Azole resistance in *Aspergillus fumigatus*: a forgotten global threat

Paul E. Verweij, MD FECMM  
Centre of Expertise in Mycology Radboudumc/CWZ  
November 2019
Disclosures

- Research grants
- Advisory boards
- Speaker
Survival

- All: 94%
- Invasive pulmonary aspergillosis: 70%
- CNS aspergillosis: 50%
- Azole Resistant: 0%
Why?

Lack of sensitive diagnostic tools for early resistance detection

Lack of alternative drugs with similar efficacy

a forgotten global threat?
Aspergillus and its habitat
The spectrum of *Aspergillus* diseases

- Angioinvasive IPA
- Non-angioinvasive IPA
- Aspergilloma: Chronic forms of pulmonary aspergillosis
- ABPA: Severe asthma with fungal sensitisation, Allergic sinusitis

*Frequency of aspergillosis* vs *Immune dysfunction* vs *Immune hyperactivity*
Fungi are eukaryotes just like humans....
Azoles are the main drug class for management of IA

- Polyene: Iv only
  - AmB (alternative) primary therapy
- Azole: Iv & oral
  - FCZ primary therapy
  - ITZ
  - VCZ primary therapy
  - PCZ
  - ISA primary therapy
- Echinocandin: Iv only
  - CAS
  - ANF
  - MIC

A. fumigatus

(prophylaxis)
12-week survival of patients with IA treated with VCZ

- VCZ vs OLAT day 84 NEJM 2002
- VCZ vs OLAT day 84 CID 2015
- VCZ vs VCZ+AFG day 84 AIM 2015
- VCZ vs ISA day 84 Lancet 2016

OLAT: 57.9% 70.8% 54.9% 60.6% 69.2% 72.3%
VCZ: 70.2% 70.7% 60.6% 70.7%
Azole resistance

Threat?
Mortality of voriconazole R IA > voriconazole S IA?

Radboudumc – LUMC – ErasmusMC

2011 - 2015

All patients with *A. fumigatus* in culture

All isolates screened with VIPcheck™

Compare mortality in R versus S
2,266 patients with positive *Aspergillus fumigatus* culture

**Study group**

- **No IA**: 2,070
- **IA**: 196

EORTC/MSG

Blot et al.

196 patients with invasive aspergillosis

Resistance phenotype

- VCZ-S: 159
- VCZ-R: 37 (19%)

**A. fumigatus** resistance phenotypes

**VCZ (>2 mg/l)**

100% cross-resistance with isavuconazole

All susceptible to AmB

196 patients with invasive aspergillosis: underlying disease

- Hematology: 103 (53%)
- SOT: 24
- Autoimmune: 24
- Lung disease: 19
- Cancer: 16
- Congenital ID: 12
- Other: 10
- None: 7

196 patients with invasive aspergillosis

Initial AF therapy

- VCZ: 154 (79%)
- L-AmB: 27
- VCZ+Ecand: Other
- VCZ+L-AmB: Other
- POS: Other

Overall mortality in *vori* R versus *vori* S (hospital wide study)

**Mortality**

**Day 42**
- VCZ-S: 28%
- VCZ-R: 49%
  - p=0.017

**Day 90**
- VCZ-S: 37%
- VCZ-R: 62%
  - p=0.0038

129 hematology patients with invasive aspergillosis

Resistance phenotype

- VCZ-S: 103
- VCZ-R: 26 (20%)

Institutions: UZ Leuven, ErasmusMC, LUMC, Radboudumc

J Antimicrob Chemother. 2019;74:2759-2766
Azole resistance and survival

84d Survival by resistance - all patients

Strata

Susceptible

Resistant

Survival probability

p = 0.064

Number at risk

<table>
<thead>
<tr>
<th>Strata</th>
<th>Susceptible</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>103</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>88</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>83</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>76</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>74</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>74</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>71</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>69</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>68</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>68</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>65</td>
<td>11</td>
</tr>
</tbody>
</table>

J Antimicrob Chemother. 2019;74:2759-2766
Putting azole-resistant associated 12-week mortality of IA in perspective

