One Health-United 2026: bridging sectors, breaking barriers, shaping solutions

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Article highlights:

- A symposium providing a discussion forum for health-related topics in mycology.
- United forces to tackle antifungal resistance and fungal disease: the first meeting that united different sectors in a One Health approach creating a broad discussion basis for pooled expertise.

Key words: AMR; AFR; antifungal resistance; antimicrobial resistance; fungal infection; zoonotic fungi; phytopathogenic agent; cross-domain pathogen; invasive fungal infection; aspergillosis; azole; antifungal.

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Introduction

The One Health-United Meeting held in Innsbruck, Austria, 23–25 September 2026, brought together early-career researchers and experts in One Health to discuss fungal infections, antifungal resistance (AFR), current gaps and challenges, and emerging therapeutic strategies. The event combined the **PhD-United One Health Meeting** (23–24 September) with the **ECMM/ISHAM-United Working Group Meeting** (24–25 September). The intentional overlap between the two events on the morning of 24 September provided an inspiring opportunity for exchange and networking, bringing together PhD students from the international mycology community with clinicians, policymakers, and researchers attending the ECMM/ISHAM One Health Meeting.

The meeting was hosted by the Medical University of Innsbruck, Austria, with Michaela Lackner and Paul Verweij serving as conference hosts. The scientific committee comprised convenors and members of the *ECMM/ISHAM One Health Working Groups* and its associated working groups affiliated to ISHAM and/or ECMM: the *Fungal One Health Coalition for Universal Strategies to Contain Antifungal Resistance* (FOCUS AFR), *One Health Sporotrichosis*, *Veterinary Mycology and One Health*, and *Environmental Fungal Exposure and Human Health*.

The program featured a variety of dynamic and interactive formats, including flash talks, poster sessions, and quizzes on antimicrobial resistance (AMR) and One Health. Another highlight was *Moving for Solutions*, a walking activity designed to encourage scientific interaction in an informal setting. Participants had the opportunity to enjoy the fresh Alpine air and explore the scenic surroundings of Innsbruck while taking part in a challenge-based game involving informal questions and activities that encouraged interaction with colleagues from different backgrounds.

The plenary sessions addressed several important themes in contemporary mycology, including equity in access to fungal disease care, the occurrence of clinical and environmental azole-resistant *Candida tropicalis* in Taiwan, and fungal infections and antimicrobial resistance (AMR) in the context of research and development investments and the WHO Blueprint for strengthening responses to fungal disease and antifungal resistance. The program also featured a non-conventional *fishbowl* discussion format designed to stimulate open dialogue on lessons learned from AMR, current challenges, and potential solutions. Discussions highlighted the need to raise awareness of AFR and addressed the limited availability of antifungal treatment options and diagnostic tools. These challenges were further explored from a veterinary perspective, including issues related to surveillance, treatment, and vaccination in feline sporotrichosis, as well as the role of water environments as potential drivers of AFR.

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Throughout the meeting, the importance of interdisciplinary collaboration was emphasized, recognizing the interconnected roles of human medicine, veterinary medicine, ecology, and agriculture in addressing the complex challenges of antifungal resistance. By bringing together perspectives from these complementary fields, the meeting provided a valuable platform for knowledge exchange, discussion, and the development of collaborative approaches to advancing One Health mycology.

Among the challenges that received particular attention was the increasing emergence and spread of antifungal resistance, which threatens the effectiveness of available antifungal treatments and complicates the management of fungal infections. The need to improve surveillance and epidemiological data collection was highlighted in presentations by Richardson *et al.* and Seidel *et al*. Particularly, Richardson *et al*. presented findings from a retrospective survey in the samples sent to the Manchester mycology reference laboratory demonstrating azole resistance in several *Candida* species and *Aspergillus fumigatus*, as well as an increasing proportion of non-*fumigatus Aspergillus* species and fungal organisms not included in the WHO Fungal Priority Pathogens List (FPPL). These findings highlight the importance of broadening surveillance efforts beyond established priority pathogens to better understand the distribution and clinical relevance of AFR.

The emergence and spread of resistant dermatophytes also represent an important challenge for clinical mycology. In particular, the increasing occurrence of *Trichophyton indotineae* infections in Asia raises concerns about the transmission of terbinafine-resistant and multidrug-resistant strains. Tsui *et al*. discussed characteristic resistance profiles and the importance of monitoring these emerging infections to improve our understanding of their epidemiology and spread. Together, these contributions emphasized the need for comprehensive surveillance systems capable of detecting emerging resistance patterns and informing appropriate public health and clinical responses. Alike, ecological dynamics underlying resistance development as a cross-domain opportunistic fungi, in which an ecologically versatile fungal species might be transiently driver of resistance by persisting across environmental, plant, animal, and human reservoirs was also discussed in the work of Roohi *et al*.

Sporotrichosis exemplifies the importance of a One Health approach to emerging fungal diseases, as animal outbreaks can drive zoonotic transmission and threaten human health. The emergence of *Sporothrix brasiliensis* and other outbreak-associated clades in Latin America and Asia highlights the need for enhanced surveillance, particularly of cats originating from Brazil. Shared itraconazole use in humans and cats raises concerns about antifungal resistance, supporting the investigation of alternative feline treatments. Control of the Brazilian epidemic was discussed during the meeting. It requires integrated interventions, including cat castration, management of free-roaming cats, public education, biosafety, and vaccine development, and it was proposed to use the outbreak preparedness framework aligned with Domain 3 of the WHO Blueprint for fungal diseases to guide actions.

Beyond surveillance and epidemiology, the meeting addressed the need to overcome the limitations of the current antifungal arsenal and platforms to test antifungal activity and specificity. The development of new therapeutic strategies and compounds, alongside improved assays and methodologies to accelerate their investigation, was explored in presentations, among others, by Alilou & Haas, Dieleman *et al.*, Sekar *et al.,* Sturm *et al.*, Smith *et al*., Pratt *et al.* and Albanich *et al.* These contributions highlighted the importance of combining innovative drug development with improved diagnostic approaches to address the growing challenges posed by antifungal resistance. In line of this, the use of AI and machine learning tools and long operon reads database was also covered, as shown by Conti *et al.* and Eisele *et al.,* respectively.

Another important theme was the need to understand AFR beyond the clinical setting. The meeting explored the role of environmental factors in the emergence and spread of resistance, including the potential contribution of agricultural waste and practices, emphasizing the importance of design an azole foot printing to our further understanding aspects of the interconnection between agricultural drivers and AFR mainly due to dual usage, as presented by Saxena *et al*. Alike this idea motivated the inspiring question that boosts the urge for accessing why agricultural waste should be integrated in global environmental mycology surveillance, as discussed by Correa-Junior *et al.*

Environmental and clinical resistance mechanisms were discussed in the context of the interconnectedness of human, animal, and ecosystem health. In this regard, the meeting emphasized the importance of integrating findings from clinical and environmental research to better understand the evolution, transmission, and distribution of resistant fungi. For instance, mapping the occurrence of fungal species in soil along a land-use gradient, or in animals from zoological environments was also further discussed by Nowak-Rainer& Neuhauser and Bordoni *et al.* The meeting also shed lights on biomass and bioprocesses conversion as an overlooked source of environmental fungal exposure, linking fungal ecology, biotechnology, and human exposure.

Ultimately, the meeting highlighted the impact on azole exposure/antifungal exposure on mycobiome and health status, and approaches that can be further exploited to check and mapping the dynamic and changing mycobiome and microbiome in health and disease, as discussed by Scheler *et al.*, Rungger *et al.,* Konrath *et al.,* Srinivasan *et al.,* Hamet *et al.,* for example.

The One Health Meeting 2026 provided a comprehensive overview of the key challenges and existing gaps in addressing AFR, while paving the way for promising actions and innovative solutions to prevent and tackle this growing threat. Discussions highlighted the urgent need to harmonize standards, methodologies, and platforms; strengthen surveillance systems; and improve awareness of emerging threats. The meeting also emphasized the importance of making One Health and antifungal resistance key priorities on the global health agenda by engaging relevant authorities, fostering new initiatives, and strengthening regional and international collaboration, partnerships, and networks.

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Metagenomic analysis revealed the fate of antibiotic resistance genes and their mobility from the impact of intense milkfish mariculture

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The rapid growth of aquaculture as a vital protein source has raised concerns due to intensive practices and extensive antibiotic use, which are increasing antimicrobial resistance (AMR). However, mechanisms of AMR gene transfer among aquatic microbes remain largely underexplored. Therefore, we used genome-centric analyses to investigate the distribution and transmission of antibiotic resistance genes (ARGs) in aquaculture, while assessing potential human health risks. Metagenomes from water samples collected at coastal aquaculture facilities in Bolinao, Philippines, were sequenced and assembled. The assembled contigs were annotated with deepARG, and mobile genetic elements were identified using geNomad to assess gene colocalisation. Our findings indicated that ARGs were widely distributed in bacterioplankton communities of the aquaculture and the surrounding coastal ecosystem. In total, 453 ARG-carrying contigs were identified, encompassing 14 unique ARG classes. The most dominant ARG classes were multidrug, peptide, tetracycline, MLS, and β-lactam, accounting for 84% of all ARGs. Furthermore, more ARGs were present on plasmids than on phages and chromosomes. Primary hosts of ARGs included *Vibrio, Stenotrophomonas, Mycobacterium, Pseudomonas, Acinetobacter*, and *Enterococcus*, which were also the main genera harbouring multiple ARGs. In conclusion, coastal areas subjected to intensive aquaculture favour (pathogenic) bacteria resistant to multiple antibiotics with potential for horizontal gene transfer, significantly increasing human and environmental health risks.

Standardized screening of antimicrobial peptides against *Candidozyma auris* and *Nakaseomyces glabrata*

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Invasive *Candida* infections remain a major clinical challenge, driven by rising antifungal resistance and a structurally narrow arsenal of approved drug classes. *Nakaseomyces glabrata* and *Candidozyma auris* are particularly concerning: both are WHO priority pathogens with rising incidence and reduced susceptibility to current therapies. We set out to identify,

repurpose and design antimicrobial peptides (AMPs) active against *Candida albicans* as templates for activity against the resistant species *N. glabrata* and *C. auris*. Starting from the 964 'Anti-*Candida*' and 'Anti-Fungal' entries of the APD3 Database, we deduplicated and manually curated by efficacy against *C. albicans*, peptide length, predicted toxicity, cysteine content, and modification requirements. The filter reduced the pool to 77 candidates, of which 67 were synthesised at greater than 97 percent purity and screened. Activity was tested by broth microdilution against two *C. auris* strains (DSM 105986, 21092), two *N. glabrata* strains (DSM 24506, 70614), and a *C. albicans* reference strain (DSM 11946); hemolysis was assessed against human red blood cells. Across the panel, peptides were consistently more potent against *N. glabrata* than *C. auris*. Eight peptides achieved activity at or below 64 µg/mL against at least one strain (excluding peptides active only against *C. albicans*), and nine were active across all five strains tested. The strongest performers, AP03957 and AP04223, reached minimum inhibitory concentrations of 64 µg/mL against N. glabrata and 128 µg/mL against *C. auris*. Nine peptides were non-hemolytic across the tested range (HC50 above 1024 µg/mL), and the top performers achieved therapeutic indices of 16 against *N. glabrata* and 8 against *C. auris*. Together, these results identify a set of repurposable AMP scaffolds and a rapid discovery framework for next-generation antifungal candidates against the WHO priority yeast pathogens.

