

# Triazole resistance in *Aspergillus fumigatus* isolates in Africa: a systematic review

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## Abstract

Emergence of triazole resistance has been observed in *Aspergillus fumigatus* over the past decade including Africa. This review summarizes the current published data on the epidemiology and reported mechanisms of triazole-resistant *Aspergillus fumigatus* (TRAF) in both environmental and clinical isolates from Africa. Searches on databases Medline, PubMed, HINARI, Science Direct, Scopus and Google Scholar on triazole resistance published between 2000 and 2021 from Africa were performed. Isolate source, antifungal susceptibility using internationally recognized methods, *cyp51A* mechanism of resistance and genotype were collected. Eleven published African studies were found that fitted the search criteria; these were subsequently analyzed. In total this constituted of 1686 environmental and 46 clinical samples. A TRAF prevalence of 17.1% (66/387) and 1.3% (5/387) was found in respectively environmental and clinical settings in African studies. Resistant to itraconazole, voriconazole, and posaconazole was documented. Most of the triazole-resistant isolates (30/71, 42.25%) were found to possess the TR<sub>34</sub>/L98H mutation in the *cyp51A*-gene; fewer with TR<sub>46</sub>/Y121F/T289A (n = 8), F46Y/M172V/E427K (n = 1), G54E (n = 13), and M172V (n = 1) mutations. African isolates with the TR<sub>34</sub>/L98H, TR<sub>46</sub>/Y121F/T289A and the G54E mutations were closely related and could be grouped in one of two clusters (cluster-B), whereas the *cyp51A*-M172V mutation clustered with most *cyp51A*-WT strains (cluster-A). A single case from Kenya shows that TR<sub>34</sub>/L98H from environmental and clinical isolates are closely related. Our findings highlight that triazole resistance in environmental and clinical *A. fumigatus* is a cause for concern in a number of African countries. There is need for epidemiological surveillance to determine the true burden of the problem in Africa.

## Lay Summary

Emergence of triazole resistance has been observed in *Aspergillus fumigatus*. TRAF was found from environmental (17.1%) and clinical (1.3%) settings in Africa. We highlighted that triazole resistance in environmental and clinical *A. fumigatus* is a cause for concern in a number of African countries.

**Keywords:** triazole-resistant mechanism, *Aspergillus fumigatus*, *cyp51A*, Africa

## Introduction

The emergence of triazole resistance, including multi-triazole resistance in *Aspergillus fumigatus* (TRAF), is a public health and clinical problem worldwide, leading to an increase of treatment failures in patients with aspergillosis.<sup>1–3</sup> Furthermore, in some patients the acquired azole resistance mechanisms emerged during therapy.<sup>4</sup> TRAF is defined as *in vitro* resistance of *A. fumigatus* to at least one triazole antifungal agent.<sup>5</sup> According to their phenotypic profiles these isolates are mainly grouped to more than one azole (multi-triazole resistant) or to all clinically available azoles (pan-triazole resistant).<sup>5</sup> Epidemiological data gathered to date show that

the triazole resistance prevalence in *A. fumigatus* varies between 3.2% and 36.3% depending on the country,<sup>6</sup> centre and underlying condition such as cystic fibrosis, which is associated with higher triazole resistance prevalence.<sup>7,8</sup> Resistances to azole in *Aspergillus* are much more prevalent in South East Asia than anywhere else, with environmental resistance rates of 65% (77/119).<sup>9</sup>

The resistance mechanisms behind TRAF, in both environmental and clinical settings, are mutations in the gene involved in the biosynthesis of ergosterol, the *cyp51A* gene.<sup>10,11</sup> From this, mutations L98H and Y121F/T289A combined with tandem repeats of 34 bp (TR<sub>34</sub>) and 46 bp (TR<sub>46</sub>), respectively,

have been frequently associated with triazole resistance.<sup>1</sup> Triazole resistance can occur through two routes, in patients receiving long-term triazole therapy<sup>1</sup> and through exposure of *A. fumigatus* to triazole compounds in the environment.<sup>12</sup>

The widespread use of azole fungicides (14a-demethylase inhibitors) in agricultural fields has been related to TRAF in Africa, yet the information provided was scarce.<sup>13,14</sup> For instance, the amount of azole fungicides sold use in agriculture was respectively 6 350 kg and 907 kg in Kenya and Tanzania in 2019.<sup>15,16</sup> Similarly, the epidemiological data related to TRAF is limited and most likely underestimated in Africa.<sup>14,17,18,19</sup> Hence, to gain more insight into the occurrence of TRAF in Africa, we performed a systematic review on current epidemiological findings and the mechanisms of multi-TRAF in both environment and clinical setting of published cases from 2000 to 2021.

## Methods

### Search strategy

We conducted a systematic search using the databases Medline, PubMed, HINARI, Science Direct, Scopus and Google Scholar for studies on TRAF cases published between 2000 and 2021 in Africa; according to the PRISMA directions indexed in international electronic databases ([http://www.prisma-statement.org/documents/PRISMA\\_2020\\_checklist.pdf](http://www.prisma-statement.org/documents/PRISMA_2020_checklist.pdf)). The methodological search was performed using the following medical subject headings (MeSH) terms: ‘triazole resistance’, ‘*Aspergillus fumigatus*’, ‘Aspergillosis’, ‘Environment’, ‘Epidemiology of aspergillosis’, ‘TR<sub>34</sub>/L98H mutations’, ‘TR<sub>46</sub>/Y121F/T289A mutations’, ‘Antifungal resistance’, ‘Africa’. The Boolean operators ‘AND’ and ‘OR’ were used to combine two or three terms. Indexed original articles in English and French of any design and sampling strategy were used for further analysis (Fig. 1).

