

Extensively Drug-resistant Tuberculosis Strains in National Tuberculosis Reference Laboratory Between 2005 And 2010, Turkey

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Abstract

Frequency, rate and trend of extensively drug-resistant [XDR] tuberculosis [TB] in Turkey are not clear and not known very well. In this study, it was aimed to try to determine and to confirm the frequency of XDR-TB strains among Multi-Drug Resistant [MDR] TB cases. A total of 4119 *M. tuberculosis* strains were analyzed. The strains were collected from 19 TB centers [hospitals or regional TB laboratories] including National TB Reference Laboratory [NTRL]. Two hundred ninety-seven 297 [7.2%] *M. tuberculosis* strains were detected as MDR-TB. Among all MDR-TB cases, 37 [12.5%] were pre-XDR-TB, and 8 [2.7%] were XDR-TB.

Keywords: Extensively drug resistant tuberculosis; Multidrug resistant tuberculosis; Tuberculosis

Introduction

TB is one of the oldest known infectious diseases, and today still remains an important health problem both worldwide and in our country. World Health Organization [WHO] estimates that one third of world's population is infected by *M. tuberculosis*. There were nearly 8.6 million cases around the world in 2012 and approximately 1.3 million of them died. TB incidence was 122 per 100,000 population, while global TB prevalence was 169 per 100,000 population in 2012 [1]. According to the 2012 report of TB Control Department in Turkey, the number of cases registered in 2010 was 16551 and TB incidence was 24 per 100 000 population, while the number of new cases was 15183 [2]. In recent years, a dramatic increase in the emergence of MDR-TB and XDR-TB cases has been observed. Globally drug resistance is a major concern for global health [1,3-6]. MDR-TB has been documented in most of the countries and regions and at least one XDR-TB has been reported in 68 countries until February 2011 [7].

XDR-TB has been identified as a significant problem in countries of the former Soviet Union [8] and the potential threat of XDR-TB for Europe has been assessed by the European Centre for Disease Centre and Prevention [ECDC] in 2006 [9]. The WHO Global Task Force on XDR-TB has recommended laboratory-based surveillance to better understand the prevalence of XDR-TB in developing countries [10]. It has not been yet notified officially of XDR-TB cases in Turkey. There are some studies on drug resistance including several geographic areas of Turkey. These studies are not comprehensive and outcome-based data have some significant limitations. There is no reliable survey to be representative of the population throughout the country. However, it has been started laboratory based surveillance by NTRL in recent years. In the this study, we aimed to analyze our laboratory data and to try to determine the frequency of XDR-TB strains among MDR-TB cases during six year-period from 2005 to 2010 in Turkey.

Materials and Methods

In the present study, *M. tuberculosis* complex strains which were isolated from National TB Reference Laboratory [NTRL] and from Regional TB laboratories and 19 TB centers located in some major cities and towns in Turkey between 2005-2010 were examined. NTRL routinely accepts cultures for further tests such as strain typing and drug

susceptibility testing [DST]. Specimens for TB cultures are requested by physicians as defined by TB control department. Treatment history was almost always not available and therefore was not included in the study. Due to the fact that DST is routinely performed in Turkey as standard patient care, no ethical approval was required for this study. Internal quality control was routinely performed during DST using reference strain *M. tuberculosis* H37Rv, and the expected results were always obtained. External quality assurance for first-line drugs was performed by the Supranational Laboratory Network of the WHO with satisfactory results. Quality assurance procedures for second-line drug susceptibility testing were not defined or developed by the Supranational Laboratory Network; thus, as a proxy for quality assurance, we studied the accuracy of second-line DST among isolates susceptible to the first-line drugs [11]. *M. tuberculosis* was isolated from the specimens by using Lowenstein-Jensen, Ogawa and/or MGIT [Becton Dickinson, Franklin Lakes, NJ, USA] media. It was then identified by using NAP-TB differentiation test [Becton Dickinson] and growth in para-nitrobenzoic acid containing medium [12]. We performed susceptibility testing by using a modified proportion method in which drug containing LJ media at the following concentrations were used: rifampicin [RIF] 40 µg/mL, isoniazid [INH] 0.2 µg/mL and 1 µg/mL, streptomycin [SM] 4 µg/mL, and ethambutol [EMB] 2 µg/mL [13]. MDR-TB strains were further tested with second-line drugs, such as capreomycin 40 µg/mL, ofloxacin 2 µg/mL [13,14] amikacin 40 µg/mL [13], kanamycin 30 µg/mL and ethionamide/prothionamide 20 µg/mL [14]. Reference strain *M. tuberculosis* H37Rv was used as a control with each susceptibility testing batch. MDR-TB is defined as TB resistant to the two most important antituberculosis drugs, INH and RIF. XDR-TB refers to TB resistant to

