Indian scenario of azole resistance in *A. fumigatus* and its emergence in Asia

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Aspergillosis

- Major cause of morbidity and mortality especially in immunocompromised patients.

- The physiologic state of the host and the *Aspergillus* species involved defines the type and severity of disease.

- Antifungals are prescribed for prophylaxis, preventive therapy for acute disease and long term maintenance therapy for chronic and allergic infection.
Magnitude of the Problem

- **IA**: most severe manifestation with highest mortality rates due to delay in recovery of immune system of the patients and delayed diagnosis (Pfaller et al., CID, 2006; Denning and Perlin, Future Microbiol. 2011; Upton et al., CID, 2007).

- **CPA** and **ABPA** cause increased morbidity affecting approx. 3 million and 4 million adults, respectively, globally (Denning et al., Med Mycol., 2013).

- **CPA** and **ABPA** are the principal patient group impacted by therapeutic failures due to triazole resistance.
Epidemiological facts of *Aspergillus* infections

**Aspergillus fumigatus—What Makes the Species a Ubiquitous Human Fungal Pathogen?**

Kyung J. Kwon-Chung*, Janyce A. Sugui

*Aspergillus fumigatus* is equipped to propagate successfully under a wide variety of environmental conditions. The growth range is 12-65 °C with a pH tolerance of 2.1-8.8.

Conidia are extremely hydrophobic, allowing for high airborne dispersibility. They are protected from UV due to the presence of melanin.

*Efficient recycler*

*Wide variety of substrates*

*Growth range 12-65 °C*

*Thermotolerance pH 2.1-8.8*
A. fumigatus

A. nidulans

Kwon-Chung and Sugui, 2014, Plos Pathog.
Aspergillus epidemiology in the ICU

- Leuven Univ. MICU (17 beds, 3 yrs, 1850 admissions)
- 127 patients with proven or probable IA (6.9%)
- When excluding cancer patients: Invasive aspergillosis incidence, 3.7%

Meersseman et al. 2004, AJRCCM
Azole resistance in *Aspergillus*: should we care?
Treatment of Aspergillosis

- **Surgical intervention**: not common-propensity to bleeding.
- **Reduction of immunosuppression**, if possible.
- **Antifungal therapy**.

**Antifungal drugs**

- **Polyenes**: Amphotericin B
- **Azole**: Triazole
- **Echinocandins**: Caspofungin, Anidulafungin, Micafungin
Azole Antifungals: Therapy and Resistance

- Azole class of drugs are mainstay of therapy/prevention of acute and chronic aspergillosis (IDSA Guidelines, 2008).

- Currently the only class of mould-active agents that can be used orally and systemically.

- *Aspergillus fumigatus* isolates harbouring mutations conferring one azole, a multi-azole or panazole-resistant phenotype have emerged in patients receiving chronic azole therapy or in azole naïve.
A global map depicting geographic distribution of multi-triazole resistant clinical and environmental *A. fumigatus* strains with Country-wise prevalence rates (%)

Country (Year of publication)
- A. Netherlands (2007)
- B. Spain (2008)
- C. United kingdom (2009)
- D. Denmark (2010)
- E. China (2011)
- F. Belgium (2011)
- G. France (2012)
- H. India (2012)
- I. Germany (2012)
- J. Iran (2013)
- K. Austria (2013)
- L. Italy (2013)
REVIEW

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Exploring azole antifungal drug resistance in *Aspergillus fumigatus* with special reference to resistance mechanisms

Anuradha Chowdhary¹, Cheshta Sharma¹, Ferry Hagen² & Jacques F Meis²,³

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**Figure 1.** Resistance mechanism in *Aspergillus fumigatus* due to (A) increased drug efflux, (B) overexpression of the target enzyme and (C) exogenous cholesterol import.
Aspergillus fumigatus cyp51A gene depicting the promoter region and mutation hot spots conferring resistance to azole antifungals.
Routes of resistance development

Treatment with azoles;
Selection in patient

Patient route

Environmental route

Selectionazole fungicides

Slide Courtesy: Prof. Paul E Verweij
Emergence of Azole-Resistant *Aspergillus fumigatus* Strains due to Agricultural Azole Use Creates an Increasing Threat to Human Health

Anuradha Chowdhary\(^1\)*, Shallu Kathuria\(^1\), Jianping Xu\(^2\), Jacques F. Meis\(^3,4\)

- The azoles could persist and remain active in many ecological niches such as in agricultural soil and aquatic environments for several months.

