



Next-Generation Sequencing in Fungal Diagnostics: Is It Ready to Be Used at the Bedside?

Amir Seyedmousavi, VMD. PhD. D(ABMM), F(ECMM)
Senior Staff, Clinical Microbiology laboratory Director
Medical Director, Mycology and Parasitology Diagnostics
Department of Laboratory Medicine, CC, NIH
Seyedmousavi@nih.gov



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Disclosure

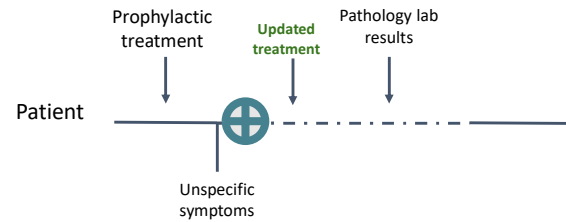
- Intramural Research Program, NIH Clinical Center, Bethesda, MD, USA.
- No outside financial relationships to disclose.

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Rapid diagnosis and treatment of infectious diseases can change the outcome

Academic and clinical perspectives of metagenome sequencing as a diagnostic tool for infectious disease



Target: Assessing *ongoing* infectious processes with point-of-care metagenomic sequencing

Benefits

- Culture negative cases
- Faster results
- Species classification (subpopulation)
- Novel or unexpected agents
- Polymicrobial infections
- Resistance prediction
- Outbreak/transmission surveillance

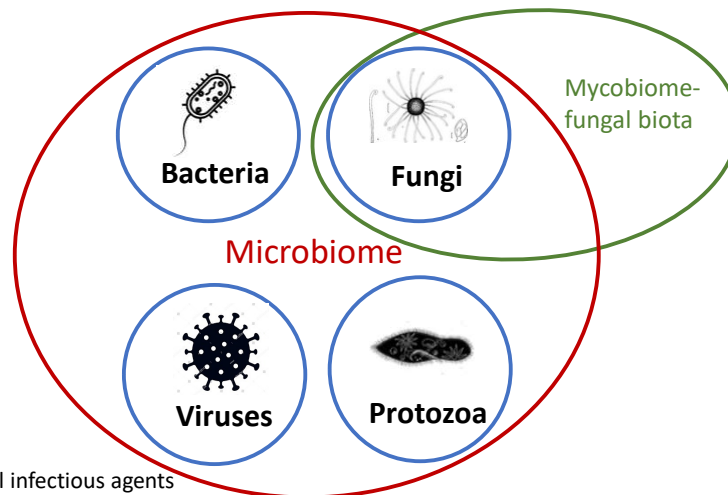
Current Issues

- Benefits seen as too "diffuse" for cost
- Interpretation complexity
- Hard to standardize
- laboratory infrastructure and trained personnel

Trivett et al. BMC Infectious Diseases (2025) 25:448

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Clinical Metagenomic Next-Generation Sequencing for Pathogen Detection



Almost all infectious agents contain DNA or RNA, making sequencing a powerful approach for pathogen detection.

Gu W, Miller S, Chiu CY. Annual Review of Pathology, 2019;14:319–338.
Chiu CY, Miller SA. Clinical metagenomics. Nature Reviews Genetics, 2019;20:341–355.
Cui L, Morris A, Ghedin E. Genome Medicine, 2013;5:63.

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NGS Sequencing – two approaches

1. Targeted NGS / Metabarcoding

PCR amplification of marker genes

- Bacteria: 16S rRNA
- Fungi: ITS1/ITS2 or 18S rRNA
- Protozoa/eukaryotes: 18S rRNA



NGS sequencing



ASV/OTU classification using reference databases (SILVA, Greengenes, UNITE, QIIME 2)



2. Shotgun metagenomics / mNGS

Non-targeted sequencing of all nucleic acids

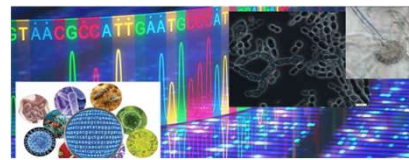
- Microbial DNA/RNA
- Host DNA/RNA
- Bacteria, viruses, fungi, parasites
- Functional genes, resistance genes, virulence genes



