Does Identification of Cryptic Aspergilli Actually Matter?

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Disclosures

Funding to FTL

- bioMerieux
- Bruker
- F2G
- Mycovia
- Scynexis
- Sfunga

Collaborations through NIH

- Amplyx
- F2G
- Fujifilm/Toyama/Appili
- Scynexis
- Viamet/Mycovia

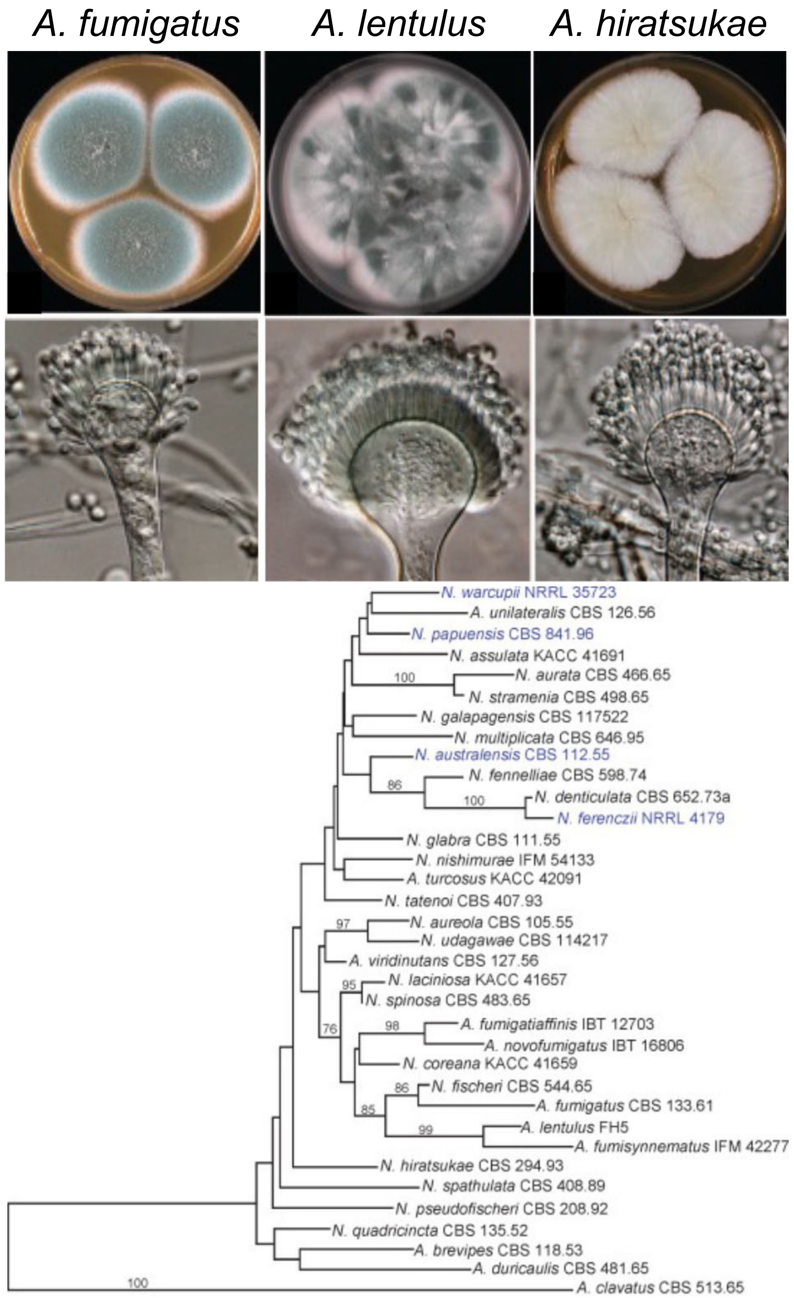
Member, CLSI Antifungal Susceptibility Subcommittee

Road Map

- What makes a species cryptic
- What is the epidemiology of cryptic *Aspergillus* species
- How are they detected and identified clinically
- What makes cryptic species clinically relevant