From mountain microbes to bioactive molecules: mining Alpine endophytes for novel antimicrobials

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Alpine plants thrive under harsh environmental conditions, including low temperatures, intense UV radiation, and limited nutrient availability. These extreme habitats are increasingly recognized as reservoirs of unique microbial diversity, particularly endophytic microorganisms that colonize healthy plant tissues without causing disease. Such endophytes represent a largely unexplored source of specialized metabolites with potential applications in agriculture and medicine use. To investigate their potential as a source of novel antimicrobial natural products, endophytic fungi were isolated from over 15 alpine plant species. Following small-scale cultivation, isolates were evaluated using an integrated natural product discovery workflow combining cultivation-based approaches, metabolomic profiling using LC-MS/MS, bioactivity assessment, prioritization strategies, and bioactivity-guided isolation. Particular emphasis was placed on identifying metabolites active against plant and human pathogens, addressing the growing need for discovery of new antimicrobials. Screening campaigns revealed a substantial proportion of isolates exhibiting inhibitory activity against for example, *Candida albicans* and *Botrytis cinerea*, highlighting the remarkable biosynthetic capacity of alpine endophytes. Comparative untargeted metabolomics revealed distinct chemical profiles across taxonomic groups, enabling prioritization of promising strains for detailed investigation. Subsequent bioprospecting led to the isolation and characterization of selected secondary metabolites associated with these bioactivities, providing insights into the chemical ecology of alpine plant–microbe interactions and their potential applications. Together, these findings underscore the value of alpine endophytes as an underexplored reservoir of natural products and bioactive leads. The combination of microbial biodiversity research, modern analytical techniques, and natural product discovery approaches offers a powerful strategy for uncovering novel chemistry and bioactive molecules from mountain ecosystems for targeting emerging pathogens.

Traditional andiroba oil production is driven by functionally specialized fungal communities: ecological succession and occupational exposure from a One Health perspective

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Traditional extraction of andiroba (*Carapa guianensis*) oil is a centuries-old Amazonian practice that relies on the natural degradation of seeds prior to oil release. While this bioprocess has long been sustained by Traditional Ecological Knowledge, the fungal communities orchestrating seed decomposition and their implications for environmental and occupational health have remained largely unknown. Environmental fungal communities were investigated using complementary culture-independent and culture-dependent approaches. Metabarcoding was employed to characterize fungal community dynamics across naturally degrading seeds collected from three Amazonian production sites. Cultivable fungi were identified through morphology, MALDI-ToF mass spectrometry, and ITS rDNA sequencing. Functional profiling included screening for hydrolytic enzymes involved in plant cell wall degradation and experimental evaluation of fungal-mediated seed softening. The andiroba mycobiome revealed a highly structured fungal community dominated by filamentous fungi with complementary degradative capacities. Members of *Aspergillus, Penicillium, Fusarium, Rhizopus, Mucor, Talaromyces, Clonostachys, Pestalotiopsis*, and other environmental genera displayed widespread cellulolytic, pectinolytic, xylanolytic, lipolytic, proteolytic, and amylolytic activities, supporting a coordinated ecological process that progressively transforms seed tissues and facilitates traditional oil extraction. Mechanical degradation assays further demonstrated that fungal colonization substantially accelerates seed softening, providing experimental evidence that this traditional production system is supported by naturally occurring fungal succession rather than passive decomposition alone. Notably, several recovered genera include opportunistic pathogens, allergenic fungi, and mycotoxin-producing species, indicating that the same microbial communities responsible for biomass conversion may also constitute an overlooked source of environmental fungal exposure for workers involved in artisanal oil production. These findings redefine traditional andiroba extraction as a naturally engineered microbial bioprocess in which ecosystem functioning, biodiversity, Traditional Ecological Knowledge, and occupational health converge. Our study expands the role of environmental mycology within the One Health framework and highlights traditional Amazonian bioeconomy systems as valuable yet underexplored interfaces linking fungal ecology, biotechnology, and human exposure.

From water systems to indoor air: monitoring fungal communities in a clinical environment

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In recent years, emerging fungal pathogens, including those exhibiting antifungal resistance, have increasingly been recognised as a global burden to human health. One of the important transmission routes of fungal pathogens is tap water, but there is limited data available on fungi present in water transport systems and associated aqueous aerosols. In our project, we are investigating the occurrence, diversity, and resistance profiles of fungi found in tap water, biofilms on materials in contact with water, indoor air, and dispersed aerosols in a clinical environment. Sampling is being conducted monthly over a one-year period at multiple locations in the selected clinic. Fungal communities from air, water and biofilms are analysed using an integrated approach that combines cultivation-based techniques, next-generation

sequencing, and antifungal susceptibility testing. Expected results include a comprehensive characterisation of fungal communities in different environmental niches in the clinic, identification of clinically relevant and antifungal-resistant species, and improved understanding of transmission pathways from the environment to the clinical setting. These findings will help evaluate the risk of fungal infections to patients residing in the clinic for prolonged periods and develop prevention strategies. Future research should focus on developing standardised surveillance protocols, investigating the role of environmental antifungal resistance in clinical outcomes, and the integration of risks posed by pathogenic fungi into broader One Health strategies linking environmental microbiology, public health, and clinical practice.

Environmental diversity of *Aspergillus* spp. in a zoological park: a One Health survey at the human–animal–environment interface

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Aspergillus spp. are ubiquitous airborne fungi occupying diverse ecological niches as decomposers, mycotoxin producers, and opportunistic pathogens. Their occurrence in zoological environments may represent a health concern for both animals and humans, particularly susceptible species such as parrots. This study investigated the presence of environmental *Aspergillus* species in an Italian zoological park using traditional and molecular identification. Environmental monitoring was conducted between May 2025 and May 2026. Passive air sampling was performed monthly in parrot aviaries using settle plates. In addition, fruit samples from aviaries, soil from different animal enclosures, and water samples were collected and cultured on Sabouraud's dextrose agar. Air samples were incubated at 26°C, whereas soil, water, and fruit samples were incubated at 37°C to favour the recovery of potentially pathogenic species. Fungal isolates were identified by sequencing the β-tubulin gene, while calmodulin sequencing was performed for isolates requiring higher taxonomic resolution. A total of 79 environmental *Aspergillus* isolates were recovered. Molecular characterization revealed a highly diverse *Aspergillus* community across the investigated matrices. Species belonging to section *Nigri* were the most frequently recovered, with *A. tubingensis* and *A. niger* predominating. Members of sections *Flavi* (*A. flavus*, *A. parasiticus*), *Circumdati* (*A. westerdijkiae*, *A. melleus*), *Terrei* (*A. terreus*, *A. floccosus*), *Fumigati* (*A. fumigatus*), *Versicolores* (*A. versicolor*), and *Nidulantes* (*A. nidulans*) were also identified, together with the xerophilic species *A. chevalieri* and *A. montevidensis*. Several isolates were identified as cryptic species, which are not reliably distinguishable by morphology alone and may display reduced susceptibility or intrinsic resistance to commonly used antifungal agents, with potential clinical relevance. These findings support a One Health mycology approach, highlighting the importance of molecular environmental surveillance to better understand shared fungal reservoirs at the human–animal–environment interface and to improve assessment of potential exposure risks relevant to both animal health and public health.

The new urban wastewater directive is out – with fungi not in!

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The recast EU Urban Wastewater Treatment Directive (EU) 2024/3019 strengthens treatment and formalises wastewater-based surveillance but does not explicitly include fungal pathogens or antifungal residues; despite exposure pathways during effluent discharge and reclaimed-water reuse. To highlight this surveillance/regulatory gap

and propose a pragmatic, risk-based addition of fungi and antifungals to wastewater monitoring. Key elements of the new EU wastewater framework with current evidence on fungal wastewater surveillance, antifungal residues (selection pressure) and the use of predicted no-effect concentrations (PNECs), complemented from hospital-sewershed monitoring. Existing reuse criteria typically rely on bacterial indicators (e.g., *E. coli*) and omit fungi/antifungals. Global initiatives (e.g., GloWACON) prioritise *Candida auris* and *Aspergillus fumigatus sensu stricto* for environmental wastewater surveillance. PNECs provide a practical basis to prioritise antifungals and define acceptable levels in effluents/reclaimed water, supporting integrated monitoring of targets plus residues. Adding fungal targets and antifungal residues as a tiered module (qPCR screening, culture/AST where feasible, sequencing as needed) would better align wastewater surveillance with One Health risk management and reclaimed-water safety, while enabling threshold-setting as QMRA evidence emerges.

From environmental reservoirs to clinical relevance: an integrative early-warning framework for emerging fungal pathogens

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Emerging fungal pathogens represent a growing challenge for global health, particularly at the interface between environmental, animal, and human systems. Unlike many bacterial or viral pathogens, most clinically relevant fungi are environmental organisms that acquire opportunistic pathogenic potential through ecological adaptation, increased host exposure, and selection in anthropogenic environments. Within a One Health perspective, understanding these transitions requires integrating ecological, veterinary, environmental, and clinical information. Here, we developed an integrative early-warning framework combining strain-isolation metadata, substrate ontology, bibliometric analysis, and trait-based interpretation to identify fungal taxa with emerging medical relevance. Using 26,459 strains representing 847 medically relevant fungal species from the MIRRI and Westerdijk Institute databases, we classified fungal isolation sources into environmental, animal-associated, and human/hospital-related categories. We then compared ecological occurrence patterns with medically oriented literature trends over time. A strong correlation was observed between host-associated isolation frequency and medical-literature representation, particularly among WHO-priority fungal pathogens. Temporal analyses identified taxa showing increasing medical attention despite limited current recognition, including *Aspergillus fischeri*, *Magnusiomyces capitatus*, *Nannizzia gypsea*, and *Trichophyton indotineae*. Many of these species occupy ecological niches bridging natural and anthropogenic environments, such as hospitals, indoor habitats, contaminated substrates, companion animals, and agricultural systems. Thermotolerance, melanization, stress tolerance, and persistence in built environments emerged as recurrent traits associated with opportunistic potential. Our results highlight how environmental reservoirs, animal hosts, and human-modified ecosystems collectively contribute to fungal emergence. By integrating ecological and bibliometric signals, this framework provides a complementary One Health surveillance strategy capable of identifying fungi transitioning from environmental obscurity to clinical relevance before they become major public-health threats. Such proactive approaches are increasingly important in the context of climate change, urbanization, global trade, antifungal resistance, and intensified human–environment interactions.

