Trends of drug resistant *Mycobacterium tuberculosis* in a tertiary tuberculosis center in Iran

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ABSTRACT

Objective: To determine the drug resistance pattern to first line antituberculous drugs in National Research Institute of Tuberculosis and Lung Disease and to compare resistant rates with previous studies.

Methods: An anterograde cross-sectional study was performed. The study includes all adults with documented pulmonary tuberculosis (TB) that were hospitalized in National Research Institute of Tuberculosis and Lung Disease in Tehran, from June 2003 to September 2004. Demographic characteristic, TB categories, and drug susceptibility test results were recorded. Two previous studies regarding drug susceptibility in Iran were selected as historical controls.

Results: One hundred and ninety-six new cases and 68 previously treated patients were enrolled in the study. The strains of 61% of new patients and 21% of previously treated patients were fully sensitive to all drugs. The most common resistance was streptomycin (27%) followed by isoniazid (23%) in new cases. Multiple drug resistant strains were noted in 2.6% (95% CI 0.8% to 5.8%) of new cases versus 56% (95% CI 43% to 68%) in previously treated group. The frequency of primary drug resistance to isoniazid was 9.8%-15% or streptomycin 9.8%-13% in the previous studies (p<0.00001).

Conclusion: While these rates may not reflect the true prevalence of drug resistance on a national scale, it does partially demonstrate some defects in the existing tuberculosis control program. The significant increase of isoniazid and streptomycin resistance in the last few years would present a serious challenge to effective management of TB.

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Drug resistant tuberculosis (TB) poses serious threats to TB control programs around the world.[1] Drug resistance of *Mycobacterium TB* was recognized shortly after the introduction of effective anti-TB agents in the late 1940s.[2-3] Therefore, very soon new definitions were formed. Drug resistant TB is defined as primary when there is no history of drug exposure and secondary when there is a history of previous treatment.[4] The problem of resistance results from inadequate treatment, often due to an irregular drug supply, inappropriate regimens, or poor compliance.[5] Ultimately, patients infected with strains resistant to multiple drugs are less likely to be cured and their treatment regimens more toxic and expensive rather than the treatment of patients with susceptible organisms.[6-8] The frequency of primary drug resistance is an important indicator of any TB control program in a population and has significant implications for public health.[9] Hence, the World Health Organization/ International Union Against TB and Lung Disease (WHO/IUATLD) urge all national TB programs to practice directly observed short course treatment strategy (DOTS), as well as ongoing monitoring of the pattern and trends in drug resistance.[10]

The complexity regarding drug resistant TB and treatment difficulties on multiple drug resistant (MDR) TB in Iran has already been shown. Among new cases of TB, the prevalence of MDR-TB had been reported 5% with a successful treatment rate of 70%.[11,12] The purpose of this study was to determine the current situation of first line anti-tuberculosis drug susceptibility and to describe how its trends have changed from past years.

Methods. We performed an anterograde cross-sectional study to reveal current drug sensitivity patterns and used historical case control studies to find its trends in last years.

The study was conducted in National Research Institute of TB and Lung Disease (NRITLD). This center located in Tehran, the capital of Iran, which acts as the reference center for the National Tuberculosis Program (NTP). It is the only center for the treatment of MDR-TB patients in Iran. The Mycobacterial laboratory of the above institute acts as a WHO-approved reference center on the national level. This study was approved in NRITLD ethical committee.

