Prevalence of Multidrug-Resistant Tuberculosis: A Six-Year Single-Center Retrospective Study in Tehran, Iran

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Received 2018 August 01; Revised 2019 October 08; Accepted 2019 October 16.

Abstract

Background: Multidrug-resistant tuberculosis (MDR-TB) is one of the main challenges to TB control, particularly in developing countries such as Iran. Continuous TB drug resistance surveillance is required for the effective management of TB patients.

Methods: The antibiotic susceptibility patterns of clinical Mycobacterium tuberculosis isolates were retrospectively analyzed from April 2012 to March 2018. Conventional and molecular methods were used for the identification and drug susceptibility testing (DST) of M. tuberculosis isolates.

Results: A total of 3,012 clinical specimens were collected from TB-suspected patients. Of them, 100 (3.3%) were culture-positive and assigned as M. tuberculosis by phenotypic and molecular methods. According to DST, 17 (17%) isolates were MDR-TB.

Conclusions: Improved diagnosis and treatment of MDR-TB may lead to better control of the disease in Iran.

Keywords: Multidrug-Resistant Tuberculosis, MDR, Iran

1. Background

Tuberculosis (TB) is still among the leading causes of death worldwide, especially in developing countries. There were more than 10 million new TB cases and 1.3 million deaths from TB in 2017 (1). The emergence of multidrug-resistant TB (MDR-TB) and inappropriate diagnosis and treatment services are among the important challenges for TB control strategies (1). Based on the World Health Organization (WHO), the treatment success rate for patients with MDR-TB is less than 60% [1]. Unfortunately, patients infected with drug-resistant strains of Mycobacterium tuberculosis have to endure long, toxic, and costly therapies with poor outcomes (2-8). The problem of MDR-TB is even more serious in many low- and middle-income countries.

In Iran, a country with a moderate annual burden of TB, the MDR-TB has not been yet brought under control, despite the government’s considerable efforts (9). According to the WHO report, the estimated proportion of TB cases with MDR-TB in new- and retreatment-TB cases was found to be 1.3% and 8.3%, respectively (1). Furthermore, statistical data from the Ministry of Health do not show a significant declining trend of MDR-TB among Iranian nationals (10). Thus, knowledge about drug resistance patterns of M. tuberculosis strains plays an essential role in the control and management of the disease.

2. Objectives

Although, the prevalence of MDR-TB has been demonstrated in several studies in Iran, continuous monitoring of MDR-TB is needed for the management of MDR-TB. Thus, our study aimed to investigate the prevalence of drug-resistant TB in Tehran, the capital of Iran.

3. Methods

3.1. Setting and Samples

In the present study, we retrospectively analyzed 3,012 clinical samples from suspected TB patients referred to the Masoud Laboratory from April 2012 to March 2018. Clinical specimens were collected either from new- or retreatment-TB cases. The average age of patients was between 18 and 89 years and all of them had clinical signs and symptoms of TB. The Masoud Laboratory is among the main laboratories for TB diagnosis in Tehran, Iran, which is
under the supervision of the Swedish Institute for Infectious Disease Control. The Ethics Committee of Shahid Beheshti University of Medical Sciences approved the study (IR.SBMU.RETECH.REC.1396.1308).

3.2. Identification of Mycobacteria

The smear, culture, and biochemical tests were performed according to the WHO protocol (11). Clinical samples were decontaminated using the N-acetyl-L-cysteine-sodium hydroxide method. All the smears were stained with Ziehl-Neelsen method. For the identification of mycobacteria, the samples were cultured in Lowenstein Jensen (LJ) medium and isolates were identified as *M. tuberculosis* using the niacin, catalase, and nitrate tests, as well as bacterial pigment production and growth rate (12).

3.3. Molecular Identification of *M. tuberculosis*

The mycobacterial DNA was extracted using the Roche DNA extraction kit according to the manufacturer’s instructions. Then, IS6110-based PCR assay was used for the molecular identification of *M. tuberculosis* (13).

