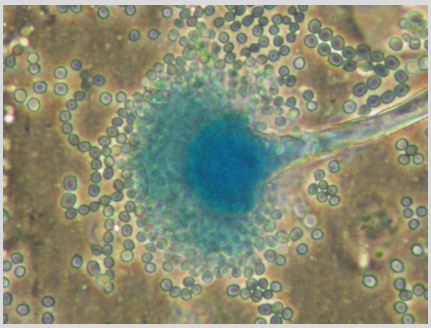
# Resistance in Aspergillus: An emerging problem?



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## Agenda

- Susceptibility testing
  - Azoles, candins and amphotericin
- *Aspergillus* and Intrinsic resistance
- Acquired resistance
  - Azole resistance
  - Candin resistance

# Susceptibility testing of moulds

- Reference methods
  - CLSI M-38A
  - EUCAST
  - Visual reading of growth
  - Ampho & azoles: MIC no growth
    Candins: MEC aberrant growth
- Alternatives
  - Etest
  - Agar-dilution


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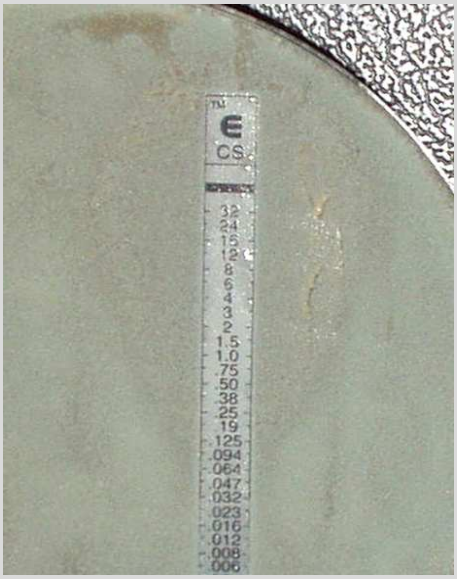
# Caspofungin S and R *A. fumigatus*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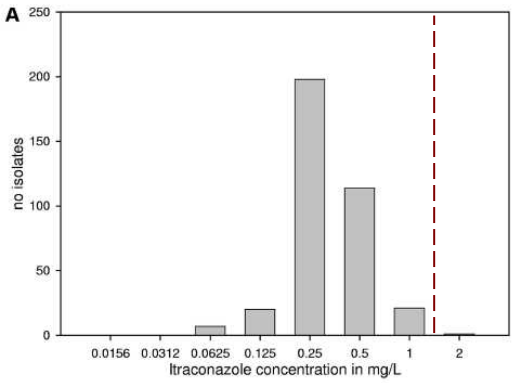
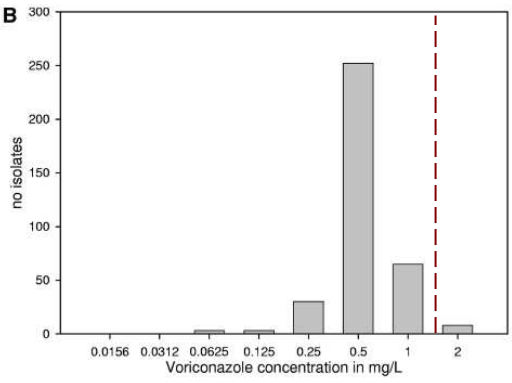
# *A. fumigatus* Epidemiological Cut off Values

Itraconazol MIC: ≤ 1 µg/ml




Voriconazole MIC: ≤ 1 µg/ml




Rodriguez-Tudela AAC 2008

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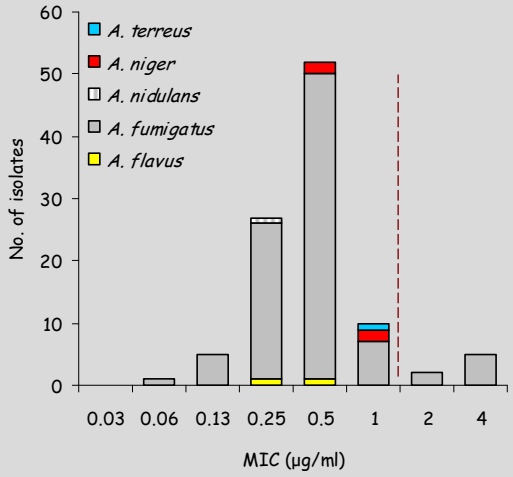
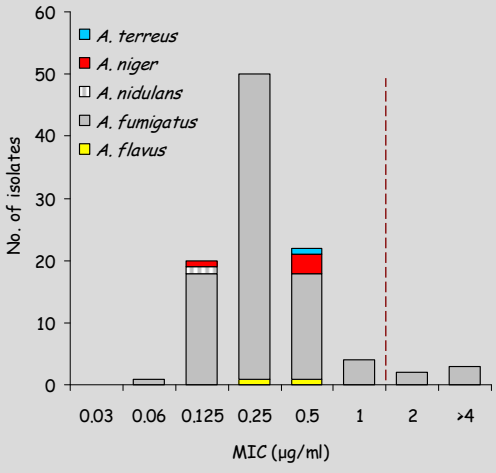
# MIC for DK *Aspergillus* isolates

