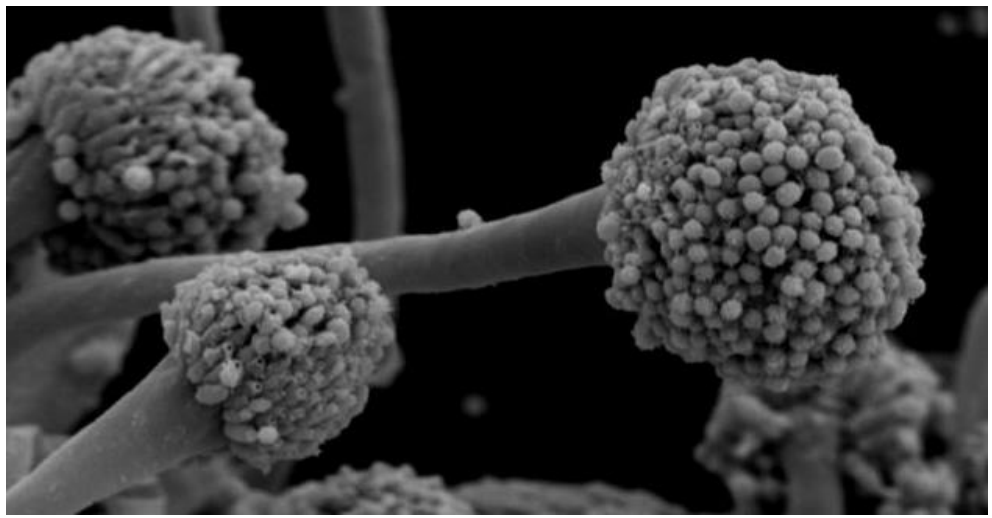
Azole resistance in *A. fumigatus*: seen from the clinical perspective



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Research grants/contract work (Paid to SSI): Amplyx, Basilea, Cidara, F2G, Gilead, Pfizer, Scynexis & T2Candida
Speaker honoraria: Astellas, Basilea, Gilead, MSD, Novartis, Pfizer & T2Candida, SEGES
Chair(wo)man for EUCAST-AFST
Past advisory board (≤ 2014): MSD, Pcovery, Pfizer

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- *Aspergillus fumigatus*
  - disease burden
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Aspergillus: Host interaction & spectrum of disease

Normal Host	Overreacting immune system	Impaired (lung) tissue architecture	Compromised immune system
Transient colonisation	ABPA Sinusitis Asthma w fungal sensitisation	TB Sarcoidosis COPD Aspergilloma Chronic forms of aspergillosis	Haematological disease HSCT, GVHD Wegeners granulomatosis etc Steroids Influenza… Semi → acute invasive aspergillosis

Aspergillus: Host interaction & spectrum of disease

Normal Host	Overreacting immune system	Impaired (lung) tissue architecture	Compromised immune system
Transient colonisation	ABPA Sinusitis As	TB Sarcoidosis	Haematological disease HSCT, GVHD Wegeners granulomatosis etc Steroids Influenza…
			Semi → acute invasive aspergillosis
			294/y

≥ 500

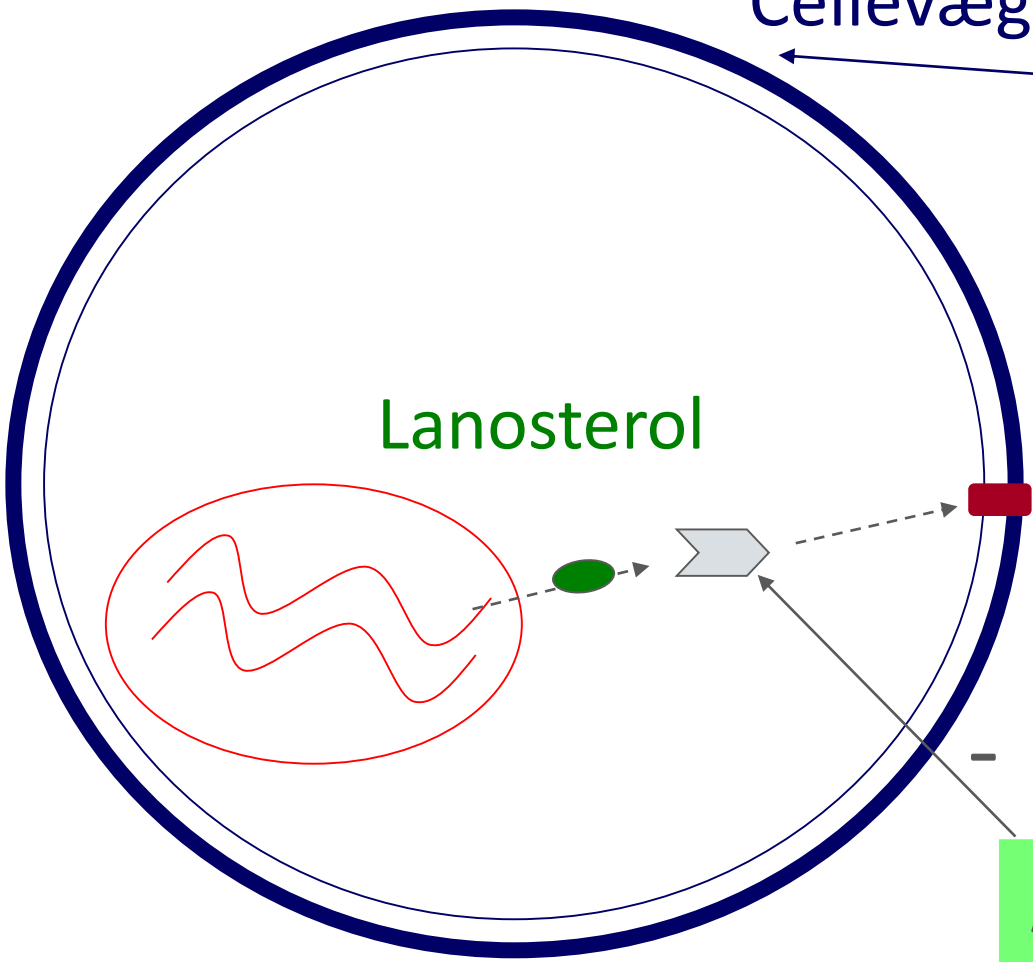
alvorlige aspergilllus infektioner

årligt i Danmark

Global burden estimates using published incidence rates

Mortensen et al Mycoses 2015; *Courtesy: Jesper Rømhild Davidsen, OUH Odense Universitetshospital

