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COMPARATIVE PROTEOMIC ANALYSIS OF SELECTED CLINICAL ISOLATES OF MDR-TB OF ANDAMAN AND NICOBAR ISLANDS

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ABSTRACT

Tuberculosis (TB) is an airborne infection caused by *Mycobacterium tuberculosis* (Mtb). The problem of TB is increasing worldwide due to prevalence of multi-drug-resistant (MDR) strains and co-infection with human immunodeficiency virus (HIV). India is the country with the highest burden of TB. The Andaman & Nicobar Islands, an archipelago of 527 islands in the Bay of Bengal, is administratively a Union Territory of India. According to the survey of Tuberculosis conducted during 2006-2009 in that place, 8%-10% were found as MDR out of the total positive isolates detected. Tuberculosis is a major public health problem in the islands, particularly among the Nicobarese tribe. This review is designed to analyze and compare the protein expression profile of *M. tuberculosis* MDR isolates from selective hospitals of Andaman & Nicobar Islands to identify as well as characterize proteins, which could be used as a drug target or diagnostic marker for future designing of valuable TB vaccine or TB rapid test.

KEYWORDS: Tuberculosis (TB), *Mycobacterium tuberculosis* (Mtb), multi-drug-resistant (MDR).

INTRODUCTION

Tuberculosis (TB) is an airborne disease caused by *Mycobacterium tuberculosis*.[1] The problem of TB is increasing worldwide due to prevalence of multi-drug-resistant (MDR) strains and coinfection with human immunodeficiency virus (HIV).[2] According to the 2014 WHO report, nine million new cases of TB and 0.36 million deaths including HIV positive cases were recorded.[3] India is the country with the highest burden of TB. TB statistics of the World Health Organisation (WHO) for the year 2016 give an estimated incidence figure of 2.79 million cases of TB for India.[5] Multi drug-resistant tuberculosis (MDR-TB) is TB that never respond to atleast isoniazid and rifampicin, the two most powerful anti-TB drugs. Inappropriate or incorrect use of antimicrobial drugs, or use of ineffective formulations of drugs (use of single drugs, poor quality medicines or bad storage conditions), and premature treatment interruption can cause drug resistance, which can then be transmitted, especially in crowded settings such as prisons and hospitals.[1]

The Andaman & Nicobar Islands, an archipelago of 527 islands in the Bay of Bengal, is administratively a Union Territory of India. The population of the territory is about 3, 79,944 (according to 2011 census) that includes six indigenous tribal groups.[7] During the period 2006-2009, a drug resistance survey of Tuberculosis was conducted by RMRC, Port Blair in these islands and it was reported that out of 74 positive isolates of *M. tuberculosis* 8%-10% were found as MDR. The results indicated that drug resistance is comparable to or slightly higher than the occurrence observed in mainland India and the median prevalence in worldwide surveys.[7] Tuberculosis is a major public health problem in the islands, particularly among the Nicobarese tribe, who were found to have almost double the occurrence and annual risk of infection of tuberculosis in the last survey carried out during RNTCP (The Revised National TB control programme) was introduced in the islands.[7]

In order to prevention of the disease, understanding of host-pathogen interactions, understanding of mechanism, which *M. tuberculosis* uses to persist in a dormant form in the human body, primarily within macrophages is necessary.[8] Once a potent method to improve new TB drugs and powerful and fast diagnosis is an analysis of individual proteins that interfering in pathogenesis and virulence. *M. tuberculosis* can code for at least 4,000 proteins, of which hardly 100 have been characterized.[4]

There are no prior data on proteome analysis in MDR *M. tuberculosis* isolates of Andaman and Nicobar Islands existed. The present study is designed to analyze and compare the protein expression profile of *M. tuberculosis* MDR isolates from selective hospitals of Andaman &

Nicobar state to identify as well as characterize proteins, which could be used as a drug target or diagnostic marker for future designing of valuable TB vaccine or TB rapid test.

