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Full Length Research Paper

Survey of extensively drug-resistant tuberculosis (XDR-TB) in Iran-Tehran: A retrospective study

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The emergence and spread of multidrug resistant (MDR) and extensively drug-resistant tuberculosis (XDR) has raised public health concern about global control of TB. Our objective was to estimate the incidence of XDR-TB as compared to susceptible controls from TB patients in Tehran-Iran between 2006 and 2009.Sputum culture and drug susceptibility testing (DST) was done for patients with known or suspected TB. The strains that were identified as MDR were subjected to susceptibility testing for second-line drugs. Of 1126 culture-positive cases with first line drug susceptibility test, 91(8.08%) were Non-tuberculosis (Atypical) isolates, 8(0.7%) *M. BOVIS* and 1027(91.2%) *M. TUBERCULOSIS.* We detected MDR-TB in 26 patients (2.5%), of whom 2(7.7%) had XDR-TB. One of the XDR-TB patients with positive HIV infection died. This study cannot give an indication of XDR-TB rates in Iran. However, the results of this study document the existence of XDR-TB in Iran and indicate a need for surveillance data to define the magnitude and trends of this serious, contagious disease.

Key words: Multidrug-resistant tuberculosis (MDR-TB), extensively drug-resistant (XDR-TB), tuberculosis

INTRODUCTION

Tuberculosis (TB) remains a grave burden to public health. Approximately two billion people world-wide are infected with *mycobacterium tuberculosis*, the pathogen responsible for tuberculosis which kills 1.7 million people each year (World Health Organization, 2006a). Multi drug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) have emerged as significant threats to global tuberculosis (TB) control. The magnitude of the problem is evidenced from the forth global report of the world health organization (WHO) which reported data from 81 countries across the globe (Sharma et al., 2009). It is estimated that 489,139 cases emerged in 2006, and the global proportion of resistance among all incident TB cases was 4.8%. China and India are estimated to carry 50% of the global burden, with the Russian Federation carrying a further 7% (World Health Organization, 2008). The high proportion of XDR-TB among MDR-TB, ranging from 4.0% to over 20% as well as the large underlying burden of MDR-TB suggests that XDR-TB is more expensive and difficult to treat than MDR-TB and outcomes for patients are much worse (Jeon et al., 2008; Cox et al., 2007), therefore understanding the magnitude and distribution of XDR-TB is important (World Health Organization, 2008).

The emergence of XDR-TB, coupled with increased use of second-line drugs, suggests that urgent measures are needed to establish population-based surveillance for second-line drug resistance and to plan public responses (Wkly Rep, 2006). Extensively drug resistant TB, XDR-TB is resistant to first line agents (Isoniazid and Rifampicin), as well as to at least one fluoroquinolone and at least one inject able agent (Wkly Epid, 2006). The exact prevalence of XDR-TB in most regions of the world is currently unknown. For this reason, we set out a retrospective survey to determine the incidence of XDR-TB among

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Table 1. Description of cases.

	Non Tuberculosis n (%)	*M. BOVIS* n(%)	*M. TB* n (%)	MDR-TB[1] n (%)	XDR-TB[2] n (%)
Total culture-positive cases N=1126	91(8.08)	8(0.7)	1027(91.2)	26(2.5)	2(7. 7)

[1]MDR-TB is defined as resistance to isoniazid and rifampicin.

[2]XDR-TB is defined as resistance to rifampicin and asoniazid. In addition to any fluroquinolone and to at least one of the three following inject able drugs: capreomycin, kanamycin and amikacin.

MDR patients who refer to TB lab of Mycobacteriology Department of Pasteur Institute of Iran between 2006 and 2009. Therefore, we retrospectively reviewed these cases and compared them to controls with susceptible disease.