- The widespread application of triazole fungicides and their persistence in the environment are significant selective forces for the emergence and spread of azole resistant *A. fumigatus*.
By molecular modeling studies these five triazole DMI fungicides were found to have similar molecular structures as medical tri-azoles and they all adopt a similar conformation while docking the target enzyme in susceptible strains of *A. fumigatus* (Chowdhary et al., PLOS Pathog, 2013)
Resistant “environmental” strains have been detected in many West-European countries as well as in Asia (India) (Chowdhary et al., PLOS One, 2012; Chowdhary et al., Future Microbiol, 2014).

Noticeably, these two continents account for the highest fungicide use in the global perspective (37% and 24%, respectively) (Chowdhary et al., PLOS pathogen, 2013).
INDIAN SCENARIO
Of the 103 A. fumigatus isolates tested, only 2 had high MIC values of ITC (>16 mg/L), VRC (2 mg/L), POSA (2 mg/L) and ISA (8 mg/L).

The resistant A. fumigatus isolates exhibited the TR/L98H genotype and showed identical patterns by microsatellite typing, but were different from 25 Dutch TR/L98H isolates.

Patients were suffering from chronic respiratory disease without any prior exposure to azoles.
Clonal Expansion and Emergence of Environmental Multiple-Triazole-Resistant *Aspergillus fumigatus* Strains Carrying the TR<sub>34</sub>/L98H Mutations in the *cyp51A* Gene in India

Anuradha Chowdhary<sup>1</sup>, Shallu Kathuria<sup>1</sup>, Jianping Xu<sup>2</sup>, Cheshta Sharma<sup>1</sup>, Gandhi Sundar<sup>1</sup>, Pradeep Kumar Singh<sup>1</sup>, Shailendra N. Gaur<sup>3</sup>, Ferry Hagen<sup>4</sup>, Corné H. Klaassen<sup>4</sup>, Jacques F. Meis<sup>4,5</sup>

- 7% of *A. fumigatus* isolates from environmental samples were found to be triazole resistant.
- Highest (33%) from soil of tea gardens, soil from flower pots of the hospital garden (20%), soil beneath cotton trees (20%), rice paddy fields (12.3%), air samples of hospital wards (7.6%).
- Cross-resistance to ITC, VRC, ISA, POSA and to six triazole fungicides used extensively in agriculture.
Minimum spanning tree showing wide genotypic diversity in the TR$_{34}$/L98H and wild type *A. fumigatus* isolates studied.

Chowdhary et al., 2012, Plos One
Dispersal of triazole resistant *A. fumigatus*: Adaptive recombinant progeny

- Indian azole-resistant *A. fumigatus* genotype was likely an extremely adaptive recombinant progeny derived from a cross between an azole-resistant strain migrated from outside of India and a native azole-susceptible strain from within India, followed by mutation and then rapid dispersal through many parts of India.

- Exposure of *A. fumigatus* to azole fungicides in the environment causes cross-resistance to medical triazoles.

*Chowdhary et al., 2012, Plos One*
Azole-resistant *Aspergillus fumigatus* with the environmental TR$_{46}$/Y121F/T289A mutation in India

Anuradha Chowdhary$^{1*}$, Cheshta Sharma$^{1}$, Shallu Kathuria$^{1}$, Ferry Hagen$^{2}$ and Jacques F. Meis$^{2,3}$

