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Original Research Article

DEVELOPMENT OF STANDARDS FOR MICROBIAL INDICATORS OF WATER BODIES USED FOR DRINKING AND RECREATIONAL WATER USE

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Abstract: Uzbekistan belongs to the regions with hot climate, where there is a deficit of water resources. As of January 1, 2025, 78.4% of urban and 26.6% of rural population is provided with centralized water supply, while the rest of the population uses underground and surface water sources for drinking purposes. Surface water bodies are widely used throughout the year for cultural and recreational (bathing and water sports) purposes. However, surface water bodies may contain pathogens of intestinal infections (Str. Faecalis) and upper respiratory tract infections (St. Aureus). Intestinal and adenovirus diseases, conjunctivitis, staphylococcal infections occur in the population after contact with contaminated water. In this regard, the purpose of the study was to develop standards for the content of enterococci and staphylococci in the water of drinking and recreational water bodies. The subject of research was the study of survival rate of sanitary-indicative microorganisms, their resistance to disinfectants chlorine and phenol, as well as the evaluation of indicator significance of sanitary-indicative microorganisms: enterococci, staphylococci, E. Coli and lactose-positive E. coli against pathogenic enterobacteriaceae of intestinal group S.Typhi, Sh.Flexneri salmonella and shigella. To achieve the goal, a set of experimental studies was conducted on model reservoirs, in which the necessary conditions were created to solve the tasks set in the work. Based on the results of the studies, the standards of permissible content of enterococci and staphylococci in water bodies were developed, which are recommended to be used for water quality monitoring of water bodies used for drinking and recreational water use.

Keywords: norms, drinking and recreational water bodies, water quality, enterococci, staphylococci, public health.

INTRODUCTION

The World Health Organization (WHO) recommends the determination of total coliform bacteria and Escherichia coli (E. coli) as the main bacteriological indicators characterizing the quality of drinking water. In a number of CIS countries, the determination of glucose-positive coliform bacteria and E. coli, which are considered indicators of recent fecal contamination of water, remains the primary regulated microbiological parameter for drinking water to date.

In Uzbekistan, the quality of water from sources of centralized domestic and drinking water supply is regulated by GOST 134:2024, "Sources of centralized domestic and drinking water supply. Hygienic and technical requirements and rules for selection" [1], while the quality of drinking water is regulated in accordance with GOST 133:2024 [2]. Water quality with respect to microbiological indicators is regulated based on established threshold values of indirect indicators and includes the determination of the index of bacteria of the Escherichia coli group (ECG) and the total microbial count (TMC) in water.

Uzbekistan belongs to regions with a hot climate and experiences acute water scarcity. In the republic, centralized water supply covers 78.4% of the urban population and 26.6% of the rural population. The remaining rural population uses groundwater and surface water sources for drinking purposes, which are also used almost year-round for cultural, domestic, and recreational activities [2,3,4,5].

The increasing importance of recreational water use, especially in regions with hot climates, raises the problem of preventing not only intestinal infections but also upper respiratory and skin diseases. Adenovirus diseases, conjunctivitis, and staphylococcal infections have been known to occur in people after contact with contaminated water. This necessitates the regulation of upper respiratory tract and skin pathogens in the water of water bodies of recreational water use. Representatives of coccal microflora (staphylococci) permanently inhabiting the skin and upper respiratory tract can be recommended as sanitary microorganisms [6,7,8].

To date, in Uzbekistan, the above-mentioned shows that the existing monitoring of water quality of water bodies is not effective, does not meet ISO requirements and does not guarantee epidemic safety of water bodies used for household and drinking needs and recreation [9,10]. In a number of surface water bodies in Uzbekistan, especially in the southern regions, the quality of water does not meet hygienic requirements in terms of microbiological indicators, which increases the risk of the spread of waterborne intestinal infections [12, 13, 14]. A similar situation is observed with regard to drinking water supplied to the population through centralized water supply systems, mainly in rural settlements of the Aral Sea region [15, 16, 17, 20]. At the same time, an increase in the level of anthropogenic pollution of rivers that serve as sources of water supply for the population of the republic has been noted [18, 19, 20]. To date, in Uzbekistan, there have been no studies devoted to the scientific substantiation of standards for the permissible content of enterococci and staphylococci in the water of surface water bodies. The aim of the research was to conduct a set of experiments to substantiate the standards for the content of new microbiological indicators: enterococci (Str. Faecalis) and staphylococci (St. Aureus) in the water of water bodies of drinking and recreational water use.