MATERIALS AND METHODS

Setting and study population

We did this study in the TB department of Pasteur Institute of Iran-Tehran. Patients, who have clinically confirmed cases or suspected TB, are referred to the Pasteur Institute (Tehran) for culture and susceptibility testing. Strains that were identified as MDR-TB were selected for further investigation. Patients receive free treatment for tuberculosis by home-based directly observed therapy, administered by ministry of health. The standard regimen, regardless of HIV status, is isoniazid, rifampicin, ethambutol and pyrazinamide for 2 months, followed by 4 months of isoniazid and rifampicin. Patients were classified as new if they had never been treated for TB >4 weeks and as previously treated if they had ever been treated for TB for ≥4 weeks. The case records of patients with MDR-TB, during the period from 2006 through 2009, at the TB laboratory of Pasteur Institute of Iran, Tehran, were retrospectively reviewed. Sputum samples (three samples per patients) were obtained from all patients for mycobacterial culture and drug susceptibility testing (DST). All MDR patients were tested for HIV infection at the AIDS and Hepatitis Department of Pasteur Institute.

Digestion-decontamination procedures

Digestion and decontamination was done with the N-acetyl-L-cysteine-sodium hydroxide method (Kent and Kubica, 1985). Cultures were done on Lowenstein-Jensen (LJ) slopes by the standard procedures manual of CDC. Acid fast microscopy, to confirm the presence of acid-fast bacilli, was done on each sputum samples. All positive cultures were identified as *M. tuberculosis* by means of Niacin test, Catalase activity, Nitrate reduction, Pigment production and growth rate (Kent and Kubica, 1985).

Drug susceptibility testing (DST)

Drug susceptibility testing (DST) for first line and second line drugs was performed by absolute concentration method (MIC) for all drugs. Drug susceptibility testing (DST) against Isoniazid, Rifampicin, Ethambutol, Streptomycin and Kanamycin was performed by the proportional method on Lowenstein-Jensen (LJ) media at a concentration of 0.2, 40, 20, 4.0 and 20 µg/ml, respectively. Drug susceptibility testing (DST) against second line drugs (Capreomycin 10 µg/ml, Amikacin 4.0 µg/ml, Ofloxacin 2 µg/ml, Para-aminosalicylic acid 5.0 µg/ml, Ethionamide 20 µg/ml and Cycloserin 30 µg/ml) was performed using 2 critical proportions of 1 and 10% (World Health Organization, 2001; Ryoken, 2002). Drugs were procured from Sigma (USA) and for each batch of DST a sensitive strain of H37Rv was used as a control.

Definition of XDR-TB

We defined XDR strains according to the World Health Organization of definition of XDR (World Health Organization, 2001 and 2006b). Positive cultures for *M. tuberculosis* were categorized on the basis of drug susceptibility results, as: fully susceptible or resistant to one or more tuberculosis drugs, but not both isoniazid and rifampicin (non-MDR tuberculosis); resistant to at least both isoniazid and rifampicin (MDR-tuberculosis); or resistant to at least isoniazid, rifampicin, fluoroquinolones and either aminoglycosides (amikacin, kanamycin) or Capreomycin or both (XDR tuberculosis)(World Health Organization, 2006 a,b and 2008).

Statistical analysis

Statistical analysis was performed with Epi Info software (2000), Centers for Disease Control and Prevention, Atlanta, GA, USA, by using test for the comparison of proportions. A p value 0.05 was considered significant.

RESULTS

1126 culture-positive cases with first line drug susceptibility test (DST) were available between 2006 and 2009. Among these 91(8.08%) were Non-tuberculosis (Atypic) isolates, 8(0.7%) *M.bovis* and 1027(91.2%) *M. tuberculosis* Table 1. Of 1027 *M. tuberculosis* cases, 26(2.5%) were MDR-TB and 2(7.7%) XDR-TB (Table 1). DST against second- line drugs was performed for all MDR-TB cases. The frequency of first line of various patterns of resistance is shown in Tables 2 and 3. The resistance rates to various first and second line drugs are shown in Table 4. Of the 26 patients, 5 patients (19.2%) showed resistance to flouroquinolones, 7 patients (26.9%) and 8 patients (30.8%) showed resistance to the injectable agent, capreomycin and Amikacin, respectively.

