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Foodborne diseases, fish and the case of Aeromonas spp.

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Food intake is an imperative need of living beings in order to obtain the nutrients and energy necessary for their growth and development. All human beings worldwide have the right to access to nutritious and safe food. Food safety is a term that has become increasingly important in food production; is defined as the set of conditions and actions necessary along all phases of the food chain (from its primary production to the consumer's table) that guarantee that the food that is ingested does not represent a risk in the health of consumers. Food can be contaminated by different types of hazards (heavy metals, pesticides, microorganisms among others) compromising their safety and functioning as vehicles for various diseases. Foodborne diseases are considered globally a serious problem in public health, and a challenge in the production and commercialization of food. The purpose of this document is to provide a general overview of foodborne diseases, causative agents with emphasis on the genus *Aeromonas* spp., methods of detection, actions of prevention and control of foodborne diseases in addition to considering the phenomenon of antimicrobial resistance detected in these pathogens of relevance in public health and production of food mainly of aquatic origin.

Key words: *Aeromonas*, ascites, fish, food safety, processing, public health, tilapia.

INTRODUCTION

The health and quality of life of people depend to a great extent on the nutritional value of the foods that are consumed. This property in turn is related to the hygienic and sanitary quality to which they are subjected along the food chain (from the field to the consumer's table) (Kopper et al., 2009; Badui, 2015). Foods play a key role in the transmission of diseases through contamination due to lack of hygiene and sanitation from air, water, soil, animals, utensils, human beings, during primary production, transport, storage, processing, distribution and elaboration (Vásquez, 2003; Kopper et al*.,* 2009).

Everybody has the right to access safe food. Food safety is defined as the set of conditions and actions necessary along the food chain to ensure when ingested, they do not represent a risk to consumers' health; food safety is not negotiable and cannot be dispensed in any food within the context of quality (Tafur, 2009; WHO, 2018a).

Foodborne Diseases (FD) are a priority in public health issues around the world due to their incidence and mortality as well as the socioeconomic burden due to high levels of productivity loss, health services costs, implementation and monitoring of food safety policies (Kopper et al*.,* 2009; Olea et al*.,* 2012; Badui*,* 2015; Forero et al.,2017).

The FDs are derived from the consumption of water and food infected with contaminants of physical, chemical or biological origin in enough quantity to affect consumers' health (Vásquez, 2003; Kopper et al*.,* 2009; Soto et al*.,* 2016). Approximately 250 causal agents have been described, including bacteria, viruses, fungi, parasites, prions, toxins and metals (Barreto et al*.,* 2010; Olea et al*.,* 2012; Jorquera et al., 2015); being the most frequent FD caused by agents of biological origin mainly bacteria such as: *Salmonella* spp., *Campylobacter* spp*., Shigella* spp*., Clostridium perfringens, Escherichia coli, S. aureus, Bacillus cereus, Vibrio* spp*., Yersinia* spp*., Aeromonas* spp*.,* and *Listeria monocytogenes* (Daskalov,2006;Barreto et al*.,* 2010; Badui, 2015; Soto et al*.,* 2016; Fernandes et al*.,* 2018; PAHO, 2018).

The purpose of this review was to do a general presentation of FD and causative agents, in particular the genus *Aeromonas* spp., the relevant considerations in public health and food production mainly of aquatic origin, like bacterial detection and isolation methodologies from foods, as well as the actions of prevention and control of foodborne diseases; also included are the aspects of the antimicrobial resistance phenomenon, an issue of relevance for food safety and public health, which has been presented for several years in this microbial genus through various studies on foods reported around the world.

FOODBORNE DISEASES

Foodborne diseases generated by the ingestion of water and food are classified as 1. Food infections are established and multiplied in the consumer and have two aspects: a) invasive infections: where the involved microorganisms colonize tissues and organs of the affected. This group includes viruses, parasitic protozoa and bacteria such as *Salmonella* spp*., Aeromonas* spp., *Campylobacter* spp., *Shigella* spp., *Vibrio parahaemolyticus, Yersinia* spp., enteroinvasive *E. coli*, among others; b) Toxi-infections: caused by non-invasive bacteria, with the ability to colonize and multiply in the intestinal tract of the host, where they excrete their toxins, as examples: *Vibrio cholerae, Bacillus cereus* (enterotoxin producers), *Clostridium botulinum, Clostridium perfringens* and enterotoxigenic *E. coli*, and 2. Food poisoning caused by toxins produced by microorganisms that have multiplied to a certain concentration in the food, controlled by a mechanism called *quorum sensing*; some causative microorganisms are: *C. botulinum, Bacillus cereus* (emetic toxin) and *Staphylococcus aureus* (Rodriguez et al*.,* 2015).

These diseases mainly affect children, the elderly, pregnant women and people with a weak immune system. Usually damaging of the gastrointestinal system leads to symptoms such as nausea, vomiting, diarrhea, abdominal pain and fever. In some cases, there may be complications such as sepsis, meningitis, abortions, Reiter syndrome, Guillan Barré syndrome or death (Kopper et al., 2009; Soto et al*.,* 2016).

It has been determined that factors such as changes in the eating habits of a society, the consumption of packaged foods, fast foods, commercial globalization, demographic movements, emergence and adaptation of pathogens (acquisition of virulence factors, development of resistance to antimicrobials, ability to survive in adverse environments), changes in population groups at risk, climate change, complex food systems and changes in food production technology have contributed to the increase of these diseases (Vásquez, 2003; Arispe and Tapia, 2007; Olea et al*.,* 2012; Rodriguez et al*.,* 2015; Jorquera et al*.,* 2015).

