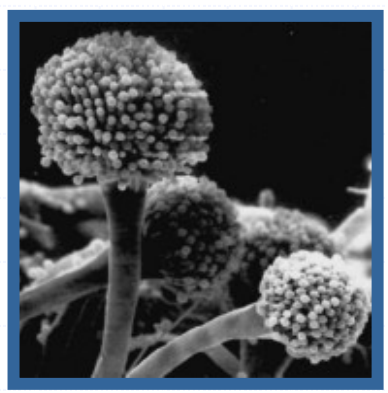
4th Congress on Trends in Medical Mycology (TIMM)

In vitro cross-resistance between azoles in *Aspergillus fumigatus*: a reason for concern in the clinic?

Emilia Mellado
Mycolgy Reference Laboratory
Centro Nacional Microbiologia
Instituto de Salud Carlos III (ISCIII)
Majadahonda, Madrid, Spain



INVASIVE ASPERGILLOSIS (IA)

- Immunocompromised patient
- Not easy to diagnose and/or treat
- High incidence (50 %) and Higher mortality rate (%)
- Treatment: amphotericin B, azoles and Echinocandins
- Triazoles seem to be key drugs in IA: Voriconazole

INVASIVE ASPERGILLOSIS (IA)

- Immunocompromised patient
- Not easy to diagnose and/or treat
- High mortality rate (38-80 %)
- Treatment: amphotericin B, azoles and Echinocandins
- Triazoles seem to be key drugs in IA: voriconazole

1.- The fungistatic nature of azole drugs have risen a considerable concern in relation to secondary resistance development.

2.- *Aspergillus fumigatus* azole resistance was first detected in 1997

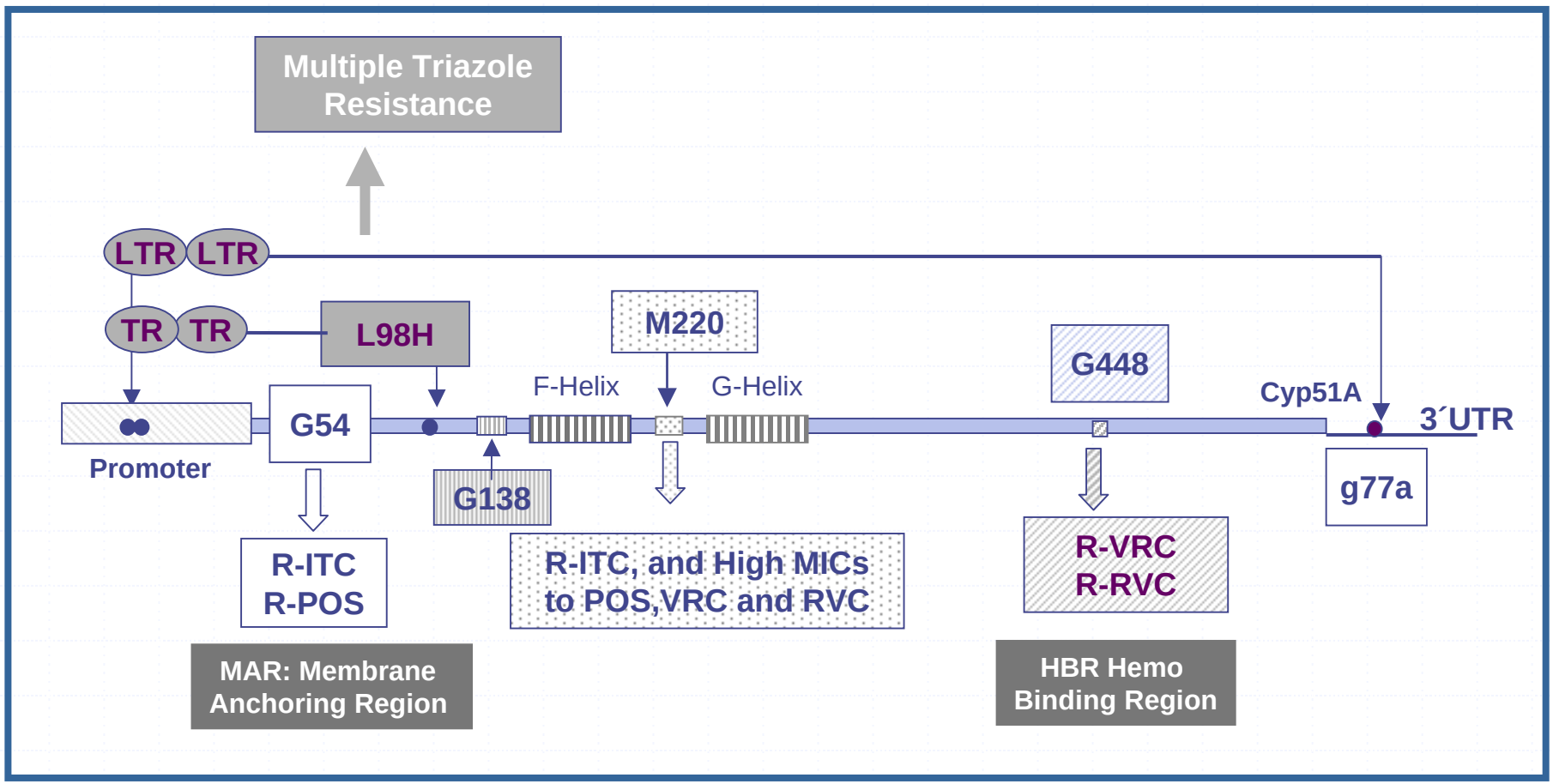
3.- The underlying molecular mechanisms of resistance have been thoroughly studied and characterized.

