

Molecular-genetic analysis of Mycobacterium tuberculosis strains spread in different patient groups in St.Petersburg (Russia)

Chernyaeva E¹., Dobrynin P.¹, Pestova N.¹, Matveeva N.², Zhemkov V.², Kozlov A.¹

1. The Biomedical Center, St.Petersburg, Russia.
2. St.Petersbutg City TB Center, St.Petersburg, Russia.

Introduction.

Spread of tuberculosis (TB) in Russia is associated with many social and medical factors, such as *HIV-infection, alcohol abuse, unemployment, homelessness, history of imprisonment*¹, *illegal labor migration, etc.* Numerous studies were conducted to discover genetic diversity of Mycobacterium tuberculosis (MTB) strains circulating in different regions of Russian Federation²⁻⁵. The majority of them studied genetic diversity of MTB strains spread among general population of TB patients^{2,3,5,6}. A little number of papers devoted to *studying the epidemiological characteristics of strains of M. tuberculosis among prisoners*^{4,7,8}. However, the *molecular epidemiological features of MTB strains circulating in high-risk groups, especially among HIV-infected individuals and homeless people, have not been studied well. Therefore, the study of genetic diversity of M. tuberculosis strains revealed among patients from different high-risk groups is of great interest. The aim of our study was to compare genotypes of MTB strains circulating among TB patients from different high risk groups (homeless, HIV-infected, prisoners) and general population of St.Petersburg citizens.*

Materials and methods.

The study was performed with 150 of M.tuberculosis complex isolates, revealed among patients from different groups: HIV-infected (56 isolates), homeless (34 isolates), prisoners (30 isolates from 2 different jails) and from general population of HIV-negative TB patients (30 isolates). All M.tuberculosis isolates were collected in St.Petersburg TB Center during the period 2007-2008.

Mycobacterial DNA was isolated from cultures cultivated on Lowenstein-Jensen medium as it was described earlier⁹. Before starting DNA isolation procedure, MTB cultures were inactivated by incubation at 80° C during 1 hour.

DNA of the strains was studied using standardized genotyping technique – spacer oligonucleotide typing (spoligotyping). Spoligotyping was performed on genomic DNA to detect presence or absence of 43 spacers by using the standard method¹⁰ with the help of commercially available kit (Isogen Biosciences). The hybridization patterns obtained were visualized, after incubating with streptavidin peroxidase (GE Healthcare) using Enhanced Chemiluminescent detection system (GE Healthcare). Proper controls (H37Rv, M.bovis BCG and Negative control)

were used with each experiment. The results were entered into MS Excel sheet in binary and octal formats and compared with the international spoligotyping database SpolDB4¹¹. Further, those spoligo patterns not found in SpolDB4 were analyzed with SOPTCUST online service¹².

Statistical analysis of spoligotype patterns was conducted using Microsoft Excel software for calculating Jaccard similarity coefficient (Kj). Chi-square test was used to compare the prevalence of M.tuberculosis Beijing spoligotype families in different patient groups. A p value of <0.05 was considered significant.

Results.

Genetic analysis using spoligotyping method was performed for 150 M.tuberculosis isolates obtained from different risk-groups and general population of St.Petersburg citizens. Cluster analysis of spoligotyping profiles was carried out in Excel package calculating Jaccard difference coefficient (1-Kj). Fingerprint patterns were identified using SpolDB4 database and SPOTCLUST online service. Results of spoligotyping of 150 isolates detected among all studied patient groups are represented in **table 1**.

The majority of M.tuberculosis isolates (56%; n=19), revealed among 34 homeless people in St.Petersburg, belonged to Beijing family. Genetic pattern ST1 was detected in 47% (n=16) of studied clinical isolates. Four isolates (11.8%) belonged to LAM9 family, 3 isolates (8.8%) – to T1 family, another 2 isolates (5.8%) belonged to MANU2 family. Single isolates belonged to families H1, H3 and T4. Genetic families of 2 isolates were not identified.

Spoligotyping of 56 isolates from HIV-infected individuals showed that 76.8% of them (n=43) belonged to Beijing family. More than half of studied samples (53.6%) belonged to spoligotype ST1 – the most prevalent pattern of all Beijing family spoligotypes. Besides Beijing we also detected genotypes belonging to LAM9 family (7.2%, n=4), H3 (3.6%, n=2), H1 (1.8%, n=1), T1 (1.8%, n=1), T2 (1.8%, n=1), T4 (1.8%, n=1), Family 34 (1.8%, n=1) and Family 35 (1.8%, n=1).

