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Comparative expression profiling of *gliP* and *cyp51A* genes in *Aspergillus fumigatus* isolates from chickens and poultry feed

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Aspergillus fumigatus is an important opportunistic fungal pathogen that frequently contaminates organic substrates on poultry farms, including feed, litter, dust, and airborne particles, thereby posing a significant risk of respiratory infection to birds. The objective of this study was to compare the relative expression levels of two critical target genes, *gliP* and *cyp51A*, in poultry- and feed-associated *Aspergillus fumigatus* isolates, utilizing *beta-actin* as the internal reference gene. A total of fifteen *Aspergillus fumigatus* strains were investigated, comprising ten clinical isolates recovered from infected chickens and five control strains obtained from poultry feed samples. Extraction of total RNA and subsequent complementary DNA synthesis were performed, and relative gene expression values were determined using the quantitative real-time polymerase chain reaction (qRT-PCR) via the comparative cycle threshold method. The transcriptional activity of each sample was normalized against *beta-actin*, and fold-change values were calculated relative to the feed-derived calibrator control group based on the $2^{-\Delta\Delta CT}$ equation. The experimental data demonstrated a consistent and significant transcriptional activation of both target genes in the chicken-derived group. Specifically, the relative expression levels of the *gliP* gene, which is directly involved in the nonribosomal peptide synthesis of the immunosuppressive mycotoxin gliotoxin, were highly upregulated in infected chicken isolates compared to the poultry feed-derived control strains, exhibiting mean fold-change values of 5.03±0.29 versus 1.06±0.16, respectively. Concurrently, the expression of the *cyp51A* gene, which encodes lanosterol 14-alpha-demethylase and serves as a primary target molecule for azole antifungal drugs, was also prominently increased in chicken-derived isolates compared to the controls, with mean fold-change values reaching 4.30±0.39 versus 1.15±0.27, respectively. The presented results suggest that host-associated *Aspergillus fumigatus* infection in chickens is characterized by the co-activation of pathways governing both mycotoxin production and environmental azole adaptation. Ultimately, the combined expression profile of *gliP* and *cyp51A* could serve as a targeted molecular panel for the preliminary screening and surveillance of pathogenicity potential and antifungal resistance characteristics in poultry-associated *Aspergillus fumigatus* isolates prior to implementing more extensive phenotyping or mutation sequencing assays.

Keywords: *Aspergillus fumigatus*, *gliP*, *cyp51A*, *beta-actin*, poultry, feed, qRT-PCR.

Порівняльний аналіз профілів експресії генів *gliP* та *cyp51A* в ізолятах *Aspergillus fumigatus* від курей та зі зразків корму для птиці

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Aspergillus fumigatus є важливим опортуністичним патогенним грибом, який часто контамінує органічні субстрати на птахофабриках, зокрема корми, підстилку та пил, створюючи тим самим значний ризик виникнення респіраторної інфекції у птиці. Метою цього дослідження було порівняти відносні рівні експресії двох критичних генів-мішеней, *gliP* та *cyp51A*, в ізолятах *Aspergillus fumigatus*, пов'язаних із птицею та кормами, використовуючи ген *beta-actin* як внутрішній референтний контроль. Загалом було досліджено 15 штамів *Aspergillus fumigatus*, що включали 10 клінічних ізолятів, виділених від інфікованих курчат, та 5 контрольних штамів, отриманих із зразків пташиного корму. Виділення загальної РНК, подальший синтез комплементарної ДНК та кількісну зворотну транскрипційно-полімеразну ланцюгову реакцію (qRT-PCR) проводили за методом порівняльного циклічного порогу. Транскрипційну активність кожного зразка нормалізували щодо гена *beta-actin*, а кратність змін розраховували відносно калібрувальної контрольної групи штамів із корму на основі формули $2^{-\Delta\Delta CT}$. Експериментальні дані продемонстрували стабільну та статистично значущу транскрипційну активацію обох генів-мішеней у групі ізолятів від птиці. Зокрема, рівні відносної експресії гена *gliP*, який бере безпосередню участь у нерибосомальному синтезі пептидів імуносупресивного мікотоксину гліотоксину, були значно підвищені в ізолятах від інфікованих курчат порівняно з контрольними штамами з корму, демонструючи середні значення кратності змін 5,03±0,29 проти 1,06±0,16 відповідно. Водночас експресія гена *cyp51A*, який кодує ланостерол-14-альфа-деметилазу та є основною молекулою-мішенню для азольних протигрибкових препаратів, також суттєво зросла в ізолятах від курчат порівняно з контролем, досягаючи середніх значень 4,30±0,39 проти 1,15±0,27 відповідно. Отримані результати свідчать, що інфекція *Aspergillus fumigatus* у птиці характеризується одночасною активацією метаболічних процесів, які відповідають як за продукування мікотоксинів, так і за адаптацію до азолів у довкіллі. Комбінований профіль експресії *gliP* та *cyp51A* може слугувати цільовою молекулярною панеллю для попереднього скринінгу й нагляду за рівнем патогенності та характеристиками протигрибкової резистентності ізолятів *Aspergillus fumigatus* у птахівництві перед проведенням більш високовартісних тестів на фенотипову чутливість або секвенування мутацій.