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at least INH and RIF, as well as to two of the most effective second-line drugs: a fluoroquinolone and at least one of the second-line injectable agents [amikacin, capreomycin, or kanamycin] [4]. Pre-XDR TB was defined as TB with resistance to isoniazid and rifampin and either a fluoroquinolone or second-line injectable agent but not both [15].

Results

Between 2005 and 2010, 4119 cultures were identified as *M. tuberculosis* complex strains from pulmonary TB cases and DST for 4119 strains was performed. While 297 [7.2%] of 4119 *M. tuberculosis* strains were detected as MDR-TB, 3270 strains were susceptible to all drugs. Ninety three [2.2%] strains were resistant to all first-line anti TB drugs. The numbers of resistant strains to SM, INH, RIF and ETB were 459 [11.1 %], 539 [13.1%], 389 [9.4 %], and 229 [5.6 %], respectively. Of 297 [7.2%] multi drug resistant *M. tuberculosis* strains, 8 [2.7%] were extensively drug resistant according to laboratory data. During the six year-period the rates of MDR- and XDR-*M. tuberculosis* varied from 5.9% to 7.9% and from 0% to 6.5% respectively (Table 1).

Discussion

The number of the resistant to second-line drugs of *M. tuberculosis* strains is increasing and to threat of MDR-TB are becoming a public health problem worldwide. This problem is man made [3,16] and it has been creating by wrong intervention such as poor prescribing practices, low drug quality [or erratic supply], and suboptimal adherence [17]. The fact that both XDR-TB and MDR-TB cases have recently been observed commonly in the world and it has been increased in the failure of TB treatment [3,16,18]. Early diagnosis and detection of resistant strains by using qualified DST facilitates introduction of an appropriate drug regimen [15,19,20], other protective measures and preventive activities [21,22].

The WHO estimated the annual burden of MDR-TB to be around 650,000 cases [1] and the prevalence of MDR-TB to be significantly higher than the annual incidence, primarily in low and middle-income countries [1,8]. MDR strains are currently found in more than

15% of all new cases of TB in some areas of the former Soviet Union [Azerbaijan, Moldova, Ukraine and Tomsk Oblast], and in more than 10% in parts of China and other areas of the former Soviet Union [Latvia, Estonia, Kazakhstan, Uzbekistan, and Ivanovo and Mari El [both Russian Federation] [8,23,24] In contrast, MDR TB in Western Europe countries with good national control programs such as Italy, is not a major health problem [25].

According to the official report of TB Control Department in Turkey, the number of cases registered in 2010 was 16,551 and TB incidence was 24 per 100,000 population, while the number of new cases was 15,183. National data on treatment outcomes for cases of MDR-TB are limited and all of these cases were not confirmed by DST in TB laboratories. MDR-TB cases reported by TB Control department between 2005 and 2009 were 191, 249, 240, 263 and 222 respectively. In 2010, 250 MDR-TB cases [5%] were detected among 4965 cases which had had DST results. Officially, no XDR-TB case was reported [2].

Until now, studies on drug resistance in Turkey have included several geographic areas around the country. However, most of these areas presented significant biases, because outcome-based measures have some significant limitations [different studies, methods, and population involved] resulting high degrees of variability between resistance levels among different settings. There is no reliable survey to be representative of the population throughout the country [26-35]. One of the first studies about drug-resistant TB in Turkey was reported by Bengisun et al. in 2000. The study retrospectively evaluated the resistance of 3319 *M. tuberculosis* strains to primary anti-tuberculosis drugs over the 21 year-period 1976-97. It was found that 60.8% of the isolates were susceptible, whereas 39.2% were resistant to at least one drug. MDR-TB was found in 194 [5.8%] cases [26]. Another study on resistance was reported by Senol et al. in 2005. The data were retrospectively collected by searching records of TB division of microbiology laboratory between 1999 and 2001. A total of 5084 TB cases were evaluated and MDR-TB rate was found to be 6.2% [27]. The other several studies showed that MDR-TB rates in Turkey varied from 3.2% to 14.7% (Table 2), while the

