

Antibiotics and phage resistant isolates of Enterobacteriaceae as a source of new infectious risks in surface water bodies

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ABSTRACT. Freshwater environments are susceptible to possible bacterial contamination by various variants, including drug-resistant ones. Such pollution occurs either by direct intake of polluted wastewater or by surface runoff from the surrounding area. The existing quantitative sanitary and hygienic assessment of aquatic ecosystems is becoming less informative, as it underestimates the risks due to antibiotic-resistant bacterial strains. In order to assess the level of resistance to antibiotics and bacteriophages among enterobacteria, water samples were taken from five surface water bodies in Ryazan during the spring and summer periods of 2016 and 2017. It is established that the absolute majority of enterobacteria were multidrug-resistant (MDR) and more than 80% were resistant to the examined bacteriophage preparations. In addition, an increase in the occurrence of extremely resistant variants (XDR) among Enterobacteriaceae was active. About half of all isolated isolates produced Extended-Spectrum β -Lactamases (ESBLs). The increase in resistance to bacteriophages occurs with an increase in the resistance of isolated enterobacteria to antibiotics.

Keywords: antibiotic resistance, resistance to bacteriophages, family Enterobacteriaceae, surface freshwater bodies, public health risks.

1. Introduction

Many members of the Enterobacteriaceae family are pathogenic and opportunistic species that can cause various diseases. Control of their distribution in water environments is based on quantitative indicators—the number of total and fecal coliforms bacteria. However, due to the widespread spread of antibiotic resistance, it is important to assess not only the quantitative abundance, but also the susceptibility to various drugs (in particular, antibiotics, as well as their alternative - bacteriophages). Because that, it is resistant to antibiotics (and bacteriophages) strains of enterobacteria that carry the maximum risks to human health in the event of infection (Lindberg et al., 2007; Nnadozie and Odume, 2019).

2. Materials and methods

Water sampling for bacteria isolation of Enterobacteriaceae family was performed in accordance with the accepted recommendations. Water samples

were taken in the spring-summer period of 2016-2017, several times per season from 5 surface freshwater bodies of Ryazan: rivers Oka and Listvyanka, three Borkovskiye quarries. The Oka river is a source of drinking water and recreation for many localities in the Ryazan region. The Listvyanka river is a tributary of the river Oka, which is a receiver of standardly treated industrial and municipal wastewater of the city of Ryazan. Borkovskiye quarries are a place of rest and bathing of the urban population.

We used the membrane filtration to count the number of isolates of the family Enterobacteriaceae. The filters were placed on Endo agar. Species identification of isolates was carried out using a test-system for rapid biochemical identification "Rapid Entero 200 M".

The susceptibility of the Enterobacteriaceae isolates to antibiotics was assessed using the disk diffusion technique on Mueller-Hinton agar (HiMedia, India). Strains were tested against the extended set of disks for evaluating the antibiotic sensitivity of enterobacteria, including 20 antibiotics (Institute of epidemiology and microbiology named after Pasteur,

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St.-Petersburg, Russia). The data were interpreted using the EUCAST criteria. Quantitative characteristics of antibiotic resistance are given by Magiorakos et al. (2012). Determination of the virulence of tested bacteriophage preparations in relation to isolated isolates of enterobacteria. The study included four commercially produced preparations of bacteriophages produced in the form of solutions of FSUE "NPO "Microgen" ("Coliproteinny Bacteriophage", "Polyvalent purified Bacteriophage", "Sexatafag", "Polyvalent Piobacteriophage" and "Intesti-bacteriophage"). The work was carried out in accordance with the Federal clinical recommendations "Rational use of bacteriophages in therapeutic and antiepidemic practice", as well as focusing on the methodological recommendations (Aslanov et al., 2014).

3. Results

The number of enterobacteria was in the vicinity or slightly exceeded the existing sanitary standards in water samples from the Oka river and Borkovskiye quarries. The average number of total coliform bacteria in the spring and summer periods was 876 CFU/100 ml in Borkovskiye quarries, in samples from river Oka – 1549 CFU/100 ml. The average seasonal amount of fecal coliform was 249 and 264 CFU / 100 ml, respectively. The values were an order of magnitude higher in samples from the Listvyanka river (total coliforms – 10234 CFU/100 ml, fecal coliforms -867 CFU/100 ml). The most diverse species composition of Enterobacteriaceae was found again in water samples of the Listvyanka river. *Escherichia coli*, *Citrobacter freundii* and *Providencia alcalifaciens* remained predominant, as in other water bodies.

The evaluation of antibiotic resistance showed that the absolute majority of enterobacteria were multidrug-resistant (MDR) in all surveyed surface water bodies. In addition, an increase in the occurrence of extremely resistant variants (XDR) among Enterobacteriaceae was active in Borkovskiye quarries. Thus, 4% of XDR variants were observed in the 1st Borkovsky quarry in the spring-summer period of 2016, and in 2017 – 11.5%, in the 2nd quarry - 21.7% and 73.1%, respectively. The number of XDR isolates ranged from 38.5 to 56% in samples from the Oka river. About half of all isolated isolates produced Extended-Spectrum β -Lactamases (ESBLs).

Most of the isolates (>80%) were resistant to the examined bacteriophage preparations, i.e., it was

recorded from the complete absence of the lysing action of the phage (-) to lysis in the form of draining areas with a large amount of secondary growth (++). It should be noted that the isolates isolated from Borkovskiye quarries are less sensitive to the action of phages, since the percentage of *Escherichia* and *Citrobacter* in the species composition of these water bodies is lower than in rivers. It is noted that the increase in resistance to bacteriophages occurs with an increase in the resistance of isolated enterobacteria to antibiotics (in the range from resistant to 1-3 antibiotics to XDR).

4. Conclusions

Antibiotics and phage resistant isolates of Enterobacteriaceae are prevalent in freshwater environments. The evidence provided in this study sustains that even reservoirs that do not receive wastewater contain a large number of drug resistant bacterial variants. Such bacterial isolates undoubtedly pose huge risks to human health. Since it is very difficult for a doctor to choose a drug therapy, and in some cases it is almost impossible if an infection caused by these bacteria occurs.

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