Contamination, Spoilage and Safety of Fisheries and Aquaculture Products by *Pseudomonas* spp

Introduction

Fish is a food widely produced, marketed, and consumed around the world, whose demand is growing significantly with the increase in the world population due to its favorable taste, efficient feed conversion, high nutritional properties as well as its commercial value. The microbial diversity in nature is extensive, with *Pseudomonas* spp. being one of the most studied due to their widespread occurrence in water, soil, or as part of the microbiota of foods such as fish. Different species, including *P. aeruginosa*, are of interest in human health, animal health, and food industry due to its relationship with food spoilage and safety. In this context, through a bibliographic search in various databases such as Scopus, Web of Science, Google Scholar, and Scielo among others, the focus of this document is to provide a general informative outline profile about food diseases, particularly fish, incorporating aspects related to biological agents such as *Pseudomonas* spp. and its relationship with the contamination and spoilage of these foods of aquatic origin, effects in human and animal health; in addition to pointing out actions to control and prevent the spoilage and health of fishery and aquaculture products in order to promote the quality and safety of food intended for human consumption and protect public health.

Keywords: Foodborne Diseases, *Pseudomonas*, Aquaculture Products, Fish, Biological Hazards, Fish Safety, Fish Spoilage

Abstract: Food safety is the guarantee that it will not cause harm to the health of those who consume it and is considered an important element of its quality. Fish is a nutritious and basic food in the human diet, and its supply occurs through fishing and aquaculture activities. Fish is susceptible to contamination and microbiological spoilage through the food chain, leading to the detriment of its quality and diseases due to consumption, the latter being considered an important public health problem worldwide. The microbial diversity in nature is extensive, bacteria of the genus *Pseudomonas* spp. are located in water, soil, or as part of the microbiota of foods such as fish, where different species, including *P. aeruginosa*, are of interest in human health, animal health, and food industry due to its relationship with food spoilage and safety. In this context, through a bibliographic search in various databases such as Scopus, Web of Science, Google Scholar, and Scielo among others. The focus of this document is to provide a general informative outline profile about food diseases, particularly fish, incorporating aspects related to biological agents such as *Pseudomonas* spp. and its relationship with the contamination and spoilage of these foods of aquatic origin, effects in human and animal health; in addition to pointing out actions to control and prevent the spoilage and health of fishery and aquaculture products in order to promote the quality and safety of food intended for human consumption and protect public health.
Foodborne diseases are considered a public health problem worldwide due to their incidence and negative effects on the health and economy of the population (Olea et al., 2012; Torrens et al., 2015; Finger et al., 2019; Fernández et al., 2021). Food safety is an essential intrinsic property of foods intended for human consumption, indicating that when ingested they will not cause harm to the consumer's health (Prieto et al., 2008; De la Fuente Salcido and Corona, 2010; Figueroa, 2018).

Food is involved in the transmission of diseases because it can be contaminated by physical, chemical, or biological hazards (bacteria, fungi, viruses, and parasites) through elements such as air, water, soil, animals, utensils, and the environment, raw materials, human manipulation during primary production, transportation, processing, storage, manufacturing and distribution (De Plata, 2003; Rosas, 2007; PAHO, 2023). Raw materials or food by itself can present pathogenic and/or saprophytic microorganisms responsible for food spoilage and consumer illnesses; in this case, the natural microbiota of a variety of foods of animal origin including fish and products may be made up of microorganisms such as *Pseudomonas* spp., *Moraxella* spp., *Flavobacterium* spp., *Bacillus* spp., *Micrococcus* spp., *Enterobacter* spp., *Salmonella* spp., *Acinetobacter* spp., *Clostridium botulinum*, *Clostridium perfringens*, *Yersinia* spp., *Vibrio* spp., *Escherichia coli*, *Staphylococcus* spp., viruses and parasites (De Plata, 2003). Thus, fish intended for human consumption are considered a health risk food as they are susceptible to microbial contamination and deterioration, frequently being bacteria that shorten their useful life and contribute to the waste of these foods (Huss, 1998; Periago et al., 2016; Kačániová et al., 2017) as well as various outbreaks of foodborne diseases globally (Alerte et al., 2012; Olea et al., 2012; Espinosa et al., 2014; Finger et al., 2019; Friesema et al., 2022).

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**Foodborne Diseases**

Foodborne Diseases (FD) are considered a public health problem worldwide due to their morbidity and mortality, negative effects on the economy and productivity of countries, costs associated with health services, development, application, and monitoring of food safety policies (Olea et al., 2012; Torrens et al., 2015; Finger et al., 2019; Fernández et al., 2021).

The World Health Organization (WHO) indicates that every year 600 million people around the world get sick after consuming contaminated food of which 420,000 are deaths (Fernández et al., 2021). Globalization that involves exports, imports, and tourism, among others, has accelerated the exchange processes of fresh and processed food products, as well as changes in food processing and preservation methods, demographic changes, changes in society's eating habits, meals outside the home, sale of prepared foods and fast foods, population aging (Cruz, 2010; Olea et al., 2012; Sotelo et al., 2019), increased resistance of causative agents (microorganisms) to antimicrobial compounds and acquisition of virulence factors are some of the multiple factors that have contributed to the appearance of diseases due to food consumption (Cruz, 2010; Marin Mendez et al., 2020).

FD are considered syndromes caused by the ingestion of food and/or water that contain etiological agents in quantities that affect the health of the consumer (De Plata, 2003; Soto Varela et al., 2016; Finger et al., 2019; Fernández et al., 2021). These diseases present various gastrointestinal symptoms such as nausea, vomiting, diarrhea, abdominal pain, or fever; where in serious situations there may be complications, such as meningitis, sepsis, abortions, Reiter syndrome, Guillan Barré syndrome, or death (Soto Varela et al., 2016; Finger et al., 2019; Fernández et al., 2021). The most susceptible populations are the elderly, children, immunocompromised, and people in high levels of poverty and insanitary conditions (De Plata, 2003; Alerta et al., 2012; Torrens et al., 2015).