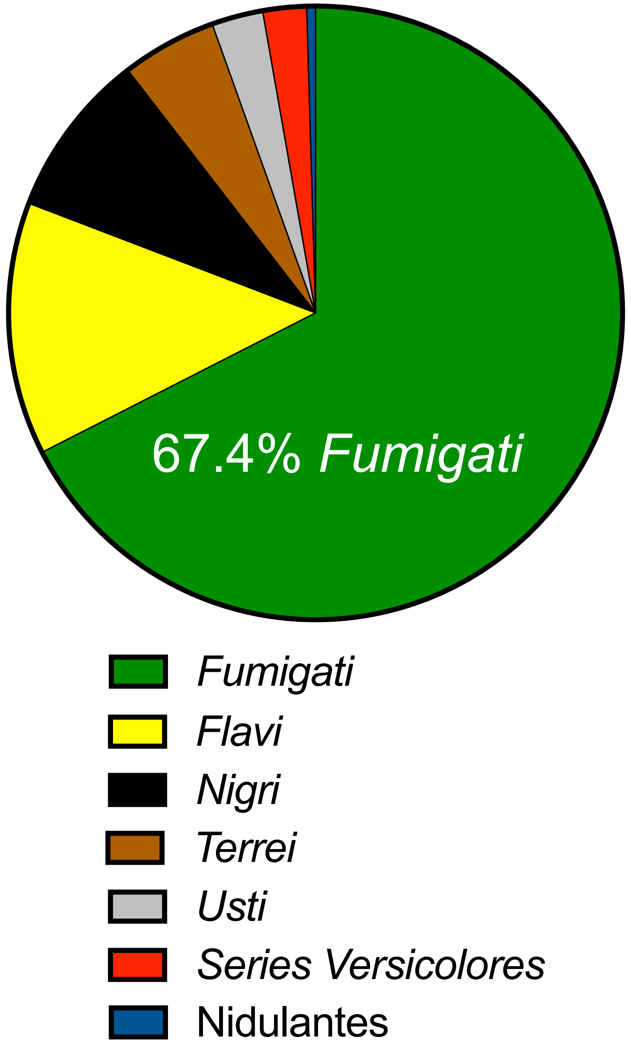
What are Cryptic Species?

- Closely related species that are non-distinguishable by morphologic characteristics
- Molecular or proteomic approaches needed to distinguish between cryptic species and others
  - DNA sequence analysis using multiple targets (β-tubulin, calmodulin [not ITS for *Aspergillus*])
  - MALDI-TOF MS
- “Sibling species”
  - Often grouped within species complex



Samson et al. *Stud Mycol* 2007;59:147-203.
Sugui et al. *J Clin Microbiol* 2014;52:3707-3721.
Samson et al. *Stud Mycol* 2014;78:141-173.

TRANSNET Study



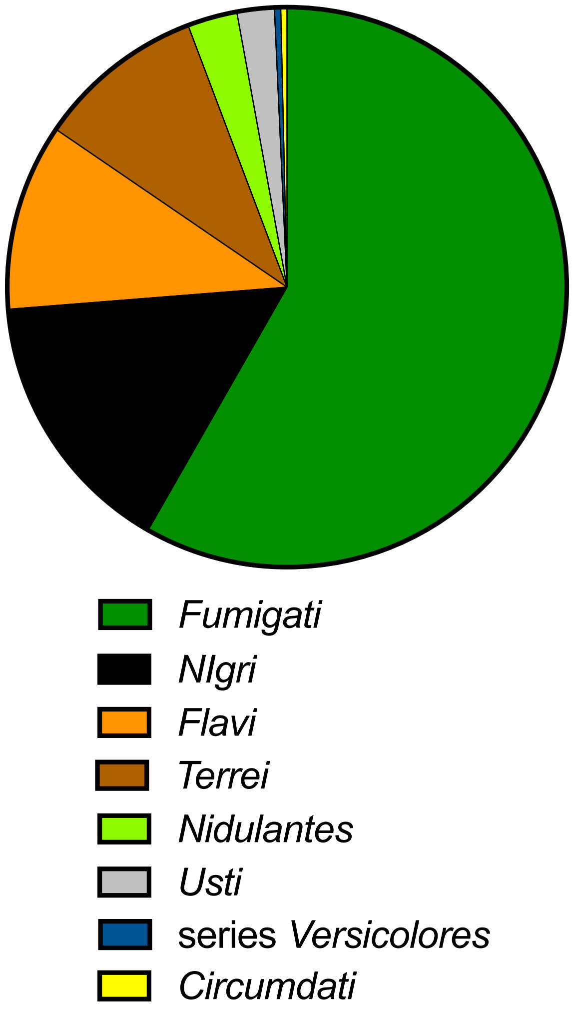
- 218 *Aspergillus* isolates
  - HSCT & SOT patients with proven or probable IA
  - 10.6% cryptic species
  - 18 in HSCT recipients, 5 in SOT recipients

Section (No.)	Cryptic Species
Fumigati (147)	139 (93.9% *A. fumigatus;* 63.4% overall) 4 *A. lentulus* (3 with elevated AMB & VRC MICs), 3 *A. udagawae* (2 with elevated AMB MICs), 1 *A. thermomutatus*
Flavi (29)	All *A. flavus*
Nigri (19)	13 *A. niger,* 6 *A. tubingensis*
Terrei (11)	All *A. terreus*
Usti (6)	All *A. calidoustus* (all with elevated ITC, VRC, & PSC MICs)
Series *Versicolores* (5)	3 *A. versicolores,* 2 *A. sydowii*
Nidulantes (1)	*A. quadrilineatus*

No clinical outcome data

Balajee et al. *J Clin Microbiol* 2009;47:3138-3141.

