GROWTH PATTERNS AND SURVIVABILITY OF THE DIFFERENT *MYCOBACTERIUM TUBERCULOSIS* SUB LINEAGES PREDOMINANT IN PERI URBAN KAMAPALA

BY

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ABSTRACT

Background: *Mycobacterium tuberculosis* (MTB), the causative agent of tuberculosis (TB) infects over 2 billion people world-wide with the vast majority of deaths from TB in the developing world. Strains of MTB vary in virulence. Different evolutionary lineages of MTB are strongly associated with specific geographical regions, suggesting that they have adapted to particular human populations. Indeed, particular MTB lineages (also known as genotype families) have shown preferential spread in certain patient populations. From previous studies done in peri-urban Kampala, it has been shown that Mtb Uganda genotype is the most dominant species in peri-urban Kampala with a prevalence rate of 63%, however, little is known why MTB Uganda genotype, is more dominant in peri-urban Kampala.

Methodology: Glycerol stocks of 10 strains (3 from each of the dominant genotypes and one from reference strain H37Rv) were purposively selected from the three dominant genotypes (MTB Uganda genotype, LAM and CAS/Delhi). These were inoculated on to 7H10 Middlebrook agar and incubated at 37^0C in a 5% carbondioxide incubator for 2 weeks. A single colony was picked for each strain and inoculated into 7H9 Middlebrook (MB) broth. This was then incubated in a shaking incubator. An aliquot was picked from the broth and adjusted to a 0.5 McFarland standard. Equal volumes of the adjusted broth were picked and inoculated individually into 7H9 MB broth. An aliquot was picked from the broth and its absorbance measured. This was done at a 24 hourly time interval. The aliquot was serially diluted and cultured on 7H10 MB agar to attain countable single colonies. From these, colony forming units were obtained and used to plot the growth curve. For assessment of survivability of the organisms, equal amounts of the MTB strains grown in 7H9 broth (1ml each) were picked and mixed in 100mls of fresh 7H9 MB broth and left to compete for the nutrients. Every day, an aliquot of the broth was picked, serially diluted (1:100, 1:1000, 1:10000 and 1:100000) and cultured on 7H10 MB agar for 3-4 weeks while monitored for discrete colony formation. The colonies were picked and diluted into 10µl of molecular grade water. LSP-PCR assay was then done on those colonies.

Results: It was observed that H37Rv grew faster than all the clinical strains. Amongst the clinical strains, CAS/Delhi strains showed a higher growth rate whereas the Uganda genotype had an intermediate growth and the difference in growth was statistically significant ($P < 0.0001$). From the survival assays, the LAM genotype showed a higher survivability as compared to the rest of the genotypes and still, the difference in the survival was statistically significant ($P < 0.0001$).

Conclusion: Our results showed an intermediate growth and the lowest survival rate for the Uganda genotype while competing with the other genotypes. Thus we speculate that the factors that make MTB

Uganda the most dominant could be related to the yet unknown biology of the organism and probably its interaction with the human host.