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Type 2 diabetes mellitus in Yakutia: prevention measures

This paper presents review of traditional feeding disorder in the North. The efficiency of the work of schools on prevention of type 2 diabetes mellitus (MD 2) in Yakutia is shown. Recommendations on nutrition of MD 2 patients and list of recommended and excepted foods of therapeutic diet #9 are given. The authors present model menu and the multiplicity of meals for MD patients. The role of physical activity and refusal from harmful habits in treatment and prevention of MD type 2 are shown.

Keywords: diabetes mellitus type 2 (MD 2), metabolic syndrome, carbohydrate metabolism, glucose, hypoglycemia, hyperglycemia, diet.

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BACTERIA ISOLATED FROM RELICT FROZEN TERRAINS OF THE CENTRAL YAKUTIA

The results of the first stage of comprehensive studies of culturable microorganisms isolated from the oldest permafrost exposed rocks of Mammoth Mountain in Yakutia are presented, and their potential scientific and applied significance is shown.

Key words: permafrost, biochemical reactions, relict microorganisms, taxonomic diversity, phenotypic properties.

Introduction

Permafrost rocks are widespread on the Earth, and in some regions their age reaches hundreds of thousands or millions of years. They present a natural store of the Earth's oldest

"preserved" natural communities of microorganisms, a bank of ancient genes and biomolecules [22, 12].

The study of viable bacteria in the Earth's cryosphere is interesting in connection with some aspects of microorganism evolution [6, 9, 18], the evaluation of microbiological diversity on the Earth [20, 26], the possibility of life on other planets [29], the potential for biochemical activity of microbial biomass of permafrost rocks [21, 17] and their potential for possible interrelations with contemporary biocenoses [19, 26]. The importance of the study of microbiote in cryosphere is also connected with the probability of the presence and preservation in them of viable pathogenic microorganisms and the need for the development of preventive measures in the case of their release as a consequence of anthropogenic activity or natural melting of permafrost rocks [28, 27]. In addition, the study of the properties of relict microorganisms is important for solution of such fundamental task as elucidation of the nature of their long-term viability and revealing the mechanisms allowing them to prevent the accumulation of damages of the genetic apparatus.

The studies described in the present work were carried out as a part of a comprehensive research of relict microorganisms isolated from permafrost terrains of different ages and geneses. The goal of the present work at this stage was to study biochemical and other properties of viable culturable microorganisms in ancient permafrost terrains of Mammoth Mountain (Yakutia), which have not been investigated in microbiological studies previously.

The permafrost sampling place and age

Mammoth Mountain is a geologically well-studied and reliably dated exposure of relict frozen terrains extending for 12 km along the left bank of the Aldan River 325 km away from its confluence with the Lena. It presents an outlier of the watershed upland of the Aldan-Amgin interfluve formed by a series of alluvial deposits of different ages at an apparent power up to 80 m and intensely eroded by the river. The lower part of deposits from which samples were collected for microbiological investigations is formed mainly by sandy sediments with abundant inclusions of fossil Neogene flora whose composition indicates that the accumulation of sediments occurred during the Middle Miocene in the time interval between 11 and 16 million years ago [16].

It is known that frozen terrains existed in this part of Eurasia already in the Early Pleistocene, 1.8 - 2 million years ago [11, 10]. A number of paleoclimatic reconstructions [3, 8] based on the results of palynological, paleogeographical, paleomagnetic, stratigraphic studies and datings reveal the climate cooling, which started in the second half of Neogene with a sharp decrease in average annual temperatures at the boundary of the Late Miocene and the Early Pliocene (5.5 million years ago). The formation of frozen terrains in this region probably began during the Late Pliocene 3.5 million years ago when the average air temperatures for July decreased to +12 ÷ +16ºC, and those for January to -12 ÷ -32ºC.

