IJM - Italian Journal of Mycology
ISSN 2531-7342 - Vol. 50 (2021): 44-51
Journal homepage: https://italianmycology.unibo.it/

Short note

Screening for M220 mutation in azole-resistant *Aspergillus fumigatus* isolates from clinical and environmental specimens in Cuba

Javier L. San Juan-Galán*, Carlos M. Fernández-Andreu, Gerardo F. Martínez-Machín, Mayda R. Perurena-Lancha, María T. Illnait-Zaragozí, Rosario E. Velar-Martínez

Department of Bacteriology-Mycology, Institute of Tropical Medicine "Pedro Kourí", autopista "Novia del Mediodía", km $6_{1/2}$, La Lisa, La Habana

* Corresponding author e-mail: jlsanjuan91@gmail.com

Article info
Received 22/4/2021; accepted 2/6/2021
https://doi.org/10.6092/issn.2531-7342/12759

Abstract
Aspergillosis is a fungal disease caused by different *Aspergillus* species and have high morbidity/mortality rates. *Aspergillus fumigatus* is the main aetiological agent related to most of *Aspergillus* infections. The use of triazoles is recommended as first line of treatment, however, the reports of azole-resistant *A. fumigatus* due to mutations in CYP51 have increased around the world. The goals were to determine the antifungal susceptibility patterns for triazoles in thirty *A. fumigatus* isolates from clinical and environmental samples and to detect mutation M220 in the resistant ones. Determination of minimum inhibitory concentrations of every isolate was performed by using the Etest commercial method for itraconazole, voriconazole and posaconazole. For the resistant isolates, a PCR was performed for amplifying a 173 bp fragment of cyp51A and the resulting amplicons were sequenced. Nine out of 30 isolates were resistant to itraconazole and none were resistant to voriconazole or posaconazole. The alignment of sequences with a CYP51 *A. fumigatus* wild type strain did not show any modifications at codon 220. Other molecular mechanisms of resistance are probably related to the resistant phenotypes isolated.

Keywords
Triazoles, Etest, drug resistance, CYP51A, sequencing

Introduction
According to the data provided by the Leading International Fungal Education (LIFE), aspergillosis has a significant impact on human health, with a global figure of 200,000 patients at risk of developing an invasive form of aspergillosis and three million people worldwide to be affected by chronic pulmonary aspergillosis (Brown et al., 2012; Anonymous, 2017). Despite there are a few *Aspergillus* species that have been reported as cause of mycosis, *A. fumigatus* Fresen. is still the most relevant species, related to 90% of all aspergillosis cases (Bonifaz, 2015). The management of this disease comprehend the use of echinocandins, polyenes and triazoles within the therapy (Jenks et al., 2018). This last group of antifungals is defined as the first line of treatment, however, in the last decades the reports of *A. fumigatus* resistant isolates have increased and the resistance issue has evolved from itraconazole resistance to panfungal resistance in just a few years (Sweileh et al., 2017). Several researchers state that triazoles resistance in *Aspergillus* is not only related to intrinsic characteristics

The journal is hosted and mantained by ABIS-AlmaDL. © 2021 The Authors. This work is licensed under CC BY 4.0

https://doi.org/10.6092/issn.2531-7342/12759

of strains but also to the deep effect of selective pressure caused by demethylase inhibitors usually used in agriculture and gardens to protect crops from plagues (Fisher et al., 2018).

The pharmacological target of triazoles is the 14-α-sterol demethylase, which is involved in ergosterol biosynthesis pathway and this enzyme belongs to the p450 cytochrome superfamily, expressed by the *cyp*51 gene (Prasad et al., 2016). Aminoacidic substitution on the backbone of this protein or modifications in the promotor region of the *cyp*51A allele can develop triazoles resistance in *A. fumigatus*. Changes at codon 220 are usually related to a diminished susceptibility to posaconazole and voriconazole and also drive to itraconazole resistance in the species (Rivero-Menendez et al., 2016). Although this polymorphism is frequently identified in *A. fumigatus* isolated from patients under azole therapy some researchers has described it at environmental screenings (Sanglard, 2016). The impact of aspergillosis in Cuba remains unknown due to the lack of epidemiological data and diagnostics methods. Few investigations suggest that the real burden of aspergillosis might be underestimated in the country for which there should be a surveillance program for detection of resistant *A. fumigatus* isolates (Beltrán et al., 2019). Although previous researches reported the presence of itraconazole resistant phenotypes distributed in the country, there is no evidence of which molecular mechanisms are involved (San Juan-Galán et al., 2017a). For that matter, the main goals of this work were to determine the susceptibility patterns of *A. fumigatus* isolates against itraconazole, voriconazole and posaconazole and to identify the M220 mutation in the resistant isolates.