Study of genetic diversity of 30 M.tuberculosis isolates revealed from general population of TB patients revealed the prevalence of Beijing spoligotypes (60%, n=17). More than 25% of studied samples belonged to spoligotype ST1. One isolate (3.3%) belonged to Beijing-like group according to SpolDB4 database. Spoligotype families LAM9, T1, T3, MANU2, Family 33, Family 35 and H1 were identified except Beijing family.

Spoligotyping of 30 isolates revealed among prisoners demonstrated comparatively low genetic diversity in this group. Overwhelming majority (83.3%, n=25) of studied samples belonged to Beijing family. In this case, 60% of studied strains (n=18) belonged to ST1 genotype. The rest of isolates belonged to LAM9, LAM3 and S/convergent, H3 and Family 33. Genetic families of 2 isolates were not identified.

Comparison of the studied patient groups in frequency of detection of M.tuberculosis Beijing Family strains (chi-squared test) determined groups with significant spoligotype differences. Significant differences in prevalence of strains of the Beijing family were found between the groups of homeless patients and prisoners (p=0.0180) and between general population and prisoners (p=0.0446)

Comparison of genetic diversity of spoligotypes within studied groups of MTB isolates was performed calculating genetic diversity (1-Kj) minimum/maximum means and quartile measurements. Results of genetic diversity analysis are represented on the **fig.1.** and **table 2.** The lowest genetic spoligotype diversity was detected among M.tuberculosis isolates revealed among prisoners – the median mean (q2) was 11%. The highest genetic diversity was shown for MTB strains circulating among general population of TB patients, homeless people and HIV-infected individuals.

Discussion.

We have detected the prevalence of Beijing family genotypes among studied M.tuberculosis strains revealed from all patient groups: homeless people, HIV-infected individuals, prisoners and general population of HIV-negative TB patients. Predominance of Beijing family MTB strains among all studied groups confirms with published data. Previously it was shown that M.tuberculosis Beijing family isolates were detected in more than 50% of TB cases ^{2,3,4}. For example, in Ural region in 2005 the proportion of Beijing family was 54.3% ². Interestingly, during the period 1995-1997 studies in Arkhangelsk region showed that Beijing family isolates were uncommon – only 8.1% of detected TB cases were due to this genotype of the pathogen. However, in 1998 and 1999 Beijing genotype isolates were detected in 41.9% and 46.1% of cases, respectively ¹³. Other molecular epidemiological studies in different regions of Russia showed that proportion of Beijing genotypes was about 50% ^{6,14}. Thus, we can assume a high degree of transmission of M.tuberculosis Beijing family strains compared with other genotypes.

M.tuberculosis Beijing family strains were previously detected not only among civilian population, but also among prisoners. The study conducted in 2001 in Arkhangelsk showed that 76% of MTB isolates revealed from prisoners belonged to Beijing family ⁸. The molecular-epidemiological research conducted in Serpukhov in 2001 showed that more than 50% of isolates detected among prisoners belonged to Beijing family. Spoligotype ST1 was found among 49% of studied clinical isolates ¹⁵.

Besides Beijing family we have also revealed M.tuberculosis strains belonging to the following spoligotype families: Beijing-like, LAM9, LAM3, T1, T3, T4, T5, T5_RUS1,

Haarlem (H)1, H2, H3, MANU2, 33, 34, 35 and 36. Beijing, Beijing-like, LAM, Haarlem and T spoligotype families are the most wide-spread genotypes all over the world ¹¹.

Spoligotype families of 4 isolates included in our study were not identified. We assume that detection of unidentified spoligotypes could be related with multiple TB infection – when *an individual becomes infected with strains of M. tuberculosis belonging to different spoligotype families. However, this hypothesis requires further confirmation using molecular-epidemiological methods with higher discriminating ability.*

We have suggested that there are significant differences by representation of Beijing and non-Beijing genotypes between different MTB groups. Statistical analysis showed that there were significant differences in Beijing genotype prevalence between “homeless” and “prisoners”, and between “general population” and “prisoners” patient groups. There were no significant differences between “homeless”, “HIV+” and “general population” patient groups on grounds of Beijing family prevalence. Each studied group of MTB isolates except “prisoners” was represented by 18 different spoligotypes. Genetic diversity analysis showed that the lowest spoligotype diversity was detected among patients from prisons ($q_2=0.11$; $q_1=0.00$; $q_3=0.27$). Three other groups of MTB isolates did not have any significant difference, however the lowest median mean was detected in “HIV+” group ($q_2=0.22$). MTB strains revealed from homeless people and general population of TB patients had very close genetic diversity median mean – 0.57 and 0.67, respectively.

