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Microbiological monitoring of causative agents of infectious diseases of salmons in the Northwest region

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Abstract. In article results of microbiological monitoring of aquatic organisms of different species in the territory of the northwest region of the Russian Federation are provided. During the period from January, 2016 to December, 2018 it was subjected to a research of 13 439 samples of salmons. As a result of bacterial researches it is allocated 235 etiologically significant strains of microorganisms. By microbiological plating gram-negative microflora such as yersiniya, mixobacteria, pseudomonas is most often detected, it has high sensitivity to cephalosporins, fluoroquinolones, chloramphenicol and it is resistant to furasolidone, piperacillin, kanamycin, amikacin, aztreonam. In a mariculture at mass death from internals and gill apparatus of fishes allocated microorganisms of *Flexibacter branchiophila* (*Flavobacterium branchiophilum*) with the accompanying coccal microflora. For the first time in the country the allocation case in an aquaculture of gram-positive bacteria of *Renibacterium salmoninarum* is described and its antibiotic resistance is studied.

1. Introduction

Every year global demand for products of an aquaculture increases more and stronger that in turn stimulates development of the industry. Ensuring enough and quality fish products is possible only in the conditions of use of the modern technologies combining use of open reservoirs and submersible tanks. Intensive cultivation requires increase in density of aquatic organisms up to 40 kg at 1 cubic meter and above. Undoubted pluses of high densities of cultivation (technological effectiveness and convenience of fish-breeding processes) are counterbalanced with serious danger of development of infectious diseases, especially bacterial and deterioration in sanitary and hygienic conditions of keeping of aquatic organisms.

Increase in the outputs, active trade in a fish stock between areas and the countries, increase in fish-holding density and the number of farms in local water areas inevitably is followed by developing of bacterial diseases. For stopping of diseases, and in some cases with the preventive purposes employees of farms begin to apply unreasonably widely and irrationally antibiotics that finally leads to selection of antibiotic-resistant strains of microorganisms, increase in costs of the subsequent treatment at decrease in their efficiency, to a long bacteriocarrier.

The purpose of the research is studying of etiological structure of causative agents of infectious diseases of salmons in the Northwest region and also the level of an antibiotic resistant of the emitted isolates.



2. Materials and methods

During the period from January, 2016 to December, 2018 10636 samples of a Rainbow trout, 1104 samples of an Atlantic salmon, 1699 samples of salmon of other species (peled, broad whitefish, muksun, pollan, bull-trout) from the enterprises of the Northwest region are subjected to researches; scientific researches were carried out in ichthyopathology laboratory to Federal State Budget Institution «Federal Scientific Centre VIEV» (FSC VIEV). Sampling and bacteriological researches were carried out according to the practical standards, according to the compendium of instructions for fish disease control and other normative documents [7,9]. For identification the schemes stated in "The guide to definition of bacteria" [6] and "Determinant of uncommon pathogenic gram-negative bacteria" [2] are used. For differentiation of microorganisms carried out inoculation of selective and accumulative mediums: SKDM and KDM-2 - for *Renibacterium salmoninarum*; Anacker & Ordal, s; FPM - for *Flexibacter columnaris*, *Flexibacter branchiophila*, *Cytophaga psychrophila*; Furunculosis agar, baktoagar Difko, TSA, TSB, SW, MPA with phenylalanine and MPB - for *Aeromonas salmonicida* (and mobile types: *Aeromonas*, *Pseudomonas*), *Yersinia*, etc. types; Medium No. 10, a serum agar, enterokokkagar - *Staphylococcus*, *Streptococcus* [1,8]. The Polymerase Chain Reaction (PCR) was carried out with use of primers, the offered OIE [10] and author's design [4].

Determination of sensitivity to antibacterial drugs was carried out by disk diffusion test with use of disks of firms of LLC NITsF (Russia), "HIMEDIA" (India), "OXOID" (Great Britain).

The sensitivity of the allocated strains of gram-negative microorganisms was defined to ofloxacin, lomefloxacin, enrofloxacin, norfloxacin, ciprofloxacin, amikacin, cefalexin, cefixim, ampicillin, piperocillin, meropenem, cefepim, chloramphenicol (levomycetinum), carbenicillin, kanamycin, polymyxin, tetracycline, furadonin, gentamycin, tobramycin, cefazolin, cefalotin, ceftriaxon, erythromycin, furasolidon, lincomycin.

The sensitivity of *Renibacterium salmoninarum* was defined to vancomycin, gentamycin, lincomycin, rifampicin, ciprofloxacin, erythromycin, streptomycin, cefoperazon, cefipim, imipenem, norsulfazol, sulfanetoxazol, sulfadimezin, trimethoprim, moxifloxacin, enrofloxacin, norfloxacin.

