Fungi are the cause of 1.7 billion human infections. Invasive fungal infections causing 1.6 million death/year, more than malaria and tuberculosis together. They are treatable if diagnosed in a timely matter. The increasing use of antifungals in agriculture/medicine leads to a rise in resistance, making fungal identification a priority at a time where not even a fifth of the 5.2 million species estimated to exist on earth have been described and no accurate species borders are in place. DNA barcoding is a culture independent approach to identification. In 2012, the primary fungal DNA barcode (internal transcribed spacer (ITS1/2) region) was selected, with BOLD containing 154,123 ITS barcodes of 29,177 species. The first quality-controlled database for human/animal pathogenic fungi (ISHAM-ITS database) was established in 2015 (4200 ITS sequences of 645 species). The ITS region is only able to correctly identify 75% of all fungi, making the introduction of the translation elongation factor 1α (TEF1α) as a secondary DNA barcode necessary (2432 TEF1α sequences of 346 species). TEF1α shows less intra-species and higher discriminatory power at inter-species level than the ITS. Next Generation Sequencing (e.g. MinION™ Oxford Nanopore Technologies) allows for a high throughput, real-time, long-read (average 10-15kb) based simultaneous identification of complex samples. To assess the ability of metagenomic sequencing the MinION™ was used to identify the unculturable fungus Pneumocystis jirovecii directly from respiratory specimens and to characterise the associated mycobiome. P. jirovecii was detected in bronchoalveolar lavage and induced sputum samples. False-positive and error-prone reads are major problems for metagenomics, calling for the development of better algorithms and reference databases.