<table>
<thead>
<tr>
<th>Locations (Samples positive for <em>A. fumigatus</em>/ Samples tested) n=117</th>
<th>Fields (common name), number of samples tested</th>
<th>No. of azole resistant <em>A. fumigatus</em></th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varanasi (10/42)</td>
<td><em>Solanum tuberosum</em> (Potato), n=12</td>
<td>5</td>
<td>TR$_{46}$/Y121F/T289A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TR$_{34}$/L98H</td>
</tr>
<tr>
<td></td>
<td><em>Trigonella foenumgraecum</em> (Fenugreek), n=9</td>
<td>1</td>
<td>TR$_{46}$/Y121F/T289A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TR$_{34}$/L98H</td>
</tr>
<tr>
<td></td>
<td><em>Solanum melongena</em> (Aubergine), n=12</td>
<td>1</td>
<td>TR$_{34}$/L98H</td>
</tr>
<tr>
<td>Delhi, Yamuna River Bank (27/63)</td>
<td><em>Brassica juncea</em> (Mustard), n=9</td>
<td>1</td>
<td>TR$_{34}$/L98H</td>
</tr>
<tr>
<td></td>
<td><em>Capsicum annuum</em> (Red chilli), n=15</td>
<td>2</td>
<td>TR$_{34}$/L98H</td>
</tr>
<tr>
<td></td>
<td><em>Rosa species</em> (Rose), n=48</td>
<td>2</td>
<td>TR$_{34}$/L98H</td>
</tr>
<tr>
<td>Himachal Pradesh (1/12)</td>
<td><em>Ganga River basin</em></td>
<td>2</td>
<td>TR$_{46}$/Y121F/T289A</td>
</tr>
</tbody>
</table>
In the present study, both TR<sub>34</sub>/L98H and TR<sub>46</sub>/Y121F/T289A coexisted in the soils where fungicides were used.

All Indian TR<sub>46</sub>/Y121F/T289A strains were similar to the Dutch clinical strains at 8 out of 9 loci.

Furthermore, it is highly likely that the mutations with multiple azole resistance could emerge from environmental sources and spread among human populations.

Chowdhary et al., 2014 JAC
The G54E mechanism was responsible for 46.4% of resistant isolates from Tanzania, followed by Romania (30.4%) and India (20%).

The G54E isolates revealed high MICs of ITC and POSA and were cross-resistant to agricultural fungicides.

The majority of the Romanian and Tanzanian G54E isolates had an identical genotype.
Table: Prevalence of triazole resistance in *A. fumigatus* isolates in environmental samples from Tanzania, Romania and India during 2013–2014.

<table>
<thead>
<tr>
<th>Location</th>
<th>Samples positive for <em>A. fumigatus</em>/<em>samples investigated</em></th>
<th>Samples positive for TRAF/<em>samples isolated</em></th>
<th>TRAF/total</th>
<th>Isolates with G54E mutation/TRAF isolates tested</th>
<th>Isolates with TR&lt;sub&gt;52&lt;/sub&gt;/L98H mutation/TRAF isolates tested</th>
<th>Isolates with TR&lt;sub&gt;52&lt;/sub&gt;/Y121F/T289A mutation/TRAF isolates tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tanzania (n = 30)</td>
<td>27/30 (90.0%)</td>
<td>10/30 (33.3%)</td>
<td>28/106 (26.4%)</td>
<td>13/28 (46.4%)</td>
<td>11/28 (39.3%)</td>
<td>4/28 (14.3%)</td>
</tr>
<tr>
<td>Romania (n = 27)</td>
<td>25/27 (92.6%)</td>
<td>5/27 (18.5%)</td>
<td>23/95 (24.2%)</td>
<td>7/23 (30.4%)</td>
<td>16/23 (69.6%)</td>
<td>Negative</td>
</tr>
<tr>
<td>India (n = 24)</td>
<td>21/24 (87.5%)</td>
<td>5/24 (20.8%)</td>
<td>5/52 (9.6%)</td>
<td>1/5 (20.0%)</td>
<td>4/5 (80.0%)</td>
<td>Negative</td>
</tr>
<tr>
<td>Total (n = 81)</td>
<td>73/81 (90.1%)</td>
<td>20/81 (24.7%)</td>
<td>56/253 (22.1%)</td>
<td>21/56 (37.5%)</td>
<td>31/56 (55.4%)</td>
<td>4/56 (7.1%)</td>
</tr>
</tbody>
</table>

TRAF, triazole-resistant *A. fumigatus*.