Urbanization impacts the fungal and bacterial community of soils and its antibiotic resistome by reshaping the diversity and network complexity

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Urbanization imposes severe anthropogenic pressures on ecosystems, yet its impact on the structural cohesion of environmental mycobiome and microbiome and the co-occurrence of antimicrobial resistance (AMR) remain poorly understood. We combined metabarcoding (16S/ITS2) analysis with cultures and *in vitro* AMR screening of isolates across soil samples in Nouvelle-Aquitaine (Southwest region of France). Co-occurrence and bipartite network inferences as well as Random Forest models (RFM) were used to identify and quantify ecological drivers and to evaluate network resilience via percolation simulations. While urbanization diversity indices did not significantly differ between urban and natural habitats, RFM revealed that microbial richness is driven by distinct, continuous local gradients. Urbanization triggered severe structural fragmentation of fungal and bacterial networks, marked by a collapse in edge density, higher modularity, and eradication of critical connectors. Percolation analysis confirmed this fragility, showing accelerated network collapse under targeted attacks in urban settings. Bipartite networks focusing on the environmental resistome revealed a striking paradox: whilst urbanization fragmented the whole fungal-bacterial networks, it significantly intensified the bipartite network when antibiotic resistance is taken into account. By structuring this new network, around nodes of cefalexin, amoxicillin and fosfomycin, it exhibited an increase in structural stability and in positive correlation, and a doubling of inter-domain (fungal-bacterial) connectivity. This study reveals that urbanization induces a trade-off between the overall cohesion of fungal and bacterial ecosystems and the stability of specialized AMR-networks. It highlights the interest of integrating high-resolution network analysis into surveillance of global AMR, and deciphering environmental mycobiome and microbiome evolution under global changes, to accurately predict and intercept the AMR spread in a One Health approach.

Molecular epidemiology of drug resistant *Trichophyton* pathogens in Singapore

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Trichophyton is the major pathogen accounting for over 70% of all dermatophytes in the world. The issue of terbinafine-resistant or even multidrug-resistant dermatophyte infections has been intensifying and raising significant concern, particularly with the widespread dissemination of *T. indotineae* originated from the Indian subcontinent. Sporadic *T. indotineae* infections have been recently described in Singapore and have received some clinical attention, but there are currently no large-scale epidemiology studies on terbinafine-resistant dermatophytosis caused by *T. indotineae* and related species. We are using a genomic approach to study the genetic relationship among 83 local *Trichophyton* isolates from two hospitals. Among them, 48 belonged to *T. indotineae*, followed by *T. interdigitale* (*n*=23) and *T. rubrum* (*n*=12). While comparing local *T. indotineae* samples to global isolates, the genetic variability was low, indicating the clonal spread of

this pathogen. The presence of genetic clusters indicated the circulation in the community. Signature mutations in SQLE (e.g. F397L) and ERG11 (e.g. Y444H) that confer resistance to terbinafine and azoles are detected. Our data reveals the importance of genomic surveillance to understand the spread and transmission of emerging terbinafine-resistant dermatophyte in Asia.

Development of operon-long read technologies and databases for yeast identification

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Over the past decades, yeast identification has largely relied on DNA barcoding approaches, with the Internal Transcribed Spacer (ITS) region emerging as the standard marker for fungal taxonomy. This framework, initially developed in the Sanger sequencing era, enabled robust species-level identification but remained constrained by limited throughput and fragment length. The advent of high-throughput sequencing technologies, particularly Illumina platforms, revolutionized microbial ecology by enabling large-scale metabarcoding studies. However, the use of short reads introduced new limitations, including: reduced taxonomic resolution, dependence on partial barcode regions, and the need for increasingly complex bioinformatic pipelines and curated reference databases. More recently, long-read sequencing technologies, such as Oxford Nanopore (MinION), have opened new opportunities by allowing the sequencing of full-length ribosomal operons (including 18S–ITS1–5.8S–ITS2–28S regions). This approach significantly enhances taxonomic resolution and enables more accurate discrimination of closely related yeast species. Our research aims to develop and implement integrated long-read sequencing pipelines, combining wet-lab protocols and bioinformatic workflows, to improve yeast identification. The focus will be also on highlighting the central role of curated and dedicated reference databases, as the accuracy and resolution of long-read-based identification approaches are inherently constrained by the quality and consistency of available reference data. Furthermore, our study explores emerging applications of long-read technologies in environmental and applied microbiology, including operon-based metabarcoding and targeted metagenomics of functional gene clusters, particularly in systems where shotgun metagenomics is limited by community complexity or low DNA input. Overall, this work emphasizes how technological innovation is reshaping both the resolution and the conceptual framework of microbial identification, calling for parallel advances in database design and data integration.

The missing link in One Health: why agricultural waste must be integrated into global environmental mycology surveillance

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Poultry litter represents a highly dynamic and nutrient-rich agricultural matrix that can select, concentrate, and disperse opportunistic fungi, posing significant challenges at the animal-environment-human interface. This study established an integrative One Health framework to map the qualitative diversity of both yeasts and filamentous molds in commercial broiler farms in Rio de Janeiro, Brazil, while evaluating routine diagnostic boundaries, microstructural fitness, and baseline antifungal susceptibility. Fungal recovery was performed using culture-dependent methods, followed by proteomic screening via MALDI-TOF mass spectrometry and molecular validation through ITS rDNA sequencing. The mycobiome profiling revealed a coexistence of high-consequence molds and highly adapted non-conventional yeasts. Filamentous mold monitoring successfully identified high-confidence isolates of *Aspergillus fumigatus* (scores 2.01–2.26), *Aspergillus niger* (1.86–1.94), *Lichtheimia corymbifera* (2.24), *Scedosporium* sp. (2.05), and *Penicillium* sp. Concurrently, 29 yeast isolates were recovered, dominated by *Trichosporon asahii* and *Candida palmioleophila*. Standard proteomic workflows failed to resolve 31% of the yeast subset, yielding low-confidence scores or 'no peak found' profiles. ITS sequencing successfully bypassed these diagnostic blind spots, unveiling rare ascomycetes including *Blastobotrys chiropterorum*, *Blastobotrys allociferrii*, and members of the *Debaryomyces hansenii* species complex. Functional profiling demonstrated that these non-conventional yeasts possess advanced fitness adaptations: dense cell wall chitin deposition (mechanical shielding), massive intracellular neutral lipid reservoirs, absolute halotolerance, and robust adherence with extracellular polymeric matrix (EPS) production on abiotic surfaces. Pharmacological screening exposed critical multidrug resistance boundaries. *Blastobotrys* strains exhibited high azole tolerance, with fluconazole MICs reaching 64 µg/mL and a prominent micafungin tolerance up to 8 µg/mL in *B. chiropterorum*. Polyene MICs remained low across all cohorts. Our findings demonstrate that poultry litter acts as a critical environmental reservoir of multidrug-resistant and diagnostically elusive fungi, emphasizing the urgency of integrating secondary agricultural waste management into global One Health surveillance programs.

Fungal surveillance in The Netherlands

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The RIVM aims to detect, control, and prevent infectious diseases to protect public health in The Netherlands. Fungal infections represent a significant and growing concern. Worryingly, resistance to antifungal agents is also increasing, largely linked to extensive agricultural fungicide use and the omnipresence of fungi in the environment. Addressing this issue requires a One Health approach. Over the past five years, RIVM has established a foundation for fungal surveillance, collaborating with Radboudumc-CWZ Center of Expertise for Fungal Infections within the National Reference Laboratory for Invasive Mycoses. Together, we developed a comprehensive surveillance infrastructure integrating clinical, diagnostic, and whole genome sequencing capabilities. Nationwide, clinical and environmental fungal isolates are collected and analyzed to monitor antifungal resistance and emerging fungal pathogens, such as *Candidozyma auris*. Several surveillance programmes now target high-priority pathogens including *Aspergillus fumigatus* and *C. auris*, with additional focus on resistant *Aspergillus nigri* species complex, dermatophytes, and non-albicans *Candida* species. Data on resistance trends in *A. fumigatus* and *C. auris* are included in the annual 'Nethmap One Health' report. An automated whole genome sequencing platform enables rapid outbreak analysis for *C. auris*, and a nationwide automated antimicrobial resistance surveillance system is being validated for candidemia surveillance. In conclusion, the Dutch National Reference Laboratory has laid the groundwork for fungal surveillance in The Netherlands. However, given the cross-border nature of infectious diseases, expanding and enhancing surveillance at national and international levels is vital. Strengthened European collaboration will facilitate comprehensive monitoring and timely detection of emerging threats, such as resistant *Trichophyton indotineae*, increasing resistance in *Candida* species, and rising infections by diverse *Aspergillus* species. A coordinated multinational approach is essential for effective fungal disease management.

Exploring efflux pump-mediated azole resistance in *Aspergillus fumigatus*

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Aspergillus fumigatus is a major fungal pathogen and poses a substantial clinical burden due to its high annual death toll. The management of *A. fumigatus*-caused aspergillosis is increasingly challenged by rising antifungal resistance, highlighting the need to better understand the underlying mechanisms of resistance. Unlike the well-studied cyp51A gene, other drivers of azole resistance like efflux remain largely understudied. Therefore, the aim of this study was to investigate the role of both known and previously uncharacterised ABC-transporters in *A. fumigatus* azole resistance. To elucidate the function of these ABC-transporters, *A. fumigatus* overexpression strains were generated for all putative transporters through protoplast-mediated CRISPR/Cas9 transformation. These respective mutant strains were screened for altered azole resistance phenotypes through spot assays. Strains exhibiting altered phenotypes were further analysed to quantify the impact of ABC-transporter overexpression on azole susceptibility and sterol content, and to study the subcellular localization of newly identified transporters. By using this approach, we identified 10 ABC-transporters – including Cdr1B and AtrF – that affected azole susceptibility upon overexpression. Interestingly, one uncharacterised transporter conferred pan-azole resistance when overexpressed. Our findings demonstrate that previously undescribed ABC-transporters affect *A. fumigatus* azole susceptibility. These transporters confer either specificity for a particular azole or broader altered azole resistance. Although speculative, mutations leading to ABC-transporter overexpression may act independently or synergistically with cyp51A-associated mutations, potentially contributing to the emergence of unique azole resistance phenotypes in both clinical and environmental contexts.

Fungal zoonoses: host immune response and challenges in surveillance, prevention and treatment of animal population (with a focus on cat-transmitted sporotrichosis)

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Fungal zoonoses are increasingly recognized as an important challenge for both veterinary and public health due to their impact on animal welfare, human health, and environmental interfaces. Among these diseases, cat-transmitted sporotrichosis, primarily caused by *Sporothrix brasiliensis* in South America and to a lesser extent by *S. schenckii* in Southeast Asia, has emerged as an important public health concern. This zoonotic form spreads efficiently and can cause outbreaks, with domestic cats serving as both highly affected hosts and main drivers of transmission to other cats and humans. This presentation will discuss the current challenges associated with surveillance, prevention, diagnosis, and treatment of fungal zoonoses affecting animal populations, with special emphasis on cat-transmitted sporotrichosis. Topics will include epidemiological trends, barriers to early diagnosis and reporting, antifungal treatment limitations,

therapeutic adherence, the emergence of antifungal resistance, and ongoing efforts to vaccine development. In addition, the talk will explore the importance of integrated One Health strategies involving veterinarians, physicians, public health authorities, and communities to improve disease control and reduce zoonotic transmission. Preventive measures such as responsible animal care, early case identification, public education, and access to veterinary treatment are essential to mitigate the spread of infection. Strengthening laboratory capacity and surveillance systems will also be highlighted as critical steps for improving outbreak monitoring and guiding evidence-based interventions. By addressing the interconnected challenges of animal, human, and environmental health, this discussion aims to contribute to broader awareness and collaborative approaches for managing fungal zoonoses worldwide.