4.- *In vitro* cross-resistance between azole drugs do exist

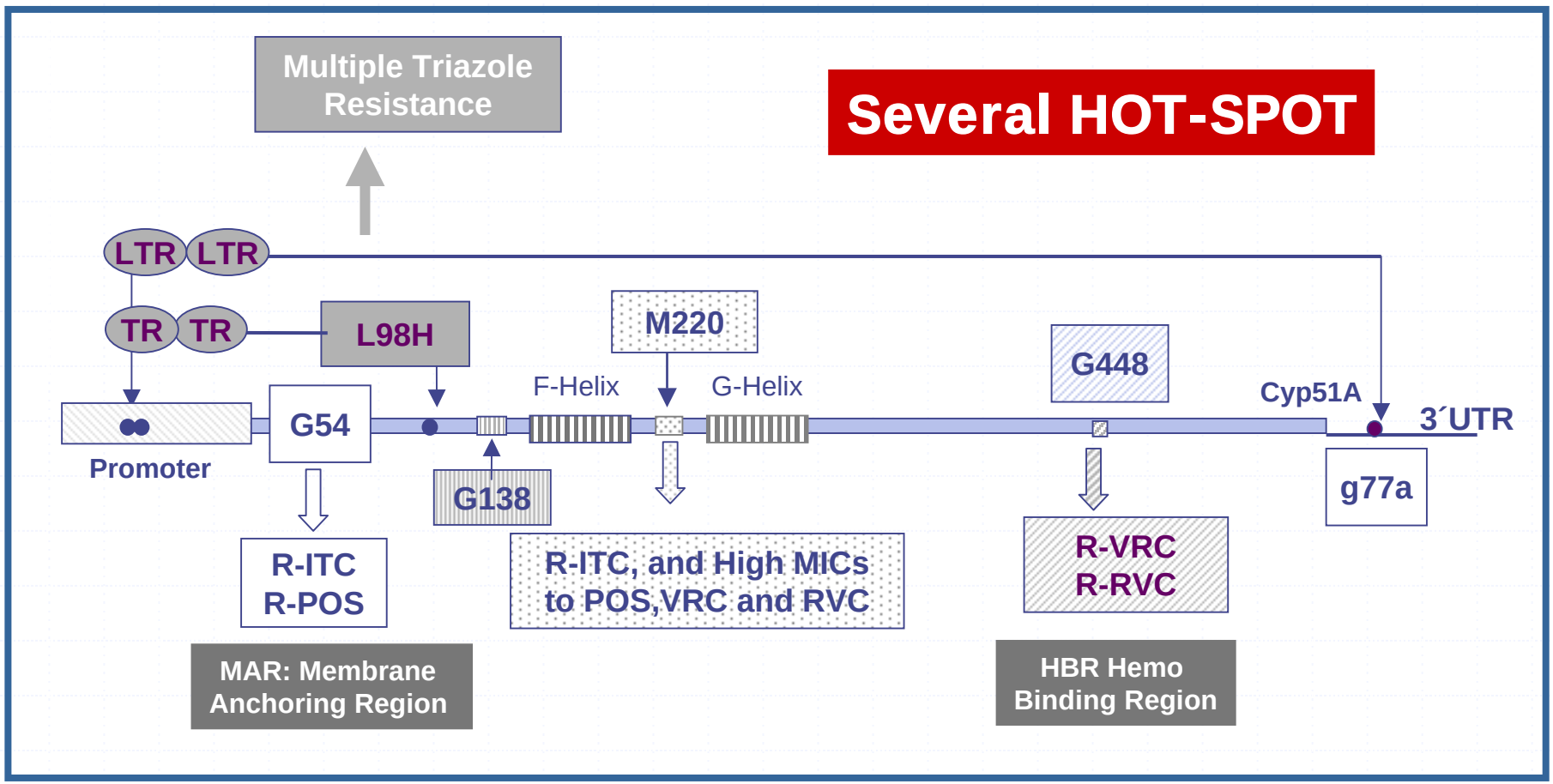
5.- Resistance Patterns:

Depends on specific mutations in **the azole target: Cyp51A**

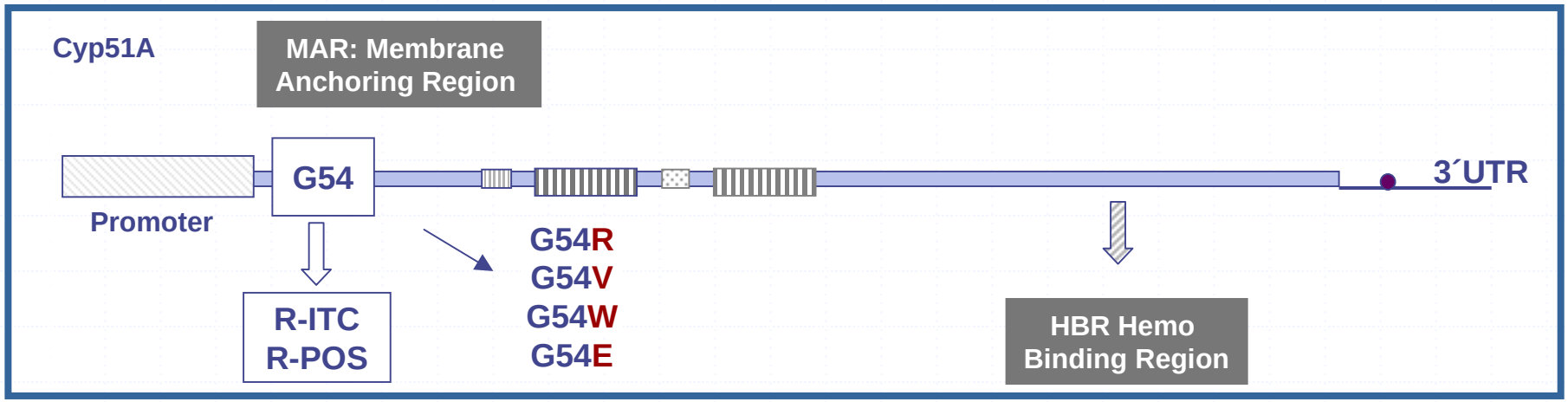
Azole Resistance Mechanisms in *A. fumigatus*



