Synopsis of the PhD Thesis Entitled
Cellular and Molecular Features of the Response of *Mycobacterium smegmatis* to Rifampicin and Moxifloxacin Upon Prolonged Exposure
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Bacterial persisters are a subpopulation of bacteria that can tolerate lethal concentrations of antibiotics. These are phenotypic variants that can give rise to drug-susceptible population upon withdrawal of the antibiotic. Persistent bacteria play a crucial role in prolonging antibiotic treatment and are responsible for the recalcitrance of many chronic bacterial diseases, including tuberculosis. Several mechanisms have been proposed for the formation of persisters, which include expression of toxin-antitoxin systems, generation of reactive oxygen species (ROS), and stochastic changes in gene expression and so on. Recent report from our laboratory has demonstrated that continuous prolonged exposure of *Mycobacterium tuberculosis* cells to lethal concentrations of antibiotics generates antibiotic persistence phase cells from which genetically resistant mutants emerge *de novo* either to the same antibiotic to which it was exposed to or to another antibiotic used for selection Sebastian *et al*., Antimicrobial Agents Chemotherapy 61(2), e01343-16, 2016). The persistence phase cells showed high levels of oxidative stress that inflicted genome-wide mutations in addition to the mutations for which the resistant mutants were selected against rifampicin and moxifloxacin. Thus, it was we demonstrated that the antibiotic persistence phase *M. tuberculosis* cells is a reservoir for the *de novo* emergence of antibiotic resistant mutants.

In the present study, the response of *Mycobacterium smegmatis* upon prolonged exposure to rifampicin was examined since the bacilli has two mechanisms to inactivate or neutralise the action of rifampicin. These mechanisms include: (i). ADP-ribosylation of rifampicin by the product of the gene ADP-ribosyltransferase (*arr*); (ii). Rescue of rifampicin-mediated transcription inhibition by MsRbpA. The question asked was whether genetically resistant mutants against rifampicin would emerge from rifampicin persister phase cells, like in the case of *M. tuberculosis* cells and if they do, what are the mechanisms by which the rifampicin-resistant mutants emerge from the persistence phase cells. For comparison and contrast purpose, and as a control sample, the response of *M. smegmatis* cells to moxifloxacin, against which the bacilli do not have any inherent inactivation or neutralisation mechanism, was studied.

The **Chapter 1**, which forms the **Introduction** to the thesis, gives an extensive literature survey on all the different aspects of the research performed on the response of mycobacterial cells to antibiotics.

The **Chapter 2** presents in detail all the materials and methods used to perform the experiments. A large number of cell biological and molecular biological methods, such as fluorescence microscopy and fluorescence measurements, flow cytometry, cloning and expression, real time RT-PCR and whole genome sequencing, and biophysical methods such as electron paramagnetic resonance spectrometry, and others were used to perform the experiments.

The data **Chapter 3** presents the data on the response of *M. smegmatis* cells to rifampicin. The data shows that exposure to MBC levels of rifampicin results in the killing of the cells to a 5-log10 reduction in the cfu of *M. smegmatis* cells but the remaining cells persist and from these cells emerge rifampicin-resistant mutants. The persistence phase cells were found to generate elevated levels of hydroxyl radical, which inflicted genome-wide mutations, and the mutants harbouring nucleotide changes at the rifampicin resistance determining region (RRDR) could regrow back. Interestingly, the killing phase and the regrowth phase showed very low levels of hydroxyl radical unlike the persistence phase cells. The mutations, which are identical to those in the rifampicin-resistant mutants have been reported in the *M. tuberculosis* cells isolated from *in vitro* cultures and from the TB patients.

The data **Chapter 4** presents the response of the *arr* knockout mutant to rifampicin. The persistence phase population of the *arr* knockout mutant showed significantly higher levels of hydroxyl radical generation than the equivalent persistence phase population of the wild type cells. While the wild type cells showed emergence of rifampicin-resistant mutants from the persistence phase, the *arr* knockout mutant showed the emergence of rifampicin-resistant mutants from the very exposure of the cells to rifampicin. In other words, the natural mutation frequency of the *arr* knockout mutant was significantly higher than that of the wild type. This indicated that the *arr* gene might have a natural role in keeping the oxidative stress at lower levels in the cells, which needs further investigation.

The data **Chapter 5** presents the response of *M. smegmatis* cells to moxifloxacin. Here also, the bacilli exposed to lethal concentrations of moxifloxacin showed a killing phase, followed by a persistence phase and a regrowth phase. The moxifloxacin-resistant mutants were found to emerge from the moxifloxacin persistence phase cells. The cells from the regrowth phase of moxifloxacin-exposed cells showed mutations in the quinolone resistance determining region (QRDR) in the gyrase gene, which is the target of moxifloxacin. The mutations, which are identical to those in the rifampicin-resistant mutants have been reported in the *M. tuberculosis* cells isolated from *in vitro* cultures and from the TB patients.

The thesis is concluded with discussion of the findings presented in the three chapters by projecting the comparison and contrast of the response of *M. smegmatis* and *M. tuberculosis* cells to rifampicin. The thesis contains an extensive bibliography.