The characteristics of patients with XDR-TB are summarized in Table 5. Both of two XDR-TB patients had pulmonary diseases and these patients were to have positive sputum smear test results. One of the XDR-TB patients (male, 45 years) with positive HIV infection had a definite history of previous treatment for TB and he

Table 2. Frequency of first- line anti-tuberculosis drug resistance profiles among positive culture isolates in Tehran-Iran, 2006-2009.

Pasteur Institute- Tehran TB Lab	
All casesN=1027(MTB)	**n (%)**
Resistance profile	
Any drug resistance	799(77.8)
ETH	410(39.9)
KM	269(26.1)
EMB	104(10.1)
RMP	110(10.7)
SM	232(22.6)
INH	116(11.3)
Mono resistance	
ETH only	90(8.8)
KM only	6(0.6)
EMB only	10(1.0)
RMP only	8(0.8)
SM only	22(2.1)
INH only	4(0.4)
Degree of drug resistance	
Susceptible to all drugs	228(22.2)
Resistance to 1 drugs	140(13.7)
Resistance to all drugs	12(1.2)
Multiple drug resistance (MDR-TB)*	26(2.5)

*MDR-TB= resistance to Isoniazid and rifampicin.
ETH=etionamide, KM=kanamycin, EMB=ethambutol, Rif= rifampicin, SM=streptomycin, INH=isoniazid.

Table 3. Frequency of second-line anti tuberculosis drug resistance profiles among MDR-TB cases in Tehran-Iran 2006-2009.

Pasteur Institute- Tehran TB Lab	
All cases N=26	**n (%)**
Resistance profile	
Any drug resistance	24(92.3)
CPM	7(26.9)
ETH	10(38.5)
AMK	8(30.8)
PAS	6(23.0)
CYC	8(30.8)
OFX	5(19.2)
XDR-TB*	2(7.7)
OFX+ETH+AMK+CPM	1(3.8)
OFX+ETH+ PAS+AMK+CPM	1(3.8)
Mono resistance	
OFX only	3(11.5)
ETH only	0(0.0)
PAS only	1(3.8)
CPM only	2(7.7)

Table 3. Contd.

CYC only	0(0.0)
AMK only	3(11.5)
Degree of drug resistance	
Susceptible to all drugs	2(7.7)
Resistance to 1 drugs	9(34.6)
Resistance to 2 drugs	11(42.3)
Resistance to 3 drugs	1(3.8)
Resistance to 4 drugs	1(3.8)
Resistance to 5 drugs	1(3.8)
Resistance to 6 drugs	0(0.0)

*XDR-TB=extensively drug-resistant TB, resistance to any fluoroquinolones and at least one inject table second –line drug.cpm=Capreomycin, ETH=Ethionamide, AMK=Amikacin, PAS=Para-aminosalicyclic acid, CYC=Cycloserine, OFX=ofloxacin

Table 4. Resistance rates to various first-line and second-line drugs among MDR-TB patients.

First-line drug	Resistance rate (%)	Second-line drug	Resistance rate (%)
Isonizied	100	Ofloxacim	19.2
Rifampicin	100	Cycloserin	30.8
Ethambutol	38.5	PAS	23.0
Streptomycin	61.5	Capreomycin	26.9
Kanamycin	42.3	Amikacin	30.8
		Ethionamide	38.5

Table 5. Characteristics of patients with XDR-TB.

Pattern of drug resistance	Previous use of second-line drugs	Previous treatment	Type of TB	Direct Smear	Sample	Age	Sex	No
CPM,ETH,AMK,OFX INH,RMP,EMB,SM,KM,PAS	N.A*	Yes	pulmonary	Pos	Sputum	45	Male	1
CPM, AMK, ETH, OFX, INH, Rif	N.A*	Yes	pulmonary	Pos	Sputum	60	female	2

*Not available CPM, capreomycin, ETH, Ethionamide, AMK,amikacin, OFX, ofloxacin, INH, Isoniazid, RMP, rifampicin, EMB, Ethambutol, SM, streptomycin, KM, Kanamycin.

Two cases of XDR-TB were detected Table 1.Thus the frequency of XDR-TB among MDR-TB patients was 7.7%. No comparisons could be made although note that one was HIV positive.

