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COMPARATIVE ANALYSIS OF BIOLOGICAL PROPERTIES OF THE MAIN FAMILIES OF *M.TUBERCULOSIS* GENOTYPES IN NEWLY DIAGNOSED PATIENTS WITH PULMONARY TUBERCULOSIS

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Using advanced molecular genetic methods for identification of *M.tuberculosis* isolates, we were able to compile detailed description of *M.tuberculosis* (MTB) population circulating in one of the underexplored northern territories of Russia. Isolates circulating in the Sakha Republic (Yakutia) were presented by 9 key heterogeneous clusters: Beijing, T, S, Ural, Lam, Haarlem, Orphan, Uganda, and X. Analysis of growth rates, colony counts, drug sensitivity of the key genotypes of MTB isolated from newly diagnosed patients with PTB showed lack of uniformity in MTB isolates, in terms of their biological properties. Viability of the causative agent can reliably be linked to emergence of primary drug resistance in MTB population; this can be clearly seen in the case of S family. Consequently, excessive MTB colony count implies that patients isolating such strains pose epidemiological risk, especially in cases where fast growth is coupled with multidrug-resistance.

Keywords: Mycobacterium tuberculosis, genotypes, biological properties, molecular genetic techniques.

Introduction. Current situation with tuberculosis (TB) is shaped in large part by the changing biological properties of the causative agent, and particularly, by *M.tuberculosis* (MTB) population composition with increasing absolute and relative proportion of drug-resistant organisms [1].

Considerably less attention is paid to exploring another changing biological property of MTB, namely its viability (in terms of growth rate and colony count), while viability has an important predictive value [3, 5]. MTB viability has been shown to be a factor determining disease severity, characteristics of disease progression, and treatment success [8, 9, 10, 13]. Also there is a correlation between such biological properties as viability, primary/secondary drug-resistance (DR) and basic epidemiological parameters (incidence, mortality, treatment effectiveness, etc.) [2, 4, 6].

Beyond that, changes in biological properties of the causative agent are defined by recently discovered genetic heterogeneity of MTB species, implying large dependence of the virulence of causative agent on its membership within specific genetic family [10].

Based on the above said, it seemed relevant to employ advanced techniques for the purpose of characterizing biological properties of MTB in newly diagnosed pulmonary tuberculosis (TB).

Aim. Define key MTB genotype families circulating in the Sakha Republic (Yakutia), and comparatively analyze their biological properties in newly diagnosed pulmonary TB.

Material and methods. The study was performed at the Bacteriological laboratory of the Phthisiatry Research-Practice Center, and at the laboratory of Epidemiology and Microbiology Institute of the Scientific Center for Family Health and Human Reproduction Problems SB RAMS (Irkutsk).

315 MTB strains were selected for the study, which were isolated from 315 newly diagnosed culture-positive patients with pulmonary tuberculosis (PTB) who underwent treatment in the in-patient clinic of the Phthisiatry Center and in rural central district hospitals.

110 (34.9%) strains were isolated from rural residents, and 205 (65.1 %) from urban residents (Yakutsk). Additionally, we used classification of rural districts to 4 socio-geographical zones first proposed by M.A. Tyrylgin [14].

Presence of MTB in culture specimens was established using Lowenstein-Jensen and Finn-2 solid egg media, after preliminary digestion and decontamination with BBLMycoPrepNALC-NaOH solution (BD, USA), in compliance with the Russian Federation Health Ministry Order no.109 (21.03.2003) [12].

To assess the growth of MTB cultures in solid media, we registered the following parameters:

1. Growth rate or the first detectable growth (based on the date of first detected growth per tube). MTB colony detection in less than 30 days was considered fast growth; growth in 30 or more days was a slow growth.

2. Load or level of growth: colony count per tube. If simultaneous growth was observed in all tubes inoculated with the same material, a total number of colony-forming units (CFU) in all of those tubes was counted. Colony count under 20 was assessed as scanty (1+); between 21 and 100 colonies – as moderate (2+); above 100 colonies – as excessive (3+).

3. Presence of contaminant flora («overgrowth»).

4. Absence of growth. This was established at week 10 since inoculation.

Viability of MTB cultures isolated from specimens before therapy was assessed based on combined assessment of growth rate and colony count, using conventional methods described elsewhere [11]: colony count less than 20 with a growth rate of longer than 30 days was considered a low viability; more than 100 colonies with a growth rate of less than 30 days was considered a high viability. Any other combinations of colony count

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and growth rate were labelled moderate viability.

