Bacteria of the genus *proteus* as sanitary indica ive microorganisms of water bodies

Nazarov Jalolitdin Sulton Erkinovich*

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ABSTRACT

The article provides information on the study of pathogenic and conditionally pathogenic microflora of water bodies in the Bukhara region, using the example of Samanid Lake, located in the western part of the city of Bukhara. During the summer reconnaissance survey of water samples from the above water reservoir, bacteria of the genus *Proteus* were found. Bacteria of this genus, in addition to other representatives of *Enterobacteriaceae,* can be fully recognized as sanitary indicative microorganisms associated with anthropogenic impact on the environment. The presence of these bacteria in water always indicates contamination of the object with decaying substrates and an extremely unfavorable sanitary condition. However, although the presence of *Proteus spp.* and indicated the contamination of this water body with organic substances, however, the absence of pathogenic enterobacteria in the Samanid Lake was proved.

Key Words: *Sanitary indicative microorganisms; bacteria of the genus Proteus; pleomorphism; Proteus meter; Allochthonous species.*

INTRODUCTION

Like *Proteus,* a character of ancient Greek mythology, who had the gift of endless metamorphoses, bacteria of the genus of the same name often show pleomorphism on a dense nutrient medium, from which they received the appropriate name of their genus [1].

According to morphology and tinctorial properties, bacteria of the genus *Proteus* are gram-negative rods with numerous flagella over the entire surface of the cell (peritrichous), due to which they are mobile. The presence of bacteria of the genus *Proteus* in water and soil may indicate faecal contamination of the environment in which these proteolytic bacteria are considered as allochthonous. *Proteus*' bacteria live in the intestines of humans and many animals, and enter the external environment along with feces. *Proteus'* bacteria in water multiply more slowly than *E. coli,* for example, in wastewater 2 times slower. The detection of *Proteus mirabilis* bacteria in water sources is considered as an indicator of faecal contamination, and *Proteus vulgaris* as an indicator of object contamination with organic substances. According to these data, *Proteus'* bacteria are classified as sanitary indicative microorganisms, and the detection of these bacteria in drinking water makes it unsuitable for consumption due to fecal contamination, which can threaten waterborne infections [2].

The type species is *Proteus vulgaris*. The genus also includes the species *P. mirabilis, P. penneri, P. hauseri*. Bacteria of this genus are widely distributed in water, soil, food products and on environmental objects surrounding a person. High adaptation to various environmental conditions is explained by their ability to swarm (creeping growth). Bacteria form many daughter colonies, and their population is constantly increasing in the environment [3].

For a long time, bacteria of the genus *Proteus* were considered unreliable sanitary indicative microorganisms, due to the fact that they were detected in the human intestine in small quantities only in 6-10% of cases. In addition, they are widely distributed in the external environment and are able to multiply in it. However, after special polymyxin media were introduced into microbiological practice, the frequency of bacterial isolation from the intestine increased to 98% of cases, and in the vast majority of cases (more than 80%), *P. mirabilis* was detected. In many ways, this discrepancy explains the long-term use of the Shchukevich method in sanitary microbiology, while this method reveals only the H-forms of bacteria. Nutrient medium containing polymyxin provides intraspecific differentiation of *Proteus*' bacteria according to their ability to form hydrogen sulfide [4].

The presence of *Proteus*' bacteria in water, food products, washings (wipe samples) always indicates contamination of the sanitary control object with decomposing substrates and an extremely unfavorable sanitary condition. Foods contaminated with *Proteus'* bacteria are usually discarded, and water containing *Proteus* bacterium should not be drunk. In many countries, water abstraction from drinking water sources containing these microorganisms is prohibited until the source of contamination is eliminated. The detection of these bacteria in the water of open reservoirs and in the study of therapeutic mud is officially called "*Proteus* meter". Water *Proteus* meter is officially recognized in the USA and some EU countries. In the Russian Federation, it is recommended for the study of water from open reservoirs, therapeutic mud, and for the examination of food products, the detection of *Proteus'* bacteria is provided for by GOST (state standards) [5].

Bacteria of the genus *Proteus* play an important role in the removal of animal organic pollutants, especially faecal ones, by decomposing dead organic matter in water or soil media. These bacteria can serve as a criterion for the sanitary well- being of water bodies, depending on their use. It is necessary to conduct a mandatory study of water reservoirs subject to sanitary and microbiological assessment for the presence of *Proteus*' bacteria [6].