Random fragmentation and library preparation



Bioinformatic classification and functional analysis (Kraken, Kaiju, InterPro)



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NGS Sequencing – two approaches

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NGS sequencing



ASV/OTU classification using reference databases (SILVA, Greengenes, UNITE, QIIME 2)

Outputs:

- Taxonomic composition
- Relative abundance
- Alpha/beta diversity
- Community comparison

2. Shotgun metagenomics / mNGS

Non-targeted sequencing of all nucleic acids

- Microbial DNA/RNA
- Host DNA/RNA
- Bacteria, viruses, fungi, parasites
- Functional genes, resistance genes, virulence genes



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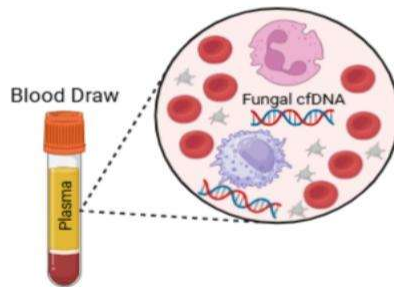
Outputs:

- Species/strain-level identification
- Pathogen detection
- Functional pathways
- Antimicrobial resistance genes
- Correlation/network analysis

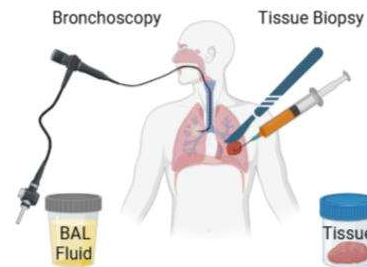
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Specimen types for diagnosis of fungal infections

Non-Invasive Specimen Testing



Invasive Specimen Testing



Anthony Lieu et al. Clin Infect Dis, Volume 80, Issue 5, 15 May 2025, Pages 1095–1102
Blauwkamp et al. Nature Microbiology. 2019;4:663–674.

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Case study 1: Investigation for possible hospital transmission



New Patient

- 27-year-old male with relapsed/refractory neuroblastoma
- Admission surveillance for *C. auris* **negative** (6/2/2026)
- Surveillance **positive** (6/23/2026)
- Inpatient overlap with colonized patient: 6/8–6/11/2026
- Outpatient visits on same unit: 6/14, 6/15, 6/18, 6/22/2026
- Shared inpatient unit again on 6/23/2026

Known Colonized Patient

- 28-year-old male with metastatic thymoma
- **Known *Candida auris* colonized patient**
- Present on same inpatient unit during overlap
- Shared inpatient unit during same period
- Receiving care on same unit
- Present on unit on 6/23/2026

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Pairwise SNP distance Matrix to determine genetic relatedness

- Pairwise SNP distance (W23 vs X96): **122 SNPs**
- 122 SNP differences indicate the isolates are not closely related, therefore not consistent with recent hospital transmission.

Conclusion: Both isolates belong to Clade I but likely represent different strains within this lineage.

MycoSNP pipeline:

	Reference	W23	X96
Reference	0	139	177
W23	139	0	122
X96	177	122	0

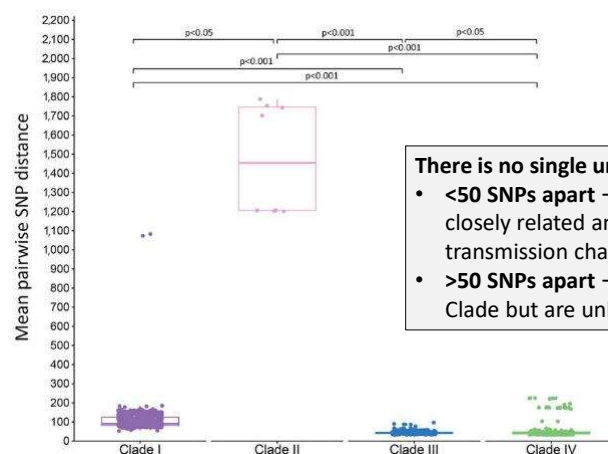


refMLST pipeline:
82 allele differences

Sample	GCF_003013715.1	W23	X96
GCF_003013715.1	0	4485	4486
W23	4485	0	82
X96	4486	82	0

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Pairwise SNP distances: Genetic relationships among cases



There is no single universal SNP cutoff:

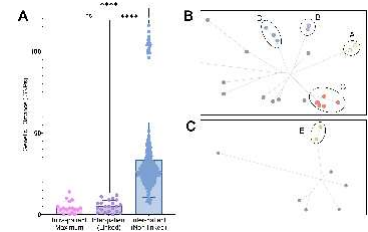
- <50 SNPs apart** → strong evidence they are closely related and likely part of the same transmission chain.
- >50 SNPs apart** → they belong to same Clade but are unlikely to be directly linked.

