ASIA FUNGAL WORKING GROUP
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ISHAM
INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

MMTN
MEDICAL MYCOLOGY TRAINING NETWORK

Testing for azole-resistant *Aspergillus*

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Disclosures

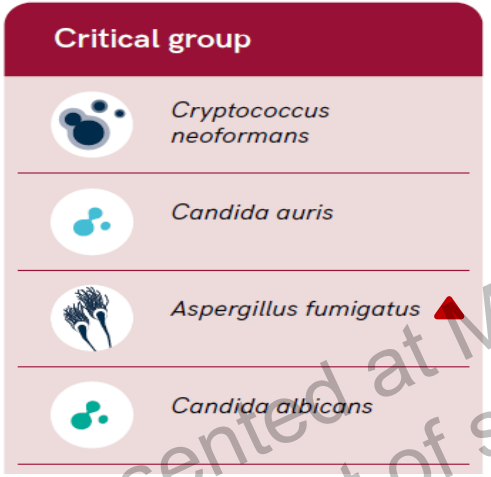
- The speaker declares no conflict of interest.

Outline

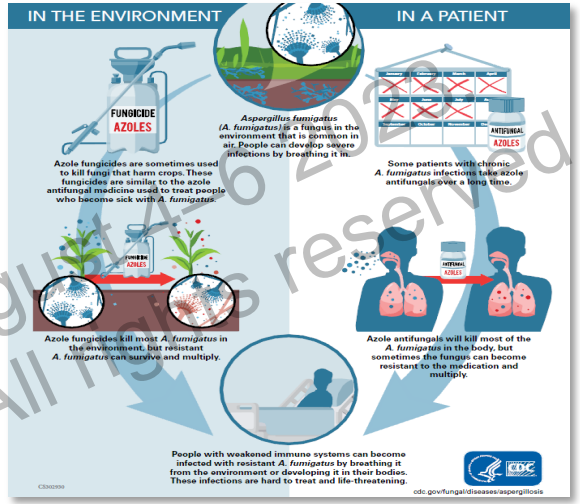
- Azole-resistant *Aspergillus* species
- Testing for azole resistance
  - ✓ Indications
  - ✓ Methods
    - Phenotypic: EUCAST, CLSI, YeastOne, Etest
    - Genotypic: detect *cyp51A* mutation
    - Proteomic: MALDI-TOF

Azole-Resistant *Aspergillus Fumigatus*

WHO Fungal Priority Pathogens



Invasive aspergillosis due to **azole-resistant *A. fumigatus*** is a life-threatening dz with high mortality (62%).



Environmental R	Patient-Acquired R
Cyp51A mutation **TR34/L98H** **TR46/Y121F/T289A**	*Cyp51A* mutation **G54**R/E/W, G138C/S, **M220**V/K/, F332K, G448S, Y431C, G432A/S Cdr1 efflux pump overexpression **HMG1** mutations: **S269**F/Y/P

Clin Infect Dis 2019;68:1463-71; J Infect Dis 2017;216(S3):S436–44

Emerging Azole-Resistant *Aspergillus* spp.

Acquired azole resistance

- ***A. flavus****:* Y119F, G441S mutation in *cyp51A*

Intrinsic azole resistance

- ***A. calidoustus***
- ***A. lentulus*** (*A. fumigatus* complex)

2017 ESCMID-ECMM-ERS Aspergillosis Guideline

Antifungal regimens in intrinsic resistance

Population	Intervention
IA due to *A. calidoustus*	Lipid formulation of AmB
IA due to *A. lentulus* (*A. fumigatus* complex)	Other than azole monotherapy

Clin Microbiol Infect 2018;24(S1):e1-e38; J. Fungi 2021;7:164; Mycoses 2023;66:711-22

MIC Matters

(minimum inhibitory concentration)

2017 ESCMID-ECMM-ERS aspergillosis guideline

Population	Intention	Intervention	SoR	QoE	
Isolate with voriconazole MIC = 2 mg/mL	To cure IA	Voriconazole + echinocandin combination therapy or L-AmB monotherapy for IA (as well as for CPA)	A	III	Strong
Isolate with voriconazole MIC >2 mg/mL	To cure IA	L-AmB	A	II_u	Strong
		AmB lipid complex	C	III	
		Voriconazole & anidulafungin	B	III	Moderate
		Posaconazole & caspofungin	C	III	
		Caspofungin or micafungin	C	III	

synergistic *in vitro* against azole-R *A. fumigatus* with voriconazole MICs of 0.5–8 mg/L*