Materials and methods

Isolates

Thirty *A. fumigatus* isolates preserved in the Fungal Pathogens Culture Collection (Institute of Tropical Medicine "Pedro Kourí", Cuba) were evaluated within the study (11 from clinical respiratory samples and 19 from environmental screenings). All isolates were cultured in malt yeast extract agar and Czapek yeast extract agar and incubated at 25 ºC and 50 ºC for 7 days to confirm correct identification (Samson et al., 2007, 2014). Macro/micro-morphological features were checked as part of the identity confirmation. *A. fumigatus* wild strain ATCC® MYA-3627 was used as reference strain for every test and was processed using the same methodology as the isolates.

Susceptibility testing

For the determination of itraconazole, voriconazole and posaconazole minimum inhibitory concentration (MIC), Etest (Biomeriéux, France) was performed. Conidial suspensions were adjusted to approximately 10^6 CFU mL^{-1} using a Neubauer chamber. For the test, each suspension was inoculated with a swab onto the surface of RPMI-1640 agar supplemented with MOPS (morpholinepropanesulfonic acid) and 2% glucose (Sigma-Aldrich, Germany). Plates were incubated at 35 ºC for 48 hours (BioMérieux).

The MIC was defined as the lowest concentration of antifungal drug for which the tangent in the elliptical zone of growth inhibition cuts the Etest strip perpendicularly. To establish susceptibility patterns (wild-type and resistant-type) for itraconazole, voriconazole and posaconazole, the epidemiological cut-off values used were 1.0 µg mL^{-1}, 1.0 µg mL^{-1} and 0.5 µg mL^{-1} respectively.

DNA extraction

Every triazole-resistant isolate was cultured in Sabouraud-dextrose broth with agitation at 37 ºC for 5 days. Subsequently each culture was filtrated and 200 mg (wet weight) of mycelium was taken for DNA extraction. Mycelium was washed two times with EDTA (0.5 M), shaked with 20-30 crystal beads (0.30 mm) for 10 minutes and then incubated at 37 ºC for two and a half hours in 400 µL of

https://doi.org/10.6092/issn.2531-7342/12759

spheroplasts buffer (Sorbitol, 1.0 M; EDTA, 0.1 M; Lyticase, 1.5 mg mL^{-1}; ditiothreitol, 0.05 M). After this pre-treatment QIAGEN UCP Pathogen MiniKit (QIAGEN Group, Germany) was used for DNA extraction.

Amplification and sequencying of cyp51A

The PCR protocol followed for *cyp*51A amplification was the one described by Spiess et al. (2012) with modifications. Briefly, for a total volume reaction of 50 µL there was added 5 µL of target DNA (~100 ng *A. fumigatus* DNA). Final concentration of 1X for TaqPol Buffer with 1.5 mM $MgCl_2$, 0.2 mM of dNTPs, 2.5 U of HotStar Taq Polymerase and 0.2 µM of forward CypA-M220-fw (5'-GCCAGGAAGTTCGTTCCAA-3') and reverse CypA-M220-rv (5'-CTGATTGATGATGTCAACGTA-3') primers. PCR was performed in a thermocycler (BIOER Technology, China) using the following program conditions: denaturation for 2 minutes at 94 ºC; followed by 40 cycles of denaturation at 94 ºC for 45 seconds, annealing at 52 ºC for 1 minute and extension at 72 ºC for 1 minute; at the end, a final extension step at 72 ºC for 10 minutes. Amplicons were checked in a 2% ethidium bromide agarose gel.