Itraconazole




Voriconazole

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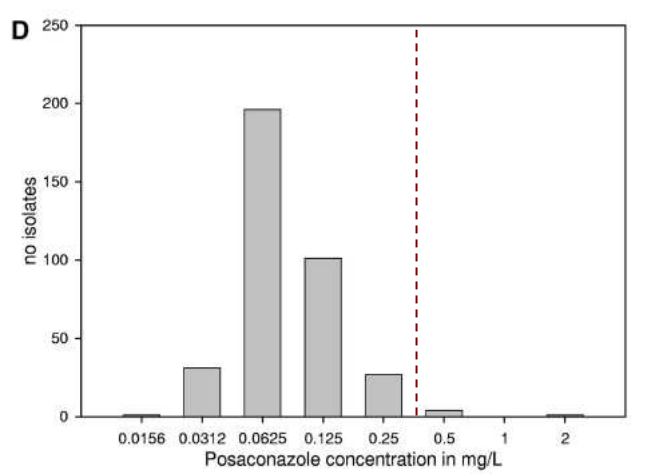
# *A. fumigatus* Epidemiological Cut off Values

Posaconazole MIC: ≤ 0.25 µg/ml

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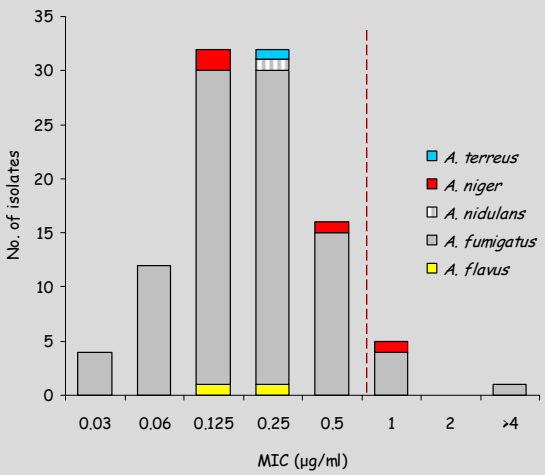
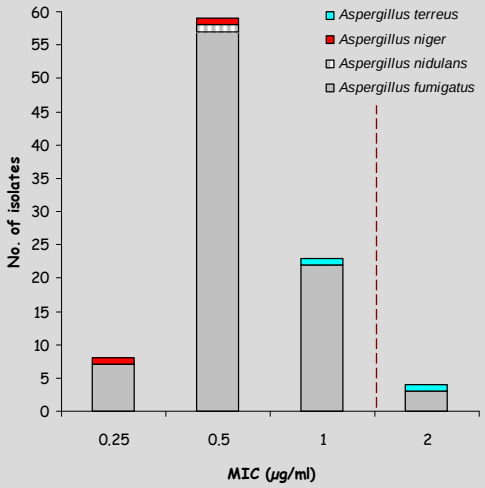
# MIC for DK *Aspergillus* isolates

Posaconazole




Amphotericin B

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# Suggestion for Cut Off values

| | Susceptible |
|---|---|
| Amphotericin B | ≤ 1 µg/ml |
| Itraconazole | ≤ 1 µg/ml |
| Voriconazole | ≤ 1 µg/ml |
| Posaconazole | ≤ 0.25 / 0.5 µg/ml |

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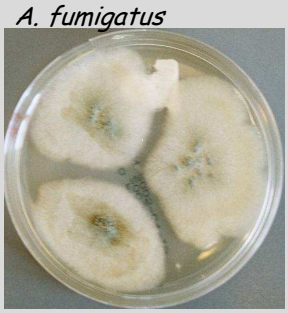
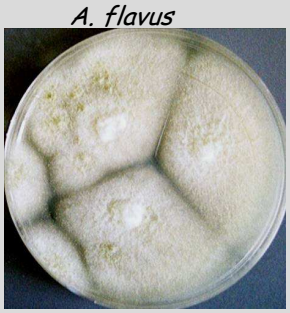
# *Aspergillus* species

