ESCMID 2025 | Poster #P2900

Phylogenomics reveals patterns of azole activity against *Aspergillus* section *Nigri* clinical isolates

John H. Kimbrough, Maura H. Karr, Marisa L. Winkler, Mariana Castanheira*

Element Materials Technology (JMI Laboratories), North Liberty, Iowa, USA

*Presenting Author

Introduction

- Antifungal resistance and infections caused by members of *Aspergillus* section *Nigri* (AsN) are increasing.
- Azole antifungal agents targeting the CYP51A and CYP51B enzymes of the ergosterol biosynthesis pathway, involved in fungal cell wall biosynthesis, represent first-line treatments for AsN-associated infections.
- Defining resistance mechanisms and resistance-conferring mutations requires comparison to closely related, susceptible reference isolates, but commonly used identification methods (e.g., morphology, MALDI-TOF MS [MALDI], PCR) cannot always resolve cryptic AsN species, potentially obscuring species-specific resistance patterns and natural sequence polymorphisms.
- To better understand antifungal resistance in AsN, we performed antifungal susceptibility testing and comparative genomics on AsN isolates collected as part of the SENTRY Antifungal Surveillance program.

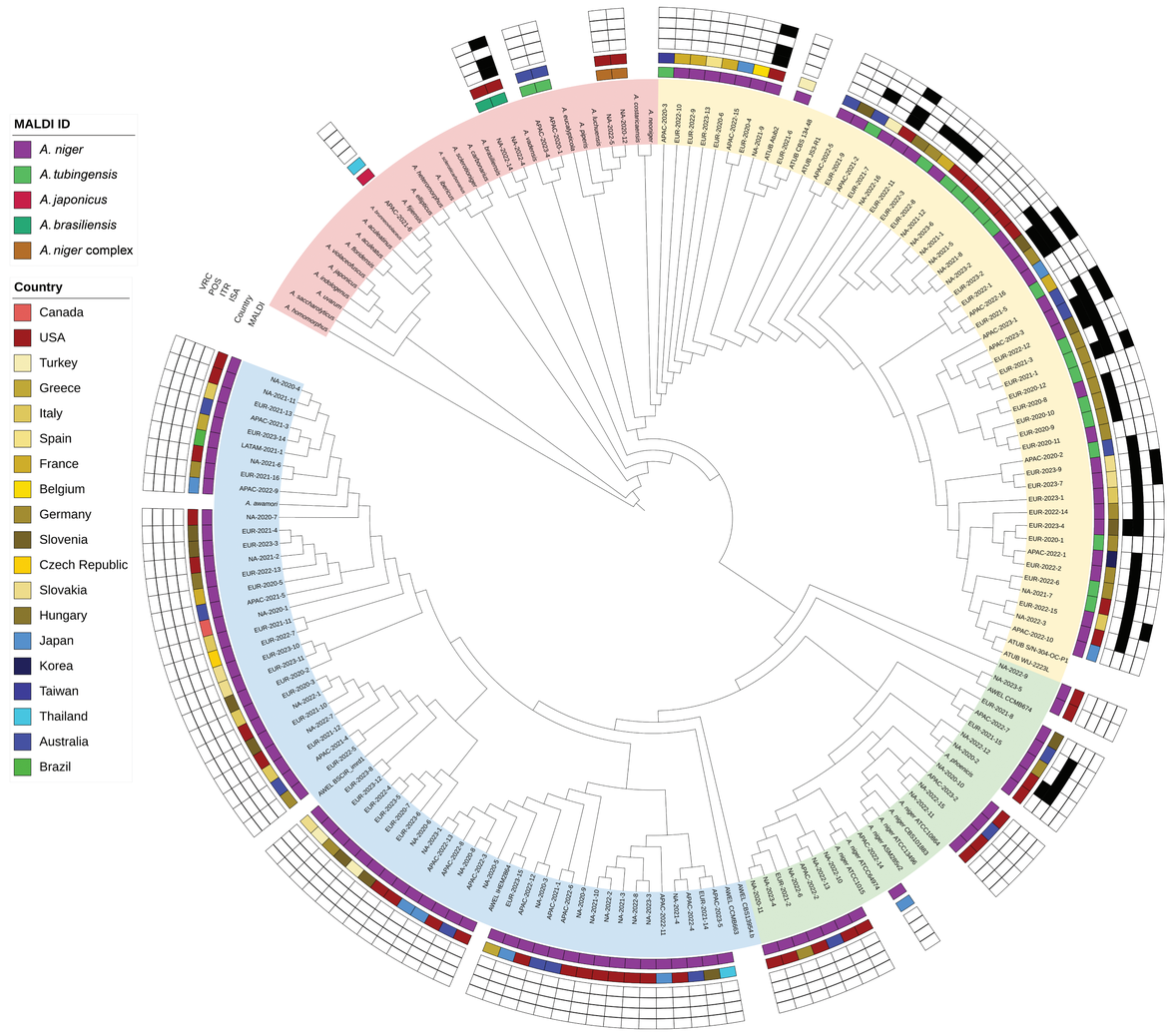
Methods

- AsN isolates (*n*=135; 1 isolate per patient episode) were collected from 2020–2023 in 19 countries (Fig. 1).
- All isolates were initially identified by MALDI and were tested by CLSI reference broth microdilution method (M38); CLSI azole ecological cutoff values (M57S) for *A. niger* (ANGR) were applied against all isolates.
