Received: 3 January 2024 | Revised: 25 April 2024 | Accepted: 29 April 2024

DOI: 10.1111/myc.13732

ORIGINAL ARTICLE

mycoses Diagnosis, Therapy and Prophylaxis of Fungal Diseases WILEY

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

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

Abstract

Background: Triazole-resistant *Aspergillus fumigatus* (TRAF) isolates are a growing public health problem with worldwide distribution. Epidemiological data on TRAF is limited in Africa, particularly in West Africa.

Objectives: This study aimed to screen for the environmental presence of TRAF isolates in the indoor air of two hospitals in Burkina Faso.

Materials and Methods: Air samples were collected in wards housing patients at risk for invasive aspergillosis, namely infectious diseases ward, internal medicine ward, nephrology ward, pulmonology ward, medical emergency ward and paediatric ward. Sabouraud Dextrose Agar supplemented with triazoles was used to screen the suspected TRAF isolates and EUCAST method to confirm the resistance of suspected isolates. Sequencing of *cyp51A* gene was used to identify the resistance mechanism of confirmed TRAF isolates.

Results: Of the 198 samples collected and analysed, 67 showed growth of *A. fumigatus* isolates. The prevalence of TRAF isolates was 3.23% (4/124). One TRAF isolate exhibited a pan-triazole resistance. Sequencing of *cyp51A* gene identified the TR34/L98H mutation for this pan-triazole resistant isolate. This study showed for the first time the circulation of the pan-azole resistant isolate harbouring the TR34/L98H mutation in Burkina Faso.

Conclusions: These findings emphasise the need to map these TRAF isolates in all parts of Burkina Faso and to establish local and national continuous surveillance of environmental and clinical TRAF isolates in this country.

KEYWORDS
Burkina Faso, *cyp51A* gene, TR34/L98H mutation, triazole-resistant *aspergillus fumigatus*

1 | INTRODUCTION

Aspergillus fumigatus is a cosmopolitan filamentous fungus that plays an important role in recycling environmental carbon and nitrogen.[1] This species is also the leading cause of *Aspergillus*-related diseases worldwide, particularly in immunocompromised patients.[2–4] The mortality of the most severe and deadly form of aspergillosis, invasive aspergillosis (IA), is high ranging from 30% to 95%.[5] The use of triazoles as a cornerstone of antifungal therapy in *A. fumigatus*-related diseases has significantly reduced this mortality.[6,7] In addition, the availability of oral formulation with triazole antifungals allows outpatient treatment, reducing the risk of nosocomial infections.[8] However, a steady increase in global reports of TRAF isolates has become a significant challenge in effective management of aspergillosis.[9] Indeed, triazole-resistant IA showed a 21%–31% higher mortality than triazole-susceptible IA.[10,11] Triazole-resistant *A. fumigatus* isolates have been reported worldwide.[12] A multicentre prospective study reported an overall prevalence of 3.2% of TRAF isolates with rates among centres ranging from 0% to 26%.[13] Data available in Africa showed a prevalence of 1.3% and 17.1% respectively in clinical and environmental settings.[14] The prevalence of TRAF isolates vary considerably between geographic regions and between hospitals.[15] Knowledge of the local epidemiology of TRAF strains being a key factor[16,17] for the choice of empirical antifungal treatment, regular monitoring of these isolates becomes essential to guide the therapeutic management of *Aspergillus*-related diseases.[18] In Burkina Faso, an initial investigative study on TRAF strains identified a prevalence of 2% in soil samples.[19] The present study aimed to screen for the presence of TRAF strains in the indoor air of hospital settings in Burkina Faso.

