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Food Safety and Fish Farming: Serious Issues for Brazil

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Abstract

Fish farming in Brazil has been growing, and a new branch that has been taking up space and evolving, every year, as a source of leisure, is fish-pay. Despite being a leisure practice, this type of fish farming has contributed to the development of Brazilian aquaculture. With the growth of aquaculture in the country, concerns about the quality of the product arise, both financially and microbiologically. Aquaculture has caused some environmental problems, such as the increase in the population growth of bacteria specific to the aquatic environment and the possible proliferation of microorganisms resistant to antibiotics due to the indiscriminate use of these drugs in nurseries. Inadequate water quality conditions can contaminate fish and favor the development of diarrheal infections due to the microbiological load present in the fish. This article aims to review the literature covering the general characteristics of fish, including aspects of nutritional importance, and mechanisms of deterioration and analysis of freshness.

Keywords

Characteristics, Deterioration, Nutritional Importance

1. Introduction

Fish farming is currently considered as a food segment with high growth rates. Indeed, fish production has increased remarkably in Brazil and worldwide. The tilapia is the most bred freshwater fish in Brazil [1]. Although fish farming is a highly profitable activity, it has caused several environmental problems such as growth of specific bacterial populations in the natural aquatic microbiota, accumulation of wastes derived from feed, and possible proliferation of microorganisms resistant to antibiotics owing to the uncontrolled usage of the latter. This practice may lead to high mortality rates in fish due to infections by pathogenic microorganisms, which is a serious issue. Many bacteria, including *Aeromonas* spp., which attack both fish and humans, are related to fish pathology [2] [3].

At present, fish farming is being increasingly practiced. Fish is one of the most commercialized food products worldwide, owing to the costs and better feeding habits [4] [5]. Since fish deteriorate rapidly, and increase in fish intake is on the rise, evaluating the sanitary and hygienic conditions of fish from the breeding phase up to the consumption phase is necessary. In fact, inadequate hygiene practices are a remarkable risk to consumer health [6].

Several studies have revealed the importance of sanitary and hygienic measures to warrant the control of fish pathogens so that the fish are devoid of diseases and can be safely consumed by humans [2] [3]. Machado *et al.* [7] identified several factors that affect fish quality in amateur fishing in the municipalities of the southern coast of the state of São Paulo, Brazil; they revealed the lack of sanitary and hygiene practices from fish capture up to its commercialization. This renders the food unsafe for consumption.

Several microorganisms, called indicator microorganisms, reveal the occurrence of fecal contamination, as well as the presence of pathogens or deterioration of food. For example, the presence of *Escherichia coli*, total and thermotolerant coliforms, and *Staphylococcus aureus* may indicate inadequate sanitary conditions during food processing, production, or storage [8].

Microbiological parameters are highly essential as they can be used to evaluate the sanitary and hygienic quality of water and food. The presence of the coliform group and *E. coli* indicate fecal contamination of food. Certain levels of *S. aureus* are considered as the bio-indicators of the sanitary quality of products by the food industry [8].

Data retrieved from the Brazilian Health Ministry have shown that animal-derived foods such as meat are involved in foodborne illness outbreaks (FIOs). *Aeromonas* are unicellular water bacteria that may cause FIOs. Water is the natural habitat of these bacteria and an important source of food contamination. Although these bacteria are prevalently detected in fish, they have also been found in swine, broilers, and humans, as they occur in the feces of ill animals and food handlers [3] [9] [10] [11].

The genus *Aeromonas*, including pathogenic strains for aquatic animals, has been indicated as one of the main etiological agents of infection in fish and other aquatic organisms, with great liabilities for Brazilian fish farming. Several bacteria of the genus have been identified as significantly pathogenic for public health, and epidemiological data have shown that consumption of contaminated food and contact or ingestion of contaminated water are one of the main causes of transmission of the pathogen. In fact, they have been described as the main etiological agents of gastroenteritis in humans [3] [12] [13].

In Brazil, regulations regarding the standard limits for microorganisms in food are lacking. The reference rates are coagulase-positive staphylococcus at

10^3/g sample and absence of *Salmonella* spp. in a 25 g sample [14] [15].

Because of the increase in the cases related to contamination by *Aeromonas*, which are ubiquitous in sea water and fresh water, irrespective of whether it is contaminated, several countries have developed quality and quantity standards for *Aeromonas* spp. These regulations are also applicable to mineral and potable water since these bacteria have been detected in public water supply as well as bottled water. Because of an increase in gastroenteritis cases and detection of *Aeromonas* spp. in water that was considered potable, Holland, Italy, and Canada established a maximum rate of 200 UFC/100 ml for potable water [12] [16] [17].

Aeromonas spp. have been characterized as an opportunistic pathogen found worldwide, especially in developing countries, and are known to be associated with traveler' diarrhea. These bacteria are mainly found in aquatic environments and in animals and are related to several infections in humans and animals [18]. Cases of human contamination by several species, mainly *Aeromonas hydrophila*, *A. caviae*, and *A. veronii* biovar *sobria*, have been reported in different countries. *A. hydrophila* is predominantly associated with watery diarrhea and dysentery. In 2010, new infection cases by wounds and gastroenteritis were shown to be associated with two new species, namely, *A. sanarellii* and *A. taiwanensis* [19]. Therefore, while evaluating the sanitary and hygiene quality of fishponds by using indicator microorganisms for fecal contamination and detecting the presence of pathogens or food deterioration, inadequate sanitary conditions during food processing, production, and storage may also be detected.

An analysis of the link between *Aeromonas* spp. and fish farming in Brazil was undertaken because of the following reasons: bacteria of the genus *Aeromonas* are pathogenic microorganisms that harm animals and humans and are important for public health owing to their high virulence and antibiotic resistance; the growing number of gastroenteritis cases in Brazil and worldwide due to these microorganisms vectored by food and contaminated water, a natural habitat for *Aeromonas*; few studies on the presence of *Aeromonas* in fish ponds in the state of Bahia; and reports on the contamination of fish caught in sporting fish ponds in Brazil.

2. Aquaculture and Fish Farming Worldwide

According to the Food and Agriculture Organization of the United Nations (FAO) [4], aquaculture is the breeding of aquatic organisms such as fish, crustaceans, mollusks, and water plants. Aquaculture comprises the culture of organisms in fresh and sea water under controlled conditions and is currently responsible for the production of one-half of fish and mollusks consumed by the world population. According to available data, aquaculture production of fish tripled between 1995 and 2007. Aquaculture is classified into segments according to the class of aquatic organisms cultivated. These segments may be divided into fish farming: fish breeding; continental fish farming: breeding of fish in freshwater; marine fish farming: breeding of fish in seawater; Mariculture: breeding of aquatic organisms in marine or estuary environments; algaculture: breeding of algae; oyster farming: breeding of oysters; and carciniculture: breeding of shrimps [20].

World fish production has grown constantly during the last fifty years, with fish feed increasing at an average annual rate of 3.2%, exceeding the growth of world population by 1.6%. World fish consumption per capita increased at an average of 9.9 kg in the 1960s to 19.2 kg in 2012. Such development has been triggered by population increase, increasing salaries, and urbanization. It has been facilitated by a strong expansion in fish production and more efficient distribution channels [4].