- Genome sequencing was performed on all clinical isolates. Twenty-nine unique AsN species reference genomes (NCBI) were used to create a set of 1,637 genes using chewBBACA v3.3.10. Genes were extracted from all clinical and reference isolates and aligned using MUSCLE v5.1. Phylogenetic reconstruction used FastTree v2.1.11 and was visualized with iTOL v6.9.1.

Results

- Phylogenetic clustering placed 58 (43.0%) and 51 (37.8%) isolates within the *A. welwitschiae* (AWEL) and *A. tubingensis* (ATUB) clades; ANGR accounted for 19 (14.7%) isolates.
- Overall, 90.4%, 77.8%, 100.0%, and 91.9% of AsN isolates displayed wildtype (WT) MICs for isavuconazole (Fig. 2A), itraconazole (ITR; Fig. 2B), posaconazole (Fig. 2C), and voriconazole (Fig. 2D), respectively.
- Azole non-WT isolates (*n*=33; 24.4%) were primarily associated with ATUB (*n*=29, 87.9%; 56.9% overall).
  - Non-WT ITR MICs were observed in 89.7% of azole non-WT ATUB, and 53.8% of azole non-WT ATUB were only ITR non-WT.
  - Isolates with >1 non-WT azole MIC were uncommon (*n*=14; 10.4%) and primarily ATUB (85.7%).
  - 100.0% of AWEL isolates were pan-azole WT.
  - Three ANGR (15.8% of ANGR) and 1 *A. brasiliensis* (ABRA) isolates were non-WT to ≥1 azole.
- Relative to the intraspecies consensus sequence, unique CYP51A alterations were identified in 1 each azole non-WT ABRA (NA-2022-4; V6I/M53I/K246Q), ANGR (EUR-2021-15; S270G), and ATUB (NA-2021-9; V17A); no azole non-WT isolates possessed unique intraspecies alterations in CYP51B.
- Interspecies variation in CYP51A (Fig. 3A) and CYP51B (Fig. 3B) consensus sequences was observed at 15 and 18 residues, respectively.
  - ATUB possessed unique residues at 100.0%/100.0% and 93.3%/100.0% of variant positions in CYP51A/CYP51B compared to ANGR and AWEL, respectively.
  - Consensus analysis identified only 1 residue lacking conservation across each species (CYP51B residue 267).