MATERIALS AND METHODS

To achieve the goal, several series of experiments were conducted, including the study of survival of sanitary-positive microorganisms, their resistance to disinfectants chlorine and phenol, as well as evaluation of the indicator significance of sanitary-positive microorganisms: enterococci, staphylococci, E. coli and lactose-positive E. coli against pathogenic enterobacteriaceae of the intestinal group S.Typhi, Sh.Flexneri salmonellae and shigellae for scientific purposes. Coli and lactose-positive E. coli in relation to pathogenic enterobacteria of intestinal group S.Typhi, Sh.Flexneri Salmonella and Shigella for scientific substantiation of norms of permissible content of enterococci (Str. Faecalis) and staphylococci (St. Aureus) in the water of water bodies of household drinking and recreational water use.

The and discussion. It was found that regardless of the degree of bacterial contamination of water, its origin, concentration of model strains, duration and type of inoculation (joint, separate) the rate of strain die-off is approximately the same **(Fig.1,2).**

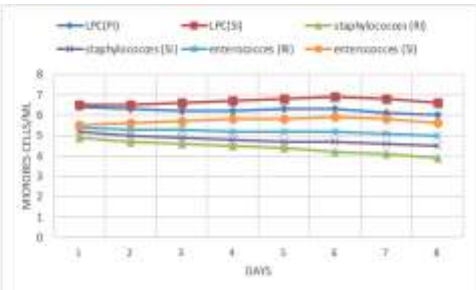

Figure 1 - Survival of (LPC), enterococci and staphylococci in autoclaved river water under separate (RI) and joint (SI) inoculation at 20^{0}C

The survival rate of lactose-positive Escherichia coli (LPC) and E.Coli does not exceed the survival rate of enterococci and staphylococci in water. Dynamics of dying off in water of model reservoirs of generally accepted sanitary-bacteriological indicators of LPC and E.Coli and newly studied enterococci and staphylococci is approximately the same and has a tendency of insignificant decrease by the end of the experiment.

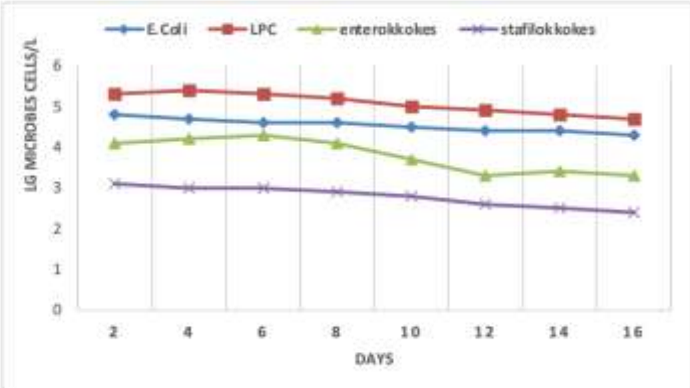

Figure 2 - Comparison of survival rates of E.Coli, LPC, Enterococci and Staphylococcus aureus in river water in reservoir models at 30^{0}C

The resistance of indicator microorganisms to the effect of disinfectants was studied in the conditions of water reservoir models under the influence of chlorine and phenol. As is known, drinking water is disinfected with different chlorine compounds depending on the initial water quality and brought to the requirements of the standard "Drinking Water".

Phenol (or carbolic acid) a hydroxyl derivative of the aromatic hydrocarbon benzene. It has a characteristic odor, antiseptic properties and is a widespread organic pollutant of surface water bodies of the republic. In addition, chlorine and phenol have the most pronounced antibacterial effect on microorganisms among chemical compounds. The above stated necessitated the study of the bactericidal effect of these widely used disinfectants on the viability of indicator microorganisms in the experiment.

