Antibiotic resistance of microorganisms isolated from a Baikal seal

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Abstract. The article presents the results of microbiological studies of the material taken from the seals' corpses. The authors studied the taxonomic characteristics of microorganisms, their virulence and antibiotic resistance. It has been established that the following types of microorganisms are found in the seal: Citrobacter braakii, S. warneri, S. aureus, Escherichia coli serotypes O2, O18 and O39, Lactobacillus sakei, Aguamicrobium lusatiense. All pathogens isolated from the gastrointestinal tract were sensitive to 52.6% of antibiotics (ampicillin, cefuroxime, cefixime, gentamicin, amikacin, ciprofloxacin, ofloxacin, furadonin, Ceftriaxone) and resistant to 15.8% of antibiotics, two representatives of cephalosporins (cefaclor and ceftazidim) and ticarcillin. Conditionally pathogenic enterobacteria (Citrobacter braakii and Escherichia coli) isolated from muscles and liver were sensitive to all antibiotics.

1. Introduction
Lake Baikal is the oldest and deepest and largest (by volume) freshwater reservoir of the Earth, in which 20% of all freshwater of lakes and rivers of the world is concentrated. A unique feature of the freshwater ecosystem of lake Baikal is the habitat of the Baikal seal. Baikal seal (Pusa sibirica Gm.) - the only endemic aquatic mammal of Baikal occupies an extremely important place in the ecosystem of the lake [1, 2].

The welfare of the seal population not only reflects the state of fish resources but also is an indicator of the well-being of the Baikal hydrogenolysis as a whole, including the level of anthropogenic impact.

The purpose of work was to study the taxonomic characteristics of microorganisms with the determination of their antibiotic resistance and virulence.

2. Objects and methods
The object of the study was 3 seals corpses of caught near the village of Listvyanka in the Irkutsk region and the village of Goryachinsk in the Republic of Buryatia. Sampling (liver, kidneys, spleen, heart, lungs, stomach, large and small intestines), isolation of cultures of microorganisms, their identification was carried out according to generally accepted methods based on the Department of diagnostics of bacterial and parasitic diseases of the Irkutsk interregional veterinary laboratory” [3-7].

The virulence determination of the tested microorganisms in vivo (intraperitoneal test) was carried out by the ability to cause the death of white mice. Laboratory animals (white mice) were intraperitoneally injected with 0.5 ml 1 billion of suspensions in the amount of three for each bacterial culture. Laboratory animals were observed at the following exposures: 24, 48, and 72 hours and 10 days.
Animals were kept at the FSBI Irkutsk Interregional Veterinary Laboratory. The experiments were carried out under the "Rules for the use of experimental animals", approved by the Ministry of Health of the USSR (1977), "International recommendations for biomedical research using animals" (1986).

To determine the sensitivity of microorganisms to antibacterial drugs, the standard disco-diffusion method was used. Evaluation of the results was carried out according to MI 4.2.1890-04 "Determination of the sensitivity of microorganisms to antibacterial drugs". The results were interpreted as follows: strains are stable (R - resistance), sensitive (S - sensitivity) and have intermediate stability (I - intermediate).

In particular, to determine the sensitivity of enterobacteria, disks with antimicrobial drugs were used, which are the most important for the treatment of infections caused by these microorganisms, i.e. first-line and reserve drugs: ampicillin, amoxicillin, cefuroxime, cefaclor, cefixime, cefepime, imipenem, gentamicin, amikacin, ciprofloxacin, ofloxacin, norfloxacin, levofloxacin, trimethoprim, furadonin, Cefotaxime, Ceftriaxone, ceftazidime, ticarcillin, benzylpenicillin erythromycin, oxacillin, clindamycin, levofloxacin, vancomycin, linezolid, fusidin, doxycycline, rifampicin, levomycetin.

To determine the sensitivity of staphylococci, disks with antimicrobial drugs were used: benzylpenicillin, oxacillin, gentamicin, erythromycin, ciprofloxacin, clindamycin, levofloxacin, vancomycin, linezolid, trimethoprim, fusidin, doxycycline, rifampicin, levomycetin.

Statistical processing of these results was performed using generally accepted statistical criteria.

Research results and discussion

It has been established that the following types of microorganisms are found in the seal: Citrobacter braakii, S. warneri, S. aureus, Escherichia coli serotypes O2, O18 and O39, Lactobacillus sakei, Aguamicrobium lusatiense.

It was established that in the seal, the microflora of the cardiovascular system was represented by two isolates: Brevundimonas diminuta and Aguamicrobium lusatiense, respectively, in 33 and 100 percent of cases. In the urogenital system were detected microorganisms of the genus Staphylococcus spp.: S. warneri and S. aureus. In the lungs, Aguamicrobium lusatiense was isolated. In the gastrointestinal tract of the seal, pathogenic enterobacteria predominantly prevailed: Citrobacter braakii, Escherichia coli serotype O18 and O39 (figure 1). Conditionally pathogenic enterobacteria were mainly found in muscle tissue and liver. Citrobacter braakii, Escherichia coli serotypes O2 and O39 and Lactobacillus sakei were isolated in the liver. Citrobacter braakii, Escherichia coli serotypes O2 and O3 were isolated in muscle tissue.