Figure 1. Evolutionary relationships among 135 globally collected *Aspergillus* section *Nigri* clinical isolates (2020–2023)



Phylogenetic tree construction (FastTree v2.1.11) based on alignment (MUSCLE v5.1) of 1,637 genes from 29 NCBI *A.* section *Nigri* reference genomes (chewBBACA v.3.3.10) and visualized with iTOL v.6.9.1. Colors from innermost to outermost indicate: top scoring MALDI-TOF MS ID (MALDI ID; Bruker MBT Filamentous Fungi Library 4.0 Rev. D [2021] and in-house custom database); epidemiological cutoff value (wildtype, open; non-wildtype, black) for isavuconazole (ISA), itraconazole (ITR), posaconazole (POS), and voriconazole (VRC); country with continental groupings sharing similar hue (North America, NA, red; Europe, EUR, yellow; Latin America, LATAM [Brazil], green; Asia-Pacific, APAC, blue). Isolates have been deidentified with their continent and year of isolation and shading of isolate identifiers corresponds to overall clade assignment as follows: Other (rose), *A. tubingensis* (yellow), *A. niger* (green), *A. welwitschiae* (blue). Reference genomes for all training-set species have been included with their respective species names and additional *A. tubingensis* (ATUB), *A. niger* (ANGR), and *A. welwitschiae* (AWEL) reference genomes have been included for comparison.

Figure 2. Azole MIC distributions for *Aspergillus* section *Nigri* isolates. Percent occurrences of MIC values for A) isavuconazole (ISA), B) itraconazole (ITR), C) posaconazole (POS), and D) voriconazole (VRC).

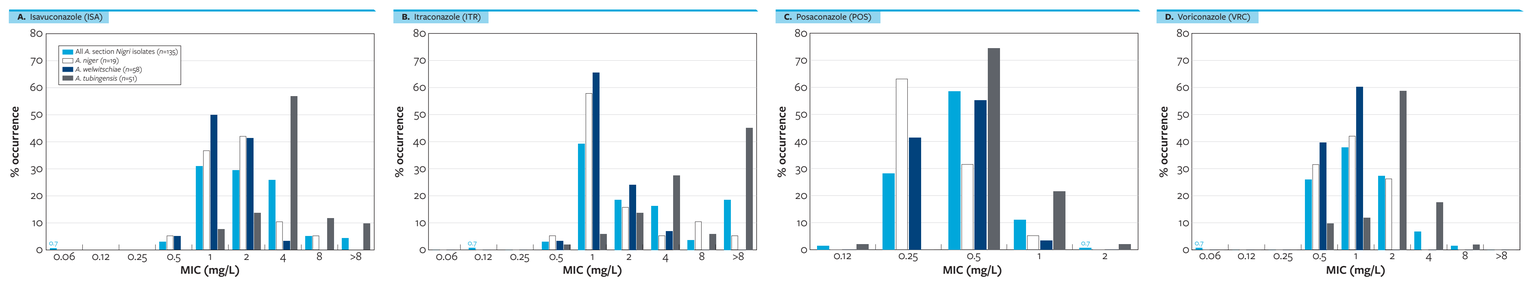


Figure 3. Positional variants in the consensus sequences of A) CYP51A and B) CYP51B among *A. tubingensis* (ATUB), *A. niger* (ANGR), and *A. welwitschiae* (AWEL). White numbers indicate position within each sequence with corresponding majority amino acid for each species with its accompanying percent conservation among isolates in the respective clade. CYP51B residue 267 (orange) is the only nonconserved position across species.

A	CYP51A AA position (% conservation)														
	3	9	24	29	57	77	270	321	343	377	382	418	492	503	504
ATUB	Y (100.0%)	A (94.0%)	A (100.0%)	V (100.0%)	A (100.0%)	K (100.0%)	N (100.0%)	T (86.0%)	L (100.0%)	V (100.0%)	R (100.0%)	E (100.0%)	L (100.0%)	I (88.0%)	Q (98.0%)
ANGR	L (100.0%)	V (100.0%)	T (100.0%)	F (100.0%)	T (63.2%)	Q (100.0%)	S (94.7%)	A (100.0%)	F (100.0%)	I (94.7%)	H (94.7%)	D (100.0%)	M (100.0%)	F (100.0%)	P (100.0%)
AWEL	L (100.0%)	V (100.0%)	T (100.0%)	F (100.0%)	A (100.0%)	Q (100.0%)	S (100.0%)	A (100.0%)	F (98.3%)	I (100.0%)	H (100.0%)	D (100.0%)	M (100.0%)	F (100.0%)	P (100.0%)