Foodborne diseases and fish

Foodborne diseases are presented within a range of illnesses, in addition to having an incidence worldwide, being considered a serious public health problem. According to data from the World Health Organization (WHO), each year 420,000 deaths are generated specifically by children under 5 years of age (WHO, 2018).

Fish as food for human consumption has its source in capture fisheries and aquaculture. These activities are

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among the most important sources of food, nutrition, income and livelihoods for hundreds of millions of people around the world; only the global per capita fish supply for 2014 was 20 kg (FAO, 2016) and it is estimated that globally by 2025 consumption will reach 21.8 kg per capita per year (Fernandes et al*.,* 2018).

Fish as a food is considered a highly nutritious product since it is a source of water (66-81%), proteins (16-21%), lipids (0.2-25%), including polyunsaturated fatty acids, carbohydrates (<5%), minerals (1.2-1.5%) and vitamins of complex B, A and D (Huss, 1999) that make it part of a healthy diet and lifestyle (Huss, 1999; Fernandes et al*.,* 2018). However, this characteristic also makes it a food highly susceptible to deterioration and putrefaction due to the action of microorganisms, oxidation reactions and endogenous enzymes as well as being a target of contamination and transmission vehicles of physical, chemical or biological hazards throughout the food chain going from its primary production, processing, storage to pre-consumer manipulation, thus becoming a high-risk food for consumers' health (Huss, 1999; Ghaly et al*.,* 2010; Ramírez et al*.,* 2011; Fernandes et al*.,* 2018).

The European Food Safety Authority (EFSA) reported that the most related foods to foodborne diseases in Europe in 2016 were chicken (9%), cheese (8%) and fish (7%) (Fernándes et al*.,* 2018). On the other hand, in countries of the continent such as Spain, through the National Network of Epidemiological Surveillance (RENAVE), it was reported that in the period corresponding to 2008 to 2011, 2,342 outbreaks had been reported; the cases associated with these outbreaks were 30219, having 1763 hospitalizations and 24 deaths; the main causal agents involved were bacteria (79%), the main foods involved were egg, egg products and mayonnaise, being 24.6% of the total, followed by meat and meat products (8%), seafood (7.4%), fish and products (6.5%). The main contributing factors to these outbreaks were cross contamination (26.5%), inadequate time or temperature (20.8%) and contaminated food (18.7%) (Espinosa et al*.,* 2014).

In the American continent, and metropolitan region of Chile, from January 2005 to July 2010, 2434 outbreaks of FD were reported with 12,196 cases; the main types of food involved correspond by 15.4% to mollusks and 15.1% to fish being the biological causal agents frequently related as: *Salmonella* spp., (20.9%), *Shigella* of unspecified type (20.4%), *Shigella sonnei* (17.7%) *Vibrio parahaemolyticus*, among others (Alerte et al*.,* 2012). For the year 2013, 1,164 outbreaks were notified with 7,841 cases. Out of the total outbreaks reported in 10% of them, the causal agent was identified. *Salmonella* spp. was responsible for 54% of the outbreaks, followed by *V. parahaemolyticus* (27%). The main foods involved in the outbreaks were meals and dishes prepared by40%, followed by fish and fish products by 32% and eggs and by-products by 10% (Jorquera et al*.,* 2015).

In Mexico, gastrointestinal diseases are derived from the consumption of contaminated food and water, mainly affecting children and adults over 60 years of age; among the pathogens involved are: *Salmonella* spp*., Shigella* spp*., E. coli, Vibrio* spp*., Aeromonas* spp. The Ministry of Health (SSA) in 2001 reported that gastrointestinal diseases, caused by bacteria or parasites, were the fourteenth cause of deaths nationwide, being the states with higher incidence: Chiapas, Oaxaca, Guanajuato, Veracruz, Federal District and Puebla (Hernandez et al., 2011). On the other hand, in 2008, the Mexican Institute of Social Security (IMSS) carried out 2 million 188 hospital consultations due to gastrointestinal diseases, where the states with the highest incidence were: Chihuahua, Coahuila, Jalisco, Michoacán, Guerrero, and Oaxaca (Hernandez et al*.,* 2011).

In Caribbean countries such as Cuba in the context of diarrheal diseases associated with *Aeromonas* spp. from February 1985 to January 2005, case studies were conducted with 2322 children under 5 years of age with acute diarrheal disease. *Aeromonas* spp*.* was isolated in 166 cases (7.15%); the most frequently isolated species were *A. caviae, A. hydrophila,* and *A. veronii bv sobria* (Bravo et al*.,* 2012).

While, in South American countries such as Venezuela, in the State of Zulia, a zone marked by aquatic environments, species of *Aeromonas* spp., have been frequently reported in individuals with episodes of diarrhea, being the main enteric bacterial pathogens prevalent in the population and where *A. caviae* and *A. hydrophila* have repeatedly been recorded as the most frequent species in fecal samples analyzed in the region. In addition, these species have been isolated in fresh commercialized foods, mainly those of vegetable origin (Rincon et al*.,* 2016).

