Two genotyping methods reveal clusters of *Aspergillus fumigatus* from chronic pulmonary aspergillosis patients in the UK

Hongwei Shen¹, Rikesh Masania², Paul Bowyer³, Riina Rautemaa-Richardson^{2,3}, Malcolm D Richardson³, LilyAnn Novak Frazer^{2,3}

¹Microbiology Department, Futian District Centre for Disease Control and Prevention, Shenzhen, Guangdong, China,

²Mycology Reference Centre Manchester, Manchester University NHS Foundation Trust, Wythenshawe Hospital, UK

³The University of Manchester, Faculty of Biology, Medicine and Health, Division of Infection, Inflammation and Respiratory Medicine, Manchester, UK.

Background

- **Chronic pulmonary aspergillosis (CPA)** patients attending the **National Aspergillosis Centre (NAC)** in the UK often require long-term antifungal therapy.
- **Resistance** to at least one of the **triazole antifungal drugs** has been found in 28% of clinical isolates of *Aspergillus fumigatus* causing exacerbations¹, yet their origin is uncertain.
- The high discriminatory power of genotyping methods (based on tandem repeats)^{2,3} may be helpful for discerning the source of *A. fumigatus* infections and may reflect antifungal resistance patterns.

Methods

- We compared the performance of two genotyping methods: nSTRAf² and TRESP³ to determine:
 - i. the relationship among isolates from the same patient; and
 - ii. whether genotypes with different resistance patterns could be distinguished.
- We identified distinct genotypes in 31 clinical isolates from 14 CPA patients located in different geographical regions in the UK and compared them to two reference isolates (ATCC46645, AF293).

Results Summary

- A wide distribution of *A. fumigatus* isolates was found across the UK: 23 genotypes according to nSTRAf and 18 according to TRESP.
- The Simpson index of discriminatory power for nSTRAf and TRESP was 0.9699 and 0.9398, respectively.
- The relationship between genotype and susceptibility patterns was variable:
 - i. in some patients, resistance developed in one genotype over time.
 - ii. in others, the same susceptibility patterns was reflected in different genotypes, suggesting the causal organisms were different.
- Isolates were not discriminated geographically.

Future Work

- Analysis of a greater number of isolates is required to decipher whether nSTRAf and TRESP typing reflects triazole resistance in the UK.
- Although technically challenging, nSTRAf may be more representative of relatedness, although addition of TRESP *ERG4b* data may allow better discrimination of these isolates.
- An expanded set of NAC CPA patient isolates, including those with verified TR34/L98H mutations, analysed by next generation sequencing will allow further discrimination of these relationships.

Genotyping analyses: 31 isolates are shown typed using both methods according to their i) patient designation (A-N); ii) the location of their domicile (Scotland, Wales or England); and the susceptibility profile of the isolate according to broth microdilution testing (using the standard EUCAST approach⁴).

References

- ¹Buied A et al. 2010. Azole antifungal resistance in *Aspergillus fumigatus*: 2008 and 2009. Journal of Antimicrobial Chemotherapy, 65:2116-2118.
- ²Araujo R et al. 2009. Simple and highly discriminatory microsatellite-based multiplex PCR for *Aspergillus fumigatus* strain typing. Clinical Microbiology and Infection, 15:260-266.
- ³Garcia-Rubio R et al. 2016. A new *Aspergillus fumigatus* typing method based on hypervariable tandem repeats located within exons of surface protein coding genes (TRESP). PlosOne:11.
- ⁴Arendrup et al. Method for determination of broth microdilution inhibitory concentrations of antifungal agents for conidia forming moulds. EUCAST E.DEF 9.3 December 2015.