# Prevalence and characterisation of azole-resistant *Aspergillus fumigatus* isolates in German cystic fibrosis patients

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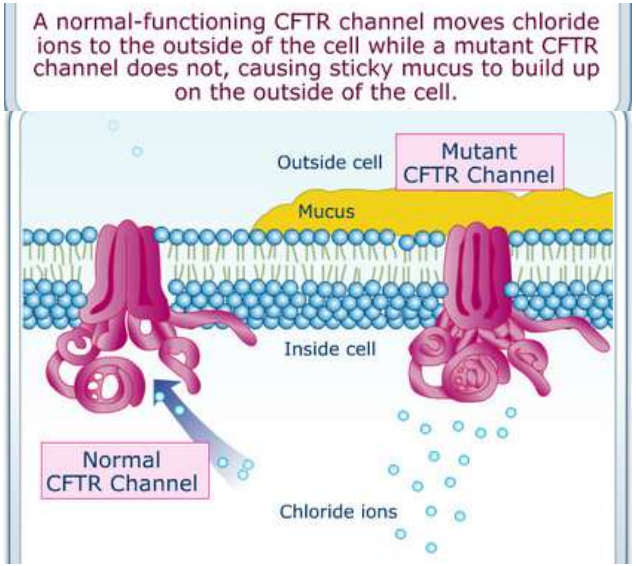
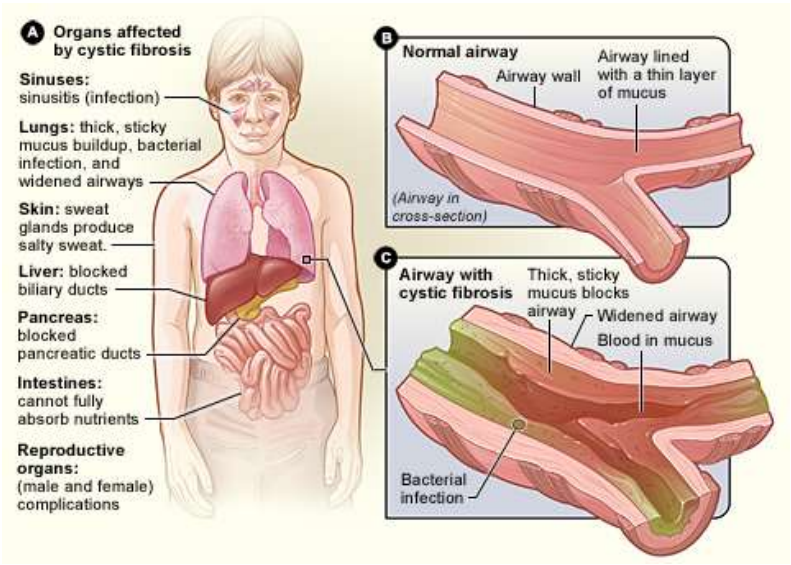
# What is cystic fibrosis (CF)?

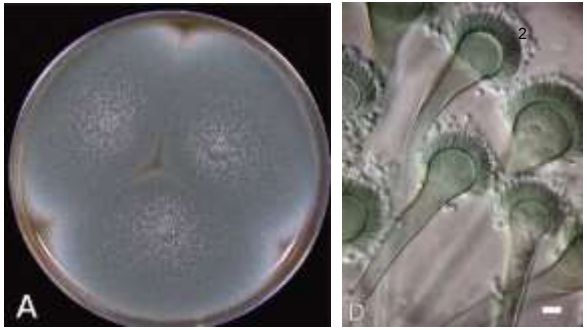
- **1:2500 newborns**
- **Autosomal recessive disorder in caucasian population**
- Prognosis of CF patients: median **40 years of life**



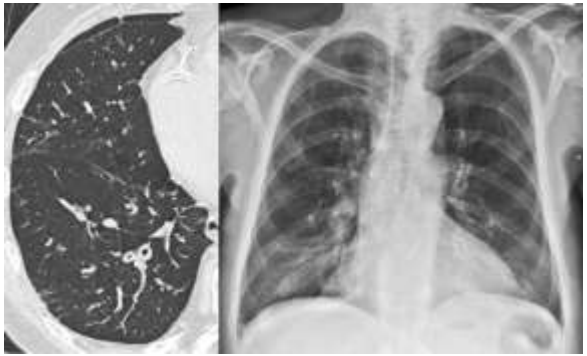



# *Aspergillus fumigatus* infections

- Airborne conidia able to get to alveolar lung structures.
- Most frequently isolated filamentous fungus of CF patients (prevalence 6% to 60%)[1].