The etiological role and virulence of the allocated microorganisms were defined in a biological test.

3. Results and discussion

During the research period 442 addresses for a bacteriological research of aquatic organisms of different types from fish farms of the Northwest region were registered. Positive results are received in 75 cases that makes 17%, in other cases of growth of virulent bacterial microflora was not revealed.

Most often myxobacteria, the causative agent of a yersiniosis and pseudomonas (tab. 1) was detected.

Table 1. A range of the allocated microorganisms.

Bacterial species	Number of cases detected	Bacterial species	Number of cases detected
<i>Cytophaga psychrophila</i> (<i>Flavobacterium psychrophilum</i>)	30	<i>Streptococcus</i> sp.	3
<i>Yersinia ruckeri</i>	28	<i>Flexibacter branchiophila</i> (<i>Flavobacterium branchiophilum</i>)	2
<i>Pseudomonas chlororaphis</i>	18	<i>Enterococcus</i> sp.	2
<i>Flexibacter columnaris</i> (<i>Flavobacterium columnare</i>)	17	<i>Enterobacter</i> sp.	1
<i>Renibacterium salmoninarum</i>	10	<i>Aeromonas salmonicida</i>	1

Aeromonas sobria	9	Mariculture	
Aeromonas caviae	7	Vibrio wodanis	4
Citrobacter freundii	6	Tenacibaculum maritimum	3
		(Cytophaga marina; Flexibacter maritimus)	
Pseudomonas fluorescens	5	Moritella viscosa	2
Pseudomonas putida	4	Flexibacter branchiophila	2
		(Flavobacterium branchiophilum)	

From gram-negative flora *Cytophaga psychrophila* was prevailed. At the same time it is established that aeromonas, pseudomonas and myxobacteria are most often detected in the form of associations with different values in colony-forming units in initial bacterial inoculations, and which pathogenicity at statement of a bioassay is usually insignificant, development of clinical signs is not observed. Contamination of internal organs of fishes by weakly virulent forms of bacteria testifies to a current of the bacterial hemorrhagic septicaemia (BHS) and the general decrease in resistance of the organisms of fishes.

Of *Yersinia ruckeri* allocated from internals it was characteristic plentiful, passing in drain, growth of microorganisms that usually testifies to an acute septicaemia. Practically in all cases the disease proceeded as monoinfection.

The part of streptococcus, enterococcus, motionless aeromonas of *Aeromonas salmonicida* - the causative agent of a furunculosis salmon in structure allocated during the analyzed period of strains of microorganisms is extremely insignificant (1-3 cases).

10 cases of allocation of gram-positive microorganisms of a type of *Renibacterium salmoninarum* - the causative agent of a bacterial kidney disease are revealed (BKD). Forming of colonies on the nutrient medium KDM-2 and SKDM was noted for 15 and 21 days (respectively) that testified to an open form of a disease, and in some cases for the 45 and 55 days as latent bacteriocarrier. It should be noted that this first message about identification of the causative agent of BKD in the aquaculture of the Russian Federation.

In a mariculture isolated such microorganisms from fishes as *Tenacibaculum maritimum* (*Cytophaga marina*; *Flexibacter maritimus*), *Vibrio wodanis*, *Moritella viscosa*. This microflora is conditional-pathogenic and its range demonstrates decrease in resistance of an organism of salmon during the winter period. Disturbance of biotechnics of cultivation, influence of other stress factors and further depression of immunity led either to development as independent diseases or to the outbreaks of the associated infection.

Table 2. The greatest sensitivity to antibacterial drugs of the gram-negative bacteria allocated from fishes (by species).

Name of an antibiotic	<i>Cytophaga psychrophila</i>	<i>Yersinia ruckeri</i>	<i>Pseudomonas chlororaphis</i>	<i>Flexibacter columnaris</i>
Ofloxacin (OF)	+	+	+	±
Enrofloxacin (EF)	+	+	+	±
Cefalexinum (CFL)	+	±	±	+
Norfloxacin (NOR)	±	±	±	±
Ciprofloxacin (CIP)	±	+	±	±
Lomefloxacin (LOM)	+	+	+	+
Ampicillin (AMP)	-	-	±	±
Cefepime (CPM)	±	±	+	+
Chloramphenicol (C)	±	±	±	+
Tetracycline (TET)	±	±	±	-

Nalidixic acid (NA)	-	-	\pm	\pm
Cefazolinum (Cz)	+	\pm	+	\pm
Ceftriaxone (CRO)	+	+	\pm	\pm
Furazolidone (FRN)	-	-	+	-

In table 2 the sensitivity to antibacterial drugs of the microorganisms which are most often allocated from fishes is provided. Aeromonas, except for A.salmonicida, pseudomonas P. putida and also streptococcus and enterococcus are conditional-pathogenic for fishes therefore isolated cases of their identification, as a part of microbic associations, allowed not to consider their individual characteristics of sensitivity at selection of antibacterial therapy.