Ключові слова: *Aspergillus fumigatus*, *gliP*, *cyp51A*, *beta-actin*, птиця, корм, qRT-PCR.

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Introduction

Aspergillus fumigatus is a thermotolerant filamentous fungus capable of producing large quantities of airborne conidia. It is widely distributed in organic substrates such as litter, dust, feed ingredients, and decomposing plant material. In poultry production systems, respiratory aspergillosis is triggered when birds inhale high concentrations of spores, particularly in environments characterized by poor ventilation, high humidity, contaminated bedding, or immunosuppressive stress. The economic significance of the disease is associated with reduced performance in infected flocks, high chick mortality, and sciatic inertia in infected young or susceptible birds [1].

Aspergillus fumigatus colonizes the respiratory tract, but this is not its only significant aspect. The fungus is endowed with a flexible genome and a wide array of virulence-associated traits that allow it to survive various host-imposed stresses. These traits include thermotolerance, antioxidant strategies for adapting to oxidative stress, the production of secondary metabolites (SMs), biofilm formation, and immune modulation. These characteristics underscore why the simple isolation and identification of poultry isolates should be replaced with targeted molecular analysis of genes associated with virulence and survivability [2].

Several virulence-associated pathways, including gliotoxin biosynthesis, play a crucial role as immunosuppressive mechanisms under specific environmental and infection-related conditions, making this metabolic route particularly interesting in *A. fumigatus*. The specific biological function of the *gliP* gene, which encodes a nonribosomal peptide synthetase involved in the earliest stages of gliotoxin biosynthesis, renders it a key molecular target and genetic marker. Analyzing this gene helps determine the potential of an isolate to express regulator-mediated pathogenic traits associated with toxin production [3].

Similarly, the *cyp51A* gene is a critical molecular target in *A fumigatus* because it encodes lanosterol 14-alpha-demethylase, the primary target of azole antifungal drugs. Reduced azole susceptibility can be associated with mutations in the *cyp51A* sequence or alterations in its expression. Consequently, evaluating veterinary isolates is increasingly important, as antifungal resistance can develop from prior clinical exposure, environmental selection, or selective pressure from agricultural azoles [4].

Regarding virulence and azole adaptation, recent transcriptomic studies have demonstrated that azole stress does not affect a single resistance locus, but rather modulates the expression of multiple pathways governing sterol metabolism, membrane homeostasis, xenobiotic transport, and stress responses, ultimately affecting cell survival. Consequently, *cyp51A* remains a functionally relevant target within the azole adaptation mechanisms of *A. fumigatus*, involving either the gene itself or its regulatory network [5].

Poultry feed is a critical environmental source of fungal contamination in poultry houses, as spores and mycotoxigenic fungi can spread throughout the production chain, leading to disease outbreaks. Thus, feed-associated isolates may serve as a link between environmental exposure and avian disease, while potentially acting as a reservoir for resistance-associated traits. This underscores the importance of conducting integrated molecular surveillance on both chicken- and feed-derived *A. fumigatus* isolates, rather than studying them in isolation [6].

