Pneumocystis pneumonia is a severe opportunistic infection due to the unusual fungus *Pneumocystis jirovecii* occurring in immunocompromised patients. Airborne transmission occurs between individuals with both immunocompromised and immunocompetent hosts as a reservoir. Numerous reports of outbreaks in renal transplant or pediatric units highlight the need for genotyping methods to characterize nosocomial acquisition. PCR-SSCP or MLST have been widely used to discriminate isolates with various propensities to detect mixtures of genotypes.

We took advantage of the recently published *P. jirovecii* whole genome sequencing (Cissé et al. mBio 2013) to develop a microsatellite-based molecular typing method, which easily detects mixtures of genotypes (de Valk et al. 2005).

From a selection of 10 pure short tandem repeats uncovered using bioinformatics tools, we finally selected six microsatellite markers whose the discriminatory power was tested on a panel of 95 *P. jirovecii* PCR-positive respiratory samples (52 BALs and 43 induced sputa) from 80 patients collected from October 2010 to August 2013.

From the 95 samples, a unique genotype was observed in 32 (33.7%) samples (29 patients) for which we obtained 23 distinct genotypes. We thus found mixed genotypes with ≥1 marker/locus in 63 (66.3%) samples from 51 (63.8%) patients, of which 44 (49.5%) samples from 36 (46.2%) patients had ≥2 markers/loci. No difference was observed considering the fungal load or the type of specimens (BAL vs. induced sputum).

The present microsatellite based molecular typing method is reliable to genotype *Pneumocystis jirovecii*. We observed a high proportion of mixed genotypes, which could not be easily achieved using MLST or SSCP.