UDC 613.31: 614.445: 614.777
DOI: 10.21668/health.risk/2024.4.08.eng

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Research article

ASSESSMENT OF POTENTIAL MICROBIAL RISK CAUSED BY SPREAD OF WATERBORNE INFECTIOUS DISEASES IN A RIVER AREA WITH INTENSIVE WATER USE

B.I. Marchenko[1], P.V. Zhuravlev[2], L.A. Deryabkina[3], O.A. Nesterova[1]

[1]Southern Federal University, 105/42 Bol'shaya Sadovaya St., Rostov-on-Don, 344006, Russian Federation
[2]Rostov Research Institute of Microbiology and Parasitology, 119 Gazetny Lane, Rostov-on-Don, 344000, Russian Federation
[3]Center for Hygiene and Epidemiology in Rostov Region, Taganrog Office, 16a Bol'shoi Av., Taganrog, 347930, Russian Federation

The necessity to raise effectiveness of activities aimed at preventing spread of waterborne intestinal infections requires improvement of methods and technologies applied in sanitary-microbiological monitoring as a part of the system for socio-hygienic monitoring.

The aim of this study was to assess dynamics of potential microbial risks caused by spread of waterborne acute intestinal infections in the Lower Don River area with intensive water use. Microbial communities in water of the Don River in Azov City over 2005–2020 were selected as research objects. The study relied on using results obtained by bacteriological tests of 540 river water samples as well as 1800 water samples taken at outlets from water treatment facilities and in distribution networks of the municipal water supply system. The tests involved identifying sanitary-indicative microorganisms (total levels, fecal and glucose-positive coliform bacteria), potentially pathogenic microorganisms (Klebsiella and Pseudomonas aeruginosa), and pathogenic enterobacteriaceae (Salmonella).

Comprehensive assessment of potential microbial risk associated with waterborne infectious diseases was performed including retrospective analysis of its trends and seasonal characteristics. Accuracy of medium-term extrapolation prediction of microbial risk was comparatively analyzed using regression and neural network models. A complex indicator was calculated for water in the Lower Don River in 2005–2020 for two sanitary-hygienic factors (Sources of Centralized Household and Drinking Water Supply and Recreational Water Use). Its value based on a five-level classifier amounted to 0.612. This made it possible to determine a very high level of potential microbial risk associated with spread of waterborne infections with its typical summer-autumn seasonal rise.

Due to optimization measures, a stable favorable trend was formed per the factor 'Centralized Household and Drinking Water Supply'. The value of the three-factor complex indicator (0.525) made it possible to establish a high level of potential microbial risk associated with spread of waterborne intestinal infections. A statistically significant ($p < 0.01$) direct medium correlation was established between incidence of acute intestinal infections and salmonellosis and the level of potential microbial risk. Neural network models were confirmed to provide higher accuracy for medium-term microbial risk predictions.

Keywords: *water of open watercourses, sanitary-microbiological indicators, potentially pathogenic microorganisms, salmonella, potential microbial risk, epidemic risk assessment, waterborne intestinal infections, multilayer forward propagation perceptron, medium-term prediction, socio-hygienic monitoring.*

Boris I. Marchenko – Doctor of Medical Sciences, Associate Professor, Professor at the Institute of Nanotechnology, Electronics and Instrument Engineering (e-mail: borismarch@gmail.com; tel.: +7 (904) 346-15-75; ORCID: http://orcid.org/0000-0001-6173-329X).

Piotr V. Zhuravlev – Doctor of Medical Sciences, Head of the Laboratory of Sanitary Microbiology of Water Bodies and Human Microbial Ecology (e-mail: pitthegreat@yandex.ru; tel.: +7 (908) 510-57-29; ORCID: http://orcid.org/0000-0002-8196-3882).

Lyudmila A. Deryabkina – Candidate of Medical Sciences, Chief Physician (e-mail: tagcgsen@pbox.ttn.ru; tel.: +7 (863) 464-29-62; ORCID: https://orcid.org/0000-0002-0790-0365).

Olesja A. Nesterova – postgraduate student of the Institute of Nanotechnologies, Electronics and Equipment Engineering (e-mail: semina@sfedu.ru; tel.: +7 (928) 610-96-15; ORCID: https://orcid.org/0000-0003-4263-5103).

The necessity to provide the country population with high quality drinking water makes assessment of health risks associated with pollution in environmental objects, water bodies included, a priority challenge to be tackled [1–3]. Water consumption and sewage volumes tend to grow and this leads to elevated levels of microbial contamination in surface water bodies used as water supply sources as well as for recreational purposes. Considerable microbial contamination of surface water supply sources, especially with antimicrobial resistant bacteria, can result in deteriorated tap water quality and create elevated risks of waterborne infections [4–9]. All this gives the highest priority to improvement of methods and technologies for microbiological control of drinking water quality, quality of natural water sources and recreational waters within the whole system of prevention activities [10–17]. Sanitary-microbiological monitoring of water environment is an integral component in the system for epidemiological surveillance and it necessarily entails health risk assessment including potential growth in incidence of waterborne intestinal infections [18–23]. Wide integration of some scientific and organizational-methodical principles of epidemiological surveillance into the national system for social-hygienic monitoring can be considered a promising trend in implementing The Concept for Development of the System for Social-Hygienic Monitoring in the Russian Federation for the Period up to 2030[1] [6].

It should be noted that the methodology for health risk assessment under variable environmental exposures has been developed quite intensively for solving relevant tasks related to providing sanitary-epidemiological safety of the population in the Russian Federation [20, 22]. Thus, various methods for microbial risk quantification have been developed and implemented into practice. They are informative instruments based on identifying indicators and parameters of fecal pollution in water, which is evidence that bacterial agents involved in gastrointestinal infection are likely to occur in it [19, 24–28]. Created mathematical models that describe cause-effect relations between frequency of acute intestinal infections (AII) and levels of bacterial contamination in water have provided theoretical grounds for microbial risk assessment [11, 12].

The aim of this study was to assess dynamics of potential microbial risks caused by spread of waterborne acute intestinal infections in the Lower Don River area with intensive water use. The research tasks included identification of the most informative sanitary-biological markers eligible to describe water quality and to assess potential microbial risks associated with spread of waterborne AII; retrospective analysis of long-term and annual dynamics of potential microbial risks together with identifying their trends and seasonal peculiarities; comparative assessment of precision achieved in middle-term prediction of potential microbial risks using a regression model and a neural network model.