AEROMONAS' GENERALITIES

The genus *Aeromonas* spp., belongs to the family of *Aeromonadaceae* which has 24 species that include *A. hydrophila, A. caviae, A. veronii biovar sobria, A. piscicola, A. jandaei, A. schubertii* and *A. trota* frequently isolated and related to clinical affectations in human beings. These bacteria are halophilic, bacilli-shaped Gram negative of 0.3-1.0 x 1.0-3.5 μm, mobile (except *A. salmonicida* and *A. media*), non-spore forming, have an optimal growth temperature of 22 to 35°C but some species grow at intervals of 0 to 45°C; their optimum pH is 5.5 to 9, are oxidase and catalase positive, facultative anaerobes, reduce nitrates to nitrites, ferment D-glucose as a carbon source and energy in addition to maltose, D-galactose, and trehalose, can grow in media containing 0 to 4% NaCl (Castro et al*.,* 2002; Parker and Shaw, 2011; Beaz et al*.,* 2012; Suárez and Herrera, 2012; Stratev et al., 2012; PHE, 2015; Priyam et al*.,*2016; Bhunia, 2018).

The habitat of these microorganisms is generally aquatic being considered autochthonous (rivers, lakes, sea, ponds, estuaries, drinking water, chlorinated water, groundwater, and wastewater) as well as located in the intestinal tract of humans and animals (Castro et al*.,* 2002; Parker and Shaw, 2011; Beaz et al., 2012; Suarez and Herrera, 2012; PHE, 2015; Priyam et al., 2016; Bhunia, 2018; Fowoyo and Achimugu, 2019). The isolation of these bacteria in water samples depends on several factors: the season of the year, concentration of organic matter, available oxygen, levels of chlorine and salinity (Castro et al*.,* 2002).

These bacteria present a variety of virulence factors to generate disease, including structural components such as flagella, pili, capsule, S layer, lipopolysaccharide (LPS), outer membrane proteins and extracellular products such as adhesins, hemolysins, cytotoxic enterotoxins, proteases, lipases, deoxyribonucleases, ribonucleases, siderophores as well as biofilm formation capacity (Castro et al*.,* 2002; Suárez and Herrera, 2012; Priyam et al., 2016; Bhunia, 2018; Fowoyo and Achimugu, 2019).

Aeromonas spp*.,* present pathogenicity towards different living beings such as: amphibians, fish, reptiles and humans. The symptoms in humans related to infection caused by *Aeromonas* spp. are gastroenteritis (diarrhea), septicemia, infection of skin and tissues, pneumonias, ocular and urinary tract infection, as well as complications in some cases such as hemolytic uremic syndrome (HUS) (Pascual and Calderón, 1999;Parker and Shaw, 2011; Beaz et al., 2012; Bravo et al., 2012; Suarez and Herrera, 2012; Stratev et al., 2012; FDA, 2012; Priyam et al., 2016; Bhunia, 2018).The strains causing diseases in fish are *A. salmonicida* and *A. hydrophila* and in humans are: *A. hydrophila, A. sobria, A. caviae, A. veronii, A. jandae* and *A. schubertii.* They affect all population groups with emphasis on children, the elderly and immunosuppressed; the infectious dose is not completely known, with an estimated increase of 10 colony-forming unit (CFU) for *A. hydrophila* up to 10^7 CFU / g of food (Parker and Shaw, 2011; Beaz et al*.,* 2012; Bravo et al*.,* 2012; Pascual and Calderón, 1999; Suárez and Herrera, 2012; FDA, 2012; Priyam et al., 2016; Bhunia, 2018).

Presence and isolation of *Aeromonas* spp., have been reported in a variety of foods such as meat products, fish, seafood, prepared foods, confectionery products, vegetables, milk and dairy products that act as potential vehicles for infection (Castro et al*.,* 2002; Castro et al*.,* 2003; Daskalov, 2006; Stratev et al., 2012; FDA, 2012; Sanchez et al*.,* 2018; Fowoyo and Achimugu, 2019).

Aeromonas and fish

The genus *Aeromonas* spp*.,* in the products of fishing and aquaculture is of relevance since it is a generator of diseases such as Aeromonas (Ascites) in fish. It presents two different symptoms, one called maculosa with red cutaneous spots with different shapes and sizes and the other ascetic of greater severity with lesions, necrosis and tissue loss. Healthy and cured carriers do not present clinical symptoms, but can transmit the disease which can appear individually or epidemic, especially in pond culture where species such as: *A. salmonicida, A. hydrophila A. veronii, A. sobria, A. caviae,* and *A. bestiarum* have been related as causal agents of high mortalities and economic losses in food production (Acha and Szyfres, 2001; Balbuena et al*.,* 2011; Stratev et al.,2012; Pridgeon and Klesius, 2011; Samal et al*.,* 2014; Zepeda, 2015). The treatment for these cases is the application of antibiotics such as streptomycin and sulfonamides orally in the feed (500 mg / kg of fish). However, it is not very effective when the disease has infested a considerable percentage (> 10% of the organisms in the pond); where it is preferable to eliminate the lot, to avoid propagation (Balbuena et al*.,* 2011).

This bacterial genus has also been related to food spoilage due to its psychrotrophic growth properties during storage stages at refrigeration temperatures (Hernández, 2016; Bhunia, 2018). Psychrotrophic microorganisms are considered as microbial indicators of food quality. Noting storage conditions, sources of contamination and possible shelf life of food mainly in those that require refrigeration conditions (Hernandez, 2016). Psychrotrophs grow at temperatures below 7°C for 7 to 10 days of culture. The genus of microorganisms most frequently isolated in foods are *Pseudomonas, Alcaligenes, Flavobacterium, Enterobacter, Proteus, Aeromonas, Lactobacillus* among others. They are involved in the alteration and decomposition and synthesis of pigments of foods of a protein nature (chicken, meat and fish) (Hernandez, 2016).