More than 250 causal agents of foodborne diseases have been described due to the variety of contaminants, which can be of physical, chemical, or biological nature (Fernández et al., 2021). Chemical contaminants can be different compounds such as antimicrobials, growth promoters, food additives, disinfectants, heavy metals, and pesticides. Physical contaminants can be pieces of glass, metal, and wood and finally, biological contaminants such as bacteria, parasites, viruses, and prions, where most cases of foodborne illness are associated with the latter contaminants to a variety of representatives bacterial (Olea et al., 2012; Torrens et al., 2015; Soto Varela et al., 2016; Fernández et al., 2021), which *Salmonella* spp., *Escherichia coli*, *Listeria monocytogenes*, *Vibrio* spp., *Aeromonas* spp., *Campylobacter* spp., *B. cereus* are frequently associated with outbreaks (Soto Varela et al., 2016; Friesema et al., 2022). Biological contaminants include zoonotic agents such as parasites, bacteria and viruses that can induce infections, which are transmitted directly or indirectly through consumption of contaminated food or direct contact with animals that may be used for food production.
FD can be classified into (1) Food infections originate when the pathogen in the food reaches and reproduces in the consumer. They have two routes: (a) Invasive infections, in which the pathogen colonizes tissues and organs of the host, and (b) Toxin infections, produced by non-invasive bacteria, which colonize and multiply in the host’s gastrointestinal system, producing and excreting toxins. (2) Food poisoning, generated by toxins synthesized during microbial growth in food (Torrens et al., 2015; Zúñiga Carrasco et al., 2017).

Fish

The denomination of fish is given to all those foods extracted from continental or oceanic waters that are intended for human or animal consumption, covering all those portions and products (De Paiva Soares and Gonçalves, 2012; Cortés-Sánchez et al., 2021). Fish is considered a highly nutritious food and a fundamental part of the human diet, as it is a source of proteins of high biological value and digestibility, polyunsaturated lipids, vitamins, and minerals (Huss, 1998; De Paiva Soares and Gonçalves, 2012; Ali et al., 2022).

It is estimated that fish constitutes approximately 17% of the animal protein intake of the world population (Dos Santos Silva and e Barros, 2020), providing important nutrients for physical and cognitive development, from fetal growth to early childhood, childhood and maintaining good nutrition and health during adolescence and adulthood (FAO, 2022) and where the trend of its consumption is increasing, related to the need for a greater number of quality, healthy and nutritious foods with actions in dietary regulation, with emphasis on cardiovascular diseases and obesity (Dos Santos Silva and e Barros, 2020).

The average composition of fish is 16-21% protein, 0.2-25% lipid, 66-81% water, 1.2-1.5% ash, and carbohydrates <0.5% (Huss, 1998) and factors such as the species, as well as age, stage of development, environment, season of the year, feeding, capture or cultivation methods and handling influence the variability of the composition of the fish (Huss, 1998).

Human beings can have access to food of aquatic origin through fishing and aquaculture activities according to estimates of the Food and Agriculture Organization (FAO) in 2022 at a global level, the production of 177.8 million tons reached (live weight equivalent) with 88.5% destined for human consumption with a per capita consumption of 20.2 kg (FAO, 2022) and where the marketing presentation of the fish is generally as live, fresh, frozen, refrigerated, smoked, dried, or salted fish (Castillo-Jiménez et al., 2017).

Table 1: Hazards related to diseases derived from the consumption of fish and products (Díaz Lorenzo and Cardona Galvez, 2008; Novoslavskij et al., 2016; Zúñiga Carrasco et al., 2017; Kačániová et al., 2019; Lehel et al., 2021; Cortés-Sánchez et al., 2021; 2022; Cortés-Sánchez, 2022)

<table>
<thead>
<tr>
<th>Hazard</th>
<th>Agent</th>
<th>Example</th>
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<tbody>
<tr>
<td></td>
<td>Virus</td>
<td>Rotavirus, hepatitis A, hepatitis E, adenovirus, norovirus, astrovirus, enterovirus</td>
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<tr>
<td>Chemical</td>
<td>Fungi</td>
<td>Copper, lead, cadmium, mercury, Polybrominated diphenyl ethers, dioxins, pesticides, microplastics, antibiotics, hormones, polycyclic aromatic hydrocarbons and polychlorinated biphenyls</td>
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<td>Heavy metals</td>
<td>Tetrodotoxin, ciguatoxin, scaritoxin, maitotoxin, palytoxin, okadaic acid, gepolitoxin, mycotoxins</td>
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<td>Organic compounds</td>
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<td>Natural toxin</td>
<td>Biogenic amines</td>
<td>Bone, thorns, crystals, porcelain, pieces of wood and metal, watches, rings jewelry, packaging, or packaging materials</td>
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<td>Biotoxins</td>
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<td>Physical</td>
<td>Object present in the food and that should not be found in it, being capable of causing harm or illness to the consumer</td>
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Foodborne Diseases: Fish and Products

The consumption of fish and products is considered high risk due to their high protein content and water activity, which are intrinsic characteristics and a determining factor in the contamination and growth of microorganisms (Fernández et al., 2021). Fish and its products have been associated with numerous cases of diseases around the world derived from its consumption, whose causal agents are mainly bacteria, viruses, parasites, fungi, biotoxins, and biogenic amines, among others (Alerte et al., 2012; Olea et al., 2012; Espinosa et al., 2014; Finger et al., 2019; Friesema et al., 2022). The risk of human infections caused by bacterial pathogens present in fish is high and its severity depends on factors such as seasonality, the immunological condition of the individual, contact with fish or the environment, and the conditions of handling and fish preservation (Kačániová et al., 2019). The factors that have commonly been associated with outbreaks of fishborne illnesses are cross-contamination, inadequate times or temperatures for processing, contaminated raw materials, use of non-potable water, contaminated container material, work equipment, and packaging, inadequate hygiene conditions and practices in handling (Alerte et al., 2012; Espinosa et al., 2014; Zúñiga Carrasco et al., 2017; Friesema et al., 2022). Table 1 describes the different contaminating hazards in fish and products associated with diseases derived from their consumption and where bacteria of the genus Pseudomonas spp. are found.

Fish Microbiology

In fish and products (whole, gutted, or fillet), the microbiological load depends on several factors such as the type of fish, season, geographical area, fish feeding, initial load in the aquatic environment, capture method, handling and storage conditions (Periago et al., 2016; Novoslavskij et al., 2016; Kačániová et al., 2017; Naef et al., 2023).