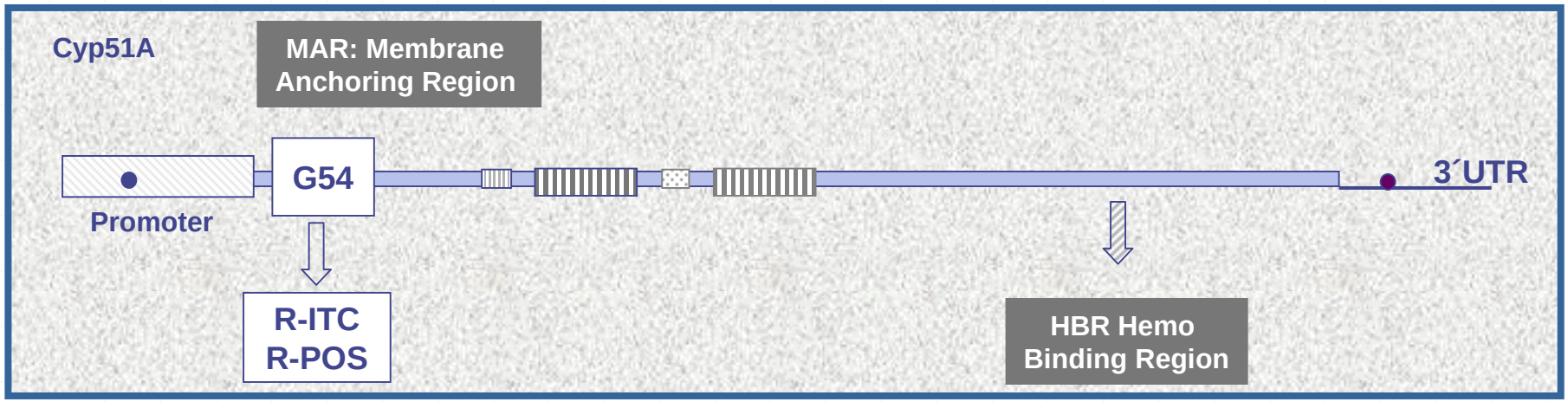
Azole Resistance Mechanisms in *A. fumigatus*