- **Allergic broncho-pulmonary aspergillosis** (ABPA) with ARAF leads to high likelihood of **therapeutic failure**[3]



ABPA: Emphysema, fibrosis, bronchiectasis
Mevis Research, Fraunhofer-Gesellschaft 2017, Bremen

[1]Medical Mycology June 2009, 47,387;97
[2]Studies in Mycology 59: 147–203. 2007
23 no. 2 299-323
[3]Eur Respir J, 2000, vol. 16, 464-471

# Azole therapy and resistance mechanisms

*14-α-(lanosterol)-demythelase*

**Lanosterol** → **Ergosterol**

**Azoles**:
- Itraconazole
- Voriconazole
- Posaconazole
- Isavuconazole
- ...

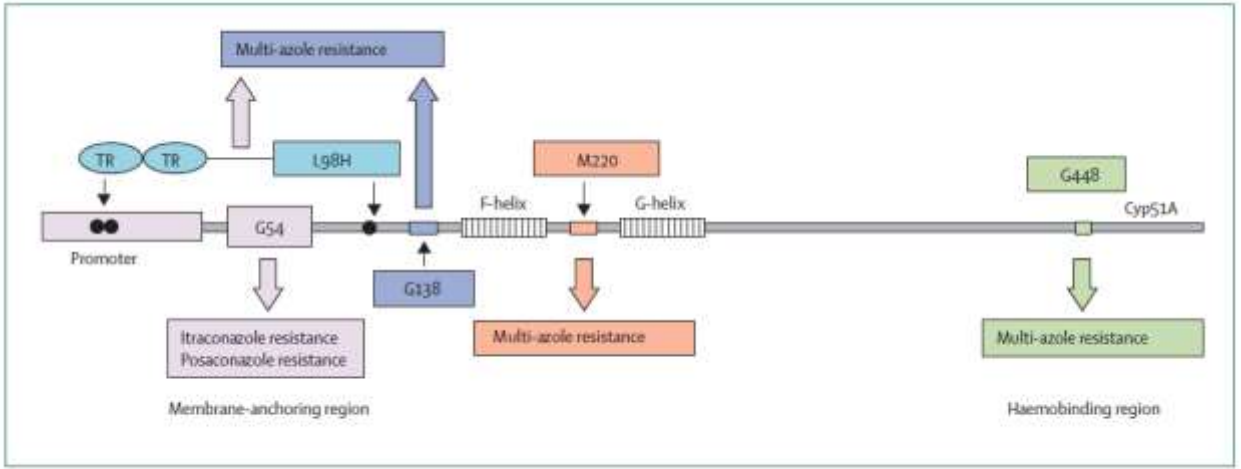



*Figure 3: Aspergillus fumigatus* cyp51A-related resistance mechanisms to azole antifungals
The position of the different mutations are shown with the associated phenotypes. MIC=minimum inhibitory concentration. TR=tandem repeat.

Lancet Infect Dis 2009; 9: 789–95

# Origin of azole resistant *A. fumigatus* (ARAF)

## Patient route

- Prolonged azole therapy
- Chronic pulmonal aspergillosis
- Cyp51A-mutation or other mechanisms
- Multiple mutations in isolates of one patient

## Environmental route

- Mostly patients without azole therapy
- Chronic infection or invasive aspergillosis
- Few molecular mechanisms (mainly $TR_{34}$/L98H)

Lancet Infect Dis 2009; 9; 789-95
Clin Infect Dis. 2016 Feb 1; 62(3): 362–368

# Aims of our study

1. Elucidation of the **prevalence** of ARAF isolates in respiratory secretions of CF patients from centres across Germany
2. Phenotypic and molecular-based **characterisation** (type of mutation, genotyping) of azole resistant *A. fumigatus* isolates
3. Analysis of **characteristics** associated with CF patients colonized by ARAF (e.g. age, sex, previous exposure to azoles etc.)