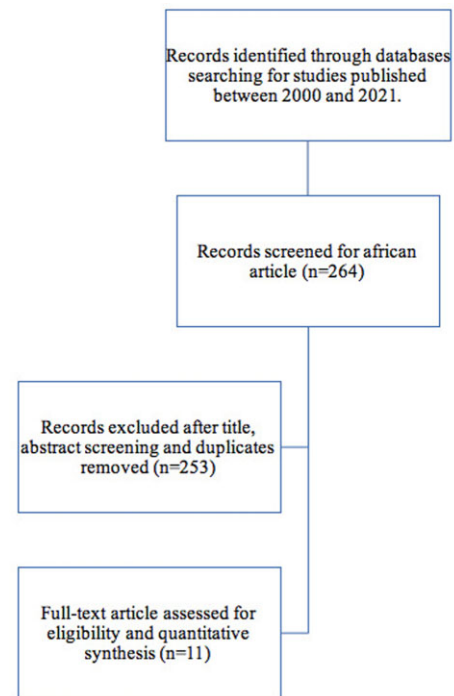
### Study eligibility criteria

Eligible studies included must have reported the epidemiology of TRAF in Africa region. Also, studies that did address this subject, duplicate publication of the same article, review literatures, meta-analysis/systematic, and narrative reviews were excluded. From obtained articles, an additional search for short tandem repeat (STR) typing results in *A. fumigatus* isolates from Africa was conducted for STR typing analysis. A phylogenetic tree based on found publicly available microsatellite markers (STRAf 3A, 3B, 3C, 4A, 4B and 4C)<sup>20</sup> data was performed and visualized (Euclidian similarity and Ward’s minimum variance linkage method) using the statistical computing and graphics software R (v4.0.3) as previously described.<sup>21–23</sup>

Triazole-resistant phenotype was considered when at least one minimum inhibitory concentration (MIC) value was above the EUCAST resistance clinical breakpoint (voriconazole > 1 mg/l, itraconazole > 1 mg/l, posaconazole > 0.25, mg/l).<sup>24</sup>

### Data extraction

The three authors compiled a database from the electronic searches and the following items were extracted: The first author’s full name, year of publication, location (country and city), sample size, sample origin and type (clinical or environmental), the number of fungi growth, number of



**Figure 1.** Flow diagram describing search strategy for triazole resistance in *A. fumigatus* isolates in Africa.

positive *A. fumigatus* samples, number of reported *A. fumigatus* isolates, type of triazole susceptibility testing methods, azole fungicides used and *cyp51A* allele (s). Furthermore, the extracted data were matched, and inconsistencies between reviewers were discussed to reach an agreement.

## Results

### Epidemiology of TRAF in Africa

The literature search revealed 11 studies from 7 African countries with reports on TRAF between 2000 and 2021 (Table 1). Overall, we summarize data from 1686 environmental and 46 clinical samples for analysis. From 46 clinical samples, 44 were suppurative otitis media samples and 2 were sputum samples; there were no bronchoalveolar lavage or sinus samples. The environmental substrates were mostly soils followed by plant debris and effluent wastewater. In total, 1732 samples were collected of which 749 (43.2%) samples had positive fungal growth on Sabouraud dextrose agar (Table 1). Of the 1732 samples, 1686 were environmental and 46 clinical samples. Furthermore, 387 samples (51.7%), including environmental (n = 380) and clinical samples (n = 7), grew *A. fumigatus* strains (Table 2). All 112 non-*fumigatus* *Aspergillus* species (14.9%) such as *A. niger* (n = 50), *A. flavus* (n = 37), *A. terreus* (n = 20), and *A. nidulans* (n = 5) were recovered from the soil.

Among *Aspergillus* isolates, triazole resistance mechanisms were intensively studied in *A. fumigatus* (n = 545). Only one study reported the resistant mechanism in non-*fumigatus* *Aspergillus* species (n = 89) including *A. niger*, *A. flavus*, and *A. terreus* across Africa.<sup>19</sup> Overall, the reported prevalence of TRAF in environmental and clinical isolates was 17.1% (66/387) and 1.3% (5/387) respectively in Africa. Analysis per country studies (Fig. 2) reported that high numbers of environmental TRAF were present in Tanzania (37% and 52%)<sup>17,18</sup>

**Table 1.** Global environmental and clinical setting, substrate, geographic origin, and *cyp51A* genotype of triazole-resistant *A. fumigatus* in African countries.

