# AIRBORNE AZOLE-RESISTANT MOULDS IN DAIRY FARMS IN NORMANDY, FRANCE: A PILOT STUDY

KENZA BOULOUIZ[1], NATHAN GOUNEL[1], ANTOINE GÉRY[1], VIRGINIE SÉGUIN[1], JULIE BONHOMME[1,2] & DAVID GARON[1]
[1]University of Caen Normandy, ABTE UR4651, ToxEMAC, France
[2]Microbiology Department, Caen University Medical Centre, France

ABSTRACT

During routine farming activities, dairy workers and animals are exposed to bioaerosols containing airborne fungal contaminants. Among them, *Aspergillus fumigatus*, *Fusarium* spp., and Mucorales are considered priority fungal pathogens by the World Health Organization because of increasing antifungal resistance and the growing burden of invasive fungal infections. The widespread use of azole compounds in agriculture may contribute to the environmental emergence and dissemination of resistant isolates. This study aimed to investigate the occurrence of airborne priority fungal pathogens in dairy farms in Normandy, France, and to assess their susceptibility to clinical and agricultural azole antifungals. A total of 119 airborne isolates, including 74 *A. fumigatus*, 13 *Fusarium* spp. and 32 Mucorales isolates, were purified and identified using microscopy and molecular sequencing. Antifungal susceptibility phenotypes were evaluated using itraconazole (1 mg/L) and, for itraconazole-resistant isolates, enilconazole (0.1 mg/L). Among Mucorales, *Lichtheimia ramosa* (43.8%), *L. corymbifera* (28.1%), and *Mucor circinelloides* (12.5%) were the predominant airborne species. Within the Fusarium genus, *Fusarium graminearum* (53.8%) and *F. culmorum* (30.8%) were the most frequently identified species. Overall, 20.2% of isolates were resistant to itraconazole, and 83.3% of these resistant isolates also exhibited resistance to enilconazole. Resistance to itraconazole reached 9.5% for *A. fumigatus*, 25% for Mucorales, and 69.2% for *Fusarium* spp. Among itraconazole-resistant isolates, all *A. fumigatus* and Mucorales isolates and 55.6% of *Fusarium* spp., were also resistant to enilconazole. These findings demonstrate the presence of airborne azole cross-resistant moulds in Normandy dairy farms and suggest that agricultural bioaerosols may represent an environmental reservoir of antifungal resistance within a One Health context. Such exposure could contribute to difficult-to-treat infections in humans and animals, while the dynamics of airborne dissemination remain poorly understood.

*Keywords: bioaerosols, airborne moulds, Aspergillus fumigatus, Fusarium spp., Mucorales, azole resistance, itraconazole, enilconazole (imazalil), One Health.*

## 1 INTRODUCTION

Europe is the second largest producer of cow's raw milk worldwide [1], with 156.1 million tonnes produced in 2024 [2]. France accounts for 15.5% of this production, making it the second largest European producer after Germany (21.6%) [2]. More than half of French milk production is concentrated in three regions, including Normandy [3], [4].

During routine farming activities such as mulching, milking, and animal feeding, workers and animals are exposed to aerosolized dust containing various airborne biological contaminants [5]–[9]. The handling of oilseed cakes, corn silage [6], lucerne, or pellets [10], which may contain bacteria and moulds [5], [6], [11], can contribute to the generation and inhalation of fungal bioaerosols by both operators and animals. Exposure to moulds may represent a health risk [7], [8]. In humans, fungal exposure can lead to a wide range of diseases, from allergies such as Farmer's lung to severe invasive fungal diseases (IFD), which are associated with high mortality [12]. Other respiratory disorders may occur, including allergic bronchopulmonary aspergillosis [12], [13], asthma [12], [13], chronic obstructive



WIT Transactions on Ecology and the Environment, Vol 266
© 2026 WIT Press
www.witpress.com, ISSN 1743-3541 (on-line)
doi:10.2495/AIR260031

bronchitis [12], and organic dust toxic syndrome [14]. Mold exposure may also exacerbate pre-existing diseases such as asthma [15], [16] and cystic fibrosis [17]. In ruminants, mycotic infections caused by species such as *Aspergillus fumigatus* (*A. fumigatus*) and Mucorales may result in mastitis, gastroenteritis, placentitis, abortion, and respiratory infections [18]. Immunocompromised workers [12] and animals [18], or poor farming conditions [19], may further increase the risk of fungal infections.

This issue is further complicated by the ability of some moulds to produce mycotoxins, including species from *Fusarium* [20], *A. fumigatus* [21], and Mucorales [22]. Some mycotoxins, such as zearalenone, are considered endocrine disruptors [23]. These compounds may enter the food chain through animal products such as milk when cattle are fed contaminated feed [23].

Because of increasing infection rates, treatment difficulties, high mortality, and rising antifungal resistance, the World Health Organization (WHO) closely monitors several fungal pathogens. Among them, *A. fumigatus* is classified as a critical priority pathogen, whereas *Fusarium* and Mucorales species are classified as high-priority pathogens [24]. These fungi are responsible for aspergillosis, fusariosis, and mucormycosis, respectively.

Azole compounds are widely used for the treatment and prevention of fungal infections in clinical, veterinary, and agricultural settings [25], [26]. Itraconazole (ITZ) is used in both clinical and veterinary medicine [25], whereas enilconazole (ENZ) is mainly used in veterinary and agricultural contexts and was recently banned from biocidal use in the EU/EEA market [25]. In Europe, agriculture represents the main sector using azole compounds, particularly for plant protection products [25]. However, azole compounds are broad-spectrum molecules [27], often overused or inappropriately used [24], [28], and may share similar molecular targets [29]. These factors are considered major drivers of the increasing emergence of azole-resistant strains, particularly in *A. fumigatus*. Several studies also suggest a link between resistant clinical isolates and resistant environmental strains [25], [28]. Especially, ITZ resistance is often associated with cross-resistance to other azoles [28].

