



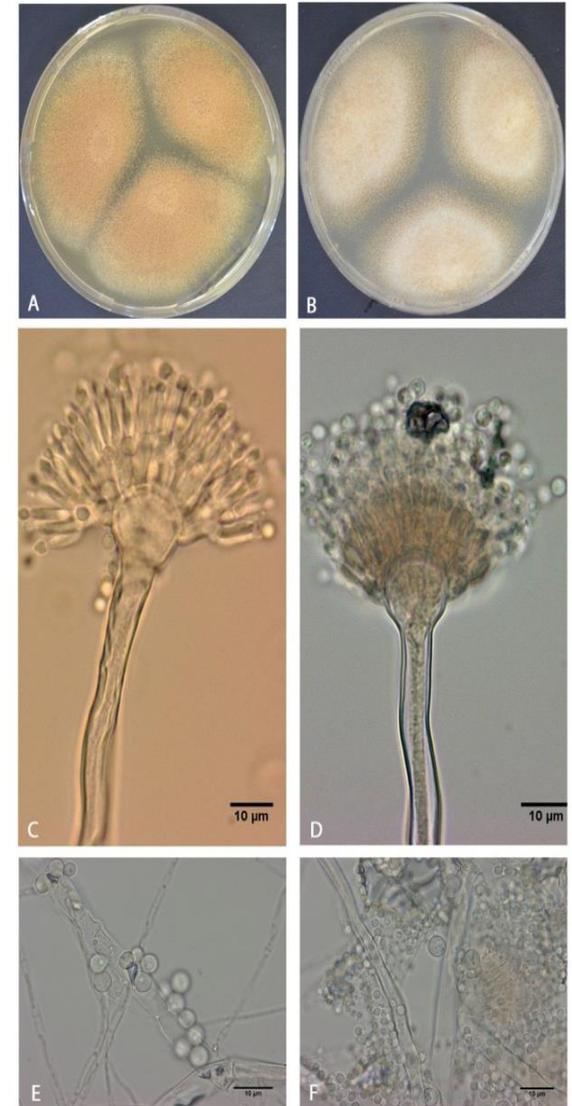
Pilzmorphologien und deren
Resistenzverhalten gegenüber



Antimykotika
Cornelia Lass-Flörl

A. *terreus* strains

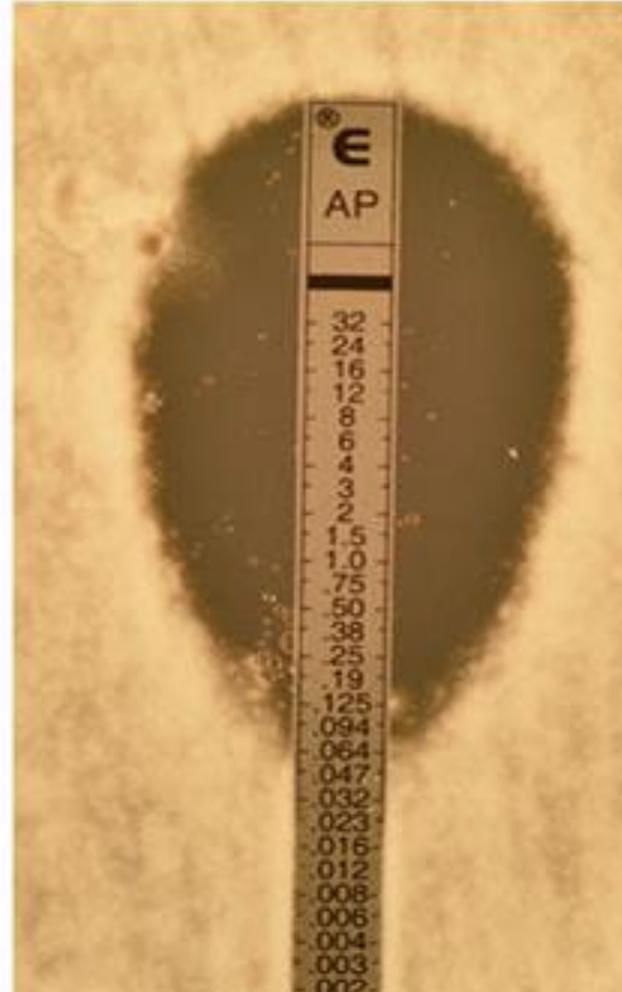
- ✓ **ATS: *Aspergillus terreus* AmB susceptible**
 - ✓ Italy and Stanford California
 - ✓ 3 strains (T77, T164, T175)
- ✓ **ATR: *Aspergillus terreus* AmB resistant**
 - ✓ Innsbruck
 - ✓ T90, T9
- ✓ Species identification via PCR sequencing
- ✓ Special feature: aleuricondida or accessory conidia



