Study of Relationship between *Mycobacterium tuberculosis* Beijing Family and Epidemiological Characteristics of Tuberculosis in the Northwestern Region of Russia

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Abstract: The study aims to investigate the relationship of genotypes and dynamics of drug resistance of MBT (*Mycobacterium Tuberculosis*) in North-West territories. Patients with pulmonary tuberculosis from areas with similar epidemical situation but different trends were selected using random sampling technique: the first group—90 patients from the Pskov region, and the second—90 patients from the Kaliningrad region. Genotyping of *Mycobacterium tuberculosis* strains was performed by test system of CJSC “Syntol” Russia “Amplitub-Beijing”. Drug sensitivity of MBT was determined by the method of absolute concentrations on Lowenstein medium. The frequency of determination of MBT Beijing family is found equal in 45.6% of cases in the Pskov and Kaliningrad regions. Excretion of drug-resistant MBT in the Pskov region was higher (61.1%) than that in Kaliningrad (45.6%). Beijing strains identified in similar high proportion of drug resistance (85.4% and 80.5%), including MDR (multidrug resistance) (58.5% or 65.9%) in the territories. Among “not Beijing” family strains in the Pskov region drug resistance was in 40.8%, which is 2.5 times more than that in Kaliningrad region (16.3%, $P < 0.01$). More significant differences are obtained in MDR cases: 32.7% and 6.1%, $P < 0.001$. MBT Beijing family dominates in the Northwest and growth of DR (drug resistance) and MDR of MBT is mainly due to this family MBT. But “not Beijing” strains also significantly often acquire drug resistance. Along with the MBT genotype, the differences in the epidemiological trends may be associated with problems in TB treatment organization.

Key words: Tuberculosis, drug resistance of MBT, Beijing genotype.

1. Introduction

Social and economic factors, the widespread decline in living standards and worsening nutrition, increase of number of people suffering from alcoholism and drug addiction, high infection and prevalence of tuberculosis in migrants, refugees and persons of no

fixed abode have a significant impact on morbidity and mortality from tuberculosis [1-3].

However, the effective implementation of epidemic surveillance also needs data on identification and differentiation of clinical strains of *Mycobacterium tuberculosis*. They allow quickly identification the source of infection, ways of transmission of infection and its distribution area [4].

Achievements in molecular biology and molecular genetics have allowed developing methods for

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genotyping of *Mycobacterium tuberculosis* strains by the differentiation of MBT strains at the level of chromosomal DNA. The most informative method was DNA (genetic) fingerprinting which allows to differentiate strains by detecting differences in copy number and localization of IS-elements in chromosome of bacteria, in particular IS6110 (method for analyzing the RFLP—restriction fragment length polymorphism) and spoligo-typing method for identifying short direct repeats and their spacers located in DR-region of MBT chromosome [5-10].

The high degree of genetic polymorphism of MBT in a specific community may indicate a significant contribution of endogenous reactivation of TB infection in the epidemic process. DNA polymorphism is less pronounced with prevalence in the community of tuberculosis infection transmission [11].

Numerous studies have shown that MBT of the Beijing family was found in the countries of Asia, South Africa, the Caribbean and the United States [12-16].

Several data show the distribution of Beijing genotype strains in our country, including in Northwest Russia [17-21].

Feature of MBT Beijing family is their high transmissibility and virulence, tendency to rapid development of drug resistance due to increased ability to accumulate mutations in the chromosomal material [22-26].

Advances in molecular genetics have allowed to clarify the mechanisms of MBT drug resistance [27-31], including the individual anti-TB drugs, especially isoniazid, rifampicin and fluoroquinolones [32, 33].

The aim of the study was to investigate the spread of MBT Beijing family in North-West Territories, relationship of genotypes and dynamics of drug resistance of MBT.

2. Materials and Methods

Two areas with similar socio-demographic characteristics, adverse epidemic situation but with different epidemiological trends were selected to study the spread of MBT of the Beijing family in the North-West Territories.

Kaliningrad region with most complicated situation was chosen for the research: in 2005 there was an epidemic of tuberculosis with total incidence—125.1 per 100 thousand populations, and incidence among the permanent residents—101.5 per 100 thousand populations. Pskov region which had similar population, where incidence rates in 2005 were respectively 86.4 per 100 thousand populations and 66.0 per 100 thousand populations was selected for comparing.

Cultures of MBT for genotyping were taken from patients with positive culture MBT new cases and relapses with pulmonary tuberculosis in 2006-2008 using random sampling technique: The first group consisted of 90 patients from the Pskov region (group 1), and the second consisted of 90 patients from the Kaliningrad region (group 2).

Groups were representative of the age and sex and reflected the age/sex structure of TB incidence with MBT in studied areas (Table 1).

Genotyping of *Mycobacterium tuberculosis* strains was performed concerning their belonging to the phylogenetic Beijing family using test system of CJSC “Syntol” Russia “Amplitub-Beijing”.

