Trends in *Aspergillus* species distribution and resistance pattern Global Vs. Indian scenario

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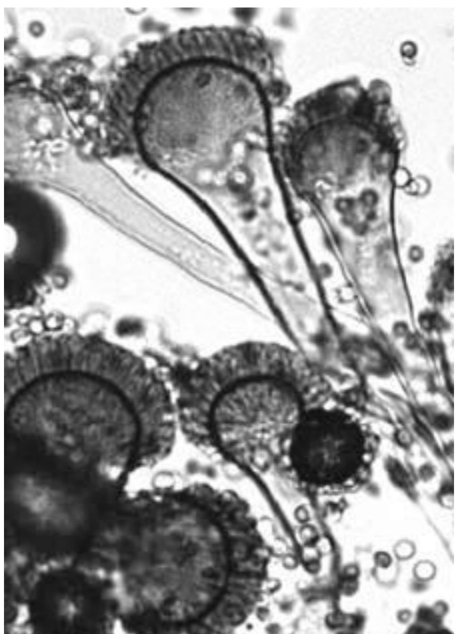
Introduction

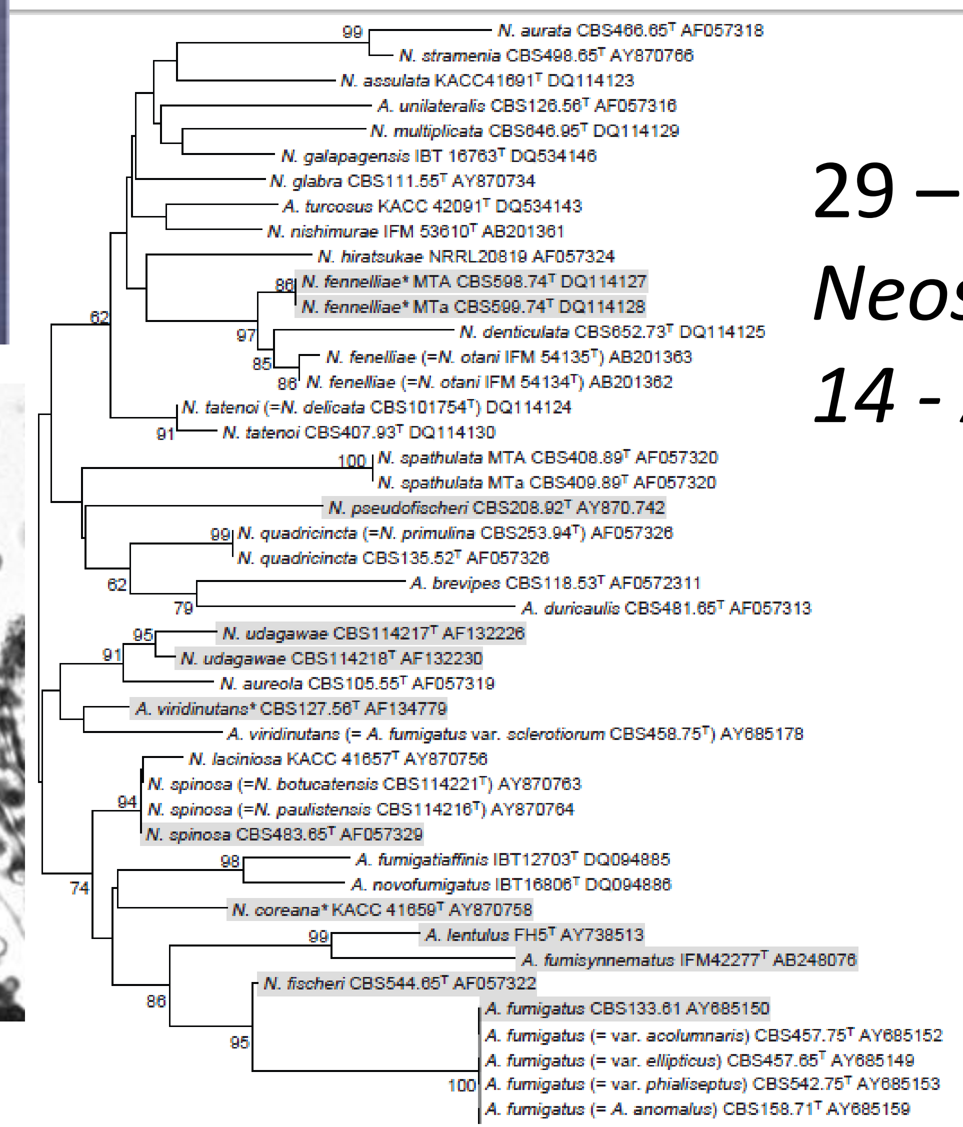
- Invasive aspergillosis (IA) remains a difficult to manage infectious disease.
- Outcome is largely determined by the persistence of the underlying disease or immunosuppression
- Factors related to the fungus also play a role.
- The taxonomy of *Aspergillus* is evolving.

Introduction

- *Aspergillus* is a large genus comprised of more *than 250 species*
- Polyphasic taxonomy has a major impact on the species concept of the genus *Aspergillus*
- New sibling species described – variable susceptibility profiles.
- *Aspergillus* sections including *Fumigati , Flavi , Terrei ,Nidulanti and Nigri*

Aspergillus section Fumigati

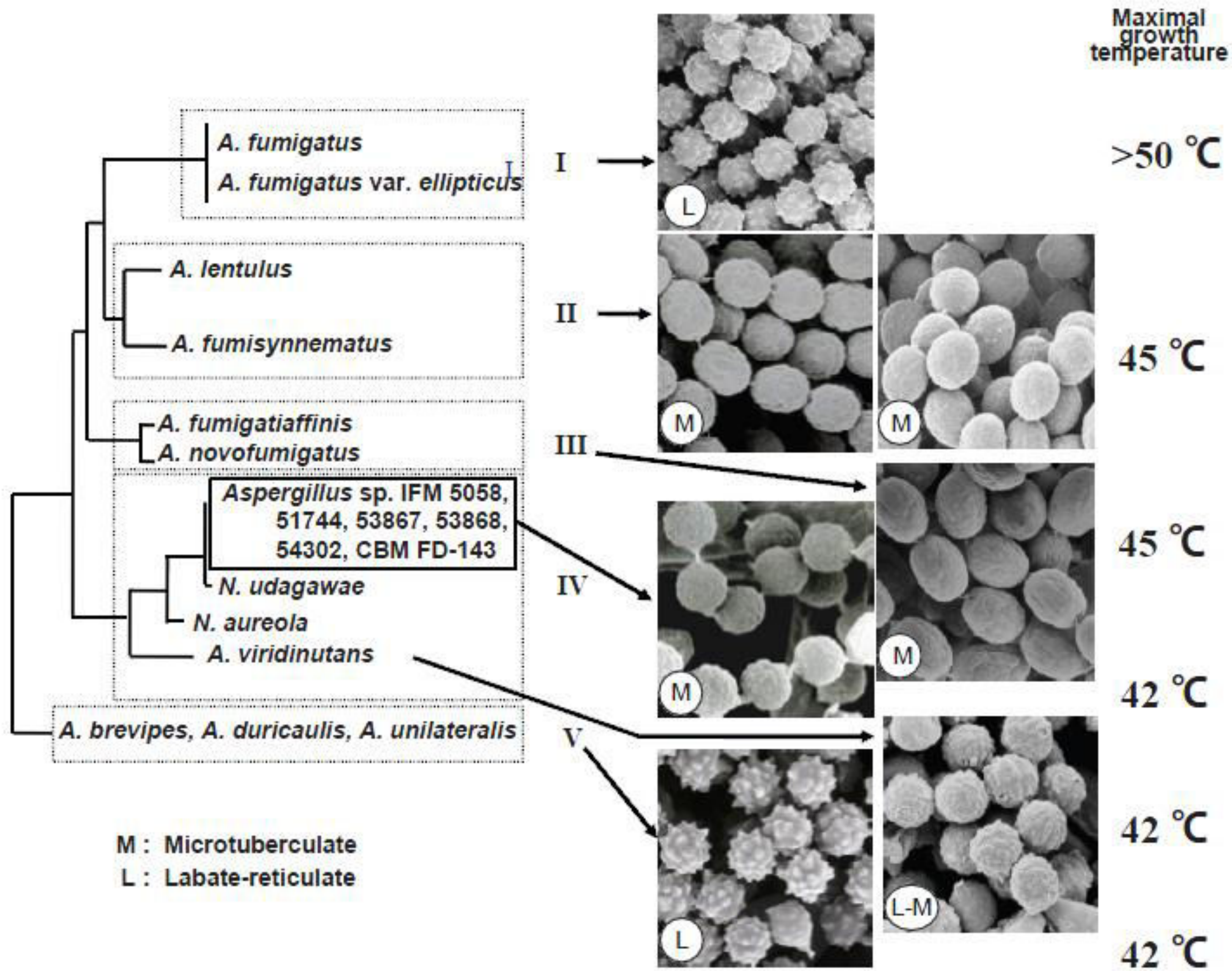




29 – *Neosartorya*

14 - *Aspergillus*

Pathogenic species in *Aspergillus* section *Fumigati*

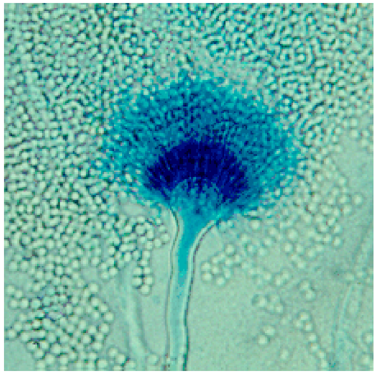


Pathogenic species in *Aspergillus section flavi*

- Second most common cause of IA
- ability to survive at higher temperatures

Aspergillus *Section Terrei*

- Until recently *A. terreus was the only recognized species in this section*
- Presence of several cryptic species
- in vivo conidia formation - a role in haematogenous dissemination