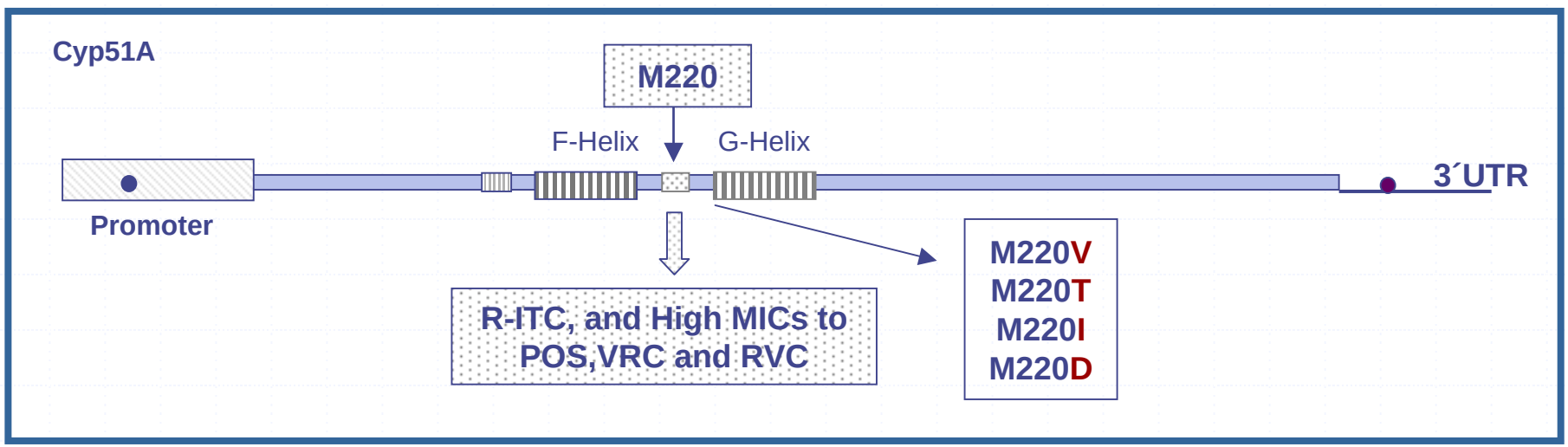


Azole Resistance Mechanisms in *A. fumigatus*

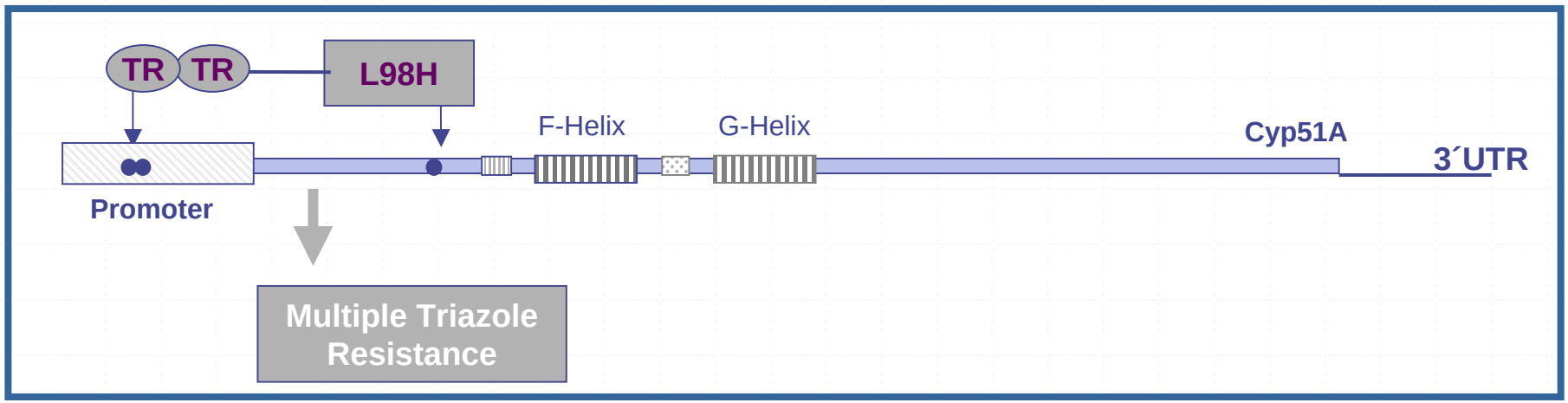


Azole Resistance Mechanisms in *A. fumigatus*

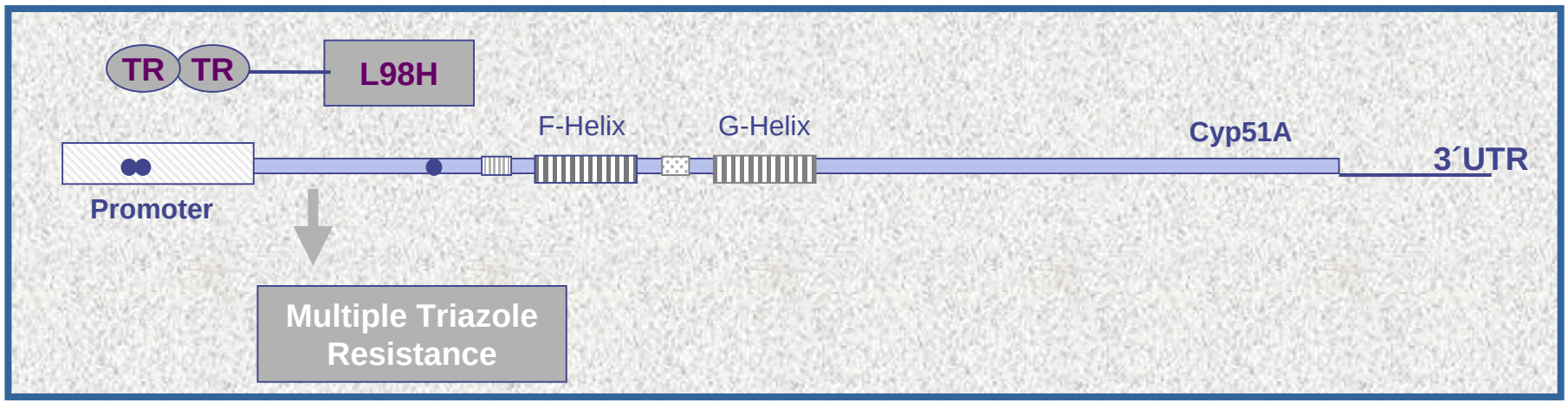




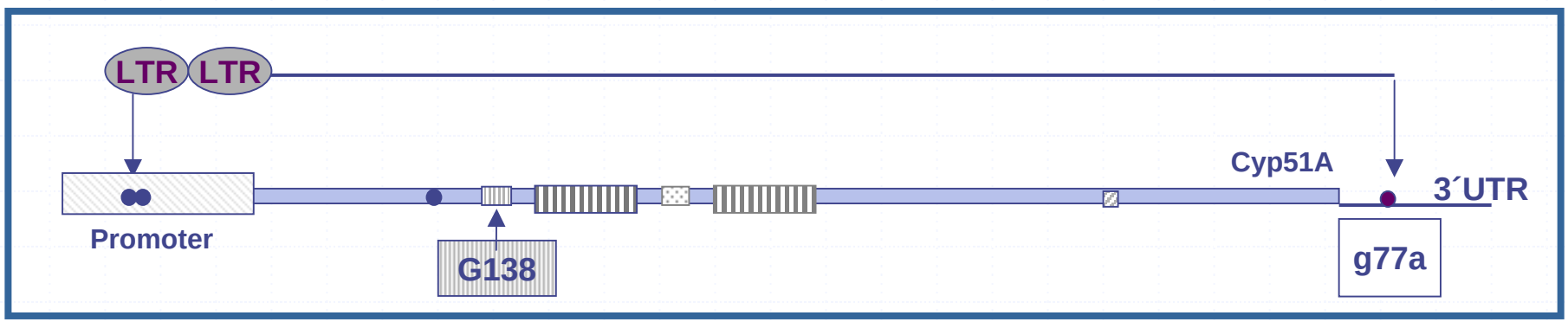
Azole Resistance Mechanisms in *A. fumigatus*



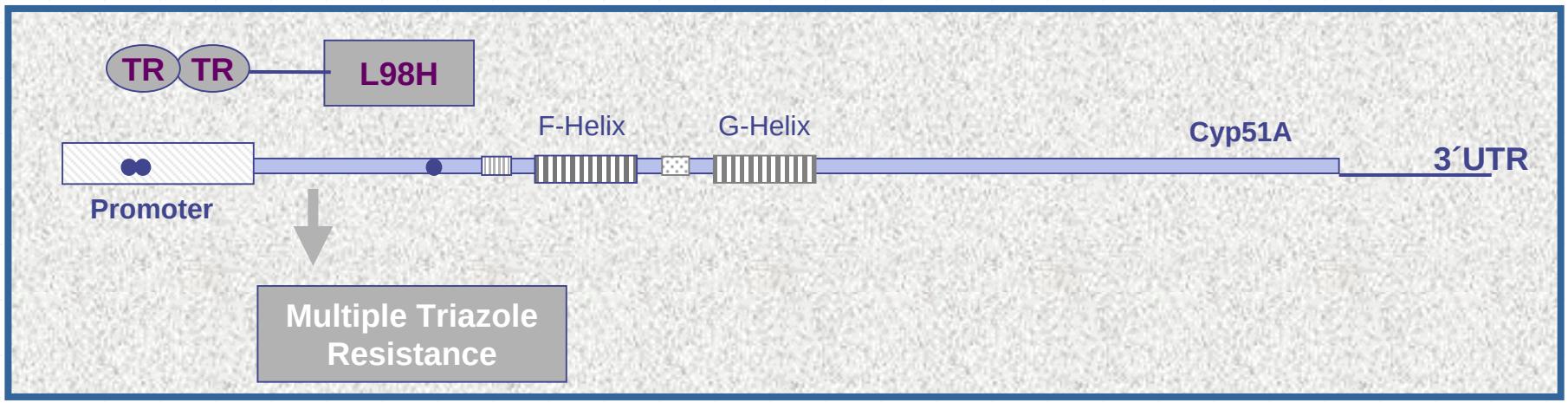
Azole Resistance Mechanisms in *A. fumigatus*



