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Determination of the Microorganisms' Resistance to Antibiotics in the Bacterioplankton Community in the Akmola Region Lakes

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ABSTRACT

The purpose of the work was to conduct biotesting for the presence of antibiotics in several lakes of the Akmola region, located in the zone of possible ingress of antimicrobial drugs into them. The adopted research method was the modified disk diffusion method, employed to determine the microorganisms' sensitivity to the used antibiotics. As a result of the study, sensitivity to antibiotics remained in the main control lakes. The antibiotics that have not been found to be resistant to microorganisms in lakes included: enronite, furagin, cefuroxime, cefoperazone, and amikacin. The most common resistance of microorganisms 90–100% was in such antibiotics as benzylpenicillin, kanamycin, streptomycin, tylosin, trimethoprim/sulfamethoxazole, metronidazole, amikacin, and spectinomycin.

Keywords: xenobiotics, pollutants, bacterioplankton, antibiotics, resistance, microorganisms.

INTRODUCTION

The problems of pollution of surface water bodies have become increasingly relevant in recent decades. Among many anthropogenic pollutants, xenobiotics are becoming increasingly common, affecting the microorganisms of aquatic ecosystems. In the research of Kazakhstani authors, various pollutants have been accumulated in the environment due to anthropogenic influences in the vicinity of industrial centers and large cities, including heavy metals and pharmaceutical pollutants [Yelkenova et al., 2023].

There is a pharmaceutical plant in the city of Shymkent and the city belongs to the metropolis in the republic. According to the authors, on the waste management rating, Shymkent is in the lower rows. The city waste is taken to landfills and disposal takes place by natural decomposition. Unauthorized landfills endanger the water environment by entering surface and groundwater [Aitimbetova et al., 2023]. Research to determine a list of priority pharmaceutical ingredients for the aquatic environment and their possible effects on aquatic biota in Kazakhstan has been conducted by several researchers [Beisenova et al., 2022; 2020; Abakirova et al., 2017]. Among them, a special place is occupied by antibiotics with a high-risk index for aquatic biota and long-term persistence in the environment: amoxicillin, clarithromycin, azithromycin, and benzylpenicillin [Aubakirova, 2017]. However, research in this area is just beginning to develop. The occurrence of antibacterial drugs in the environment is of great interest to researchers, since their impact on biota can cause the most unpredictable consequences, and it is also rare to find studies on the content of more than 20 pollutants using one method [Wilkinson et al., 2022; Bereznyak et al., 2018].

The impact of antibiotics can lead to a change in the species composition of bacterioplankton, up to an imbalance in the aquatic biocenosis. However, prolonged exposure to antibiotics should trigger the mechanisms of selection and adaptation of bacteria towards resistance to xenobiotics entering water bodies [Vaz-Moreira et al., 2014; Zhuravljov et al., 2015]. In the Republic of Kazakhstan, there are no domestic wastewater treatment plants where antibiotics are purposefully purified from water, and there are very few works on monitoring antibiotics in water resources. At the same time, there is a large consumption of antibiotics in the country, which are used to treat people (annually, volumes), as well as for the treatment of pets (from 2015 to 2019, the volume of antibiotic-containing medicines imported into Kazakhstan ranges from 504 to 675 million US dollars) [Beisenova et al., 2022]. Thus, according to the SK Pharmacy, among the consumed medicinal substances, systemic antibacterial drugs are in the first place (97% of the market share in US dollars), and there is no strict control over the sale of these drugs by prescription, especially for animals.

There is every reason to believe that many of these substances pass through the stages of water treatment undestroyed and remain in surface waters [Balcioglu et al., 2003; Subbiah et al., 2011]. At the same time, the usual chemical-analytical determination of drugs cannot show the picture of the disturbance of the microbial community in surface waters. The purpose of the study was to conduct biotesting for the presence of antibiotics in several water bodies of the Akmola region, located in the zone of possible ingress of antimicrobial drugs into them.

MATERIALS AND METHODS

The object of the study were lakes into which excrements from private livestock farms are constantly or periodically released when animals are freely grazing, or domestic wastewater from nearby populated areas may enter through underground routes. The subject of the study was filtered water samples from lakes, where the bacterioplankton community represents a single test system in which general resistance to a particular drug is formed.