MATERIALS AND METHODS

In July 2021, water samples were taken from Samanids' Lake, located in the western part of the city of Bukhara, to detect *Salmonella*. *Salmonella* in the water of open reservoirs can persist for up to 3 months and are pathogenic for humans. Bacteria of the genus *Salmonella* cause diseases such as typhoid fever, paratyphoid fever A,B,C and other acute intestinal infections (salmonellosis) [7].

For monitoring the water of this surface water body, 2 samples of 500 ml each were taken at three location points. Samples were taken in the southwestern, northeastern and central parts of the lake. After the water samples were delivered to the laboratory, they were subjected to membrane filtration. The analyzed water samples were passed through membrane

Department of Environmental Microbiology, Bukhara State Medical Institute named after Abu Ali ibn Sino, Uzbekistan

Correspondence: *Nazarov Jalolitdin Sulton Erkinovich, Department of Environmental Microbiology, Bukhara State Medical Institute named after Abu Ali ibn Sino, Uzbekistan, Tel: 998931400027; E-mail: sultan.nazarov73@gmail.com*

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filters, after which these filters were placed in the Endo nutrient medium and incubated in a thermostat at a temperature of 37°C.

When sowing on Endo's medium, colorless colonies characteristic of lactose-negative bacteria were found the next day. *Salmonella* are lactose-negative gram-negative rods that do not ferment lactose, have peritrichous (over the entire surface of the cell) flagella. In terms of these properties, *Proteus*' bacteria are similar to *Salmonella*, which, unlike the former, can break down carbamide. However, in the study of inoculations, the growth of colonies in the form of a veil-like plaque, which is inherent in *Proteus*' bacteria, was noted.

Bacteria of the genus *Proteus* growing on agar demonstrate a swarming type of colony growth. The swarm cells of these microbes with many flagella move along the edges of the colony and then turn into normal cells with few flagella and reduced motility. Periodically, new generations of highly mobile swarm cells develop, and this process is repeated. As a result, a *Proteus* colony has the characteristic appearance of a series of concentric rings (Dienes lines).

In order to fully verify the results of the microbiological examination, some of the suspicious colonies were introduced into a magnesium selective enrichment medium. This pursued the goal of differentiating *Proteus*' bacteria from *Salmonella*, if the latter were present in the studied water samples. The magnesium medium provides nutritional requirements for the growth and accumulation of *Salmonella*, while it has an inhibitory effect on a number of microorganisms, including *Proteus*' bacteria [8].

A day after the incubation of the inoculations in a magnesium medium at a temperature of 37°C, a bacteriological loop was used for streak passages on the Endo medium. Magnesium chloride suppressed the growth and development of *Proteus'* bacteria, therefore, after further incubation on a nutrient medium, colony growth was visually absent. In the presence of *Salmonella* after cultivation, colonies with a characteristic morphology for these microorganisms would be identified. This growth was not detected, which once again served as evidence of the absence of *Salmonella* in water samples [9].

At the same time, the initially obtained colonies were isolated on Olkenitsky's medium (the triple sugar iron medium with carbamide).

The culture was inoculated by piercing the medium with a microbiological loop in the center of the agar column to the very bottom of the tube. The samples were placed in a thermostat at 37°C for 24 hours. After that, an assessment was made of the change in the appearance of the nutrient medium. Initially, the medium was red in color, blackening of the medium upon injection was observed in the column, while the slanted part of the nutrient medium was bright crimson. In Olkenitsky's medium, with the growth of a culture that hydrolyzes carbamide, the medium acquires a diffuse bright red-crimson color. These facts indicated the absence of *Salmonella* and the presence of *Proteus spp*.

Bacteria of the genus *Salmonella* break down glucose in Olkenitsky's medium, with the formation of acid and gas. The fermentation of glucose with the formation of acid is manifested by a change in the color of the column of the medium to yellow, while the red color of the "tongue" of the medium is preserved. With the production of hydrogen sulfide by *Salmonella*, the presence of a black precipitate is observed at the border of the column and the sloping part of the Olkenitsky medium. Yellowing of the medium and rupture of the agar column with gas bubbles, signs of the presence of *salmonella*. In this case, culture growth in Olkenitsky's medium contributed to the hydrolysis of carbamide, due to which the medium acquired a diffuse bright red-crimson color [10].

In addition, in the presence of a bright crimson color of the slanted part of the medium, blackening of the medium was observed in the agar column at the injection, which indicated the production of hydrogen sulfide. *Proteus vulgaris* tends to produce large amounts of hydrogen sulfide by hydrolyzing carbamide. The medium acquires a crimson-black color. These facts indicated the absence of *Salmonella* and the presence of *Proteus*' bacteria in the studied water samples [11].