Svampemidler til behandling af aspergillus infektioner



Echinocandiner

- Caspofungin
- Micafungin
- Anidulafungin

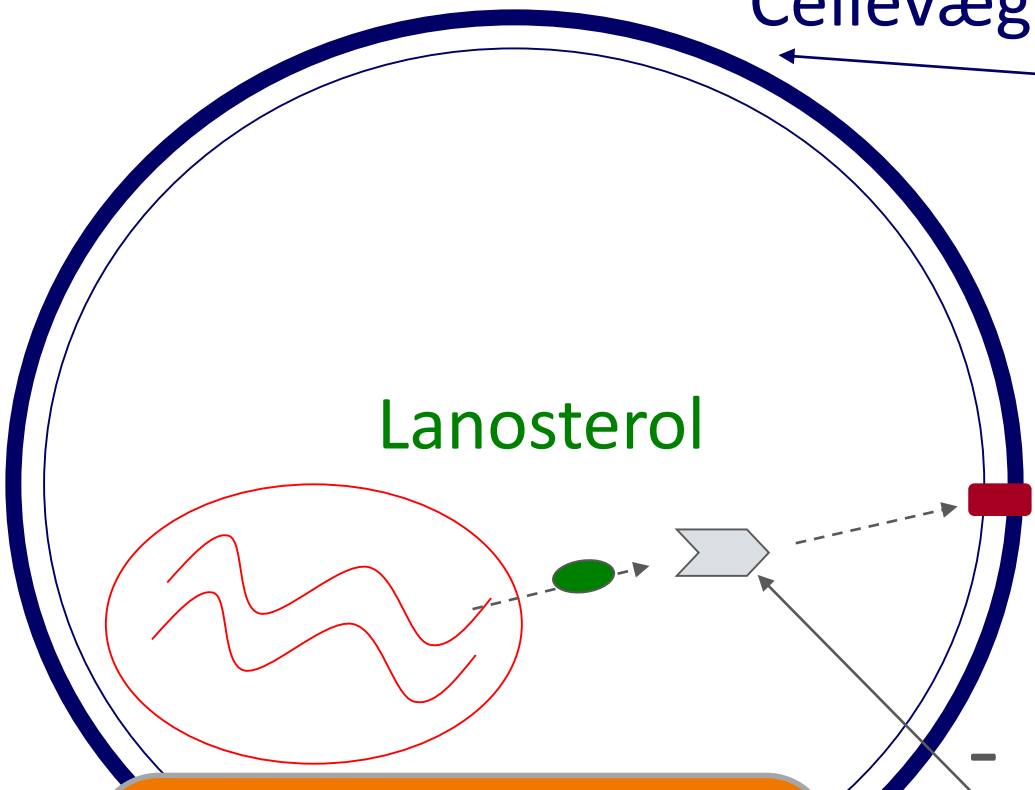
Polyener

- Amphotericin B

Azoler

- Itraconazol
- Vori-, posa- & isavuconazol

Svampemidler til behandling af aspergillus infektioner



Echinocandiner
- Caspofungin
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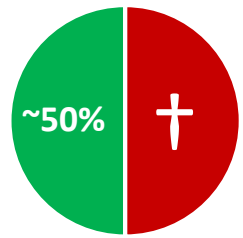
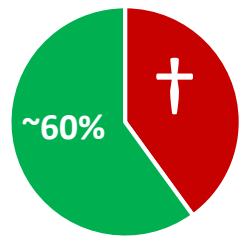


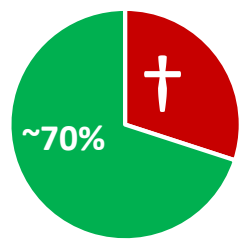
Polyener
- Amphotericin B



1ste valgsbehandling

Eneste stofgruppe der kan gives som tabletter

Azoler
- Itraconazol
- Vori-, posa- & isavuconazol



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Azole resistant Asp: *In vivo* Origin – "human route"

- Cystic Fibrosis (CF) patient, long term azole therapy

Date	ITC	cyp51A alteration	2A	2B	2C	3A	3B	3C	4A	4B	4C
17.07.2007	**1**	Not found	18	23	16	35	13	18	15	9	10
05.12.2007	**0.5**	Not found	18	23	16	35	13	18	15	9	10
09.07.2009	**>4**	**M220I** + V101F	18	23	16	35	13	18	15	9	10
16.07.2009	**>4**	**M220I** + V101F	18	23	16	35	13	18	15	12	10
21.07.2009	**>4**	**M220I** + V101F	18	23	16	35	13	18	15	12 & 9	10
10.08.2009	**>4**	**M220I** + V101F	18	23	16	35	13	18	15	9	10
07.09.2009	**>4**	**M220I** + V101F	18	23	16	35	13	18	15	12	10
23.10.2009	**>4**	**M220I** + V101F	18	23	16	35	13	18	15	12	10
24.11.2009	**>4**	**M220I** + V101F	18	23	16	35	13	18	15	9	10
26.11.2009	**0.5**	Not found	18	23	16	35	13	18	15	9	10
14.12.2009	**>4**	**M220I** + V101F	18	23	16	35	13	18	15	9	10

Azole resistant Asp: *In vivo* Origin – "environmental route"

- 2007- 2012: TR_{34}/L98H found only in CF patients
- 2012→ TR_{34}/L98H in 3 non-CF patients 2 of whom = azole naive

CASE	Day	Species	MIC (µg/mL)*)					*CYP51A* genotype	STR*Af* (2A-2B-2C-3A-3B-3C-4A-4B-4C)
			POS	VOR	ITRA	AMB	CAS		
2012	7	*A. fumigatus*	0.06	1	0.5	0.5	0.064	wt	18-19-8-26-10-21-9-9-5
	7	*A. fumigatus*	**1**	**2**	**>8**	0.5	0.064	TR_{34}/L98H +S297T+F495I	14-10-9-30-9-6-8-10-20
	17	*A. fumigatus*	**0.5**	1	**>8**	0.5	0.064	TR_{34}/L98H+S297T +F495I	14-10-9-30-9-6-8-10-20
2013	5	*A. fumigatus*	**0.5**	**4**	**>8**	0.25	0.064	TR_{34}/L98H	20-20-28-32-9-6-8-10-20
2013	44	*A. fumigatus*	0.03	0.25	0.25	0.25	0.064	wt	14-20-11-34-9-7-8-10-12
	90	*A. fumigatus*	**0.5**	**4**	**>8**	0.5	0.032	TR_{34}/L98H	25-10-12-79-9-9-8-10-11
	90	*R. pusillus*	0.25	>4	2	0.5	>32	NA	NA
	106	*R. pusillus*	0.125	>4	0.5	0.5	NA	NA	NA
	110	*R. pusillus*	0.125	>4	0.5	0.5	NA	NA	NA
	117	*A. fumigatus*	≤0.03	0.5	0.5	1	0.064	wt	25-16-19-48-17-23-8-9-5
	117	*R. pusillus*	0.25	>4	0.5	0.5	>32	NA	NA

