Detection of drug resistant mycobacterium tuberculosis using genotypic method in positive cases admitted in Abbassia Chest Hospital

Thesis

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Abbreviations

AFB	Acid fast bacilli
Ag	Antigen
AII	Airborne infection isolation
AMK	Amikacin
AMTD	Amplified mycobacterium tuberculosis direct test
BSA	Bovine serum albumin
CAP	Capreomycin
CDATs	Commercial direct amplification Tests
CFP	Culture filtrate protein
CRP	C-reactive protein
CT	Computed tomography
Ct	Cycle threshold
DNA	Deoxy ribo nucleic acid
dNTPs	Deoxynucleoside triphosphates
DOTS	Directly observed therapy strategy
DR	Direct repeat
DST	Drug susceptibility test
EDTA	Ethylene diaminete traacetic acid
ELISA	Enzyme-linked immunosorbent Assay
ELISPOT	Enzyme-linked immunospot

EMB	Ethambutol
EQA	Eternal quality assurance
ESAT	Early secretary antigenic target
ESR	Erythrocyte sedimentation rate
ETR	Exact tandem repeat
FDA	Food and drug administration
FNAB	Fine needle aspiration biopsy
FQ	Fluroquinolones
Hb	Hemoglobin
HIV	Human immunodeficiency virus
HPLC	High performance liquid Chromatography
HPTLC	High performance thin liquid Chromatography
IFN	Interferon
IGRAs	Interferon gamma release assays
INH	Iso nicotinic acid hydrazide
IS	Insertion segment
IUATLD	International union against tuberculosis and lung disease
KAN	Kanamycin
KDa	Kilo dalton
L J	Lowenstein–jensen
LAM	Lipo-arabinomannan

LAMP	Loop-mediated isothermal amplification
LPA	Line probe assay
LTBI	Latent tuberculosis infection
MDDR	Molecular detection of drug resistance
MDR	Multiple drug resistance
MGIT	Mycobacterial growth indicator tube
MIRU-VNTR	Mycobacterial interspersed repetitive-unit-variable-number tandem-repeats
MLVA	Multi locus variable-number tandem-repeat analysis
MODS	Microscopic observation of drug susceptibility assay
MPTR	Major polymorphic tandem repeat
mRNA	Messenger ribonucleic acid
MTB	Mycobacterium tuberculosis
MTBC	Mycobacterium tuberculosis complex
NAA	Nucleic acid amplification
NPV	Negative predictive value

NRA	Nitrate reductase assay
NTPs	National TB programmes
PAS	Para-amino salicylic acid
PCC	Probe check control
PCR	Polymerase chain reaction
PGRS	Polymorphic guanine-cytosine rich sequence
PPD	Purified protein derivative
PPV	positive predictive value
PSQ	Pyrosequencing
PZA	Pyrazinamide
PZase	Pyrazinamidase enzyme
QFT-Gold	Quanti feron-TB gold
RFLP	Restriction fragment length Polymorphism
RIF	Rifampicin
RMF	Rifampicin
RNA	Ribonucleic acid
RRDR	Rifampin resistance determining Region
rRNA	Ribosomal ribonucleic acid
RR-TB	Rifampicin resistance tuberculosis
SBE	Single-base extension

SGOT	Serum glutamate oxaloacetate Transaminase
SGPT	Serum glutamate pyruvate Transaminase
SM	Streptomycin
SNPs	Single nucleotide polymorphism
SPC	Sample processing control
SuPAR	Soluble urokinase type plasminogen activator receptor
TB	Tuberculosis
TMA	Transcriptase-mediated Amplification
TNF	Tumor necrosis factor
TST	Tuberculin skin test
WBC	White blood cells
WGS	Whole genome sequencing
WHO	World Health Organization
XDR	Extensive drug resistance
ZN	Ziehl–Neelsen

Introduction

In developing countries, tuberculosis (TB) considered as a foremost infectious disease that killing nearly 2 million person every year. The incidence of Mycobacterium tuberculosis resistance specially towered the first line treatment was increased and contributed in the present TB epidemic. ***(WHO, 2014)***

In spite of the organization efforts in controlling TB infection, the mortality and morbidity of it still high. From all factors that associated with its spread, the expansion of human immunodeficiency virus (HIV) infection was the major one as it increases the possibility of TB strains to become resistance. ***(WHO, 2014)***

Another vital cause that facilitating the spread of new strains is imagination from countries with higher incidence to another with low, besides the overcrowding in hospitals and other public places, expansion of population and spread of poverty, intravenous drug abuse and homelessness. ***(WHO, 2014)***

Patients with active state (sputum positive) are the main source for infection spread. ***(WHO, 2014)***

Drug resistance towered the first line regimen is defined as drug resistance TB (DR-TB), which include multi drug resistance form (MDR-TB) while the strains is resisance at least isoniazid (INH) and rifampcin (RIF). ***(WHO, 2014)***

The susceptible TB patients showed successful response towered their regimens depend on many involved factors as drug combination, duration, side effect and costs. The conversion state from positive to negative sputum within 2 month of treatments is considered as good sign for improvement, beside radiological and clinical picture, on the other hand positive sputum culture after 4 month of treatment denote failure of treatment. **(ATS, 2003)**

Incomplete treatment or interrupted one shard in developing acquired resistance, while primary resistance happens with infection by resistance strain from the begging. **(ATS, 2003)**

Due to lack of commitment with adequate regimen protocol, the acquired resistance developed, and thus spread of resistance strain from patients to another become easy which lead to the primary resistance. Some factors associated with development of drug resistance as non-adherence to therapy; due to its longer duration, several combinations and side effects, co infection with HIV and poor resources. **(WHO, 2014)**

TB infection; whatever pulmonary or extra pulmonary, is usually diagnosed by clinical suspicious which followed by so many investigation tools to confirm it as chest radiology, direct smear evaluation for Acid fact bacilli (AFB), sputum culture in liquid and solid one, and nucleic acid detection of TB bacteria using PCR hybridization and amplification. **(Mitchison, 2005)**

Diagnosis of TB or drug resistance TB improved recently either by using conventional (phenotypic) and molecular (genotypic) methods. **(WHO, 2014)**

The conventional DST technique needs culture of organisms and evaluate their growth in

the presence of anti-TB drugs, while molecular (genotypic) DST methods depend on detecting the resistance associated mutation in specific gene of organism without any need for bacterial growth evaluation, so it can be happen rapidly and with any specimen type. **(Palomino, 2005)& (Traore et al, 2006)**

Aim of the work

The study designed to investigate the genetic mutation in TB organisms that responsible for drug resistance pattern that isolated from patients with positive sputum aiming for early detection of MDR-TB.