3.4. Drug Susceptibility Testing of *M. tuberculosis*

The drug susceptibility testing (DST) was performed by the proportional method as described previously (14, 15). Resistance was expressed as the percentage of colonies that grew on critical concentrations of the drugs including 0.2 µg/mL for isoniazid (INH) and 40 µg/mL for rifampicin (RIF). *Mycobacterium tuberculosis* H37Rv strain (ATCC 27294) was used for quality control testing in DST.

3.5. DNA Sequencing

The DNA sequencing of genes katG and rpoB was conducted to confirm the results as described previously (15).

4. Results

Of 3,012 clinical specimens, 100 (3.3%) were culture-positive and assigned as *M. tuberculosis* by phenotypic and molecular methods. Among the investigated *M. tuberculosis* isolates, 17 were MDR (Table 1). Based on DNA sequencing analysis, drug-resistant strains had a mutation in their hot spot region.

| Table 1. Drug Resistance Patterns of Mycobacterium tuberculosis Isolates |
|-----------------------------|-----------------------------|
| Number of clinical isolates | 3,012                      |
| Number of confirmed *M. tuberculosis* isolates | 100 |
| Drug resistance patterns, % |                            |
| MDR | 17 |
| INH | 15 |
| RIF | 6 |

Abbreviations: INH, isoniazid; MDR, multidrug-resistant; RIF, rifampicin.

5. Discussion

The relatively high prevalence of MDR-TB (17.0%) makes this study relevant to all physicians treating patients with TB. The rate of MDR-TB in the current study was higher than those of previous studies from Iran. The rate of MDR-TB in studies conducted in different cities of Iran ranged from 1% to 26% (16-22).

The history of incomplete TB treatment, inadequate supply of drugs, lack of treatment supervision, and inappropriate intake of prescribed anti-TB drugs are among the critically important factors contributing to acquiring MDR-TB (23). If MDR-TB arises from incomplete treatment, improving treatment adherence can prevent further spread of resistant forms of TB (23).

In Iran, patients with MDR-TB are placed on the MDR-TB treatment regimen based on either DST results or the history of drugs taken, for a minimum of 12 months (24). The regimen usually contains an injectable aminoglycoside, a quinolone, para-aminosalicylic acid, prothionamide, pyrazinamide, ethambutol, etc. (24). However, prolonged therapy often results in a low TB treatment completion rate. To deal with this problem, some countries are evaluating other treatment regimens given for 9-12 months to shorten the duration of MDR-TB therapy (25-27).

The treatment success rate of MDR-TB is low in Iran, as well. This can be due to the following important factors: unavailable treatment regimens in many parts of the country, high treatment costs, ineffective treatment management and finally, limited numbers of equipped laboratories with DST capability. Thus, better treatment management and using new technologies can improve the current situation.

One of the key limitations of this study is that the information regarding the previous TB treatment was not available; thus, the prevalence of MDR-TB could not be separately analyzed in new cases or previously treated TB cases.

In conclusion, there was a high rate of MDR-TB in the current study. Improving the diagnosis and management of MDR-TB should be highly prioritized.
Acknowledgments

We would like to thank Masoud Laboratory’s Staff, Microbiology Unit, for their kind help in providing the data.

Footnotes

Conflict of Interests: None.

Ethical Approval: The Ethics Committee of Shahid Beheshti University of Medical Sciences approved the study.

Funding/Support: The article was financially supported by Shahid Beheshti University of Medical Sciences, Tehran, Iran (grant no. 11308).

