International Journal of
Health Systems and Medical Sciences

ISSN: 2833-7433
Volume 2 | No 9 | Sep -2023

Peculiarities of Seeding of Gram-Positive Cocci and Gram-Negative Bacteria from Water Samples of Different Types of R Eservoirs

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Abstract: The goal was to determine and compare the microbial landscape of water samples from different types of reservoirs in Uzbekistan. It has been established that, according to microbiological indicators, the quality of water samples from the Tuyamuyun hydroelectric complex is significantly worse than the same indicators from the Kattakurgan and Charvak reservoirs. It is recommended to use Enterococcus spp and Staphylococcus spp as an addition to reservoir water quality hygiene regulations.

Keywords: Enterococcus, Kattakurgan, Charvak, inoculability, microflora.

The significance of the degree of inoculation of pathogenic microorganisms in the water of various reservoirs is important for carrying out preventive and ongoing sanitary surveillance, as well as for organizing effective anti-epidemic measures [3, 4, 5].

The study and assessment of the inoculability of pathogenic and sanitary-indicative microorganisms from water samples of reservoirs is of general biological and theoretical interest, since it allows us to determine the limits of viability of microorganisms and their relationships, which are found in abundance in reservoirs [1, 6, 7, 9].

Modern sanitary and microbiological laboratory diagnostics of environmental objects, including reservoir water, is of great importance, since the correct determination of the presence of a pathogenic microorganism, the quantitative and qualitative composition of the microflora of water is the key to the prevention of various sporadic diseases and outbreaks of intestinal diseases transmitted by water among the population [6, 10].

It is known that the most complete assessment of the sanitary condition of a surface reservoir, including reservoirs, is achieved by conducting a complex of hydrochemical, sanitary-hygienic, hydrobiological and bacteriological studies [8].

Considering the above, **the purpose** of this study was to conduct microbiological studies to determine and comparatively characterize the microbial landscape of the water of various reservoirs in Uzbekistan.

Materials and methods. To achieve the goal, we studied water samples from the Kattakurgan, Charvak reservoirs and the Tuyamuyun hydroelectric complex, which includes the Ruslovoe, Sulton Sanzhar and Kaparas reservoirs. Selection and delivery of water samples was carried out according to Alieva S.K. and co-authors [2].

The following microbiological indicators were studied: determination of the total number of

mesophilic aerobic and facultative anaerobic microorganisms (FAM); determination of common and thermotolerant coliform bacteria (TCB and TCB); o identification of pathogenic bacteria (*Salmonellaspp* , *Schigellaspp*) and opportunistic microorganisms (*Escherichiaspp* , *Enterococcus spp* , *Staphylococcus spp*).

All microbiological studies on the identification and differentiation of microorganisms, as well as recording the results, were carried out using traditional methods [2, 10, 11]. For homogeneity of the studies, they were carried out under the same conditions, instruments and used the same nutrient media from HiMedia (India).

Statistical processing was carried out using the method of variation statistics. All calculations were carried out on a personal computer based on PentiumIV processors using a package of application programs for biomedical research.

Research results and discussion. To compare the results obtained, microbiological indicators given in SanPiN No. 0172-04 and O´ zDSt 950-2011 were used. "Drinking water. Hygienic requirements and quality control", in the form of a standard.

The study and analysis of microbiological indicators of water samples from the Kattakurgan reservoir showed that the total number of TCB and TCB were the smallest in water samples below the dam - 500 CFU/100 ml, which corresponds to normal values for water bodies of category I water use, but 2.0 times lower than normal for water bodies of water use category II ($P<0.05$). In other samples obtained from different places of the reservoir, this indicator was 2-9 times higher ($P<0.01$) than the indicators below the dam (500 CFU/100 ml) - respectively 1100 CFU/100 ml (middle of the reservoir), 5200 CFU/ 100 ml (above the dam) and 9400 CFU/100 ml (recreational area).

It is known that the determination of TMC in the water of open reservoirs is carried out when it is necessary to know the degree of contamination of a water source and when studying water according to epidemic indicators [6]. In all water samples taken from different places, TMC was higher than the selected norm - no more than 100 CFU/100 ml [12]. This was especially clearly expressed in water samples from the recreational area (9400 CFU/100 ml).

