Objectives: Extensively drug-resistant tuberculosis (XDR-TB) is more expensive and difficult to treat than multidrug-resistant tuberculosis (MDR-TB), and outcomes for patients are much worse; therefore, it is important that clinicians understand the magnitude and distribution of XDR-TB. We conducted a retrospective study to compare the estimated incidence of XDR-TB with that of susceptible TB controls.

Methods: Sputum culture and drug susceptibility testing (DST) were performed in patients with known or suspected TB. Strains that were identified as MDR were subjected to DST for second-line drugs using the proportion method.

Results: Among 1,442 TB patients (mean age, 46.48 ± 21.24 years) who were culture-positive for *Mycobacterium tuberculosis*, 1,126 (78.1%) yielded isolates that were resistant to at least one first-line drug; there were 33 isolates (2.3%) of MDR-TB, of which three (0.2%) were classified as XDR-TB. Ofloxacin resistance was found in 10 (0.7%) isolates. Women were 15% more likely than men to yield M/XDR-TB isolates, but this difference was not significant. In a multivariate analysis comparing susceptible TB with X/MDR-TB, only one variable—the number of previous treatment regimens—was associated with MDR (odds ratio, 1.06; 95% confidence interval, 1.14–21.2).

Conclusion: The burden of M/XDR-TB cases is not sizeable in Iran. Nonetheless, strategies must be implemented to identify and cure patients with pre-XDR-TB before they develop XDR-TB. Our results provide a greater understanding of the evolution and spread of M/XDR-TB in an environment where drug-resistant TB has a low incidence.

Key Words: extensively drug-resistant tuberculosis, multidrug-resistant tuberculosis, risk factors, second line drugs

INTRODUCTION

Tuberculosis remains a major global health problem. In 2012, an estimated 8.6 million people developed TB, and 1.3 million died from the disease [1]. Moreover, the increasing incidences of multidrug and extensively drug-resistant tuberculosis (M/XDR-TB) are a major concern.
concern for TB control programs worldwide [2]. Concomitantly, the estimated global incidence of TB is declining slowly by less than 2% per year. The TB elimination target for 2050 is less than 1 case/1,000,000 population, which would necessitate an annual decline in incidence by 20% [3].

By the end of 2012, 92 countries had reported at least one case of XDR-TB. On average, an estimated 9.6% of MDR-TB cases involve XDR-TB [4]. Treatment for XDR-TB is usually more complex, toxic, and costly than treatment for other forms of TB, and it is less effective [2]. To better understand epidemiological trends in drug resistance at the global and national levels, researchers must carry out repeated surveys and, ultimately, establish continuous surveillance based on routine drug susceptibility testing (DST) of all confirmed TB cases [5].

When countries with a low TB burden experience an epidemic that is concentrated within specific at-risk groups, they focus their care and prevention efforts on those groups. In particular, when a country is striving to eliminate TB, it must invest additional resources in effectively reaching the least accessible populations; in addition, screening selected high-risk groups may be a key component of TB response. In any case, the primary objective of screening is to ensure that active TB is detected early and, thus, to reduce the risk of poor disease outcomes, adverse social and economic consequences, or disease transmission [4].

Conversely, the TB incidence is one of the most important indicators of the disease's impact on public health [6]. For these reasons, we conducted a population-based, retrospective study to ascertain M/XDR-TB incidence rates among all patients with clinically confirmed or suspected TB. We also sought to determine the risk factors for TB in Iran, the effect of these risk factors on the overall outcome of treatment, and the association between these risk factors and drug resistance. All the patients studied had been referred to the Pasteur Institute in Tehran.