Materials and methods. The study relied on using the results obtained by microbiological tests of 540 water samples taken from the Don River, 1800 water samples taken at outlets from water treatment facilities and from the centralized drinking and household water supply system, as well as data on incidence of AII and salmonellosis in the Azov City in the Rostov region over 2005–2020. Water samples were taken at three monitoring points along the Don River stream; in a water intake area where water from the river was taken into the centralized water supply system of the Azov City; in a recreational area (the community beach), and 500 meters downstream from the municipal sewage drainage (Figure 1).

[1] Ob utverzhdenii kontseptsii razvitiya sistemy sotsial'no-gigienicheskogo monitoringa v Rossiiskoi Federatsii na period do 2030 goda: Prikaz rukovoditelya Federal'noi sluzhby po nadzoru v sfere zashchity prav potrebitelei i blagopoluchiya cheloveka (Rospotrebnadzora) ot 26.08.2019 № 665 [On Approval of the Concept for Development of the System for Social-Hygienic Monitoring in the Russian Federation for the Period up to 2030: the Order by the Head of the Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing (Rospotrebnadzor) dated August 26, 2019 No. 665]. Moscow, Rospotrebnadzor, 2019, 27 p. (in Russian).

Figure 1. Points for sanitary-biological monitoring of water in the Lower Don River within the Azov City boundaries

River and drinking water was analyzed in the laboratory for sanitary microbiology of water bodies and human microbial ecology of the Rostov Research Institute of Microbiology and Parasitology. The aim was to identify the occurrence of sanitary significant microorganisms in it including total coliforms (TCs) and thermotolerant coliforms (TTCs); potentially pathogenic microorganisms such as Klebsiella and Blue pus bacilli; pathogenic enterobacter salmonellas; as well as glucose positive coliforms (GPCs) in conformity with the valid sanitary rules and norms[2] and methodical guidelines[3]. A liquid accumulation medium was applied to isolate bacteria of Salmonella species from water bodies (RNS medium). It promotes accumulation of Salmonella for various serological groups, inhibits growth of concomitant microflora and helps obtain objective information about bacteriological contamination in water objects [29]. GPCs levels were analyzed in river water because this indicator combines a wide range of both pathogenic (Salmonella and Shigella) and potentially pathogenic microorganisms.

This study continues the research based on the method for microbial risk assessment that employs mathematical models for calculating integral and complex indicators of health hazards associated with intestinal infections depending on sanitary-hygienic conditions of water use[4].

Factor-related integral indicators of potential microbial risk (R_i) were determined for three sanitary-hygienic factors (Sources of Centralized Drinking and Household Water Supply, Recreational Water Use and Centralized Drinking and Household Water Supply), for which 5, 4 and 7 primary indicators (X_i) were considered respectively. Thus, Centralized Drinking and Household Water Supply as a factor included such primary indicators as

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[2] SanPiN 1.2.3685-21. Gigienicheskie normativy i trebovaniya k obespecheniyu bezopasnosti i (ili) bezvrednosti dlya cheloveka faktorov sredy obitaniya; utv. postanovleniem Glavnogo gosudarstvennogo sanitarnogo vracha Rossiiskoi Federatsii ot 28 yanvarya 2021 goda № 2 (s izmeneniyami na 30 dekabrya 2022 goda) [Hygienic Standards and Requirements to Providing Safety and (or) Harmlessness of Environmental Factors for People; approved by the Order of the RF Chief Sanitary Inspector on January 28, 2021 No. 2 (as of last edited on December 30, 2022)]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/573500115 (May 05, 2024) (in Russian).

[3] MUK 4.2.1884-04. Sanitarno-mikrobiologicheskii i sanitarno-parazitologicheskii analiz vody poverkhnostnykh vodnykh ob"ektov: metodicheskie ukazaniya, utv. i vved. v deistvie Glavnym gosudarstvennym sanitarnym vrachom, Pervym zamestitelem Ministra zdravookhraneniya Rossiiskoi Federatsii G.G. Onishchenko 3 marta 2004 g. [Sanitary-microbiological and sanitary-parasitological analysis of water in surface water bodies: methodical guidelines, approved and enacted by G.G. Onishchenko, the RF Chief Sanitary Inspector, First Deputy to the Minister of Health of the Russian Federation on March 03, 2004]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/1200039680 (May 05, 2024) (in Russian).

[4] MR 2.1.10.0031-11. Kompleksnaya otsenka riska vozniknoveniya bakterial'nykh kishechnykh infektsii, peredavaemykh vodnym putem: metodicheskie rekomendatsii, utv. Rukovoditelem Federal'noi sluzhby po nadzoru v sfere sluzhby zashchity prav potrebitelei i blagopoluchiya cheloveka, Glavnym gosudarstvennym sanitarnym vrachom Rossiiskoi Federatsii G.G. Onishchenko 31.07.2011 [Complex assessment of risks of waterborne bacterial intestinal infections: methodical guidelines, approved by G.G. Onishchenko, Head of the Federal Service for Surveillance over Consumer Rights Protection and Human Wellbeing, the RF Chief Sanitary Inspector on July 31, 2011]. Moscow, Federal Center for Epidemiology of Rospotrebnadzor, 2012, 47 p. (in Russian).

the share of drinking water samples with TCs contamination prior to intake into the water supply system and within it; average TCs index in the water supply system; the share of water samples from the centralized water supply system with the TCs level equal to 2 CFU/100 cm^3 and higher; the average quantity of microorganisms in 1 cm^3 of water in the centralized water supply system; occurrence of pathogenic bacteria and the share of water samples with potential pathogens identified in them. Complex indicators of potential microbial risk associated with waterborne intestinal infections were calculated in two versions using the outlined factor-related weight coefficients (G_i) based on integral indicators. Two versions depended on sanitary-hygienic conditions and were two-factor ones (R_{ab}) that considered sanitary-bacteriological indicators of river water (Sources of Centralized Drinking and Household Water Supply and Recreational Water Use) and three-factor ones that also considered Centralized Drinking and Household Water Supply as another factor (R_k). Scope of epidemiological hazard associated with waterborne bacterial intestinal infections (the microbial risk level) was established based on the factor-related integral indicators (R_i) and the complex microbial risk indicators (R_{ab} and R_k) using the five-level classifier. Very low level of microbial risk was matched with the calculated integral and complex microbial risk indicators equal to $0 \leq R < 0.15$; low (acceptable), $0.15 \leq R < 0.20$; medium, $0.20 \leq R < 0.40$; high, $0.40 \leq R < 0.60$; very high, $0.60 \leq R <= 1.00$.