In contrast, less is known about the susceptibility of *Fusarium* and Mucorales species to azole compounds. Susceptibility profiles are highly variable depending on the species and antifungal molecule considered [30]–[33]. This variability may be related to conserved mutations in azole target genes, which could explain the intrinsic resistance observed in some *Fusarium* spp. and Mucorales species [32], [34].

In addition, the European Food Safety Authority (EFSA) and associated agencies have highlighted the lack of detailed environmental data regarding antifungal resistance, as reported in a *Nature Medicine*'s comment [25], [35]. Important knowledge gaps remain concerning the quantities, locations, persistence, accumulation, and ecological dissemination of resistant fungi. These limitations complicate risk assessment, preventive strategies, and regulatory decision-making [25], [35].

In this context, and within a One Health framework, this study aimed to investigate the resistance of airborne *A. fumigatus*, *Fusarium*, and Mucorales isolates to itraconazole and enilconazole in dairy farms from Normandy, one of the main French milk-producing regions.

## 2 MATERIALS AND METHODS

### 2.1 Prerequisites

A total of 119 airborne isolates (74 *A. fumigatus*, 13 *Fusarium* spp. and 32 Mucorales isolates) collected during the BIOESTROGEN-AIR project, a previous study conducted in


© 2026 WIT Press
www.witpress.com, ISSN 1743-3541 (on-line)

12 dairy farms located in Calvados (Normandy, France), were investigated. Isolates were conserved at −80°C in 10% glycerol (Sigma Aldrich, Jakarta Timur, Indonesia).

Air samples were collected using a Coriolis® air sampler (Bertin Technologies, Montigny-le-Bretonneux, France) in outdoor and indoor open areas of the barns during resting, milking, feeding, and straw mulching activities. Fungal samples were collected in 15 mL of 0.02% Tween 80 solution (300 L/min for 10 min), diluted to $10^2$, and the $10^1$ and $10^2$ dilutions were cultivated using the pour plate method on Malt Extract Agar supplemented with chloramphenicol. Microfungi were isolated daily after incubation at 25°C for one week.

#### 2.1.1 Mold identification

After seven days of incubation at 25°C, *A. fumigatus*, *Fusarium*, and Mucorales isolates were examined macroscopically and microscopically. DNA extraction was then performed using the NucleoSpin Plant II kit (Macherey-Nagel, Düren, Germany).

Scraped colonies were transferred into microtubes containing glass beads (1 and 5 mm) and 300 µL of lysis buffer 1. Samples were frozen at −80°C for 20 min and mechanically disrupted using a Bead Mill Max (VWR) (4 m/s; 3 cycles of 3 min; 10 s intervals). Next, 10 µL of proteinase K (10 mg/mL) and 5 µL of RNase A (0.01 mg/mL) were added, and incubated at 65°C for 10 min. After vortexing, 400 µL of chloroform was added to the lysed samples. DNA extraction was then completed according to the manufacturer's instructions.

For molecular identification, the *benA* gene, *tef-1α* gene, and internal transcribed spacer (ITS) region were respectively amplified for *A. fumigatus*, *Fusarium* spp., and Mucorales isolates. PCR amplification was performed in a final volume of 50 µL containing 2 µL of DNA template, $MgCl_2$-enriched buffer (5 µL), dNTPs 10 mM (1 µL), FastStart Taq polymerase 5 U/µL (0.5 µL) (Roche Diagnostics GmbH and Co. KG, Switzerland), primers (100 µM; Eurogentec, Belgium), and DNase-free water up to 50 µL. Amplification was carried out using an Eppendorf Mastercycler Personal thermocycler (Eppendorf, Germany). PCR products were mixed with BlueJuice 10× loading buffer (Fisher Scientific, USA) and loaded onto a 2% agarose gel containing 6% Midori Green (Genetics EUROPE GmbH, Germany). After electrophoresis (20 min at 110 V), gels were visualized under UV light. Samples were finally sequenced using Illumina paired-end technology at Eurofins Genomics Germany GmbH (Germany) [36].

#### 2.1.2 *cyp51A* sequencing and analysis

The *cyp51A* gene and its promoter region, known to be involved in azole resistance in *A. fumigatus*, were amplified using the *cyp51A*-F2/*cyp51A*-R2 primers as previously described by Chen et al. [37]. DNA concentration was measured using an Invitrogen™ Qubit™ 4 Fluorometer (Fisher Scientific, USA) prior to amplification. Sequences were aligned to the *A. fumigatus cyp51A* reference sequence AFUB_063960 [37], and exonic regions were translated into amino acid sequences using CLC Genomics Workbench version 12.0 [38] to identify mutations potentially associated with azole resistance.

#### 2.1.3 Antifungal susceptibility tests

Antifungal susceptibility tests were performed according to EUCAST recommendations [39]. For each isolate, a spore suspension adjusted to 0.5 McFarland (± 0.15) was prepared in 0.02% Tween 80 using a DEN-1B densitometer (Grant Instruments, UK), without filtration. After a tenfold dilution, isolates were tested with ITZ at 1 mg/L, corresponding to the EUCAST breakpoint for *A. fumigatus*. Isolates resistant to ITZ were subsequently tested with ENZ at 0.1 mg/L. All strains were tested in technical triplicate. At least one reference strain was included for each priority taxon (Table 1).


