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DISEASES CAUSED BY BACTERIA OF THE *AEROMONAS* AND *PSEUDOMONAS* GENUS WHEN REARED FISH IN CONTROLLED SYSTEMS

Aquaculture represents a higher share of world fish supply and a strong influence on price formation in the sector overall (both production and trade). The total sale value of fisheries and aquaculture production in 2016 was estimated at USD 362 billion, of which USD 232 billion was from aquaculture production. Between 1961 and 2016, the average annual increase in global food fish consumption (3.2 percent) outpaced population growth (1.6 percent) and exceeded that of meat from all terrestrial animals combined (2.8 percent). By creating optimal growing conditions such as temperature, oxygen and hydrochemical regimes, the growth and sexual maturation of fish is significantly reduced, which contributes to the development of industrial aquaculture. However, the rapid development of aquaculture is accompanied by outbreaks of diseases caused by bacterial infection, which lead to high mortality and catastrophic economic losses. Mass outbreaks can occur suddenly under the influence of stress factors arising in connection with a sharp change in conditions of detention, as well as an increase in planting densities of rearing objects. The most severe bacterial diseases in aquaculture are infections caused by members of the Aeromonas and Pseudomonas genus. Bacteria of the Aeromonas and Pseudomonas genus are the causative agents of hemorrhagic septicemia (Aeromonas hydrophila, Pseudomonas fluorescens), furunculosis (Aeromonas salmonicida), which annually cause economic losses in aquaculture. Nowadays, due to widespread and often uncontrolled use of antibiotics, the number of bacteria resistant to antibiotics has increased dramatically and is the main cause of morbidity and mortality. This phenomenon can not only lead to the failure of antimicrobial therapy, but also raise concerns about the safety of fish products.

This review examines the current data on fish diseases caused by bacteria of the Aeromonas and Pseudomonas genus, dominant virulence factors, problems of identification and antibiotic resistance.

Key words: aquaculture, bacterial diseases, Aeromonas spp., Pseudomonas spp., resistance.

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Реттелетін жүйелер жағдайында балық өсіру кезінде *Aeromonas* және *Pseudomonas* тұқымдарының бактериялары тудыратын аурулары

Әлемдік балық өндірісіндегі аквакультураның үлесі өте жоғары және осы экономика секторындағы (өндіріс және сауда саласында) өнімнің жалпы құнының қалыптасуына әсері де жеткілікті дәрежеде. 2016 жылы балық аулау және аквакультура өнімдерін сатудың жалпы құны 362 млрд АҚШ долларын құраса, оның 232 млрд АҚШ доллары аквакультура өнімінен түскені анықталды. 1961-2016 жылдар аралығында азық-түлік балықтарын әлемдік тұтынудың орташа жылдық өсуі (3,2 пайыз) популяцияның өсуінен 1,6 пайызға асып, барлық құрлықтағы жануарлардың етін тұтынудан 2,8 пайызға асып түсті. Температура, оттегі және гидрохимиялық режимдер сияқты оңтайлы өсіру жағдайларын жасау арқылы балықтардың өсуі мен жыныстық жетілу уақыты едәуір қысқарады, бұл өндірістік аквакультура саласының дамуына ықпал етеді. Алайда, аквакультураның қарқынды дамуы бактериялық инфекциядан туындаған аурулардың өршуімен үйлесіп отырды, бұл балықтардың жоғары деңгейде өлуіне, ал ол өз кезегінде шектен тыс экономикалық шығындарға әкелді. Инфекциялық аурулардың өршуі, балықтарды өсіру жағдайларының күрт өзгеруіне, сондай-ақ балықтардың белгілі бір көлемге қатысты тығыздығының артуына байланысты туындайтын стресстік факторлардың әсерінен тез арада пайда болуы мүмкін. Аквакультурадағы ең ауыр бактериялық аурулар бұл Aeromonas және Pseudomonas тұқымдасына жататын бактерия түрлері тудыратын инфекциялар. Геморрагиялық септицемия (Aeromonas hydrophila, Pseudomonas fluorescens), фурункулоз (Aeromonas salmonicida) ауруларын қоздыратын Aeromonas және Pseudomonas тұқымдасының бактерия түрлері аквакультура өндірісіндегі шектен тыс экономикалық шығындардың себептері болып табылады. Қазіргі уақытта антибиотиктердің еш бақылаусыз кеңінен және жиі қолданылуына байланысты,

антибиотиктерге төзімді бактериялардың саны күрт өсті және осы бактериялар аквакультура өндірісіндегі балық өлімінің негізгі себебі болып табылады. Бұл құбылыс микробқа қарсы терапияның сәтсіздігіне ғана емес, сонымен қатар балық өнімдерінің адамға қатысты қауіпсіздігі алаңдатушылық тудырып отыр.