As saprophytic microorganisms natural microflora of river water taken in the experiment was used. At the same time, the physical and chemical composition of river water met the requirements of the standard 951:2011 "Sources of centralized domestic drinking water supply. Hygienic, technical requirements and selection rules" The volume of water in the model reservoirs was not less than 3 liters. Antibacterial effect of disinfectants was studied at the temperature of 23-25^{0}C, which corresponds to the water temperature in the summer-autumn period of the year. The concentration of microorganisms was calculated by determining the density of suspension of daily agar cultures in isotonic solution according to the turbidity standard with subsequent dilution in sterile tap water to the required concentration. The suspension of each strain was prepared separately. The initial number of microorganisms in the model reservoirs was taken as the actual number, determined by sowing water immediately after the introduction of test cultures

Antibacterial effect of chlorine was studied at concentrations of 0.5; 1.5; and 3.0 mg/l. It was taken into account that during disinfection of water in urban water pipelines, the concentration of residual chlorine in water should be at the level of 1.5 mg/l, and in rural water pipelines are often carried out hyperchlorination and in such conditions, the concentration of chlorine in water far exceeds its permissible level. The effect of phenol on microbial die-off was studied at concentrations of 0.02; 0.1 and 0.5 mg/l. It was taken into account that MPC of phenol in water bodies was set at the level of 0.1 mg/l. The results were evaluated by comparing the rate of microorganisms dying off in model water bodies in relation to the control. Studies have established that chlorine at a concentration of 3 mg/L has a high bactericidal effect (Fig.3,4.).

By the first day of observation, the number of LPC decreased 4-fold, E.Coli - 2.72-fold, enterococci - 2.35-fold and staphylococci - 2.31-fold. The dynamics of microorganisms dying off under the influence of chlorine in concentration of 1.5 mg/l was similar, but less pronounced. Even less pronounced dynamics of microorganisms dying off on models of water bodies was noted under the influence of chlorine in concentration 0.5 mg/l. It was found that the most resistant are enterococci (Str. Faecalis) and staphylococci (St. Aureus). The least resistant are

LPC.

The obtained results were confirmed in the following series of experiments in which the antibacterial effect of phenol was studied.

Phenol at a concentration of 0.1 mg/l (at the MAC level) had a noticeable bactericidal effect. The number of LCPs by the end of the experiment decreased 2.8 times; E.Coli - 3 times; enterococci - 1.69 times and staphylococci - 1.76 times. In this series of experiments it was also found that the less resistant microorganisms to phenol are LPC .

The performed experimental studies give grounds to believe that the studied indicator microorganisms have different resistance to the action of disinfectant chemicals used in water supply practice. Direct interrelation of microorganisms dying off in water depending on time and concentration of chemical water disinfectants is noted. Among the studied test microorganisms the most resistant to the bactericidal effect of chemicals is Str.Faecalis, the indicator value of which is more pronounced in comparison with LPC and E.Coli.

Figure 3: Dynamics of die-off in water of OMFs under the influence of 3 mg/l chlorine.

Figure 4: Dynamics of die-off of enterococci in water under 3 mg/l chlorine.

The least resistant microorganisms to chlorine and phenol, regardless of exposure time and concentration of chemicals, are lactose-positive Escherichia coli. In the next series of experiments we carried out rationing of microbial contamination of household and recreational water use. As is known, the criterion of epidemic safety of water is the absence of changes in the state of public health caused by pathogens of intestinal infections spread by water. The main principle of rationing of microbial contamination of water at the present stage of development of water epidemiology and methods of sanitary and bacteriological research is the absence of intestinal pathogens of bacterial etiology in certain volumes of water for each type of water use. This principle should be used in justification and development of regulations on microbial contamination of water. Documents of water sanitary legislation should define microbiological indicators and their permissible levels at which water is considered safe in epidemic terms. Norms of indicator microorganisms are determined on the basis of quantitative relationships between the level of microbial contamination of water and the incidence of intestinal waterborne infections in the population, as well as on the basis of ratios between indicator and pathogenic microorganisms in water. Determination of microbial contamination levels by indicator microorganisms allows assessing the potential threat of waterborne transmission of pathogens of intestinal infectious diseases. The effectiveness of microbial contamination regulation is largely determined by the degree of representativeness of indicators and their permissible levels, which necessitates the development of methodological approaches to the scientific substantiation of indicators of epidemic water safety. Indicator microorganisms: enterococci (Str.Faecalis) and staphylococci (St. Aureus) were used as normalized indicators.

