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Research Article

To What Extent Can Viable Bacteria in Atmospheric Aerosols be Dangerous for Humans?

Multi-year monitoring of atmospheric bioaerosol in Southwestern Siberia revealed the presence of a large number of various culturable microorganisms. It is known that viable microorganisms can cause directly or provoke different human diseases. It's very difficult to evaluate the danger represented by each microorganism to man directly. Therefore, a relatively simple method is required for evaluation of potential danger represented to man by the whole assembly of culturable microorganisms in an atmospheric aerosol sample. For bacteria, the method can be based on a number of individual characteristics of each microorganism determined in the course of biochemical and other test required for identification of the detected bacterium, and a number of other tests. It is proposed to classify the measured individual characteristics of bacteria under four groups of indices responsible for: *(i)* potential pathogenicity for man; *(ii)* the numbers of bacteria in the sample; *(iii)* resistance to unfavorable environmental factors; *(iv)* drug resistance of bacteria. Each of four groups of indices is numerically evaluated by a certain integral index, which quantitatively reflects the contribution of experimentally determined characteristics of bacteria. Expert evaluation of the contribution of each characteristic of microorganisms to the corresponding group of indices is performed. The generalized index of potential danger of culturable bacteria in atmospheric aerosols for human health is presented as the product of four integral indices summarizing the normalized individual integral indices for all bacteria detected in the sample. The work presents the results of measuring the variations of all the above indices for atmospheric air samples collected during one year.

Keywords: Bioaerosols; Atmospheric aerosols; Culturable bacteria; Health effects

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1 Introduction

In accordance with literature data, various bioaerosols including viable microorganisms can be found in all atmospheric layers, in the near-ground layer and at altitudes higher than 70 km [1 – 9]. Their concentration varies from several species and less to several thousands and even tens of thousands in 1 m^3 of atmospheric air [1 – 17]. Among viable microorganisms, there are the so-called culturable and non-culturable microorganisms, i.e., to be more exact, microorganisms, which do not grow under standard cultivation conditions. Bioaerosols also include dead and destroyed microorganisms and the associated debris. All bioaerosols represent a certain danger to human health since they can provoke infectious diseases, allergic reactions and a general worsening of the state of health. As well as the aforementioned species, viable microorganisms are especially dangerous and can directly cause infectious diseases.

When the atmosphere contains known pathogens, the determination of their concentration, particle size distribution and the dependence, i.e., the active factor dose or the organism reaction, one can predict the risks of these microorganisms on humans. A similar situation is also observed in the case when known biological toxicants and allergens affect humans. The situation radically changes when atmospheric air simultaneously contains different viable microorganisms of unknown origin or non-identified microorganisms. There are no methods for predicting the risk for man or at least no method for the quantitative comparison of the potential dangers presented to human health by the whole assembly of microorganisms detected in different atmospheric air samples described in the literature. The present work is devoted to the development of such a method for bacteria present in atmospheric aerosols.

Firstly, it should be noted that the proposed method is based on the use of different morphological, biological and biochemical indices. Therefore, it can be used only for culturable bacteria. Moreover, if it is possible to identify any bacteria present in atmospheric aerosols using non-cultural methods, e.g., methods based on the use of PCR [18, 19], the proposed approach cannot be used since non-cultural methods usually do not give an answer to the question of whether the detected microorganisms are viable or not.

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It is proposed to use four groups of tests for quantitative evaluation of the potential dangers represented by all culturable microorganisms detected in atmospheric aerosols. Firstly, microorganisms in atmospheric aerosols must be pathogenic or conventionally pathogenic for man in order to pose a health risk. Therefore, the first group of tests should characterize the potential danger represented by bacteria to humans. Secondly, bioaerosols are more dangerous when they have a higher concentration of culturable microorganisms and therefore, have a larger portion of microorganisms that are pathogenic or conventionally pathogenic for humans. Consequently, the second group of tests should evaluate the number of various culturable microorganisms in an atmospheric air sample. Thirdly, the danger represented by certain microorganisms increases in the case where they display increased resistance to unfavorable environmental factors causing inactivation of the microorganisms. Consequently, the third group of tests should evaluate their resistance to unfavorable environmental factors. Fourthly, if potentially pathogenic microorganisms, which are not inactivated in the environment, affect humans then those displaying drug resistances are the most dangerous. Consequently, the fourth group of tests should characterize the antibiotic resistance of bacteria. The results obtained in each of the four groups of tests, can be numerically characterized by a certain integral index quantitatively reflecting the contribution of the experimentally determined characteristics of microorganisms.

Quantitative evaluation of integral indices for each of the four groups of tests requires expert evaluation of numeric indices determined in the experiments with methods that characterize different properties of bacteria, on the basis of literature data analysis. The full list of determined properties of bacteria is given in the Materials and Methods section.