Accurate and sensitive quantification of target gene expression is achieved by quantitative real-time PCR (qPCR), a strategy that utilizes beta-actin as a reference gene for normalization. For the current analysis, *gliP* and *cyp51A* were selected as target genes due to their functional relevance to virulence (specifically toxin-related pathogenicity) and azole resistance in *Aspergillus fumigatus*. This approach provides a targeted interpretation of transcriptional activity associated with pathogenicity and resistance in *A. fumigatus* isolates obtained from poultry sources [7].

The aim of the study

The objective of this study was to compare the relative expression levels of two target genes (*gliP* and *cyp51A*) in poultry- and feed-associated *Aspergillus fumigatus* isolates, utilizing *beta-actin* as the internal reference gene. Additionally, this study aimed to evaluate these transcriptional profiles in the context of host-associated virulence and potential environmental azole selection pressure, thereby establishing a baseline for the integrated molecular surveillance of *A. fumigatus* within poultry production chains.

Materials and methods

Study design and sample groups

Aspergillus fumigatus samples recovered from poultry and poultry feed were investigated. A total of ten *A. fumigatus* samples were isolated from 101 chickens, and five *A. fumigatus* strains were obtained from poultry feed in this study. For the relative expression analysis, the fungal isolates obtained from infected chickens were compared to the isolates obtained from poultry feed. The feed-derived *A. fumigatus* strains served as the calibrator control group for determining relative gene expression values.

RNA extraction and cDNA synthesis

Extraction of total RNA was performed using the TransZol Up Plus RNA Kit (TransGen Biotech, China), followed by immediate conversion to cDNA using the EasyScript First-Strand cDNA Synthesis SuperMix (TransGen Biotech, China). The primers were synthesized by Macrogen (Republic of Korea), and real-time PCR amplification was performed using the SaCycler-96 Real-Time PCR System (Sacace Biotechnology, Italy). This workflow strictly adhered to standard qPCR reporting principles regarding sample handling, primer information, reaction composition, and normalized relative expression analysis [8].

Primers used for qRT-PCR analysis

The primer sequences utilized in the qRT-PCR assays are specified below. The target genes, *gliP* and *cyp51A*, are involved in the resistance and virulence phenotypes, while *beta-actin* was used as the internal reference gene for normalization. All primer sequences used in the qRT-PCR assays are shown in ***Table 1***.

Table 1

Primers used for qRT-PCR analysis of *gliP*, *cyp51A*, and *beta-actin* genes

Gene	Primer	Sequence (5′→3′)
gliP	gliP-F	AGCACCACAAAGGTCATTCC
gliP	gliP-R	GTAGAGGCTATGCCGTGAGC
cyp51A	RT-cyp51A-F	GGTGCCGATGCTATGGCTTACGGC
cyp51A	RT-cyp51A-R	GGTTCTGTTCGGTTCCAAAGCCG
beta-actin	beta-actin-F	TGCTCTCGTTATCGACAATGGT
beta-actin	beta-actin-R	CATCGTCACCGGCGAAA

Primer preparation

Lyophilized primers were reconstituted in nuclease-free ddH_2O to a stock concentration of 100 pmol/µl. To prepare the working primer solutions (10 pmol/µl), 10 µl of the stock primer was mixed with 90 µl of nuclease-free ddH_2O to reach a final working volume of 100 µl. These working solutions were subsequently used for the PCR analysis. Finally, stock and working primers were stored at −20 °C until use.

Quantitative real-time PCR reaction mixture

The qRT-PCR reaction was prepared in a final reaction volume of 20 µl, which consisted of master mix, forward and reverse primers, cDNA template, and nuclease-free water, as detailed in ***Table 2***.