Fishing and aquaculture are remarkable relevant for world hunger eradication, health promotion, and poverty mitigation. In no other epoch, fish consumption or dependence on this economic sector for well-being of people has been so high. Fish is an extremely nutritive product since it is a plentiful source of protein and essential nutrients, especially for the poor, worldwide. In a planet where 800 million people suffer from chronic malnutrition and in which human population concentrated in coastal urban areas is estimated to increase by another two billion, reaching 9.6 billion people in 2050, feeding the population is a remarkable challenge, in addition to the safeguarding of the natural resources for future generations. In 2012, approximately 58.3 million people were involved in the fishing sector, including capture and aquaculture. The FAO estimates revealed that, as a rule, fishing and aquaculture guarantee the living of 10% - 12% of the world population [4].

China has been responsible for the greatest growth rate in fish availability, because of its expansion in fish production, especially through aquaculture. The proportion of fish production by humans increased from 71% in the 1980s to more than 86% (136 million tons) in 2012. The remaining 21.7 million tons comprised non-food usages such as fish flour and fish oil. China alone produced 43.5 million tons of fish as food and 13.5 million tons of aquatic algae in 2013 [4] [5].

According to studies by FAO, all continents have shown an increasing trend in aquaculture-produced fish. Since 2008, in general, in Asia, the numbers for fish production are more than those for captured ones [4] [5]. Globally, aquaculture reveals a predominance of cyprinids, with several species of the common carp ranking first in production, whereas the Nile tilapia ranks fourth as the most important fish produced worldwide. Fish farming in Brazil may be divided into tilapia (43%), tambaqui (23%), and tambacu and tambatiga (15%). The production of the Nile tilapia accounts for 35% of fish farming in Brazil [20]. Fish breeding in Brazil is on the increase, with a 10% increase in 2016 [4]. According to the Brazilian Association of Fish Farming, Brazil is one of the four major producers of tilapia in the world. China ranks first, with 1.8 million tons, followed by Indonesia, with 1.1 million tons, and Egypt. Brazil comes next, followed by the Philippines and Thailand [21].

Fish constitute approximately 17% of animal protein ingestion in the world population and 6.7% of all consumed protein. Furthermore, fish provide more than 3.1 billion people with 20% of the mean average content of animal protein. Remarkable increase in fish consumption worldwide is related to the need for healthier, better-quality, diversified, and more nutritive food. In fact, such nutritional assets may have an important role in dietary regulation, especially to control obesity and cardiovascular diseases [4] [5] [22] [23].

3. Fish Farming in Brazil

Fish production and consumption have remarkably increased in Brazil during the last few years. Because of its climatic conditions and territory, Brazil has been considered to have remarkable potential to develop aquaculture especially with regard to its hydric potential and favorable conditions for the construction of reservoirs. Research comparing data on meat products revealed that the aquaculture sector had the best increase between 2004 and 2014 [1] [24]. Among the various species bred in Brazil, the tilapia is the main fish species, with an average annual production of 14.2%. According to IBGE [25] [26] [27], the tilapia was the most produced species in 2014 and represented 41% of fish production, with an increase of 17.3% compared to that in 2013.

In 2014, fish production rendered R$3.87 billion, of which 90.0% was from fish, *i.e.*, 70.2% and 20.5% from fish and shrimp breeding, respectively. Fish farms in Brazil produced 474.33 thousand tons of fish in 2014. The highest fish production was noted in the northern region of Brazil in 2013 and 2014, with the state of Rondônia totaling 75.02 thousand tons [25] [26] [27].

Brazilian fish production continued to rise in 2015, with 640.510 tons and a 4.4% increase compared to that in 2016. In 2017, the growth reached 8%, or 691,700 tons, with the tilapia being the most bred species in Brazil, or 51.7% of Brazilian fish farming, with 357.639 tons [21] [25] [26] [27] [28] [29].

The state of Paraná is the largest producer of tilapia in Brazil, accounting for 94% of total fish production, whereas tilapia constitutes 95% of fish production in the state of São Paulo. The state of Santa Catarina ranks third in total production (74%), followed by Minas Gerais (94%) and Bahia (81%). The five states as a whole make up 64.0% of Brazilian fish production [21].

In addition to sport fishing, sports fish farming is a new branch of activity that is remarkably growing annually in Brazil. It is currently a highly profitable sector producing employment and assets for the regions concerned. It includes catching and immediately releasing the fish. This type of sports, called catch and release, started in the US in the 1970s [30] [31].

Brazilian aquaculture tourism has always been associated with catch-and-release activities, which, although for leisure, are included within the production chain because of their demand with regard to fish, meals, equipments, and contribution toward the development of Brazilian aquaculture. Catch-and-release activity became profitable and triggered the rapid growth of fish chains and the establishment of several entrepreneurships encouraging this practice, especially in the states of São Paulo, Paraná, and Espirito Santo [32].

According to studies on fish farming in Brazil, fish production in the state of Bahia focuses on the tilapia [1] [24]. Brazil is one of the seven major tilapia producers in the world, with more than 250,000 tons. Tilapia is one of the three most bred species on the planet [33]-[39] Reports by residents in the Bahia Concavo region who frequent the catch-and-release fishing modes suggest that the type of breeding varies highly across farms. Fish species also vary across farms: *Colossoma macropomum* (tambaqui), *Oreochromis niloticus* (tilapia), Siluriformes (catfish), *Pseudoplatystoma fasciatum* (surubins), *Piaractus mesopotamicus* (pacu), *Cyprinus carpio* (carp), and *Leporinus friderici* (piaus) (**Figure 1**).

The growth of fish farming in Brazil increases concerns regarding product quality at the financial and microbiological levels. Problems such as an increase in the natural microbiological load and more resistant bacteria may be associated with the spread of fish pathogenic microorganisms. *Aeromonas*, a fish pathogen, is a case in point; it causes economic liabilities and risks to people who may consume the fish. Another issue associated with fish farming is the excessive use of commercial antibiotics that may generate the production of resistant strains. The above factors may affect fish and people [40] [41] [42].

4. Water- and Foodborne Diseases and Microorganisms Indicating Contamination

Water- and foodborne diseases (WFBDs) are normally related to not only merely bad hygiene and sanitary habits but also cross-contamination by infected handlers and contaminated utensils [43]. According to the Brazilian Health Ministry

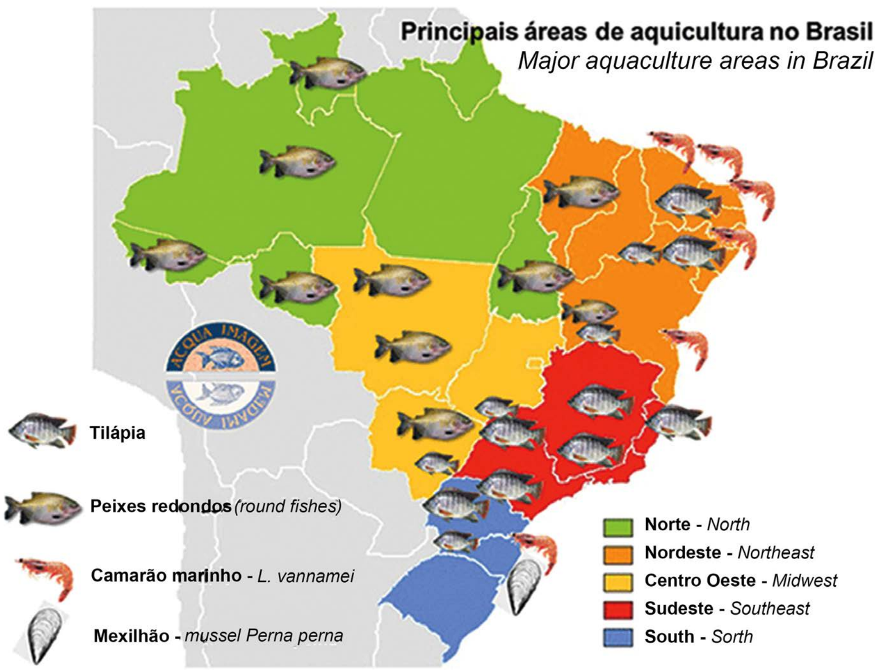

Figure 1. Aquaculture in Brazil.