The presence and composition of culturable microorganisms in the atmospheric aerosols of southwestern Siberia have been observed since 1999 [20 – 23]. Data have been obtained on the annual and altitude variations of the concentrations of culturable microorganisms and the total protein concentrations have been used to characterize the presence of biogenic material in the atmosphere. Apart from the data on the concentrations of culturable microorganisms in the atmosphere, some reports [20 – 23] also provide data on the representation of different microorganisms in samples. Since the summer of 2006, microorganisms detected in atmospheric aerosol have been characterized in more detail by using the four groups of tests mentioned above.

Analysis of the literature [24 – 30] shows that the most important indirect characteristics of bacteria determining their pathogenicity are as follows: plasmocoagulation activity (the significance index of the characteristic is estimated at 7, see below), the presence of capsules in the bacterium and hemolytic activity (the significance index is estimated at 5), the presence of endonucleases in the microorganism (the significance index is estimated at 4), the presence of lecitinase, fibrinolytic and lipase activities in the microorganism (the significance index for all of these is estimated at 3), and the presence of gelatinase activity (the significance index is estimated at 2).

In addition, there exist a number of microorganism characteristics, which also influence the potential pathogenicity of defined strains, but to a smaller extent. These include the presence of proteolytic activity (the significance index is estimated at 1), the presence of pigments in cells (the significance index is estimated at 0.5) and the presence of amylolytic activity and mobility of bacteria on the growth medium (the significance index for all of them is estimated at 0.1). All these characteristics are determined quantitatively or qualitatively in experiments according to the methods described below, and generally speaking, are unique for each microorganism genus, species or strain. At the same time, the literature analysis performed shows that with some entries it is possible to construct a unified integral index of potential pathogenicity, which can be used for all bacteria detected in the samples. In this case, the value of potential pathogenicity for a sample will be determined as the average for all the bacteria detected in the sample.

Expert evaluation of the significance factors was performed from published works considering the factors and conditions for pathogenicities of different bacteria, e. g., *Bacillus anthraxes*, *Bordetella pertussis*, *Candida*, *Corynebacterium diphtheriae*, *Legionella pneumophila*, *Mycobacteria tuberculosis*, *Neisseria meningitidis* as well as some representatives of the genera *Staphylococcus*, *Streptococcus*, etc., [24 – 30]. As noted above, the sets of characteristics determining pathogenicity are different for different microorganism strains. However, they also have common characteristics. The above expert evaluations of their significance are based on the comparability of characteristics determining pathogenicity, which are common for many microorganisms. The value of the integral index of potential pathogenicity was then calculated as the sum of the characteristics present in a microorganism multiplied by the significance coefficients (it was considered that if this characteristic was present, its index equaled 1, if not, the index equaled zero) divided by the maximally possible value of this sum. In other words, the most dangerous bacteria have a value of the integral index of conventional pathogenicity that equals 1, and microorganisms, which do not represent danger, have a value that equals 0. Other integral indices (except for the second one, which was standardized by the maximal value of culturable microorganism concentration detected in atmospheric air samples during the year of measurement) were calculated and standardized in a similar way.

The methods to determine the concentrations and representation of different microorganisms in air samples are then presented in the next section of this work.

The degrees of increased resistance to unfavorable environmental factors as well as the manifestation of pathogenicity were determined by performing the expert evaluation of significance of a certain bacterial characteristic constructed on the basis of literature data analysis [24 – 30] and influencing bacterial resistance to environmental factors. The most significant characteristics determining the resistance of bacteria to unfavorable environmental factors are the presence of restriction endonucleases and plasmid DNAs as well as the ability to form quiescent forms, in particular, endospores allowing a species to survive under unfavorable environmental conditions (the significance index for all of these is estimated at 2). The ability to grow at increased NaCl concentrations (the significance index is estimated at 1) and the presence of pigmentation of cells (the significance index is estimated at 0.5) are less significant for survival.

The evaluation of drug resistance only included the study of bacterial resistance to the effect of 15 antibiotics widely used in medicine. All the antibiotics were considered to have equal significance.

The goal of the present work is the use of the proposed method on an array of data obtained from atmospheric aerosol monitoring in southwestern Siberia for the purpose of evaluating the annual variation in the degree of danger represented by culturable bacteria in the atmospheric air of southwestern Siberia for humans.

