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► To cite this version:

Isidore W. Yerbanga, Katrien Lagrou, Rita Merckx, Seydou Nakanabo-Diallo, Jean-Pierre Gangneux, et al.. First detection of triazole-resistant *aspergillus fumigatus* harbouring the TR34/L98H Cyp51A mutation in Burkina Faso. *Mycoses*, 2024, 67 (5), pp.e13732. 10.1111/myc.13732 . hal-04574367

HAL Id: hal-04574367

<https://hal.science/hal-04574367>

Submitted on 2 Jul 2024

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1 **First detection of triazole-resistant *Aspergillus fumigatus* harbouring the TR34/L98H**
2 ***Cyp51A* mutation in Burkina Faso**

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28 ABSTRACT

29 **Background:** Triazole-resistant *Aspergillus fumigatus* (TRAF) isolates are a growing public
30 health problem with worldwide distribution. Epidemiological data on TRAF is limited in
31 Africa, particularly in West Africa. **Objectives:** This study aimed to screen for the
32 environmental presence of TRAF isolates in the indoor air of two hospitals in Burkina Faso.
33 **Materials and methods:** Air samples were collected in wards housing patients at risk for
34 invasive aspergillosis, namely infectious diseases ward, internal medicine ward, nephrology
35 ward, pulmonology ward, medical emergency ward, and pediatric ward. Sabouraud Dextrose
36 Agar supplemented with triazoles was used to screen the suspected TRAF isolates and
37 EUCAST method to confirm the resistance of suspected isolates. Sequencing of *cyp51A* gene
38 was used to identify the resistance mechanism of confirmed TRAF isolates. **Results:** Of the 198
39 samples collected and analysed, 67 showed growth of *A. fumigatus* isolates. The prevalence of
40 TRAF isolates was 3.23% (4/124). One TRAF isolate exhibited a pan-triazole resistance.
41 Sequencing of *cyp51A* gene identified the TR34/L98H mutation for this pan-triazole resistant
42 isolate. This study showed for the first time the circulation of the pan-azole resistant isolate
43 harbouring the TR34/L98H mutation in Burkina Faso. **Conclusions:** These findings emphasize
44 the need to map these TRAF isolates in all parts of Burkina Faso and to establish local and
45 national continuous surveillance of environmental and clinical TRAF isolates in this country.

46 **Keywords:** Triazole-resistant *Aspergillus fumigatus*; *cyp51A* gene; TR34/L98H mutation;
47 Burkina Faso

48 1. INTRODUCTION

49 *Aspergillus fumigatus* is a cosmopolitan filamentous fungus that plays an important role in
50 recycling environmental carbon and nitrogen.¹ This species is also the leading cause of
51 *Aspergillus*-related diseases worldwide, particularly in immunocompromised patients.²⁻⁴ The
52 mortality of the most severe and deadly form of aspergillosis, invasive aspergillosis (IA), is
53 high ranging from 30 to 95%.⁵ The use of triazoles as a cornerstone of antifungal therapy in *A.*
54 *fumigatus*-related diseases has significantly reduced this mortality.^{6,7} In addition, the
55 availability of oral formulation with triazole antifungals allows outpatient treatment, reducing
56 the risk of nosocomial infections.⁸ However, a steady increase in global reports of TRAF
57 isolates has become a significant challenge in effective management of aspergillosis.⁹ Indeed,
58 triazole-resistant IA showed a 21–31% higher mortality than triazole-susceptible IA.^{10,11}
59 Triazole-resistant *A. fumigatus* isolates have been reported worldwide.¹² A multicentre

60 prospective study reported an overall prevalence of 3.2% of TRAF isolates with rates among
61 centres ranging from 0% to 26%.¹³ Data available in Africa showed a prevalence of 1.3% and
62 17.1% respectively in clinical and environmental settings.¹⁴ The prevalence of TRAF isolates
63 vary considerably between geographic regions and between hospitals.¹⁵ Knowledge of the local
64 epidemiology of TRAF strains being a key factor^{16,17} for the choice of empirical antifungal
65 treatment, regular monitoring of these isolates becomes essential to guide the therapeutic
66 management of *Aspergillus*-related diseases.¹⁸ In Burkina Faso, an initial investigative study on
67 TRAF strains identified a prevalence of 2% in soil samples.¹⁹ The present study aimed to screen
68 for the presence of TRAF strains in the indoor air of hospital settings in Burkina Faso.