Microbial spoilage is the main mechanism of deterioration in the quality of fresh and frozen fish (Parlapani et al., 2013). The microorganisms are located in the skin, gills and intestines of live and freshly caught fish. The microbiota in freshly caught fish depends more on the capture environment than on the species (Huss, 1999). In warm water fish, there is a variety of mainly gram-negative psychotropic bacteria such as *Pseudomonas* spp., *Acinetobacter* spp*., Moraxella* spp*., Flavobacterium* spp*., Shewanella* spp*., Vibrionaceae* spp*.* and *Aeromonas* spp., which are part of the initial fish microbiota; being only a small fraction of fish microbiota identified in deterioration processes such as: *Pseudomonas* spp*., Shewanella* spp*., Photobacterium* spp*., Moraxella* spp*., Acinetobacter* spp., and *Aeromonas* spp. (Huss, 1999; Parlapani et al., 2013; Buhnia, 2018).

In the deterioration of fish, microbial activity plays an important role in their shelf life, where it can be included that the fish may be contaminated with pathogenic microorganisms that put the health of consumers at risk. Microorganisms of a pathogenic character can be species whose natural habitat is water and where the temperature has a selective effect, such as *Vibrio* spp*., Aeromonas* spp*., Plesiomonas* spp*., C. botulinum*, among others, and those microorganisms that are present in the water due to contamination of fecal origin and associated to the manipulation process to which the fish is later subjected as: *E. coli*, *Salmonella* spp., *Shigella* spp., *L. monocytogenes*, *S. aureus* (Huss, 1997; Ramírez et al*.,* 2011; Sánchez and Delgado, 2016; Soto et al*.,* 2016). It should be noted that factors such as the time of year of capture or harvest, the characteristics of the diet, geographical area, species of fish, sanitary quality of the water and the capture system influence qualitatively and quantitatively microbiological aspects present initially in fish; the handling and storage hygiene conditions can influence the microbiota responsible for deterioration and pathogenicity (Ramirez et al*.,* 2011; Sánchez and Delgado, 2016; Soto et al*.,* 2016, Fernandes et al*.,* 2018).

In the case of bacteria of the genus *Aeromonas* spp*.,* the detection of species such as *A. hydrophila, A. jandaei, A. veronii, A. popoffii, A. eucrenophila, A. caviae/A. media, A. schubertii, A. eucrenophila,* and *A. salmonicida* has been reported in the microbiological analysis of fish products (Suárez and Herrera, 2012; Soto et al*.,* 2016; Samal et al*.,* 2014; Praveen et al*.,* 2016). Being indigenous environmental microorganisms of water, pathogens of fish and humans, capable of tolerating low temperatures (-2 to 10°C), this genus has been considered important for animal health (fish) and public health (Balbuena et al*.,* 2011; Hernández et al*.,* 2011; Samal et al*.,* 2014; Zepeda, 2015; Praveen et al*.,* 2016).

MICROBIOLOGICAL ANALYSIS FOR THE ISOLATION AND DETECTION OF *AEROMONAS* SPP.

The isolation and identification of microorganisms in a traditional way is through different techniques such as the observation of macroscopic, microscopic morphology, and biochemical or phenotypic characteristics (Bou et al*.,* 2011; Sánchez et al*.,* 2017). Different procedures have been developed for the determination in meat foods of species of *Aeromonas* spp., some of them standardized as it is developed by the Department of Agriculture of the United States of North America (USDA, 2018) or the Department of Public Health of England (PHE, 2015).

Aeromonas spp*.* are microorganisms that can grow in different common culture media including those differential and selective media used in the isolation of Gram-negative bacteria (Castro et al*.,* 2002; Sanchez et al*.,* 2017). In the isolation and detection in fecal matter, water and / or food, for example, the cefsulodin irgasan novobiocin agar, ampicillin blood agar, ampicillin-dextrin agar and *Aeromonas* agar which contain the selective agents such as bright green and irgasan, can be used. Direct plate count or previous enrichment in liquid medium followed by sowing in selective solid culture medium (Pascual and Calderon, 1999; Sharma et al*.,* 2010; Zepeda, 2015; Sánchez et al*.,* 2017) and subsequent identification by complementary biochemical tests of genus and species (Table 1). Figures 1 and 2 show the methods used for the isolation and identification of *A. hydrophila*; for food samples, a pre-enrichment broth is suggested, an option may be alkaline peptone water to pH 8.7, incubated at 37°C / 18-24 h and then sown on bile-irgasan-bright green agar (Castro et al*.,* 2002; Sharma et al*.,* 2010). While for the analysis of water samples, the use of the membrane filtration technique and subsequent culture in agar dextrin ampicillin agar, without pre-enrichment is effective and allows isolation (Castro et al*.,* 2002). Likewise, for the isolation and rapid identification of *Aeromonas* species, chromogenic culture media have been developed and used with good results (Viera et al*.,* 2016) (Table 2).

For the biochemical identification of isolates, in addition to traditional tests, commercial systems can be used, such as API, or automated systems such as Vitek, GNI-CARD, BBL Crystal, DD Enteric/Nonfermenter (Feng, 2001; Castro et al*.,* 2002; Priyam et al., 2016).

The profile of sensitivity to antibiotics of the bacterial isolates obtained can be made through the disc diffusion method using Mueller-Hinton agar and microbroth dilution method using cation-adjusted Mueller-Hinton Broth as per the Clinical and Laboratory Standards Institute (CLSI) guidelines (Priyam et al., 2016). These tests in recent years have become a must as part of the characterization of pathogenic isolates of clinical, environmental and food origin.

