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Full Length Research Paper

Primary drug resistance patterns in newly diagnosed tuberculosis patients in Yazd, Southern Province of Iran

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Prevalence of multidrug resistant (MDR) and extensively drug-resistant tuberculosis (XDR) has been a cause of concern for tuberculosis (TB) control in both developed and developing countries. The objective of this study was to investigate the prevalence of drug resistance patterns among newly diagnosed tuberculosis cases, in Yazd, the southern province of Iran, during 2009 to 2010. The drug susceptibility testing (DST) was performed for 31 culture-positive individuals. The strains that were identified as MDR were subjected to susceptibility testing for second line drugs. The highest rate of resistance was to ethinoamide (51.61%), followed by kanamycin (29.03%), streptomycin (25.8%), rifampine (22.58%), isoniazid (6.45%), and ethambutol (3.2%). Drug resistance to both isoniazid and rifampin was identified in 2 cases.

Key words: Tuberculosis, drug resistance, multidrug-resistant tuberculosis, extensively drug-resistant tuberculosis.

INTRODUCTION

Tuberculosis is still an epidemic in many regions of the world, claiming the lives of millions of people each year (Matteelli et al., 2007), with more than 9 million cases annually and with 1.7 million death from TB in 2009 (including 3, 80,000 people with HIV), equal to about 4,700 deaths a day (World Health Organization, 2006a). TB is a disease of poverty, affecting mostly young adults in their most productive years. The vast majority of TB deaths are in the developing world, with more than half occurring in Asia. 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 caring a further 7% (World health Organization, 2008a). 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

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Abbreviations: MDR, Multidrug resistant; **TB,** tuberculosis; **XDR,** extensively drug-resistant tuberculosis; **DST,** drug susceptibility testing.

Organization, 2008 b). During the last ten years there has been a renewed global interest for research in TB, and this has resulted in the launch of several new initiatives by national and international organizations, private charities and pharmaceutical companies (Matteelli et al., 2007). The struggle against tuberculosis (TB) is still far from over, co-infection with human immunodeficiency virus (HIV), the emergence of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) strains have further increased the burden for this disease. The new focus on TB has partly been triggered by the persistent high number of TB cases in poor countries, and partly by the increased occurrence of MDR- and XDR-Tuberculosis. In recent years, international attention has turned toward the evolving burden of drug resistance. Multi-drug resistant tuberculosis (MDR- TB) has emerged in epidemic proportions in the wake of widespread HIV infection. MDR TB is defined as laboratory-confirmed resistance to the two most potent first-line medications isoniazid and rifampin. Since 2007, XDR TB has been defined as resistance to both isoniazid and rifampin with additional resistance to at least one fluoroquinolone and one inject able agent (WHO Report, 2008c).Primary drug resistance is defined as cases which have not been previously treated, and secondary drug resistance occurs in pre-treated patients. The world is on track to achieve two TB targets set for 2015, which aims to halt and reverse global incidence (in comparison with 1990) and Stop TB Partnership target of halving deaths from TB (also in comparison with 1990). Forty one million TB patients have been successfully treated in DOTs programs and up to six million lives saved since 1995, five million more lives could be saved between now and 2015 by fully funding and implementing The Global Plan to Stop TB 2011 to 2015. The World Health Organization (WHO) reported an estimated 9.4 million incident TB cases and 1.7 million deaths (WHO Report, 2009). Existing evidence-based interventions for TB control that have been successfully implemented from 1995 through 2009 have saved 6 million lives and alleviated much human suffering (WHO, 2010a) yet, by 2009 only an estimated 63% of annual incident TB cases were being detected and reported; of these, 86% were successfully treated. World Tuberculosis (TB) Day, which falls on March 24 every year, aims to raise public awareness about tuberculosis, a preventable disease, to accelerate progress against the global effect of disease caused by TB and to achieve its elimination. Co-infection with HIV and the emergence of resistant and extremely resistant strains of the bacteria have led to a worldwide effort to combat this disease and efforts remain the central hope in finding improved therapeutics for this purpose (WHO, 2008c). Overall, XDR TB has now been reported in 55 countries from all geographic regions of the world. MDR- , XDR-, and TDR- TB are now emerging problems of great importance to public health (WHO, 2009; M/XDR-TB, 2010b). Therefore, the aim of this study was to investigate the primary drug resistance patterns in newly diagnosed tuberculosis patients in Yazd, the southern province of Iran.