© 2026 WIT Press
www.witpress.com, ISSN 1743-3541 (on-line)

Table 1: List of the fungal reference strains used in the antifungal susceptibility tests.

| **Priority taxa** | **Species** | **Reference strain** | **Characteristics** |
|---|---|---|---|
| *A. fumigatus* | *A. fumigatus* | ATCC 204305 | Susceptible to the ITZ clinical breakpoint |
| | | CCUG 74258 | TR34/L98H mutation Associated with pan-azole resistance [40]–[43] |
| | | CCUG 74259 | G54W mutation associated with cross-resistance to ITZ and posaconazole [40], [44] |
| Mucorales | *Lichtheimia corymbifera* | CBS 115811 | Unknown |
| | *A. cylindrospora* var. *rhizomorpha* | CBS 153.63 | Unknown |
| *Fusarium* | *Fusarium poae* | CBS 128536 | Unknown |

Incubation was performed at either 25°C or 37°C according to the optimal growth temperature reported for each taxon [45] and for the durations presented in Table 2. No optimal growth temperature for *Circinella chinensis* was identified in the literature. Because of the environmental nature of this isolate, incubation was performed at 25°C.

Table 2: Incubation parameters for the antifungal susceptibility tests for genus and/or species.

| **Incubation time** | **Mold genus/species** | **Incubation temperature** |
|---|---|---|
| Up to 2 days | *A. fumigatus* | 37°C |
| | *Circinella* | 25°C |
| | *Lichtheimia ramosa* and *L. corymbifera* | 37°C |
| | *Mucor* | 25°C |
| Up to 3 days | *Fusarium* | 25°C |
| Up to 4 days | *Absidia healeyae* | 25°C |

The maximum incubation duration was determined according to fungal growth in both control and antifungal-containing wells, following EUCAST recommendations. In cases of ambiguous growth results, incubation was extended by one additional day without exceeding the 3-day limit recommended by EUCAST. However, *Absidia healeyae* growth was only observed after four days in the presence of ENZ.


© 2026 WIT Press
www.witpress.com, ISSN 1743-3541 (on-line)

#### 2.1.4 Statistical analysis

Fisher's exact test ($\alpha = 0.05$) was used to evaluate associations between the presence of priority taxa (*Aspergillus fumigatus*, Mucorales, and *Fusarium*) and season, as well as between susceptibility phenotypes and sampling characteristics, including sampling location (outdoor vs. indoor), farming activity during indoor air sampling, and sampling season. Analyses were performed for all moulds and separately for *A. fumigatus*, Mucorales, and *Fusarium* isolates.

To support the interpretation of Fisher's exact test results, post-hoc power analyses based on contingency table effect sizes (Cramér's V/φ) were additionally performed to evaluate whether the sample size and study design provided sufficient statistical power to detect potential associations. Statistical analyses were conducted using XLSTAT (Addinsoft, Paris), version 2023.2.1413 [46]. Differences were considered statistically significant at $p < 0.05$.

## 3 RESULTS AND DISCUSSION

### 3.1 Fungal diversity in airborne aerosols

Among the *Aspergillus* species isolated from air samples, *Aspergillus fumigatus* represented approximately 47% of the isolates. This species is the most common *Aspergillus* species involved in invasive pulmonary diseases [47]. A French clinical study also identified the *Fumigati* section as the most prevalent *Aspergillus* section [48].

Among Mucorales isolates, *Lichtheimia ramosa* (43.8%), *L. corymbifera* (28.1%), and *Mucor circinelloides* (12.5%) were the predominant airborne species. A systematic review on mucormycosis reported that approximately 75% of cases worldwide were caused by *Rhizopus* (48%), *Mucor* (14%), and *Lichtheimia* (13%) species [49], [50]. According to a French epidemiological study, *Rhizopus arrhizus* was the most frequent species involved in human mucormycosis (21.2% of cases), followed by *L. corymbifera* and *M. circinelloides* (13.3%), and *L. ramosa* (10.8%) [51]. These species were also among the most prevalent Mucorales identified in Normandy dairy farm bioaerosols.

Regarding mortality, *L. corymbifera* is associated with the highest mortality rate (70%), followed by *R. arrhizus* (53.1%), *L. ramosa* (37.5%), and *M. circinelloides* (32.4%) [51]. Consequently, the presence of these pathogenic airborne species in dairy farms may represent a risk for immunocompromised individuals. Even species associated with lower mortality rates, such as *M. circinelloides*, should not be neglected.

In a previous study conducted by our laboratory in Normandy dairy farms [6], *A. fumigatus*, *L. corymbifera*, and *M. circinelloides* were detected in ambient air during cattle feed preparation and distribution, as well as in personal air samples collected during two sampling periods (October–December and February–April). These findings confirm the exposure of farmers and animals to opportunistic airborne fungal pathogens.

Within the *Fusarium* genus, *F. culmorum* (42%) and *F. graminearum* (32%) were the most frequently isolated and are considered as major pathogens in the agri-food sector [52] because of their ability to simultaneously produce several mycotoxins, including deoxynivalenol and zearalenone [20], [52]–[54], the latter presenting estrogenic effects. Some *Fusarium* species are also responsible for major agricultural losses and, in some cases, the disappearance of cultivars [52], [53].