FungAI: using machine learning for the identification of fungi in patient isolates

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Currently, morphological identification of fungi relies on expert knowledge. Molecular methods allow faster identification with rapid throughput but are typically associated with higher cost. AI image classification offers the advantage of low costs and high throughput. Therefore, this project aimed to train specialized artificial intelligence (AI) models to enable a high confidence genus-level discrimination of fluorescence microscopy images of three clinically important fungal pathogens, *Aspergillus* spp., *Candida* spp. and *Mucorales* spp. The AI models were trained on retrospective data. Original patient material containing fungi was stored at –80°C between 2018 and 2026. Fungal elements in patient samples were analysed using Calcofluor White fluorescence microscopy and a 100× oil-immersion objective. 2006 images of *Aspergillus* spp. isolates of 70 patients, 1030 images of *Candida* spp. of 75 patients and 327 images of *Mucorales* spp. of 7 patients were split into training, validation and test sets and pre-trained convolutional neural networks (CNNs) were trained. When combining the output of the three best performing CNNs using a soft majority vote classifier, 97% (232/239) of *Aspergillus* spp. and 93% (145/156) of *Candida* spp. and 89% (31/35) of *Mucorales* spp. images in the test set were correctly identified. Misidentified images typically had low contrast, artifacts and/or little fungal material in the image. When looking at the isolate level instead of the image level, 100% (18/18) of *Aspergillus* spp. and 100% (20/20) of *Candida* spp. and 100% (4/4) of *Mucorales* spp. were identified correctly. Altogether, the majority vote of different CNNs enables reliable discrimination between *Aspergillus* spp., *Candida* spp. and *Mucorales* spp.

Evidence that increased azole persistence and stress resistance precede the in vivo evolution of azole resistance in *Aspergillus fumigatus*

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Aspergillus fumigatus is the major causative agent of human aspergillosis. Azoles are the first-line therapy, but resistance is increasing. Here, we characterized voriconazole persistence by screening a global collection of 495 clinical and environmental *A. fumigatus* isolates. We demonstrate two persistence phenotypes: non-growth persisters (NGPs) and slow-growth persisters (SGPs). We focused on nine sequential clinical isolates from a single patient treated

with voriconazole for 2 years. Genome sequencing and phylogenomic analyses show that these isolates form a single genetically related population exhibiting within-host diversification. While temporal isolation order does not strictly correlate with genetic relatedness, we observe that increased azole persistence and stress resistance are early adaptive steps in the *in vivo* evolution of azole resistance, preceding the emergence of voriconazole resistance. Our findings indicate that increased persistence and stress resistance may create a permissive adaptive landscape within the host, highlighting the complex evolutionary dynamics underlying antifungal treatment failure.

Aquatic fungi: occurrence and response to environmental stressors

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Aquatic fungi play an important role in many aquatic systems as saprotrophic fungi are central for carbon and nutrient cycles and parasitic fungi for food web interactions and control of mass blooms, e.g. of toxic cyanobacteria. To better understand the controlling factors of fungal communities in lentic and lotic ecosystems across Europe, thereby comparing protected *vs.* non-protected ecosystems. Furthermore, we conducted lab experiments to study the role of parasitic fungi for food algal bloom development and control of, for example, toxic cyanobacteria and flow of carbon, nutrients, and energy through aquatic food web under changing environmental conditions. Our results highlight that natural fungal communities in aquatic ecosystems are exposed to numerous environmental stressors including fungicides used in agriculture. Furthermore, parasitic fungi have the potential to control or to prolong toxic cyanobacteria blooms in relation to environmental factors such as nutrient availability. Consequently, aquatic fungi are key to aquatic ecosystem functioning and health, but their ecological functions are to a large extent determined by environmental factors and anthropogenic activities. As a result, aquatic fungi are essential for human health as they are essential for water quality and subsequently human health.

Early inflammatory macrophage reprogramming by *Scedosporium apiospermum* determines fungal survival

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Scedosporium is the second most prevalent airway colonization fungal agent after *Aspergillus* in cystic fibrosis. We hypothesized that disparities in terms of cell wall structure and conidia morphology between both fungi, may affect interactions with host innate cells, that may ultimately affect fungal survival within the host. The objectives of this study were i) to perform the first comparative transcriptomic analysis of human macrophages challenged with *S. apiospermum* (Sap) or *A. fumigatus* (Afu) conidia, at two distinct time points, *i.e.* 4 h and 12h, ii) to measure the inflammatory cytokine response and iii) to assess the survival of Sap and Afu conidia after macrophage challenge. All experiments were performed with conidia harvested from 9 day-cultures of the wild-type 14462 *Scedosporium apiospermum* strain (Sap) and the wild-type Af 293 *Aspergillus fumigatus* strain. Macrophages derived from human peripheral blood mononuclear cells (PBMCs). RNAseq was performed from macrophage lysates after 4h and 12h infection at 37°C with Sap or Afu conidia (MOI

10:1). Bioinformatics analysis was conducted to identify differentially expressed genes (DEGs - log2fold-changeI>1). Cytokines were measured in culture supernatants after 12h infection at 37°C using a 13plex-bead-based immunoassay LEGENDplex® assay (BioLegend). After co-incubation at 37°C for 6h, the killing macrophage of Af and Sap conidia by macrophages was determined by cytometry assay after propidium iodide staining. In this work, we observed that at 4h post-infection, a total of 15 pathways involved in inflammatory processes were differentially expressed in Sap-infected macrophages (FDR of 4.10^{-2} to 7.10^{-13}), of which 12 were concomitantly enriched in Afu-infected macrophages (FDR of 4.10^{-2} to 0), independently of the sense of variation. The four more strongly dysregulated pathways were signaling pathways associated with TNF, IL-17, NF-kappa B. Interestingly, Sap triggered a stronger and earlier activation of pro-inflammatory pathways. Sap infection also triggered a higher release of IL-6, IL-1β, TNF-α, IL-23, IL-10 ($p<0.001$) and IFN-α2 ($p<0.05$) in culture supernatants. If the rate of infected macrophages was similar at 4h between both fungi, the killing of Sap conidia was higher than Afu conidia after 6h co-incubation (46.6% *vs* 34.5%, $p<0.05$), reflecting a lower ability of Sap to survive macrophage antifungal mechanisms. In conclusion, *S. apiospermum* tailored a stronger and quicker inflammatory macrophage response, which could mediate a facilitated clearance, compared to *A. fumigatus*. An excessive inflammatory response could be deleterious for the host in the long term, especially in the specific context of cystic fibrosis.

Pulmonary microbiota and mycobiota during *Pneumocystis jirovecii* pneumonia in immunocompromised patients not infected with HIV

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The detection of *P. jirovecii* by quantitative PCR in bronchoalveolar lavage fluid (BALF) does not distinguish between colonisation and disease. Identifying a bacterial or fungal signature within the microbiota that accompanies, or even promotes, the transition from colonisation to pneumocystosis would provide a useful indicator for modifying patient management. Here, we analysed a cohort of Immunocompromised patient with cancer (n=30) or chronic inflammatory diseases (CID) (n=30) who were classified as uninfected, colonised or infected with *P. jirovecii* were included. Microbiome analysis using targeted metagenomics (V3-V4 region of 16S rRNA for bacteria, and ITS2 for fungi) was performed on BAL samples. Taxonomic identification was conducted using the Kraken2 software allowing to evaluate bacterial and fungal alpha-diversity (observed OTUs and Shannon index) and relative fungal or bacterial abundance (RFA and RBA) in each group of patients. 6,728 and 2,425 bacterial and fungal OTUs were identified within the 60 BALF. Fungal alpha diversity was lower (< 300 Observed OTUs; Shannon index < 2) than bacterial diversity (Observed OTUs > 500; Shannon index > 3). Further analysis reveals dysbiosis dependent on the underlying pathology. In terms of fungi, pneumocystosis is accompanied with an over-representation of *Malassezia*, particularly in the CID group (RFA= 53.7%). Besides, the microbiota from colonised patients is enriched with opportunistic bacteria, *Stenotrophomonas* in cancer patients (RBA= 3.04%), and *Haemophilus* in both groups (RBA= 3.34% and 17.49% in cancer and MIC patients respectively). Altogether, these specific microbial signatures could either correspond to an early marker of the transition or be directly involved in the process. These preliminary findings require comparison with other patient groups and prompt further exploration of the mechanisms involved in the colonisation-to-infection transition

Paracellular transmigration and intracellular localization of *Stenotrophomonas maltophilia* in polarized human airway epithelial cells

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Chronic airway diseases often require repeated and long-term antibiotic therapy, which promotes the emergence and persistence of antimicrobial resistance. *Stenotrophomonas maltophilia* is an opportunistic multidrug-resistant pathogen that is increasingly isolated from patients with cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD). Although its clinical importance is well recognized, its contribution to airway infection and its interaction with the host epithelium remain incompletely understood. We established an infection model using differentiated Calu-3 human airway epithelial cells cultured at the air-liquid interface to investigate host-pathogen interactions of the clinical *S. maltophilia* isolate Sm314. Confocal laser scanning microscopy (CLSM) and transmission electron microscopy (TEM) images revealed that *S. maltophilia* cells were located in the intercellular space between neighboring Calu-3 cells. Quantification of the bacterial CFU in the basolateral chamber indicated that *S. maltophilia* is able to cross the Calu-3 epithelial barrier. Interestingly, this process is dependent on the expression of two putative proteases, DegP and DegQ, as demonstrated using corresponding *S. maltophilia* knockout mutants. *S. maltophilia* DegP and DegQ are homologs of HtrA family proteases which are involved in degradation of host extracellular matrix proteins, proteoglycan, or E-cadherin in various pathogens. Notably, we also identified intracellular bacterial cells either in membrane-bound compartments, in LAMP1-positive compartments or as free single cells in the host cell cytoplasm. Our data suggest that cell-cell contacts of polarized host cells serve as an entry portal for *S. maltophilia* Sm314, leading to the disruption of the junctional complex, opening of the intercellular space, and paracellular transmigration of bacterial cells within the first hours after infection. Together, these findings reveal previously unrecognized aspects of *S. maltophilia* pathogenicity and establish a foundation for future bacterial–fungal co-infection studies.

Azole resistance in *Fusarium* strains isolated from maize in Lower Austria

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Azoles are essential antifungals used in medicine, veterinary medicine and agriculture. However, the continuous application of azoles, especially in agriculture, drives the selection for azole resistant fungi. Between 2015 and 2024 *Fusarium* strains were isolated from maize grown in Lower Austria. For a preliminary screening we randomly selected 5 strains from each year (except 2022) for an initial test on resistance towards the triazoles propiconazole and tebuconazole which are widely used in agriculture. In a first setup growth was assessed in presence of azole concentrations between 12.5 µg/mL and 200 µg/mL and there we found that several isolates are still able to grow in presence of 200 µg/mL propiconazole. Even though the susceptibility towards tebuconazole was generally higher, two isolates showed growth beyond 100 µg/mL tebuconazole. While *F. proliferatum*, *F. awaxy*, and *F. annulatum* isolates showed moderate resistance (up to 50 µg/mL) towards the tested azoles, several strains such as *F. tricinctum*, *F. solani*, *F. campestre*, and *F. annulatum* showed high resistance levels (from 100 µg/mL to 200 µg/mL). The strain with the highest resistance level beyond 200 µg/mL against propiconazole as well as tebuconazole was an *F. solani* isolate, which is also an opportunistic human pathogen.