We suggest the following interpretation of the results. Prevalence of Beijing genotype strains circulating among high-risk groups is not different from the general population of TB patients in St.Petersburg. The group of homeless TB patients is not isolated from the rest of the city population. Genetic diversity of M.tuberculosis strains among homeless patients is not different from the control group (general population). The proportion of M.tuberculosis Beijing genotype detected among HIV/TB co-infected individuals is also similar with general population of TB patients. Thus we can suppose that HIV-infected and homeless patients have got TB infection independently of the intra-group contacts. However, according to published data, group of HIV-infected patients consists mainly of drug users ¹⁶ and quite separate from the general population of St. Petersburg.

Similarities in the genetic diversity of M.tuberculosis strains circulating among general population of St.Petersburg citizens, as well as among homeless and HIV-infected people, suggest an influence of patients from high-risk groups on TB spread in the city. This fact is probably caused by the lack of preventive control activities in high-risk groups and low TB treatment adherence among homeless and HIV-infected patients, leading to high morbidity and TB dissemination.

We have observed the lowest variety of *M.tuberculosis* spoligotypes among prisoners. Compared to all other studied groups, “prisoners” was the most isolated, physically separated from the rest of population. Low genetic diversity of MTB strains shows that TB infection happens within small groups of prisoners

Results of our study demonstrate that *M.tuberculosis* strains circulating among homeless and HIV-infected people are also spread among general population of St.Petersburg citizens. Thus, we have investigated the participation of high-risk groups in the TB infection spread in the city. High prevalence of drug resistant TB among prisoners ⁷, HIV-infected ¹⁷ and homeless patients (reports of St.Petersburg TB dispensaries, unpublished) pose a risk to increase of TB drug resistance level among general population in the city.

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Table 1.

Mycobacterium tuberculosis spoligotypes revealed among different patient groups.

Spoligotype	ST (SpolDB4)	Number of isolates	%	Family	
				SPOTCLUST	SpolDB4
Isolates detected among homeless TB patients					
000000000003371	ST265	1	2,94	Beijing	Beijing
000000000003661	ST1651	1	2,94	Beijing	Beijing
000000000003771	ST1	16	47,06	Beijing	Beijing
000002000003671	NA	1	2,94	Beijing	NA
515047607760661	NA	1	2,94	LAM9	NA
777477607700771	NA	1	2,94	LAM9	NA
777741003760771	NA	1	2,94	LAM9	NA
777761007760771	ST766	2	5,88	LAM9	T1
77777607760771	ST42	1	2,94	LAM9	LAM9
774777777423771	ST1481	1	2,94	33	MANU2
77777607763771	ST1247	1	2,94	33	MANU2
77777777760771	ST53	1	2,94	T1	T1
177777657760771	ST1255	1	2,94	T1	T5
77777777770000	NA	1	2,94	NA	NA
777737677420731	NA	1	2,94	H3	NA
774002000760771	NA	1	2,94	NA	NA
777761007763771	NA	1	2,94	T4	NA
777777774020771	ST47	1	2,94	H1	H1
Isolates detected among HIV-infected TB patients					
000000000003371	ST265	3	5,36	Beijing	Beijing
000000000003661	ST1651	5	8,93	Beijing	Beijing
000000000003671	ST255	4	7,14	Beijing	Beijing
000000000003771	ST1	30	53,57	Beijing	Beijing
000002000003661	NA	1	1,79	Beijing	Beijing
775741003760771	NA	1	1,79	LAM9	NA
711041003760661	NA	1	1,79	LAM9	NA
711041007740661	NA	1	1,79	LAM9	NA
77777607760771	ST42	1	1,79	LAM9	LAM9
77777777760771	ST53	1	1,79	T1	T1
711007676420621	NA	1	1,79	H3	NA
774777777420771	ST262	1	1,79	NA	H4
775147677700000	NA	1	1,79	T2	NA
777760007760771	ST254	1	1,79	T4	T5_RUS1
77777644020771	NA	1	1,79	H1	NA
77777704020771	ST283	1	1,79	NA	H1
777000000000371	ST560	1	1,79	35	U
377737670000000	NA	1	1,79	34	NA
Isolates detected among general population of TB patients					
000000000003371	ST265	1	3,33	Beijing	Beijing
000000000003661	ST1651	4	13,33	Beijing	Beijing
000000000003601	NA	1	3,33	Beijing	Beijing
000000000003771	ST1	8	26,67	Beijing	Beijing
000002000003771	ST1184	3	10,00	Beijing	U
000000000000771	ST269	1	3,33	Beijing	Beijing-like
555047607760661	NA	1	3,33	LAM9	NA
777761007760651	NA	1	3,33	LAM9	NA
777477607760771	ST252	1	3,33	LAM9	LAM9
515047636760661	NA	1	3,33	T1	NA
515047236760661	NA	1	3,33	T1	NA
77777777760771	ST53	1	3,33	T1	T1
757737777420771	NA	1	3,33	H3	NA
570000636760601	NA	1	3,33	T3	NA
506044034000461	NA	1	3,33	H1	NA

