P0024
Poster Session I
News from the fungal frontier

ULTRA DEEP PYROSEQUENCING OF PNEUMOCYSTIS JIROVECII REVEALS HIGHER DIVERSITY THAN PREVIOUSLY STATED

A. Alanio[1], J. Menotti[1], S. Mercier-Delarue[2], S. Bretagne[1]

[1]Laboratoire de parasitologie mycologie, Hôpital Saint Louis, Paris Cedex 10, France ; [2]Laboratoire de microbiologie, Hôpital Saint Louis, Paris Cedex 10, France

Objectives : *Pneumocystis jirovecii* is an opportunistic uncultivable fungal pathogen responsible for severe pneumonia in HIV and non-HIV immunocompromised patients. Primoinfection occurs in childhood and transmission occurs between individuals with both immunocompromised and immunocompetent as a reservoir. Traditional PCR-based methods have been widely used to investigate outbreaks but there is a need for detecting minoritary genotypes. The recent ultra-deep pyrosequencing method (GsJunior System, 454 Life Sciences, Roche) is already used in virology for this purpose.

Methods : We designed a 314-bp PCR for screening 3 single nucleotide polymorphisms (SNPs) at positions 13,159; 13,215; and 13,378 (GenBank JX499143) in the mitochondrial Large Subunit ribosomal RNA gene. We then quantified mitochondrial variability in 36 *P. jirovecii* PCR-positive respiratory samples (29 patients). We obtained a mean of 3998 (+/- 2084) sequences per sample. Reproducibility of the technique was about 2%.

Results : We could select haplotypes based on the SNP combinations (AAC, ACC, ACT, ATT, ATC, AAT, and GNN). The mean number of haplotypes detected in a given sample was 4/sample (range 2-7) with various proportions of each haplotype. From 6 patients with >1 samples at different time points, the haplotypes were conserved for a given patient although the proportion of the haplotypes can vary over time in a given patient.

Conclusion : Ultra deep pyrosequencing is a promising tool to allow genotyping fungal pathogens especially to detect minoritary haplotypes when mixtures are present, which cannot be apprehended using Sanger sequencing. This could have some impact on the comprehension of the pathophysiology to resolve the question of latency or recent acquisition in pneumocystosis.