Drug sensitivity was determined based on absolute concentration method, using Lowenstein-Jensen medium, following the procedure described in the Russian Federation Health Ministry Order no. 109 (21.03.2003) [12]. The following resistance types were determined: monoresistance; poly-resistance (resistance to 2 or more drugs, with the exception of simultaneous resistance to isoniazid and rifampicin); multidrug-resistance (MDR, resistance to at least isoniazid and rifampicin); extensive drug resistance (XDR, resistance to rifampicin + isoniazid + fluoroquinolone + capreomycin/kanamycin).

Further, the isolated MTB strains were genotyped, using molecular genetic methods; this was performed at the laboratory of Epidemiology and Microbiology Institute of the Scientific Center for Family Health and Human Reproduction Problems SB RAMS (Irkutsk). DNA was extracted from inactivated cultures. For inactivation, one or several colonies from Lowenstein-Jensen medium were re-suspended in 500 mL of 1% mixture of N-acetyl-N,N,N-trimethyl ammonium bromide (CTAB) with 50% isopropanol, as described in the literature [17]. DNA isolation was performed using DNA-sorb-B extraction kit (Interlabservis, Russia), following the manufacturer's protocol.

To define key genetic families forming MTB population in Yakutia, MIRU-VNTR genotyping was performed using the protocol described at MIRU VNTR plus website [18]. Primary determination of MTB genotypes was done using phylogenetic software available at the web-site. Prevalence of MTB strains was reconfirmed using the publicly available SITVIT database [15]. Beijing family genotypes were additionally sub-typed based on RD105/RD207 genome region, as described earlier [7]. Strain profiles, which were verified as Beijing genotype using the above methods, were then compared with the database published by M.Merker and colleagues, against 24 MIRU-VNTR loci [16].

Statistical data processing was done using conventional software (Microsoft Excel, StatSoft Statistica 6), and calculated based on mean values (M±m) and the significance of differences observed between variables compared (p).

Results and discussion. Based on the results of MIRU-VNTR typing (Table 1), of 315 isolates of MTB, Beijing family was detected in the highest number of strains (152; 48.2%) over entire republic: of them, 95 (30.1%) from Yakutsk, and 57 (18.1%) from rural administrative districts. Proportions of genotypes belonging to Beijing family among urban and rural patients made 46.3% (95/205) and 51.8% (57/110), respectively. Based on distribution by socio-geographical zones, the following variations were observed: 42.8% (9/21) in Arctic districts, 44.0% (11/25) in hybrid districts, 44.4% (4/9) in industrial districts; 60.0% (33/55) in agrarian districts.

The second largest group comprising 37 isolates (11.7%) consisted of genotypes referred to family T, based on their spoligoprofiles. Individual strains were identified as MANU 2, H37Rv, SIT 877, SIT 1562. Family T was detected in 12.2% (25/205) of urban residents (Yakutsk), and in 10.9% (12/110) of rural residents. By socio-geographic zones, T family was absent in industrial zone (including 5 administrative districts); 7.3% (4/55) were detected in agrarian zone, 12.0% (3/25) in hybrid zone, and 23.8% (5/21) in the Arctic zone.

The third most frequent in Yakutia was a clustered S family (32; 10.1%) with a single spoligotype (SIT 1253). The family formed a distinctive cluster on phylogenetic tree, and was presented by similar MIRU-VNTR profiles. Proportions of S family in Yakutsk and in rural districts were 10.2% (21/205) and 6.4% (7/110), respectively. Family S showed an interesting distribution by socio-geographic zones: it was absent in Arctic zone (consisting of 11 districts); proportions in agrarian, hybrid, and industrial zones were respectively, 7.3% (4/55), 8.0% (2/25), 11.1% (1/9).

The fourth by occurrence was Ural family (26; 8.2%). Profiles identified included MIT 197, 756 and some other ones, not registered in SITVIT database, but characterized by typical MIRU-VNTR features distinguishing Ural family. Ural family was found in 8.8% (18/205) of the residents of Yakutsk, and in 7.3% (8/110) of rural residents. Distribution by socio-geographic zones was as follows: 5.4% (3/55) in agrarian zone; 8.0% (2/25) in hybrid zone; 14.8% (3/21) in Arctic zone; no cases in industrial zone.