The study included all adult patients (>15 years) with documented pulmonary tuberculosis that were hospitalized in the TB wards from June 2003 to September 2004. Demographic characteristics (age, gender, nationality, place of residency), drug susceptibility test results, disease category (new case, previously treated), referral status (other hospitals, private clinics, national TB program centers, or by him/herself), close contact to smear positive pulmonary TB (who often lives in very close contact with relatives and neighbors), and smoking history (current smokers are defined as those who have ever smoked 100 cigarettes and currently smoke some days or everyday) were recorded for each patient by questionnaire. Questionnaires were completed for all cases via face to face interview by one expert physician after signing written consent by patients. Human immunodeficiency virus (HIV) tests were requested by infectious disease specialists for each patient who had suspected symptoms to HIV/AIDS, or patients who displayed high risk behaviors (unprotected sex practice, intravenous drug abusers, sex workers, homosexuality, concomitant sexually transmitted infections). How did we define TB cases in the study? Identifiable groups included: Confirmed cases were considered confirmed if the patient shows clinical and radiological characteristics of the disease as well as at least an *M. tuberculosis* positive culture. New patients were defined as those who had received less than one month of anti-tuberculosis drug treatment or no treatment before diagnosis. Primary drug resistance was defined as resistance to strains of *M.tuberculosis* in patients without a history of previous treatment. Acquired drug resistance was defined as resistance in a patient who had previously received anti-TB treatment for at least one month, including those with treatment failures and relapse. First line anti-TB drugs include isoniazid (INH), rifampin (RIF), ethambutol (EMB), pyrazinamid (PZA) and streptomycin (STM). Monoresistance was defined as resistance to only one of the first line anti-TB medication. Multi drug resistance (MDR) TB was defined as resistance to at least isoniazid and rifampicin. Poly-resistance or combined resistance was defined as at least 2 drug resistances other than isoniazid and rifampicin combination. Laboratory evaluation was carried out by standard methods. In summary, the sputum culture was carried out in Lowenstein–Jensen (L-J) medium. The standard proportional method was employed for drug susceptibility test (DST).[13,14] The isolated strains were tested for susceptibility to all first line drugs, namely INH, RIF, STM, PZA and EMB. The resistant results were expressed as a colony growth of at least 1% at the following critical concentrations: 0.2 µg/mL INH, 40 µg/mL RIF, 2 µg/mL EMB, 4 µg/mL STM and 900 µg/ml PZA.[15] To assess trends and provide a more representative estimation of the magnitude of the drug resistance TB problem, we compared our finding with previous reports. Mansoori et al[16] had shown the pattern of drug resistance TB in the same institute. Their studies had been conducted from September 1996 to March 2000. The setting (cross-sectional study, adult TB patients, and the same TB wards) and definitions (Inclusion criteria and case definitions) were similar. The laboratory methods (smear, culture and drug susceptibility test) and even laboratory staffs were similar to our study. However, they did not evaluate PZA susceptibility due to laboratory limitation in that time. We compared the above results with the sole existing nationwide study carried out as part of WHO/IUATLD working group on anti-TB drug resistance surveillance.[11]

The Statistical Package for Social Sciences (SPSS) for Windows (version 11, SPSS, Chicago) was used for the analysis. Mean values were calculated for the quantities of demographic variables. The standard chi-squared test and Fisher's exact test were used for the comparison of 2 data points (Armitage test was used for trend evaluation in proportions). Mantel-Hanszel test was used for odd's ratio. A *p*-values less than 0.05 was considered significant.

Results. The study included 264 TB patients of which 196 were new TB cases (74.2%) and 68 were previously treated (25.7%). Patients ranged in age from 15 - 90 years, with a mean (±SD) of 48 ± 21.5 and a median of 50 years for new cases and 16 - 58 years, with a mean 45.4 ± 68, 33.5 years in the previously treated group. Overall, 58% of the patients were male in the new cases and 68% for the previously treated group respectively. Of the total patients, 191 (72%) had Iranian nationality and 73 (28%) were Afghani refugees. The frequency of drug resistant TB in Iranian new cases was 57 (37%) versus 20 (49%) in Afghani refugees. Although this is a numeric difference between the 2 groups, it was not statistically significant (p=0.08). A history of smoking did not show significant differences

in susceptibility patterns comparing new cases with the previously treated group (p>0.05).

The epidemiologic variables of new and previous cases are shown in **Table 1**.

Results on resistance to the 5 first-line drugs tested are shown in **Table 2**.

