Journal of Advances in Science and Technology

Vol. VIII, Issue No. XV, November-2014, ISSN 2230-9659

RECOGNITION OF MYCOBACTERIUM TUBERCULOSIS RESISTANCE: MUTATIONS TO RIFAMPIN AND ISONIAZID BY REAL-TIME PCR

AN INTERNATIONALLY INDEXED PEER REVIEWED & REFEREED JOURNAL

www.ignited.in

Journal of Advances in Science and Technology
Vol. VIII, Issue No. XV, November-2014, ISSN 2230-9659

Recognition of Mycobacterium Tuberculosis Resistance: Mutations to Rifampin and Isoniazid by Real-Time PCR

Md Nurul Hoda

Research Scholar

Abstract – Control of tuberculosis is threatened by widespread emergence of drug resistance in Mycobacterium tuberculosis. Understanding the molecular basis of resistance might lead to development of novel rapid methods for diagnosing drug resistance. We set out to determine the molecular basis of resistance to rifampicin, a major component of multidrug regimens used for treating tuberculosis.

Keywords: Ant tuberculosis, World Health Organization, Mycobacterium tuberculosis

----------♦----------

INTRODUCTION

The need to limit the transmission of drug-resistant strains and to reduce the time between diagnosis and effective therapy requires rapid identification of resistance. Classical phenotypic determination of resistance may take up to 10 weeks after referral of a sample to the laboratory. Nucleic acid amplification assays can greatly shorten the detection time. Due to this major advantage, in the last few years, a lot of effort has been invested in designing performance protocols for genotyping MTB strains. Real-time PCR came to be the main approach because of its special features: high sensitivity and specificity as well as speed, with no need for any post-PCR sample manipulation. The results from fundamental research (such as the sequencing of the complete MTB genome) were used to design specific primers and probes that would allow the identification of gene mutations associated with drug resistance in MTB.

It is known that RMP interferes with RNA synthesis by binding to bacterial RNA polymerase. Resistance to RMP is conferred by mutations resulting in at least eight amino acids substitutions in the *rpoB* subunit of RNA polymerase. Mutations in a limited region of *rpoB* have been found in >95% of RMP-resistant clinical isolates of MTB and has been shown to result in high-level resistance (MIC >32 µg/mL) to RMP and cross-resistance to all rifamycins [2].

INH acts by inhibiting an oxygen-sensitive pathway in the mycolic acid biosynthesis of the cell wall. At least four genes have been described to be involved in resistance to isoniazid: the katG gene, which encodes a catalase; the *inhA* gene, whose product is a target for INH; and the *oxyR* gene and the neighboring *aphC* gene, as well as their intergenic region [3]. Several real-time PCR-based methods targeting these specific genomic regions have been described [4-9]. The purpose of the present study was to evaluate the LightCycler instrument in the detection of these mutations associated with resistant MTB strains isolated from Romanian patients.

REVIEW OF LITERATURE:

Uses of drug resistance:

Multi Drug Resistant Tuberculosis is a man-made problem. Blame for this goes to the government, the pharmaceutical industry, doctors, patients and their families, each of whom contributes in his/her own way to this problem. The government plays its share by providing poor infrastructure in the National Tuberculosis Control Programme, unnecessary administrative control on purchase with no proper mechanism on quality control and bioavailability tests.

The pharmaceutical industry contributes by manufacturing drugs of uncertain bio-availability in fixed dose or inappropriate drug combinations, poor storage condition of drugs and substitution by inferior quality drugs by pharmacies.

Risk factors of drug resistance:

Three most important risk factors, identified in the causation of drug resistant tuberculosis are- inappropriate previous treatment with anti-tubercular drugs, high prevalence of drug resistant tuberculosis in the community and contact with patients known to have drug resistant tuberculosis. However standardized short course chemotherapy carries a

little risk of emergence of MDR-TB. Other factors that may be responsible for increased risk of resistant tuberculosis are: Co-infection with HIV, socio-economically deprived groups in slums, prisons, correctional facilities, day care centres, intravenous drug abusers and other immuno-compromised states as in transplant recipients, anti-cancer chemotherapy recipients and patients with diabetes mellitus.

