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

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INVASIVE ASPERGILLLOSIS (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)

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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*

- **Multiple Triazole Resistance**
- **LTR**
- **LTR**
- **TR**
- **TR**
- **L98H**
- **G54 (Promoter)**
- **G138**
- **M220**
- **G448**
- **Cyp51A**
- **3'UTR**
- **g77a**

**MAR**: Membrane Anchoring Region

**HBR**: Hemo Binding Region

Azole Resistance Mechanisms in *A. fumigatus*

**Multiple Triazole Resistance**

- **L98H**
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**Several HOT-SPOT**

- **MAR: Membrane Anchoring Region**
- **F-Helix**
- **G-Helix**
- **R-ITC**
- **R-POS**
- **R-ITC, and High MICs to POS, VRC and RVC**
- **R-VRC**
- **R-RVC**
- **HBR Hemo Binding Region**
- **3’UTR**
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**Promoter**

**Cyp51A**

**G54**
Azole Resistance Mechanisms in *A. fumigatus*

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- **MAR: Membrane Anchoring Region**
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- **3´UTR**
- **G54R**
- **G54V**
- **G54W**
- **G54E**
Azole Resistance Mechanisms in *A. fumigatus*

**Cyp51A**

**MAR: Membrane Anchoring Region**

**G54**

**R-ITC, R-POS**

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**R-ITC, and High MICs to POS, VRC, and RVC**

**M220**

**F-Helix**

**G-Helix**

**M220V**

**M220T**

**M220I**

**M220D**
Azole Resistance Mechanisms in *A. fumigatus*

- **L98H** mutation
- **Promoter**
- **F-Helix**
- **G-Helix**
- **Cyp51A**
- **3’UTR**
- **Multiple Triazole Resistance**

**Images and Diagrams:**
- A diagram showing the localization of resistance mechanisms involving the L98H mutation in the promoter region, connected to F-Helix and G-Helix, leading to Cyp51A and the 3’UTR.
Azole Resistance Mechanisms in *A. fumigatus*

**F-Helix G-Helix**

- **L98H**
- **Promoter**
- **Multiple Triazole Resistance**

**Cyp51A**

**3’UTR**

**Multiple Triazole Resistance**

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Azole Resistance Mechanisms in *A. fumigatus*

**Promoter**

1. **L98H**
2. **G138**

**3’UTR**

**Multiple Triazole Resistance**
Azole Resistance Mechanisms in *A. fumigatus*

**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 Failure¹

Susan J. Howard, Dasa Cesar, Michael J. Anderson, Ahmed Albarrag, Matthew C. Fisher, Alessandro C. Pasqualotto, Michel Laverdure, 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

New mutations:

Frequency and Evolution of Azole Resistance in *Aspergillus fumigatus* Associated with Treatment Failure¹

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- Study made in UK, Manchester
- Clinical collection 519 *A. fumigatus*
- 1st resistant isolate in 1999
- Before 2004 - 1 %
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- Differences.......
- Most patients under azole treatment
- Opposite to mainly one single mechanism
- Multiple *cyp51A* 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

The important facts are:

- Azole Resistance seems to be EMERGING

- towards multiple Azole Cross-resistance
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Does this has any meaning to clinicians?
- 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 forazole drugs

**Itraconazole**

**Voriconazole**

**Ravuconazole**

**Posaconazole**
Aspergillus fumigatus: MICs distribution for azole drugs

**Itraconazole** seems to be the guide for azole resistance detection. Azole cross-resistance depends on the resistance mechanism.
Epidemiological Cutoffs and Cross-Resistance to Azole Drugs in *Aspergillus fumigatus*

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

Servicio de Micología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Spain

**EUCAST methodology**

**Epidemiological cutoff for the wild-type populations**

Itraconazole and/or voriconazole \( \leq 1 \text{ mg/L} \)

Posaconazole \( \leq 0.25 \text{ mg/L} \)
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. Pfaffer,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. Molyl13 L. Ostrosky-Zeichner,14 D. J. Sheehan,15
and T. J. Walsh16 for the Clinical and Laboratory Standards
Institute Antifungal Testing Subcommittee

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

<table>
<thead>
<tr>
<th>Antifungal agent</th>
<th>No. of isolates tested</th>
<th>MIC (μg/mL)</th>
<th>ECV (%)</th>
<th>No. of isolates tested</th>
<th>MIC (μg/mL)</th>
<th>ECV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>Mode</td>
<td></td>
<td>Range</td>
<td>Mode</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>637</td>
<td>0.015–2</td>
<td>0.25</td>
<td>1 (99.8)</td>
<td>393</td>
<td>0.06–2</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>637</td>
<td>0.06–1</td>
<td>0.03</td>
<td>0.25 (99.8)</td>
<td>393</td>
<td>0.015–2</td>
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<tr>
<td>Voriconazole</td>
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*Values in parentheses represent the percentage of MICs ≤ ECV.
Itraconazole and/or voriconazole $\leq 1 \text{ mg/L}$

Posaconazole $\leq 0.25 \text{ mg/L}$
Setting Clinical Breakpoints

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

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

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Proposed Interpretative Breakpoints

<table>
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<tr>
<th>Drug</th>
<th>&lt; 2 mg/L (S)</th>
<th>2 mg/L (I)</th>
<th>&gt; 2 mg/L (R)</th>
</tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posaconazole</td>
<td>&lt; 0.25 (S)</td>
<td>0.25-0.5 (I)</td>
<td>&gt; 0.5 (R)</td>
</tr>
</tbody>
</table>

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.

- **other factors:**
  - Azole drugs pharmacokinetics
  - doses and drugs timing
  - drugs interaction
  - host response.
- 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)