Thus, the study of microbiological indicators of water samples from the Kattakurgan reservoir showed that the total amount of TCB and TCB exceeded the selected normal values in water samples from the middle of the reservoir by 2.2 times ($P<0.05$), and water samples obtained above the dam by 10 .4 times ($P<0.001$), from the recreational zone 18.8 times ($P<0.001$), only the indicators below the dam were within the normal range. Similar indicators were obtained for TMC parameters ($P<0.05$) from water samples below the dam.

Studies to study microbiological parameters were also carried out with water samples from the Tuyamuyun hydroelectric complex. The results show that the highest parameters of the total amount of CFU and TCB were determined in water samples below the dam of the Ruslovoe reservoir and in the outlet channel (Amu Darya River) - 13,000 CFU/100 ml, respectively. The smallest amount was found in water samples from the Sult on Sanzhar reservoir (500 CFU/100 ml), this amount corresponds to the standard values [SanPiN RUz No. 0172-04; 12].

Water parameters from the Kaparas and Ruslovoe reservoirs (above the dam) were also higher than normal ($P<0.05$), but significantly lower than the parameters below the dam and the outlet channel ($P<0.01$). TMC indicators in all water samples, regardless of the place of collection, were more than 300 CFU/100 ml, which exceeded the standard values.

Thus, microbiological studies of water samples from different parts of the Tuyamuyun hydroelectric complex showed that the total amount of OKB and TKB exceeded the standard from 4.5 to 26.0 times ($P < 0.001$), only in water samples from Sulton Sanzhar this parameter did not exceed the selected norm ($P>0.05$). This fact is explained by the fact that the water in the Sulton Sanzhar reservoir comes from the Ruslovoe reservoir and settles there. Apparently, microorganisms, together with chemicals and suspended particles of clay and sand, settle to the bottom of the Sulton Sanzhar reservoir. In addition, in this reservoir (Sulton Sanzhar) there is practically no water movement and this eliminates the rise of suspended particles from the bottom of the reservoir. According to

microbiological indicators, the quality of water samples from the Tuyamuyun hydroelectric complex is significantly worse than the same indicators from the Kattakurgan reservoir ($P < 0.05$).

Microbiological studies were also carried out with water samples from the Charvak reservoir. The results obtained are interesting because in water samples from the middle of the reservoir and above the dam, the results were below the upper limits of the norm for water bodies of water use category II. Indicators of the total number of TCB and TCB of water samples from the recreational area (1200 CFU/100 ml) and below the dam (800 CFU/100 ml) were within the normal range for water bodies of category I water use (norm - 1000 CFU/100 ml).

TMC indicators in all water samples, regardless of the place of collection from the Charvak reservoir, were within the specified standard values. Only the TMC parameters of the recreational zone and below the dam were at the level of the upper limits of the norm (120 CFU/100 ml and 70 CFU/100 ml, respectively), and in other water samples the TMC was 3.3 and 5.0 times lower than the upper limits norms. If we compare the microbiological indicators of this reservoir with other water bodies described above, it is noted that in terms of the total number of OKB and TKB, OMC indicators are many times reduced and are within the specified standards. This is the main difference between the water of the Charvak reservoir compared to the water from the Kattakurgan reservoir, and especially the water of the Tuyamuyun hydroelectric complex.

Thus, the determination of the total amount of OKB and TKB, TBC from water samples of the Charvak reservoir showed that these parameters were at the level of the upper limits of the norm for water bodies of the II category of water use (water samples above the dam and the middle of the reservoir) and the upper limits of the norm for water bodies of the I category of water use (water samples from the recreational area and below the dam). It was established that the water quality of the Charvak reservoir in terms of microbiological indicators was many times higher than the similar indicators of the Kattakurgan reservoir and the Tuyamuyun hydroelectric complex.

The next stage of our research was to study the inoculability of pathogenic and opportunistic microorganisms from water samples of the studied reservoirs. Microbiological studies were devoted to the identification and differentiation of *Shigellaspp, Salmonellaspp, Staphylococcusspp* and *Enterococcusspp*. The values of Staphylococcus *spp and Enterococcus spp were* studied as sanitary indicator microorganisms in order to justify additions to hygienic regulations for water quality in reservoirs.