2 Materials and Methods

2.1 Atmospheric Air Sampling

Atmospheric air sampling was performed in three points of the studied region in southwestern Siberia, i.e., on the site of FSRI SRC VB Vector 4 times daily in the middle of the month, in the Klyuchi settlement once daily for 7 successive days once a season, and monthly on a day during the last ten days of each month at a point 50 km to the south of Novosibirsk with the Optic-E laboratory. The Optic-E laboratory mounted on an Antonov-30 airplane, shown in the photo in Fig. 1a) (see also [31 – 34]), includes devices for determining environmental characteristics, e.g., temperature, relative humidity, overboard pressure, radiation characteristics of the atmosphere, the concentration and particle size distribution of atmospheric aerosol measured with a photoelectric counter, navigation parameters, e.g., altitude relative to the relief, the flight direction and speed relative to the ground surface, the wind direction and speed at the flight altitude, samplers for collecting samples for chemical, biochemical and biological analyzes of atmospheric aerosol samples, gas analyzers for determining concentrations of basic gas pollutants of the atmosphere including hothouse gases, e.g., CO_2, CO, NH_3, SO_3, SO_4, O_3. In addition, a LIDAR was mounted on the airplane for remote probing of the atmosphere [31, 35]. Sampling onto different samplers was performed with a specially constructed sampler shown in the photo in Fig. 1b) [31, 36], which provided isokinetic sampling and increased pressure at the samplers' inlets as compared with the pressure outside the cabin. The cruising airspeed at the time of sampling was ca. 360 km/h. The airplane was flying in the daytime over forests successively at altitudes of 7000, 5500, 4000, 3000, 2000, 1500, 1000, and 500 m. Atmospheric air samples were collected on stainless steel impingers with a critical nozzle (its structure is described in the a published review [37]) maintaining a constant flow rate at a pressure differential of more than $4 \cdot 10^4$ Pa of air through the device (manufactured by JSC "Experimental-design bureau of biological precision engineering", Kirishi, Russia) for 30 min for above-ground samples and for ca. 10 min for altitude samples. The difference in sampling time is due to high price of aircraft hire. Particles were deposited into fluid twisted by incoming flow at a flow rate of 50 ± 5 L/min over the device walls. 50 mL of noncolored Hanks' solution (ICN Biomedicals) was used as a sorbing fluid. The retention efficiency of this device for aerosols of more than 0.3 μm (minimal size of known bacteria) exceeds 80% making up a constant value of 90 ± 15% for particles with a diameter of > 2 μm. An A-D1-04 pump (JSC "Kot", St. Petersburg, Russia) was used to pump air samples through the impinger. The pump outlet was connected to overboard air, which provided the required pressure differential for the critical nozzle.

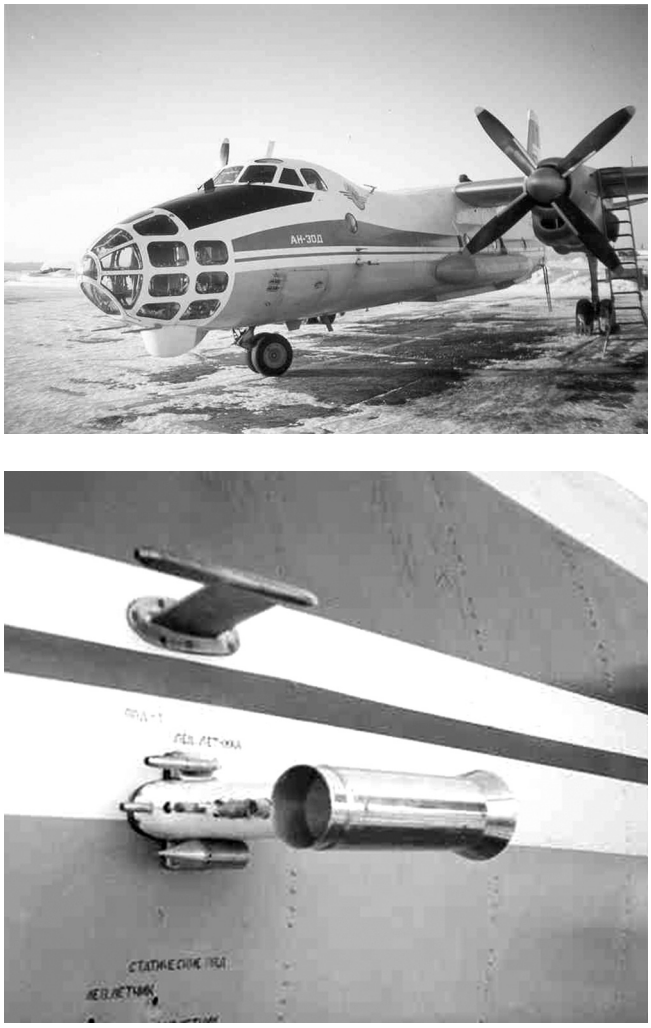

Figure 1. (a) "Optic-E" laboratory mounted on an Antonov-30 airplane, and (b) "Optic-E" laboratory samplers' inlets. See references [31, 36] for details.