Azole resistant Asp: *In vivo* Origin – "environmental route"

- 2014
  - TR_{46}/Y121F/T289A (LTX patient w agammaglobulinaemia)

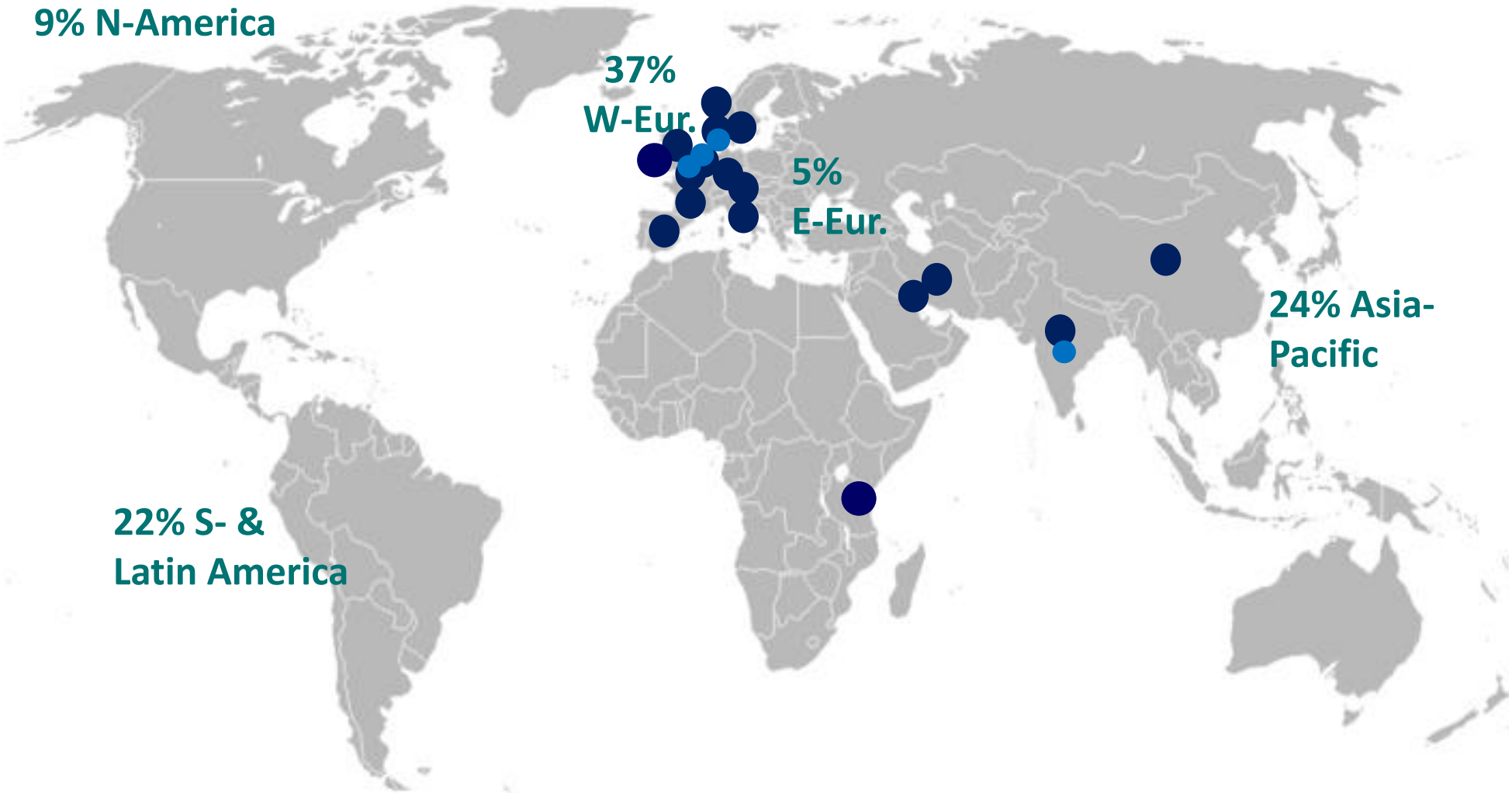
CASE	Day	Species	MIC (µg/mL)*)					*CYP51A* genotype	STR*Af* (2A-2B-2C-3A-3B-3C-4A-4B-4C)
			POS	VOR	ITRA	AMB	CAS		
	0	*A. fumigatus*	0.06	0.5	0.25	0.75	0.125	wt	18-25-15-26-11-7-26-30-8
2014	43	*A. fumigatus*	**0.125**	**>4**	0.5	1	0.032	TR_{46}/Y121F/T289A	26-21-16-32-9-10-8-14-10
	62	*A. fumigatus*	**0.25**	**>4**	1	1	0.032	TR_{46}/Y121F/T289A	26-21-16-32-9-10-8-14-10

- 2/4 were azole naïve
- 3/4 polyfungal infection

All 4 died †

Fungicide use & Azole "R" *A. fumigatus* reports - Oct 2014

Global market share of pesticide use in agriculture;



Stensvold Curr Fungal Inf Reports 2012, Rath AAC 2012, Chowdhary Plos One 2012, Badali Mycoses 2013; Chowdhary JAC 2014.