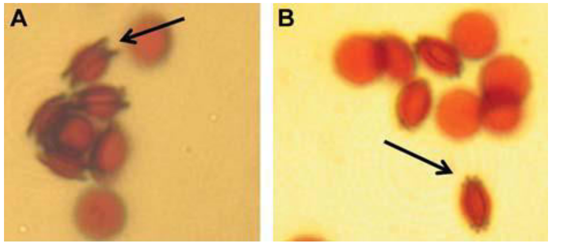


Aspergillus section Nidulanti

- reported in patients with chronic granulomatous disease
- *A. nidulans*
- *Emericella quadrilineata* anamorph *Aspergillus tetrazonus*

Aspergillus section Usti

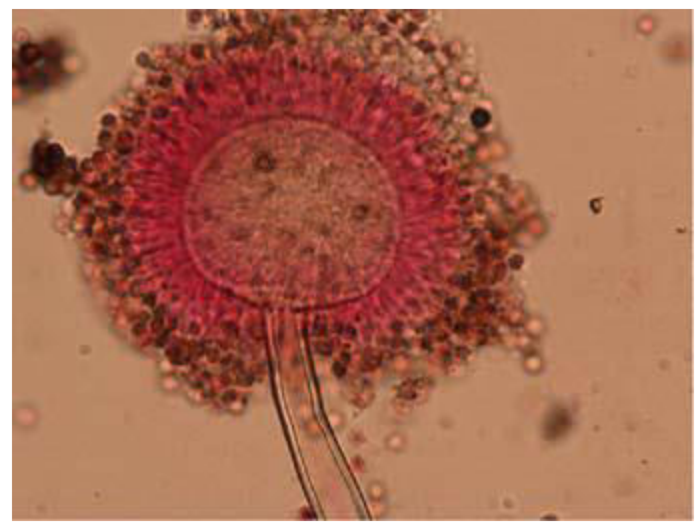
- *A. ustus, A. puniceus ,A. granulosus, A. pseudodeflectus, A. calidoustus , A. insuetus A. keveii sp.*



A. calidoustus	CBS 112452	Indoor air, Germany
A. calidoustus	CBS 113228	ATCC 38849, IBT 13091
A. calidoustus	CBS 114380	Wooden construction material, Finland
A. calidoustus[T]	CBS 121601, 677	Bronchoalveolar lavage fluid, proven invasive aspergillosis; Nijmegen, The Netherlands[a]
A. calidoustus	CBS 121602, 678	Bronchial secretion, proven invasive aspergillosis; Nijmegen, The Netherlands[a]
A. calidoustus	CBS 121589, 682	Autopsy lung tissue sample, proven invasive aspergillosis; Nijmegen, The Netherlands[a]
A. calidoustus	CBS 121603, 741	Elevator shaft in hospital, Nijmegen, The Netherlands
A. calidoustus	CBS 121604, 924	Patient room, Nijmegen, The Netherlands
A. calidoustus	CBS 121605, 943	Laboratory, Nijmegen, The Netherlands
A. calidoustus	CBS 121606, 2725	Sputum, Nijmegen, The Netherlands
A. calidoustus	CBS 121607, 3297	Feces, Nijmegen, The Netherlands
A. calidoustus	CBS 121608, 6989	Bronchoalveolar lavage, Nijmegen, The Netherlands
A. calidoustus	7843	Pasteur Institute, Paris, France
A. calidoustus	8623	Oslo, Norway
A. calidoustus	9331	Mouth wash, Nijmegen, The Netherlands
A. calidoustus	9371	Mouth wash, Nijmegen, The Netherlands
A. calidoustus	9420	Bronchial secretion, Nijmegen, The Netherlands
A. calidoustus	9692	Hospital ward, Nijmegen, The Netherlands
A. calidoustus	V02-46	Tongue swab, Nijmegen, The Netherlands
A. calidoustus	V07-21	Bronchial secretion, Nijmegen, The Netherlands
A. calidoustus	V17-43	Bronchial secretion, Nijmegen, The Netherlands
A. calidoustus	V22-60	Skin biopsy, Nijmegen, The Netherlands

Aspergillus section Nigri

- *A. niger, A. tubingensis*
- Mainly cause otomycoses.
- Third leading cause of IPA aspergillosis



Aspergillus species isolated from immunocompromised patients in USA 1980-1997

Species	Percentage
Aspergillus fumigatus	68.7
Aspergillus flavus	17.0
Aspergillus niger	5.7
Aspergillus terreus	3.9
Aspergillus nidulans	1.1
Aspergillus ustus	1.1
Neosartorya spp.	0.7
Each of the rest of species	≤ 0.5

Journal of Clinical Microbiology, Oct. 2009, p. 3138–3141 Vol. 47, No. 10
0095-1137/09/$08.00+0 doi:10.1128/JCM.01070-09

Molecular Identification of *Aspergillus* Species Collected for the Transplant-Associated Infection Surveillance Network▽

S. Arunmozhi Balajee,[1]* Rui Kano,[1] John W. Baddley,[2,11] Stephen A. Moser,[3] Kieren A. Marr,[4,5] Barbara D. Alexander,[6] David Andes,[7] Dimitrios P. Kontoyiannis,[8] Giancarlo Perrone,[9] Stephen Peterson,[10] Mary E. Brandt,[1] Peter G. Pappas,[2] and Tom Chiller[1]

Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, Georgia[1]*; Department of Medicine*[2] *and Department of Pathology,*[3] *University of Alabama at Birmingham, and Department of Medicine, Birmingham Veterans Affairs Medical Center,*[11]

Species complex	Frequency (%)	Sequence-based identification	Frequency (%)
A. fumigatus	147/218 (67.4)	*A. fumigatus*	139/147 (93.9)
		A. lentulus	4/147 (2.7)
		A. udagawae	3/147 (2.0)
		N. pseudofischeri	1/147 (0.8)
A. flavus	29/218 (13.2)	*A. flavus*	29/29 (100)
A. niger	19/218 (8.7)	*A. niger*	13/19 (68)
		A. tubingensis	6/19 (32)
A. terreus	11/218 (7.4)	*A. terreus*	11/11 (100)
A. ustus	6/218 (2.7)	*A. calidoustus*	6/6 (100)
A. versicolor	5/218 (2.3)	*A. versicolor*	3/5 (60)
		A. sydowii	2/5 (40)
A. nidulans	1/218 (0.5)	*A. tetrazonus*	1/1 (100)

CANADIAN ANTIMICROBIAL RESISTANCE ALLIANCE CARA

Alberta Health Services

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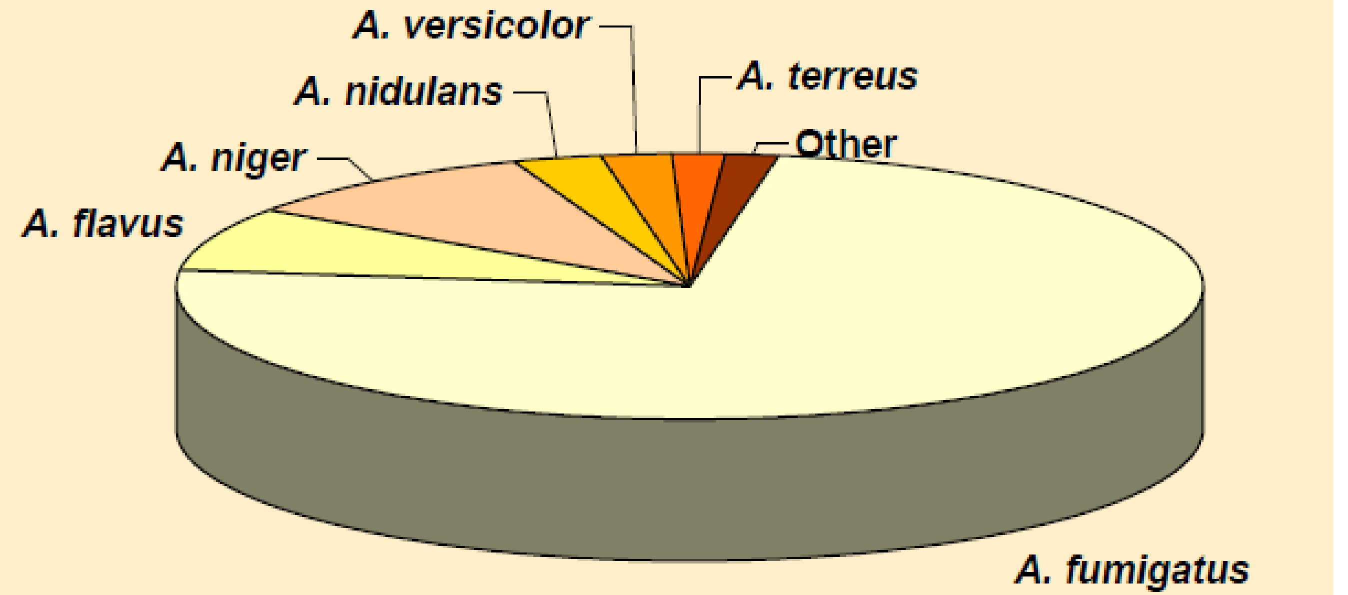
bannerGraphics



ıre 1. *Aspergillus* species distribution of respiratory isolates.