Even more heterogeneous Haarlem family was established in 21 (6.7%) cases. Haarlem was present in 7.3% (15/205) of Yakutsk cases, and in 5.4% (6/110) of rural cases. By socio-geographic zones, the incidence was as follows: 8.0% (2/25) in hybrid zone; 9.5% (2/21) in Arctic zone; 22.2% (2/9) in industrial zone; no cases in agrarian zone.

MTB belonging to LAM family were detected in 21 (6.7%) cases. Of them, 14 (6.8%) in Yakutsk, and 7 (6.4%) in rural districts. Distribution of LAM family by socio-geographic zones was as follows: 4.0% (1/25) in hybrid zone; 7.3% (4/55) in agrarian zone; 22.2% (2/9) in industrial zone; no cases in Arctic zone. LAM cluster included both registered profiles (MIT 1, 140, 326), and some strains with MIRU-VNTR codes proximal to LAM family. This heterogeneous group comprised different spoligotypes (SIT 42, 254, 1337, new), assigned to different sub-families, based on Spol tool of SITVIT database (LAM 9, LAM 5). Also, strains within single MIT or SIT type had variations in a number of loci (Mtub 4, Mtub 30, Qub 26), which may imply long-time circulation of LAM family in the territory of Sakha Republic (Yakutia).

Next group (15 isolates) was presented by a cluster formed by Orphan family (15; 4.8 %): 8 (2.5%) in Yakutsk, and 7 (2.2%) in rural districts. Orphan family was found in the following socio-geographic zones: agrarian (3.6%; 2/55), Arctic (4.8%; 1/21), hybrid zone (16.0%; 4/25). No cases in industrial zone were detected.

The rest of the strains belonged to families Uganda (7; 2.2%) and X (4; 1.3%). Incidences of these families in Yakutsk and in rural districts were, respectively: 5 (1.6 %) and 2 (0.6 %); 2 (0.6 %) and 2 (0.6 %).

Biological properties, such as growth rate and colony count, were analyzed for key genotypes identified in MTB cultures (Table 2).

As is seen in Table 2, out of 315 cultures, 194 (61.6%) showed scanty colony count, 95 (30.1%) had moderate colony count, and 26 (8.3%) showed excessive colony count. Fast growth rate was observed in 238 (75.5%), and slow growth rate in 77 (24.4%) cases. Mean growth rate was 27.4±1.2 days.

Scanty, moderate and excessive growth rates were observed in the following genotype families, respectively: Beijing: 92 (60.5%), 51 (33.6%), 9 (5.9%); T: 19 (51.4%), 16 (43.2%), 2 (5.4%); S: 21 (65.6%), 3 (9.4%), 8 (25.0%); Ural: 18 (69.2%), 4 (15.4%) 4 (15.4%); Orphan: 11 (73.3%), 3 (20.0%), 1 (6.7%); Uganda: 3 (42.9%), 2 (28.6%), 2 (28.6%). Scanty and moderate growth rates were observed for genotypes Lam (14 (66,7%); 7 (33.3%)) and Haarlem (12 (57.1%); 9 (42.9%)). Genotype X showed scanty growth rate in all 4 (100%) cases.

Higher colony counts occurred statistically more often ($p<0.001$; $p<0.05$) in genotype S (8-25.0%), then in genotypes Beijing (9-5.9%) and T (5-5.4%), based on detections of excessive colony counts in culture media, but compared to Ural, Orphan, Uganda genotypes, S family

Table 1

Prevalence of key genetic families of *M. tuberculosis* population in the entire region of Yakutia, in rural socio-geographical zones, and in Yakutsk