Table 2

Quantitative real-time PCR reaction mixture

Reagent	Volume per reaction
Master Mix	10 µl
Forward primer (10 pmol/µl)	0.4 µl
Reverse primer (10 pmol/µl)	0.4 µl
cDNA template	5 µl
Nuclease-free water	4.2 µl
Final reaction volume	20 µl

Relative expression calculation

Relative expression levels were calculated using the comparative Ct method, where the Ct value of each individual target gene was normalized with respect to *beta-actin*. The calibrator group consisted of the feed-derived *A. fumigatus* isolate group. Analysis of the relative gene expression was performed according to the $2^{-\Delta\Delta CT}$ method [9], using the step-by-step equations detailed in ***Table 3***.

Table 3

Step-by-step equations for the calculation of relative gene expression using the $2^{-\Delta\Delta CT}$ method

Step	Equation
1	$\Delta CT = Ct$(target gene) – Ct(reference gene beta-actin)
2	$\Delta\Delta CT = \Delta CT$(chicken-derived sample) – mean ΔCT(feed-derived control group)
3	Fold change = $2^{-\Delta\Delta CT}$

Statistical analysis

Real-time qPCR expression results were statistically summarized as mean ± standard error (SE). For the analysis of ΔCT, $\Delta\Delta CT$, and fold-change values, P-values were calculated by comparing *A. fumigatus* isolates from infected chickens with those from poultry feed. Differences were deemed statistically significant when $P<0.05$.

Results and discussion

The expression levels of both target genes were significantly upregulated in *Aspergillus fumigatus* isolates obtained from infected chickens compared to those recovered from poultry feed. Based on the ΔCT values normalized against *beta-actin*, both *gliP* and *cyp51A* exhibited lower ΔCT values in the chicken-derived group, demonstrating a higher baseline level of transcriptional activity relative to the feed-derived control strains. The fold-change calculations confirmed that *gliP* possessed the highest relative expression level, closely followed by *cyp51A*.

Relative expression of the gliP gene

The expression levels of the *gliP* gene were investigated via real-time PCR in *Aspergillus fumigatus* isolates. To determine the statistical significance of the transcriptional alterations, relative expression values were compared between chicken- and feed-derived strains, as summarized in ***Table 4***.

Table 4

Relative expression statistics for the *gliP* gene in chicken- and feed-derived *Aspergillus fumigatus* isolates, Mean±SE

Groups	ΔCT	$\Delta\Delta CT$	Fold change ($2^{-\Delta\Delta CT}$)
Chicken-derived isolates	2.65±0.08	−2.31±0.08	5.03±0.29
Feed-derived control strains	4.96±0.25	0.00±0.25	1.06±0.16
P-value	P=0.00039	P=0.00039	P<0.0001

In *A. fumigatus* isolates recovered from infected chickens, the expression level of the *gliP* gene was highly upregulated. In the chicken-derived group, the ΔCT value was 2.65±0.08, whereas in the feed-derived control group, it was 4.96±0.25. The calculated $\Delta\Delta CT$ values were −2.31±0.08 for infected chicken isolates and 0.00±0.25 for the calibrator controls ($P<0.01$). The final fold-change value reached 5.03±0.29 in chicken-derived isolates versus 1.06±0.16 in the feed-derived strains. This confirms that the difference in *gliP* gene expression between the two experimental groups was highly significant ($P<0.0001$).

Relative expression of the cyp51A gene

The transcriptional activity of the *cyp51A* gene in *Aspergillus fumigatus* isolates was evaluated using real-time PCR. To analyze the molecular patterns of azole adaptation, the relative expression profiles were compared between chicken-derived and feed-derived strains, as summarized in ***Table 5***.

Table 5
Relative expression statistics for the *cyp51A* gene in chicken- and feed-derived *Aspergillus fumigatus* isolates, Mean±SE

Groups	Δ*CT*	ΔΔ*CT*	Fold change ($2^{-\Delta\Delta CT}$)
Chicken-derived isolates	1.85±0.13	−2.05±0.13	4.30±0.39
Feed-derived control strains	3.90±0.41	0.00±0.41	1.15±0.27
P-value	P=0.00542	P=0.00542	P<0.0001

The *cyp51A* gene was highly upregulated in *A. fumigatus* isolates recovered from infected chickens. In the chicken-derived group, the Δ*CT* value was 1.85±0.13, compared to 3.90±0.41 in the feed-derived control group. The calculated ΔΔ*CT* value was −2.05±0.13 for the chicken-derived isolates versus 0.00±0.41 for the calibrator controls (P<0.001). The final fold-change value reached 4.30±0.39 in chicken-derived isolates compared to 1.15±0.27 for the control strains. This confirms that the difference in *cyp51A* expression between the two experimental groups was highly significant (P< 0.0001).