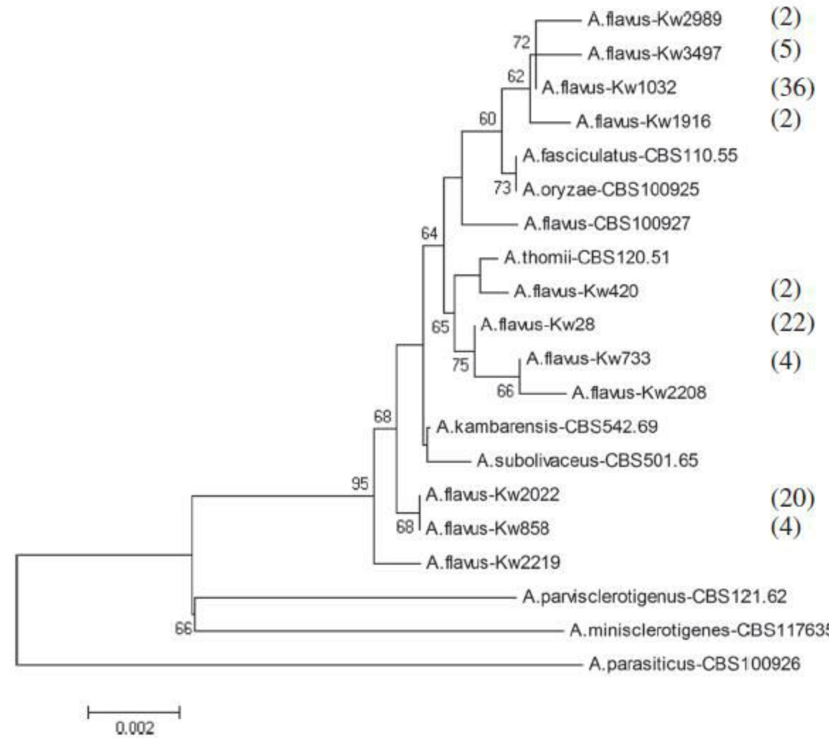
RESEARCH ARTICLE Open A

Molecular identification and antifungal susceptibility profile of *Aspergillus flavus* isolate recovered from clinical specimens in Kuwait

Faten Al-Wathiqi, Suhail Ahmad and Ziauddin Khan*

Sources	No. of isolates
Ear swabs	31
Nasal biobsy	10
Respiratory secretion*	32
Wound swabs	9
Rectal swab	2
Peritoneal abscess	3
Blood	1
Corneal plate	1
Urine	1
Cutaneous infections	2
Environment	7
Total	99

*Include sputum, endotracheal secretion and bronchoalveolar lavage.



Indian Journal of Medical Sciences, Vol. 58, No. 12, December, 2004, pp. 513-519

Original Article

Prevalence of *Aspergillus* species in clinical samples isolated in tertiary care hospital

Xess Immaculata, Mohanty Srujana, Jain Neena, Banerjee Uma

Department of Microbiology,All India Institute of Medical Sciences, New Delhi - 29

Table 1: Distribution of culture positive Aspergillus species in clinical samples (2000-2004)

Specimen	*A. fumigatus*	*A. flavus*	*A. niger*	*Total*
Respiratory specimens	66	58	26	150
Blood	73	26	14	113
Nasal polyps	10	99	3	112
Nail	5	10	19	34
Tissue biopsy	12	13	1	26
Urine	4	5	2	11
Sterile fluids	2	3	5	10
	172	214	70	456

Aspergillus spp 2012 & 2013

- Total -445
  - 223 isolates from pus and tissue sample
  - 169 isolates from respiratory samples
  - 53 isolates from eye samples (from 2013 only)

Respiratory samples

Isolates / Samples	*A. flavus* (90)	*A. fumigatus* (57)	*A. terreus* (2)	*Aspergillus* spp. (20)	Total
Sputum (77)	41/77	24/77	1/77	11/77	77
ETA (45)	22/45	18/45	1/45	4/45	45
BAL (30)	20/30	7/30	-	3/30	30
Others* (17)	7/17	8/17	-	2/17	17
No of Isolates /(percentage)	90/169 **(53.25%)**	57/169 **(33.72)**	2/169 (1.18%)	20/169 (11.83%)	169

* Majority of the samples were from pleural fluid and Gastric aspirates

Pus / Tissue samples

Isolates / samples	*A. flavus* (204)	*A. fumigatus* (11)	*A.spp* (8)	Total
Fungal muck/ nasal tissue	173/186	7/186	6/187	186
Other samples*	31/37	4/37	2/37	37
No of Isolates /(percentage)	204/223 **(91.47%)**	11/223 **(4.93%)**	8/223 **(3.58%)**	223

* Includes deep tissue samples, aneurysmal wall, brain tissue/ abscess, pus

Eye sample (2013)

- Total - *Aspergillus spp* isolated- 53
- *Aspergillus flavus*- 47
- *Aspergillus fumigatus*- 3
- *Aspergillus niger*-3

Aspergillus keratitis – Aravind eye hospital

Table 1 Microbiological profile of the examined keratitis cases diagnosed between September 2005 and August 2008 at the Aravind Eye Hospital, Coimbatore, South India.

Diagnosis	Total number	%
Direct microscopy (10% KOH and Gram staining) (*Aspergillus* keratitis)		
Positive (both or either one)	147	73.5
Negative (both)	52	26.0
Not done (both)	1	0.5

Type of organism	Number of cases	Prevalence % (95% CI[1])
Only fungi	887	51.0 (48.7–53.4)
Only bacteria	560	32.2 (30–34)
Aspergillus spp.	200	11.5 (10–13)
Mixed (bacteria and fungi)	75	4.3 (3–5)
Acanthamoeba spp.	15	0.9 (0.005–0.01)
Total	1737	100

[1]Confidence interval.

Aspergillus keratitis – Aravind eye hospital

Table 2 Uncommon/rare species of *Aspergillus* isolated from corneal ulcers at the Aravind Eye Hospital, Coimbatore, South India between September 2005 and August 2008.

Species	Number of isolates deposited	GenBank accession numbers (locus)	Culture collection number	MIC values (µg/ml)	Ref.
A. tubingensis[1]	2	EU600388(β-tubulin) EU600389(β-tubulin)	CBS 122719 CBS 122725	I: 1–2[5]; K: 0.5–1[5]; V: 0.064[5]; A: 0.032–0.25[5]; E: 0.5–1[6]; CL: >32[6]; F: >256[5]; N: 0.5–1[6]; CA: 0.064–0.25[5]	[27]
A. brasiliensis[1]	2	EU600387(β-tubulin) EU600386(β-tubulin)	CBS 122724 CBS 122723	I: 0.25–1[5]; K: 0.125–0.5[5]; V: 0.032–0.064[5]; A: 0.064–0.125[5]; E: 0.06–0.032[6]; CL: >32[6]; F: >256[5]; N: 1[6]	[29]
A. nomius[2]	1	GQ221261 (ITS) GQ221262 (β-tubulin) GQ221263 (calmodulin)	CBS 123901	I: 4; K: 1[5]; V: 0.125[5]; A: 15; E: 2[6]; CL: 1[6]; F: >256[5]; N: 128[6]; CA: 0.25[5]	[28]
A. tamarii[2]	1	EF525554 (ITS) EF525555(β-tubulin)	CBS 121598	I: 0.064[5]; K: 0.25[5]; V: 0.125[5]; A: 0.125[5]; E: 0.064[6]; F: >256[5]; N: >1024[6]	[26]
A. tamarii	7	ITS, β-tubulin, calmodulin	SZMC 2393, 2404, 2428, 2434, 2438, 2439, 2465	ND	–
A. pseudonomius[2]	1	identical calmodulin and β-tubulin sequences with the type strain NRRL 3353[50]	CBS 123902	ND	–
A. sydowii[3]	1	ITS, β-tubulin, calmodulin	SZMC 2483	ND	–
Eurotium amstelodami[4]	1	ITS, β-tubulin, calmodulin	SZMC 2459	ND	–

Manikandan et al Mycoses 2012

FUNGAL ENDOPHTHALMITIS

Fourteen Years' Experience From a Center in India

ARUNALOKE CHAKRABARTI, MD,* M. R. SHIVAPRAKASH, MD,*
RAMANDEEP SINGH, MS,† BANSIDHAR TARAI, MD,*
VARGHESE K. GEORGE, PhD,* BASHIR A. FOMDA, MD,* AMOD GUPTA, MS†

1402 RETINA, THE JOURNAL OF RETINAL AND VITREOUS DISEASES • 2008 • VOLUME 28 • NUMBER 10

Table 1. Spectrum of Fungal Agents Isolated From Patients With Fungal Endophthalmitis