Administrative districts by socio-geographical zones	Genotypes, n (%)								
	Beijing	T	S	Ural	Haarlem	Lam	Orphan	Uganda	X
Arctic zone: 21 (6.7%)									
Abyysky	-	2	-	-	-	-	-	-	-
Allaikhovsky	1	-	-	-	-	-	-	-	-
Anabarsky	1	-	-	-	-	-	-	-	-
Bulunsky	-	-	-	2	1	-	-	-	-
Zhigansky	1	1	-	-	-	-	-	-	-
Momsky	1	-	-	-	1	-	-	-	-
Nizhnekolymsky	2	-	-	-	-	-	-	-	-
Olenyoksky	-	2	-	1	-	-	-	-	-
Srednekolymsky	2	-	-	-	-	-	1	-	-
Ust-Yansky	-	-	-	-	-	-	-	-	-
Eveno-Bytantaysky	1	-	-	-	-	-	-	1	-
Total, Arctic zone:	9 (2.8)	5 (1.6)	-	3 (0.9)	2 (0.6)	-	1 (0.3)	1 (0.3)	-
Industrial zone: 9 (2.8%)									
Aldansky	1	-	1	-	1	-	-	-	-
Lensky	-	-	-	-	-	1	-	-	-
Mirninsky	-	-	-	-	-	-	-	-	-
Neryungrinsky	2	-	-	-	-	-	-	-	-
Oymyakonsky	1	-	-	-	1	1	-	-	-
Total, industrial zone:	4 (1.3)	-	1 (0.3)	-	2 (0.6)	2 (0.6)	-	-	-
Agrarian zone: 55 (17.5%)									
Amginsky	3	-	-	1	-	-	1	-	1
Verkhnevilyuysky	7	-	1	-	-	-	-	-	-
Vilyuysky	3	2	-	-	-	-	-	-	-
Gorny	1	-	-	-	-	-	-	-	-
Megino-Kangalassky	7	-	-	1	-	1	-	-	-
Namsky	1	-	-	-	-	-	-	-	-
Nyurbinsky	4	-	4	-	-	-	1	-	-
Suntarsky	3	-	-	-	-	1	-	-	-
Tattinsky	2	-	-	-	-	1	-	-	-
Ust-Aldansky	-	1	-	1	-	1	-	1	-
Churapchinsky	2	1	1	-	-	-	-	-	1
Total, agrarian zone:	33 (10.5)	4 (1.3)	6 (1.9)	3 (0.9)	-	4 (1.3)	2 (0.6)	1 (0.3)	2 (0.6)
Hybrid zone: 25 (7.9%)									
Verkhnekolymsky	-	-	-	-	-	-	-	-	-
Verkhoyansky	2	1	-	-	1	-	-	-	-
Kobyaysky	3	2	-	-	-	-	-	-	-
Olyokminsky	2	-	-	-	-	-	-	-	-
Tomponsky	-	-	1	-	-	1	-	-	-
Ust-Maysky	-		-	1	1	-	-	-	-
Khangalassky	4	-	1	1	-	-	4	-	-
Total, hybrid zone:	11 (3.5)	3 (0.9)	2 (0.6)	2 (0.6)	2 (0.6)	1 (0.3)	4 (1.3)	-	-
Total, all rural socio-geographical zones n=110 (34.7%)	57 (18.1)	12 (3.8)	9 (2.8)	8 (2.5)	6 (1.9)	7 (2.2)	7 (2.2)	2 (0.6)	2 (0.6)
City of Yakutsk n=205 (65.3%)	95 (30.1)	25 (7.9)	23 (7.3)	18 (5.7)	15 (4.8)	14 (4.4)	8 (2.5)	5 (1.6)	2 (0.6)
Total, Sakha	152 (48.2)	37 (11.7)	32 (10.1)	26 (8.2)	21 (6.7)	21 (6.7)	15 (4.8)	7 (2.2)	4 (1.3)

showed no statistically meaningful differences.

Genotypes Haarlem, Lam, Uganda, S, T, Beijing, and Orphan showed fast growth rates, with mean durations varying between 22.9±1.5 and 28.8±2.0 days. Ural and X genotypes were characterized by slow growth rates (30.9±2.0 and 34.3±2.1 mean number of days, respectively). Predominating Beijing cluster did not differ from other genotypes, in terms of growth rate or colony count.

Table 3 shows comparative analysis of culture viability for key MTB genotypes.

As is seen in Table 3, out of 315 cultures, viability (combined assessment of growth rate and colony count) was high in 25 (7.9%), moderate in 206 (65.4%), and low in 84 (26.7%) cultures, respectively.