Бұл шолуда Aeromonas және Pseudomonas тұқымдасының бактерия түрлері тудыратын аурулар, вируленттіліктің доминантты факторлары, бактерияларды идентификациялау және антибиотиктерге төзімділіктің қалыптасуы туралы проблемаларға қатысты қазіргі заманғы мәліметтер қарастырылады.

Түйін сөздер: аквакультура, бактериялық аурулар, Aeromonas spp., Pseudomonas spp., төзімділік.

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Заболевания, вызываемые бактериями родов *Aeromonas* и *Pseudomonas* при выращивании рыб в условиях регулируемых систем

Аквакультура представляет более высокую долю мирового производства рыбы и оказывает сильное влияние на ценообразование в секторе в целом (как производстве, так и торговле). Установлено, что общая стоимость продажи продукции рыболовства и аквакультуры в 2016 году оценивалась в 362 млрд долларов США, из которых 232 млрд долларов США приходилось на продукцию аквакультуры. В период с 1961 по 2016 год среднегодовое увеличение мирового потребления пищевой рыбы (3,2 процента) опережало рост популяции (на 1,6 процента) и превышало потребление мяса всех наземных животных, вместе взятых (на 2,8 процента). За счет создания оптимальных условий выращивания таких как температурный, кислородный и гидрохимический режимы, значительно сокращаются сроки роста и полового созревания рыб, что способствует развитию индустриальной аквакультуры. Однако быстрое развитие аквакультуры сопровождается вспышками заболеваний, вызванных бактериальной инфекцией, которые приводят к высокой смертности и катастрофическим экономическим потерям. Массовые вспышки могут происходить внезапно под действием стрессовых факторов, возникающих в связи с резким изменением условий содержания, а также увеличением плотностей посадок объектов выращивания. Наиболее тяжелыми бактериальными заболеваниями в аквакультуре являются инфекции, вызываемые представителями родов Aeromonas и Pseudomonas. Бактерии родов Aeromonas и Pseudomonas являются возбудителями геморрагической септицемии (Aeromonas hydrophila, Pseudomonas fluorescens), фурункулеза (Aeromonas salmonicida), ежегодно являющиеся причинами экономических потерь в аквакультуре. В настоящее время из-за широкого и часто неконтролируемого использования антибиотиков количество бактерий, устойчивых к антибиотикам, резко возросло и является основной причиной заболеваемости и смертности. Это явление может не только привести к неудаче антимикробной терапии, но и вызвать опасения относительно безопасности рыбных продуктов.

В настоящем обзоре рассматриваются современные данные о заболеваниях рыб, вызываемые бактериями родов Aeromonas и Pseudomonas, доминирующих факторах вирулентности, проблемы идентификации и устойчивости к антибиотикам.

Ключевые слова: аквакультура, бактериальные заболевания, Aeromonas spp., Pseudomonas spp., резистентность.

Introduction

Growing fish in aquaculture contributes to the conservation of biological diversity, and is also aimed at providing the world's population with additional protein as a necessary daily nutritional component of the human diet.

It should be noted that aquaculture represents a higher share of world fish production and has a strong influence on pricing in the sector as a whole (both production and trade). Thus, the total sales value of fishery and aquaculture products in 2016 was estimated at US $ 362 billion, of which US $ 232 billion accounted for aquaculture products. Between 1961 and 2016, the average annual increase in global food fish consumption (3.2 percent) outpaced population growth (1.6 percent) and exceeded the consumption of meat of all land animals combined (by 2.8 percent) [1].

The most severe bacterial diseases in sturgeon aquaculture are infections caused by bacteria of the genera *Pseudomonas* and *Aeromonas*. Today, due to the widespread and often uncontrolled use of antibiotics, the number of antibiotic-resistant bacteria has

increased dramatically and is the leading cause of morbidity and mortality. This phenomenon can not only lead to the failure of antimicrobial therapy, but also raise concerns about the safety of fish products. For this reason, new strategies to combat these drug-resistant pathogens are urgently needed.

