Study of Mycobacterium Tuberculosis resistance to first-line anti-tubercular drugs in three regions of Kosova during the period 2003-2014

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Abstract

Aim: Drug-resistant tuberculosis (TB) continues to threaten global TB control and remains a major public health concern in many countries. Despite lengthy treatment with costly second-line drug regimens, curing multidrug-resistant TB (MDR - bacillary resistance to at least Isoniazid and Rifampicin) remains a challenge. The aim of this study was to assess the prevalence of different patterns of resistance to four first-line anti-TB drugs, according to the history of treatment and describe the most common forms of TB resistance in transitional Kosova.
Methods: Retrospective study of all cases notified with bacillary tuberculosis in Prishtina, Ferizaj and Gjilan over the course of 2003-2014. We studied 2112 strains of *M. tuberculosis* isolated at the National TB Reference Laboratory in Prishtina. Drug sensitivity testing (DST) was performed with the proportion method on LJ media. The current analysis includes only DST results on isolates taken at the start of treatment.
Results: The prevalence of total resistance to any of the four drugs tested was 20.6% and according to new and previously treated cases was respectively 19.9% and 28.2%. The drug to which the resistance was most frequent in the new cases was Streptomycin 15.2%, whereas the other resistance rates were Ethambutol 2.3%, Rifampicin 2.0% and Isoniazid 0.4%, respectively. In previously treated cases, the resistance rates were 17.6% for Streptomycin, 6.9% for Ethambutol, 3.2% for Rifampicin and 0.5% for Isoniazid. The prevalence of MDR was 1.2%. Multi-drug resistance was statistically significantly associated ($p<0.01$) with a history of previous TB treatment.
Conclusion: Resistance to Streptomycin was the most common phenomenon. The prevalence of MDR tuberculosis is low in Kosova. Even though Kosova went through political and socioeconomic constraints, the prevalence of anti-TB drug resistance remains low. Resistance is associated with a history of previous TB treatment.

Keywords: DST, Kosova, MDR, prevalence rate, TB drugs, tuberculosis

Introduction

Tuberculosis (TB) in 2015 was one of the top 10 causes of death worldwide, ranking above HIV/ AIDS and one of the leading cause of death from infection diseases (1). In addition, the emergence of drug-resistant tuberculosis, especially multidrug-resistant (MDR) TB (2,3) and extensively drug-resistant (XDR) TB (4,5) poses a substantial threat to TB control programmes worldwide. Precise information about the global incidence of drug-resistant tuberculosis is difficult to obtain, since routine sputum culture and drug susceptibility testing are not performed routinely in resource-limited settings, where the disease occurs most frequently (6). The WHO 2014 Global Tuberculosis Report estimated that, worldwide, approximately 3.5 percent of all new tuberculosis cases and 20.5 percent of previously treated cases are multidrug resistant; it also noted that 55 percent of patients with multidrug-resistant tuberculosis were not detected in 2013 (7). Despite these limitations, surveys of worldwide drug resistance indicate that drug-resistant TB is a large and increasing problem (8,9). MDR-TB occurs worldwide particularly in China, India, Russia, and the countries of the former Soviet Union are estimated to carry the highest number of MDR-TB cases (6,10,11). The most affected MDR-TB areas in Balkan are Rumania, Bulgaria and Bosnia (12).
The scale of prevalence of drug resistance was not known in Kosova because there were not data collected and registered for TB drug resistances in Kosova for many decades before the establishment of the National Tuberculosis Programme (NTP) in 2000. With the NTP started the implementation of Direct Observed Short Treatment Strategy (DOTS) which enabled the condition for performing drug resistance surveillance and its accurate registration. This is the first study conducted in Kosova regarding drug resistant tuberculosis surveillance in three main districts representative at nationwide scale surveys. The population in Kosova was estimated 1.739.825, last census in 2011. The country is divided into seven regions and the survey was carried out in three district, representing 34% of population. The incidence of tuberculosis in Kosova during the study period ranged from 62.7 in 2003 to 44.9 in 2014 per 100 000 population for all forms of tuberculosis. The incidence of smear positive cases ranged from 14.9/ 100 000 in 2003 to 13/ 100.000 in 2014 for all TB pulmonary cases. The data of human immunodeficiency virus (HIV) infection among general population was not known during the study period but the latest data in 2014 confirmed 94 cases with HIV disease. The total number of co-infected individuals with HIV and TB and treated with anti TB drugs were 15 cases during the study period.
The objective of the survey was to assess the prevalence of different patterns of resistance to four first-line anti-TB drugs, Rifampicin (R), Isoniazid (H), Streptomycin (S) and Ethambutol (E) in the general population according to the history of treatment and describe the most common forms of TB resistance in transitional Kosova.