69 **2. MATERIALS AND METHODS**

70 **2.1. Study design (type, site and period)**

71 A cross-sectional study was carried out in two reference university hospital centres in Burkina
72 Faso namely “Centre Hospitalier Universitaire Sourô Sanou” (CHUSS) and “Centre Hospitalier
73 Universitaire Régional de Ouahigouya” (CHUR-OHG). These reference hospitals have 550 and
74 311 beds, respectively. Air samples were collected in wards housing patients at risk for IA
75 (infectious diseases ward, internal medicine ward, nephrology ward, pulmonology ward,
76 medical emergency ward, and pediatric ward). The samples were collected from September
77 2021 to February 2022.

78 **2.2. Sampling**

79 Air samples were collected using portable air sampler (SpinAir®, IUL S.A., USA) with a flow
80 rate of 100 L·min⁻¹ (collected volume was 0.5 m³) in 90 mm diameter Petri dishes containing
81 Sabouraud Dextrose Agar with Chloramphenicol (50mg L⁻¹) (LIOFILCHEM®, Italy). Prior to
82 collecting each sample, the sampler head was rinsed in 70% ethyl alcohol. Air sampling was
83 performed in a dry and clean area between 1:00 pm and 4:00 pm at a height of 1.5 m above the
84 floor.

85 **2.3. Identification of *A. fumigatus***

86 The collected plates were incubated at 30°C for 2 to 4 days and if necessary up to 5-7 days.
87 Based on the macroscopic features mainly the colour (white initially, then characteristic dark
88 green and finally smoky grey on old cultures),²⁰ the suspected *A. fumigatus* isolates were sub-
89 cultured on another Petri dishes containing Sabouraud Dextrose Agar with Chloramphenicol
90 (50 mg L⁻¹). Formal identification of *A. fumigatus* was done using the microscopic features.²⁰

91 The results were reported as colony forming unit (CFU/m³). Confirmed *A. fumigatus* isolates
92 were then stored at -80°C in purified water.

93 **2.4. Screening for triazole-resistance isolates**

94 **2.4.1. Sub-culture of *A. fumigatus* isolates**

95 *Aspergillus fumigatus* isolates were sub-cultured on potato dextrose agar at 30°C. Inoculum
96 suspensions were prepared from fresh and mature cultures (2–5 days old). In some cases,
97 prolonged incubation was necessary to allow proper sporulation of conidia.

98 **2.4.2. Screening for triazole-resistant *A. fumigatus***

99 The colonies were covered with 5 mL of purified water then carefully wiped with a sterile
100 cotton swab and transferred into a sterile tube. This suspension was adjusted to the optical
101 density of a 0.5 McFarland. After homogenization of the suspension (pipetting up and down 5
102 times), 100 microliters were plated onto three Sabouraud dextrose agar (SDA) media plates;
103 one without triazole antifungal (control plate) and two supplemented with triazoles,
104 itraconazole (4 mg L⁻¹) and voriconazole (2 mg L⁻¹). Samples were incubated at 30°C for 2 to
105 7 days. Isolates that grew on media supplemented with triazole were considered as suspected
106 TRAF isolates. These suspected isolates were stored at -80°C in purified water then shipped to
107 the department of microbiology, immunology and transplantation in Leuven (Belgium) where
108 further laboratory analysis were carried out. Suspected TRAF isolates were categorized as *A.*
109 *fumigatus* sensu-strictu via Matrix-assisted laser desorption ionization-time of flight mass
110 spectrophotometry (MALDI-TOF) (Bruker Daltonics, Bremen, Germany) and the Mass
111 Spectrometry Identification platform (MSI) reference spectra database.²¹ If the identification
112 with the MALDI-TOF was inconclusive, beta-tubulin gene sequencing was performed as
113 previously described.²²

114 **2.4.3. Triazole-resistant *A. fumigatus* phenotype determination and *Cyp51A* gene 115 sequencing**

116 The European Committee on Antimicrobial Susceptibility Testing (EUCAST) broth
117 microdilution reference method for moulds was used to determine the minimal inhibitory
118 concentrations (MIC) values of itraconazole, voriconazole, posaconazole and isavuconazole.
119 An isolate was considered as resistant if the MIC value was > 1 mg L⁻¹, > 1 mg L⁻¹, > 0.25 mg
120 L⁻¹ and > 2 mg L⁻¹ respectively for itraconazole, voriconazole, posaconazole and
121 isavuconazole.²³ Sequencing of the *cyp51A* gene and its promoter region was performed in