Correlation coefficients for the level of potential microbial risk associated with the spread of waterborne intestinal infections and AII incidence as well as incidence of salmonellosis among the population over 2005–2020 were calculated based on dynamic series of relevant monthly indicators including 192 levels in a series.

Creation of databases and statistical data analysis were accomplished using the information-analytical software Microbiology of Surface Waters, version 1.65, Turbo Dinamics, version 2.51, our own software for calculating dynamic series statistics and extrapolation prediction, as well as the expert statistical software IBM SPSS Statistics version 20.0. Variation and dynamic data series were tested to identify anomalies (spikes) based on the Chauvenet's criterion. Models to describe long-term dynamics were created by fitting approximating functions with estimation of significance ($p < 0.05$). When examining seasonal peculiarities of the potential microbial risk, we calculated the upper bounds of its year-round levels and typical annual dynamics curves with differentiated year-round and seasonal components[5], monthly indexes of seasonal variations, the seasonality coefficient that described the share of periods with a seasonal rise in the total, as well as the seasonality index per Warringer. Middle-term predictions of the potential microbial risk in 2019 and 2020 were calculated using neural network models and regression models; their precision was comparatively estimated on the basis of 14-year long dynamic data series covering average annual indicators (2005–2018). Prediction relied on using two types of two-layer artificial neural networks (ANN), a cascade-forward back-propagation network (Cascade-Forward backprop) and a feed-forward back-propagation network (Feed-Forward backprop) with the number of neurons in hidden layers equal to 23 and 30 accordingly. ANNs were created using the Matlab R2021a applied software package with Neural Network Toolbox for neural network synthesis and analysis. They were trained using the Levenberg – Marquardt method, which is applied to optimize parameters of nonlinear regression models in solving nonlinear least square problems [30, 31].

Results and discussion. This study continues the research with its focus on assessing epidemiological significance of indicators that describe bacterial contamination in water of

[5] Degtiarev A.A., Khodyrev A.P. Method for analyzing the annual dynamics of infectious incidence. *Zhurnal mikrobiologii, epidemiologii i immunobiologii*, 1976, vol. 53, no. 2, pp. 97–102 (in Russian).

the Lower Don River [32]. The research results obtained per 5-year periods give evidence of substantially deteriorated indicators of microbial contamination in water along the river stream within the Azov City water area. Thus, the average TCs level was 2.36 – 6.04 times higher in the recreational area and 6.5 – 7.06 times higher in the sewage drainage area than in the water intake area. The maximum detected TCs level of $2.4{\cdot}10^8$ CFU/100 cm^3 was also identified in water at the municipal sewage drainage. The ratio between the water intake area, recreational area and the sewage drainage area per the TCs level was 1.00 : 1.57–5.51 : 2.64–29.64 with the maximum level being equal to $2.4{\cdot}10^6$ CFU/100 cm^3. The average GPCs was 1.43–5.95 times higher in the recreational area and 1.62-5.9 times higher in the sewage drainage area than in the water intake area. Klebsiella and Blue pus bacilli were found in 100.00 and 99.81 % of the analyzed samples respectively; their highest average levels were equal to $1.3{\cdot}10^7$ and $2.4{\cdot}10^5$ CFU/100 cm^3 (Table 1).

Table 1

Sanitary-bacteriological indicators of water in the Lower Don River within the Azov City boundaries in the Rostov region per 5-year periods over 2006–2020

Monitoring points	Indicators	Observation periods		
		2006–2010	2011–2015	2016–2020
1	2	3	4	5
Total coliforms (TCs, colony-forming units (CFU) in 100 cm^3)				
Water intake area	Average	7767.76	8131.32	9560.00
	Maximum	700,000	700,000	240,000
	The share (%) of samples with quantity over 1000	85.51	81.67	97.73
Recreational area	Average	18,326.10	49,124.21	24,322.50
	Maximum	7,000,000	7,000,000	240,000
	The share (%) of samples with quantity over 500	98.55	96.67	100.00
Sewage drainage area	Average	50,522.58	54,979.26	67,520.93
	Maximum	240,000,000	2,400,000	7,000,000
	The share (%) of samples with quantity over 1000	97.10	96.67	100.00
Thermotolerant coliforms (TTCs, colony-forming units (CFU) in 100 cm^3)				
Water intake area	Average	812.29	1265.51	2190.26
	Maximum	24,000	24,000	24,000
	The share (%) of samples with quantity over 100	76.81	91.67	100.00
Recreational area	Average	1811.78	6975.55	3447.11
	Maximum	240,000	2,400,000	70,000
	The share (%) of samples with quantity over 100	89.86	96.67	100.00
Sewage drainage area	Average	2142.91	37,515.17	10,874.39
	Maximum	2,400 000	2,400 000	240,000
	The share (%) of samples with quantity over 100	97.10	98.33	100.00
Glucose positive coliforms (GPCs, colony-forming units (CFU) in 100 cm^3)				
Water intake area	Average	81,418.75	75,896.43	75,668.18
	Maximum	2,400,000	2,400,000	240,000
	The share (%) of samples with quantity over 1000	97.10	100.00	100.00
Recreational area	Average	411,513.4	451,858.6	108,404.65
	Maximum	24,000,000	24,000,000	700,000
	The share (%) of samples with quantity over 500	100.00	100.00	100.00
Sewage drainage area	Average	460,677.6	453,305.0	122,275.0
	Maximum	240,000,000	2,400,000	2,400,000
	The share (%) of samples with quantity over 1000	100.00	100.00	100.00
Klebsiella spp., (colony-forming units (CFU) in 100 cm^3)				
Water intake area	Average	11,808.73	10,529.39	20,400.00
	Maximum	700,000	2,100,000	240,000
	The share of samples (%) with found bacteria	100.00	100.00	100.00