- VCZ vs OLAT:
  - Day 84: NEJM 2002

- VCZ vs VCZ+AFG:
  - Day 84: AIM 2015

- VCZ vs ISA:
  - Day 84: Lancet 2016

- VCZ vs OLAT:
  - Day 84: CID 2015

- VCZ vs VCZ+AFG:
  - Day 84: AIM 2015

- VCZ vs ISA:
  - Day 84: Lancet 2016

- Radboudumc
Activity of antifungal agents: azole resistance

Polyene
- AmB (primary therapy)
  - Alternative primary therapy
  - CNS
  - Oral

Azole
- FCZ
- ITZ
- VCZ
- PCZ
- ISA
- CAS
- ANF
- MIC
  - primary therapy
  - prophylaxis

Echinocandin
- Iv only

A. fumigatus
How are patients infected?

Medical triazoles

Propiconazole; tebuconazole; epoxiconazole; difenoconazole; bromuconazole

TR\textsubscript{34}/L98H
TR\textsubscript{53}
TR\textsubscript{46}/Y121F/T289A

Lancet Infect Dis. 2009;9:789-95
<table>
<thead>
<tr>
<th>Disease</th>
<th>Route of resistance</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF</td>
<td>E &gt; P</td>
<td></td>
</tr>
<tr>
<td>ABPA</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Aspergilloma</td>
<td>P &gt;&gt; E</td>
<td>azole therapy, cavity, multiple R-mutations, fitness cost</td>
</tr>
<tr>
<td>CPA</td>
<td>P &gt;&gt; E</td>
<td>azole therapy, cavity, multiple R-mutations, fitness cost</td>
</tr>
<tr>
<td>IA - pulmonary</td>
<td>E</td>
<td>2/3 no azole therapy, TR$<em>{34}$ &gt; TR$</em>{46}$, mixed infections</td>
</tr>
<tr>
<td>IAA</td>
<td>E</td>
<td>TR$<em>{34}$ &gt; TR$</em>{46}$, mixed infections, tracheobronchitis</td>
</tr>
<tr>
<td>CNS-IA</td>
<td>E</td>
<td>TR$<em>{34}$&amp;TR$</em>{46}$, sanctuary site</td>
</tr>
</tbody>
</table>

P = patient route   E = environmental route
Mixed infections

- Azole susceptible
- Azole resistant
37 patients with VCZ-R invasive aspergillosis

Cyp51A resistance mutations

- TR34: 19
- TR46: 14
- WT: 5

7 pts (19%) with mixed infection:
- 6 x S/R
- 1 x R/R

87% environmental

Resistance diagnosis and appropriateness of antifungal therapy

- Diagnosis IA (154)
- VCZ therapy
- Culture A. fumigatus
- MIC-test
- VCZ-S
- VCZ-R

- continue VCZ (124)
- Switch to appropriate AF therapy (30)

- appropriate
- inappropriate

Intensive R-screening of cultures

log-rank test, p=0.0049

appropriate

23%

inappropriate

10 days

The promise of commercial resistance PCRs

MycoGenie® (AdemTech, Pessac, France)

- quadriplex real-time PCR assay
- *A. fumigatus* (*28S* rRNA gene)
- Cyp51A-gene: TR$_{34}$, L98H
- internal control

AsperGenius® assay (PathoNostics, Maastricht, The Netherlands)

- 2 different real-time quadriplex amplification mixtures
- Aspergillus species multiplex assay targets the 28S rRNA multicopy gene
- *A. fumigatus* complex (Af), *Aspergillus terreus* and *Aspergillus* spp. (Asp sp)
- *Cyp51A* gene: TR$_{34}$, L98H, Y121F and T289A
- The distinction between wild-type and mutant *A. fumigatus* strains is performed by melting curve analysis
Resistance PCR and appropriateness of antifungal therapy

AzorMan study – B. Rijnders

Sensitivity of resistance PCR
Number of mutations detected
Strategy when R-frequency is low

Diagnosis IA
VCZ therapy
Resistance PCR
VCZ-S
VCZ-R
continue VCZ
Switch to appropriate AF therapy

appropriate
inappropriate

2 Days
Management strategy........New SWAB guideline

Maximum effort to diagnose

Appropriate drug

MIC-testing / resistance PCR

Prevent overuse

Unknown
VCZ / ISA + L-AmB
VCZ / ISA + Ecand
L-AmB

Confirmed S
VCZ / ISA
L-AmB
L-AmB / POS

Confirmed R
L-AmB
Ecand

Mixed
VCZ / ISA + L-AmB
VCZ / ISA + Ecand
Multidisciplinary mycology team

ID physician
Pharmacist
Microbiologist
Pulmonologist
Hematologist
Threat? yes

Global?
Acquired resistance frequency in A. fumigatus 2013 - 2017

- Screening for resistance of unselected clinical isolates using VIPcheck™
- Includes clinically not-relevant isolates
- Number of patients screened 600 to 814 per annum

