Molecular detection of *mycobacterium tuberculosis* in environmental samples and mutational analysis of *pncA* gene by multiplex allele specific PCR

By
Saema Salim

14002140004

ADVISOR:

Dr. Nouman Rasool

DEPARTMENT OF CHEMISTRY
SCHOOL OF SCIENCE
UNIVERSITY OF MANAGEMENT AND TECHNOLOGY,
LAHORE, PAKISTAN
2016

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In the name of ALLAH the beneficent, the merciful Read! Thy Lord is most honorable most benevolent, who taught (to write) by pen; He taught man that which he knew not.

(Sura AL-ALAQ 30:3-5)AL-QURAN

RESEARCH COMPLETION CERTIFICATE

Certified that the research work contained in this thesis titled, "Molecular detection of *Mycobacterium tuberculosis* in environmental samples and mutational analysis of *pncA* gene by multiplex allele specific PCR''has been carried out and completed by Saema Salim **ID: 14002140004.** The quantum and the quality of the work contained in this thesis is adequate for the award of Degree of MS/M.Phil.

Supervisor	**External Examiner**
Dr. NoumanRasool	**Dr. Nasir Mehmood**
Assistant Professor	Assistant Professor
Department of Chemistry,	Department of chemical pathology
UMT, Lahore.	UHS, Lahore

Dr. SammiaShahid	**Dr. Muhammad Azhar Iqbal**
Chairperson,	**Dean**
Department of Chemistry,	School of Science,
UMT, Lahore.	UMT, Lahore

DECLARATION

I Saema Salim D/O Muhammad Salim Khan ID: 14002140004Session **2014-2016** hereby declare that the matter printed in the thesis title **"Molecular detection of *Mycobacterium tuberculosis* in environmental samples and mutational analysis of *pncA* gene by multiplex allele specific PCR"** is my own work and has not been printed, published and submitted as research work, thesis or publication in any form in any University, Research institution etc. in Pakistan or Abroad.

Dated: _____ _____

( )

This thesis is dedicated to my beloved father

Muhammad Salim Khan

ABSTRACT

Tuberculosis (TB) has reemerged as one of the main sources of death in the most recent decade. Tuberculosis (TB), one of the basic human diseases, brought about by species from *Mycobacterium tuberculosis* complex, creating 3,000,000 deaths for each year around the world (WHO, 1996). Five species are included in MTBC. These are *M. tuberculosis*, *M. canetti*, *M. bovis*, *M. microti*and *M. africanum.* The spread of TB is because of migration, the rise of drug resistant strains.

This studyis aimed to identify the *M. tuberculosis* and *M. bovis*in environmental samples (drinking water, sewerage water, hospital waste) and bovine milk samples using PCR method. DNA was extracted from bovine milk samples, drinking water samples (tape water), hospital waste (waste water) and sewerage samples. The primers M and S were used to generate amplicons of sizes 318 and 291 bp which represented *M. tuberculosis* and *M. bovis*respectively. 55 samples of milk were tested and the prevalence of TB in these samples was 5.4%. 30 samples of drinking water were tested in this study and the prevalence of TB in these samples was 3.3%. 5 samples of waste water from hospitals were tested and the prevalence of TB in these samples was 40%. 10 samples of sewerage water were tested and the prevalence of TB in these samples was 40%.

The mutations of *pncA*gene were analyzed in these positive TB samples. Pyrazinamide (PZA) is an important first-line drug in the treatment of MDR-TB. These TB positive samples were subjected to multiplex allele specific PCR. Two mutations were targeted in this study with one being the leucine replaced by proline in the amino acid position 85 and the other being the aspartate changing to alanine in the amino acid position 12. The substitution being the as partate to alanine at amino acid 12 was more frequent as compared to substitution being the leucine replaced by proline in the amino acid position 85.

Presence of TB in these samples is a great health hazard. So control measures are required to stop the transmission of TB.