2.2 Determining the Concentration of Culturable Microorganisms

Standard methods were employed to determine the concentration of culturable microorganisms. Samples were seeded onto Petri dishes containing agarized media as follows: LB [38] was used to detect saprophyte bacteria, depleted LB medium (diluted 1:10) was used to isolate microorganisms inhibited by the excess of organic substances, starch-ammoniac medium (SAA) [39] was used to detect actinomyces, soil agar was used for soil microorganisms, and Saboraud medium [39] was used for lower fungi and yeast. Successive sample dilutions were prepared as required. The seedings were incubated in a thermostat at a temperature of 28 – 30°C for 3 – 14 days. Morphological peculiarities of the detected bacteria were studied visually and with light microscopy. Fixed preparations of gram-stained cells and live preparations of cell suspensions observed with the phase contrast method were prepared for this purpose. The taxonomic groups of the detected microorganisms were determined according to published methods [40 – 42], and the analysis of nucleotide sequences of PCR products corresponding to the fragments of 16S rDNA gene was performed for some bacterial strains [43, 44]. The calculation of the number of culturable microorganisms in samples was performed according to standard methods [45]. The number of microorganisms was averaged over 3 – 4 parallels of samples 4 and 5 seeded on different media.

The determination of pathogenic properties of the strains included testing the studied microorganisms for the presence of hemolytic, plasmocoagulation, fibrinolytic and gelatinase activities.

2.3 Hemolytic Activity

The hemolytic activity was determined on blood agar by clarification zones around colonies according to a method described in the literature [42].

2.4 Plasmocoagulation Activity

In order to determine plasmocoagulation activity, dry plasma of rabbit blood was diluted 1:5 with sterile physiological solution and used as a test reaction. 0.5 mL of diluted plasma was placed into a sterile test tube, and one loop of 18 – 20-hour culture was suspended in it. The test tubes were placed into a thermostat at 37°C, and the presence of plasma coagulation was observed within 1, 2, 3, 18 and 24 h of incubation.

2.5 Fibrinolytic Properties of Microorganisms

In order to reveal fibrinolytic properties of microorganisms, sterile test tubes were filled with 0.1 mL of citrate plasm, 0.4 mL of physiological solution, 0.25 mL of 18 – 20-hour broth culture of the tested strain, and 0.25 mL of 0.25% calcium chloride solution. The test tubes were shaken and placed into a thermostat at 37°C for 15 to 20 min. If a clot was formed in the tube (as in the control case where nutrient medium was added instead of broth culture), the tested culture was considered to have no fibrinolytic properties. If fluidization of the clot was observed within 2 h, the culture was characterized by the presence of fibrinolysin.

2.6 Gelatinase Activity

Gelatinase activity was determined at seeding microorganisms in beef-peptone broth containing 12% gelatin. A positive reaction was the fluidization of the gelatin column [40].

2.7 Growth Characteristics of Bacteria at Increased Salt Concentration

The growth characteristics of bacteria at increased salt concentration were determined by growing microorganisms on RPA medium with increased NaCl concentration (5 or 10%). The growth of colonies provided indirect evidence of bacterial resistance to drying.

2.8 Determination of Enzymatic Activity of Isolated Bacteria

The determination of enzymatic activity of isolated bacteria was performed by the following tests.

2.8.1 Determination of Proteolytic (Caseinolytic) Activity

The determination of proteolytic (caseinolytic) activity of the tested strains was performed using milk agar (MA). The composition of MA was as follows: 3% starved agar (tap water + 3% agar), which was sterilized at 1 atm for 30 min and 12% non-fat milk sterilized at 1 atm for 20 min. The starved agar was melted and cooled to 50 to 55°C before use, and thoroughly mixed with sterile milk warmed to the same temperature and poured into dishes. The tested microorganisms were seeded onto the medium surface by stroking and incubated under optimal conditions. The presence and size of substrate hydrolysis zones were determined in mm by measuring them [40].

2.8.2 Amylolytic Activity

Amylolytic activity was determined by the ability of the strains to secrete amylolytic enzymes during growth on SAA medium containing starch. The studied cultures were seeded onto the medium surface by stroking or injection with a bacteriological loop. After incubation, iodine solution was poured onto the dish, which was used to detect colonies producing hydrolysis zones. A positive test result was indicated by the appearance of a colorless area around the growth zone.

2.8.3 Lecitinase and Lipase Activities

The determination of lecitinase and lipase activities of the strains was performed on yolk agar (RPA + yolk). Yolk was placed into 400 mL of molten agar and mixed to obtain uniform suspension under aseptic conditions. Then, the yolk medium was poured into dishes and left until it became hard. The cultures were seeded by stroking onto the medium surface and incubated for 3 to 7 days. Positive lecitinase reaction was expressed as the appearance of turbid zones at clarification of the yolk agar around colonies of tested cultures [40]. The presence of lipase was revealed by viewing the grown colonies in inclined light. A positive result was indicated by the formation of an oily, glittering and shimmering or pearl layer above the colony or around it on the agar surface [40].