**MATERIALS AND METHODS**

1. **Setting**

We performed this study in the Tuberculosis Department of the Pasteur Institute in Tehran, which is a government TB laboratory. A retrospective study was performed among all patients who had clinically confirmed or suspected TB between January 2006 and October 2013. The study was approved by the institutional review board at the Pasteur Institute-Tehran (the approval No. PII-439). All such cases were referred to the TB laboratory for culture and susceptibility testing. In addition, screening selected high-risk groups may be a key component of TB response. In any case, the primary objective of screening is to ensure that active TB is detected early and, thus, to reduce the risk of poor disease outcomes, adverse social and economic consequences, or disease transmission [4]. Conversely, the TB incidence is one of the most important indicators of the disease's impact on public health [6]. For these reasons, we conducted a population-based, retrospective study to ascertain M/XDR-TB incidence rates among all patients with clinically confirmed or suspected TB. We also sought to determine the risk factors for TB in Iran, the effect of these risk factors on the overall outcome of treatment, and the association between these risk factors and drug resistance. All the patients studied had been referred to the Pasteur Institute in Tehran.

**Figure 1. Study population.**

Any resistance (resistance to any of the anti-TB drugs) + and mono-resistance (resistance to only one drug) + poly-resistance (resistance to at least two drugs, excluding the isoniazid and rifampin combination).

MDR, multidrug resistant; XDR, extensively drug-resistant; TB, tuberculosis; DST, drug susceptibility testing.
viewed follow-up health workers to obtain additional information about the patients.

Figure 1 shows a summary of the recruitment process. Before commencing treatment, sputum samples (three samples per patient) were obtained from all patients and used for mycobacterial culture and DST. In cases involving positive culture results, the MDR-TB working group recommends that patients be followed up for 12-months after treatment has begun. All patients with MDR had been tested for human immunodeficiency virus (HIV) at the AIDS Department of the Pasteur Institute.

2. Decontamination procedures

Decontamination was performed using the N-acetyl-L-cysteine-sodium hydroxide method. Cultures were grown on Lowenstein-Jensen slopes according to the guidelines outlined in the Communicable Disease Control Manual. To confirm the presence of bacilli, each sputum sample was subjected to acid fast microscopy. All positive cultures were then identified for the presence of Mycobacterium tuberculosis using the niacin test, as well as by analyzing catalase activity, nitrate reduction, pigment production, and growth rate [8].

3. Drug susceptibility testing

DST for all first- and second-line drugs was performed using the absolute concentration method. More specifically, DST against first-line drugs (rifampicin, streptomycin, ethambutol, and isoniazid,) was performed using the proportional method at concentrations of 40, 4.0, 2.0, and 0.2 µg/mL. DST against second-line drugs (kanamycin, capreomycin, amikacin, ofloxacin, para-aminosalicylic acid, ethionamide, and cycloserine) was performed using two critical proportions (1% and 10%) [9]. Drugs were sourced from Sigma-Aldrich (Sigma, St. Louis, MO, USA), and for each batch of DST, a sensitive strain of H37Rv was used as a control.

4. Definition of XDR-TB

XDR-TB is defined as TB that has developed resistance to at least rifampicin and isoniazid, as well as to any member of the quinolone family, and at least one of the following second-line, anti-TB, injectable drugs: kanamycin, capreomycin, or amikacin [10].

5. Statistical analysis

Risk factors were defined according to the World Health Organization definition of risk groups [4]. A risk group for TB is any group of people within which the prevalence or incidence of TB is significantly higher than in the general population. The following risk factors were examined: age, gender, HIV, diabetes, smoking, high-risk job (e.g., health-care worker), cavitations on chest X-ray, extrapulmonary TB, previously treatment for TB. The last variable (previous treatment) was treated as a binary (yes or no), and the “yes” patients were labeled as “received prior treatment” [4]. Data analysis was conducted using IBM SPSS Statistics version 19.0 (IBM Co., Armonk, NY, USA). Descriptors and risk factors were compared using the independent samples t-test. Logistic regression was performed to compare M/XDR-TB cases to susceptible TB cases, and categorical variables were compared using the \( \chi^2 \) test, with odds ratios (ORs) and 95% confidence intervals (95% CIs). A \( p \)-value < 0.05 was considered significant.