One of the main reasons of the fact that relict frozen terrains of Mammoth Mountain did not melt during later periods of geological development is the absence of terrestrial glaciation in this region throughout the whole Quarternary period [8]. The results of some studies [15, 11, 1, 5, 2, 4] allow us to conclude that during complete Pleistocene glaciations of eastern Eurasia and partial ones of Western Siberia this part of Asia was free of ice sheets contributing to the increase of the mean annual temperature of rocks and melting of the previously formed frozen terrains.

The climate, which was more continental as compared with current conditions, along with extremely low annual precipitation (below 250 mm) provided the preservation of Neogene sediments in frozen state throughout the whole Pleistocene. They did not melt during the Holocene climatic optimum either, which is evidenced by the studied cryogenic structure of the upper portion of the Miocene terrain and younger sediments covering it.

In addition, due to the direction of tectonic motions during the Late Cainozoe [10], this territory was not subjected to the effect of sea transgressions and related periodic thawing of relict frozen terrains as was the case in more northern coastal lowlands of Yakutia and Eurasia on the whole. Thus, the age of relict Neogene permafrost terrains of Mammoth Mountain, which did not melt after their formation in the Late Pliocene, probably reaches 3-3.5 million years.

Samples of frozen rocks were collected for microbiological studies in areas of the maximal intensity of river erosion from newly destroyed vertical walls of the exposure (Fig. 1) in its medium and lower parts between 15 and 30 m higher than the river's edge and 40-50 m below the ground surface. According to the data of our routine observations, the rate of thermal erosive destruction of the exposure in sampling places exceeds 4-5 m per year in the upper part and reaches 1-1.5 m in the medium part. Sampling was performed from the depths exceeding the power of the seasonally thawed layer by 1-1.5 m, which prevented previously thawed rocks from getting into the sampling area.

Fig. 1. One of sampling places of frozen rocks with undisturbed structure

Research methods

Under field conditions, samples of frozen rocks with undisturbed structure weighing 4-6 kg consisting mainly of sand with rare interlayers of fine-dispersed grounds and inclusions of organic debris were collected from permafrost terrains using alcohol- and flame-sterilized instruments. The collected monoliths were stored in frozen state at a near-natural temperature (-5°C). Transportation of samples to the laboratory was also carried out without thawing in cold boxes with cooling agents.

Under sterile laboratory conditions, a specimen of approximately 3x4 cm was taken from the center of the sample, placed in alcohol for 2-3 seconds followed by burning in the flame of a spirit lamp. Thus treated material was transferred to an empty sterile Petri dish and left at room temperature (20ºC) for 1 hour for further thawing.

Five milliliters of sterile physiologic solution was added to the thawed ground with a pipette followed by thorough mixing. Smears prepared from the obtained soil suspension were Gram-stained [7].

Petri dishes with GRM agar and tubes with GRM broth and minimal synthetic medium were inoculated with 0.1 ml of the obtained soil suspension each. The seedings were incubated at 28 and 37ºC. The remaining soil suspension was left at room temperature for 14 days.

Biochemical properties of the strains were determined with traditional methods [14].

Antagonistic properties of isolated strains with respect to different test cultures (*Escherichia coli* 113-13, *Bacillus cereus* 8035, *Staphylococcus aureus* 209) were determined with the agar block method. For this purpose, the studied culture was seeded in the form of a

solid lawn on the surface of GRM agar in Petri dishes and incubated at 28°C for 7 days. Then agar blocks with bacterial lawn were cut with a sterile drill and transferred to the surface of GRM agar preliminarily inoculated with the test microorganisms. The dishes were placed for 24 h into a thermostat at a temperature favorable for the development of the test microorganism. The sensitivity of test cultures to antibiotic substances of the studied strains was determined by the formation of areas with no growth.

The resistance of isolated strains to groups of antibiotics with different chemical composition was determined by the disk method. Aminoglycosides (streptomycin, neomycin), macrolides (erythromycin, oleandomycin), beta-lactams (benzylpenicillin, oxacillin, carbenicillin), and aromatic antibiotics (levomycetin) were used in the work. The studied strains were seeded in the form of a solid lawn onto the surface of AGV medium in Petri dishes. Then disks were placed with sterile pincers onto the lawn surface and incubated in a thermostat for 24 h at 37°C followed by the evaluating the formation of areas with no growth and measuring their diameters. The diameters of areas with delayed growth were compared with boundary values in reference tables [9], and the studied strains were classified under one of three categories of sensitivity: resistant, moderately resistant and sensitive [14, 13].