Resistance frequency = number of patients with azoleR isolate / number of screened patients

5 university medical centers

Anticipated risk: Aspergillus resistance surveillance

Unselected isolates

Clinical data are not collected

Only culture positives

Variation between hospitals

80% to 90% environmental
Acquired resistance: global?

Resistance %
- >10
- Unknown/not yet detected
- Environmental only
- 1-5
- 5-10
- 0-1

Does your country have a resistance surveillance program in Aspergillus?

No international surveillance programs

Most clinical microbiology laboratories do not perform MIC testing of molds
Threat? yes

Global? yes

Forgotten?
An antibiotic is an antimicrobial drug used to treat bacterial infections in humans and animals. Like humans, animals (including farm animals) carry bacteria. When antibiotics are given to animals, the drugs kill most of the bacteria, but resistant bacteria can survive and multiply.

Do you worry about AMR?
Figure 2.6 Antibiotic use and AMR from 1990–2000 in selected countries

DDD: Defined Daily Doses
Total antibiotic use in outpatients versus prevalence of penicillin-nonsusceptible *Streptococcus pneumoniae* in 20 industrialized countries.
AMR ⊄ ABR
<table>
<thead>
<tr>
<th>Program</th>
<th>Period</th>
<th>Budget (M€)</th>
<th>Molds included?</th>
<th>No projects awarded</th>
<th>No fungal projects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infectieziektebestrijding</td>
<td>2006 - 2011</td>
<td>12.6</td>
<td>Tolerated</td>
<td>33</td>
<td>1</td>
</tr>
<tr>
<td>Q-koorts</td>
<td>2010-2014</td>
<td>3</td>
<td>No</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Infectieziektebestrijding</td>
<td>2014 - 2017</td>
<td>17</td>
<td>Fungal resistance excluded</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>Ronde 1 (Non-alimentaire zoönosen)</td>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Ronde 2</td>
<td></td>
<td></td>
<td></td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Ronde 3 (Non-alimentaire zoönosen)</td>
<td></td>
<td></td>
<td></td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Ronde 4</td>
<td></td>
<td></td>
<td></td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Priority Medicines Antimicrobiële Resistentie</td>
<td>2009 - 2018</td>
<td>14.76</td>
<td>Fungi excluded</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>Antibioticaresistentie</td>
<td>2016 - 2023</td>
<td>16</td>
<td>Fungi excluded</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Ronde 1</td>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Ronde 2</td>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td><strong>Totaal</strong></td>
<td></td>
<td><strong>63.36</strong></td>
<td></td>
<td><strong>129</strong></td>
<td><strong>1 (0.8%)</strong></td>
</tr>
</tbody>
</table>
Fungal disease not perceived as a public health threat

Fungal disease unit?

Cib/RIVM  ❌
eCDC  ❌
WHO  ❌
CDC  ✔
Azole resistance in *A. fumigatus*: Under the radar........

- **Medical**: Not a public health threat....
- **Agricultural**: Not a plant pathogen....
- Lack of awareness
Change?  Influenza-associated aspergillosis

Am J Respir Crit Care Med. 2017;196:524-527
An very unfavorable combination......
Influenza-associated aspergillosis

- Occurs in 1 in 5 patients admitted to the ICU with influenza
- 1 in 3 patients were previously healthy
- 1 in 3 patients had azole resistance
- 1 in 2 patients died

Emergence of drug resistance in *Candida*

Emergence of drug resistant *Candida auris*

*Am J Respir Crit Care Med. 2017;196:524-527*
Fungal disease not perceived as a public health threat

Fungal disease expertise?