*A. fumigatus*



*A. flavus*



*A. terreus*

*A. niger*

***Aspergillus:***

- 662 asexual forms
- 45 with medical significance

**Most frequent:**

*A. fumigatus, A. flavus, A. terreus* and *A. niger*

***Aspergillus* section *Fumigati***

- 10 strict anamorphs
- 23 teleomorphs

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# *Aspergillus* section *Fumigati*

- 10 anamorphs
  - *A. brevipes*
  - *A. duricaulis*
  - ***A. fumigatiaffinis***
  - ***A. fumigatus***
  - ***A. fummisynnematus***
  - ***A. lentulus***
  - *A. novofumigatus*
  - *A. turcosus*
  - *A. unilateralis*
  - ***A. viridinutans***
- 23 telemorphs (*Neosartorya*)
  - *N. assulata*
  - *N. aurata*
  - *N. aureola*
  - *N. australensis*
  - *N. coreana*
  - *N. denticulata*
  - *N. ferenczii*
  - *N. fennelliae*
  - ***N. fischeri***
  - *N. galapagensis*
  - *N. glabra*
  - ***N. hiratsukae***
  - *N. laciniosa*
  - *N. mulplicata*
  - *N. papuensis*
  - ***N. pseudofischeri***
  - *N. quadricincta*
  - *N. spinosa*
  - *N. stramenia*
  - *N. spathulata*
  - *N. tatenoi*
  - *N. udagawa*
  - *N. warcupii*

Isolates in red have been isolated from humans

Samson in "*Aspergillus fumigatus* and Aspergillosis" 2008

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# Intrinsic and Primary resistance

Intrinsic: √ Primary: (√)

| | AMB | Azoles | Echinocandins |
|---|---|---|---|
| *Aspergillus* section *fumigati* | | | |
| *A. fumigatiaffinis* | √ | √ | |
| *A. lentulus* | √ | √ | (√) |
| *N. pseudoficheri* | | √ | |
| *A. viridinutans* | | √ | |
| *A. terreus* | √ | | |
| *A. ustus* | √ | √ | √ |
| *A. flavus* | (√) | | (√) |
| *A. allilaceus* | (√) | | (√) |

Alcazar-Fuoli AAC 2008; Perlin & Mellado in "*Aspergillus fumigatus* and Aspergillosis" 2008

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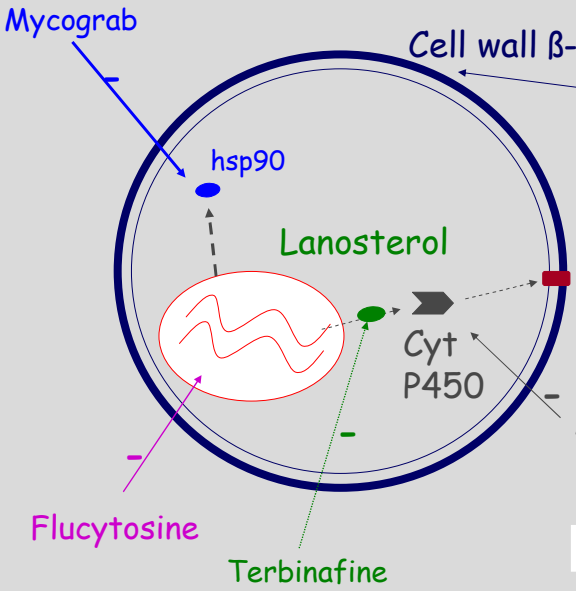
# Systemic Antifungals: Mode of Action




**Candins**
- Anidulafungin
- Caspofungin
- Micafungin

**Polyenes**
- Amphotericin B

**Azoles**
- Ketoconazole
- Flu- & itraconazole
- Voriconazole & posaconazole

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# Resistance mechanisms in *Aspergillus*

| | Target | Target Gene Mutations | Target up regulation | Efflux pumps |
|---|---|---|---|---|
| Azoles | P450 demethylase | *CYP51A* | *CYP51A*+ Promotor | ✓ |
| Candin | Glucan synthase | *FKS1* | ✓ | |
| Amph | Ergosterol | ? | ? | |