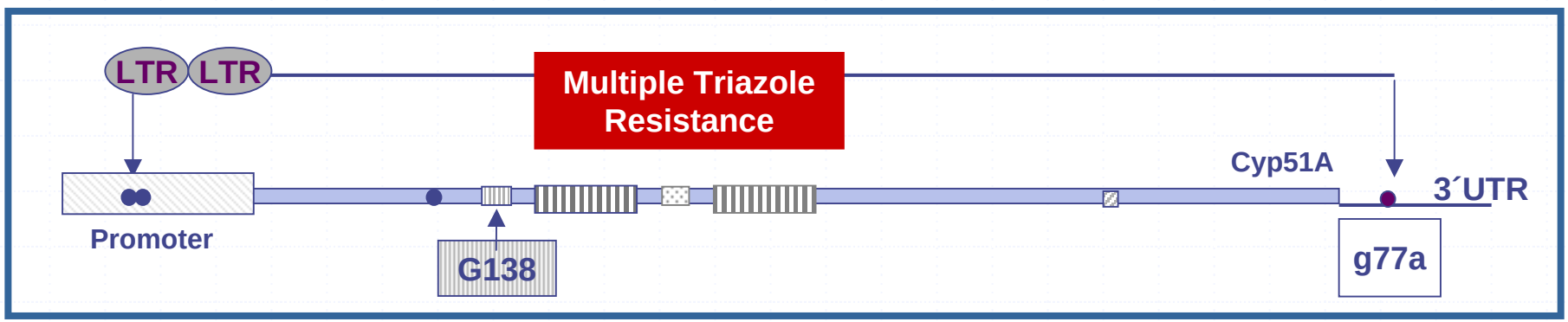
Multiple Triazole Resistance



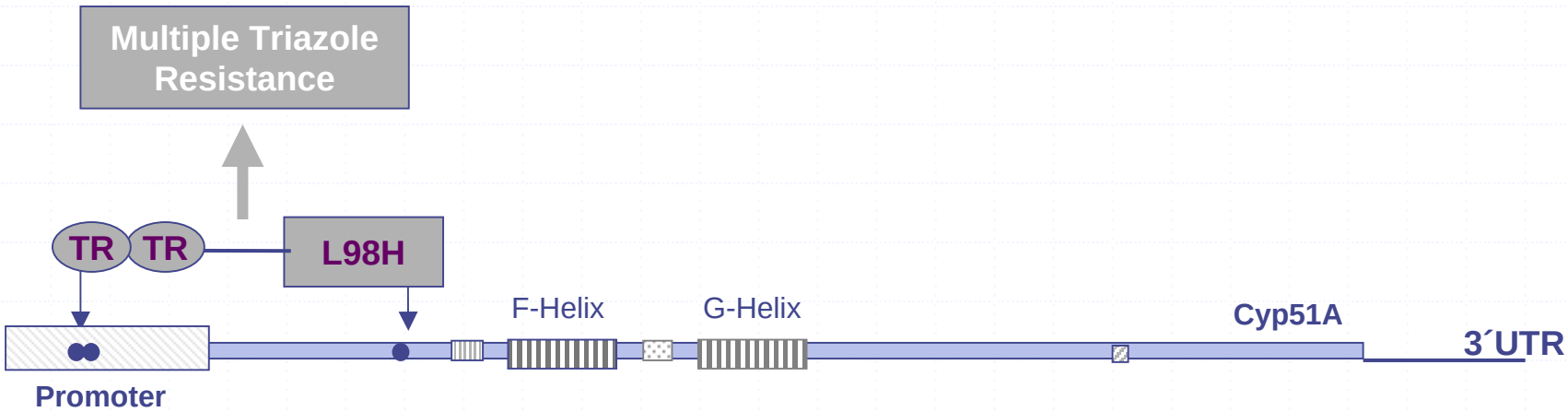
Azole Resistance Mechanisms in *A. fumigatus*



Multiple Triazole Resistance



Azole Resistance Mechanisms in *A. fumigatus*

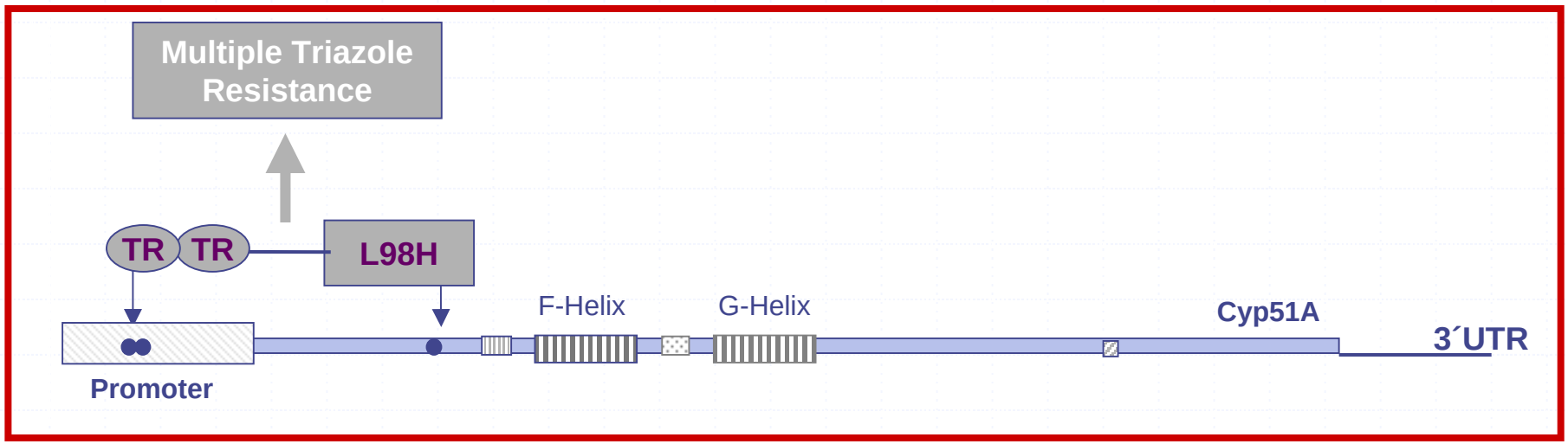


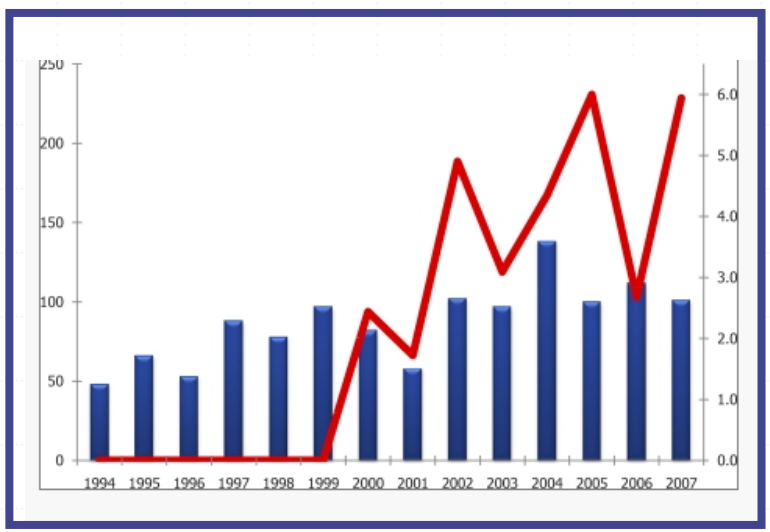
PLoS Medicine | November 2008 | Volume 5 | Issue 11 | e219

Emergence of Azole Resistance in *Aspergillus fumigatus* and Spread of a Single Resistance Mechanism

Eveline Snelders[1,2], Henrich A. L. van der Lee[1,2], Judith Kuijpers[1,2], Anthonius J. M. M. Rijs[1,2], János Varga[3,4], Robert A. Samson[3], Emilia Mellado[5], A. Rogier T. Donders[6], Willem J. G. Melchers[1,2], Paul E. Verweij[1,2*]

NEW TRENDS ……..