B	CYP51B AA position (% conservation)																	
	20	23	26	29	38	40	45	168	171	172	181	262	267	285	328	351	431	522
ATUB	I (100.0%)	L (100.0%)	I (100.0%)	S (100.0%)	L (100.0%)	V (100.0%)	L (100.0%)	N (100.0%)	D (100.0%)	D (100.0%)	Q (100.0%)	V (100.0%)	T (100.0%)	V (100.0%)	E (100.0%)	E (100.0%)	N (100.0%)	V (100.0%)
ANGR	V (100.0%)	V (100.0%)	F (100.0%)	L (100.0%)	F (100.0%)	I (100.0%)	F (100.0%)	G (100.0%)	E (100.0%)	N (100.0%)	K (100.0%)	I (100.0%)	N (84.2%)	I (94.7%)	Q (100.0%)	D (100.0%)	S (94.7%)	I (100.0%)
AWEL	V (100.0%)	V (100.0%)	F (100.0%)	L (100.0%)	F (100.0%)	I (100.0%)	F (87.9%)	G (100.0%)	E (100.0%)	N (100.0%)	K (100.0%)	I (100.0%)	I (84.5%)	I (86.2%)	Q (100.0%)	D (100.0%)	S (100.0%)	I (100.0%)

Conclusions

- Overall, *A. tubingensis* was the predominant species displaying azole non-WT MIC values, including non-WT MICs to ≥2 azoles, while the most common species, *A. welwitschiae,* was 100% pan-azole WT.
- Unique alterations in azole targets relative to the species-specific consensus sequences were generally absent in azole non-WT *A. tubingensis* and *A. niger.*
- *A. tubingensis* displayed few variable residues in CYP51A and CYP51B compared to *A. niger* and *A. welwitschiae*; further examination of the impact of nonconserved residues may shed light on a potential role governing naturally reduced azole activity against this species.
- The results of this study corroborate those of previous studies of *A.* section *Nigri* isolates with reduced azole responses, and suggest mechanisms beyond alterations in CYP51A/B may play a larger role in azole susceptibility in this species group than in other species causing invasive aspergillosis (e.g., *A. fumigatus*).

References

CLSI, *M38 Ed3. Reference method for broth microdilution antifungal susceptibility testing of filamentous fungi*. 2017, Clinical and Laboratory Standards Institute: Wayne, PA.

CLSI, *M38M51S Ed3. Performance standards for antifungal susceptibility testing of filamentous fungi*. 2022, Clinical and Laboratory Standards Institute: Wayne, PA.

CLSI, *M57S Ed4. Epidemiological cutoff values for antifungal susceptibility testing*. 2022, Clinical and Laboratory Standards Institute: Wayne, PA.

Letunic I and Bork, P. 2021. *Nucleic Acids Res.* 49e:W293–W296.

Price MN, *et al.* 2010. *PLoS One.* 5:e9490.

Silva M, *et al*. 2018. *Microb. Genom.* 4.

Funding

JH Kimbrough, MH Karr, ML Winkler, and M Castanheira were employees of Element Materials Technology (JMI Laboratories) at the time of this study, which was a paid consultant to Pfizer in connection with the development of this poster.

Contact

Mariana Castanheira
345 Beaver Kreek Centre, Suite A
North Liberty, IA 52317
Phone: (319) 665-3370
Fax: (319) 665-3371
Email: mariana.castanheira@element.com

To obtain a PDF of this poster:
Scan the QR code or visit https://www.jmilabs.com/data/posters/ESCMID2025_24-PZR-07_A1_GMB.pdf

SCAN ME

Charges may apply. No personal information is stored.