The microorganisms inhabit in variable proportions all the external surfaces of life and recently captured fish such as skin, gills, and intestines with a normal range of $10^2$-$10^7$ CFU/cm$^2$ on the skin surface while in gills and intestines, they contain between $10^3$-$10^6$ CFU/g. In life, microorganisms do not invade the organs and muscle tissue of healthy fish; however, when they are caught and die, microorganisms penetrate and are involved in the spoilage of fish (Huss, 1998; Novoslavskij et al., 2016).

In a qualitative and quantitative way, the microbiota of fish is a function of the temperature and salinity of the water where they live (Huss, 1998; Romero-Jarero and Negrete-Redondo, 2011). In warm waters, a greater number of Gram-positive mesophiles such as Clostridium, Bacillus, Micrococcus and Coryneformes can be isolated; meanwhile, in cold or temperate waters, the microbiota is dominated by Gram-negative psychrophilic bacteria of the genera Pseudomonas, Moraxella, Acinetobacter, Shewanella, Flavobacterium, members of the Vibrionaceae (Vibrio and Photobacterium) and Aeromonas spp., Gram positives such as Bacillus, Micrococcus, Clostridium, Lactobacillus, Coryneformes, as well as molds and yeasts that are common microorganisms of the fish microbiota (Huss, 1998; Pascual and Calderón, 2000). Pseudomonas species are frequently associated with fish, being isolated from the skin, gills, and intestines and may comprise a predominant part of the psychrotrophic microbiota of fish (Pascual and Calderón, 2000; Ardura et al., 2013; Novoslavskij et al., 2016; Kačániová et al., 2017). It should be noted that Aeromonas spp., are typical bacteria of freshwater fish, while Vibrio, Photobacterium, and Shewanella of fish from marine waters (Huss, 1998).

In polluted waters, the microbiota of fish will depend on what exists in the aquatic environment where they live: Large numbers such as $10^7$ CFU/cm$^2$ on surfaces, correspond to fish from highly polluted waters, mostly Enterobacteriaceae such as Serratia spp., Proteus spp., Escherichia coli and Salmonella spp., (Huss, 1998; Romero-Jarero and Negrete-Redondo, 2011).

In aspects focused on the microbiological quality of fish and products, bacteria of interest to human health have been divided into two groups: (I) Autochthonous, which are common and are located in aquatic environments around the world, with water temperature being a selective factor. Among the bacteria present are Vibrio spp., Aeromonas spp., Plesiomonas spp., (tropical climates), C. botulinum and Listeria (cold climates), and (II) non-native groups such as Salmonella spp., Shigella spp., E. coli and S. aureus that are the result of contamination in processing, handling, conservation, transportation or fecal contamination of the aquatic environment (Huss, 1997; Pascual and Calderón, 2000; Romero-Jarero and Negrete-Redondo, 2011).

Pseudomonas

Bacteria of the genus Pseudomonas belong to the Pseudomonadaceae family and correspond to straight or curved bacilli, 0.5-1 µm wide by 1.5-4 µm long, Gram-negative, chemoheterotrophic, aerobic, non-fermenting glucose metabolism (metabolize glucose via Entner-Doudoroff as the main route), are oxidase and catalase positive, with polar flagella and do not form spores (Madigan et al., 2004; García and Iannacone, 2014; Estupiñan-Torres et al., 2017; Pinzón-Junca, 2019).

The genus is broad, comprising 5 groups according to their DNA and rRNA homology, as well as culture characteristics: Group I, which is subdivided into fluorescent ones that produce pigments, where P. aeruginosa, P. fluorescens, and P. putida are included; a non-fluorescent group which includes P. stutzeri, P.
mendocina, P. alcaligenes and P. pseudoalcaligenes; Group II: Burkholderia species pseudomallei, mallei, cepacia; Group III: Comamonas and Acidovorax; Group IV: P. diminuta V. vesicularis and Group V: Stenotrophomonas (Romero Cabello, 2005).

Pseudomonas are ubiquitous microorganisms in nature capable of using a wide variety of compounds such as carbon, nitrogen, and energy sources to adapt and grow in various natural environments, including waters that contain only minimal amounts of nutrients (Franzetti and Scarpellini, 2007; García and Iannacoone, 2014; Estupiñán-Torres et al., 2017; Kačániová et al., 2017). The genus Pseudomonas includes numerous species with importance for human and animal health, where P. aeruginosa, P. fluorescens and P. putida stand out, with a mesophilic character; however, some members of the genus are psychrophilic (Lopardo, 2016; Kačániová et al., 2017; Pinzón-Junca, 2019). Pseudomonas are sensitive to heat and dehydration, with poor or no growth above 43°C (Frazier and Westhoff, 1978), but they are inherently resistant to various antimicrobial agents, becoming a serious public health problem (García and Iannacoone, 2014; Estupiñán-Torres et al., 2017; Lopardo, 2016; Kačániová et al., 2017).

For the food industry, the interest of these microorganisms is in aspects of quality, safety and consumer health (Jiménez et al., 2004; Maia et al., 2009; Hernández Urzúa, 2016), being generally involved in product deterioration processes (Camacho et al., 2006; Franzetti and Scarpellini, 2007; González et al., 2007; Escalante et al., 2008; Zhang et al., 2012) due to their ability to metabolize different sources of nitrogen, carbon and energy, in addition to carbohydrates producing unpleasant substances associated with odor and taste, changes in appearance, texture, generation of pigments and their growth in aerobic and refrigerated conditions can generate oxidation compounds and/or mucilage in food (Frazier and Westhoff, 1978; Camacho et al., 2006; González et al., 2007; Escalante et al., 2008).

The genus Pseudomonas is found together with other Gram-negative (Alcaligenes, Moraxella, Flavobacterium, Aeromonas, Escherichia, Serratia, Enterobacter, Yersinia, Proteus) and Gram-positive (Bacillus, Lactobacillus, Streptococcus, Staphylococcus, and Corynebacterium) microorganisms within the group of psychrophots, capable of growing at temperatures of 7°C for 7-10 days and having optimal growth at temperatures above 20°C. This microbial group is considered an indicator of the hygienic quality of food, generally among those of a protein nature such as fish, whose presence can indicate sources of contamination, inadequate aerobic storage conditions, as well as being an indicator of shelf life since the increase of psychrotrophic during refrigerated storage, even at temperatures close to 0°C, accelerate deterioration and reduce food’s useful life (Pascual and Calderón, 2000; Hernández Urzúa, 2016).