In the conditions of industrial aquaculture, where artificial conditions for fish farming are created, there is no dependence on the climatic and geographical characteristics of the area, which allows fish to be raised in fairly optimal conditions [2]. The main fish species that are objects of cultivation in industrial aquaculture are representatives of the families: sturgeon *(Acipenseridae)*, salmon *(Salmonidae)*, cyprinids *(Cyprinidae)*, catfish *(Siluridae)*, etc. [3-6]. The objects of cultivation – fish are cold-blooded animals, the body temperature of which depends on the temperature regime of the habitat, thus water as a habitat for fish has a direct effect on their general physiological state. In aquatic conditions, bacteria spread faster than in soil and air [7], in this regard, fish are most vulnerable to bacterial pathogens that cause fish diseases. In the conditions of industrial aquaculture, when fish are constantly in a confined space limited by pools and other containers, the risk of bacterial diseases in fish increases by several orders of magnitude [8]. In this regard, enterprises focused on the artificial reproduction of fish pay special attention to measures for the prevention and treatment of fish. Identifying a pathogenic agent takes a lot of time and money. It is often impossible to clinically identify the pathogen, since bacteria can have an indistinguishable negative effect on the fish organism. Unqualified identification of the pathogen followed by inappropriate treatment can negatively affect the development of the disease and lead to the death of fish.

Biology of bacteria of the *Aeromonas* genus

Aeromonas hydrophila is one of the most widespread representatives of the *Aeromonas* genus, which includes 36 species [9]. Bacteria of the *Aeromonas* genus are characterized as non-spore-forming gram-negative bacilli, the majority *(A.hydrophila, A.caviae, A.sobria, A.veronii)* are mobile except for *A.salmonicida*, facultative anaerobes, oxidase-positive, the optimal temperature is in the range from 22 to 28°C, grow at 37°C [10]. In a study by Altwegg M. et al. (1990), it was found that 96% of *A.hydrophila*, 96% of *A.sobria*, 94% of *A.caviae* showed motility. About 80% of *A.hydrophila* and *A.sobria* and only 5% of *A.caviae* are capable of fermenting glucose to form gas [11].

Bacteria of the *Aeromonas* genus are ubiquitous in soil, in water, and are also found in food [12]. The soil serves as a so-called reservoir for cytotoxic and invasive strains of bacteria of the *Aeromonas* genus. Bacterial strains *A.hydrophila, A.caviae, A.sobria* being in the soil are able to maintain virulence factors for up to 5 months, and therefore there is a risk of human infection, directly related to land work [13]. While the risk of human contamination through soil may be indirect, food has a direct impact on health. Bacteria of the *Aeromonas* genus inhabit foods such as raw meat, raw fish, milk and dairy products. It has been shown that bacteria isolated from 56.8% and 43.1% products are identified as *A.hydrophila* and *A.sobria*, respectively [14].

In the studies of Burke V. et al. (1984), bacteria of the genus *Aeromonas* were identified in the samples both from open water bodies and from drinking water, and it was also shown that chlorination under drinking water standards reduces the number of bacteria, but does not completely exclude them [15].

The bacteria are well adapted to aquatic life, making them dangerous to most aquatic animals. Fish species exposed to diseases caused by aeromonads are presented in Table 1.

Table 1 – Fish species exposed to diseases caused by bacteria of the *Aeromonas* genus in aquaculture

Causative agent of the disease	Object of study	References
Aeromonas hydrophila	*Acipenser baerii* *Acipenser gueldenstaedtii* *Acipenser schrenckii* *Acipenser sinensis* *Oncorhynchus mykiss* *Cyprinus carpio L.* *Clarias gariepinus*	[16] [17] [18] [19] [20] [21] [22]
Aeromonas caviae	*Clarias gariepinus* *Oreochromis niloticus*	[23] [24]
Aeromonas sobria	*Acipenser baerii* *Clarias betrachus*	[17] [25]
Aeromonas veronii	*Acipenser stellatus,* *Huso huso* *Oreochromis niloticus* *Carassius gibelio*	[26] [27] [28, 29]
Aeromonas salmonicida	*Oncorhynchus masou* *Oncorhynchus kisutch* *Carassius auratus*	[30] [31] [32]