- Cib/RIVM: ✘
- eCDC: ✘
- WHO: ✘
- CDC: ✓

Center of Expertise in Mycology
Radboudumc/CWZ

antibiotic resistance threats
CDC – antibiotic resistance threats

Antibiotic Resistance Threats in the United States 2019

Urgent Threats
- Carbapenem-resistant *Acinetobacter*
- *Candida auris* (C. auris)
- *Clostridioides difficile* (C. difficile)
- Carbapenem-resistant Enterobacteriaceae (CRE)
- Drug-resistant *Neisseria gonorrhoeae* (N. gonorrhoeae)

Serious Threats
- Drug-resistant *Campylobacter*
- Drug-resistant *Candida*
- Extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae
- Vancomycin-resistant *Enterococci* (VRE)
- Multidrug-resistant *Pseudomonas aeruginosa* (P. aeruginosa)
- Drug-resistant nontyphoidal *Salmonella*
- Drug-resistant *Salmonella* serotype Typhi
- Drug-resistant *Shigella*
- Methicillin-resistant *Staphylococcus aureus* (MRSA)
- Drug-resistant *Streptococcus pneumoniae* (S. pneumoniae)
- Drug-resistant Tuberculosis (TB)

Concerning Threats
- Erythromycin-resistant group A *Streptococcus*
- Clindamycin-resistant group B *Streptococcus*

Watch List
- Azole-resistant *Aspergillus fumigatus* (A. fumigatus)
- Drug-resistant *Mycoplasma genitalium* (M. genitalium)
- Drug-resistant *Bordetella pertussis* (B. pertussis)

November 2019
Threat? yes
Global? yes
Forgotten? yes
Deadly flower power? Imported tulip bulbs spread antifungal resistance

**Date:** May 18, 2017  
**Source:** Trinity College Dublin  
**Summary:** Tulip and narcissus bulbs imported into Ireland from the Netherlands may be acting as vehicles for the international spread of a drug-resistant fungus -- with potentially fatal consequences. Experts advise people not to plant bulbs near hospitals or to gift them to at-risk patients.

**RELATED TOPICS**  
Health & Medicine  
- Diabetes  
- HIV and AIDS  
- Pharmacology  
- Today's Healthcare  
Plants & Animals

**FULL STORY**

Tulip bulbs imported into Ireland from the Netherlands may be helping to spread resistance to vital antifungal medicines called Triazoles that treat potentially fatal fungal infections. That is according to research showing that the inter-country transfer of bulbs of Holland’s iconic flowers may inadvertently have opened up a new transport route for a particularly nasty fungal pathogen called Aspergillus fumigatus.
The tsunami of resistance mechanisms involving TR

- 1998: 34 bp L98H
- 2000: -
- 2005: 53 bp
- 2010: -
- 2006: 46 bp Y121F/T289A
- 2009: 46 bp Y121F/T289A
- 2012: 46 bp
- 2012: Y121F/T289A/G448S

mBio 2017;8(3)
A. fumigatus is able to complete its life cycle in the presence of azole residues.

Hot spot

- Flower bulb waste
- Green waste
- Wood chippings waste
- Strawberry waste
Where?

application of fungicides

collection of organic waste

mature compost

Emerg Infect Dis. 2019;25:1347-1353
Commercial composting

- Grass: >500, >200
- Leaves: 0, 0
- Wood chippings: >500, 200
- Processing: >200, >200
- Processing: >100, 10
- Mature compost: 60, 0

Temperatures:
- 65-70 °C
- 50-60 °C

Emerg Infect Dis. 2019;25:1347-1353
Where?

application of fungicides

mature compost

collection of organic waste

composting
Discuss at EU-level

Intensify surveillance and develop better diagnostic tests

Source control – re-evaluate authorized azole fungicides

Prevent storage and facilitate early composting

de schimmel *Aspergillus fumigatus* tegen azolen

Geachte Voorzitter,

Op 17 juni jl. heb ik uw Kamer geïnformeerd over het onderzoeksrapport van het RIVM over de ontwikkeling van resistentie bij de schimmel *Aspergillus fumigatus* tegen azolen (Kamerstuk 27858, nr. 460). Ik heb in deze brief toegezegd uw Kamer te informeren over het advies van het College voor de toelating van
How are patients infected? – potential sources

other?
How are patients infected? – potential sources

tea

pepper

onions
Conclusions

Threat?  yes  Develop better diagnostic tests
          New drug targets

Global?  yes  Set up international surveillance
          Include fungal resistance in One-
          health programs

Forgotten?  yes  Prioritize fungal resistance
              research
              Include fungal resistance in AMR
              initiatives