The strains of 61% of new patients and 21% of previously treated patients were fully sensitive to all drugs. The most common mono-resistance pattern was to STM (12%) and INH (11%) in new cases. Streptomycin resistance was partially frequent in new case patients (27%). Isoniazid resistance rate was 23% in all new case patients. However, in the previously treated group, INH showed the highest level of resistance overall (71%) followed by STM (65%). The level of PZA resistance was not found in new cases, but 14% was seen in the previously treated group. The MDR-TB strain was 2.6% (95% Cl 0.8% to 5.8%) in new cases versus 56% (95%Cl 43% to 68%) in the previously treated group. The proportion of MDR-TB was significantly higher in the previously treated group (p<0.0001).

The results of drug resistance in previous studies are shown in **Table 3**. Mansoori et al[16] and Espinal et al[11] had already shown 25% and 15.9% resistance to any first line anti-TB drugs in the new case group respectively, which had significant differences with our finding (39%) (p=0.0001). There were also differences with the previously treated group (p=0.006). This study revealed 79%, Mansoori 56% and Espinal MA 57% resistance strains to any first line anti-TB drugs in the previously treated group. There were no differences in any Rifampicin, Etambutal and MDR-TB frequency in any groups. They reported 9.8%-15% INH resistance frequency in new case and 9.8%-13% in the previously treated group. This study revealed 23% and 27% resistance frequency for INH and streptomycin in new cases and 71% and 65% in previous treated group. Resistance to INH and STM therefore showed significant increasing trends in the last few years (p<0.0001 in both groups). The level of PZA resistance is only reported in our study. No primary PZA resistance was found but 14% of previous treated patients showed resistance. Only 5 cases were HIV infected, 2 of them were new cases and 3 were previously treated. None of them were pan sensitive. One showed mono-resistance to INH and another to combination of INH and STM in the new case group. In the previous treated group, one had monoresistance to RIF and one was involved by MDR-

Table 1 - Demographic and epidemiological characteristics of tuberculosis patients.

Variables	New cases (196) No. (%)		*P*-value	Previous treated (68) No. (%)		*P*-value
	Susceptible (n=119)	Resistance (n=77)		Susceptible (n=15)	Resistance (n=53)	
Gender			0.738			0.152
Male	69 (58)	47 (61)		8 (53)	38 (72)	
Female	50 (42)	30 (39)		7 (47)	15 (28)	
Nationality			0.08			0.217
Iranian	98 (82)	57 (74)		10 (67)	27 (51)	
Afghani refugees	21 (18)	20 (26)		5 (33)	26 (49)	
Place of Residence			0.931			0.246
City	106 (89)	71 (92)		13 (87)	39 (74)	
Village	13 (11)	6 (8)		2 (13)	14 (26)	
Referral status			0.801			0.051
Direct	69 (58)	48 (62)		4 (27)	31 (58)	
Other hospital clinics	45 (38)	25 (32)		5 (33)	5 (10)	
NTP centers	5 (4)	4 (6)		6 (40)	17 (32)	
Contact history			0.53			0.12
No	99 (83)	69 (90)		9 (60)	42 (79)	
Yes	19 (17)	8 (10)		6 (40)	11 (21)	
Smoking			0.95			0.319
No	78 (66)	51 (66)		11 (73)	33 (62)	
Yes	41 (34)	26 (34)		4 (27)	20 (38)	

nationality - place of birth, contact history - close contact to tuberculosis patients,
smoking - current tobacco user, NTP - National Tuberculosis Program

Table 2 - First-line anti-tuberculosis drug resistance results.

