A Proposed Method for Identification of Streptomycin Resistance from 16s rRNA Sequence by Co-localization Analysis of Fluorescent Signals: A Step Towards Detection of Streptomycin Resistant *Mycobacterium* Species in Culture Free and Gene Amplification Independent Technique

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Dear Editor,

Mycobacterium tuberculosis is the causative organism of Tuberculosis, a major contributor to health burden globally. The increasing incidence of this disease is further compounded by the rise of multi drug resistant & extensively drug resistant strains of the bacillus and lack of adequate therapy options for these strains. Rapid and reliable testing in suspected patients is valuable to physicians for making important therapy decisions. Popular techniques such as sputum smear microscopy and drug susceptibility testing are time-consuming and tedious, whereas modern techniques such as BACTEC and GeneXpert are limited by their availability in poor resource settings [1]. Therefore, development of on-spot tests for rapid detection of drug resistant tuberculosis pathogens is the need of the hour. Here, we propose to conceptualize microscopy based identification of streptomycin resistant *Mycobacterium* species based on sequence analysis of the 16s rRNA of the bacteria which helps to identify the bacteria and predict the antibiotic resistance [2].

The 16s rRNA contains the antibiotic binding site that can be identified by in situ hybridization in combination with confocal laser scanning microscopy [3]. It is reported that streptomycin binds at conserved site localized at 915 stem loop region of 16s rRNA and mutation at this binding site results in resistance [4]. Here we propose to design two oligonucleotide anti-sense probes with different fluorescent emission signals but specific for two separate conserved regions- one streptomycin binding site and other for *Mycobacterium* species identification in 16s rRNA [5]. Such probes are likely to permeate into the pathogens. Co-localization of both the probes in one 16s rRNA sequence is expected to generate co-localized signals which in turn will prove the presence of streptomycin sensitive *Mycobacterium* species in the tissue sample. The Detection of the *Mycobacterium* species identification signal alone will hint the presence of resistant strains of bacteria. We feel that experimental validation of our proposed protocol will pave the path of understanding the presence of streptomycin resistant *Mycobacterium* species in tissue samples in a culture independent as well as gene amplification independent manner, although live and dead bacteria distinction may not be possible by this method.

In order to understand the actual presence of the mutation(s) in streptomycin binding region of 16s rRNA, we have examined for the presence of same in public databases available through World Wide Web. 16s rRNA sequences of *Mycobacterium* species are identified from established databases such as EBI (<https://www.ebi.ac.uk/metagenomics/>) and Greengene (http://greengenes.secondgenome.com/downloads/database/13_5). Bacteria showing identification regions and streptomycin binding site identical to that reported in the literature are identified using PERL programming and labeled as wild-type. Those showing mutations at the same site are labeled as mutant. Sample

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secondary structures of wild-type and mutant *Mycobacteria* are given in the supplementary material (Fig. 1.1 & Fig. 1.2), highlighting the identification region and streptomycin binding site. So we feel that our proposed method is suitable to be worked upon at bench-top.

Compliance with Ethical Standards

Conflict of interest All the authors declare that they do not have any conflict of interest.

Human and Animal Participants This article does not contain any studies with human participants or animals performed by any of the authors. The study does not involve any human participants or experimental animals or any micro organisms or a hazardous chemical. It only requires browsing and analyzing data available in the public domain accessible through world wide web.

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