DISCUSSION

We found a low incidence of resistance to second-line drugs including XDR-TB (7.7%). In this study, we have estimated the incidence and not the prevalence of XDR-TB. An estimate of prevalence can be made by multiplying incidence by the average duration of the disease (World Health Organization, 2008). Our study population is not representative of TB patients in Iran due to selection bias and selective testing bias. For this reason, we do not report a prevalence rate of XDR-TB. By July 2010, 58 countries and territories had reported at least one case of extensively drug-resistance TB (XDR-TB) (World Health Organization, 2008). There have been only a few reports of XDR-TB published from Iran. The first report that describes the transmission of XDR-TB among patients with secondary cases of TB, Masjedi et al. (2006) showed that 12(10.9%) of 113 Iranian MDR-TB strains were resistant to all 8 second- line drugs tested.

In further studies, Velayati et al. (2009) recognized that, of 146 MDR-TB strains 8 XDR isolates (5.4%) were identified. In addition, population-based data on drug

susceptibility of TB isolates were obtained from the United States (1993-2004), Latvia (2000-2002) and South Korea (2004), where 4, 19 and 15% of MDR-TB cases, respectively, were XDR (Wkly Rep, 2006). Results of a hospital-based study in Japan and Russia were identified a high proportion of the XDR-TB strains 31% and 21%, respectively (Yoshiro et al., 2010; Punga et al., 2009).

These rates are not similar to our findings. The discrepancy in findings between the present and an earlier study (Wkly Rep, 2006; Yoshiro et al., 2010; Punga et al., 2009) can be explained by the difference in the numbers of MDR-TB cases. Our study has a small underlying population of MDR-TB cases. Using MDR-TB cases tested for second-line drugs as denominator is problematic in survey settings where the number of MDR-TB cases detected in the nationwide survey sample may be small and may not reflect the true proportion of XDR-TB among all MDR-TB cases (World Health Organization, 2008). In addition, only a single fluroquinolones and two injectable agents were tested. Thus, XDR patients infected with strains resistant to fluroquinolone or injectable agents other than those tested could be misclassified as MDR or drug-susceptible TB (Balaji et al., 2010). Due to our retrospective design, some of the variables examined were inconsistently recorded or missing. Second line drug susceptibility testing is not well standardized (Balaji et al., 2010), so we cannot refer to a standard laboratory method to certify our fluroquinolone or injectable susceptibility results. Additionally, there is the insufficient quality assurance of drug susceptibility testing for second-line drugs (World Health Organization, 2008). However, existing tests for susceptibility to second line drugs are less reproducible than tests for susceptibility to isoniazid and rifampicin, and better methods are needed (Heifets and Cangelosi, 1999).

Another interesting observation was that one of the patients with XDR-TB was HIV co- infected in the present study that he died. Our data suggests no association between HIV infection and drug resistance.

Although outbreaks of drug resistant forms of tuberculosis among HIV infected patients have been widely documented in nosocomial and other congregate settings (Edlin et al., 1992; Moro et al., 1998), our data suggests no association between HIV infection and drug resistance. Furthermore, little information is available about the association of the HIV and drug resistant TB on a population level (Kenyon et al., 1999; Mac-Arthur et al., 2001).

In conclusion, we do not give an indication of XDR-TB rates in Iran. Because, the findings in this report are subject to at least two limitations. First, second line drug testing methods and results have varied because of the lack of international standards and the limited reproducibility of drug-susceptibility testing for certain drugs (World Health Organization, 2006c). Second, our data were drawn from a convenience sample of isolates and might reflect a referral bias. Regardless, these data indicate that XDR-TB is geographically widespread. However, this should be interpreted with caution. Moreover, although our sample size of drug resistant isolates is small, most of the data available from our study reveal a low proportion of XDR-TB among MDR-TB cases. Data from this study indicate that XDR-TB is widespread in the majority of countries that reported were low TB burden countries or very few cases of MDR. This study highlights the need to strengthen capacity for both diagnosis and surveillance of resistance to second- line-drugs and XDR-TB.

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