Comparison of genotypes by their viability showed high, moderate, and low viability as follows: Beijing: 37 (24.3%), 106 (69.7%), 9 (5.9%); T: 11 (29.7%), 24 (64.9%), 2 (5.4%); S: 6 (18.7%), 18 (56.2%), 8 (25.0%); Ural: 11 (42.3%), 12 (46.1%), 3 (11.5%); Orphan: 11 (73.3%), 3 (20.0%), 1 (6.7%). Low and moderate viability, respectively, was observed in genotypes LAM (2 (9.5%); 19 (90.5%)) and Haarlem (2 (9.5%); 19 (90.5%)). Genotype Uganda showed moderate (5; 85.7%) and high (2; 28.6%) viability. All isolates with X genotype (4; 100%) showed low viability. Interestingly, cultures with S genotype (8-25.0%) demonstrated reliably ($p<0.001$; $p<0.05$) higher viability compared to Beijing (9-5.9%) or T (5-5.4%), but showed no meaningful differences compared to Ural, Orphan, Uganda.

Shown in Fig. 1 is distribution of phenotypic MTB drug resistance types by key genotypes.

As is seen, out of 315 cultures, 207 (65.7%) were drug sensitive. Monoresistance was detected in 10 (3.2%) cases, and poly-resistance in 20 (6.3%) cases. MDR was present in 78 (24.8%) cases, of them, 5 (1.6%) were XDR.

Preserved sensitivity to all drugs was observed in the following genotypes: Beijing (101; 66.4%), T (31; 83.8%), S (3; 9.4%), Ural (14; 53.8%), LAM (17; 80.9%), Haarlem (20; 95.2%), Orphan (12; 80.0%), Uganda (6; 85.7%), X (3; 75.0%). Monoresistance was observed in Beijing (2; 1.3%), T (4; 10.8%), Ural (1; 3.8%), LAM (1; 4.8%), Haarlem (1; 4.8%), Uganda (1; 14.3%) genotypes. Poly-resistance was established in Beijing (2; 1.3%), S (6; 18.6%), Ural (10; 38.5%), LAM (1; 4.8%), X (1; 25.0%) genotypes. MDR was found in Beijing (49; 30.9%), T (2; 5.4%), S (23; 71.9%), Ural (1; 3.8%), LAM (2; 9.5%), Orphan (3; 20.0%). Of

Table 2

Comparative analysis of colony count and growth rate of *M.tuberculosis* strains by genotypes

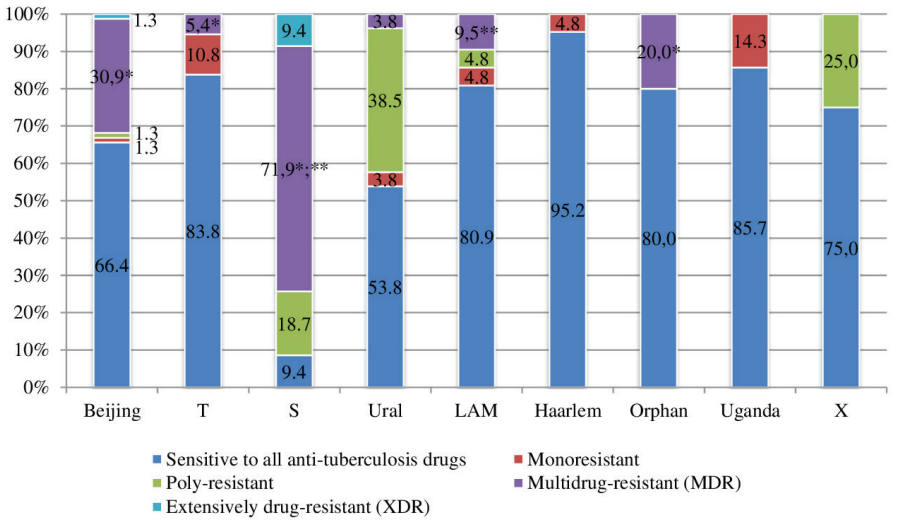
Genotype	Colony count, n (%)			Growth rate, n (%)		Mean growth rate, days (M ± m)
	1+	2+	3+	<30 days	>30 days	
Beijing n=152	92 (60.5)	51 (33.6)	9* (5.9)	116 (76.3)	36 (23.7)	27.6±0.8
T n=37	19 (51.4)	16 (43.2)	2** (5.4)	25 (67.6)	12 (32.4)	27.4±1.5
S n=32	21 (65.6)	3 (9.4)	8*; ** (25.0)	25 (78.1)	7 (21.9)	26.5±1.1
Ural n=26	18 (69.2)	4 (15.4)	4 (15.4)	14 (53.8)	12 (46.2)	30.9±2.0
Lam n=21	14 (66.7)	7 (33.3)	-	19 (90.5)	2 (9.5)	23.6±1.2
Haarlem n=21	12 (57.1)	9 (42.9)	-	19 (90.5)	2 (9.5)	22.9±1.5
Orphan n=15	11 (73.3)	3 (20.0)	1 (6.7)	12 (80.0)	3 (20.0)	28.8±2.0
Uganda n=7	3 (42.9)	2 (28.6)	2 (28.6)	7 (100.0)	-	25.0±1.9
X n=4	4 (100.0)	-	-	1 (25.0)	3 (75.0)	34.3±2.1
Total n= 315	194 (61.6)	95 (30.1)	26 (8.3)	238 (75.5)	77 (24.4)	27.4±1.2