FILPOP Study – Spain, October 2010 & May 2011



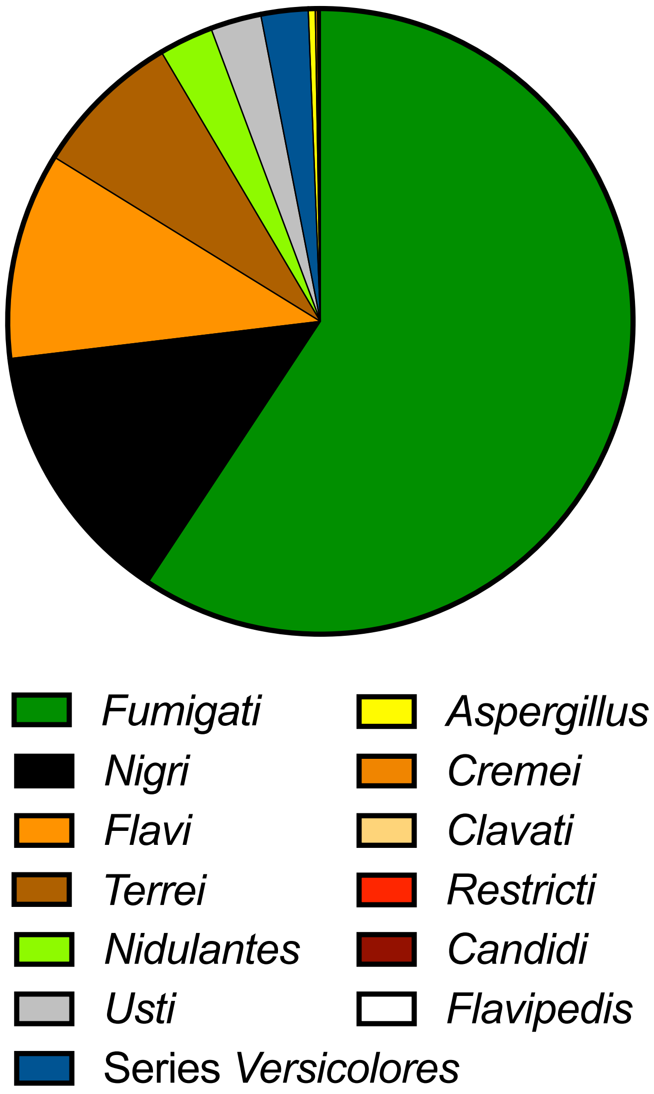
- 278 *Aspergillus* isolates
  - 14.4% (40) cryptic species
  - 16 isolates of cryptic species resistant to at least 1 antifungal (5.76% overall, or 40% of cryptic species)

Section (No.)	Cryptic Species
Fumigati (162)	3 *A. lentulus* (all ITC resistant), 1 *A. viridinutans,* 1 *A. fumigatiaffinis,* 1 *A. thermomutatus*
Nigri (43)	22 *A. tubingensis*
Flavi (30)	3 *A. alliaceus* (all AMB resistant)
Terrei (27)	1 *A. carneus*
Usti (8)	4 *A. calidoustus* (all VRC & PSC resistant)
Versicolores (1)	*A. sydowii*
Circumdati (1)	*A. westerdijkiae*

No clinical outcome data

Alastruey-Izquierdo et al. *Antimicrob Agents Chemother* 2013;57:3380-3387.