**Pseudomonas Aeruginosa**

*P. aeruginosa* is an aerobic, motile bacillus, that does not decarboxylate lysine, and does not produce hydrogen sulfide, but it does generate catalase and oxidase, as well as ammonia from arginine; it reduces nitrates to nitrites, does not ferment lactose and it can use citrate as a carbon source (Farias, 2015; García and Iannacoone, 2014; Pachori et al., 2019). It grows at a temperature range of 4-42°C (Farias, 2015) and a pH of 5.6-8 (De Plata, 2003). A metabolic characteristic is that it produces two pigments such as pyocin, which can color blue-green and pyoverdine (fluorescence), a yellow-green pigment that fluoresces under ultraviolet light, a property that can be used in identification by culture (García and Iannacoone, 2014; Kačániová et al., 2017; Pachori et al., 2019; Pinzón-Junca, 2019).

**Pseudomonas aeruginosa** is considered an opportunistic pathogen associated with infections in plants, animals, and humans, the latter in the hospital and community environment, affecting the urinary tract, gastrointestinal system, cardiovascular, respiratory, and bone systems; it can generate diarrheal episodes, septicemia with a high mortality rate, being the most susceptible patients newborns, the elderly and generally those with immunosuppression, chronic infections, metabolic disorders, cancer, burns, or treatment with immunosuppressants (Esnard et al., 2004; Romero Cabello, 2005; Maia et al., 2009; García and Iannacoone, 2014; Pachori et al., 2019).

Infections caused by *P. aeruginosa* can be toxigenic as well as invasive, involving various virulence factors and taking place in three stages: (1) Colonization and survival: Mediated by structural components (pilis, fimbrae, flagella, lipopolysaccharide, capsule), secretion of proteolytic enzymes, alginate and biofilm generation. (2) Invasion: Ability to invade tissues that depends on the host defense process that involves the generation of biofilm that protects the bacterium from opsonization, complement, and phagocytosis, the activity of synthesized extracellular proteases such as elastase destroys collagen, IgG, IgA, complement and lyases fibronectin, the alkaline protease that interferes with the generation of fibrin, the cytotoxic that affects neutrophil activity, phospholipase and lecinthin’s that degrade lipids and lecithin. (3) Systemic disseminated disease derived from capsular components that generate resistance of the pathogen to phagocytosis inhibiting the immune response by means of secreted enzymes, dissemination is established, being capable of producing disease in different organs and systems; two pigments are also produced (pyocyanin and pyoverdine) that can produce toxic forms of oxygen.
stimulate the production of cytokines, regulate the formation of biofilms and secretion of extracellular toxins (exoenzyme S and Exotoxin A), which necrotize colonized tissue and inhibit the activity of phagocytic cells favoring dissemination (Esnard et al., 2004; Rojas, 2005; Navia et al., 2010; Farias, 2015; Murray, 2018).

*P. aeruginosa* is cosmopolitan, being found in soil, food, and water, and is even considered part of the microbiota of plants, animals, and humans (Maia et al., 2009; Pachori et al., 2019). The presence of *Pseudomonas* spp., in water and food can pose a risk to the health of the population (Maia et al., 2009; García and Iannacone, 2014). Transmission of this pathogen occurs mainly through contact of injured skin and mucous membranes with contaminated water or objects, inhalation of bioaerosols of contaminated water or fluids, and contact or ingestion of contaminated water (INSSST, 2022). The incidence of *Pseudomonas aeruginosa* infections in humans and their resistance to antimicrobials are considered an important health problem, especially in the hospital environment (Maia et al., 2009; García and Iannacone, 2014; Pachori et al., 2019).

In fish, *Pseudomonas aeruginosa* has been isolated presenting resistance to various antimicrobials, such a finding compromises not only the quality of the food but also becomes a risk to consumer health; therefore, the phenomenon of drug resistance requires the analysis and surveillance of this microorganism in fish and products, the adoption of hygiene measures in food production and the importance of the appropriate use of antimicrobials in human and animal health (Maia et al., 2009; Romero García et al., 2015; Boss et al., 2016; Bernie et al., 2017a-b; Gaigher, 2022). *P. aeruginosa* is also resistant to different adverse conditions, being able to multiply under refrigeration, with high salt concentrations, properties that contribute to its presence in various environments (Maia et al., 2009). In the food industry, this is of great importance since its presence at high levels reduces the shelf life of products stored at low temperatures, generating superficial mucus, unpleasant odors, and flavors (Maia et al., 2009; Zhang et al., 2021). Additionally, it can generate biofilms on equipment, materials, and on any type of surface (plastic, glass, wood, metal, and on food) affecting food safety, hindering production processes by generating energy loss, flow reduction (pipes), and heat transfer, blockage of membrane pores, among others (Navia et al., 2010; Meliani and Bensoltane, 2015; Gutiérrez et al., 2017).

**Animal Health: Fish and Pseudomonas**

*Pseudomonas* are found naturally in aquatic, terrestrial, and atmospheric environments, as well as being part of the intestinal microbiota of healthy fish (Balbuena and Rios, 2011; Puello-Caballero et al., 2018; Alcántara-Jauregui et al., 2022). However, *Pseudomonas* can cause infection in fish (Ardura et al., 2013; Kačániová et al., 2017) known as *Pseudomonadiosis* which is septicemic in fish that live in fresh and brackish waters (Balbuena and Rios, 2011; Puello-Caballero et al., 2018; Alcántara-Jauregui et al., 2022).

In aquaculture activities, processes such as the management of individuals, batch classification, application of cleaning procedures, use of chemical products, inadequate culture density and water quality, poor nutrition, transport between facilities, or alteration of environmental conditions can generate a state of stress in fish, favoring infection and disease outbreaks by various causal agents, including *Pseudomonas* (Balbuena and Rios, 2011; Bautista Covarrubias et al., 2011; Ardura et al., 2013; Cepa et al., 2019; Duman et al., 2021). Within the genus, *P. aeruginosa*, *Pseudomonas anguilliseptica*, *P. baetica*, *P. chlororaphis*, *P. fluorescens*, *P. koreensis*, *P. luteola*, *P. plecoglossicida*, *P. pseudoalcaligenes* and *P. putida* have been reported to cause disease in several species of fish (Austin et al., 2007; Duman et al., 2021; Alcántara-Jauregui et al., 2022). Conducted studies have reported almost a 100% mortality from infection with *Pseudomonas* spp., fish such as sea bream, sea bass, Ayu, and rainbow trout in aquaculture conditions (Duman et al., 2021).