A. fumigatus with TR46/Y121F/T289A mutation usually has voriconazole MICs >8 mg/L.

Clin Microbiol Infect 2018;24(S1):e1-e38

Indications for Testing Azole Resistance in *Aspergillus*

2017 ESCMID-ECMM-ERS aspergillosis guideline

Known resistance

Treatment failure

Surveillance

Population	Intention	Intervention	SoR	QoE
All clinically relevant *Aspergillus* isolates (in patient groups or regions with known azole resistance)	Identify azole resistance	Reference MIC testing	A	II
Clinically relevant *Aspergillus* isolates in patient groups with high prevalence of azole resistance or patients unresponsive to treatment	Identify isolates with intrinsic resistance	Species identification to complex level	A	III
Clinically relevant *A. fumigatus* isolates	Identify azole-resistant *A. fumigatus*	Routine azole agar screening	B	III
All isolates – resistance surveillance	Determine the local epidemiology of azole resistance	Periodical reference MIC testing of *A. fumigatus* complex	A	II
Azole-resistant isolates	Determine nature and trends in Cyp51A mutation distribution	Cyp51A-gene mutation analysis	A	II

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Clin Microbiol Infect 2018;24(S1):e1-e38

Azole Susceptibility Testing:

timing, methods, and number colonies

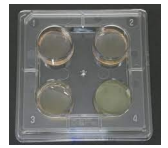
2017 ESCMID-ECMM-ERS aspergillosis guideline

Population	Intention	Intervention	SoR	QoE
Any	Confirm or reject azole resistance in clinical *A. fumigatus* isolates when antifungal treatment is considered	Azole agar screening test followed by reference MIC test where needed	A	III
	Detect azole-resistant *A. fumigatus* genotypes in a single culture	Reference MIC testing of multiple colonies (up to five colonies)	B	III
		Routine azole agar screening (up to five colonies)	B	III
	Confirm or reject azole resistance by a validated method	MIC test using EUCAST method and EUCAST BPs (S, I, R)	A	III
		MIC test using CLSI method and CLSI ECVs (wild-type/non-wild-type)	B	III
	MIC testing of various *Aspergillus* spp.	Etest®	C	III

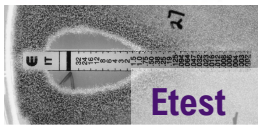
mixed susceptible–resistant colonies could be found in 20% of clinical specimens

Reference MIC testing

azole agar screening test



broth microdilution

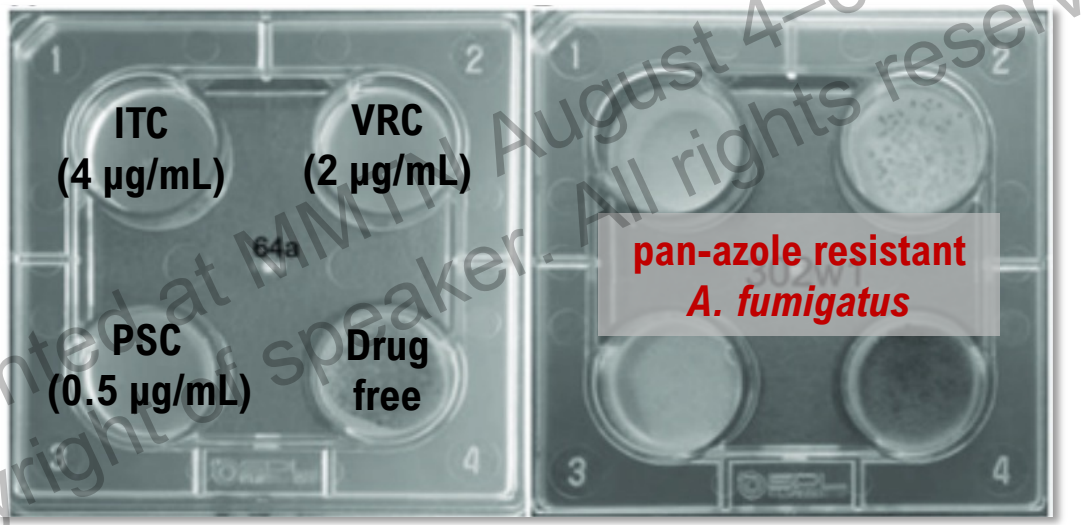


Clin Microbiol Infect 2018;24(S1):e1-e38

Detection of Azole Resistance in *Aspergillus fumigatus* Using Antifungal Containing Agar Plates

EUCAST Definite Document E.Def 10.2 (2022.6)

VIPcheck™
CE approved



Multiple (up to five) *Aspergillus fumigatus* colonies from a patient culture can be tested using a single plate.