Contemporary U.S. Data

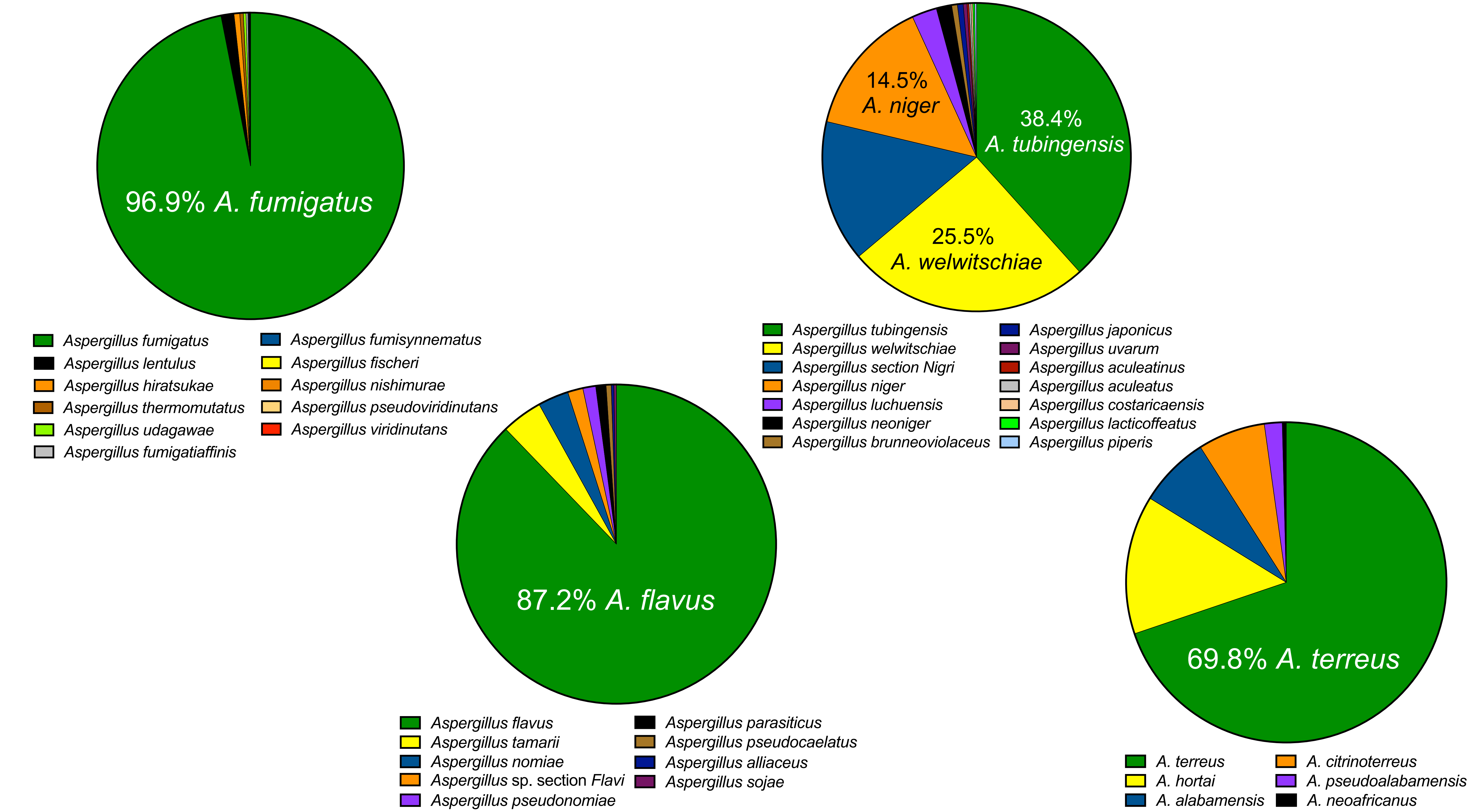


3606 *Aspergillus* isolates over 52-month period (Oct. 2015-Jan. 2020)

- 59.3% section *Fumigati*
- 13.8% section *Nigri*
- 10.7% section *Flavi*
- 7.7% section *Terrei*

All other sections < 5%
(< 9% combined)

Fungus Testing Laboratory UT Health San Antonio internal data



Fungus Testing Laboratory UT Health San Antonio internal data

Cryptic *Aspergillus* & Clinical Outcomes

Prospective, multicenter observation study

(13 French & Danish centers over 27-month period)

- Clinical involvement recorded for 369 cryptic *Aspergillus* isolates (67 species from 13 sections)
  - *Nidulantes* – 119 isolates, 16 species
  - *Nigri* – 53 isolates, 7 species
  - *Fumigati* – 49 isolates, 7 species
  - *Circumdati* – 48 isolates, 8 species
- Only 15 linked to invasive aspergillosis
  - 17 involved in chronic pulmonary aspergillosis
  - 225 bronchial colonization
  - 53 in superficial infection (onychomycosis & otomycosis)

3-month mortality in cryptic IA patients 60%
(4 of 5 infected with *A. sublatus*)

Imbert et al. *Mycoses* 2021;64:1346-1353.