The disease manifests with an acute or chronic course, with hemorrhagic lesions on the skin and internal tissues, darkening of the skin, hemorrhages, ulcerations (which extend to the fins and tail), desquamation, abdominal ascites and exophthalmia (Balbuena and Rios, 2011; Alcántara-Jauregui et al., 2022). In case of the appearance of disease, it is recommended to reduce the density of the fish in the culture ponds and to carry out the necessary actions to maintain water quality at appropriate levels (Balbuena and Rios, 2011). There are foods medicated with broad-spectrum antibiotics such as terramycin or oxytetracycline for the treatment of the disease; however, it is important to consider that prevention is better than cure, through the implementation of good aquaculture practices (Balbuena and Rios, 2011).

**Spoilage of Fish by Pseudomonas spp., in Post-Harvest or Post-Capture Phases**

The spoilage of food can have physical, chemical, and/or biological causes, such as physical injuries (due to abrasion, pressure, freezing, drying), the enzymatic activity of the food itself, and chemical reactions specific to its chemical composition, growth of microorganisms, the action of insects, rodents, birds and other animals, where frequently these causes are not presented separately (Gómez, 2010).

Spoilage is characterized by a series of sensory (unpleasant taste or odor), physical, and chemical changes in food that make it unacceptable to the consumer (Gram et al., 2002). The result of microbial growth and production of various unwanted metabolites in the product is considered a serious economic problem
(it has been estimated that 25% of all food produced worldwide is lost after harvest or slaughter) and is also the main cause of deterioration, manifesting itself in signs such as visible growth, texture changes (polymer degradation), gas generation, sliminess, colors, odors, and unpleasant flavors. These signs will depend on the type of food, microbiological load, and environmental conditions (Gómez, 2010; Ali et al., 2023).

It should be noted that many raw materials used in food processing contain their own natural microbiota. Additionally, during various phases of processing and distribution, food can be contaminated with a wide variety of microorganisms, where their chemical composition and conditions can favor the growth and predominance of the microbial population. In general, the higher the initial microbial load of the food, the shorter its shelf life, and there will be a higher risk to the health of consumers (Gómez, 2010).

Fish is a food with an almost neutral pH, high water activity, and a source of proteins, lipids, vitamins, and minerals (Gómez, 2010; Ghaly et al., 2010; Ali et al., 2022). These qualities also make fish very susceptible to contamination and spoilage, mainly due to autolysis, lipid oxidation, and microbiological activity (Ghaly et al., 2010; Mei et al., 2019).

Growth and microbiological activity are considered the main causes of fish quality spoilage, causing up to 25-30% loss of these products, where intrinsic properties (high level of free amino acids, pH, water content, and non-protein nitrogenous compounds such as trimethylamine oxide) of fish promote bacterial growth over a wide temperature range (Mei et al., 2019). Bacteria on the skin and gastrointestinal system of live fish do not invade the muscle, they are sterile due to their natural defenses. Therefore, when the fish dies, bacteria proliferate freely on the surface and penetrate the muscle tissues, favoring their spoilage (Huss, 1998; Romero-Jarero and Negrete-Redondo, 2011).

The type and rate of spoilage of fish vary depending on various factors such as (1) The type and size of fish where flat fish, high in lipids and trimethylamine oxide are more susceptible than cylindrical, fatty fish spoil faster than lean; (2) If it is native or cultivated, in addition to the capture conditions where the fish are exhausted due to shaking, lack of oxygen and improper handling, as well as those with a full digestive tract that spoil more quickly; (3) Type and microbiological load, where the greater the load the deterioration occurs at a greater speed and (4) Storage temperature where the higher the temperature, the shorter its shelf life (Frazier and Westhoff, 1978; Naef et al., 2023).

Microbiological activity in fish occurs due to a wide variety of pathogenic and/or saprophytic bacteria that affect its sanitary quality and deterioration. These bacteria can be present naturally or through contamination when introduced in the different stages of processing and conservation (Tirado et al., 2005; Boari et al., 2008; Romero-Jarero and Negrete-Redondo, 2011; Periago et al., 2016; Mei et al., 2019). During fish processing, the most common contamination comes from direct handling, from the transfer of intestinal bacteria from gills, or the skin, to the surface of products (fillets), the transfer to fish of bacteria from the environment (contaminated surfaces, knives, machines, etc.) as well as ship loading and unloading operations, public auctions and sale (sellers, buyers and spectators) that contribute to the microbiological contamination of fish (Gómez, 2010). The handling and processing activities to which fish is subjected to obtain various products (fillets) can increase the risk of contamination with deficient hygienic practices, favoring the presence and multiplication of pathogenic or spoilage microorganisms responsible for the formation of nitrogenous compounds as ammonia and trimethylamine, volatile sulfides, ketones, aldehydes, esters, hypoxanthine compounds related to flavors and odors typical of altered fish, (some of them considered as indicators of the state of freshness/deterioration) (Huss, 1998; Gram et al., 2002; Periago et al., 2016; Parlapani et al., 2023) directly affecting the sensory quality, safety and useful life of fishery products (Tirado et al., 2005; Boari et al., 2008; Gómez, 2010; Romero-Jarero and Negrete-Redondo, 2011; Periago et al., 2016).

There is a wide variation in the bacterial microbiota present in fish and associated with deterioration, which is a function of factors such as geography, type of fish, handling conditions, conservation (salted, cured, among others), storage (temperature, atmosphere), intrinsic properties (pH, aW, chemical composition) (Huss, 1998; Gram et al., 2002; Boari et al., 2008; Romero-Jarero and Negrete-Redondo, 2011; Periago et al., 2016) and production system since, for example, for aquaculture systems some genera that have been commonly associated with the fish microbiota are Aeromonas spp., Micrococcus spp., Staphylococcus spp., Bacillus spp., Pseudomonas spp., Plesiomonas spp., Listeria spp., Moraxellaceae and Enterobacteriaceae (Boari et al., 2008; Dos Santos Silva and e Barros, 2020).

