Presence of antibiotic-resistant bacteria in the environment

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Abstract. The emergence and spread of antibiotic-resistant bacteria in the environment is a serious medical and environmental problem worldwide. An important reservoir of such bacteria are aquatic ecosystems, which are polluted by human waste, agricultural and industrial wastewater. Antibiotic-resistant bacteria are able to transfer their genes to other aquatic bacteria, which thus acquire new resistance genes. The aim of this study is to assess the antibiotic resistance of bacteria isolated from the rivers of the Kaluga region, which are most susceptible to the effects of urbanization and agriculture. Water samples were taken from the Oka, Yachenka, Kaluzhka, Ugra rivers. Most often, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis, Enterobacter aerogenes, Bacillus subtilis, Clostridium perfringens, Klebsiella spp., Alcaligenes faecalis, Aeromonas spp. Were sown from water samples. Studies have shown that all isolated microorganisms have multi-resistance to one degree or another. But Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis, and Alcaligenes faecalis had the greatest resistance to various antibiotics.

1. Introduction
The intensive use of antimicrobial agents is one of the main factors in the formation of resistant bacteria. Infections caused by resistant organisms and the emergence of pathogenic and non-pathogenic bacteria with multidrug resistance are global problems since they increase morbidity and mortality and reduce infectious diseases treatment effectiveness [1, 2].

About 25,000 of European citizens die each year from infections caused by bacteria that have developed resistance to antimicrobial drugs. In the United States nosocomial infections cause 12,000 deaths each year. More than 70% of the bacteria that cause these infections are resistant to at least one of the commonly used antibiotics for treatment. One of the three patients receive at least one antimicrobial drug every day. Every 15th patient in European hospitals has at least one of the nosocomial infections caused by multidrug-resistant bacteria [3].

Between 1940 and 1970 the growing pharmaceutical industry produced more than 160 new antibiotics and semi-synthetic drugs which later become the basis for infectious diseases treatment. The global use of antibiotics in medicine and agriculture has grown steadily over the past decades. For example, consumption of antibiotics in livestock production reached 63,151 tons in 2010 and is projected to grow by another 67% by 2030 [4].

Antibiotic resistance develops as a result of antimicrobial agents’ exposure on bacteria populations and communities. These populations include human and animal microbiome which includes more than 500 bacteria species. They interact with antibiotics used for therapeutic or prophylactic purposes. In public institutions, in hospitals and in crowded places antibiotic-resistant bacteria are exchanged between people. A similar exchange at the genetic level occurs between antibiotic-resistant
microorganisms that have entered the environment with biological secretions from humans and animals and the natural bacterial microflora. Bacteria of various types, antimicrobial substances and heavy metals mix in the environment which contributes to the development and spread of antibiotic resistance [5]. Water plays a key role in these processes.

Many antibiotics enter the aquatic environment with untreated wastewater and wastewater from pollution control facilities. As micro-pollution they enter surface and groundwater potentially altering microbial ecosystems. Aquatic habitat creates ideal conditions for the acquisition and spread of antibiotic resistant microorganisms. Aquatic habitat is not only a way of spreading antibiotic-resistant microorganisms among humans and animals but also a way through which resistance genes are introduced into natural bacterial ecosystems [6, 7].

Most bacteria in natural aquatic ecosystems form biofilms that help them survive and spread. Biofilms are clusters of bacteria that live in a well-structured and organized community [8]. Biofilms contribute to the formation of resistance to antibiotics which is associated with a high density of cells and active horizontal gene exchange between them.

Bottom sediments of fresh water bodies and rivers also represent an important ecological matrix in which genetic exchange between microorganisms takes place. Antibiotics are sorbed by sediments which function as complex and dynamic microorganisms interface community facilitating the transfer, maintenance and spread of resistant microorganisms exposed to antibiotics [9, 10].

Antibiotics sorption by bottom sediments slows down the processes of their biodegradation which contributes to the long-term presence of antibiotics in the aquatic ecosystem. Antimicrobial agents are retained in sediment and soil through their association with soil chemicals. For example, soil humic acids form complexes with antibiotics. Antibiotic-resistant bacteria spread in water sources is facilitated by manure removal to fields with washing away by rain streams that’s followed [11].