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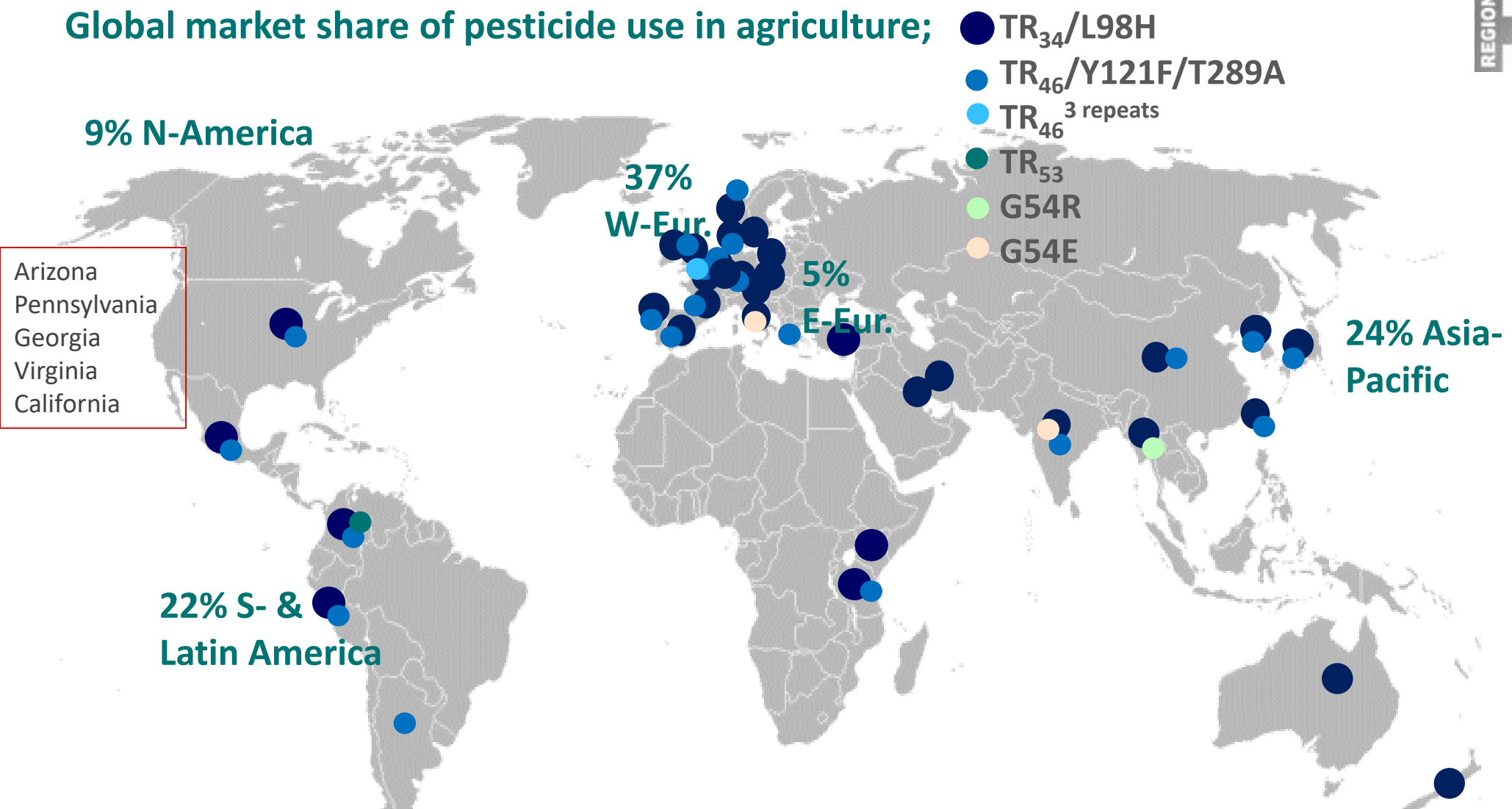
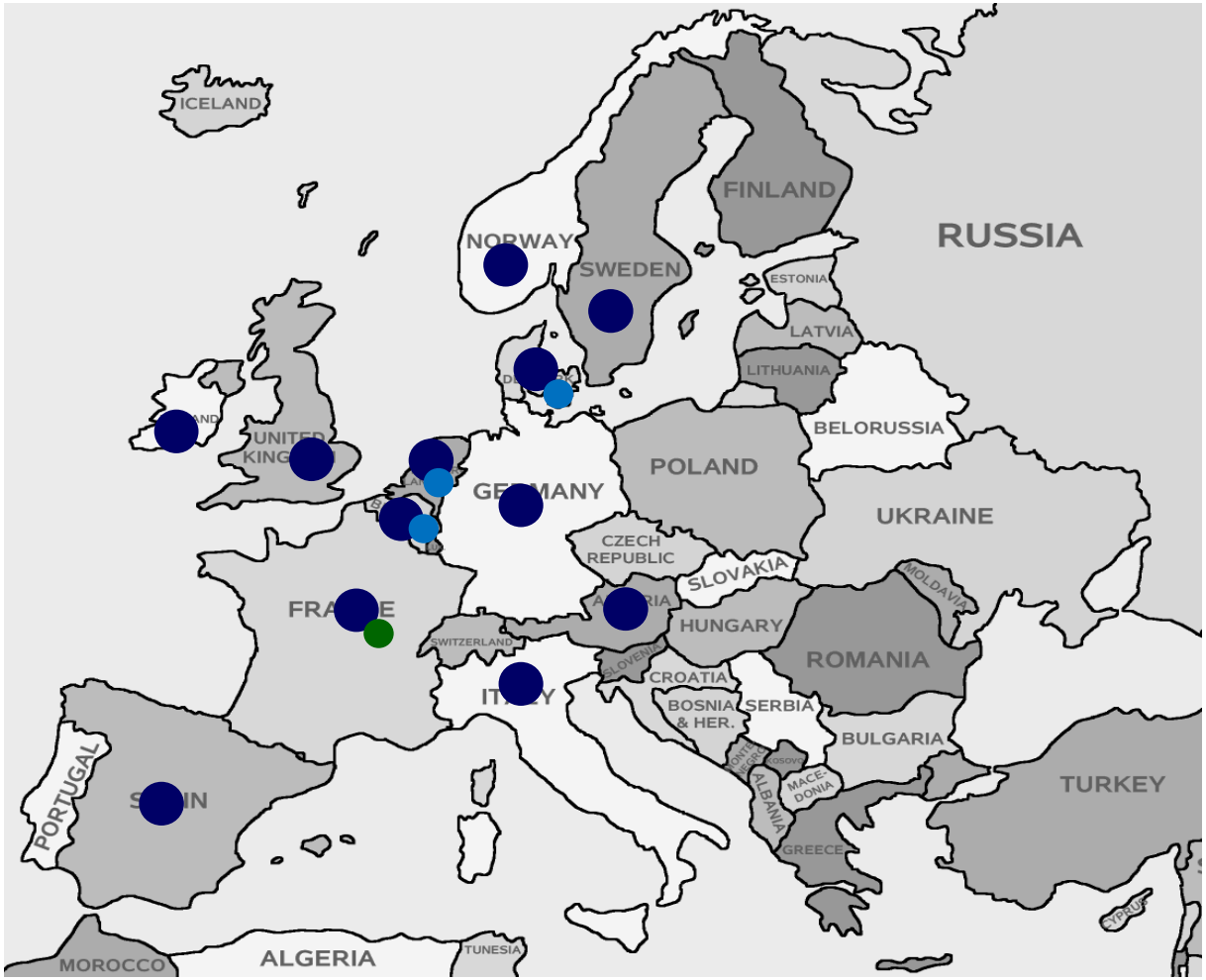