References

1. World Health Organization. Global tuberculosis report. Geneva, Swiss: WHO; 2018.
2. Dawson R, Diacon AH, Everett D, van Niekerk C, Donald PR, Burger DA, et al. Efficiency and safety of the combination of moxifloxacin, pretomanid (PA-824), and pyrazinamide during the first 8 weeks of antituberculosis treatment: A phase 2b, open-label, partly randomised trial in patients with drug-susceptible or drug-resistant pulmonary tuberculosis. Lancet. 2015;385(9979):473-47.
3. Diel R, Vandeputte J, de Vries G, Stillo J, Wanlin M, Nienhaus A. Costs of tuberculosis disease in the European Union: A systematic analysis and cost calculation. Eur Respir J. 2013;42(2):554-65. doi: 10.1183/09031936.00139.0.
4. Ahmad N, Javaid A, Basit A, Afriadi AK, Khan MA, Ahmad I, et al. Management and treatment outcomes of MDR-TB: Results from a setting with high rates of drug resistance. Int J Tuberc Lung Dis. 2015;19(9):1309-14. doi: 10.5588/ijtld.15.0067.
5. Weyer K, Dennis Falzon D, Jaramillo E, Zignol M, Mirzayev F, Raviglione M. Drug-resistant tuberculosis: What is the situation, what are the needs to roll it back. AMR control. 2017;20:60-7.
6. Turkova A, Tebruegge M, Brinkmann F, Tsolia M, Mouchet F, Kampmann B, et al. Management of child MDR-TB contacts across countries in the WHO European Region: A survey of current practice. Int J Tuberc Lung Dis. 2017;21(7):774-7. doi: 10.5588/ijtld.16.0294.
7. Nasiri MJ, Dabiri H, Darban-Sarakholil H, Rezadehbashi M, Zamani S, Prevalence of drug-resistant tuberculosis in Iran: Systematic review and meta-analysis. Am J Infect Control. 2014;42(11):2228-8. doi: 10.1016/j.ajic.2014.07.017.
8. Iranian Center for Disease Control and Prevention. Ministry of health and medical education, Iran. Providing electronic health services. 2010. Available from: http://www.cdc.hbi.ir.
9. Harries AD, Maher D, Graham S. TB/HIV: A clinical manual. Geneva, Swiss: World Health Organization; 2004.
10. Giri A, Gupta S, Safi H, Narang A, Shrivastava K, Kumar Sharma N, et al. Polymorphisms in rpoB gene in relation to ethambutol resistance in clinical isolates of Mycobacterium tuberculosis from North India. Tuberculosis (Edinb). 2018;108:41-6. doi: 10.1016/j.tube.2017.10.003.
11. Eisenach KD, Cave MD, Rates JH, Crawford JT. Polymerase chain reaction amplification of a repetitive DNA sequence specific for Mycobacterium tuberculosis. J Infect Dis. 1990;161(5):977-81. doi: 10.1093/infdis/161.5.977.
12. Kim SJ. Drug-susceptibility testing in tuberculosis: Methods and reliability of results. Eur Respir J. 2003;22(3):564-9. doi: 10.1183/09031936.03.003104.
13. Lipin MY, Stepanshina VN, Shemyakin IG, Shinnick TM. Association of specific mutations in katG, rpoB, rpsL and rrs genes with spoligotypes of multidrug-resistant Mycobacterium tuberculosis isolates in Russia. Clin Microbiol Infect. 2007;13(6):620-6. doi: 10.1111/j.1469-0691.2007.01711.x.
14. Varahram M, Nasiri MJ, Farnia P, Mozafari M, Velayati AA. A retrospective analysis of isoniazid-monoresistant tuberculosis: Among Iranian pulmonary tuberculosis patients. Open Microbiol J. 2013;7:5-10. doi: 10.2174/1874285808408010001.
15. Namaei MH, Sadeghian A, Naderinasab M, Ziaee M. Prevalence of primary drug resistant Mycobacterium tuberculosis in Mashhad, Iran. Indian J Med Res. 2005;122(1):77-80. doi: 10.11648/ijmri.20140240401001.