MATERIALS AND METHODS

Bacterial strains

Mycobacterium tuberculosis isolates were obtained from sputum samples of patients with active pulmonary tuberculosis (TB) referred to Nikopoor hospital in city of Yazd, the southern province of Iran. This center is the only referral center for TB evaluation and treatment in this province. Then, specimens were transferred to the mycobacteriology, and pulmonary laboratory in Pasteur Institute in Tehran for drug susceptibility testing. The primary isolation and culture was done after NaOH N-acetylcysteine (Becton Dickinson Diagnostic Systems) treatment in accordance with standard solid-culture procedures (KentandKubica, 1985). The isolates were cultured on Löwenstein-Jensen medium and the resulting colonies were identified according to standard biochemical tests, including production of niacin, catalase activity, and nitrate reduction, as well as pigment production. 2-thiophene carboxylic acid (TCH) was used to determine the species, and two sensitive isolates were used as negative controls.

Susceptibility testing

Drug susceptibility testing (DST) for first line and second line drugs was performed by absolute concentration method (MIC) for all drugs. Drug susceptibility testing 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. All drugs in this study were purchased from Sigma Chemical (St. Louis, MO). The drug-susceptibility testing against isoniazid (0.2 µg/ml), streptomycin (4 µg/ml), pyrazinamide (2 µg/ml), ethambutol (2 µg/ml), ethionamide (2 µg/ml), rifampicin (40 µg/ml), kanamycin (20 µg/ml) were performed according to the CDC standard conventional proportional method on slants (Kubicaand Dye, 1967), using H_{37}Rv strain as positive control. The drug-susceptibility testing against second-line anti-tuberculosis drugs kanamycin (20 µg/ml), amikacin, capreomycin(10 µg/ml), ciprofloxacin (2 µg/ml), cycloserine, ethionamide (20 µg/ml), and para-aminosalicylic acid were performed using two critical proportions of 1% .

Definition of new case, MDR, XDR TB

New case tuberculosis is TB in a patient who has never received treatment for TB, or who has taken anti-TB drugs for less than one month (WHO, 2009). MDR-TB was defined when isolates were resistant at least to both isoniazid and rifampicin. XDR TB was considered resistance to the best second-line medications: fluoroquinolones and at least one of three injectable drugs that is, amikacin, kanamycin, or capreomycin (World Health Organization, 2006 a, b, 2008). The results of biochemical tests are shown in Table 1.

Table 1. Biochemical testing results of collected clinical specimens.

Specimen number	Niacin production	Nitrate reductase	Catalase at 68	Catalase at 22	TCH
1	+5	+	_	+	R
2	+3	+	_	+	R
3	+4	+	_	+	R
4	+3	+	_	+	R
5	+4	+	_	+	R
6	+4	+	_	+	R
7	+5	+	_	+	R
8	+5	+	_	+	R
9	+5	+	_	+	R
10	+4	+	_	+	R
11	+5	+	_	+	R
12	+4	+	_	+	R
13	+5	+	_	+	S
14	+4	+	_	+	R
14	+4	+	_	+	R
16	+5	+	_	+	R
17	+3	+	_	+	R
18	+3	+	_	+	R
19	+3	+	_	+	R
20	-	-	+	+	R
21	+5	+	_	+	R
22	+4	+	_	+	R
23	+4	+	_	+	R
24	+3	+	_	+	R
25	+3	+	_	+	R
26	+3	+	_	+	R
27	+3	+	_	+	R
28	+3	+	_	+	S
29	+4	+	_	+	R
30	+1	+	_	+	R
31	+3	+	_	+	R
32	+4	+	_	+	R

TCH = 2-Thiophene carboxylic acid.

As shown in Table 1, strain number 20 was atypical mycobacterium tuberculosis, and resistance to all the tested antibiotics.

The results of antibiotic susceptibility testing are shown in Table 2. The highest rate of resistance was to ETH 16(51.61%), followed by KM 9 (29.03%), SM 8(25.08%), RIF 7 (22.58%), INH 2(6.45%), EMB 1 (3.22%). Two strains were MDR andnone were found to be XDR or TDR.

The percentage rates of susceptibility of tested strains are shown in Table 3. As shown in Table 3, the highest rate of resistance was seen with ETH, followed by KM, SM, and RIF respectively. The total distribution results of MDR, XDR, and TDR are shown in Table 4. As shown in Table 4, only one out of thirty two strains was a typical mycobacterium tuberculosis, and it was MDR.