Results and discussion

One of the main problems of any paleomicrobiological study is the possibility of contamination. A model experiment was conducted to control the penetration of contemporary microbiote or DNA into the collected monolith of frozen rocks. During this experiment, the monolith surface was treated with solution of specially synthesized amplicon (D-loop of mitochondrial DNA 1100 bp long). The results of the analysis of amplicon concentrations at different depths of the monolith after 3 months of storage allow us to speak about practically complete impossibility of penetration of surface pollutants into the collected frozen soil samples with undisturbed structure.

No microorganism colonies, vegetative cells or bacterial spores were not detected by microscopic examination of smears of thawed ground. This indicates their scarcity and, possibly, close contact with ground particles [7]. However, microscopic examinations of frozen soil samples detected individual cells separated by polysaccharide (polypeptide) films and attached to soil particles.

Visible bacterial growth on all media was observed on day 3 of cultivation. On GRM agar, the growth was weak, often semi-transparent. Slight turbidity was observed in liquid media. Small and large bacilli, gram-positive nonsporiferous rods and gram-positive cocci of irregular shape were found in smears.

Then the cultures isolated on solid and liquid media were seeded onto dishes with GRM agar to obtain isolated colonies. The seedings were cultivated at 28 and 37ºC for 3 days. Most cultures did not show growth when re-seeded onto a medium. Strains # 6, 13, 14 and 15 yielded a pure culture.

After 2-week incubation of soil suspension at room temperature, gram-positive rods of different sizes and cocci of irregular shape were detected in Gram-stained smears. Soil suspension was seeded according to the above scheme. This indicates that elevated temperature conditions enabled more active metabolism and, probably, division of the cells. Weak growth was observed on all media on day 1 of cultivation, and abundant growth was observed on day 3. Small and large bacilli, gram-positive nonsporiferous rods were found in smears. As distinct from the first variant of the experiment, a greater portion of cultures showed visible growth at re-seeding onto a medium. Strains # 17, 20, 27, 29, 30, 32, 33, 34, 37, 39 and 40 yielded a pure culture.

Some isolated strains had similar cultural and morphological characteristics and were divided into conventional groups.

The largest group was comprised of strains (# 13, 15, 17, 30) producing shiny wrinkled colonies of irregular shape on GRM agar (Fig. 2a). Uniform short gram-positive sporiferous rods with rounded ends were detected in smears. The second typical group included bacterial strains (# 20, 27, 40, 47) forming large round colonies with opaque surface on agar (Fig. 2b).