| Year | Number of <i>M. tuberculosis</i> isolates | Number of MDR [*] isolates, (%) | Number of Pre-XDR ^{**} isolates, (%) | Total Number of XDR isolates | XDR as % of total MDR |
|--------------|---|--|---|------------------------------|-----------------------|
| 2005 | 644 | 47 (7.3) | 5 (10.6) | 0 | 0 |
| 2006 | 716 | 42 (5.9) | 3 (7.1) | 2 | 4.8 |
| 2007 | 636 | 50 (7.9) | 7 (14.0) | 2 | 4.0 |
| 2008 | 670 | 53 (7.9) | 4 (7.5) | 0 | 0 |
| 2009 | 638 | 46 (7.2) | 14 (30.4) | 3 | 6.5 |
| 2010 | 815 | 59 (7.2) | 4 (6.8) | 1 | 1.7 |
| Total | 4119 | 297 (7.2) | 37 (12.5) | 8 | 2.7 |

*MDR: multidrug resistant; **XDR: extensively drug resistant.

Table 1: MDR and XDR *M. tuberculosis* isolates, in a six year period from 2005 to 2010.

| Authors (Reference) | City/Region(s) | Period of study | Tested/evaluated | <i>M. tuberculosis</i> cases/strains MDR rates (%) |
|--------------------------|-------------------------|---------------------|------------------|--|
| Bengisun JS, et al. [26] | Ankara | 1976-1997 | 3319 | 5.8 |
| Şenol G, et al. [27] | Izmir | 1999-2001 | 5084 | 6.2 |
| Saral ÖB, et al. [28] | Trabzon | 1998-2004 | 442 | 14.7 |
| Özekinci T, et al. [29] | Diyarbakir | 2001-2003 | 100 | 8.0 |
| Erturan Z, et al. [30] | Istanbul | 1992-2002 | 1843 | 3.2 |
| Uçar E, et al. [31] | Seven regions of Turkey | 2003-2006 | 1091 | 9.98 |
| Karabay O, et al. [32] | Edirne | 2004 | 214 | 11.6 |
| Karadağ A, et al. [33] | Samsun | 2004 | 50 | 4.0 |
| Gönlüğü U, et al. [34] | Sivas | 2004-2006 | 158 | 3.8 |
| Aydın F, et al. [35] | Trabzon | Jan. 2005-Mar. 2010 | 212 | 4.7 |

Table 2: Reports of studies on *M. tuberculosis* resistance in Turkey (1974-2010).

XDR-TB rate from 2005 to 2010 was variable without any evidence of increasing (Table 1).

Conclusions

Despite the existence of Directly Observed Treatment Strategies [DOTS] and TB Control program, Turkey couldn't exclude the problems of emergence of drug-resistant *M. tuberculosis* strains.

The present study which was conducted from 2005 to 2010 demonstrated that there is no evidence of raising of XDR-TB cases in 19 defined centers in Turkey. Statistical analysis could not apply, due to low numbers of XDR-TB cases in this study. Average rates were 7.2% for MDR-TB and 2.7% for XDR-TB which was below the global XDR-TB average [6.6-23.7%] [36].

The results discussed in this study suggest that Turkey needs to strengthen strategies to prevent the emergence of new drug-resistant *M. tuberculosis* strains, not only for MDR-TB, but also for XDR-TB cases.

Study limitations include unavailability of data to assess trends in specimen collection practices, so we could not evaluate how specimen collection practices may have influenced the increase in reporting of MDR-TB over time. Also the data were unavailable for categorizing MDR-TB cases as new or retreatment. Therefore, it is necessary and urgent to estimate the magnitude of the resistance of *M. tuberculosis* at a national level in order to evaluate the progress of DOTS under recent health care reforms. According to WHO/IUATLD [7], NTRL has started a sub nationwide survey [Ankara, 2011, and Istanbul, 2012] and has been tested to test MDR-TB isolates for second-line drug resistance. NTRL has established capacity to perform survey by technical coordination and cooperation with Supranational Tuberculosis Reference Laboratory in Italy. This survey will be a priority to consider the future management of MDR-TB and XDR-TB in Turkey.

Summary

In this retrospective study, our results suggest that Turkey needs to strengthen strategies to prevent the emergence of drug-resistant *M. tuberculosis* strains, not only for MDR-TB, but also for XDR-TB.

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