2.8.4 Lipolytic Activity

The screening of the strains for lipolytic activity was also performed at room temperature by seeding them by injection onto *L*-agar containing 1% Tween-20 or Tween-40 supplemented with 0.01% $CaCI_2$. The results were determined within 3 – 4 days of incubation of the seedings by the presence of turbid zones in agar around the colonies. The relative activity was determined by measuring the diameters of the colony and the zone [40].

2.8.5 Alkaline Phosphatase Detection

In order to detect alkaline phosphatase, 0.3 mL of suspension in physiological solution (0.85% NaCl) was added to 0.3 mL of substrate solution containing 0.04 M glycine buffer at pH 10.5 and with 0.01 M disodium-*n*-nitrophenyl phosphate (Sigma). The mixture was incubated for 3 h at 37°C. Positive reaction manifested itself as yellow staining of the reaction mixture [42]. The enzyme activity was determined within 3 h of incubation by absorption on a Uniplan apparatus (Russia) with a color filter at a wavelength of 450 nm.

2.8.6 Endonuclease Activity

The endonuclease activity was determined on a solid medium with thymus DNA and toluidine blue. The reaction was evaluated by the appearance of a bright-pink zone around the bacterial colony [42].

2.9 Presence of Restriction Endonucleases

When screening the strains for the presence of restriction endonucleases, individual colonies collected from a solid culture were suspended in 100 to 200 μL of TEN-buffer (0.1 M Tris, pH 7.5, 0.01 M EDTA and 0.05M NaCl). Lysocime and triton X-100 were used to destroy the cell walls of the bacteria. The cell extract obtained was used for analysis of the presence of restriction endonucleases. DNAs of phages λcI857 and T7 were used as substrates for hydrolysis. The

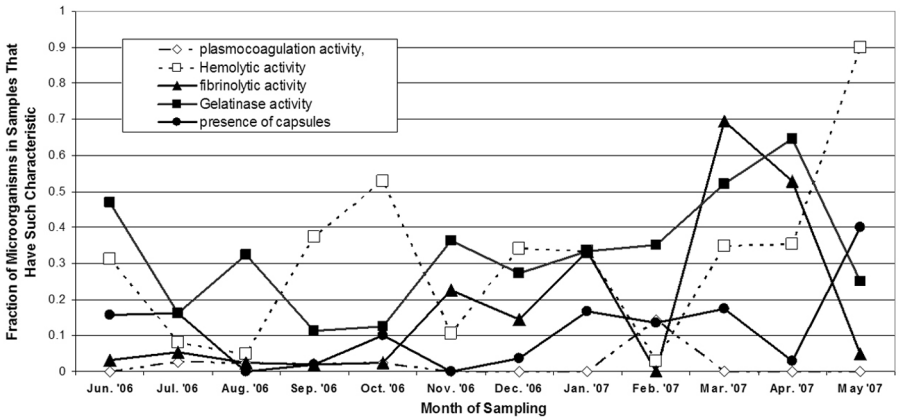

Figure 2. The main average characteristics referring to the integral index of potential pathogenicity of bacteria for individual bacteria detected in atmospheric air samples at heights of 500 to 7000 m. Standard deviations are not shown.

electrophoresis of DNA after restriction was performed in 1% agarose (Sigma) [46]. The presence of restriction endonucleases in the microorganism strains was revealed by the appearance of discrete fragments of substrate DNA in the electrophoregram under UV light.

2.10 Plasmid DNA Concentration

The concentration of plasmid DNA in the strains was determined with the screening method using a standard procedure. Cells from a solid medium were suspended with a loop in 100 μL of buffer (50 mM Tris pH 8.0, 50 mM Na_2-EDTA and 15% sucrose), 200 μL of alkaline solution (0.2 N NaOH and 1% SDS) and 150 μL of 3 M sodium acetate of pH 5.0 were added, and centrifugation was performed for 5 min on a desktop centrifuge. Then 1 mL of 96% ethanol was added to the sediment. The DNA obtained was analyzed in 0.8% agarose in tris-borate buffer pH 8.0 [43].

