

Azole resistant *Aspergillus fumigatus* in Denmark: a laboratory based study on resistance mechanisms and genotypes

Rasmus H Jensen¹, Ferry Hagen², Karen MT Astvad¹, Alexander Tyron¹, Jacques F Meis^{2,3}, *Maiken C Arendrup¹

¹) Unit of Mycology, Statens Serum Institut, Copenhagen, Denmark; ²) Dept Med Microbiol & Inf Dis, Canisius-Wilhelmina Hospital, Nijmegen, the Netherlands and

³) Dept Med Microbiol, Radboudumc, Nijmegen, the Netherlands.

Introduction

Azole resistant *Aspergillus fumigatus* was first found in clinical samples in 2007 in Denmark.

In order to investigate the contemporary epidemiology of azole resistant *A. fumigatus* a laboratory based retrospective study was performed including all samples received at the national mycology reference laboratory in 2010-2014.

The underlying resistance mechanisms were described and the accumulated microsatellite genotypes of Danish isolates compared with those of foreign isolates.

Materials & Methods

Clinical isolates: 1162 *A. fumigatus* isolates.

Environmental isolates: 133 *A. fumigatus* isolates from air and soil, autumn of 2014.

Identification: morphology, thermo-tolerance (48°C), \pm beta-tubulin sequencing.

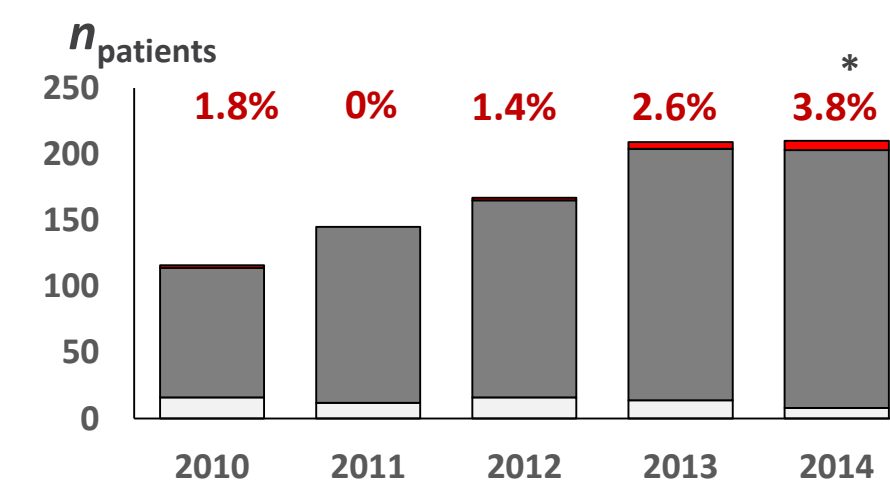
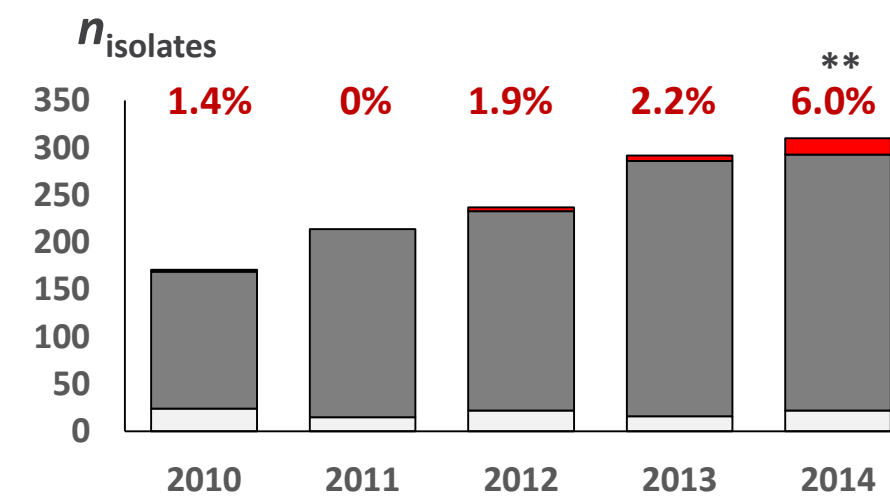
Susceptibility testing: 1098 (94.5%) isolates were screened using azole agars (itraconazole 4 mg/L, voriconazole 1 mg/L and posaconazole 0.5 mg/L) and susceptibility tested when relevant by the EUCAST E.Def 9.2 method.

Molecular tests:

- *CYP51A* sequencing for azole "R" isolates (64).
- STRAf genotyping for azole "R" isolates (64) & a representative collection (120) of clinical and environmental wild-type DK isolates as well as 1822 genotypes from *A. fumigatus* isolates obtained from 15 countries around the world.