EUCAST; Front Microbiol 2018;9:1395

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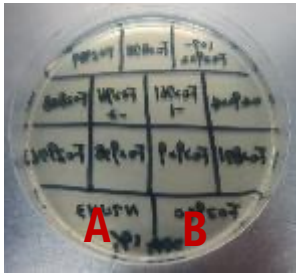
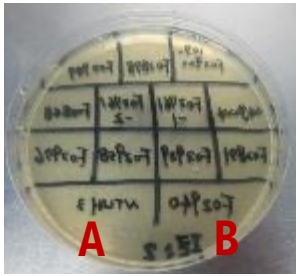
In House Azole Agar Screening Plates

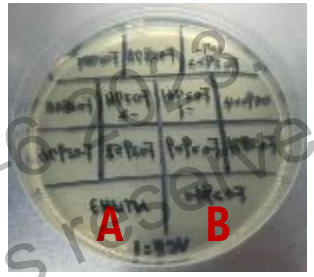


SDA
(drug free)



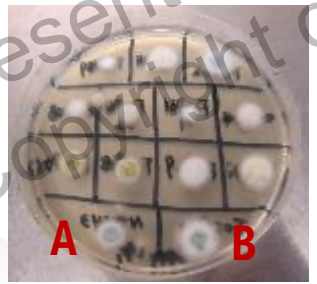
itraconazole
(2 µg/ml)

posaconazole
(0.25µg/ml)

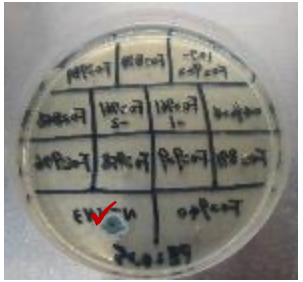


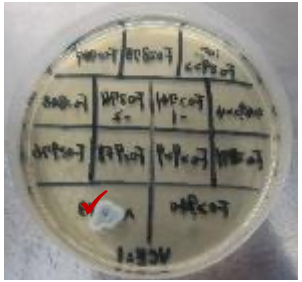
voriconazole
(1 µg/ml)

Inoculate
35°C; 24-48 hr



Growth control



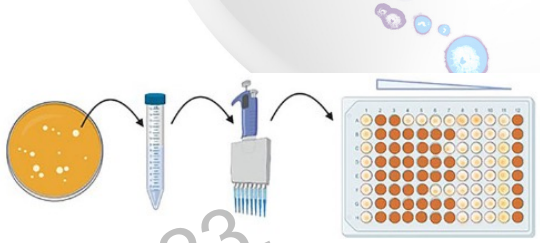


✓ Select for reference MIC testing to confirm azole resistance

The European Committee on Antimicrobial Susceptibility Testing – EUCAST

Susceptibility testing in moulds

EUCAST method for susceptibility testing of moulds (version 9.4 valid from 1 April, 2022)



Front Cell Infect Microbiol 2021;11:720609

Epidemiological Cut-Off Value (ECOFF or ECV) & Clinical Breakpoints (CBP) (Sep 2020)

A. fumigatus

Species	Drug	ECOFF (mg/L)	Clinical Breakpoints (mg/L)			
		WT ≤	S ≤	I	R >	ATU
A. fumigatus	Amphotericin B	1	1		1	
	Anidulafungin	ND	ND		ND	
	Micafungin	ND	ND		ND	
	Fluconazole	ND	ND		ND	
	Itraconazole	1	1		1	2
	Posaconazole	0.25	0.125	#	0.25	0.25
	Voriconazole	1	1		1	2
	Isavuconazole	2	1	#	2	2

A. flavus

Species	Drug	ECOFF (mg/L)	Clinical Breakpoints (mg/L)			
		WT ≤	S ≤	I	R >	ATU
A. flavus	Amphotericin B	4	-		-	
	Anidulafungin	ND	ND		ND	
	Micafungin	ND	ND		ND	
	Fluconazole	ND	ND		ND	
	Itraconazole	1	1		1	2
	Posaconazole	0.5	ND		ND	
	Voriconazole	2	ND		ND	
	Isavuconazole	2	1	#	2	2

CBP & ECOFF also proposed for ***A. terreus***, ***A. niger***, ***A. nidulans***.

CLSI M38-A3 (2017)

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

M38 3rd Edition

Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi

Front Cell Infect Microbiol 2021;11:720609

CBP by CLSI M61 2nd ed (2020)

Antifungal	Species	MIC Breakpoints and Interpretive Categories, µg/mL		
		S	I	R
Voriconazole[a]	*A. fumigatus*	≤0.5	1	≥2

ECV by CLSI M59 3rd ed (2020)

Antifungal Agent	Species	ECV, µg/mL[a,b,c]
Amphotericin B	*A. flavus*	4
	A. fumigatus	2
	A. niger	2
	A. terreus	4
	A. versicolor	2
Caspofungin[d]	*A. flavus*	0.5
	A. fumigatus	0.5
	A. niger	0.25
	A. terreus	0.12
Isavuconazole	*A. flavus*	1
	A. fumigatus	1
	A. niger	4
	A. terreus	1
Itraconazole	*A. flavus*	1
	A. fumigatus	1
	A. niger	4
	A. terreus	2
Posaconazole	*A. flavus*	0.5
	A. niger	2
	A. terreus	1
Voriconazole	*A. flavus*	2
	A. niger	2
	A. terreus	2

Abbreviation: ECV, epidemiological cutoff value.