In spite of having a variety of biochemical tests, it is sometimes necessary to have faster and more specific results. In addition, the absence of correlation between the observable morphological and / or phenotypic characteristics of the isolate under study and those corresponding to the strain of the type species can be presented in the obtaining of results, making the phenotypic methods perform the most probable identification and not definitive. To solve these cases genetic methods have been developed in the microbiological analysis, these being considered complementary or alternative procedures to the traditional procedure (Bou et al*.,* 2011; Sanchez et al*.,* 2018).

A variety of genes have been used as molecular targets in taxonomic studies or phylogeny in different genre and different bacterial species including *Aeromonas;* so the identification and genetic differentiation can be made by using markers such as: 16S rRNA, *gyr*B (subunit B DNA gyrase), *rpo*D (factor σ), *rpo*B (subunit β, DNA dependent on RNApolymerase) and DnaJ (head of protein shock 40) (Porteen et al., 2007; Sharma et al*.,* 2010; Zepeda, 2015; Yang et al.,

Table 1. Biochemical tests for the identification of microorganism of the genus *Aeromonas* spp. (Castro et al., 2002; Romero, 2007; PHE, 2015).

Test	Result
Gram stain	Negative Gram
Oxidase	(+)
Catalase	(+)
Growth in nutritious broth with 3 or 6% of NaCl	Difference of *Vibrio* spp., which are positive growth in 6% NaCl, *Aeromonas* spp., only grows at 3% NaCl
Growth on thiosulfate citrate bile sucrose agar (TCBS)	Positive growth yellow colonies.
Sensitivity to pteridine O129	Difference from *Vibrio* spp., where *Aeromonas* spp., are resistant.
Production of acid from inositol	Negative reaction for *Aeromonas* spp., difference of *Plesiomonas* spp., which give a positive reaction
Oxidation-fermentation in Hugh Leifson medium (O/F) with glucose	Difference of *Pseudomonas* spp., glucose fermentation is positive for *Aeromonas* spp.
Voges-Proskauer	Difference of *Vibrio* spp., *Aeromonas* spp., have positive reaction

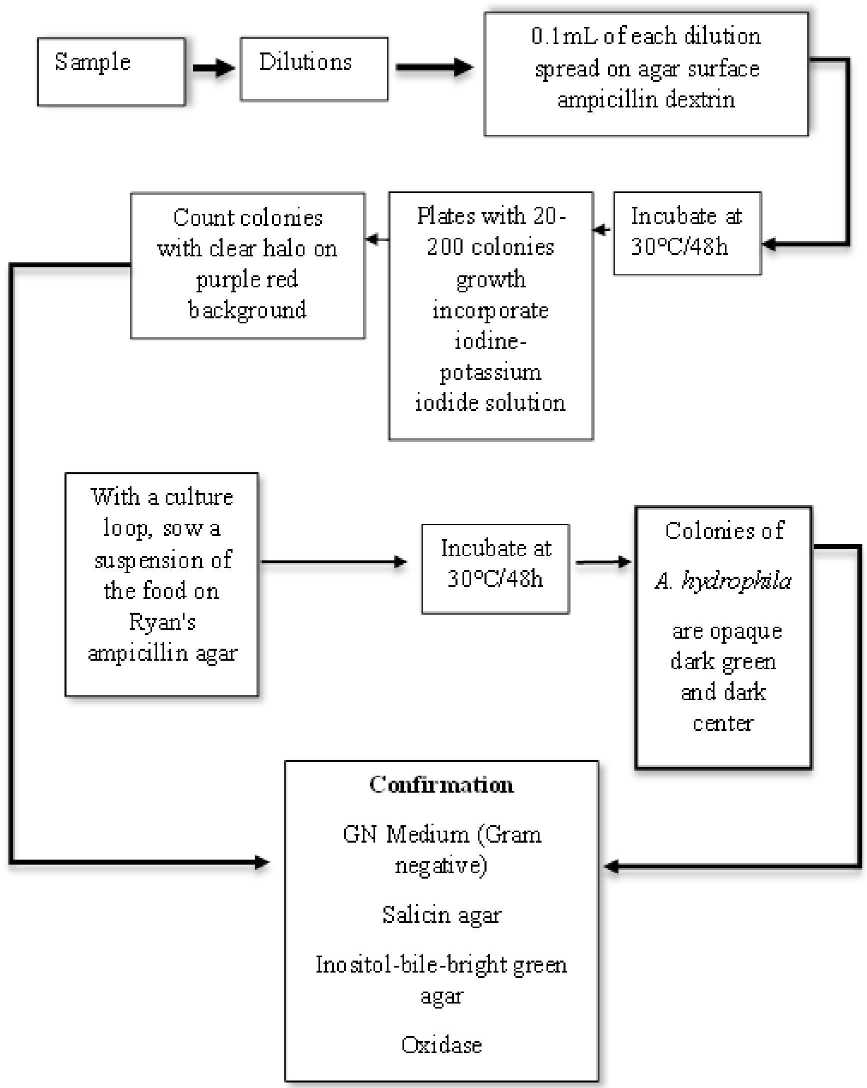

Figure 1. Direct plate count of *A. hydrophila* in food (Pascual and Calderon, 1999).