122 confirmed TRAF isolates, as described previously.²⁴ Briefly, primers specifically designed for
123 the *cyp51A* gene of *A. fumigatus* and the BigDye-Terminator-v3.1 cycle sequencing kit
124 (Applied-Biosystems, Vilnius, Lithuania) were used to sequence the amplified DNA obtained
125 from confirmed TRAF isolates. The manufacturer's protocol was used to purify, dry and
126 reconstitute the reaction products. ABI3730xl Genetic Analyser (Applied-Biosystems, Foster
127 City, CA, USA) was used to analyse the reconstituted products. The consensus sequences were
128 obtained by aligning the resulting sequences using the CLC-Genomics-Workbench software
129 (CLC-bio, Aarhus, Denmark). Finally, the consensus sequences were compared to the reference
130 sequence of the *A. fumigatus cyp51A* gene (ATCC366071).¹⁹

131 **2.5. Data management**

132 Microsoft office excel 2013 software was used to data entry and to calculate the proportions.

133 **2.6. Ethical statements**

134 The authors confirm that the ethical policies of the journal, as noted on the journal's author
135 guidelines page, have been adhered to. No ethical approval was required as the research in this
136 article related to microorganisms.

137 **3. RESULTS**

138 Of the 198 samples collected and analysed, the overall rate of positive cultures for *A. fumigatus*
139 was 33.84%. (67/198). This rate of positive cultures for *A. fumigatus* were 37.04% (30/81) and
140 31.62% (37/117) for the CHUR-OHG and CHUSS, respectively. Contamination of the indoor
141 air of the hospital by *A. fumigatus* conidia varied from 1.43 to 30 CFU/m³ and from 2.5 to 40
142 CFU/m³ for the CHUR-OHG and CHUSS, respectively. Within positive cultures, 124 *A.*
143 *fumigatus* isolates were identified, including 51 isolates at CHUR-OHG and 73 isolates at
144 CHUSS (Table 1). The screening onto SDA supplemented with triazole antifungals identified
145 two suspected TRAF isolates in each hospital. These four suspected isolates were confirmed as
146 TRAF isolates by EUCAST method (Table 2). The overall prevalence of TRAF isolates was
147 3.23% (4/124) (Table 1). The prevalence of TRAF isolates was 3.92% (2/51) at CHUR-OHG
148 and 2.74% (2/73) at CHUSS. These TRAF isolates were identified in three wards' namely
149 infectious diseases ward, internal medicine ward and medical emergencies ward. One TRAF
150 isolate identified in the medical emergencies ward at CHUR-OHG exhibited a pan-triazole
151 resistance (Table 2). The sequencing of the *cyp51A* gene was identified the TR34/L98H

152 mutation for this pan-triazole resistant isolate. The others TRAF isolates showed the
153 F46Y/M172V/N248T/ D255E/E427K mutations in the *cyp51A* gene (Table 1).

154

Table 1 Distribution of triazole susceptibility profile and resistance mechanism of *A. fumigatus* isolates by hospitals and wards

Ward	N° of <i>A. fumigatus</i> isolates	CHUSS			<i>cyp51A</i> gene mutation	N° of <i>A. fumigatus</i> isolates	CHUR-OHG		
		N° of suspected TRAF isolates	N° of confirmed TRAF isolates				N° of suspected TRAF isolates	N° of confirmed TRAF isolates	<i>cyp51A</i> gene mutation
Infectious diseases	17	1	1	F46Y/M172V/N248T/D255E/ E427K	13	1	1	F46Y/M172V/N248T/D255E/ E427K	
Internal medicine	11	1	1	F46Y/M172V/N248T/ D255E/E427K	7	0	0	-	
Medical emergencies	16	0	0	-	10	1	1	TR34/L98H	
Nephrology	15	0	0	-	11	0	0	-	
Paediatric	9	0	0	-	6	0	0	-	
Pulmonology	5	0	0	-	4	0	0	-	
Total	73	2	2	-	51	2	2	-	

CHUSS: “Centre Hospitalier Universitaire Sourô Sanou”; CHUR-OHG: “Centre Hospitalier Universitaire Régional de Ouahigouya”, TRAF: Triazole-resistant *A. fumigatus*,
N°: Number

Table 2 Minimum inhibitory concentration values of confirmed TRAF isolates by hospital

Hospital name	MIC (mg L ⁻¹)				
	Isolate ID	Itraconazole	Voriconazole	Isavuconazole	Posaconazole
CHUSS	9	0.5	2	2	0.125
	77	1	2	4	0.25
CHUR-OHG	40	1	2	2	0.25
	123	> 16	8	8	1

CHUSS: “Centre Hospitalier Universitaire Sourô Sanou”; CHUR-OHG: “Centre Hospitalier Universitaire Régional de Ouahigouya” ;

ID: Identity; MIC: Minimal inhibitory concentration; TRAF: Triazole-resistant *A. fumigatus*

