

Antifungal Resistance in *Aspergillus*

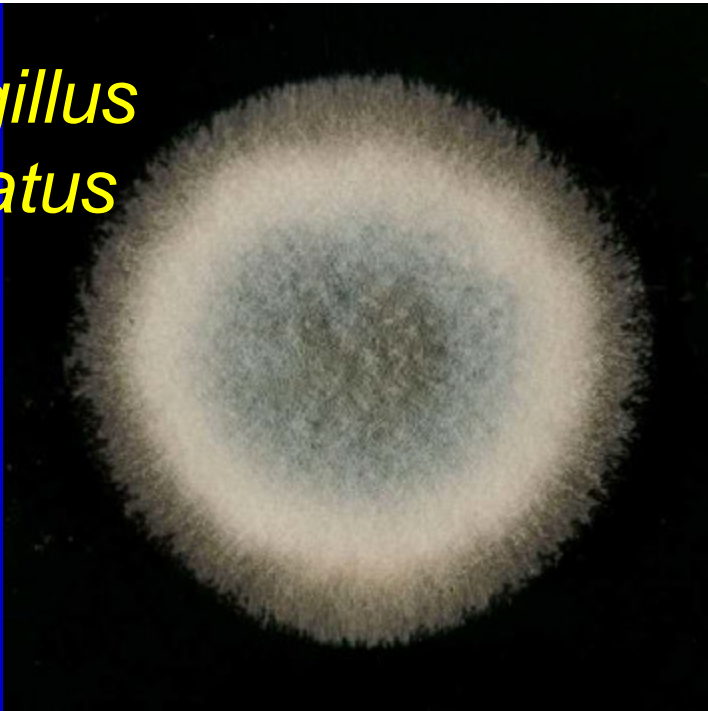
Dr Susan J Howard

*The University of Manchester
Manchester Academic Health Science Centre
NIHR Translational Research Facility in Respiratory Medicine
University Hospital of South Manchester NHS Foundation Trust*

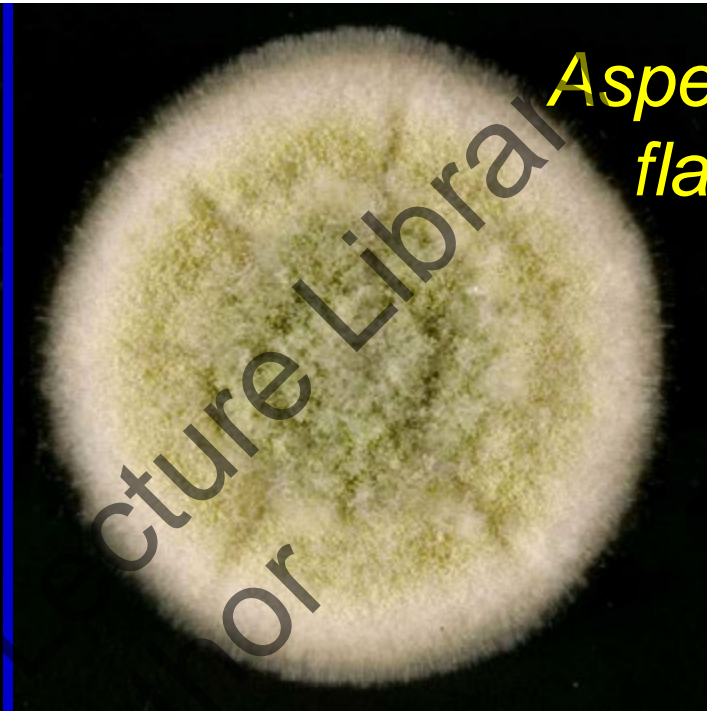
Agenda

- How common are resistant infections?
- What are the clinical risk factors?
- How does resistance occur?
- Is cross-resistance a clinical problem?
- How can we detect resistance?

Aspergillus fumigatus



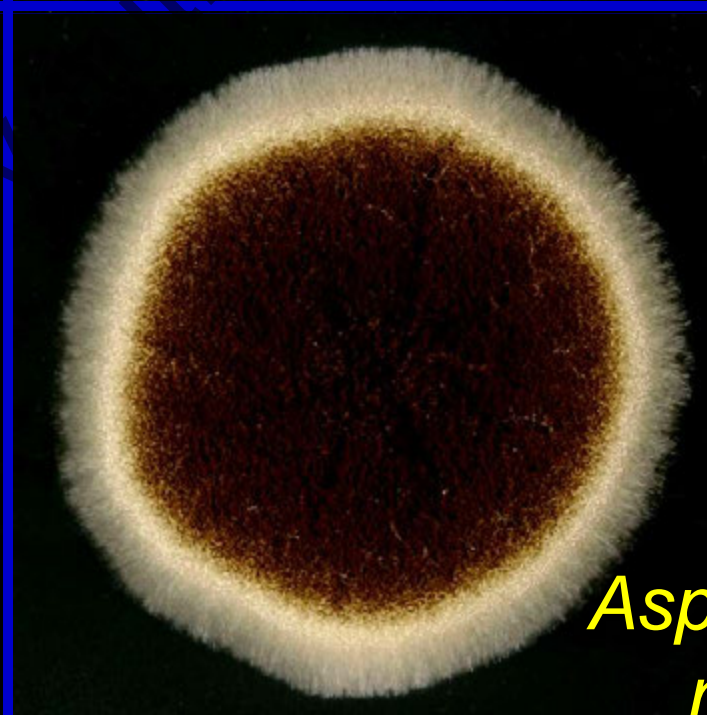
Aspergillus flavus



Aspergillus terreus



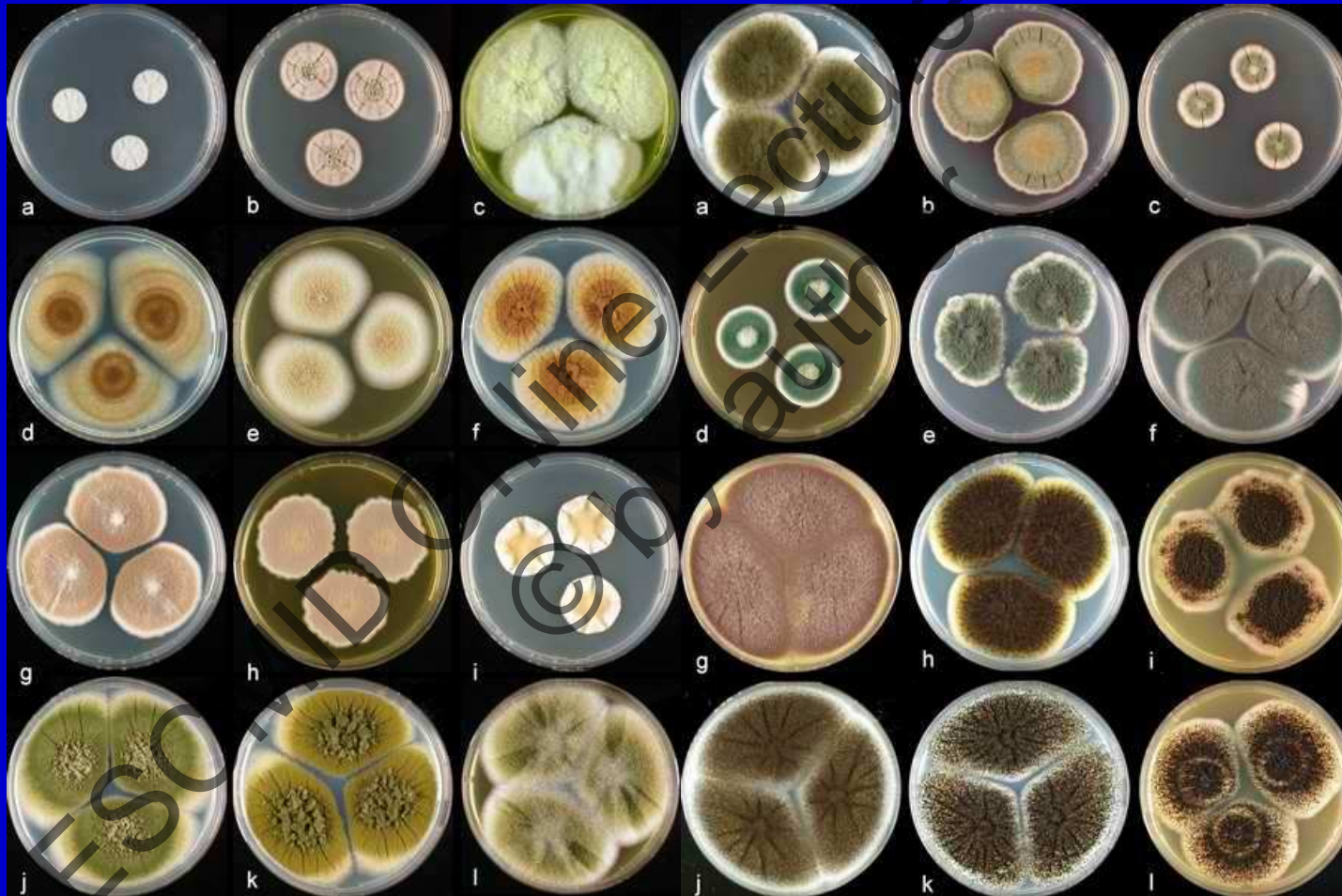
Aspergillus niger

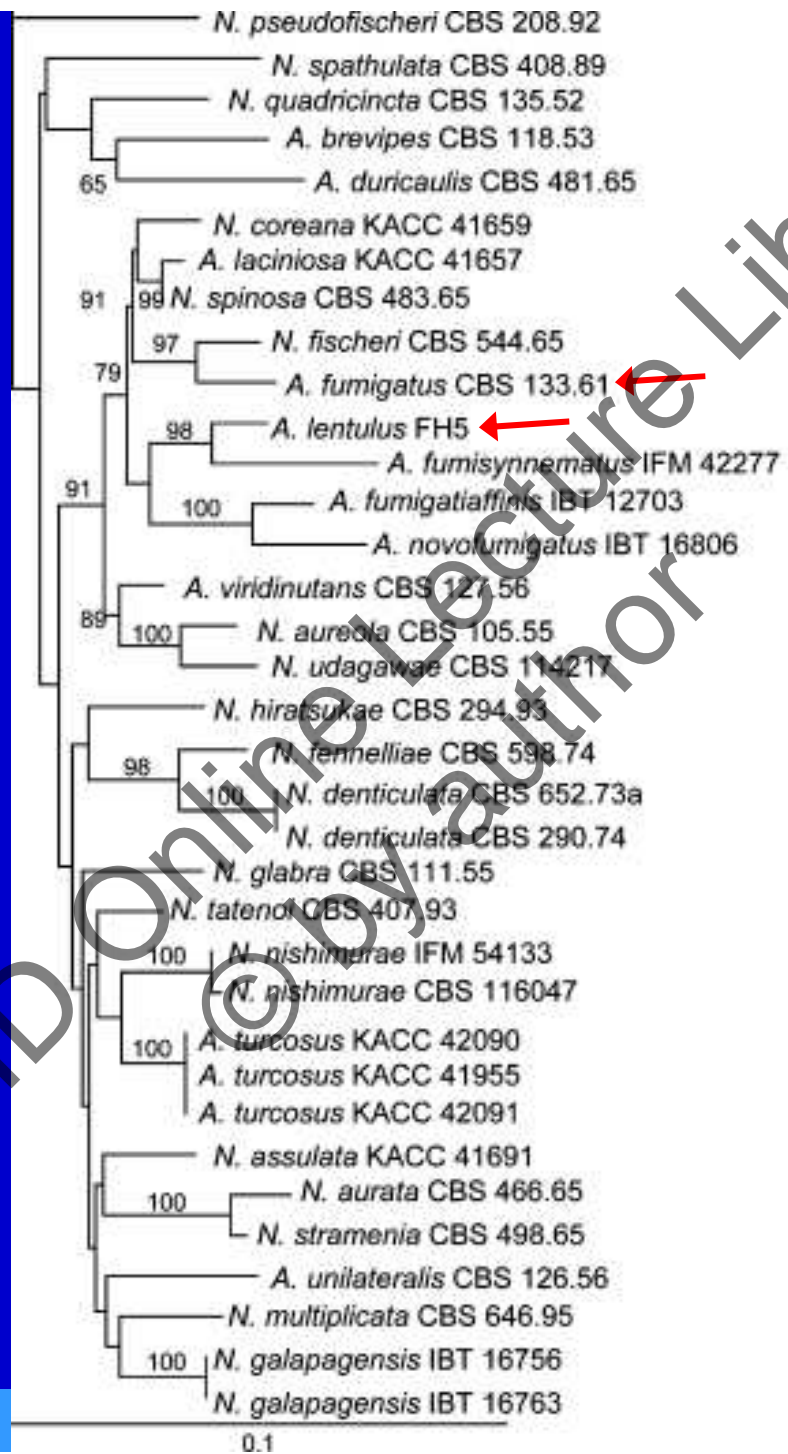


In vitro susceptibility of *Aspergillus*

	FLU	ITR	VOR	POS	RAV	AMB	5FC	CANDINS
<i>fumigatus</i>	-	++	++	++	+	++	+/-	++
<i>terreus</i>	-	++	++	++	+	-	-	++
<i>flavus</i>	-	++	++	++	+	++	-	++
<i>niger</i>	-	++	++	++	+	++	-	++