It is established that the most part of strains keeps sensitivity to drugs of the cephalosporins (ceftriaxone, cefalexinum, cefepime), the fluoroquinolones (enrofloxacin, norfloxacin, ofloxacin, etc.), to chloramphenicol, at the same time all strains differed in high resistance to furasolidone, a piperacillin, kanamycinum, amikacin, aztreonam, except for several strains of Pseudomonas chlororaphis.

Table 3. Sensitivity of *Renibacterium salmoninarum* to antibacterial drugs.

Name of an antibiotic	Sensitivity	Name of an antibiotic	Sensitivity
Rifampicin	\pm	Norsulfazolum	+
Cefoperazone	\pm	Sulfamethoxazole	\pm
Cefepime	\pm	Sulfadimezin	\pm
Imipenem	+	Trimethoprim	+
Moxifloxacin	\pm	Erythromycin	+

Apparently from table 3, all allocated strains of *Renibacterium salmoninarum* were moderate and highly sensitive to synthetic derivatives of sulphamid, carbapenem, erythromycin, showed good activity synthetic bacteriostatic drug, derivative a diaminopyrimidine - Trimethoprimum. The high level of resistance is established for gentamycin, vancomycinum, streptomycin, the tetracyclins and fluoroquinolones I of generation, penicillinic group. In a mariculture the serious danger is constituted by identification from internals (kidneys) of fishes and the gill of microorganisms of *Flexibacter branchiophila* (*Flavobacterium branchiophilum*) with the accompanying coccal microflora. Infection of gills demonstrates development of a bacterial gill disease (BGD) at which for a short time there is a mass death of fish without visible pathology. Over time infectious process passes into a chronic stage that is followed by the high mortality of fishes with manifestation of clinical signs of a disease: refusal of a forage, a disorientation in space (fish accepts vertical position, chaotic swimming), lack of reactions to external irritants, increase in number of respiratory movements, open gill covers. The fish struck and weakened by a bacterial gill disease is susceptible to infection with conditional-pathogenic microorganisms of the bacterial and/or fungus nature.

For the treatment of a mixobacteriosis (flavobacteriosis, BGD) can apply drugs, however in the conditions of sea fish-breeding complexes it is physically impossible. Therefore, disease control and prevention of further distribution of the activator consists in observance of veterinary health regulations: timely collecting and utilization of corpses and fishes with clinical signs of a disease, regular cleaning dels from the remains of a forage, waste products.

4. Conclusion

In the absence of symptoms of bacterial diseases independent purpose and use of antibacterial drugs is irrational and inefficient as the part of true virulent causative agents of diseases in actively developing aquaculture of the Northwest is still small - 17%. The great influence on forming of economic losses in the industry is exerted now by adverse ecological conditions, non-compliance with conditions of keeping, stocking density, fodder toxicoses, etc. Uncontrolled use of drugs in the conditions of the unconfirmed

diagnosis and a certain antibiogram promotes forming of antibiotic-resistant strains of microorganisms that limits further the choice of medicamentous means of disease control, creates the center of a bacteriocarrier and, in general, has negative effect on the environment, an organism of fishes and consumer qualities of products.

Knowledge of etiological structure of an infectious disease and resistance of the revealed microbic association at the enterprise is a basis for elaboration of strategy of antibacterial therapy. Use of drugs has to be based on results of a microbiological research. In case of mass death of fishes with objective suspicions of the bacterial disease (cankers on a body, an exophthalmia, etc.) caused by gram-negative microflora for immediate appointment it is necessary to use drugs of the cephalosporins (ceftriaxson, cefalexin, cefepime), the fluoroquinolones (enrofloxacin, norfloxacin) or chloramphenicol. At the same time an antibioticotherapy does not guarantee 100% of elimination of a yersiniosis, mixobacteriosis and some other and also lack of repeated flashes, owing to high stability of causes agents in external environment, especially with a large amount of organic matters (ground silt deposits, stillwater, contaminated dells, etc.) that is inevitable by intensive production. It is much more reasonably world practice of disease control of bacterioses of fishes which consists in universal vaccinal prevention and observance of veterinary health regulations at cultivation. In Russia development and deployment of vaccinal drugs from endemic strains of bacteria as to authors it is important that prevention promoted steady biological production at insignificant, strictly reasonable consumption of antibiotics is also conducted [3,5].

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