T9



T77

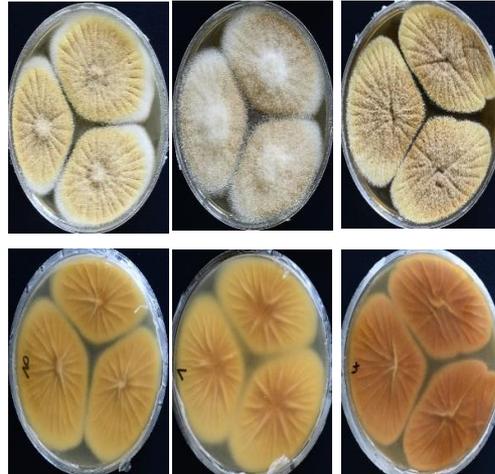
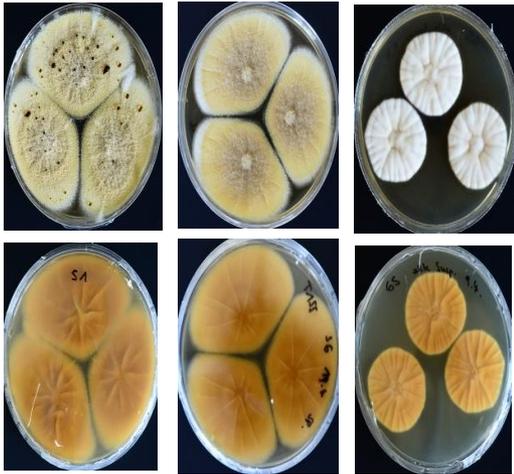
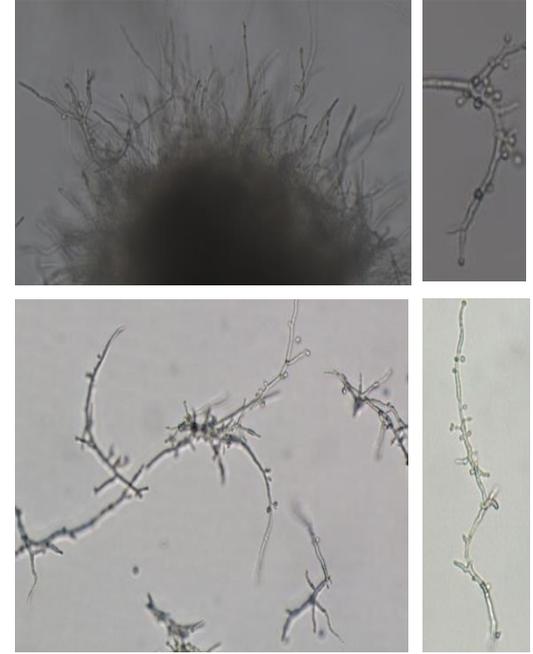




A. neoafricanus 262
A. terreus s.s. T152
A. floccosus 204



A. hortai 142
A. alabamensis 121
A. citrinoterreus 263



- release of pigment into growth media was strain specific rather than species specific
- all species except *A. floccosus* formed accessory conidia in Sabouraud media after 3- 5 days



T109

225

320

119



T130

T113

T92

T154



175

262



morphological sectors in molds

- ▶ (i) reduced sporulation
- ▶ (ii) reduced pigmentation
- ▶ (iii) increased growth rates



morphological sectors in molds

- ▶ Such morphological heterogeneity was reported to be a result of mutations or environmental adaptations, the clinical relevance is unknown.



morphological sectors in molds

- ▶ we aimed to investigate the frequency of sectorization in three-point inoculation subcultures or primary *Aspergillus* cultures and analyzed the impact on antifungal susceptibility testing.