© 2026 WIT Press
www.witpress.com, ISSN 1743-3541 (on-line)

### 3.2 Antifungal susceptibility level

#### 3.2.1 Antifungal susceptibility of *Aspergillus fumigatus* isolates

The majority of *A. fumigatus* isolates (90.5%) were susceptible to ITZ, similarly to the reference strain ATCC 204305 (Tables 3 and 4). Among the seven ITZ-resistant isolates, all were also resistant to ENZ. This phenotype was comparable to that observed for the pan-azole-resistant reference strain CCUG 74258 carrying the TR34/L98H mutation. However, *cyp51A* sequencing revealed sequence variations in resistant isolates, but none corresponded to previously described resistance-associated mutations. These findings may suggest the involvement of potentially novel alterations contributing to azole resistance, or alternative resistance mechanisms independent of *cyp51A*, such as overexpression of efflux pumps, mutations in regulatory pathways, or alterations in sterol biosynthesis. In contrast, strain CCUG 74259, carrying the G54W mutation, was only resistant to ITZ. The TR34/L98H mutation is known to confer resistance to multiple azoles [40]–[43], whereas the G54 substitution is mainly associated with cross-resistance between ITZ and posaconazole [55]–[57]. These observations are consistent with a previous study evaluating ATCC 204305 and TR34/L98H mutant isolates [58]. In this work, ENZ remained effective against both wild-type and mutant environmental isolates collected from agricultural soils and compost.

Table 3: Results of antifungal susceptibility testing of the reference strains used.

| Taxa | Reference strains | ITZ phenotype | ENZ phenotype |
|---|---|---|---|
| *A. fumigatus* | *A. fumigatus* ATCC 204305 | S | S |
| | *A. fumigatus* CCUG 74258 | R | R |
| | *A. fumigatus* CCUG 74259 | R | S |
| *Fusarium* | *F. poae* CBS 128536 | R | R |
| Mucorales | *L. corymbifera* CBS 115811 | S | / |

S: susceptible; R: resistant; /: not tested.

Table 4: Frequency of the different sensitivities to itraconazole (1 mg/L; $N$ = 119 isolates) and enilconazole (0.1 mg/L; $N$ = 24 isolates) depending on the studied isolates.

| Taxa | Isolates ($N$) | ITZ-resistant isolates (%) | ENZ-resistant isolates among ITZ-resistant isolates (%) |
|---|---|---|---|
| ***A. fumigatus*** | **74** | 9.5 | 100 |
| ***Fusarium*** | **13** | 69.2 | 55.6 |
| *F. avenaceum* | **1** | 100 | 100 |
| *F. culmorum* | **4** | 75 | 66.7 |
| *F. graminearum* | **7** | 57.1 | 50 |
| *F. sibiricum* | **1** | 100 | 0 |
| **Mucorales** | **32** | 25 | 100 |
| *A. healeyae* | **1** | 100 | 100 |
| *C. chinensis* | **1** | 0 | / |
| *L. corymbifera* | **9** | 0 | / |
| *L. ramosa* | **14** | 0 | / |
| *M. circinelloides* | **4** | 100 | 100 |
| *M. hiemalis* | **1** | 100 | 100 |
| *M. racemosus* | **2** | 100 | 100 |

S: susceptible; R: resistant; /: not tested.


© 2026 WIT Press
www.witpress.com, ISSN 1743-3541 (on-line)

On the dairy farms studied in Calvados, the prevalence of ITZ-resistant *A. fumigatus* isolates reached 9.5%. In comparison, resistance rates to azoles ranging from 15% to 20% have been reported in some European regions according to WHO data [24], while a study conducted in Italy reported an ITZ-resistance rate of 16.9% in environmental soil samples [59]. In Asia, environmental resistance rates to ITZ and/or voriconazole may reach 80% [24], [60], with approximately 33% of isolates exhibiting cross-resistance phenotypes [60]. In Portugal, 8.1% of *A. fumigatus* isolates collected from air, dust, and respiratory protective devices were resistant to at least one azole [41]. Overall, although resistance rates observed in this study were lower than those reported in several previous investigations, they remain noteworthy and raise concerns regarding the environmental spread of azole resistance and its potential public health implications.

### 3.2.2 Antifungal susceptibility of Mucorales isolates

Among Mucorales isolates, 25% were resistant to ITZ, reaching 100% within the *Mucor* genus and 0% for *Lichtheimia* isolates. All ITZ-resistant isolates were also resistant to ENZ. An environmental study reported ITZ-resistant Mucorales at 4 mg/L, mainly belonging to the *Mucor* and *Rhizopus* genera, with a resistance rate of 3% [61]. This proportion was substantially lower than that observed in the present study.

A study based on CLSI methods and clinical isolates reported ITZ resistance rates reaching 52.6% within the *Mucor* genus [33]. In contrast, no resistance was observed for *L. corymbifera* [33], in agreement with the present results (Tables 3 and 4).

Another study also using the CLSI method reported ITZ MICs ranging from 0.25 to 16 mg/L for *M. circinelloides*, with a modal MIC of 4 mg/L [62]. For *L. corymbifera*, MIC values ranged from 0.06 to 8 mg/L, with a mean MIC of 0.25 mg/L [62]. Overall, our observations are consistent with these previous findings.

### 3.2.3 Antifungal susceptibility of *Fusarium* isolates

Among *Fusarium* isolates collected from Normandy dairy farms, 69.2% were resistant to ITZ (Table 4). However, unlike *A. fumigatus* and Mucorales isolates, not all ITZ-resistant *Fusarium* isolates were resistant to ENZ, as approximately 30.8% remained susceptible.

For the predominant species, *F. graminearum* and *F. culmorum*, susceptibility rates to ENZ reached 42.9% and 25%, respectively, whereas *F. sibiricum* showed susceptibility to ENZ despite ITZ resistance. However, these observations should be interpreted cautiously because of the limited number of isolates studied.

The reference strain *F. poae* (FFSC complex) was resistant to ITZ and ENZ (Table 3).