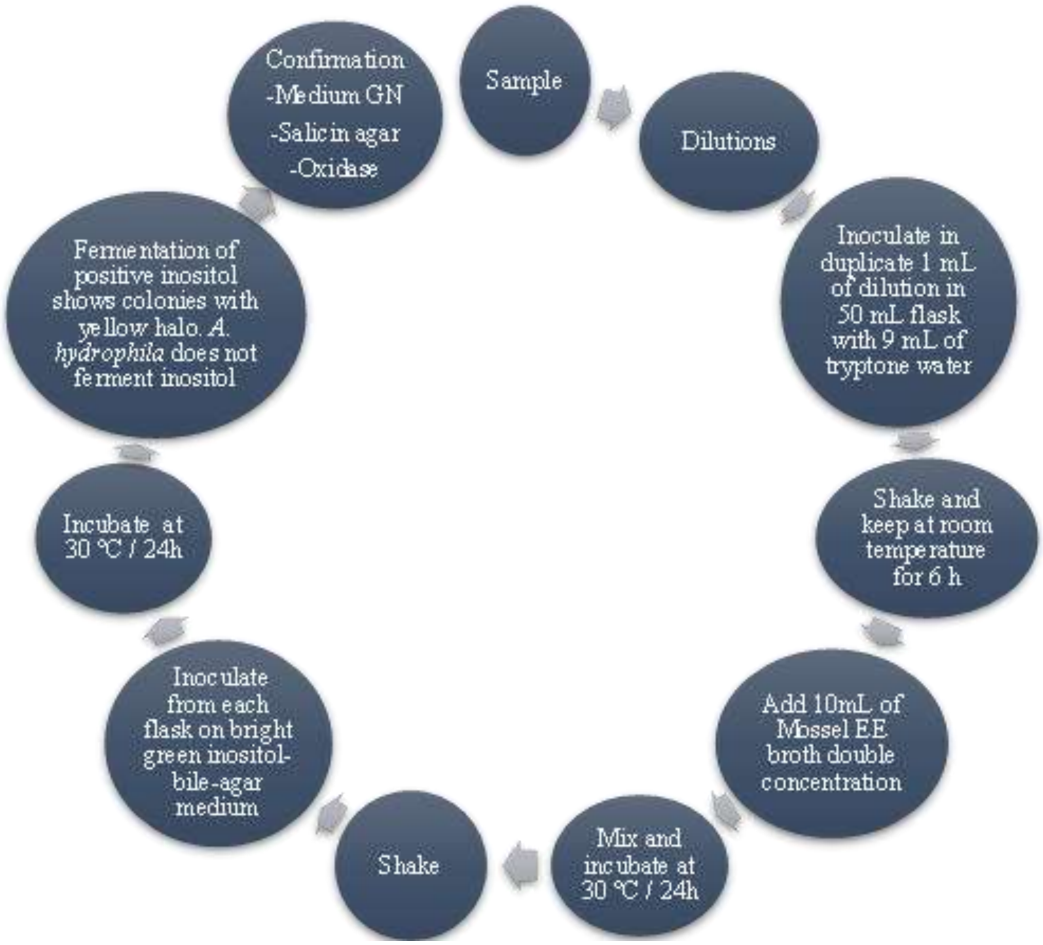

Figure 2. Presence-absence by selective enrichment of *A. hydrophila* in food (Pascual and Calderon, 1999).

2017; Sánchez et al*.,* 2018; Fowoyo and Achimugu, 2019). Also, molecular methods such as: Pulsed Field Gel Electrophoresis (PFGE), Multilocus Sequence Typing (MLST), Multiple-Locus Variable Number Tandem Repeat Analysis (MVLA), Fluorescent Amplified Fragment Length Polymorphism (FAFLP) and Whole Genome Sequencing (WGS) have been used as epidemiological analysis tools for the strain typing (PHE, 2015; Teng et al., 2017). Also, through the study and characterization of the set of proteins expressed by a genome (proteomics) using the Mass Spectrometry by Matrix Assisted by Laser Desorption-Ionization of Flight Time (MALDI-TOF) is able to identify species of *Aeromonas* for clinical diagnosis and a rapid decision making (PHE, 2015; Sánchez et al*.,* 2017; Sanchez et al*.,* 2018).

Likewise, the analysis of *A. hydrophila* based on systems for rapid detection in culture media (<2 h) has been developed, with the use of an electronic device, detecting the changes of patterns of volatile organic compounds generated during growth. Considering the authors that this methodology has potential for the detection of microorganisms; where rapid detection of pathogens is essential for the diagnosis of associated infections and food safety (Fujioka et al., 2013).

Control and prevention of diseases by *Aeromonas* spp.

Agricultural production is considered a key point in the economy worldwide, mainly in developing countries, and food safety is of vital interest for its economic and social development. The safety of food has been established as a fundamental attribute of quality which is generated in primary production and mobilizes the other phases of the food chain such as processing, packaging, transport and preparation of the product (Tafur, 2009).

Food safety is currently one of the main challenges in the agri-food industry; producers and health regulatory

Table 2. Biochemical tests for the identification and selection between species of genus *Aeromonas* (Pascual and Calderon, 1999; Castro et al*.,* 2002; Priyam et al., 2016).

Test	*A. hydrophila*	*A. caviae*	*A. media*	*A. salmonicida*	*A. jandaei*	*A. schubertii*
Oxidase	+	+	+	+	+	+
Lysine decarboxylase	+	-	-	V	+	V
Ornithine decarboxylase	-	-	-	-	-	-
Voges-Proskauer	+	-	-	V	+	V
Arginine hydrolase	+	+	+	V	+	+
Glucose gas	+	-	-	V	+	-
D-mannitol	+	+	+	+	+	-
Sacharose	+	+	+	+	-	+
Indol	+	+	+	+	+	V

V, variable reaction; +, positive reaction ; -, negative reaction.

agencies are in charge of its surveillance, so within this context there is a continuous demand for the strengthening of regulatory frameworks, quality standards, inspection and control (Tafur, 2009; Gutiérrez et al*.,* 2017).

