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Multiple antibiotic resistance of heterotrophic bacteria in the littoral zone of Lake Shira as an indicator of human impact on the ecosystem

Tatiana I. Lobova[a,*], Yuri V. Barkhatov[b], Ol'ga V. Salamatina[c], Lyudmila Yu. Popova[d]

[a]*LTD "Territory-oriented information systems", Institute of Computational Modeling, off.501-1, Russian Academy of Sciences, Siberian Branch, Akademgorodok 50, Krasnoyarsk 660036, Russian Federation*
[b]*Institute of Biophysics, Russian Academy of Sciences, Siberian Branch, Akademgorodok 50, 660036, Russian Federation*
[c]*Center of Hygiene and Epidemiology in Krasnoyarsk Region, Sopochnaya 38, Russian Federation*
[d]*Krasnoyarsk Scientific Centre, Akademgorodok 50, Krasnoyarsk 660036, Russian Federation*

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Summary

Resistance to Ampicillin and Kanamycin displayed by heterotrophic bacteria isolated in Summer and in Spring from the littoral and the central parts of Lake Shira (a therapeutic lake in the Khakasia Republic, Russia) has been investigated. It has been found that in Summer, human and animal microflora featuring multiple antibiotic resistance (to Ampicillin and Kanamycin) predominates in all the studied stations of the littoral zone of the lake. In Spring, concentrations of bacteria featuring multiple antibiotic resistance decrease significantly and bacteria sensitive to antibiotics predominate in the lake. Emergence of multiple antibiotic resistance in bacteria of Lake Shira is caused by the input of allochthonous bacteria into the lake; this feature of heterotrophic bacteria of Lake Shira can be used to monitor the impact on the ecosystem made by health resorts.

Introduction

Human impact frequently causes undesirable and, sometimes, irreversible changes in natural aquatic ecosystems. One example is adverse effect of human activity on the ecosystem structure and biodiversity. It is especially important to preserve natural biodiversity of the lakes with health resorts on their shores. Ecosystems subjected to human impact should be continuously monitored and, whenever necessary, measures should be taken to

*Corresponding author.
E-mail address: yanalobova@mail.ru (T.I. Lobova).

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avoid adverse effects. A conventional method of estimating the state of aquatic environments is to measure the levels of enterobacteria, specifically pathogenic bacteria which are indicators of the level of water pollution (Tchounwou and Warren, 2001; Chao et al., 2003; Mohandass and Bharathi, 2003; Zmirou et al., 2003; Byamukama et al., 2005). One of the approaches that continues to attract more and more attention of scientists is biological monitoring (biomonitoring), which is based on living organisms being sensitive to certain toxic agents. Bacteria can quickly adapt to the presence of human-induced factors in the ecosystem, acquiring the features that allow them to survive, such as resistance to antibiotics, xenobiotics, etc. (Lemke and Leff 1999; McArthur and Tuckfield, 2000; Summers, 2002; Springale and Top, 2004; Kummerer, 2004; Mudryk, 2005). Thus, the properties of bacteria that develop in response to human impact can be used as indicators in ecological monitoring.

In our earlier studies we investigated antibiotic resistance of heterotrophic bacteria isolated from Lake Shira in different seasons of 1997–2001 and found that in Summer, when the health resorts situated on its shores are functioning, the lake water contains increased concentrations of allochthonous bacteria featuring multiple antibiotic resistance (Lobova et al., 2002a). The input of allochthonous bacteria entering the lake with waste effluents of the resorts increases the concentration of bacteria resistant to antibiotics in the part of Lake Shira most distant from the resort (the center) (Lobova et al., 2004). In Winter and Spring, when human impact on the lake decreases significantly, the concentration of autochthonous bacteria featuring multiple antibiotic resistance is much lower (Lobova et al., 2002b). Hence, multiple antibiotic resistance is a specific feature of autochthonous bacteria of Lake Shira, which can be used as an indicator of human impact on the ecosystem.

Allochthonous bacteria that enter the lake with waste effluents first accumulate in the littoral zone and after that as a result of water mixing, are transported to the zones that are more distant from the shore. Thus, a study of antibiotic resistance of heterotrophic bacteria isolated from the littoral and the central parts of Lake Shira can provide the basis for both assessing human impact on the ecosystem as a whole and determining its intensity. The purpose of this study was to investigate antibiotic resistance of the bacteria isolated from the littoral zone of the lake in Summer and Spring and to estimate the contribution of the health resorts to impairment of bacterial diversity in the central part of Lake Shira.

Materials and methods

Sampling sites

Lake Shira is a brackish lake in the South of the Khakasia Republic (Russia) (90°14′E, 54°30′N). The lake is 9 km long and 5 km wide. The lake has no outflow. The physicochemical parameters of the Lake Shira water are described in greater detail in the paper by Kalacheva et al. (2002). Samples of Lake Shira water were taken from the littoral zone and from the central part of the lake (Fig. 1):

Stations (1, 2, 13, 14, 15, 16, 17, 18, 19, 20) – parts of the lake subjected to the impact of the health resorts and tourist camps;

Stations (7, 8, 9, 10, 11, 12) – parts of the lake infrequently visited by vacationers;

Stations (3, 4, 5, 6) – parts of the lake inaccessible to vacationers because of the rough terrain;

Station 21 – the central part of Lake Shira.

Samples from the stations were taken in the Spring (March) of 2003 and Summer (July) of 2002. Samples were taken from the depth of 0.5 m.

Media

Water samples were plated on mineral medium (M9) with peptone that contained per liter of distilled water: 6 g Na_2HPO_4 , 3 g KH_2PO_4, 0.5 g NaCl, 1 g NH_4Cl, 5 g peptone and 20 g agar. After autoclaving, 1 ml of 20% $MgSO_4$ and 1 ml of 0.5% $CaCl_2$ were added (Miller, 1972).

Isolation of bacterial cells

To isolate heterotrophic bacteria, water samples were plated on agar medium M9 containing peptone and cultivated thermostatically at 25 °C.

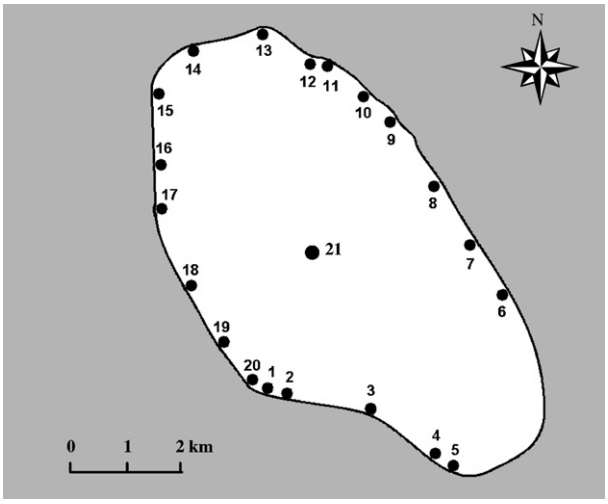

Figure 1. Sampling sites in Lake Shira.