITC), voriconazole (VRC), posaconazole (PSC), amphotericin B (AMB) and caspofungin (CSF).[3] *CYP51A* gene (encoding the 14-α-sterol demethylase, target of azole drugs) was PCR amplified including 5′ UTR, ORF and 3′ UTR regions. The obtained amplification fragment was sequenced as described before.[4,5]

The *A. fumigatus* strain MICs and MEC were ITC >8 μg/ml, VRC 0.5 μg/ml, PSC 0.12 μg/ml, AMB 0.5 μg/ml and CSF 0.5 μg/ml. According to the ECV values published by Espinel-Ingroff et al., the strain was considered as ITC-resistant and susceptible to all the other antifungal agents tested.[6,7] *CYP51A* sequencing showed two nucleotide mutations (G161A and G162A) when compared with the one published under the GenBank sequence AF338659.1. These mutations lead to a substitution at codon 54 (G54E) (Fig. 1) which was already described and associated to ITC-resistant phenotypes, but was never seen in South America before.[4,8–11] Thus, to the best of our knowledge, this is the first ITC-resistant *A. fumigatus* strain reported in this part of the world.

Azole-resistant *A. fumigatus* isolation frequency is increasing. These resistant strains were isolated with or without previous azole exposure.[8,9,12–14] Our patient, who had never been treated previously with azole drugs, suffered an infection with an ITC-resistant *A. fumigatus* strain due to an accidental inoculation with vegetal detritus. This fact suggests an environmental origin of the strain. There have been no reports in Argentina demonstrating the existence of environmental azole-resistant *A. fumigatus* strains. However, these strains might be being selected since azole antifungal agents are widely used in agriculture for plant protection. Such environmental route of resistance development in *A. fumigatus* was firstly proposed in 2001 and molecularly confirmed later in the Netherlands for a common azole resistance mechanism involving L98H substitution at Cyp51Ap coupled with a promotor modification.[15–17]

The emergence and spread of the resistance mechanism described here in *A. fumigatus* is of major concern because ITC is a highly used azole drug in developing countries. It would be useful to analyze environmental sources to detect these strains.

Conflict of interest

None to declare.

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