| Authors                              | Country/city                 | Year | Substrate sampled   | Collected Samples (No.) | Fungi Growth No (%)    | Sample type and origin   | Triazole susceptibility testing methods | Azole fungicides used <sup>a</sup> | <i>cyp51A</i> allele(s)                                     |
|--------------------------------------|------------------------------|------|---------------------|-------------------------|------------------------|--|---|------------------------------------|---|
| Chowdhary et al. <sup>17</sup>       | Tanzania/Moshi               | 2014 | Plant debris, soil  | 30                      | 29 (96.7)              | Woody debris and wood samples from trees near a medical centre, hospital parking areas and gardens (flowers not specified) | CLSI M38-A2                             | Suggested                          | TR <sub>34</sub> /L98H, TR <sub>46</sub> /Y121F/T289A       |
| Sharma et al. <sup>18</sup>          | Tanzania/Moshi               | 2015 | Plant debris, soil  | 30                      | 73 (90)                | Woody debris from tree trunk hollows, hospital parking areas and gardens (flowers not specified)                           | CLSI M38-A2                             | Suggested                          | TR <sub>34</sub> /L98H, TR <sub>46</sub> /Y121F/T289A, G54E |
| Mushi et al. <sup>25</sup>           | Tanzania                     | 2016 | Clinical sample     | 44                      | 23 (5.6)               | Suppurative otitis media   | EUCAST                                  | No                                 | TR <sub>34</sub> /L98H                                      |
| Kemoui et al. <sup>13</sup>          | Kenya/Nairobi and Naivasha   | 2017 | Soil                | 492                     | 186 (37.8)             | Flower, agricultural, horticultural, and unplanted fields and greenhouses (plants not specified)                           | EUCAST                                  | Yes                                | MIC   |
| Ashu et al. <sup>5</sup>             | Cameroon                     | 2017 | Soil                | 495                     | -                      | Farmlands  | CLSI M38-A                              | Unknown                            | MIC, WT   |
| Kemoui et al. <sup>19</sup>          | Kenya                        | 2018 | Soil, sputum        | 158 <sup>b</sup>        | 50 <sup>b</sup> (32.1) | Horticultural and greenhouse soil (crops not specified)  | CLSI M38-A2                             | No                                 | TR <sub>34</sub> /L98H, MIC                                 |
| Assress et al. <sup>26</sup>         | South Africa/Gauteng         | 2021 | Effluent wastewater | -                       | 41                     | Clinical sputum of suspected aspergillosis patients  | CLSI M38-A2                             | No                                 | MIC   |
| Campbell et al. <sup>32</sup>        | Nigeria/Lagos                | 2021 | Soil                | 168                     | 117 (69.6)             | 5 L of effluent wastewater   | EUCAST                                  | Uncertain                          | MIC   |
| Resendiz-Sharpe et al. <sup>22</sup> | Nigeria/Lagos                | 2021 | Soil                | 100                     | 93 (93)                | Urban settlements, roadsides and markets   | EUCAST                                  | No                                 | M172V   |
| Yerbanga et al. <sup>14</sup>        | Burkina Fasso/Bobo-Dioulasso | 2021 | Soil                | 120                     | 51 (42.5)              | Parks, city and hospital flowerbeds, agricultural fields, greenhouses, gardens, compost, and commercial soil               | EUCAST                                  | Yes                                | F46Y/M172V/E427K  |
| Resendiz-Sharpe et al. <sup>22</sup> | Benin                        | 2021 | Soil                | 95                      | 86 (90.5)              | Hospital, residential, university, and nurseries, agricultural (cotton, rice, and market gardening field) and forest       | EUCAST                                  | No                                 | MIC   |
|                                      |                              |      |                     |                         |                        | Parks, city and hospital flowerbeds, agricultural fields, greenhouses, gardens, compost, and commercial soil               | EUCAST                                  | No                                 | MIC   |

<sup>a</sup> Yes = azole fungicide uses either clearly stated or measured as residual azole values present in the substrate. Azole use was suggested when information was provided about the country's azole use overall, but not the specific site. No azole use was when studies clearly stated no azoles were used.

<sup>b</sup> Two clinical samples.

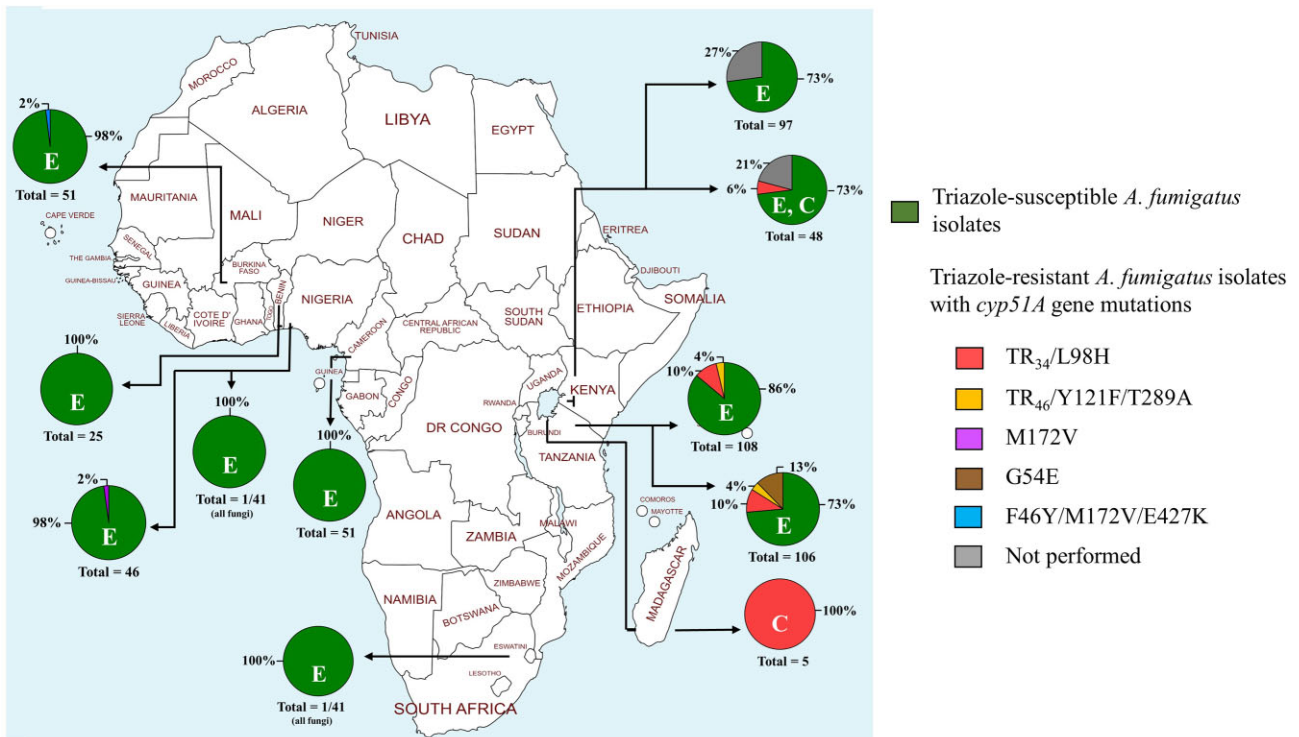
MIC = minimum inhibitory concentration; WT = Isolates with a wild type *cyp51A*; CLSI = Clinical and Laboratory Standards Institute's, M38- A reference method; EUCAST = European Committee on Antimicrobial Susceptibility Testing