# Study design

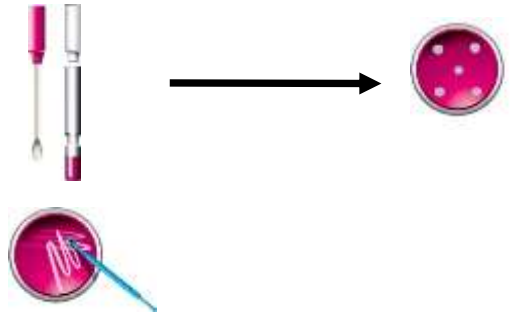
**Study period**: 2012-2016

Multicenter study to determine prevalence of ARAF in German CF patients

(**13** CF centres in Germany, **2893** isolates of **966** CF patients)

**Specimen collection and culture**

**4 mg/l itraconazole agar screening**



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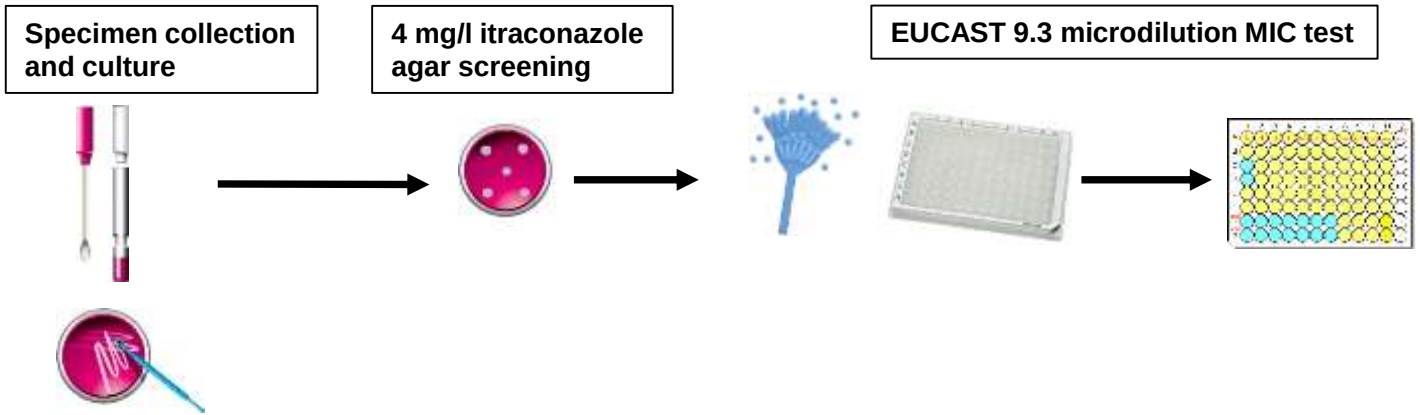