Note: Colony count per tube was scored using 3-grade scale:
(1+): 1-20 CFU – scanty bacterial load;
(2+): 21-100 CFU – moderate bacterial load;
(3+): >100 CFU – excessive bacterial load;
*statistically significant difference between variables compared, $p<0.001$;
**statistically significant difference between variables compared, $p<0.05$.

Table 3

Comparative analysis of *M.tuberculosis* strains by genotypes and viability

Genotypes	Viability level					
	Low		Moderate		High	
	Абс. ч.	%	Абс. ч.	%	Абс. ч.	%
Beijing (n=152)	37	24,3	106	69,7	9*	5,9
T (n=37)	11	29,7	24	64,9	2**	5,4
S (n=32)	6	18,7	18	56,2	8*; **	25,0
Ural (n=26)	11	42,3	12	46,1	3	11,5
Lam (n=21)	2	9,5	19	90,5	-	-
Haarlem (n=21)	2	9,5	19	90,5	-	-
Orphan (n=15)	11	73,3	3	20,0	1	6,7
Uganda (n=7)	-	-	5	85,7	2	28,6
X (n=4)	4	100,0	-	-	-	-
Total: n=315	84	26,7	206	65,4	25	7,9



Comparative characteristics of drug resistance types between *M. tuberculosis* genotypes

them, XDR was determined in Beijing (2; 1.3%) and S (3; 9.4%) genotypes.

In terms of drug-resistance, genotypes Haarlem, Uganda, and X were regarded as favorable, due to absence of MDR cases. Genotypes Ural, Orphan, LAM, and T were deemed less favorable, as 53.8% to 83.8% cases with theses genotypes proved drug-sensitive or else showed minimal spectrum of DR. Genotypes Beijing and S were considered unfavorable, based on the incidence of MDR (47 (30.9%) for Beijing; 23 (71.9%) for S), including cases with XDR (1.3% and 9.4%, respectively). Moreover, genotype S showed statistically higher incidence of MDR ($p<0.001$; $p<0.05$) among all the rest.

Conclusion. Using advanced molecular genetic methods for identification of MTB isolates, we were able to compile detailed description of MTB population circulating in one of the underexplored northern territories of Russia. Isolates circulating in the Sakha Republic (Yakutia) were presented by 9 key heterogeneous clusters: Beijing, T, S, Ural, Lam, Haarlem, Orphan, Uganda, and X. Beijing genotype was the most prevalent in the MTB population (152; 48.2%).

Analysis of growth rates, colony counts, drug sensitivity of the key genotypes of MTB isolated from newly diagnosed patients with PTB showed lack of uniformity in MTB isolates, in terms of their biological properties. Assessment of colony counts showed the following variations: scanty colony count in 43.9% to 100% cases; moderate colony count in 9.4% to 43.2% cases; excessive colony count ranging from 5.4% to 28.6% cases. Assessment of growth rates showed fast growth in 25.0% to 100%, and slow growth in 9.5% to 75.0% of cases. Mean growth rate was 27.4±1.2 days. Viability, based on combined assessment of growth rate and colony count, was assessed as high in 25 (7.9%) cultures, moderate in 206 (65.4%), and low in 84 (26.7%) cultures. By drug sensitivity, 9.4% to 95.2% were sensitive, and 5.4% to 71.9% were multidrug-resistant. Beijing genotype, along with S family, are the two epidemiologically significant families in a region of Yakutia, based on the incidence of MDR.