Methods

A retrospective cohort study was conducted in Prishtina, Ferizaj and Gjilan from the beginning of January 2003 to the end of December 2014. Patients included in the cohort were confirmed by culture and performed drug susceptibility test. The data were obtained by TB registries in lung diseases dispensary at regional level and National Institute of Public Health of Kosova-Microbiological Laboratory / Reference Laboratory. The study population was defined as the selection of all cases with cultures growing M. Tuberculosis (n=2112) in three above mention districts. To classify drug resistance as primary or acquired (secondary) information on previous treatment were obtained for all cases through district registers. Definitions for describing the different types of drug-resistant tuberculosis were based on the WHO classification system (13).

- Drug-resistant tuberculosis refers to TB caused by an isolate of Mycobacterium tuberculosis that is resistant to one or more antituberculous drugs.

- Monoresistance: resistance to one first-line anti-TB drug only
- Poly drug resistance: resistance to more than one first-line anti-TB drug (other than both Isoniazid and Rifampicin).
- Multidrug resistance: resistance to at least both Isoniazid and Rifampicin.
- Primary resistance patients with TB resistant to one or more anti-tuberculosis drugs, but who have never been previously treated for TB.
- Acquired drug resistance refers to the development of drug resistance during or following antituberculous therapy in patients who had previously had drug-susceptible TB and have not been reinfected by a different drug-resistant organism.

Drug sensitivity testing of first line drug was performed with proportion method (Canetti et al.,1963,1969; Vestal,1975) in Lowenstein Jensen terrain (14,15). Critical proportion of antituberculous drugs and critic proportion of for sensitivity used in this study are shown in Table 1.

Table 1. Concentration and critical proportion of antituberculous drugs

Antituberculous drugs	Concentration	Critical proportion
Isoniazid	0.2 μg/ml	1%
Rifampicin	40.0 μg/ml	1%
Streptomicin	4.0 μg/ml	1%
Ethambutol	2.0 μg/ml	1%

Statistical analysis

The χ^2 test was used to compare percentages when the respective assumptions were met (for each expected count no less than five), and Fisher's exact test for small sample sizes. All analyses were performed using Stata statistical software (version 9.0SE, Stata Corporation, College Station, TX, USA).

Results

The drug susceptibility test results were available for 2112 cases with positive culture of complex M. tuberculosis isolated in the National Reference Laboratory of TB in NIPHK during 2003-2014. All positive cultures belong to the samples taken before initial treatment (or within month of started treatment). All cases were classified into two groups: i) new cases (patients never had treatment for TB, or have taken anti-TB drugs for less than 1 month); ii) retreated cases (previously treated patients have received 1 month or more of anti-TB drugs in the past).

The prevalence of total resistance to any of the four drugs tested was 20.6% and according to new and previously treated cases was respectively 19.9% and 28.2%. The drug to which the resistance was most frequent in new cases was Streptomycin 15.2%, and less resistance rates was respectively Ethambutol 2.3%, Rifampicin 2.0% and Isoniazid 0.4%. In previously treated cases the resistance rates was respectively 17.6% Streptomycin, Ethambutol 6.9%, Rifampicin 3.2% and Isoniazid 0.5%. The prevalence of MDR was 1.2%.