Real-time PCR using the Lightycler

The MTB drug-resistance genotyping was performed by adapting a previously described protocol [2]. The method published by Torres *et al*. was designed as a single-tube method capable of detecting RMP and INH resistance mutations; one set of primers and two fluorescently labeled hybridization probes were used for each targeted region. One set of primers and two sets of probes (rpoB1 and rpoB2) that targeted the *rpoB* gene were used for detection of RMP resistance and one set of primers and probes each for the *katG* and *inhA* genes in order to test for INH resistance. All primers and probes were synthesized by TIB Molbiol (DNA Synthesis Service; Roche Diagnostics, Berlin, Germany). The real-time PCR was followed by melting curve analysis, both performed on the LightCycler instrument (Roche Diagnostics, Mannheim, Germany). We used the same PCR conditions (components concentration, cycling, and melting programs) as previously described, but we added 10 more cycles of amplification to the 35 recommended [3]. We included into each experimental run one negative control (the DNA template was replaced with PCR-grade water) and one positive control (the DNA template was isolated from *M tuberculosis* H37Rv, a strain susceptible to both INH and RMP).

Mycobacterium tuberculosis and resistance mutations:

Global prospects for TB control are challenged by the emergence of mono-resistant strains, multidrug resistant and extensively drug resistant strains. *Mono-resistance* has been encountered against all anti-TB drugs and it arises by chromosomal mutation in a small proportion of bacilli in any wild strain. These resistant strains are selected by mono-therapy in conditions where the bacterial population is sufficiently large, as in cavitatory pulmonary disease.

Multidrug resistant (MDR) tuberculosis is caused by strains which are generally considered to be resistant to at least two drugs, such as isoniazid and rifampin. The term for *extensively drug resistant* (XDR) tuberculosis appeared in the literature in 2006 to describe a severe form of disease, presently defined as MDR-TB with additional bacillary resistance to any fluoroquinolone and at least one of three second-line injectable drugs: capreomycin, kanamycin and amikacin. XDR-TB is not yet the final product in the treatment of TB; amplification of drug resistance is still likely to occur if we do not manage XDR-TB properly [3].

There is also important to differentiate between primary and acquired resistance which is usually more severe. *Primary resistance* occurs in persons who have not received any anti-TB therapy and they are initially infected with drug resistant strains. *Acquired resistance* occurs in patients who have previously received anti-TB therapy and resistance develops as a result of inadequate regimen. Also, drug resistance among previously treated cases may not be truly acquired resistance, but contains a combination of several types of resistance: patients who have acquired resistance during TB treatment, patients who have been primarily infected with a resistant strain and subsequently failed therapy and patients who have been reinfected with a resistant strain.

The most recent estimates on the prevalence of anti-TB drug resistance come from surveys conducted by the WHO and the International Union against Tuberculosis and Lung Disease [4]. These organizations investigated both new and previously treated TB cases in 93 geographical settings between 2002–2006. In these surveys, the prevalence of MDR-TB ranged from 0% to 22% among newly diagnosed cases and from 0% to 60% among previously treated cases. Rates of MDR-TB are especially high in some countries, including the former Soviet Union [1] and the frequency of acquired resistance to multi drugs is more common than primary resistance. In addition, since 2002, 45 countries have reported cases of XDR-TB. Of the MDR isolates tested for second-line drugs, 0%–30 % were found to be XDR.

Findings from modeling exercise warn that if MDR-TB case detection and treatment rates increase to the WHO target of 70%, without simultaneously increasing MDR-TB cure rates, XDR-TB could increase exponentially [3]. Clearly, control of drug resistant TB relies on preventing the emergence and amplification of drug resistance as well as timely diagnosis and proper management of drug-resistant disease.

Mycobacterium strains genotyping methods

Historically, the microbiological tools used to differentiate or subspeciate clinical isolates of Mycobacterium tuberculosis were based on phage and/or drug susceptibility patterns. The comparison of strains has evolved from analysis of protein products (phenotyping) to the analysis of genetic content (genotyping).

TB *genotyping* is a laboratory-based approach used to analyze DNA of *Mycobacterium tuberculosis* due to specific sections of the *M. tuberculosis* genome that form distinct genetic patterns which help to distinguish different strains of *M. tuberculosis* [17]. Genotyping is important in research of genes and gene variants associated with disease. Genotyping results combined with epidemiologic data are used in a wide range of epidemiological, clinical and basic studies: to

demonstrate foci of transmission in cites, nosocomial outbreaks, and congregate settings; to study transmission among high-risk populations such as HIV-positive and homeless persons; to evaluate cross-contamination in the clinical laboratory; and to analyze sequence changes as they reflect rates of molecular evolution.