Viability of the causative agent can reliably be linked to emergence of primary drug resistance in MTB population; this can be clearly seen in the case of S family. Consequently, excessive MTB colony count implies that patients isolating such strains pose epidemiological risk, especially in cases where fast growth is coupled with multidrug-resistance.

Further in-depth studies are necessary to keep looking into the prevailing MTB genotypes circulating in Yakutia, for the purposes of epidemiological surveillance and monitoring of TB infection.

Литература

1. Борисов С. Е. Комментарий к статье Филинюк О. В., Яновой Г. В., Стрелиса А. К. и др. Множественная лекарственная устойчивость – туберкулез легких: медико-социальные особенности и эффективность стационарного этапа лечения // Проблемы туберкулеза. – 2008. – № 8. – С. 28. [Borisov SE. Commentary to article by Filinyuk OV, Yanova GV, Strelis AK, et al. Multidrug-resistant pulmonary tuberculosis: socio-medical features and effectiveness of in-patient treatment phase. *Problemy tuberkuleza (Issues of Tuberculesis)*. 2008; 8: 28].

2. Вишневский Б.И. Эпидемиологическое значение жизнеспособности и лекарственной устойчивости МБТ / Б. И. Вишневский, А. В. Васильев, Т. М. Голубева, [и др.] // 5-й Национальный конгресс по болезням органов дыхания. – М., 1995. – №1747. [Vishnevskiy BI, Vasilyev AV, Golubeva TM et al. Epidemiological significance of the viability and drug resistance of MTB. The 5th National congress on respiratory diseases. Moscow, 1995; 1747].

3. Вирулентность микобактерий туберкулеза / Б. И. Вишневский, О. В. Нарвская, С. Н. Васильева [и др.] // Проблемы туберкулеза. – 2002. – № 10. – С. 33-36. [Vishnevskiy BI, Narvskaya OV, Vasilyeva SN et al. Virulence of M.tuberculosis. *Problemy tuberkuleza (Issues of tuberculosis)*. 2002; 10: 33-36].

4. Вишневский Б. И. Основные направления работы лаборатории микробиологии туберкулеза/ Вишневский Б.И. // Туберкулез: проблемы диагностики, лечения и профилактики: тр. Всеросс. науч.-практ. конф. – СПб., 2003. – С. 34-38. [Vishnevskiy B. I. Main focus areas in the work of Tuberculosis microbiology laboratory. Tuberculosis: issues in diagnostics, treatment and prevention.: proceedings of All-Russian research-practice conference. Saint-Petersburg, 2003: 34-38].

5. Вишневский Б.И. Вирулентность Mycobacterium tuberculosis / Б. И. Вишневский, О. А. Маничева, П.К. Яблонский // Инфекция и иммунитет. – 2014. – № (4)4. – С. 319-330. [Vishnevskiy BI, Manicheva OA, Yablonskiy PK. Mycobacterium tuberculosis virulence. *Infektsiya i immunitet (Infection and immunity)*. 2014; (4)4: 319-330].

6. Гращенкова О.В. Совершенствование эпидемиологического анализа при туберкулезной инфекции / О. В. Гращенкова, А. В. Васильев // Проблемы туберкулеза. – 2000. – № 6. – С. 33-36. [Grashchenkova OV, Vasilyev AV. Advancements in epidemiological analysis of tuberculosis infection. *Problemy tuberkuleza (Issues of tuberculosis)*. 2000; 6: 33-36].

7. Жданова С.Н. Применение делеционного анализа по RD105 для выявления генотипа Пекин Mycobacterium tuberculosis / С.Н. Жданова, О.Б. Огарков, Л.А. Степаненко, [и др.] // Бюл. Восточно-Сибирского научн. центра СО РАМН. – 2011. – № 2. – С. 194-197 [Zhdanova SN, Ogarkov OB, Stepanenko LA et al. The deletion analysis of RD105 is a useful tool in the evaluation of Beijing strains of Mycobacterium tuberculosis. Bulletin of the East Siberian Scientific Center SB RAMS. 2011; 2: 194-197].

8. Корецкая Н.М. Сравнительная характеристика диссеминированного туберкулеза легких у больных, выделяющих микобактерии туберкулеза с высокой и низкой жизнеспособностью / Н. М. Корецкая, И. В. Ярыгина // Проблемы туберкулеза. – 2007. – № 2. – С. 17-20 [Koretskaya NM, Yarygina IV. Comparative characteristics of disseminated pulmonary tuberculosis in patients isolating M.tuberculosis with high and low viability. *Problemy tuberkuleza (Issues of tuberculosis)*. 2007; 2: 17-20].

