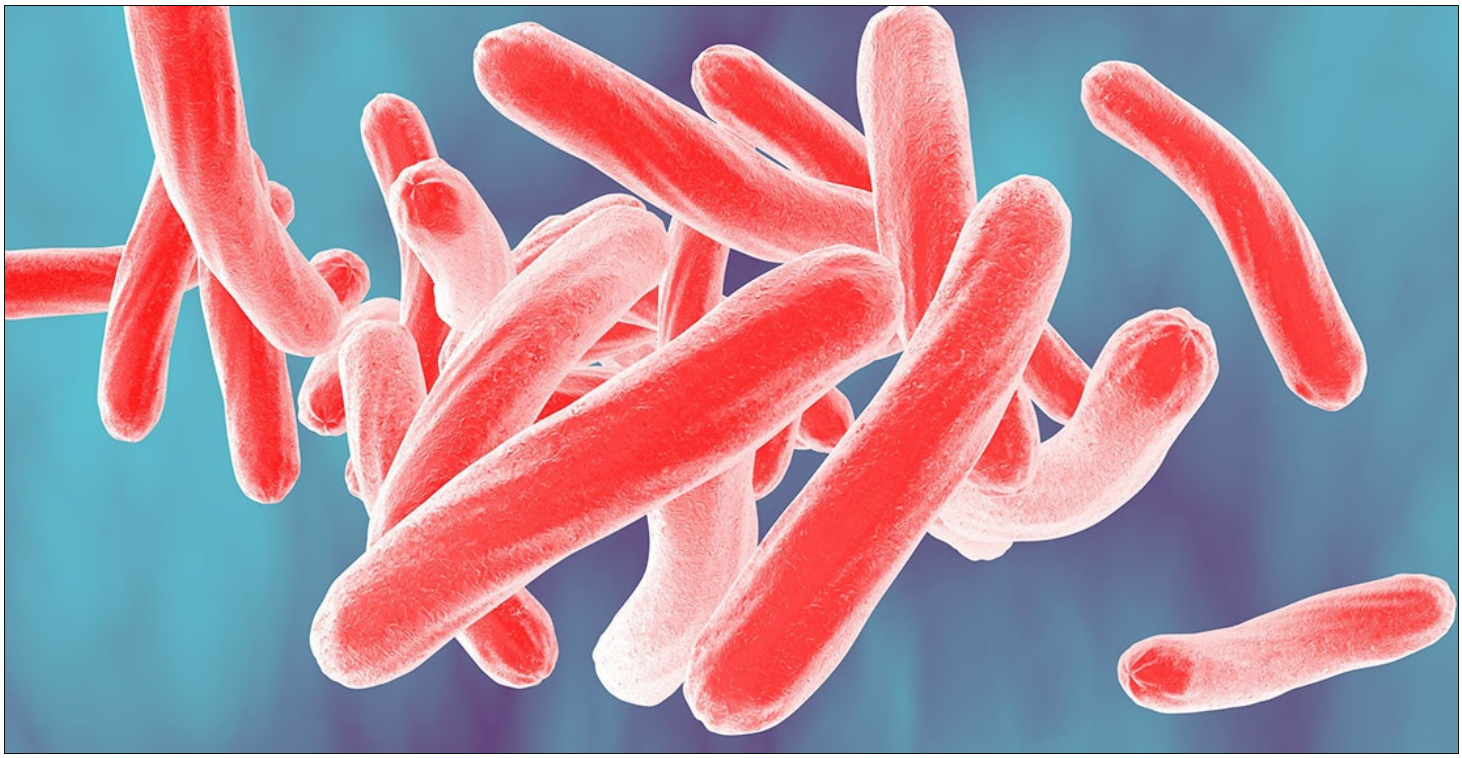
PhD defence Nana Akua Boatema Ofori-Anyinam

Towards improved understanding of M. tuberculosis complex strain differences in The Gambia.

23 Oct 2017
Promotiezaal Grauwzusters, University of Antwerp - Antwerpen
Booking not required



Dit is de omschrijving

Supervisors: Prof. Dr. Bouke de Jong (ITM), Prof. Dr. Leen Rigouts (University of Antwerp), Prof. dr. Martin Antonio (Medical Research Council, The Gambia)

Summary:

In West Africa, tuberculosis (TB) is caused by *Mycobacterium tuberculosis sensu stricto* (Lineages (L) 1-4) and two geographically restricted *M. africanum* lineages (L5 and L6). *M. africanum* contributes significantly to the disease burden yet the biology of both *M. africanum* lineages is relatively underexplored.