Fungal Isolates	Mean Latent Period (days)	No. (%)	Postoperative (%)	Posttrauma (%)	Endogenous (%
***Aspergillus* spp**	**9.8**	**31 (54.4)**	**15 (51.7)**	**14 (60.9)**	**2 (40.0)**
A. flavus	5.0	14 (24.6)	8	4	2
A. fumigatus	8.7	8 (14.0)	2	6	0
A. niger	30	5 (8.8)	2	3	0
A. terreus	4.5	2 (3.5)	2	0	0
A. ustus	2	1 (1.8)	0	1	0
A. versicolor	4	1 (1.8)	1	0	0
Yeasts	**17.7**	**14 (24.6)**	**9 (31.0)**	**3 (13.0)**	**2 (40.0)**
C. tropicalis	7	5 (8.8)	2	2	0
C. albicans	2	4 (7.0)	4	1	0
C. guilliermondii	2	1 (1.8)	1	0	0
C. parapsilosis	80	1 (1.8)	1	0	0
C. glabrata	4	1 (1.8)	1	0	0
Trichosporon cutaneum	30	1 (1.8)	0	0	1
Cryptococcus neoformans	90	1 (1.8)	0	0	1
Melanized fungi	**24.6**	**6 (10.5)**	**3 (10.3)**	**3 (13.0)**	**0**
Fonsecaea pedrosi	30.6	3 (5.3)	2	1	0
Curvularia lunata	25	2 (3.5)	1	1	0
Colletotrichum dematium	6	1 (1.8)	0	1	0
Others	**9.8**	**6 (10.5)**	**2 (6.9)**	**3 (13.0)**	**1 (20.0)**
Fusarium solani	2.6	3 (5.3)	1	2	0
Paecilomyces lilacinus	10.5	2 (3.5)	1	0	1
Pseudallescheria boydii	30	1 (1.8)	0	1	0
Total	**14.5**	**57 (100)**	**29 (50.9)**	**23 (40.4)**	**5 (8.8)**

Aspergillus species sent for identification to NCCPF

STRAINS	DATE	NCCPF NO.
A.niger	15/11/2012	790039
A.niger	15/03/2013	790038
A.niger	01-10-2013	790037
A.versicolor	23/3/2012	820016
A.versicolor	19/9/2012	820017
A.versicolor	19/9/2012	820018
A.versicolor	19/9/2012	820019
A.versicolor	19/9/2012	820020
A.versicolor	17/4/2012	820021
A.tetrazonous	06-07-2012	840010
A.tetrazonous	28/12/2012	840011
A.terreus	03-01-2012	860030
A.terreus	05-05-2012	860031
A.terreus	06-07-2012	860032
A.terreus	28/12/2012	860033
A.terreus	28/2/2013	860034
A.terreus	25/6/2013	860035

Pathogenic *Aspergillus* Species Recovered from a Hospital Water System: A 3-Year Prospective Study

Elias J. Anaissie,[1] Shawna L. Stratton,[1] M. Cecilia Dignani,[1] Richard C. Summerbell,[3] John H. Rex,[4] Thomas P. Monson,[2] Trey Spencer,[1] Miki Kasai,[5] Andrea Francesconi,[5] and Thomas J. Walsh[5]

[1]Myeloma and Transplantation Research Center, Arkansas Cancer Research Center, and [2]John L. McClellan Memorial Veterans Hospital, University of Arkansas for Medical Sciences, Little Rock; [3]Centraalbureau voor Schimmelcultures, Baarn, The Netherlands; [4]Center for Infectious Diseases, University of Texas–Houston Medical School, Houston; and [5]Immunocompromised Host Section, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

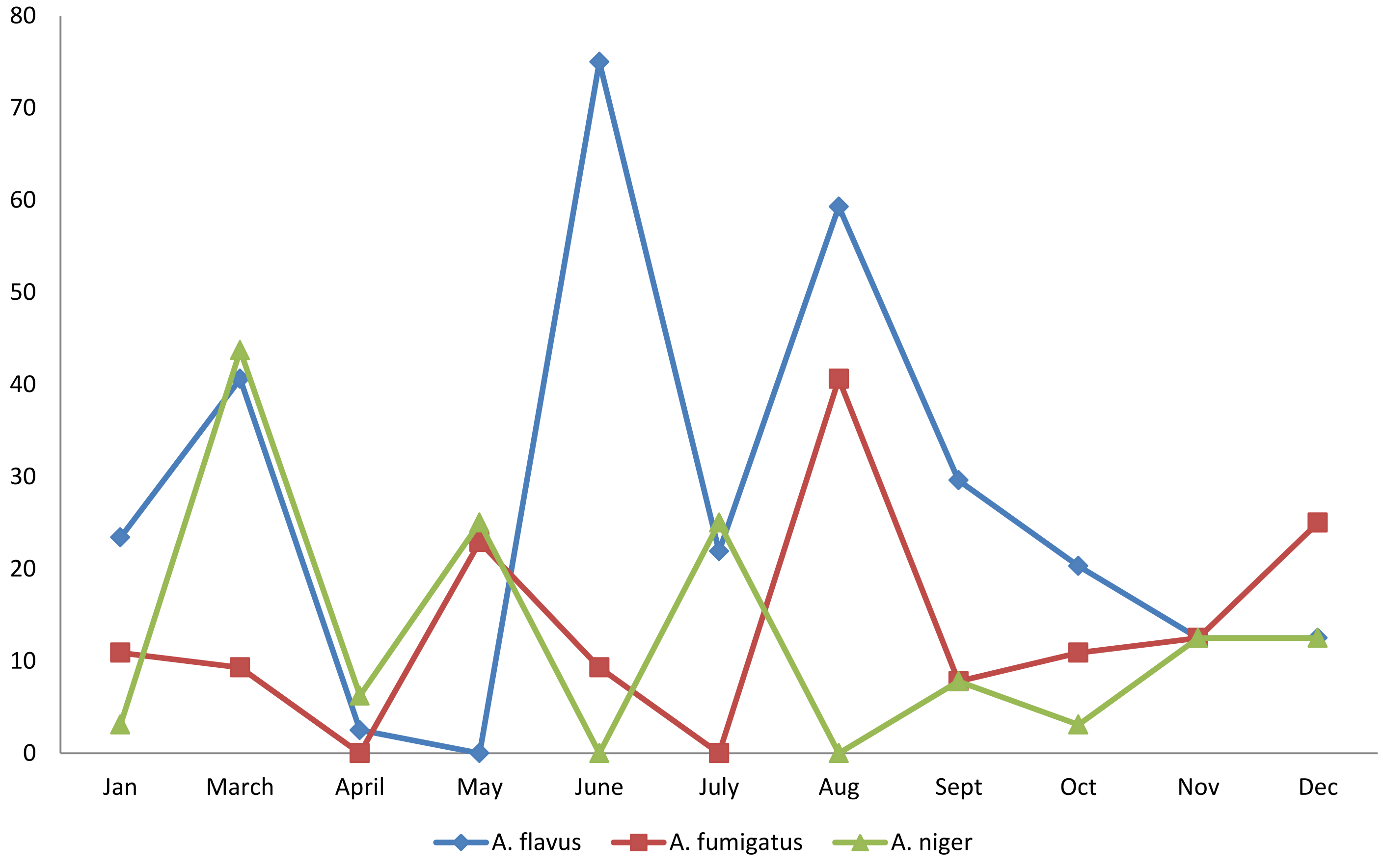
Table 1. *Aspergillus* species detected in 416 samples of hospital water by use of water-filtration sampling methods.

	Aspergillus recovered			
Source of water sample	No. of samples positive/no. of samples (%)	Species	No. of samples that yielded species/ no. of positive samples	Mean concentration,[a] cfu/L
Municipal water	6/18 (33)	*A. niger*	5/6	1.86
		A. flavus	1/6	
Hospital water storage tanks				
Cold water[b]	15/16 (94)	*A. niger*	9/15	6.43
		A. fumigatus	8/15	
		A. terreus	6/15	
Hot water[c]	7/24 (29)	*A. niger*	6/7	1.14
		A. flavus	1/7	
Patient care areas				
Cold water tap	17/81 (21)	*A. niger*	17/17	1.44
		A. terreus	1/17	
Hot water tap	12/81 (15)	*A. niger*	10/12	3.42
		A. fumigatus	1/12	
		A. flavus	1/12	
Cold water, shower	22/98 (22)	*A. niger*	18/22	2.39
		A. fumigatus	2/22	
		A. terreus	2/22	
Hot water, shower	23/98 (23)	*A. niger*	19/23	1.83
		A. fumigatus	1/23	
		A. terreus	1/23	

Aspergillus in our hospital

Months	Aspergillus		Other Hyalo		Mucor		Dematiaceous		Unidentified	
	AC	NON-AC	AC	NON-AC	AC	NON-AC	AC	NON-AC	AC	NON-AC
Jan	37	64	13	4	2	2	17	10	0	0
Mar	94	133	0	0	38	2	9	0	0	0
Apr	9	65	0	5	3	0	58	162	3	5
May	48	115	0	0	0	0	10	0	0	0
June	84	90	0	0	6	10	0	0	0	0
July	47	89	0	0	6	10	3	0	0	0
Aug	100	178	16	4	0	6	0	0	0	0
Sept	45	76	0	0	0	0	16	8	0	2
Oct	34	118	2	4	2	0	70	28	8	4
Nov	38	54	13	42	0	0	9	0	3	0
Dec	50	44	0	6	0	2	0	0	6	0

Aspergillus in our hospital



Epidemiology of antifungal resistance in Aspergillus

- Current treatment options of *Aspergillus* diseases include three classes of antifungal agents
  - polyenes (amphotericin B), echinocandins (caspofungin) and azoles.