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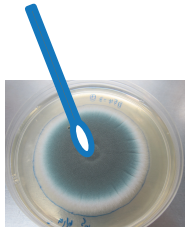
Antifungal Susceptibility Testing of *Aspergillus* spp. by CLSI M38-A3

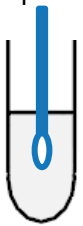
Class II biosafety cabinet



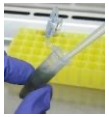
Subculture *Aspergillus* on PDA for 48 hrs or until good sporulation

conidia

heavy conidia suspension



sterile saline w/Tween 20 settle 3-5 min



Upper suspension

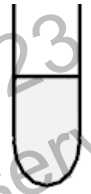
conidia suspension OD (530nm) 0.09-0.13, or 0.5 McFarland

sterile saline w/Tween 20

100 ul

(1:50x dilution)

adjusted inoculum

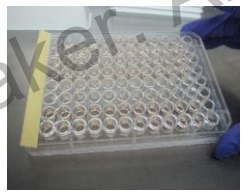


5 ml RPMI
2x (0.4 x10^4- 5 x 10^4)/CFU/ml

transfer 100 µl

Prepared drug panels (100 µl in

Final inoculum & volume 0.4-5x10^4 CFU/ml; 200 µl



Cover with adhesive seal

35˚C

incubation

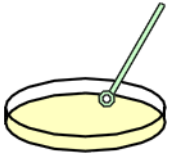
Reading

Azoles MIC reading

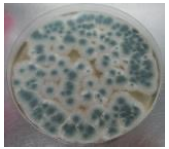
- **48 hrs**
- **100% inhibition**

Minimum Inhibitory Concentration:
the lowest conc. which prevents any visible growth (100% inhibition)

Inoculum quantification



Plating 10 µl from adjusted inoculum (or 1: 10 dilution) on SDA at 28-30˚C
80-1000 colonies (or 8-100 colonies)



Growth control (drug free)
Reference strain: *A. fumigatus* ATCC MYA-3626
QC strains: *Candida parasilosis* ATCC 22019
Candida krusei ATCC 6258

EUCAST vs. CLSI

Broth microdilution	EUCAST E.DEF 9.4	CLSI M38-A3
microplate well shape	flat-bottom 	U-shape bottom 
Medium	RPMI 1640	RPMI 1640
Glucose content	2%	0.2%
Final inoculum	1-2.5 x 10^5 cfu/mL	0.4-5 x 10^4 cfu/mL
Temperature	34 - 37°C	35°C
Endpoint for MIC	no growth	no growth
Reading	Visual or Spectrophotometric (*A. fumigatus*)	Visual
CBPs	*A. fumigatus, A. flavus, A. terreus, A. niger, A. nidulans*	*A. fumigatus*-voriconazole
	High essential agreement (97%~100%, ± 2 dilutions) between azole MIC values using the CLSI and EUCAST methods.	

J Clin Microbiol 2011;49:1110-2; Diagn Microbiol Infect Dis 2011;71:370-7

Commercial Assays for Azole Susceptibility Testing

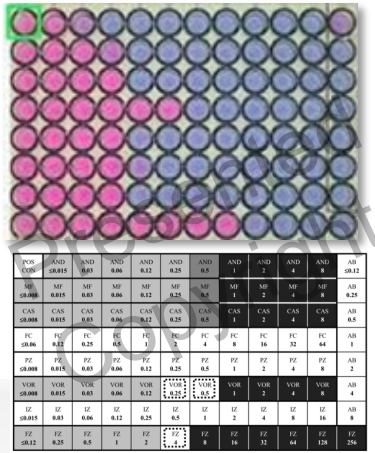
Essential agreement (± 2 two-fold dilutions) between MIC values by YO/Etest and CLSI M38A2

YeastOne vs. CLSI

Itraconazole (92%)

Voriconazole (100%)

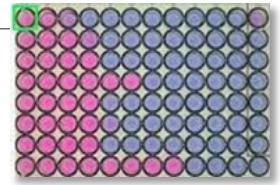
Posaconazole (95%)

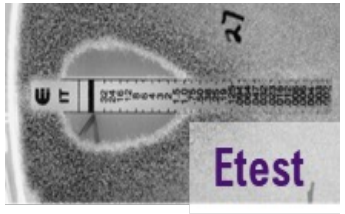


YeastOne: voriconazole (96%), posaconazole (94%)

Etest: itraconazole (83%), voriconazole (87%)

Method	Antifungal Drug	Essential Agreement (*n* Tests)
Etest (*n* = 84)	AMB	82.1% (69/84)
Sensititre YeastOne (*n* = 46)	AMB	93.5% (43/46)
Etest (*n* = 55)	VCZ	87.3% (48/55)
Sensititre YeastOne (n = 46)	VCZ	95.7% (44/46)
Etest (n = 64)	ITC	82.8% (53/64)
Sensititre YeastOne (*n* = 46)	ITC	78.3% (36/46)
Sensititre YeastOne (*n* = 46)	POS	93.5% (43/46)





J Clin Microbiol 2018;25;56:e00780-18; Pharmaceutics 2022;14:2161

Molecular Detection of Azole Resistance Genes in *A. fumigatus*

Commercial assays with CE-IVD certification

TR_{34}/L98H & TR_{46}/Y121F/T289A
in *cyp51A* (single copy) as targets.