155 **4. DISCUSSION**

156 Triazole-resistant *A. fumigatus* harbouring the TR34/L98H mutation in *cyp51A* gene first
157 described in The Netherlands is now reported worldwide among clinical and environmental
158 strains.²⁵⁻²⁷ Triazole-resistant IA due to isolates harbouring the TR34/L98H mutation has
159 been shown to be associated with poor outcomes with 88% mortality, compared to the 30–
160 50% mortality found with triazole-susceptible IA.²⁸ In this context, the first detection of this
161 pan-azole-resistant isolate carrying the TR34/L98H mutation in Burkina Faso is worrying.
162 Although the origin of the emergence of this pan-azole resistant strain has not been formally
163 identified, an environmental route could be suspected due to the use of azole fungicides in
164 agricultural fields in this country.²⁹ International travel to and from Burkina Faso could also
165 favour the emergence of this pan-azole resistant isolate.¹⁹ Following a previous study
166 conducted in Bobo-Dioulasso on soil samples which found a TRAF prevalence of 2%,¹⁹ the
167 present study shows the circulation of TRAF isolates also in Burkina Faso hospital settings.
168 This situation adds to the diagnostic difficulties of IA in Burkina Faso, a huge therapeutic
169 challenge. Indeed, no IA case has yet been reported in Burkina Faso, despite data estimating
170 its annual incidence at 54 cases.³⁰ TRAF isolates having been detected in wards housing
171 patients at risk for IA (e.g., patients with hematological malignancies, patients with
172 HIV/AIDS, or patients with COVID-19) highlight the urgent need to evaluate their
173 prevalence in the occurrence of IA cases in Burkina Faso. The detection of these resistant
174 isolates in two different areas in Burkina Faso (North and West area of Burkina Faso) also
175 highlights the need to establish a national surveillance program for these resistant strains.
176 Finally, this situation underscores the dire need to map the distribution of these TRAF
177 isolates in this country. The prevalence of TRAF in the present environmental study
178 (3.92%) is consistent with findings reported in Nigeria (2.2%).³¹ However, this prevalence,
179 was lower compared with data previously recorded in Eastern Africa (20-33%).³²⁻³⁴
180 Furthermore, no case of TRAF has been isolated from African countries such as Benin and
181 Cameroon.^{31,35} These data indicate the variability of the prevalence of TRAF isolates across
182 regions. Consistent with previously reported studies, the TR34/L98H mutation was
183 identified in environmental settings.¹² This mutation was found in 29.3%, 37.4%, 77%, and
184 58.8% of the azole-resistant environmental isolates in Africa, East Asia, Europe, and North
185 America, respectively.¹² Understanding the fitness cost of TRAF isolates will provide
186 valuable insights into the spread of these resistant isolates and their tendencies to expand in
187 terms of frequency and geographical range.¹² Finally, raising awareness among national

188 health authorities and medical personnel is essential and urgent in order to promote the
189 establishment of a national protocol for the management of TRAF isolates in Burkina Faso.

190 This study showed for the first time the circulation of the pan-azole resistant isolate
191 harbouring the TR34/L98H mutation in Burkina Faso. This study also showed the presence
192 of TRAF isolates in two different areas in this country. These findings emphasize the need
193 to map these TRAF isolates in all parts in Burkina Faso and to establish local and national
194 continuous surveillance programs of environmental and clinical TRAF isolates in this
195 country.

196 **AUTHOR CONTRIBUTIONS**

197 **Isidore W. Yerbanga, Sanata Bamba, Olivier Denis and Isabel Montesinos** designed the
198 study. **Isidore W. Yerbanga** and **Seydou Nakanabo Diallo** collected the data. **Isidore W.**
199 **Yerbanga, Katrien Lagrou and Rita Merckx** analysed and interpreted the data. **Isidore**
200 **W. Yerbanga** wrote the manuscript. **All authors** read and agreed to the published version
201 of the manuscript.

202 **ACKNOWLEDGMENTS**

203 We would like to thank the staff of “Centre Hospitalier Universitaire Sourô Sanou” and
204 “Centre Hospitalier Universitaire Régional de Ouahigouya”.

205 **CONFLICTS OF INTEREST**

206 The authors declare no conflict of interest.

207 **FUNDING**

208 This study was funded by the Belgian government « (Académie de Recherche et
209 d’Enseignement Supérieur, Commission pour la coopération au développement ARES-
210 CCD) » through his program «Programme de Formation Sud (PFS) » managed by the «
211 Université Libre de Bruxelles » and the «Université Nazi Boni ».

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