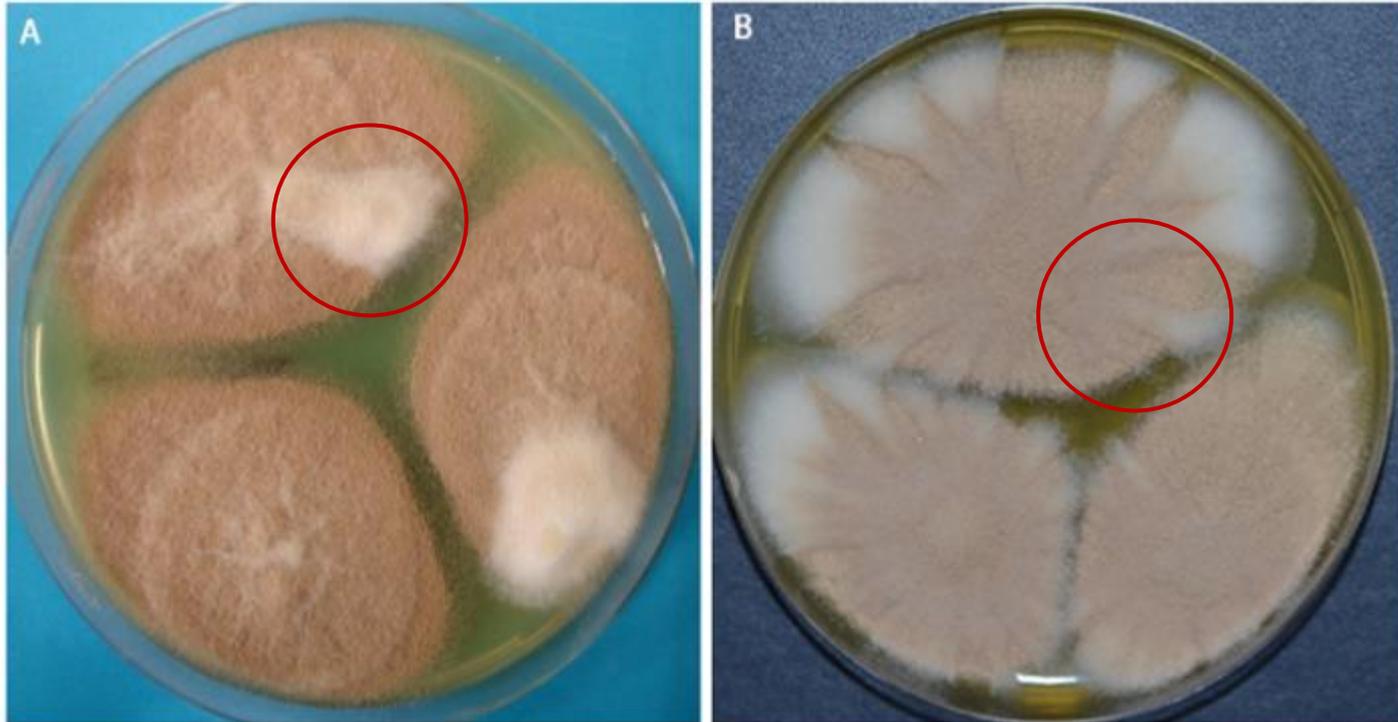




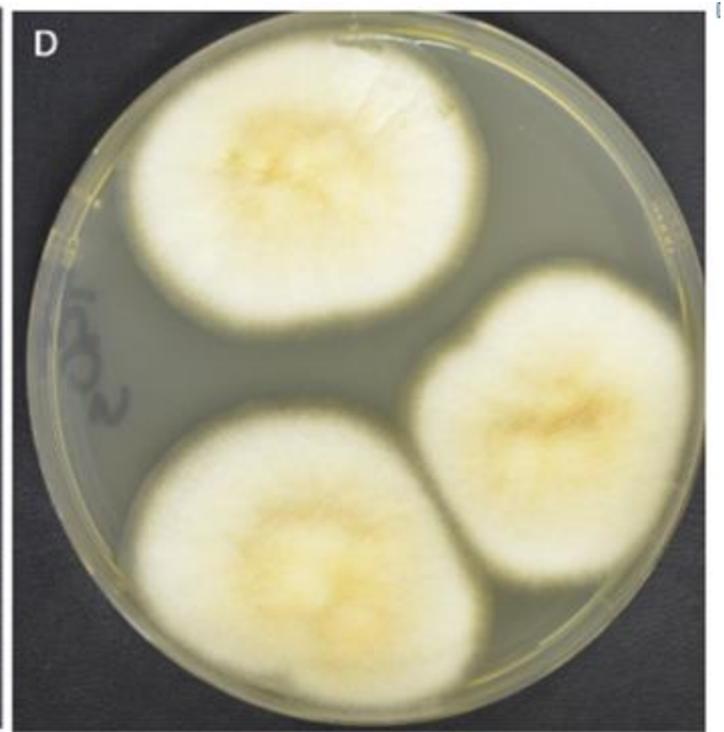
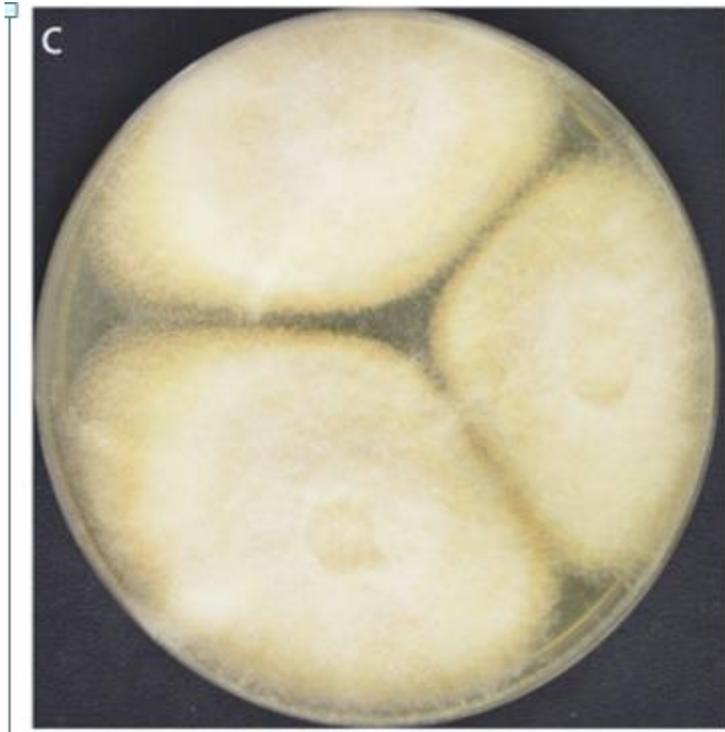








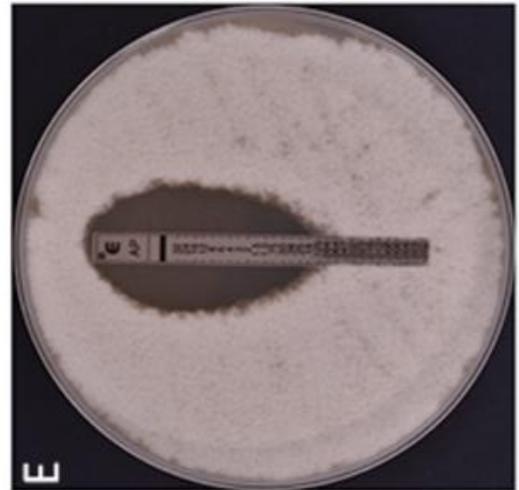