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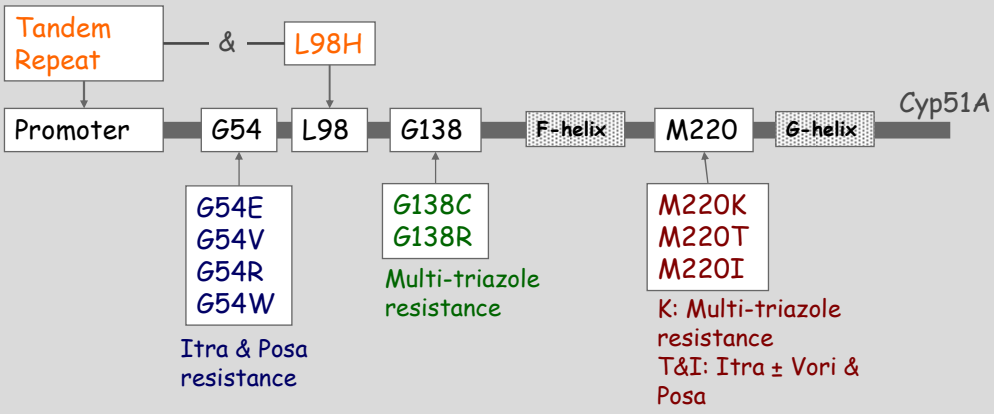
# Azole resistance: Cyp51A mutations

Up-regulation of gene expression
Multi-triazole resistance




New mutations are continuously reported
Azole resistant isolates without Cyp51A mutations have been reported

Howard Int J AA 2006; Perlin & Mellado in "*Aspergillus fumigatus* and Aspergillosis" 2008

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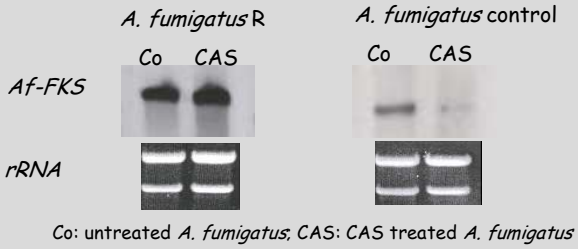
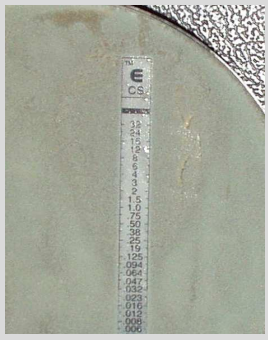
# Echinocandin resistance

- Mutations in FKS1 gene coding glucan synthase
- Over-expression of the gene




Co: untreated *A. fumigatus*; CAS: CAS treated *A. fumigatus*



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# Elevated MICs to azoles and/or AMB

6/169 (3.5%) *Aspergillus* isolates in DK 2007

| | ITR | POS | VOR | AMB |
|---|---|---|---|---|
| *A. fumigatus* [a] | >4 | >4 | 2 | 0.5 |
| *A. fumigatus* | 2 | 0.5 | 2 | 2 |
| *A. fumigatus* | >4 | 0.125 | 1 | 0.38 |
| *A. fumigatus* | 0.25 | 0.25 | 0.25 | 1.5 |
| *A. terreus* | 2 | 2 | 2 | 0.75 |
| *A. terreus* | 2 | 0.125 | 1 | 1.5 |

[a] M220K mutation


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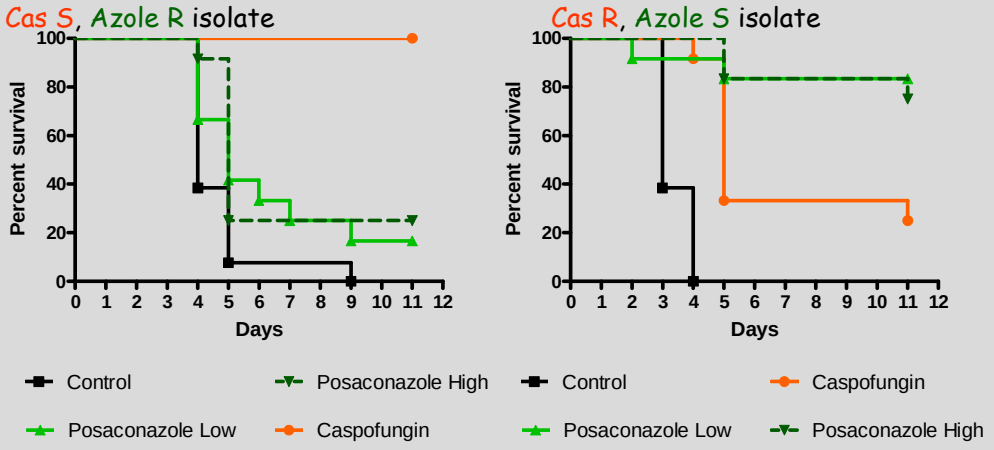
# Caspofungin S and R *A. fumigatus*