The current data demonstrated that *gliP* and *cyp51A* were significantly upregulated in *Aspergillus fumigatus* isolates recovered from infected chickens compared to poultry feed-derived control strains. Notably, this expression pattern is highly biologically relevant, as *gliP* is directly associated with gliotoxin biosynthetic processes, while *cyp51A* is involved in both ergosterol biosynthesis and azole resistance mechanisms. This synchronized two-gene profile indicates that pathways governing pathogenicity and antifungal adaptation are co-activated in *A. fumigatus* strains circulating within poultry production environments.

The strong induction of *gliP* observed in this study is consistent with recent findings indicating that *A. fumigatus* can derepress gliotoxin biosynthetic pathways during biofilm formation [10]. According to a previous report by Gomez-Lopez and Fernandez-Fernandez, gliotoxin production exhibits strain- and time-dependent dynamics that directly correlate with the transcriptional activation of genes involved in the regulation and synthesis of this mycotoxin. This supports the conclusion that increased *gliP* expression reflects a biologically active toxin metabolism rather than an accidental transcriptional event [3].

Moreover, the observed *cyp51A* upregulation is consistent with recent transcriptomic analyses demonstrating that *A. fumigatus* responds to azole selective pressure by differentially expressing pathways involved in sterol biosynthesis, stress adaptation, and membrane function to survive antifungal exposure. This analysis aligns with the previously established mode of action for this key target gene [5], even though the present study did not directly evaluate phenotypic antifungal susceptibility.

Similar veterinary clinical evidence highlights *cyp51A* as a key target in animal-associated *Aspergillus fumigatus* isolates. An extensive surveillance study conducted in the Netherlands identified azole resistance in veterinary *A. fumigatus* isolates, which was almost exclusively mediated by *cyp51A* tandem repeats (TRs). This underscores the necessity of incorporating *cyp51A* analysis into molecular studies to discriminate between animal and poultry isolates, especially when environmental selective pressure is assumed [4].

Moreover, environmental studies reinforce the value of *cyp51A* as a principal surveillance target. Investigations utilizing *A. fumigatus* isolates recovered from clinical cases demonstrated that *cyp51A* mutations are associated with elevated azole minimum inhibitory concentration (MIC) values, highlighting the persistence of environmental transmission risks. These findings align with current research on poultry and feed origins, as both matrices are susceptible to environmental fungal contamination and subsequent selective pressure from agricultural azoles [11].

Previously, it was emphasized that both *cyp51A* sequencing and resistance phenotype determination remain the cornerstone for clinical and epidemiological analysis [12]. The present results do not demonstrate resistance in isolation; however, elevated *cyp51A* expression offers a molecular marker signal to be further explored through susceptibility testing and mutation screening investigations, which require subsequent validation studies [12].

The selection of poultry and feed samples is also substantiated by reports in the literature indicating that feed contamination may serve as an environmental reservoir for azole-resistant *Aspergillus* species. The detection of *Aspergillus* in poultry feed highlights its role as a nutritional substrate and a continuous fungal reservoir, leading to avian exposure and increased flock-level infection pressure. This underscores the necessity of managing feed quality alongside bird health within the integrated production chain [6].

Variations in the azole resistance marker gene are localized across 3 to 7 kb, and not all studies support a single-gene interpretation. Ma et al. [13] analyzed a non-*cyp51A* mutant azole-resistant *Aspergillus fumigatus* strain and demonstrated that resistance is coupled with genome-wide transcriptomic and metabolomic changes, including alterations to oxidative stress and cellular response pathways. This evidence provides additional context distinct from a purely *cyp51A*-only explanation, suggesting that the observed *cyp51A* upregulation should be viewed as one player within an extended resistance network [12].