Exploring the molecular mechanism of small antifungal peptides derived from the γ-core of the *Penicillium chrysogenum* antifungal protein (PAF)

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Despite the common occurrence of fungal infections, antifungal treatment is limited to only four classes of antifungals and resistance has become increasingly common in recent years. Hence there is an urgent need for novel antifungals with unique properties and mode of actions (MOA). Promising candidates are antimicrobial peptides derived from natural sources. This study aimed to investigate the mechanism underlying the antifungal action of the synthetic peptide Pγopt, which mimics the structural γ-core motif of the *Penicillium chrysogenum* antifungal protein (PAF). The antifungal mode of action was investigated by studying the cellular effects of Pγopt in *Candida albicans* as well as the cellular localization of fluorescently labelled Pγopt. Our data showed no membrane depolarization or sustained permeabilization was observed for Pγopt when compared to a membrane-active antimicrobial peptide that had been previously studied in our lab and was used as a control. Instead, Pγopt was taken up by *C. albicans* cells in a concentration- and energy-dependent manner. Furthermore, an increase in intracellular reactive oxygen species was observed, which might contribute to the Pγopt-specific fungicidal effect. While many antimicrobial peptides act on the membrane of pathogens, our results indicate a different MOA behind the antifungal activity of Pγopt. This peptide seems to exhibit no membrane-activity. Instead, it is taken up by the yeast cells and induces cell death via an unknown mechanism. Hence, a further characterization of the uptake mechanism and the cellular response of *C. albicans* cells to peptide treatment is needed to achieve a deeper understanding of the antifungal MOA of Pγopt on a molecular level. This might also help uncover novel targets or mechanisms that could be used to develop new therapeutic options against fungal infections.

Latin American *Aspergillus fumigatus* environmental azole resistance survey 2021–2024 in 12 countries / 26 laboratories

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Aspergillosis is caused by 4 of the more than 250 *Aspergillus* species described (*A. fumigatus*, *A. flavus*, *A. terreus*, and *A. niger*), with most infections caused by *A. fumigatus*. Aspergillosis is primarily treated with azoles, with voriconazole being the drug of choice for treatment. However, over the last decade, increasing rates of azole resistance have been reported globally. As infections are typically caused via inhalation of fungal spores from the environment, the increasing use of azole fungicides in agriculture, which closely resemble those used to treat human disease, is thought to be a major driver of resistant strains. To close the knowledge gap, a survey of azole resistance *A. fumigatus* throughout Latin America, using the existing Latin American Medical Mycology Network, has been carried out using a citizen science approach from 2021 to 2024. Air sampling was conducted by ~1000 volunteers across Chile, Colombia, Costa Rica, Ecuador, Guatemala, Mexico, Paraguay, Peru, and Uruguay, yielding 6954 air samples. Isolates were identified

to species level by MALDI-ToF or β-tubulin sequencing. 2152 *A. fumigatus* strains were isolated. MICs against clinically relevant azoles were determined. All resistant isolates and representative non-resistant isolates were further studied via microsatellite (STRAf loci) typing and sequencing of the cyp51A gene. Whole-genome sequencing was performed on 300 isolates. 3.9% of environmental *A. fumigatus* isolates were resistant to azoles. Phylogenomics analysis identified all three previously described major *A. fumigatus* clades in Latin America. Population structure analysis identified multiple subclades representing recombinants among the major clades. Recombination is one of the pathways of the spread of pan-azole resistance within the population, besides the spread of known resistant genotypes. This study is the first covering most of Latin America, indicating a relatively low prevalence of environmental resistance in the context of widespread usage of agricultural azoles.

Azole-induced modulation of reactive oxygen species and nuclear factor erythroid 2-related factor 2 activation in hepatic cells

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Fungal infections represent a growing public health concern. Azoles are widely used antifungal agents, but their clinical use is limited by hepatotoxicity, endocrine disruption, and drug–drug interactions. This study investigated the redox-modulating properties of selected azoles and their impact on cellular stress responses, focusing on ROS dynamics and nuclear factor erythroid 2-related factor 2 (Nrf-2)-dependent antioxidant responses. HepG2 and HepG2-derived reporter cell lines were exposed to ketoconazole and miconazole. Cytotoxicity was assessed using a resazurin reduction assay following 24-, 48-, and 72-hour exposures. Intracellular ROS generation and/or scavenging were quantified using dichlorodihydrofluorescein diacetate under basal conditions and following chemically induced oxidative stress. Activation of the Nrf-2/antioxidant response element (ARE) pathway was determined using a β-lactamase reporter assay. Cytotoxic effects were observed at concentrations of 80 µM and above for ketoconazole and 120 µM and above for miconazole, becoming apparent after 48 and 72 hours of exposure. Acute exposure to ketoconazole induced a concentration-dependent increase in intracellular ROS levels under chemically induced oxidative stress conditions, whereas no effect was observed under basal conditions. In contrast, miconazole did not affect intracellular ROS levels under either basal or oxidative stress conditions. Although the acute effects on intracellular ROS differed between the two compounds, both exhibited a comparable concentration–response pattern of Nrf-2 activation. Maximal Nrf-2 activation occurred at concentrations below those associated with subsequent cytotoxicity. Ketoconazole and miconazole modulated oxidative stress responses and induced delayed cytotoxicity, with early changes in Nrf-2 activity potentially preceding later toxic effects. Understanding azole-induced redox modulation may provide a basis for future studies investigating the contribution of redox dysregulation to azole-associated hepatotoxicity, immunomodulation, and resistance development.

Occurrence and abundance of azole resistant fungi in soil along a land-use gradient

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Fungal pathogens threaten human life directly and indirectly as they are responsible for about 2.5 million human deaths annually and for up to 30% pre- and post-harvest yield losses in agriculture. Azoles function as antimycotic agents for agricultural and medical purposes, resulting in the risk of cross-resistance development within azole antifungals. The development of azole resistance has been repeatedly discussed to be a result of agricultural exposure. Yet, the role of other environmental factors, environmental azole burden as well as the diversity of azole resistant fungi in soils remain unclear. Closing this research gap was the aim of this work. Sixteen soils across a land use gradient have been analyzed for the overall fungal diversity and the diversity of azole resistant fungi, which were isolated from all studied soils regardless of land-use. The biodiversity and abundance of azole resistant fungi varied between the studied regions, however, so far, no statistically significant influence of abiotic soil parameters on fungal diversity and abundance was identified. As this is work in progress, the azole burden still needs to be determined and included in those analysis. Interestingly, some soils showed higher CFU counts on azole amended media leading to the assumption that the suppression of susceptible species may influence the abundance and diversity of azole resistant species. To date 294 isolates were tested on their thermotolerance and 20.7 % (61 isolates) grew at 37°C on azole amended medium. This result provides plausible evidence that soil can serve as a potential reservoir for fungi that are adapted to the thermal niche of the human body and resistant to azoles. Among the further steps are the sequencing of the cyp51 azole resistance gene in representative isolates to establish a gene database for resistance gene variety and amplicon sequencing of the azole resistome of soils to generate a bigger picture.

Microbiome-derived cecacins as One Health antifungal candidates against multidrug-resistant *Nakaseomyces glabratus*

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Antimicrobial resistance (AMR) is a major One Health challenge, with projections suggesting that resistant infections could contribute to more than 10 million deaths annually by 2050 and substantial global economic losses. While bacterial AMR has received considerable attention, drug-resistant fungal pathogens are increasingly recognised as an urgent public health concern. Among these, *Nakaseomyces glabratus*, formerly *Candida glabrata*, is a high-priority fungal pathogen associated with intrinsic fluconazole resistance and reduced susceptibility across multiple antifungal classes. In this study, we assessed six novel antimicrobial peptides, cecacins 9, 55, 56, 77, 94 and 103, originally identified from the chicken cecal microbiome, for activity against multidrug-resistant *N. glabratus* strains. The cecacins demonstrated strong anticandidal activity, with minimum inhibitory concentrations ranging from 16–128 µg/mL against *N. glabratus* ATCC 2001 and DSM 11950. Several peptides also enhanced the activity of existing antifungal drugs. Cecacins 9, 55, 77 and 103 acted synergistically with amphotericin B, reducing amphotericin B MICs by four-fold. Notably, cecacins 55 and 77 also restored fluconazole activity against intrinsically resistant *N. glabratus*, lowering the effective fluconazole concentration into the susceptible range. The most active peptide-antifungal combinations produced rapid fungicidal effects, achieving >4-log reductions in CFU/mL within 60–180 minutes compared with single-agent treatments. These combinations also significantly inhibited biofilm formation and promoted eradication of established biofilms. Electron microscopy of treated cells suggested membrane disruption and leakage of intracellular contents as a likely mode of action. Importantly, the cecacins showed minimal cytotoxicity against human red blood cells and

multiple mammalian cell lines, supporting their potential therapeutic index. Together, these findings highlight chicken microbiome-derived cecacins as promising One Health-informed candidates for further development as antifungal therapies against *N. glabratus* and other drug-resistant *Candida* infections.

ISCAID guidelines for the diagnosis and management of fungal urinary tract disease in dogs and cats

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Fungal urinary tract disease in dogs and cats is uncommon but clinically significant, with a spectrum ranging from subclinical funguria to disseminated infection. Unlike bacterial urinary tract infection, no evidence-based management guidelines have previously existed for this condition. To develop consensus, evidence-informed recommendations for the diagnosis and management of fungal urinary tract disease in dogs and cats using a formal guideline development process. A multidisciplinary panel of veterinary internal medicine specialists, and human medical mycologists developed twelve clinical questions in PICO format. Evidence was drawn from a scoping review (28 studies, 82 animals) and a multicentre retrospective cohort (95 animals) of fungal urinary tract disease in dogs and cats, supplemented by human clinical practice guidelines and primary literature where veterinary data were absent. Certainty of evidence was assessed using GRADE, and recommendations were formulated using the GRADE evidence-to-decision framework and finalised by panel vote. Certainty of evidence was very low for eleven of twelve PICOs and low for one. The panel recommends systemic antifungal treatment for symptomatic fungal cystitis and presumed fungal pyelonephritis, and against treatment of subclinical candiduria in the absence of disseminated disease or indwelling devices. Fluconazole is suggested as first-line therapy for susceptible Candida species in both lower and upper urinary tract disease; itraconazole is not recommended for cystitis given negligible urinary excretion. Intra-vesicular therapy is not recommended as initial treatment but may be considered for refractory cystitis. Surgical or interventional management is suggested for fungal balls causing or threatening ureteral obstruction, and device management is central to treating device-associated funguria. Despite a severely limited evidence base, these guidelines provide a practical framework for clinical decision-making in fungal urinary tract disease and identify priority areas, including standardised case definitions and veterinary-specific pharmacokinetic and susceptibility data, for future research.

Evidence for environmental azole resistance selection in *Aspergillus* series *Nigri*

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Aspergillus section *Nigri* (black aspergilli) are frequently isolated from clinical samples and are increasingly reported to be resistant to azole antifungal agents. However, the antifungal susceptibility profiles and resistance mechanisms of these species remain insufficiently characterized. Given that azole resistance in *A. fumigatus* often originates from environmental sources, this study hypothesizes that a similar environmental route of resistance acquisition may occur in black aspergilli. A total of 243 *Aspergillus* section *Nigri* isolates were collected from both hospitals (clinical samples) and environmental sources. Minimum inhibitory concentrations (MICs) for azoles were determined using EUCAST methodology. Due to the cryptic diversity within section *Nigri*, whole-genome sequencing was performed for accurate species identification, population genomic analysis, and investigation of azole resistance loci, including the Cyp51 genes. MIC testing revealed that 47.5 % (79/166) of clinical isolates and 71.4 % (55/77) of environmental isolates exhibited MICs at or above epidemiological cut-off values for at least one triazole. Whole-genome sequencing revealed that *A. tubingensis* and *A. niger* are the most prevalent black aspergilli in the analyzed study set. Population genomics showed limited genetic differentiation between clinical and environmental isolates, suggesting an environmental origin of clinical infections. Analysis of the Cyp51 genes identified known and novel mutations, however association with elevated azole MICs remains unclear. This study demonstrates that azole resistance is present in both clinical and environmental *Aspergillus* section *Nigri* isolates in The Netherlands, with genomic data supporting the hypothesis of environmental acquisition of resistance. These findings underscore the need for surveillance of azole resistance in black aspergilli and provide a foundation for future studies investigating the molecular mechanisms underlying antifungal resistance.