Mice inoculated with A fumigatus and subsequently treated for 10 days

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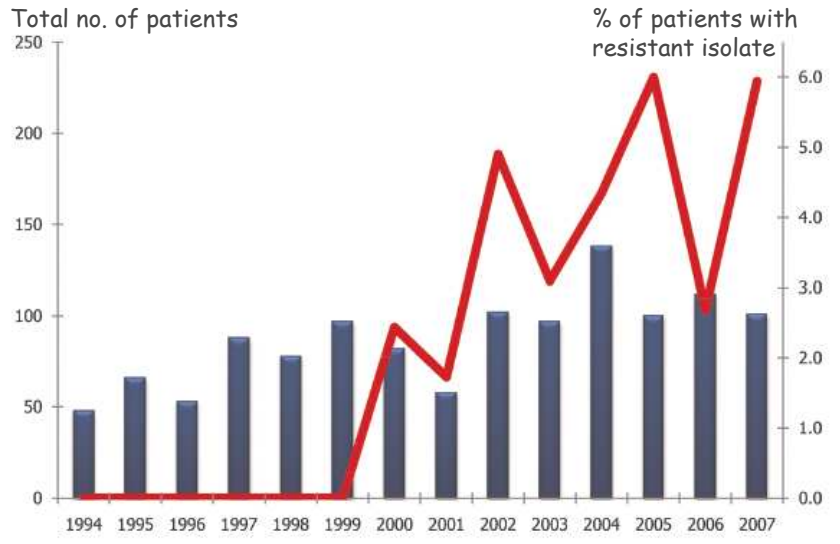
# Azole resistance in *A. fumigatus* NL




Mechanism:

94% L98H + TR

Snelders PLoS Med 2008

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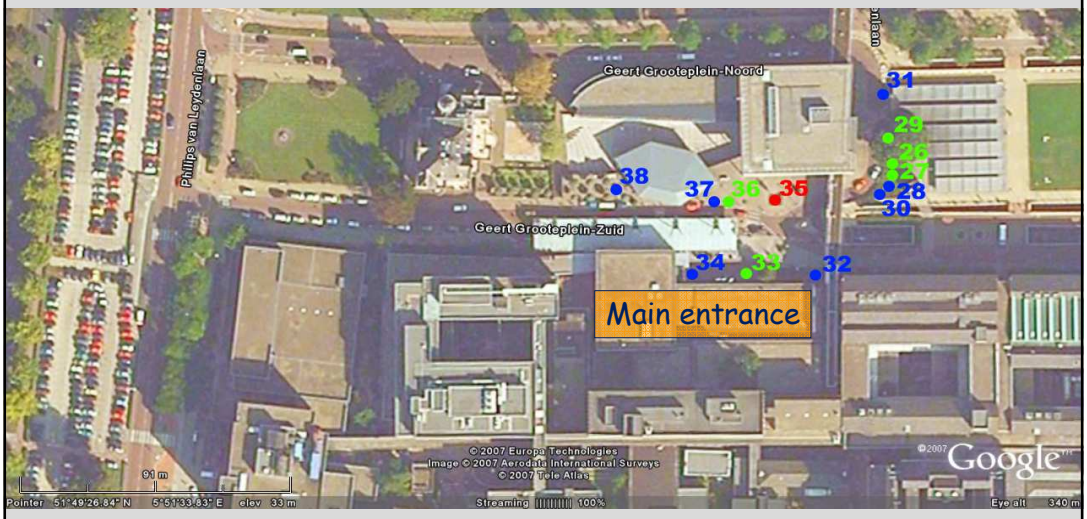
# Environmental cultures




*A. fumigatus* Azole S

Azole R (L98H + TR)

Courtesy Verweij ECCMID 2008

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# Conclusion

- Increasing no of reports on clinical resistance
  - Uncommon species with intrinsic resistance
  - Isolates with acquired resistance
- Azole resistance
  - Cyp51A mutations
  - Promoter TR + L98H mutation – overexpression
  - Others as well
  - Itraconazole alone or also Posa / Voriconazole
- Echinocandin resistance
  - FKS1 mutations
  - Over expression of the target enzyme

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