The frequency of excretion of Beijing strains in patients with various amounts of excretion of MBT was evaluated. The massiveness MBT excretion was determined in points: 1 point corresponded to a meager; 2 points to moderate; 3 points to abundant excretion.

Drug sensitivity of MBT to antitubercular drugs was determined by the method of absolute concentrations on Lowenstein-Jensen medium [34]. Drug resistance was considered multiple (MDR), if it was defined to at least two major anti-TB drugs—isoniazid and rifampicin [35].

2.1. Ethics

The study protocol was approved by ethics committee of Saint-Petersburg Research Institute of Phthisiopulmonology of Ministry of Health of Russia. All patients provided written informed consent.

Table 1 Age and sex structure of the studied patients.

Groups	Sex	Total		Age (years)				
		Abs.	%	< 30	31-40	41-50	51-60	>61
Group 1	m	59	65.6%	17.0%	27.1%	27.1%	18.6%	10.2%
	f	31	34.4%	38.7%	32.3%	16.1%	6.5%	6.5%
	all	90	100.0%	24.4%	28.9%	23.3%	14.4%	8.9%
Group 2	m	50	55.6%	10.0%	40.0%	20.0%	28.0%	2.0%
	f	40	44.4%	42.5%	30.0%	17.5%	7.5%	2.5%
	all	90	100.0%	24.4%	35.6%	18.9%	18.9%	2.2%

2.2 Statistical Analysis

The database was created. The portion of every index in groups and confidence probabilities between groups were calculated to assess the results.

3. Results

The analysis of epidemic situation dynamics in both areas has shown that the epidemic process was developing in opposite directions from 2005 to 2009. Territorial incidence in Kaliningrad region was reducing since 2006, and its growth rate in 2009 compared to 2005 was (-19.8%), while the proportion of persons discharging bacteria was simultaneously decreasing (-7.6%). The disease incidence of permanent residents of the territory decreased even more rapidly, and in 2009 it was 78.3 per 100 thousand per population.

In the Pskov region, where the situation in 2005 was more favorable for the specified period, the territorial incidence continued to rise (+0.4%), and the disease incidence of the resident population continued to grow (1.0%). In 2009 (compared with 2005), the growth rate in the detection rate of tuberculosis with excretion of MBT general in Pskov region was 2.7%, in the Kaliningrad region (-7.6%), and among the permanent residents, respectively (-5.0%) and (-15.0%). 28.0% increasing of posthumous tuberculosis indicated the deterioration of the epidemiological situation in the Pskov region for 5 years whereas in the Kaliningrad region posthumously detected tuberculosis incidence decreased by 61.0%.

Genotypic similarity of 173 *Mycobacterium* cultures

was determined in a study of 180 patients with pulmonary tuberculosis with MBT excretion: 90 patients from Kaliningrad—group 1 and 90—from the Pskov region, group 2. MBT genotype has not been determined in 7 patients (3.9%).

MBT strains of the genetic family (genotype) Beijing were disease pathogens in 45.6% of TB patients, MBT of the remaining patients belonged to other genotypes (50.6%). The proportion of patients excreting MBT of the Beijing genotype was similar in studied groups. Results of the study are consistent with the notion of the general circulation of Beijing genotype strains in the territories of the North-West Russia [16, 20].

In the group 1, middle growth was found (2 points) in 52.2% MBT cultures and the slight pronounced growth (1 point) in 38.9% cultures.

Among the patients in group 2 massive growth of the culture MBT was poorly expressed (1 point)—44.4%, or expressed much (3 points)—36.7%.

Limited bacterial excretion is documented with the same frequency in comparison between groups, but it moderately predominated in the group 1 (52.2% vs. 17.8%, $P < 0.001$). The rates of massive of MBT excretion in group 2, in contrast, was recorded significantly higher than that in group 1—36.7% against 8.9%, $P < 0.001$.

Comparison of the massiveness of MBT excretion with frequency of recording of Beijing and “not Beijing” strains found that massiveness of growth of the MBT culture among patients in the Pskov Region was determined in the same proportions regardless of the MBT genotype. Patients of the Kaliningrad region

also did not show any difference in the massiveness of MBT excretion.

However, it should be noted that the incidence of abundant bacterial excretion among patients excreting MBT of the Beijing family and other families in group 2 was significantly higher than that in group 1: respectively 31.7% vs. 9.8% and 40.8% vs. 8.2% ($P < 0.001$). At the same time moderate bacterial excretion was more often reliably determined among the patients with tuberculosis of the Pskov region identified as in family of Beijing (53.7% vs. 19.5%, $P < 0.001$), and in other families (51.0% vs. 16.3%, $P < 0.001$) (Table 2).