Virulence factors of bacteria of the *Aeromonas* genus

The pathogenicity of bacteria of the *Aeromonas* genus is due to the presence of virulence genes encoding a large number of extracellular proteins, such as aerolysin (AerA), hemolysin (HlyA), cytotonic heat-labile enterotoxin (Alt), cytotonic heat-resistant toxin (Ast), cytotoxic heat-labile enterotoxin (Act), lipase (Lip), elastase (Ela), serine protease (Ser), DNase (Exu), polar flagellum (fla), and lateral flagella (laf) [33]. The pathogenic mechanism of their action is based on the use of a special protein secretion system (T2SS and T3SS), which exports virulence factors directly into the host cells. The widely conserved T2SS secretion system is present in all known representatives of *A. hydrophila* and is an integral part of the extracellular secretion of a wide range of virulence factors, including aerolysin, amylases, DNases and proteases [34, 35].

Type III Secretion Systems T3SS, or injectosome, one of several types of bacterial secretion systems, is a protein complex found in several gram-negative bacteria, including members of the *Aeromonas* genus. Through the needle-like structure, the secreted effector proteins toxins are transported directly from the bacterial cell to the eukaryotic host cell, where they have a number of effects that help the pathogen to survive and avoid an immune response. The T3SS secretion system is more common in clinical *Aeromonas* isolates than in aqueous isolates [36, 37].

Isolates of *A.hydrophila* isolated from fish are characterized by the presence of the following virulence genes: Lip (100%), Ela (100%), Exu (30%), Ast (30%), Act (95%), Hly (76), Aer, Ser (100%) [34]. Six virulence genes (aer, alt, ahyB (Ela), gcaT (gene encoding cholesterol acyltransferase), lip and ser) were found in the isolated *A.veronii* strain (CFJY-623) from infected with *Carassius auratus gibelio* [29]. It is noted that the most common virulence factors among bacteria of the *Aeromonas* genus are pore-forming toxin – aerolysin and exotoxin – hemolysin [36, 38].

Fish diseases caused by bacteria of the *Aeromonas* genus

Aeromonosis (bacterial hemorrhagic septicemia, motile *Aeromonas* septicaemia (MAS)) and furunculosis are among the most common diseases of fish, both in the natural environment and in artificial conditions. The causative agents of aeromonosis (also hemorrhagic septicemia, rubella of the fins and tail) are the following types of bacteria of the *Aeromonas* genus: *Aeromonas hydrophila, Aeromonas sobria, Aeromonas caviae*, etc., *Aeromonas salmonicida* (furunculosis). Bacteria can enter the fish organism through open damage to the skin and gills, as well as during food consumption [39, 40].

When sturgeons are infected with the *A.hydrophila* bacterium, mortality can reach 100%, which represents large economic losses in the fishing industry [41]. For example, for the Amur sturgeon *(Acipenser schrenckii)* the lethal dose of *Aeromonas hydrophila* was $1.17x10^7$ CFU/ml^{-1} [18], the mean lethal dose of the *Aeromonas veronii* isolate (CFJY-623) for the silver Prussian carp *(Carassius auratus gibelio)* was $1.31x10^7$ CFU/ml [29].

Aeromonosis or bacterial hemorrhagic septicemia (motile *Aeromonas* septicaemia, MAS) in fish can manifest itself clinically as bleeding, abscesses in different parts of the body, mainly in the fins and tail, accumulation of ascitic fluid in internal organs, anemia, and also in more severe cases of infection, the formation of numerous ulcers on the fish body is noted [39]. The formation of ulcers on the body is a consequence of muscle necrosis, which occurs due to the presence of the virulence factor exotoxin A in representatives of bacteria of the *Aeromonas* genus, which promotes tissue decomposition [42]. It is noted that in the USA from motile *Aeromonas* septicaemia (MAS), which is characterized by a massive outbreak in catfish raised in aquaculture, the losses amounted to $ 12 million [43]. Among the bacterial isolates isolated from sturgeon fish, the largest percentage 38.71 falls on *Aeromonas hydrophila*, 13.98% *Aeromonas sobria*, 13.98% were representatives of the *Pseudomonas* genus [44]. In sturgeon fish infected with *Aeromonas hydrophila*, the following clinical signs are noted: darkening of the skin, numerous hemorrhages in the head and abdomen, as well as on the fins [45]. When *Ictalurus punctatus* is infected with the bacterium *Aeromonas hydrophila*, 50% of the mortality is achieved within 12 hours after infection, and by 72 hours the mortality rate increases to 95% [46].