[35] more than 250 types of WFBDs exist. They are currently considered as one of the major causes of morbidity and mortality worldwide. WFBDs are a serious health issue in Brazil and in the world. Epidemiological research in Brazil by the Health Ministry revealed 10,666 WFBD outbreaks between 2000 and 2015, with 154 deaths. Most outbreaks occurred in the southeastern region of the country, with 39.9% of notified cases; in the northeastern region of the country, the prevalence reached 14.7%, ranking third, with the highest number of notified outbreaks [34] [35] [36].

WFBDs are diseases caused by the ingestion of contaminated food or water containing microorganisms or toxins produced by them. Several pathogens are related to WFBD outbreaks. They may occur in one or more persons, according to the virulence of the pathogens. Outbreaks may occur, for example, by *Clostridium botulinum* and *E. coli* O157:H7. These microorganisms contaminate food, but fail to modify the foods' characteristics such as smell or taste. Hence, it is difficult to identify contamination by these pathogens. Several studies have shown that these outbreaks occur because the infecting dose is lower than the amount of pathogens required for degrading foods [44].

One of the reasons for the occurrence of WFBDs is the habit of eating street food, a trend common in modern society. This practice may increase the transmission of foodborne diseases to consumers since cross-contamination may occur during food processing and handling [45] [46].

Etiological agents causing notified WFBD outbreaks have not yet been identified, even though the most commonly identified agent is *Salmonella* spp. In addition to *Salmonella* spp., indicator microorganisms are employed in the evaluation of the microbiological quality of water and food [36]. This group of microorganisms comprises total and thermotolerant coliforms, mesophyll aerobic microorganisms, and psychrotrophic aerobic agents [47]. According to Ribeiro (2008), the genus *Aeromonas* may be used as an indicator of potable water [48].

Adequate procedures during fish handling are highly relevant to guarantee the microbiological quality of fish from the moment of capture up to intake by the consumer. Several studies have shown that the microbiological quality of pond water used to culture fish may affect the microbiological quality of fish and its byproducts. Fish have been associated to human illnesses since they are the transmitters of pathogenic microorganisms and thus a public health issue. Foodborne diseases associated with fish have been related to hygiene and sanitary conditions and the lack of knowledge by employees on breeding and good handling practices [4] [5] [49] [50].

5. Total Coliforms and Thermotolerant Bacteria

Total coliforms are classified as non-sporulating gram-negative bacilli formed by species of the family Enterobacteriaceae, which are capable of fermenting lactose with the formation of gas bubbles when incubated at 35˚C - 37˚C during 48 h. Thermotolerant coliform is a subgroup that includes microorganisms capable of fermenting lactose at 44˚C - 45˚C. *E. coli* is the main representative of this group

[47].

Water is colonized by several microorganisms, with special reference to those derived exogenously from human and animal fecal materials, such as total coliforms, thermotolerant coliforms, and enterococci. The genera *Escherichia*, *Enterobacter*, *Citrobacter*, and *Klebsiella* constitute the group. Some even constitute water' natural microorganisms, which include the mobile bacteria of the genera *Aeromonas* and *Pseudomonas* [47].

Although total coliforms can be used to evaluate hygiene conditions, thermotolerant coliforms reveal fecal contamination and suggest deficient hygiene and sanitary conditions. The presence of thermotolerant coliforms in food suggests high *E. coli* rates and requirement of the assessment of the contamination levels and sanitary quality of food [47]. *E. coli* in processed food indicate that they may have been contaminated at a post-processing stage under improper handling and hygiene conditions [51].

Coliforms may indicate fecal contamination and reveal the sanitary and hygiene conditions of food and water. They are not a part of the natural intestinal microbiota of fish even though they have been frequently isolated from fish gastrointestinal tract. One may conclude that microbiological evaluation of fish may reveal the microbiological conditions of water in which fish inhabit [52]. Several studies have detected thermotolerant coliforms and total aerobic microorganisms in fishponds. This is a factor of remarkable concern and shows that strict hygiene control during management and manipulation of the products produced in fish farming should be adopted to prevent the transference of bacteria from the water to the fish and its byproducts [52] [53].

A study on fish and the water of sports fish farming in the microbasin of Corrego Rico, the state of São Paulo, was undertaken to determine the number of total and thermotolerant coliforms, positive *Staphylococcus* coagulase, and bacteria of the genus *Salmonella* in the muscle, surface tissue, and gastrointestinal tract. Total and thermotolerant coliforms and *Salmonella* were found in one of the samples, even though no contamination by *Staphylococcus* coagulase was detected. Therefore, fish may be the vector of cross-contamination, since the skin and gastrointestinal tract may be a source of contamination for the muscles [52].

Santos *et al.* [54] evaluated the microbiological quality of water and tissue changes in the gills of Nile tilapias (*Oreochromis niloticus*) and the hybrid tambacu (*Colossoma macropomum* female × *Piaractus mesopotamicus* male), in the municipality of Itapecuru-Mirim, Maranhão, Brazil. The microbiological analysis of water revealed pollution by total coliforms, *E. coli*, and heterotrophic bacteria. Several gram-positive and gram-negative bacteria were present in the gills. In fact, monitoring of water quality is an important factor to guarantee the production of fish with hygiene and sanitary quality for human consumption [52].

6. *Escherichia coli*

The genus *Escherichia* includes gram-negative facultative anaerobia bacteria belonging to the family Enterobacteriaceae. They are rod-shaped and may be immobile or mobile owing to the presence of peritrichious flagella, with respiratory and fermentative metabolism. They are lactose fermenters, negative for oxidase, positive for catalase, grow in non-enriched media, and are not non-sporulating. The primary habitat of *E. coli* is the gastrointestinal tract of humans and hot-bloodied animals. The strains that colonize the intestinal tract of human beings are not harmful even though some may be pathogenic for humans and cause diseases such as urinary infections, diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome [8] [45] [46] [47] [55].

E. coli strains may be classified by antigenic differences (serum typing) and virulent factors. Antigen O characterizes the serum group and flagellum H characterizes the isotype. Flagellum-less strains are identified as non-motile (NM). Several strains have a K capsular antigen, which is also used as a classifier [8]. More than 180 serum types have been derived from *E. coli*. A certain co-relationship exists between the serum group and virulence [47] [56].