RESULTS

Between January 2006 and January 2014, 1,442 patients had culture proven M. tuberculosis infections (Figure 1). The DST results showed that 1,090 isolates had mixed resistance (any resistance, mono-resistance, and poly-resistance combined), 33 contained MDR-TB, and three contained XDR-TB; 316 isolates were susceptible to all drugs. From among the 1,126 drug-resistant specimens (mixed resistance, MDR, and XDR-TB), the MDR-TB and XDR-TB isolates were submitted for analysis; the remaining specimens were excluded. Of the 316 patients with susceptible TB, 195 were excluded because of unclear or incomplete clinical records, or because no second-line drug-susceptibility test results

| Table 1. Demographic data and description of all patients studied |
|-----------------------------|----------------|
| Characteristic | Data |
| Age (y) | 46.5 ± 21.2 |
| Susceptible | 46.6 ± 22.0 |
| MDR | 46.6 ± 18.8 |
| XDR | 54.0 ± 13.1 |
| Frequency per age-group (y) | |
| < 20 | 19 (12.1) |
| 20–40 | 47 (29.9) |
| 40–60 | 44 (28.0) |
| > 60 | 47 (29.9) |
| Range | 88.0 |
| Gender | |
| Male | 66 (42.0) |
| Female | 91 (58.0) |
| Total | 157 (100.0) |

Values are presented as mean ± standard deviation or number (%). MDR, multidrug resistant; XDR, extensively drug-resistant.

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were available and could not therefore be classified into one category. Ultimately, 121 cases of susceptible TB were available for further analysis. Thus, of the 1,442 positive culture cases, a total of 157 isolates (121 susceptible, 33 MDR-TB, and three XDR-TB) were submitted for analysis.

The demographic characteristics of the patients are shown in Tables 1, 2, and 3; they had a mean age of 46.48 ± 21.24 years, and 91 (58.0%) of them were women. Forty-one patients only had extra-pulmonary TB (42.2%). Seventy-one were tested for HIV, among which 12 (17%) were HIV positive; 25 (34.2%) patients showed cavitations on chest X-ray. Among the 157 patients analyzed, 129 (82.1%) were newly diagnosed patients, while 28 (17.9%) were previously treated cases. There were 33 (21.1%) cases of MDR-TB, of which 16.6% were newly treated cases, 1.3% were relapse cases, and 3.2% were failure cases. The XDR-TB rate was 0.6% among new cases and 1.3% among treated cases.

Table 4 shows the distribution of patients and resistance rates among positive culture isolates and MDR-TB cases. Among the culture-positive cases, 58.4% showed resistance to at least one of the tested anti-TB drugs. Ofloxacin resistance was found in 10 (0.7%) of the positive cultures. Three cases of XDR-TB (9.0% of MDRs) were detected (Table 4). Thus, the frequency of XDR-TB among MTB patients was 0.2%. All three XDR-TB patients had pulmonary disease, and positive sputum smear test results. One of the XDR-TB patients a 45-year-old man was HIV positive and

Table 2. Frequency of drug resistance subjects according to positive culture isolates, MDR and XDR cases

| Drug resistance pattern | New case | Previously treated case | Total of isolates |
|-------------------------|----------|-------------------------|------------------|
|                         |          | Relapse | Failure     |                   |
| Susceptible             | 102 (65.0) | 8 (5.1) | 11 (7.0) | 121 (77.1)       |
| MDR                     | 26 (16.5)  | 2 (1.3) | 5 (3.2)  | 33 (21.0)        |
| XDR                     | 1 (0.7)   | 0 (0.0) | 2 (1.2)  | 3 (1.9)          |
| Total                   | 129 (82.2)| 10 (6.4)| 18 (11.4) | 157 (100.0) |

Values are presented as number (%).

MDR, multidrug resistant; XDR, extensively drug-resistant.