Parnell et al. Emerg Infect Dis. 2026;32(5):728-739

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Candida auris genetic variation within and between patients in a healthcare system

	n	MycoSNP (SNPs)		refMLST (alleles)		Significance ^c
		Median	Range	Median	Range	
Intra-clade (Clade I)	23	27	0–48 (outlier: 116)	25	0–42 (outlier: 94)	*
Intra-clade (Clade III)	8	22.5	5–51	18.5	6–35	n.s.
Maximum intra-patient ^b	17	4	0–14	4	0–14	n.s.
Inter-patient (linked)	15	5	0–12	3	0–9	n.s.
Inter-patient (unlinked)	16	27	7–48 (outlier: 116)	25	6–42 (outlier: 94)	**



- Study- and pipeline-specific thresholds
- Interpret with epidemiologic information

Murphy et al. J Clin Microbiol. 2026, 64:e01348-25.c

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Detection of Genetic Markers Predicting Antifungal resistance matching phenotypic AFST

Genotypic Determinants

Copy table Violin plot Export as CSV... Showing 1/2 rows.

Sample Name	ERG11_Y132F
W23	Detected
X96	Detected

ERG11_Y132F mutation

Genotypic Determinants Stratified by Antimicrobial and Organism

Note: Genotype does not necessarily predict phenotypic antimicrobial resistance. Laboratory and clinical correlation are required.

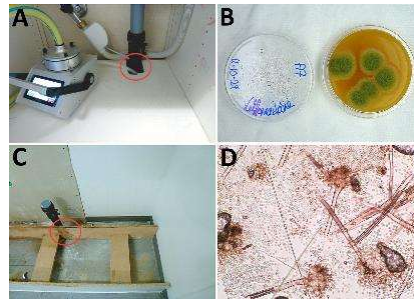
Copy table Configure columns Scatter plot Violin plot Export as CSV... Showing 1/4 rows and 3/3 columns.

Antimicrobial	Class	W23	Isolate	Isolate# 01
		Candidozyma auris	Antibiotics	MIC Interp
Fluconazole	Azole	Resistant	Amphotericin B	2 NI D1
Anidulafungin	Echinocandin	Susceptible	Anidulafungin	0.12 NI D1
Caspofungin	Echinocandin	Susceptible	Caspofungin	0.12 NI D1
Micafungin	Echinocandin	Susceptible	Fluconazole	>64 NI D1
Rezafungin	Echinocandin	Susceptible	Isavuconazole	0.015 NI D1
Amphotericin B	Polyene	Susceptible	Itraconazole	0.06 NI D1
			Micafungin	0.06 NI D1
			Posaconazole	0.015 NI D1
			Voriconazole	0.5 NI D1

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Aspergillus flavus Outbreak Linked to Contaminated Building Materials, Denmark, 2025

- Outbreak investigation
- WGS of 167 isolates + core-genome SNP analysis
- Genetic clustering of outbreak isolates from patients and contaminated building materials
- Importance of environmental source investigation and outbreak control measures

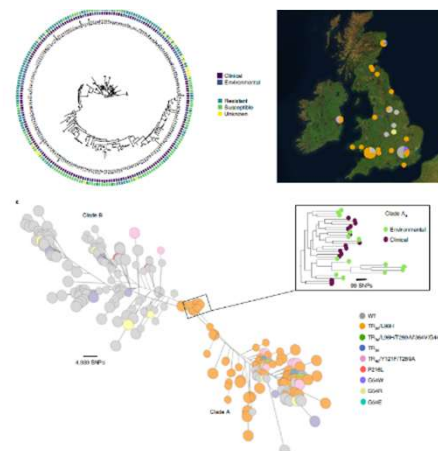


Gewecke et al. Emerg Infect Dis. 2026 Mar;32(3):376-387.