Around the world, regulations have been established on hygiene and sanitary quality of food. In Europe through Regulation (CE) 178/2002 of the European Parliament and the Council of the European Union, the principles and general requirements of food legislation were established, creating the European Food Safety Authority (EFSA) and procedures related to food safety subsequently through Regulation CE 853/2004 established the hygiene standards of foods of animal origin, including fish. Likewise, in Regulation CE 854/2004 the specific rules for the organization of official controls of products of animal origin destined for human consumption were presented. Moreover, Regulation CE 2074/2005 and Regulation CE 2406/96 set out the visual examinations and freshness criteria for fishery products and establish common marketing standards for certain fishery products, respectively.

Specifically, in pathogens such as *Aeromonas* spp., in the Netherlands, health authorities established maximum values for the density of these bacteria in drinking water, being for water in purification plants 20 CFU/100ml and for distribution water 200 CFU/100 ml (Acha and Szyfres, 2001).

In Mexico, the official Mexican standard "NOM-242-SSA1-2009", establishes the sanitary requirements of mandatory observance in the national territory for producers engaged in the capture, extraction, processing, conservation, storage, distribution, transportation, sale or import of fishing products (NORMA Oficial Mexicana, 2009). It is mentioned that the sanitary microbiological specifications for microbiological indicators and pathogens such as fecal coliforms and / or *E. coli* must be as maximum limit of 400 MPN / g, *Salmonella* spp.*,* must be absent in 25 g, as well as *Vibrio cholerae* O:1 and No O:1 absent in 50 g. However, this regulation does not mention specifications regarding *Aeromonas* spp.

As part of the measures for the prevention of diseases by *Aeromonas* spp. and other pathogens specifically in primary production, basic and documentary operating conditions and practices are required, such as good agricultural, livestock and aquaculture practices in the latter, with an emphasis on avoiding overpopulation, adverse environmental factors (increased organic matter and decreased dissolved oxygen), change and monitor water quality, regulation of antibiotic use, adequate climate control and nutrition necessary for food production (Acha and Szyfres, 2001; Tafur, 2009; Balbuena et al*.,* 2011; Fernandes et al*.,* 2018). While in the food industry involving the stages of fish handling and processing, it is required to establish control and prevention procedures such as good manufacturing practices and hazard analysis and critical control point (HACCP) systems (Tafur, 2009; PAHO, 2016; Fernandes et al*.,* 2018). Likewise, different food safety management systems have been developed and implemented at a global level within the scope of food production and commercialization, including fish products such as: Global Food Safety Initiative (GFSI), British Retail Consortium (BRC), International Food Standards (IFS), ISO 22000, Quality Certification Services (QCS), FSSC 22000, Safety Quality Food (SQF), Global Aquaculture Alliance (GAA)/Aquaculture Certification Council (AAC), GlobalGAP or PrimusGFS (Racua, 2018).

Within the processes of control and prevention of diseases involve the continuous participation of government authorities, agro-industry, academia and consumers and should be considered a priority (Kopper et al*.,* 2009; Tafur, 2009; Arispe and Tapia, 2007). The foregoing should involve the adoption of practical measures in the businesses themselves relating to the technical innovations of the processes, models of productive organization, administrative management, and investment for the improvement of the work infrastructure. In addition, health authorities and other related institutions should promote training and propaganda campaigns for the adoption and implementation of actions for the safe manipulation or processing at the commercial or household level of food (Kopper et al*.,* 2009).

In this sense, the prevention through health education of consumers and food handlers is key to preventing the occurrence of diseases by *Aeromonas* spp., so it is promoted to avoid the consumption of raw fish and shellfish, avoid cross contamination between foods raw and cooked, do not use seawater, use potable water, maintain cleanliness of food preparation area, food handler must maintain personal hygiene, complete cooking of vegetables and meats, keep food at safe temperatures, consume milk and pasteurized derivatives (Acha and Szyfres, 2001; WHO, 2007;Priyam et al.,2016; González et al*.,* 2018).

Resistance to antimicrobials by *Aeromonas* spp.

In general, diarrheal processes derived from infection by *Aeromonas* spp*.,* in healthy individuals are self-limiting and are cured in a few days with diet and oral rehydration; of requiring treatment with antibiotics, cephalosporins, quinolones, tetracyclines, chloramphenicol and aminoglycosides can be used in treatment of infections (Castro et al*.,* 2002; Romero, 2007; Priyam et al., 2016). However, the resistance of these microorganisms to several antibiotics such as beta-lactams, including penicillin, ampicillin, carbenicillin, ticarcillin and cephalothin, has been reported for several years (Castro et al*.,* 2002; Priyam et al., 2016).

Antimicrobial resistance is the ability of a microorganism to resist the inhibitory or killing activity of an antimicrobial growth beyond the normal susceptibility of specific bacterial species (Verraes et al*.,* 2013). At present, this phenomenon constitutes a threat and a global issue in human and animal health due to the increase in morbidity and mortality rates due to infections, dilation of the disease and increased health costs (Cabello, 2004; Puig et al*.,* 2011; FAO, 2018). This phenomenon affects food safety, food security, social and economic well-being (Verraes et al*.,* 2013; FAO, 2018).

It has been identified that resistance in bacteria to antimicrobials is generated by the combination of several factors such as the inappropriate use of antimicrobials in humans and animals; where in primary animal production these compounds are used for therapeutic, prophylactic and / or growth promotion purposes (Cabello, 2004; Puig et al., 2011; Verraes et al., 2013; Stratev and Odeyemi, 2016). It has been reported that in livestock activities including aquaculture, the use of antibiotics as prophylactic is probably the main purpose; and it has also been shown that the prophylactic use of antibiotics is unnecessary and dispensable and can be replaced by good hygiene practices, without consequences for animal health and industrial economy (Cabello, 2004).