Mellado AAC 2007, Denning CID 2011, Snelders PLoS Med 2008, Mortensen AAC 2010, Kuipers AAC 2011, Lockhart AAC 2011, Rath AAC 2012, Stensvold Curr Fungal Inf Reports 2012, Bader AAC 2013, van der Linden CID 2013 & Emerg Inf Dis 2015, Prigitano Eurosurv 2014, Chowdhary Plos One 2012, JAC 2014 Jan & Nov., Badali Mycoses 2013; Seyedmousavi Emerg Inf Dis 2013, Astvad AAC 2014, Steinmann JAC 2015, Brillowska-Dabrowska Acta Biochim Pol. 2015, Kidd Mycoses 2015, Lavergne AAC 2015, Wu Mycoses 2015, Chen AAC 2015, Pelaez New microbes New Inf 2015; Sharma J Global Antimicrob Res. 2015, Özmerdiven J Inf Chem 2015, Verweij CID 2016, Hagiwara J Infect Chemother 2016, Wiederhold JCM 2016, Le Pape Emerg ID 2016, Castanheira DMID 2016, Tangwattanachuleeporn Med Mycol In press 2016, Moore JIA 2017, Alvarez-Moreno Sci Rep. 2017, Toyotome J Inf Chemother 2017, Hurst JAC 2017, Zhang mBio 2017, Riat AAC 2018, Isla AAC 2018, Kemoi Int J Microbiol 2018, Lee Mycopath 2018, Monteiro J Glob Antimicrob Resist 2018; Pinto Front Microbiol 2018, Beer MMWR 2018, AzoleResNet report 2019, Siopi ECCMID 2019, Resendi-Sharpe ECCMID 2019, Chen Mycoses 2019, Korfanty Mycopathol 2019, Prigitano JGAR 2019..

“Environmental” Azole R *A. fumigatus* in Europe Oct 2014



● TR_{34}/L98H detected

“New” azole resistance mechanisms detected in azole naïve patients or in the environment

NL:

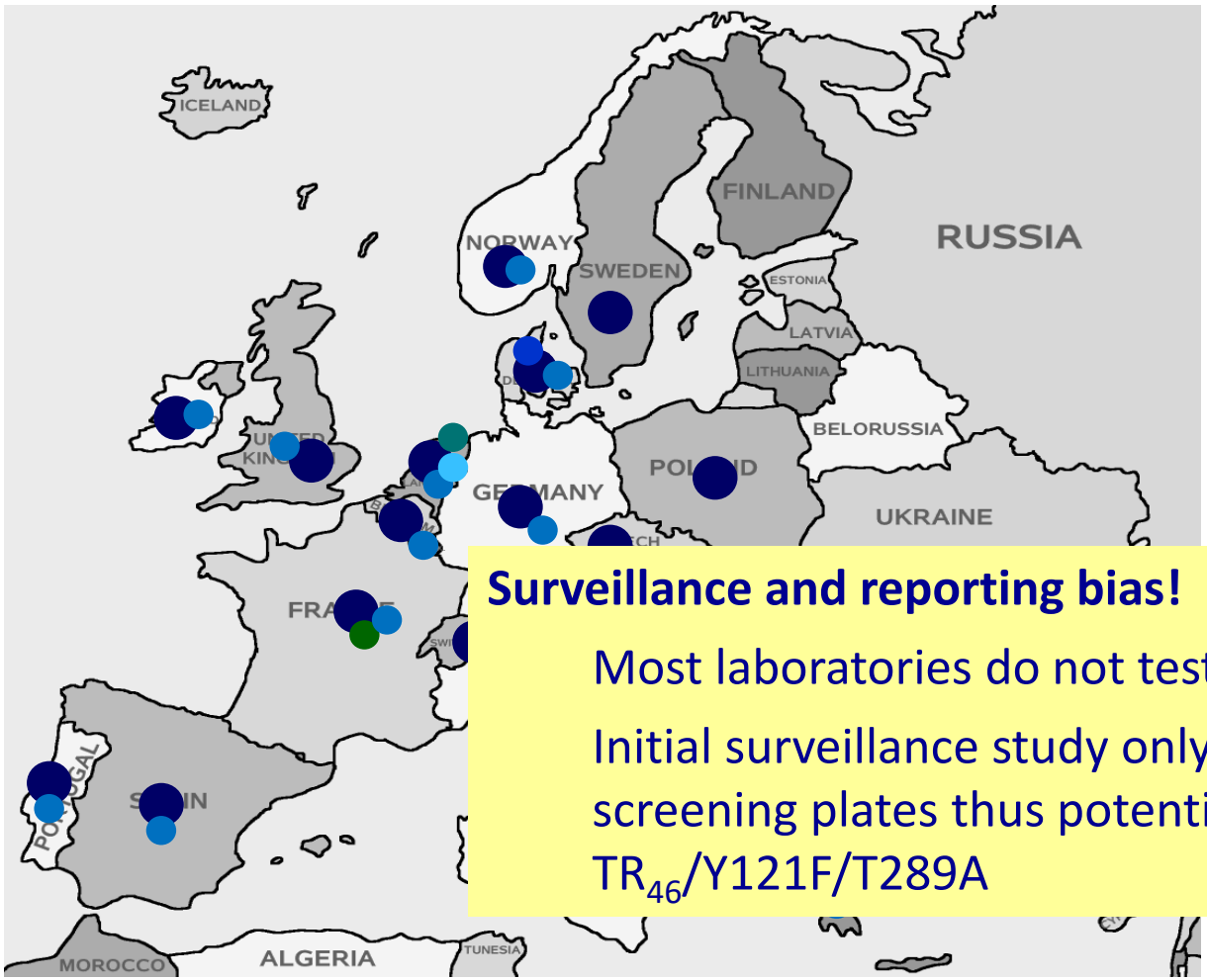
● TR_{46}/Y121F/T289A

France:

● G432S

Scare Study, Denning CID 2011, Snelders PLoS Med 2008, Mortensen AAC 2010, Verweij ICAAC 2010, Mellado AAC 2007, Rath AAC 2012, Stensvold Current Fungal Infection Reports 2012, Vermeulen Eorosurveillance 2012 , Meiss personal communication

“Environmental” Azole R *A. fumigatus* in Europe Nov 2019



- TR_{34}/L98H
- TR_{34}[3]/L98H
- TR_{46}/Y121F/T289A
- TR_{46}[3]/Y121F/M172I/T289A/G448S)
- TR_{53}
- G432S
- G54E

Surveillance and reporting bias!

Most laboratories do not test

Initial surveillance study only used itraconazole screening plates thus potentially missing TR_{46}/Y121F/T289A

Denning CID 2011, Snelders PLoS Med 2008, Mortensen AAC 2010, Kuipers AAC 2011, Mellado AAC 2007, Rath AAC 2012, Stensvold Curr Fungal Inf Reports 2012, Bader AAC 2013, van der Linden CID 2013 & Emerg Inf Dis 2015, Prigitano Eurosurv 2014, Astvad AAC 20140, Ozmerdiven ICAAC 2014, Steinmann JAC 2015, Pelaez New Microbes New Infect. 2015, Brillowska-Dabrowska Acta Biochim Pol. 2015, Lavergne AAC 2015, Verweij CID 2016, Castanheira DMID 2016, Sharma JGAR 2016, Zhang mBio 2017, Rogers CID 2017, Riat AAC 2018, Unpublished data (Czech republic), AzoleResNet report 2019, Risum ECCMID 2019, Siopi ECCMID 2019, Prigitano JGAR 2019.

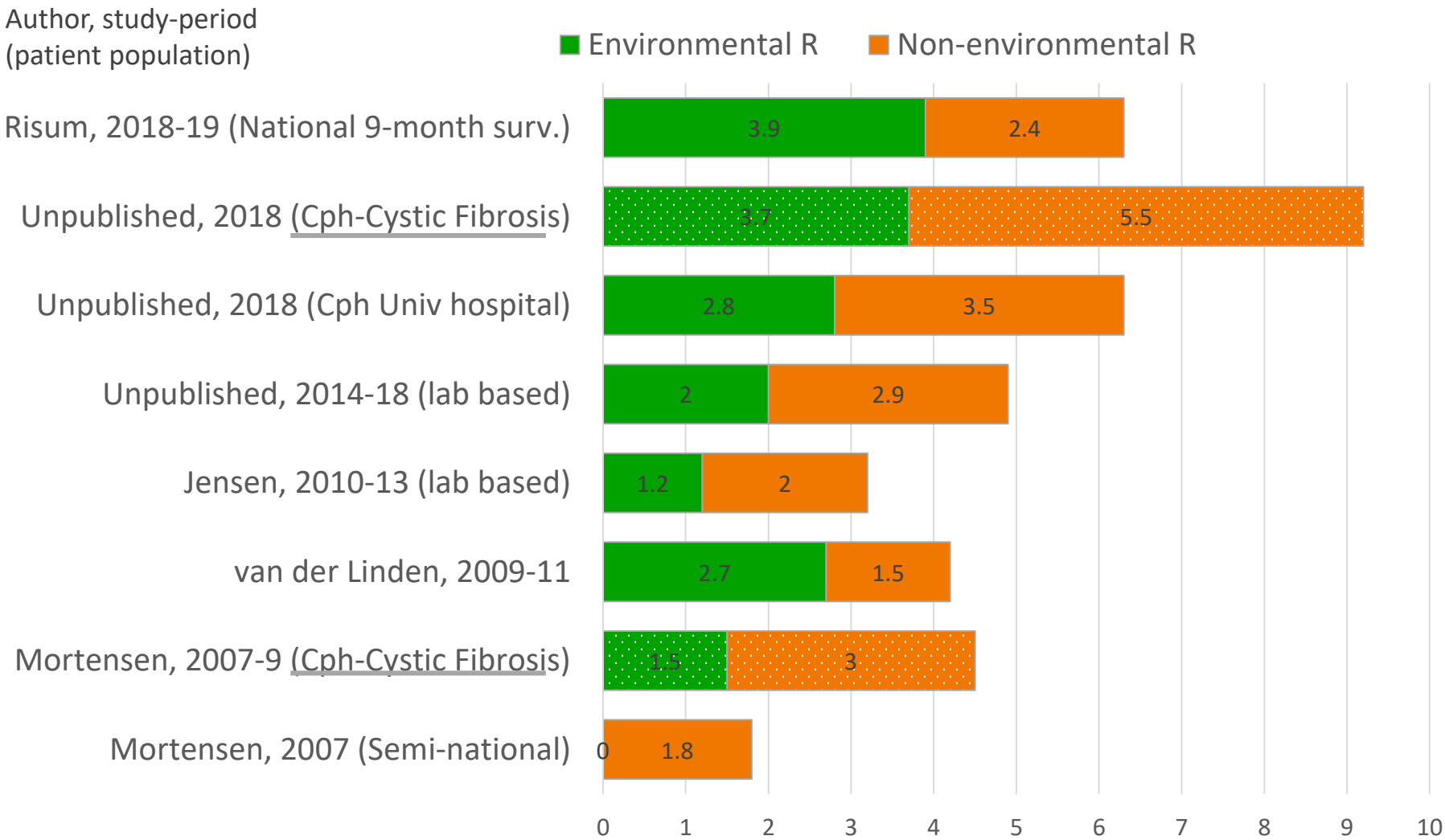
Nationwide surveillance: Azole R *A. fumigatus*

- Voluntary surveillance programme
- Inclusion criteria were:
  - a) isolates regarded clinically significant and
  - b) isolates detected on a Monday regardless of clinical significance.
  - Isolates from same patients were defined as unique if found >30 days apart or with a different susceptibility pattern.
- 9 months' data (Oct 2018 – June 2019)
  - 703 isolates from 508 patients
    - 53 isolater (7.5%) from 51 patients (10%) were marked "Monday samples"