Cryptic *Aspergillus* & Clinical Outcomes

Section	Species (No.)	Susceptibility (MIC_{50} / MIC_{90}; Range)				
		Itraconazole	Voriconazole	Posaconazole	Isavuconazole	Amphotericin B
Flavi	*A. alliaceus* (12)	0.12 / 0.12 0.06-0.12	0.25 / 0.25 0.25-0.5	0.06 / 0.12 0.015-0.25	0.5 / 1 0.25-1	>16 / >16 4->16
Fumigati	*A. fisheri* (3)	>8	2	0.25-0.5	2	1-2
	A. udagawae (4)	2->8	2-4	0.25-0.5	2-4	1-8
	A. lentulus (9)	0.5->8	2-8	0.25-0.5	0.5-1	1-8
Nidulantes	*A. sydowii* (34)	>8 / >8 0.25->8	2 / 2 0.25-4	0.5 / 1 0.25-1	1 / 2 0.25-2	4 / >16 1->16
Nigri	*A. tubingensis* (10)	>8 / >8 0.5->8	2 / 2 1-2	0.25 / 0.25 0.12-0.5	4 / 4 1-4	0.5 / 0.5 0.12-0.5
Usti	*A. calidoustus* (18)	>8 / >8 >8	8 / 16 4-16	>8 / >8 >8	4 / 4 2->8	1 / 2 0.25-2

- 10 of 15 isolates associated with IA exhibited high MICs to at least one antifungal
  - Except for the 5 *A. sublatus* isolates (3-month mortality 80% [4 of 5 patients])

Imbert et al. *Mycoses* 2021;64:1346-1353.

Aspergillus section *Usti*

Pathogens/Colonizers/ Environmental	Species (at least 26 unique species)
Pathogens Capable of growth at 37°C	***A. calidoustus****, A. pseudodeflectus, A. granulosus* *A. ustus* – proven case soft tissue IA (unable to grow at 37°C)
Colonizers Unable to grow at 37°C	*A. insuetus, A. keveii, A. puniceus*
Environmental Specimens Unable to grow at 37°C	*A. amylovorus, A. asper, A. baeticus, A. californicus, A. carlsbadensis, A. cavernicola, A. colinsii, A. deflectus, A. egyptiacus, A. elongatus, A. germanicus, A. heterothallicus, A. kassunensis, A. lucknowensis, A. monodii, A. pseudoustus, A. subsessilis, A. thessauricus, A. turkensis*

Grayish-brown colonies

Biseriate phialides

Hülle cells

Samson et al. *Stud Mycol* 2011;69:81-97.
Glampedakis, Erard, Lamoth. *J Fungi* 2020;6. doi:10.3390/jof6020084.

Clinical Characteristics IA Secondary to *A. calidoustus*

- Retrospective review of 72 proven/probable cases
  - 45 previously published in literature
  - 27 identified from screening of clinical microbiology databases in European hospitals
- Most patients non-neutropenic transplant recipients
  - 47% HSCT
  - 33% SOT (lung)
  - Receiving long-term immunosuppressive therapies
    - Calcineurin inhibitors +/- corticosteroids

Organ Involvement	
Organ	**Percent**
Lungs	76%
Skin/soft tissue	28%
CNS	14%

Disseminated infection in 33% of cases

Overall mortality 58% at 24 weeks

- IA contributor to death in 81%
- Higher mortality in HSCT vs. SOT recipients (79% vs. 47%; $p = 0.01$)

Glampedakis et al. *Clin Infect Dis* 2021;72:1379-1385.

Clinical Characteristics - Treatment

Treatment difficult with frequent use of multiple antifungals (consecutively or in combination)

- Failure (stable disease or progression) in 55%
- Voriconazole 1st line agent in non-HSCT and less severely ill patients with non-disseminated disease
- Higher mortality in those receiving amphotericin B (more proven & disseminated disease)

Characteristic	AMB	VRC
HSCT	64%	30%
Proven IA	72%	26%
Disseminated Dz.	56%	13%

47% were receiving mold-active antifungal prophylaxis at time of diagnosis (primarily posaconazole). Consistent with previous literature –

- Egli et al. *Transplantation* 2012;94:403-410.
- Small case-control study in lung SOT recipients with *A. calidoustus* IA
- Antifungal prophylaxis (3rd gen. azole) in 83.3% cases & 33.3% controls ($p = 0.13$)

Glampedakis et al. *Clin Infect Dis* 2021;72:1379-1385.

Alignment of *A. calidoustus* Cyp51A-like protein with other *Aspergillus* species showed M220V replacement M220V mutations in *A. fumigatus* associated with azole-resistance in *A. fumigatus*

Harigawa et al. *Front Microbiol* 2016;7:1382.

