KU LEUVEN

Department of Microbiology, Immunology and Transplantation

Hmg1 gene mutation in triazole-resistant *Aspergillus fumigatus* clinical isolates without *cyp51A* gene mutations

Resendiz-Sharpe Agustin[1], Merckx Rita[1], Verweij Paul[2], Maertens Johan[1, 3], Lagrou Katrien[1, 4]

[1] Department of Microbiology, Immunology and Transplantation, KU Leuven, Leuven, Belgium. [2] Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, The Netherlands. [3] Department of Hematology, University Hospitals Leuven, Leuven, Belgium. [4] Department of Laboratory Medicine and National Reference Center for Mycosis, University Hospitals Leuven, Leuven, Belgium.

Introduction

- Reports of resistance in *Aspergillus fumigatus (A. fumigatus)* to triazole antifungals, the recommended first line therapy for prophylaxis and treatment of Aspergillus related-diseases, are increasing worldwide.
- Triazole-resistance is most commonly associated to mutations in the *cyp51A* gene and its promotor region.
- Recently, mutations in the *3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase*-encoding gene (*hmg1*), an upstream gene involved in the ergosterol production pathway, were described and associated to triazole-resistance in *A. fumigatus.*

Objective

- In this study, we determined the prevalence of mutations in the *hmg1* gene in triazole-resistant *A. fumigatus* isolates lacking *cyp51A* gene associated triazole-resistant mutations in our collection.

Methodology

- Clinical *A. fumigatus* isolates collected between March 2016 and July 2019 with a confirmed triazole-resistant phenotype and no *cyp51A* gene associated-resistance mutation were selected for analysis. Ten triazole-susceptible isolates were included as controls (Wild type).
- After DNA extraction and PCR amplification, sequencing of the *hmg1 g*ene using 16 designed primers based on the *hmg1* genomic reference sequence of *A. fumigatus* (Fungi DB ascension number AFUB_020770, A1163) was performed.
- Attained isolates *hmg1* sequences were assembled to create a sequence consensus and subsequently aligned to the *hmg1* reference sequence to determine genetic variances.

Results

- A total of 13 triazole-resistant isolates without *cyp51A* gene mutations and 10 triazole-susceptible *A. fumigatus* clinical isolates were sequenced (Table 1).

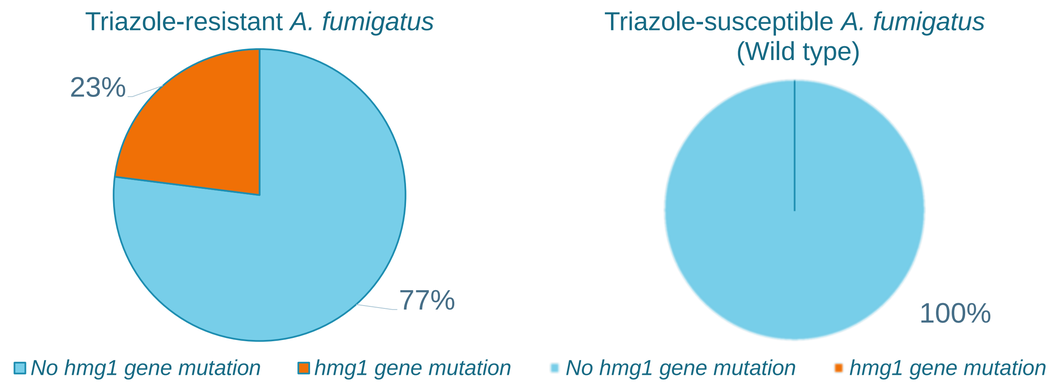
Table 1.- *Hmg1* gene mutations and susceptibility results of triazole-susceptible and triazole-resistant *A. fumigatus* clinical isolates without *cyp51A* gene mutations.

***Aspergillus fumigatus* clinical isolates**	***hmg1* gene mutation (amino acid substitution)**	**EUCAST (MIC = mg/L) ***		
		Voriconazole	**Posaconazole**	**Itraconazole**
Triazole-resistant isolates				
CYP-15-18	-	8	0.5	1
CYP-15-27	E105K	4	0.5	8
CYP-15-33	H564Y	4	0.125	0.125
CYP-15-41	W273S, S541G	4	0.5	1
CYP-15-75	-	4	1	8
CYP-15-93	-	4	1	>16
CYP-15-106	-	4	0.5	>16
CYP-15-108	-	8	1	>16
CYP-15-109	-	>8	1	2
CYP-15-115	-	4	0.5	>16
CYP-15-117	-	0.25	0.5	>16
CYP-15-146	-	4	0.5	>16
CYP-15-147	-	4	1	>16
Triazole-susceptible isolates (Wild type)				
ASFU 4058	-	0.5	0.06	0.125
ASFU 4361	-	0.25	0.06	0.125
ASFU 4425	-	0.25	0.125	0.06
ASFU-4701	-	0.25	0.125	0.06
ASFU-5291	-	0.06	0.25	0.125
ASFU-5458	-	0.25	0.06	0.25
ASFU-5496	-	0.25	0.06	0.125
ASFU-5771	-	0.5	0.06	0.125
ASFU-5774	-	0.5	0.125	0.25
ASFU-5779	-	0.25	0.06	0.125

- EUCAST broth microdilution reference method for filamentous fungi. " - " = not detected
- Triazole-resistance: at least one minimal inhibitory concentration (MIC) value above EUCAST clinical breakpoints for *A. fumigatus* (voriconazole >2, itraconazole >2, posaconazole>0.25 mg/L).

- Sequencing of the *hmg1* gene revealed two isolates with one (E105K, H564Y) and one isolate with two (W273S, and S541G) amino acid substitutions (n= 3, 23%) among the triazole-resistant isolates without *cyp51A* gene mutations (Table 1, Figure 1).
- No *hmg1* amino acid substitutions were observed in the triazole-susceptible isolates.

Figure 1.- Prevalence of *hmg1* gene mutations in *A. fumigatus* clinical isolates without *cyp51A* gene mutations



- All isolates with a *hmg1* mutation were resistant to voriconazole (MIC 4 mg/L), MIC values for itraconazole and posaconazole differed, ranging from 0.125 to 8 mg/L and 0.125 to 0.5 mg/L respectively.

Conclusion

- We report a prevalence of 23% *hmg1* gene mutations in our collection of triazole-resistant clinical *A. fumigatus* isolates without *cyp51A* gene mutations associated with triazole-resistance.
- Two previously described (E105K and S541G), as well as two novel mutations, (W273S and H564Y) were found.
- Further investigation is required to determine the precise role of these mutations.