Other *Aspergilli* species



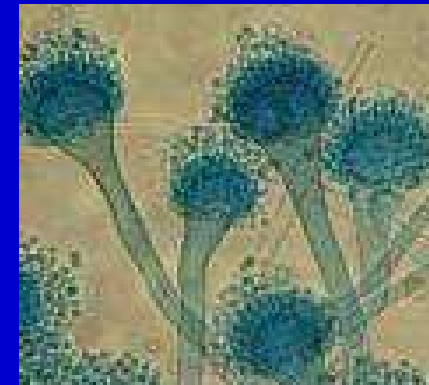


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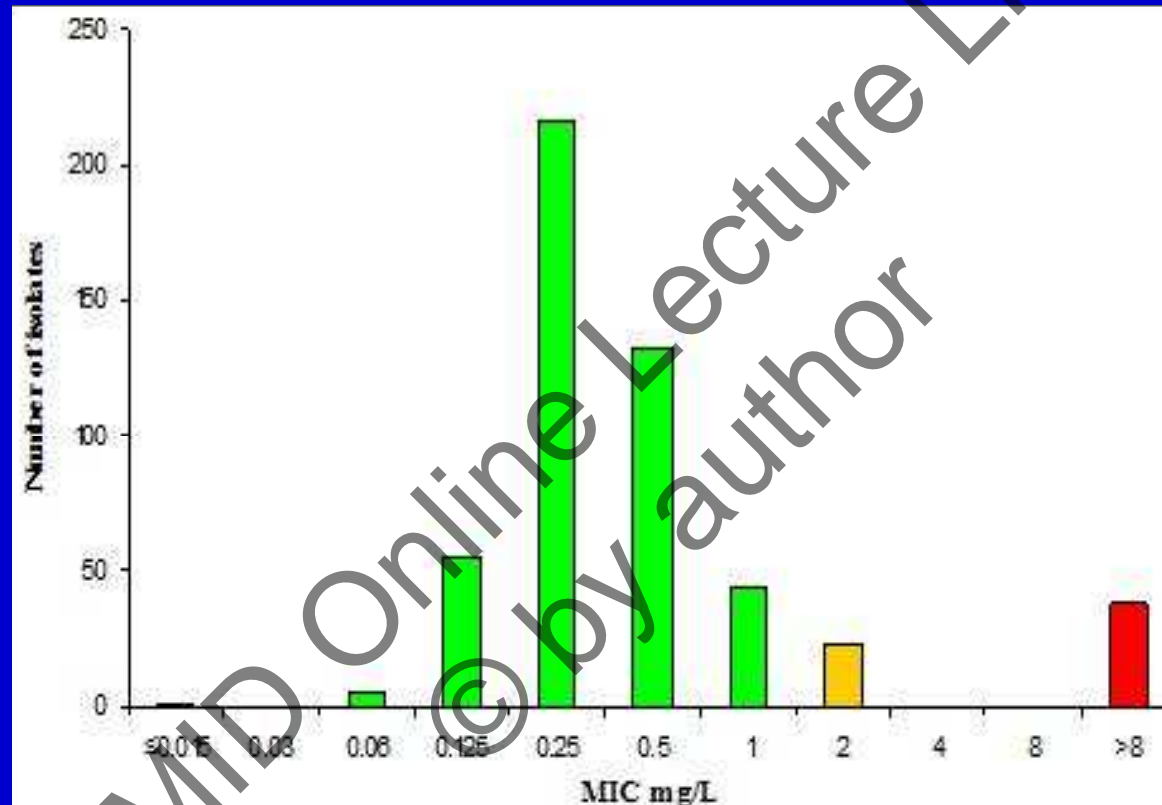
Hong Antonie Van Leeuwenhoek
2008 93;87-98

Acquired resistance

- Predominantly azole resistance in *A. fumigatus* (primary therapy)
- Standardised methodology
- First resistant case late 1980s but most >2000
- Frequency 0 – 12% (2%)



Itraconazole breakpoints



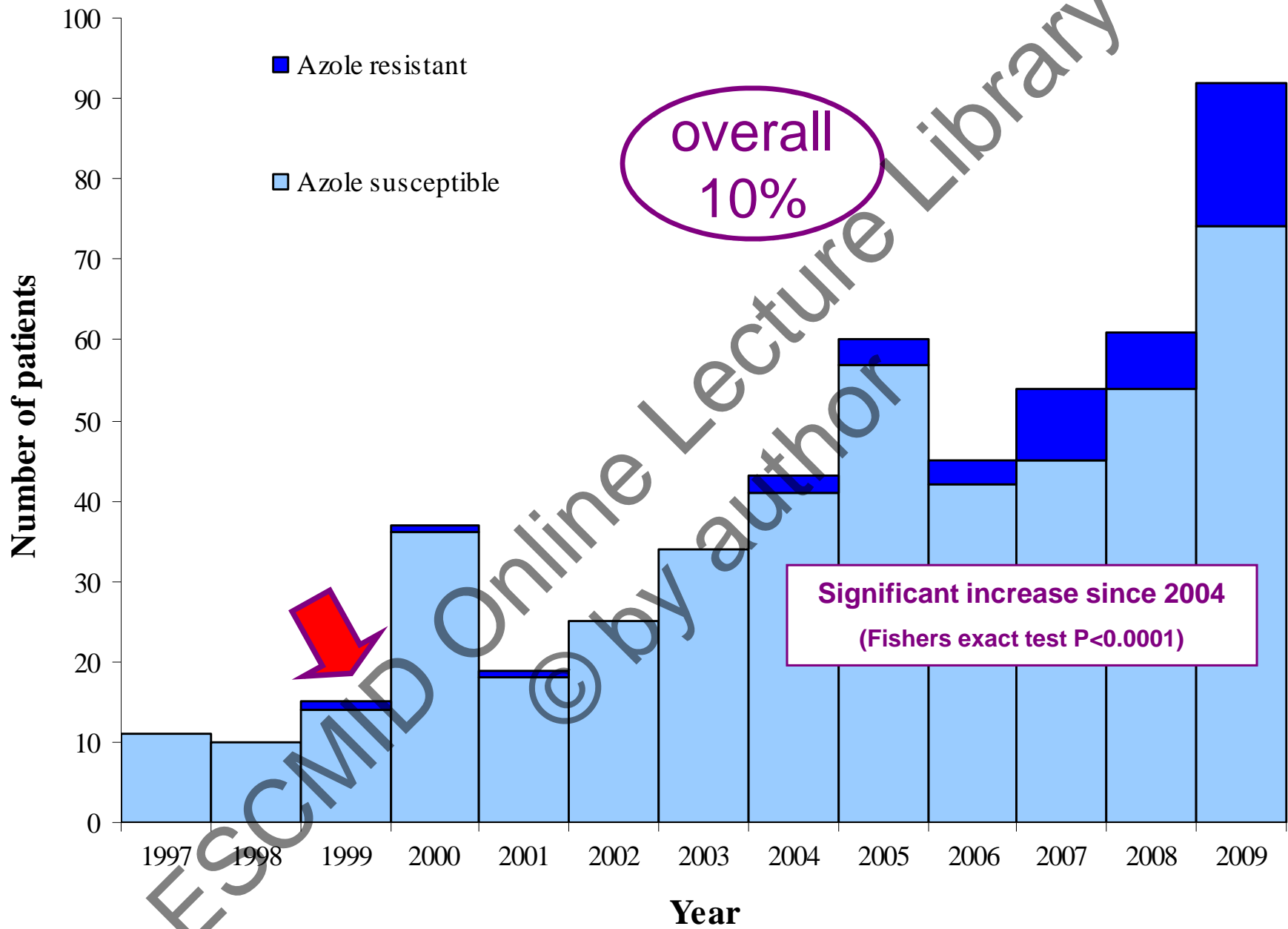
Susceptible
<2

Intermediate
2

Resistant
>2mg/L

Agenda

- How common are resistant infections?
- What are the clinical risk factors?
- How does resistance occur?
- Is cross-resistance a clinical problem?
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Manchester as a centre

→ Specialist service for the management of aspergillosis

2009 National Aspergillosis Centre

www.nationalaspergillosiscentre.org.uk

→ Susceptibility testing is routinely conducted

may explain high frequency of itra resistance

but does not explain the change in frequency

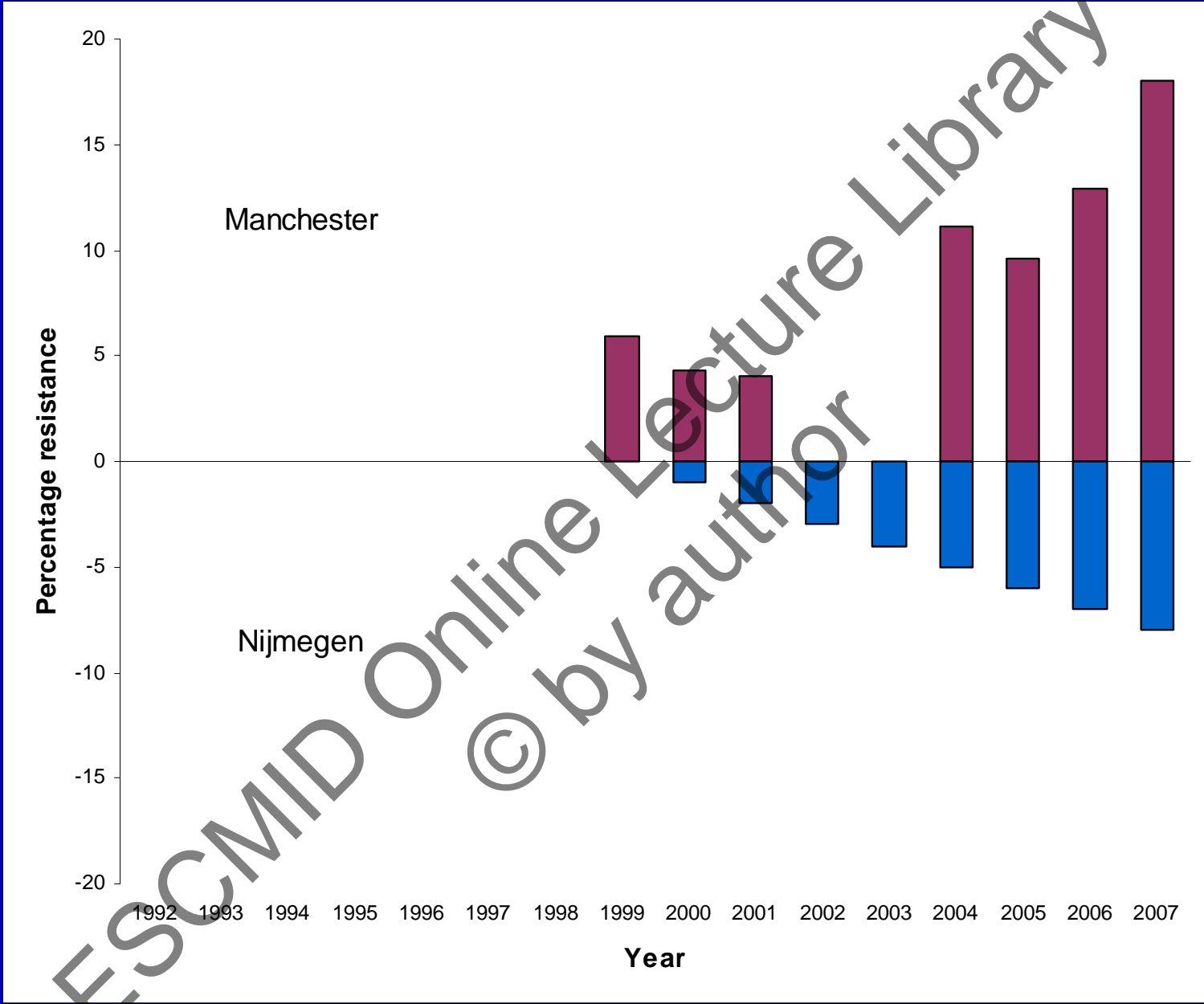
why?