Several lakes of the Akmola region were selected, which are located near rural settlements where there is no sewage treatment system or near poultry and livestock farms. These are the lakes Small Chebache, Ulken Shabakty, Kopa, Zhaltyrkol (Arhaniya), Malaya Saryoba, Koygeldy, Zhalanash, Big Taldykol and, Kumkol (Table 1). Lakes located remotely from settlements were selected as control reservoirs: Bolshaya Saryoba, Zhangula, Esei, Kokay, and Sultankeldy (Table 2).

We selected the area of the study of lakes for antibiotic resistance, which is in Akmola region of the Republic of Kazakhstan [Azbantayeva et al., 2022] (Fig. 1). The location of the studied lakes by region can be seen in Fig. 2 (a, b, c, d, e). The map shows the location of water bodies by region, on an enlarged scale. When choosing antibiotics, the authors were guided by their commercial demand, since this should determine the volume of their use. In water bodies, resistance to the antibiotics, which are in the greatest commercial demand – including amoxicillin, clarithromycin,

Table 1. Lakes of the Akmola region that have contact with settlements and livestock facilities

Name of lakes/Cordinates	Lakes area, km^2	Remoteness from the settlement or livestock complex
Small Chebache/53°04'40" N 70°08'20" E	16.8 km^2	Burabay district. 6.5 km north of Shchuchinsk
Ulken Shabakty/53°07'06" N 70°16'11" E	23 km^2	Burabay district. 16.5 km north of Shchuchinsk
Kopa/53°18′34″N 69°20′56″ E	13.1 km^2	Kokshetau city. Northwestern part of the city
Zhaltyrkol (Arhaniya)/ 51°59'33" N 71°49'41" E	2 km^2	Arshaly district. 5 km from the rural district of Zhibek Zholy
Malaya Saryoba/51°11'05.2" N 72°11'56.8" E	2 km^2	Arshaly district. South-eastern part of the village of Saryoba
Koygeldy/51°05'38" N 71°56'38,1" E	9 km^2	Arshaly region. In the northwest of the village of Koigeldy
Zhalanash/51°03'34" N 71°00'35" E	8.83 km^2	Tselinograd district. On the banks are the villages of Akmol and Zhanazhol
Big Taldykol/51°07'27" N 71°20'20" E	5 km^2	In the city of Astana, Yesil district
Kumkol / 50°45'36" N 70°04'11"E	6.5 km^2	Korgalzhyn district. In the southeast of the village of Kumkol

Table 2. Lakes of the Akmola region, located remotely from populated areas

Name of lakes/Cordinates	Lake's area, km^2	Remoteness from the settlement or livestock complex
Bolshaya Saryoba/51°10'15"N 72°5'32"E	8 km^2	Arshaly district. 5 km west of the settlement Saryoba
Zhangula/51°11'6"N 71°58'16"E	1.44 km^2	Arshaly district, near the village of Bereke
Esei/50°28'58"N 69°38'8"E	36.46 km^2	Korgalzhyn district. The territory of the Korgalzhyn State Natural Reserve
Kokay/50°27′55″ N 69°26′03″ E	24.04 km^2	Korgalzhyn district. The territory of the Korgalzhyn State Natural Reserve
Sultankeldy/50°29'30"N 69°30'39"E	33.66 km^2	Korgalzhyn district. The territory of the Korgalzhyn State Natural Reserve