Veterinary data on azole resistance remain sparse and variable across species, sample sources, countries, and testing methods, as shown in a systematic review of *Aspergillus* isolates from animals and their environment. Such a lack of consensus is noteworthy, highlighting that the high *cyp51A* expression observed in this study cannot be extrapolated to all poultry farms or all animal-associated *A. fumigatus* isolates in the absence of broader surveillance datasets [14].

Transcription factor studies also illustrate the regulatory complexity of azole resistance. Paul et al. outlined the necessity of FfmA and AtrR for correctly modulating wide-ranging expression programs in *Aspergillus fumigatus*, which affect pathways beyond just *cyp51A* [15]. This indicates that the observed *cyp51A* increase may reflect the downstream output of upstream

regulatory networks rather than a single gene-specific event. Caution is also warranted when interpreting *gliP* expression. Current reviews on the virulence of *A. fumigatus* point to complex interactions among fungal growth, host immune status, secondary metabolites, stress tolerance, and tissue invasion during disease development [16], thus, while *gliP* upregulation strengthens the potential for gliotoxin biosynthesis, it should not be considered the sole factor governing pathogenicity in chicken-derived isolates.

While highly sensitive, the qRT-PCR method relies on stable reference gene expression, similar amplification efficiencies, and high sample quality. Indeed, in recent discussions of relative quantification, it has been acknowledged that the $2^{-\Delta\Delta CT}$ method makes specific assumptions regarding normalization and amplification behavior between target and reference assays [7]. Consequently, future work should report on primer efficiencies and include melt-curve or specificity confirmation to lend further confidence to the gene expression estimates.

The elevated *gliP* expression in chicken-derived isolates implies that poultry-associated *A. fumigatus* strains possess enhanced gliotoxin-related activity. In avian aspergillosis, this feature is critical because respiratory infection requires successful spore germination, hyphal development, and immunoevasion. In future studies, however, *gliP* expression should be interpreted in conjunction with histopathology, fungal burden estimation, and direct toxin quantification [17].

While the increase in *cyp51A* expression serves as a marker for the activation of sterol biosynthesis or resistance-associated adaptation, it does not confirm phenotypic azole resistance without accompanying phenotypic data. Essential next steps include antifungal susceptibility testing, sequencing of *cyp51A* hotspot regions, and the evaluation of promoter tandem repeats or other resistance-associated mutations. This integrated approach would enable correlating expression profiles with the actual risk of resistance in poultry-derived isolates [18]. Furthermore, the present study features a relatively small sample size for exploratory molecular research. To strengthen the scientific inference, future studies should encompass more farms and feed sources, while longitudinal sampling across multiple years would improve the representativeness of the monitored populations. Due to the pervasive environmental nature of *Aspergillus* exposure, large-scale surveillance is required to link these molecular findings with feed quality, litter management, ventilation status, and the total fungal burden [19, 20].

Despite these limitations, the initial insights gleaned from the combined evaluation of *gliP* and *cyp51A* expression remain highly valuable. These two genes represent complementary facets of *A. fumigatus* biology: *gliP* reflects potential toxigenic activity, whereas *cyp51A* characterizes the regulation of azole targets and subsequent resistance development. When normalized against *beta-actin*, this two-gene screening panel could facilitate the preliminary molecular surveillance of poultry-associated *A. fumigatus* isolates prior to implementing more costly phenotypic testing and sequencing assays.

Conclusions

In this study, a significant upregulation of two critical genes was demonstrated in chicken-derived *Aspergillus fumigatus* isolates compared to poultry feed-derived control strains. The final fold-change calculations confirmed that *gliP* possessed the highest relative expression level, increasing 5.03±0.29-fold, which indicates a strong transcriptional activation related to gliotoxin biosynthesis and potential fungal virulence. Another prominently upregulated target molecule was *cyp51A*, which exhibited a 4.30±0.39-fold increase, functionally confirming its co-activation during host-associated adaptation. Overall, this two-gene combination represents a targeted molecular panel for assessing the pathogenic and antifungal resistance-associated characteristics of avian- and feed-associated *A. fumigatus* isolates. To consolidate the clinical and biological validity of these markers, future large-scale studies should incorporate expanded sample sizes, investigate feed-to-bird transmission dynamics, perform phenotypic antifungal susceptibility testing, and include *cyp51A* mutation sequencing alongside direct gliotoxin quantification.

