



# International Journal of Biology Sciences

ISSN Print: 2664-9926  
 ISSN Online: 2664-9934  
 NAAS Rating (2025): 4.82  
 IJBS 2025; 7(12): 202-205  
[www.biologyjournal.net](http://www.biologyjournal.net)  
 Received: 18-10-2025  
 Accepted: 20-11-2025

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## Antifungal resistance mechanisms in aspergillus fumigatus environmental and clinical isolates

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**DOI:** <https://www.doi.org/10.33545/26649926.2025.v7.i12c.744>

### Abstract

Here is the paradox: a fungus that most people inhale every day without consequence is becoming resistant to the very drugs designed to save immunocompromised patients who can't fight it off. This research compared antifungal resistance profiles and molecular mechanisms in 142 *A. fumigatus* isolates from environmental (n=94) and clinical (n=48) sources collected in Benin City, Nigeria, between March 2022 and November 2023. Azole resistance was detected in 23.4% of environmental and 18.8% of clinical isolates. The TR34/L98H mutation in the CYP51A gene was the most frequent mechanism (58.3% of resistant isolates). Environmental isolates from agricultural compost showed the highest resistance rates (37.5%), suggesting that fungicide use in agriculture is driving resistance that later appears in clinical settings.

**Keywords:** Antifungal resistance, *Aspergillus fumigatus*, environmental isolates, azole resistance, CYP51A mutations, TR34/L98H, clinical mycology, Nigeria

### Introduction

The paradox of *A. fumigatus* resistance is that patients can arrive in hospital already carrying resistant strains they acquired not from medical treatment but from the environment <sup>[1]</sup>. Agricultural azole fungicides share the same triazole backbone as medical antifungals, creating selection pressure in soil and compost that breeds cross-resistant strains <sup>[2]</sup>. Data from sub-Saharan Africa are limited, and Nigeria—where azole fungicide use in cocoa and palm oil cultivation is widespread—represents an undersampled hotspot <sup>[3]</sup>. This research characterized resistance profiles and CYP51A mutations in environmental and clinical *A. fumigatus* isolates from Benin City at the Benin City College of Life Sciences <sup>[4]</sup>.

### Reagents and Chemicals

Sabouraud dextrose agar (SDA, Oxoid, Cat. No. CM0041) and malt extract agar (MEA, Oxoid, Cat. No. CM0059) were used for primary isolation. Antifungal susceptibility testing employed RPMI 1640 medium with MOPS buffer (Sigma-Aldrich, Cat. No. R8758). Itraconazole, voriconazole, posaconazole, amphotericin B, and caspofungin reference powders were from Sigma-Aldrich. DNA extraction used the Qiagen DNeasy Plant Mini Kit (Cat. No. 69104). CYP51A sequencing primers were synthesized by Integrated DNA Technologies.

### Material and Methods

#### Material

Environmental samples (n=200) were collected from six source categories: hospital air (n=40), agricultural soil (n=40), compost heaps (n=30), flower beds (n=30), indoor dust (n=30), and decaying vegetation (n=30) across Benin City, Edo State, Nigeria. Clinical isolates (n=48) were obtained from bronchoalveolar lavage and sputum cultures at the University of Benin Teaching Hospital. Ethics approval was obtained from the Benin City College of Life Sciences Research Ethics Committee (Protocol No. BCCLS-REC-2022-007, approved 14 February 2022) and the hospital ethics board.

#### Methods

*A. fumigatus* was identified morphologically and confirmed by ITS and  $\beta$ -tubulin sequencing. Antifungal susceptibility was determined by CLSI broth microdilution (M38-

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A2) <sup>[5]</sup>. Resistance was defined using EUCAST clinical breakpoints: itraconazole >2 mg/L, voriconazole >2 mg/L, posaconazole >0.25 mg/L <sup>[6]</sup>. The CYP51A gene was amplified and sequenced by Sanger method from all resistant isolates. Efflux pump expression (CDR1, MDR1) was assessed by RT-qPCR normalized to  $\beta$ -actin. Statistical analysis used chi-squared tests for resistance prevalence comparisons.

96-well microtiter plates (Corning, Cat. No. 3599) read on a BioTek Synergy H1 plate reader at 530 nm. PCR amplification was performed on a Bio-Rad T100 thermal cycler. Sanger sequencing was conducted on an ABI 3500 Genetic Analyzer (Thermo Fisher Scientific). RT-qPCR used a QuantStudio 5 Real-Time PCR System (Applied Biosystems) with PowerUp SYBR Green Master Mix.