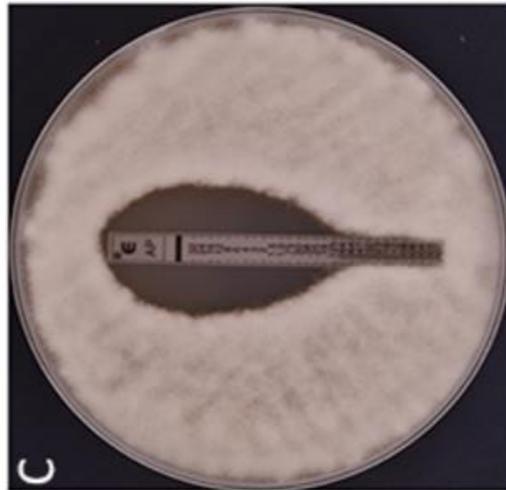
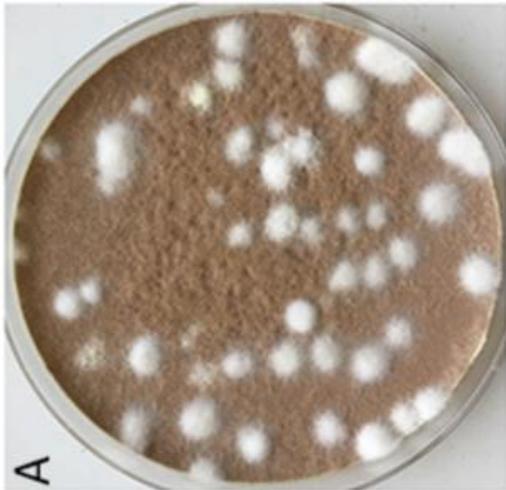
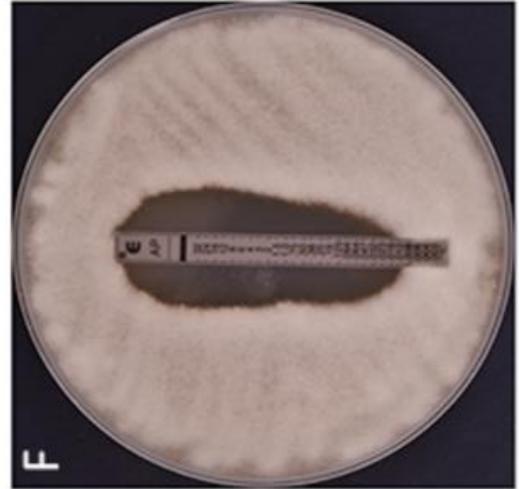
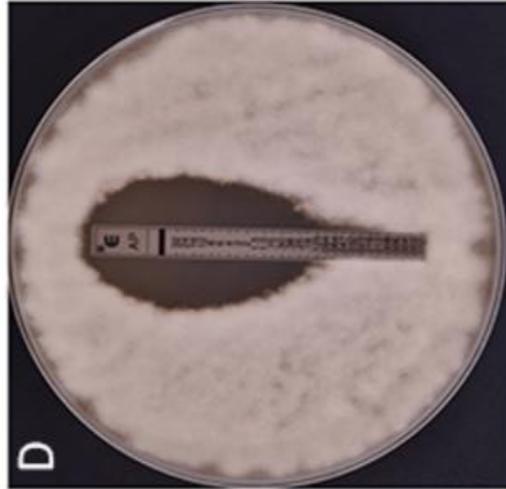
Culture degeneration sectoring



