Potentially dangerous to human pathogenic thermophilic microorganisms of fish in recirculation aquaculture systems

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Abstract. This article discusses the pathogenic threat to humans thermophilic microorganisms encountered in recirculation aquaculture systems for growing fish. This article discusses such genera as — Escherichia, Salmonella, Staphylococcus, Vibrio, Pseudomonas, Aeromonas and some of their subspecies, the most characteristic and representing the greatest distribution and danger to humans, including golden and methicillin-resistant Staphylococcus aureus and MRSA (S. aureus and MRSA), hydrophilic aeromanad (A. hydrophilia), Pseudomonas aeruginosa, choleric (V. cholerae) and non-choleric (V. parahaemolyticus, V. vulnificus and others) vibrians. Their pathogenic mechanism, the diseases they cause in humans, their danger and resistance to environmental conditions, as well as their sensitivity to antimicrobials and antibiotics also considered. In addition, the topic of preventing them from entering the ultrasound system and possible preventive measures discussed.

1. Introduction
Now, fish farming in recirculation aquaculture system actively used in many regions due to the increased convenience and efficiency of this method. Fish breeding technology in this way greatly facilitates the maintenance of the breeding environment, monitoring the state of the system and the fish bred. This method also allows you to install systems of aggregates that meet certain economic needs. In addition, this type of economy has to base in regions that initially do not have the necessary natural resources for breeding the desired species of fish [1].

Because of fish breeding and the functioning of the recirculation, aquaculture systems, specific microflora appears in it is the water. This is due to the complexity of the presence and content of fish in the system, which is accompanied by its own set of microorganisms (agents of its microflora, parasites and symbionts), as well as due to the presence of various biogenic elements in the water [2].

Among these, there are also potentially dangerous thermophilic microorganisms that are pathogens of various diseases not only in fish, but also in humans [3]. For example: enteropathogenic Escherichia coli (ETEC), Pseudomonas aeruginosa, various Salmonellae sp., and others [4].

Pathogenic microorganisms can separated into indicators of two types of contamination: the first is a violation of the operation of the recirculation aquaculture system is cleaning system, which leads to increased activity of the natural microflora, and the second is the pervasion of parasitic microorganisms into the recirculation aquaculture systems.
2. Materials and methods

To determine the most pathogenic types of bacteria found in closed water supply systems, information from scientific articles was used, using such search engines as, Web of Science, PubMed and Google Scholar. The following keywords used in the search: pathogenicity, recirculation aquaculture system, fish, microorganisms, thermophilic; and others. In addition, for more information, the cited links from the found articles used. In some cases, we guided by our own scientific works [3; 5-7].

The information received was structured, and the works with the highest number of citations (scientific significance) selected among the sources.

3. Results

An important indicator of fecal contamination in the conditions of sanitary and veterinary assessment of water quality in the recirculation aquaculture systems: Escherichia coli - mobile gram-negative rods, aerobes belonging to the species Escherichia coli, the genus Escherichia, the family Enterobacteriaceae. Under normal conditions, they are present in water in small concentrations, since their presence accompanied by fecal contamination. An important aspect is that water treatment systems in such systems do not allow increasing water contamination with these microorganisms and prevent their accumulation. Otherwise, if the water treatment system does not work correctly or the conditions for keeping fish violated, their concentration inevitably increases, which indicates that the water contaminated with fish products. Among these, there may be pathogenic thermophilic species for humans, such as the Escherichia coli strain O104:H4 [8] — a highly pathogenic strain that can cause intestinal infections in humans and some animals, as well as in the course of severe disease thrombocytopenia with kidney damage and unstable CNS disorders. As well as the Escherichia coli strain O157:H7 [9], which is often the cause of food poisoning. Among the genus Escherichia, there are also less virulent strains, but even their presence in the water of recirculation aquaculture systems in case of excess of the norm indicates fresh fecal contamination, which does not exclude the presence of high concentrations of more dangerous strains for fish and humans. These stereotypes are primarily dangerous due to the release of an abundance of extracellular toxins that poison the human body and lead to disorders of the gastrointestinal tract.