In the Gambia alone, *M. africanum* L6 , a relatively more attenuated and slower growing pathogen than *M. tuberculosis*, causes about a third of TB. Biological factors driving the differences observed are largely unknown. The dormancy regulon is known to increase intracellular survival and virulence of *M. tuberculosis* by facilitating rapid adaptation to the hypoxic environment encountered in the human host. Impaired function of the regulon has been associated with *M. tuberculosis* attenuation. In this thesis, ex-vivo gene expression in sputa revealed significantly lower expression of dormancy regulon genes in *M. africanum* L6 when compared to *M. tuberculosis* L4. Our findings pointed towards lesser aerobic requirements of L6 for survival, indicating L6 was likely pursuing a different survival strategy within the host, compared to *M. tuberculosis*. As impaired function of the regulon leads to reduced virulence, our findings provide reasons for the attenuated clinical phenotype of *M. africanum* L6. Weak induction of the dormancy regulon in *M. africanum* L6 and the preference for hypoxic growth could indicate an adaptation of L6 to latent survival and lower virulence enabling a stable association with its restricted human host range.

Moreover, *mpt64*, encoding the virulence associated and immunogenic MPT64 protein, the target of a commonly used rapid diagnostic test for identifying MTBC strains in culture was significantly underexpressed in *M. africanum* L6 compared to *M. tuberculosis* L4. Beyond providing further reasons for relative attenuation of *M. africanum* L6 compared to *M. tuberculosis*, as identification of the *M. tuberculosis* complex (MTBC) in AFB positive cultures increasingly relies on MPT64 based tests, and misidentification of MTBC as atypical mycobacteria leads to diagnostic and consequently treatment errors, a prospective study was designed to determine if *mpt64* gene underexpression translated to lower sensitivity of MPT64 tests in detecting *M. africanum* L6. In this analysis, we indeed found lower sensitivity of the MPT64 tests for detecting L6 compared to *M. tuberculosis* lineages. To prevent misclassification, MPT64 negative cultures, particularly in West Africa, should be confirmed as MTBC or Non-tuberculous mycobacteria with more robust molecular tools. Generally, in every setting, novel tests and diagnostics should be evaluated before laboratory implementation.

Studying pathogens at the genomic level enables the discovery of genetic factors responsible for specific phenotypes and provides insights into disease etiology. Using bacterial whole genome sequencing and bioinformatics, *mpt64* was interrogated for genomic variations that could drive *mpt64* underexpression in *M. africanum* L6 or reduce the sensitivity of MPT64 rapid test for detecting L6. Several tools for analyzing whole genome sequences have been developed yet there are currently no standardized guidelines or protocols specifying cut-offs and thresholds for whole genome sequence analysis. Therefore, we carried out an evaluation of different bioinformatics pipelines for the best combination of tools for analysis. This evaluation also led to a basic guide for researchers in Africa and other low resource settings for analyzing bacterial whole genome sequencing data locally. In a limited resource setting, Snippy, a wrapper for BWA-MEM, Samtools and Freebayes, was the most suitable analysis pipeline, given its high accuracy in detecting important genomic variations, fast analysis runtimes, economical use of disk space and low computational complexity.

In *M. africanum* L6, gene underexpression and impaired MPT64 secretion could not be directly attributed to (a) distinctive mutation(s) in *mpt64*. However, in genes with predicted regulatory interactions with *mpt64*, multiple non-synonymous mutations, with predicted deleterious effects on protein function, were found. In *M. africanum* L5, on the other hand, a characteristic mutation in *mpt64*, also predicted to be deleterious, was detected.

Because nutrient starvation was previously shown to induce *mpt64* underexpression and to reduce antigen secretion, genes encoding enzymes required for metabolism of nutrients in the BACTEC MGIT 960 growth media - media used to culture MTBC prior to confirmation with the MPT64 diagnostic - were interrogated for mutations that could affect nutrient metabolism, cause nutrient starvation in L6 and ultimately, reduce *mpt64* secretion. This analysis confirmed *M. africanum* L6 had multiple mutations in key carbon metabolism genes required for optimum use of nutrients in the MGIT 960 growth medium, providing reasons for MPT64 underexpression and reduced antigen secretion by L6 in the MGIT growth system. An improved formulation of the BACTEC MGIT Growth Supplement could increase isolation and detection of *M. africanum* L6 with the MGIT 960 system and MPT64 rapid tests respectively.

Date: 20/10/2017