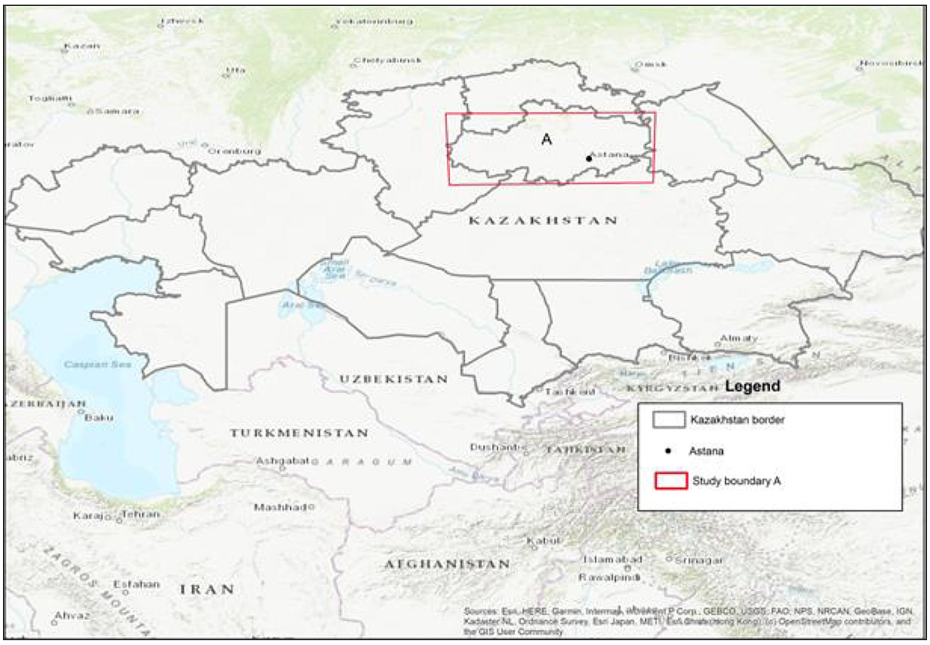


Figure 1. Area of study of lakes for antibiotic resistance on the map of the Republic of Kazakhstan

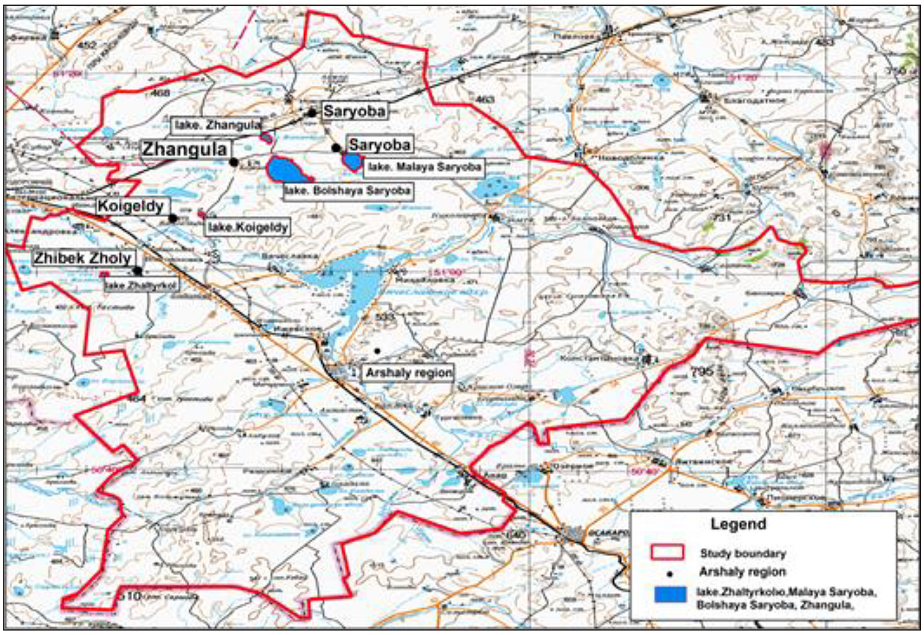
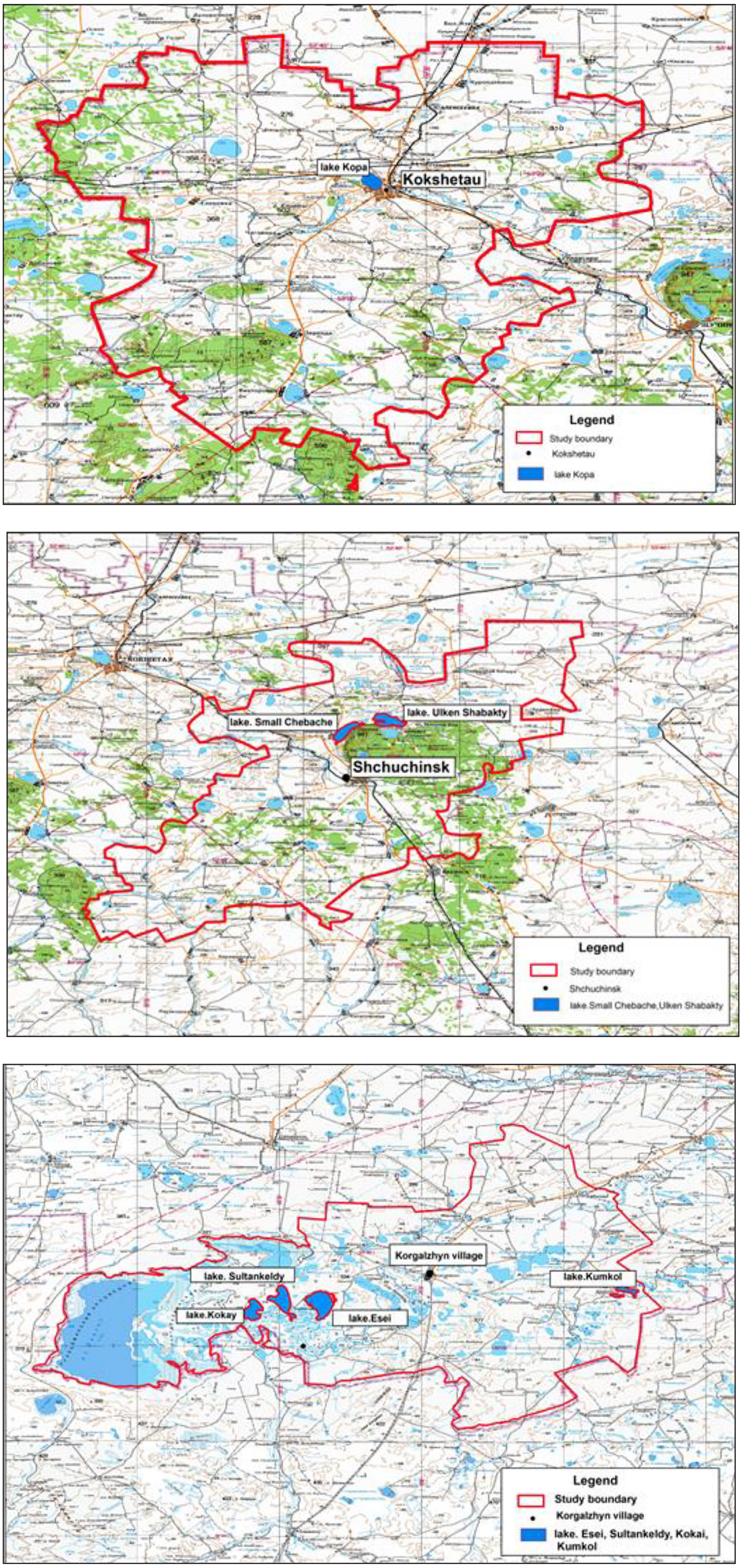
a)