**Table 2.** Prevalence of multi-triazole resistance in *A. fumigatus* isolates in environmental and clinical samples in Africa.

| Authors                              | Country/city                 | Year | Source                | No. of samples positive for <i>A. fumigatus</i> /No. of samples collected (%) | Prevalence No. of samples positive for               |   |                                    |                                    |                                    | Isolates with TR46/Y121E/T289A mutation/ TRAF isolates tested | Isolates with TR34/ L98H, mutation/ TRAF isolates tested | Isolates with G54E mutation/ TRAF isolates tested | Isolates with TR46/Y121E/T289A mutation/ TRAF isolates tested | Isolates with F46Y/M172V/ E427K mutation/ TRAF isolates tested |
|--------------------------------------|------------------------------|------|-----------------------|---|--|---|------------------------------------|------------------------------------|------------------------------------|---|--|---|---|--|
|                                      |                              |      |                       |   | TRAF/No. of samples positive for <i>A. fumigatus</i> | Total no. of <i>A. fumigatus</i> isolated | Isolates with TRAF isolates tested | Isolates with TRAF isolates tested | Isolates with TRAF isolates tested |   |  |   |   |  |
| Chowdhary et al. <sup>17</sup>       | Tanzania/Moshi               | 2014 | Environment           | 29/30 (96.7%)   | 15/29 (52%)  | 108                                       | 0/15 (0)                           | 11/15 (73.3%)                      | 4/15 (26.7%)                       | NT  | NT   | NT  | NT  |  |
| Sharma et al. <sup>18</sup>          | Tanzania/Moshi               | 2015 | Environment           | 27/30 (90.0%)   | 10/27 (37%)  | 106                                       | 13/28 (46.4%)                      | 11/28 (39.3%)                      | 4/28 (14.3%)                       | NT  | NT   | NT  | NT  |  |
| Mushi et al. <sup>25</sup>           | Tanzania                     | 2016 | Clinical              | 5/44 (11.4%)  | 5/5 (100%)   | 5   | 0/5 (0)                            | 5/5 (100%)                         | 0/5 (0)                            | NT  | NT   | NT  | NT  |  |
| Kemoi et al. <sup>13</sup>           | Kenya/Nairobi and Naivasha   | 2017 | Environment           | 97/492 <sup>a</sup> (19.7%)   | 26/97 (27%)  | 97  | 0/26 (0)                           | 0/26 (0)                           | 0/26 (0)                           | NT  | NT   | NT  | NT  |  |
| Ashu et al. <sup>19</sup>            | Cameroon                     | 2017 | Environment           | 51/495 (10.3%)  | 0/51 (0)   | 51  | 0/51 (0)                           | NT                                 | NT                                 | NT  | NT   | NT  | NT  |  |
| Kemoi et al. <sup>19</sup>           | Kenya                        | 2018 | Environment, clinical | 50 <sup>b</sup> /158 (32%)  | 13/50 (26%)  | 50  | 0/25 (0)                           | 3/25 (12%)                         | 0/25 (0)                           | NT  | NT   | NT  | NT  |  |
| Assress et al. <sup>26</sup>         | South Africa/Gauteng         | 2021 | Environment           | 1/- (2.4%)  | 0  | 1   | NT                                 | NT                                 | NT                                 | NT  | NT   | NT  | NT  |  |
| Campbell et al. <sup>32</sup>        | Nigeria/lagos                | 2021 | Environment           | 5/168 (3%)  | 0/5 (0)  | 5   | NT                                 | NT                                 | NT                                 | NT  | NT   | NT  | NT  |  |
| Resendiz-Sharpe et al. <sup>22</sup> | Nigeria/lagos                | 2021 | Environment           | 46/100 (46%)  | 1/46 (2.2%)  | 46  | NT                                 | NT                                 | NT                                 | NT  | NT   | NT  | 1/46 (2.2%)   |  |
| Yerbanga et al. <sup>14</sup>        | Burkina Fasso/Bobo-Dioulasso | 2021 | Environment           | 51/120 (42.5%)  | 1/51 (2%)  | 51  | NT                                 | NT                                 | NT                                 | NT  | NT   | NT  | 1/51 (2%)   |  |
| Resendiz-Sharpe et al. <sup>22</sup> | Benin                        | 2021 | Environment           | 25/95 (26.3%)   | 0/25 (0)   | 25  | NT                                 | NT                                 | NT                                 | NT  | NT   | NT  | NT  |  |