AsperGenius® (PathoNostics)
Resistance multiplex

Resistance TR multiplex	Resistance multiplex
• *Aspergillus fumigatus TR34*	• L98H
• *Aspergillus fumigatus TR46*	• Tandem repeat 34
• *Aspergillus fumigatus cyp51A (WT)*	• T289A
• Internal Control (IC)	• Y121F

Fungiplex® Aspergillus Azole-R

IVD Real-Time PCR **Detect tandem repeat TR34 &TR46**

MALDI-TOF could detect azole resistance in *Aspergillus*

- **Accurate identification of species with intrinsic azole resistance**
- **Determining the Minimal Profile Change Concentration (MPCC)**

  MS measures susceptibility by detecting proteome modification in the presence of the antifungals.
  - Fungal suspension was added in to voriconazole solutions with concentrations 0.125-16 g/mL.
  - After incubation for up to 48 hr, the fungal materials were processed, and the supernatants were spotted onto the MALDI-TOF target plate and analyzed by MS.
  - MPCC is the lowest drug concentration that alters the microorganism protein profile.
  - High agreement between MICs by CLSI BMD and MPCC by MALDI-TOF.

MIC **MPCC**

Strain	Species	Cyp51A amino acid substitution	BMD MIC (μg/ml) at 48 h (WT identification)	MALDI-TOF MS MPCC (μg/ml) at 24 h Trial[a] 1	24 h Trial 2	30 h Trial 1	30 h Trial 2	48 h Trial 1	48 h Trial 2
F13747	*A. fumigatus*	G434C/G138C	4 (non-WT)	4	0.5	2	2	2	2
F15122	*A. fumigatus*	G448S	4 (non-WT)	8	16	8	16	16	8
F17294	*A. fumigatus*	L98H+TR[c]	4 (non-WT)	4	2	2	2	8	8
F11628	*A. fumigatus*	G138C	4 (non-WT)	0.5	2	2	2	2	2
Af958	*A. fumigatus*	WT	0.12 (WT)	0.5	0.25	0.5	0.25	1	0.5
Af982	*A. fumigatus*	WT	0.12 (WT)	0.25	0.125	0.25	0.25	0.25	1
Af983	*A. fumigatus*	WT	0.12 (WT)	0.5	0.25	0.25	0.125	0.5	0.25
Af987	*A. fumigatus*	WT	0.25 (WT)	0.5	1	1	0.25	1	0.25
Af919	*A. fumigatus*	WT	0.12 (WT)	0.25	0.25	0.5	0.125	0.5	0.25
Au204	*A. ustus*	ND	8	4	4	8	4	8	16
Au960	*A. ustus*	ND	8	8	8	8	8	16	8
Ac366	*A. calidoustus*	ND	8	8	4	8	8	16	16

Clin Microbiol Infect 2022:260-6
J Clin Microbiol 2017;55:2030 –2034

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Clues for Azole-Resistant *Aspergillus*

Clinical: unresponsive to or breakthrough during azole treatment

Laboratory

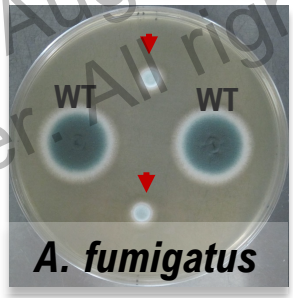
✓ **Atypical growth**

- Patient-acquired resistance

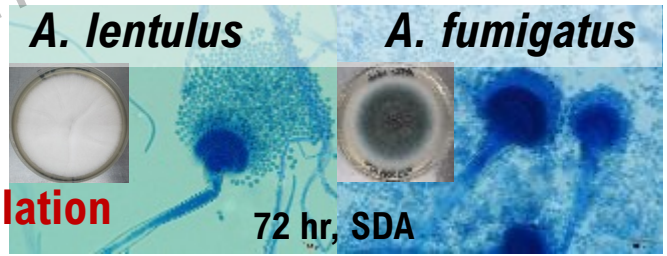
Patient-Acquired Resistance	Environmental Resistance
Aspergillus fumigatus colonies may show an abnormal colony morphology, lack of sporulation or reduced growth rate	No apparent fitness cost (TR34, TR46)