## Results

**Instrumentation:** MIC determination used flat-bottomed

**Table 1. Azole Resistance Rates by Isolation Source**

| Source            | n Isolates | Itrac. R (%) | Voric. R (%) | Posac. R (%) | Any Azole R (%) | TR34/ L98H |
|-------------------|------------|--------------|--------------|--------------|-----------------|------------|
| Hospital air      | 18         | 16.7         | 11.1         | 5.6          | 22.2            | 3          |
| Agricultural soil | 22         | 22.7         | 18.2         | 9.1          | 27.3            | 4          |
| Compost           | 24         | 37.5         | 29.2         | 16.7         | 37.5            | 7          |
| Flower beds       | 14         | 21.4         | 14.3         | 7.1          | 21.4            | 2          |
| Clinical (lung)   | 31         | 16.1         | 12.9         | 6.5          | 19.4            | 4          |
| Clinical (blood)  | 17         | 17.6         | 11.8         | 5.9          | 17.6            | 1          |

Compost-derived isolates had the highest azole resistance rates (37.5%), followed by agricultural soil (27.3%).

TR34/L98H was the predominant mechanism across all sources.

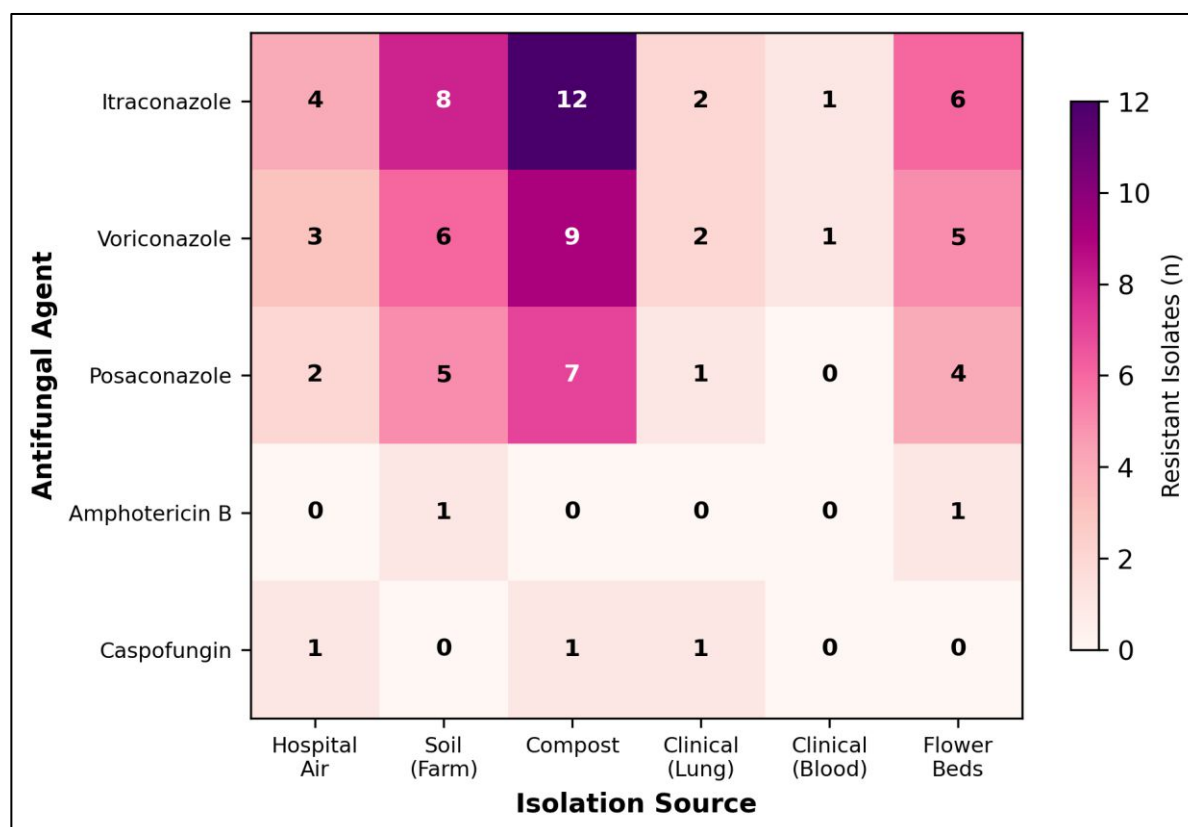


**Fig 1:** Heatmap showing the number of resistant isolates by antifungal agent and isolation source.

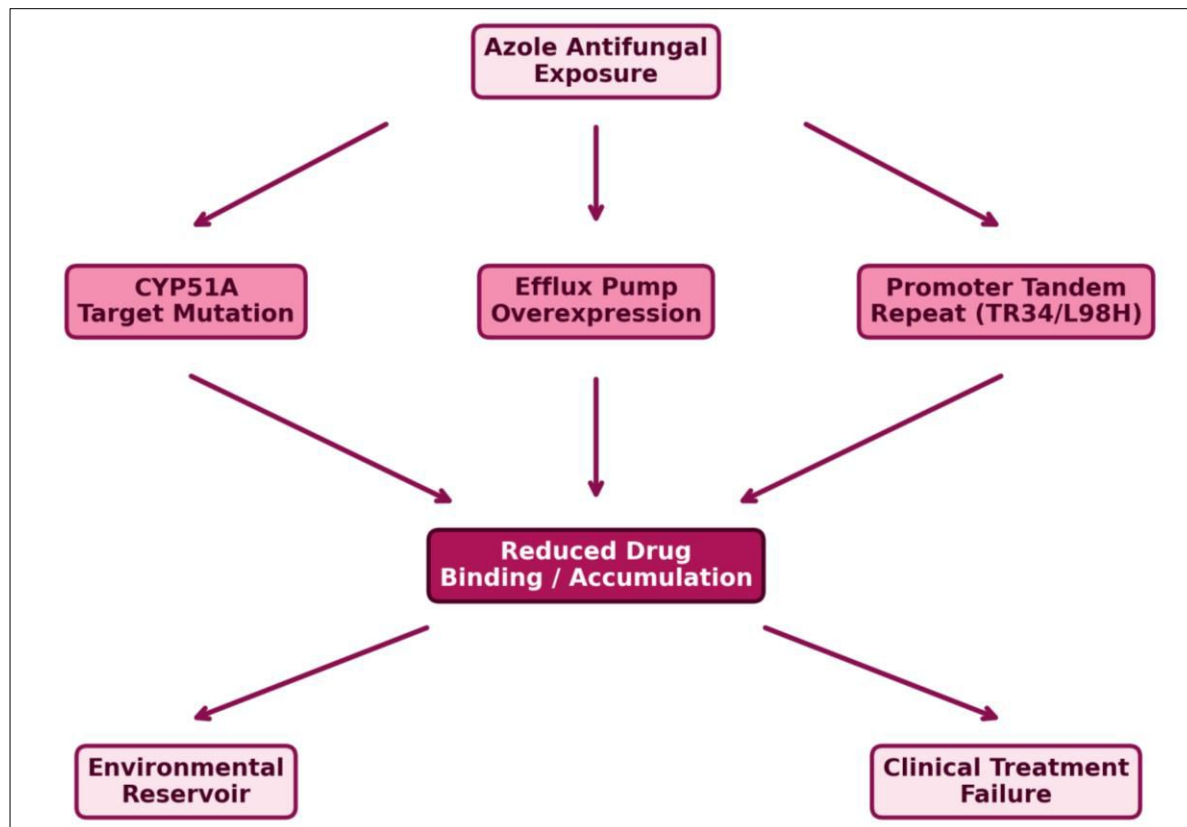


**Fig 2:** Concept map of azole resistance mechanisms in *A. fumigatus*, from environmental fungicide exposure through CYP51A mutation, efflux pump overexpression, and clinical treatment failure.