2.11 Antibiotic Resistance

Antibiotic resistance was studied with the disk diffusion method [40], at seeding microorganisms onto solid media followed by application of paper disks (Research Center of Pharmacology, Saint-Petersburg, Russia) with antibiotics widely used in medical practice being utilized for the seeding: ampicillin (10 μg/disk), neomycin (30 μg/disk), benzylpenicillin (100 U/disk), levomycetin (30 μg/disk), carbenicillin (100 μg/disk), canamycin (30 μg/disk), oleandomycin (15 μg/disk), rifampicin (5 μg/disk), streptomycin (30 μg/disk), polymxin (300 U), erythromycin (15 μg/disk), lincomycin (15 μg/disk), oxacillin (10 μg/disk), gentamycin (10 μg/disk), and tetracycline (30 μg/disk).

3 Results and Discussion

Figures 2 – 4 show annual variations of different indices measured for all culturable bacteria detected in atmospheric air samples collected at heights of 500 to 7000 m. As shown in Figs. 2 – 4, each individual characteristic for the bacteria varies during the year rather randomly, and it does not seem possible to draw any conclusions on regularities of variations for each of these values.

The situation changes considerably when one turns from the individual characteristics of bacteria to integral indices constructed on their basis. Figure 5 presents the annual dynamics of variation in the number of culturable bacteria detected in atmospheric air samples at heights of 500 to 7000 m. A comparison with data obtained previously at the same sampling places by the current authors [20 – 23] shows that the numbers of bacteria for the year do not differ from those determined for the previous eight years, within the acceptable error ranges. For measurements performed in the Klyuchi settlement and on the site of the FSRI SRC VB Vector, the annual dynamics are also seen to behave in a similar manner. Both of the curves in Fig. 5 have their maximum from late spring to early autumn and their minimum from late autumn to early spring.

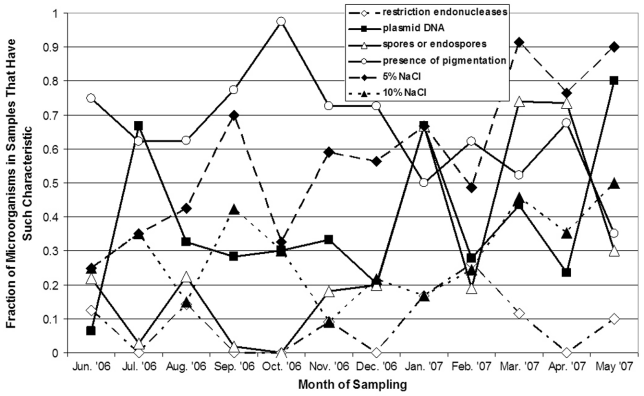

Figure 3. The main average characteristics referring to the integral index of bacterial resistance to environmental factors for individual bacteria detected in atmospheric air samples at heights of 500 to 7000 m. Standard deviations are not shown.

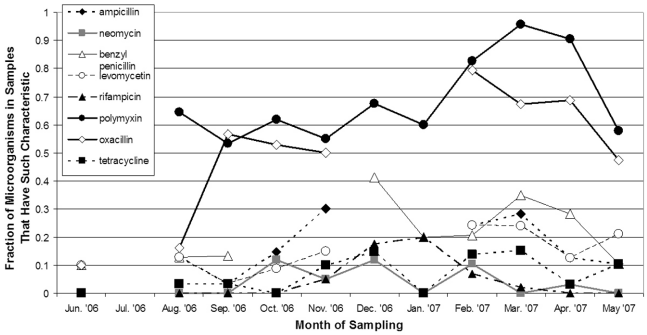

Figure 4. The main average characteristics referring to the integral index of antibiotic resistance of bacteria for individual bacteria detected in atmospheric air samples at heights of 500 to 7000 m. Standard deviations are not shown.

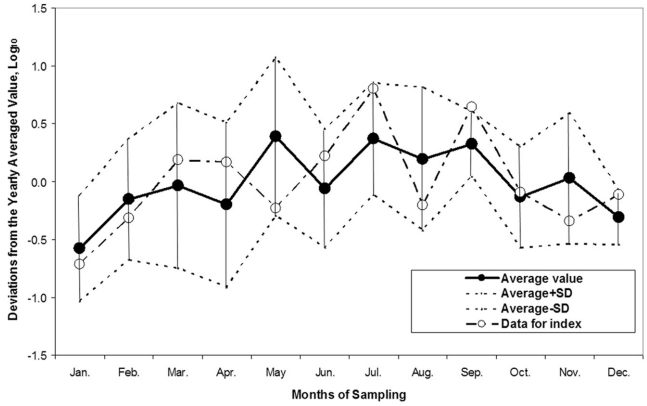

Figure 5. Standardized data over the annual averages values concentrations of culturable microorganisms for 8 years of research of in the atmosphere at altitudes of 500 to 7000 m and standardized over the annual average value data taken for determination of integrated index of bacteria concentration in the air.