Table 3. Distribution of risk factor rates among positive culture isolates

| Description of cases | Data |
|----------------------|------|
| HIV infection        | 12/71 (16.9) |
| Cigarette use        | 30/79 (38.0) |
| Extra pulmonary involvement | 41/97 (42.3) |
| Cavity (chest X-ray)  | 25/73 (34.2) |
| Received prior treatment | 28/81 (34.6) |
| Treatment with fluoroquinolone/ injectable second-line drugs | 4.0/47 (8.5) |
| High risk jobs        | 15/57 (26.3) |
| Diabetes              | 34/87 (39.1) |

Values are presented as number (%).
HIV, human immunodeficiency virus.
*More than 25% data unavailable.

Table 4. Frequency of first-line and second-line anti-TB drug resistance profiles among positive culture isolates and MDR-TB cases, respectively, in Tehran, Iran between 2006 and 2013 (n = 1,442)

| Drug resistance pattern | Frequency (%) |
|-------------------------|---------------|
| Any drug resistance     |               |
| First-line rates        |               |
| EMB                     | 169 (11.7)    |
| SM                      | 330 (22.9)    |
| RIF                     | 176 (12.2)    |
| INH                     | 168 (11.6)    |
| MDR-TB*                 | 33 (2.3 vs. MTB) |
| INH+RIF                 | 2 (0.1)       |
| INH+RIF+EMB             | 7 (0.5)       |
| INH+RIF+SM              | 9 (0.6)       |
| INH+RIF+SM+EMB          | 15 (1.1)      |
| XDR-TB*                 | 3 (0.2 vs. MTB) |
| Susceptible             | 316 (21.9)    |
| Mixed resistance        | 1,090 (75.6)  |
| Second-line rates       |               |
| OFX                     | 10 (30.3 vs. MDR) |
| PAS                     | 16 (48.5)     |
| CPM                     | 13 (39.4)     |
| KM                      | 12 (36.3)     |
| AMK                     | 15 (45.4)     |
| CYC                     | 15 (45.4)     |
| ETH                     | 13 (39.4)     |
| XDR-TB                  | 3 (9.0 vs. MDR) |
| OFX+PAS+CPM+KM+AMK+ETH  | 1 (3.0)       |
| OFX+CPM+AMK+ETH         | 1 (3.0)       |
| OFX+AMK                 | 1 (3.0)       |

Values are presented as number (%).
TB, tuberculosis; MDR, multidrug resistant; XDR, extensively drug-resistant; EMB, ethambutol; SM, streptomycin; RIF, rifampin; INH, isoniazid; OFX, ofloxacin; PAS, para-aminosalicylic acid; CPM, capreomycin; KM, kanamycin; AMK, amikacin; CYC, cycloserine; ETH, ethionamide.
*Resistance to isoniazid and rifampicin; **MDR-TB that is also resistant to any fluoroquinolone and at least one injectable, second-line drug; ‘any resistance (resistance to any anti-TB drug) + mono-resistance (resistance to only one drug) + poly-resistance (resistance to at least two drugs, excluding the isoniazid and rifampicin combination).
had a definite history of previous treatment for TB; he died before the end of the follow-up period.

The risk factors for M/XDR-TB are summarized in Table 5. The odds of M/XDR-TB were 25% higher among patients with HIV than among those without; however, this difference was not statistically significant (data not shown). In bivariate analysis, diabetes was positively associated with MDR (as compared with sensitive TB: OR, 1.01; 95% CI, 1.09–30.7; Table 5), while age between 20 and 40 years was negatively associated with MDR (OR, 0.17; 95% CI, 0.07–0.60), as was male gender (OR, 0.27; 95% CI, 0.18–0.78). In the multivariate analysis, only one variable number of previous treatment regimens was associated with MDR (as compared with susceptible TB: OR, 1.06; 95% CI, 1.14–21.2; multivariate regression, data not shown). None of ten variables analyzed was significantly associated with XDR-TB.