2 | MATERIALS AND METHODS

2.1 | Study design (type, site and period)

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

2.2 | Sampling

Air samples were collected using portable air sampler (SpinAir®, IUL S.A., USA) with a flow rate of 100 L·min-1 (collected volume was 0.5 m^3) in 90 mm diameter Petri dishes containing Sabouraud Dextrose Agar (SDA) with Chloramphenicol (50 mg L^{-1}) (LIOFILCHEM®, Italy). Prior to collecting each sample, the sampler head was rinsed in 70% ethyl alcohol. Air sampling was performed in a dry and clean area between 1:00 pm and 4:00 pm at a height of 1.5 m above the floor.

2.3 | Identification of *A. fumigatus*

The collected plates were incubated at 30°C for 2–4 days and if necessary up to 5–7 days. Based on the macroscopic features mainly the colour (white initially, then characteristic dark green and finally smoky grey on old cultures),[20] the suspected *A. fumigatus* isolates were sub-cultured on another Petri dishes containing SDA with Chloramphenicol (50 mg L^{-1}). Formal identification of *A. fumigatus* was done using the microscopic features.[20] The results were reported as colony forming unit (CFU/m^3). Confirmed *A. fumigatus* isolates were then stored at −80°C in purified water.

2.4 | Screening for triazole-resistance isolates

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

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

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

The colonies were covered with 5 mL of purified water then carefully wiped with a sterile cotton swab and transferred into a sterile tube. This suspension was adjusted to the optical density of a 0.5 McFarland. After homogenization of the suspension (pipetting up and down 5

times), 100 microliters were plated onto three SDA media plates; one without triazole antifungal (control plate) and two supplemented with triazoles, itraconazole (4 mg L^{-1}) and voriconazole (2 mg L^{-1}). Samples were incubated at 30°C for 2–7 days. Isolates that grew on media supplemented with triazole were considered as suspected TRAF isolates. These suspected isolates were stored at −80°C in purified water then shipped to the department of microbiology, immunology and transplantation in Leuven (Belgium) where further laboratory analysis were carried out. Suspected TRAF isolates were categorised as *A. fumigatus* sensu-strictu via Matrix-assisted laser desorption ionisation-time of flight mass spectrophotometry (MALDI-TOF) (Bruker Daltonics, Bremen, Germany) and the Mass Spectrometry Identification platform (MSI) reference spectra database.[21] If the identification with the MALDI-TOF was inconclusive, beta-tubulin gene sequencing was performed as previously described.[22]

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

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) broth microdilution reference method for moulds was used to determine the minimal inhibitory concentrations (MIC) values of itraconazole, voriconazole, posaconazole and isavuconazole. An isolate was considered as resistant if the MIC value was >1 mg L^{-1}, >1 mg L^{-1}, >0.25 mg L^{-1} and >2 mg L^{-1} respectively for itraconazole, voriconazole, posaconazole and isavuconazole.[23] Sequencing of the *cyp51A* gene and its promoter region was performed in confirmed TRAF isolates, as described previously.[24] Briefly, primers specifically designed for the *cyp51A* gene of *A. fumigatus* and the BigDye-Terminator-v3.1 cycle sequencing kit (Applied-Biosystems, Vilnius, Lithuania) were used to sequence the amplified DNA obtained from confirmed TRAF isolates. The manufacturer's protocol was used to purify, dry and reconstitute the reaction products. ABI3730xl Genetic Analyser (Applied-Biosystems, Foster City, CA, USA) was used to analyse the reconstituted products. The consensus sequences were obtained by aligning the resulting sequences using the CLC-Genomics-Workbench software (CLC-bio, Aarhus, Denmark). Finally, the consensus sequences were compared to the reference sequence of the *A. fumigatus* *cyp51A* gene (ATCC366071).[19]

2.5 | Data management

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

2.6 | Ethical statements

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No

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

Ward	CHUSS				CHUR-OHG			
	N° of *A. fumigatus* isolates	N° of suspected TRAF isolates	N° of confirmed TRAF isolates	*cyp51A* gene mutation	N of *A. fumigatus* isolates	N of suspected TRAF isolates	N° of confirmed TRAF isolates	*cyp51A* 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	-

Abbreviations: CHUSS, Centre Hospitalier Universitaire Sourô Sanou; CHUR-OHG, Centre Hospitalier Universitaire Régional de Ouahigouya; N, number; TRAF, Triazole-resistant *A. fumigatus*.