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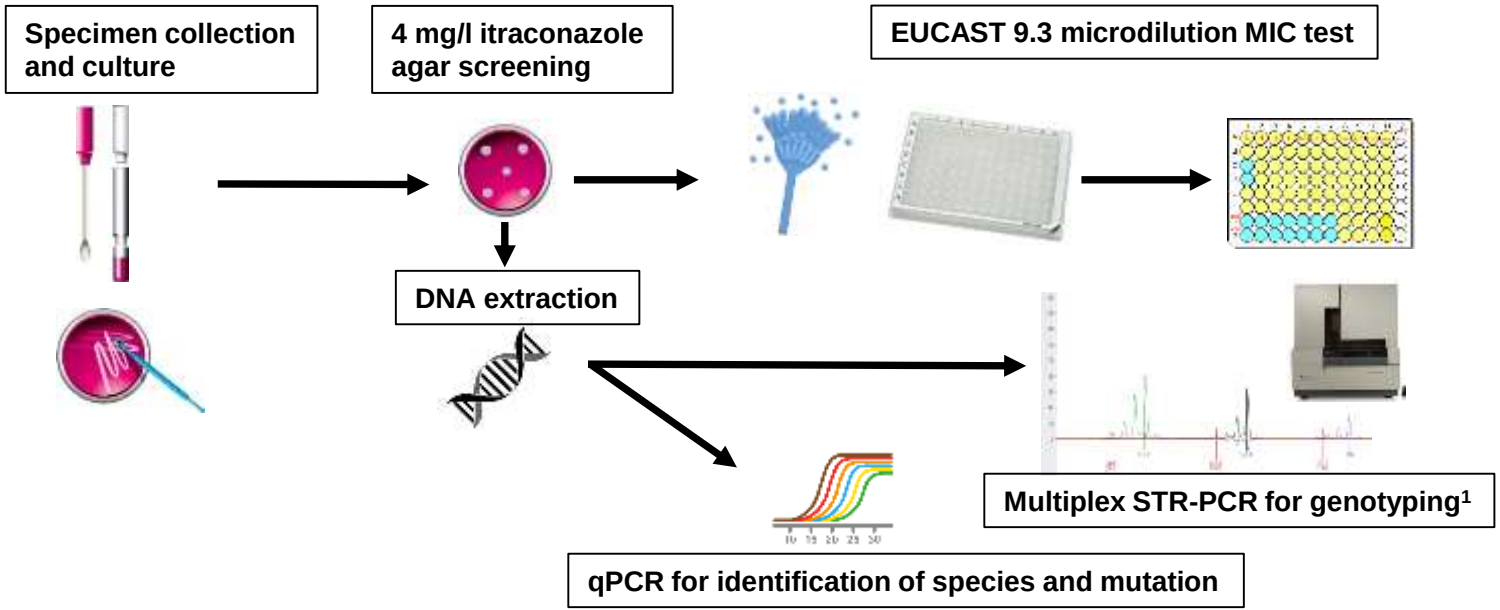
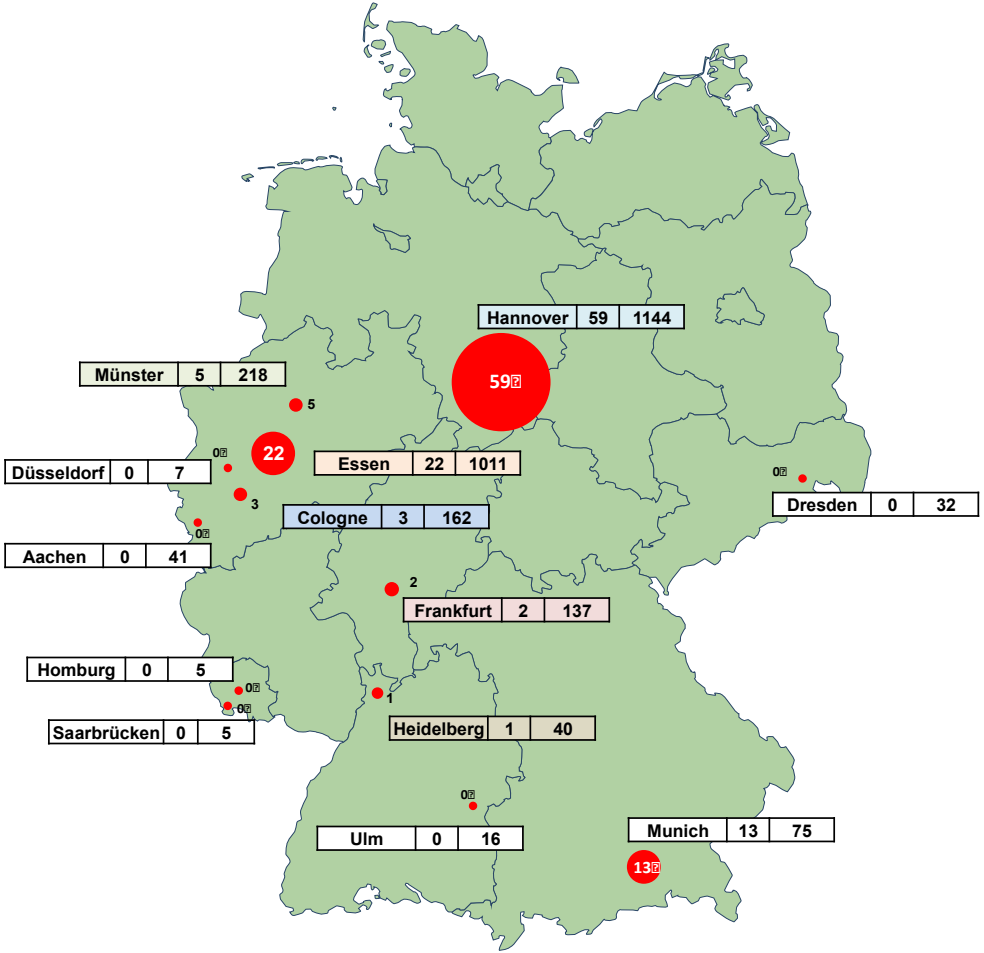