The study of the results of DR (drug resistance) examination has shown that in strains excreted from patients of group 1 drug resistance of MBT was found in the 61.1%, whereas among patients of group 2 it was found in 45.6% of cases. According to the frequency of mono-poly resistance and MDR (multidrug resistance) there was not found any differences between groups: Mono resistance was observed in 9.1% in group 1 and 7.3% in group 2; poly resistance—respectively 18.2% and 19.5% of cases, MDR—respectively 72.7% and 73.2%.

The frequency of distribution of drug-resistant

cultures of MBT of Beijing genetic families almost did not differ in group 1 (85.4%) and group 2 (80.5%) and was significantly higher in both groups, than MBT “not Beijing” families—respectively 40.8% and 16.3%. Similar extent of the differences was observed concerning MDR of MBT (Table 3).

Identified differences in genotype strains “not Beijing” are of special interest if there is no difference in the incidence of drug resistance in strains of Beijing genotype in the studied areas. In the group 1 of “not Beijing” strains drug resistance was noted in 40.8% of cases, which is 2.5 times more than in the group 2 (16.3%, $P < 0.01$). More significant differences were obtained by the detection rate of MDR of MBT of not Beijing family: group 1—in 32.7% of cases, which is 5.4 higher than in group 2 (6.1%, $P < 0.001$).

In this connection it should be noted that according to the literature, unlike MBT strains of Beijing genotype other strains have much lesser original genetic predisposition to the drug resistance development. Consequently, it can be assumed that the development of drug resistance, including MDR in MBT of genetic families “not Beijing” is not due to genetic predisposition, but it is a “by-product” of

Table 2 The massiveness of growth of MBT culture of different genetic families.

Genotypes recording frequency (in %) / massiveness of growth		1 point	2 points	3 points	Total
Group 1					
Beijing	1	36.6	53.7	9.8	41
“Not Beijing”	2	40.8	51.0	8.2	49
Group 2					
Beijing	3	48.8	19.5	31.7	41
“Not Beijing”	4	42.9	16.3	40.8	49
<i>P</i>		$p_{1,3}; p_{2,4} > 0.05$	$p_{1,3}; p_{2,4} < 0.001$	$p_{1,3}; p_{2,4} < 0.001$	

Table 3 The frequency of MBT drug resistance in different genetic families.

		Sensitivity is saved		Drug resistance		Multidrug resistance		Total
		Abs.	%	Abs.	%	Abs.	%	
Group 1								
Beijing	1	6	14.6	35	85.4	24	58.5	41
“Not Beijing”	2	29	59.2	20	40.8	16	32.7	49
Group 2								
Beijing	3	8	19.5	33	80.5	27	65.9	41
“Not Beijing”	4	41	83.6	8	16.3	3	6.1	49
$P < 0.05$		$p_{1,2}; p_{3,4}; p_{2,4}$		$p_{1,2}; p_{3,4}; p_{2,4}$		$p_{1,2}; p_{3,4}; p_{2,4}$		

suboptimal antituberculous events, primarily due to intermittent, incomplete and/or inadequate chemotherapy.

4. Discussion

It is established that frequency of determination of the most dangerous strains of MBT Beijing family is found equal—in 45.6% of cases in the Pskov and Kaliningrad regions, despite the differences in epidemic trends on tuberculosis incidence. Dependence of the massiveness of bacterial excretion and clonal belonging of MBT was not established. Differences in the frequency of massive bacterial excretion in the two areas could be due to the different virulence of MBT, according to data of B.I. Wisniewski, massive bacterial excretion is one of the indirect signs of high virulence of the MBT [20].

Frequency of excretion of MBT with DR as a marker of epidemic trouble in the Pskov region with an unfavorable trend of the epidemic situation was higher (61.1%) than in Kaliningrad region (45.6%). Thus genotypes of Beijing family were identified at the same frequency in both areas, and Beijing strains identified as high proportion of DR (85.4% and 80.5%), including MDR (58.5% or 65.9%) without significant differences in the territories. All this suggests that these Beijing strains have significant impact on the spread of drug-resistant tuberculosis.

At the same time, among the strains that do not belong to the family of Beijing, the differences between the territories were substantial: in the Pskov region among strains of the families “not Beijing” drug resistance was noted in 40.8% of cases, which is 2.5 times more than in Kaliningrad region (16.3%, $P < 0.01$). More significant differences are obtained in MDR frequency: In areas with poor epidemiological trends it is detected 5.4 times more frequently (32.7% and 6.1%, $P < 0.001$), which indicates that factors not related to the genetic characteristics of MBT can influence on the development of drug resistance.

5. Conclusions

The obtained data suggest that the MBT of the Beijing family dominating in the Northwest Territories do not affect at massiveness of MBT excretion in patients, and therefore at the intensity of the possible contamination. Growth of DR and MDR of MBT is mainly due to MBT of this family. At the same time “not Beijing” strains without high genetic predisposition to development of drug resistance at adverse epidemic situation significantly more often acquire the property of drug resistance, including plural. These data show that except of MBT genotype, the differences in the epidemiological situation may be associated with difference in efficiency of primary treatment management in the TB service organization.

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