DISCUSSION

Responding to drug-resistant tuberculosis is possibly one of the most profound challenges facing global health. The past 20 years have seen the world wide appearance of multidrug-resistant (MDR) tuberculosis (Gandhi et al., 2006, 2010; WHO, 2010; Upshur et al., 2009; CDC, 1991; Frieden et al., 1993; Ritacco et al., 1997; Rulln et al., 1996), followed by extensively drug-resistant (XDR) tuberculosis, and most recently strains that are resistant to all anti-tuberculosis drugs (TDR) (Migliori et al., 2007; Velayati et al., 2009; Shah et al., 2009). Data are insufficient to indicate whether incidence of MDR tuberculosis is rising or falling globally. However, the fact that only 7% of the estimated 440 000 cases of MDR disease worldwide were reported to WHO in 2008, and of these, only a fifth (1.2% of the total) treated according to WHO recommended standards is of major concern (WHO, 2008d). India and China together carry nearly 50% of the global burden, followed by Russia (9%). MDR tuberculosis caused an estimated 150 000 deaths in

Table 2. Drug resistance patterns of *Mycobacterium tuberculosis* isolated from new case tuberculosis patients in Yazd.

Specimen	INH	RIF	SM	EMB	KM	ETH
1	S	S	S	S	S	S
2	S	S	S	S	R	R
3	S	S	S	S	S	R
4	S	S	R	S	R	R
5	S	S	S	S	S	R
6	S	S	R	S	S	R
7	S	S	S	S	R	R
8	R	R	R	R	R	R
9	S	S	S	S	S	S
10	S	S	S	S	S	S
11	S	S	S	S	S	S
12	S	S	S	S	S	S
13	S	S	S	S	S	S
14	S	S	S	S	S	R
14	S	S	S	S	S	S
16	S	S	S	S	S	S
17	S	S	S	S	S	S
18	S	S	S	S	R	R
19	S	S	S	S	R	R
20	R	R	R	R	R	R
21	S	S	S	S	S	S
22	S	R	R	S	R	R
23	S	R	R	S	R	R
24	S	S	S	S	S	S
25	S	S	S	S	S	S
26	S	R	R	S	S	S
27	S	R	R	S	S	R
28	S	S	S	S	S	S
29	S	S	S	S	S	S
30	R	R	R	S	S	R
31	S	R	S	S	S	R
32	S	S	S	S	R	R

INH: Isoniazid, SM: Streptomycin, RIF: Rifampicine, EMB: Ethembutol, KM: Kanamycin, ETH: Ethinoamide, S: Sensitive, R: Resistance.

Table 3. Percentage of drug susceptibility testing.

INH	RIF	SM	EMB	KM	ETH	INH +RIF	INH+RIF+SM	INH+EMB +SM	INH+EMB +SM+RIF
6.45%	22.58%	25.8%	3.2%	29.03%	51.61%	6.45%	6.45%	3.2%	3.2%

2008. These estimates are based on surveys done by local organizations, but coordinated, supported, and analyzed by the Global Project on anti-tuberculosis drug resistance surveillance (WHO, 2010).

According to a nationwide survey conducted in Iran, among all Mycobacterium tuberculosis isolates tested for drug susceptibility, 5.2% were resistant to both isoniazid and rifampin (Tabarsi et al., 2005). These finding is similar to our study. Overall, we found drug resistance to SM, RIF, KM< and ETH were more frequent than to the other agents. Similarly other study conducted in Iran have demonstrated the rate of 2.8% MDR-TB and the rates of INH, RIF, EMB, SM 11.6, 3.9, 3.0, and 23.1% in new cases respectively (Shamaei et al., 2009), compare to our finding of 6.45% MDR, and resistance rate of 6.45, 22.58, 3.2, and 25.8% for INH, RIF, EMB, SM, which are

higher than their results. This might be due to variation in the location of research, and the influence of foreign immigrants especially from Afghanistan. Other patterns of anti-TB resistance also conducted in Iran showed that 43% of XDR and TDR strains belonged to immigrants living in Iran or who had visited the country. These patients did not have a proper clinical history, and the possibility they had received second-line drugs could not be ignored, the majority of them (58%) had either resistance to any drug or to a drug combination including MDR-TB. This might explain the relatively high level of resistance in new cases (Velayati et al., 2009). More epidemiological studies are needed to identify areas of high and increasing levels of drug resistance, and to identify risk factors that promote drug resistance. Risk factors to be evaluated include type and quality of first-line treatment supervision; access to TB drugs outside TB programs; infection control practices; use of rifampin in the continuation phase of the Category 1 regimen; composition of and referral to retreatment regimens; drug quality; M. tuberculosis genotype; HIV prevalence; and level of use of antiretroviral treatment. Such analyses should help elucidate the factors with the greatest impact on the drug resistance situation and thereby the most effective interventions. In addition, any intervention should be monitored for its impact on the drug resistance situation.

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