Microbiological studies to study the inoculability of the above microorganisms in water samples of the Kattakurgan reservoir showed that, regardless of the location of water sampling, it was not possible to identify *Shigellaspp, Salmonellaspp, Staphylococcusspp* and *Enterococcusspp*.

Apparently, this is due to the fact that the sanitary protection zones of the reservoir were strictly observed and prescribed anti-epidemic measures; moreover, the time of taking water samples coincided with the filling of the reservoir, where the water movement was significant.

Similar studies were carried out with water samples from the Tuyamuyun hydroelectric complex. The results obtained show that *Shigellaspp, Salmonellaspp, Staphylococcusspp* and *Enterococcusspp* were not sown in water samples from the Kaparas and Sulton Sanzhar reservoirs; in addition, these microorganisms could not be differentiated in water samples above the dam of the Ruslovoye Reservoir. *Shigellaspp* and *Salmonellaspp were not detected* in water samples below the dam, but *Escherichiaspp, Staphylococcusspp* and *Enterococcusspp* were cultured in some water samples - 33.0% and 66.0%, respectively. The same microorganisms were identified from water samples from the outlet channel - the Amu Darya River (66.0% respectively).

Thus, *Escherichiaspp, Staphylococcusspp* and *Enterococcusspp were sown from water samples of the Tuyamuyun hydroelectric complex*, but they were found only in water samples below the dam and the diversion channel within the range of 33.0-66.0%. In the Kaparas, Sulton Sanzhar and Ruslovoe reservoirs (above the dam), these microorganisms did not differentiate. As in the Kattakurgan reservoir, *Shigellaspp and Salmonellaspp* were not found in water samples from the Tuyamuyun hydroelectric complex, regardless of the sampling location.

Escherichiaspp were identified in water samples from the recreational area and below the dam (100.0% and 33.0%, respectively), as well as *Enterococcusspp* in the recreational area in 33. 0% cases. *Shigellaspp* and *Salmonellaspp* were not identified.

Thus, it was established that *Escherichiaspp, Enterococcusspp* and *Staphylococcusspp* were sown only on the banks (at a distance of 1 meter from the shore) of reservoirs where there were recreational areas or livestock grazing (P<0.05). Starting from a distance of 5 meters and further, as well as at a depth of 20 cm and more, pathogenic and opportunistic microorganisms were not sown in all studied reservoirs (P>0.05). Apparently, *Enterococcusspp and Staphylococcusspp* can be used as an addition to the hygienic regulations for water quality in reservoirs, and their detection even in small quantities can be considered a factor of microbial contamination and a risk factor for recreational areas of reservoirs.

Conclusions.

1. It was established that the total number of OKB and TKB water samples of the Tuyamuyun hydroelectric complex exceeded the standard from 4.5 to 26.0 times, only in the Sulton Sanzhar water samples this parameter did not exceed the selected norm. This fact is explained by the fact that the water in the Sulton Sanzhar reservoir comes from the Ruslovoe reservoir, where the water settles and clarified water is formed in Sulton Sanzhar. Apparently, microorganisms, along with chemicals and suspended particles, settle to the bottom of this reservoir.
2. According to microbiological indicators, the quality of water samples from the Tuyamuyun hydroelectric complex is significantly worse than the same indicators from the Kattakurgan and Charvak reservoirs (P<0.001) .
3. It was revealed that *Enterococcusspp* and *Staphylococcusspp* were sown only on the banks (at a distance of 1 meter from the shore) of reservoirs where there were recreational areas or livestock grazing (P<0.05). Starting from a distance of 5 meters and further, as well as at a depth of 20 cm and more, pathogenic and opportunistic microorganisms, including *Enterococcus spp* and *Staphylococcus spp*, were not sown in all the studied reservoirs.
4. *Enterococcusspp* and *Staphylococcusspp* are recommended to be used as an addition to the hygienic regulations for water quality in reservoirs and their detection can be considered as a factor of microbial contamination and a risk factor for recreational areas of reservoirs.

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