Furunculosis is a disease of mainly salmonids *(Salmonidae)* caused by the bacteria *Aeromonas salmonicida*, a member of the *Aeromonas* genus. As well as *Aeromonas hydrophila, Aeromonas salmonicida* causes economic losses in fish farming, especially in the cultivation of Atlantic salmon *(Salmon salar)* in offshore farms, as well as in open water [47]. Furunculosis is characterized by a high proportion of morbidity and mortality rates in fish. During the period of infection with *Aeromonas salmonicida*, so-called boils are formed in fish, which

are necrotic formations with a purulent exudate inside. In sick fish, there is a darkening of the skin, hemorrhages in the internal organs and on the fins, as well as a noticeable decrease in activity, a drop in the consumption of compound feed [40].

The serine protease encoded by the AspA gene in *Aeromonas salmonicida* liquefies the muscle tissue of diseased fish, thereby facilitating the formation of boils [48].

Thus, in the studies of Lin Q. et al. (2019), the death of the Chinese perch *(Siniperca chuatsi)* occurred within 2 weeks, with infection at a dose of $1.2x10^6$ CFU/fish, the mortality rate was about 70%, 90% of the mortality rate occurred at a dose of $1.2x10^7$ CFU/fish [49].

The risk of fish contamination with *Aeromonas spp.* increases when the objects of cultivation are in a state of stress, which can arise in connection with the occurrence of many factors, including abrupt changes in temperature, hydrochemical composition, high planting densities, etc. Wedemeyer G. (1970), Mateus A.P. et al. (2017) state that during the period when the fish is under stress the risk of infection with bacterial pathogens increases several times, so-called outbreaks of mass infections occur [50, 51]. Therefore, in the studies conducted by Gao J. (2019) it was shown that the stress hormone norepinephrine affects the virulence of the bacteria *Aeromonas hydrophila*. It was also determined that norepinephrine has a positive effect on the expression of the following virulence genes (ompW, ahp, aha, ele, ahyR, ompA, fur) *Aeromonas hydrophila*, which increases the level of bacterial pathogenicity [52].

Biology of bacteria of the *Pseudomonas* genus

Bacteria of the *Pseudomonas* genus represent a large group of 144 species. *Pseudomonas* are characterized as gram-negative movable rods, the mobility of which is carried out due to the presence of a single or several polar flagella. Representatives of the *Pseudomonas* genus are able to survive in a fairly wide temperature range from 4 *(P.fluorescens)* to 41°C *(P.aeruginosa)*. *P.aeruginosa, P.fluorescens, P.putida* are united in the group of bacteria with fluorescent diffusing pigments [10, 53]. Thus, *P.aeruginosa, P.fluorescens*, and others are capable of producing a yellow-green fluorescent pigment known as pyoverdin, this feature is often used to differentiate bacteria of the *Pseudomonas* genus [54]. Bacteria of the *Pseudomonas* genus are ubiquitous in soil and water. And also bacteria are causative agents of various diseases in plants, animals and humans [55].

P.aeruginosa is one of the most widespread species of bacteria of the *Pseudomonas* genus in the soil. Therefore, in the studies of Green S. et al. (1974), 58 soil samples were taken in various agricultural areas of California, of which 24% of the sample contained the *P.aeruginosa* bacterium. Thus, the soil is a reservoir for bacteria of the *Pseudomonas* genus [56]. The authors note that the widespread prevalence of bacteria of the *Pseudomonas* genus in soil represents a large community of bacteria, which is an important factor in stimulating plant growth and biological control of pathogenicity [57, 58].

Bacteria of the *Pseudomonas* genus are also common in water, for example, *P.aeruginosa* isolates were isolated from rivers, lakes, and open ocean waters [59]. Besides natural bodies of water, *Pseudomonas spp.* quite often found in drinking water and water supply systems, and therefore there is a risk of human infection with a bacterial pathogen. In addition, isolates isolated from drinking water samples often exhibit a high level of antibiotic resistance, which is the cause of many clinical diseases [60, 61]. For example, bacteria, *Pseudomonas aeruginosa* and *Pseudomonas maltophilia*, represent about 80% of all clinical human diseases caused by representatives of the *Pseudomonas* genus [53].