Clin Infect Dis 2016;62:362-8

VRC-R after 3- month VRC



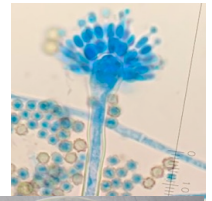
- Intrinsic resistance

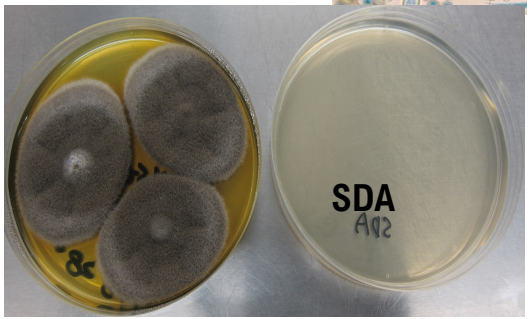


✓ **Yellowish pigment**

- Intrinsic resistance

A. calidoustus





Take Home Message

Azole-resistant *Aspergillus*

- Acquired: *A. fumigatus*
- Intrinsic: *A. lentulus*, *A. calidoustus*

Indications for testing azole resistance

- Surveillance
- Clinically relevant *Aspergillus* isolates in patient groups/regions with **known azole resistance** or patients **unresponsive to treatment**

Methods

- Azole agar **screening** testing
- MICs by reference **EUCAST** or **CLSI** BMD
- MICs by **YeastOne** or **Etest** as alternatives
- **Molecular detection** of azole resistance genes
- **MALDI-TOF**: need optimization & standardization

Aspergillosis Guideline, Taiwan

4. Identification of causing etiology to species level and saving the isolate for future antifungal susceptibility testing are recommended.

J Microbiol Immunol Infect 2018;51:1-16

Thank you for your attention!

Thank you

Identification of *Aspergillus* species

2017 ESCMID-ECMM-ERS guideline for aspergillosis

Species identification to the **complex level** should be carried out for

(1) **clinically relevant isolates** from patients who need antifungal treatment

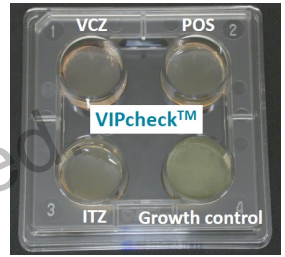
(2) **epidemiological purposes**

Intention	Intervention	SoR	QoE	Comment
Identification of species complex	Macroscopic and microscopic examination from primary cultures	A	II	Colony colour, conidium size, shape and septation. Colour of conidia and conidiophore and conidiogenesis (tease or tape mounts are preferred); expertise needed for interpretation
Identification of species complex (and species identification of *A. fumigatus* specifically)	Culture on identification media at 25–30°C, 37°C and 50°C (2% MEA and Czapek-Dox Agar) and microscopic examination	A	II	Thermotolerance test (growth at 50°C for species confirmation of *A. fumigatus*)
Identification at species level	MALDI-TOF MS identification	B	II	In-house databases are often used to improve identification rates
Identification at species level	Sequencing of ITS, β-tubulin and calmodulin	A	III	Not necessary in organisms with typical growth, but in cases of atypical growth
To study outbreaks	Microsatellite and CSP analysis	C	II	To study outbreaks (which in general may comprise more than one genotype)
		B	II	To study colonization patterns

Clin Microbiol Infect. 2018;24 Suppl 1:e1-e38

Antifungal Susceptibility Testing

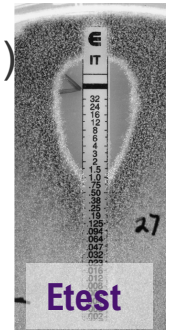
2017 ESCMID-ECMM-ERS guideline

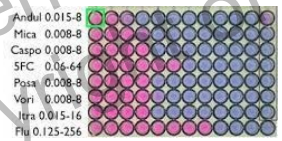


Identify azole resistance for all clinically relevant *Aspergillus* isolates in patient groups or regions with known azole resistance (AII)

- Azole agar screening tests (in house & commercial) followed by reference MIC test (AIII)
- Routine azole agar screening or reference MIC testing of multiple colonies (up to **5 colonies**) (BIII)

  (mixed susceptible–resistant colonies could be found in 20% of clinical specimens)
- CLSI-M38A2 broth microdilution method & CLSI-M59 epidemiological cut-off value (ECV)(BIII)
- EUCAST broth microdilution method & ECV and clinical breakpoints (AIII)
- Etest (CIII)





Alternative methods

- YeastOne
- Molecular detection of azole resistance genes (TR34, L98H, TR46, Y121F, T289A, M220...)

  (in house & commercial real-time PCR assays [AsperGenius, MycoGENE])

J Antimicrob Chemother 2015;70:2894-8; Stud Mycol 2021;100:100115; Clin Microbiol Infect 2018;24(S1):e1-e38; J Clin Microbiol 2018;25;56:e00780-18

Emerging Azole-Resistant *Aspergillus* spp.