Among 2112 tested cases, 1924 (91%) were new cases and 188 (9%) cases were previously treated. Among 1924 new cases, 1456 (75.6%) of them did not show resistance in any antituberculous drugs after testing. The prevalence of primary resistance to any of the four drugs tested was 468 (24.4%) cases.

Among 188 previously treated cases, 114 (60.6%) didn't show resistance. Whereas, the prevalence among previously treated cases to any of the four drugs tested was 74 cases (39.4%).

Results of different antituberculosis drugs are shown in table No.2 regarding treatment status new cases or previously treated.

Table 2. Sensitivity for different antituberculosis drugs

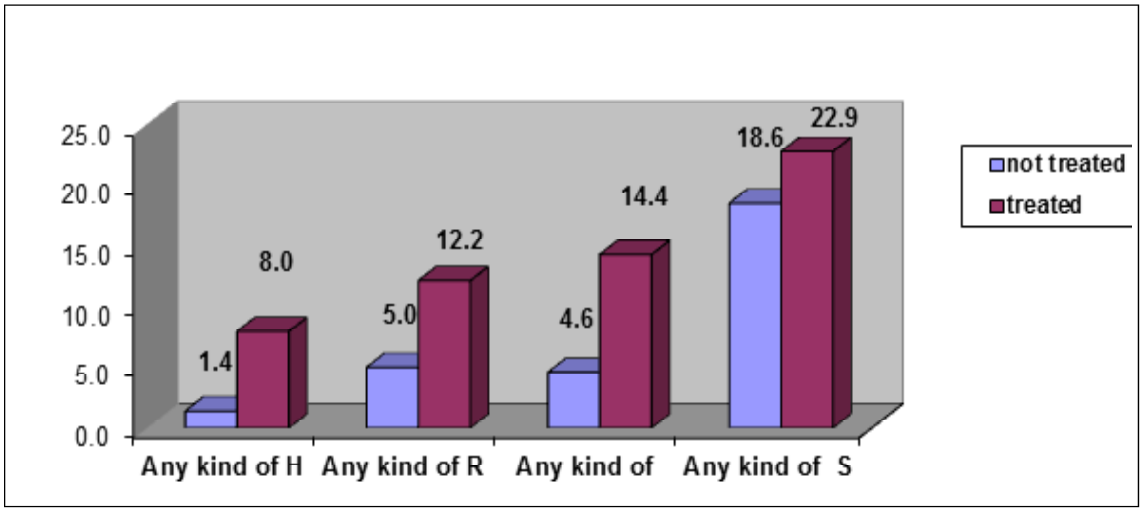
	PT*		T†		TOTAL	
	N	%	N	%	N	%
Tested patients	1924		188		2112	
I. Only H	8	0.4	1	0.5	9	0.4
Only R	38	2.0	6	3.2	44	2.1
Only E	45	2.3	13	6.9	58	2.7
Only S	292	15.2	33	17.6	325	15.4
Total Monoresistance	**383**	19.9	**53**	28.2	**436**	**20.6**
II. H+R	3	0.2	4	2.1	7	0.3
H+R+E	3	0.2	3	1.6	6	0.3
H+R+S	5	0.3	0	0.0	5	0.2
H+R+E+S‡	2	0.1	5	2.7	7	0.3
Multi resistance MDR§	**13**	**0.7**	**12**	**6.4**	**25**	**1.2**
III. H+E	2	0.1	1	0.5	3	0.1
H+S	1	0.1	1	0.5	2	0.1
H+E+S	3	0.2	0	0.0	3	0.1
R+E	12	0.6	3	1.6	15	0.7
R+S	33	1.7	2	1.1	35	1.7
R+E+S	0	0.0	0	0.0	0	0.0
E+S	21	1.1	2	1.1	23	1.1
Total polyresistanceII	**72**	3.7	**9**	**4.8**	**81**	**3.8**
Any kind of H	27	1.4	15	8.0	42	2.0
Any kind of R	96	5.0	23	12.2	119	5.6
Any kind of E	88	4.6	27	14.4	115	5.4
Any kind of S	357	18.6	43	22.9	400	18.9