Reference	No. Isolates MIC param.	ITR	VRC	PSC	ISC	AMB	AFG	CFG	MFG	TRB
Varga et al. *Euk Cell* 2008	N = 20 Range	**>16**	**8-16**	**>16**	---	1-2	---	0.25-4	---	0.03-0.125
Alastruey-Izquierdo et al. *Med Mycol* 2010	N = 8 GM MIC (range)	**15.54 (8-16)**	**7.13 (4-8)**	**13.07 (4-16)**	---	0.93 (0.25-2)	0.02 (0.015-0.12)	3.08 (0.5-32)	0.06 (0.015-0.5)	---
Buil et al. *J Antimicrob Chemother* 2017	N = 25 MIC_{50}/MIC_{90} (range)	**>16/>16 (1->16)**	**8/16 (8-16)**	**>16/>16 (>16)**	**4/8 (4-8)**	1/2 (0.5-2)	1/4 (0.125-16)	---	---	---
Wiederhold et al. *J Antimicrob Chetother* 2018	N = 11 MIC_{50}/MIC_{90} (range)	---	**4/4 (2-8)**	**4/4 (2-8)**	---	---	0.12/4 (0.06-4)	0.12/4 (0.06-4)	≤0.015/ 0.03	---
Glampedakis et al. *Antimicrob Agents Chemother* 2018	N = 10 Range	---	**2-8**	**4->16**	**2-4**	0.25-2	---	---	---	0.5-1
Rivero-Menendez et al. *J Antimicrob Chemother* 2019	N = 20 MIC_{50}/MIC_{90} (range)	---	**4/8 (4-16)**	**16/16 (4-16)**	---	0.5/2 (0.12-2)	---	---	0.12/4 (0.004-4)	---
Glampedakis et al. *Clin Infect Dis* 2021	N = 44 MIC_{50}/MIC_{90} (range)	---	**8/8 (2-16)**	**16/>16 (4->16)**	**2/4 (0.5->16)**	0.5/1 (0.25-2)	---	---	---	---

Unanswered Questions

- How frequently do cryptic species cause disease?
  - No information from clinical trials - most patients now enrolled based on antigen +/- imaging results
    - Herbrecht et al. *N Engl J Med* 2002 - 53.8% culture positive
    - Marr et al. *Ann Intern Med* 2015 - 23.3% culture positive
    - Maertens et al. *Lancet* 2016 - 33.8% culture positive
    - Maertens et al. *Lancet* 2021 - 21.4% culture positive

- Cultures of tissue & respiratory tract specimens - sensitivity 30-58% for invasive mold infections
  - Autopsy-confirmed cases = 53% culture positive (MD Anderson Cancer Center)
  - Cytologic evidence of fungus = 58% culture positive
  - Histologic evidence of fungus = 30% culture positive

Tarrand et al. *Am J Clin Pathol* 2003;119:854-858.

Surrogate Markers/Biomarkers

- (1,3)-β-D-glucan
  - Component of fungal cell wall
  - Species identification not possible
  - Pan-fungal marker
  - False-positives problematic

- Galactomannan (GM)
  - Component of *Aspergillus* cell wall
  - Species identification not possible
  - Other fungi with GM
    - *Penicillium/Talaromyces/Paecilomyces*
    - *Fusarium*
    - *Histoplasma*

PCR-Based Diagnostic Tools

AsperGenius (Pathonostics)

28S rRNA gene
- *A. fumigatus*
- *A. flavus*
- *A. terreus*
- *A. fumigatus* TR$_{34}$, TR$_{46}$, & WT *cyp51A*

MycoGENIE (Ademtech)

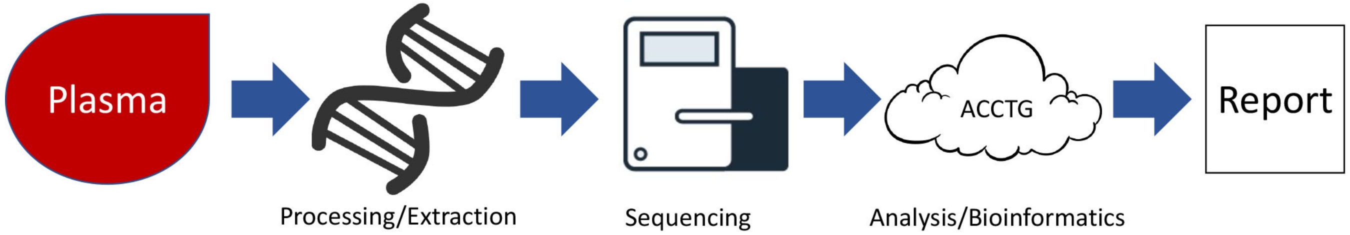
28S rRNA gene
- *A. fumigatus*
- *A. fumigatus* TR34/L98H

Fungiplex (Bruker)