End of the Table 1

1	2	3	4	5
Recreational area	Average	60,769.38	57,552.50	30,063.16
	Maximum	2,400,000	13,000,000	240,000
	The share of samples (%) with found bacteria	100.00	100.00	100.00
Sewage drainage area	Average	85,127.10	70,036.15	77,172.09
	Maximum	2,400,000	2,400,000	700,000
	The share of samples (%) with found bacteria	100.00	100.00	100.00
Blue pus bacilli (Pseudomonas aeruginosa, colony-forming units (CFU) in 100 cm^3)				
Water intake area	Average	769.84	575.28	141.22
	Maximum	7000	2400	2400
	The share of samples (%) with found bacteria	100.00	100.00	97.73
Recreational area	Average	869.52	829.33	797.37
	Maximum	70,000	240,000	7000
	The share of samples (%) with found bacteria	100.00	100.00	100.00
Sewage drainage area	Average	952.56	963.11	1698.95
	Maximum	70,000	24,000	7000
	The share of samples (%) with found bacteria	100.00	100.00	100.00
Salmonella, (Most-probable-number (MPN) in 1000 cm^3)				
Water intake area	Average	141.68	2.41	6.58
	Maximum	2100	24	70
	The share of samples (%) with found bacteria	79.71	31.67	36.36
Recreational area	Average	524.70	21.48	41.57
	Maximum	24,000	240	240
	The share of samples (%) with found bacteria	89.86	81.67	97.73
Sewage drainage area	Average	440.12	20.88	262.93
	Maximum	7000	240	6200
	The share of samples (%) with found bacteria	97.10	95.00	100.00

Over the whole observation period from 2005 to 2020 inclusively, bacterial agents involved in intestinal infections were not found in river water, Salmonella excluded. Salmonella were identified in 433 of the analyzed samples. The comparison made per 5-year periods established that the share (specific weight) of river water samples taken near the municipal water intake with six identified Salmonella serotypes in them varied within the range between 0.32 in 2011–2015 and 0.80 in 2005–2010 (31.67–79.71 %). The same indicator identified for water in the recreational area near the community beach turned out to be considerably higher and varied between 0.82 in 2011–2015 and 0.98 in 2016–2020 (81.67–97.73 %). The maximum most-probable-number (MPN) of eight isolated salmonella serotypes registered over the analyzed period was equal to $2.4 \cdot 10^4$ MPN/100 cm^3. In 2016-2020, the share of water samples with detected 10 Salmonella serotypes reached 1.00 (100 %) in the sewage drainage area, which indicates that the sanitary-epidemiological situation is extremely unfavorable in this area. Although such a serotype of Salmonella enterica subsp. Enterica as Salmonella typhimurium was prevalent among Salmonella isolated from river water, contrast spatial differences were established for all other Salmonella serotypes. Thus, Salmonella derby and Salmonella Heidelberg serotypes were typical in water from the water intake area but Salmonella london and Salmonella essen prevailed in the recreational area whereas Salmonella london and Salmonella derby were the most typical for sewage water. Salmonella enteritidis were found only in river water taken downstream from the sewage drainage area (Tables 1 and 2).

We analyzed the correlation matrix that combined quantitative data on levels of all identified microorganisms in 540 analyzed river water samples, As a result, an authentic (at $p < 0.01$) pair correlation was identified; a strong direct correlation was established between such sanitary-significant microorganisms as total coliforms and glucose positive

Table 2

Salmonella serotypes isolated from river water in the Azov City water area in the Rostov region over 2005–2020

Salmonella serotypes	Monitoring points		
	Water intake area	Recreational area	Sewage drainage area
S. typhimurium	32.99	34.16	19.43
S. heidelberg	14.43	4.97	9.71
S. derby	22.68	6.21	12.57
S. essen	8.25	16.77	10.29
S. reading	12.37	3.11	Not found
S. bredeney	9.28	7.45	8.57
S. london	Not found	18.01	12.00
S. brandenburg	Not found	9.32	Not found
S. enteritidis	Not found	Not found	19.43
S. montevideo	Not found	Not found	1.14
S. chester	Not found	Not found	2.86
S. mission	Not found	Not found	4.00

coliforms (TCs and GPCs) as well as between potentially pathogenic Klebsiella and Pseudomonas aeruginosa; a medium direct correlation was established between thermotolerant coliforms (TTCs) and Klebsiella, TTCs and Pseudomonas aeruginosa, GPCs and TTCs, TCs and TTCs. Salmonella levels in the analyzed river water samples were not established to correlate with levels of any other identified microorganisms (Table 3).

Overall, over the analyzed 16-year period, very high microbial risk was established for water in the water intake area and high risk – for water in the recreational area with their R_a and R_b values equal to 0.655 and 0.574 respectively. These values were established relying on the five-level classifier per the integral indicators of the microbial risk associated with the spread of waterborne intestinal infections. The value of the calculated two-factor complex indicator of the microbial risk (*Rab*) was equal to 0.612, which means very high level. This risk was caused entirely by sanitary-bacteriological indicators of water quality and combines such sanitary-hygienic factors as Sources of Centralized Drinking and Household Water Supply and Recreational Water Use (Table 4).

We assessed share contributions made by primary sanitary-microbiological indicators (X_i) to the structure of Sources of Centralized Drinking and Household Water Supply sanitary-hygienic factor. As a result, the priority contribution (46.96 %) was established to be made by the primary indicator ‘The share of river water samples with identified potential pathogens (Klebsiella and Blue pus bacilli)’ (*X9*). The next three rank places in the structure of this

Table 3

Correlation matrix of sanitary-bacteriological indicators of water in the Lower Don River within the Azov City territory in the Rostov region over 2006–2020

Indicator	TCs	TTCs	GPCs	Klebsiella	Blue pus bacilli	Salmonella
TCs	1.000	0.513	0.993	0.048	0.030	0.083
TTCs	0.513	1.000	0.547	0.611	0.475	0.038
GPCs	0.993	0.547	1.000	0.126	0.103	0.088
Klebsiella	0.040	0.611	0.126	1.000	0.817	0.011
Blue pus bacilli	0.030	0.475	0.103	0.817	1.000	0.023
Salmonella	0.083	0.038	0.088	0.011	0.023	1.000

Note: TCs means total coliforms; TTCs, thermotolerant coliforms; GPCs, glucose positive coliforms.