RESULTS AND DISCUSSION

Microbiological investigations of water and bottom sediments of the *Vrelo Cave* (the Republic of Macedonia) revealed the presence of *allochthonous* strains *of Proteus penneri*. Water samples were taken from a place located 400 m from the cave entrance and at a depth of 100 m. Despite the fact that various parameters indicated high water quality, a large number of fecal coliform bacteria were found both in the water and in samples of bottom sediments, indicated the pollution of this water source [12].

P. vulgaris was claimed to be the main microbial pollutant of drinking water in India's largest state, Rajasthan. In water from wells in Mysore city, strains of *P. mirabilis* and *P. vulgaris* dominated over other strains of H_2 S-producing bacteria (which are considered to be associated with coliforms) [13].

Multidrug resistant strains of *P. mirabilis* and *P. vulgaris* have been found in drinking water from rural springs and streams in the Indian state of Sikkim. In Nigeria, bacteria of the genus *Proteus* were present in two of the five studied well waters considered as a source of drinking water. In Brazil's southernmost state of Rio Grande do Sul, patches of rice fields are supplied with water from irrigation canals in which antibiotic-resistant strains of *P. mirabilis, P. vulgaris,* and *P. penneri* have also been found indicative of fecal water contamination [14].

Among other things, bacteria of the genus *Proteus* can serve as indicators of faecal pollution not only in freshwater reservoirs, but also in marine ecosystems. In the waters of the salt lake of the *El Golea oasis* (Algerian Sahara), halotolerant strains of bacteria of the genus *Proteus* were found, indicating a high plasticity to the habitat of these bacteria, which helps them survive in sea water. The *sponges Spongia* officinalis are animal filter feeders that absorb various bacteria, including the genus *Proteus*. Bacteria are spreading in the marine food chain. Aquatic animals can absorb these microorganisms from sea water, for example, oysters (*Ostreidae*), loggerheads (*Caretta caretta*), green turtles (*Chelonia mydas*).

In addition, the marine environment appears to be a reservoir of antibiotic resistance genes in bacteria, as many antibiotic resistant strains, including *Proteus spp*. Have been isolated from sea water and marine animals. From water samples of the Douala lagoon (Cameroon), contaminated with industrial and household waste, antibiotic-resistant strains of *P. vulgaris* were isolated in the course of research, which pose a threat to human health. Another example is the Jiaozhou Bay, a bay in the Yellow Sea off the southern coast of the Shandong Peninsula (China) [15].

The waters of the bay are heavily polluted due to intensive industrial development and urbanization. Wastewater from hospitals and sewers entering seawater is a source of drug-resistant bacteria, allowing antibiotic resistance genes to be passed on to environmental microflora. Thus, strains of *P. mirabilis* resistant to tetracycline and chloramphenicol were isolated from the sea water of the bay.

Being opportunistic inhabitants of the intestines of humans and many animals, these microorganisms indicate fecal contamination of water or soil. Thus, strains of *P. mirabilis*, *P. vulgaris,* and *P. penneri*, in addition to other representatives of *Enterobacteriaceae,* can be fully recognized as sanitary indicative microorganisms associated with anthropogenic impact on the environment [16].

CONCLUSION

Bacteria of the genus *Proteus* are distributed as sanitary indicative microorganisms for the characteristics of the consumption mineralization process. The reservoir, located in the Samanids' Park of the city of Bukhara, is actively visited by citizens in the summer. Adjacent to the lake, homeowners use this water reservoir to raise waterfowl. There are catering points along the banks. The closed structure of the reservoir and the mass attraction of people create conditions for contamination of this water body with various organic wastes [17].

Anthropogenic pollution causes changes in the composition and structure of aquatic communities, expressed in a change in the dominant complexes of organisms, a simplification of the ecological structure, and the appearance of highly saprobic species in the dominants [18].

Pollution of the reservoir with organic matter creates the prerequisites for the reproduction of diatoms, and as a result of the "blooming" of the water source. In addition, a large amount of organic substances in the water of freshwater reservoirs creates conditions for the growth and reproduction of pathogenic microorganisms in it.

Although the presence of *Proteus vulgaris* indicated the pollution of this water body with organic substances, it was nevertheless proved that there were no pathogenic enterobacteria in the Samanids' Lake [19,20]

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