

EP0002

ePoster Session

Advances in medical mycology

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

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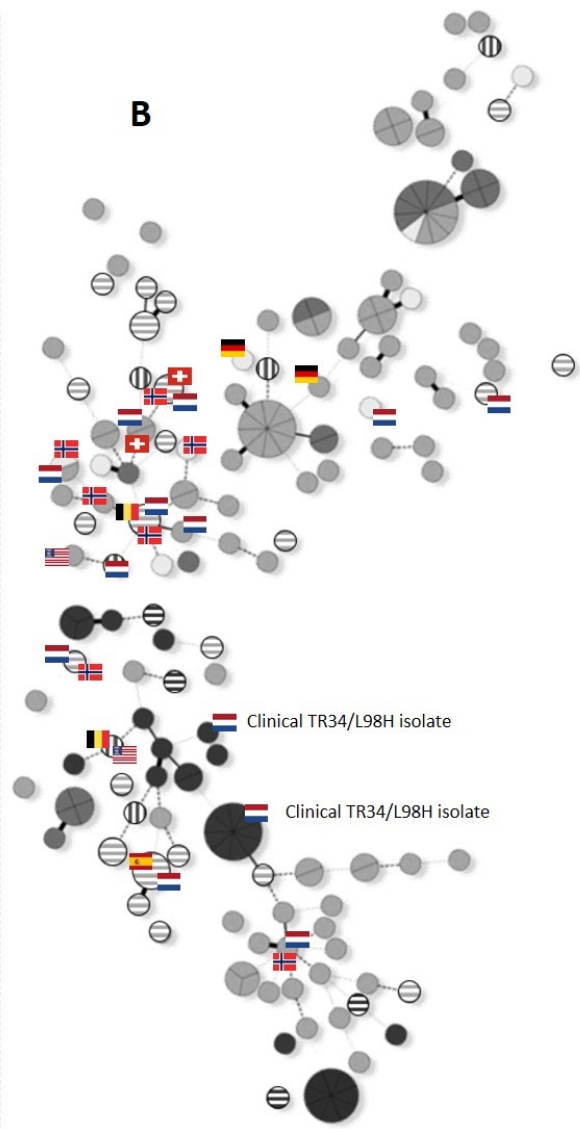
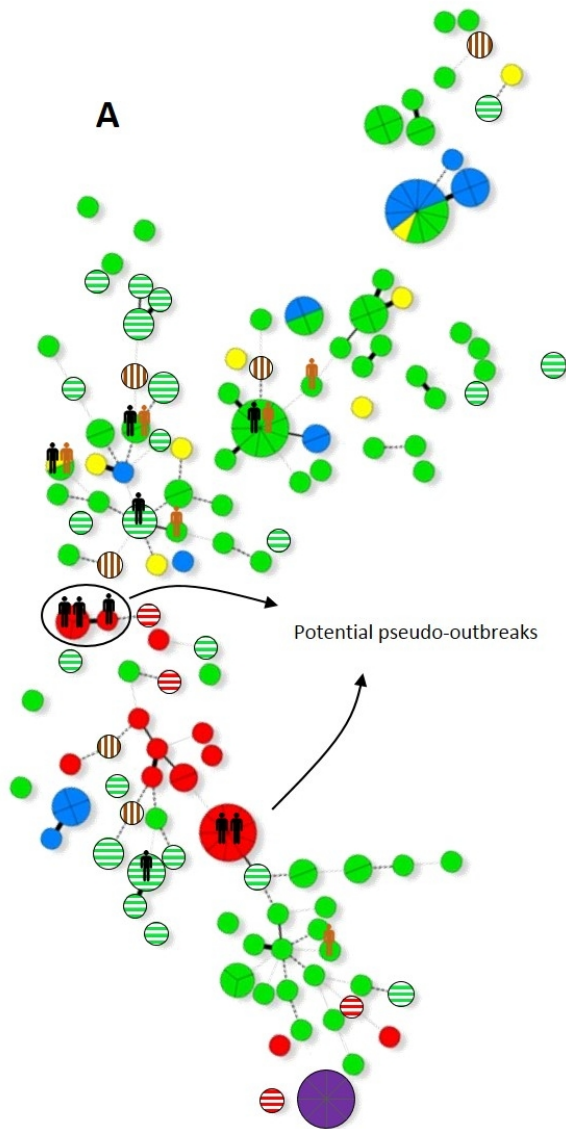
Background: Azole resistant *Aspergillus fumigatus* was first found in Danish clinical samples in 2007. In order to investigate the contemporary epidemiology of azole resistant *A. fumigatus* in Denmark a laboratory based retrospective study was performed including all samples received at the national 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