morphological sectors in molds

- ▶ *Aspergillus fumigatus* complex (n=75)
- ▶ *Aspergillus terreus* complex (n=32),
- ▶ *Aspergillus flavus* complex (n=19)
- ▶ *Aspergillus niger* complex (n=14)
- ▶ investigated during routine cultivation and processing of various respiratory specimens on Sabouraud dextrose agar (48-72 hours at 37°C)
- ▶ Sector and non-sector areas of pure cultures affected were again subcultured and further analyzed using E-test®
- ▶ the inoculum prepared consisted either of sector or non-sectored spores
- ▶ Amphotericin B (AMB), posaconazole (POS), voriconazole (VOR), anidulafungin, caspofungin, and
- ▶ micafungin





Species	Group	MIC Etest (mg/L)		MIC EUCAST (mg/LI)	
		Mean ± SD	Range	Mean	Range
<i>A. terreus</i>	ATR	32 ± 0.0	32	3.2 ± 1.1	2-4
	ATS	0.14 ± 0.09 ^d	0.012- 0.25	0.55 ± 0.27 ^d	0.5-1
	ATSec	0.18 ± 0.19 ^d	0.012-0.5	0.8 ± 0.27 ^e	0.5-1



morphological sectors in molds

- ▶ 27% of *Aspergillus* cultures showed sectorization including smooth, star, irregular wrinkle, mottled, fuzzy, or volcano-like colonies.
- ▶ Volcano-like phenotypes were restricted to specimens obtained from cystic fibrosis patients only.
- ▶ 45% of sectoring phenotypes were reversible, 55% (mainly in *A. terreus* complex) were permanent.
- ▶ The majority of sector-subcultures (63%) showed significant differences in MICs when compared to non-sectoring cultures.



morphological sectors in molds

- ▶ *A. terreus* and AMB were strongly affected, resulting in AMB susceptible sector isolates
- ▶ *A. fumigatus* and the various echinocandins displayed both, resistant and susceptible sector isolates
- ▶ azoles were restricted to a lesser extent showing minor differences for VOR and POS.



morphological sectors in molds

- ▶ Sector progenies differed significantly from sector-free isolates
- ▶ Were highest in CF patients or chronically infected pts
- ▶ hence we raise the awareness that the presence of sector formation in cultures might strongly impact antifungal susceptibility patterns
- ▶ Colonies selected for inoculum preparation for antifungal susceptibility testing should be free of any sectors to avoid MIC discrepancies and unreproducible MIC values.





Vielen Dank für die Aufmerksamkeit!