9. Корецкая Н.М. Изменения биологических свойств возбудителя при инфильтративном туберкулезе легких / Н. М. Корецкая, А. А. Чушкина // Туберкулез и болезни легких. – 2010. – № 9. – С. 33-38 (Koretskaya NM, Chushkina AA. Changes in the biological properties of the pathogen in infiltrative tuberculosis. *Tuberkulez i bolezni legkikh* (*Tuberculosis and lung diseases)*. 2010; 9: 33-38.

10. Лекарственная чувствительность Mycobacterium tuberculosis в сопоставлении с их жизнеспособностью, цитотоксичностью, генотипом и течением процесса у больных туберкулезом органов дыхания / О. А. Маничева, Е. Б. Ласунская, В. Ю. Журавлев, [и др.] // Проблемы туберкулеза. – 2008. – № 12. – С. 18-22. [Manicheva OA, Lasunskaya EB, Zhuravlev VY et

al. Drug sensitivity of Mycobacterium tuberculosis in relation to its viability, cytotoxicity, genotype, and clinical disease progression in patients with pulmonary tuberculosis. *Problemy tuberkuleza (Issues of Tuberculosis).* – 2008. – No. 12. – P. 18-22.

11. Методы математического анализа эпидемиологической ситуации по туберкулезу: пособие для врачей. – СПб., 1998. – 24 с. [Methods of mathematical analysis of the epidemiological situation for tuberculosis: guide for physicians. Saint-Petersburg, 1998: 24].

12. О совершенствовании противотуберкулезных мероприятий в Российской Федерации: приказ МЗ РФ №109 (21.03.2003). Приложение №11 «Инструкция по унифицированным методам микробиологических исследований при выявлении, диагностике и лечении туберкулеза». [On improving anti-tuberculosis measures in Russian Federation: Order of the Russian Federation Ministry of Health no.109 (21.03.2003). Appendix 11 'Instructions on unified techniques for microbiological tests to detect, diagnose, and treat tuberculosis'].

13. Сапожникова Н.В. Особенности течения туберкулеза легких в зависимости от биологических свойств возбудителя: автореф. дис. … канд. мед. наук / Сапожникова Н.В. – СПб., 2003. – 24 с. [Sapozhnikova N.V. Characteristics of pulmonary tuberculosis disease progression in relation to biological properties of the causative agent. Extended abstract of Cand.Sci.(Medicine) thesis. Saint-Petersburg, 2003; 24].

14. Тырылгин М.А. Проблемы охраны здоровья населения Крайнего Севера: на примере региона Якутия / М.А. Тырылгин. – Новосибирск: Наука, 2008. – 304 с. [Tyrylgin M. A. Health protection problems in the population of the Extreme North: case of Yakutia. Novosibirsk: Nauka Publ., 2008: 304].

15. Demay C, Liens B, Burguière T et al. "SITVITWEB – a publicly available international multimarker database for studying Mycobacterium tuberculosis genetic diversity and molecular epidemiology". *Infection, Genetics and Evolution*. 2012; Iss. 12(4): 755-766. http://www.pasteur-guadeloupe.fr:8081/SITVIT_ONLINE/query

16. Merker M, Blin C, Mona S, Duforet-Frebourg N, Lecher S, Willery E et al. Evolutionary history and global spread of the Mycobacterium tuberculosis Beijing lineage. *Nat Genet.* 2015; Iss. 47(3): 242-249. doi: 10.1038/ng.3195.

17. Ogarkov O, Zhdanova S, Mokrousov I, Sinkov V, Antipina S. Lethal combination of mycobacterium tuberculosis Beijing genotype and human CD209-336G allele in Russian male population. *Infection, genetics and evolution*. 2012; Iss. 12(4): 732-736.

18. Weniger T, Krawczyk J, Supply P, Niemann S, Harmsen D. MIRU-VNTRplus: a web tool for polyphasic genotyping of Mycobacterium tuberculosis complex bacteria. *Nucleic Acids Res.* 2010; Iss. 38. Suppl. W326-331. – http://www.miru-vntrplus.org/MIRU/miruinfo.faces;jsessionid=89112F274226E781C7B0B0D9118FDD70