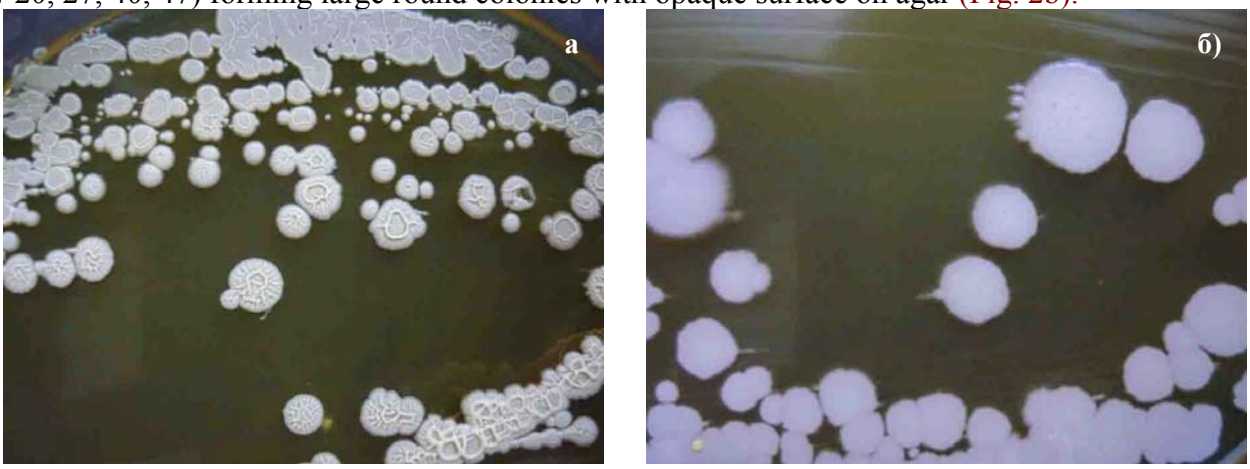

Fig 2. The colony morphology for strains #17 (a) and 40 (b) on GRM agar on day 3 cultivation

This group of strains also differed from the previous one in cell morphology (Fig. 3) and presented long sporiferous rods with lopped ends. According to cell morphology, strain # 29 was similar to the group of sporiferous bacteria, but they somewhat differed in cultural properties (Table 1). The rest of the isolated strains presented gram-positive nonsporiferous rods differing in cell morphology (of regular or irregular shape, with rounded or lopped ends) or cultural properties (smooth or wrinkled, with the presence or absence of pigment).

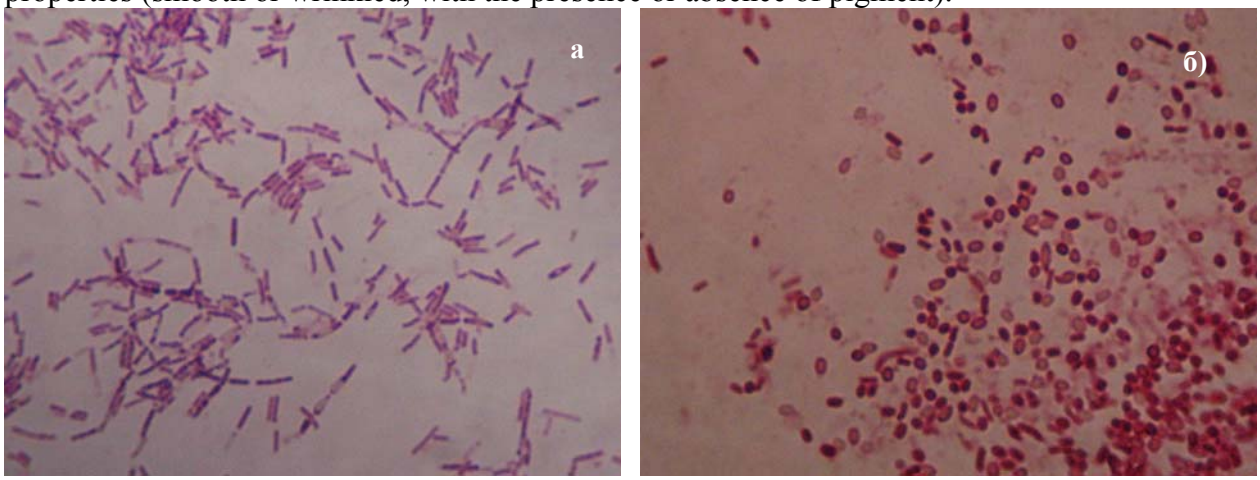

Fig. 3. Cell morphology of strains # 17 (a) and 40 (b) on day 3 of cultivation

The study of biochemical activity of bacteria isolated from permafrost rocks revealed that isolated strains included both aerobes and facultative anaerobes (Table 1). Not a single culturable obligate anaerobe was detected.