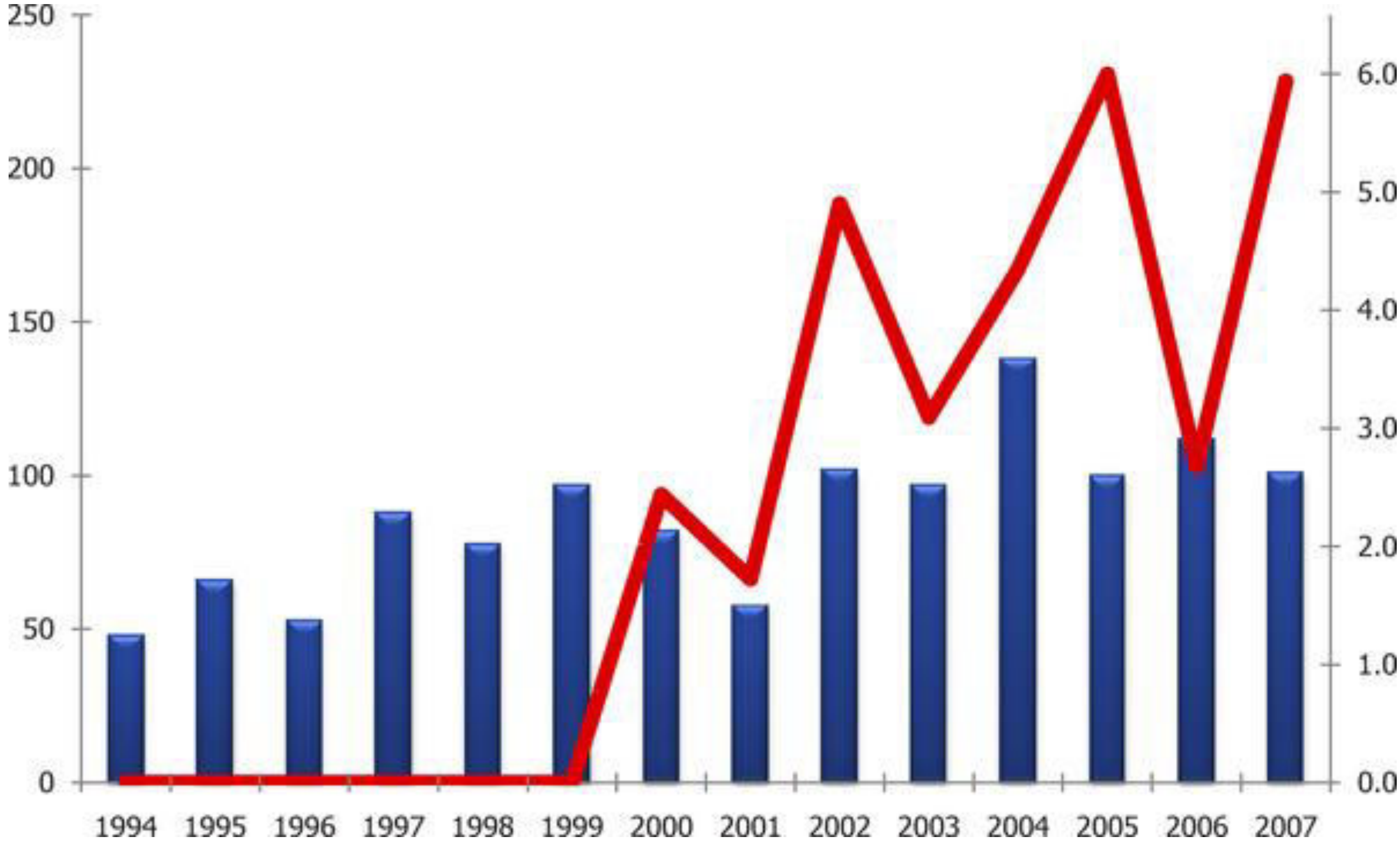
Epidemiology of antifungal resistance in Aspergillus

- Voriconazole is the recommended first choice therapy for invasive aspergillosis.
- Itraconazole is used for the treatment of chronic and allergic conditions
- Posaconazole is used prophylactic agent to prevent IA in patients with certain hematologic malignancies.

Itraconazole Resistance

- The first cases of itraconazole resistance were reported in late 1980s
- Vast majority have been detected since the turn of the millennium.
- The frequency is largely undefined
  - many centres do not routinely perform AFST for *Aspergillus*
- Resistance has currently been reported in Belgium, Canada, China, Denmark, France, Norway, Spain, Sweden, The Netherlands, UK and the USA.
- The frequency in these reports (~ 5,000 isolates) ranged from 0 – 6%, with an arithmetic mean of 2%

Epidemiology of ITZ Resistance in the A. fumigatus Isolates



Snelders E, van der Lee HAL, Kuijpers J, Rijs AJMM, et al. (2008) Emergence of Azole Resistance in Aspergillus fumigatus and Spread of a Single Resistance Mechanism. PLoS Med 5(11): e219. doi:10.1371/journal.pmed.0050219
http://www.plosmedicine.org/article/info:doi/10.1371/journal.pmed.0050219

PLOS | MEDICINE

Azole resistance in the A. fumigatus

- The frequencies in UK (Manchester) and The Netherlands (Nijmegen) is 5 and 6% respectively.
- Cross-resistance to other azoles are common
  - Among itraconazole resistant isolates -74% resistant to posaconazole and 65% to voriconazole

Howard et al *Emerg Infect Dis 2009*

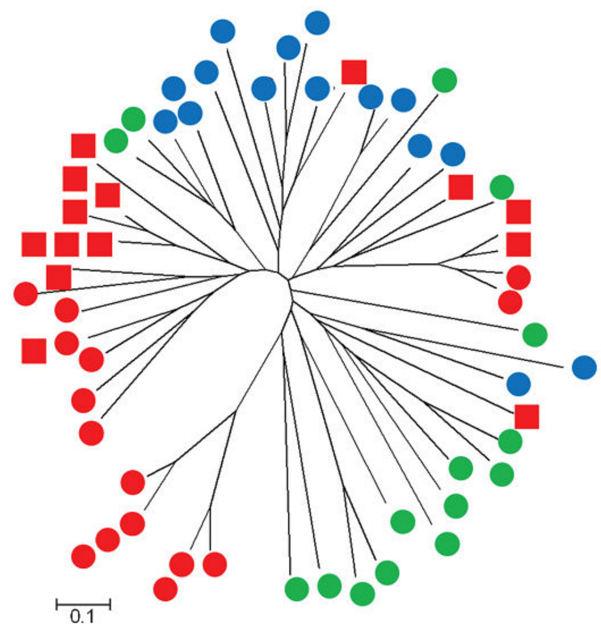
- Current data suggests azole resistance is acquired by two routes
  - *In- situ* within the lung (chronic aspergillosis cases on long term therapy)
  - Acquisition of a resistant *A. fumigatus* from the environment driven by agricultural use of 14^{α}- demethylase inhibitors (DMI).

Aspergillosis due to Voriconazole Highly Resistant *Aspergillus fumigatus* and Recovery of Genetically Related Resistant Isolates From Domiciles

Jan W. M. van der Linden,[1,2,a] Simone M. T. Camps,[1,2,a] Greetje A. Kampinga,[3] Jan P. A. Arends,[3] Yvette J. Debets-Ossenkopp,[4] Pieter J. A. Haas,[5] Bart J. A. Rijnders,[6] Ed J. Kuijper,[7] Frank H. van Tiel,[8] János Varga,[9] Anna Karawajczyk,[10] J. Zoll,[1,2] Willem J. G. Melchers,[1,2] and Paul E. Verweij[1,2]

Clinical Infectious Diseases 2013;57(4):513–20

- Study period 1315 *A. fumigatus* isolates from 921 patients screened for resistance.
- Prevalence of azole resistance was 6.8% (63 of 921 patients)
- TR34/L98H - 74.6% (47/63)
- TR46/Y121F/T289A –**20.6%** (13 patients)
- No mutation in Cyp51A - 3 patients



- Microsatellite genotypes of the clinical and environmental resistant TR 46 /Y121F/T289A isolates, compared with TR 34/L98H and wild-type controls

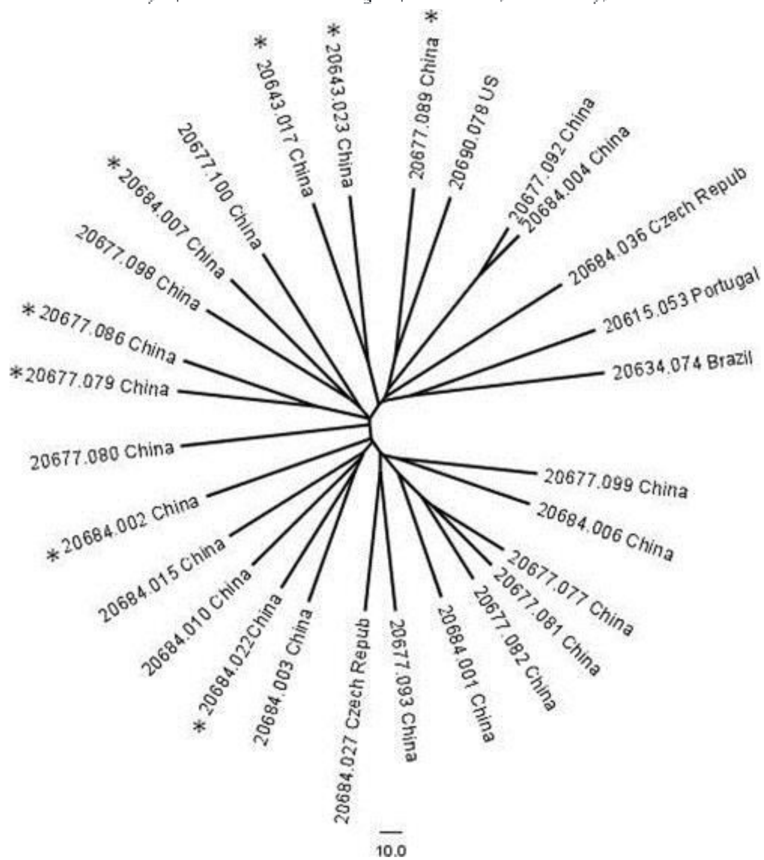
Antimicrobial Agents and Chemotherapy, Sept. 2011, p. 4465–4468 Vol. 55, No. 9
0066-4804/11/$12.00 doi:10.1128/AAC.00185-11