The methodology of regulating enterococci and staphylococci in water bodies provided for two series of experiments. In the first series of experiments, microorganisms were observed for 30 days (1, 5, 10, 20, 30) in model reservoirs. As test microorganisms were studied: LKP, E. Coli, Str.Faecalis, S.Typhi, Sh.Flexneri. In the second series of experiments, the test microorganisms were: LPC, E. Coli, St. Aureus, S. Typhi, Sh.Flexneri. Concentrations of microorganisms in the water of model reservoirs were created based on the results of field studies on quantitative distribution of indicator and pathogenic microorganisms in the water of surface reservoirs. For this purpose, maximum (Zaravshan River), minimum (Chirchik River) and average (Ahangaran River) concentrations of sanitary-indicative microorganisms and pathogenic Enterobacteriaceae were tested in the experiment in their joint presence at temperature 23-25^0 C.

River water upstream of industrial and domestic wastewater discharge was used as a dilution water. In this case, the water quality met the requirements of SanPiN 0172-04 "Hygienic requirements for the protection of surface water in the Republic of Uzbekistan" and GOST 134:2024 "Sources of centralized domestic and drinking water supply. Hygienic, technical requirements and selection rules". Dynamics of microorganisms dying off was calculated in relation to the control. Concentrations of microorganisms were expressed in logarithms (lg) of cell-forming units (CFU) in 1 liter of water. The results of experimental studies showed that in the I series of experiments on models of water bodies a pronounced dynamics of dying off of the studied strains of microorganisms was established, which depends on the infectious concentration of pathogens and duration of the experiment. By 30 days the number of lactose-positive Escherichia coli decreased to tens at the initial concentration (1 day of the experiment) in water lg KOE 7.6. Decrease of initial concentration of LPC in water by 1-2 orders of magnitude leads to its decrease at 20 days within lg KOE 1.8-2.3. And on the 30th day negative growth of microorganisms on nutrient media was observed.

E. coli are more stable in comparison with LCP. So if on the 1st day of the experiment its values were lg KOE 6.1, at the end of the experiment - lg KOE 1.7. When the infectious concentration decreased by an order of magnitude, the value of lg KOE by 30 days was 0.8.

Enterococci are even more resistant to the influence of unfavorable environmental factors. By the end of the experiment, their presence in water was observed within lg KOE 0.7-2.2. Salmonella and especially Shigella are the least resistant and die off already on 20 and 10 days of the experiment, respectively, depending on the value of the initial concentration in water. Similar results were obtained in the second series of experiments (Table 10).
In these studies, staphylococci were found to be less resistant than E. coli, but more viable than lactose-positive E. coli. By 30 days of observation, the values of staphylococci in water were several tens in 1 liter. On the basis of the performed experimental studies it was established that not only lactose-positive bacilli and E.coli but also enterococci, which are more resistant to physical, chemical and biological factors, can be indicators of Salmonella and Shigella pathogens.

Staphylococcus aureus is an indicator indicator of water quality in water bodies of recreational water use of the population. The norm of enterococci (Str. Faecalis) in the water of water bodies of economic and drinking water use is recommended at the level of 100 microbial bodies in 1 liter of water, the norm of staphylococci (St. Aureus) - at the level of 500 microbial bodies in 1 liter of water. Their presence in the water in concentrations exceeding the permissible levels indicates the need to prohibit further use of the reservoir for economic, drinking and recreational water use or special water treatment.

CONCLUSION

1.The permissible content of enterococci (Str. Faecalis) in the water of water bodies of economic and drinking water use is recommended at the level of 100 microbial bodies in 1 liter of water (m.t. in 1 dm3 of water), and staphylococci (St. Aureus) - at the level of 500 m.t. in 1 dm3 in water bodies of recreational use.
2. Presence of enterococci (Str. Faecalis) and staphylococci (St. Aureus) in the water of water bodies in concentrations exceeding the recommended norms indicates the restriction or prohibition of further use of the water body
3.For hygienic assessment of water quality of water bodies of water bodies intended for drinking and recreational water use, along with the mandatory microbiological analysis for the presence of LPS and E. coli. Coli, we recommend also determination of enterococci (Str. Faecalis) and staphylococci (St. Aureus) in water.

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