Variables	New cases n (%)		Previous treated cases n (%)		Total n (%)		*P*-value	Odds ratio
Total tested	196		68		264			
Any resistance	77	(39.0)	53	(78.0)	131	(50.0)	<0.0001	OR = 5.5 95% CI =2.8 - 10.4
Mono resistance								
H only	22	(11.0)	4	(6.0)	26	(10.0)		
R only	3	(1.5)	1	(1.5)	4	(1.5)		
E only	3	(1.5)	0		3	(1.0)		
S only	24	(12.0)	3	(4.0)	27	(10.0)		
Z only	0		0		0			
H and R resistance								
MDR	5	(2.6)	38	(56.0)	45	(16.0)	<0.0001	OR = 48 95% CI=17.6– 132
HR only	0		5	(7.0)	5	(2.0)		
HRE only	0		0		0			
HRS only	3	(1.5)	6	(9.0)	9	(3.0)		
HRZ only	0		0		0			
HRES only	2	(1.0)	24	(35.0)	26	(10.0)		
HREZ only	0		0		0			
HRSZ only	0		0		0			
HRESZ	0		2	(3.0)	2	(0.7)		
E + other resistance								
E + S	0		2	(3.0)	2	(0.7)		
H + other resistance								
HE only	0		0		0			
HS only	18	(9.0)	5	(7.0)	23	(9.0)		
HZ only	0		0		0			
HES only	0		2	(3.0)	2	(0.7)		
HEZ only	0		0		0			
HESZ only	0		0		0			
R + other resistance								
RE only	0		0		0			
RS only	3	(1.5)	1	(1.5)	4	(1.5)		
RZ only	0		1	(1.5)	1	(0.4)		
RES only	0		0		0			
RESZ only	0		0		0			
Any drug resistance								
Any H resistance	45	(23.0)	48	(71.0)	93	(35.0)	<0.0001	OR = 8 95% CI =4.3 - 14.9
Any R resistance	11	(6.0)	41	(60.0)	52	(20.0)	<0.0001	OR = 25.4 95% CI=11.7 - 55.3
Any E resistance	7	(4.0)	28	(41.0)	35	(13.0)	<0.0001	OR = 18.8 95% CI = 7.7 - 46
Any S resistance	52	(27.0)	44	(65.0)	96	(36.0)	<0.0001	OR = 5.4 95% CI = 2.8 - 9.1
Any Z resistance	0		10	(14.0)	10	(4.0)	0.027	

OR - odds ratio, CI - confidence interval, H - isoniazid, R - rifampin, E - ethambutol, Z - pyrazinamide, S - streptomycin

TB (Isoniazid, Rifampicin, Streptomycin) strain and the latest showed resistance to INH and STM together (data were not showed in table).

Discussion. We attempted to study trends in resistance to anti-TB drugs at our institute by statistical means and assess trends by comparative analysis.

Since the National Research Institute of TB and Lung Disease in Tehran is the only referral center that is involved in the management of drug resistant patients, all known drug resistant TB cases are referred here from the entire country. Consequently, the demonstrated trend may have the ability to expand in the Iranian TB community. There were no significant differences in primary drug resistance TB between Iranian and Afghani refugees' populations (p=0.08). This finding indicates that drug resistant TB is prevalent in both Iranians and Afghani refugees too. The results show that drug resistant strains of *Mycobacterium TB* are not limited to immigrants and refugees in Iran. The creation of drug resistant TB in previously treated patients has not had any relation to gender, nationality, resident place, and contact history to a TB patient. This manner is acceptable for new case patients. (As data in **Table 1** shows). So, we can assert that epidemiologically, Iranian and Afghani people separately propagate TB.

The frequency of 39% in any resistance strains in the new case group is an indicator of a poor national TB program in Iran, and this high resistance level will engender great pressure on the health care system in coming years. The results of any resistance strains in previously treated group are of even more concern. Our study reveals high rates of primary drug resistance to INH (23%). This has very important implications for chemoprophylaxis strategies in the community, as it would make INH less effective in treating latent TB infection. The rate of primary drug resistance of Mycobateriuym TB to STM is high (27%). In comparison to other limited resource countries, we found an equivalent or less frequent resistance pattern,[17-19] that seems to be high compared to global rates.[20-23] Our study emphasizes the need for sputum cultures and DST in all new TB case not only in this institute but also in all of the NTP centers of Iran. It is important to consider a strategy for TB treatment in patients who have failed a first line anti-TB regimen. So, we suggest putting an obligation in national TB guidelines for sputum culture and DST on all patients selected for the WHO retreatment regimen and to add at least 2 new drugs before receiving DST results. As a result of high frequency of STM resistance strains, we do not recommend STM in any empirical anti-TB treatment in Iran. The cause of the high prevalence of resistant TB in newly diagnosed TB patients is difficult to explain. The high prevalence of STM resistant strains may be explained by previous usage of other aminoglycosidal drugs in humans and animals for other reasons resulting in cross-resistance.[24] An alternative explanation may be the availability of anti-TB drugs outside the national TB program in Iran. They are available on the open market and each physician can prescribe them easily. We strongly recommend changing this practice. This study indicates a major problem in the quality of TB control in recent years. There are significant increases in INH and STM resistance in the last 4 years. Studies

Table 3 - First-line anti- tuberculosis drug resistance results in different studies.