Using CLSI methodology, a previous study reported that 98.2% of 488 tested *Fusarium* isolates were resistant to ITZ concentrations above 1 mg/L [33]. Another study based on EUCAST methodology reported ITZ susceptibility values ranging from 2 to >16 mg/L within the FFSC complex [30].

## 3.3 Study limitations

Comparisons with previous studies should be interpreted cautiously because the studies differed in geographic scale, environmental matrices, and sampling methodologies. For example, some studies relied on impactor samplers, which collect lower air volumes than the Coriolis® air sampler used in the present study. In addition, cultivated sample volumes in some studies were approximately 6.7-fold lower than those used here.

Most studies used for comparison investigated soil or plant matrices rather than airborne samples collected in dairy farms. Nevertheless, these comparisons remain relevant because


© 2026 WIT Press
www.witpress.com, ISSN 1743-3541 (on-line)

air is considered an important vector for biological contaminants, and these matrices are also present in agricultural environments.

Finally, ITZ resistance thresholds varied between studies. While the present work used the EUCAST breakpoint of 1 mg/L, other studies used thresholds up to 2 or 4 mg/L, particularly when antifungal agar screening methods were applied.

All of these elements can explain the differences in resistance rates between studies.

### 3.4 Statistical analysis

None of the Fisher's exact tests showed statistically significant associations, possibly because of the limited number of isolates included in this study. Post-hoc power analyses revealed low statistical power across all analyses (power range: 0.055–0.089), well below the commonly accepted threshold of 0.80, suggesting a limited ability to detect potential associations. Increasing the sample size would improve the robustness and statistical power of future analyses.

A study conducted in cattle barns in Türkiye showed that seasonal climatic conditions significantly influence airborne fungal biodiversity [63]. *Aspergillus* and *Mucor* species were detected throughout the year, whereas *Rhizopus* species were mainly detected during spring and *Fusarium* species during summer months [63]. In contrast, no association between *Fusarium* occurrence and summer sampling was observed in the present study.

The same study also reported that *Aspergillus* and *Mucor* species were able to grow across a broad range of temperatures (5.5°C–34.7°C) and humidity levels (27%–83%), unlike *Fusarium* species, which appeared more restricted to specific environmental conditions [63]. Temperature and humidity were significantly associated with airborne fungal density ($p < 0.05$).

## 4 CONCLUSION

In conclusion, this study aimed to improve knowledge on the resistance of airborne A. fumigatus, *Fusarium*, and Mucorales isolates to itraconazole (ITZ) and enilconazole (ENZ) in Normandy dairy farms. The results demonstrated the presence of ITZ-resistant isolates among the twelve investigated farms. Overall, 20.2% of isolates were resistant to ITZ, and 83.3% of these resistant isolates also exhibited cross-resistance to ENZ.

The detection of airborne resistance to azoles used in both clinical and agricultural settings represents a potential public health concern, particularly because the environmental dissemination and airborne transmission of resistant fungi remain poorly understood. In addition, important knowledge gaps persist regarding the mechanisms underlying azole resistance, especially in *Fusarium* and Mucorales species.

These preliminary findings suggest that dairy farm bioaerosols may constitute an environmental reservoir of azole-resistant moulds and highlight the need for further investigations within a One Health framework to better understand the emergence, dissemination, and consequences of antifungal resistance for animal and human health. Resistance to azoles is a global challenge, as fungal spores can spread over long distances through the air and potentially contaminate other environments.

From a practical perspective, strengthening azole stewardship in agricultural settings appears essential. Farmers and azole prescribers should follow official recommendations regarding azole use (dosage, timing, and treatment frequency) while avoiding both excessive and insufficient application, as both may contribute to resistance selection. Integrating resistance monitoring and promoting more targeted fungicide use could help reduce selective


© 2026 WIT Press
www.witpress.com, ISSN 1743-3541 (on-line)

pressure. Occupational hygiene should also be reinforced through appropriate personal protective equipment and increased awareness of bioaerosol-associated risks.

Policymakers also play a key role by supporting antifungal stewardship through regulation, surveillance programs, and investment in sustainable alternatives such as biocontrol strategies and novel approaches with different mechanisms of action. Given the socioeconomic constraints faced by farmers, tackling antifungal resistance will require coordinated efforts involving researchers, healthcare professionals, agricultural stakeholders, and policymakers.

## ACKNOWLEDGEMENT

The authors sincerely thank the farmers and farm owners for their participation and cooperation throughout this study. The authors declare no conflicts of interest.

## REFERENCES

[1] Our World in Data, Milk production. https://ourworldindata.org/grapher/milk-production-tonnes. Accessed on: 18 Apr. 2026.

[2] Eurostat, Production and utilization of milk on the farm: Annual data, https://ec.europa.eu/eurostat/databrowser/view/apro_mk_farm__custom_18792604/bookmark/bar?lang=en&bookmarkId=527f32c3-7321-4223-be30-334d9b7903a2&c=1762512571000. Accessed on: 4 May 2026.

[3] Agreste, La statistique agricole. Enquête annuelle laitière 2024. https://agreste.agriculture.gouv.fr/agreste-web/disaron/Chd2602/detail/. Accessed on: 4 May 2026.

[4] DRAAF Pays-de-la-Loire, Direction régionale de l'alimentation, de l'agriculture et de la forêt 2025. Les Pays de la Loire, troisième région de production de lait de vache. https://draaf.pays-de-la-loire.agriculture.gouv.fr/fiche-filiere-lait-de-vache-a2074.html. Accessed on: 18 Apr. 2026.

[5] Pfister, H. et al., Granulometry, microbial composition and biological activity of dusts collected in French dairy farms. *Environmental Research*, **158**, pp. 691–702, 2017. https://doi.org/10.1016/j.envres.2017.07.025.