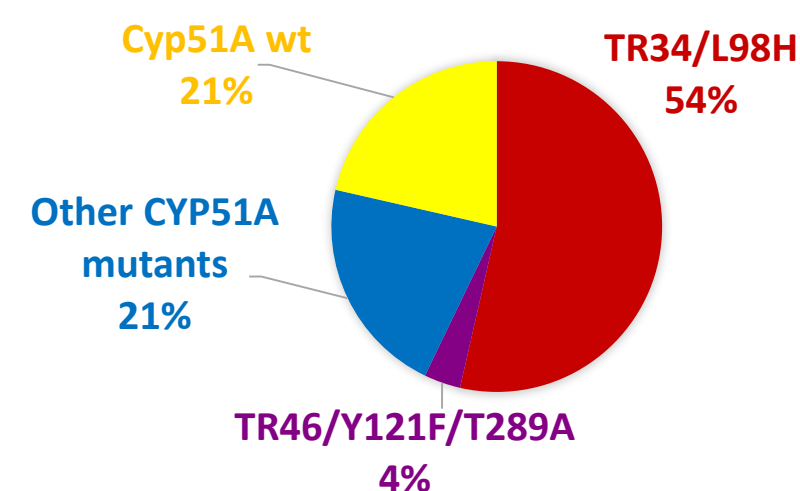
Results: Azole Susceptibility

Clinical isolates 2010-14:



* $p < 0.05$, ** $p < 0.001$ (Chi-square test)

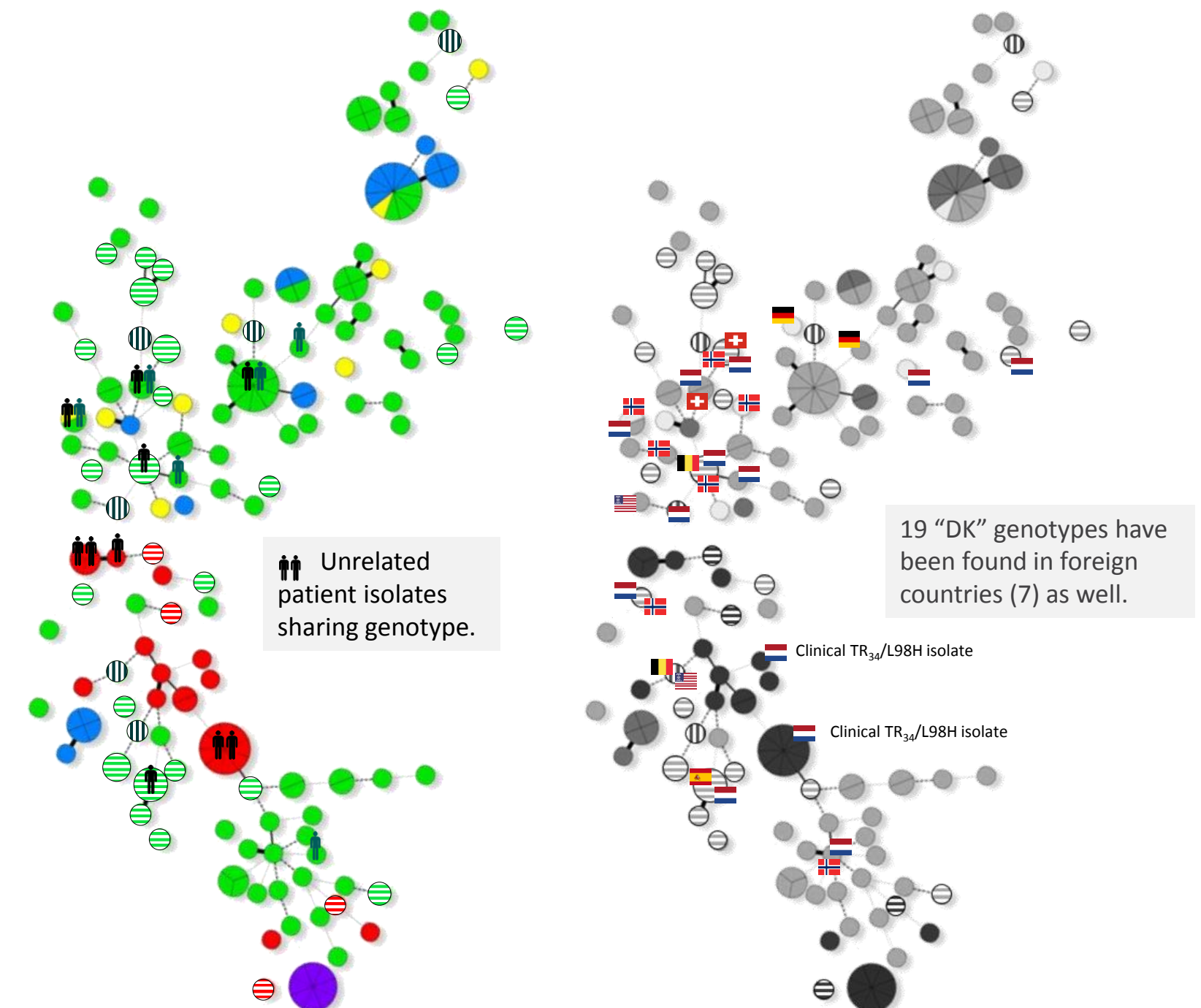
CYP51A Genotype



Environmental isolates 2014

All were susceptible (wild type).

Results: STRAf Typing



Conclusion

1. Azole resistance among clinical *A. fumigatus* isolates in DK is increasing.
2. Environmental resistance mechanisms dominate.
3. Susceptibility testing is demanded for treatment requiring infections.
4. Shared genotypes among isolates with different origin were frequent.
5. Our data are in agreement with the hypotheses on clonal expansion and of one single ancestor of the TR₃₄/L98H clones.