Basically, Enterobacteriaceae have moderate survivability — in water in the temperature range of 19-24 °C up to 7 days, on a dry surface at the same temperatures from 3 hours to 3 days, they are sensitive to antibiotics and antimicrobials, so their suppression both in water and on dry surfaces and when ingested is quite effective.

The family Enterobacteriaceae also includes Salmonellae - gram-negative mobile rod-shaped bacteria that have a high pathogenic danger to humans. Under normal conditions, these bacteria should not be present in the water of recirculation aquaculture systems due to their high virulence for fish and humans. However, in natural conditions, this bacterium occurs in water, but, at the same time, its presence in fish breeding systems for food purposes is unacceptable [5]. Like Escherichia, Salmonella is also a causative agent of intestinal infections and diarrhoeal diseases, as well as gastroenteritis, about 10% of the world's population suffer from salmonellosis every year, which makes this genus one of the most common diseases in the world associated with the use of poor-quality water and fish. However, the cases recorded in statistics are approximately 10-20 times lower than the actual cases [10]. Among the large abundance of pathogenic species for humans, we should note the two most common - Salmonella bongori and Salmonella enterica. The pathogenic mechanism of Salmonella is similar to that of Escherichia in terms of ancestry. The presence of Salmonella in the water of recirculation aquaculture systems indicates primarily the lack of measures to ensure sanitary safety, in such cases, fish products considered unfit for sale, and the contaminated system is subject to cleaning from possibly accumulated pathogens and disinfection of bacteria.

Salmonella’s bacteria are highly resistant - in water in the temperature range of 19-24 °C survives from 3 weeks to several months, on a dry surface at the same temperatures from 2 to 4 weeks. There are also stereotypes that are resistant to antibiotics and antimicrobials, which complicates the fight against them [11]. In the context of fisheries, special attention should paid to working tools such as
scalpels, nets, trays for carrying and storing fish. Since Salmonella has a high survivability in the open air in a dry environment. This contributes to the infection of obviously clean fish production material through contaminated tools, which is not always possible to track and/or control.

The next agent of pathogenic aquatic microflora is Staphylococcus – gram-positive pathogenic bacterium of coccoid morphology of the genus Staphylococcus. Now, there are about 27 types of staphylococcus known in the world, but the most dangerous for humans is Staphylococcus aureus. Under normal conditions, these bacteria should not be present in the water of recirculation aquaculture systems [7] due to their danger to humans. Staphylococcus aureus bacteria often found on human skin, as well as household items, but the pathogenic activity of Staphylococcus aureus shows only after penetration into the human body. Staphylococcus aureus is a pathogen of inflammatory and purulent diseases in humans. The most dangerous from the point of view of treatment is methicillin-resistant Staphylococcus aureus (Staph infection MRSA), these bacteria are significantly more resistant to antibiotics and antimicrobials [12-13], and are more difficult to treat, in addition, MRSA are more pathogenically active, and have a greater chance of relapse in the future and the appearance of chronic diseases [14].

Staphylococcus aureus and MRSA are highly resistant and can survive in water at a temperature range of 19-24 °C from 3 weeks to several months, on a dry surface at the same temperatures from 1 to 3 weeks [12; 15]. Also, Staph. Aureus and MRSA are resistant to most antibiotics and antimicrobials [12] that are safe for humans, which complicates treatment, and have the possibility of developing recurrent diseases.