Acquired azole resistance

- ***A. flavus**:* Y119F, G441S mutation in *cyp51A*

Intrinsic azole resistance

- ***A. calidoustus***
- ***A. lentulus*** (*A. fumigatus* complex)
- ***A. niger* complex:** reduced susceptibility to itraconazole & isavuconazole

2017 ESCMID-ECMM-ERS Aspergillosis Guideline

Antifungal regimens in intrinsic resistance

Population	Intervention
IA due to *A. calidoustus*	Lipid formulation of AmB
IA due to *A. lentulus* (*A. fumigatus* complex)	Other than azole monotherapy
IA due to *A. niger* complex	Other than itraconazole and isavuconazole

Clin Microbiol Infect 2018;24(S1):e1-e38; J. Fungi 2021;7:164; Mycoses 2023;66:711-22

Aspergillus calidousus (*Aspergillus* section *Usti*)

- an uncommon but emerging cause of invasive aspergillosis
- displays intrinsic resistance to medical azoles

	MIC_{50}/MIC_{90}; Range, µg/mL			
	Voriconazole	Posaconazole	Isavuconazole	Amphotericin B
A. calidoustus	8/8; 2–16	16/>16; 4 to >16	2/4 2/4; 0.5 to >16	0.5/1; 0.25–2

2017 ESCMID-ECMM-ERS guideline

Antifungal regimens in intrinsic resistance

Population	Intention	Intervention	Comment
IA due to *A. calidoustus*	To cure IA	Lipid formulation of AmB	Avoid azoles

Atlas of Clinical Fungi; Clin Infect Dis 2021;72:1379–85; Clin Microbiol Infect 2018;24(S1):e1-e38

Aspergillus lentulus (*Aspergillus* section *Fumigati*)

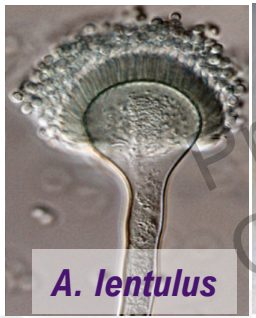
- Newly identified spp. to cause invasive aspergillosis
- Intrinsic resistance to both azoles & amphotericin B

A. lentulus (26)	MIC (mg/L)	
	AMB	VCZ
GM	2.98	3.41
Range	0.25–32.00	0.25–16.00
MIC_{50}	4.00	4.00
MIC_{90}	16.00	8.00

2017 ESCMID-ECMM-ERS guideline

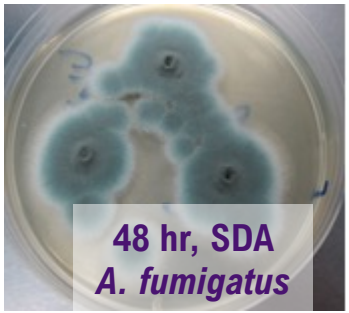
Antifungal regimens in intrinsic resistance

Population	Intention	Intervention
IA due to *A. lentulus*	To cure IA	Other than azole monotherapy



A. lentulus

- whitish, pale green-blue
- reduced sporulation
- not grow at 50°C



MMTN MEDICAL MYCOLOGY TRAINING NETWORK

Mycopathologia 2014:178:427-33; Med Mycol 2011;49(Suppl1):S82–S89; Clin Microbiol Infect 2018;24(S1):e1-e38

ELSEVIER

Diagnostic Microbiology and Infectious Disease 71 (2011) 370–377

DISEASE

www.elsevier.com/locate/diagmicrobio

Mycology

In vitro activity of isavuconazole against 208 *Aspergillus flavus* isolates in comparison with 7 other antifungal agents: assessment according to the methodology of the European Committee on Antimicrobial Susceptibility Testing☆,☆☆

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Received 31 May 2011; accepted 4 August 2011

Abstract

Aspergillus flavus is the second most common species causing invasive aspergillosis after *A. fumigatus*. In certain countries like India, Sudan, and Saudi Arabia, *A. flavus* is most frequently isolated from patients with fungal rhinosinusitis and endophthalmitis. *A. flavus* exhibit an increased resistance to antifungal agents compared to *A. fumigatus*. We determined the in vitro activity of isavuconazole, voriconazole, posaconazole, itraconazole, amphotericin B, caspofungin, micafungin, and anidulafungin against 208 isolates of *A. flavus* by the EUCAST method and compared with the results obtained by the CLSI method. Isavuconazole and voriconazole MICs were ≤2 μg/mL in 99% and 95%, respectively. Posaconazole and itraconazole MICs were ≤0.5 and ≤1 μg/mL, respectively, for all isolates. MICs of amphotericin B were ≥2 μg/mL in 91%; 36% of them exhibited MICs of ≥8 μg/mL. All echinocandins demonstrated good anti–*A. flavus* activity. The essential agreement of the MIC/MEC results by EUCAST with CLSI broth dilution method assessed at ±2 dilutions was good for itraconazole (97.8%), voriconazole (100%), posaconazole (98.3%), isavuconazole (98.9%), caspofungin (99.4%), and anidulafungin (100%), but poor for amphotericin B (53.5%) and micafungin (79.1%).