Variables	New cases			*P*-value	Previous treated cases			*P*-value
	Current study	Mansoori et al[16]	Espinal et al[11]		Current study	Mansoori et al[16]	Espinal et al[11]	
Years	2003-2004	1996-2000	1998		2003-2004	1996-2000	1998	
Total tested	196	187	666		68	86	56.0	
Any (%) resistance	39.0	26.0	15.9	<0.00001	79	56	57.1	0.006
Any H resistance (%)	23.0	15.0	9.8	<0.00001	71	55	50.0	0.017
Any R resistance (%)	6.0	6.5	6.2	0.96	60	43	50.0	0.19
Any E resistance (%)	4.0	5.5	4.7	0.92	41	20	32.0	0.18
Any S resistance (%)	27.0	13.0	9.8	<0.00001	65	18	39.0	0.0008
Any Z resistance (%)[#]	0.0	-	-	-	14	-	-	-
MDR (%)	2.6	0.4	5.0	0.13	56	58	48.0	0.43

OR - odds ratio, CI - confidence interval, H - Isoniazid, R - Rifampin, E - Ethambutol, Z - Pyrazinamide, S - Streptomycin.
[#]there is no data in previous studies

in Czechoslovakia,[25] Algeria,[26] Korea,[27] Baltimore,[28] and New York[29] have shown that sound control policies are associated with decreases in drug resistance levels. However, we believe the relation between drug resistance and the quality of control is complex.[30] For example, the outbreaks of the W strain of *Mycobacterium TB*, a variant of the Beijing genotype, could alter the resistance pattern in each community even with a well run TB control program.[24,31-34] The transmission of TB between humans and animals is another control challenge. Infection and disease related to *M.tuberculosis* have been reported for a variety of species ranging from birds to primates.[35,36] The uncontrolled usage of antibiotics for birds and domestic animals may increase drug resistant TB in animals, which could transmit to humans. We have had some major limitations in this study. Distinguishing between new cases and previously treated patients is not always reliable. In the absence of nationwide TB registries, this distinction depends on the patient's history. Sometimes patients may be unaware about previous drug taking. Unfortunately, we have not had sputum culture and DST results routinely in the new cases of TB in Iran (except at this institute). This limitation may have erroneously classified new patients in the previously treated group as drug resistant. This bias may have been present in the original episode and has falsely contributed to failure of treatment. In the comparative portion of the study, we may not be able to evaluate accurately any differences between any increases in drug resistant TB between the historical control and the new study. It is true that the setting of the previous study had several similarities, especially in laboratory methods, but the sample size was different in both of the previous studies compared to the recent study. The Espinal MA study was also a community based project. Despite these limitations, our study provides important data on the frequency of drug resistance in the only drug resistance TB treatment center in Iran. We attempted to show trends of drug resistance in our center as a representative sample of the situation in the rest of the country. Our study has indicated a need for a nationwide survey on drug resistance TB with standard epidemiologic and laboratory methods in Iran. High STM resistance in new cases of TB indicates that the WHO retreatment regimen is not suitable in Iran and should be revised as soon as possible. High rates of drug resistance indicate the need for more centers specialized in the care of these patients. Drug resistant TB is going to be a serious threat to our TB control programs in Iran. Streptomycin is the most common resistance pattern in new cases and INH in the previously treated group.

The trends of resistant *Mycobacterium TB* showed a significant increase from 1998 to the present for INH and STM. The MDR-TB frequency, however, has not changed significantly.