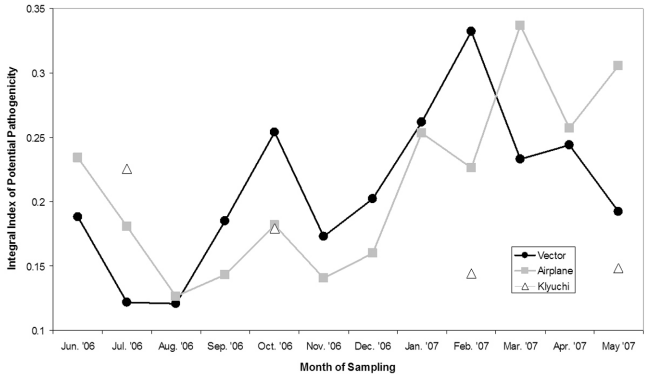

Figure 6. Annual dynamics of variation of the integrated index of potential pathogenicity determined for bacteria detected in three sampling points of atmospheric air.

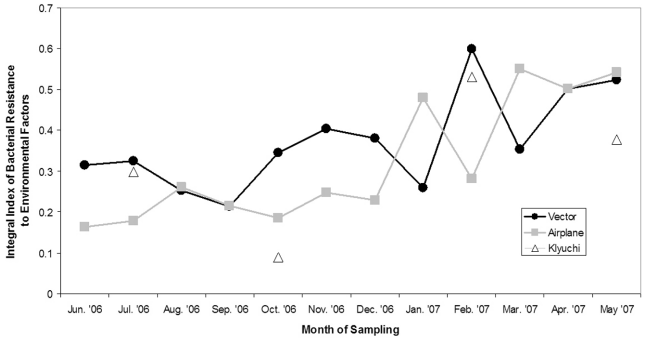

Figure 7. Annual dynamics of variation of the integral index of bacterial resistance to environmental factors determined for bacteria detected in three sampling points of atmospheric air.

With regard to the average values, the maximal and minimal values of concentrations of culturable bacteria differ by more than an order of magnitude. Thus, the value of the second integral index (the number of culturable bacteria in the atmospheric aerosols) varies during the year by ca. an order of magnitude.

The data on the diversity of culturable microorganisms observed in atmospheric air samples obtained during the year do not suggest the existence of statistically significant regularities of variation in the numbers of microorganisms of different groups in the atmospheric aerosols of southwestern Siberia. The same situation is observed for the diversity of culturable microorganisms in the long-term altitude samples described elsewhere [20, 21, 47].

The analyses of the variation of three other integral indices are detailed in Figs. 6 – 8. Unlike the second integral index (the number of culturable bacteria in the atmospheric aerosol), the dependences presented in these figures do not show such a sharp change, e.g., the integral index of potential pathogenicity during the year changes smoothly from ca. 0.12 at the end of summer to ca. 0.28 at the end of winter and the beginning of spring, Fig. 6. Thus, the atmosphere contains twice as many microorganisms that are harmful for human health in the most dangerous periods than in the least dangerous periods. The integral index of bacterial resistance to environmental factors also changes smoothly during the year by ca. a factor of 2 from 0.25 at the end of summer and the beginning of autumn to 0.5 at the end of winter and the beginning of spring, Fig. 7. In addition, the integral index of antibiotic resistance is ca. 0.12 from May to September and increases stepwise to ca. 0.27 from October to April, Fig. 8. The increased resistance of culturable bacteria detected in atmospheric air samples to oxacillin and especially to polymyxin is unexpected. As far as the current authors are aware, oxacillin ointment is used in the region for respiratory disease prophylaxis, which could account for the increased resistance of culturable bacteria detected in atmospheric air samples to oxacillin. Polymyxin is not a priority antibiotic used for disease treatment in the region, and there are no prophylactic ointments available based on it. As a result, the reasons for the increased resistance of culturable bacteria detected in atmospheric air samples to polymyxin are not clear.

It is necessary to interpret the above-formulated integral index of potential pathogenicity of bacteria for humans by comparison with the existing approaches to risk evaluation proceeding from the simple dependence on "the active factor dose – the organism reaction". The value of the integral index of potential bacterial pathogenicity for humans is a certain value of the probability that the organism reaction can be caused by a single microorganism, i.e., the p value in the formula given in Eq. (1):

$$P = 1 - \exp(-pD) \tag{1}$$

where P is the probability of causing a reaction in the organism, and D is the active factor dose. This formula describes the dependence "the active factor dose – the organism reaction" in the supposition that all microorganisms getting into the organism and acting independently and with equal probability, p, can cause a corresponding reaction in it [48, 49]. Higher values of p or the value of the integral index, indicate a higher potential bacterial pathogenicity for humans, and a greater probability of causing the organism reaction as well as a higher potential for bacterial pathogenicity, respectively. The values for the proposed index calculated from experimental data vary from 0 to 0.68. In each of the air samples studied, the maximal value of this index for individual bacteria exceeds 0.36, and in 50% of cases it exceeds 0.48. In other words, in addition to potentially quite non-pathogenic bacteria (with an integral index of potential pathogenicity strictly equal to 0 – note that an average sample contains ca. only 2.5% of such bacteria), there are potentially medium-pathogenic bacteria. Thus, practically all bacteria detected