Most *E. coli* do not cause any risk for the host, even though some are pathogenic and may trigger diarrhea. They are classified according to virulence factors, pathogenic mechanisms, and clinical and serological symptoms. Enteropathogenic *E. coli* (EPEC), enterotoxigen *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), and enterohemorrhagic *E. coli* (EHEC), as well as *E. coli* O157:H7, are the main pathogenic *E. coli* groups associated with food consumption. They are the main *E. coli* pathotypes that cause diarrhea in humans [8] [41] [47] [57].

Since 1892, *E. coli* has been used to indicate fecal contamination in water and food and the eventual presence of enteropathogens [47]. According to FAO [4] [5] *E. coli* is a non-indigene microorganism for fish and does not constitute the normal microbiota of fish. The isolation of *E. coli* from fish food may be associated with fecal contamination at the site of capture, cross-contamination during transport or handling or via utensils or ice that come in contact with fresh fish. Their presence suggests unsatisfactory hygiene and sanitary conditions [4] [5] [47] [51] [58].

Ferreira [58] analyzed fish and ice samples from the state of Maranhão and detected *E. coli* and thermotolerant coliforms in the ice samples. Presence of microorganisms in ice indicates that the water used was in direct or indirect contact with fecal contamination, thereby leading to malfunction during food preparation caused by inadequate hygiene habits, cross-contamination by utensils and equipments, inadequate cleansing, and disinfection. This causes risk to consumer health since *E. coli* have five human pathogenic groups. Another research in Cruz das Almas BA, Brazil, revealed the isolation of *E. coli* resistant to antibiotics such as erythromycin 08 (100%), amycacin 02 (25%), ampicillin, cephalothin, and tetracycline 01 (12.5%) from fish at a municipal market [59].

Enterohemorrhagic *E. coli* have several serum types, among which the notorious O157:H7 has remarkable relevance to public health. *E. coli O*157:H7 was classified as a food-originated pathogen in the 1980s, with several outbreaks caused by the etiological agent [60]. Serum type O157:H7 is a highly important microorganism as it is associated to human pathology. Infection caused by the serum type is associated with the development of hemorrhagic colitis, bloody diarrhea, and uremic hemolytic syndrome [45] [46]. *E. coli* O157:H7 is currently considered as an emerging pathogen. Since it is a foodborne pathogen, it may be found anywhere. Its resistance to several environments has been widely investigated and discussed [59].

7. Mesophyll Aerobic Microorganisms

Mesophyll aerobic microorganisms are bacteria that multiply at a temperature between 10˚C and 45˚C, with best growth temperature between 30˚C and 40˚C, whereas psychrotrophic aerobic microorganisms grow in food at 0˚C - 7˚C, with the best growth temperature above 20˚C [2].

The total count of mesophyll aerobic microorganisms is not useful for the identification of species types. It is used as a general indicator of bacterial populations in food. Although regulations regarding the limits of these microorganisms in any fish are lacking, their presence in food needs to be monitored to guarantee food quality. Their presence is used as general information on product quality, production practices, quality of prime matter employed, processing conditions, handling, and shelf life [2] [61].

The total number of aerobic mesophylls is used as an indicator of cleanliness and serves as a way for controlling and monitoring the processes involved in production. This value reveals whether the product has been handled in an adequate manner to ensure food safety. Thermophilic microorganism count evaluates the degree of deterioration of refrigerated foods or of those foods that are subjected to thermal treatment [62].

Lanzarin *et al.* [63] investigated the hygiene and sanitary quality of *Pseudoplatystoma fasciatum* (cachara) retrieved from fish farms and isolated strains of *Aeromonas* and mesophyll aerobic heterotrophic bacteria. Since high counts of *Aeromonas* spp. were reported, remarkable concern ensued since the genus has been known to be involved in human pathologies. The authors underscored the care that needs to be taken to control and improve the quality of water and fish used for consumption.

Staphylococcus aureus

Staphylococcus species belong to the Micrococcaceae family. They have a spherical form (0.5 - 1.5 m); they are gram-positive facultative anaerobic bacteria that may be isolated in pairs and in irregular groups. They are immobile and non-sporulated, chemo-organotrophic agents, with the best growth temperature between 30˚C and 37˚C. *S. aureus* strains grow at pH 7, with the best temperature of 3˚C [64] [65].

S. aureus is a pathogenic agent that causes several food poisoning outbreaks. When these microorganisms inhabit favorable growth conditions, they may be related to opportunistic infections. *S. aureus* cause food poisoning by releasing enterotoxins during their growth in food. These microorganisms are found in several sites since they have a wide distribution in nature. Because of their toxin-infection potential, they are a remarkable risk to public health [8] [47].

S. aureus is a human pathogen that causes several diseases such as intense intestinal infection, along with vomiting and diarrhea; it may also cause skin infections. The bacterium is one of the most common causes of food infection worldwide. The consumption of *S. aureus*-colonized food and the production of enterotoxins lead to food poisoning *S. aureus* may be found in foods derived from raw animal origin. They require strict handling procedures and effective processes for food safety. In fact, infected humans are an important source of recontamination [67].

S. aureus is often considered as the causative agent in the outbreaks of *Staphylococcal* food diseases. It is frequently isolated from the skin surface, human mucosa, and food. It is used as a bio-indicator of a product's sanitary quality [47] and is one of the most researched pathogenic food microorganisms since it causes outbreaks of intoxication. *Staphylococcal* enterotoxin differs from that produced by other microorganisms because of its thermostability, and the species can remain alive in food even after cooking. This makes *S. aureus* resistant, leading to the establishment of food poisoning conditions and a public health issue [47] [68].

Contamination of fish with *S. aureus* is commonly reported in the literature and in the statistical data of Brazil and worldwide. Contamination indicates inadequate handling, directly related to a handler's personal hygiene and to the utensils used. Staphylococcal intoxication occurs from toxins released by the microorganisms growing on the food surface. Intoxication causes nausea, vomiting, abdominal pain, and diarrhea within a short incubation period, between 1 to 6 h after the ingestion of contaminated food. Good manufacturing practices, healthy sanitary conditions, and temperature control are essential to avoid contamination, proliferation, and the production of toxins, particularly in pre-cooked fish [8] [66] [68].

Fish is an animal-origin product that is the most liable to microbial proliferation owing to its physiological characteristics such as pH close to neutral, high water activity in tissues, and high nutrient availability. Consequently, inadequate hygiene conditions during production phases associated with favorable conditions may trigger the growth of unwanted microorganisms [68] [69] [70] [71]. Rocha *et al.* [68] quantified coagulase-positive staphylococci in tilapia fillets (*Oreochromis niloticus*) marketed and showed that all samples were positive for *S. aureus*. Furthermore, in 73.3% of cases, the bacterial counts were above the standard established by the current legislation, suggesting low hygiene conditions and inadequate sanitary conditions of the analyzed material.

Viana [72] assessed the microbiological quality of 16 tambaqui samples commercialized at a municipal market of Ariquemes RO, Brazil and detected total coliform contamination of all samples. *S. aureus* was isolated in 37.5% of the samples. As their level was higher than the permissible limit, Viana concluded that the analyzed tambaqui were exposed to contamination owing to inadequate sanitary conditions.