While various species of bacteria such as Pseudomonas spp., S. putrefaciens, Photobacterium phosphoreum, Aeromonas spp., Vibrio sp., and Enterobacteria they are indicated as part of the altering microbiota of fresh and processed fish (Huss, 1998; Periago et al., 2016; Kačániová et al., 2017) where their total number is related to the storage time and shelf life of the fish (Huss, 1998; Gram et al., 2002). Pseudomonas spp. and other gram-negative psychrotrophic organisms (Alteromonas, Shewanella, and Aeromonas) predominate in protein foods such as fish stored in aerobic conditions at low temperatures or ice (Gram et al., 2002; Franzetti and Scarpellini, 2007; Gómez, 2010; Parlapani et al., 2023). Pseudomonas is considered part of the specific spoilage organisms in fish stored aerobically at low and ambient...
temperatures, being an important producer of the volatile compounds responsible for bad smell and taste, such as aldehydes, ketones, esters, and sulfides, through protein metabolism (Huss, 1998; Gram et al., 2002; Franzetti and Scarpellini, 2007; Parlapani et al., 2015; 2023; Naef et al., 2023). Additionally, if intrinsic parameters in the food such as high pH are combined, organisms such as S. putrefactions can develop at the same time, becoming dominant spoilage organisms for frozen fish (Gram et al., 2002).

Table 2 shows the different microbial groups associated with the deterioration of fishery products under different intrinsic and conservation conditions. The influence of bacterial activity on the deterioration of fish at low temperatures begins after the death of the animal; however, the spoilage and biochemical changes generated by microbial growth are not marked until the specific spoilage organisms reach certain proportions (Naef et al., 2023). It has been mentioned that aerobic storage levels of 10⁷-10⁸ CFU/g are usually indicative of spoilage fish (Huss, 1998; Gram et al., 2002; Parlapani et al., 2018), where even in studies carried out in cephalopods stored at 2°C for 5 days, these levels are even lower, with aerobic plate count ratios of 6.6 log CFU/g and specifically for Pseudomonas spp., of 4.8 log CFU/g, which present rejection in sensory evaluation due to deterioration (Parlapani et al., 2018).

Within the group of psychrophiles, there are also microorganisms of interest in the food industry and public health such as Listeria monocytogenes (Pascual and Calderón, 2000). It has been pointed out that the presence of Pseudomonas in protein foods can enhance the growth of L. monocytogenes due to the proteolytic activity and the release of highly metabolizable peptides in contaminated products, so the production and preservation conditions of products must be strict since contamination by both microorganisms can make them a serious risk to the health of consumers (De Fernando et al., 1995).

On the other hand, in the spoilage of fish, the presence and growth of different species of the Vibronaceae, Enterobacteriaceae, and Pseudomonadaceae families have been associated with the formation and accumulation of biogenic amines such as histamine, putrescine, cadaverine, tyramine, agmatine, and tryptamine due to the activity of decarboxylase enzymes on free and tissue amino acids such as histidine, ornithine, lysine, tyrosine, arginine and tryptophan respectively, affecting the health of consumers of fish and products through poisoning in particular due to the presence of high histamine levels (Bermejo, 2003; Barba Quintero et al., 2012; 2013; Escalante Reséndiz et al., 2014; Del Rio et al., 2020).

Control and Prevention of Fish Spoilage and Diseases Transmitted by Consumption

The most frequent causes reported in cases of foodborne illnesses are inadequate food handling, cross-contamination, contamination from the environment, contaminated water and raw materials, poor processing and preservation of food (refrigerated temperatures), poor cleaning procedures, materials, inadequate design of facilities and equipment (Tirado et al., 2005; Alerte et al., 2012; Espinosa et al., 2014).

The useful life and safety of food under refrigeration conditions are determined by the cumulative effects of temperature during the phases of the food chain such as the handling of raw materials, production processes in all stages of storage and transport required for its commercialization. Therefore, inadequate temperature management is reflected in serious problems of microbiological stability and risk to the health of consumers (Tirado et al., 2005; Gómez, 2010).

The main measures adopted for the control and prevention of diseases transmitted by food are inspection of the premises, health education, control of handlers, and cold chain (Tirado et al., 2005; Espinosa et al., 2014). For food, constant handling in the cold chain is recommended within a temperature range between -1 and 2°C and not higher than 5°C (Tirado et al., 2005).

In the case of fish contamination with biogenic amines (histamine), this is reduced by applying good hygiene conditions and practices in the handling and conservation of fish, specifically adequate temperature control (close to 0°C) from capture until consumption (Barba Quintero et al., 2012). Through food legislation around the world, to safeguard the health of consumers, the European Union sets maximum limits for histamine content with 100 mg/kg of fish, the Food and Drug Administration (FDA) 50 mg/Kg and in Mexico, health authorities establish the maximum limit of 100 mg/Kg of fish to avoid poisoning (NOM-242-SSA1, 2009; Pérez and Jimenez-Colmenero, 2010; Barba Quintero et al., 2013).

Characteristics of Microorganisms Associated with Fish Spoilage

Table 2: Microorganisms and typical conditions associated with the spoilage of fish and products (Gram et al., 2002)  

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>F</th>
<th>T</th>
<th>A</th>
<th>pH</th>
<th>AW</th>
<th>N/S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shewanella, Pseudomonas</td>
<td>Fish</td>
<td>Low</td>
<td>Aerobic</td>
<td>High</td>
<td>High</td>
<td>Amino acids</td>
</tr>
<tr>
<td>Photobacterium</td>
<td>Fish</td>
<td>Low</td>
<td>Anaerobic</td>
<td>High</td>
<td>High</td>
<td>Amino acids</td>
</tr>
<tr>
<td>Shewanella LAB</td>
<td>Smoked fish</td>
<td>Low</td>
<td>Anaerobic</td>
<td>High</td>
<td>Low</td>
<td>Amino acids</td>
</tr>
<tr>
<td>Enterobacteriaceae, LAB</td>
<td>Marinated fish</td>
<td>Low</td>
<td>Anaerobic</td>
<td>High</td>
<td>Low</td>
<td>Amino acids, simple carbs</td>
</tr>
</tbody>
</table>


Table 2: Microorganisms and typical conditions associated with the spoilage of fish and products (Gram et al., 2002)
In the meat industry, one of the main causes of deterioration and decreased shelf life is the growth of psychrotrophic bacteria capable of growing between 0 and 4°C at a very slow rate, but growth can be accelerated when there are abuses of temperature at some point in the cold chain. The shelf life of a refrigerated product can be shortened in half if it remains between 7 and 10°C. In addition, the risk of contamination and the growth of pathogens of sanitary interest at temperatures between 10°C and 5°C, such as Yersinia enterocolitica and Listeria monocytogenes, must be considered (Tirado et al., 2005).