<sup>a</sup>Collected sample (492) of both naïve (246) and experience soils (246).<sup>b</sup>Two *A. fumigatus* from clinical sputum.

NT = not tested.



**Figure 2.** Geographic distribution of reported triazole-susceptible and triazole-resistant *A. fumigatus* environmental and clinical isolates from African countries. Pie charts depict prevalence of triazole-susceptible (= green) and triazole-resistant isolates harboring *cyp51A* gene mutations (TR<sub>34</sub>/L98H = red, TR<sub>46</sub>/Y121F/T289A = yellow, M172V = purple, G54R = brown, F46Y/M172V/E427K = blue, not performed = gray) from each reported epidemiological study. Letters within pie charts represent the origin of the studied isolates; 'E' for environmental and 'C' for clinical isolates. The figure was adapted using a free license map from mapchart.net.

and Kenya (27%),<sup>19</sup> whereas Nigeria and Burkina Faso samples harboured only 2% resistant isolates (Table 2). Concerning clinical isolates, all analysed samples report resistance to triazoles, yet the number of analysed samples was low limiting prevalence determination, with only two isolates from Kenya (2/2 TRAF)<sup>13</sup> and five from Tanzania (5/5 TRAF) all carrying TR<sub>34</sub>/L98H resistance allele.<sup>25</sup>

**Molecular determination of the triazole resistance mechanisms in Africa**

Five different *cyp51A* mutations were reported in Africa, namely the TR<sub>34</sub>/L98H, TR<sub>46</sub>/Y121F/T289A, G54E, F46Y/M172V/E427K and M172V. From the 11 included studies, 4 undertook sequencing of the *cyp51A* gene and reported TRAF isolates harbouring TR<sub>34</sub>/L98H (n = 30) and TR<sub>46</sub>/Y121F/T289A (n = 8) mutations.<sup>13,17,18,25</sup> Single cases of TRAF harbouring either a F46Y/M172V/E427K (n = 1), G54E (n = 13), or M172V (n = 1) mutations were reported respectively (Table 2).<sup>14,18,22</sup> In total, 66 environmental and 5 clinical TRAF culture-positive samples were reported. Of these, 25 environmental (35.2%) and 5 clinical samples (7%) harboured isolates with a TR<sub>34</sub>/L98H mutation. The G54E triazole resistance mutation was found in 18.3% of samples (13/71) from Tanzania. Furthermore, the G54E mutation resistance mechanism coexisted with TR<sub>34</sub>/L98H in five samples originating from tree trunk hollows and two parks areas. Moreover, a single soil sample from the same parking area harboured TRAF isolates (n = 7) was carrying G54E, TR<sub>34</sub>/L98H and TR<sub>46</sub>/Y121F/T289A mutations.<sup>18</sup>

**Mycological characteristics of triazole-resistant *A. fumigatus* isolates**

All 71 triazole-resistant isolates were confirmed to be resistant to at least 1 of the 4 medical triazoles tested specifically itraconazole (ITC), voriconazole (VRC), posaconazole (POS), and isavuconazole (ISA) (Table 3). Antifungal susceptibility (AST) was performed using EUCAST (n = 6) and CLSI M38-A2 (n = 5) recommendations. Strains with TR<sub>34</sub>/L98H mutations had high VRC MICs > 16 mg/l in Tanzania (n = 22), while MICs for ITC (n = 5) were high (>32 mg/l) in Tanzania (n = 3) and Kenya (n = 3).<sup>17,18</sup> TRAF isolates from Tanzania harbouring the G54E (n = 13) mutation presented with high ITC (>16 mg/l) and POS MICs (>2 mg/l) with low VRC (0.29 mg/l) and ISA (0.2 mg/l) MICs.<sup>18</sup> The TR<sub>46</sub>/Y121F/T289A (n = 4) isolates from Tanzania revealed high VRC-MIC of >16 mg/l.<sup>18</sup> In Burkina Faso, one isolate (2%, 1/51), from an urban location, was confirmed as resistant to voriconazole (MIC 2 mg/l), isavuconazole (MIC = 4 mg/l), and posaconazole (MIC = 0.5 mg/l), but susceptible to itraconazole (MIC = 1 mg/l).<sup>14</sup> In Nigeria, one sample of urban origin with a M172V mutation presented elevated MICs to ITC (4 mg/l) and VRC (2 mg/l).<sup>22</sup> A study from South Africa reported that *A. fumigatus* (n = 9) showed no increased resistance to any of the tested drugs in the wastewater samples.<sup>26</sup>

**Genetic diversity of triazole-resistant *A. fumigatus* isolates in Africa**

Based on short tandem repeats *A. fumigatus* analysis, isolates were clustered in two different groups, group A and B at a genetic height of 11.5 (Fig. 3). Most TRAF isolates