8. Taxonomy and Classification of the Genus Aeromonas

Zirmmerman was the first to identify *Aeromonas* species more than 120 years ago. Since then, the species has undergone numerous taxonomic classifications. Although several authors tried to describe the new species, studies were not accurate owing to the current identification and failed to define the species as a new or the same one [15]. Aeromonas were classified into several genera and were included in the genus *Proteus*, *Escherichia*, *Pseudomonas*, *Vibrium*, and *Aeromonas* and the family Pseudomonadaceae [42].

During the last decade, the genus *Aeromonas* has undergone an extensive review in nomenclature and taxonomy. First, *Aeromonas* were classified in the Vibrionaceae family. However, phylogenetic, genetic, and molecular studies showed that the genus did not have the characteristics similar to other microorganisms in this family. A new family, called Aeromonadaceae, was subsequently established [42].

At present, this family includes 31 acknowledged species of the genus *Aeromonas* and 12 subspecies. *A. veronii*, *A. caviae*, and *A. hydrophila* are the species commonly related to pathologies in human beings [11] [73]. Thus, *Aeromonas* may have been undergoing classifications in the last decades.

Bacteria of the genus *Aeromonas* are classified according to their multiplication ability and motility temperature. The first group (such as *A. hydrophila*, *A. caviae*, and *A. sobria*) comprises heterogeneous mesophyll species and are pathogenic for human beings and fish. They are mobile species, with multiplication temperature ranging between 5˚C and 45˚C, with the best temperature of 28˚C [2] [11]. The second group (*A. salmonicide* and *A. media*) is formed by non-mobile psychrophilic bacteria. *A. media* does not multiply at 37˚C, and *A. salmonicide* does not multiply at temperatures below 22˚C and 25˚C. Although the latter is not pathogenic to humans, it may cause furunculosis in fish [2].

Two new species of Aeromonas of clinical importance (*A. sanarellii* and *A. taiwanensis*) were discovered in 2010. These were classified from the strains isolated from the wounds of a patient in Taiwan. After this first report, the species was also identified and isolated from wastewater and environmental samples in Portugal. In Israel, *A. taiwanensis* was isolated from the feces of a patient with diarrhea and in pond water residue [74].

Bacteria of the genus *Aeromonas* have been classified as facultative anaerobic, gram-negative rods, with 0.3 - 1.0 μm diameter and 1.5 - 3.5 μm in length. Isolates are identified as bacilli or coccobacilli and may be found isolated in pairs or in short chains, with oxidase reactions and positive for catalase [2]. The literature underscores the optimum growth temperature at 30˚C, with the minimum and maximum temperature ranging between 4˚C and 45˚C, respectively, and pH rates between 4.5 and 9.0 [75].

The abovementioned bacteria are mobile. Their structure comprises flagella, either as lateral flagella or in a polar monotric form. Several authors have shown that young specimens may have peritrichous flagella. Furthermore, *Aeromonas* may have other structures such as pili, layer S, and a capsule, which are responsible for the pathogenicity [2] [75].

Although most *Aeromonas* are encapsulated and non-sporulating, they produce a wide range of exo-enzymes that establish their pathogenicity and virulence levels. In the case of biochemical factors, *Aeromonas* are glucose fermenter microorganisms, with the production of acids, followed or not, by gas production derived from de-nitrification. When oxygen is lacking, they reduce nitrate to nitrite and are not inhibited by the vibriostatic Agent 2.4-diamino-6.7-Diisopropylpteridine [2] [74] [75].

The genus *Aeromonas* is associated with aquatic environments. Strains have been isolated from rivers, lakes, seawater (estuaries), fish farming tanks, and all types of drinking and ground water. They are also found in wastewater and sewage [2] [76].

Several studies have shown that *Aeromonas* spp. in potable waters in river marine waters, fish farming tanks, and mainly in water for consumption from distribution systems are a serious public health issue. This is true because *Aeromonas* are an indicator of environmental quality, and the species is pathogenic to humans. Several Aeromonas strains have been shown to have the ability to survive even in climatic variations and under different physical and chemical conditions of water [2] [7] [15].

Few studies have focused on the isolation of *Aeromonas* spp. in soils. Several studies have insisted that *Aeromonas* strains may multiply and preserve their virulence in the soil. The latter may be classified as a possible reservoir of the infection-causing bacterium [76] [78].

Aeromonas spp. may be isolated from vertebrates and insects. These bacteria have been shown to trigger serious diseases in animals and human beings. Therefore, animals involved in the *Aeromonas* dissemination cycle within the environment may transmit pathogens to humans. In fact, pathogenic strains have been detected in the carcasses and meat consumed by humans. It may also be associated with infectious wounds caused by bites of infected animals and to a generalized infection situation (septicemia) in immunodepressed patients [76] [77] [78] [79].

The pathogenicity mechanism of *Aeromonas* spp. needs to be defined. The virulence of the strains is multifactorial since *Aeromonas* produce several extracellular enzymes such as amylase, hemolysin (aerolysin), cytotoxins, gelatinase, enterotoxins, proteases, lipase, leukocyte, phospholipases, chitinase, nuclease, lecitinase, elastases, DNAses, adesins, cholesterases, and endotoxins, which influence the degree of virulence of each species. They are directly related to the pathogenesis of the disease and produce several virulence factors [3] [80] [81].

Several factors have been shown to cause the development of diseases by *Aeromonas* infection. They cause diseases in humans, with water and food as relevant vectors. Studies have shown that animal-derived foods are one of the main factors contributing toward the epidemiology of pathogenic species in human beings [11].

In Italy, 142 Aeromonas strains were isolated from patients with diarrhea caused by food and surface water; *A. hydrophila* and *A. caviae* were found to be the most frequent species in clinical samples, whereas *A. salmonicide* was the most commonly detected in food. Tests on virulence factors showed that 86 strains were positive for more than one virulence factor. *Aeromonas* may cause diseases in humans, and food and water are the vectors for disease development [11] [79] [80].

A study on the water from a public supply company was conducted in Spain: 132 samples were collected, and 35 isolates of *A. caviae* and *A. media* were identified. The identified strains showed more than one virulence factor correlated to genes with a high degree of pathogenicity. The study also showed that *Aeromonas* strains could be recovered at 14˚C, or that the proliferation of the bacterium even occurs at lower temperatures. Thus, the transmission of microorganism to human populations might occur directly and indirectly through the ingestion (drinking) of contaminated water, or through contaminated water used in the food cooking process [2] [70].

9. Bacteria of the Genus *Aeromonas* spp. Associated to Pathologies in Humans

Infections caused by *Aeromonas* spp. are a public health issue. Outbreaks of diseases by these microorganisms trigger economic consequences, in addition to personal and public costs associated with the impact of contaminated water. Several studies have revealed that the prevalence of *Aeromonas* infections may be underestimated in developing nations through underreporting, and that exposure to foodborne pathogenic agents may occur frequently [15].

According to Igbinosa *et al.* [15] *Aeromonas* spp. are found in terrestrial and aquatic environments. Recent research indicates that these species cause serious public concern owing to their resistance to antibiotics and to several virulence factors associated with infections and other pathologies in humans, such as gastroenteritis, diseases of the soft tissue, muscular infections, and septicemia.