For sequencying, PCR products were purified using QIAquick PCR Purification kit (QIAGEN Group, Germany). Sanger sequencing was performed with CEQ DTCS QuickStart commercial kit (Beckman Coulter, USA) for a final volume reaction of 20 µL. Amplification was performed in a thermocycler (BIOER Technology, China) with the following protocol: 35 cycles of denaturation at 94 ºC for 20 seconds, annealing at 52 ºC for 20 seconds and extension at 72 ºC for 4 minutes. The sequencer used was GenomeLab GeXP (Beckman Coulter, USA).

Sequence analysis

Sequences were edited using MEGA X ver. 10.0.5. For alignment of sequences and detection of M220, a BLASTx ver. 2.9.0+ was performed. The reference sequence used for comparison was Af293, an *A. fumigatus* wild-type 14-α-sterol demethylase (Accession Number: XP752137) available in the GenBank database (http://www.ncbi.nlm.nih.gov/).

Statistical analysis

Antifungal susceptibility data was processed for descriptive analysis using GraphPad Prism software ver. 6.01 (La Jolla, CA, United States). Also, a Kruskal-Wallis test was performed for determine variations between the medians. Statistical significance was set at $P < 0.05$.

Results

From the *A. fumigatus* isolates evaluated, nine out of 30 were resistant to itraconazole and none were resistant to voriconazole or posaconazole. Statistics from the descriptive analyses are presented in Table 1. MIC values for each of the isolates in evaluation against all three azoles tested are presented in Figure 1. Results from the Kruskal-Wallis showed a P value < 0.0001, confirming the differences between the medians of the MIC values for the three tested triazoles. All PCR products from the *A. fumigatus* itraconazole-resistant isolates were consistent with the *cyp*51A region selected for the study.

The BLASTx analysis performed did not show any evidence of modifications of methionine at codon 220 neither at any other codon contained in the same 173 bp region.

https://doi.org/10.6092/issn.2531-7342/12759

Table 1 - Descriptive statistics of MICs data for *A. fumigatus* isolates evaluated against itraconazole, voriconazole and posaconazole.

Isolation source (No. of strains)	Antifungal	Range	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	Geometric mean
Total (30)	ITZ	0.38–2.0	0.63	1.5	0.70
	VCZ	0.064–0.38	0.13	0.25	0.15
	PCZ	0.016–0.19	0.094	0.13	0.083
Clinical (11)	ITZ	0.38–2.0	1.0	1.90	0.91
	VCZ	0.094–0.25	0.19	0.25	0.16
	PCZ	0.047–0.19	0.094	0.19	0.095
Environmental (19)	ITZ	0.38–1.5	0.50	1.5	0.60
	VCZ	0.064–0.38	0.13	0.25	0.14
	PCZ	0.016–0.13	0.094	0.13	0.076