Bacteria of the *Pseudomonas* genus, being widespread inhabitants of water, pose a great danger to most aquatic organisms, for example, such fish species (Table 2) and marine mammals *(Zalophus californianus, Phoca vitulina)* [62]. In fish, the most common pathogens from the *Pseudomonas* genus are the following species: *P.fluorescens, P.putida* and *P.aeruginosa*.

Table 2 – Major pathogens causing pseudomonosis disease in aquaculture

Causative agent of the disease	Object of study	References
Pseudomonas aeruginosa	*Cyprinus carpio, Oreochromis niloticus, Clarias gariepinus*	[63]
Pseudomonas fluorescens	*Acipenser baerii*	[64, 17]
	Clarias gariepinus, Oreochromis niloticus, Liza ramada	[65]
Pseudomonas putida	*Acipenser baerii*	[17]
	Oncorhynchus mykiss	[66]
	Liza ramada	[65]

Virulence factors of bacteria of the *Pseudomonas* genus

As pathogenic microorganisms of many animals and humans, bacteria of the *Pseudomonas* genus are characterized by the presence of the following virulence factors: endotoxin, thermostable hemolysin, proteases (elastase, alkaline), exoenzyme S, toxin A, etc. About 90% of all species of bacteria of the *Pseudomonas* genus are capable of producing toxin A, the most dangerous from virulence factors [53]. In the studies of Haghi F. et al. (2018), the isolated *P.aeruginosa* isolates contained 14 virulent genes. For example, 97.8% of the strains contained toxA (encoding toxin A), 96.7% plcH (hemolytic phospholipase C), 96.7% phzI (phenazine operons), 93.1% exoY (adenylate cyclase), 20.4% exoT (exotoxin T) [67]. Studies of *P.aeruginosa* isolates from food are also pathogenic. So isolates isolated from meat contained the following virulence genes 96.7% of the strains studied contained the lasB (LasB elastase) and exoS (exozyme S) gene, 74.5% algD (alginate), 72.1% plcH (phospholipase C). Strains isolated from fresh fish contained 71.4% lasB, 77.5% algD, 75.5% plcH and 67.3% exoS [68].

Fish diseases caused by bacteria of the *Pseudomonas* genus

Pseudomonosis (also fin rot, hemorrhagic septicemia) is characterized as an infectious disease of fish that live in natural reservoirs, as well as those grown in regulated systems. The main causative agents of pseudomonosis are bacteria: *Pseudomonas fluorescens, Pseudomonas putida, Pseudomonas aeruginosa, Pseudomonas plecoglossicida, Pseudomonas anguilliseptica* [69, 70]. Bacteria of the *Pseudomonas* genus are representatives of the intestinal microflora of many fish. Sivakami R. revealed that in the studied species of cyprinids *(Catla catla, Labeo rohita, Cirrhinus mrigala, Cyprinus carpio)*, more than 50% of intestinal bacteria are bacteria *Escherichia coli* and *Pseudomonas aeruginosa* [71]. Under these conditions, there is a risk of mass fish diseases caused by stress in connection with changes in the environment, which include changes in temperature, oxygen, and hydrochemical regimes [72].

Hemorrhagic septicemia is the cause of high fish mortality in aquaculture. The pathogenicity of most bacteria of the *Pseudomonas* genus is characterized by the presence of virulence factors (protease, elastase, phospholipase C, and exotoxin A), which, in turn, are the main agents in the destruction of muscle tissue and the occurrence of bleeding in fish [73]. When rainbow trout *(Oncorhynchus mykiss)* is infected with the bacterium *Pseudomonas putida*, darkening of the skin, exophthalmia, and deep penetrating ulcers in the back are noted in fish, while no changes from the norm in the internal organs were noted. The mortality rate of rainbow trout in water contaminated with a bacterium (*Pseudomonas putida*, $5x10^6$ CFU/ml^{-1}) was 35% [66].

Pseudomonosis and aeromonosis caused by bacteria of the *Pseudomonas* and *Aeromonas* genus are one of the most common diseases in sturgeon rearing in recirculating aquaculture systems (RAS). Thus, in the studies of Sergaliyev N. et al. (2017), of all the studied species of sturgeon fish (*Acipenser gueldenstaedtii, Huso huso, Acipenser ruthenus, Acipenser baerii, Acipenser nudiventris* and their hybrids), about 43.7% of diseases account for aeromonosis and pseudomonosis. In the studied sturgeon fish with pseudomonosis, the following clinical signs are noted: blood clots on the body, pupillary constriction, and in severe forms of the disease, necrosis of muscle tissue and deep penetrating tongues are found in fish [74]. In addition, it is noted that the mortality of the Siberian sturgeon *(Acipenser baerii)* from the disease caused by the bacterium *Pseudomonas fluorescens* in 2005 was 40% in one of the fish farms in Italy. At the same time, the Siberian sturgeon *(Acipenser baerii)* shows changes in internal organs, namely, swelling of the swim bladder and hemorrhages in the intestine [64].