Agenda

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Clinical data

- Clinical data were available for 14 patients
- 2 invasive aspergillosis (IA)
9 chronic pulmonary aspergillosis (CPA)
2 allergic bronchopulmonary aspergillosis (ABPA)
1 *Aspergillus* bronchitis
- Highest frequency in those with aspergillomas
- 13 had prior azole exposure (1 – 30 months)
6 had low drug exposures
- 8 patients failed therapy and 5 failed to improve
(1 not treated)



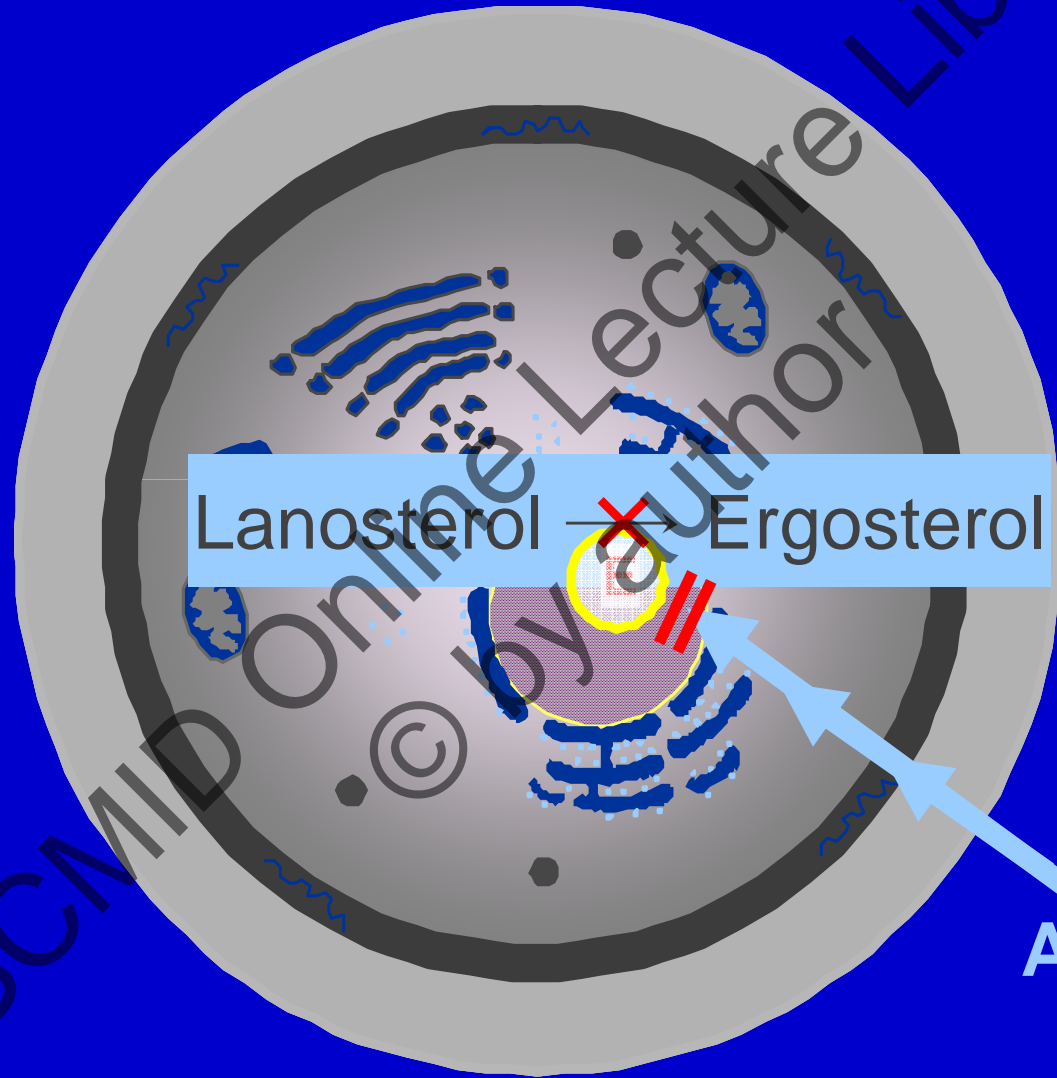
Clinical azole resistance reported



Agenda

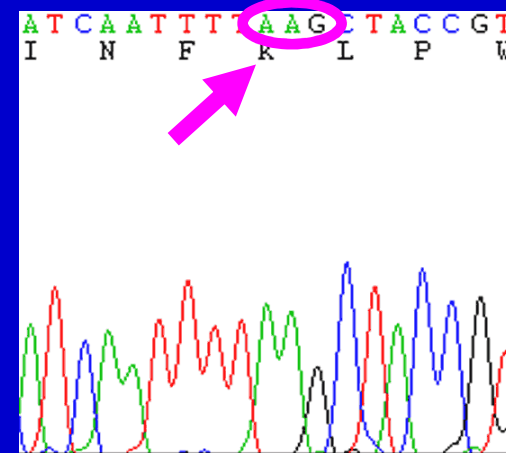
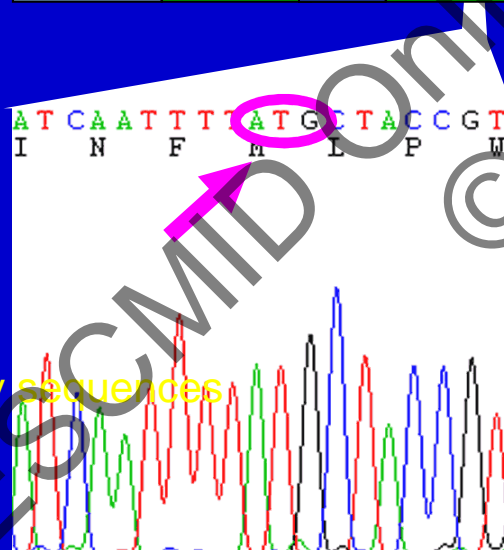
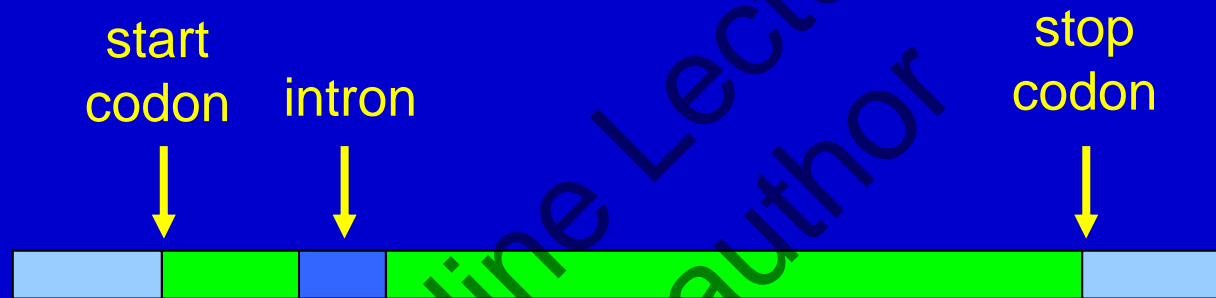
- How common are resistant infections?
- What are the clinical risk factors?
- **How does resistance occur?**
- Is cross-resistance a clinical problem?
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Resistance mechanism



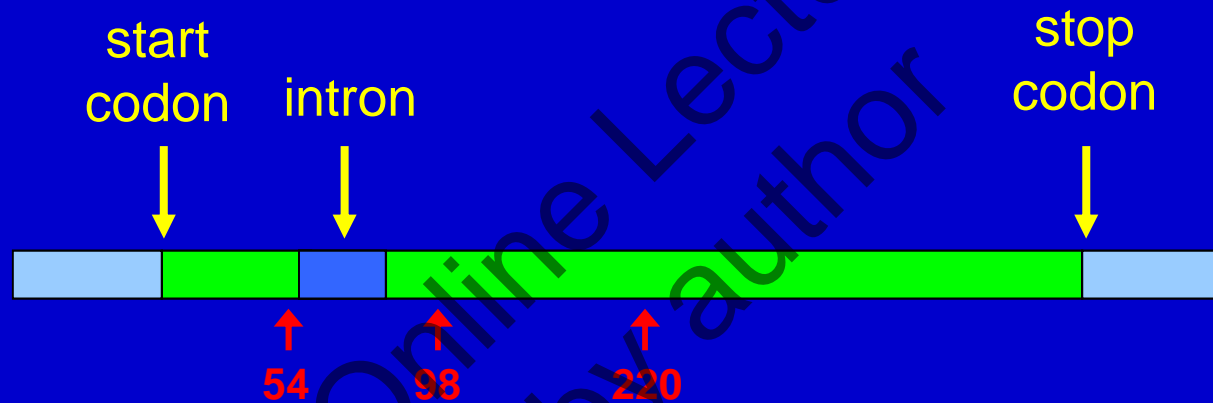
Azole drug

cyp51A mutations



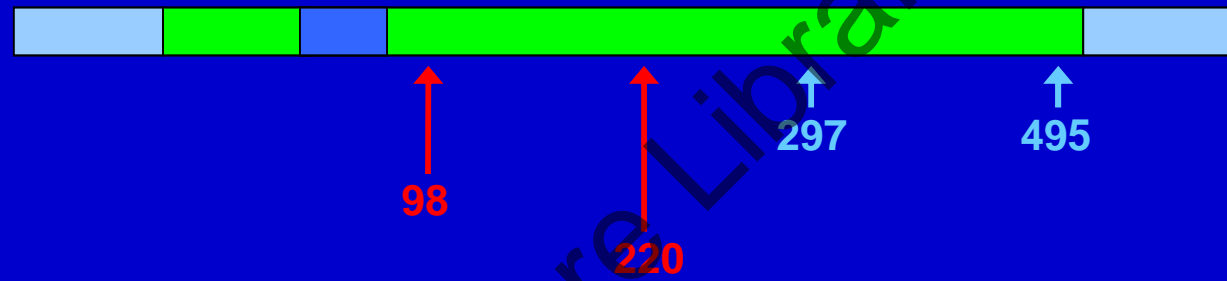
- Regulatory sequences
- Intron
- Exons

cyp51A mutations



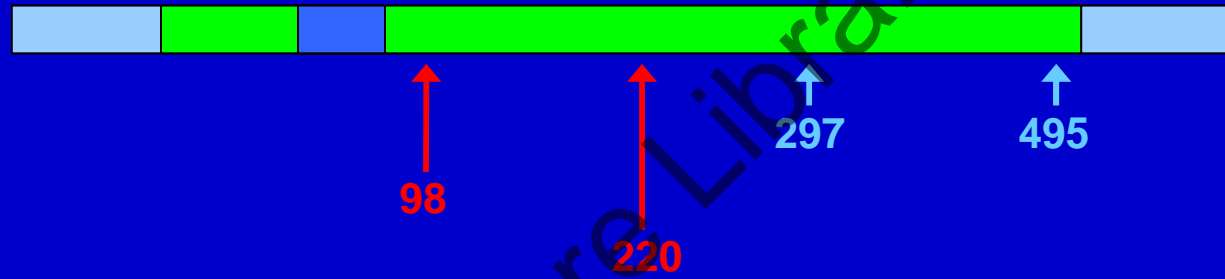
“not-spots”