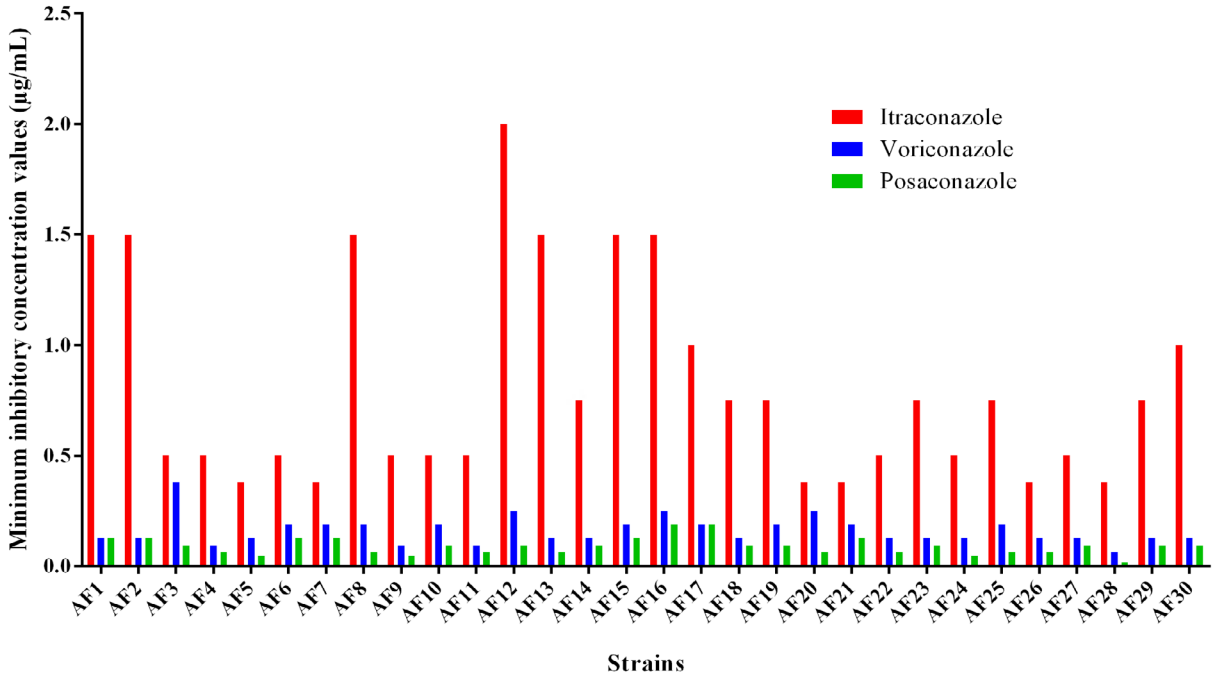

Fig 1 - MIC values distribution for itraconazole (ITZ), voriconazole (VCZ) and posaconazole (PCZ) in *Aspergillus fumigatus* isolates. Epidemiological cut-off values used were 1.0 µg mL^{-1}, 1.0 µg mL^{-1} and 0.5 µg mL^{-1} for itraconazole, voriconazole and posaconazole respectively. Isolates AF7 to AF17 are from clinical samples and the rest of isolates are from environmental screenings.

https://doi.org/10.6092/issn.2531-7342/12759

Discussion

In the last decade the reports on azole-resistant *A. fumigatus* research have increased exponentially while new molecular mechanisms of resistance to these antifungals have come into sight. This fact has become a world-wide subject, however, there are few studies of antifungal susceptibility in *A. fumigatus* in Latin America and lesser in the Caribbean region (Gonçalves, 2016). Cuba has a broad fungi diversity and *Aspergillus* genus is vastly represented all across its capital city, Havana (Almaguer et al., 2013). Previous studies performed by researchers from the Institute of Tropical Medicine "Pedro Kourí" stated the presence of azole-resistant phenotypes in *A. fumigatus* and other species isolated from environmental and clinical specimens (San Juan-Galán et al., 2017a, b).

In the present study, the number of itraconazole-resistant *A. fumigatus* isolates (30%) was similar to reports from other countries. A susceptibility study performed by Badiee et al. (2012) at the University of Medical Sciences in Shiraz, Iran, showed MIC_{50} and MIC_{90} values for itraconazole of 0.75 and 1.00 µg mL^{-1}, respectively during the evaluation of thirty *A. fumigatus* isolates. In Netherlands, a group of researchers found a 37% (155/420) of azole resistant *A. fumigatus* isolates and a high prevalence of mutations on *cyp*51 gene within a screening on clinical samples (Buil et al., 2018).

In Cuba, the clinical triazole available for the treatment of aspergillosis is itraconazole (Morales Ojeda, 2018). This might be related to the absence of resistant phenotypes to voriconazole or posaconazole because of the lack of selective pressure. Also commercialization and development of these latter drugs has been limited for longer than for itraconazole, under patent conditions and quality controls required by international regulatory entities, thus restraining their employment in therapy (Maertens, 2004). On the other hand, these triazoles have other alternative pharmacological targets that reinforce their antifungal effect, as it is the case of voriconazole which inhibits different enzymes from the ergosterol biosynthesis pathway besides the 14-α sterol demethylase (Chandrasekar and Manavathu, 2009).