Vibrions — straight or curved mobile rod-shaped bacteria, gram-negative, among the representatives of the most widespread facultative anaerobes. Vibrions are highly pathogenic for humans and fish, as well as for various crustaceans and mollusks, which makes them unacceptable for the presence in the recirculation aquaculture systems. In the natural environment, vibrions found in open water parasitizing the aquatic flora. For humans pathogenic are V. cholerae, V. parahaemolyticus, V. vulnificus, for fish-the same V. splendidus, V. anguillarum, V. tubiashi. What is more dangerous in the case of myxobacteriosis, when a person is already a carrier of pathogenic vibrions, the excitation of which occurs after the activity of fish pathogens. It recorded that V. vulnificus has the greatest fatality for humans — this species excites septicemia, acute urethritis, acute cardiovasculitis [14; 16]. V. parahaemolyticus has similar symptoms [17]. V. cholerae (V. cholerae 01/0139) it is the causative agent of typical chola in humans [13] (V. cholerae non01/non0139 — the causative agent of atypical chola) [18], however, Vibrio cholerae is quite rare, and infection of fish-producing products with it is possible during transportation, as well as in the case of establishing a recirculation aquaculture systems in unfavorable regions and when taking poor-quality water [6; 19-20]. The rest of the non-cholerogenic vibrions mainly have a striking property due to their extracellular substances, such as proteases, chitinases, hemolysins, and others. The presence of vibrions in the water of recirculation aquaculture systems or fish products is a serious danger for the enterprise, in this case, it is necessary to take a full range of measures to clean up the systems and eliminate the infected products.

From the point of view of durability, vibrions are moderately resistant — they do not survive outdoors in a dry environment, but in water in the temperature range of 19-24 °C, they survive on average from 2 weeks to 1 month [21]. Vibrions are sensitive to basic antibiotics, sensitive to the absence of nitrogen in water (approximately less than 1 mg per 1L of water) and sensitive to vibriostatics (these include 2, 4-dimanino-6, 7-disopropylpteridine and its homologues) [21-22] which simplifies the fight against them in the conditions of fisheries. Pathogenic species for humans are less sensitive to external conditions, in particular to the nitrogen content. Fight against them complicated by the condition of using vibriostatics not in all cases, but they are still sensitive to antibiotics.

The following agents of the aquatic microflora that represent pathogenicity for humans are Pseudomonas - gram-negative rod-shaped bacteria often found in water and soil. Among which should be noted - Pseudomonas aeruginosa, which is conditionally pathogenic for humans and is one of the most common pathogens of nosocomial infections (due to the significant relief of damage to persons
with weakened immunity), abscesses and purulent wounds and is associated with enteritis, but has less virulence compared to MRSA described above. Its pathogenicity is due to its high mobility, toxin formation, and the production of hydrolytic enzymes. The main problem of treatment of this bacterium is its high resistance to antibiotics, including aminoglycosides and quinols [23], as well as having signaling molecules that form a sense of quorum [24-25], which allows it to make coordinated common decisions and quickly adapt to environmental conditions, as well as resist the effects of antibiotics and antibacterial drugs. For example, Pseudomonas aeruginosa colonies can form a biofilm [26-27] that protects the entire colony from getting antibiotics, which makes treatment much more difficult. However, in modern medicine, substances that inhibit the "social" behavior of Pseudomonas aeruginosa used for therapeutic and preventive purposes, which greatly simplifies the fight against it.

Pseudomonas aeruginosa is highly resistant, in water in the temperature range of 19-24 °C they survive on average from 4 weeks to 1 month (some strains can survive up to a year at lower temperatures), on a dry surface at the same temperatures from 5 to 12 days [28]. They are also insensitive to antibiotics and antimicrobials, highly mobile and have a quorum sensing mechanism, which significantly complicates the fight against it, but, at the same time, for a person with a strong immune system, it should not pose a huge danger due to its low virulence compared to MRSA.