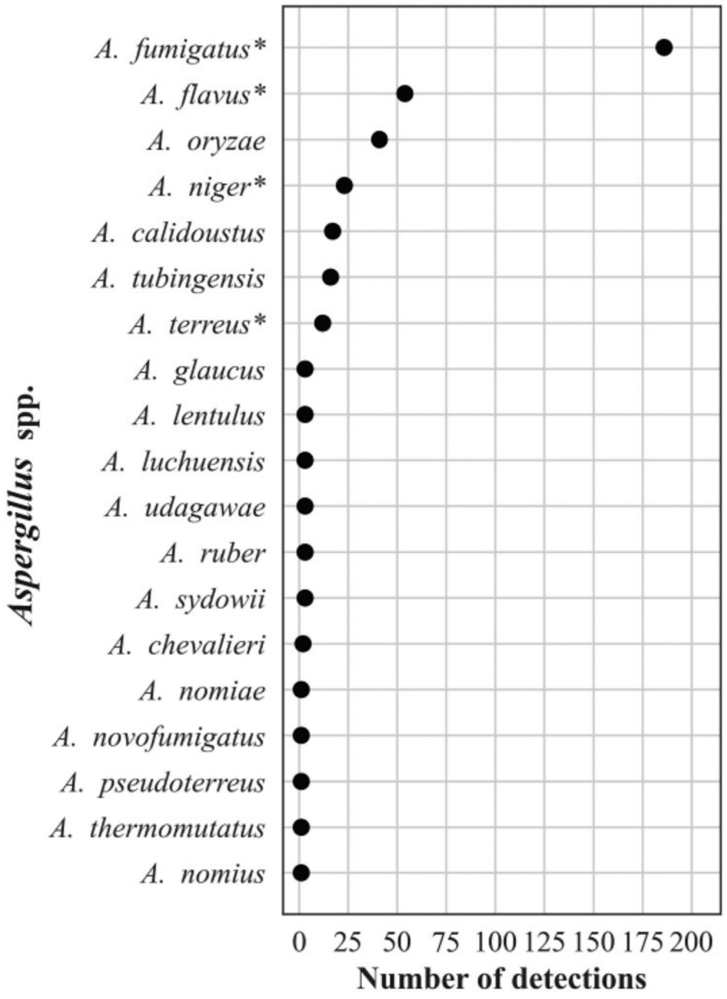
Aspergillus identified
- *Aspergillus* spp. (*A. fumigatus, A. flavus, A. niger*)
- *A. terreus*
- *A. fumigatus* TR$_{34}$, TR$_{46}$

Do not identify cryptic species; unavailable in some areas

Microbial cell-free DNA Sequencing
(mcf-DNA-Seq; liquid biopsy approach)



- Advantages
  - Non-invasive (plasma sample – liquid biopsy)
  - Species-level identification
- Challenges/limitations
  - Not widely available
  - Performance in different patient populations undefined
  - Interpretation in context of multiple microbial species (different fungi & bacteria)



Park et al. *J Clin Microbiol* 2023;61:e018552.

