

**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

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DEPARTMENT OF CHEMISTRY  
SCHOOL OF SCIENCE  
UNIVERSITY OF MANAGEMENT AND TECHNOLOGY,  
LAHORE, PAKISTAN  
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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****Dr. Nouman Rasool**

Assistant Professor

Department of Chemistry,

UMT, Lahore.

---

**External Examiner****Dr. Nasir Mehmood**

Assistant Professor

Department of chemical pathology

UHS, Lahore

---

**Dr. Sammia Shahid****Chairperson,**

Department of Chemistry,

UMT, Lahore.

---

**Dr. Muhammad Azhar Iqbal****Dean**

School of Science,

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 study is 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 (tap 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 aspartate 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.

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## 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 <sub>2</sub>	Magnesium Chloride
MDR-TB	Multi-drug resistance tuberculosis
MIC	Minimum inhibitory concentration
mPCR	Multiplex Polymerase chain reaction
<i>Mtb</i>	<i>Mycobacterium tuberculosis</i>
MTBC	<i>Mycobacterium tuberculosis</i> Complex
<i>M. africanum</i>	<i>Mycobacterium africanum</i>
<i>M. canetti</i>	<i>Mycobacterium canetti</i>
<i>M. leprae</i>	<i>Mycobacterium leprae</i>
<i>M. microti</i>	<i>Mycobacterium microti</i>
<i>M. tuberculosis</i>	<i>Mycobacterium tuberculosis</i>
MIRUs	Mycobacterial interspersed repetitive units

MTBDRplus	<i>M. tuberculosis</i> -multidrug resistantplus
PCR	Polymerase Chain Reaction
POA	Pyrazinoic acid
PZA	Pyrazinamide
PZase	Pyrazinamidase
RIF	Rifampicin
REMA	Resazurinmicrotitre plate assay
STR	Streptomycin
TAE	Tris Acetate EDTA
TB	Tuberculosis
$\mu\text{L}$	Microliter
V	Volts
WHO	World Health Organization
XDR	Extremely-drug Resistance

## Table of contents

<b>Abstract</b> .....	i
<b>Acknowledgment</b> .....	ii
<b>List of symbols and abbreviations</b> .....	iii
<b>Table of contents</b> .....	v
<b>List of tables</b> .....	viii
<b>List of figures</b> .....	ix
<b>1. Introduction</b> .....	1
1.1. Tuberculosis.....	1
1.2. Types of tuberculosis.....	1
1.3. Prevalence in World.....	2
1.4. Prevalence in Pakistan.....	2
1.5. Route of infection.....	3
1.6. Pathogenesis of tuberculosis.....	3
1.7. Comparison of active disease and latent TB infection.....	4
1.8. <i>Mycobacterium tuberculosis</i> complex.....	5
1.8.1. <i>Mycobacterium tuberculosis</i> .....	5
1.8.1.1. Morphology of <i>M. tuberculosis</i> .....	8
1.8.1.2. Classification of <i>M. tuberculosis</i> .....	8
1.8.1.3. Cell wall of <i>M. tuberculosis</i> .....	8
1.8.2. <i>Mycobacterium bovis</i> .....	9
1.8.3. <i>Mycobacterium africanum</i> .....	10
1.8.4. <i>Mycobacterium leprae</i> .....	10
1.9. Tuberculosis strains.....	10
1.9.1. Multidrug-resistant tuberculosis.....	11
1.9.2. Extremely drug-resistant tuberculosis.....	11