The M220 mutation comprises several different aminoacidic changes and emerges mostly in *A. fumigatus* species isolated from patients with chronic infections who are under long-terms azole therapy. Furthermore, it is associated to itraconazole resistance and wide-ranging susceptibility to voriconazole and posaconazole (Arikan-Akdagli et al., 2018). In this work, six out of nine resistant isolates came from clinical samples, specifically from patients with pulmonary infections caused by *A. fumigatus*, reason why it was decided to search for M220 instead of other mutations like TR34/L98H with higher frequencies, especially in environmental isolates.

Although there is no evidence of M220 in the present study, the isolation of itraconazole resistant phenotypes implies the existence of molecular mechanisms linked to them. While *cyp*51 mutations are the most common cause of reduced susceptibility against triazoles there are other causes with a probable association to this issue such as the expression of MFS (Major Facilitators Superfamiliy) and ABC (ATP-binding cassettes) transporters (Cannon et al., 2009). Both of them inhibit the effect of antifungals by pumping the drug out from the cytoplasm, in the first case through an antiporter ion H^+ exchange and in the second case through an efflux pump ATP-dependent. Within the MFS, Mfs56 have been described with an important role on triazole resistance and some authors suggests that cluster 10 of antifungal/H^+ antiporter transporters may contain other molecules with similar functions (Costa et al., 2014). There is very few data on ABC transporters in *A. fumigatus*: *cdr*1B gene expression has a proven connection to triazole resistance and ABC transporters like AtrF, AtrI are suggested to be involved in antifungal resistance (Berger et al., 2017).

There are only two conjectures about the causes for the detection of *A. fumigatus* resistant isolates: i) different *cyp*51 mutations such as TR34/L98H and TR46/Y121F/T289A, which are related to

https://doi.org/10.6092/issn.2531-7342/12759

antifungal overuse in the environment, are expressed in Cuban phenotypes; ii) resistant phenotypes were naturally introduced in the country via airborne or by accidental importation through people, animals, plants or traded goods.

As a reference for the first outline, according to official documentation published by the Cuban Health and Agriculture Ministries there are 11 different demethylation inhibitors approved and distributed all across the country which also can lead to cross-resistance to clinical triazoles (Snelders et al., 2012; Cuba, 2016). There is no certainty about which of these chemicals have been used in the sampled areas or in what proportions they have been used, however this is a hypothesis that can lead to a high selective pressure in the environment and, consequently, to emergence of resistant phenotypes. Thus this approach should be explored.

As for the second case, *A. fumigatus* is an anemophilous fungus that can be spread through large distances, and spores can keep viability for long periods of time (Magyar et al., 2016). Cuba is an archipelago located in the middle of the three regions that constitute the American Continent. For species different from *Aspergillus,* such as *Peronospora tabacina* D.B. Adam, there are published articles about its natural introduction in the western region of Cuba due to strong winds from hurricanes in the South and Central American coasts (Lopetegui et al., 2006). Reports on antifungal resistance linked to TR34/L98H in *A. fumigatus* isolates from United States and Colombia indicate that resistant genotypes are circulating in the nearby area (Gonçalves et al., 2016; Verweij et al., 2016). We suggest that resistant phenotypes could have a natural and accidental influx favoured by atmospheric phenomena like hurricanes, so often in the Mexican Gulf. Multi-locus sequence typing, microsatellite analysis or other molecular tools for epidemiological studies are needed in order to shed light on this statement.

This work is the first step for future investigations on molecular epidemiology and resistance distribution in Cuba. It also supports the inclusion of voriconazole and posaconazole to the national treatment scheme for fungal infections. As a final conclusion, new mechanisms of resistance need to be studied focusing on those related to environmental sources.

Acknowledgments

The authors thanks to Liset Pérez, MSc. and Yanet Pintos, MSc. for the laboratory assistance on the sequencing procedures.

