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Title of Thesis: Molecular Characterisation and Immunological Analysis of Clinical and Environmental Isolates of *Mycobacterium kansasii* from South African Gold Mines

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**ABSTRACT**

The South African gold-mining workforce has an unusually high incidence of *Mycobacterium kansasii* disease, yet little is known about the possible sources of *M. kansasii* infection, genetic diversity and the basis for this organism's pathogenicity. The purpose of this study was to investigate these issues in a gold-mining environment. Five *M. kansasii* isolates and 10 other potentially pathogenic mycobacteria were cultured mainly from showerhead biofilms. PCR-restriction analysis (PRA) of the *hsp65* gene on 191 clinical and on the 5 environmental *M. kansasii* isolates revealed 160 subtype I (157 clinical and 3 environmental), 8 subtype II (clinical) and 6 subtype IV (5 clinical and 1 environmental) strains. Twenty-two isolates (21 clinical and 1 environmental) did not show the typical *M. kansasii* PRA patterns. After confirmation by DNA sequencing as belonging to the *M. kansasii* species, the results suggested that these isolates were probably new subtypes of *M. kansasii*. In contrast to the clonal population structure found amongst the subtype I isolates from studies in other countries, DNA fingerprinting of 114 subtype I clinical and environmental isolates showed genetic diversity amongst the isolates. One of the

environmental isolates showed 100% identity with a clinical isolate, suggesting that water distribution systems are the possible sources of *M. kansasii* infection for the miners. An investigation into the genetic differences between clinical (subtype I) and environmental (III, IV and V) isolates, using Hybridisation Monitored Differential Analysis (HMDA), identified 45 open reading frames (ORFs) encoding predominantly membrane-associated proteins that include six potential virulence factors, two family members of transcription regulators for drug and xenobiotic metabolism, three family members of multidrug efflux systems, a number of proteins associated with lipid and carbohydrate metabolism and transport, and a number of hypothetical proteins with unknown function. Immunological analysis of *M. kansasii* isolates, using the Lymphocyte Transformation and Cytometric Bead Array assays, showed that *M. kansasii* modulates immune responses through suppression of lymphocyte blastogenesis and by altering the expression of Th1/Th2/Th17 cytokines by human lymphocytes *in vivo* for its own survival. This study demonstrated for the first time that water distribution systems in South Africa are possible sources of *M. kansasii* infection, and showed that subtype I strains of *M. kansasii* from the study region display genetic diversity and have unique or divergent genes not found in other subtypes. It also demonstrated that immunosuppression is one of the pathogenic mechanisms employed by *M. kansasii*.