DK *Aspergillus* surveillance group

Jan Berg Gertsen
Lise Kristensen
Flemming S Rosenvinge
Sofia Sulim
Ea Marmolin
Bent L Røder
Jette Bangsborg
Esad Dzajic
Michael Pedersen
Karen MT Astvad
Steen L Andersen

Azole resistance in *A. fumigatus* in Denmark



Mortensen EJCMID 2011, Mortensen JCM 2011, van der Linden Emerg Inf Dis 2015, Jensen CMI 2016, Risum TIMM abstract 2019

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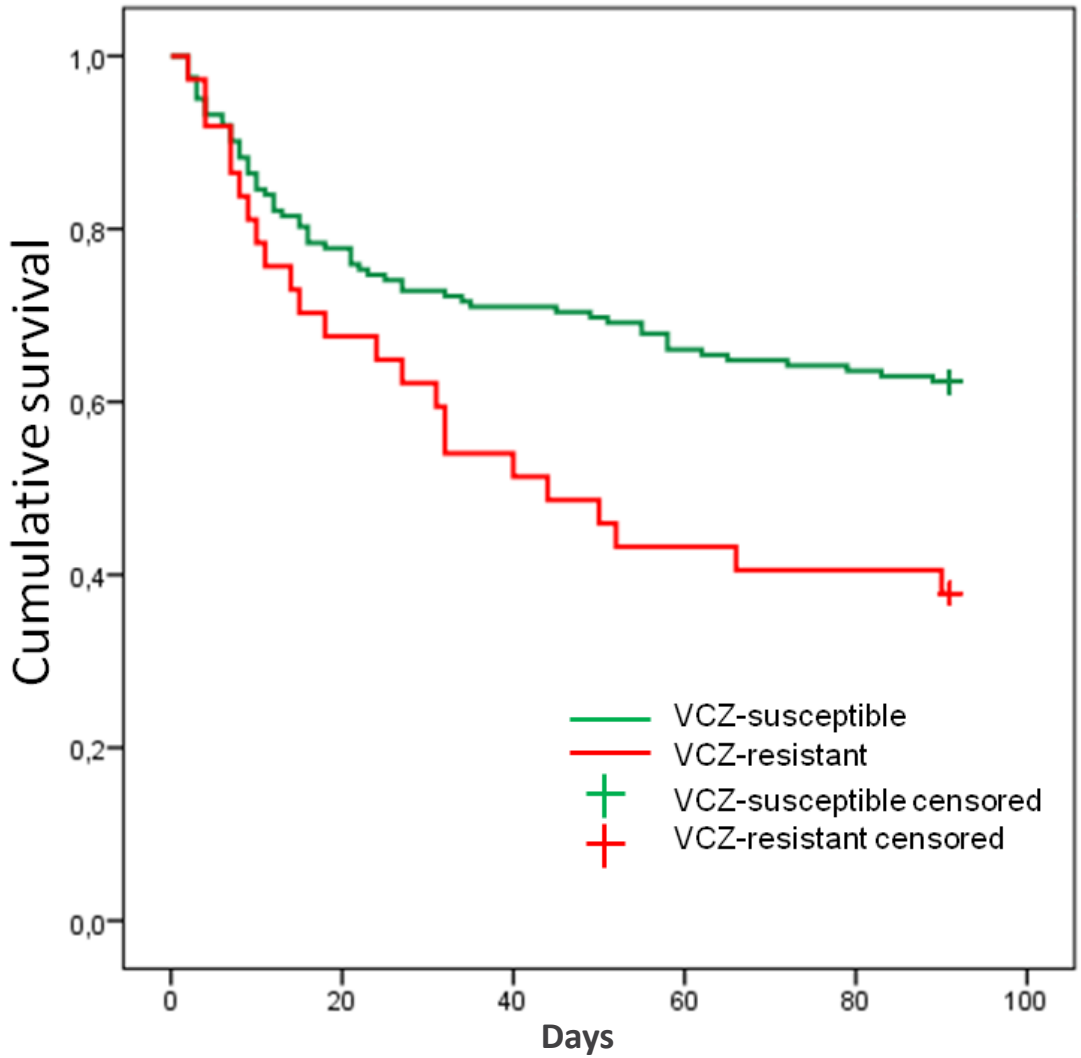
Overall mortality: voriconazole R >< S inv. aspergillosis

- A Dutch 5-year retrospective cohort study (2011–2015; 196 patients)



Mortality

Day 42

VCZ-S 28%

VCZ-R 49%

p=0.018

21% ↑

Day 90

VCZ-S 37%

VCZ-R 62%

p=0.004

25% ↑

Azole R Surveillance in Haematology Belgium & NL 2012-17

- 4 hospitals, 129 haematology patients w probable/proven inv. aspergillosis

-

- Azole R *A. fumigatus* 26/129 (20.2%)
  - 96% (25/26) environmental resistance:
    - 13 TR_{34}/L98H
    - 12 TR_{46}/Y121F/T289A

- Outcome
  - Initial therapy= voriconazole in 75% (97/129, incl. 5 w combination therapy)
  - Mortality:

	6 weeks		12 weeks	
	Azole R	Azole S	Azole R	Azole S
All (129)	42%	28%	58%	37%
Non-ICU (100)	36%	22%	54%*	31%*

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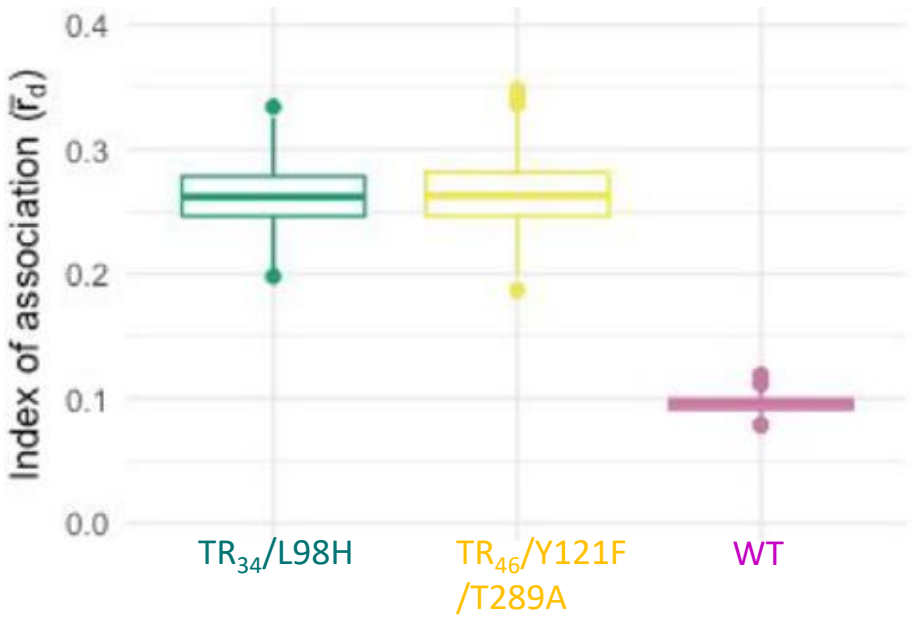
Environmental sampling