References

Almaguer M, Aira MJ, Rodríguez-Rajo FJ, Rojas TI (2013) Study of airborne fungus spores by viable and non-viable methods in Havana, Cuba. Grana 52(4):289-298. https://doi.org/10.1080/00173134.2013.829869

Anonymous (2017) The burden of fungal disease: new evidence to show the scale of the problem across the globe. LIFE [Online]. Available: http://go.nature.com/2sMKpuN

Arikan-Akdagli S, Ghannoum M, Meis JF (2018) Antifungal resistance: specific focus on multidrug resistance in *Candida auris* and secondary azole resistance in *Aspergillus fumigatus*. Journal of Fungi 4(129):1-13. https://doi.org/10.3390/jof4040129

Badiee P, Alborzi A, Moeini M, Haddadi P, Farshad S, Japoni A, Ziyaeyan M (2012) Antifungal susceptibility of the *Aspergillus* species by etest and CLSI reference methods. Archives of Iranian Medicine 15(7):429-432.

Beltrán-Rodríguez N, San Juan-Galán JL, Fernández-Andreu CM, Yera DM, Pita MB, Perurena-Lancha MR, Velar-Martínez R, Illnait-Zaragozí MT, Martínez-Machín GF (2019) Chronic

https://doi.org/10.6092/issn.2531-7342/12759

pulmonary aspergillosis in patients with underlying respiratory disorders in Cuba - a pilot study. Journal of Fungi 5(18):1-8. https://doi.org/10.3390/jof5010018

Berger S, El-Chazli Y, Babu AF, Coste AT (2017) Azole resistance in *Aspergillus fumigatus*: a consequence of antifungal use in agriculture? Frontiers in Microbiology 8(1024):1-6. https://doi.org/10.3389/fmicb.2017.01024

Bonifaz JA (2015) Micología Médica Básica. McGraw-Hill, México D. F., México.

Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC (2012) Hidden killers: human fungal infections. Science Translational Medicine 4(165):165rv113. https://doi.org/10.1126/scitranslmed.3004404

Buil JB, Hagen F, Chowdary A, Verweij PE, Meis JF (2018) Itraconazole, voriconazole, and posaconazole CLSI MIC distributions for wild-type and azole-resistant *Aspergillus fumigatus* isolates. Journal of Fungi 4(103):1-9. https://doi.org/10.3390/jof4030103

Cannon RD, Lamping E, Holmes AR, Niimi K, Baret PV, Keniya MV, Tanabe K, Niimi M, Goffeau A, Monk BC (2009) Efflux-mediated antifungal drug resistance. Clinical Microbiology Review 22(2):291-321. https://doi.org/10.1128/CMR.00051-08

Chandrasekar PH, Manavathu EK (2009) Antifungal resistance: *Aspergillus*. In: Antimicrobial Drug Resistance (Mayers DL ed). Humana Press, New York, pp 953-965. https://doi.org/10.1007/978-1-60327-595-8_19

Costa C, Dias PJ, Sa-Correia I, Teixeira MC (2014) MFS multidrug transporters in pathogenic fungi: do they have real clinical impact? Frontiers in Physiology 5(197):1-8. https://doi.org/10.3389/fphys.2014.00197

Cuba GdlRd (2016) Lista oficial de plaguicidas autorizados. Plaguicidas RCd (16):147.

Fisher MC, Hawkins NJ, Sanglard D, Gurr SJ (2018) Worldwide emergence of resistance to antifungal drugs challenges human health and food security. Science 360(6390):739-742. https://doi.org/10.1126/science.aap7999

Gonçalves SS (2016) Global aspects of triazole resistance in *Aspergillus fumigatus* with focus on Latin American countries. Journal of Fungi 3(5):1-10. https://doi.org/10.3390/jof3010005

Gonçalves SS, Souza ACR, Chowdhary A, Meis JF, Colombo AL (2016) Epidemiology and molecular mechanisms of antifungal resistance in *Candida* and *Aspergillus*. Mycoses 59: 198-219. https://doi.org/10.1111/myc.12469

Jenks JD, Hoenigl M (2018) Treatment of aspergillosis. Journal of Fungi 4(98):1-17. https://doi.org/10.3390/jof4030098

Lopetegui CM, Menéndez GMM, Pérez YT, López ME, Castillo EMG (2006) Aplicaciones de la aerobiología en el sistema de predicción y vigilancia de la enfermedad moho azul del tabaco en la provincia Pinar del Río, Cuba. Avances 8(4):1-11.