OBJECTIVES

1. Isolation of *M. tuberculosis* from clinical specimens collected from TB patients (MDR and Sensitive) of Andaman & Nicobar Islands and their biochemical test followed by drug Susceptibility test.
2. Extraction of protein from isolated strains of *Mtb* (MDR and Sensitive).
3. Study the expression profile of each protein sample and characterize them.

METHODOLOGY

1. Isolation of *M. tuberculosis* according to the methods described in ref.[6,1] Three specimens of sputum (two on spots and one in the morning) will be collected over two consecutive days from each subject of identified patients of tuberculosis (sensitive and MDR-TB isolates) (as per RNTCP guidelines) will be sent to the laboratory maintaining cold-chain conditions at 4^{o}c. Sputum smears have to prepare and stained by Ziehl Neelsen (Z-N) method for acid-fast bacilli identification. Homogenization & decontamination of sputum specimens for culture will be performed by using 4% Sodium hydroxide (NaOH) solution following Modified Petroff's method. To the sputum specimens 4% NaOH needs to be added in double the volume and shake to digest the sputum, let stand for 15 min at room temperature with occasional shaking. After subsequent centrifugation supernatant needs to be decanted and kept sediment for L-J medium (Löwenstein–Jensen medium) inoculation. Colonies on L-J medium slopes will be identified as *M. tuberculosis* or Non-tuberculosis Mycobacterium using biochemical test. Susceptibility of the *M. tuberculosis* isolates to first-line drugs Streptomycin, Isoniazid, Rifampicin, and Ethambutol will be tested by proportion sensitivity test (PST) method.
2. *Protein extraction procedures*; *M. tuberculosis* strains will be cultured in Middlebrook 7H9 medium for 4 weeks at 37°C. The bacteria will be separated from the culture medium by centrifugation and Protein extraction will be performed by sequential extraction method.[9] Their protein concentration will be determined by using the Bradford method (Bradford, 1976).[11] Extracted proteins from each strain (sensitive and MDR-TB isolates) need to be loaded in the gradient polyacrylamide gel. Proteins will be visualized with Coomassie Brilliant Blue stain. The proteins need to be lyophilized.
3. *Two-dimensional gel electrophoresis (2DE);* the extracted protein samples with rehydration buffer will be used for IPG strip passive rehydration. The required amount of proteins need to be loaded on IPG strip. The gel strips will be focused on an isoelectronic focusing unit according to the program required. In order to transition the IPG strips to 2-D PAGE, they need to be incubated two times (10 min) in the equilibration solution. To solubilize focused proteins and to allow SDS binding in preparation for the second dimension, IPG strips need to be placed one strip in each channel and will be filling it with equilibration buffer followed by subsequent incubation. Finally, IPG strips will be removed and embed it on to second dimension electrophoresis (2-D PAGE). The proteins will be visualized with Coomassie Brilliant Blue stain for detection in polyacrylamide gels.
4. The identification of proteins by mass spectrometry is one of the most widely used techniques in proteomics. Automated two-dimensional electrophoresis coupled with MALDI-TOF-TOF (matrix-assisted laser desorption/ionization) has proven to be very efficient in the analysis of complex protein/peptide mixtures in comparison to conventional protein sequencing procedures.[1] Protein spots of polyacrylamide gels electrophoresis need to be excised in 1×1 mm pieces. Purified peptides will be applied to an Anchor Chip and need to be subjected in MALDI-TOF MS analysis.
5. Protein profiles will be compared by a 2DE run in triplicates for each isolate.

Significance

In this review, it needs to be focused on the proteins, which either upregulated/expressed or only exist in MDR-TB by comparative proteome analysis of MDR-TB and sensitive isolates. It is a complementary approach to study proteomics, which will reveal the functional information, since proteins are the workhorses of cellular metabolism, ultimately. The proteomic tools can be lead to the identification of different antigens between MDR-TB and sensitive isolates, which can form the basis of immunodiagnostic reagents. Since there no any reported work has done on a proteomic study on MDR-TB of the patients of Andaman & Nicobar, It will be an initial step towards proteomics on it.

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