Holland

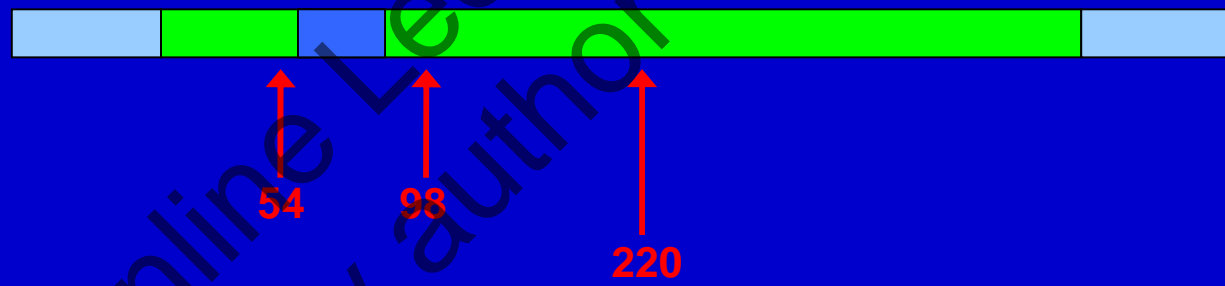


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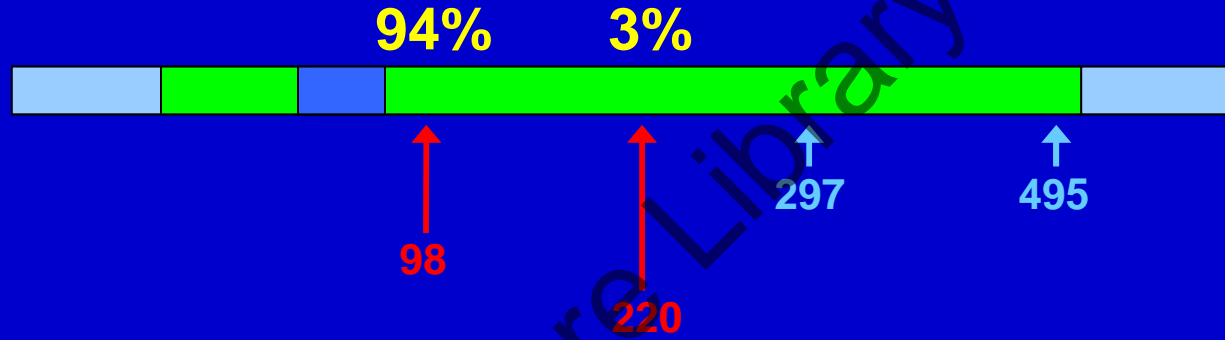
Holland



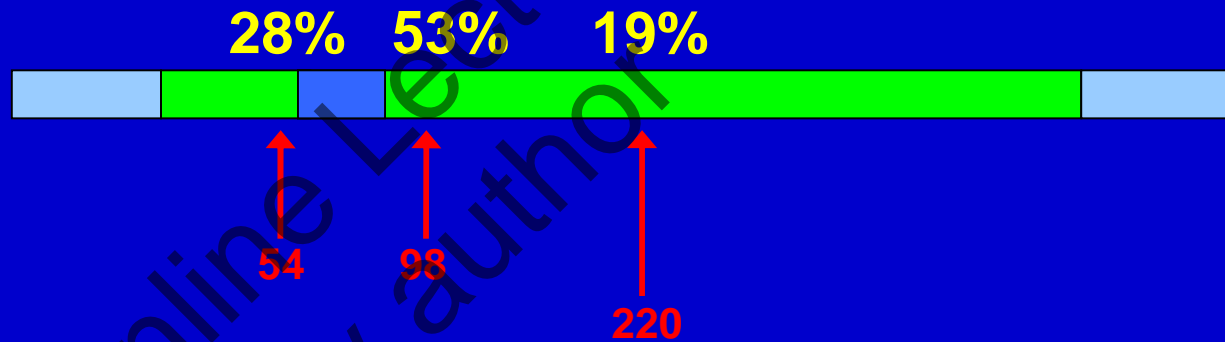
Spain



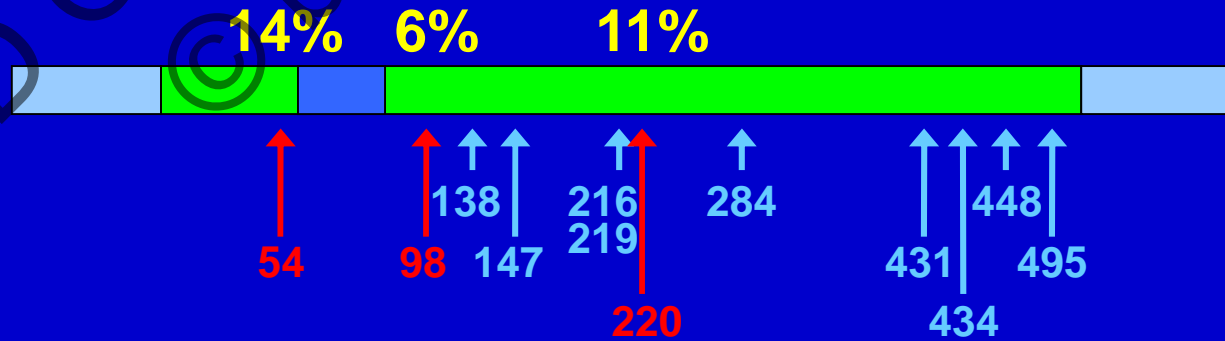
Holland



Spain



Manchester

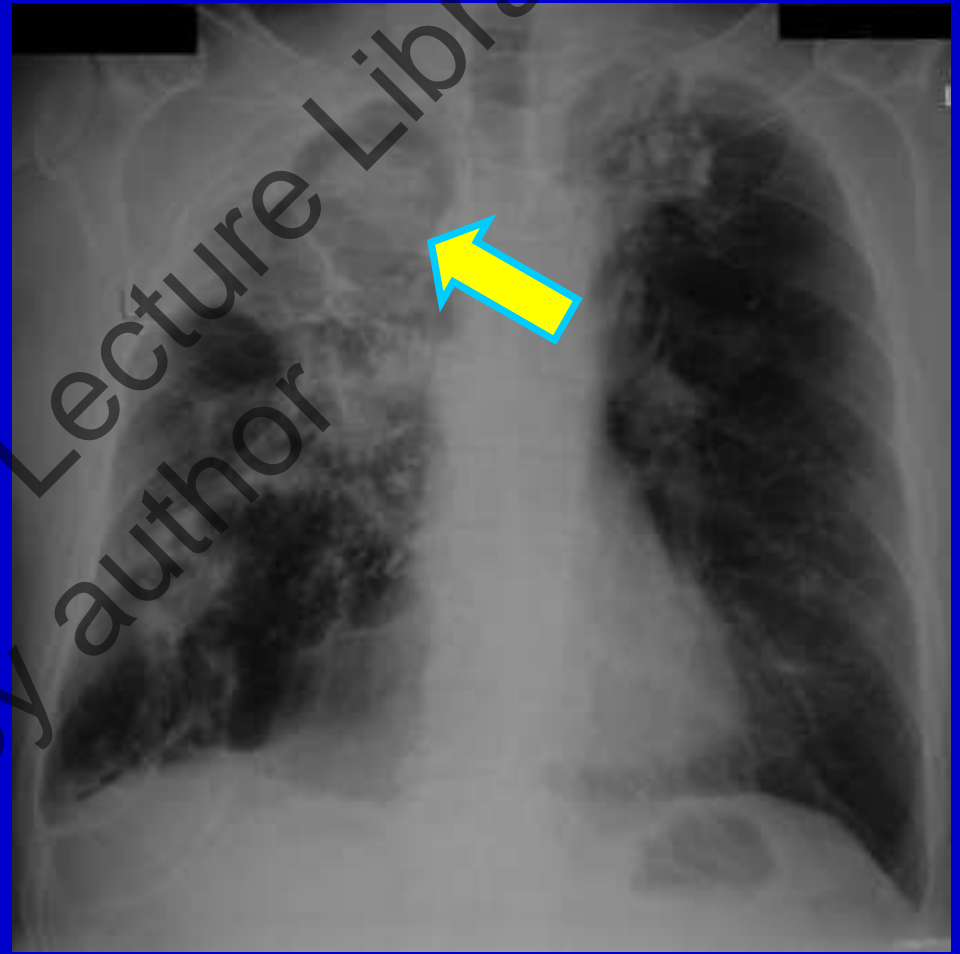


Manchester findings

- Striking variety of *cyp51A* mutations
- Including previously reported mutations (including the hot-spots)
- Some novel (147, 216, 431 & 434) – as yet uncharacterised
- Of 7 patients with multiple resistant isolates, 4 revealed different mutations over time

Patient case

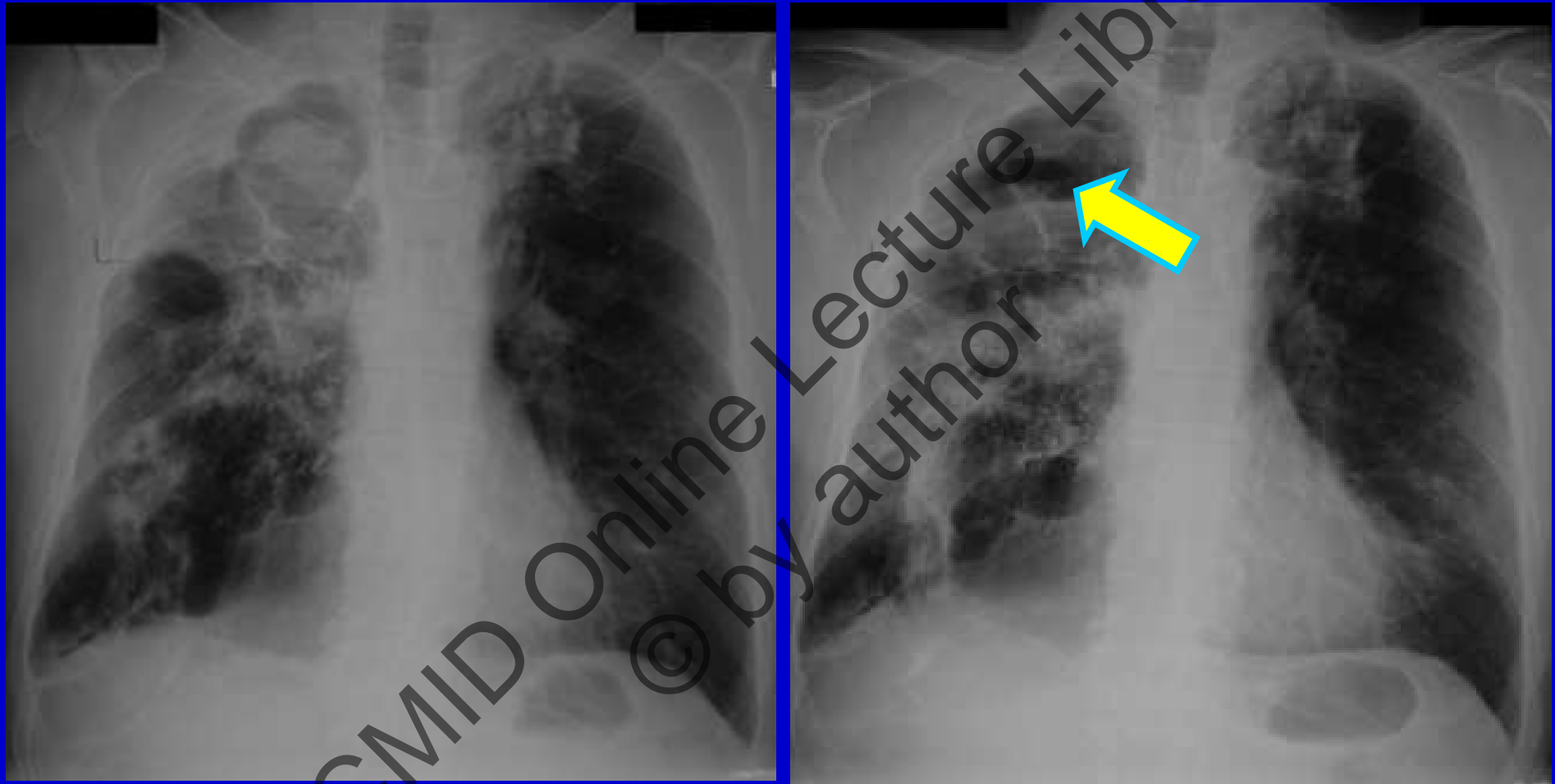
- 64 M
- COPD, bronchiectasis, *Mycobacterium avium* pulmonary infection
- Chronic pulmonary aspergillosis 2003
- Azole susceptible *A. fumigatus*
- Itra therapy
- Low itra drug exposure (rifabutin)
- Ambisome twice for 2wk - some clinical improvement
- 4 mo itra resistant isolate (G54R)
- 4 mo later, another itra res isolate (G54E)
- Increased precipitins titre, radiological progression



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Patient case

- Oct 2004 vori, 500 > 400 mg daily
- Good levels (0.72-1.66mg/L)
- Radiological and serological improvement



