TITLE: "THE MOLECULAR EPIDEMIOLOGY OF *PNEUMOCYSTIS JIROVECII* IN CAPE TOWN, SOUTH AFRICA"

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Background: *Pneumocystis jirovecii* is an opportunistic fungal pathogen that causes *Pneumocystis* pneumonia (PCP) in immunocompromized hosts**.** PCP is associated with substantial morbidity, and mortality rates range from 10% to 40%. The diagnosis of PCP relies on the microscopic detection of *P. jirovecii* in stained clinical samples. Polymerase chain reaction (PCR) may provide better sensitivity than microscopy; therefore, evaluation and implementation of PCR assays are required for the detection of *Pneumocystis* infection. *P. jirovecii* is not cultivatable, therefore molecular tools are used for characterizing *P. jirovecii* genotypes; common targets are the dihydropteroate synthase (DHPS) and mitochondrial large subunit rRNA (mtLSU rRNA) genes. DHPS is a therapeutic target; mutations may be associated with co-trimoxazole prophylaxis and treatment failure. Polymorphisms in mtLSUrRNA have been used for phylogenetic studies.

Aims: 1) to evaluate a real time PCR (rtPCR) assay for diagnosis of PCP by comparing the performance to immunofluorescence (IF) and 2) to describe the molecular epidemiology of *P. jirovecii* isolates from Tygerberg Hospital by analyzing DHPS and mtLSU rRNA genes.

Methods: Clinical samples from 305 children and adult patients at Tygerberg Hospital were collected, after testing using IF. DNA was extracted using the NucliSens easyMAG platform (Biomérieux). The rtPCR assay targeting the major surface glycoprotein (MSG) gene was evaluated to detect *P. jirovecii* DNA. The DHPS and mtLSU rRNA genes were amplified by nested PCR and analyzed by DNA sequencing.

Results: The SYBR Green rtPCR detected *P.jirovecii* in 57% of samples (175/305) compared to the 7% (21/305) detected by IF. Our rtPCR had a sensitivity of 100% and specificity of 46%, although this increased if the detection threshold increased. Of the 50 negative control samples used in this study, none tested positive for *P.jirovecii*. There were 237 lower respiratory tract (LRT) and 58 upper respiratory tract (URT) samples. The yield of PCR in LRT samples was 55.3% (131/237) compared to 70.6% (41/58) in URT samples (p=0.03). In contrast, none of the URT samples were positive using IF, and 8.9% (21/237) of

LRT samples were positive on IF. Stellenbosch University https://scholar.sun.ac.za iii DHPS was successfully amplified in 123 (70.3%) samples; and mtLSU in 126 (72%) samples. Genotype 1 (wild type) was the predominant DHPS genotype, and a mutation rate of 42.3% was recorded for this gene. The mtLSU genotype 3 was present in 50.8% of samples, genotype 1 (42%) was the next most common genotype. Mixed genotypes were detected in 2.4% of the samples analyzed for each gene. There was no clear association between DHPS polymorphisms and mtLSU genotype.

Conclusions: The SYBR Green rtPCR was more sensitive than IF for detection of *P. jirovecii*; especially in URT samples, which is comparable to previous studies. The DHPS mutation rate increased to 42% from 27% recorded in 2013 from our division. The increase in DHPS mutation rate may be a result of on-going co-trimoxazole use, for prophylaxis or treatment of PCP or other infections. Our findings need to be linked to clinical data to better understand transmission dynamics and potential impact of strain variation on clinical outcome, and further studies are required to better describe the local strain diversity.

Biography

Mr DERRICK BANDA has completed his MSc at the age of 31 years from Stellenbosch University, Faculty of Medicine and Health Sciences, Cape Town, South Africa. He is the Lecturer of Biological Sciences and Microbiology at Mulungushi University in Zambia.

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