In post-capture fish operations, proper hygienic handling can reduce the spoilage microbiota responsible for the formation of nitrogenous compounds related to the appearance of typical spoiled fish odors, such as the formation of ammonia and trimethylamine, as well as other low-level, molecular weight, volatile nitrogenous components (Periago et al., 2016). Furthermore, to control and reduce the microbiological load of fish and products, different technologies, compounds, and preparations have been used during storage, extending their commercial life and favoring their safety, being the most common use of ice, refrigeration, freezing, salting, acidification, use of essential oils, the addition of chitosan, packaging in modified atmospheres, high pressure, barrier technologies, biopreservation, among others (Periago et al., 2016; Castillo-Jiménez et al., 2017; Naef et al., 2023).

Pseudomonas species, despite being associated with spoilage processes in fish and products, which in some conditions can become human pathogens, causing infections through consumption, are not classified as foodborne pathogens in various regions of the world, for which the control tests for fish and shellfish include some species such as Escherichia coli, Enterobacter sakazakii; C. botulinum, Listeria monocytogenes, Staphylococcus aureus and Salmonella sp., but not Pseudomonas spp., (Ardura et al., 2013; Kačániová et al., 2019). Since they are cosmopolitan microorganisms and part of the fish microbiota and have an impact on human and animal health, their incorporation into the detection of pathogens in these foods of global distribution and consumption is advised.

**Microbiological Analysis of Fish and Products: Pseudomonas**

The group of psychrophilic bacteria to which Pseudomonas spp., belongs are the main ones responsible for food spoilage (fish) at low storage temperatures and these can be fully quantified, acting as microbiological indicators of quality (Huss, 1998; Pascual and Calderón, 2000; Gram et al., 2002; Franzetti and Scarpellini, 2007; Hernández Urzúa, 2016; Parlapani et al., 2023; Naef et al., 2023). In addition to aspects associated with deterioration by psychrophiles, there is a health interest, since within this group there are pathogenic species for humans such as Yersinia enterocolitica, Listeria monocytogenes, and Clostridium botulinum type E, where their isolation requires specific techniques (Pascual and Calderón, 2000). Therefore, the detection and quantification of psychrotrophic, including Pseudomonas, in refrigerated protein foods, contributes to the evaluation of quality, potential shelf life, and influence of processing procedures on the contamination of food products (Mead, 1985; El-Aziz, 2015).

For the quantification of total psychrophilic in foods, different methods have been developed, such as the one proposed by Pascual and Calderón (2000), where the dilution of the sample is carried out in 1/4 Ringer’s diluent solution and the sowing in a selective medium for Pseudomonas aeromonas (GSP agar), incubation at 17°C/5 days and subsequent counting. Additionally, there is the method proposed by the official Mexican standard (NOM-092-SSA1, 1994) that involves the dilution of the sample in peptone water, inoculation on standard agar beads or tryptone-yeast extract agar, incubation at 5±2°C/7-10 days and subsequent count.

On the other hand, Pseudomonas spp., are microorganisms that can be isolated and identified by using various enriched, differential, and/or selective culture media frequently used in the laboratory (peptone water, blood agar, McConkey agar, eosin methylene blue agar, cetrimide agar, Cystine-Lactose-Electrolyte-Deficient agar (CLED) and Cetrimide-Fucidin-Cephaloridine (CFC) agar (Jiménez et al., 2004; Romero Cabello, 2005; Farias, 2015; Lopardo, 2016; Benie et al., 2017b; Murray, 2018). For P. aeruginosa, during cultivation, it can generate the production of pigments (pyocyanin and/or pyoverdine) in addition to a typical fruity odor for identification (Lopardo, 2016). Gram staining is a characteristic test for the identification of potential isolates, as well as biochemical tests such as positive β-hemolysis, negative indole, and methyl red, negative hydrogen sulfide, no lysine decarboxylation, positive gelatin liquefaction, citrate, catalase, and oxidase positive (Jiménez et al., 2004; Romero García, 2005; Farias, 2015; Benie et al., 2017a-b; Murray, 2018). Likewise, through different biochemical tests displayed in Table 3, the identification of species of the fluorescent group associated with diseases can be conducted.

**Table 3:** Biochemical analysis of species identification of the fluorescent group of Pseudomonas (Lopardo, 2016)

<table>
<thead>
<tr>
<th>Analysis</th>
<th>P. putida</th>
<th>P. fluorescens</th>
<th>P. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelatine</td>
<td>-</td>
<td>+</td>
<td>V (46%)</td>
</tr>
<tr>
<td>42°C growth</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Nitrates</td>
<td>-</td>
<td>V (19%)</td>
<td>V (74%)</td>
</tr>
<tr>
<td>Pyocyanin</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Pyoverdine</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

: Variable, + positive reaction, - negative reaction
The specific detection and isolation methods of *Pseudomonas* spp., in food is the one proposed by Jiménez et al. (2004), where the dilution of the sample is carried out in peptone water, inoculation in ceramid agar and incubation at 44.5°C/48 h. for subsequent growth analysis. Likewise, there is the method of the International Organization for Standardization ISO 13720: 2010 for the enumeration of presumed *Pseudomonas* spp., in meats and products.

The method (Mead, 1985), which involves the use of selective media Cetrimide-Fucidin-Cephaloridine (CFC), allows the quantitative recovery of pigmented and non-pigmented *Pseudomonas*, while inhibiting most other organisms by incubating at 30°C/1 weeks (Mead, 1985; Chabela et al., 2008). It has been reported that the use of CFC agar and variants is the most appropriate for the isolation and growth of *Pseudomonas* (Stanbridge and Board, 1994; Tryfinopoulou et al., 2001; Parlapani et al., 2015; Benie et al., 2017a), where the differentiation with *Enterobacteriaceae* occurs through the application of various biochemical tests such as oxidase or metabolism of organic compounds such as arginine (Stanbridge and Board, 1994; Tryfinopoulou et al., 2001). However, it has been noted that this medium has drawbacks since it does not discriminate colonies of *S. putrefaciens*, there is no rapid effective method for their differentiation and it involves the application of biochemical tests such as trimethylamine oxide reduction and H₂S formation (both negative for *Pseudomonas*). Therefore, it has been considered that the use of CFCs may not be the most appropriate for fish, where the selectivity of this medium is affected by the storage conditions of the product and the proportion of interfering organisms (*S. putrefaciens* and *Enterobacteria*) (Tryfinopoulou et al., 2001).