[6] Lanier, C., Richard, E., Heutte, N., Picquet, R., Bouchart, V. & Garon, D., Airborne molds and mycotoxins associated with handling of corn silage and oilseed cakes in agricultural environment. *Atmospheric Environment*, **44**(16), pp. 1980–1986, 2010. https://doi.org/10.1016/j.atmosenv.2010.02.040.

[7] Basinas, I., Cronin, G., Hogan, V., Sigsgaard, T., Hayes, J. & Coggins, A.M., Exposure to inhalable dust, endotoxin, and total volatile organic carbons on dairy farms using manual and automated feeding systems. *Annals of Work Exposures and Health*, **61**(3), pp. 344–355, 2017. https://doi.org/10.1093/annweh/wxw023.

[8] Basinas, I. et al., Exposure-affecting factors of dairy farmers' exposure to inhalable dust and endotoxin. *The Annals of Occupational Hygiene*, **58**(6), pp. 707–723, 2014. https://doi.org/10.1093/annhyg/meu024.

[9] Mbareche, H., Veillette, M., Bilodeau, G.J. & Duchaine, C., Fungal aerosols at dairy farms using molecular and culture techniques. *Science of The Total Environment*, **653**, pp. 253–263, 2019. https://doi.org/10.1016/j.scitotenv.2018.10.345.

[10] Adelusi, O.A. et al., Seasonal diversity and occurrence of filamentous fungi in smallholder dairy cattle feeds and feedstuffs in South Africa. *Journal of Fungi*, **8**(11), p. 1192, 2022. https://doi.org/10.3390/jof8111192.


© 2026 WIT Press
www.witpress.com, ISSN 1743-3541 (on-line)

[11] Pfister, H. et al., Factors determining the exposure of dairy farmers to thoracic organic dust. *Environmental Research*, **165**, pp. 286–293, 2018. https://doi.org/10.1016/j.envres.2018.04.031.
[12] Oliveira, M., Oliveira, D., Lisboa, C., Boechat, J. & Delgado, L., Clinical manifestations of human exposure to fungi. *Journal of Fungi*, **9**(3), p. 381, 2023. https://doi.org/10.3390/jof9030381.
[13] Denning, D.W. & Perlin, D.S., Azole resistance in *Aspergillus*: A growing public health menace. *Future Microbiology*, **6**(11), pp. 1229–1232, 2011. https://doi.org/10.2217/fmb.11.118.
[14] Madsen, A.M., Zervas, A., Tendal, K. & Nielsen, J.L., Microbial diversity in bioaerosol samples causing ODTS compared to reference bioaerosol samples as measured using Illumina sequencing and MALDI-TOF. *Environmental Research*, **140**, pp. 255–267, 2015. https://doi.org/10.1016/j.envres.2015.03.027.
[15] Chen, Z., He, Y. & Bu, H., Association between sensitization to common fungi and severe asthma. *Frontiers in Public Health*, **13**, 1582643, 2025. https://doi.org/10.3389/fpubh.2025.1582643.
[16] Réau, R. et al., Impact of fungal sensitization on long-term outcomes in severe asthma. *The Journal of Allergy and Clinical Immunology: In Practice*, **14**(1), pp. 196–204, 2026. https://doi.org/10.1016/j.jaip.2025.10.025.
[17] Madrid-Carbajal, C.J. et al., Influence of fungal colonization on exacerbations in patients with cystic fibrosis. *Journal of Fungi*, **10**(12), p. 875, 2024. https://doi.org/10.3390/jof10120875.
[18] Seyedmousavi, S. et al., Fungal infections in animals: A patchwork of different situations. *Medical Mycology*, **56**(suppl_1), pp. S165–S187, 2018. https://doi.org/10.1093/mmy/myx104.
[19] Seyedmousavi, S. et al., *Aspergillus* and aspergilloses in wild and domestic animals: A global health concern with parallels to human disease. *Medical Mycology*, **53**(8), pp. 765–797, 2015. https://doi.org/10.1093/mmy/myv067.
[20] Ryu, D. & Bullerman, L.B., Effect of cycling temperatures on the production of deoxynivalenol and zearalenone by *Fusarium graminearum* NRRL 5883. *Journal of Food Protection*, **62**(12), pp. 1451–1455, 1999. https://doi.org/10.4315/0362-028X-62.12.1451.
[21] Boudra, H. & Morgavi, D.P., Mycotoxin risk evaluation in feeds contaminated by *Aspergillus fumigatus*. *Animal Feed Science and Technology*, **120**, pp. 113–123, 2005. https://doi.org/10.1016/j.anifeedsci.2005.01.006.
[22] Soliman, S.S.M. et al., Mucoricin is a ricin-like toxin that is critical for the pathogenesis of mucormycosis. *Nature Microbiology*, **6**(3), pp. 313–326, 2021. https://doi.org/10.1038/s41564-020-00837-0.
[23] World Health Organization, Mycotoxins. https://www.who.int/news-room/fact-sheets/detail/mycotoxins. Accessed on: 27 Apr. 2026.
[24] World Health Organization, *WHO Fungal Priority Pathogens List to Guide Research, Development and Public Health Action* 1st ed., WHO: Geneva, 2022.
[25] European Food Safety Authority (EFSA), European Centre for Disease Prevention and Control (ECDC), European Chemicals Agency (ECHA), European Environment Agency (EEA), European Medicines Agency (EMA) & European Commission's Joint Research Centre (JRC), Impact of the use of azole fungicides, other than as human medicines, on the development of azole-resistant *Aspergillus* spp. *EFSA Journal*, **23**(1), 2025. https://doi.org/10.2903/j.efsa.2025.9200.