Table 4

Potential microbial risk of waterborne intestinal infections depending of sanitary-bacteriological indicators of water in the Lower Don River within the Azov City area in 2005–2020

Indicator	Points for sanitary-bacteriological monitoring of water in the Don River	
	Water intake area	Recreational area
Integral indicators of the microbial risk associated with the spread of waterborne intestinal infections per the results of sanitary-bacteriological monitoring of river water (estimated factors are Sources of Centralized Drinking and Household Water Supply and Recreational Water Use)		
The share of river water samples with TCs levels being higher than safe standards – 1000 CFU/cm^3 for the water intake area (*X1*, %) and 500 CFU/cm^3 for the recreational area (*X2*, %)	87.78	98.33
Adjusted values (*P1* and *P2*) at weight coefficients *V1* = 0.7 for the water intake area and *V2* = 0.8 for the recreational area	1.00	1.00
Weighted microbial risk indexes for river water in the water intake area (*A1 = P1·V1*) and the recreational area (*A2 = P2·V2*)	0.70	0.80
Average TCs level (CFU/100 cm^3) in river water in the water intake area (*X3*) and the recreational area (the community beach) (*X4*)	13,601.95	42,916.88
Adjusted values (*P3* and *P4*) at weight coefficients *V3* = 0.8 for the water intake area and *V4* = 0.9 for the recreational area	1.00	1.00
Weighted microbial risk indexes for river water in the water intake area (*A3 = P3·V3*) and the recreational area (*A4 = P4·V4*)	0.80	0.90
The share of river water samples with identified pathogenic bacteria involved in acute intestinal infections in the water intake area (*X5*, %) and the recreational area (*X6*, %)	0.00	0.00
Adjusted values (*P5* and *P6*) at weight coefficients *V5* = 2.0 for the water intake area and *V6* = 2.0 for the recreational area	0.00	0.00
Weighted microbial risk indexes for river water in the water intake area (*A5 = P5·V5*) and the recreational area (*A6 = P6·V6*)	0.00	0.00
The share of river water samples with identified Salmonellas in the water intake area (*X7*, %) and the recreational area (*X8*, %)	53.89	89.44
Adjusted values (*P7* and *P8*) at weight coefficients *V7* = 0.8 for the water and *V8* = 1.0 for the recreational area	1.00	1.00
Weighted microbial risk indexes for river water in the water intake area (*A7 = P7·V7*) and the recreational area (*A8 = P8·V8*)	0.80	1.00
The share of river water samples with identified potential pathogens (Klebsiella and Blue pus bacilli) in the water intake area (*X9*, %)	100.00	—
Adjusted value (*P9*) for the water intake area at weight coefficient *V9* = 1.5	1.00	—
Weighted microbial risk index for river water in the water intake area (*A9 = P9·V9*)	1.50	—
Sums of weight coefficients for the water intake area (*Wa = V1+V3+V5+V7+V9*) and the recreational area (*Wb = V2+V4+V6+V8*)	5.80	4.70
Integral microbial risk indicators for the water intake area (*Ra*) and the recreational area (*Rb*), calculated per the following formulas: *Ra = 1 / Wa · (A1+A3+A5+A7+A9)* and *Rb = 1 / Wb · (A2+A4+A6+A8)*	0.655	0.574
Assessment of integral microbial risk indicators for the water intake area (*Ra*) and for the recreational area (*Rb*) Per the five-level classifier	very high	very high
Complex indicator of the microbial risk associated with intestinal infections per the results of sanitary-bacteriological monitoring of river water (estimated factors are Sources of Centralized Drinking and Household Water Supply and Recreational Water Use)		
Weight coefficients for the estimated factors Sources of Centralized Drinking and Household Water Supply (*Ga*) and Recreational Water Use (*Gb*)	0.7	0,8

End of the Table 4

The sum of weight coefficients (*Wab*) for the estimated factors Sources of Centralized Drinking and Household Water Supply and Recreational Water Use: $Wab = Ga + Gb$	1.5
Complex indicator of potential microbial risk (*Rab*) of intestinal infections depending on sanitary-bacteriological indicators of river water quality: $Rab = 1 / Wb \cdot (Ga \cdot Ra + Gb \cdot Rb)$	0.612
Assessment of complex potential microbial risk indicator associated with intestinal infections for the estimated factors Sources of Centralized Drinking and Household Water Supply and Recreational Water Use per five-level classifier	very high

factor belonged to 'Average TCs level' (X_3), 'The share of river water samples with TCs levels above 1000 CFU/cm^3' (*X1*) and 'The share of river water samples with identified Salmonellas' (*X7*) with their contributions being equal to 21.73, 19.32 and 11.99 % respectively. As regards the integral microbial risk indicator for Recreational Water Use factor (R_b), primary indicators were ranked as follows: 'Average TCs level' (X_4), 'The share of river water samples with identified Salmonellas' (X_8) and 'The share of river water samples with TCs levels above 500 CFU/cm^3' (X_2) with their contributions being equal to 34.81, 33.90 and 31.29 % respectively.

Periods with elevated risks of waterborne intestinal infections were determined using the results obtained by analyzing annual dynamics. Thus, calculated upper bounds of the year-round level of its integral indicators amounted to 0.558 for the Sources of Centralized Drinking and Household Water Supply factor (R_a), 0.545 for the Recreational Water Use factor (R_b) and 0.554 for the two-factor complex indicator (R_{ab}) at the seasonality coefficients being 53.50, 52.06 and 52.87 % respectively. We compared typical annual curves built for potential microbial risk with the upper bounds of the year-round level. As a result, we established that a seasonal rise in it occurred in a period from June to November in the water intake area and from July to November in the recreational area, which was located downstream. The results obtained by analyzing the annual dynamics of sanitary-bacteriological indicators give evidence that a seasonal increase in microbial risk in June-August is due to growing levels of TCs, GPCs, Klebsiella and Blue pus bacilli in river water at the seasonality indexes per Warringer, which are determined as a ratio between the indicators in months with maximum and minimum levels, 6.93, 23.94, 12.43 and 15.01 respectively. The second seasonal rise in microbial risk in October-November correlates with growing Salmonella levels in river water at the seasonality index per Warringer being equal to 12.26 (Table 5, Figure 2).