**DISCUSSION**

The present study constituted a comprehensive description of second-line drug resistance among MDR-TB isolates in a low-burden country. Only a few reports of M/XDR-TB in Iran have been published. For example, in one study describing the transmission of XDR-TB among patients with secondary TB, Masjedi et al [11] showed that 12/113 (10.6%) Iranian MDR-TB strains were resistant to all eight second-line drugs tested [11]. Similarly, Metanat et al [12] recently reported the prevalence of M/XDR-TB in Zahedan, southeastern Iran. Among 88 patients, 12.2% had MDR-TB, while one had pre-XDR-TB. There were no cases of XDR-TB. Another study diagnosed only seven cases of the 105 patients with XDR-TB among those treated with the standardized regimen. The same report showed a poor prognosis among patients with XDR-TB [13]. The discrepancies in resistance rates between the present and earlier studies [11,13] are likely due to differences in the number of MDR-TB cases. Specifically, the present study involved a small underlying population of MDR-TB cases.

A cohort study in eight countries reported that 43.7% of MDR-TB cases showed resistance to at least one second-line drug, and that 12.9% were resistant to fluoroquinolone. The same study showed that previous treatment using second-line drugs was consistently the strongest risk factor for resistance to these drugs, increasing the risk of XDR-TB more than fourfold [14]. A hospital-based study from Thailand determined that the occurrence rates of MDR-TB and XDR-TB were 1.2% and 0.38%, respectively [15]. Meanwhile, 8.6% of TB cases in China showed ofloxacin resistance [16]. Using the US National Tuberculosis Surveillance System, Ershova et al [17] recently reported the risk

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**Table 5. Risk factors in patients with MDR-TB and XDR-TB (bivariate analysis)**

| Factor*                                      | MDR-TB (OR, 95% CI) | XDR-TB (OR, 95% CI) |
|---------------------------------------------|---------------------|---------------------|
| Age (y)                                     |                     |                     |
| < 20                                        | 0.28 (0.27–2.88)    | 0.97 (0.35–1.86)    |
| 20–40                                       | 0.17 (0.07–0.60)    | 0.99 (0.31–3.06)    |
| 40–60                                       | 0.84 (0.19–7.49)    | 0.52 (0.19–25.00)   |
| > 60                                        |                     |                     |
| Male gender                                 | 0.27 (0.18–0.78)    |                     |
| HIV infection                               | 0.09 (0.66–17.20)   | 0.00                |
| Cigarette use                               | 0.62 (0.10–3.74)    | 0.83 (0.72–25.50)   |
| Extra pulmonary involvement                 | 0.30 (0.47–10.80)   | 0.61 (0.12–31.70)   |
| Cavity (chest X-ray)                        | 0.56 (0.004–1.07)   |                     |
| Received prior treatment                    | 0.87 (0.17–4.33)    | 0.45 (0.11–7.70)    |
| Treatment with fluoroquinolone/Injectable   | 0.74 (0.15–3.18)    | 0.16 (0.005–2.40)   |
| High-risk jobs                              | 0.13 (0.66–24.10)   | 0.68 (0.78–48.80)   |
| Diabetes                                    | 1.01 (1.09–30.70)   | 0.48 (0.13–70.70)   |

MDR, multidrug resistant; XDR, extensively drug-resistant; TB, tuberculosis; OR, odds ratio; 95% CI, 95% confidence interval; HIV, human immunodeficiency virus.

*More than 25% data unavailable.
factors for acquired resistance to injectable second-line drugs and fluoroquinolones. The baseline prevalences of MDR-TB and XDR-TB were 12.6% and 0.38%, respectively, which differ markedly from the rates in the present study. However, these discrepancies are subject to at least three limitations. Firstly, the number of XDR-TB cases was a minimum estimate in the present study, because we had incomplete DST data. That is, initial TB isolates that have any resistance to rifampin or to any two first-line drugs should be tested for susceptibility to a full panel of anti-TB drugs, and the results should be reported accordingly [18]. Secondly, aggregate reporting of drug resistance has traditionally been based on initial DST results, not on drug resistance that develops during treatment. Thirdly, in the present study, several reported TB cases did not have positive cultures, so DSTs could not be performed.