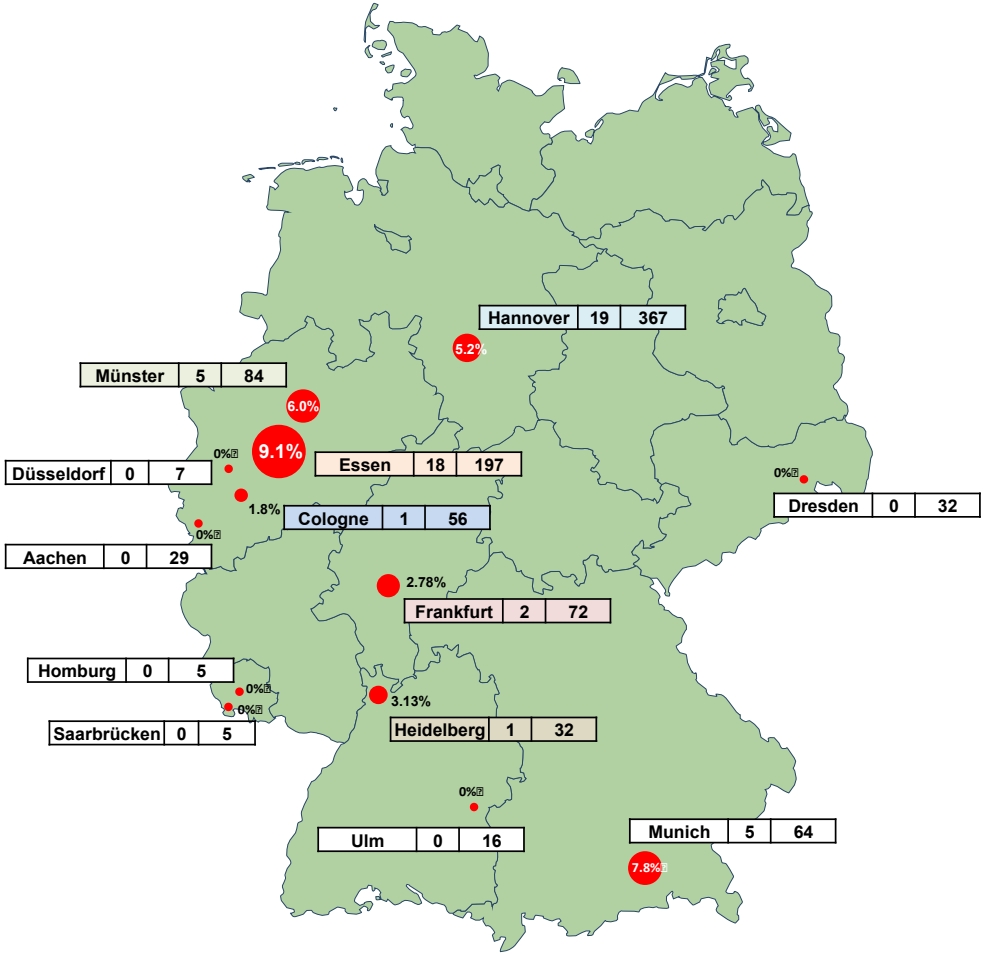

[1]JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2005, p. 411

# Results 1 – Number of isolates collected




- Most isolates were collected from centres **Hannover** and **Essen**
- **101** isolates showed resistance to itraconazole

