ABSTRACT

Pneumocystis pneumonia (PCP) is one of the most prevalent diseases in HIV-positive and other immunosuppressed patients. It is caused by the opportunistic fungal pathogen *Pneumocystis jirovecii*. Dihydropteroate synthase (DHPS) mutations in *P. jirovecii* have been linked to resistance to trimethoprim-sulphamethoxazole (cotrimoxazole), the main treatment and prophylaxis used for PCP. DHPS mutations have been identified globally, predominantly in developed countries. This study investigated the *P. jirovecii* DHPS genotypes in PCP-positive patients from Chris Hani Baragwanath Hospital in Gauteng Province, South Africa.

During the period March 2005 through June 2009, 266 patients were enrolled in the study and 306 specimens were collected. *P. jirovecii* was identified in 67% (205/306) of these specimens with quantitative real-time PCR (qPCR). The qPCR had a sensitivity of 98% and a specificity of 70%, compared with the immunofluorescence assay (IFA). Using sequencing and cloning techniques, 64% (110/173) of the nested PCR-positive specimens contained *P. jirovecii* with mutant DHPS genotypes. There was no association between patients harbouring *P. jirovecii* with mutant DHPS genotypes and in-hospital patient outcome (p-value = 0.19). As part of this project the Roche MagNA Pure Compact (RMPC) instrument and technology was validated for use as a new DNA extraction method. The RMPC was quick and easy to use compared to the Qiagen manual extraction method.

The specificity of the qPCR was compromised by the high number of apparent false positive results obtained by the assay. However, as the IFA is an imperfect gold standard, these are probably true cases of infection or colonisation. This study found a higher proportion of *P. jirovecii* with DHPS mutant genotypes than wild type in PCP patients, which is unusual. The most probable reason for this is the widespread use of sulfa drugs, which are thought to select for these mutations. While this study did not find an association between DHPS mutations and adverse patient outcome, there have been contradictory findings. If further investigations reveal that DHPS mutations affect patient treatment or outcome, it will have major implications for the management of PCP in the country.