We performed retrospective analysis of long-term dynamics over 2005–2020 with qualitative and quantitative assessment of the existing trends; the analysis results are evidence of stably high two-factor integral indicators of potential microbial risk associated with the spread of waterborne intestinal infections per the sanitary-hygienic factors Sources of Centralized Drinking and Household Water Supply (*Ra*) and Recreational Water Use (*Rb*) with their average long-term growth rates equal to +0.06 % and 0.00 % respectively. Aiming to raise drinking water quality in the centralized water supply system in the Azov City, a set of optimization activities was implemented; technical equipment at water treatment facilities was brought in conformity with the modern requirements and safe standards, water treatment technologies were optimized and repairing and maintenance works were accomplished in considerable scopes at the municipal water supply system. Due to these activities, since 2009, there have been stable descending trends in microbial risk indicators, both the integral one per the Centralized Drinking and Household Water Supply factor (*Rc*) and the three-factor complex indicator (*Rk*) with their annual decline rates being equal to -4.87 and -1.63 % respectively (Table 6, Figure 3).

Table 5

Seasonal peculiarities of potential microbial risk associated with waterborne intestinal infections in the Azov City over 2005–2020

Indicator	Months of observation											
	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
Integral indicators of potential microbial risk per estimated factor Sources of Centralized Drinking and Household Water Supply (*Ra*) The seasonality coefficient is 53.50 % / the seasonality index per Warringer is 1.279 / The upper bound of the year-round level is 0.558												
Typical annual curve *Ra*	0.514	0.499	0.521	0.525	0.536	0.601	0.611	0.569	0.569	0.587	0.599	0.478
Seasonal variation index (%)	0.921	0.894	0.933	0.941	0.960	1.076	1.095	1.020	1.019	1.052	1.073	0.856
Integral indicators of potential microbial risk per estimated factor Recreational Water Use (*Rb*) The seasonality coefficient is 52.06 % / the seasonality index per Warringer is 1.242 / The upper bound of the year-round level is 0.545												
Typical annual curve *Rb*	0.462	0.516	0.535	0.531	0.535	0.544	0.574	0.574	0.561	0.548	0.574	0.528
Seasonal variation index (%)	0.848	0.946	0.982	0.974	0.982	0.998	1.053	1.053	1.029	1.005	1.053	0.969
The complex indicator of potential microbial risk associated with intestinal infections for the estimated factors Sources of Centralized Drinking and Household Water Supply and Recreational Water Use (*Rab*) The seasonality coefficient is 52.87 % / the seasonality index per Warringer is 1.366 / The upper bound of the year-round level is 0.554												
Typical annual curve *Rab*	0.433	0.508	0.550	0.536	0.538	0.570	0.592	0.572	0.565	0.566	0.586	0.511
Seasonal variation index (%)	0.782	0.917	0.992	0.968	0.971	1.030	1.068	1.032	1.019	1.022	1.057	0.923

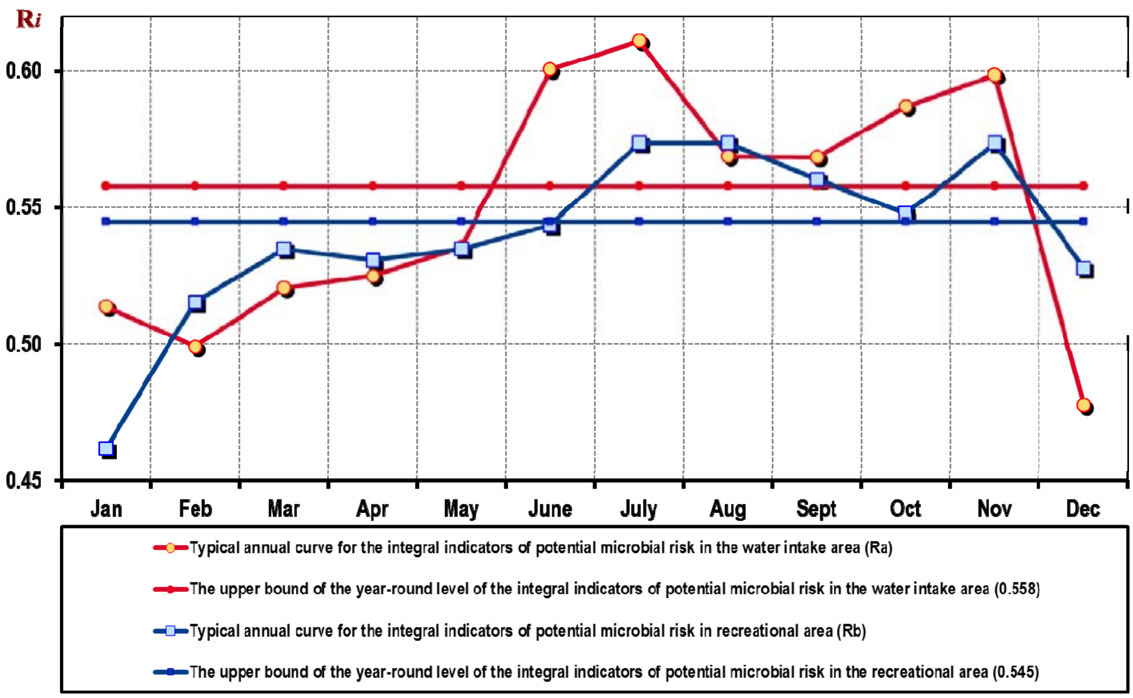

Figure 2. Seasonal peculiarities of potential microbial risk associated with waterborne intestinal infections in the Azov City over 2005–2020

Table 6

Dynamics over 2005–2020 and predictions of indicators of potential microbial risk associated with waterborne intestinal infections in the Azov City in the Rostov region