© 2026 WIT Press
www.witpress.com, ISSN 1743-3541 (on-line)

[26] Verweij, P.E., Chowdhary, A., Melchers, W.J.G. & Meis, J.F., Azole resistance in *Aspergillus fumigatus*: Can we retain the clinical use of mold-active antifungal azoles? *Clinical Infectious Diseases*, **62**(3), pp. 362–368, 2016. https://doi.org/10.1093/cid/civ885.
[27] Price, C.L., Parker, J.E., Warrilow, A.G., Kelly, D.E. & Kelly, S.L., Azole fungicides: Understanding resistance mechanisms in agricultural fungal pathogens. *Pest Management Science*, **71**(8), pp. 1054–1058, 2015. https://doi.org/10.1002/ps.4029.
[28] Arastehfar, A. et al., *Aspergillus fumigatus* and aspergillosis: From basics to clinics. *Studies in Mycology*, **100**, 100115, 2021. https://doi.org/10.1016/j.simyco.2021.100115.
[29] Snelders, E., Huis In 'T Veld, R.A.G., Rijs, A.J.M.M., Kema, G.H.J., Melchers, W.J.G. & Verweij, P.E., Possible environmental origin of resistance of *Aspergillus fumigatus* to medical triazoles. *Applied and Environmental Microbiology*, **75**(12), pp. 4053–4057, 2009. https://doi.org/10.1128/AEM.00231-09.
[30] Blaize, M. et al., Antifungal susceptibility of 182 *Fusarium* species isolates from 20 European centers: Comparison between EUCAST and gradient concentration strip methods. *Antimicrobial Agents and Chemotherapy*, **65**(12), 2021. https://doi.org/10.1128/AAC.01495-21.
[31] Almyroudis, N.G., Sutton, D.A., Fothergill, A.W., Rinaldi, M.G. & Kusne, S., *In vitro* susceptibilities of 217 clinical isolates of zygomycetes to conventional and new antifungal agents. *Antimicrobial Agents and Chemotherapy*, **51**(7), pp. 2587–2590, 2007. https://doi.org/10.1128/AAC.00452-07.
[32] Caramalho, R., Tyndall, J.D.A., Monk, B.C., Larentis, T., Lass-Flörl, C. & Lackner, M., Intrinsic short-tailed azole resistance in mucormycetes is due to an evolutionary conserved aminoacid substitution of the lanosterol 14α-demethylase. *Scientific Reports*, **7**(15898), 2017. https://doi.org/10.1038/s41598-017-16123-9.
[33] Borman, A.M. et al., MIC distributions and evaluation of fungicidal activity for amphotericin B, itraconazole, voriconazole, posaconazole and caspofungin and 20 species of pathogenic filamentous fungi determined using the CLSI broth microdilution method. *Journal of Fungi (Basel)*, **3**(2), p. 27, 2017. https://doi.org/10.3390/jof3020027.
[34] Vermeulen, P., Gruez, A., Babin, A.-L., Frippiat, J.-P., Machouart, M. & Debourgogne, A., CYP51 mutations in the *Fusarium solani* species complex: First clue to understand the low susceptibility to azoles of the genus *Fusarium*. *Journal of Fungi*, **8**(5), p. 533, 2022. https://doi.org/10.3390/jof8050533.
[35] Verweij, P.E. et al., Closing the gap on antifungal resistance. *Nature Medicine*, **32**, pp. 1586–1591, 2026. https://doi.org/10.1038/s41591-026-04334-5.
[36] Eurofins Scientific, Eurofins Genomics Germany. https://www.eurofins.com/contact-us/worldwide-interactive-map/germany/eurofins-genomics-germany/. Accessed on: 1 Jun. 2025.
[37] Chen, P., Liu, M., Zeng, Q., Zhang, Z. & Sang, H., Uncovering new mutations conferring azole resistance in the *Aspergillus fumigatus cyp51A* gene. *Frontiers in Microbiology*, **10**, p. 3127, 2020. https://doi.org/10.3389/fmicb.2019.03127.
[38] QIAGEN, CLC Genomics Workbench.
[39] EUCAST, AST of moulds. https://www.eucast.org/astoffungi/methodsinantifungalsusceptibilitytesting/ast_of_moulds. Accessed on: 21 Apr. 2024.


© 2026 WIT Press
www.witpress.com, ISSN 1743-3541 (on-line)