- Now the more frequently reported R mechanism
- In the Netherlands can go up to 6 -12 %
- Just reported in UK
- It has been described in azole naive patients
- Probably related to the use of antifungals in the field
- still need to be probed

Frequency and Evolution of Azole Resistance in *Aspergillus fumigatus* Associated with Treatment Failure[1]

Susan J. Howard, Dasa Cerar, Michael J. Anderson, Ahmed Albarrag, Matthew C. Fisher, Alessandro C. Pasqualotto, Michel Laverdiere, Maiken C. Arendrup, David S. Perlin, and David W. Denning

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 15, No. 7, July 2009

- Study made in UK, Manchester
- Clinical collection 519 *A. fumigatus*
- 1st resistant isolate in 1999
- before 2004 - 1 %
- After 2004 - 8 %

Mutations found in Cyp51A

Table 3. Cyp51A amino acid substitutions and associated cross-resistance patterns in azole-resistant RMLM *Aspergillus fumigatus* isolates*

Cyp51A codon	No. patients	No. isolates	Amino acid substitutions	MIC, mg/L†		
				Itraconazole	Voriconazole	Posaconazole
F46‡	3	4‡	Y	>8	2–4	0.125–0.5
G54	4	5	E, R, V	>8	0.125–1	1–>8
L98+TR	2	2	H	>8	8	1–2
G138	1	10	C	>8	8–>8	2–>8
H147§	1	1§	Y	>8	>8	0.5
M172‡	3	4‡	V	>8	2–4	0.125–0.5
P216	1	1	L	>8	1	1
M220	3	4	K, T	>8	1–4	0.5–>8
N248‡	1	1	T	>8	2	0.25
D255‡	1	1	E	>8	2	0.25
E427‡	4	5‡	G, K	>8	2–4	0.125–0.5
Y431	1	1	C	>8	4	1
G434	1	1	C	>8	4	1
G448	2	2	S	>8	>8	0.5–1
No substitutions	2	3	NA	>8	2–8	0.25–1

New mutations:

F46Y, H147Y, M172V, P216L, N248T,D255E, E427G/K, Y431C, G434C, G448S

Frequency and Evolution of Azole Resistance in *Aspergillus fumigatus* Associated with Treatment Failure[1]

Susan J. Howard, Dasa Cerar, Michael J. Anderson, Ahmed Albarrag, Matthew C. Fisher, Alessandro C. Pasqualotto, Michel Laverdiere, Maiken C. Arendrup, David S. Perlin, and David W. Denning

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 15, No. 7, July 2009

- Study made in UK, Manchester
- Clinical collection 519 *A. fumigatus*
- 1st resistant isolate in 1999
- before 2004 - 1 %
- After 2004 - 8 %

- Differences…….
- most patients under azole treatment
- opposite to mainly one single mechanism
- multiple *cyp*51A mutations (18 aa)

AZOLE RESISTANCE..... PROBLEM ?

- **Whatever the resistance mechanism found:**

  - **Might be influenced by the country under study**
  - **by the sampling type and size**
  - **the underlying disease of patient under study**

<u>The important facts are</u>:

- **Azole Resistance seems to be EMERGING**

- **towards multiple Azole Cross-resistance**

AZOLE RESISTANCE..... PROBLEM ?

- **Whatever the resistance mechanism found:**

  - **Might be influenced by the country under study**
  - **by the sampling type and size**
  - **the underlying disease of patient under study**

<u>The important facts are</u>:

- **Azole Resistance seems to be EMERGING**

- **towards multiple Azole Cross-resistance**

Does this has any meaning to clinicians?

Mould Antifungal Susceptibility Testing (AST)

- **AST standardization:**

  Europe (EUCAST)
  United States (CLSI).

Mould Antifungal Susceptibility Testing (AST)

- **AST standardization:**

  Europe (EUCAST)
  United States (CLSI).

The availability of *A. fumigatus* azole resistant strains with known resistance mechanisms have been used to define:

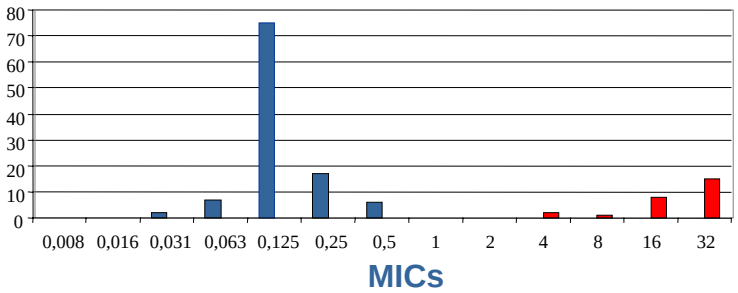
- **wild-type populations**

- **epidemiological cut-offs**

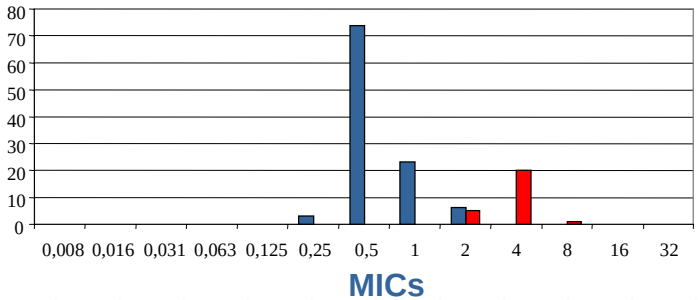
- **cross-resistance between azole drugs.**