A. hydrophila are pathogenic gram-negative bacilli for aquatic animals and an opportunistic pathogen for humans. Humans are infected either through skin wounds, which are a gateway for the species, or through ingestion, causing gastroenteritis derived from the ingestion of contaminated water or food, either of animal (fish, seafood, and meat) or plant origin. *Aeromonas* are found in all water environments—salt water, wastewater, and freshwater [2] [82] [83] [84].

Aeromonas are opportunistic pathogens of remarkable relevance to public health. They are potentially responsible for gastroenteritis and extra-intestinal infections. Wound infections caused by *Aeromonas* often develop into cellulite, which is another health issue related to septicemia that may be associated with wound infection or systemic diseases such as hemolytic uremic syndrome, peritonitis, and pneumonia [2] [75]. Recent studies have indicated that extra-intestine infections may also occur in healthy individuals [15].

Aeromonas hydrophila, *A. caviae*, and *A. veronii* biovar *sobria* are key human pathogens among the clinical manifestations by infections caused by the genus *Aeromonas*. *A. hydrophila* strains are associated with watery diarrhea and dysentery. These species particularly attack immunosuppressed people and may develop into a general infection case. Neonatal patients develop gastrointestinal disease [12] [16] [82] [83].

Mukhopadhyay [85] investigated *Aeromonas* as a pathogen causing extra-intestinal infections and detected that all patients with infections by *Aeromonas* also had other illnesses such as liver diseases, diabetes mellitus, and pneumonia. They also revealed the importance of isolating *A. hydrophila* from intestine samples, since the microorganism causes severe infections in immunocompromised patients and immunocompetent people.

In the literature, controversies exist regarding the etiology of gastroenteritis related to infection by *Aeromonas*. In fact, few studies have revealed outbreaks of gastroenteritis caused by *Aeromonas* and its presence in the feces of asymptomatic individuals. *Aeromonas* has been shown to be the etiological agent of gastrointestinal diseases, and the virulent characteristics of each species that causes infection and disease varies [12] [86].

A study conducted in Pudong, Shanghai, with 101 hospitalized patients with diarrhea, on their state of infection and *Aeromonas*' virulent genes, revealed that 71 patients suffered from diarrhea caused by *Aeromonas* infection. The occurrence of infection by *Aeromonas* was higher among the microorganisms. Most strains harbored the virulent genes, although the species distribution varied considerably [87].

Pereira *et al.* [88] investigated the presence of *Aeromonas* in 2,323 samples of rectal swabs from hospitalized neonates in Rio de Janeiro, Brazil, and detected 94.6% prevalence for *Aeromonas* species, namely, *A. caviae*, *A. veronii* biovar *sobria*, *A. hydrophila*, *A. veronii* biovar *veronii*, *A. sobria*, *A. hydrophila*, and *A. schubertii*. *A. caviae* was the most prevalent (42.8%) in the samples. Furthermore, 26.8% of the strains were found to be resistant to antibiotics.

An outbreak of gastroenteritis occurred in a college in Xingyi, China, in 2012. The case was reported to the Guizhou Center for Disease Control and Prevention (CDC) and 200 students were evaluated. Investigations revealed that the outbreak was caused by *Aeromonas hydrophila* as the etiological agent. The possible cause was the consumption of salads infected with contaminated water [89].

The best way to avoid contamination, either by contaminated food or cross-contamination, is to employ good manipulation practices, as well as adequate sanitize utensils. Therefore, the exposure of human beings to these diseases may be avoided or minimized. People who have direct contact with fish, such as aquaculture employees, sellers, and sport fishing amateurs, are remarkably exposed to this pathogenic agent, some of which are pathogenic to humans. These products should be handled with care since fish may be infected by *A. hydrophila* strains that cause skin infections [2].

Studies on frozen and fresh fish on *Aeromonas* strains related to the deterioration process of fresh and frozen fish, in frozen foods could be recovered [67]. A survey conducted in the state of São Paulo evaluated salmon microbiology by quantifying microorganisms in the flesh of 31 salmon samples (frozen and chilled) and detected *Aeromonas* ssp. and other microorganisms in the retail network of several cities in the state of São Paulo, Brazil. *Aeromonas* ssp. have been detected in 41.95% of the samples, with population variation from 2.0×10^2 to 8.0×10^3 CFU/G [90]. Another study in the state of Rio Grande do Sul, Brazil, investigated the occurrence of *Aeromonas hydrophila* and *Campylobacter jejuni* in the samples of fresh tuna (*Thunnus* spp.) captured on the coast of Santa Catarina and evaluated 85 samples of tuna fillets by using bacteriological analysis and PCR. Eleven (13%) samples were positive for *Aeromonas* spp., with 10/11 (90.9%) proved to be *Aeromonas hydrophila* by PCR [10] [90]. The sawfish was contaminated by *A. hydrophila*, vectoring the pathogenic agent when the fish was consumed [58].

The above-mentioned study collected 60 samples of live (common carps) and frozen fish from 15 local markets in Baghdad, Iraq, to isolate *Aeromonas hydrophila* and determine hemolytic activity, isolates' cytotoxicity, and their susceptibility to antibiotics. Furthermore, 65% of the samples had *Aeromonas hydrophila*, 76.6% were found in samples of live fish and 53.3% in frozen fish; hemolysis α e β was detected in 94.87% of the samples; 100% of the isolates from live fish had β hemolysis, whereas 85.7% showed β hemolysis, and 14.3% showed α hemolysis; 97.43% of the isolates had cytotoxic effect on Vero cells. All isolates were 100% resistant to penicillin, ampicillin, cloxacillin, and bacitracin, according to the sensitivity test, whereas resistance to other antibiotics was 56.5% to oxitetraciclina; 33.4% to tetracycline, 30.8% to cefoxetin, and 28.2% to chloramphenicol and kanamycin. Isolates were resistant to streptomycin and rifampicin, respectively, at 23.1% and 15.4%. The presence of *A. hydrophila* with multiple resistance to antibiotics was detected in fish sold in the markets of Baghdad [91].

Four species of *Aeromonas* spp. (*A. hydrophila*, *A. caviae*, *A. veronii* biovar *sobria*, and *A. schubertii*) were identified in all samples analyzed in sports fish farms in the state of Maranhão, Brazil. These fish farms were a possible risk in the transmission of pathogenic *Aeromonas* and a risk factor for consumers of fish bred on these farms [2]. *Aeromonas* are gastroenteritis-causing bacteria that are transmitted to humans by contact with the etiological agent and the intake of contaminated meat, vegetables, and water. Several studies suggested that infection by *Aeromonas* (gastroenteritis) was one of the causes of traveler's diarrhea [11] [92].

A. hydrophila, free-living bacteria, were found to cause diseases in fish; they are an opportunistic pathogen in humans. *A. hydrophila* infections in animals and humans in Thailand first occurred in 1976 and 1979, respectively, with fish mortality between 0% and 20%. Diarrhea is prevalent in the cases of human infection [93]. Vila *et al.* [92] showed that *A. veronii* biovar *sobria* and *A. caviae* are mostly associated with traveler's diarrhea. Pereira *et al.* [88] investigated the presence of *Aeromonas* in neonates hospitalized in Rio de Janeiro, Brazil and obtained 94.6% prevalence rate for *Aeromonas*. *A. caviae* was the most prevalent (42.8%) in the samples.