Keywords: Antifungal susceptibility; *Aspergillus flavus*; EUCAST; Wild-type cut-off value

JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2011, p. 1110–1112 Vol. 49, No. 3
0095-1137/11/$12.00 doi:10.1128/JCM.02432-10

Comparison of the Broth Microdilution Methods of the European Committee on Antimicrobial Susceptibility Testing and the Clinical and Laboratory Standards Institute for Testing Itraconazole, Posaconazole, and Voriconazole against *Aspergillus* Isolates▽

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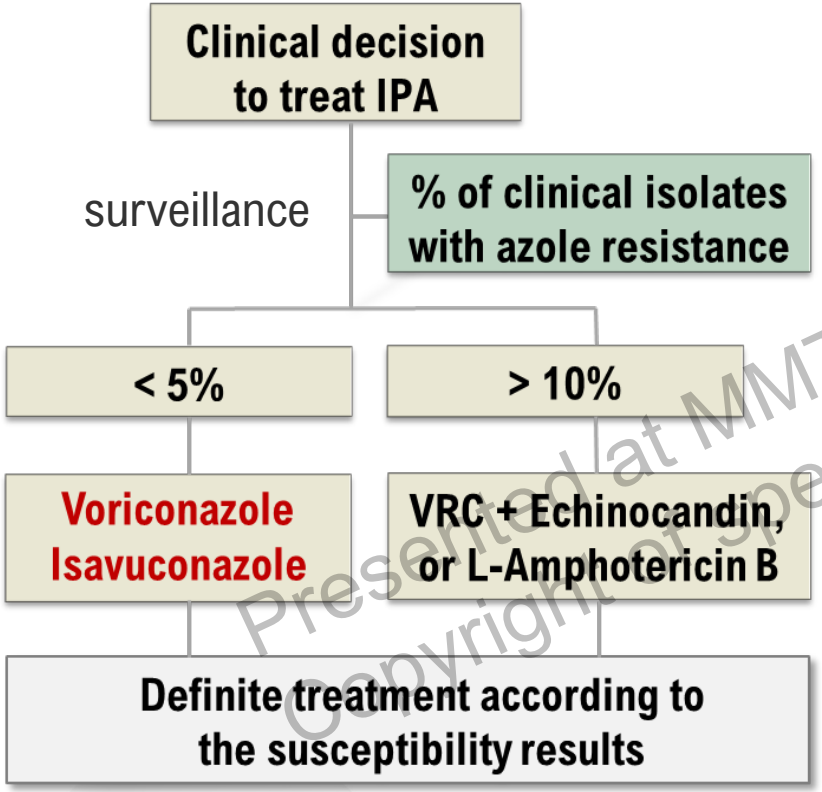
Received 30 November 2010/Accepted 27 December 2010

We compared EUCAST and CLSI antifungal susceptibility testing methods for itraconazole, posaconazole, and voriconazole by testing 245 *Aspergillus* clinical isolates. The essential agreement (EA) between methods was excellent: 100% (itraconazole), 98.4% (posaconazole), and 99.6% (voriconazole) assessing EA at ±2 dilutions and 99.6% (itraconazole), 87.7% (posaconazole), and 96.3% (voriconazole) at ±1 dilution.

Take Home Message



Drug Resistance Updates 2015; 21-22: 30-40

Be aware of azole-resistant *Aspergillus*

- Acquired: *A. fumigatus*, *A. flavus*
- Intrinsic: *A. lentulus*, *A. calidoustus*

AFST

- screening azole-agar
- MICs by reference CLSI or EUCAST
- MICs by YeastOne or Etest as alternatives
- Molecular detection of azole resistance genes
- MALDI-TOF: promising; need clinical validation

Invasive Aspergillosis Guideline, Taiwan

4. Identification of causing etiology to species level and saving the isolate for future antifungal susceptibility testing are recommended.

J Microbiol Immunol Infect 2018;51:1-16

Strength of recommendation and quality of evidence

Strength of recommendation	**Definition**
Grade A	Societies strongly support a recommendation for use
Grade B	Societies moderately support a recommendation for use
Grade C	Societies marginally support a recommendation for use
Grade D	Societies support a recommendation against use
Quality of evidence	**Definition**
Level I	Evidence from at least one properly* designed randomized, controlled trial (oriented on the primary end point of the trial)
Level II	Evidence from at least one well-designed clinical trial (including secondary end points), without randomization; from cohort or case–controlled analytic studies (preferably from more than one centre); from multiple time series; or from dramatic results of uncontrolled experiments
Level III	Evidence from opinions of respected authorities, based on clinical experience, descriptive case studies, or reports of expert committees