* Woody debris of tree trunk hollows (n = 12); soil from hospital parking areas (n = 12); garden soil (n = 6).

* Soil from *Helianthus annuus* (sunflower) field (n = 15); sunflower woody debris (n = 6); soil from *Solanum tuberosum* (potato) field (n = 6).

* Garden soil (n = 24).
ASIAN SCENARIO
A total of 497 *A. fumigatus* isolates submitted from 62 medical centers worldwide were collected and screened.

Eight out of 29 *A. fumigatus* with elevated MICs to one or more triazoles harbored TR$_{34}$/L98H mutation.

All originated from China. This was the first time the TR/L98H mutation was identified outside Europe.
A total of 41 *A. fumigatus* isolates were obtained from 150 soil samples processed.

Overall 3.3% (5/150) of the Iranian soil samples mostly from north-east of Iran had the resistant *A. fumigatus* isolates with TR$_{34}$/L98H mutation.

Azole resistant *A. fumigatus* isolates from hospital surroundings in Sari and Tehran had the same TR$_{34}$/L98H STRAf genotype and were related to some resistant clinical and environmental TR$_{34}$/L98H isolates from the Netherlands and India.
Eight of 115 *A. fumigatus* isolates were resistant ITC, POSA and VRC.

All ITC-resistant isolates contained TR$_{34}$/L98H mutation in *cyp51A*.

Three microsatellite patterns were observed among resistant isolates with one pattern clustering with Indian clinical and environmental isolates.
Fig. 2. Genotypic relationship between 8 environmental triazole-resistant A. fumigatus isolates from Kuwait with environmental and/or clinical isolates from The Netherlands, Germany, France, India, China and Iran. The dendrogram is based on a categorical analysis of 9 microsatellite markers in combination with UPGMA clustering. The scale bar represents the percentage identity.
China (C)
AAC 2011;55:4465-8

India (C+E)
JAC 2012;67:362-6
PLoS One, 2012;7:e52871

TR_{34}/L98H

Iran (C+E)
EID 2013;19:832-4
Mycoses 2013;56:659-63

Kuwait (C+E)
JCM 2014;52:2223-7
JAC 2015,70:412-5
Indonesia
Issues with ARAF

- Early detection of azole resistance, especially in culture negative patients. (Denning et al., CID, 2011).

- Direct detection of resistance mutations by PCR in clinical specimens has been reported, but these tests are not widely available. (Denning et al., CID, 2011; Zhao et al., JAC, 2013)

- Surveillance, a case registry, improved diagnostics and better understanding of resistance selection in the environment are areas where research is urgently needed.
Management of Infections caused by ARAF

- Although preclinical data suggest that an azole-echinocandin combination can render an azole-resistant *A. fumigatus* strain more susceptible (synergy), no clinical evidence exists to support this combination in azole-resistant IA. *(Seyedmousavi et al., JAC, 2013; Lepak et al., AAC, 2013)*

- Experimental models indicate LAMB may be effective or a combination of voriconazole or posaconazole with an echinocandin. *(Seyedmousavi et al., JAC, 2013; AAC, 2013; Lepak et al., AAC, 2013)*

- Currently no guidelines or recommendations for ARAF aspergillosis.
The ECDC recommends

- increased surveillance for clinical and environmental azole resistant pathogens

- A more judicious use of azoles in patients, in agriculture settings, and alternative strategies such as chemosensitization could help lower the dosage levels of fungicides in the environment and minimize the emergence and spread of TRAF.
The environmental route of azole resistance selection is of major concern and has global dimensions.

Culprits
- Bromuconazole
- Epoxiconazole
- Tebuconazole
- Difenoconazole
- Propiconazole

Take-home messages
- More research is necessary to understand how resistance arises in the environment and which measures might be effective.
- It would be beneficial to:
  - Perform active multi-azole susceptibility testing of *A. fumigatus*.
  - Reduce agricultural use of triazole DMI fungicides, and
  - Use combination drug therapy.

Rapid diagnostic tools and new treatment strategies are urgently warranted.
Take time to know the “FUNGUS” in your hospital....