

Correlation between phenotypic and genotypic drug resistance to kanamycin and amikacin in clinical isolates of *Mycobacterium tuberculosis*

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Dear Editor,

Tuberculosis is still an important hygienic problem in developing countries (1). XDR bacilli are not only resistant to isoniazid and rifampin but also to fluoroquinolones and at least one of the three injectable drugs of amikacin, kanamycin and capreomycin (second line tuberculosis drugs) (2). Kanamycin and amikacin are indeed attached to 16S rRNA component of 30S subunit which leads to wrong coding of genetic transcription and therefore increase the level of mis-folded proteins in the cell that leads to cell death (3, 4). Mutations in codons 1401 and 1402 of *rrs* gene lead to resistance to the aforementioned drugs. In the present work, the sequencing method was used to examine the mutations causing resistance to injectable drugs.

In this descriptive study, a total number of 41 samples, including 23 resistant and 18 susceptible samples from isolates with phenotypically confirmed resistance to injectable drugs of kanamycin and amikacin were obtained from genome bank of Infectious Diseases Research Center (IDRC) of Arak University of Medical Sciences. The desirable amplicon containing mutant and wild-type codons were amplified using specific primers. The primers were designed with different specific software. Performing PCR on *rrs* gene (NC_000962.3) on the entire studied sam-

ples resulted in 422bp band which reflects the correct selection of primers and determines the appropriate amplification program.

To assess the accuracy of the PCR results, all of 41 *Mycobacterium tuberculosis* isolates were sequenced to examine probable mutations. As a result, from 23 resistant isolates, 9 isolates harbored mutations at 1401 and 1402 codons of *rrs* gene and 14 isolates had not such the mutations. Interestingly, all of 18 susceptible isolates had not any mutations in the codons. Sensitivity of this method was 40.9% while its specificity was 100%.

In the present study, the sequencing method was used in order to determine mutation in codons 1401 and 1402 of *rrs* gene. Phenotypic results and sequencing method were used as gold standard of resistance and gold standard of molecular methods, respectively.

According to the literature, examination of identical nucleotide sequences in other bacteria suggested the key role of mutation in codon 1402 in establishment of resistance in kanamycin-resistant strains (5, 6). Studies conducted in various countries included carrying out a PCR followed by sequencing of samples which is a time-consuming and costly technique for patients while it also calls for skilled personnel and laboratory facilities in routine tests.

Kanchanjani (2011) sequenced 150 XDR-TB strains in order to determine mutation causing codons in *rrs*, *gyrB*, *gyrA*, *katG*, *inhA* and *rpoB* genes which suggested mutation in codon 1401 of *rrs* gene in 71% of strains. In his study, 106 strains showed mutation

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in codon 1401 of *rrs* gene, while 42 strains showed mutation in codon 1484 and 13 samples showed mutation in codon 1484 of *rrs* gene and codons 205 of *thyA* gene and 3 samples had mutation in *tylA* gene (7). In another study, mutation in codon 1401 of *rrs* gene in 26 strains sequence the resistance of which to at least one of the three injectable second line drugs had been determined through application of sensitivity tests. Using this method, mutation was observed in 19 out of 21 resistant strains to kanamycin, 19 out of 24 resistant strains to amikacin and 11 out of 14 resistant strains to capreomycin. It seems that mutation diagnosis in 1401 is 100% specific to diagnosis of resistance to kanamycin and amikacin (8).

The results of our study are consistent with those of above mentioned scholars in terms of relevance of phenotype resistant to mutation in 1401. The sequencing method is recommended as a valid technique applied to diagnose drug resistance in *Mycobacterium tuberculosis* which is of high precision and reliability and used as a standard reference for detection of resistance to injectable second line drugs because of its phenotypic and genotypic association. It used to be conducted in the past according to instructions but is being carried out nowadays with automatic sequencing systems.

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REFERENCES

1. Ali MK, Karanja S, Karama M. Factors associated with tuberculosis treatment outcomes among tuberculosis patients attending tuberculosis treatment centres in 2016-2017 in Mogadishu, Somalia. *Pan Afr Med J* 2017; 28: 197.
2. Bennett S, Lienhardt C, Bah-Sow O, Gustafson P, Manneh K, Del Prete G, et al. Investigation of environmental and host-related risk factors for tuberculosis in Africa. II. Investigation of host genetic factors. *Am J Epidemiol* 2002;155:1074-1079.
3. van Ingen J, Simons S, de Zwaan R, van der Laan T, Kamst-van Agterveld M, Boeree MJ, et al. Comparative study on genotypic and phenotypic second-line drug resistance testing of *Mycobacterium tuberculosis* complex isolates. *J Clin Microbiol* 2010;48:2749-2753.
4. Weinstein MP, Wilson ML. Recent advances in the laboratory detection of *Mycobacterium tuberculosis* complex and drug resistance. *Clin Infect Dis* 2011;52:1350-1355.
5. Kahbazi M, Sarmadian H, Ahmadi A, Didgar F, Sadrnia M, Poolad T, et al. Novel mutations in *pncA* gene of pyrazinamide resistant clinical isolates of *Mycobacterium tuberculosis*. *Sci Pharm* 2018;86:15.
6. Suzuki Y, Katsukawa C, Tamaru AK, Abe C, Maki-no M, Mizuguchi Y, et al. Detection of kanamycin-resistant *Mycobacterium tuberculosis* by identifying mutations in the 16S rRNA gene. *J Clin Microbiol* 1998;36:1220-1225.
7. Ajbani K, Rodrigues C, Shenai S, Mehta A. Mutation detection and accurate diagnosis of extensively drug-resistant tuberculosis: report from a tertiary care center in India. *J Clin Microbiol* 2011;49:1588-1590.
8. Rodwell TC, Valafar F, Douglas J, Qian L, Garfein RS, Chawla A, Torres J, Zadorozhny V, Kim MS, Hoshide M, Catanzaro D. Predicting extensively drug-resistant *Mycobacterium tuberculosis* phenotypes with genetic mutations. *J Clin Microbiol* 2014;52:781-789.