A. fumigatus: MICs distribution for azole drugs

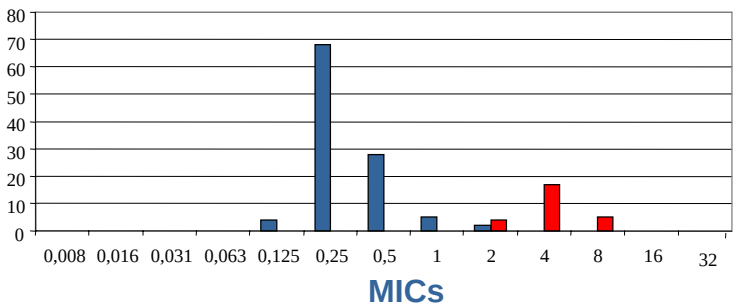
Itraconazole



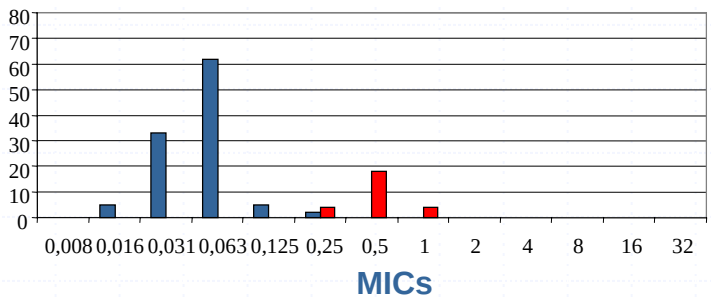
Voriconazole



Ravuconazole

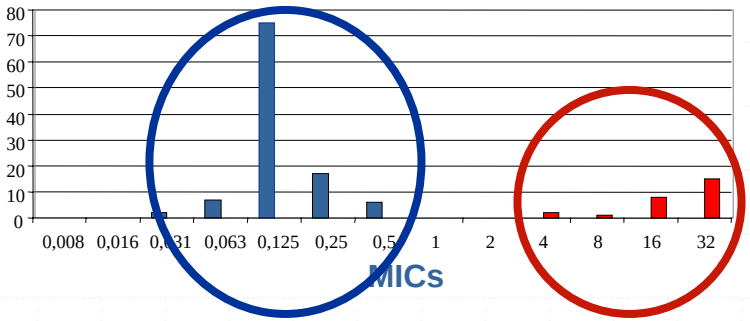


Posaconazole

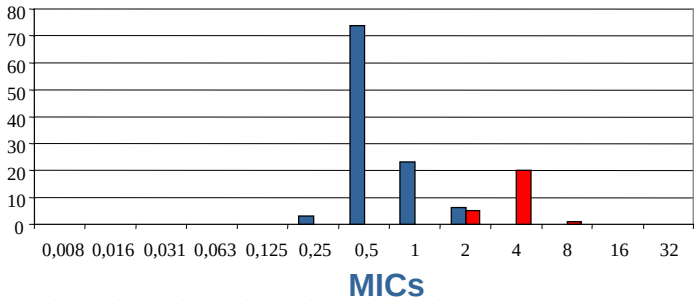


Aspergillus fumigatus: MICs distribution for azole drugs

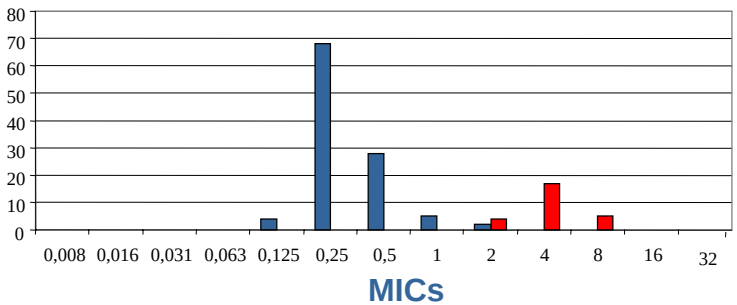
Itraconazole



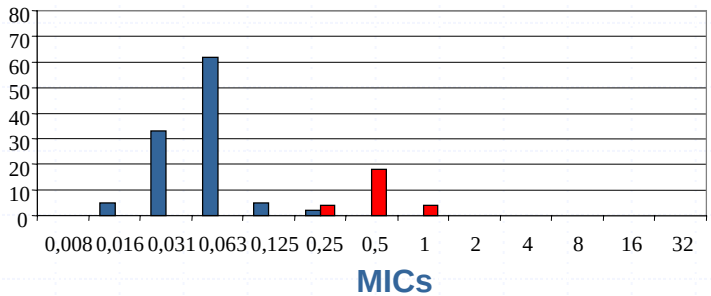
Voriconazole



Ravuconazole



Posaconazole



Itraconazole seems to be the guide for azole resistance detection.
Azole cross-resistance depends on the resistance mechanism.

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 2008, p. 2468–2472 Vol. 52, No. 7
0066-4804/08/$08.00+0 doi:10.1128/AAC.00156-08

Epidemiological Cutoffs and Cross-Resistance to Azole Drugs in *Aspergillus fumigatus*▽†

Juan Luis Rodriguez-Tudela,* Laura Alcazar-Fuoli, Emilia Mellado, Ana Alastruey-Izquierdo, Araceli Monzon, and Manuel Cuenca-Estrella

Servicio de Micologia, Centro Nacional de Microbiologia, Instituto de Salud Carlos III, Majadahonda, Spain

EUCAST methodology

Epidemiological cutoff for the wild-type populations

⬇

Itraconazole and/or voriconazole ≤ 1 mg/L
Posaconazole ≤ 0.25 mg/L

JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2009, p. 3142–3146 Vol. 47, No. 10
0095-1137/09/$08.00+0 doi:10.1128/JCM.00940-09

Wild-Type MIC Distribution and Epidemiological Cutoff Values for *Aspergillus fumigatus* and Three Triazoles as Determined by the Clinical and Laboratory Standards Institute Broth Microdilution Methods[▽]

M. A. Pfaller,[1,2]* D. J. Diekema,[1] M. A. Ghannoum,[3] J. H. Rex,[4] B. D. Alexander,[5] D. Andes,[6] S. D. Brown,[7] V. Chaturvedi,[8] A. Espinel-Ingroff,[9] C. L. Fowler,[10] E. M. Johnson,[11] C. C. Knapp,[12] M. R. Motyl,[13] L. Ostrosky-Zeichner,[14] D. J. Sheehan,[15] and T. J. Walsh[16] for the Clinical and Laboratory Standards Institute Antifungal Testing Subcommittee