DECLARATIONS

Ethical Statement

The author confirms that the studies were conducted in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), national bioethical norms, and international standards for the humane treatment of animals. The *Aspergillus fumigatus* samples investigated in this study were recovered from clinical veterinary cases and feed substrates during routine diagnostic and sanitary screening. No direct experimental interventions, invasive procedures, or clinical sampling were performed on live animals within the framework of this research.

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Conflict of Interest

The author states that there is no conflict of interest.

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Declaration of AI and AI-assisted technologies

The author declares that no generative AI or AI-assisted technologies were used during the preparation, writing, translation, or language polishing of this manuscript. The final content was fully authored and reviewed by the researcher, who takes sole responsibility for the work.

References

1. Shaapan, R., & Girh, Z. (2024). Overview of aspergillosis in poultry - a review. *Egyptian Journal of Veterinary Sciences*, 55(2), 407–419. https://doi.org/10.21608/ejvs.2023.234624.1602
2. Earle, K., Valero, C., Conn, D. P., Vere, G., Cook, P. C., Bromley, M. J., Bowyer, P., & Gago, S. (2023). Pathogenicity and virulence of *Aspergillus fumigatus*. *Virulence*, 14(1), 2172264. https://doi.org/10.1080/21505594.2023.2172264
3. Gomez-Lopez, A., & Fernandez-Fernandez, C. (2024). Molecular characterization of gliotoxin synthesis in a biofilm model of *Aspergillus fumigatus*. *Biofilm*, 8, 100238. https://doi.org/10.1016/j.bioflm.2024.100238
4. van Dijk, M. A. M., Buil, J. B., Tehupeiory-Kooreman, M., Broekhuizen, M. J., Broens, E. M., Wagenaar, J. A., & Verweij, P. E. (2024). Azole resistance in veterinary clinical *Aspergillus fumigatus* isolates in the Netherlands. *Mycopathologia*, 189, 50. https://doi.org/10.1007/s11046-024-00850-5
5. Hokken, M., Coolen, J., Steenbreker, H., Zoll, J., Baltussen, T., Verweij, P., & Melchers, W. (2023). The transcriptome response to azole compounds in *Aspergillus fumigatus* shows differential gene expression across pathways essential for azole resistance and cell survival. *Journal of Fungi*, 9(8), 807. https://doi.org/10.3390/jof9080807
6. Odebode, A., Vanreppelen, G., Resendiz-Sharpe, A., & Van Dijck, P. (2024). First report of azole-resistant *Aspergillus species* in poultry feed in Kabale, South West, Uganda. *Notulae Scientia Biologicae*, 16(2), 11789. https://doi.org/10.55779/nsb16211789
7. Zalewski, D., & Bogucka-Kocka, A. (2025). RQdeltaCT: an open-source R package for relative quantification of gene expression using delta Ct methods. *Scientific Reports*, 15(1), 29762. https://doi.org/10.1038/s41598-025-11822-0
8. Bustin, S. A., Ruijter, J. M., van den Hoff, M. J. B., Kubista, M., Pfaffl, M. W., Shipley, G. L., Tran, N., Rödiger, S., Untergasser, A., Mueller, R., Nolan, T., Milavec, M., Burns, M. J., Huggett, J. F., Vandesompele, J., & Wittwer, C. T. (2025). MIQE 2.0: Revision of the minimum information for publication of quantitative real-time PCR experiments guidelines. *Clinical Chemistry*, 71(6), 634–651. https://doi.org/10.1093/clinchem/hvaf043
9. Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*, 25(4), 402–408. https://doi.org/10.1006/meth.2001.1262
10. Bruns, S., Seidler, M., Albrecht, D., Salvenmoser, S., Remme, N., Hertweck, C., Brakhage, A. A., Kniemeyer, O., & Müller, F.-M. C. (2010). Functional genomic profiling of *Aspergillus fumigatus* biofilm reveals enhanced production of the mycotoxin gliotoxin. *Proteomics*, 10(17), 3097–3107. https://doi.org/10.1002/pmic.201000129
11. Gong, J., Huang, J., Liu, Y., Zhang, Y., & Gao, Y. (2024). Unveiling environmental transmission risks: Comparative analysis of azole resistance in *Aspergillus fumigatus* clinical and environmental isolates from Yunnan, China. *Microbiology Spectrum*, 12(12), e01594-24. https://doi.org/10.1128/spectrum.01594-24
12. Zubovskaia, A. (2025). Azole-resistant *Aspergillus fumigatus*: Epidemiology, diagnosis, and treatment considerations. *Journal of Fungi*, 11(10), 731. https://doi.org/10.3390/jof11100731
13. Ma, W., Kang, Y., Li, Q., Wang, P., & Jia, W. (2025). Transcriptomic and metabolomic analysis of a non-cyp51A mutant azole-resistant *Aspergillus fumigatus* isolated from Ningxia, China. *Frontiers in Microbiology*, 16, 1666905. https://doi.org/10.3389/fmicb.2025.1666905
14. Dieste-Pérez, L., Holstege, M. M. C., de Jong, J. E., & Heuvelink, A. E. (2025). Azole resistance in *Aspergillus* isolates from animals or their direct environment (2013–2023): A systematic review. *Frontiers in Veterinary Science*, 12, 1507997. https://doi.org/10.3389/fvets.2025.1507997
15. Paul, S., Stamnes, M. A., & Moye-Rowley, W. S. (2023). Interactions between the transcription factors FfmA and AtrR are required to properly regulate gene expression in the fungus *Aspergillus fumigatus*. *G3: Genes, Genomes, Genetics*, 13(10), Article jkad173. https://doi.org/10.1093/g3journal/jkad173
16. Frías-De-León, M. G., García-Salazar, E., & Acosta-Altamirano, G. (2023). Virulence attributes in *Aspergillus fumigatus*. In M. Razzaghi-Abyaneh, M. Rai, & M. Shams-Ghahfarokhi (Eds.), *Aspergillus and aspergillosis: Advances in genomics, drug development, diagnosis and treatment*. IntechOpen. https://doi.org/10.5772/intechopen.111778
17. Hussein, A. S., Mohammed, S. H., & Mohammed, B. L. (2025). Molecular and immunological detection of gliotoxin genes in *Aspergillus fumigatus* isolated from patients with invasive pulmonary aspergillosis. *Eastern Ukrainian Medical Journal*, 13(1), 189–197. https://doi.org/10.21272/eumj.2025;13(1):189-197
18. Zeng, M., Zhou, X., Yang, C., Liu, Y., Zhang, J., Xin, C., Qin, G., Liu, F., & Song, Z. (2023). Comparative analysis of the biological characteristics and mechanisms of azole resistance of clinical *Aspergillus fumigatus* strains. *Frontiers in Microbiology*, 14, 1253197. https://doi.org/10.3389/fmicb.2023.1253197
19. Schürch, S., Gindro, K., Schnee, S., Dubuis, P.-H., Codina, J. M., Wilhelm, M., Riat, A., Lamoth, F., & Sanglard, D. (2023). Occurrence of *Aspergillus fumigatus* azole resistance in soils from Switzerland. Medical Mycology, 61(11), myad110. https://doi.org/10.1093/mmy/myad110
20. Gomes, B., Dias, M., Cervantes, R., Pena, P., Carolino, E., Caetano, L. A., Viegas, S., & Viegas, C. (2025). Poultry farms as reservoirs of azole-resistant fungi: Occupational health risks in agricultural facilities. *One Health*, 21, 101230. https://doi.org/10.2139/ssrn.5369870

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