16. Merza MA, Farnia P, Tabarsi P, Khazampour M, Masjedi MR, Velayati AA. Anti-tuberculosis drug resistance and associated risk factors in a tertiary level TB center in Iran: A retrospective analysis. Infect Dev Cites. 2011;5(2):51-9. doi: 10.3855/jidc.1259.
17. Nasiri MJ, Rezaei F, Zamani S, Darban-Sarakholil H, Fooladli AA, Shohaj E, et al. Drug resistance pattern of Mycobacterium tuberculosis isolates from patients of five provinces of Iran. Asian Pac J Trop Med. 2017;10:393-6. doi: 10.6004/japmt.2015.0201.
18. Metanat M, Sharifi-Mood B, Shahreki S, Dowoudi SH. Prevalence of multidrug-resistant and extensively drug-resistant tuberculosis in patients with pulmonary tuberculosis in zahedan, southeastern iran. Iran Red Crescent Med J. 2012;14(1):31-5. doi: 10.5812/ircmj.2273557.
19. Mansouri SD, Mirabolhasani Z. The pattern of drug resistance among newly diagnosed and old cases of pulmonary tuberculosis in NRITLD. Arch Iranian Med. 2003;6(4):225-60.
20. Bahrmand AR, Velayati AA, Bakayev VV. Treatment monitoring and reliability of results. Eur Respir J. 2015;46(5):129-38. doi: 10.1183/13993003.00091315.
21. Mansouri SD, Mirabolhasani Z. The pattern of drug resistance among newly diagnosed and old cases of pulmonary tuberculosis in NRITLD. Arch Iranian Med. 2003;6(4):225-60.
22. Bahrmand AR, Velayati AA, Bakayev VV. Treatment monitoring and reliability of results. Eur Respir J. 2015;46(5):129-38. doi: 10.1183/13993003.00091315.
23. Kendall EA, Fofana MO, Dowdy DW. Burden of transmitted multidrug resistance in epidemics of tuberculosis: A transmission modelling analysis. Lancet Respir Med. 2015;3(12):963-72. doi: 10.1016/S2213-2600(15)00458-0.
24. Mirsaidi SM, Tabarsi P, Khoshnood K, Pooramiri MV. Treatment monitoring and reliability of results. Eur Respir J. 2015;46(5):129-38. doi: 10.1183/13993003.00091315.
25. Van Deun A, Declercq E, Sarker MR, Daal M, Hussain MA, et al. Successful ‘9-month Bangladesh regimen’ for multidrug-resistant tuberculosis among over 500 consecutive patients. Int J Tuberc Lung Dis. 2005;9(6):317-22. doi: 10.5588/ijtld.04.09.012.
26. Van Deun A, Maeg AM, Daal M, Dossain MA, et al. Successful ‘9-month Bangladesh regimen’ for multidrug-resistant tuberculosis among over 500 consecutive patients. Int J Tuberc Lung Dis. 2005;9(6):317-22. doi: 10.5588/ijtld.04.09.012.
27. Van Deun A, Maeg AM, Daal M, Dossain MA, et al. Successful ‘9-month Bangladesh regimen’ for multidrug-resistant tuberculosis among over 500 consecutive patients. Int J Tuberc Lung Dis. 2005;9(6):317-22. doi: 10.5588/ijtld.04.09.012.
28. Van Deun A, Maeg AM, Daal M, Dossain MA, et al. Successful ‘9-month Bangladesh regimen’ for multidrug-resistant tuberculosis among over 500 consecutive patients. Int J Tuberc Lung Dis. 2005;9(6):317-22. doi: 10.5588/ijtld.04.09.012.
29. Van Deun A, Maeg AM, Daal M, Dossain MA, et al. Successful ‘9-month Bangladesh regimen’ for multidrug-resistant tuberculosis among over 500 consecutive patients. Int J Tuberc Lung Dis. 2005;9(6):317-22. doi: 10.5588/ijtld.04.09.012.
30. Van Deun A, Maeg AM, Daal M, Dossain MA, et al. Successful ‘9-month Bangladesh regimen’ for multidrug-resistant tuberculosis among over 500 consecutive patients. Int J Tuberc Lung Dis. 2005;9(6):317-22. doi: 10.5588/ijtld.04.09.012.