Figure 2. Maps of the studied lakes for antibiotic resistance of microorganisms in the next regions: (a) Arshaly district, Akmola region



b) c) d)

Figure 2. Cont. Maps of the studied lakes for antibiotic resistance of microorganisms in the next regions: (b) Kokshetau, Akmola region; (c) Burabay district, Akmola region; (d) Korgalzhyn district, Akmola region;

e)

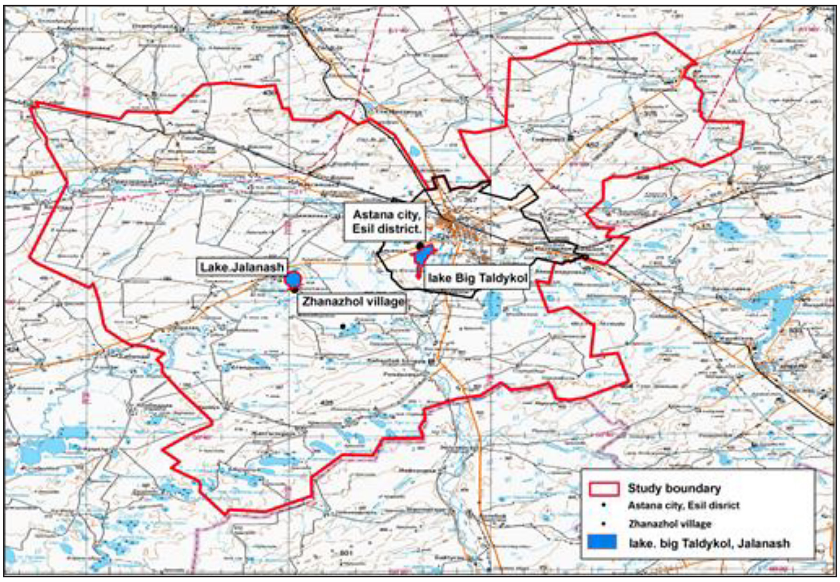


Figure 2. Cont. Maps of the studied lakes for antibiotic resistance of microorganisms in the next regions: (e) Astana and Tselinograd district, Akmola region

azithromycin, trimethoprim/sulfamethoxazole, oxytetracycline, and benzylpenicillin – was studied [Aubakirova, 2017]. The authors also studied the antibiotics for which a long period of resistance to the environment has been established, since pollutants of long persistence can cause the greatest damage in the biocenosis. A total of 40 commercially successful drugs were selected from antibiotics: amoxicillin, benzylpenicillin, nitox forte, kanamycin, lomefloxacin, streptomycin, polymyxin M sulfate, doxycycline, furazolidone, norfloxacin, enrofloxacin, tylosin, rifampicin, chloramphenicol, novobiocin, lincomycin, ampicillin, thiamisol, imipenem, clarithromycin, enronit, ciprofloxacin, gentamicin, vancomycin, furagin, nalidixic acid, cefuroxime, cefoperazone, amikacin, netilmicin, trimethoprim/sulfamethoxazole, metronidazole, cefazolin, ofloxacin, oxytetracycline, piperacillin, spectinomycin, azithromycin, eriprimum, and tromexin.