Fifteen years of fungal surveillance: changing epidemiology and antifungal resistance in clinical isolates from the U.K.

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The Mycology Reference Centre Manchester (MRCM) is a UKAS ISO 15189-accredited national reference laboratory providing specialised mycological diagnostic services for hospitals across the U.K. and supporting the National Aspergillosis Centre. As an ECMM Centre of Excellence, a EUCAST Antifungal Susceptibility Testing Collaborative Laboratory, and an ISHAM Fungal PCR Initiative Collaborative Centre, the MRCM delivers integrated conventional and molecular diagnostics for fungal infections. A comprehensive diagnostic approach is employed, incorporating culture-based methods, microscopy, biochemical testing, MALDI-TOF mass spectrometry and antifungal susceptibility testing for both yeasts and moulds as well as targeted PCR and panfungal sequencing. In cases of positive *Aspergillus* PCR

with culture-negative specimens, pyrosequencing of the CYP51A gene is performed to detect mutations associated with azole resistance. The MRCM monitors long-term trends in fungal species distribution and antifungal susceptibility. Recently, a retrospective database search of all final reports for fungal identification and susceptibility testing was undertaken for a 15-year period. One isolate per species per patient per year was included, selecting the isolate with the highest level of azole antifungal resistance where multiple isolates were available. A total of over 34,000 isolates were analysed and the main trends noted were: a notable level of azole antifungal resistance in a number of *Candida* species and *Aspergillus fumigatus*, and a rising proportion of non-fumigatus *Aspergillus* species, and organisms not included in the WHO FPPL in culture-positive disease.

Whole-genome sequencing for fungal public health: investigating *Candida auris* transmission and emerging *Trichophyton mentagrophytes* genotype VII in the U.K.

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Whole-genome sequencing (WGS) is increasingly being incorporated into fungal surveillance and outbreak investigation, providing a powerful One Health tool to understand pathogen emergence, transmission and spread. We present two applications of WGS undertaken at the UK Health Security Agency (UKHSA) National Mycology Reference Laboratory that demonstrate its value for public health action. The first study focused on *Candida auris*, an emerging multidrug-resistant healthcare-associated pathogen. Following a rise in cases in England and ongoing outbreaks in two tertiary referral hospitals, 120 isolates collected nationally were sequenced using Illumina technology and analysed using the CDC MycoSNP pipeline. WGS demonstrated that the two major hospital outbreaks were genetically unrelated, while identifying limited onward transmission from both centres to other hospitals. Phylogenetic analysis revealed two previously unrecognised clusters among geographically dispersed cases, suggesting either undetected healthcare-associated transmission or repeated introductions from common overseas sources. Epidemiological investigation linked one cluster to travel from Kuwait and another to intensive care repatriations from Greece. WGS also identified the first known South American clade isolate detected in England. These findings informed national incident management by distinguishing outbreak continuation from new introductions and enabling assessment of outbreak containment. The second study examined *Trichophyton mentagrophytes* ITS-genotype VII (TMVII), a globally emerging sexually transmitted dermatophyte associated with severe inflammatory skin infections. We generated the first draft genomes of four TMVII isolates recovered from UK patients between 2021 and 2025. Whole-genome sequencing, *de novo* assembly and SNP-based phylogenetic analyses were performed to investigate relatedness and place UK isolates within the wider European emergence of TMVII. Cases were identified in London and Edinburgh; two patients had travel links to Spain and the Middle East. Despite their temporal and geographical separation, the isolates formed a highly clonal cluster, consistent with the recent emergence and expansion of a closely related lineage. Comparison with 40 published TMVII genomes showed similarly limited diversity. All isolates remained susceptible to terbinafine and itraconazole. Together, these studies demonstrate how fungal WGS supports surveillance, outbreak response and investigation of emerging fungal threats within a One Health framework.

Cross-domain opportunistic fungi: expanding the One Health perspective on azole resistance

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Azole antifungals are indispensable across human medicine, veterinary care, and crop protection, yet their extensive use has contributed to the emergence of antifungal resistance across interconnected ecological systems. Current discussions have largely focused on resistance in well-established pathogens such as *Aspergillus fumigatus*. However, this perspective may not fully capture the broader ecological dynamics underlying resistance development. We propose the concept of cross-domain opportunistic fungi, defined as ecologically versatile fungal species capable of persisting across environmental, plant, animal, and human reservoirs. Rather than being restricted to a single host or niche, these fungi occupy overlapping ecological niches and exploit shared exposure pathways. As outlined in our recent synthesis, members of the *Fusarium oxysporum* species complex, *Neocosmospora solani*, and selected *Aspergillus* species, among others, exemplify this ability to bridge sectors and contribute to disease across multiple domains. We synthesize evidence demonstrating how azole use across medicine, agriculture, and veterinary practice contributes to shared environmental selection pressures. Environmental reservoirs, including soil, agricultural waste, and aquatic environments, expose fungal populations to azoles and may promote the emergence and maintenance of resistance traits with relevance across sectors. By framing antifungal resistance through ecological connectivity rather than isolated host-specific models, this perspective expands the One Health framework to better reflect the complexity of fungal disease emergence. Recognizing cross-domain opportunistic fungi as shared ecological actors may improve surveillance strategies across environmental and clinical settings while supporting coordinated antifungal stewardship to preserve the long-term effectiveness of azole compounds.

Integrative transcriptomic profiling of the intratumoral microbiome and its role in antitumor immunity

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The role of intratumoral microbiota in tumor biology and anticancer immunity is increasingly recognized and provides potential applications in diagnosis and immunotherapy. However, its effects are largely species-specific, and

underlying mechanisms remain poorly understood. To address this, we are developing an automated pipeline to identify intratumoral microorganisms, characterize their composition, and discover potential immunoregulatory mechanisms. Our workflow is implemented in Nextflow to ensure scalable and reproducible results. It processes reads from metatranscriptomic and metagenomic sequencing that cannot be mapped to the host genome. Taxonomic classification is performed using Centrifuge or Kraken2, followed by quality control of alignments using Recentrifuge. Contaminants are identified using negative control samples and based on the inverse relationship between microbial abundance and nucleic acid concentration. Downstream analyses include microbial diversity, core microbiome identification, co-occurrence inference, and metabolite prediction. From bulk RNA sequencing data various immune related parameters and immune cell fractions can be estimated and correlated with microbial abundance. Furthermore, microbial impact on patient prognosis and potential molecular mimicry with known tumor-associated antigens can be assessed. We applied this pipeline to RNA sequencing data from pancreatic ductal adenocarcinoma (PDAC) in The Cancer Genome Atlas (TCGA) and to selected PDAC samples from an independent cohort. Among the identified fungal species were well-described *Malassezia restricta* and *Malassezia globosa*, whose metabolites have been shown to activate the immunomodulatory aryl hydrocarbon receptor (AhR) signaling pathway. In fact, the inferred AhR signature, together with the presence of *Malassezia* spp., was significantly associated with shorter patient survival. To further investigate these interactions, we perform single cell and spatial transcriptomics analyses of PDAC samples to characterize the spatial distribution of intratumoral microbes and their impact on the local tumor immune-microenvironment. We have designed a custom gene panel targeting genes involved in microbial-immune interactions, together with probes for key intratumoral bacterial and fungal species, including *M. globosa*. The spatial localization of *M. globosa* is further validated using fluorescence *in situ* hybridization (FISH).The integration of computational approaches with single cell and spatial transcriptomics analyses, together with FISH validation, provides a robust framework to characterize the intratumoral microbiome and generate testable hypotheses regarding microbiome-immune interactions.

Chemical footprints: prevalence of azole antifungals in Tyrolean blood donors

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Azole antifungals are essential for the treatment of invasive fungal infections and are extensively used as agricultural fungicides. Their widespread application has raised concerns about environmental contamination and the emergence of antifungal resistance, yet population-level biomonitoring data on human azole exposure remain limited. This study aims to characterize background exposure to azole antifungals among healthy adults in Tyrol, Austria, using blood donors as a proxy for the general population, and to identify environmental, demographic, and dietary factors associated with exposure. Between November 2025 and July 2026, 1,000–2,000 consenting blood donors are being recruited across 16 donation events selected to represent regions with varying agricultural intensity and seasons of pesticide application. Residual EDTA blood samples are processed, stored at –80°C, and analysed for azole antifungals using validated liquid chromatography-mass spectrometry (LC-MS). Participants complete a standardized REDCap questionnaire capturing age, sex, residence, dietary habits, personal azole use, and other potential exposure sources. Dietary information, including fruit and vegetable consumption, organic food preference, produce washing practices, and meal frequency, will be integrated with biomonitoring data to investigate whether specific dietary patterns are associated with the presence of detectable azoles and with higher circulating azole concentrations. Exposure will be evaluated using two complementary outcomes: the prevalence of detectable azoles in blood and the absolute concentrations among individuals with detectable levels. Logistic regression will be used to identify predictors of detectable exposure, while multiple linear regression will assess factors associated with concentration levels, with analyses stratified by age, sex, geography, season, dietary characteristics, and personal azole history. Autumn and winter recruitment has been

completed successfully, with participation rates increasing from approximately 50% to 60–70% across donation events, demonstrating the feasibility of large-scale population biomonitoring. The study will provide the first baseline data on azole antifungal exposure in an Austrian population and improve understanding of the environmental and dietary determinants of exposure, informing future exposure surveillance, risk assessment, and strategies to preserve the effectiveness of azole antifungals.

Fungal bioluminescent reporter strains for studying fungal colonization of the mammalian gut

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Fungal organisms are increasingly recognized as components of the mammalian gut microbiome, with roles in gut homeostasis and diseases such as inflammatory bowel disease (IBD). However, it remains unclear which fungi are transient luminal passengers and which are capable of stable colonization. Bioluminescent (BL) reporter strains, developed for *in vivo* mouse models of fungal infection, enable fungal load monitoring via photon emission. Here, we adapt this system to study gut colonization and metabolic activity using established BL reporter strains of *Aspergillus fumigatus*, *Candida albicans*, and *Cryptococcus neoformans*. We further evaluate alternative luciferin substrates to standard D-luciferin – not previously tested in fungal BL reporter strains – for improved signal transduction. Photon emission of all three BL strains was compared across cell densities (10^4–10^7 cells/mL) and substrate concentrations (0.5–500 µM) for D-luciferin and CycLuc1, in black 96-well plates and extracted mouse organs, using an IVIS Lumina III In Vivo Imaging System (Revvity, U.S.A.). To distinguish gavaged BL-reporter strains from endogenous colonizers and environmental passengers, we developed a culturomics system using luciferin agar (Sabouraud agar + 0.4 mM D-luciferin). Serial dilutions of fungal cells were mixed with stool and intestinal mucosal scrapings, filtered, and plated on luciferin agar. BL colony-forming units (CFUs) and signal intensity were imaged using a Fusion Absolute Imager (Vilber, France) and quantified with the Cactus CFU counter module. CycLuc1 outperformed D-luciferin at lower substrate concentrations and cell densities in both the cell culture and ex vivo gut colonization models, establishing it as a promising substrate for enhanced BL fungal detection in vivo. The BL-culturomics method reliably detected BL-positive colonies even at low initial fungal input (10^1 cells). BL fungal reporter strains offer a promising tool for studying colonization capacity and fungal effects on intestinal homeostasis. These proof-of-concept experiments will next be extended to *in vivo* mouse systems to investigate colonization across different host genetic backgrounds and dietary regimes.