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Near-identical **azole-resistant** clinical and environmental isolates

- Population genomics study
- WGS of 218 *A. fumigatus* isolates + SNP-based phylogeny
- Linked environmental azole resistance to human infection and transmission risk



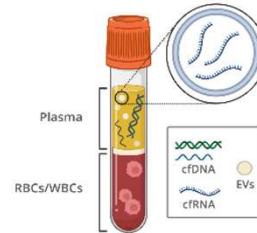
Rhodes et al. Nat Microbiol. 2022 May;7(5):663-674

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Case study 2:

Mold Cell-Free DNA PCR in Plasma for the Detection of Invasive Fungal Infections

- 506 patients underwent testing with non-invasive Mold plasma cfDNA PCR and invasive specimen (tissue and or BAL fluid) fungal assays
- Invasive specimens: 123 tissues, 426 BAL fluids (43 patients had both)



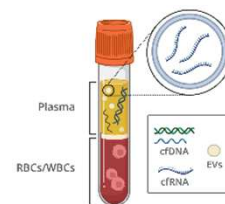
- Retrospective single-center cohort study
- 7 days before or 2 days after invasive specimen collection.
- Test results were **88.5%** (448/506) concordant.
- In proven cases, 64.7% (11/17) of negative PCR results occurred in patients with fungal sinusitis and limb infection.
- **Non-hematologic malignancy and nonneutropenic state were statistically associated with negative PCR in patients with proven or probable IMD.**

Anthony Lieu et al. Clin Infect Dis, Volume 80, Issue 5, 15 May 2025, Pages 1095–1102

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Cell-free DNA PCR: High sensitivity and specificity for invasive mold infections, particularly *Mucorales*

	Sensitivity	Specificity
<i>Aspergillus</i> spp (<i>A. fumigatus</i> , <i>A. flavus</i> , <i>A. niger</i>)	56.25% (9/16)	97.2% (104/107)
<i>Aspergillus</i> spp. (<i>A. terreus</i> , <i>A. ustus</i> , <i>A.</i> <i>nidulans</i>)	66.7% (2/3)	99.2% (121/122)
<i>Mucorales</i> spp. (<i>Rhizopus</i> spp, <i>Rhizomucor</i> spp, <i>Mucors</i> spp)	91.7% (11/12)	98.2% (111/113)
<i>Fusarium</i> spp	66.7% (2/3)	99.2% (121/122)



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Senchyna et al. Clinical Infectious Diseases, Volume 73, Issue 9, 1 November 2021, Pages 1677–1684

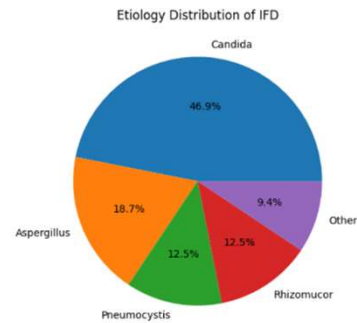
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mNGS for diagnosis of IFDs in patients with hematological diseases

A total of 67 patients were included in the study cohort:

- 32 were diagnosed with IFDs based on mNGS and culture.
- 35 showed no detectable fungal pathogens.

	Detection Yield
mNGS	48 %
Culture	15 %
β-D-glucan assay	12%
Galactomannan assay	3 %



Independent risk factors for IFD:

- CD4+ T cell count <400 cells/μL → OR 9.45
- Elevated CRP → OR 3.18
- Elevated IL-6 → OR 5.75
- Elevated IL-10 → OR 3.31
- Hypoproteinemia → OR 42.17
- Neutropenia >10 days → OR 4.11

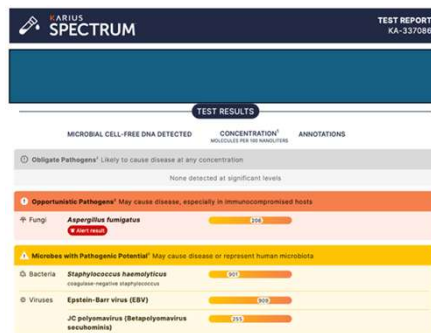


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Chen et al. IJID Regions 17 (2025) 100780