# Results 2 – Prevalence of ARAF in CF centres

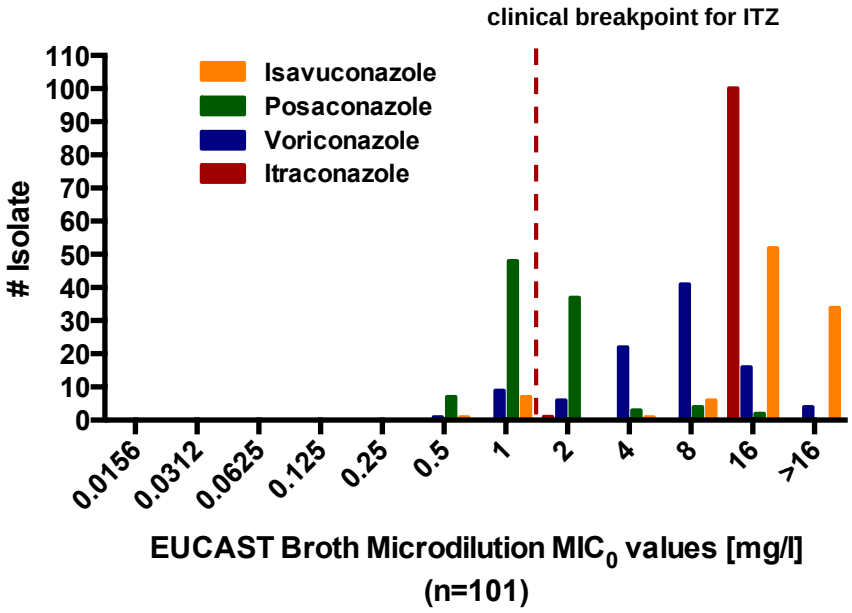



| | ARAF pat. | Pat. total | Prevalence |
|---|---|---|---|
| Hannover | 19 | 367 | 5,2% |
| Essen | 18 | 197 | 9,1% |
| Münster | 5 | 84 | 6,0% |
| Munich | 5 | 64 | 1,8% |
| Frankfurt | 2 | 72 | 7,8% |
| Cologne | 1 | 56 | 2,8% |
| Heidelberg | 1 | 32 | 3,1% |
| Düsseldorf | 0 | 7 | 0,0% |
| Homburg | 0 | 5 | 0,0% |
| Dresden | 0 | 32 | 0,0% |
| Saarbrücken | 0 | 5 | 0,0% |
| Ulm | 0 | 16 | 0,0% |
| Aachen | 0 | 29 | 0,0% |
| | 51 | 966 | 5,3% |

- Prevalence of ARAF in German CF patients is **5.3%.**
- **45%** of ARAF colonized patients received **previous azole therapy**
- **22%** of patients suffered from ABPA
- **24%** underwent lung transplantation

# Results 3 – MICs and mutations

- All isolates showed cyp51A mutations, $TR_{34}$/L98H most frequent
- We found **one** codon substitution not yet described