It is known that of the antibiotics used in the Republic of Kazakhstan, the most resistant to the environment are benzylpenicillin, metronidazole, ceftriaxone, and trimethoprim/sulfamethoxazole [Aubakirova, 2017]. In the general list of drugs, antibiotics most used in livestock farms include: nitox forte, enronit, ceftiosan, amoxicillin 150, eriprim, tromexin, etc. Microbiological water samples from water bodies were taken on August 1–2, 2022, in accordance with ST RK 3468-2019 “Methods for sanitary and microbiological analysis of water in surface water bodies” [ST. RK 3468, 2019]. For microbiological studies, water samples of 300 ml were taken using the envelope method and mixed into one sample. Fresh water samples were studied using a modification of the disk diffusion method for determining the sensitivity of microorganisms, which is usually used in clinical microbiology [ST RK 3643-2020, 2020; Mamytova 2021].

Agar was used in the work (“Nutritional medium for determining the sensitivity of microorganisms to antibacterial drugs, dry” TU 9398-132-78095326-2011, State Research Center for Applied Microbiology and Biotechnology, Russia). The dry mixture in the amount of 11.375 g was dissolved in 200 ml of distilled water, brought to a boil, and autoclaved in SPVA-75-I-NN at a pressure of 1 atm. within 1 hour. Cooled to body temperature, 10 ml of human blood was added in a jet. The prepared medium was poured into sterilized Petri dishes, 25 ml each. Petri dishes were seeded with a bacterial sample from a reservoir, after which they were incubated for 24 hours at a temperature of 37 °C. The grown culture was reseeded on a nutrient medium with a lawn and disks of various antibiotics were placed. Petri dishes were incubated again at 37 °C for 24 hours. After that, resistance to antibiotics was determined.

The diameter of the zone of growth inhibition was measured considering the diameter of the disk itself with an accuracy of 1 mm. When the measuring zones of growth inhibition, the authors were guided by the complete inhibition of visible growth. If the diameter of the zones of growth inhibition for the culture was less than 6 mm, this meant that at a given concentration of antibiotic in the microbial community sample, there are strains that have resistance to the antibacterial drug. This indicates that selection for antimicrobial resistance has already taken place in the reservoir. Before the start of the experiment, Escherichia coli strain ATCC25922 – Sensitive, wild type from collections of type cultures was used in separate Petri dishes to control the quality of the technique. At the same time, the culture was sown in a freshly prepared medium and disks of the tested antibiotics were used to detect sensitivity to them.

RESULTS

The control type culture of Escherichia coli strain ATCC25922 after incubation revealed clear zones of growth inhibition to antibiotic disks; therefore, it showed high sensitivity to those antibiotics that were planned for testing in samples from natural waters.

Further studies were carried out according to the scheme for each lake – 40 antibiotics. In total, 93 series of the experiment were delivered (6 antibiotics in 1 cup) in 3 repetitions. A positive result was counted if at least one of the replicates showed a zone of growth inhibition around the antibiotic disk. In the control lakes (Bolshaya Saryoba, Zhangula, Esei, Kokay, Sultankeldy). the response to all listed antibiotics was sensitivity (+). The results of the experiment of lakes in the contaminated zone are listed in Table 3. In the lakes located in the zone of suspected contamination, which are located near runoff from settlements and livestock farms, different results of sensitivity to antibiotics were found. In this group of lakes, almost all water bodies are resistant to such antibiotics as benzylpenicillin, kanamycin, streptomycin, tylosin, trimethoprim/sulfamethoxazole, metronidazole, amikacin, spectinomycin.