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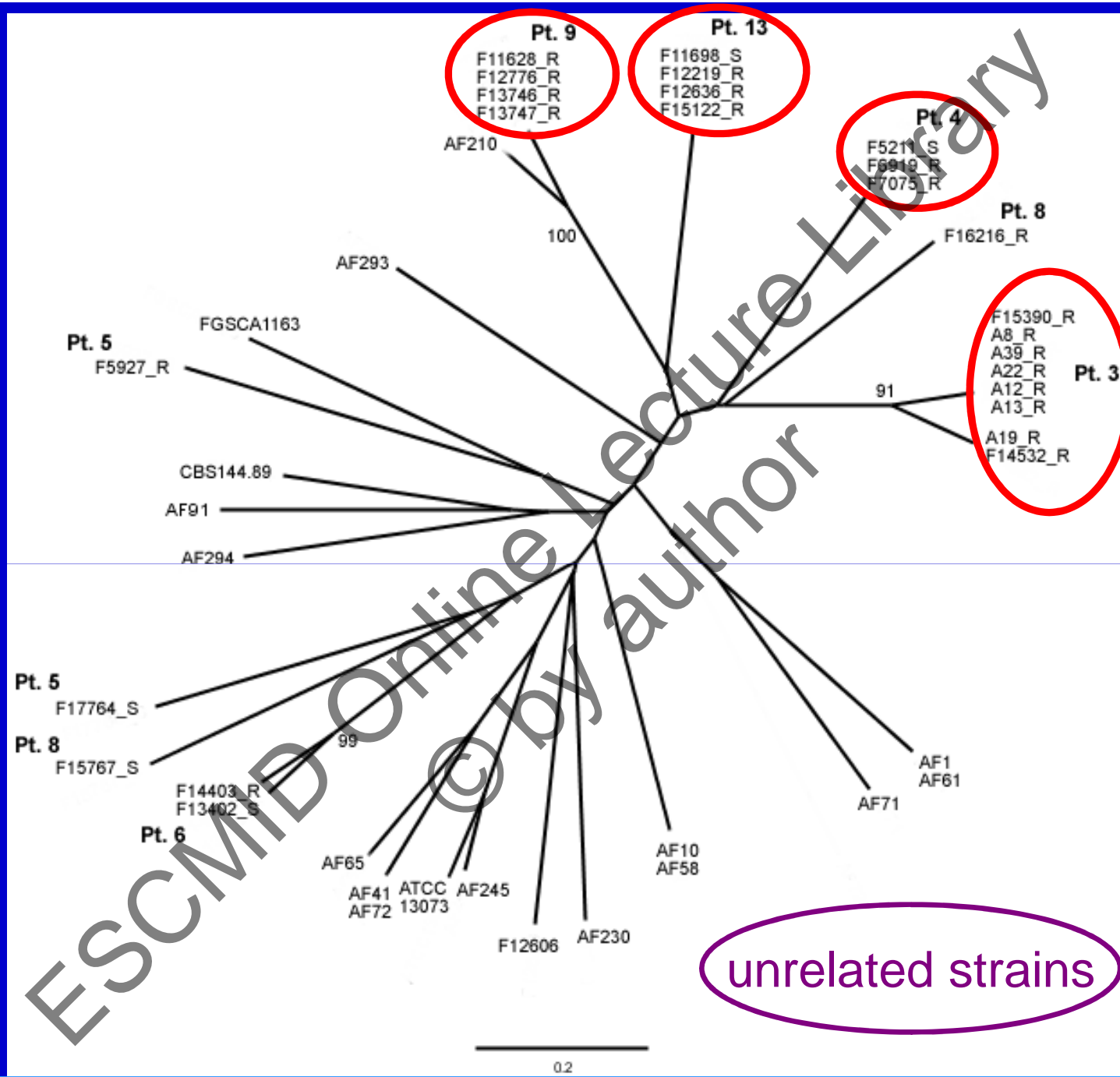
Patient case

- Oct 2004 vori, 500 > 400 mg daily
- Good levels (0.72-1.66mg/L)
- Radiological and serological improvement
- 20 mo isolate vori resistant (G448S), posa MIC 1mg/L

- Sept 2006 posa therapy 800mg daily
- Good levels (1.18-1.9mg/L)
- Slow continued improvement

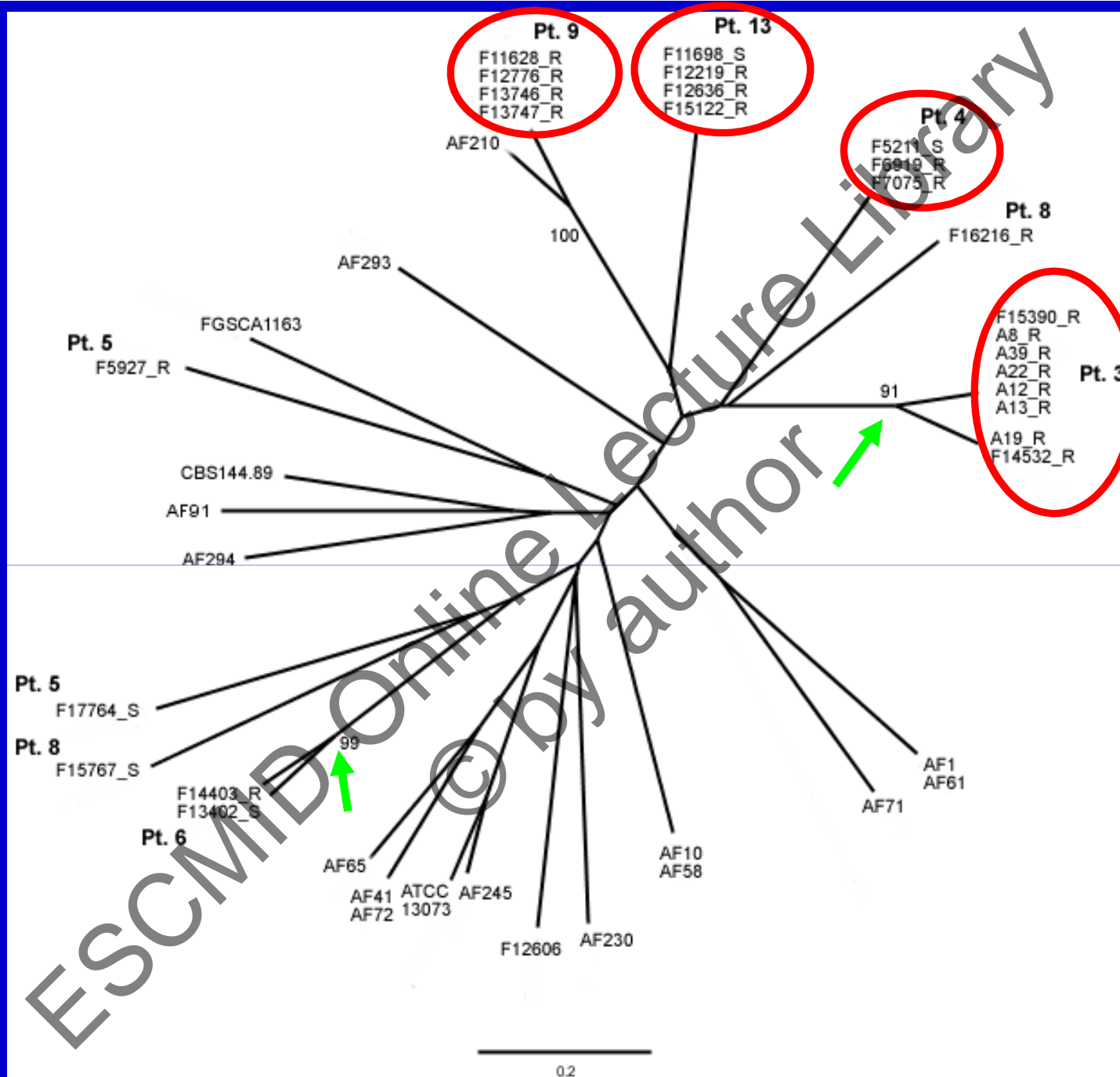
- ?same/different genetic type → microsatellite typing

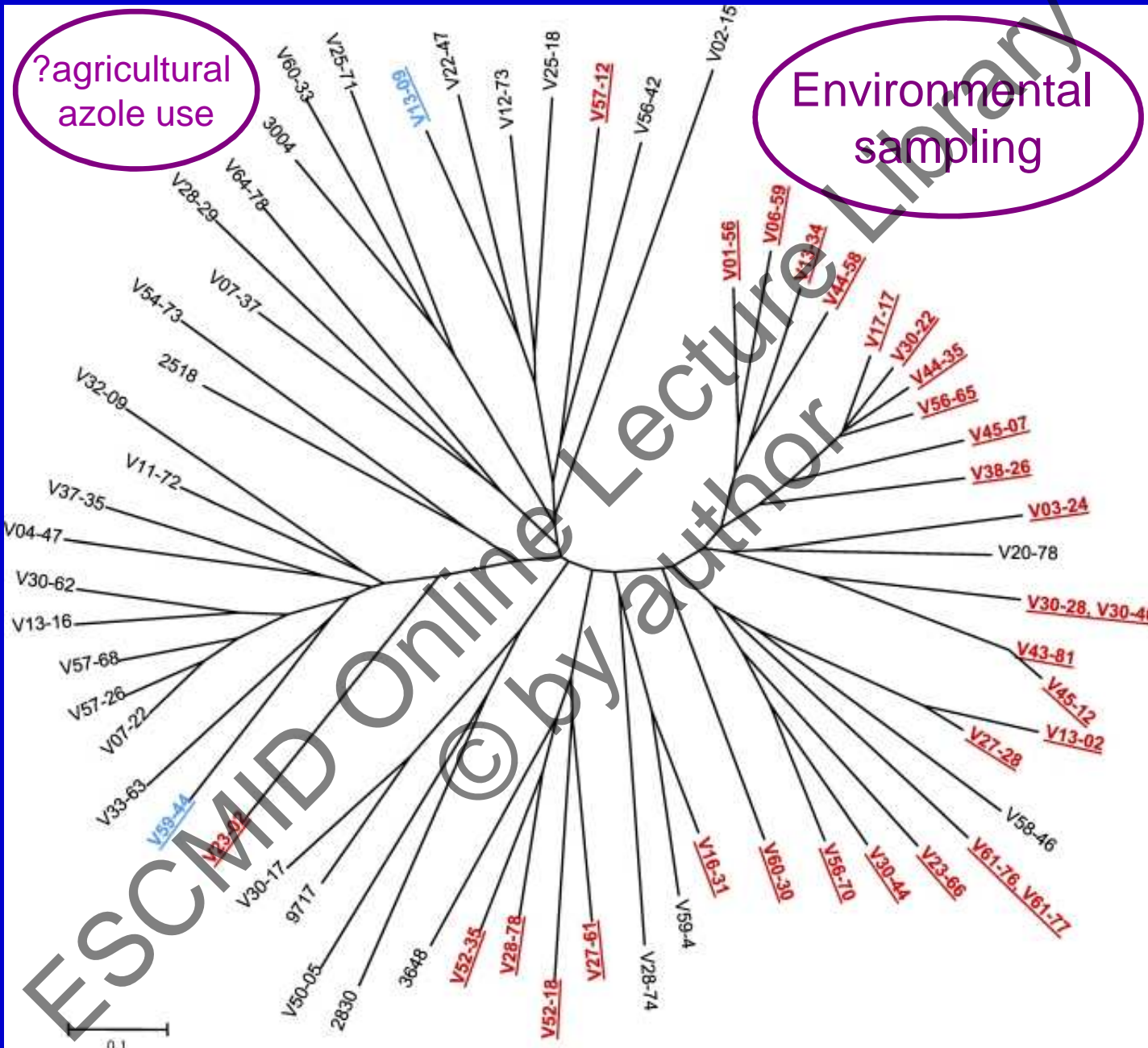
**keep
checking
MICs!**



Evolution in the lung

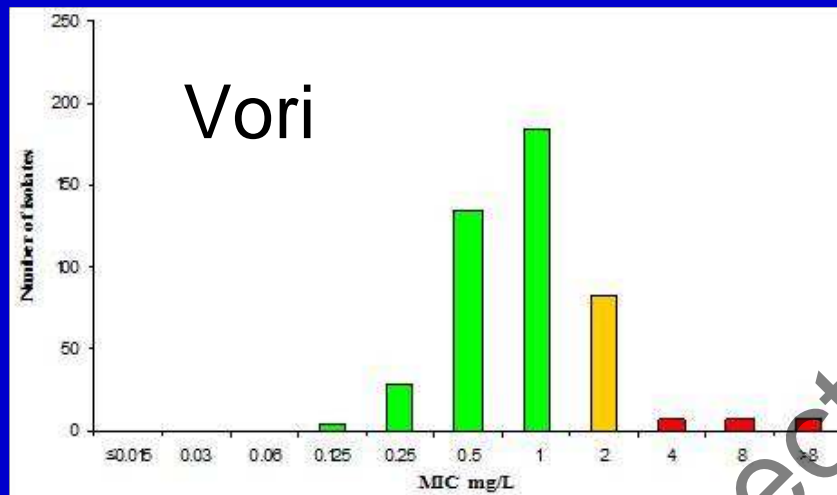
- 3 matched sets
→ emergence of resistance from susceptible isolates in situ
- 4 patients with >1 *cyp51A* mutation found to be the same strain
- Microevolution of microsatellite loci