Biochemical properties of the strains isolated

Strains	Anaerobic grows	Catalase	Oxidase	Voges-Proskaue test	Citrate using	Nitrate reduction	Hydrolisis			Acid production							Gas production			N_2 fixation
							Caseinase	Gelatin	Starch	Glucose	Mannit	Arabinose	Xilose	Lactose	Mannose	Sorbitol	Ammonia	Indole	Hydrogen	
6	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
13	-	+	+	+	+	+	-	+	-	-	+	-	-	-	+	-	-	-	-	-
14	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	+	+	+	+	+	-	+	-	-	+	-	-	-	+	-	-	-	+	+
17	-	+	+	+	+	+	-	+	-	-	+	-	-	-	+	-	-	-	+	+
20	+	+	-	-	+	+	-	+	-	-	-	-	-	-	+	-	-	-	+	+
27	+	+	-	-	-	+	-	+	-	-	-	-	-	-	+	-	-	-	-	+
29	-	+	-	+	+	+	-	+	-	-	+	-	-	-	+	-	-	-	+	+
30	-	+	+	+	+	+	-	+	-	-	+	-	-	-	+	-	-	-	+	+
32	+	+	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	+	+
33	-	+	+	+	+	+	-	+	+	-	+	-	-	-	-	-	-	-	+	+
34	+	+	+	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	+	-
37	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
39	+	+	+	-	+	+	-	+	-	-	+	-	-	-	+	-	-	-	-	+
40	+	+	-	-	+	+	-	+	-	-	-	-	-	-	+	-	-	-	-	+

All isolated microorganisms were catalase positive, reduced nitrates to gaseous products and did not possess caseinase. The results of the other biochemical tests varied in different strains. Low sachharolytic activity of isolates is noteworthy: only three strains of nonsporiferous rods possessed amylase, and some strains of the 7 proposed sugars used only mannitol and mannose. The study of peptolytic activity revealed the ability of most strains to release hydrogen sulfide at peptone decomposition. None of the studied strains was able to produce ammonia or indole. Most isolates (10 of 15) fixed atmospheric nitrogen and showed abundant growth on Ashby's nitrogen-free medium.

It is interesting to compare biochemical activities of strains combined by us into groups by cultural and morphologic characteristics. Biochemical properties of strains of the first group were practically identical, only strain # 13 differed from the other isolates of this group in the ability to produce hydrogen sulfide and fix nitrogen. The second group also proved to be sufficiently uniform by biochemical activity: differences were noticed only in the ability to use citrate as the only carbon source as well as in hydrogen sulfide production. Strains # 33 and 37 having similar cultural and morphological properties considerably differed by biochemical activity, and therefore, we considered them separately in further investigations.

The study of the range of resistance of isolated strains to different physicochemical factors revealed that the lower temperature limit for growth of most strains was +8°C. Incubation at +2°C did not result in the formation of visible colonies for 2 months. High temperatures (+43°C) inhibited the growth of four strains. Thus, most studied strains grew equally well in the temperature range from +8 to +43°C.

High sodium chloride concentrations had a detrimental effect on most isolated strains. The presence of 6.5% sodium chloride in the medium inhibited the growth of seven strains, and none of the studied strains showed visible growth at 10% sodium chloride concentration in the

medium. The lower limit of pH values at which the growth of isolated cultures was observed varied from 5.0 to 6.0. Resistance to high pH values (11.0) was revealed for nine strains. No growth of isolated cultures was observed at pH 12.0 (Table 2).

When comparing the limits of the strains' tolerance within our groups to different physicochemical factors it was established that the resistance of the strains of the first group was absolutely identical, and the strains of the second group had insignificant differences in sensitivity to 6.5 % NaCl and acidity (pH 5.0).