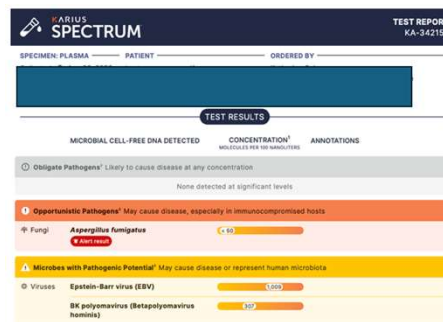
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Early detection, monitoring of progress of infection & efficacy of treatment



Aspergillus fumigatus
206 mol/100 nl

(July 24, 2026)



Aspergillus fumigatus
<50 mol/100 nl

(Aug 28, 2026)

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Complement to, or replacement for, conventional microbiologic and fungal diagnostic testing ?

KARIUS
CLARITY. AT SPEED.

	Year	Study Type	Patient Population	Key Results	Clinical Impact	Major Limitations
Akkad	2025	Review	Solid organ and hematopoietic stem cell transplant recipients	Adjunct diagnostic tool for invasive fungal infections.	May support earlier diagnosis when invasive sampling is not feasible.	Limited prospective data; heterogeneous studies.
Bell	2024	Review	Immunocompromised and general infectious disease patients	Summarized evidence supporting plasma cfDNA sequencing for fungal diagnosis.	Useful as a complementary test in selected patients.	No pooled performance analysis.
Huang	2024	Review	Patients with suspected invasive fungal infections	Potential for rapid fungal detection.	May shorten time to diagnosis and treatment.	Requires further clinical validation.
Huygens	2024	Retrospective multicenter cohort	Hematologic malignancies and suspected invasive fungal infection	Improved diagnostic yield vs. conventional methods. Detected fungi missing by culture.	Helpful in culture negative IFI.	Moderate sensitivity; should complement standard tests.
Hyser	2024	Single case report	Advanced HIV patient	Identified a mixed fungal infection missed by routine testing.	Guided targeted antifungal therapy.	Single case report.
Wolf	2026	Prospective cohort	Pediatric oncology and hematopoietic cell transplant patients	Predicted bloodstream ~3 days earlier; 50% sensitivity and 94% specificity	Potential for earlier antimicrobial therapy.	Fungal-specific performance was not analyzed separately.

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NGS Performance depends on underlying status of patients

Neutropenic

Non-neutropenic:

- ☐ Lung transplant recipients
- ☐ Critically ill patients
- ☐ Patients on steroids
- ☐ ...



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Case study 3: identification of novel fungal species

- A sputum sample from a 32-year-old patient with Burkitt lymphoma was collected 35 days after allogeneic bone marrow transplant with graft-versus-host disease.
- CT chest imaging the day prior showing interval development of patchy ground-glass opacities throughout the lungs, some areas of which are nodular in configuration.
- Posaconazole prophylaxis.
- Methylprednisolone, tacrolimus, and ruxolitinib.
- Afebrile.

Li, et al. mBio. 2024 Aug 14;15(8):e0014424.

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Microbiology Work-Up: unusual laboratory finding

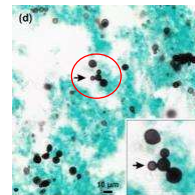
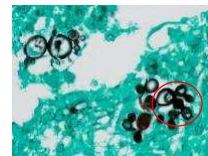


- Abundant squamous epithelial cells
- Mixed oropharyngeal flora
- Rare neutrophils

Multiple spherical buds all over the surface



a "Mickey Mouse" shape budding like structure.

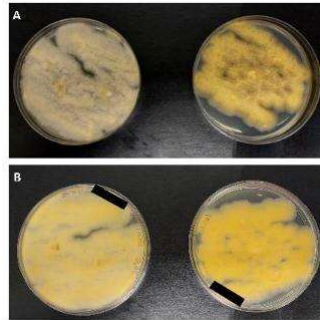


Presumptive ID: *Paracoccidioides* ?