## Discussion

The finding that compost harbors the most resistant isolates aligns with European data showing agricultural waste as a reservoir for triazole-resistant *A. fumigatus* [7]. In Nigeria, azole fungicides are applied heavily in cocoa processing and palm oil storage—both of which generate large compost volumes that create ideal conditions for resistance selection [8]. The TR34/L98H genotype was identical between environmental and clinical isolates, strongly suggesting environmental acquisition rather than independent clinical evolution [9].

Amphotericin B and caspofungin resistance remained rare (0–1 isolates per source), indicating that these drug classes retain clinical utility in azole-resistant cases [10]. A limitation is that our sampling covered a single city; regional variation across Nigeria's diverse agricultural zones could reveal different resistance patterns [11].

## Conclusion

This research identified azole resistance in 23.4% of environmental and 18.8% of clinical *A. fumigatus* isolates from Benin City, Nigeria, with agricultural compost as the highest-risk source. The TR34/L98H CYP51A mutation was the dominant mechanism. These findings highlight the environmental route of resistance acquisition and call for integrated antifungal stewardship spanning agricultural and medical settings.

## Acknowledgements

### Funding Sources

Supported by the Benin City College of Life Sciences mycology research programme.

## Institutional Support

The Department of Medical Mycology provided BSL-2 laboratory and sequencing facilities.

## Contributions Not Qualifying for Authorship

Microbiology laboratory staff at the University of Benin Teaching Hospital provided clinical isolates.

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