Table 2

Survival of isolated strains under extreme conditions

Strains	Grow under														
	+2 °C	+8°C	+43°C	6.5%NaCl	10%NaCl	pH 4.0	pH 5.0	pH 5.5	pH 6	pH 8.5	pH 9.0	pH 10.0	pH10.5	pH 11.0	pH 12.0
6	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-
13	-	+	+	+	-	-	+	+	+	+	+	+	+	+	-
14	-	+	-	-	-	-	-	-	+	+	+	-	-	-	-
15	-	+	+	+	-	-	+	+	+	+	+	+	+	+	-
17	-	+	+	+	-	-	+	+	+	+	+	+	+	+	-
20	-	+	+	-	-	-	-	+	+	+	+	+	+	+	-
27	-	+	+	-	-	-	-	+	+	+	+	+	+	+	-
29	-	+	+	+	-	-	+	+	+	+	+	+	+	+	-
30	-	+	+	+	-	-	+	+	+	+	+	+	+	+	-
32	-	+	-	-	-	-	+	+	+	-	-	-	-	-	-
33	-	+	+	+	-	-	-	+	+	+	+	+	+	+	-
34	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-
37	-	+	-	-	-	-	-	-	+	+	+	-	-	-	-
39	-	-	+	-	-	-	-	-	+	+	+	-	-	-	-
40	-	+	+	+	-	-	+	+	+	+	+	+	+	+	-

We have studied antagonistic properties of isolated strains with respect to standard test cultures: *E. coli* 113-13, *S. aureus* 209-P, *B. cereus* 8035 (Table 3). Only one strain (# 29) displayed antagonistic activity with respect to *E. coli*. Strains # 13, 15, 30, 39 inhibited the growth of gram-positive bacteria (*S. aureus and B. cereus*). Interestingly that three of them belonged to group I. Strain # 17 also belonging to group I displayed antagonistic activity only with respect to *B. cereus*. Strain # 33 possessed similar activity. Strain # 29 displaying antagonistic activity with respect to gram-negative rods was also active with respect to gram-positive cocci. Strains # 6, 14, 20, 27, 32, 34, 37, 40 did not inhibit the growth of test cultures, and strain # 37 had a stimulating effect on the growth of *B. cereus*.

The study of phenotypic properties (antibiotic resistance) of isolated strains revealed that strains # 6, 15, 17, 30 were sensitive to all the used antibiotics except for levomycetin. Strains # 14, 37, 39 displayed the maximal resistance. The other isolates were characterized by varying

sensitivity to antibiotics of different groups. Neomycin had a strong antibacterial effect with respect to all isolated strains. Levomycetin displayed the weakest biologic activity, only strain # 27 proved to be sensitive to it (Tables 4, 5).

The obtained data significantly differ from the results of similar studies of microorganisms isolated from Antarctic ice cover where high resistance of isolates to most antibiotics was revealed [14]. This can be associated with much younger age of Antarctic ices as compared with ancient permafrost terrains of Central Yakutia as, in spite of taxonomic similarity of microorganisms from natural ices [24], the spectrum of their antibiotic resistance varies depending on isolation places, samples ages and the probability of contact with contemporary microorganisms [23, 25].

Conclusions

Ancient permafrost terrains rocks of the exposure of Mammoth Mountain contain relict viable microorganisms, which were present in permafrost terrains from the moment of freezing of deposits 3-3.5 million years ago.

Culturable bacteria are not numerous and are present in frozen rocks in the form of individual surviving cells, no spores and colonies were detected at microscopic examinations of soil samples.

The degree of taxonomic diversity of microorganisms is not high, and most of them are not available for cultivation, which is confirmed by the cessation of growth of bacterial cells after their transfer to artificial media. No dominant cultures were detected. All isolated strains are gram-positive and differ in an insignificant set of characteristics.

The characteristic features of isolates of Mammoth Mountain allowing us to distinguish them from other relict microorganisms isolated from the youngest permafrost rocks of other regions are high ability for nitrogen fixation, antibiotic sensitivity, insignificant antagonistic properties and ability for active growth over a wide temperature and pH range and under other extreme conditions.

The revealed biological properties of bacteria, along with viability preserved by them over a long period of time, allow us to speak about the need for their more detailed study and the prospects for using isolated strains in biotechnology and medicine including epidemiology.