2. Research methods

Sampling of water from the Oka, Yachenka, Kaluzhka, Ugra rivers was carried out downstream of the city treatment facilities or the discharge of polluted effluents in accordance with NSS 31942-2012 (ISO 19458: 2006) “Water. Sampling for microbiological analysis”.

Microbiological study of water was carried out in accordance with the methodological instructions MG 4.2.1884-04 "Sanitary-microbiological and sanitary-parasitological analysis of surface water bodies”

Bacteria were identified in the following sequence:

- Cultural characteristics description of the isolated microorganism;
- Obtaining a clean daily culture by sowing on nutrient media;
- Gram’s stain and specimen microscopy
- use of MIKROLATEST test system for microorganism’s identification

Bacteria sensitivity determination to antibiotics was carried out by the diffusion method using discs with antibiotics (table 1).

| № | Title       | Symbol | № | Title       | Symbol |
|---|-------------|--------|---|-------------|--------|
| 1 | Oflagacin   | OF     | 9 | Levoflagacin| LFC    |
| 2 | Clarithromycin | KTM   | 10| Phosphomycin| FOS    |
| 3 | Benzylpenicillin | PEN   | 11| Tobramycin  | TOB    |
| 4 | Ciproflagacin| CIP    | 12| Optokhin    | OP     |
| 5 | Cefoperazone | CPR    | 13| Lincomycin  | LIN    |
| 6 | Novobiocin  | NB     | 14| Azithromycin| ARN    |

Table 1. List of antibiotics used.
3. Results and discussion

Totally ten species of bacteria were isolated from various analyzed samples. Among them are Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis, Enterobacter aerogenes, Bacillus subtilis, Clostridium perfringens, Klebsiella spp., Alcaligenes faecalis, Aeromonas spp. The total number of microorganisms in water from various sources ranged from $0.7 \times 10^4$ to $6.6 \times 10^5$ CFU / ml. The isolated microorganisms are relatively evenly distributed in water samples from various sources.

Occurrence percentage frequency of bacterial isolates is shown in figure 1. Most often Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis were inoculated from water samples. The number of coliform bacteria ranged from $0.5 \times 10^4$ to $1.9 \times 10^4$ CFU / ml. The predominance of representatives of the intestinal microflora in river water indicates an intense anthropogenic, fecal pollution of aquatic ecosystems. The analyzed water samples showed a high percentage of Pseudomonas aeruginosa occurrence. These microorganisms negatively affect water bacteriological quality and can be pathogenic for humans and animals.

The isolated microorganisms to one degree or another have multiresistance to the studied antibiotics (figure 2). Among the antimicrobial agents tested optoquin (OP) and ampicillin (AMP) had the highest bacterial resistance response (86.7% and 67.7%, respectively). They are followed by claritomycin (KTM), tylosin (TLZ), benzylpenicillin (PEN), lincomycin (LIN) and novobiocin (NB) with a lower bacterial resistance response (45.1%, 40.0%, 30.0%, 26, 6%, respectively). A similar picture albeit to a lesser extent is observed for other studied antibiotics. A low level of resistance in aquatic bacteria was noted in relation to ciprofloxacin (CIP), cefoperazone (CPR) and tobramycin (TOB).

![Figure 1. Microorganisms occurrence frequency of isolated from water bodies.](image-url)
Figure 2. Total resistance of aquatic microorganisms to antibiotics.

The study results show that all isolated microorganisms were resistant or relatively resistant to all antibiotic tests. Microorganisms of the intestinal group (Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis, Enterobacter aerogenes, Alcaligenes faecalis) and Staphylococcus aureus (Staphylococcus aureus) were characterized by the greatest resistance to antibiotics (figures 3 a and b). The most frequently observed resistance of aquatic microorganisms to optochin, ampicillin, gentomycin, tetracycline. Bacillus subtilis and Clostridium perfringens showed less resistance to test antibiotics.