Years	Integral indicators of potential microbial risk per specific sanitary-hygienic factors: Sources of Centralized Drinking and Household Water Supply (*Ra*)	Recreational Water Use (*Rb*)	Centralized Drinking and Household Water Supply (*Rc*)	The complex indicator of potential microbial risk associated with waterborne intestinal infections (*Rk*)
2005	0.655	0.574	0.560	0.591
2006	0.655	0.574	0.497	0.566
2007	0.566	0.574	0.500	0.542
2008	0.655	0.574	0.820	0.695
2009	0.655	0.574	0.560	0.591
2010	0.655	0.574	0.570	0.595
2011	0.655	0.574	0.492	0.564
2012	0.652	0.574	0.355	0.508
2013	0.655	0.574	0.412	0.532
2014	0.653	0.574	0.347	0.505
2015	0.655	0.574	0.292	0.484
2016	0.655	0.574	0.318	0.494
2017	0.655	0.574	0.284	0.481
2018	0.655	0.574	0.282	0.480
2019	0.655	0.574	0.277	0.478
2020	0.594	0.574	0.298	0.469
2005–2020	Average long-term levels of integral and complex indicators of potential microbial risk associated with waterborne intestinal infections			
	0.645 ± 0.014	0.574 ± 0.000	0.403 ± 0.066	0.525 ± 0.026
	Models of long-term dynamics (2005–2020) of potential microbial risk associated with waterborne intestinal infections (*X* is the ordinal number of a year in a dynamic series)			
	$Ra = 0.641+0.005 \cdot log(X)$	$Rb = 0.574+0.000 \cdot log(X)$	$Rc = 0.657–0.287 \cdot log(X)$	$Rk = 0.625–0.113 \cdot log(X)$
	Average annual growth / decline rates in trends of potential microbial risk associated with waterborne intestinal infections (%)			
	+ 0.06	0.00	-4.87	-1.63

Artificial two-layer neural networks			Cascade-Forward backprop	Feed-Forward backprop
Artificial two-layer neural networks	Neural network type		Cascade-Forward backprop	Feed-Forward backprop
	The number of neurons in hidden layer		23	30
Predictions based on ANN models using data collected over 2005–2018		2019	0.291	0.488
		2020	0.279	0.481
Regression prediction models over 2005–2018			$Rc = 0.605–0.026 \cdot X$	$Rk = 0.621–0.104 \cdot log(X)$
Predictions based on regression models and data over 2005–2018		2019 ($X = 15$)	0.243	0.498
		2020 ($X = 16$)	0.217	0.496
Inaccuracies in ANN-based predictions	abs.	2019	0.014	0.010
	%		5.05	2.09
	abs.	2020	0.019	0.012
	%		6.38	2.56
Inaccuracies in predictions based on regression models	abs.	2019	0.034	0.020
	%		12.27	4.18
	abs.	2020	0.081	0.027
	%		27.18	5.76
Ration between absolute inaccuracies in predictions based on ANN and regression models		2019	2.429	2.000
		2020	4.263	2.250
Difference between relative inaccuracies in predictions based on ANN and regression models (%)		2019	7.22	2.09
		2020	20.81	3.20

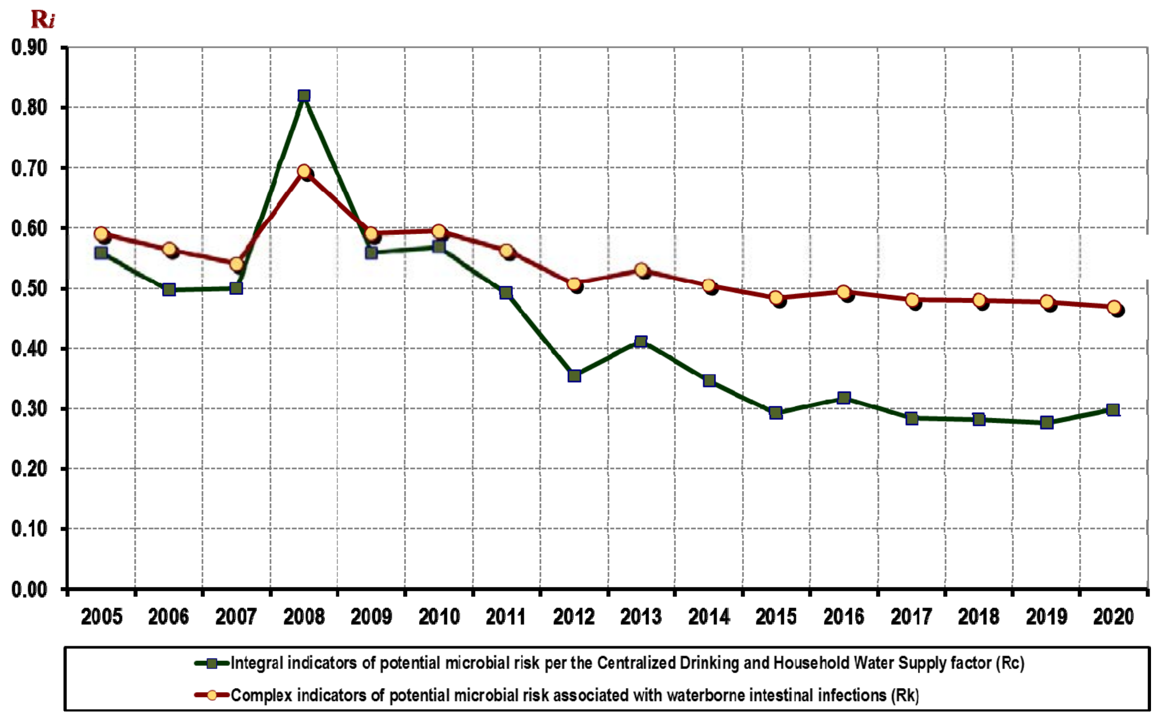

Figure 3. Long-term dynamics of potential microbial risk associated with waterborne intestinal infections in the Azov City over 2005–2020

We comparatively assessed precision of middle-term predictions of microbial risk associated with waterborne intestinal infections, which were obtained by using two alternative methods, a conventional extrapolation per regression lines and artificial neural networks. The assessment was based on their absolute and relative inaccuracies against the actual indicators calculated for 2019 and 2020. ANN-based predictions were proven to be more precise both for the Centralized Drinking and Household Water Supply factor (R_c) and for the three-factor complex indicator (R_k) of potential microbial risk associated with the spread of waterborne intestinal infections (Table 6).

The results obtained by retrospective analysis over 2005–2020 are evidence of a relatively unfavorable epidemiological situation with incidence of total AII and salmonellosis among the Azov City population, which was respectively 1.2 and 2.18 times higher than the regional average (Table 7).