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Table 3

Antagonistic activity of bacteria (d areas of inhibition, mm)

Test cultures	Strains														
	6	13	14	15	17	20	27	29	30	32	33	34	37	39	40
E. coli 113-13	0	0	0	0	0	0	0	24±0.8	0	0	0	0	0	0	0
S. aureus 209-P	0	30±0.9	0	34±1.0	0	0	0	18±1.2	22±0.9	0	0	0	0	22±1.0	0
B. cereus 8035	0	14±1.3	0	27±1.2	20±1.2	0	0	0	16±0.2	0	22±1.1	0	стимул	16±1.0	0

Table 4

Antibioticogramm of microorganism strains (d areas of inhibition, mm)

Group of antibiotics in chem. structure	Antibiotic	Strains													
		6	13	14	15	17	20	27	29	30	33	37	39	40	47
aminoglycosides	streptomycin	21±0.3	18±0.8	18±0.9	23±0.6	20±0.1	25±2.2	28±1.8	26±2.1	17±0.6	0	12±0.1	12±0.8	26±2.1	29±2.6
	neomycin	43±1.5	30±1.4	36±2.1	41±3.9	44±3.6	37±2.2	30±2.4	45±3.0	45±4.4	34±2.8	43±3.6	37±3.0	41±3.4	42±3.5
macrolides	erythromycin	43±2.1	12±0.2	16±1.6	26±2.2	27±1.6	36±4.0	37±2.6	28±2.0	19±0.6	48±3.4	0	0	42±3.8	37±3.1
	oleandomitsin	27±0.6	13±0.2	0	20±2.3	22±1.8	23±2.2	25±2.4	15±1.7	22±1.3	0	0	0	30±2.4	24±3.5
beta-lactams	Benzilpenitsilin	19±0.5	20±1.3	0	40±4.6	32±2.3	11±0.2	0	24±0.6	36±3.0	32±4.0	0	0	0	0
	oxacillin	20±0.8	24±1.2	0	32±2.5	30±2.9	0	11±0.3	26±0.8	30±2.5	30±1.8	0	0	0	0
	carbenicillin	35±2.4	41±3.2	14±0.7	40±4.6	45±3.4	15±0.7	15±1.0	39±3.2	40±3.7	43±3.4	25±2.0	24±1.8	16±1.0	17±1.0
aromatic antibiotics	Levomicetin	0	0	0	0	0	0	16±0.3	0	0	0	0	0	0	0

Table 5

Antibiotic resistance of microorganism strains

Group of antibiotics in chem. structure	Antibiotic	Strains													
		6	13	14	15	17	20	27	29	30	33	37	39	40	47
aminoglycosides	streptomycin	++	+	+	++	++	++	++	++	+	–	–	–	++	++
	Neomycin	++	++	++	++	++	++	++	++	++	++	++	++	++	++
macrolides	erythromycin	++	–	–	++	++	++	++	++	+	++	–	–	++	++
	oleandomitsin	++	–	–	+	++	++	++	–	++	–	–	–	++	++
beta-lactams	Benzilpenitsilin	++	++	–	++	++	+	–	++	++	++	–	–	–	–
	Oxacillin	++	++	–	++	++	–	–	++	++	++	–	–	–	–
	carbenicillin	++	++	–	++	++	+	+	++	++	++	++	++	+	+
aromatic antibiotics	Levomicetin	–	–	–	–	–	–	+	–	–	–	–	–	–	–

Note: – - resistant strains; + - moderately resistant strains; ++ - sensitive strains.

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Features of the epidemic process of viral hepatitis B in the Altai region in prior to the vaccination and during the immunization

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Hepatitis B – a global problem of public health services in the world.

For the purpose of studying of development of epidemic process of sharp virus hepatitis B the analysis of dynamics of indicators of disease by the given infection in Altay territory during the period with 1986 for 2009 is carried out

As a result of the spent analysis it is revealed: decrease in disease by an acute hepatitis B in 2009 to a maximum level 1996; presence of return statistically significant correlation dependence between disease of the population of an acute hepatitis B and coverage by preventive inoculations against the given disease, with more expressed dependence among adult population till 55 years; presence of return statistically significant correlation dependence between disease of an acute hepatitis B and coverage by preventive inoculations of children till 17 years; change of age structure of ill children till 17 years towards decrease B relative density of children of