Figure 3. Differential antibiotic resistance of aquatic bacteria.

The existence of such a number of antibiotic-resistant strains of intestinal bacteria is alarming since these microorganisms are not only capable of causing disease under certain circumstances but also actively carry out horizontal transfer of resistance genes between other microorganisms.

Thus, our studies confirm other authors conclusions that the aquatic environment is a potential reservoir of microorganisms resistant to antibiotics and capable of transmitting resistance genes to other members of the bacterial community.

The widespread consumption of antibiotics and the development of resistant bacteria are of great concern. Therefore, monitoring and surveillance programs as well as further studies of environmental
reservoirs are needed to better understand antibiotic resistance mechanisms and develop management solutions to this serious problem.

4. Conclusion
The predominance of representatives of intestinal microflora in river water indicates intensive anthropogenic, fecal pollution of studied aquatic ecosystems.

Resistance to antibiotics is widespread among representatives of the intestinal microflora and Staphylococcus aureus found in water samples from the rivers Oka, Yachenka, Kaluzhka, Ugra.

All isolated microorganisms were multi-resistant to test antibiotics. The most common was the resistance of aquatic microorganisms to optochine (up to 86.7%), ampicillin (up to 67.7%), gentomycin (GENT) (up to 61.9%), tetracycline (TETR) (up to 62.1%).

References
[1] 2018 Antibiotic Resistance – an Increasing Threat to Human Health https://antibiotic.ecdc.europa.eu/en/publications-data/antibiotic-resistance-increasing-threat-human-health, last accessed 06/11/2020
[2] Vaz-Moreira I, Nunes O C and Manaia C M 2014 Bacterial diversity and antibiotic resistance in water habitats: searching the links with the human microbiome FEMS Microbiology Reviews 38(4) 761-78
[3] 2020 The European Union Summary Report on Antimicrobial Resistance in Zoonotic and Indicator Bacteria from Humans, Animals and Food in 2017/2018 https://www.ecdc.europa.eu/en/publications/EU-summary-report-antimicrobial-resistance-zoonoses-2017-2018, last accessed 06/11/2020
[4] Thomas P Van Boeckel, Charles Brower and Ramanan Laxminarayan 2015 Global trends in antimicrobial use in food animals Proc. Natl. Acad. Sci.i USA 112(18) 5649-54 doi:10.1073/pnas.1503141112
[5] Callan A C, Aitken B, Shearn R, Koenders A and Hinwood A 2017 Relationship between antibiotic resistance genes and metals in residential soil samples from Western Australia Environ. Sci. Pollut. Res 24 2484–94 DOI 10.1007/s11356-016-7997-y
[6] Kümmerer K 2009 Antibiotics in the aquatic environment – a review – part I Chemosphere 75 417-34
[7] Kümmerer K 2009 Antibiotics in the aquatic environment – a review – part II Chemosphere 75 435–41 doi: 10.1016/j.chemosphere.2008.12.006
[8] Lykov I N and Shestakova G A 2014 Microorganisms: Biology and Ecology (Kaluga: Publishing house “SerNa”) p 451
[9] Lykov I N 2020 Pharmaceutical pollution of the environment Problems of regional ecology 3 23-7 DOI 10.24411/1728-323X-2020-13023
[10] Nick G H Taylor, David W Verner-Jeffreys and Craig Baker-Austinl 2011 Aquatic systems: maintaining, mixing and mobilising antimicrobial resistance? Trends Ecol. Evol 26 278-84
[11] Heuer H, Schmitt H and Smalla K 2011 Antibiotic resistance gene spread due to manure application on agricultural fields Curr. Opin. Microbiol 14 236-43 DOI: 10.1016/j.mib.2011.04.009
[12] Kristiansson E, Fick J, Janson A, Grabic R, Rutgersson C, Weijdegård B et al 2011 Pyrosequencing of Antibiotic-Contaminated River Sediments Reveals High Levels of Resistance and Gene Transfer Elements PLoS ONE 6(2) e17038 https://doi.org/10.1371/journal.pone.0017038