Azole Resistance in *Aspergillus fumigatus* Isolates from the ARTEMIS Global Surveillance Study Is Primarily Due to the TR/L98H Mutation in the *cyp51A* Gene▿

Shawn R. Lockhart,[1]* João P. Frade,[1] Kizee A. Etienne,[1] Michael A. Pfaller,[2] Daniel J. Diekema,[2,3] and S. Arunmozhi Balajee[1]

Mycotic Diseases Branch, Division of Foodborne, Waterborne and Environmental Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333,[1] and Department of Pathology[2] and Department of Internal Medicine,[3] University of Iowa Carver College of Medicine, Iowa City, Iowa 52242

28 isolates with high triazole MICs
From: Brazil, China, Czech Republic, Portugal, USA



Survey of 497 A. fumigatus

- Years 2008-2009
- Part of ARTEMIS global surveillance study

Dendrogram of all 28 isolates with elevated triazole MIC values. Isolates with the TR/L98H mutation are marked with an asterisk

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Journal of Antimicrobial Chemotherapy

Isolation of multiple-triazole-resistant *Aspergillus fumigatus* strains carrying the TR/L98H mutations in the *cyp51A* gene in India

Anuradha Chowdhary[1*], Shallu Kathuria[1], Harbans S. Randhawa[1], Shailendra N. Gaur[2], Corné H. Klaassen[3] and Jacques F. Meis[3,4]

2 isolates **(1.9%)** with high triazole MICs
Itra MIC: >16
Vori MIC: 2
Posa MIC: 2
Isavu MIC: 8
Isolates are from patients with chronic respiratory disease who are **azole-naive**

JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2003, p. 403–409 Vol. 41, No.
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.1.403–409.2003

Evaluation of Broth Microdilution Testing Parameters and Agar Diffusion Etest Procedure for Testing Susceptibilities of *Aspergillus* spp. to Caspofungin Acetate (MK-0991)

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Received 21 August 2002/Returned for modification 24 September 2002/Accepted 14 October 2002

TABLE 1. Caspofungin, amphotericin B, and itraconazole in vitro susceptibility data for 200 *Aspergillus* isolates[a]

Species (no. tested)	Incubation time (h)	Caspofungin				Amphotericin B MIC (μg/ml)		Itraconazole MIC (μg/ml)	
		MEC (μg/ml)		MIC (μg/ml)					
		Range	G (90%)[b]	Range	G (90%)	Range	G (90%)	Range	G (90%)
A. flavus (22)	24	0.12–2	0.3 (0.5)	0.06–2	0.31 (0.5)				
	48			0.12–>8	1.0 (0.5)	0.2–2	1.26 (2)	0.03–0.2	0.11 (0.2)
A. fumigatus (137)	24	0.12–>8	0.64 (0.5)	0.12–4	0.31 (0.5)				
	48			0.12–>8	0.75 (0.5)	0.2–4	1.2 (1.0)	0.03–>8	0.71 (0.5)
A. nidulans (13)	24	0.2–2	0.42 (0.5)	0.2–4	0.44 (0.5)				
	48			0.2–4	0.51 (0.5)	0.5–4	0.88 (2)	0.06–0.2	1.6 (0.2)
A. niger (13)	24	0.06–0.5	0.16 (0.2)	0.06–0.5	0.14 (0.2)				
	48			0.12–0.5	0.2 (0.5)	0.5–1.0	0.7 (1.0)	0.12/1.0	0.48 (1.0)
A. terreus (15)	24	0.06–0.5	0.12 (0.2)	0.06–0.2	0.12 (0.2)				
	48			0.2–0.5	0.27 (0.5)	0.5–4	1.4 (4)	0.03–0.5	0.14 (0.2)

[a] Results obtained with RPMI and an inoculum size of approximately 10^4 CFU/ml. MIC, ≥50% inhibition for caspofungin and itraconazole.
[b] G, geometric mean; 90%, MIC_{90} or MEC_{90}.

Transplant Associated Infection Surveillance Network

		Range	50%/90%
Aspergillus fumigatus[2] (181)	Amphotericin B	0.125-2	0.5/1
	Itraconazole	0.125-4	0.25/0.5
	Voriconazole	0.125-8	0.5/0.5
	Posaconazole	0.03-1	0.06/0.125
	Ravuconazole	0.25-1	0.5/1
Aspergillus niger (28)	Amphotericin B	0.125-0.25	0.125/0.25
	Itraconazole	0.25-1	0.5/1
	Voriconazole	0.5-1	1/1
	Posaconazole	0.06-0.5	0.25/0.25
Aspergillus flavus (27)	Amphotericin B	0.5-1	1/1
	Itraconazole	0.06-0.25	0.125/0.25
	Voriconazole	0.125-1	0.5/0.5
	Posaconazole	0.06-0.125	0.06/0.125
	Ravuconazole	0.25-0.5	0.5/0.5
Aspergillus terreus (22)	Amphotericin B	0.25-4	2/2
	Itraconazole	0.03-0.25	0.125/0.25
	Voriconazole	0.25-0.5	0.5/0.5
	Posaconazole	0.03-0.06	0.06/0.06
	Ravuconazole	0.5	0.5/0.5
Aspergillus versicolor (7)	Amphotericin B	0.125-1	0.5/1
	Itraconazole	0.125-16	0.25/16
	Voriconazole	0.25-2	1/2
	Posaconazole	0.06-16	0.25/16
	Ravuconazole	0.25-4	1/4
Apergillus calidoustus (5)	Amphotericin B	0.5-1	1/1
	Itraconazole	16	16/16
	Voriconazole	4-8	4/8
	Posaconazole	16	16/16
	Ravuconazole	4	4/4
Apergillus other(4)[3]	Amphotericin B	0.5-4	0.5/4
	Itraconazole	0.06-0.25	0.125/0.25
	Voriconazole	0.5-1	0.5/1
	Posaconazole	0.06-0.25	0.06/0.25

Baddely et al JCM 2009

Antimicrobial Agents and Chemotherapy, Feb. 2009, p. 794–795 Vol. 53, No. 2
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In Vitro Activities of Various Antifungal Drugs against *Aspergillus terreus*: Global Assessment Using the Methodology of the European Committee on Antimicrobial Susceptibility Testing▽

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isolates of *A. terreus* to antifungal agents

Strain group (n^a)	Drug	MIC (µg/ml) of indicated agent for *A. terreus* strains[b]			
		Range	50%	90%	Mean
Clinical (48)	AMB	0.5–8	2	2	1.67
	ITC	0.06–0.5	0.125	0.5	0.21
	VRC	0.5–4	1	2	1.54
	RVC	0.5–4	1	2	1.42
	POS	0.03–0.5	0.12	0.12	0.11
	TBF	0.06–1	0.25	0.5	0.28
	CPF	0.03–4	1	2	1.34
	MCF	0.015–0.06	0.015	0.03	0.03
	ADF	0.015–0.06	0.015	0.03	0.02
Environmental (31)	AMB	0.5–8	2	2	1.77
	ITC	0.12–0.5	0.125	0.5	0.23
	VRC	0.5–4	1	2	1.62
	RVC	0.5–4	1	2	1.32
	POS	0.06–0.25	0.12	0.12	0.10
	TBF	0.12–0.5	0.25	0.5	0.29
	CPF	0.12–2	1	2	1.24
	MCF	0.015–0.06	0.015	0.03	0.03
	ADF	0.015–0.06	0.015	0.03	0.02

Table 1 Characteristics and intrinsic resistance profiles of *Aspergillus* species.