Identification of bacterial pathogens of fish

In microbiology, one of the main research tasks is to determine the belonging of microorganisms and their systematization [75]. Identification is complicated primarily by the size of the microorganisms. In aquaculture, microorganisms are widespread, some of them are used purposefully to solve the problems of purification, mineralization, and disinfection of water, others are less useful or have a completely negative effect on fish, which often include bacteria [76, 77]. The main methods for identifying bacterial pathogens are the determination of morphobiological (staining followed by microscopy), biochemical characteristics (response chemical reactions of bacteria), as well as the use of molecular genetic analysis (PCR, sequencing) [78].

For example, identification of bacteria of the *Aeromonas* and *Pseudomonas* genus at the level of morphology is quite difficult, the difficulty lies in the fact that bacteria of these genera have almost identical morphology, since bacteria of the *Aeromonas*

and *Pseudomonas* genus are characterized as gram-negative rods, stained pink and red (staining by the method Gram), bacteria are also characterized by the presence of flagella [79]. Therefore, for a more detailed study of pathogenic bacteria in aquaculture with pseudomonosis and aeromonosis of fish, along with morphobiological signs, biochemical characteristics are determined. For example, standard tests and responses are used to identify gram-negative bacteria, which include: oxidase and indole tests, determination of carbohydrate fermentation, ONPG test, amino acid decarboxylation and hydrolysis tests, gelatin dilution test, Voges-Proskauer test, oxidative-fermentative (OF) test, etc. [10]. In addition, to determine the taxonomy, bacteria use differential (selective) nutrient media, with which you can separate bacteria by genus. For example, *Aeromonas* agar is used to identify aeromonads (proteose peptone, yeast extract, lactose, inositol, sorbitol, xylose, lysine monohydrochloride, arginine monohydrochloride, sodium chloride, bile salts no. 3, sodium thiosulfate, ammonium iron citrate, bromolthymol blue, thymol blue, ampicillin and agar). When *Aeromonas hydrophila* grows, colonies are presented as green with a dark center; fermentation of trehalose is also noted [80, 81]. *Pseudomonas* agar (gelatin peptone, casein hydrolyzate, potassium sulfate, anhydrous, magnesium chloride, anhydrous, agar, cetrimide, fusidic acid and cephaloridin), King's A medium (gelatin peptone (pancreatic), magnesium chloride and agar) the principle of media is based on the production of blue-green pigment by bacteria (fluorescent pyoverdin) [82-85]. Biochemical characteristics of bacteria of the *Aeromonas* and *Pseudomonas* genus isolated from diseased fish are presented in Table 3.

Table 3 – Biochemical characteristics of isolates of bacteria of the *Aeromonas* and *Pseudomonas* genus from sick fish [65]

Characteristics (tests)	*Aeromonas*	*Pseudomonas*
Gram stain	-	-
Cell shape	rod	Rod
Motility	+	+
Colony color	dark green	yellow-green
Oxidase test	+	+
Indole production	+	-
Methyl red	-	+
Voges-Proskauer	+	-
Hydrolysis of gelatin	+	+/-
Utilization of glucose	+	-
Mannitol	+	+
Maltose	+	+
Catalase	+	+