**Table 3.** *In vitro* antifungal susceptibility profile of triazole-resistant *A. fumigatus* isolates in Africa.

| Authors                              | Years | Source                   | Isolates                      | n  | EUCAST MIC (mg/l) and CLSI Triazole drugs |      |      |      |
|--------------------------------------|-------|--------------------------|-------------------------------|----|---|------|------|------|
|                                      |       |                          |                               |    | ITC                                       | VRC  | ISA  | POS  |
| Chowdhary et al. <sup>17</sup>       | 2014  | Environment              | TR <sub>34</sub> /L98H        | 11 | 16  | 3.52 | 8    | 1.13 |
|                                      |       |                          | TR <sub>46</sub> /Y121F/T289A | 4  | 1.68                                      | 16   | 8    | 0.42 |
| Sharma et al. <sup>18</sup>          | 2015  | Environment              | G54E                          | 13 | 16  | 0.29 | 0.20 | 2.14 |
|                                      |       |                          | TR <sub>34</sub> /L98H        | 11 | 16  | 4.9  | 8    | 1.1  |
|                                      |       |                          | TR <sub>46</sub> /Y121F/T289A | 4  | 1.68                                      | 16   | 8    | 0.42 |
| Mushi et al. <sup>25</sup>           | 2016  | Clinical                 | TR <sub>34</sub> /L98H        | 5  | 32  | 1.74 | NT   | 1    |
| Kemoui et al. <sup>13</sup>          | 2018  | Environment and clinical | TR <sub>34</sub> /L98H        | 3  | 32  | 4    | NT   | 2    |
| Resendiz-Sharpe et al. <sup>22</sup> | 2021  | Environment              | M172V                         | 1  | 4   | 2    | NT   | 0.25 |
| Yerbanga et al. <sup>14</sup>        | 2021  | Environment              | F46Y/M172V/E427K              | 1  | 1   | 2    | 4    | 0.5  |

Triazole-resistant phenotype was confirmed if at least one MIC value was above the EUCAST resistance clinical breakpoint (voriconazole > 1, itraconazole > 1, posaconazole > 0.25, mg/l).

NT = not tested; ITC = itraconazole; VRC = voriconazole; POS = posaconazole; ISA = isavuconazole.

harbouring a *cyp51A* resistance mechanism grouped together in clade B, while most triazole susceptible isolates were distributed mostly in clade A. The *cyp51A*-M172V mutation, originating from an isolate from Nigeria, was the only *cyp51A* mutation located in clade A. Based on our phylogenetic analysis, African TRAF isolates with the most commonly reported *cyp51A* gene mutations are closely related (Fig. 3).

## Discussion

Triazole antifungal agents are the workhorse antifungals for the treatment of all forms of aspergillosis, and the only orally active class. Given the global emergence of TRAF around the world, further environmental and clinical monitoring is required in order to fully appreciate the threat of TRAF, particularly in African countries where this information is limited and the burden of fungal diseases is a huge public health problem.<sup>14,19</sup>

Many studies from different areas of the world such as the European,<sup>2,27</sup> Asian,<sup>28,29</sup> and American regions<sup>22</sup> have reported multiple sources of TRAF from both environment and clinical samples. Here, we reviewed all published cases of TRAF from environmental and clinical samples from Africa. We reveal that the prevalence of TRAF was 17.1% in the environmental samples and 1.3% in *A. fumigatus* culture positive clinical samples growth. When analysed by region, epidemiological environmental data from countries in Western Africa (Nigeria, Benin and Burkina Faso) present with none to low triazole resistance in *A. fumigatus* positive samples with an average prevalence of 1.6% (2/127). In contrast, a high triazole-resistance prevalence was observed in Eastern African countries of 31% (64/203), where Tanzania (37% and 52%) and Kenya (27%) reported the highest prevalence of TRAF among *A. fumigatus* culture-positive samples in Africa.<sup>17-19</sup> This higher prevalence in both East African countries may be due to the intense flower farming with extensive use of azole fungicides and substantial international trade, which can increase the risk of selection and dissemination of resistant isolates. Chowdhary and colleagues reported a genetic relatedness of Tanzanian TR<sub>46</sub>/Y121F/T289A strains to Dutch isolates, and the TR<sub>34</sub>/L98H isolates were identical to the Indian TR<sub>34</sub>/L98H genotype. These similarities in molecular epidemiology suggest possible migration of isolates harbouring resistance traits from distant locales.<sup>30</sup> In Kenya, although

the prevalence of TRAF was higher in fungicide-exposed soil, TRAF was also present in fungicide-naive soil samples.<sup>19</sup> This finding implies that TRAF might spread locally from areas of fungicide use to places where fungicides have not been used. Nigeria and Burkina Faso samples harboured only 2% resistant isolates, whereas no resistant strains were evident in their neighbouring countries (Cameroon and Benin) or in South Africa. Globally, azole fungicides comprise increasing proportion of all fungicides used (~16% in 2020).<sup>31</sup>

Most positive fungi cultures (n = 387) found *A. fumigatus* as a common *Aspergillus* species (n = 545) in Kenya, Benin and Tanzania<sup>17,19,22</sup> and non-*fumigatus* *Aspergillus* such as *A. niger* (n = 50) and *A. flavus* (n = 37). Similar results in other countries have reported *A. fumigatus* as a common *Aspergillus* species.<sup>9</sup> In contrast, Campbell and colleagues reported non-*fumigatus* *Aspergillus* such as *A. niger* (42.7%) and *A. flavus* (31.6%) to be mostly isolated in Nigeria.<sup>32</sup> However, this was not the case in the study from Resendiz-Sharpe and colleagues where *A. fumigatus* was the most frequent species cultured (n = 46), including one TRAF isolate.<sup>22</sup> Their study was performed in a different region of Nigeria (Lagos Island) during the time of year when the season is good for fungi growth, which might be the reason for the observed differences.