References

1. World Health Organization/Stop TB partnership. Stop TB annual report 2001. Geneva: The organization; 2002. Available from URL: http://www.stoptb.org/resource_center/assets/documents/Final_report2001.pdf
2. Crofton J, Mitchison DA. Streptomycin resistance in pulmonary tuberculosis. ***BMJ*** 1948; 2: 1009-1015.
3. Canetti G. Present aspects of bacterial resistance in tuberculosis. ***Am Rev Respir Dis*** 1965; 92: 687-703.
4. Treatment of Tuberculosis, American Thoracic Society, CDC, and Infectious Diseases Society of America, Morbidity and Mortality Weekly Report, June 20, 2003 / Vol. 52 / No. RR-11.
5. Kochi A, Vareldis B, Stybb K. Multidrug–resistant tuberculosis and its control. ***Res Microbiol*** 1993; 144: 104-110.
6. Mitchison DA, Nunn AJ. Influence of initial drug resistance on the response to short-course chemotherapy of pulmonary tuberculosis. ***Am Rev Respire Dis*** 1986; 133: 423-430.
7. Goble M, Iseman MD, Madsen LA, Waite D, Ackerson L, Horsburgh CR Jr. Treatment of 171 patients with pulmonary tuberculosis resistant to isoniazid and rifampin. ***N Engl J Med*** 1993; 328: 527-532.
8. Mahmaudi A, Isman MD. Pitfalls in the care of patients with tuberculosis: common errors and their association with the acquisition of drug resistance. ***JAMA*** 1993; 270: 65-68.
9. Raviglione MC, Dye C, Schmidt S, Kochi A. Assessment of worldwide tuberculosis control. ***Lancet*** 1997; 350: 624-629.
10. De Kantor IN, Kim SJ, Frieden T, Laszlo A, Luelmo F, Norval PY, et al. Laboratory services in tuberculosis control. Geneva: World Health Organization; 1998. Available from URL: http://www.who.int/bookorders/anglais/detart1.jsp?sesslan=1&codlan=1&codcol=93&codcch=1167
11. Espinal MA, Laszlo A, Simonsen L, Boulahbal F, Kim SJ, Reniero A, et al. Global Trends in resistance to antituberculosis drugs. ***N Engl J Med*** 2001; 344: 1294-1303.
12. Mirsaeidi SM, Tabarsi P, Khoshnood K, Pooramiri MV, Rowhani-Rahbar A, Mansoori SD, et al. Treatment of Multiple Drug-Resistant Tuberculosis (MDR-TB) in Iran (Preliminary Report). ***Int J Infect Dis*** 2005; 9: 317-322.
13. World Health Organization. Standardization of methods for conducting microbic sensitivity tests. ***WHO Tech Rep Ser*** 1961; 210: 3-24.
14. Canetti G, Fox W, Khomenko AA, Mitchison DA, Rist N, Smelev NA. Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity tests in tuberculosis control programmes. ***Bull World Health Organ*** 1969; 41: 21-43.
15. Heifets L, Sanchez T. New Agar Medium for Testing Susceptibility of Mycobacterium tuberculosis to Pyrazinamide. ***J Clin Microbiol*** 2000; 38: 1498-1501.
16. Mansoori SD, Agha alaei SY, Arami S. Comparative study of initial and acquired drug resistance in pulmonary tuberculosis, ***International Review of the Armed Forces Medical Services*** 2003; 79: 45-49.