* Patient not treated before with antituberculosis drugs or treated less than 1 month.
† Patient treated before with antituberculosis drugs for 1 or more months, except patents that has before undergone chemoprophylaxis.
‡ Total number of case tested for DST for 1-st line of antituberculosis drugs (H+R+E+S)
§ Resistance in H and R with or without other TB drugs resistance.
II Resistance for all kinds of H, R, E and S

From 2215 cases tested with culture in any kind of Isoniazid (H), resistance showed 27 cases or 1.4%, in any kind of Rifampicin (R) 96 cases or 5.0 %, in any kind of Ethambutol (E) 88 cases or 4.6% and in any kind of Streptomycin (S) 357 cases or 18.6%. From 1924 (91%) cases not treated before: 18.6% showed resistance in S, 1.4 % in H, 5.0% in R, and 4.6 % in E.

From 188 (9.0%) cases treated before: 8.0% showed resistance in H, 12.2 % in R, 22.9% in S and 14.4% in E (Figure 1).

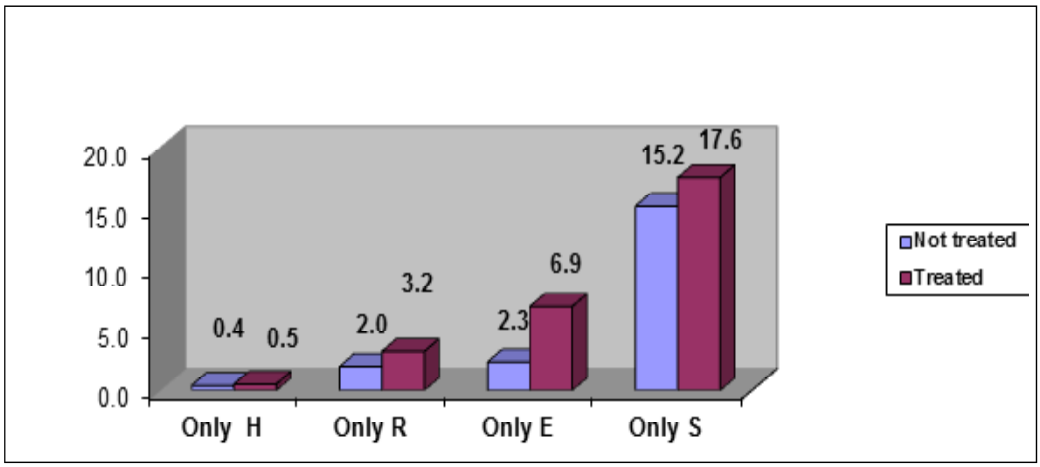
Figure 1. Treated patients and Resistance in any kind of TB drugs



Regarding monoresistance among new cases (NC): 15.2% showed resistance to Streptomycin, 0.4% to Isoniazid, 2.0% to Rifampicin and 2.3% to Ethambutol.

Retreated cases: 17.6% showed resistance to Streptomycin, 0.5% to Isoniazid, 3.2% to Rifampicin and 6.9% to Ethambutol (Figure 2).

Figure 2. Monoresistence of TB drugs in untreated and treated patients



MDR TB in NC was at 0.7% and 6.4% of cases treated before (Figure 3).

Poly-resistance was found in 3.7% of NC, the mostly was combination (R+S) and 4.8% of treated cases mostly was combination of (R+E) (Figure 4).