Cross-resistance between ITC, VRC, and POS is common in 71.8% (51/71) of isolates. Most of the triazole-resistant isolates reported in Africa were found to possess the TR<sub>34</sub>/L98H mutation in the *cyp51A*-gene (30/71, 42.25%), a similar proportion reported previously in the Latin American,<sup>22</sup> European<sup>33,34</sup> and Asia.<sup>9,35</sup> All African TRAF isolates harboring the TR<sub>34</sub>/L98 mutation were resistant to itraconazole. This is of concern as itraconazole is only authorized triazole-antifungal among most countries having investigated epidemiology of TRAF (Burkina Faso, Cameroon, Kenya, and Tanzania). The TR<sub>34</sub>/L98H mutation is worldwide distributed and is commonly associated with a pan-triazole resistance phenotype to all clinical azoles and it has spread worldwide.<sup>36,37</sup> In Africa, *A. fumigatus* isolates harboring the TR<sub>34</sub>/L98H resistance mechanism were mostly recovered from the environment. Only one proper study has analyzed clinical samples in Africa with reports of TR<sub>34</sub>/L98H mutations.<sup>25</sup> Additional clinical studies are warranted to show if TR<sub>34</sub>/L98H resistance mechanism is present in clinical isolates in more countries.

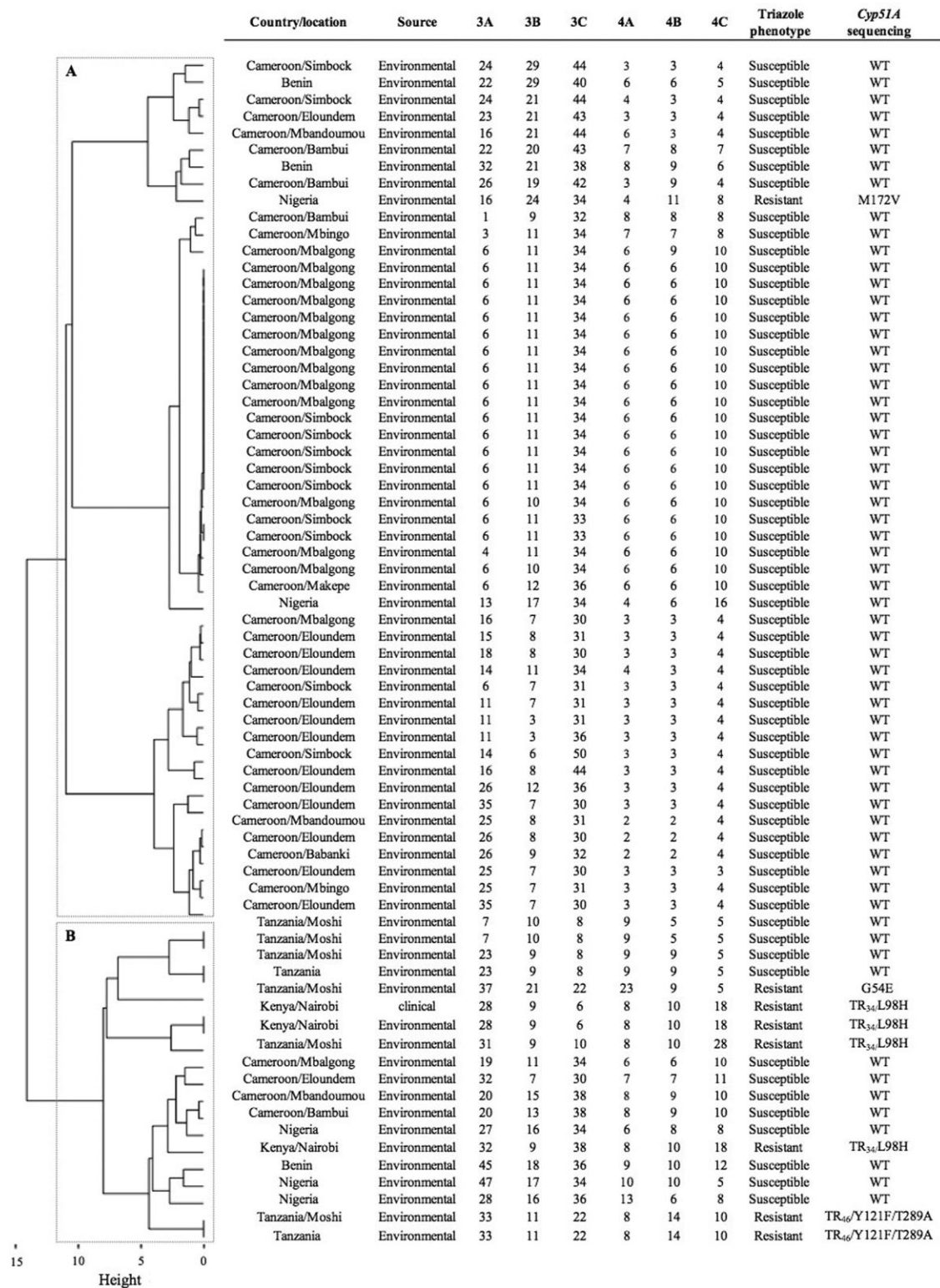


Figure 3. Genetic relationship and characteristics of selected *A. fumigatus* isolates according to short tandem repeats typing in seven African countries.