1.10. Treatment of TB.....	12
1.10.1. Standard TB drugs .....	12
1.11. Pyrazinamide.....	14
1.11.1. Mode of action of PZA .....	15
1.12. Background of problem .....	16
1.13. Aims and objectives .....	16
<b>2. Review of literature .....</b>	<b>18</b>
<b>3. Materials and Method.....</b>	<b>27</b>
3.1. Sample collection .....	27
3.1.1. Bovine milk samples.....	27
3.1.2. Drinking water samples.....	27
3.1.3. Hospital waste samples .....	27
3.1.4. Sewerage samples.....	28
3.2. Isolation of genomic DNA .....	28
3.2.1. Quantification of DNA .....	29
3.2.2. Analysis of DNA isolation .....	29
3.3. Detection of the presence of <i>M. tuberculosis</i> and <i>M .bovis</i> in various samples .....	29
3.3.1. Primer designing .....	29
3.3.2. Amplification of gene.....	30
3.3.3. Analysis of amplicons.....	30
3.4. Analysis of <i>pncA</i> gene mutations by multiplex allele specific PCR.....	31
3.4.1. Primer designing .....	31
3.4.2. Amplification of <i>pncA</i> gene.....	31
3.4.3. Analysis of amplicons.....	32
<b>4. Result.....</b>	<b>33</b>

4.1. Isolation and quantification of DNA from various samples .....	33
4.1.1. Isolation and quantification of DNA from bovine milk samples.....	33
4.1.2. Isolation and quantification of DNA from drinking water samples .....	35
4.1.3. Isolation and quantification of DNA from waste water from hospitals.....	37
4.1.4. Isolation and quantification of DNA from sewerage samples .....	38
4.2. Analysis of DNA extracted from various samples by electrophoresis .....	39
4.3. Detection of the presence of <i>M. tuberculosis</i> and <i>M. bovis</i> in various samples .....	41
4.4. Analysis of <i>pncA</i> gene mutation .....	43
4.4.1. Frequency of targeted mutations in various samples .....	45
<b>5. Discussion.....</b>	<b>46</b>
<b>Conclusion .....</b>	<b>49</b>
<b>References.....</b>	<b>50</b>

## List of Tables

Table 1.1: Pulmonary TB disease and <i>M. tuberculosis</i> infection .....	5
Table 1.2: Standard anti-TB drugs, target, discovery year and activity .....	13
Table 3.1: Sequence of primers used for the detection of presence of <i>M. tuberculosis</i> & <i>M. bovis</i> in various samples.....	30
Table 3.2: Sequence of primers for <i>pncA</i> gene amplification.....	31
Table 4.1: Total DNA obtained from bovine milk samples.....	33
Table 4.2: Total DNA obtained by drinking water samples .....	36
Table 4.3: Total DNA obtained from hospital waste samples .....	38
Table 4.4: Total DNA obtained from sewerage samples .....	39
Table 4.5: Detection of the presence of <i>M. tuberculosis</i> and <i>M. bovis</i> by PCR using various samples.....	43
Table 4.6: Mutations in various samples .....	45

## List of Figures

Fig 1.1: Steps of <i>M. tuberculosis</i> infection .....	7
Fig 1.2: Structure of mycobacterium cell wall.....	9
Fig 1.3: Recent group of TB drugs consisting front-line defense .....	14
Fig 1.4: Mode of action of PZA against <i>M. tuberculosis</i> .....	16
Fig 4.1: Ethidium bromide stained 0.8% gel extracted DNA.....	40
Fig 4.2: Ethidium bromide stained 0.8% gel extracted DNA.....	40
Fig 4.3: Ethidium bromide stained 0.8% gel extracted DNA.....	41
Fig 4.4: Ethidium bromide stained 1.5% agarose gel demonstrating PCR amplified 291 bp fragments of <i>M. bovis</i> .....	42
Fig 4.5: Ethidium bromide stained 1.5% agarose gel demonstrating PCR amplified 318 bp fragments of <i>M. tuberculosis</i> .....	42
Figure 4.6:Ethidium bromide stained 1.5% agarose gel demonstrating PCR amplified 250bp and 447bp fragments of <i>pncA</i> gene .....	44
Figure 4.7: Ethidium bromide stained 1.5% agarose gel demonstrating PCR amplified 250bp fragments of <i>pncA</i> gene .....	44

# 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