Figure 3. MDR TB in untreated and treated patients

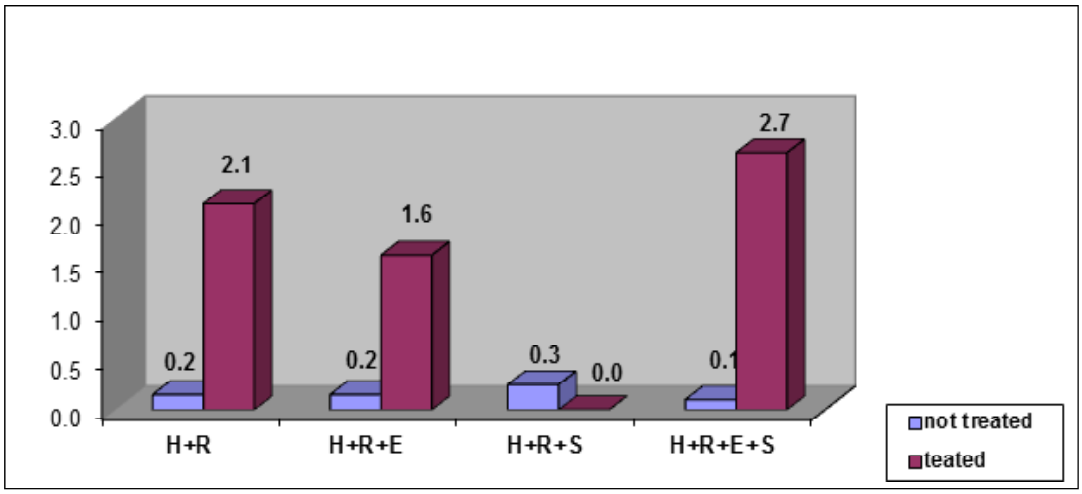
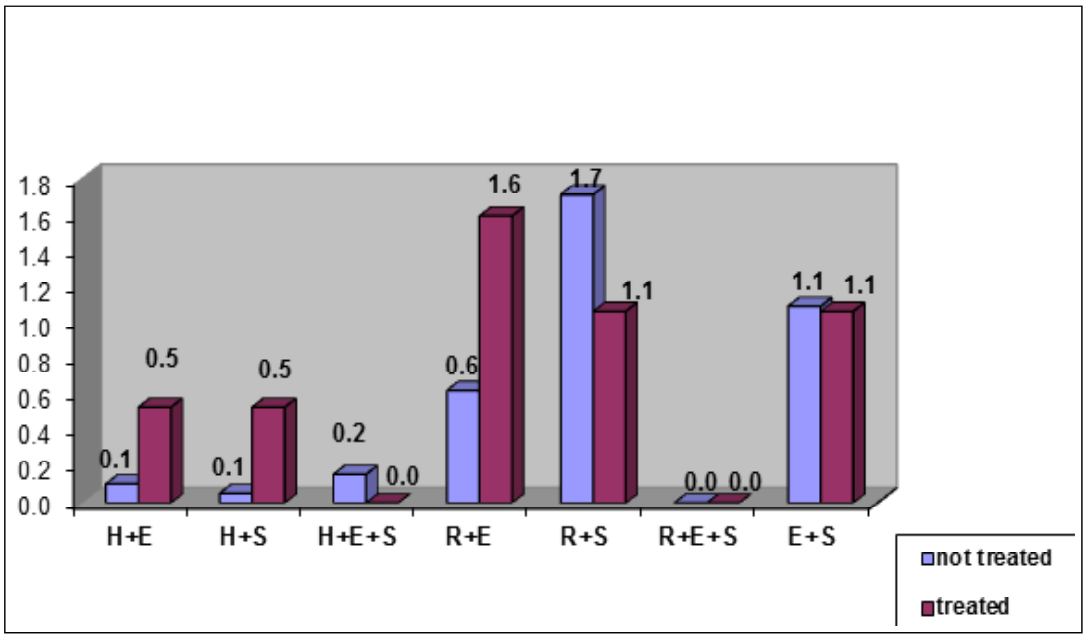