TABLE 1. MIC distribution and ECVs for azoles and *A. fumigatus*

Antifungal agent	Result from: This study				Study by Rodriguez-Tudela et al. (44)			
	No. of isolates tested	MIC (μg/ml) Range	Mode	ECV (%)[a]	No. of isolates tested	MIC (μg/ml) Range	Mode	ECV (%)[a]
Itraconazole	637	0.015–2	0.25	1 (99.8)	393	0.06–2	0.25	1 (99.7)
Posaconazole	637	0.06–1	0.03	0.25 (99.8)	393	0.015–2	0.06	0.25 (98.6)
Voriconazole	637	0.12–4	0.25	1 (99.2)	393	0.06–2	0.5	1 (97.8)

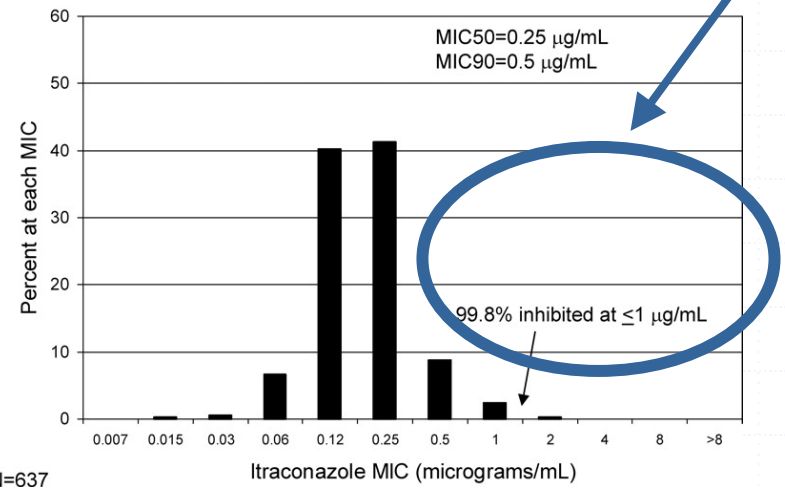
[a] Values in parentheses represent the percentage of MICs ≤ ECV.

CLSI = EUCAST

Itraconazole and/or voriconazole ≤ 1 mg/L

A

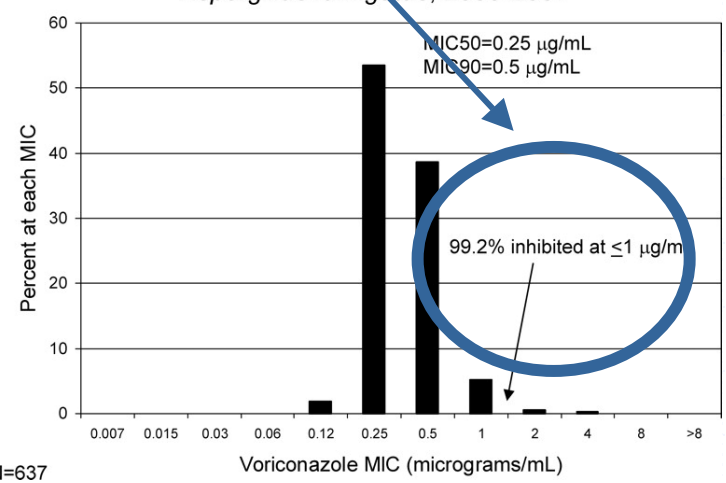
Itraconazole MIC Distribution
Aspergillus fumigatus, 2005-2007



N=637

C

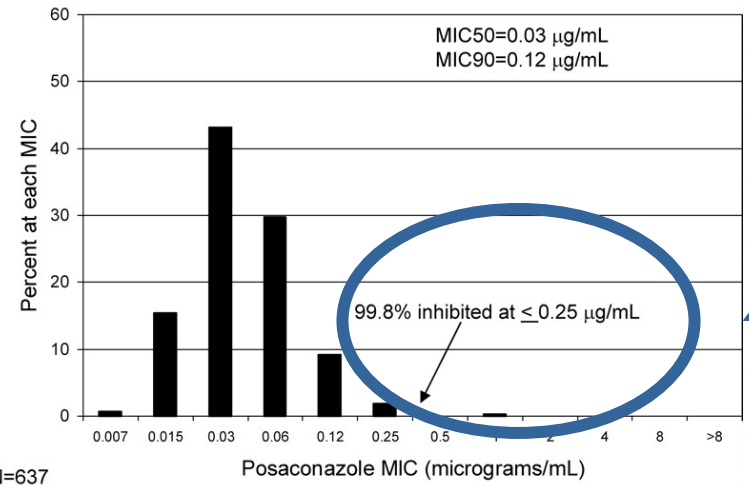
Voriconazole MIC Distribution
Aspergillus fumigatus, 2005-2007



N=637

B

Posaconazole MIC Distribution
Aspergillus fumigatus, 2005-2007



N=637

Posaconazole ≤ 0.25 mg/L

Setting Clinical Breakpoints

- difficult.....
- controversial.....!

⇩

- **Microbiological information**
- **Clinical data - success**
  - **failure**
- **Animal models, to confirm clinical observations**

Setting Clinical Breakpoints

- difficult.....
- controversial.....!

⇩

- **Microbiological information**
- **Clinical data - success**
  - **failure**
- **Animal models, to confirm clinical observations**

Proposed Interpretative Breakpoints

Itraconazole / voriconazole	**< 2 mg/L (S)**	**2 mg/L (I)**	**> 2 mg/L (R)**
Posaconazole	**< 0.25 (S)**	**0.25-0.5 (I)**	**> 0.5 (R)**

Verweij P, Howard S, Melchers W and Denning D. (in press)

Will That Change the CLINICAL OUTCOME ?

- **Influenced by the fungus susceptibility**

In general, there is the agreement that MICs correlates better with clinical resistance than with susceptibility prediction.

- **<u>other factors</u>:**

  - **Azole drugs pharmacokinetics**
  - **doses and drugs timing**
  - **drugs interaction**
  - **host response.**

IMPROVING THE FACTS

- we need more Epidemiological studies:
  - local epidemiology

- testing new antifungals in development:

  - Isavuconazole
  - Albaconazole

- they are azole derivatives:
  - check cross-resistance with them (expected?)

- Development New antifungals
- Discovery of New targets
- Combined therapy (complementary targets)