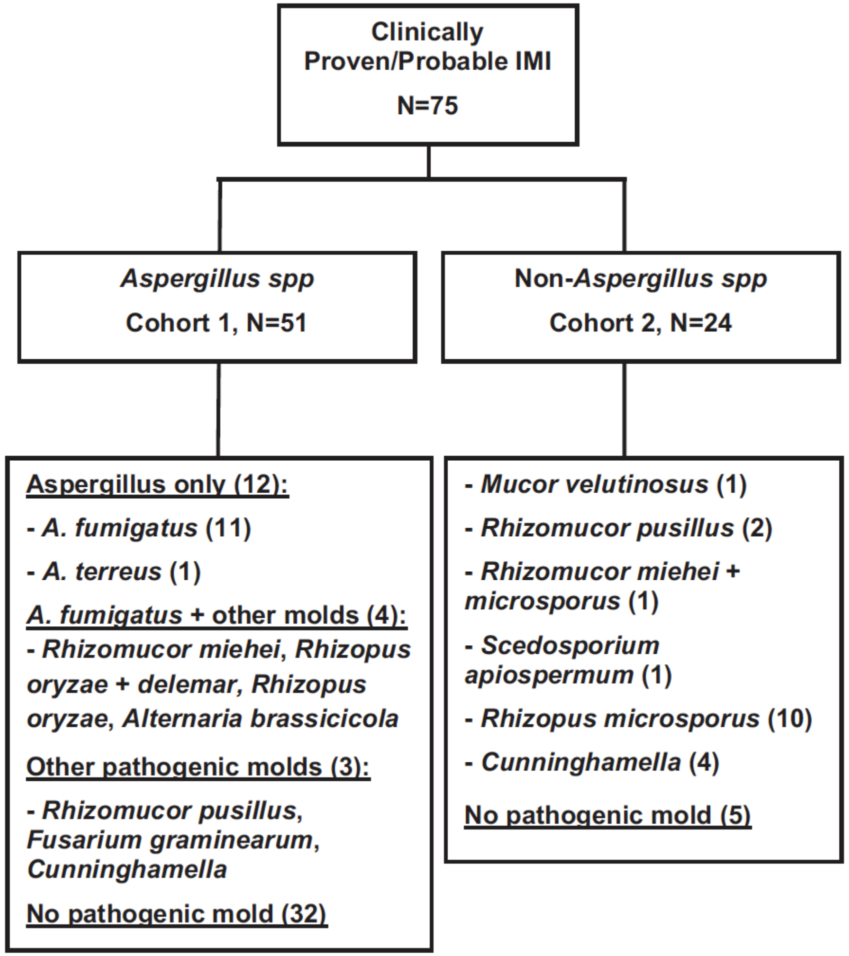
Validated *Aspergillus* Species

Aspergillus (26)
Candida (16)
Fusarium (10)
Mucor (4)
Penicillium (5)
Rhizomucor (3)
Rhizopus (4)
Scedosporium (4)
Blastomyces dermatitidis
Coccidioides immitis
Cryptococcus gattii VGI (Cryptococcus gattii)
Cryptococcus neoformans
Histoplasma capsulatum
Pneumocystis jirovecii

Aspergillus aculeatus
Aspergillus brasiliensis
Aspergillus calidoustus
Aspergillus candidus
Aspergillus chevalieri
Aspergillus clavatus
Aspergillus fischeri
Aspergillus flavus
Aspergillus fumigatus
Aspergillus glaucus
Aspergillus lentulus
Aspergillus luchuensis
Aspergillus nidulans
Aspergillus niger
Aspergillus nomiae
Aspergillus novofumigatus
Aspergillus oryzae
Aspergillus persii
Aspergillus sclerotiorum
Aspergillus terreus
Aspergillus thermomutatus
Aspergillus tubingensis
Aspergillus udagawae
Aspergillus ustus
Aspergillus versicolor
Aspergillus wentii

https://kariusdx.com/karius-test/pathogens

Microbial cell-free DNA Sequencing & IA



- Well-characterized cohort of HSCT recipients with pulmonary IMI (Hill et al. - Karius assay)
  - 38 of 75 with ≥1 pathogenic mold – (Sensitivity 51%)
  - *Aspergillus* detected in 16 of 51 with P/P IA – (Sensitivity 31%)
  - Mold detected in 19 of 24 patients with P/P non-*Aspergillus* IMI (Sensitivity 79%)
  - No false-positives in 19 negative controls – (Specificity 100%)

- Patients with suspected CAPA (Hoenigl et al - Karius assay)
  - Fungal pathogens detected in 16 plasma samples from 11 patients (*A. fumigatus* in 10 samples)
  - *A. fumigatus* detected in 8 samples from 4 patients with probable CAPA (1 sample *R. microsporus*)
  - 3 of 106 patients without CAPA or other mold infection plasma samples positive for *A. fumigatus* or *Alternaria* sp. (97% specificity)

- IA in HM patients & those with COVID-19 (Lee et al. - non-Karius assay)
  - *Aspergillus* DNA detected in 100% hematologic malignancy pts with proven IA and 91.7% those with probable IA
  - *Aspergillus* DNA detected in 50% of COVID-19 pts with probable IA
  - *A. fumigatus* predominant species identified in 89.5% (1 case each of *A. chevalieri* and unspecified *Aspergillus* sp.)

Hill et al. *Clin Infect Dis* 2021;73:e3876-3883. Hoenigl et al. *J Clin Microbiol* 2023;61:e0185922. Lee et al. *J Infect Dis* 2023;228:444-452.

Unanswered Questions

- Do patients infected with cryptic species infections respond less favorably?
  - No clear answer since no controlled studies
  - Although mortality rates are high, patients immunosuppressed (HSCT or SOT) with multiple comorbidities
    - Cannot underestimate underlying disease

- Best treatment remains unknown
  - Should you change pre-emptive treatment practices?
  - Investigational agents with promising activity
    - Olorofim
    - Fosmanogepix

Nedel & Pasqualotto. *Mycopathologia* 2014;178:441-445.

Unanswered Questions

- Are cryptic species more resistant to antifungals?
  - Many cryptic species do have reduced susceptibility to clinically available antifungals
    - TRANSNET, FILPOP, others
  - But these species often represent a very low percentage of total isolates (e.g., ~5% in FILPOP study)
  - Rate of azole-resistant *A. fumigatus* similar to or exceeds this in many areas

- Should we systematically search for them?
  - Based on reduced susceptibility of many species, easy to assume that patients may benefit from proper identification
    - Useful for epidemiologic purposes and in outbreak investigations
    - But is it cost-effective to do so?

Nedel & Pasqualotto. *Mycopathologia* 2014;178:441-445.