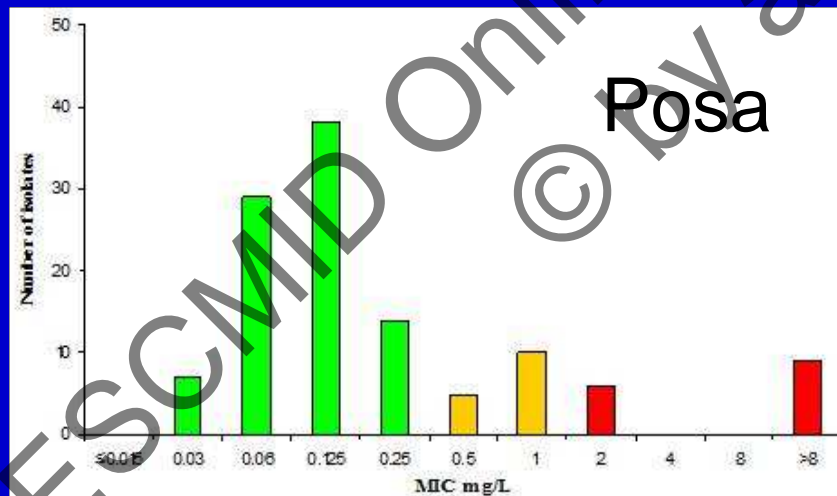


Agenda

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- How does resistance occur?
- **Is cross-resistance a clinical problem?**
- How can we detect resistance?

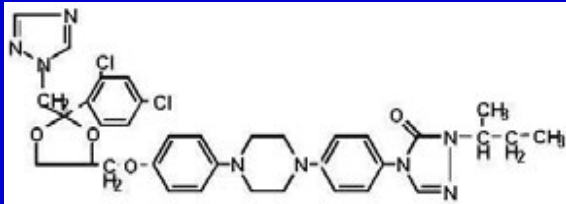


Resistant
>2mg/L

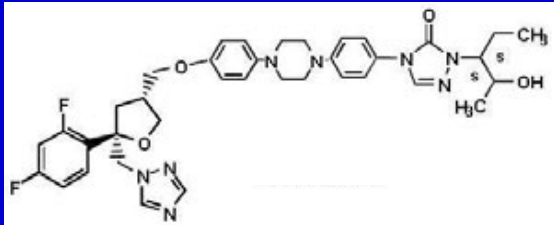


Resistant
>0.5/1mg/L

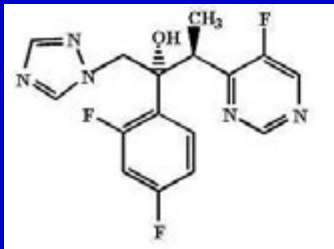
Azole cross-resistance



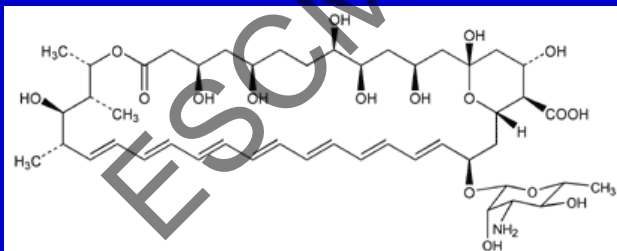
Itra resistance = almost all



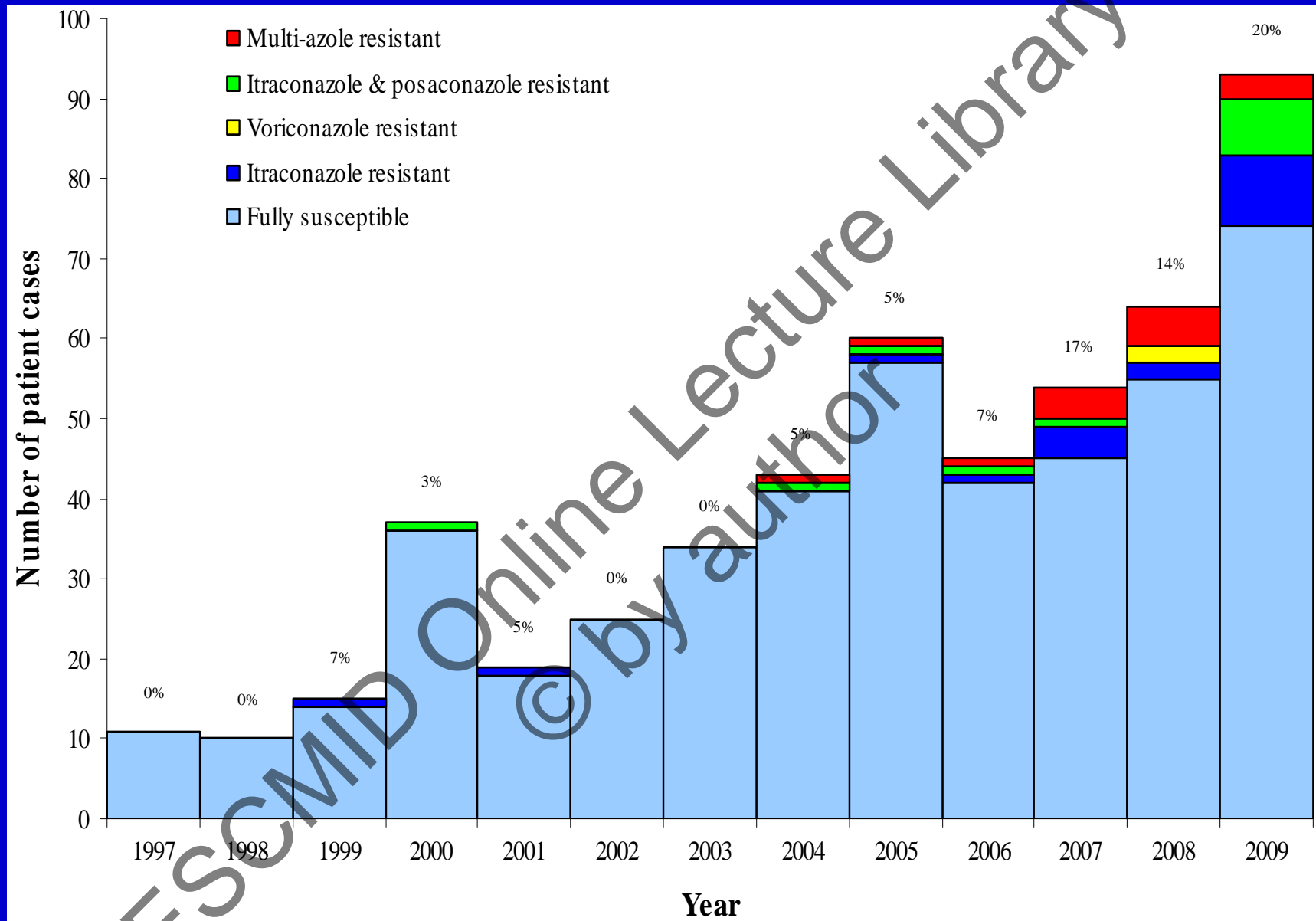
Posa resistance = 74%



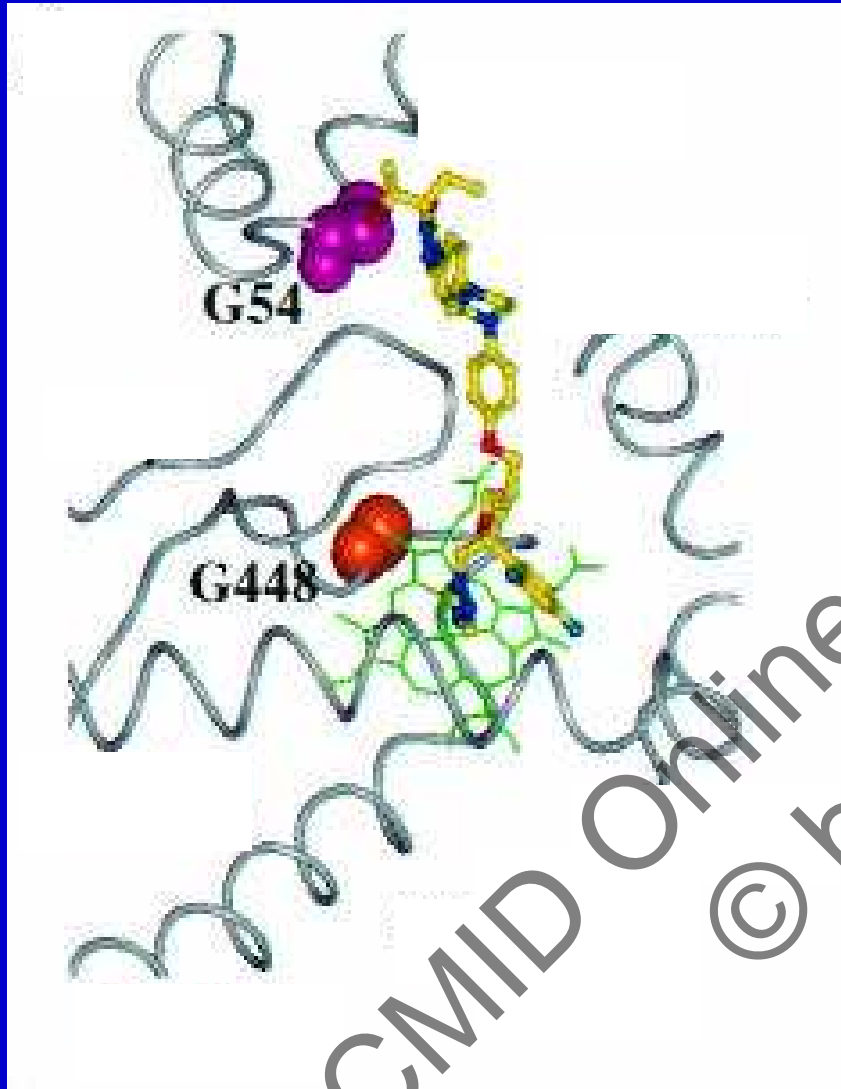
Vori resistance = 65%



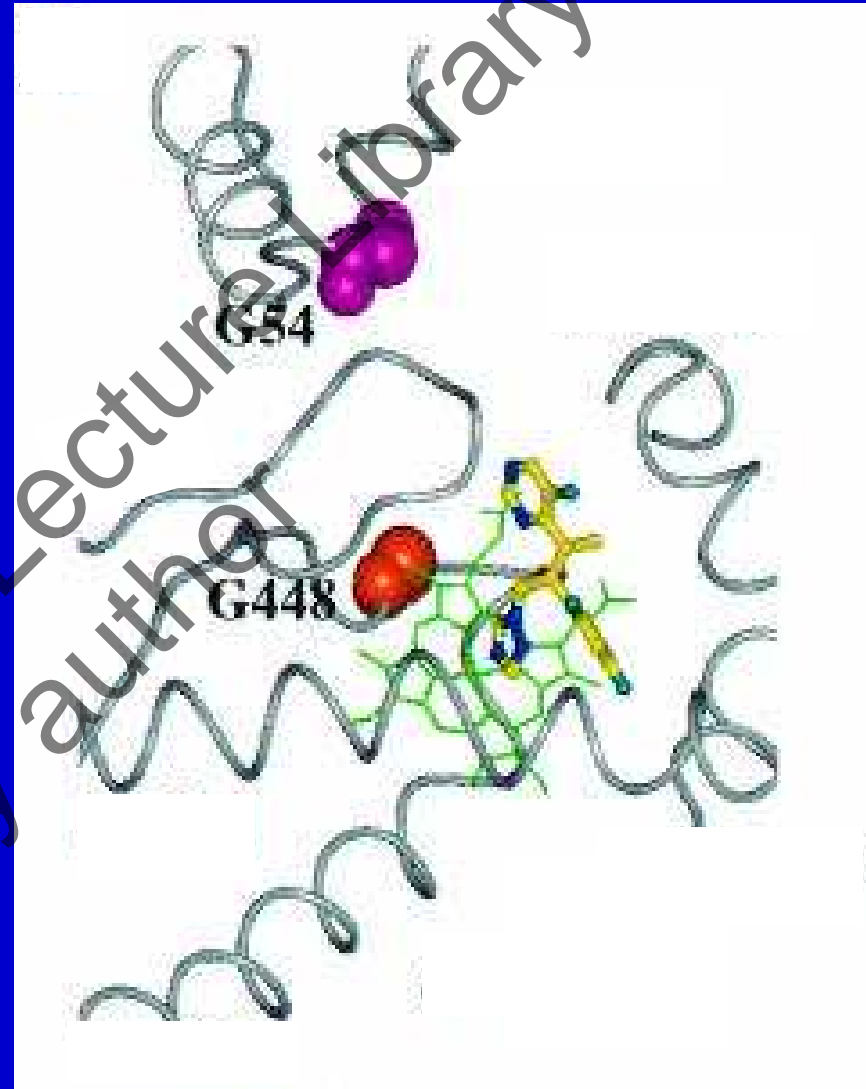
Amb resistance = 0%



<i>cyp51A</i> alteration (n=)	Susceptibility (mg/L)		
	Itraconazole	Voriconazole	Posaconazole
G54 (5)	>8	0.125-1	1->8
L98+TR^a (2)	>8	8	1-2
G138 (10)	>8	8->8	2->8
P216 (1)	>8	1	1
M220 (4)	>8	1-4	0.5->8
Y431 (1)	>8	4	1
G434 (1)	>8	4	1
G448^b (2)	>8	>8	0.5-1