TABLE 2 Minimum inhibitory concentration values of confirmed TRAF isolates by hospital.

	MIC (mg L^{-1})				
Hospital name	**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

Abbreviations: 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*.

ethical approval was required as the research in this article related to microorganisms.

3 | RESULTS

Of the 198 samples collected and analysed, the overall rate of positive cultures for *A. fumigatus* was 33.84%. (67/198). This rate of positive cultures for *A. fumigatus* were 37.04% (30/81) and 31.62% (37/117) for the CHUR-OHG and CHUSS, respectively. Contamination of the indoor air of the hospital by *A. fumigatus* conidia varied from 1.43 to 30 CFU/m^3 and from 2.5 to 40 CFU/m^3 for the CHUR-OHG and CHUSS, respectively. Within positive cultures, 124 *A. fumigatus* isolates were identified, including 51 isolates at CHUR-OHG and 73 isolates at CHUSS (Table 1). The screening onto SDA supplemented with triazole antifungals identified two suspected TRAF isolates in each hospital. These four suspected isolates were confirmed as TRAF isolates by EUCAST method (Table 2). The overall prevalence of TRAF isolates was 3.23% (4/124) (Table 1). The prevalence of TRAF isolates was 3.92% (2/51) at CHUR-OHG and 2.74% (2/73) at CHUSS. These TRAF isolates were identified in three wards' namely infectious diseases ward, internal medicine ward and medical emergencies ward. One TRAF isolate identified in the medical emergencies ward at CHUR-OHG exhibited a pan-triazole resistance (Table 2). The sequencing of the *cyp51A* gene was identified the TR34/L98H mutation for this pan-triazole resistant isolate. The others TRAF isolates showed the F46Y/M172V/N248T/ D255E/E427K mutations in the *cyp51A* gene (Table 1).

4 | DISCUSSION

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

This study showed for the first time the circulation of the pan-azole resistant isolate harbouring the TR34/L98H mutation in Burkina Faso. This study also showed the presence of TRAF isolates in two different areas in this country. These findings emphasise the need to map these TRAF isolates in all parts in Burkina Faso and to

establish local and national continuous surveillance programs of environmental and clinical TRAF isolates in this country.

AUTHOR CONTRIBUTIONS

Isidore W. Yerbanga: Conceptualization; investigation; writing – original draft; methodology; validation; visualization; writing – review and editing; software; formal analysis; data curation; project administration; supervision; resources. **Katrien Lagrou:** Methodology; validation; writing – review and editing; visualization. **Rita Merckx:** Methodology; validation; visualization; writing – review and editing; resources. **Seydou Nakanabo Diallo:** Investigation; writing – review and editing; supervision. **Jean-Pierre Gangneux:** Writing – review and editing; resources. **Aymeric Delabarre:** Writing – review and editing; resources. **Olivier Denis:** Conceptualization; funding acquisition; writing – review and editing; resources. **Hector Rodriguez-Villalobos:** Conceptualization; funding acquisition; writing – review and editing; resources. **Isabel Montesinos:** Conceptualization; funding acquisition; writing – review and editing; resources. **Sanata Bamba:** Conceptualization; funding acquisition; writing – review and editing; supervision; resources.

ACKNOWLEDGEMENTS

We would like to thank the staff of 'Centre Hospitalier Universitaire Sourô Sanou' and 'Centre Hospitalier Universitaire Régional de Ouahigouya'.

FUNDING INFORMATION

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

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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How to cite this article: Yerbanga IW, Lagrou K, Merckx R, et al. First detection of triazole-resistant *aspergillus fumigatus* harbouring the TR34/L98H *Cyp51A* mutation in Burkina Faso. *Mycoses*. 2024;67:e13732. doi:10.1111/myc.13732