The most effective method for differentiating bacterial pathogens is molecular genetic, as it is characterized by high identification accuracy at the genus and even species level. Since today there are many databases with the nucleotide sequences of most microorganisms, the use of the polymerase chain reaction (PCR) method is one of the most common, time-consuming method for determining the genus and species of microorganisms. The use of universal bacterial primers helps differentiate bacteria from other microorganisms. The principle of PCR identification is based on the use of specially synthesized primers (for example, genus- or species-specific), which, by means of complementarity between the studied template and primers, amplify the studied gene region for further research. For PCR identification, the most commonly used primers of the 16S rRNA gene, virulent genes, etc. For example, to identify such bacterial pathogens of fish as *Aeromonas hydrophila, Aeromonas veronii, Pseudomonas aeruginosa* and *Pseudomonas putida*, specific primers are used to amplify 16S rRNA and 16S rDNA genes [20, 29, 86]. In addition, to build a phylogenetic tree of the studied bacterial strains, a specific gene (16S rRNA, gyrB, etc.) or a complete genome is sequenced. It is noted that the sequence of the gyrB gene encoding the B protein of DNA gyrase, like the 16S rRNA gene, can be used to construct a phylogenetic tree and species identification of bacteria [87]. It was also determined that the rate of molecular evolution of the gyrB gene is higher than that of 16S rRNA; in turn, the gyrB gene is ubiquitous among bacterial species [88]. So in the studies of Di J. et al. (2018), Chen F. et al. (2019) to determine the relationship and origin of isolated strains of bacteria *Aeromonas hydrophila, Aeromonas veronii,* they sequenced certain regions of the 16S rRNA, gyrB, and rpoD genes [19, 29].

Antibiotic resistance of bacteria

The acquisition of genetic resistance by bacteria to antibiotics used against them and therapeutic agents based on them is today one of the leading problems not only in aquaculture, but also in medicine, as well as in the food industry. In this regard, the increasing level of bacterial resistance poses a serious danger to humans, animals, and plants [89]. The high resistance of bacteria to antibiotics is due to the presence of antibiotic resistance genes (ARG) in the genome of microorganisms, which are responsible for resistance to a specific antibacterial agent. It is noted that one of the main genetic mechanisms for the propagation of ARG is mobile genetic elements,

the so-called integrons, which are capable of propagating antibiotic resistance genes through transmissible plasmids and transposons [90]. Thus, bacterial resistance to antibiotics is increasingly observed in bacterial pathogens of fish reared in recirculating aquaculture systems, the main reason being the excessive unregulated use of antibiotics in the prevention and treatment of fish. The authors of Lulijwa R. et al. (2019) note that in the period from 2008 to 2018, from 15 countries with developed fisheries in 11 countries (China, Indonesia, India, Vietnam, the Philippines, Bangladesh, South Korea, Egypt, Norway, Japan, Chile, etc.) 67 antibiotics were used, of which 73% were oxytetracycline, sulfadiazine and florfenicol [91]. Bacteria of the *Aeromonas* and *Pseudomonas* genus are representatives of multiresistant organisms that exhibit resistance to several antibiotics [73, 92]. Thus, in studies conducted by Matyar F. et al. (2010) it was determined that 66.6% of isolates of the *Aeromonas* genus showed resistance to cefazolin, 66.6% to trimethoprim-sulfamethoxazole, at the same time, isolates of the genus *Pseudomonas* showed a high level of resistance to nitrofurantoin (86, 2%), cefazolin (84.8%) and cefuroxime (71.7%) [93]. According to the latest results of the analysis carried out by Preena P. et al. (2020), it is noted that among bacterial pathogens in aquaculture that exhibit antibacterial resistance are bacteria of the *Vibrio* (23%), *Aeromonas* (20%), *Enterobacteriaceae* (10%), *Pseudomonas* (5%) genus and others (Figure 1) [94].

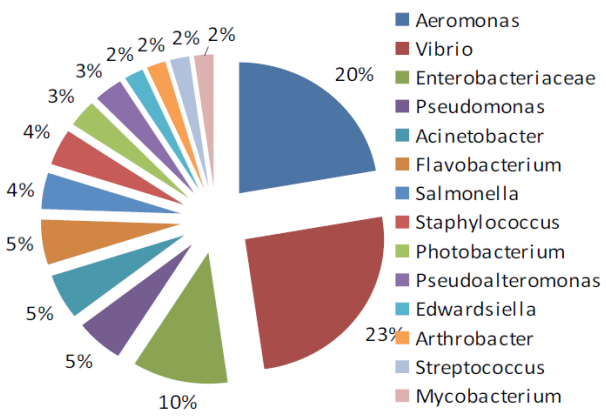

Figure 1 – Percentage of antimicrobial resistance exhibited by various fish pathogens [94]

Thus, the presented review characterizes bacteria of the *Aeromonas* and *Pseudomonas* genus as one of the most common causative agents of bacterial diseases of fish grown in industrial aquaculture, possessing a wide arsenal of virulence factors and high resistance to antibiotics, as well as increasingly showing multiresistance.

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