Material/methods: A total of 1162 *A. fumigatus* isolates were identified by morphology, thermo-tolerance (48°C) and beta-tubulin sequencing. The majority, comprising 1098 isolates (94.5%), were screened for azole resistance 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 reference method. In addition, an environmental survey was carried out during autumn of 2014 and 133 *A. fumigatus* isolates were isolated from air samples and characterised as described above. Resistant isolates were *CYP51A* sequenced and genotyped using the Short Tandem Repeat *Aspergillus fumigatus* (STRAf) microsatellite assay. STRAf genotypes were compared to those of a representative collection of clinical and environmental wild-type isolates as well as 1822 genotypes from *A. fumigatus* isolates obtained from 15 countries around the world.

Results: Through 2010-2014 an increasing prevalence of azole resistance was observed in Denmark, 1.4%-6% isolates ($P<0.001$) and 1.8%-3.8% patients ($P<0.05$). Azole resistance mechanisms were dominated by the *CYP51A* variants TR₃₄/L98H and TR₄₆/Y121F/T289A (57%), while the remaining isolates harboured five different *CYP51A* mutations (21%) or were *CYP51A* wild-type (21%). All 133 environmental *A. fumigatus* isolates were azole susceptible. STRAf analysis revealed 120 unique genotypes among 184 genotyped Danish *A. fumigatus* isolates (Fig). One potential hospital outbreak involving six patients was examined using STRAf genotyping of six clinical and six air-filter isolates but no shared genotypes were identified. Overall, seven (5.8%) Danish genotypes were shared between isolates with different origin (Figure, A), 19 (15.8%) were shared with foreign genotypes and two out of 17 (11.8%) genotypes of isolates carrying the TR₃₄/L98H resistance mechanisms were identical to two Dutch TR₃₄/L98H isolates (Figure, B).

Conclusions: Azole resistance among clinical *A. fumigatus* isolates in Denmark is increasing and dominated by resistance mechanisms derived from the environment. This may complicate the

management of patients with invasive *A. fumigatus* infections and emphasises the demand for susceptibility testing. Genotyping revealed a high degree of shared genotypes among isolates with different origins (clinical, environmental and/or geographical), which could support previous hypotheses on clonal expansion. The hypothesis of one single ancestor of the TR₃₄/L98H clones may still be relevant to pursue and would require further and more thorough genetic analyses such as whole genome sequencing.



- Azole susceptible (⊖ environmental isolate from RH)
- Azole resistant, *CYP51A*: wild-type
- Azole resistant, *CYP51A*: G54W, M220K, P216L etc.
- Azole resistant, *CYP51A*: TR₃₄/L98H (⊖ environmental isolate)
- Azole resistant, *CYP51A*: TR₄₆/Y121F/T289A
- 👤 Roskilde outbreak patient isolate (⊖ air filter isolate)
- 👤👤 Unrelated patient isolates sharing genotype
- 👤⊖ Patient and environmental isolates sharing genotype

Country (number of identical STRAf types/total in %)

- 🇳🇴 NO (7/209 = 3.3%)
- 🇳🇱 NL (13/768 = 1.7%)
- 🇩🇪 DE (1/103 = 1%)
- 🇧🇪 BE (2/108 = 1.9%)
- 🇪🇸 ES (1/236 = 0.4%)
- 🇺🇸 US (1/133 = 0.8%)
- 🇨🇭 CH (2/72 = 2.8%)