Traveler's diarrhea is the most common health issue among international travelers. A study conducted in Spain at the Tropical Medicine Unit of the Clinical Hospital of Barcelona, between 1999 and 2001, determined the prevalence of *Aeromonas* in patients with traveler's diarrhea. The pathogen was the cause of traveler's diarrhea in 18 (2%) of 863 patients. Species *A. veronii* biovar *sobria*, *A. caviae*, *A. jandaei*, and *A. hydrophila* were isolated in the samples [93].

In a study on an outbreak of diarrhea in São Bento do Una, state of Pernambuco, Brazil, in 2004, in which 2170 cases were reported, 145 (25%) out of the 582 stool tests performed had bacterial enteropathogenic agents. Furthermore, 114 cases (19.5%) were caused by *Aeromonas*. When the occurrence was analyzed by gender, *Aeromonas* spp. was found to be prevalent among females (54% - 59.3%), even though, in the study, the most compromised age group was in the 1 - 5 years age bracket (50% - 34.4%). *A. caviae* (57/9.8%), *A. veronii* biovar sober (23/3.9%), and *A. veronii* biovar *veronii* (15/2.6%) were more associated with diseases in humans [18].

10. Virulence Factors

Aeromonas segregate several toxins that usually cause gastroenteritis. They may also cause skin damage to infected individuals. Bacteria cause intestinal infections associated with severe diarrhea and may be lethal if it infects other regions of the body, such as an open wound. The presence of *Aeromonas* spp. in foods of animal origin suggests that these species persists even when hygiene and sanitary control is employed, leading to a risk to consumers' health [3] [12] [13].

Aeromonas spp. are important pathogenic microorganisms. Since they originate from water and other environments, they can adapt themselves to a wide variety of environments owing to several virulence factors. The above is directly linked to the microorganism's pathogenicity for human beings [2] [12]. In fact, water and food play an important role in the transmission of the pathogen [3] [11].

Because of the remarkable diversity of ecosystems in which *Aeromonas* inhabit, they have become resistant to antimicrobial agents and become highly virulent. In fact, several *Aeromonas* species secrete extracellular proteins such as amylase, chitinase, elastase, aerolysine, nuclease, gelatinase, lecitinase, lipase, and protease, which, according to the literature, are known to be virulent factors that cause disease in fish and human beings [69] [70] [81] [90].

Aeromonas spp. can form biofilms that allow their survival in water distribution systems, food processing surfaces, and food [94]. Several studies indicate that biofilms efficiently adhere to the gastrointestinal tract and thus increase the microorganisms' pathogenicity [44] [54]. As *Aeromonas* spp. even form a biofilm in stainless steel discs, the importance of cleaning procedures and disinfection of food processing environments is realized [44] [94].

According to Puthucheary *et al.* [79], the pathogenicity of *Aeromonas* is related to several factors, namely, thermolabile cytotonic enterotoxin (ALT), thermostable cytotonic enterotoxin (AST), cytotoxic thermolabile enterotoxin (ACT), aerolysine, flagella A and B, lipase, elastase, serine, and protease. Several studies have shown that some factors determine the evolution of the bacterium-caused infections [11]. In Spain, among 800 feces samples retrieved from patients with diarrhea, 32 (4%) were tested positive for *Aeromonas* spp. Most isolates had one or more virulence genes. The occurrence of *ALT*, *hlyA*, *aerA*, *AST*, and *LAF* genes was 71.9%, 28.1%, 25.0%, 18.8%, and 9.4%, respectively [70].

Research on the virulence degree of *Aeromonas* has shown its potential in strains isolated from water and from the environment and wounds and feces of infected patients. A study on the importance of flagella and enterotoxins for the virulence of *Aeromonas* in rats was revealed by evaluating 55 samples of drinking water and 9 clinical isolates; 16 strains of *A. hydrophila*, 7 strains of *A. veronii*, and 7 strains of *A. caviae* exhibited different combinations of virulence factor genes in immunocompromised rats. Further, only strains with one or more of flaA, flab, and flaG or lafA enterotoxins showed any signs of virulence. As an association has been reported in 97% of the strains, *Aeromonas* isolates in water might be considered to have a pathogenic potential for immunocompromised individuals [95].

A study conducted in southern Taiwan between 2004 and 2011 showed that *A. dhakensis* is the most common species among wound isolates. This species is more virulent than *A. hydrophila*. In fact, *A. dhakensis*, phenotypically identified as *A. hydrophila*, is an important human pathogen. Both may cause serious infections of the skin and soft tissues [12].

Aravena-Román *et al.* [96] revealed that 96% of the strains contained at least one virulent gene. The general distribution of virulent genes was aerA/haem (77%), Alt (53%), lafA (51%), AST (39%), flaA (32%), aspA (29%), vasH (26%), ascV (16%), and aexT (13%). Furthermore, 48% of *A. hydrophila* and 42% of *A. dhakensis* had five or more virulent genes, with 19% for *A. veronii* biovar *sobria* and none in the isolates of *A. caviae*. Thus, the strains of *A. dhakensis* and *A. hydrophila* in Western Australia are more virulent than *A. veronii* biovar *sobria* and *A. caviae*.

Manna *et al.* [84] indicated that gastroenteritis induced by *Aeromonas* ssp. is common in India. In fact, enterotoxigenic *Aeromonas* in different raw and processed foods posit a serious threat to human health. Antimicrobial-resistant

A. hydrophila in foods is a threat to public health and aquatic animals [13].

11. Resistance to Antimicrobials in *Aeromonas* spp.

A. hydrophila is a gram-negative bacterium with virulence factors such as hemolysins, aerolysins, adesins, and enterotoxins. Although an opportunistic aquatic pathogen, it has been isolated from vegetables, meat, milk, and their derivates. Several studies have shown that this species is resistant to commercial antibiotics by genetic mutations (plasmids or horizontal gene transfer) owing to the indiscriminate use of antibiotics in fish breeding and other aquatic food products. Since *A. hydrophila* is resistant to antimicrobial agents and frequently occurs in food, it is a threat to public health [13].

Martineli *et al.* [97] [98] assessed bovine carcasses in the state of São Paulo, Brazil, and reported that 38 out of 285 samples were positive for *Aeromonas* spp. Antimicrobial resistance tests revealed that all isolates were resistant to ampicillin and cephalexin. Resistance to antimicrobial agents is a concern, because, in the case of *Aeromonas* spp., their indiscriminate use may lead to the development of multidrug-resistant bacteria. In addition, several species are pathogenic to humans. In this specific analysis, the authors focused on the care that must be taken to overcome the occurrence of *A. caviae*, the most prevalent in the study and one of the species defined in the literature as an etiological agent of gastroenteritis in humans. A study conducted in the types of cheese showed total multidrug resistance to 15 antimicrobial agents in *Aeromonas* spp. isolates. These findings suggest concern due to threats to public health [99].

Clinical investigations conducted in Malaysia evidenced resistance to antimicrobial agents by the genus *Aeromonas*. A study by Odeyemi & Ahmad [100], in 2015, on *Aeromonas* strains retrieved from aquatic environments showed a pattern of multi-resistance between isolates and 21 different phenotypes. Among the antimicrobial agents analyzed, ampicillin, novobiocine, sulfamethoxazole, and trimethoprim resistance was noted in all the isolates. They are sensitive to other antimicrobial agents such as tetracycline. The indiscriminate use of antibiotics may have caused the emergence of multi-drug resistant bacteria of the genus *Aeromonas*. The above constitutes a public health issue, since these species are pathogenic for humans and aquatic animals [100] [101] [102]. Furthermore, as several *Aeromonas* species are pathogenic and may cause diarrhea, they should be included in routine bacteriological tests [74].