We revealed a direct medium significant ($p < 0.01$) correlation between monthly indicators over 2005–2020: prevalence of total AII and TCs levels in water in the water intake area at the pair correlation coefficient being equal to 0.513; prevalence of total AII and salmonellosis, on one hand, and values of calculated complex indicators of potential microbial risk associated with waterborne intestinal infections (*Rk*), on the other hand, at the relevant pair correlation coefficients being equal to 0.547 and 0.494 respectively.

The accomplished study focuses on the topical issue of minimizing risks associated with epidemic spread of waterborne intestinal infections. The basic results of the present study include objective description of water microbiocenosis in the Azov City water area as the most contaminated section of the Lower Don River as well as in the centralized drinking and household water supply system based on the data obtained by sanitary-bacteriological monitoring over 2005–2020. In addition to analyzing river and drinking water in order to identify indicative microflora (total and thermotolerant coliforms) in them, we tested river and drinking water to identify pathogenic enteric bacteria in them including Salmonella as well as the most epidemically significant common non-fermenting gram-negative potential pathogens, which are typical representatives of Enterobacteriaceae and Pseudomonadaceae –

Table 7

Incidence of acute intestinal infections and salmonellosis in the Azov City in dynamics over 2005–2020

Year		Dysentery	Other AII	Total AII	Salmonellosis
2005		71.20	322.30	393.50	19.30
2006		22.42	348.08	370.50	34.86
2007		17.32	355.08	372.40	44.07
2008		18.48	399.82	418.30	48.17
2009		21.86	364.84	386.70	54.25
2010		16.78	385.32	402.10	49.20
2011		18.00	330.50	348.50	54.08
2012		9.64	287.94	297.58	68.67
2013		7.24	373.00	380.24	38.62
2014		2.40	503.50	505.90	40.95
2015		3.60	623.10	626.70	37.58
2016		0.00	470.60	470.60	37.80
2017		1.22	613.20	614.42	56.19
2018		2.46	529.90	532.36	50.41
2019		7.43	517.80	525.23	48.31
2020		3.74	277.72	281.46	7.47
2005–2020	Azov City	14.67 ± 10.04	428.33 ± 61.27	443.00 ± 56.24	45.50 ± 6.58
	Rostov region	14.02 ± 2.15	354.19 ± 30.16	368.20 ± 28.99	20.90 ± 2.11

Klebsiella spp. and Blue pus bacilli (Pseudomonas aeruginosa). Additionally, we determined such a highly informative integral indicator of potential hazard associated with acute intestinal infections as glucose positive coliforms (GPCs). The accomplished tests established excessive microbial contamination in river water, which were higher than safe standards and tended to deteriorate substantially along the river stream in the Azov City water area. The results obtained by analyzing the correlation matrix are evidence that GPCs levels in the Lower Don River water can be considered a universal indicator of risk associated with waterborne intestinal infections. This is in line with opinions expressed by some other authors that use of this indicator in sanitary-bacteriological monitoring ensures reliable control over epidemiological safety of water due to the fact that bacteria included in the GPCs indicator are able to survive for a long time in water objects and drinking water and are highly resistant to disinfectants. These properties of the said bacteria are either equal or even surpass the same characteristics of pathogens and potential pathogens [6, 7, 11, 14, 21, 25].

Use of mathematical models for calculating integral and complex indicators that describe levels of potential epidemic hazard associated with waterborne intestinal infections depending on sanitary-hygienic conditions of water use are an effective instrument for assessing health risks caused by bacterial pathogens. This is evidenced by the results obtained by long-term research both in Russia and abroad [14, 18, 19, 21, 24, 25]. Our study established differentiated contributions made by specific sanitary-bacteriological indicators to the structure of potential risks associated with waterborne intestinal infections. This risk was estimated as very high both per the value of the two-factor complex indicator of microbial risk, which was a derivative from the values of its integral indicators for the Sources of Centralized Drinking and Household Water Supply and Recreational Water Use factors, and per the three-factor complex indicator of microbial risk calculated considering values of the integral indicators per the Centralized Drinking and Household Water Supply factor. Analysis of typical annual curves and upper bounds of the year-round level of potential microbial risk made it possible to reveal and quantify its summer-autumn seasonality caused by high levels of potentially pathogenic microflora in June – August and Salmonella in October – November in river water. The results obtained by analyzing long-term dynamics in levels of pathogens (Salmonella) and potential pathogens isolated from water in the Lower Don River are largely consistent with data reported by some other authors [6, 7, 14]. We established that though sanitary-bacteriological indicators of river water were stably poor, still a favorable descending trend occurred for the complex three-factor indicator of potential microbial risk associated with the spread of waterborne intestinal infections. This was due to improving quality of water in the centralized drinking and household water supply system in the Azov City. We performed comparative testing of methods for predicting potential microbial risks, which employed regression models and ANN-based models. The testing confirmed higher precision of predictions derived from using artificial neural networks. Hydrosphere pollution is a serious challenge since the occurrence of pathogens and potential pathogens in water is evidence of potential risks associated with outbreaks and epidemic spread of intestinal infections as reported in multiple studies by Russian and foreign researchers [6, 7, 18, 21, 25–27]. The results obtained by retrospective analysis of incidence of acute intestinal infections and salmonellosis in the Azov City using the pair correlation analysis confirm its dependence on levels of potential microbial risk.

Conclusion. Therefore, the study has established stably excessive bacterial contamination in the Azov City water area, which is higher than safe standards. This allows estimating potential microbial risk associated with waterborne intestinal infections as very high. It is primarily caused by potentially pathogenic microorganisms (Klebsiella and Blue pus bacilli) as well as Salmonella.

Technologies for building mathematical models that describe outbreak and spread of waterborne bacterial intestinal infections should be implemented in social-hygienic monitoring activities. This is a reliable instrument for accomplishing prospective analysis of an epidemic situation relying on data about sanitary-microbiological quality indicators of water in open watercourses and water bodies as well as drinking water. Assessment of potential microbial risk based on a complex of considered sanitary-hygienic factors is a significant component in information and analytical support in developing optimization management decisions aimed at preventing epidemic spread of waterborne intestinal infections. High precision in predicting how an epidemic situation might develop can be achieved by using artificial neural networks.

Funding. The research was not granted any sponsor support.

Competing interests. The authors declare no competing interests.

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Received: 22.07.2024
Approved: 28.11.2024
Accepted for publication: 19.12.2024