Figure 1. Taxonomic characteristics of the microorganisms of the gastrointestinal tract of the seal.
The study of antibiotic resistance pathogenic enterobacteria isolated from the gastrointestinal tract showed that resistance to various antibiotics varied significantly. All microorganisms were sensitive to 52.6% of antibiotics (ampicillin, cefuroxime, cefuroxime, gentamicin, amikacin, ciprofloxacin, ofloxacin, furadonin, Ceftriaxone) and resistant to 15.8% of antibiotics, two representatives of cephalosporins (cefaclor and ceftazidime) and ticarcillin. High resistance was shown to antibiotic in trimethoprim, used for the prevention and treatment of diseases of the urinary system - 87.5 percent. 62.5% are resistant to cefotaxime; norfloxacin - 50%; imipenem - 25%; cefepime and amoxicillin - 12.5% of isolates (figure 2).

Conditionally pathogenic enterobacteria (Citrobacter braakii and Escherichia coli) isolated from muscles and liver were sensitive to all antibiotics.

Microorganisms isolated from the heart were sensitive to all antibiotics in 38.7% of cases (ampicillin, amoxicillin, gentamicin, amikacin, ciprofloxacin, ofloxacin, furadonin, benzylpenicillin, oxacillin, erythromycin, clindamycin, linezolyl); in 6.5% of cases, all microorganisms were resistant to ticarcillin and fusidine.

Resistance to imipenem was established - 75%, cefaclor - 50%, norfloxacin, trimethoprim, levofloxacin, cefixime, cefepime, cefotaxime, trimethoprim, doxycycline - 25% of isolates (tables 1, 2).

Microorganisms isolated from the lungs were characterized by resistance to all antibiotics in 3.2% of cases and 48.4% of cases by sensitivity to all antibiotics. So, about 66% of isolates were resistant to cefaclor, imipenem and fusidine, 33% to norfloxacin, trimethoprim, cefotaxime, levofloxacin and doxycycline.
**Table 1.** Antibiotic resistance of microorganisms isolated from the heart and lungs of a seal. (Ampicillin- Furadonin).

| Name of the microorganism/place of isolation | Name of the antimicrobial drug (antibiotic) extended set |
|---------------------------------------------|---------------------------------------------------------|
| **Brevundimonas diminuta / heart**           | S* S S I S I R S S S I S I S |
| A. lusatiense (isolate 1) / heart            | S S S I S I R S S S S I S I S |
| A. lusatiense (isolate 2) / lung             | S I S R S I I S S S S R S R S |
| A. lusatiense (isolate 3) / Heart            | S S S R S R I S S S S R I R S |
| A. lusatiense (isolate 4) / lung             | S S S R S I R S S S S I S I S |
| A. lusatiense (isolate 5) / Heart            | S S S R S I R S S S S R S Y S |
| A. lusatiense (isolate 6) / lung             | S S S I S I R S S S S I S I S |

*S – sensitive, I – intermediate, R – resistant.

**Table 2.** Antibiotic resistance of microorganisms isolated from the heart and lungs of a seal. (Cefotaxime - Levomycetin).

| Name of the microorganism/place of isolation | Name of the antimicrobial drug (antibiotic) extended set |
|---------------------------------------------|---------------------------------------------------------|
| **Brevundimonas diminuta/ heart**            | S R I R S S S R S S R S I S |
| A. lusatiense (isolate 1) / heart            | S R I R S S S S I S R R S I |
| A. lusatiense (isolate 2) / lung             | I S R R S S S S S I S I R S I |
| A. lusatiense (isolate 3) / Heart            | I S R R S S S S R S S R S I S |
| A. lusatiense (isolate 4) / lung             | R S R R S S S S R S S R S I S |
| A. lusatiense (isolate 5) / Heart            | R S R R S S S S I S S R S I S |
| A. lusatiense (isolate 6) / lung             | S R I R S S S S I S S R S I S |
When testing staphylococci, isolates resistant to oxacillin (class of penicillins) were not detected. However, all tested staphylococci were resistant to another representative of this class (benzylpenicillin). All isolates were also resistant to trimethoprim. There were no isolates resistant to gentamicin, erythromycin, ciprofloxacin, levofloxacin, vancomycin, fusidin, rifampicin, and levomycetin.

**Table 3.** Antibiotic resistance of microorganisms isolated from the heart and lungs of a seal.

| Name of the microorganism | Benzylpenicillin | Oxacillin | Gentamicin | Erythromycin | Ciprofloxacin | Clindamycin | Levofloxacin | Vancomycin | Linezolid | Trimethoprim | Fusidin | Doxycline | Rifampicin | Levomycetin |
|---------------------------|------------------|-----------|------------|--------------|---------------|-------------|--------------|-------------|-----------|--------------|---------|-----------|------------|-------------|
| S. warneri                | R* S S S S R S S I R S I S S |
| S. aureus – isolate 1    | R S S S S R S R R S I S S |
| S. aureus - isolate 2    | R S S S S I S S R R S I S S |
| S. aureus – isolate 3    | R S S S S I S S I R S I S S |

* R- resistant, S – sensitive, I – intermediate.

3. Conclusions

It has been established that the following types of microorganisms are found in the seal: Citrobacter braakii, S. warneri, S. aureus, Escherichia coli serotypes О2, О18 and О39, Lactobacillus sakei, Aguamicrobium lusatiense.

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Microorganisms isolated from the heart were sensitive to all antibiotics in 38.7% of cases (ampicillin, amoxicillin, gentamicin, amikacin, ciprofloxacin, ofloxacin, furadonin, benzylpenicillin, oxacillin, erythromycin, clindamycin, linezolyl); in 6.5% of cases, all microorganisms were resistant to ticarcillin and fusidine.

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