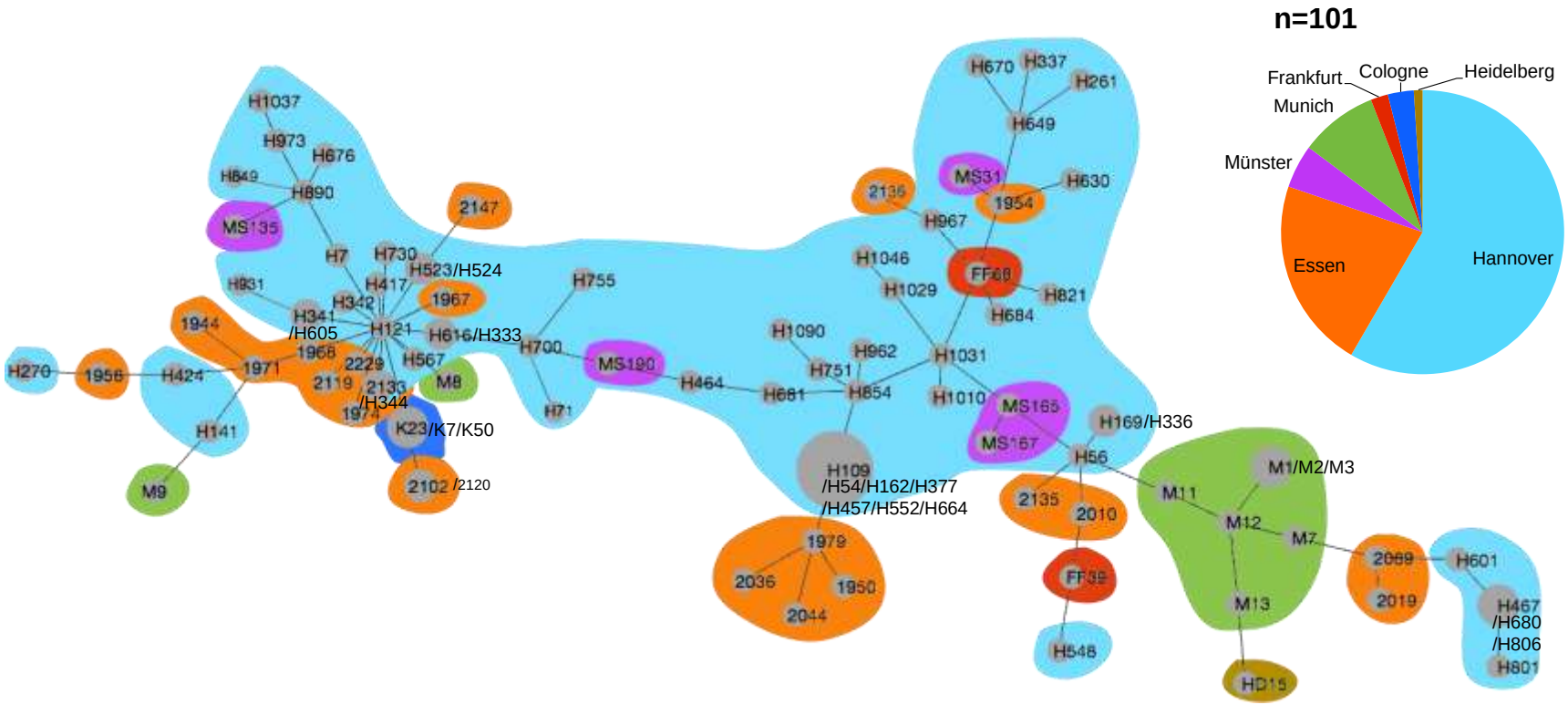



| Mutation | Prevalence (n= 101) |
|---|---|
| $TR_{34}$/L98H | 92/101 (≈91%) |
| $TR_{46}$/Y121F/T289A | 1/101 (≈1%) |
| G54E | 7/101 (≈7%) |
| other cyp51A mutation (Q166S) | 1/101 (≈1%) |

| Mutation | ITZ | VOR | POS | ISA | Publication |
|---|---|---|---|---|---|
| $TR_{34}$/L98H | R | R | R | R | Verweij (2007),... |
| $TR_{46}$/Y121F /T289A | R | R | R | R | Wiederhold(2016), Steinmann(2015),... |
| G54E | R | S | R | S | Wiederhold(2016) |
| Q166S | (R) ? | (S) ? | (R) ? | (I) ? | |