Also in the lakes of the first group, resistance to amoxicillin and nitox forte is found selectively (Kopa, Zhaltyrkol, Koygeldy, Zhalanash, Bolshoi Taldykol, Kumkol); lomefloxacin (Kopa, Malaya Saryoba, Zhalanash, Big Taldykol); polymyxin M sulfate (Kopa, Zhaltyrkol, Malaya Saryoba, Koygeldy, Zhalanash, Big Taldykol); doxycycline (Small Chebache, Ulken Shabakty, Kopa, Malaya Saryoba, Koygeldy, Zhalanash, Bolshoi Taldykol); furazolidone (Kopa, Zhaltyrkol, Koygeldy, Zhalanash, Big Taldykol); norfloxacin (Kopa, Zhalanash); enrofloxacin (Small Chebache, Kopa, Bolshoi Taldykol); rifampicin (Small Chebache, Ulken Shabakty; Kopa, Zhaltyrkol, Koygeldy, Zhalanash; Bolshoi Taldykol); chloramphenicol (Small Chebache, Zhalanash, Bolshoi Taldykol, Kumkol); novobiocin (Kopa, Zhalanash); lincomycin, eriprim and vancomycin (Small Chebache, Ulken Shabakty, Kopa, Zhaltyrkol, Malaya Saryoba, Koygeldy, Zhalanash, Bolshoi Taldykol); ampicillin (Kopa, Zhaltyrkol, Zhalanash, Big Taldykol, Kumkol); thiamisolu (Kopa, Malaya Saryoba, Zhalanash); imipenem (Zhalanash, Big Taldykol); clarithromycin (Kopa, Zhalanash, Big Taldykol, Kumkol); ciprofloxacin (Kopa, Malaya Saryoba, Zhalanash, Bolshoi Taldykol,); gentamicin (Kopa, Zhaltyrkol, Zhalanash, Big Taldykol); nalidixic acid (Kopa, Bolshaya Saryoba); netilmicin (Kopa, Big Taldykol, Zhalanash); cefazolin (Small Chebache, Ulken Shabakty, Kopa, Zhaltyrkol, Malaya Saryoba, Koygeldy, Kumkol); ofloxacin (Small Chebache, Ulken Shabakty, Zhaltyrkol, Malaya Saryoba, Koygeldy, Kumkol); oxytetracycline (Kopa, Big Taldykol); piperacillin, tromexin and azithromycin (Kopa, Zhalanash, Big Taldykol).

Thus, the lakes with the largest number of types of antibiotics are Kopa, Zhalanash, Big Taldykol, Zhaltyrkol, Malaya Saryoba, and Koigeldy. In the first group of lakes in all reservoirs, microorganisms showed sensitivity to such antibiotics as enronit, furagin, cefuroxime, cefoperazone, and amikacin. This fact proves the absence of the studied antibiotics in the lakes. In total, out of 360 discs tested in 1 group of lakes (9 lakes with 40 discs each), resistance was found in 54 % (in 196 cases). According to the frequency of occurrence of antibiotics in lakes (frequency of resistance), a series can be compiled in descending order of types of antibiotics in lakes (Fig. 3). The most common resistance to antibiotics in the 1st group of lakes – 90–100% of cases are: resistance to benzylpenicillin, kanamycin, streptomycin, tylosin, trimethoprim / sulfamethoxazole, metronidazole, amikacin, and spectinomycin.Less often, in 80–89% of cases, resistance to lincomycin, vancomycin and eriprim occurs. In the range of 70–79% of cases, resistance to doxycycline, rifampicin, cefazolin is observed. In these variants, there are mainly universal drugs for both humans and animals.

Then, there was resistance in 60–69% of cases to polymyxin M sulfate, and ofloxacin. In 50–59% of cases, resistance to amoxicillin, nitox forte, furazolidone, ampicillin was found.

Table 3. Sensitivity (+) and resistance (-) to antibiotics in the lakes of the Akmola region