The last agent of the aquatic microflora of recirculation aquaculture systems that pose a pathogenic danger to humans are Aeromonas - a genus of gram-negative rod-shaped bacteria, among which there are both mobile and stationary species. Conditional pathogens for humans are A. hydrophila, A. caviae, and A. sobria. Among which the first type — hydrophilic aeromanad should be considered especially because of its high toxicity to many warm-blooded and cold-blooded organisms, including both fish and humans. These species rarely affect healthy people, but in the case of a weakened immune system or interaction with large volumes of bacteria and myxobacteriosis are quite dangerous. The described species are the initiators of toxic poisoning and gastroenteritis. In the case of the pathogenic mechanism of hydrophilic aeromanad consists in fixing inside the host body through the bloodstream in the first available organ. Second - accompanying release of various extracellular proteins, such as lipase, hemolysin, chitinase and others, as well as the release of cytotoxic enterotoxin and aerolysin the pathogenic mechanism based on the secretion of proteins that export virulence factors directly to the host cells. The pathogenic mechanism of A. caviae, A. sobria is similar to that of A. hydrophila. In addition, hydrophilic aeromanad can produce a toxin similar to cholera Vibrio, and some strains and cytotoxin, which will complicate the diagnosis and treatment of victims.

From the point of view of stability, aeromanads are moderately resistant, in water in the temperature range of 19-24 °C, they survive on average from 4 weeks to 1 month, on a dry surface at the same temperatures they practically do not survive [28, 29]. Aeromonads are resistant to low temperatures and most antibiotics and antimicrobials, but they rarely infect healthy people. The main risk group is children under 7 years of age and people with weak immune system.

Separately, some sanitary aspects of the fisheries should note, such as the need to sterilize equipment and tanks for carrying fish, scalpels, and other tools due to the possibility of the transfer of pathogens between cages and/or infection of fish during transport, which is much more difficult to track. In addition, attention should be paid to the quality of feed and mineral additives, since it is often with them that pathogens introduced into the recirculation aquaculture systems. In addition, a specialist epidemiologist and take precautions should monitor employees of the company during work and monitor the workspace. The recirculation aquaculture systems itself should be regularly checked for the presence of pathogenic microflora and remain in good condition.

4. Conclusion
The conducted research allows us to draw the following conclusions:

- Recirculation aquaculture systems are able to accumulate various pathogenic microorganisms belonging to the genera Escherichia, Salmonella, Staphylococcus, Vibrio, Pseudomonas, Aeromonas, which pose a pathogenic hazard to both humans and fish;
The considered pathogens pose a significant danger to humans and can cause a number of diseases accompanied by serious symptoms and entail violations of the natural work of the human body, violating the correctness of the immune system;

- The Pathogenic mechanism of the considered species Escherichia, Salmonella, Vibrio, Aeromonas. microorganisms are based on the synthesis of extracellular proteins and toxins in the host body, which leads to poisoning, gastroenteritis, various intestinal infections and diarrheal diseases;
- The Pathogenic mechanism of the considered Staphylococcus and Pseudomonas species is based on the formation of inflammatory and purulent processes in the host body;
- Some of the considered species, such as S. salmonella, S. bongori, S. enterica, S. aureus (MRSA), V. vulnificus, P. aeruginosa, have a high resistance to antibiotics and antibacterial drugs, which makes it difficult to fight them in the conditions of recirculation aquaculture systems, as well as in the case of treatment of already affected people;
- Ways of getting into the recirculation aquaculture system of the considered pathogens can be violations of sanitary norms (made with non-sterile tools, nets, trays for carrying fish, etc., due to the survivability of the considered microorganisms on dry surfaces for a long period of time) as well as during transportation (due to violations of sanitary norms during transportation and use of bacterial-infected equipment, low-quality water and due to the fact that it is difficult to maintain optimal conditions for fish-producing material in the conditions of transportation), as well as with low-quality feed, dietary supplements, infected with pathogenic microflora;
- In case of detection of the considered pathogens in the recirculation aquaculture systems, a set of measures should take to eliminate the current infection, prevent the spread of pathogens by means of tools and personnel to other systems of the complex, as well as take measures to prevent such incidents in future.

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