Species	Characteristics	Resistance profile	Comments	References
A. lentulus	Newly recognized to cause IA	Reduced susceptibility to amphotericin B, azoles and variable susceptibility to caspofungin	Sibling species of *A. fumigatus*	9,17,20,22–24
A. udagawae	Uncommon cause of IA	Reduced susceptibility to amphotericin B and voriconazole	Sibling species of *A. fumigatus*	9,17–19,25
N. pseudofisheri	Uncommon cause of IA	Variable susceptibility to amphotericin B and reduced susceptibility to azoles	Sibling species of *A. fumigatus*	10,17,20
A. fumigatiaffinis	No cases of IA reported	Reduced susceptibility to amphotericin B and azoles	Sibling species of *A. fumigatus*	20
A. viridinutans	Newly recognized to cause IA in patients with primary immunodeficiencies	Reduced susceptibility to amphotericin B and azoles	Sibling species of *A. fumigatus*	20,21
A. flavus	Common in dry climates	Reduced susceptibility to amphotericin B		17,18,26–31
A. nidulans	Primarily causes IA in patients with CGD	Reduced susceptibility to amphotericin B		16,17,32–34
A. tetrazonus (*E. quadrilineata*)	Newly recognized to cause IA in CGD	Susceptible to amphotericin B but reduced susceptibility to caspofungin	Sibling species of *A. nidulans*	17,34
A. terreus	Propensity to disseminate with positive blood cultures	Reduced susceptibility to amphotericin B		18,41–46
A. alabamensis	No cases of IA reported	Reduced susceptibility to amphotericin B	Sibling species of *A. terreus*	55
A. niger	Uncommon cause of IA; Common cause of otomycosis	Variable susceptibility patterns with reduced activity of azoles		27,52–54,56
A. tubingensis	Newly recognized to cause keratitis and IA	Variable susceptibility patterns with reduced activity of azoles	Sibling species of *A. niger*	17,53,57
A. calidoustus	Uncommon cause of IA; Propensity to disseminate	Resistant to the triazoles and variable susceptibility to caspofungin	Previously reported as *A. ustus*	17,35–40
A. versicolor	Uncommon cause of IA; Common cause of onychomycosis	Reduced susceptibility to amphotericin B and variable susceptibility to azoles		18,56,58
A. sydowii	Newly recognized to cause onychomycosis and peritonitis in patients undergoing dialysis; Uncommon cause of IA.	Reduced susceptibility to amphotericin B and variable susceptibility to azoles	Sibling species of *A. versicolor*	17,54,59,60
A. persii	Newly recognized to cause onychomycosis	Reduced susceptibility to amphotericin B and caspofungin	Recently recognized as being a new species in section *Circumdati*	61

mycoses
Diagnosis, Therapy and Prophylaxis of Fungal Diseases

Original article

In vitro susceptibility of 188 clinical and environmental isolates of *Aspergillus flavus* for the new triazole isavuconazole and seven other antifungal drugs

M. R. Shivaprakash,[1] Erik Geertsen,[2] Arunaloke Chakrabarti,[1] Johan W. Mouton[2,*] and Jacques F. Meis[2]

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Table 2 *In vitro* susceptibilities of 188 isolates of *Aspergillus flavus* for isavuconazole and seven other antifungal agents.

Antifungal agent	MIC/MEC (mg l^{-1})				Cumulative % of isolates inhibited at MIC (mg l^{-1}) of										
	Range	GM	50%	90%	≤0.008	0.016	0.031	0.062	0.125	0.25	0.5	1	2	4	8
Amphotericin B	0.25–2	0.592	0.5	1	0	0	0	0	0	4.3	72.9	98.4	100	100	100
Itraconazole	0.062–0.5	0.177	0.125	0.25	0	0	0	2.1	50.0	97.3	100	100	100	100	100
Voriconazole	0.5–4	1.167	1	2	0	0	0	0	0	0	5.3	73.9	98.4	100	100
Posaconazole	0.062–0.25	0.123	0.125	0.25	0	0	0	14.9	87.2	100	100	100	100	100	100
Isavuconazole	0.125–2	0.697	1	1	0	0	0	0	0.5	3.7	48.4	99.5	100	100	100
Caspofungin	0.25–1	0.506	0.5	0.5	0	0	0	0	0	1.1	97.3	100	100	100	100
Anidulafungin	≤0.008–0.016	0.004	≤0.008	≤0.008	96.3	100	100	100	100	100	100	100	100	100	100
Micafungin	≤0.008–0.125	0.025	0.016	0.062	2.1	64.9	72.3	94.7	100	100	100	100	100	100	100

GM, geometric mean; MEC, minimum effective concentration; MIC, minimal inhibitory concentration.

Epidemiology of *Aspergillus* keratitis at a tertiary care eye hospital in South India and antifungal susceptibilities of the causative agents

Palanisamy Manikandan,[1] János Varga,[2] Sándor Kocsubé,[2] Raghavan Anita,[1] Rajaraman Revathi,[1] Tibor Mihály Németh,[2] Venkatapathy Narendran,[1] Csaba Vágvölgyi,[2] Kanesan Panneer Selvam,[3] Coimbatore Subramanian Shobana,[4] Yendremban Randhir Babu Singh[1,4] and László Kredics[2]

[1]*Aravind Eye Hospital and Postgraduate Institute of Ophthalmology, Coimbatore, Tamilnadu, India,* [2]*Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary,* [3]*Department of Microbiology, MR Govt. Arts College, Mannargudi, Thiruvarur District, Tamilnadu, India and* [4]*Department of Microbiology, Dr. GRD College of Science, Coimbatore, Tamilnadu, India*

	A. flavus (n = 74)			*A. fumigatus* (n = 14)		
	MIC (µg/ml)			MIC (µg/ml)		
Antifungal agent	MIC range	MIC_{50}[1]	MIC_{90}[1]	MIC range	50%	90%
Amphotericin B	0.5–16	2	8	0.25–1	0.5	1
Natamycin	4–128	128	128	4–64	4	16
Voriconazole	0.25–1	0.5	1	0.5–1	0.5	0.5
Itraconazole	1–4	2	4	1–4	2	2
Econazole	0.5–4	2	4	1–8	4	8
Clotrimazole	1–4	1	2	1–8	2	8
Ketoconazole	1–4	2	2	8–16	8	16
Fluconazole	256–512	512	512	256–512	512	512

In vitro antifungal activity of Indian liposomal amphotericin B against clinical isolates of emerging species of yeast and moulds, and its comparison with amphotericin B deoxycholate, voriconazole, itraconazole and fluconazole

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Department of Medical Microbiology, Division of Mycology, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India

Organism	n	Lip AMB			AMB			VOR			ITR		
		Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Aspergillus species	37	0.06-2	0.25	0.5	0.5-4	1	1	0.125-4	0.25	0.5	0.125-16	0.25	>16
A. flavus	23	0.06-0.5	0.25	0.5	0.5-2	0.5	1	0.125-1	0.25	0.5	0.125-16	0.125	>16
A. fumigatus	12	0.125-0.25	0.125	0.5	0.5-1	1	1	0.125-0.5	0.125	0.25	0.125-0.5	0.25	0.5
A. oryzae	2	1-2	1-2	ND	2-4	2-4	ND	0.5-4	0.5-4	ND	0.5-4	0.125	ND

EDITORIAL COMMENTARY

Voriconazole Resistance in *Aspergillus fumigatus*: Should We Be Concerned?

David W. Denning and Paul Bowyer

National Aspergillosis Centre, University Hospital of South Manchester; University of Manchester and Manchester Academic Health Science Centre, United Kingdom

(See the Major Article by van der Linden et al on pages 513–20.)

Keywords. fungicide; posaconazole; triazole; *fumigatus*; *Mycosphaerella*.

The triazole class of antifungal agents provides the backbone of human antifungal therapy. Resistance is therefore problematic. The clinical impact depends on

containing agar for primary culture of clinical specimens from all over the Netherlands and from many different patient types. The Nijmegen group has

infection (*Aspergillus* bronchitis, recently rediscovered [5]), and allergic bronchopulmonary and rhinosinus disease. Worldwide, approximately 300 000 people are estimat-