Antibiotics and active ingredients**	Group 1 - Lakes in the contaminated zone*								
	Small chebache	Ulken shabakty	Kopa	Zhaltyrkol	Malaya saryoba	Koygeldy	Zhalanash	Big taldykol	Kumkol
1	+	+	-	-	+	-	-	-	+
2	-	-	-	-	-	-	-	-	-
3	+	+	-	-	+	-	-	-	+
4	-	-	-	-	-	-	-	-	-
5	+	+	-	+	-	+	-	-	+
6	-	-	-	-	-	-	-	-	-
7	+	+	-	-	-	-	-	-	+
8	-	-	-	+	-	-	-	-	+
9	+	+	-	-	+	-	-	-	+
10	+	+	-	+	+	+	-	+	+
11	-	+	-	+	+	+	+	-	+
12	-	-	-	-	-	-	-	-	-
13	-	-	-	-	+	-	-	-	+
14	-	+	+	+	+	+	-	-	-
15	+	+	-	+	+	+	-	+	+
16	-	-	-	-	-	-	-	-	+
17	+	+	-	-	+	+	-	-	-
18	+	+	-	+	-	+	-	+	+
19	+	+	+	+	+	+	-	-	+
20	+	+	-	+	+	+	-	-	-
21	+	+	+	+	+	+	+	+	+
22	+	+	-	+	-	+	-	-	+
23	+	+	-	-	+	+	-	-	+
24	-	-	-	-	-	-	-	-	+
25	+	+	+	+	+	+	+	+	+
26	+	+	-	+	+	+	+	+	+
27	+	+	+	+	+	+	+	+	+
28	+	+	+	+	+	+	+	+	+
29	-	-	-	-	-	-	-	-	-
30	+	+	-	+	+	+	-	-	+
31	-	-	-	-	-	-	-	-	-
32	-	-	-	-	-	-	-	-	-
33	-	-	-	-	-	-	+	+	-
34	-	-	+	-	-	-	+	+	-
35	+	+	-	+	+	+	+	-	+
36	+	+	-	+	+	+	-	-	+
37	-	-	-	-	-	-	-	-	-
38	+	+	-	+	+	+	-	-	+
39	-	-	-	-	-	-	-	-	+
40	+	+	-	+	+	+	-	-	+

Note: *sensitivity (+) and resistance (-) to antibiotics. **List of 40 commercially successful antibiotics: amoxicillin (1), benzylpenicillin (2), nitox forte (3), kanamycin (4), lomefloxacin (5), streptomycin (6), polymyxin M sulfate (7), doxycycline (8), furazolidone (9), norfloxacin (10), enrofloxacin (11), tylosin (12), rifampicin (13), chloramphenicol (14), novobiocin (15), lincomycin (16), ampicillin (17), thiamisol (18), imipenem (19), clarithromycin (20), enronit (21), ciprofloxacin (22), gentamicin (23), vancomycin (24), furagin (25), nalidixic acid (26), cefuroxime (27), cefoperazone(28), amikacin (29), netilmicin (30), trimethoprim/sulfamethoxazole (31), metronidazole (32), cefazolin (33), ofloxacin (34), oxytetracycline (35), piperacillin (36), spectinomycin (37), azithromycin (38), eriprimum (39), tromexin (40).

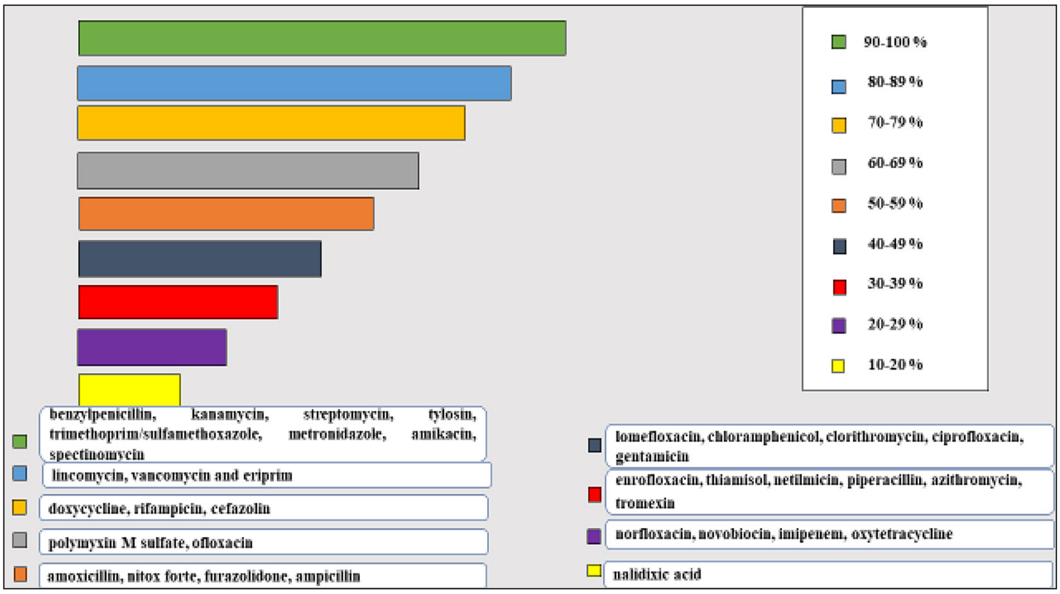


Figure 3. Comparison of resistance of microorganisms to antibiotics in the lakes of the Akmola region, %