On the other hand, the use of methods that involve traditional (phenotypic) analysis using culture media and evaluation of morphology, biochemical and metabolic properties of microorganisms, is common because its implementation and cost are feasible, but it may lead to limitations such as a longer time to obtain results, dependence on the analyst's experience. In addition, there may be a lack of correspondence between the phenotypic characteristics of the isolate under study and the reference strains, thus traditional methods can perform the isolation and identification with greater probability but may not be definitive. To improve the inherent problems of traditional identification systems, modern genotypic methods have been developed for bacterial detection and identification, considered complementary or alternative procedures to traditional ones (Bou et al., 2011). The advantages of modern methods are greater speed, good detection limit, specificity and sensitivity, easy automation, and the ability to process a larger number of samples for analysis (Palomino-Camargo and González-Muñoz, 2014).

Modern methods of analysis based on the Polymerase Chain Reaction (PCR) and its different variants have been proposed for the detection and quantification of *Pseudomonas* in fish and products, either in implications associated with aspects of safety and/or deterioration using genus-or species-specific site markers (Reynisson et al., 2008; Ardura et al., 2013). In the case of *Pseudomonas* spp., the 16S rRNA gene (Böhme et al., 2010; Ardura et al., 2013; El-Aziz, 2015; Kačáňová et al., 2019), rpoB gene (RNA polymerase β subunit), genes encoding virulence factors lasB (elastase), exoS (exoenzyme S), algD (alginate) and plcH (phospholipase) in the case of *Pseudomonas aeruginosa* (Benie et al., 2017b). Likewise, the carA gene (carbamoyl phosphate synthase, subunit (a) has been used, which provides the glutamine aminotransferase activity necessary for the elimination of the ammonia group of glutamine in the biosynthesis of pyrimidines and purines that is present in the genus *Pseudomonas* spp. (Reynisson et al., 2008).

Additionally, genome sequencing has been used for the analysis of pathogenic bacterial communities and/or those associated with the deterioration of aquatic products such as *Pseudomonadales*, *Moraxellaceae*, *Shewanella*, *Comamonas*, *Carnobacterium*, *Vagococcus*, *Vibrio* sp., *L. monocytogenes* or *C. jejuni*. genome sequencing can complement the study through classical microbiological methodologies or even contribute to the detection of microorganisms when methodologies that involve culture are difficult, short-term or simultaneous results are required (Ferrario et al., 2017; Parlapani et al., 2018; Parlapani, 2021; Liu et al., 2022). Another modern fast and reliable analysis method is Matrix-Assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) mass spectrometry, which is based on the study and characterization of the protein profile of a bacterial isolate for identification (Bou et al., 2011; Ashfaq et al., 2022). This method has been used for the diagnosis and microbiological identification in samples of wild fish and from aquaculture activities (Kačáňová et al., 2019; Cardoso et al., 2021), environmental samples (Ashfaq et al., 2022) and various foods of aquatic origin (Böhme et al., 2010).

**Conclusion**

Fish is considered a nutritious food, marketed and consumed globally, being an important part of the human diet and whose consumption trend is increasing due to the population's search for a healthier lifestyle and diet. Due to the intrinsic properties of fish, it is susceptible to contamination along the food chain by various bacteria, which are associated with its spoilage or outbreaks of diseases in humans, either through direct contact or consumption.

For bacteria of the genus *Pseudomonas* spp., factors such as the pathogenic nature of its different members,
including *Pseudomonas aeruginosa*, the diverse number of diseases they cause in the community, hospital, and animal production (aquaculture) environment, their resistance to antimicrobials, in addition contributing to the spoilage and waste of food has led them to be identified as being of interest in human health, animal health and the food industry.

*Pseudomonas* spp., are microorganisms of a cosmopolitan nature, even associated with the natural microbiota of fish and as contaminants in stages after the capture or harvest of fish. Contamination by these hazards can give rise to diseases that cause detriment to animal health, fishing, and aquaculture production, and negative effects on human health due to contact or consumption of contaminated food, especially in individuals belonging to vulnerable groups (chronic infections, metabolic disorders, complications in the immune system, among others) and finally economic losses due to the short shelf life and availability of food due to the spoilage that they cause.

Until now, to reduce the contamination of fish and products by various pathogenic microorganisms and to ensure that these foods do not become vehicles for diseases, various basic actions have been established throughout the different phases of the food chain, such as good hygiene practices and implementation of microbiological criteria for products, but, in the particular case of *Pseudomonas* ssp., it has not been contemplated to introduce information regarding microbiological criteria in fish and products intended for human consumption, despite the information in microbiological studies referring to this group microbial and its implications on human health and food spoilage; in addition, it has been reported in research around the world that the incidence of foodborne diseases has been favored by multiple factors such as climate change, demographic changes, changes in dietary habits in the population, the emergence of pathogens resistant to antimicrobials, globalization in food production and marketing, changes in food processing and preservation methods, among others.

To safeguard the health of a growing population and demand for food, the quality and safety of fish become an essential and non-negotiable factor. For this, it is necessary to generate more research regarding the safety of fish and products and the analysis of risk to the health of consumers regarding *Pseudomonas* ssp., this information could be supported and will promote the consideration of being incorporated as a criterion or specification microbiological contamination in related products and will contribute to the emphasis on the implementation or reinforcement of hygiene practices in the food chain to control and reduce this or other biological contamination.

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**Author’s Contributions**

Alejandro De Jesús Cortés-Sánchez: Conceptualization and organization of the study, search, and analysis of information, written.


**Ethics**

This article has not been published elsewhere before. The author has also confirmed to all authors involved in this study to read and accept the content of this article and that there are no ethical issues involved.

**Conflict of Interest**

The authors declare that they have no conflict of interest in the publication of this manuscript.

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