The murine mycobiome in IBD with changing environment and diet – a time course experiment

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Fungal organisms are increasingly recognized as components of the mammalian gut microbiome, with roles in gut homeostasis and diseases such as inflammatory bowel disease (IBD). However, it remains unclear which fungi are transient luminal passengers and which are capable of stable colonization, and how host genetic susceptibility, diet, and housing environment influence the occurrence of specific fungal species. X-box binding protein 1 (Xbp1), a transcription factor regulating the unfolded protein response (UPR) during endoplasmic reticulum stress, drives Crohn's disease-like intestinal inflammation in mice fed a transfat-enriched Western diet – providing a platform for studying diet-driven effects on intestinal homeostasis in mice. Xbp1-knockout ($Xbp1^{\Delta IEC}$) and wildtype mice (n=3 per diet) were fed standard chow (ssniff), low-fat control, cis-fat-enriched, or transfat-enriched Western diets (Brogaarden) for three months. Fresh rectal stool was collected at four timepoints: (1) before leaving the sterile barrier facility, (2) before switching to experimental diets, (3) after two months on the experimental diet, and (4) at sacrifice, when luminal and mucosal samples were also taken from all intestinal compartments (duodenum, jejunum, ileum, colon). DNA was extracted using the DNeasy PowerSoil Pro Kit (Qiagen), and the ITS2 region was amplified and sequenced on Illumina MiSeq. Demultiplexing, quality filtering, and OTU construction were performed using LotuS2; downstream analysis used the microbiome, phyloseq, and MaAsLin2 packages in R. Stool and intestinal samples across four timepoints and compartments were collected from $Xbp1^{\Delta IEC}$ and wildtype mice across four dietary regimens. ITS2 sequencing and mycobiome profiling are ongoing; preliminary analyses indicate that fungal community composition shifts with diet and genotype over the time course. Full results, including diversity metrics and taxon-level associations, will be presented at the conference. This time-course design will help disentangle the relative contributions of host genetics, diet, and environment to gut fungal community assembly, and determine whether Xbp1-dependent ER stress signaling shapes mycobiome composition during diet-induced inflammation – clarifying how gene-diet-environment interactions influence fungal colonization dynamics relevant to IBD pathogenesis.

Impact of azole resistance in invasive aspergillosis: a multinational European cohort in hematological patients

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Azole-resistant invasive aspergillosis (ARIA) is increasingly recognized in patients with hematological malignancies, yet its clinical impact remains uncertain outside high-prevalence settings. We evaluated outcomes of ARIA *versus* azole-susceptible invasive aspergillosis (ASIA) across diverse European settings. We performed a retrospective multicenter analysis including adult hematology patients with culture-positive proven or probable IA. A hierarchical cohort design was applied: (1) a primary multinational cohort without Dutch cases (2016–2020), (2) a sensitivity analysis including Dutch ARIA cases from high-resistance settings, and (3) an expanded non-Dutch cohort (2010–2024) integrating FungiScope registry data. ARIA was defined by phenotypic non-wild-type susceptibility, growth on azole screening media, or cyp51A mutations. The primary endpoints were all-cause mortality at day 42 and 84. A total of 178 patients were included (138 ASIA, 40 ARIA) from six European countries. In the primary cohort (13 ARIA; 66 ASIA), ARIA was associated with higher crude mortality compared with ASIA (day 42: 69.2% *vs* 40.9%; day 84: 76.9% *vs* 50.0%; ~25 30% difference); however, this was not observed after adjustment. ICU admission was the main predictor for mortality. Addition of Dutch ARIA cases attenuated mortality differences (day 42: 52.4% *vs* 40.9%; day 84: 57.1% *vs* 50.0%), with no independent association seen between resistance and outcome. In the expanded non-Dutch cohort (*n*=170), ARIA remained associated with increased mortality (day 42: 65.6% *vs* 39.9%; adjusted HR 1.78, 95% CI 1.03-3.08); strongest in non-ICU patients, whereas differences were attenuated in ICU populations. Environmental resistance mechanisms, predominantly TR34/L98H, were most frequently identified. The impact of azole resistance on IA mortality is context-dependent. Excess mortality in lower-awareness settings attenuates with optimized diagnostics and treatment, suggesting partly modifiable risk. These findings support resistance surveillance and management adapted to local epidemiology.

Adapting a mammalian CYP450 fluorescence assay for screening fungal CYP51 inhibition

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Fungal pathogens and agricultural fungi represent growing health and crop-protection challenges, while the antifungal drug pipeline remains limited. Azole antifungals target CYP51, a cytochrome P450 enzyme required for ergosterol biosynthesis. However, resistance and possible inhibition of human CYP enzymes can compromise their therapeutic selectivity. Therefore, scalable assays that compare fungal CYP inhibition with mammalian CYP activity are valuable for early antifungal screening. In this study, a commercially available Vivid™ CYP450 fluorescence assay was adapted for detecting fungal CYP activity and evaluating azole-dependent inhibition in yeast-derived membrane preparations. *Saccharomyces cerevisiae* strains heterologously expressing fungal CYPs and their cognate cytochrome P450 reductases were used, including *Aspergillus fumigatus* CYP51A and *Rhizopus arrhizus* CYP51F1. Crude membrane fractions were generated by mechanical cell disruption followed by differential centrifugation. Enzymatic activity was measured using the fluorogenic substrate BOMCC together with an NADPH-regeneration system. Human CYP3A4 Baculosomes were included as a mammalian CYP control. The adapted assay produced measurable and reproducible fluorescence signals from crude membranes expressing *A. fumigatus* CYP51A and *R. arrhizus* CYP51F1, supporting functional detection of fungal CYP activity in a repurposed mammalian assay format. CYP3A4 Baculosomes confirmed assay performance. Azole compounds showed distinguishable inhibitory profiles across the fungal CYPs tested, reflecting differences in CYP–azole interaction and species-dependent azole response. These findings provide proof-of-concept that a commercial human CYP450 fluorescence kit can be redirected toward fungal CYP screening. This approach may support early antifungal discovery by identifying compounds with stronger fungal CYP inhibition while enabling parallel assessment of potential mammalian CYP cross-reactivity.

Monoclonal antibodies targeting yeast and mould cell wall remodelling enzymes: next generation antifungal therapeutics against drug resistant infections

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Invasive fungal infections (IFI) are responsible for >2.5 million global deaths annually. Treatment of IFI is complicated by increasing prevalence of antifungal drug resistant infections, drug toxicities and drug-drug interactions. In parallel there are increasing numbers of at-risk patients. Mortality rate associated with IFI remain unacceptably high, >40% even with treatment. We have identified novel, surface-exposed protein targets that are more abundant in drug-resistant fungi and overexpressed in response to treatment with current antifungals. The selected cell wall protein targets Pga31 (pan-*Candida*) and Utr2 (pan-fungal) are critical for cell wall remodeling and pathogenicity and are expressed during infection. Fully human monoclonal antibodies (mAbs) targeting Pga31 and Utr2 are in late-stage preclinical development leading to candidate nomination. Our mAbs bind to clinically significant fungal species, including drug-resistant isolates of *Candida albicans, C. auris, C. parapsilosis*, *C. tropicalis,* and *Aspergillus fumigatus*. mAb binding was enhanced when cells were stressed with echinocandins and azoles, due to increased expression of the mAb targets. There was strong binding to the invasive hyphal morphology of *C. albicans* and *A. fumigatus* swollen conidia, germlings and hyphae even without antifungal treatment. Antibody-mediated opsonisation of fungal cells enhanced phagocytosis by murine J774.1 macrophages compared with controls. The mAbs have demonstrated significant *in vivo* efficacy in clinically predictive, murine models of invasive candidiasis representing both immunocompetent and immunosuppressed status of patients. For example, a pan-fungal mAb protected 80% immunosuppressed mice infected with echinocandin resistant *C. albicans* when delivered as a co-therapy with caspofungin, whereas caspofungin monotherapy was ineffective. Novel approaches are desperately needed to tackle drug resistant IFI. There is huge potential for fungal-specific cell wall-targeting mAbs to improve clinical outcomes in complex patients either as monotherapy or co-therapy with existing antifungals.

Impact of iron availability and azole treatment on the mycobiome and resistance development

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Fungal infections are a major complication in immunocompromised patients, including those with myelodysplastic syndromes (MDS). Azole antifungal medications are widely used for prophylaxis and treatment; however, they have been linked to rising antifungal resistance. Iron availability also affects fungal growth and host-pathogen interactions. In MDS, iron overload and chelation therapy may influence the mycobiome and susceptibility to antifungals. However, the combined effects of iron modulation and azole exposure remain poorly understood. This project will investigate how iron availability and azole therapy shape the mycobiome and resistance development in patients with MDS. Stool samples collected from a previous MDS patient cohort were processed for internal transcribed spacer (ITS) sequencing to characterize the gut mycobiome composition. Sequencing data were analyzed to assess fungal diversity, relative abundance, and potential resistance-associated taxa. Clinical treatment data, including iron chelator use and cardiovascular drug use were integrated with the analysis to explore the associations between therapeutic interventions and mycobiome alterations.This cohort had a non-significant group of patients who underwent azole treatment, which made it implausible for sequencing analysis. Overall, alpha diversity is largely comparable across groups, with no consistent differences between conditions. No clear beta-diversity clustering by group or condition was observed. However, significant differences were detected at the genus level, including increased abundance of *Candida* and *Nakaseomyces* in iron-treated conditions, whereas no significant associations were observed at the species level. In this initial cohort, gut mycobiome composition was largely homogeneous, with no robust differences attributable to iron or azole therapy. The findings emphasize the need for larger, prospectively sampled cohorts to validate subtle treatment-related effects and to improve detection of therapy-associated fungal signatures.