The next group is 40–49% resistance for lomefloxacin, chloramphenicol, clorithromycin, ciprofloxacin, and gentamicin. 30–39% resistance was recorded for enrofloxacin, thiamisol, netilmicin, piperacillin, azithromycin, and tromexin. Less common is resistance to such antibiotics as norfloxacin, novobiocin, imipenem, oxytetracycline, which occurred in 20–29% of cases. Least of all, up to 10%, resistance to nalidixic acid was detected. Among the antibiotics to which resistance was not detected in any case, there were enronit, furagin, cefuroxime, and cefoperazone. In the control group of lakes, resistance to some of the above-mentioned antibiotics is found only in lakes Bolshaya Saryoba and Zhangula.

In the lakes of the control group, resistance to benzylpenicillin (Bolshaya Saryoba, Zhangula); kanamycin, streptomycin, tylosin, enronit, nalidixic acid and oxytetracycline (Bolshaya Saryoba) is found selectively. This indicates that the remoteness of the last two lakes from settlements sometimes does not prevent antibiotics from infiltrating into them. Perhaps this occurs through other migration routes of water pollutants. Most likely, antibiotics enter the lakes from the leaching of organic fertilizers applied in catchment areas.

Thus, the presence of resistance to these antibiotics indicates a large number and possibly their long-term persistence in the aquatic environment. Among them, a significant place of 87.5% is occupied by antibiotics, which, along with drugs for humans, are also used for animals and birds, or only for animals and birds – 35 items from the list of antibiotics studied. The resistance to this group of antibiotics becomes an argument for poor control of the use of antibiotics in animals. In the region, these medicines most often directly enter the environment past the treatment facilities [Sabily et al., 2020]. The largest share in this case is made up of antibiotics from manure, which is popular in vegetable farms. Antibiotics accumulate in the soil of these fields and migrate to surface waters with storm runoff [Kenneth et al., 2023].

If several resistant antimicrobials are present in the lake, it is rather difficult to study their synergistic effect since they can interact with various intermediate and final products. However, even in this case, the removal of sensitive members of the microbial community can occur, which causes serious disturbances in the balance between different types of bacterioplankton, especially the fraction of heterotrophic bacterioplankton [United Nations Environment Programme, 2017; Akbayeva et al., 2014]. Violation of the ratio of functional groups of bacteria can affect the decrease in self-cleaning capacity in the reservoir, triggering the processes of accelerating eutrophication [Akbayeva et al., 2020].

CONCLUSIONS

Thus, in the conducted work, a rather sensitive qualitative method of biotesting for the presence of antibiotics in natural waters was used. An area with a large territory was studied, on which lakes were located at different distances from pollution sources. Sensitivity to antibiotics persisted in the main control lakes located far from settlements, in the Korgalzhyn nature reserve. On the basis of the results obtained, the following conclusions were drawn:

1. Antibiotics that have not been found to be resistant to microorganisms in lakes include: enronit, furagin, cefuroxime, cefoperazone, and amikacin.
2. Antibiotics in descending order of their resistance in natural waters can be divided into 9 groups:
   - 90–100% – benzylpenicillin, kanamycin, streptomycin, tylosin, trimethoprim/sulfamethoxazole, metronidazole, amikacin, and spectinomycin;
   - 80–89% – lincomycin, vancomycin and eriprim;
   - 70–79% – doxycycline, rifampicin, and cefazolin;
   - 60–69% – polymyxin M sulfate, and ofloxacin;
   - 50–59% – amoxicillin, nitox forte, furazolidone, and ampicillin;
   - 40–49% – lomefloxacin, chloramphenicol, clorithromycin, ciprofloxacin, and gentamicin;
   - 30–39% – enrofloxacin, thiamisol, netilmicin, piperacillin, azithromycin, nd tromexin;
   - 20–29% – norfloxacin, novobiocin, imipenem, and oxytetracycline;
   - 10–20% – nalidixic acid.
3. In the control group of lakes, sensitivity to antibiotics remains (except for lakes Bolshaya Saryoba and Zhangula), which indicates the absence of the studied drugs in them.
4. Lakes with the largest number of types of antibiotics include: Kopa, Zhalanash, Big Taldykol, Zhaltyrkol, Malaya Saryoba, and Koigeldy.
5. Antibiotics that are used simultaneously for both humans and animals, or only for animals, are most often found to be resistant.

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