**University of Leicester**

**Future 50 PhD Scholarship**

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| **Project Reference** | RS Pearl |

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**Section 2 – *Project Information***

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| **Project Title** | **Hypoxia-mediated modulation of antibiotic resistance and susceptibility in clinical non-tuberculous mycobacteria** | |
| **Project Highlights:** | 1. | Clinical Non-tuberculosis mycobacteria [NTM] isolates will be screened for antibiotic resistance/susceptibility under oxic, hypoxic and anoxic conditions. |
| 2. | For those isolates that exhibit increased resistance to frontline drugs under conditions of oxygen limitation, gene expression and phenotypic analysis will identify the mechanistic basis for their enhanced resistance. |
| 3. | Human and mouse macrophage cell lines will be used to investigate the impact of hypoxia on cellular activation leading to antimycobacterial effector function in the presence of frontline antibiotics. |
| **Project Summary** | | |
| **Clinical problem.** Antibiotic treatment of pulmonary disease caused by non-tuberculous mycobacteria [NTM] often requires months of multidrug therapy yet treatment failure remains a significant risk (*1, 2*). Pulmonary NTM disease is caused by *M. avium,* *M. intracellulare, M. chimaera* and/or *M. abscessus,* all of which share formidable innate resistance to antibiotics (*3, 4*) and whose treatment failure rates correlate with greater tissue involvement and persistence of mycobacteria in the sputum (*2*). Meta-analyses report that failure rates can reach 61% (*5*) while long-term recrudescence (*6*) can occur in 84.3% of patients (*7*). It is well-known that pulmonary NTM disease usually presents with radiologic evidence of noncavitary, cavitary or fibrocavitary nodular bronchiectasis which strongly correlates with treatment failure (*8*). These lesions are known to be hypoxic (*9*). Therefore, we hypothesize that treatment failure is related to adaptation of mycobacteria to this oxygen-limited environment which impacts antibiotic resistance and susceptibility as we have previously suggested (*10*).  **Preliminary Data.** We have discovered that *M. avium* is capable of survival and growth in the absence of oxygen. Our working model is that *M. avium* [and likely other NTM species, too] are capable of survival and replication in conditions where oxygen is highly limited. We have demonstrated that *M. avium* can replicate exponentially in a nearly oxygen-free environment under conditions that kill *M. tuberculosis* and *M. smegmatis.*    In order to better understand the mechanisms involved in antibiotic treatment failure for NTM, we propose to address three investigative questions:   * Do clinical NTM isolates demonstrate modulated antibiotic resistance/susceptibility under oxic, hypoxic and anoxic conditions? This question will be addressed using minimal inhibitory concentration [MIC] assays with clarithromycin, azithromycin, rifabutin, ethambutol, streptomycin and amikacin. * For those isolates that exhibit modulated resistance/susceptibility, are patterns of gene expression and phenotypic change similar or different between species? We will describe these changes using gene expression analysis and proteomics. * Does the environment within the activate macrophage under physiological hypoxia impact drug resistance/susceptibility? The question tests whether additional stimuli aside from oxygen limitation are necessary for modulation of drug resistance/susceptibility and will use activated human and mouse macrophage cell lines.   **References.**  1. Gopalaswamy, R., et al., DOI: 10.1186/s12929-020-00667-6  2. Min, J., et al.,DOI: 10.5588/ijtld.14.0139  3. Li, G., et al., DOI: 10.1016*/*j.ijantimicag.2016.10.024  4. Zitko, J., et al., DOI: 10.3390/molecules181214807  5. Xu, H.B., et al., DOI: 10.1007/s10096-013-1962-1  6. Henkle, E., et al., DOI: 10.1513/AnnalsATS.201610-801OC  7. Jo, K.W., et al., DOI: 10.1016/j.jiac.2014.05.010  8. Koh, W.J., et al., DOI: 10.1183/13993003.02503-2016  9. Maycher, B., et al., Can Assoc Radiol J, 2000. **51**(2): p. 93-102.  10. Pearl, J.E., M. Das, and A.M. Cooper, *Immunological roulette: Luck or something more? Considering the connections between host and environment in TB.* Cell Mol Immunol, 2018. **15**(3): p. 226-232. | | |