Maertens JA (2004) History of the development of azole derivatives. Clinical Microbiology and Infection 10(1):1-10. https://doi.org/10.1111/j.1470-9465.2004.00841.x

Magyar D, Vass M, Li DW (2016) Dispersal Strategies of Microfungi. In: Biology of Microfungi (Li D-W ed) Springer International Publishing, Switzerland, pp 315-371. https://doi.org/10.1007/978-3-319-29137-6_14

Morales Ojeda R (2018) Cuadro básico de medicamentos y productos naturales. MINSAP (Resolución No. 536):1-38.

Prasad R, Shah AH, Rawal MK (2016) Antifungals: mechanism of action and drug resistance. In: Advances in experimental medicine and biology (Ramos J ed) Springer International Publishing, Switzerland, 22. https://doi.org/10.1007/978-3-319-25304-6_14

https://doi.org/10.6092/issn.2531-7342/12759

Rivero-Menendez O, Alastruey-Izquierdo A, Mellado E, Cuenca-Estrella M (2016) Triazole resistance in *Aspergillus* spp.: a worldwide problem? Journal of Fungi 2(21):1-20. https://doi.org/10.3390/jof2030021

Samson RA, Hong S, Peterson SW, Frisvad JC, Varga J (2007) Polyphasic taxonomy of *Aspergillus* section *Fumigati* and its teleomorph *Neosartorya*. Studies in Mycology 59:147-203. https://doi.org/10.3114/sim.2007.59.14

Samson RA, Visagie CM, Houbraken J, Hong SB, Hubka V, Klaassen CHW, Perrone G, Seifert KA, Susca A, Tanney JB, Varga J, Kocsubé S, Szigeti G, Yaguchi T, Frisvad JC (2014) Phylogeny, identification and nomenclature of the genus *Aspergillus*. Studies in Mycology 78:141-173. https://doi.org/10.1016/j.simyco.2014.07.004

Sanglard D (2016) Emerging threats in antifungal-resistant fungal pathogens. Frontiers in Medicine 3(11):1-10. https://doi.org/10.3389/fmed.2016.00011

San Juan-Galán JL, Fernández-Andreu CM, Martínez-Machín G, Perurena-Lancha MR, Velar-Martínez R, Illnait-Zaragozí MT (2017a) Emergencia en Cuba de especies de *Aspergillus* resistentes a antifúngicos. Boletín Epidemiológico IPK 27(13):97-104.

San Juan-Galán JL, Fernández-Andreu CM, Almaguer M, Perurena-Lancha MR, Martínez-Machín G, Velar-Martínez R, Illnait-Zaragozí MT (2017b) Susceptibilidad in vitro de cepas cubanas de *Aspergillus* de origen clínico y ambiental. Biomédica 37(4):452-459. http://www.scielo.org.co/pdf/bio/v37n4/0120-4157-bio-37-04-00452.pdf

Snelders E, Camps SMT, Karawajczyk A, Schaftenaar G, Kema GHJ, van der Lee HA, Klaassen CH, Melchers WJG, Verweij PE (2012) Triazole fungicides can induce cross-resistance to medical triazoles in *Aspergillus fumigatus*. PLoS ONE 7(3):1-11. https://doi.org/10.1371/journal.pone.0031801

Spiess B, Seifarth W, Merker N, Howard SJ, Reinwald M, Dietz A, Hofmann WK, Buchheidt D (2012) Development of novel PCR assays to detect azole resistance mediating mutations of the *Aspergillus fumigatus cyp*51A gene in primary clinical samples from neutropenic patients. Antimicrobial Agents and Chemotherapy 56(7):3905-3910. https://doi.org/10.1128/AAC.05902-11

Sweileh WM, Sawalha AF, Al-Jabi S, Zyoud SH (2017) Bibliometric analysis of literature on antifungal triazole resistance: 1980–2015. Germs 7(1):19-27. https://doi.org/10.18683/germs.2017.1104

Verweij PE, Lestrade PPA, Melchers WJG, Meis JF (2016) Azole resistance surveillance in *Aspergillus fumigatus*: beneficial or biased? Journal of Antimicrobial Chemotherapy 71(8):2079-2082. https://doi.org/10.1093/jac/dkw259