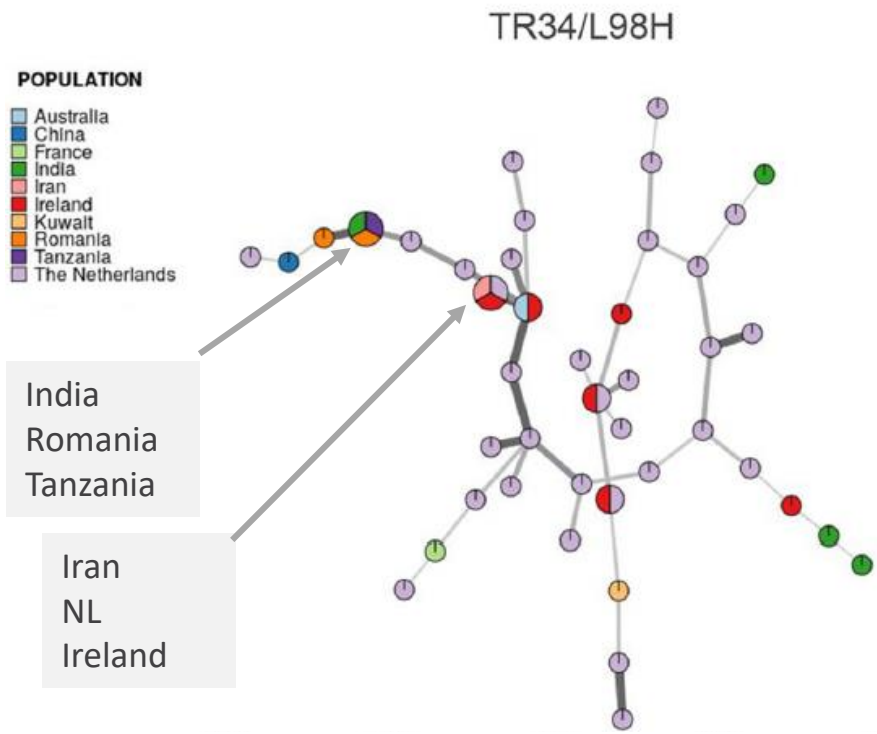
Country, ref.	Sampling period	Sampling location(s)	Method	Resistance prevalence	TR_{34}/L98and/or TR_{46}/Y121/T289A*
DK	June-Aug 2009	RH&Tivoli flowerbeds	Soil, ITR agar	8% (4/50) soil DK	100% TR_{34}/L98H
DK	March-April (frosty) 2010	Tivoli, fields, indoor hospital	Soil, azole agar	None	None
DK	Sept-Oct 2013	Conventional and organic fields	Soil & air, azole screening agar	None	None
NL	Jul-Aug 2009	Hosp flowerbeds and natural soil)	Soil, ITR agar	7.6% (6/79) 0% in natural soil.	83% (5/6) TR_{34}/L98H (6.3% of soil samples)
NL	Oct 2010 & Mar 2011	Domiciles/ hospitals, indoor/outdoor	Air 14.000L Azole agar.	140 azole-R Af isolates.	90% TR_{34}/L98H 10% TR_{46}/Y121F
UK	Fields: June 2011. Urban: 3 years	Fields Manchester	Soil and air	Azole-R fields (1.7%)	TR_{34}/L98H detected.
UK	May-July 2018	Urban Rural (S-UK)	Soil	U: 13.8% R: 1.1% (tot 6.7%)	65% TR_{34}/L98H, 30% TR_{46}/Y121F
Italy	May 2011; Oct-Dec 2011; Mar-June 2012	Fields, gardens, pot compost	Soil, ITR agar	12.8% (6/47) soil 15.5% (9/58) of Af.	78% (7/9)TR_{34}/L98H Only 5.6% of soil Oct-Dec pos
BE	June-July 2012	Outdoor	Air 14.000L	NA	TR_{46}/Y121F
IN	NA Published 2012.	Outdoor, div	Soil, few air. ITR agar.	5% (24/486) soil; 7 % of Af (44/603)	100% TR_{34}/L98H
IN	2012-2013	Outdoor Yamana River fields, Delhi+ Varanasi	Soil, ITR agar	5.7% of soil	57% TR_{34}/L98H 43% TR_{46}/Y121F

Astvad AAC 2014, Sewell AAC 2019

A. fumigatus Genetic relationship among isolates

- Str*Af* typing: 4,049 isolates from 26 countries and 4 continents
  - 2,293 genotypes in 2 clades, both incl. WT, TR_{34}/L98H & TR_{46}/Y121F/T289A
  - TR_{34}/L98H and TR_{46}/Y121F/T289A more related than wt isolates
    - emerged more recently
  - Isogenic strains of TR_{34}/L98H found in distant countries → isolates travel
    - with goods, people & wind





Summary

- Quantifying Azole Resistance *A. fumigatus* rates is complex
  - Bias include (the following amongst others)
    - sample size
    - patient mix
    - culture techniques (affects sensitivity and specificity)
    - azole resistance screening methods

- DK situation
  - 0 → ≤ 5% Azole-R in DK
  - ↑ incidence in the CF population (TR mechanisms doubled)
  - but only few found in environmental samples
    - in part potentially due to diagnostic sensitivity issues

- Globally
  - Increasing, in rural as well as urban areas
  - Increasing number of mechanisms involved
  - Driven by fungicide use & expansion (wind, crops, travel)
  - Significant impact on mortality for affected patients

Acknowledgements

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The EFISG study group