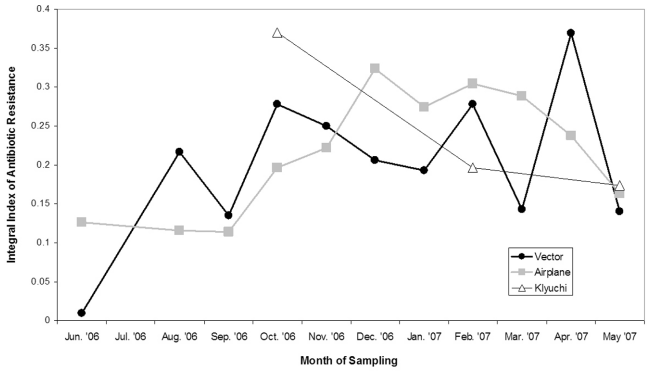

Figure 8. Annual dynamics of variation of the integral index of antibiotic resistance determined for bacteria detected in three sampling points of atmospheric air. Determination of this index was not performed in July 2006.

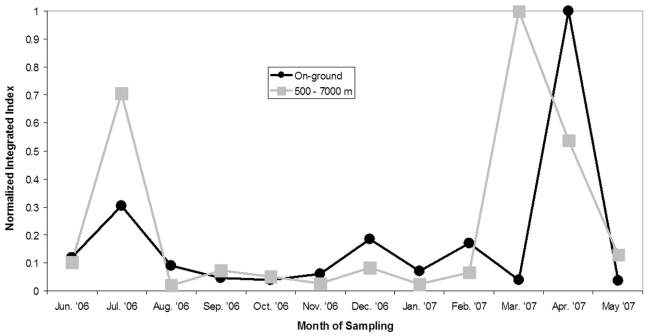

Figure 9. Annual dynamics of variation of the integral index of atmospheric aerosol bacterias' potential dangers for humans.

in atmospheric air samples are to some extent potentially pathogenic for humans. No known highly pathogenic bacteria were detected in the studied atmospheric air samples.

The integral index of microorganisms' resistance in the environment provides a certain generalized characteristic of bacterial resistance. It is obvious that in comparison with other integral indices it does not reflect all nuances of bacterial resistance in the environment. At the same time, as literature analysis shows, detailed characteristics of bacterial resistance to unfavorable environmental effects such as temperature, relative humidity, freezing/thawing, irradiation at different wavelengths, mechanical injuries, sampling-related stresses, etc., have only been determined for a few strains from the total number of currently known microorganisms (it should be noted that according to the data presented by Oren [50], it has been estimated that not more than 10% of bacteria existing on the Earth are known to scientists). Data on bacterial resistance to unfavorable environmental effects can undoubtedly be used for more precise definition of the integral index of bacterial resistance, but only in cases where it has been possible to determine the presence of a definite strain in an atmospheric air sample and to prove its viability.

Superposition (multiplication) of all of the four integral indices for each sampling allows one to evaluate the dangers represented by culturable bacteria, which are present in atmospheric aerosols of the region at the moment of sampling, and to trace its variation during the year, as shown in Fig. 9. In particular, for the studies conducted, the maximal value of this index was observed in February – April 2007 and comprised ca. 0.045, and the minimal value was observed in August – September 2006 and comprised 0.003.

It should be noted that the proposed procedure of expert evaluation does not attempt to be definitive. It is quite possible that not all significant indices have been included in the above groups of test. In addition, the performed expert evaluation of significance of various bacterial characteristic may not be quite accurate. However, the proposed method allows one to compare the potential dangers represented to humans by all bacteria detected in an atmospheric air sample with other samples, with significantly different concentrations and representation of bacteria present.

4 Conclusions

The present work proposes an approach to the evaluation of danger represented by culturable bacteria in atmospheric aerosol for the population of southwestern Siberia. The results obtained by the realization of this approach allow the evaluation for the first time of the dangers represented by atmospheric microorganisms for humans. The annual dynamics of variations of four integral indices reflecting different components of this degree of danger were observed. At the same time, since the observations were performed during one year, it was impossible to reveal the annual recurrence and inter-year differences between these indices. In addition, the dependences obtained are not statistically significant due to the small number of samplings performed. Only the continuation of the work will facilitate the reliable determination of the dynamics of the values studied and their use for reasonable prediction of variations in the dangers represented by culturable bacteria in atmospheric aerosols for the region's population.

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