775743003763771	NA	1	3,33	33	NA
777777777723771	ST1634	1	3,33	33	MANU2
777402000000171	NA	1	3,33	35	NA
Isolates detected among TB patients from prisons					
00000000003671	ST255	2	6,67	Beijing	Beijing
00000000003771	ST1	18	60,00	Beijing	Beijing
000002000003771	ST1184	2	6,67	Beijing	U
000002000003671	NA	3	10,00	Beijing	NA
77777607760771	ST42	1	3,33	LAM9	LAM9
77767777720771	NA	1	3,33	NA	NA
77477737423731	NA	1	3,33	33	NA
00000007760771	ST4	1	3,33	36	LAM3 and S /convergent
00000000503771	NA	1	3,33	NA	NA

Table 2.

Spoligotype diversity of M.tuberculosis strains revealed in different patient groups.

Patient Group	Genetic diversity between isolates					Number of isolates
	median	min	max	lower quartile	upper quartile	
Homeless	0.57	0.00	1.00	0.10	0.80	34
HIV+	0.22	0.00	1.00	0.00	0.78	56
General population	0.67	0.00	0.95	0.22	0.81	30
Prisoners	0.11	0.00	0.85	0.00	0.27	30

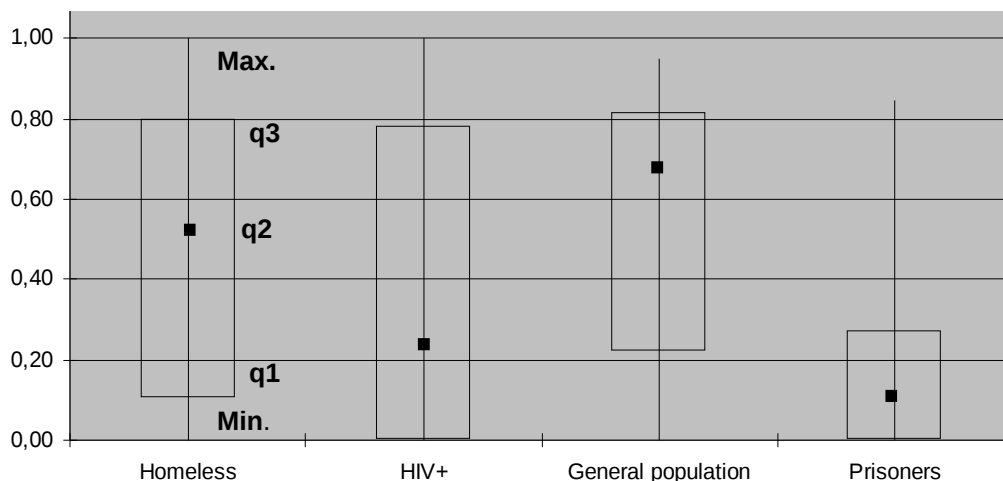


Fig. 1. Spoligotype diversity of M.tuberculosis strains revealed among different patient groups: “homeless”, “HIV-infected” (HIV+), “General population” and “Prisoners”. The diagram shows maximum (Max.) and minimum (Min.) values of genetic differences, median mean (q2), lower (q1) and upper (q3) quartiles.