itra/posa



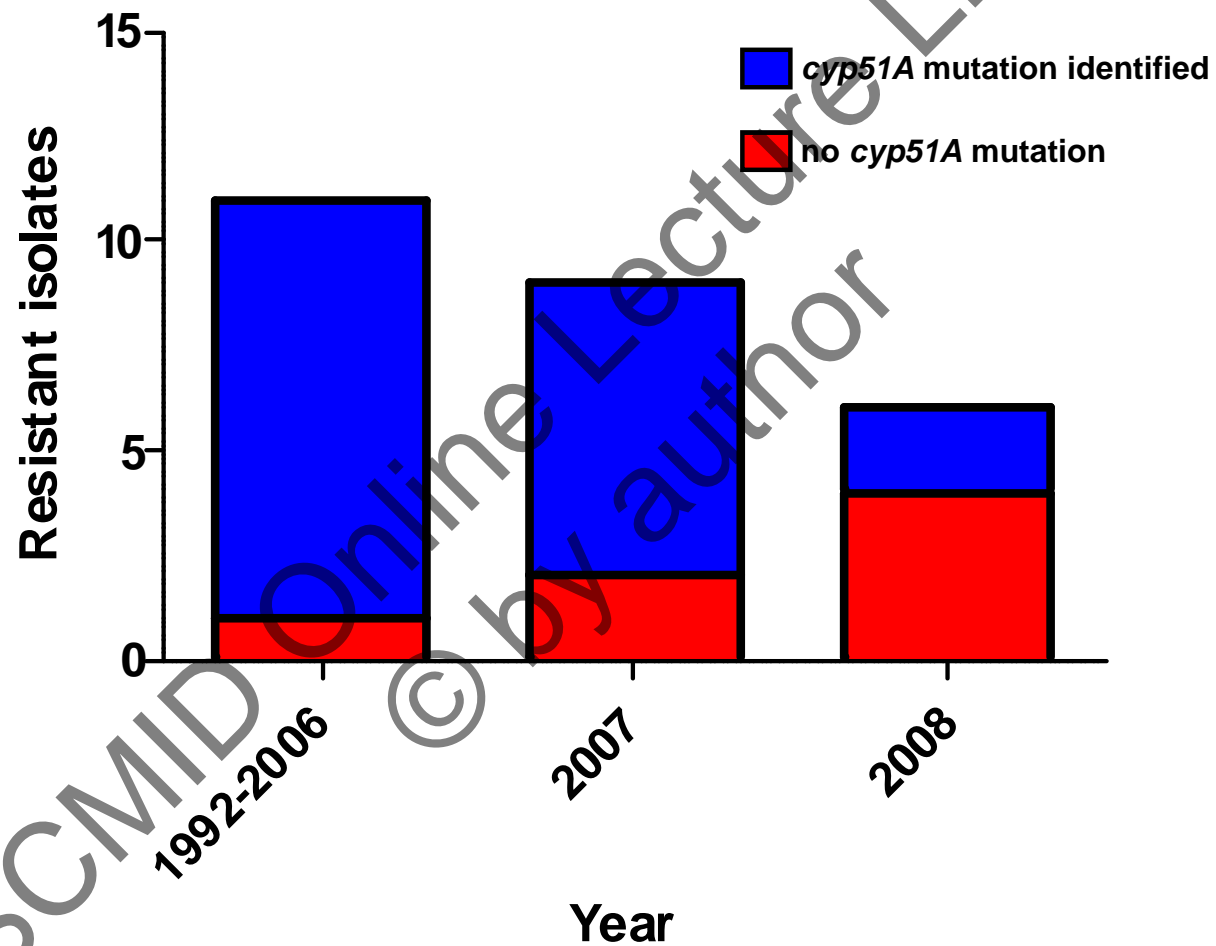
vori

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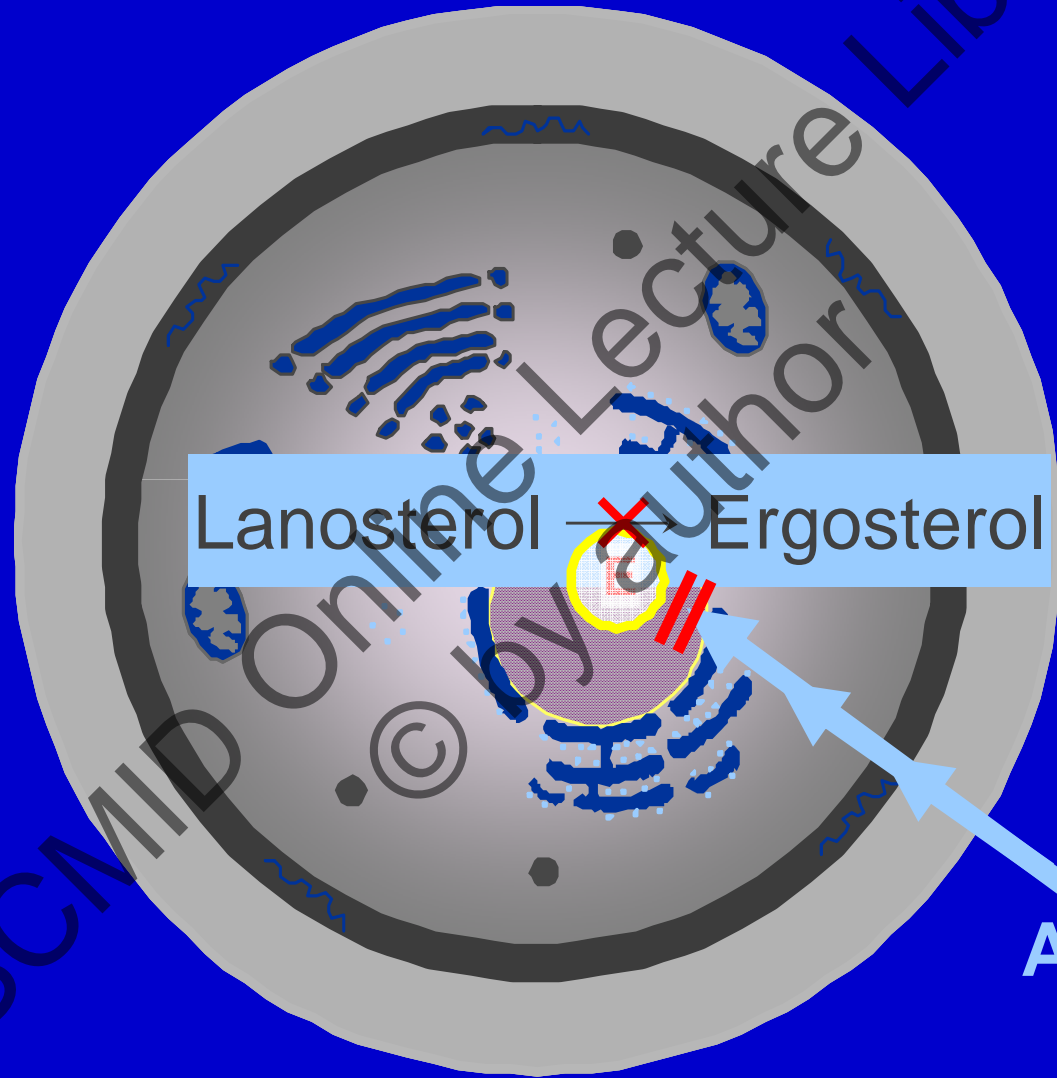
Evidence for multiple mechanisms of resistance

- Isolates with unpredictable cross-resistance pattern
- Isolates with *cyp51A* mutations that don't confer resistance
- Resistant isolates with no *cyp51A* mutation