[40] Serrano-Lobo, J., Gómez, A., Rodríguez-Sánchez, B., Muñoz, P., Escribano, P. & Guinea, J., Azole-resistant *Aspergillus fumigatus* clinical isolate screening in azole-containing agar plates (EUCAST E.Def 10.1): Low impact of plastic trays used and poor performance in cryptic species. *Antimicrobial Agents and Chemotherapy*, **65**(8), 2021. https://doi.org/10.1128/AAC.00482-21.
[41] Gonçalves, P. et al., Azole-resistant *Aspergillus fumigatus* harboring the TR34/L98H mutation: First report in Portugal in environmental samples. *Microorganisms*, **9**(1), p. 57, 2021. https://doi.org/10.3390/microorganisms9010057.
[42] Schürch, S. et al., Occurrence of *Aspergillus fumigatus* azole resistance in soils from Switzerland. *Medical Mycology*, **61**(11), 2023. https://doi.org/10.1093/mmy/myad110.
[43] van Dijk, M.A.M. et al., Azole resistance in veterinary clinical *Aspergillus fumigatus* isolates in the Netherlands. *Mycopathologia*, **189**(4), p. 50, 2024. https://doi.org/10.1007/s11046-024-00850-5.
[44] Sharma, C., Hagen, F., Moroti, R., Meis, J.F. & Chowdhary, A., Triazole-resistant *Aspergillus fumigatus* harbouring G54 mutation: Is it de novo or environmentally acquired? *Journal of Global Antimicrobial Resistance*, **3**(2), pp. 69–74, 2015. https://doi.org/10.1016/j.jgar.2015.01.005.
[45] Domsch, K.H., Gams, W. & Anderson, T.H., *Compendium of Soil Fungi*. IHW-Verlag (Eching), p. 672, 2007.
[46] Lumivero, XLSTAT statistical and data analysis solution. 2025.
[47] Lass-Flörl, C., How to make a fast diagnosis in invasive aspergillosis. *Medical Mycology*, **57**(Supplement_2), pp. S155–S160, 2019. https://doi.org/10.1093/mmy/myy103.
[48] Bretagne, S. et al., Active surveillance program to increase awareness on invasive fungal diseases: The French ressif network (2012 to 2018). *mBio*, **13**(3), 2022. https://doi.org/10.1128/mbio.00920-22.
[49] Skiada, A., Pavleas, I. & Drogari-Apiranthitou, M., Epidemiology and diagnosis of mucormycosis: An update. *Journal of Fungi*, **6**(4), p. 265, 2020. https://doi.org/10.3390/jof6040265.
[50] Jeong, W. et al., The epidemiology and clinical manifestations of mucormycosis: A systematic review and meta-analysis of case reports. *Clinical Microbiology and Infection*, **25**(1), pp. 26–34, 2019. https://doi.org/10.1016/j.cmi.2018.07.011.
[51] Gouzien, L. et al., Epidemiology and prognostic factors of mucormycosis in France (2012–2022): A cross-sectional study nested in a prospective surveillance programme. *The Lancet Regional Health – Europe*, **45**, 101010, 2024. https://doi.org/10.1016/j.lanepe.2024.101010.
[52] Tupaki-Sreepurna, A. & Kindo, A.J., *Fusarium*: The versatile pathogen. *Indian Journal of Medical Microbiology*, **36**(1), pp. 8–17, 2018. https://doi.org/10.4103/ijmm.IJMM_16_24.
[53] Akpinar Ergün, I., Sar, T., Şah, I. & Ekinci, H., The importance of *Fusarium* species in agricultural products and potential applications of bioactive agents. *Science and Art in 21st Century Turkey*, **2**, pp. 1672–1686, 2017.
[54] Drouault M. et al., *Ex vivo* effects of bisphenol A or zearalenone on the prepubertal rat testis. *Environmental Toxicology and Pharmacology*, **101**, 104203, 2023. https://doi.org/10.1016/j.etap.2023.104203.
[55] Zhang, J., Li, L., Lv, Q., Yan, L., Wang, Y. & Jiang, Y., The fungal CYP51s: Their functions, structures, related drug resistance, and inhibitors. *Frontiers in Microbiology*, **10**, p. 691, 2019. https://doi.org/10.3389/fmicb.2019.00691.


© 2026 WIT Press
www.witpress.com, ISSN 1743-3541 (on-line)

[56] Rodriguez-Tudela, J.L., Alcazar-Fuoli, L., Mellado, E., Alastruey-Izquierdo, A., Monzon, A. & Cuenca-Estrella, M., Epidemiological cutoffs and cross-resistance to azole drugs in *Aspergillus fumigatus*. *Antimicrobial Agents and Chemotherapy*, **52**(7), pp. 2468–2472, 2008. https://doi.org/10.1128/AAC.00156-08.

[57] Howard, S.J. et al., Frequency and evolution of azole resistance in *Aspergillus fumigatus* associated with treatment failure. *Emerging Infectious Diseases*, **15**(7), pp. 1068–1076, 2009. https://doi.org/10.3201/eid1507.090043.

[58] Sanseverino, I. et al., Activity of azole and non-azole substances against *Aspergillus fumigatus* in clinical and environmental samples to address antimicrobial resistance. *International Journal of Molecular Sciences*, **26**(3), p. 1033, 2025. https://doi.org/10.3390/ijms26031033.

[59] Prigitano, A., Esposto, M.C., Romanò, L., Auxilia, F. & Tortorano, A.M., Azole-resistant *Aspergillus fumigatus* in the Italian environment. *Journal of Global Antimicrobial Resistance*, **16**, pp. 220–224, 2019. https://doi.org/10.1016/j.jgar.2018.10.017.

[60] Zhou, D. et al., Extensive genetic diversity and widespread azole resistance in greenhouse populations of *Aspergillus fumigatus* in Yunnan, China. *mSphere*. **6**(1), 2021. https://doi.org/10.1128/mSphere.00066-21.

[61] Caetano, L.A., Faria, T., Springer, J., Loeffler, J. & Viegas, C., Antifungal-resistant Mucorales in different indoor environments. *Mycology*, **10**(2), pp. 75–83, 2018. https://doi.org/10.1080/21501203.2018.1551251.

[62] Espinel-Ingroff, A. et al., Multicenter evaluation of MIC distributions for epidemiologic cutoff value definition to detect amphotericin B, posaconazole, and itraconazole resistance among the most clinically relevant species of Mucorales. *Antimicrobial Agents and Chemotherapy*, **59**(3), pp. 1745–1750, 2015. https://doi.org/10.1128/AAC.04435-14.

[63] Giray, G. & Şimşek, A., The impact of seasonal climate conditions on fungal biodiversity in barn air. *Journal of the Hellenic Veterinary Medical Society*, **77**(1), pp. 10127–10134, 2026. https://doi.org/10.12681/jhvms.41076.


© 2026 WIT Press
www.witpress.com, ISSN 1743-3541 (on-line)