The first genotyping methods of *M. tuberculosis* were introduced in 1990. In the ensuing years, more than 30,000 strains have been analyzed and cataloged, and thousands of unique patterns have been identified. Current methods of genotyping include restriction fragment length polymorphism identification (RFLP) of genomic DNA, random amplified polymorphic detection (RAPD) of genomic DNA, amplified fragment length polymorphism detection (AFLPD), polymerase chain reaction (PCR), DNA sequencing, allele specific oligonucleotide (ASO) probes and DNA microarrays hybridization. Some of the techniques used nowadays are characterized below [18].

CONCLUSION:

One-third of the world's population is thought to be infected with the tuberculosis (TB) bacillus [10]. Despite the availability of highly efficacious treatment for decades, TB remains a major global health problem. In 1993, the World Health Organization (WHO) declared TB to be a global public health emergency, at a time when an estimated 7–8 million cases and 1.3–1.6 million deaths occurred each year. In 2010, there were approximately 8.5–9.2 million cases and 1.2–1.5 million deaths from TB worldwide [11]. TB is the second leading cause of death from an infectious disease (after HIV, which caused an estimated 1.8 million deaths in 2008).

REFERENCES:

1. Cho SN, Brennan PJ. Tuberculosis: Diagnostics. Tuberculosis (Edinb) 2007; 87:S14-7.

2 Marin M, Garcia de Viedma D, Ruiz-Serrano MJ, Bouza E. Rapid direct detection of multiple rifampin and isoniazid resistance mutations in Mycobacterium tuberculosis in respiratory samples by real-time PCR. Antimicrob Agents Chemother 2004;48:4293-300.

3. Torres MJ, Criado A, Palomares JC, Aznar J. Use of real-time PCR and fluorimetry for rapid detection of rifampin and isoniazid resistance-associated mutations in Mycobacterium tuberculosis. J Clin Microbiol 2000;38:3194-9.

4. Garcia de Viedma D, del Sol Diaz Infantes M, Lasala F, Chaves F, Alcala L, Bouza E. New real-time PCR able to detect in a single tube multiple rifampin resistance mutations and high-level isoniazid resistance mutations in Mycobacterium tuberculosis. J Clin Microbiol 2002;40:988-95.

5. Kocagoz T, Saribas Z, Alp A. Rapid determination of rifampin resistance in clinical isolates of Mycobacterium tuberculosis by real-time PCR. J Clin Microbiol 2005;43:6015-9.

6. Espasa M, Gonzalez-Martin J, Alcaide F, Aragon LM, Lonca J, Manterola JM, et al. Direct detection in clinical samples of multiple gene mutations causing resistance of Mycobacterium tuberculosis to isoanizid and rifampicin using fluorogenic probes. J Antimicrob Chemother 2005;55:860-5.

7. Wada T, Maeda S, Tamaru A, Imai S, Hase A, Kobayashi K. Dual-probe assay for rapid detection of drug-resistant Mycobacterium tuberculosis by real-time PCR. J Clin Microbiol 2004;42:5277-85.

8. Ruiz M, Torres MJ, Llanos AC, Arroyo A, Palomares JC, Aznar J. Direct detection of rifampin- and isoanizid-resistant Mycobacterium tuberculosis in auramine-rhodamine-positive sputum specimens by real-time PCR. J Clin Microbiol 2004;42:1585-9.

9. Hillemann D, Weizenegger M, Kubica T, Richter E, Niemann S. Use of the genotype MTBDR assay for rapid detection of rifampin and isoanizid resistance in Mycobacterium tuberculosis complex isolates. J Clin Microbiol 2005;43:3699-703.

10. World Health Organization. Tuberculosis Fact sheet N°104. – Mode of access: http://www.who.int/mediacentre/factsheets/fs104/en/index.html

11. World Health Organization. Global tuberculosis control 2011. – Mode of access: http://www.who.int/tb/publications/global_report/en/

12. Guide to the Application of Genotyping to Tuberculosis Prevention and Control. – Mode of access: http://www.cdc.gov/tb/programs/genotyping/Chap3/3_CDCLab_2Description.htm

13. Conell N.D., Kreiswirth B.N. Mycobacterial Strain Genotyping. *Tuberculosis A*

Comprehensive International Approach. Marcel Dekker, 2000

14. http://www.ijmm.org/article.asp?issn=0255-0857;year=2010;volume=28;issue=3;spage=211;epage=216;aulast=Hristea

15. http://www.resmedjournal.com/article/S0954-6111%2809%2900244-3/abstract?cc=y=