In Israel, a study evaluated the prevalence and virulence of *Aeromonas* by using molecular methods by analyzing 1033 samples of diarrheal feces. The etiological agent was identified to be *Aeromonas* spp. in 17 samples by using *rpoD* gene sequencing. The first clinical record of diarrhea by *A. taiwanensis* was identified in other species. The species were resistant to beta-lactam antibiotics, with susceptibility to third-generation cephalosporin antibiotics [74] [103].

Studies have also been undertaken to identify and characterize *A. sanarellii* and *A. taiwanensis* strains. Chironomid egg masses were identified in a waste pond in the same region in Israel where Senderovich *et al.* [74] conducted the *rpoD* gene sequencing study. Data failed to characterize the virulence and antimicrobial resistance of the new species with pathogenicity in humans. *A. sanarellii* and *A. taiwanensis* were subjected to an antimicrobial susceptibility test. They were sensitive to amikacin, aztreonam, cefepime, cefotaxime, ceftazidime, ciprofloxacin, gentamicin, piperacillin-tazobactam, tigecycline, tobramycin, sulphamethoxazole, trimethoprim, and imipenem. Thus, this factor is responsible for the infections caused by the species [19].

Evangelista-Barreto *et al.* [104] conducted a study in the Cocó River, CE, Brazil, and detected *Aeromonas* spp. in 60% of the 38 strains evaluated. *A. caviae*, *A. veronii* biovar *sobria*, *A. veronii* biovar *veronii*, *A. trota*, *A. media*, *A. sobria*, *A. hydrophila*, and *Aeromonas* sp. were identified. All strains showed resistance to at least one of the tested antibiotics, with several species presenting multiple resistance. *A. caviae* showed the highest resistance rate (four antibiotics). *A. caviae* is a pathogenic strain for humans, and its presence in contaminated water and food may cause gastroenteritis mainly in children and immunosuppressed individuals [16].

In Mumbai, India, 154 ready food samples were analyzed for *Aeromonas* spp. during a 2-year period (2006 to 2008). The study identified the bacterium in 18 (11.7%) samples, including 22 *Aeromonas* isolates of seven different species. Since the isolated strains of *Aeromonas* were positive for virulence factors with high antimicrobial resistance potential, they were a risk to the health of individuals who consumed contaminated raw or cooked food products [105].

Aeromonas strains are known for their increased capacity to acquire and exchange antibiotic resistance genes. There is a strong correlation between aquaculture, *Aeromonas* diversity, and antibiotic resistance. In addition, robust links exist between the prophylactic and systemic use of antibiotics in aquaculture and the propagation of resistance to antibiotics [71] [105].

12. Brazilian Legislation on Fish

There is only a single reference for positive staphylococcus coagulase/g 10^3, with a rate of 5×10^2 and maximum of 10^3, with absence in the case of *Salmonella* spp. in 25 g. Microbiological standards are regulated by Brazilian legislation through RDC 12, published in January 2001, which determined the standards of microorganisms in fish-derived food and from fish—fresh, cooled or frozen, and not consumed raw [14]. No microbiological standard exists in the Brazilian legislation for *Aeromonas* spp. in food [80].

According to Lanzarin *et al.* [63] brazilian legislation fails to define the limits for heterotrophic aerobic psychrotrophic microorganism counts in fresh fish. However, international legislation establishes a maximum limit of 7.0 log per gram. Furthermore, no known minimal infection doses are known for *A. hydrophila*.

Resolution 357, published in 2005, states that thermotolerant coliforms should not exceed 1000 thermotolerant coliforms/100 ml in water for aquaculture and fishing activity [36] [42]. There is no legislation for total coliform limits in fish. However, the presence of this group in food should be investigated to guarantee the sanitary quality of fish. The total coliform rates have been shown to be above 50 and up to 100 NMP per gram of fish meat. This mandates the implementation of control measures [14] [51].

Aeromonas are pathogenic bacteria classified as emerging ones by the WHO. Studies have revealed that Brazilian legislation fails to establish parameters for the identification or quantification of *Aeromonas* for research involving food and water. The genus has been shown to be neither routinely investigated nor considered under environmental and sanitary legislation. Its analysis in water and farmed fish is an issue of public health and high economic relevance. In fact, the bacterium may cause remarkable liabilities for fish farms and serious illnesses in humans. Liabilities are also associated with shelf time owing to the high concentrations of bacteria [63].

Infection dose for *Aeromonas* in human beings has still to be defined. Cooking process may inactivate these bacteria; however, cross-contamination remains a threat against health, especially in the case of high-risk groups such as children, elderly people, and immunodepressed individuals. This microorganism has frequently been detected in the feces of patients with HGS-gastroenteritis [12] [106].

13. Aquaculture Challenges in Brazil

Although Brazil has native aquaculture species with great productive and economic potential, none of them, yet, has scientific and technological information that allows the structuring of the production chain. Thus, the main challenge of national aquaculture research is to generate knowledge about genetics and breeding, reproduction, physiology, nutrition, health, production systems, slaughter, processing and the market related to these species. For this, it is essential to bring research bodies closer to the productive sector and coordinated interaction between the researchers, generating knowledge and technologies for the agents of the production chain, in an increasingly efficient manner.

In this sense, there is already research in the areas of reproduction and genetic improvement of fish, nutrition and feeding of aquaculture species with the production of more sustainable rations that minimize the environmental impact, conservation and management of fishery resources, health of aquaculture species, agro-industrial processing of fish, aquaculture production systems, effluent treatment and reuse and sustainable development of continental artisanal fishing.

14. Conclusions

The term "environmentally sustainable" has predominated in aquaculture debates, although such debates have not yet generated wide-ranging practical measures. At least in Brazil, sustainability studies are still focused exclusively on the environmental aspects of production.

Impacts may arise, for example, from conflict with the use of water bodies, sedimentation and obstruction of water flows, through hypernutrification and eutrophication, discharge of effluent from nurseries and pollution by chemical residues employed in the different stages of cultivation, introduction and dissemination of exotic species. Another frequent criticism that aquaculture receives from environmentalists is related to the excessive use of captive-bred carnivorous species, which consume food consisting in part of fish caught in the wild. According to these environmentalists, in a short time "there simply won't be enough fish in the seas to be crushed and made into fish feed and oil." In this respect, aquaculture is seen as a direct consumer of wild stocks. Food security must be provided by a state which, in its responsibility as the guardian of individual guarantees, must offer democracy, to its fullest extent, to its governed, assuming responsibility for the realization of human rights. Food security as a right to adequate food is a goal to be met by the state, which, if unable to do so, must seek international help for its fulfillment. In the world, food security is the agenda of developed countries and has been on the political agenda since the First World War. Since the creation of the UN, FAO was created to discuss supply policies to end world hunger and misery. For a long time, it was thought that the concern was with the production of food, that it might be lacking, and the world could perish by the absence of food. However, technology has improved and found to be the most serious: that hunger in the world.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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