The present study found three TB cases that met the case definition of XDR-TB. One of these three patients was newly diagnosed, while two had experienced treatment failure. No significant difference in resistance rates was found between the new and previously treated cases (data not shown).

A small number of Iranian TB patients have XDR-TB, indicating that resistance to second-line drugs is related to both the spread of resistant strains and the development of resistance to such drugs during treatment.

Even though there was insignificant heterogeneity among the ORs in the present study, all factors had an OR < 1, indicating that there was a decreased risk of M/XDR-TB. Conversely, some risk factors may have been underestimated, and others may not have been identified. In more than 25% of cases, data were not available for all factors. Therefore, interpretation of the comparison was hampered.

Numerous risk factors for MDR-TB have been identified, and the epidemics of MDR-TB and HIV have converged. For this reason, there must be stronger collaboration between clinicians carrying out TB and HIV control, and innovative measures to accelerate detection of TB resistance and improve treatment adherence must be implemented [19]. The present study did not identify HIV infection as a risk factor for drug-resistant TB in Iran. Among the three patients with XDR-TB, one had HIV, and he died before the end of the follow-up period. However, HIV testing was carried out in less than half of the patients; this was among the limitations of the present study, and the association between gender and HIV positivity should be interpreted with caution.

Many countries with low TB prevalence may show fluctuating trends in the prevalence of resistance because their overall TB burden is low; nonetheless, most such countries report small absolute numbers of MDR-TB per year [20]. Based on the classification of the World Health Organization, a TB incidence or notification rate of ≥ 25 cases per 100,000 per year is defined as high/intermediate [21]. In 2012, a total of 10,987 TB cases were reported in Iran—a rate of 14.43 cases per 100,000. In 2011, the number of TB cases and the rate were 11,030 and 14.56 cases per 100,000, respectively. This constitutes a 0.9% decrease between 2011 and 2012. Thus, TB case notifications are stable in Iran, and although TB rates increased in some regions, such as the Golestan province in northern Iran, more recent data have shown a stabilizing trend [22].

The prevalence of diabetes mellitus is increasing rapidly worldwide and the disease is significantly associated with an increased risk of TB [6]. In the present study, the association between diabetes and MDR-TB was significant (OR, 1.01; 95% CI, 1.09–30.7); other studies have reported similar findings. For instance, in a cohort of patients in Hong Kong aged ≥ 65 years, diabetes mellitus was associated with TB [23]. Another study in India indicated that diabetes makes a substantial contribution to the burden of incident tuberculosis, and the association was particularly strong in the case of infectious TB [24]. This may have serious implications for tuberculosis control.

In the present study, the MDR-TB incidence was negatively associated with male gender (OR, 0.27; 95% CI, 0.18–0.78). Differences in both infection risks and lifestyle may account for this gender discrepancy. Moreover, men and women may experience different contact types (in terms of intensity and duration), and their exposure to M. tuberculosis may differ, resulting in incidence discrepancies [6].

Resistance to fluoroquinolones (ofloxacin) was low (0.7%) in the present study. This result contrasts with reports from both the United States and eight countries with high rates of fluoroquinolone-resistant TB [14,17]. However, the sample size of drug-resistant isolates was small in the present study, and only a single fluoroquinolone was tested. Thus, patients with XDR-TB who were infected with strains resistant to a fluoroquinolone other than ofloxacin may have been misclassified as having MDR-TB or even drug-susceptible TB.

The data in the present study are not the first representative information available regarding M/XDR-TB in Iran; nonetheless, the study had some limitations. For instance, DST for second-line drugs has insufficient quality assurance. Furthermore, DST is not available for all drugs. On a separate note, the absolute number of M/XDR-TB cases is low in Iran, and the present study was designed to estimate the proportion of XDR-TB, as well as the risk factors, among patients with MDR-TB. For this reason, the study could not detect differences among other patient subgroups or the administrative divisions in Iran. Therefore, statistically significant differences between regions could only be discerned in
the case of extreme values. Efforts to diagnose, treat, and prevent the spread of M/XDR-TB need to be scaled up [25].

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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