Eastern African countries have reported intense flower farming with the extensive use of azole fungicides mainly 14a- demethylase inhibitors<sup>38</sup> in Kenya, Tanzania, and Nigeria, with a use per area of cropland of 0.43, 0.1, and 0.1 kg per used-hectare.<sup>13,15,39</sup> This could favor the selection of azole-resistant isolates of *A. fumigatus* with cross-resistance to med-

ical azoles.<sup>40,41</sup> In contrast, studies from Benin and Burkina Faso refer to the use of pesticides, but no data concerning used amounts per area could be found.<sup>42,43</sup> Triazole resistance due to a mutation at codon 54 in the *cyp51A* gene of *A. fumigatus* has been reported among patient isolates on long-term azole therapy for chronic pulmonary aspergillosis.<sup>2,44,45</sup> G54

substitution is the most described in patients after treatment with itraconazole or posaconazole.<sup>46,47</sup> In this study, the 13 *Aspergillus fumigatus* isolates with a G54E mutation in Tanzania were reported from environmental setting. Similarly, Prigitano and colleague reported only one environmental isolate of G54E mutation in Italy.<sup>48</sup> Furthermore, TR<sub>34</sub>/L98H (n = 5) were the only resistant mechanism found in clinical patient in Africa.<sup>25</sup> It may be anticipated that long-term exposure of saprobic *A. fumigatus* to the triazole fungicides used extensively in agriculture may induce selection of G54, TR<sub>34</sub>/L98H and TR<sub>46</sub>/Y121F/T289A mutants in the environment.

The remaining *cyp51A* mutations were the F46Y/M172V/E427K and M172V in one isolate from Burkina Faso and Nigeria respectively. Isolates with a F46Y/M172V/E427K mutation have been reported for in both clinical and environmental strains in Europe and Australia since 2001.<sup>49</sup> The F46Y/M172V/E427K amino acid substitutions are located in non-conserved areas, mainly at the surface of the protein, and they were predicted neither to interact with triazole compounds nor to affect structural integrity.<sup>49</sup> This suggests that the mutations found might play no role or only a minor role in reduced triazole susceptibilities and other mechanisms are responsible for conferring the observed triazole-resistant phenotype.

Our phylogenetic analysis revealed that the TR<sub>34</sub>/L98H, TR<sub>46</sub>/Y121F/T289A and G54E *cyp51A*-mediated mechanisms were closely related (cluster B), inferring that these isolates have developed from a reduced set of clonally related strains (ancestors) with shorter genetic distances among themselves, particularly in the TR<sub>34</sub>/L98H and TR<sub>46</sub>/Y121F/T289A mutations, as it has been previously suggested.<sup>22,50</sup> This was not the case for the *cyp51A*-M172V mutation located in cluster A where most *cyp51A*-WT strains were found with greater genetic diversity than cluster B, where the rest of TRAF isolates located. Interestingly, a study from Kenya shows a close genetic relationship between environmental and clinical isolates with TR<sub>34</sub>/L98H mutations, further supporting the link between environment-patient transmission in this country.<sup>13</sup> This result is in agreement with those of previous works showing the similar findings.<sup>3,11,12,22,44</sup>

Both itraconazole and voriconazole are used clinically in many countries in Africa. Resistance in *A. fumigatus* is problematic as patients infected with these strains will fail therapy. Fungal culture is often undertaken in university and private hospitals in most countries in Africa, but susceptibility is rarely undertaken for *Aspergillus* spp. Given the high levels of azole resistance encountered in East Africa, this procedure probably needs be implemented, at least for regular surveillance purposes.

## Conclusion

This review shows a high TRAF prevalence (17.1%) in Africa, with most of the data from just Tanzania and Kenya. Five mutations in the *cyp51A* gene have been reported as the resistant mechanism in Africa. The isolates tested revealed multi-triazole resistance to ITC, VRC, and POS with elevated MICs. Our findings highlight that triazole resistance in environmental and clinical *A. fumigatus* is a cause for concern in African, thus highlighting the dire need for epidemiological surveillance studies in a region with significant population of tuberculosis patients.

## Author's contributions

F.M.A., D.W.D, R.O.O: conceptualization, data curation, methodology, project administration, resources, validation; F.M.A., A.R.-S., L.H., H.Z.: formal analysis; F.M.A: investigation, writing—original draft; A.L.-S., L.H., H.Z.: software; D.W.D, R.O.O: supervision; F.M.A., R.O.O., A.R.-S., D.W.D., C.K., K.L., H.Z., L.H.: writing—review and editing. All authors have read and agreed to the published version of the manuscript.

## Informed Consent statement

This analysis did not source with individual patient data but with published data, which does not require ethical approval.

## Data Availability Statement

All data is contained within the article.

## Declaration of interest

The authors declare no conflict of interest.

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