Figure 4. Polyresistence of TB drugs in untreated and treated cases



From 2112 tested cases, 91% were cases with no history of previous treatment. Among patients with no prior treatment: 15.2% were resistant to Streptomycin, 2.3% to Ethambutol, 0.2% to Rifampicin and 0.4% to Isoniazid. Whereas, among previously treated patients: 17.7 % showed resistance to Streptomycin, 6.9% to Ethambutol, 3.3% to Rifampicin and 0.5% to Isoniazid.

The prevalence of primary multidrug resistance was 0.2% and among them prior treated 2.1%. The overall resistance for single drug was 19.9% at the patient with no prior treatment and 28.8% at the patients with history for prior treatment. Multidrug resistance was 0.7% at the patient with no prior treatment and 6.4% at the patient with prior treatment. Resistance was associated with a history for of previous treatment.

Discussion

The microbiological detection of TB and drug susceptibility testing allows patients to be correctly diagnosed and started on the most effective treatment regimen as early as possible.

The present study on *M. tuberculosis* resistance to anti-tuberculosis drugs is the first to be performed at national level. The results of the study are representative because the sample size was calculated before the study and three districts cover more than half of pulmonary TB cases in Kosova. The number of TB patients during 2003–2014, a total of 2112 pulmonary TB patients were diagnosed in these three regions and resistance to any first-line drug and MDR was much higher among previously treated cases than among new cases (Table 2); 28.2% versus 19.9 % for monoresistance and 6.4% versus 0.7 for multidrug resistance. Total multi drug resistance was 1.2 or 25 MDR cases among 2112 total cases. This difference is statistically significant for MDR cases between new and retreated cases ($p<0.01$) but not significant for monoresistance.

Globally in 2014, an estimated 3.3% of new cases and 20% of previously treated cases have MDR-TB; these levels have remained virtually unchanged in recent years (12). The percentage of MDR in Kosova is much lower than globally MDR data but also if we compare to the some neighbouring countries like Bulgaria where MDR was 2.3% for new cases and 23% for retreated cases in 2014, 7.8% in Romania. The figures were more or less similar with other countries like Albania where MDR was 0.6% and Serbia 1.3% MDR in 2014 (13). The MDR data analysis shows that MDR is not a problem for the time being and this is one of the best indicators proving that TB situation and TB program are performing at good standard.

Conflicts of interest: None declared.

Resistance to any anti TB drugs was relatively high 20.6% but the main contributor to this figure was resistance to Streptomycin 15.4% and the resistance of the rest of TB drugs was small and doesn't represent problem for TB treatment and control. The resistance to Streptomycin is related to use of this drugs for other purpose besides TB. Streptomycin was the main antibiotic used in Kosova for other banal bacterial infection as well as in others countries in the first decades after its discovery. Compared to new cases, previously treated confirmed pulmonary TB cases were almost the same for Isoniazid, Rifampicin and Streptomycin and two times more resistant for Ethambutol. TB resistance in Kosova are more or less the same with TB resistance data in Albania (16). This study shows that MDR and other TB drug resistance are low in Kosova and comparable with other countries that have good National TB Program.

A concern remains the low percentage of bacteriological confirmation of TB diagnosis during the study period, the incidence of smear positive cases ranged from 14.9/100,000 in 2003 to 13/ 100,000 in 2014 for all TB pulmonary cases and this problem needs to be addressed urgently in order to improve bacteriological confirmation.

Conclusion

Resistance to Streptomycin and Ethambutol was the most common. Resistance to Streptomycin might be related to common use in the past decades for other purpose besides TB. The prevalence of MDR tuberculosis is low. Even though Kosova is going through political and socioeconomic constraints, the prevalence of anti-TB drug resistance is low. Resistance is associated with a history of previous anti TB treatment.

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