17. Zaman K, Rahim Z, Arifeen S, Baqui AH, Sack DA, Hossain S, et al. Drug resistance of *Mycobacterium tuberculosis* in selected urban and rural areas in Bangladesh. ***Scand J Infect Dis*** 2005; 37: 21-26.
18. Dewan P, Sosnovskaja A, Thomsen V, Cicenaite J, Laserson K, Johansen I, et al. High prevalence of drug–resistant tuberculosis, republic of Lithuania 2002. ***Int J Tuberc Lung Dis*** 2005; 9: 170-174.
19. Pereira M, Tripathy S, Inamdar V, Ramesh K, Bhavsar M, Date A, et al. Drug resistance pattern of mycobacterium tuberculosis in seropositive and seronegative HIV-TB patients in Pune, India. ***Indian J Med Res*** 2005; 121: 235-239.
20. De Riemer K, Garcia-Garcia L, Boladilla-del-valle M. Does DOTS work in populations with drug resistant tuberculosis? ***Lancet*** 2005; 365: 1239-1245.
21. Centers for disease control and prevention (CDC). Trends in tuberculosis-United States, 2004. ***MMWR Morb Mortal Wkly Rep*** 2005; 54: 245-249.
22. Lumb R, Bastian I, Crighton T, Gilpin C, Haverkort F, Sievers A. Tuberculosis in Australia: bacteriologically confirmed cases and drug risistance, 2003. A report of the Australian mycobacterium reference laboratory Network. ***Commun Dis Intell*** 2004; 28: 474-480.
23. Pablos-Mendez A, Raviglione MC, Laszlo A, Binkin N, Rieder HL, Bustreo F, et al. Global surveillance for anti tuberculosis-drug resistance 1994-1997. ***N Engl J Med*** 1998; 338: 1641-1649.
24. Horsburgh CR Jr. The global problem of multidrug resistant tuberculosis. ***JAMA*** 2000; 283: 2575-2576.
25. Styblo K, Dankova D, Drapela J, Galliova J, Jezek Z, Krivanek J, et al. Epidemiological and clinical study of tuberculosis in the district of Kolin. Czechoslovakia report for the first 4 years of the study (1961–64). ***Bull World Health Organ*** 1967; 37: 819-874.
26. Boulahbal F, Khaled S, Tazir M. The interest of follow up of resistance of the tubercle bacillus in the evaluation of a programme. ***Bull Int Union Tuberc Lung Dis*** 1989; 64: 23-25.
27. Kim SJ, Bai GH, Hong YP. Drug-resistant tuberculosis in Korea 1994. ***Int J Tuberc Lung Dis*** 1997; 1: 302-308.
28. Chaulk CP, Moore-Ricek, Rizzo R, Chaisson RE. Eleven years of community based directly observed therapy for tuberculosis. ***JAMA*** 1995; 274: 945-951.
29. Frieden TR, Fujiwara PI, Washko RM, Hamburg MA. Tuberculosis in New York City: history, implementation, results and challenges. ***Clin Chest Med*** 1997; 18: 135-148.
30. Barnes PT. The influence of epidemiologic factors or drug resistance rate in tuberculosis. ***Am Rev Respire Dis*** 1987; 136: 325-328.
31. Bifani PJ, Plikaytis BB, Kapur V, Stockbauer K, Pan X, Lutfey ML, et al. Origin and in interstate spread of a New York City multi drug–resistant *Mycobacterium tuberculosis* clone family. ***JAMA*** 1996; 275: 452-457.
32. Frieden TR, Sherman LF, Maw KL, Fujiwara PI, Crawford JT, Nivin B, et al. A multi-institutional outbreak of highly drug resistant tuberculosis: epidemiology and clinical outcomes. ***JAMA*** 1996; 276: 1229-1235.
33. Borgdorff MW, Hass PD, Kremer K. *Mycobactereium tuberculosis* Beijing genotype, the Netherlands. ***Emerg Infect Dis*** 2003; 9: 1310-1313.
34. Hjillemann D, Kubicat T , Rusch-Gerdes S , Niemann S. Disequilibrium in distribution of resistance mutations among *Mycobacterium Tuberculosis* Beijing and non-Beijing strains isolated from patients in Germany. ***Antimicrob Agents Chemother*** 2005; 49: 1229-1231.
35. Ackerman LJ, Benbrook SC, Walton BC. Mycobacterium tuberculosis infection in parrot (Amazona forinosa). ***Am Rev Respir Dis*** 1974; 109: 388-390.
36. Oh P, Granich R, Scott J, Sun B, Joseph M, Stringfield C, et al. Human Exposure following *Mycobacterium tuberculosis* infection of multiple animal species in a metropolitan zoo. ***Emerg Infect Dis*** 2002; 8: 1290-1293.