Clin Infect Dis 2013

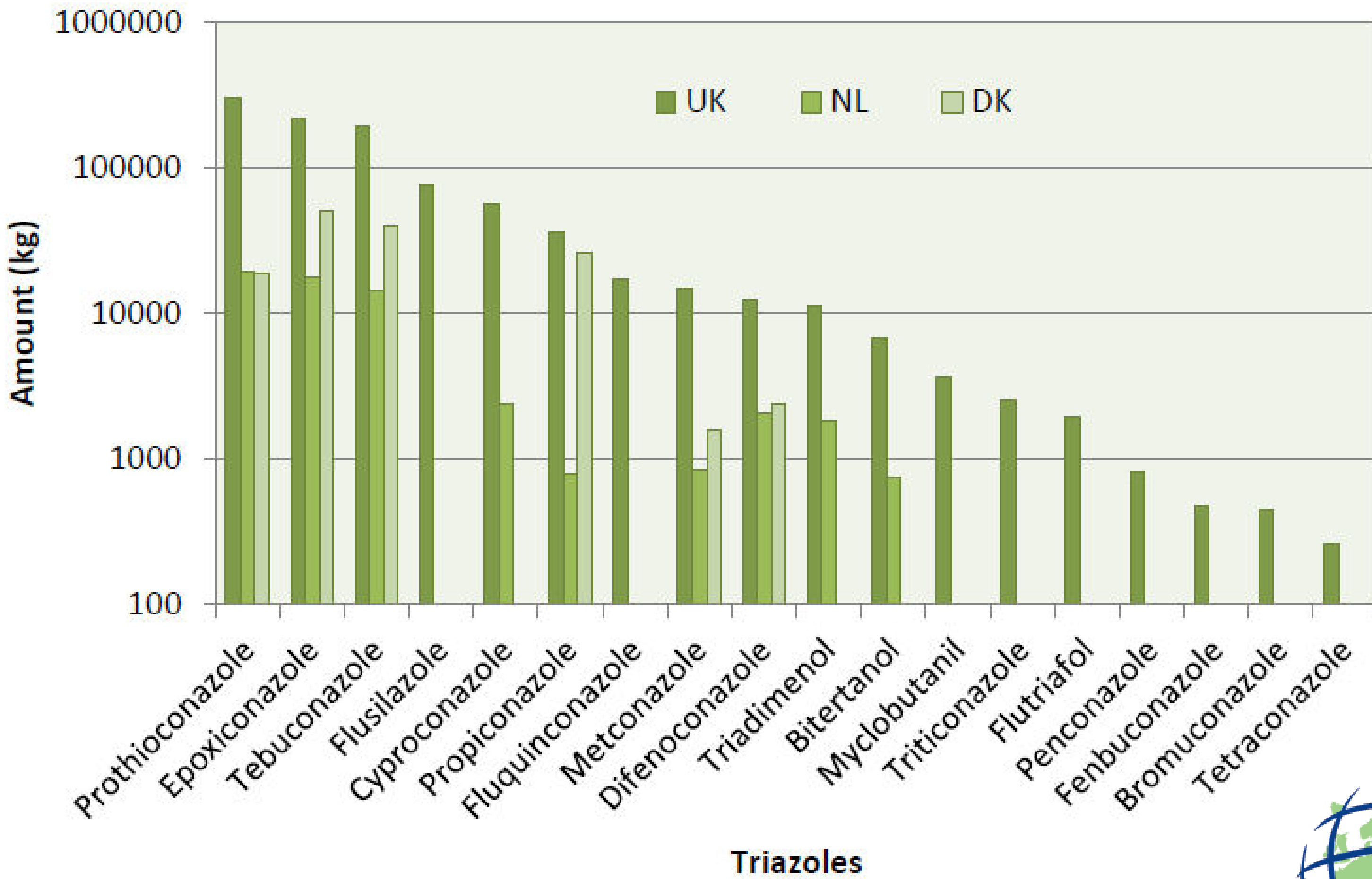
ECDC TECHNICAL REPORT

on

Risk assessment on the impact of environmental usage of triazoles on the development and spread of resistance to medical triazoles in *Aspergillus species*

EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

Use of triazoles and other sterol demethylation inhibitor (DMI) fungicides in agriculture



EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

Use of triazole fungicides in the production of selected crops/commodities in the United Kingdom

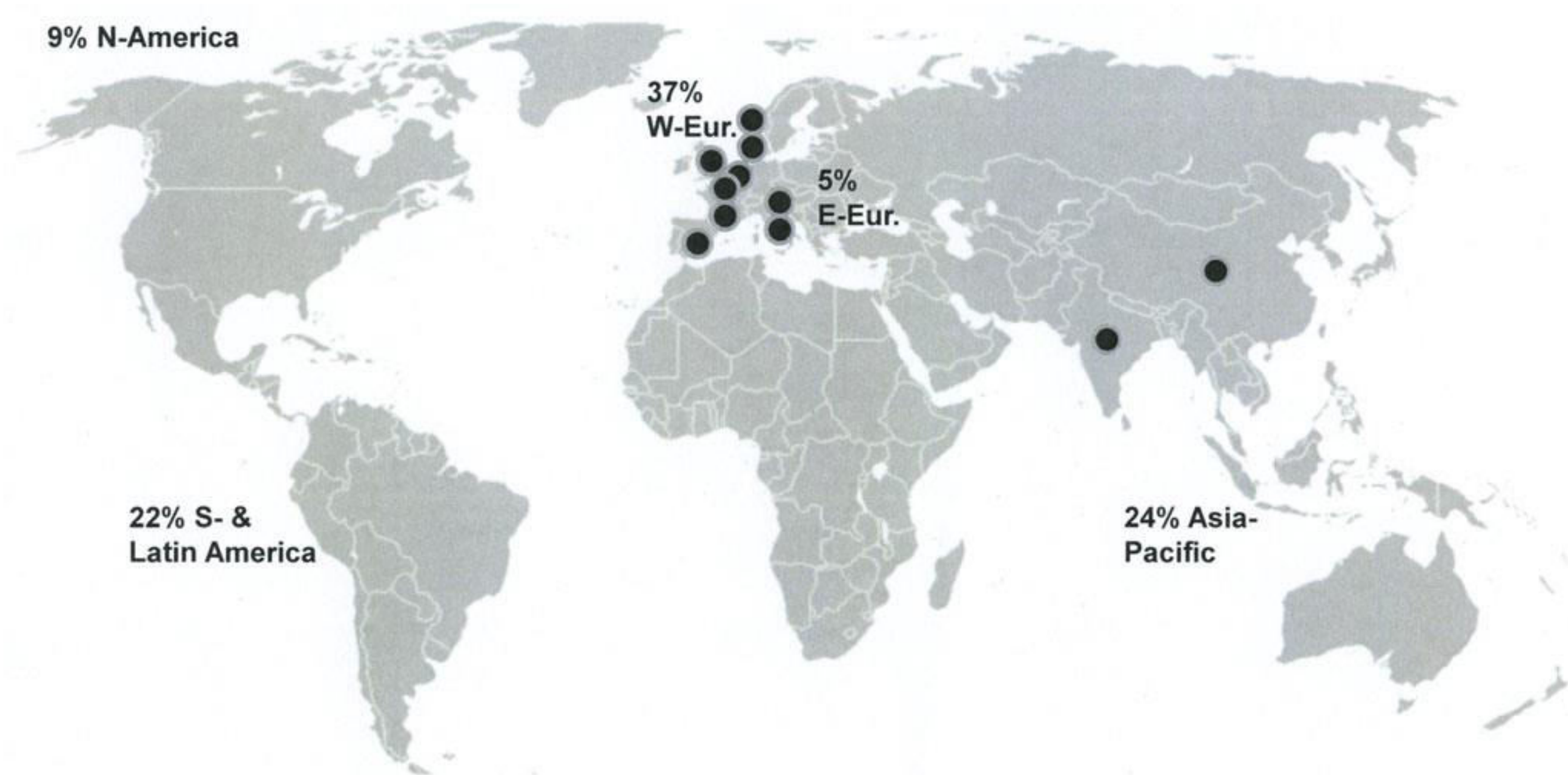
Crop	Year[a]	Production area (ha)	Key triazole fungicide products[b]	Area treated (ha)[c]	Amount used (kg)	Average spray number[d]	Average dose rate[e]
Beans	2008	118 462	Tebuconazole	65 691	10 222	1.32	0.62
			Cyproconazole/chlorothalonil	24 899	14 938	1.19	0.72
Carrots	2007	15 380	Tebuconazole	11 131	2 328	1.56	0.84
Wheat	2008	2 068 104	Epoxiconazole	1 242 366	75 598	1.46	0.49
			Prothioconazole/tebuconazole	571 313	89 840	1.57	0.54
Dessert apples	2008	5 577	Myclobutanil	22 356	1 309	4.70	0.64
			Penconazole	11 987	386	3.30	0.65
Field roses	2009	324	Myclobutanil	446	20	2.47	volumetric
Herbaceous plants	2009	910	Prochloraz/propiconazole	163	39	4.00	0.39
Hops	2008	1 071	Myclobutanil	4 139	162	4.86	volumetric
Oilseed rape	2008	597 706	Flusilazole/carbendazim	286 508	44 283	1.26	0.52
			Prothioconazole	225 120	22 877	1.24	0.58
			Metconazole	180 784	7 442	1.15	0.57
Outdoor bulbs	2009	4 875	Tebuconazole	6 311	966	1.65	0.61
Strawberries	2006	4 480	Myclobutanil	8 084	634	2.35	0.85
Vines	2006	856	Myclobutanil	1 577	80	2.62	1.02

Non-agricultural use of triazole fungicides

Non-agricultural use of triazole fungicides

The triazoles difenoconazole, tebuconazole and propiconazole are used for the control of key diseases on lawns (*Fusarium* patch, anthracnose and dollar spot) and ornamentals (mildew and rusts) by professionals (greens and golf courses) and amateurs (gardening). Tebuconazole and propiconazole complement each other with regard to efficacy against wood decaying fungi (e.g. *Gloephyllum trabeum* and *Poria* spp.). Together with copper carbonate, these fungicides/biocides are the main components of copper organic wood preservatives used in industry to pressure treat timbers to achieve long-lasting preventive protection of fencing, cladding, plywood, roofing and garden decking. Copper-triazole-based preservatives are widely marketed under the Wolmanized® brand in North America and the Tanalith® brand across Europe. Wood preservatives containing propiconazole and tebuconazole are also available for domestic curative use. Propiconazole for example is registered for use in adhesives, paints, leather, paper and textiles [84] and is available in the UK as the active ingredient in an antifouling agent, biocidal paints and surface biocides.

Countries where *A. fumigatus* with the TR34/L98H and percentage of agricultural fungicide use



Stensvold et al, Curr Fungal Infect Rep 2012

What happens if we do nothing?

- If left unchecked, azole resistance could continue to spread in the environment
- development of novel resistance mechanisms in the environment
- acquisition of an azole-resistant strain of Aspergillus spp. will likely go unnoticed until

the patient fails initial treatment

Recommendations

- Improve epidemiological surveillance through
  - Routine triazole susceptibility testing for clinical isolates
  - Increased and continuous surveillance of triazole resistance in A. fumigatus in each EU Member State

- Develop molecular methods to detect triazole resistance in culture-negative specimens and implement them in laboratory practice.
- Investigate the environmental origin through environmental and laboratory studies
  - Field studies with different triazoles which are putative resistance generators
  - Extensive and continued environmental studies
  - Non-agricultural studies
  - Studies on the reversal of rising resistance rates by restricting certain triazole applications


Thank you!