ACKNOWLEDGMENT

Thanks to **ALLAH**, who pulled us through the times, when every stone was turned against us, guide us in the light and darkness and show the right path in the right direction, provided us with the opportunity, courage and ability to complete this humble contribution towards knowledge.

All respects for the **Holy Prophet Muhammad (PBUH**), who has sent as greatest educator for the guidance forever and whose teaching enables to see what is beautiful, to know what is true and to love what is good.

I express my deepest gratitude to my research supervisor**, Dr. NoumanRasool** for the encouragement, guidance, unfailing patience, masterly advice and inspiring attitude, without which this study would not be taken.

I would like to thank **Dr. Nasir Mehmood,** whose guidance greatly helped me in the completion of my project. I thank to him for giving me encouragement and organizing the necessary infrastructure for my Project to be possible.

And a special thanks to **Dr. FouziaTabbassum** whose kind attitude always encouraged me.

I also thanks to **Safoora** for helping me in executing this project.

I would take this prospect to extend my admiration and appreciation to my research fellow **Iram Afzal** for her cordial harmonization.

I really under high obligation for expressing my best wishes for my friends **Hafsa, Iram and Fouzia**who encourage me and provide nice company and love.

This acknowledgment would be incomplete if I do not pay my sincere and hearty thanks to my cherished and loving parents for their sacrifices, prayer, and affection without which it would have been just a dream to achieve any goal.

List of symbols and abbreviations

%	Percentage
°C	Degree of Celsius
ACM	Absolute concentration method
BMM	Broth micro dilution method
bp	Base pairs
CAS	Central Asian Strain
CDC	Centers for Disease Control and Prevention
CMI	Cell-mediated immunity
CFU	Colony forming unit
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide
DTH	Delayed-type hypersensitivity
EMB	Ethambutol
HPOA	Protonated POA
HIV	Human Immunodeficiency Virus
INH	Isoniazid
$MgCl_2$	Magnesium Chloride
MDR-TB	Multi-drug resistance tuberculosis
MIC	Minimum inhibitory concentration
mPCR	Multiplex Polymerase chain reaction
Mtb	*Mycobacterium tuberculosis*
MTBC	*Mycobacterium tuberculosis* Complex
M. africanum	*Mycobacterium africanum*
M. canetti	*Mycobacterium canetti*
M. leprae	*Mycobacterium leprae*
M. microti	*Mycobacterium microti*
M. tuberculosis	*Mycobacterium tuberculosis*
MIRUs	Mycobacterial interspersed repetitive units

MTBDRplus	*M. tuberculosis*-multidrug resistantplus
PCR	Polymerase Chain Reaction
POA	Pyrazinoic acid
PZA	Pyrazinamide
PZasePyrazinamidase	
RIF	Rifampicin
REMA	Resazurinmicrotitre plate assay
STR	Streptomycin
TAE	Tris Acetate EDTA
TB	Tuberculosis
μL	Microliter
V	Volts
WHO	World Health Organization
XDR	Extremely-drug Resistance

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1. INTRODUCTION

1.1.Tuberculosis

Tuberculosis (TB) has reemerged as one of the main sources of death in the most recent decade (Bloom and Murray, 1992). Tuberculosis (TB), one of the basic human disease, brought about by species from *Mycobacterium tuberculosis* complex, creating 3,000,000 deaths for each year around the world (WHO, 1996). Five species are included in *M. tuberculosis* complex. These are *M. tuberculosis*, *M. canetti*, *M. bovis*, *M. microti*and *M. africanum.* The spread of TB is because of migration, the rise of drug resistant strains (WHO, 1997). From the inactive stage (infection without active disease, LTBI) to active TB, TB ordinarily advances gradually while in HIV co-infected patients, the progression can be quick and lethal. Development from LTBI to active TB more often occurs in just a little proportion of individuals (WHO, 2011). To resist the activity of antimicrobial agents, *M. tuberculosis* and different species from the *M. tuberculosis* complex uses a few procedures