P1100

Paper Poster Session V

Improving fungal diagnostics

Detection and quantification of microbial minority variants by ultra-deep pyro-sequencing (UDPS): application for resistance mutations detection in *Aspergillus*

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Objectives : *Aspergillus fumigatus* is an opportunistic environmental fungal pathogen that causes invasive aspergillosis in immunocompromised patients. Azoles are major agents for the treatment of aspergillosis but *A. fumigatus* resistance has appeared recently and increased dangerously in the last decade. The mechanism of azole-resistance in *A. fumigatus* is mainly linked to mutation in the *Cyp51A*, the molecular target of azole drugs. Azole-resistance may be related to antifungal exposure in treated patients but also to environmental exposure to fungicides. Among resistant isolates, the TR_{34}/L98H mutation in *cyp51A* was the most prevalent, but isolates with others substitutions were also found. In patients, co-existence of different populations of wild-type and mutated azole-resistant isolates may be present in various proportions. Thus, resistant isolates may represent minority populations, which require a highly sensitive technology such as Ultra-Deep Pyro-Sequencing (UDPS) to be quantified.

Our aim was to develop and validate a sensitive UDPS technique for the detection and quantification of *cyp51A* genetic variants in *A. fumigatus* obtained in cultures from respiratory samples.

Methods: One plasmid containing the wild type *cyp51A* gene associated with its promoter (PWT) and another containing different known resistance mutations (PM) were synthetized. PWT and PM were mixed in different proportions to reach 100%, 10%, 5%, 2%, 1%, 0% of PM and used as control to detect minority alleles of *cyp51a.* In parallel, 32 cultures from respiratory samples of patients were analyzed after extraction of fungal DNA (QIAsymphony©). Each preparation was then amplified by 5 different PCR along *cyp51a* and promotor. The libraries were prepared and sequenced according to the protocol for the GS sequencer (454/Roche). **Sequences** generated were analysed using an in-house software "PyroMIC©" to allow quantification of mutations. The QuRe© software was used to perform reconstruction of the gene and analyze the microbial populations diversity of *cyp51A* by haplotype approach.

Results: Over 100,000 sequences of good quality (average length: 439 bp) were generated for controls and used for technical validation. For the PM, the expected mutations were correctly identified. A detection limit of 0.4% and a limit of quantification of 1.3% were established for tested mutations. In parallel, more than 400,000 sequences were generated for isolates obtained from respiratory samples (2625X coverage on average). Among these samples, two had several minority variants of *cyp51A*. For one sample, these minority variants showed mutations related to azole-resistance.

Conclusions: We have set-up an original and sensitive method of analysis of *Cyp51A* diversity in *A. fumigatus*. This method allowed the detection of azole-resistant isolates even if present as a small proportion and mixed with WT isolates. The presence of azole-resistant isolates in small amount must be further explored because it could affect the success of treatment of patients and modify our laboratory practice.