# Results 4 – Clustering genotypic relations

- MLVA-Clusteranalysis of all isolates using minimum spanning tree



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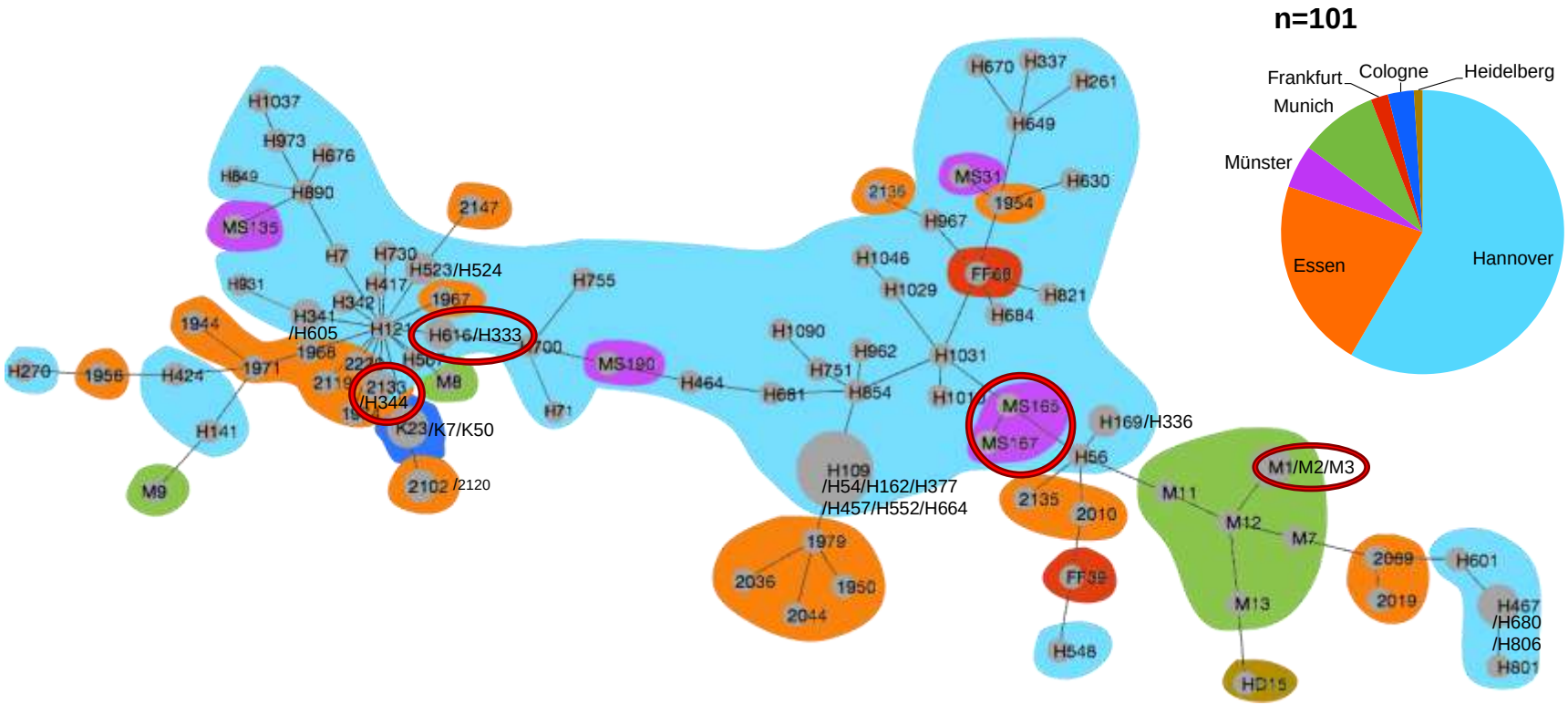

# Conclusion

This is the **first multicentre** study on azole resistance of *A. fumigatus* in CF patients in Germany.

- With a **prevalence of 5.3%,** susceptibility screening, as **cheap** and **quick**, should be done before any decision about antifungal treatment
- With STR-genotyping, we could demonstrate **possible transmission** of ARAF between patients or an unknown **common source**

# Acknowledgement

**ARAF in CF Study Group**

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