The transfer of resistance to antibiotics by microorganisms such as bacteria is through genetic material through plasmids, insertion sequences (IS), integrons, transposons (Tn) and bacteriophages through conjugation, transformation or transduction, which can occur in any environment from soil, water, food, human and animal digestive system (Sánchez et al*.,* 2012; Verraes et al*.,* 2013); it generates different mechanisms to resist the action of antibiotics such as the alteration of membrane permeability, ejection pumps, enzymatic inhibition and the modification of the attack target (Tafur et al*.,* 2008; Puig et al*.,* 2011).

Several studies have been conducted indicating the incidence of different pathogenic microorganisms with this characteristic from foods such as *Salmonella* spp*., E. coli, Campylobacter* spp., *Listeria* spp., *S. aureus, Aeromonas* spp., among others, highlighting the potential risk to health in the consumption of these foods generated under inadequate hygiene practices (Puig et al*.,* 2011; Sasidharan et al*.,* 2011; Nagar et al*.,* 2011; Rahimi et al*.,* 2012; Stratev and Odeyemi, 2016). Microbiological studies in foods around the world, in the particular case of fish, have reported the isolation of strains of *Aeromonas* spp., with resistance to different antimicrobials. Castro et al*.* (2003) in an analysis of samples of frozen Tilapia (*Oreochromis niloticus*) sold in markets in Mexico City, reported 82 strains of *Aeromonas* spp*.,* with *A. salmonicida* being the most frequent followed by *A. hydrophila, A. veronii bv. sobria, A. bestiarum, A. encheleia* and *A. caviae.* These strains showed resistance to antibiotics such as ampicillin, penicillin, cephalothin, and clindamycin. The studies concluded that the presence of these microorganisms in food, especially when consumed raw, represents an important risk to public health (Castro et al*.,* 2003). Ashiru et al*.* (2011) carried out the microbiological analysis and antibiotic profile of *Aeromonas* spp*.,* isolated from species in Tilapia and Bagre fish commercialized in markets of Makoko, Nigeri, for which they reported the presence of *A. caviae, A. hydrophila* and *A. sobria* where all of those were resistant to tetracycline, nitrofurantoin and augmentin but susceptible to pefloxacin, ofloxacin and ciprofloxacin.

On the other hand, in a study of 60 samples of live fish (common carp) and frozen fish collected from 15 local markets in the city of Baghdad, focused on the isolation of *A. hydrophila* to determine susceptibility to antibiotics, it was reported that 65% of samples were positive for the isolation of *A. hydrophila*, 76.6% were samples of live fish and 53.3% in frozen fish, and all isolates were 100% resistant to penicillin, ampicillin, cloxacillin and bacitracin. Other antibiotics were oxytetracycline with 56.5%, tetracycline 33.4%, cefoxitin 30.8%, chloramphenicol, kanamycin 28.2%, and finally streptomycin and rifampicin in 23.1 and 15.4% respectively (Alzainy, 2011). On the other hand, Roy et al. (2013) collected samples of the fish *Lepidocephalicthys guntea* and water from the Lotchka River of Darjeeling District, West Bengal, India, with the purpose of isolating strains of *Aeromonas spp.* and analyzing their resistance to antimicrobials, reporting the isolation of 49 strains of *Aeromonas* spp., which showed high resistance to ampicillin, penicillin, cephalothin and erythromycin and minimal resistance to ciprofloxacin and tetracycline.

In all the previous studies, the authors agree on the potential risk to public health posed by the handling and consumption of these foods (mainly raw), which are contaminated with antibiotic-resistant pathogens and are marketed in different regions of the world. This involves the need to implement research actions and regular surveillance of the phenomenon of antimicrobial resistance in fish and aquatic production environments intended for human consumption by various social sectors such as government, nongovernmental and academic organizations.

CONCLUSION

Foodborne diseases are considered as serious and significant public health problem at a global level due to their incidence and mortality, mainly in children, together with the fact that different causative agents of biological origin, mainly bacteria, have shown resistance to different antibiotics used in the treatment of diseases.

The food safety is considered a preponderant factor in the production, processing, conservation, distribution and handling of food, which is put at risk due to the presence of various causative agents, which may be of physical, chemical or biological origin, the latter being the most frequently related to disease outbreaks.

Fish and products around the world are considered a source of food of good nutritional quality and economic development for human beings; however, due to its composition, fish is highly susceptible to deterioration and contamination along the food chain, making it a potential vehicle for disease transmission.

The biological agents that cause diseases through foods that are mostly related are bacteria; species of the genus *Aeromonas* spp., whose natural habitat is an aquatic environment, is considered important in terms of human health, animal health and food due to the different diseases generated, sources and forms of transmission.

In order to reduce, control and prevent the incidence of diseases through food by *Aeromonas* spp., recommendations and practices have been developed and proposed around the world by different international organizations in the field of health and food that involve the control and prevention of diseases along the food chain (from the farm to the consumer's table); they range from good aquaculture practices, good hygiene practices, traditional and molecular microbiological analysis methods in the laboratory to foods with a risk of contamination as well as specifications or microbiological limits of these microorganisms in food or water for human use or consumption. Likewise, actions focused on the handling and hygienic preparation of food at the household level have been developed and promoted in such a way that the final manipulators and consumers, along with governmental entities, academia and food industry, contribute to the protection of public health with a supply of nutritious and safe foods for people.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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