Resistances in environmental *Candida* isolates

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Recently revised ascomycetous genus *Candida* includes clinically important species. While their pathogenic potential and involvement in diseases is well explored, the missing parts often remain their natural habitats, transmission routes and enroute development of the resistances. To explore possible connections between habitat and phenotypic resistances we tested strains of *Candida parapsilosis*, *C. tropicalis* and *C. albicans*, isolated from seawater, salterns, groundwater, drinking water, kitchens, bathrooms, household appliances, and wastewater against nine most used antifungal agents, including echinocandins, polyenes and azoles. Obtained results showed the intermediate resistance against echinocandins in majority of *C. parapsilosis* isolated from water. Surprisingly high percentage of *C. parapsilosis* from human-made indoor environments was showing borderline resistances against 5-flucytosine, often combined with resistances to either fluconazole, posaconazole, anidulafungin or micafungin. Dishwashers and bathrooms appeared as habitats with the highest possibility to recover strains resistant to at least one antimycotic agent. In accordance with the general reports, also *C. tropicalis* isolates from our study showed trailing growth and resistances towards azoles. Multiple intermediate resistances were common for this species. The raising concern in our project was also the result for *C. albicans*, isolated from wastewater. Besides trailing growth this strain showed azole resistances above designated breakpoints. Our results

suggest that the wild strains present in different water types and indoor environment likely acquire resistances enroute through prolonged stress, like higher temperatures, salts, and detergents or contact with antimycotic agents. Future attention should thus focus on natural habitats that accumulate antimycotics due to agriculture in urban areas: seawater, surface water, and wastewater. Indoor niches, like household appliances and bathrooms should be taken into consideration in cases of possible resistant strains in hospitals.

Biofilm-driven rezafungin tolerance in clinical and environmental isolates of *Candida parapsilosis*: a One Health concern

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Candida parapsilosis is a major cause of healthcare-associated candidemia, particularly in settings involving intravascular devices, where biofilm formation may reduce antifungal efficacy. Rezafungin, a next-generation echinocandin with once-weekly dosing potential, represents an important therapeutic option; however, its activity against *C. parapsilosis* biofilms remains insufficiently characterized. Here, we evaluated 119 *C. parapsilosis* isolates from host-derived and non-host-derived sources for biofilm formation and susceptibility to rezafungin, micafungin, and liposomal amphotericin B under planktonic and sessile conditions. Biofilm formation was markedly enriched among host-derived isolates, especially clinical isolates, whereas most non-host-derived strains were weak or non-biofilm producers. Planktonic rezafungin MICs remained generally low, although host- derived isolates showed a modest but significant shift toward higher MIC values, and four clinical isolates displayed intermediate MICs. In contrast, sessile testing revealed biofilm-associated tolerance that was not captured by conventional MIC assays. Rezafungin retained the strongest antibiofilm activity among the tested agents, inhibiting most biofilm-forming isolates at concentrations at or below the clinical breakpoint, and showed a smaller MIC-to-SMIC increase than micafungin and liposomal amphotericin B. Nevertheless, rezafungin SMICs increased with biofilm biomass and metabolic activity, indicating that reduced sessile susceptibility is driven primarily by biofilm-related traits rather than intrinsic planktonic resistance. These findings support rezafungin as a promising option against *C. parapsilosis* biofilms while highlighting the need for sessile susceptibility testing and One Health surveillance of biofilm-forming isolates from clinical and environmental reservoirs.

Towards photoconazole, a dual-active CYP51 photodynamic inhibitor

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Fungal infections pose a non-negligible threat to public health, with recent trends indicating rising incidence, increasing mortality, and growing antifungal resistance. In response, five EU health and environment agencies issued a call for novel antimycotics operating under mechanisms less prone to resistance development. Azoles remain first-line therapeutics for most mycoses and act by inhibiting 14α-demethylase (CYP51), a key enzyme in the ergosterol biosynthetic pathway essential to fungi. However, mutations within the protein's active site can markedly reduce the binding affinity of common azoles and are a major driver of clinical resistance. Here, we present a new concept of treating fungal infections via dual-mode inhibition and the first steps towards photoconazole, a next-in-class agent that integrates a conventional azole pharmacophore with a photosensitizing substructure to enable photodynamic action. Photosensitizers generate reactive oxygen species upon light excitation, causing localized oxidative damage to the host leading to cell death. Through chemical linkage of azole and photosensitizer, the resulting compound proved to be fungistatic in the dark and fungicidal upon irradiation across a test set of nearly 50 clinically relevant fungal isolates. Control studies showed that the azole motif alone produced no measurable inhibition under the assay conditions, whereas the photosensitizer alone exhibited activity only when illuminated. Ongoing work focuses on optimizing the scaffold to improve pharmacodynamics, increase singlet oxygen quantum yield, achieve bathochromic shifts in absorption, and refine physicochemical properties. These advances aim to support future applications in human or animal health and crop protection in a One Health context.

Azole-resistant *Candida tropicalis* clade 4 genotype persistence in both clinical and environment setting in Taiwan

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A predominant azole-resistant *Candida tropicalis* clade 4 genotype, resulting from overexpressing mutated azole target (ERG11), causing candidemia in humans has been detected not in clinical settings but in environments in several countries. Moreover, the use of azole fungicides was associated with the detection of azole-resistant *C. tropicalis* in grape orchards. Yeasts isolated from fruits, soils, water, and farmers from 30 grape orchards in 2023 were identified by ribosomal DNA sequencing. The susceptibilities to clinical and agricultural azole compounds were determined. Residues of azole-fungicides in soil were determined. Of 471 individual yeasts, 449 (64.3%) were from environments and 249 (35.7%) from farmers. Among the common human pathogenic yeast species recovered, *Candida albicans* (18/20, 90%), followed by *Candida parapsilosis* (4/5, 80%) were prevalent in famers, while all 11 *Candida palmioleophila* and most of *C. tropicalis* (24/25, 96%), *Pichia terricola* (12/13, 92.3%), *Pichia kudriavzevii* (*Candida krusei*) (10/11, 90.9%), and *Hanseniaspora opuntiae* (11/13, 84.6%) were from environment. The rate of fluconazole-resistant *C. tropicalis* from the present study survey (21/25) was significantly higher than that from grape orchards in the 2012 survey (4/29) ($p < 0.0001$). The detection of fluconazole-resistant *C. tropicalis* was positive association with the residue of azole fungicides. The observation that residues of azole fungicides in soils lead to select azole-resistant pathogenic fungi in environments strongly suggests that reducing/stopping azole fungicides may be a solution to prevent the spread of azole-resistant pathogenic fungi, including azole-resistant clade 4 *C. tropicalis*.

Emergence of a high-risk ST11 lineage and early azole resistance in clinical *Candida parapsilosis* isolates

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Candida parapsilosis is a major cause of invasive candidiasis and is notable for its ability to spread in healthcare settings and develop azole resistance. Following a rise in detection at our institution, from 18 isolates in 18 patients in 2022 to 47 isolates in 24 patients during 2023–2024, a detailed investigation was undertaken. The study aimed to investigate antifungal susceptibility, genetic relatedness, and resistance mechanisms among *C. parapsilosis* isolates. Susceptibility to fluconazole and anidulafungin was determined using the Clinical & Laboratory Standards Institute (CLSI) broth microdilution and gradient diffusion (Etest) methods. Whole-genome sequencing (WGS) was applied for SNP-based phylogeny and multilocus sequence typing (MLST). Resistance-associated mutations were investigated in non-susceptible isolates. WGS of the 47 isolates revealed no single clonal outbreak but a genetically heterogeneous population belonging to nine sequence types (STs), six SNP clusters and ten singletons. A notable cluster included closely related ST11 isolates detected in three patients and linked to previously described epidemic lineages. Fluconazole non-susceptibility was confined to isolates from one patient within this cluster. These isolates harbored predominantly heterozygous variants consistent with early polygenic resistance. Method-dependent discrepancies were observed for anidulafungin, while fluconazole showed higher concordance. Integration of whole-genome sequencing (WGS) with antifungal susceptibility testing differentiated increased prevalence from clonal transmission, identified a high-risk ST11 lineage, and revealed early resistance evolution. These findings support molecular surveillance and careful interpretation of susceptibility results for infection control and antifungal stewardship.

Effects of plant-derived bioactive compounds on anaerobic ruminal fungi, microbial interactions, and methane mitigation: an *in vitro* study

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Anaerobic ruminal fungi are key members of the rumen microbiome and play an essential role in the degradation of lignocellulosic plant biomass, thereby supporting nutrient utilization in ruminant animals. Through their interactions with bacteria, protozoa, and methanogenic archaea, these fungi contribute to rumen fermentation and influence microbial ecosystem dynamics. Despite their ecological importance, anaerobic fungi remain one of the least studied microbial groups in the rumen, and limited information is available regarding their responses to plant-derived bioactive compounds and their potential role in methane mitigation. This ongoing study investigates the effects of selected plant-derived bioactive compounds, including allicin and saponins, on anaerobic ruminal fungi and their interactions with associated bacterial communities under controlled in vitro conditions. Pure cultures of anaerobic fungi have been successfully established under strict anaerobic conditions, and experiments evaluating the effects of these bioactive compounds are currently in progress. Fungal growth, viability, and fermentation gas production will be evaluated following exposure to different concentrations of the compounds. In parallel, bacterial community composition will be characterized using amplicon-based sequencing to investigate how modulation of anaerobic fungi influences microbial interactions within the rumen ecosystem. These analyses will provide insight into the relationship between fungal activity, bacterial community dynamics, and fermentation characteristics. It is expected that selected plant-derived bioactive compounds will selectively modulate anaerobic fungal growth and alter microbial interactions without adversely affecting fiber degradation. Furthermore, changes in microbial community structure are anticipated

to improve rumen fermentation efficiency and reduce methane production by influencing hydrogen availability and microbial metabolic pathways. The study is also expected to identify promising bioactive compounds that can beneficially manipulate the rumen microbiome while maintaining digestive function.The findings will advance current understanding of the ecological role of anaerobic fungi in rumen fermentation and their interactions with bacterial communities. Ultimately, this research may contribute to the development of sustainable nutritional strategies that improve feed utilization, reduce greenhouse gas emissions, and enhance the environmental sustainability of ruminant livestock production.

Identification and phenotypic characterization of airborne yeasts in the U.S. cornbelt: a potential threat to human health?

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Airborne fungal and yeast spores are a critically understudied component of atmospheric particulate matter. Ongoing global changes in climate, land use, and human activity are likely intensifying selective pressures that favor fungal and yeast persistence, thermotolerance, and anti-fungal resistance. The objective of this study is to characterize the abundance, distribution, antifungal responses, and thermotolerance of environmental airborne fungal and yeast isolates within the intensive agricultural ecosystems of the US cornbelt. Here, we focus on airborne yeasts with particular interest in basidiomycete yeasts that may be responding the most strongly to increasing temperatures. Air sampling was conducted from April 2024 through December 2025 at four Iowa sites: Dairy (DY), Ames (AM), Northwest Research Farm (NWRF; Sutherland, IA), and Southeast Research Farm (SERF; Crawfordsville, IA). These locations provided geographic and climate diversity based on elevation, weather patterns, and proximity to the Mississippi River. Environmental spores were collected using spore traps and cultured at room temperature, 32°C, 37°C, and 42°C. To date, approximately 1,800 isolates have been recovered. Initial phenotypic screening revealed substantial morphological diversity, including pigmentation ranging from white and yellow to pink, orange, and black, with many isolates displaying heavy encapsulation. Matrix-assisted laser desorption/ionization (MALDI) identification confirmed the presence of *Filobasidium magnum*, *Hannaella luteola*, *Tausonia pullulans*, and *Rhodotorula mucilaginosa*. With the exception of *Rhodotorula* species, most isolates tested do not growth at 37°C. However, broad antifungal resistance is observed across many of the tested isolates. Ongoing genotypic characterization and additional anti-fungal susceptibility testing aim to further assess the public health relevance of these findings. Collectively, these data suggest that airborne yeasts may serve as an under-recognized reservoir of thermotolerance and potentially anti-fungal-resistant organisms, posing increased risks to vulnerable populations in agricultural settings.