cyp51A genotype in azole resistant isolates

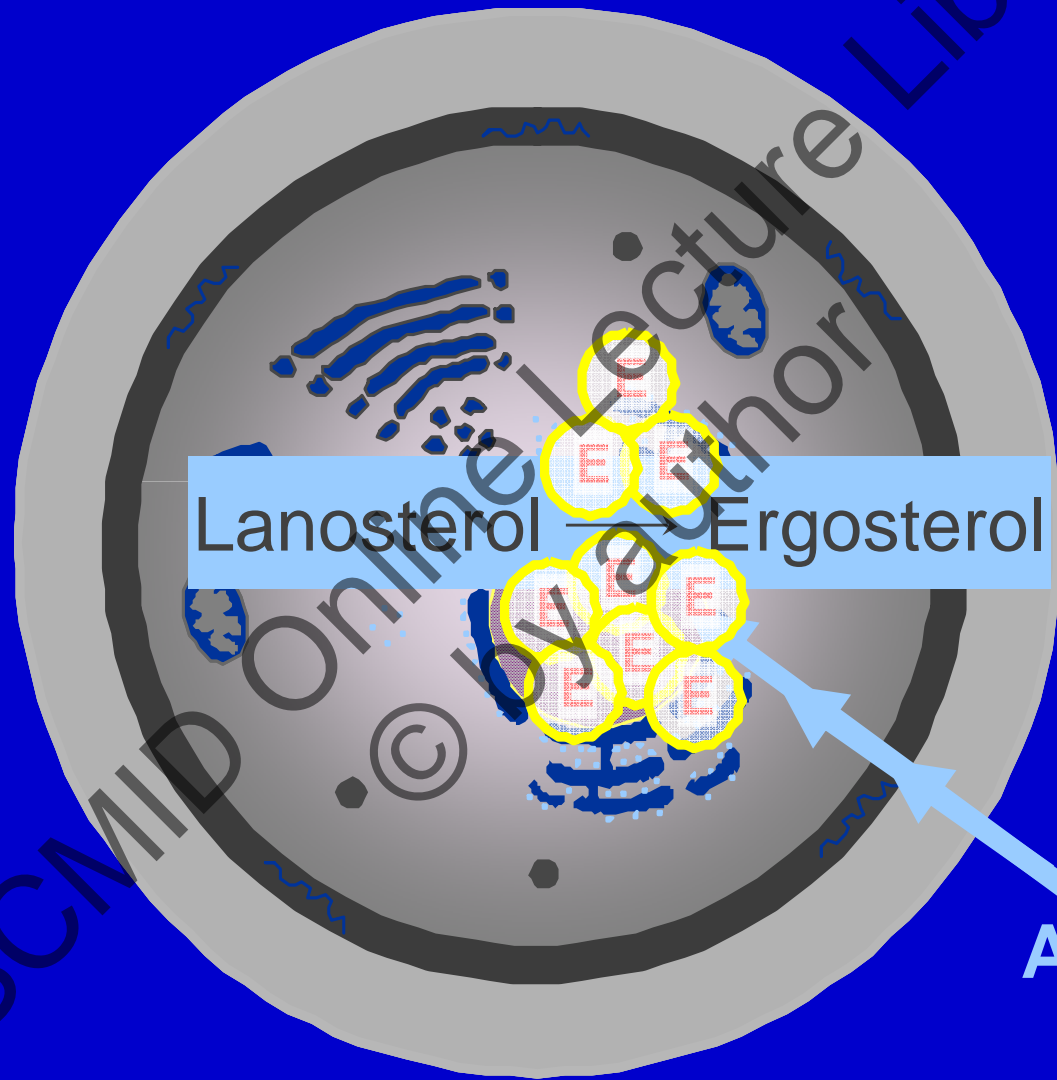


Resistance mechanism



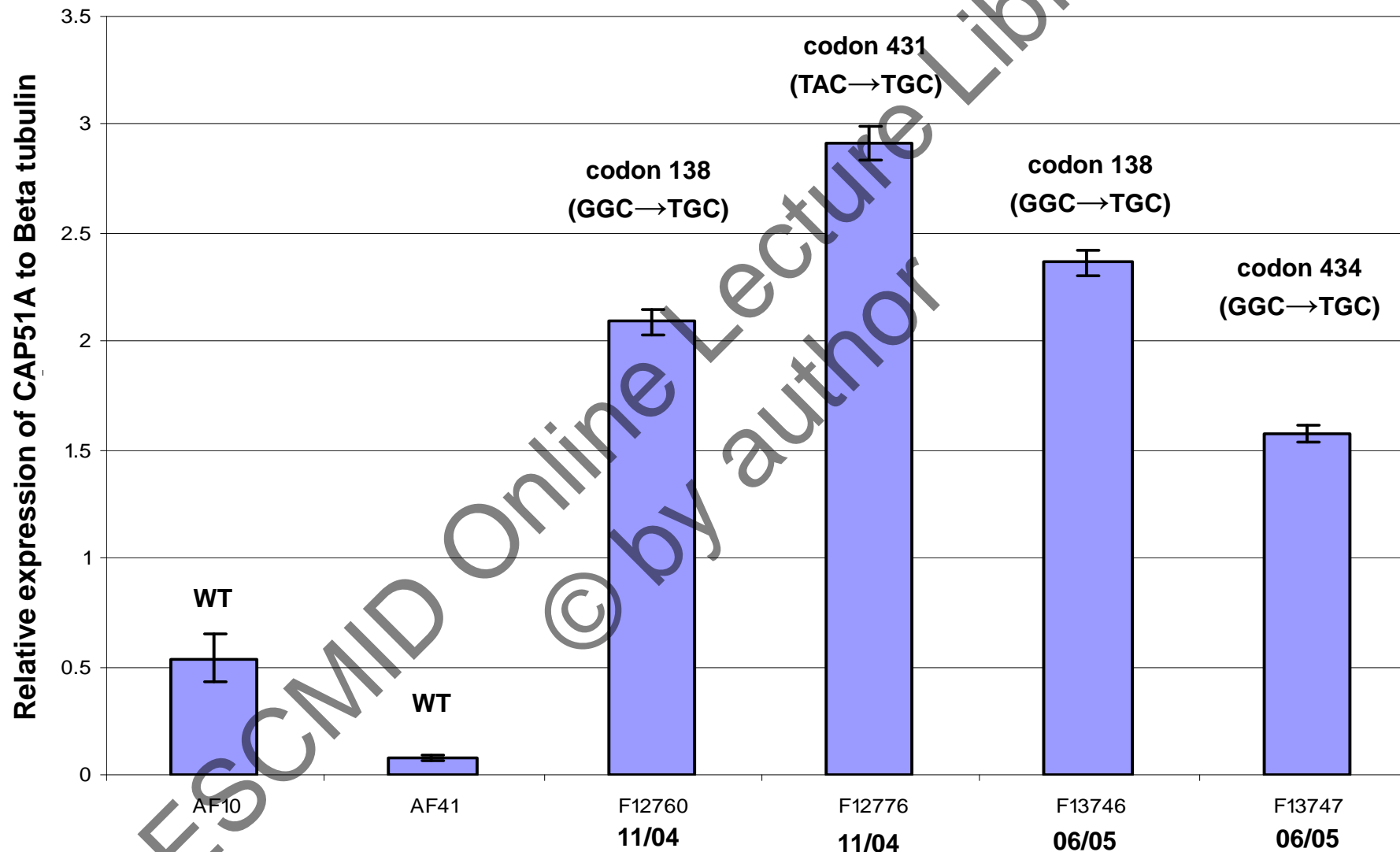
Azole drug

Resistance mechanism

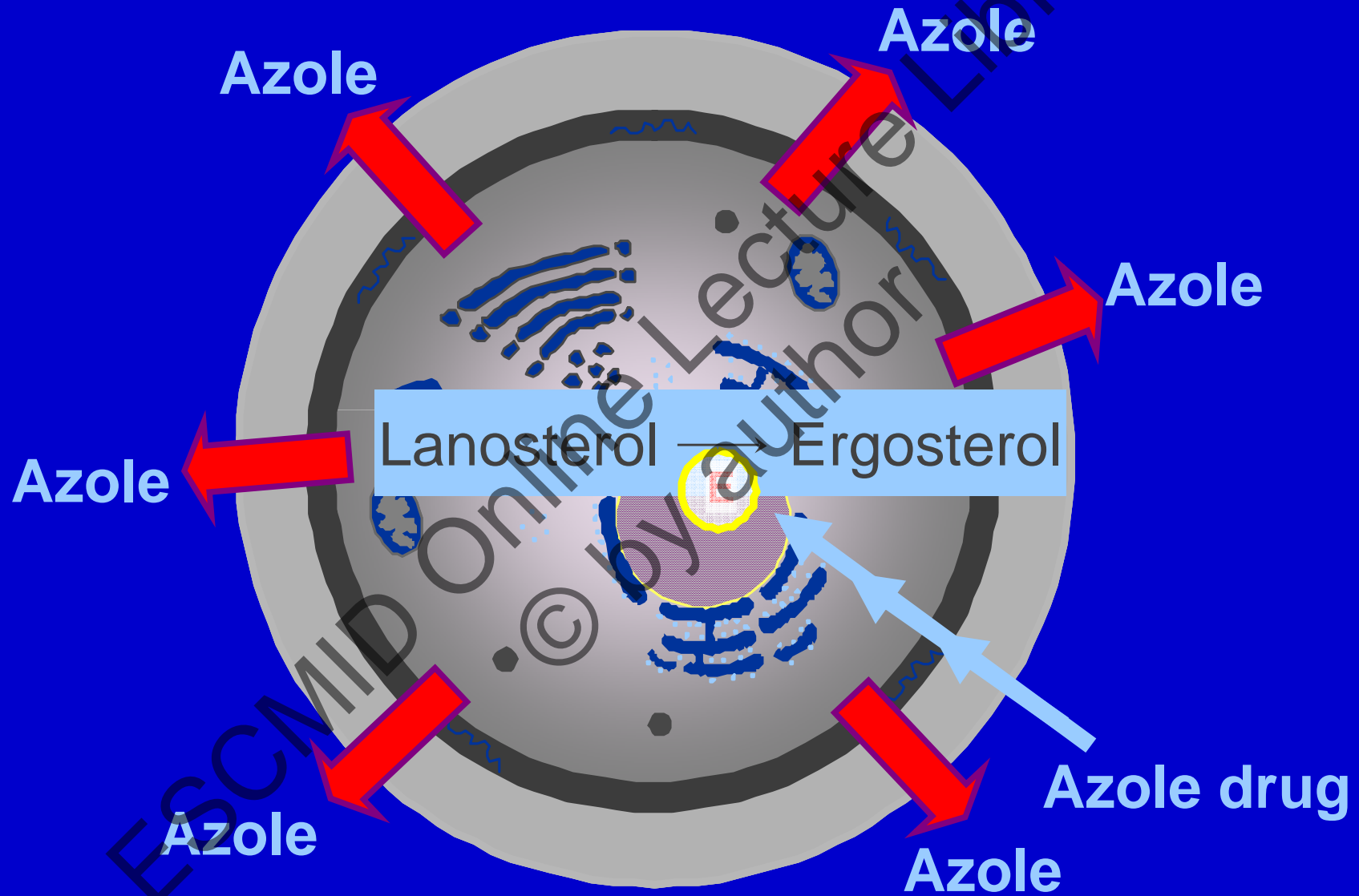


Azole drug

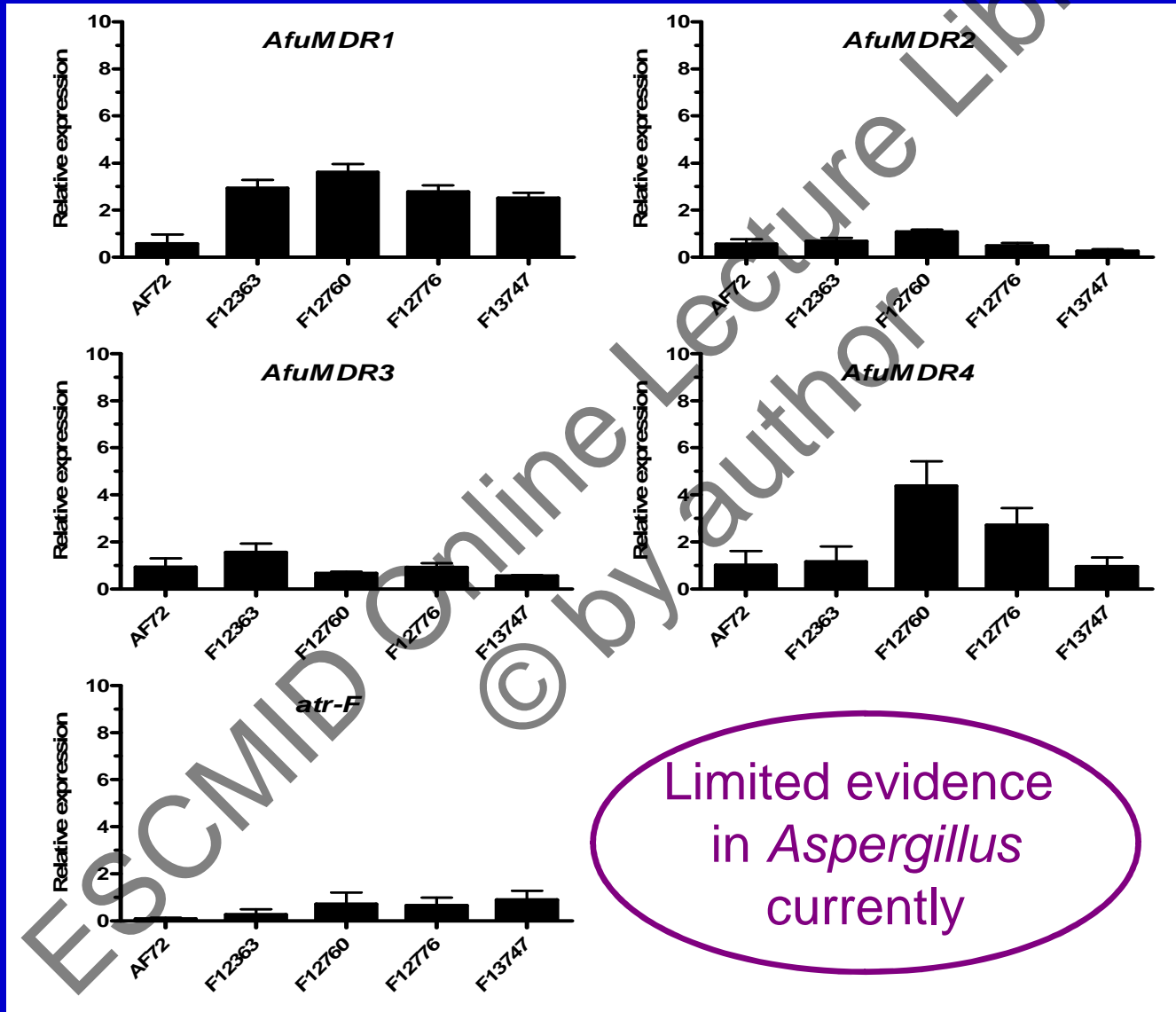
CYP51A overexpression with target mutations



Resistance mechanism



expression of efflux transporters



Other as yet
un-identified
mechanisms??

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Detection options

- MICs slow
- Cultures frequently falsely negative in all forms of aspergillosis
- Direct *cyp51A* mutation detected by real-time PCR
→ look for the most common mutations;
G54, L98, G138, M220, TR
- 55.1% mutations in culture –ve samples
- Pro's and con's
(speed vs other mutations/no mutations/cost)

Conclusions

- Resistance is of significant clinical import
- Evidence of both environmental acquisition and emergence of resistance in the lung
- Currently low frequency but increasing
- Risk of cross-resistance is high
- Routine susceptibility testing now required

Thank you

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