Li, et al. mBio. 2024 Aug 14;15(8):e0014424.

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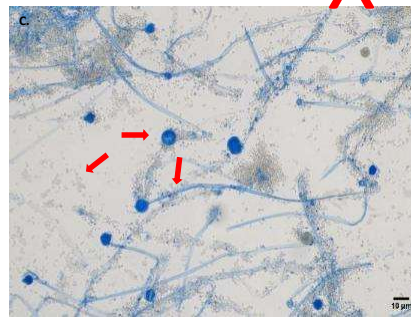
Mucoralean-like fungus after 2 days



30°C on SGA

Macroscopy:

- Fast growing
- Cottony to fluffy
- White to yellow colonies
- Multiple sporangia



Microscopy:

- Hyaline non-septate hyphae
- Terminal, globose to spherical sporangia
- Multi-spored sporangia

Presumptive ID: ~~Parasaccidioides~~ ?

Li, et al. mBio. 2024 Aug 14;15(8):e0014424.

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MALDI Biotyper® (Bruker Daltonics Inc.)



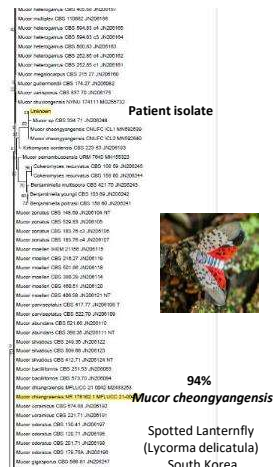
No match

ITS PCR-Sequencing



577 bp fragment
Blast GenBank and MycoBank
databases

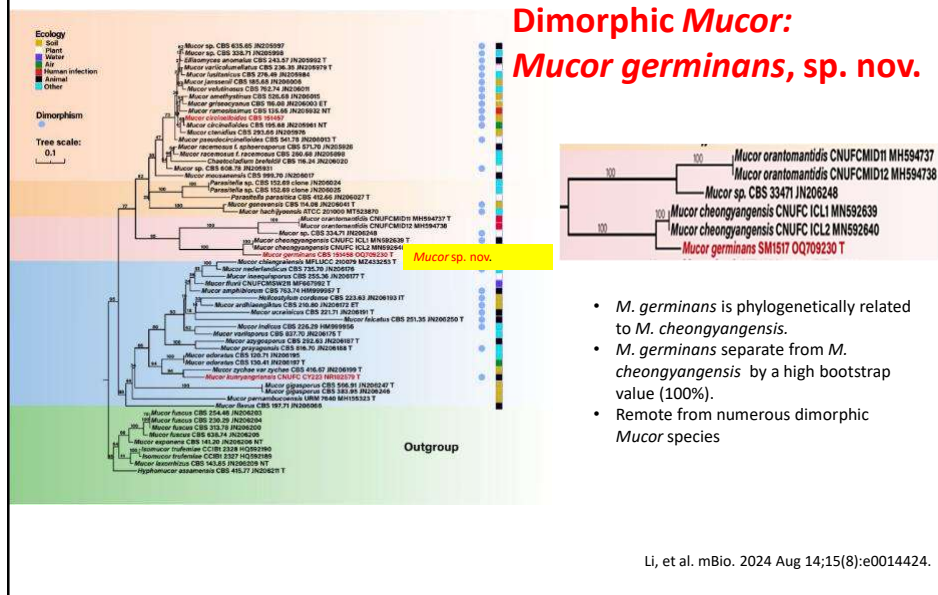
Alignment and phylogenetic tree



The isolate was reported as *Mucor* species.

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Phylogenetic analysis and relationship with other dimorphic *Mucor* species



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Take-home message

NGS is the routine clinical practice:

- valuable tool for (early) diagnosing complex, invasive, and culture-negative fungal infections: **its performance varies with the patient's underlying clinical status.**
- reveals cryptic and previously unrecognized fungal species, improving species identification beyond conventional diagnostics.
- has an expanding role in detecting antifungal resistance and resistance-associated mutations.
- can distinguish relapse from reinfection with high precision.
- the gold standard for outbreak investigations, providing high-resolution evidence of fungal transmission to support infection prevention and surveillance.

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Thank you!



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